DETERMINING THE ROLE OF PULMONARY FIBROSIS IN THE ETIOLOGY OF LUNG CANCER

FINAL REPORT

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ABSTRACT

The purpose of this study was to determine the type and extent of pulmonary fibrosis experimentally induced in hamsters by intratracheal instillations of quartz, fibrous glass, hydrated alumina, or a 1:1 mixture of quartz and ferric oxide. The objective was to determine a dose of each material which would induce a pronounced pulmonary fibrosis without compromising the life expectancy of the animal.

Four dose levels of each of the materials were administered in 15 weekly intratracheal instillations to groups of 25 hamsters beginning at 11 weeks of age. Survival and microscopic evidence of pulmonary fibrosis were determined and the results compared to similar data from a group instilled with saline only and a cage control group.

Dose-related decreases in survival were evident for the groups instilled with the two highest doses of quartz or quartz and ferric oxide. A dose-related increase in lung weight was most apparent in those groups instilled with quartz or quartz and ferric oxide. A correlation between the incidence and severity of alveolar septal fibrosis and the dose of instilled material was evident in most groups, being strongest in those groups exposed to quartz or quartz and ferric oxide. These materials induced the most intense pulmonary fibrotic response.

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INTRODUCTION

Epidemiological studies have suggested that pulmonary fibrosis induced by inhalation of certain particulate materials may contribute to the development of lung cancer. Egan et al. (1979) reported evidence of a significant increase in the incidence of lung cancer in foundry workers, an occupation that involves potential respiratory exposure to mineral dusts, including ferric oxide, and to potentially carcinogenic polycyclic aromatic hydrocarbons. Boyd et al. (1970) noted an increased lung cancer incidence in hematite miners occupationally exposed to aerosolized ferric oxide, silica, and radon. Other occupations which involve high potential for exposure to iron and/or silica, and for which increased lung cancer incidence has been reported, include asbestos workers (McLaughlin, 1956), metal grinders (Kennaway and Kennaway, 1947), and chromate workers (Bidstrup and Case, 1956).

Particulate materials have played a key role in the experimental induction of pulmonary tumors. The success of Saffiotti's intratracheal instillation model (1968) was attributed in part to the role of the ferric oxide carrier dust in transporting benzo(a)pyrene across the barrier of the bronchiolar and alveolar epithelial cell membranes. A considerable amount of research has been done in attempting to determine the role of the carrier dust in the production of respiratory tract tumors using Saffiotti's method (Creasia and Nettesheim, 1974; Stenback, 1974; Port et al., 1973; Harris et al., 1971). However, the exact role of the carrier dust in the induction of pulmonary neoplasia remains unclear.

Although it apparently possesses some cocarcinogenic properties, ferric oxide dust is relatively innocuous in the induction of pulmonary irritation and fibrosis (Creasia and Nettesheim, 1974). One proposed mechanism by which ferric oxide exerts its cocarcinogenic effect is stimulation of cellular proliferation (Creasia and Nettesheim, 1974). If this proposed mechanism is correct, it seems logical that particulate materials such as silica, which stimulate a much more pronounced proliferative and inflammatory response in the lung (Spencer 1977) may also possess much greater cocarcinogenic potential than ferric oxide. Pylev (1979) recently reported pulmonary tumors induced in rats intratracheally exposed to silica before or simultaneously with benzo(a)pyrene, whereas no tumors were induced with benzo(a)pyrene or silica alone. However, the method of instilling the benzo(a)pyrene without silica was not provided in this paper.

Further study is clearly needed on the role of particulate-induced pulmonary fibrosis in the etiology and/or pathogenesis of pulmonary cancer. A logical first step is development of an animal model in which intense pulmonary fibrosis is induced by particulate material, but the lifespan of the experimental animal (and thus the period of time during which the animal may

develop a neoplastic response) is not affected by the instilled material. This animal model should also have a predictable neoplastic response to intratracheally instilled carcinogens such as benzo(a)pyrene. If the particulate materials induce pulmonary fibrosis in conjunction with the presence of a known carcinogen such as benzo(a)pyrene, then the role of these particulate materials as potential pulmonary cocarcinogens may be assessed.

The objective of this study was to carry out the first step of the model described above, i.e., to induce varying degrees of pulmonary fibrosis using different concentrations of four particulate materials (quartz, ferric oxide, hydrated alumina, and fibrous glass), all of which have potential for occupational exposure. Doses of each material were chosen, based upon data in the literature, in an attempt to obtain dose-response data and to determine a dose of each material which would produce pronounced pulmonary fibrosis without compromising the life expectancy of the animal.

MATERIALS AND METHODS

The experimental protocol required intratracheal instillation of fifteen weekly individual doses to each of 25 hamsters per group to obtain the total doses indicated in Table 1. These doses were chosen on the basis of information available from the literature, from data previously generated at our laboratory, and from personal communication with scientists at other laboratories. Very little information was available on experimental pulmonary fibrosis in hamsters, and most of the data used in choosing the doses were from fibrosis studies in rats or guinea pigs. In attempting to extrapolate from another species to hamsters, total lung volumes of approximately 5 ml, 9 ml, and 15 ml were used for the hamster, rat and guinea pig, respectively.

The range of doses of quartz was based on studies in our laboratory using multiple intratracheal instillations of quartz in rats at weekly intervals (Renne et al., 1980), in which a total dose of 90 mg (30 mg weekly) produced a pronounced fibrotic response in the lungs and tracheobronchial lymph nodes by 8 months postexposure, but no deaths attributable to fibrosis occurred. Individual weekly doses of 3.3 mg quartz produced a total dose of 50 mg, the approximate equivalent dose in the hamster lung of 90 mg in the rat lung.

The range of doses of quartz and ferric oxide was based on the amount of quartz present, since the fibrogenic potential of quartz was considered to be much greater than that of ferric oxide. Lesion development and morphology was predicted to parallel that of the quartz-only groups.

The range of doses of hydrated alumina was based on two published studies. (a) Engelbrecht et al. (1959) induced "intense" fibrosis (and some mortality) in one year in rats with a single intratracheal instillation of 500 mg/kg of aluminum hydroxide. This represents a total dose of 40 mg for an 80-gram hamster, or fifteen doses of 2.67 mg each. (b) Stacy et al. (1959) induced in rats a "grade 3 fibrosis, collagenous and still somewhat cellular" with a single instillation of 70 mg of aluminum hydroxide. This represents a dose of 350 mg/kg, or 28 mg total dose for an 80-gram hamster, corresponding to 1.87 mg/dose if given in fifteen instillations. The data in (a) and (b) above indicated an "ideal" individual dose of aluminum hydroxide should be between 1.87 mg and 2.67 mg; however, if one takes into account the likelihood that hamsters, like guinea pigs and mice (Engelbrecht et al., 1959), may be less susceptible than rats to dust-induced fibrosis, this range could be conservative. Also, fractionating the total dose into fifteen weekly doses could decrease the fibrotic response. For these reasons, the chosen dose range of hydrated alumina (Table 1) had a 100-fold difference between the highest and lowest doses, with the calculated "ideal" dose range near the "M" or next-to-lowest dose, to take into account the considerations noted above.

The range of doses of fibrous glass was based on two published studies. (a) Wright and Kuschner (1975) induced "significant peribronchiolar fibrosis" in guinea pigs by intratracheal instillation of a total of 12 mg of fibrous glass with fiber dimensions of 0.1 to 1.0 μm diameter by 10 μm length. The equivalent dose for a hamster, based on interspecies differences in lung volume (5 ml vs. 15 ml for guinea pigs), is 4 mg which, divided into 15 doses, represents 0.27 mg per dose. (b) Pickrell, et al. (1978) induced biochemical changes indicative of early fibrosis (increased total pulmonary collagen) in hamsters sacrificed 3.5 months after instillation of a total of 2.0 mg of glass fibers, of which only 10% had a fiber diameter less than 5 μm. Another group of hamsters in this study, instilled with a total of 7 mg of glass fibers, of which 24% had a diameter of less than 5 μm, had 100% mortality within four weeks postexposure due to acute pulmonary injury. The difference in the data noted in (a) and (b) above indicated a wide variation in predicted ideal dose range of fibrous glass. We chose a 200-fold dose range as indicated in Table 1.

The hydrated alumina, ferric oxide, and crystalline quartz samples were physically characterized by NIOSH as to median, average, mass median, and aerodynamic diameters. The fibrous glass sample was characterized by median diameter, median fiber length, number of fibers of various lengths (0.5 to 30 μm), and number of fibers of various diameters (0.25 to 6.0 μm). These dimensions were to verify the specification in the contract that the fibrous glass sample was to consist of fibers 10 to 20 μm in length and 1 to 2 μm in diameter.

Samples of the particles for characterization were prepared from the bulk powders by dispersing a small quantity of the powder in a 0.05% solution of Aerosol OT* in deionized water. The solutions were placed in an ultrasonic bath for 10 min and then stirred magnetically for 30 min. Aliquots of these suspensions were then filtered through a 0.1-μm pore size Nuclepore filter.† The filters were attached to carbon planchets with colloidal graphite and examined directly with a scanning electron microscope (JEOL, Model JXA-50A), equipped with an energy dispersive x-ray spectrometer system (EG&G Ortec Model EEDS II) and an image analysis system (LeMont Scientific, Model B-10) using a backscattered electron image. A minimum of 1,000 particles of each sample was sized.

Five hundred male outbred Syrian Golden hamsters (Lak:LVG), 6 weeks of age, were received in two lots on 11/7/79 and 11/9/79 from Charles River Breeding Laboratories (Lakeview), Wilmington, Massachusetts. Ten hamsters were necropsied upon arrival. Lung and nasopharynx were cultured for

*American Cyanamid Co.
†General Electric Co.

Table 1. Individual doses instilled weekly (mg) in 0.5 ml saline, and total amounts instilled (mg).

Material	Dose (mg) of Material in 0.5 ml Saline			
	Low (L)*	Medium (M)*	High (H)*	Very High (V)*
Min-U-Sil Quartz				
Individual	0.03	0.33	3.3	6.0
Total	0.45	4.95	49.5	90.0
Min-U-Sil Quartz and Ferric Oxide				
Individual	0.03 each	0.33 each	3.3 each	6.0 each
Total	0.45 each	4.95 each	49.5 each	90.0 each
Fibrous Glass				
Individual	0.05	0.5	1.0	10.0
Total	0.75	7.5	15.0	150.0
Hydrated Alumina				
Individual	0.2	2.0	5.0	20.0
Total	3.0	30.0	75.0	300.0

*Dose-level groups with 25 hamsters in each group

bacterial pathogens; histopathologic examination was performed on tissue samples from lung, trachea, liver, ileum, and any gross lesions observed at necropsy. Serum samples were screened for viral antibody titers by Microbiological Associates Laboratory in Bethesda, Maryland. During quarantine the hamsters were group-housed, five per cage, in solid-bottom cages with bedding.* They were fed a standard laboratory rodent diet† and were provided water ad libitum. During the instillation and postexposure periods the hamsters were individually housed in stainless steel wire cages. They were fed the same diet as during quarantine in slot feeders, and were provided water ad libitum. Temperature in the animal room was controlled to 23 ± 2°C; relative humidity was maintained at 50 ± 15 percent.

Four hundred fifty (450) hamsters, approximately 11 weeks of age, were divided (no formal randomization) into 18 groups of 25 animals each (four dose levels each of four materials plus one saline control group and one cage control group), and individually identified by ear notching. Intratracheal instillations began at approximately 11 weeks of age for all groups except those instilled with hydrated alumina; due to a delay in shipping of material, instillations of hydrated alumina began at approximately 12 weeks of age. Instillations began on December 12 and December 21, 1979 and concluded on March 19 and March 28, 1980.

For the repeated intratracheal instillations, each hamster was anesthetized with methohexital sodium.‡ Initial instillations were performed using an anesthetic dose range of 0.30 ml to 0.44 ml of a 1% solution of methohexital sodium per 100 grams body weight administered intraperitoneally. After encountering problems of inconsistency in plane of anesthesia at this range of anesthetic dose, we found that a dose of 0.42 ml of a 1% solution of methohexital sodium per 100 gram body weight administered intraperitoneally provided a level of anesthesia sufficient to allow intratracheal instillation without danger of death from respiratory distress. Test materials were instilled as the animals began to recover from surgical anesthesia. An intratracheal speculum was inserted through the larynx into the upper section of the trachea, and the test material, diluted in sterile saline to a total volume of 0.5 ml, was instilled into the lungs with a syringe via a catheter inserted into the speculum.

Immediately before each weekly instillation procedure, the appropriate amount of each particulate material was removed from its container, weighed and placed into a sterile flask; sterile saline solution was then added. To insure adequate dispersal of the material in the saline solution, the suspension was sonicated for 15 min at 200 watts. During the intratracheal instillation procedure, the suspension being instilled was kept homogenous by stirring on a magnetic stirrer.

A number of acute deaths occurred during and shortly after the first instillations. There appeared to be no real correlation between death and the type or amount of material instilled. Some animals appeared to

*San-I-Cel, Paxton Processing Company, Paxton, IL
†Wayne Lab Blox, Allied Mills, Chicago, IL
‡Brevital Sodium; Eli Lilly & Company, Indianapolis, IN

die from an overdose of anesthetic, others were found at necropsy to have damage to the trachea, still others appeared to suffocate from the inability to respire through the instilled material. All 31 animals that died during the week after the first instillation of materials were replaced by other animals from the same shipment. These replacements were given the identical animal number to the dead animals they replaced. The only data used from the original animals were the initial body weights and body weights at instillation; survival, histopathology, and all other data do not include these original animals. During week seven, twelve more animals, again from the same shipment, were added to the study to replace some of the animals dying between weeks two and seven. These additional animals were given the same animal number as animals from their group that had died, with the addition of the letter "A". All data from all animals dying during weeks 2-7 are included in this report. The selection of appropriate animals to replace during weeks 2-7 was based on the number of animals remaining in each group, since only a limited number of appropriate replacement animals were available. All replacement animals were instilled a total of 15 times on a weekly basis as specified in the protocol. Since some of these early acute deaths appeared to be due to anesthetic overdose, we changed our anesthetic injection method to a more accurate mg/kg dose, which resulted in a decrease in mortality. We also began using a smaller (#5 French) outer catheter for intratracheal instillation; originally we used a #8 French catheter.

Due to mortality resulting from anesthesia or improper instillation technique (i.e., evidence of perforation, rupture, or massive hemorrhage of the larynx, trachea, or lungs), the number of "effective animals" was determined for each group for the purpose of accurately determining the effect of the instilled materials on survival. The effective number of hamsters per group was equal to the original group size of 25, minus the number of technique- or anesthesia-related deaths, plus the number of animals replaced per group. The resultant size of each group for the purpose of survival analysis varied from 23 to 26 hamsters. The group size of 26 resulted from the erroneous assumption that one animal died due to the instillation technique; necropsy revealed that this animal died from other causes.

Hamsters were observed daily by animal care personnel for clinical signs, with special attention given to respiratory signs such as labored breathing, coughing, or nasal discharge. Each animal was weighed at the time of ear notching, weekly during the fifteen instillations, biweekly throughout the postexposure period, and immediately before necropsy. Each group of hamsters was held until survival within the group reached 20%, at which time all remaining animals within the group were killed. All remaining groups, regardless of survival rate within the group, were killed when the animals reached 24.5 months of age. Any moribund animal was killed.

Hamsters were killed by intraperitoneal injection of a lethal dose of pentobarbital sodium.* All animals dying spontaneously or killed were

*Nembutal sodium, Abbott Laboratories, Chicago, IL

necropsied. The thoracic cavity was opened and the trachea and lungs examined in situ. The entire larynx, trachea, and thoracic cavity contents were removed intact. Representative gross lesions were photographed. The heart was removed and the lungs, larynx, trachea, and associated mediastinal tissues were weighed as a unit; the lungs were then inflated with 10 percent neutral buffered formalin to approximately the normal inspiratory volume, and immersed in 10 percent neutral buffered formalin. The remaining organs were then examined grossly, and representative tissue samples from the heart, stomach, liver, kidney, spleen, and any gross lesions observed were fixed in 10 percent neutral buffered formalin.

Following fixation for at least 48 hours, the lungs were trimmed away from the trachea, and a 5-mm-thick horizontal section through the widest part of each lobe was processed for microscopic examination; the tracheobronchial lymph nodes, tracheal bifurcation, and proximal trachea were also processed. These tissues were embedded in paraffin and stained with hematoxylin and eosin. Representative samples of gross lesions in other tissues were processed and stained in a similar manner. Additional sections of selected tissues were cut and stained with hematoxylin and eosin in a few instances to clarify morphologic changes, and special stains for collagen (Masson's trichrome) and amyloid (Congo Red) were done on selected tissues.

All histopathologic evaluation of tissues was done by the principal investigator. This evaluation included "blind" comparison of selected lung sections from animals in various treated and control groups to eliminate bias. This was accomplished by first examining the tissues from animals in all groups with the groups identified to determine the types of lesions being produced, then re-evaluating pertinent lesions by randomizing slides from all animals from instilled and control groups, and re-evaluating these tissues without knowing the group from which each animal originated. Severity of pertinent lesions was graded according to a numerical system from one to five, indicating whether the lesion was minimal (grade 1), mild, moderate, moderate to severe, or severe (grade 5). Pulmonary lesions were also classified as to the area of involvement, using the term "focal" to indicate a single area or focus of involvement, "multifocal" to indicate multiple foci or areas affected, and "diffuse" to indicate that the entire area of all lung sections examined was affected.

A pathology record was maintained on each individual animal indicating date and cause of death, lung weight and body weight at death, and gross and microscopic lesions observed.

Individual lung weight data were analyzed statistically using analysis of covariance. The analysis used dose of instilled material as a main effect, with body weight and survival as covariates.

Survival differences were examined statistically using the BMDP life-table program PIL (Dixon and Brown, 1970). The Kaplan-Meier estimate of the survival function was used. The survival function of the saline controls was compared to the survival function of each of the dose groups with both the Mantel-Cox and Breslow statistics. The Breslow statistic tends to place greater weight on early differences than does the Mantel-Cox statistic. This

estimate of the survival function and these two statistics are appropriate so long as the censoring mechanism (in this case, terminal sacrifice) is conditionally independent of mortality. The particular censuring scheme that was used (20% mortality or 24.5 months of age) does result in independent censoring (Kalbfleisch and Prentice, 1980).

The analysis of the prevalence and grade of pulmonary fibrosis cannot be accomplished by straightforward application of common statistical techniques. The discrete nature of the grade scale indicates that the distributional assumptions of analysis of variance will probably not be satisfied. Additionally, the differential mortality of the treatment groups must be accounted for in the analysis. Because of these potential difficulties, the data were analyzed by two different statistical methods. Consistent results by the two methods reinforce the validity of the conclusions.

Mantel (1963) has extended the Mantel-Haenszel procedure for computing a summary chi-square to the case of a multi-level response to a multi-level factor. This procedure utilizes the natural orderings of the study factor (dose level) and the response (lesion grade). The method may be used with either a discrete or continuous response. The differential mortality is accounted for by stratifying over time, as in the application of the usual Mantel-Haenszel procedure. A test of the resulting summary chi-square is equivalent to a test of the pooled regression coefficient, without the distributional assumptions associated with the usual F test of regression. The test provides power for detecting any progressive association between lesion grade and dose level.

The statistic was computed separately for each material. The time stratification that was used divided the data into three strata defined by $t \leq 440$ days, 440 days $< t \leq 600$ days, and $t > 600$ days, where t is the time on study. The division points correspond to approximately 20% and 50% mortality in the saline controls. The procedure is applied by conceptually dividing the data into three tables (one for each time stratum) with five levels (rows) of the study factor (saline controls plus four dose levels) and six levels (columns) of response (lesion severity grades 0 through 5). The presence of a large number of empty cells in the table does not affect the validity of the statistic, since the table is only a conceptual device for arranging the data.

The second statistical method used was Snell's (1964) technique for scaling ordered categorical data so that analysis of variance can be used. Several analyses were carried out with this technique. First, dose-response was examined by scaling and analyzing each material separately. Because the scaling was different for each material, the adjusted means were not comparable across materials. Therefore, a second analysis was performed with a common scale for all materials. For all of the analyses, the data was stratified as above, and time was used as one factor in a two-way analysis of variance.

RESULTS

Table 2 gives the results of the physical characterization, performed at NIOSH, of the four instilled materials. The ferric oxide sample was highly aggregated; the ultimate particle size appeared to be 0.02 μm. The large mass median and aerodynamic diameter for the hydrated alumina sample indicate the presence of a few very large particles in the sample, which on a weight basis were a considerable part of the sample. These large particles found in the ferric oxide and hydrated alumina samples were not dissociated by the low-level ultrasonication used to prepare the sample for microscopic examination. Since this sonication technique was similar to that used to prepare the suspension for intratracheal instillation, the size data obtained probably reflected the size of the particles the hamsters received.

Forty percent (407/1017) of the particles characterized from the fibrous glass sample had aspect ratios greater than 3:1 and therefore may be considered as fibers. The median length of these fibers was 4.30 μm with a median diameter of 0.75 μm. Fifteen percent (61/407) of the fibers were greater than 10 μm in length. Approximately twelve percent (47/407) of the fibers characterized were greater than 10 μm in length and 2.0 μm or less in diameter.

Necropsy and bacterial culture results from the ten hamsters necropsied upon arrival were not remarkable. At microscopic examination, the majority of lung sections from these animals contained foci of minimal to mild bronchiolar epithelial hyperplasia and alveolar bronchiolization. Of samples from eight hamsters screened for viral antibody titers, all eight had significant (>1:10) titers to Sendai virus, with the highest titer 1:640. These serology and histopathology results indicated the presence of an active Sendai virus infection in the hamsters when received.

The incidence of the most frequently observed clinical signs during the exposure and postexposure periods is summarized in Table 3. The most frequently observed clinical sign was acute, severe loss of body weight (loss of over 10 grams between biweekly weighings). This was frequently observed in conjunction with diarrhea. The incidence of weight loss and diarrhea was similar in instilled and control groups. Samples were taken from the intestinal tracts of several animals with diarrhea for microbiological culture, but no bacterial pathogen was isolated. Subcutaneous edema, not observed in controls, was observed most frequently in the groups exposed to the highest dose of quartz or quartz and ferric oxide. Dyspnea was observed in a small percent of animals instilled with test materials or saline.

Mean body weight data for each instilled group and the saline controls for the entire duration of the study are compared graphically in Figures 1-4. Figure 5 compares mean body weight data from the saline control and cage control groups for the same time period. There is some indication of a dose-related decrease

Table 2. Physical sizing of materials. (mean ± 1 SD)

Compound	Particles	Diameters (μm) Median	Average	Mass Median	Mass Aerodynamic
Quartz (6 samples)	1048 ± 70	0.84 ± 0.07	1.06 ± 0.07	3.14 ± 0.24	5.13 ± 0.40
Ferric Oxide (1 sample)	1754	0.27	0.29	0.60	1.37
Alumina (1 sample)	1002	0.50	0.81	6.31	9.81

		Length	No. of Fibers with Lengths and Diameter ≤ Indicated Values (μm)									
		(μm)	0.25	0.5	0.75	1.0	1.25	1.5	2.0	3.0	4.0	5.0	6.0
Fibrous Glass	1017	0.5	1										
		1.0	7	5									
		2.0	4	45	7								
		3.0	5	17	32	13							
		4.0	1	16	15	18	5						
		5.0		7	3	10	12	8	1				
		7.5		5	14	5	23	12	11	6			
		10.0		1	7	4	2	4	10	10			
		12.5		2	4	5	4	2	1	4			
		15.0		1	2	4	2	3	2	2	2		
		17.5			1	0	1	0	1	1	1	1	1
		20.0			2	1	0	2	1	0			
		25.0				1	4		0	1		1	
		30.0							1				

Table 3. Summary of pertinent clinical observations

Groups	No. of Animals[A]	Severe, Acute Weight Loss[B]	Diarrhea	Subcutaneous Edema	Dyspnea	Total Animals Affected[C]
Quartz, 6.0 mg	25	5 (20)[D]	2 (8)	5 (20)	0 (0)	10
3.3 mg	26	6 (23)	3 (12)	1 (4)	2 (8)	10
0.33 mg	27	8 (30)	7 (26)	1 (4)	1 (4)	13
0.03 mg	25	6 (24)	1 (4)	0 (0)	1 (4)	7
Quartz + Fe_2O_3, 6.0 mg ea	26	7 (27)	3 (12)	3 (12)	0 (0)	12
3.3 mg ea	28	7 (25)	5 (18)	1 (4)	0 (0)	11
0.33 mg ea	25	4 (16)	7 (28)	1 (4)	0 (0)	12
0.03 mg ea	26	6 (23)	5 (19)	1 (4)	0 (0)	10
Glass, 10.0 mg	25	5 (20)	6 (24)	1 (4)	2 (8)	12
1.0 mg	26	5 (19)	2 (8)	2 (8)	0 (0)	8
0.5 mg	25	4 (16)	4 (16)	2 (8)	2 (8)	9
0.05 mg	26	9 (35)	7 (27)	0 (0)	1 (4)	12
Alumina, 20.0 mg	25	8 (32)	9 (36)	0 (0)	0 (0)	15
5.0 mg	25	6 (24)	4 (16)	0 (0)	1 (4)	8
2.0 mg	25	10 (40)	8 (32)	1 (4)	0 (0)	14
0.2 mg	25	10 (40)	9 (36)	1 (4)	0 (0)	16
Saline Controls	27	7 (26)	6 (22)	0 (0)	1 (4)	11
Cage Controls	25	8 (32)	6 (24)	0 (0)	0 (0)	11

[A]Total number of animal per group
[B]Loss >10 g between biweekly weighings
[C]Total number of animals per group affected with one or more of the clinical signs listed.
[D]Percentage of total in parentheses

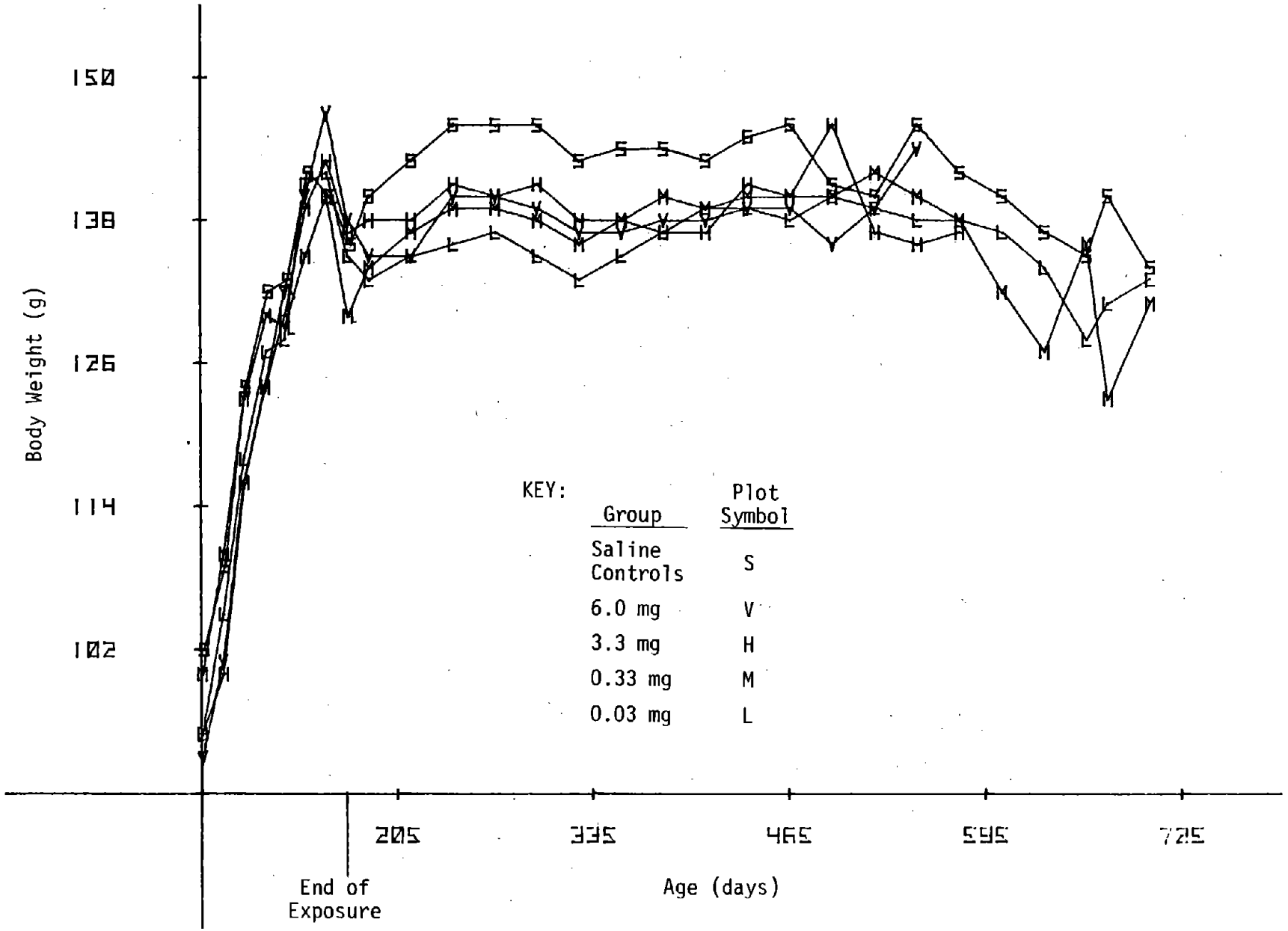

Figure 1. Comparative body weights of quartz groups and saline controls

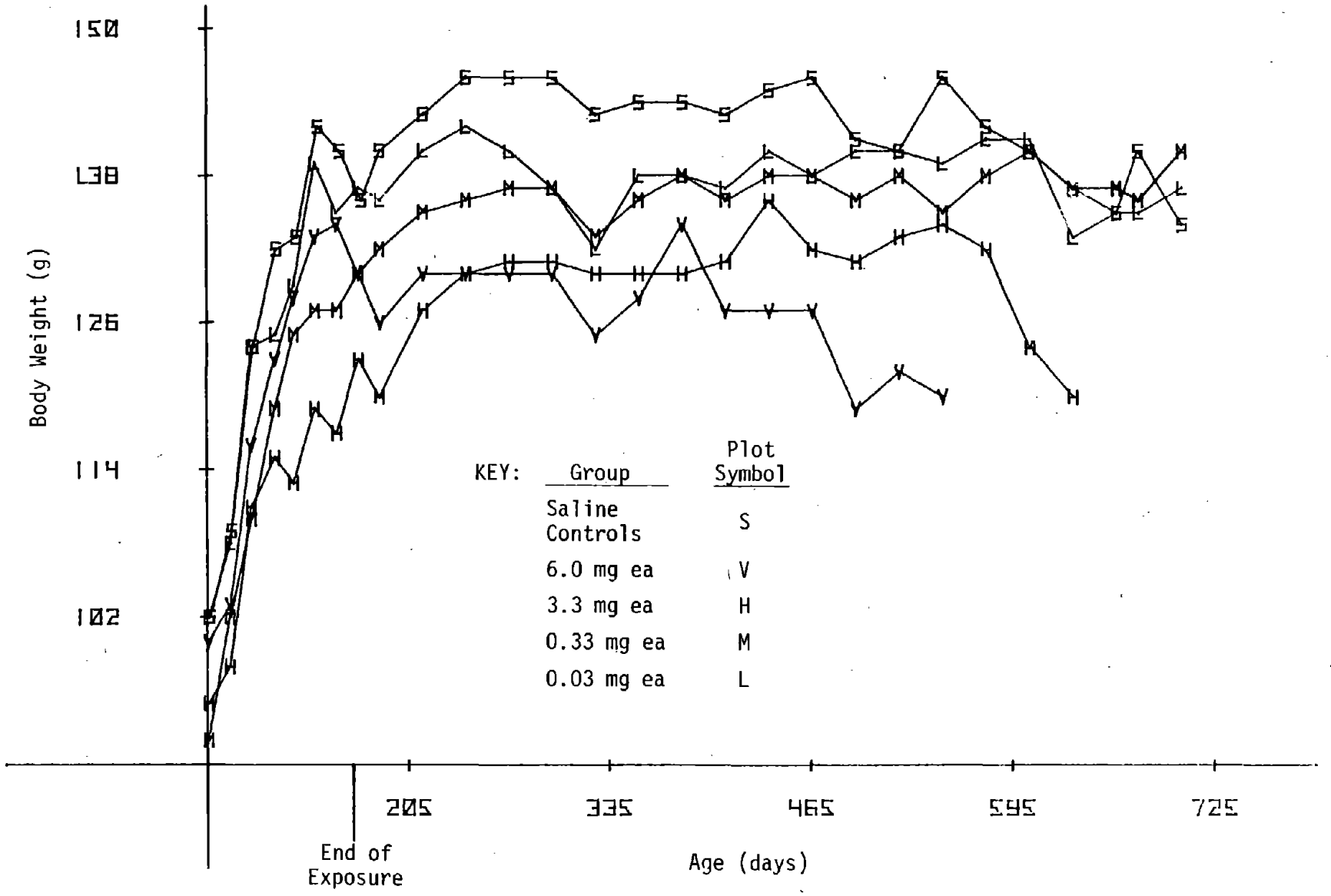

Figure 2. Comparative body weights of quartz and ferric oxide groups and saline controls

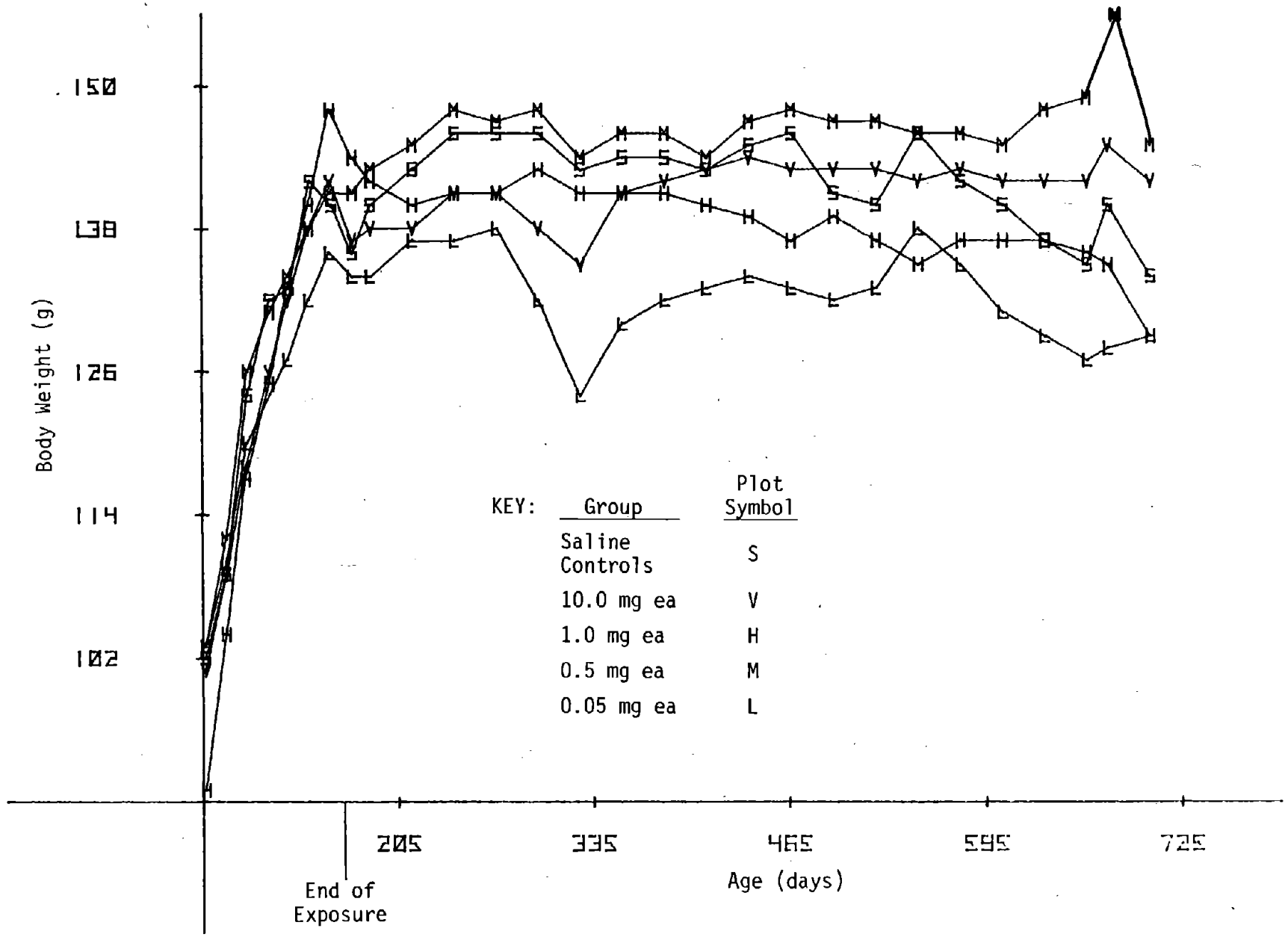

Figure 3. Comparative body weights of glass groups and saline controls

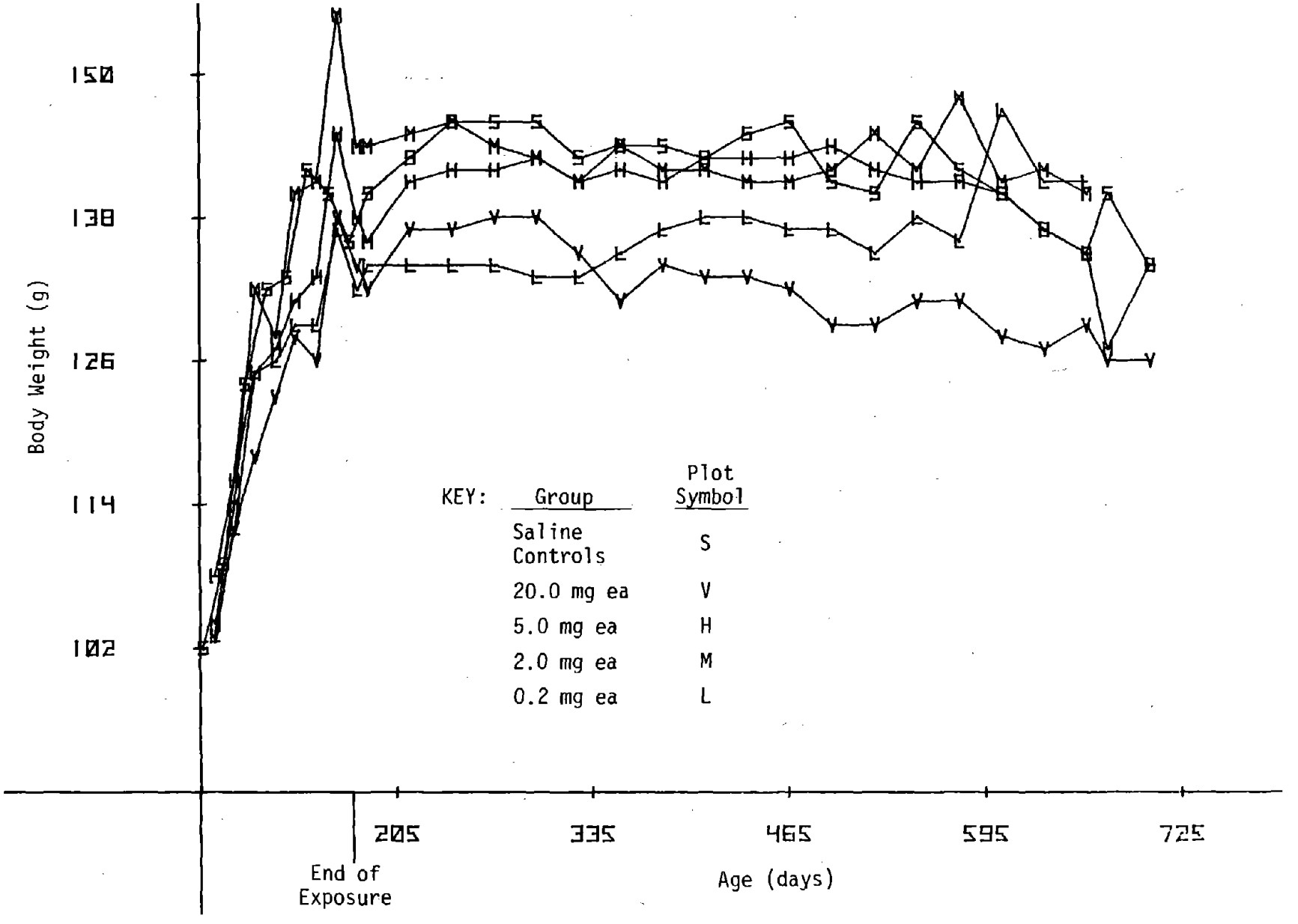

Figure 4. Comparative body weights of alumina groups and saline controls

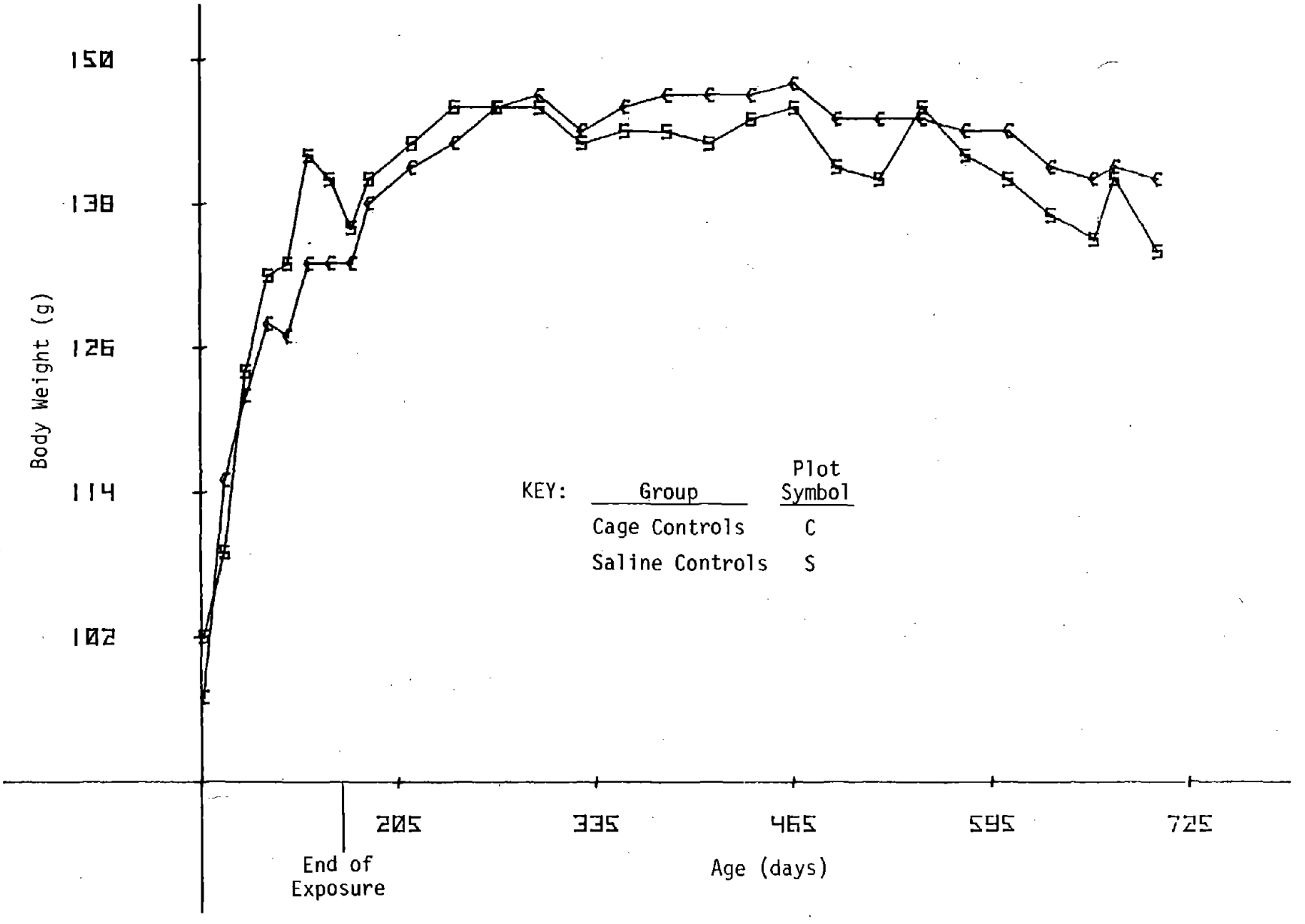

Figure 5. Comparative body weights of saline and cage controls

in mean body weight in the groups exposed to the two highest doses of quartz and ferric oxide and the group exposed to the highest dose of hydrated alumina. The mean body weights of the two control groups and the remaining instilled groups were relatively similar throughout the study.

Tabulations of group mean lung weight raw data and group mean lung weight adjusted for body weight and survival, calculated from survival data and lung and body weights at necropsy of animals dying throughout the study and those terminally sacrificed, are presented in Table 4 and Figure 6. Analysis of covariance indicated significant ($p < 0.0001$) dose-related increases in mean lung weight of groups instilled with quartz or quartz and ferric oxide. There were no significant dose-related differences in lung weight for groups instilled with alumina or fibrous glass. The adjustment for body weight was significant ($p < 0.01$) for all materials, and the adjustment for survival was significant ($p < 0.05$) for the quartz and quartz plus ferric oxide exposed groups. The covariate by dose interaction was not significant for any material.

Survival rates for all groups are shown in Table 5. The mortality of the two control groups is compared graphically in Figure 7. Excluding those deaths that occurred during the exposure period, mortality in the saline control group was similar to that in the cage controls. Comparative mortality of the four dose groups of each material and the two control groups are shown in Figures 8-11. Dose-related mortality rates are evident only in the two highest-dose groups instilled with quartz or quartz and ferric oxide.

The life table analysis is summarized in Table 6. The survival of the two high-dose groups instilled with quartz or quartz and ferric oxide was highly significantly decreased from the survival of the saline controls. The low- and medium-dose groups instilled with hydrated alumina were significantly different from the saline controls. No other significant differences were found.

The incidence of pertinent gross lesions observed at necropsy is summarized in Table 7. Mottling (patchy discoloration) of the lungs was observed in a high percentage of animals exposed to the higher two doses of quartz or quartz and ferric oxide, and in lower percentages of other groups, including controls. The lungs of the groups exposed to quartz and ferric oxide had an orange discoloration from the ferric oxide; the lungs of the group exposed to the two highest doses of quartz were most often described as having yellow mottled areas. In the other groups, the mottling was most often described as dark red, brown, or grey. Discrete pulmonary masses were described at necropsy in eleven hamsters. Enlargement of tracheobronchial lymph nodes was observed in a high percentage of hamsters instilled with the two highest doses of quartz or quartz and ferric oxide, and much less frequently in other groups. An uneven, pale, granular-appearing renal capsular surface was observed with about equal frequency in both instilled and control groups. Generalized edema was noted frequently in both exposed and control groups; however, there was a slightly higher incidence in the group instilled with the highest dose of quartz. The incidence of pale kidneys in the quartz- or quartz plus ferric oxide-exposed groups was indicative of a dose-response. Dilatation of the right ventricle, mottling of the left atrium, and accumulation of clear fluid in the pleural cavity (hydrothorax) were noted with about equal frequency in exposed and control groups.

Table 4. Lung weight data summary

	Mean Lung Weights, g (± SD)			
	Quartz	Quartz + Fe_2O_3	Glass	Alumina
Very High	3.45 ± 0.91 (20)*	3.74 ± 0.58 (19)	2.24 ± 0.71 (23)	2.01 ± 0.71 (22)
High	2.82 ± 0.84 (18)	3.00 ± 0.68 (19)	1.97 ± 0.62 (23)	2.28 ± 1.00 (20)
Middle	2.04 ± 0.78 (20)	2.07 ± 0.80 (24)	2.16 ± 1.08 (21)	2.04 ± 1.36 (21)
Low	1.82 ± 0.74 (20)	1.81 ± 0.69 (21)	1.70 ± 0.69 (20)	1.79 ± 0.79 (22)
Saline Controls	1.72 ± 0.58 (21)			
Cage Controls	2.27 ± 1.06 (25)			

	Mean lung weights adjusted for body weights and survival			
	Quartz	Quartz + Fe_2O_3	Glass	Alumina
Very High	3.50 (20)	3.63 (19)	2.22 (23)	2.04 (22)
High	2.81 (17)	2.98 (19)	1.97 (23)	2.32 (20)
Middle	1.98 (20)	2.10 (24)	2.00 (21)	1.95 (21)
Low	1.83 (20)	1.84 (21)	1.82 (20)	1.70 (23)
Saline Controls**	1.74 (21)	1.78 (21)	1.79 (21)	1.81 (21)

* Sample size in parentheses

** The adjusted mean for the saline control group varies with each test material due to differences in dependency of lung weight on the body weight and survival covariates.

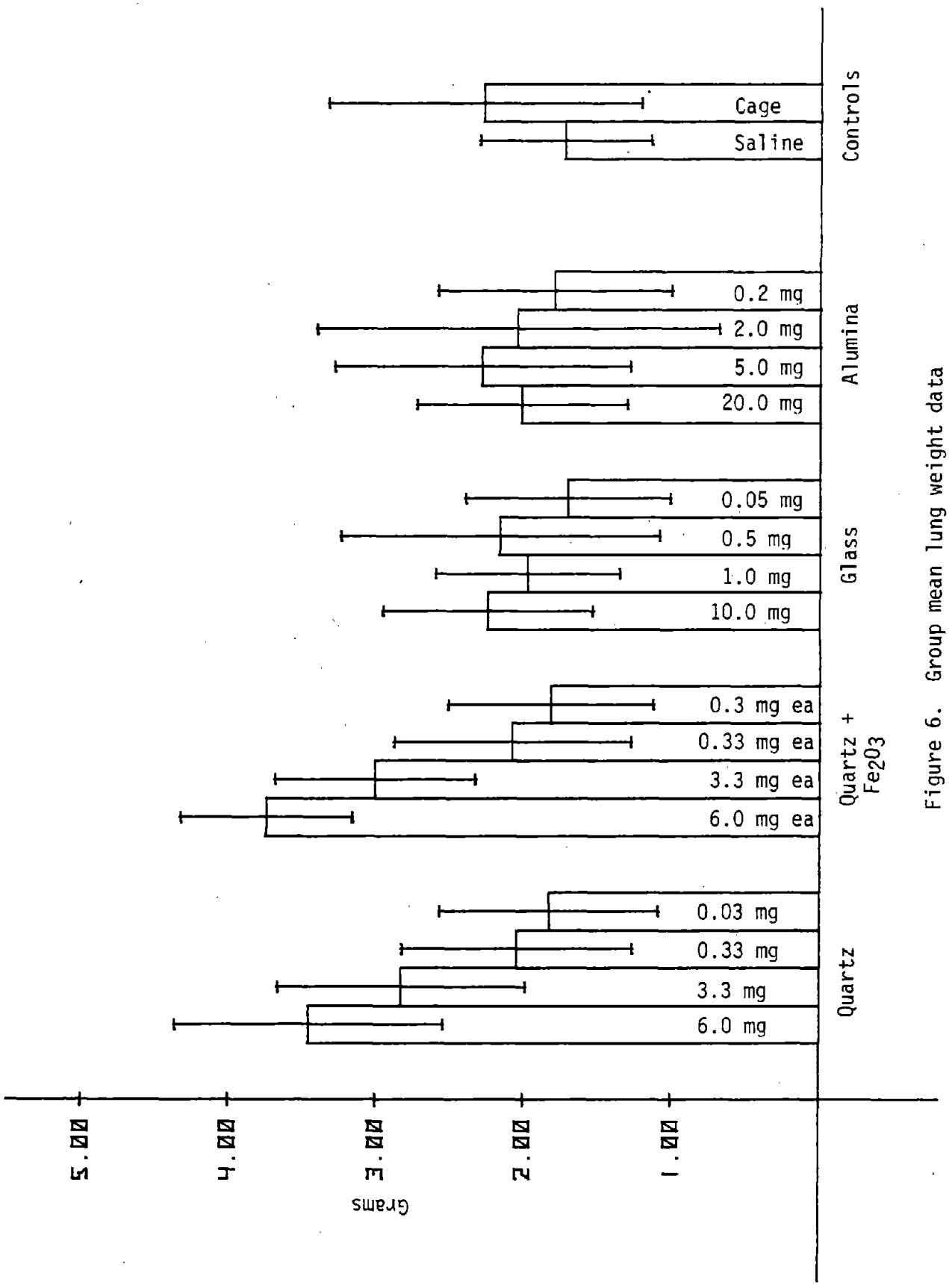

Figure 6. Group mean lung weight data

Table 5. Survival data for all groups

Group	Total Animals	Effective Animals*	Survival, weeks 0	5	10	15	20	30	40	50	60	70	80	90	95
Quartz, 6.0 mg	25	25	25	25	25	23	21	21	20	16	8	0	0	0	0
Quartz, 3.3 mg	25	23	25	24	23	21	21	20	19	18	13	6	0	0	0
Quartz, 0.33 mg	25	25	25	24	23	22	21	21	21	21	19	18	16	9	0
Quartz, 0.03 mg	25	24	25	24	22	20	20	20	20	19	19	17	13	9	0
Quartz + Fe_2O_3, 6.0 mg ea	25	26	25	23	23	22	21	20	16	12	7	0	0	0	0
Quartz + Fe_2O_3, 3.3 mg ea	25	25	25	22	22	22	21	20	18	16	12	10	0	0	0
Quartz + Fe_2O_3, 0.33 mg ea	25	25	25	24	24	24	24	24	24	24	23	21	19	12	0
Quartz + Fe_2O_3, 0.03 mg ea	25	24	25	24	23	23	23	23	22	21	20	19	15	10	0
Glass, 10.0 mg	25	25	25	25	25	24	23	23	22	22	20	20	17	12	0
Glass, 1.0 mg	25	25	25	24	24	23	23	23	22	22	18	16	13	10	0
Glass, 0.5 mg	25	25	25	24	24	24	24	23	22	22	21	21	13	9	0
Glass, 0.05 mg	25	25	25	24	24	24	24	20	19	19	18	13	13	7	0
Alumina, 20.0 mg	25	24	25	24	23	22	22	22	22	19	19	15	12	7	0
Alumina, 5.0 mg	25	25	25	24	24	23	22	20	20	19	18	16	16	6	0
Alumina, 2.0 mg	25	24	25	24	23	22	22	21	20	17	14	12	8	0	0
Alumina, 0.2 mg	25	25	25	24	23	23	23	22	22	19	17	12	8	0	0
Saline, 0.5 ml	25	23	25	23	22	22	22	21	21	21	20	15	13	10	0
Cage Controls	25	25	25	25	25	25	25	25	25	25	25	23	17	10	0

*Effective animals = 25 minus the number of technique- or anesthesia-related deaths plus the number of animals replaced per group.

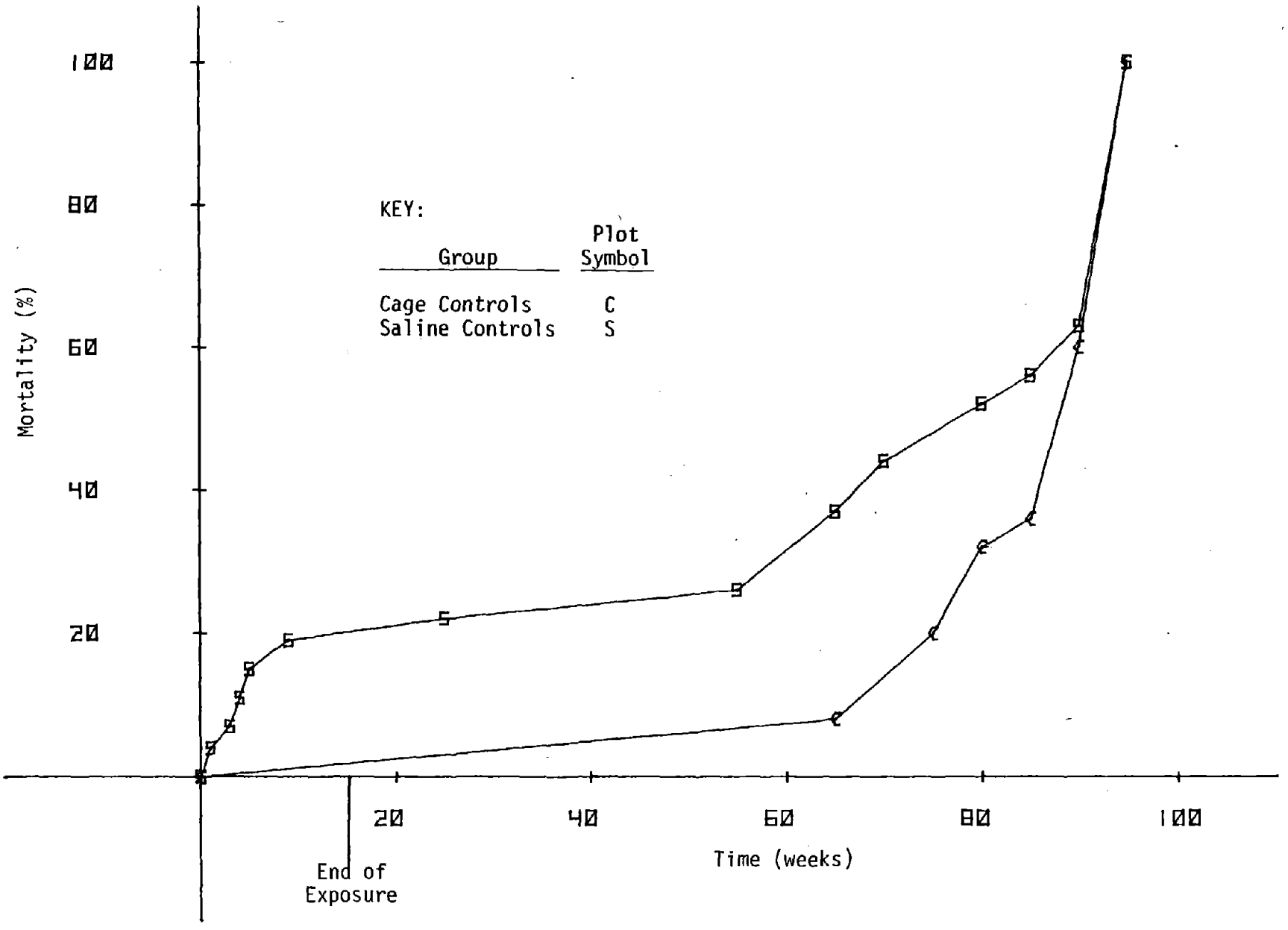

Figure 7. Comparative mortality of saline and cage controls

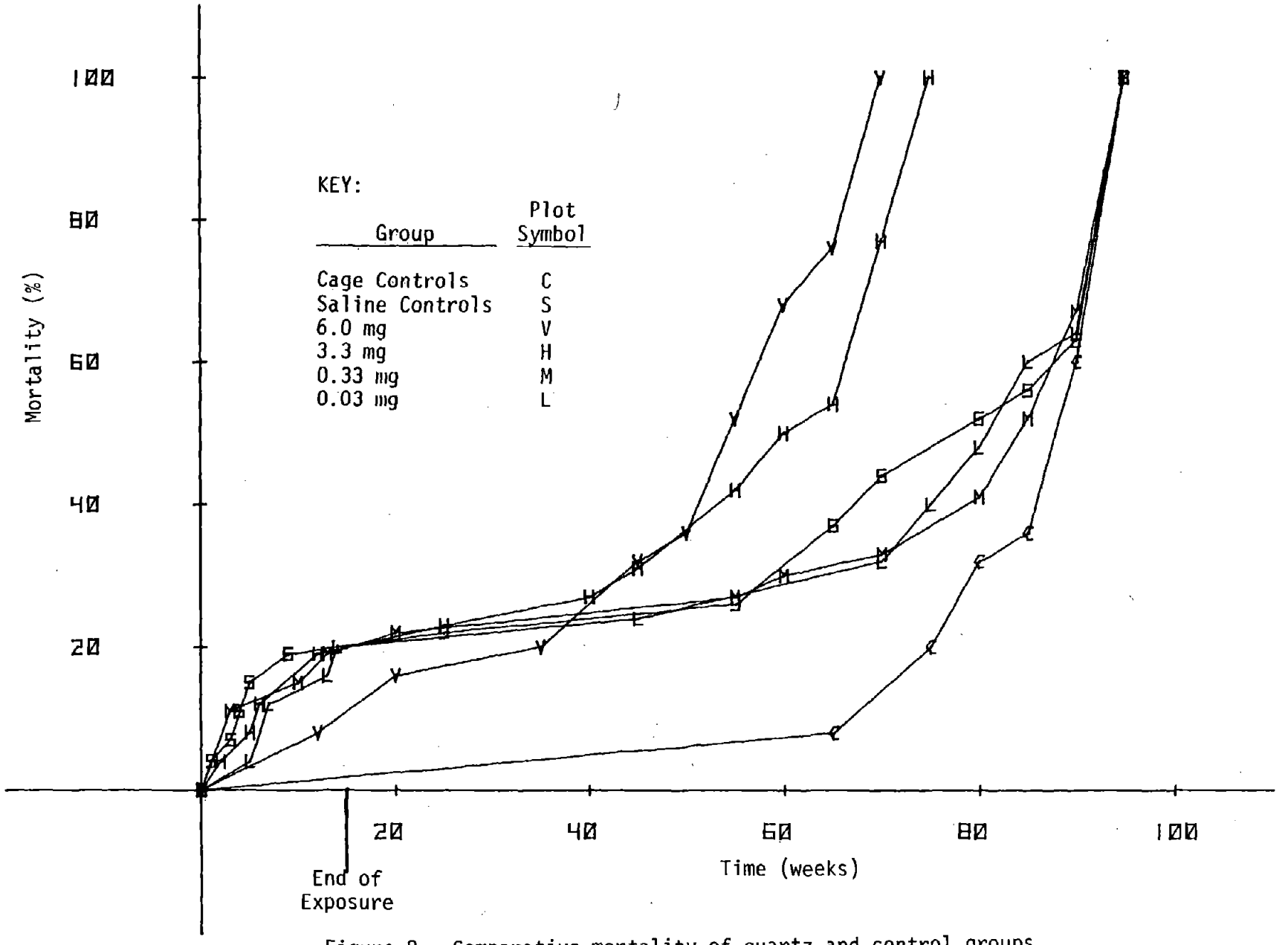

Figure 8. Comparative mortality of quartz and control groups

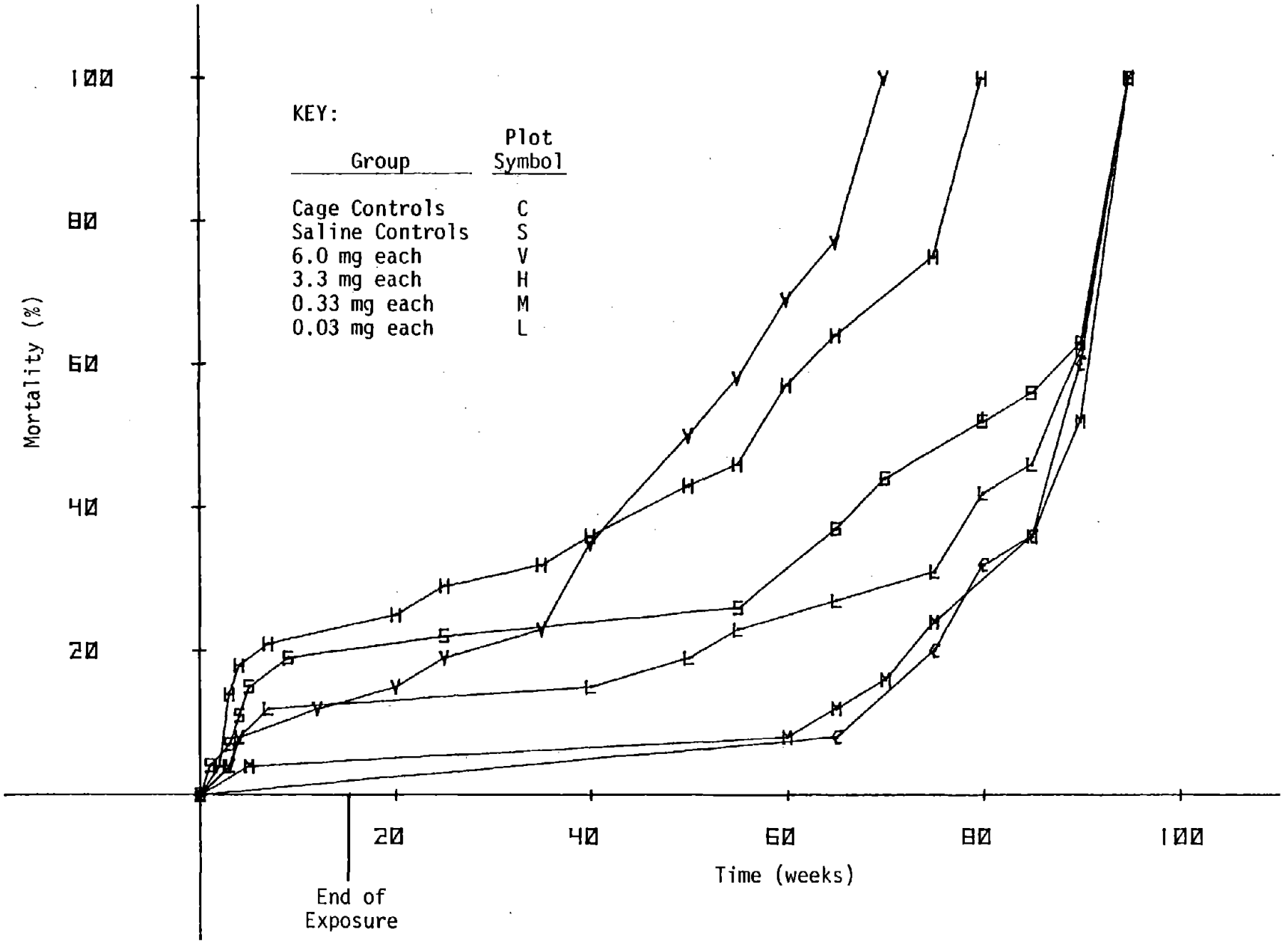

Figure 9. Comparative mortality of quartz + ferric oxide and control groups

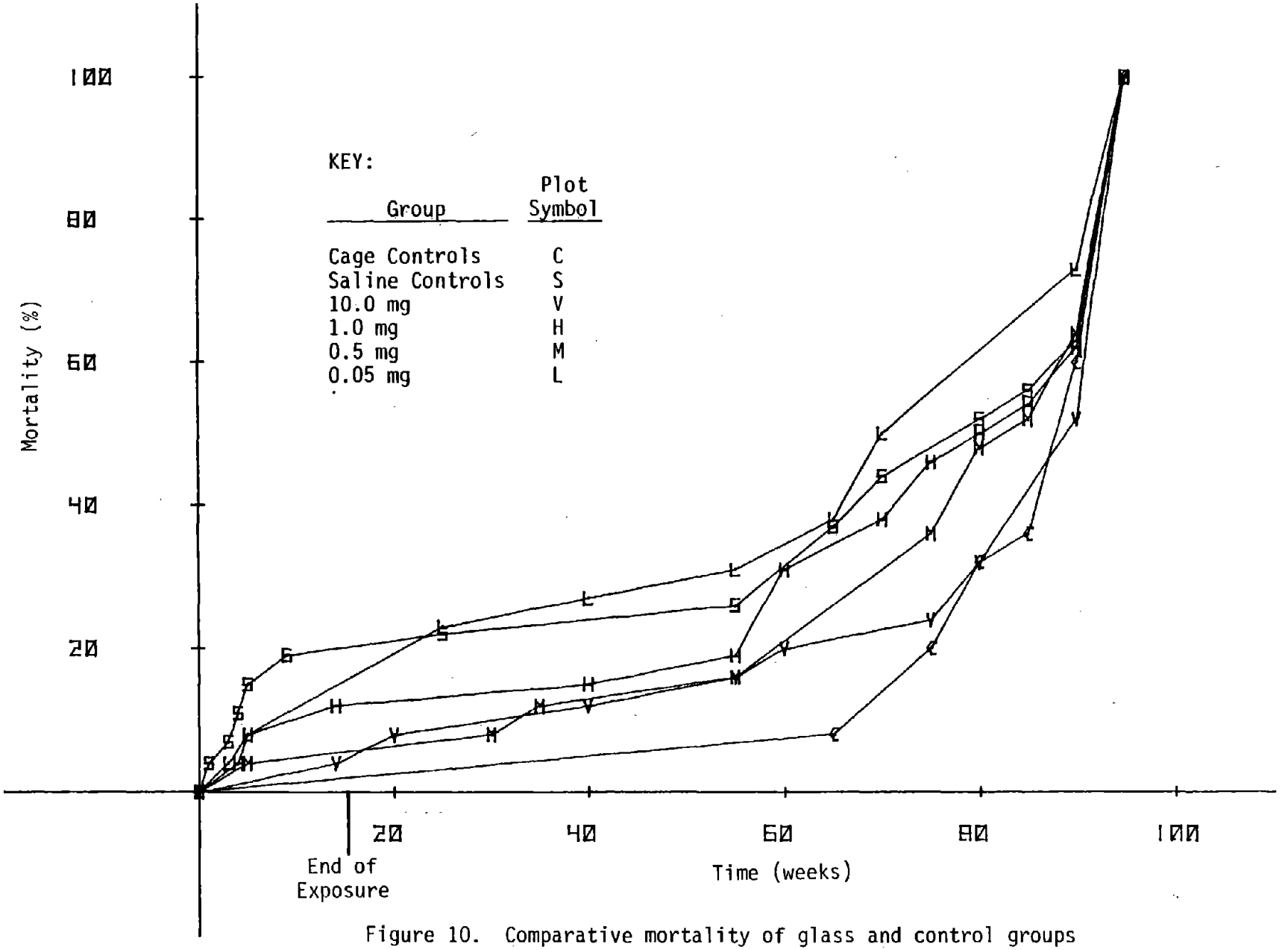

Figure 10. Comparative mortality of glass and control groups

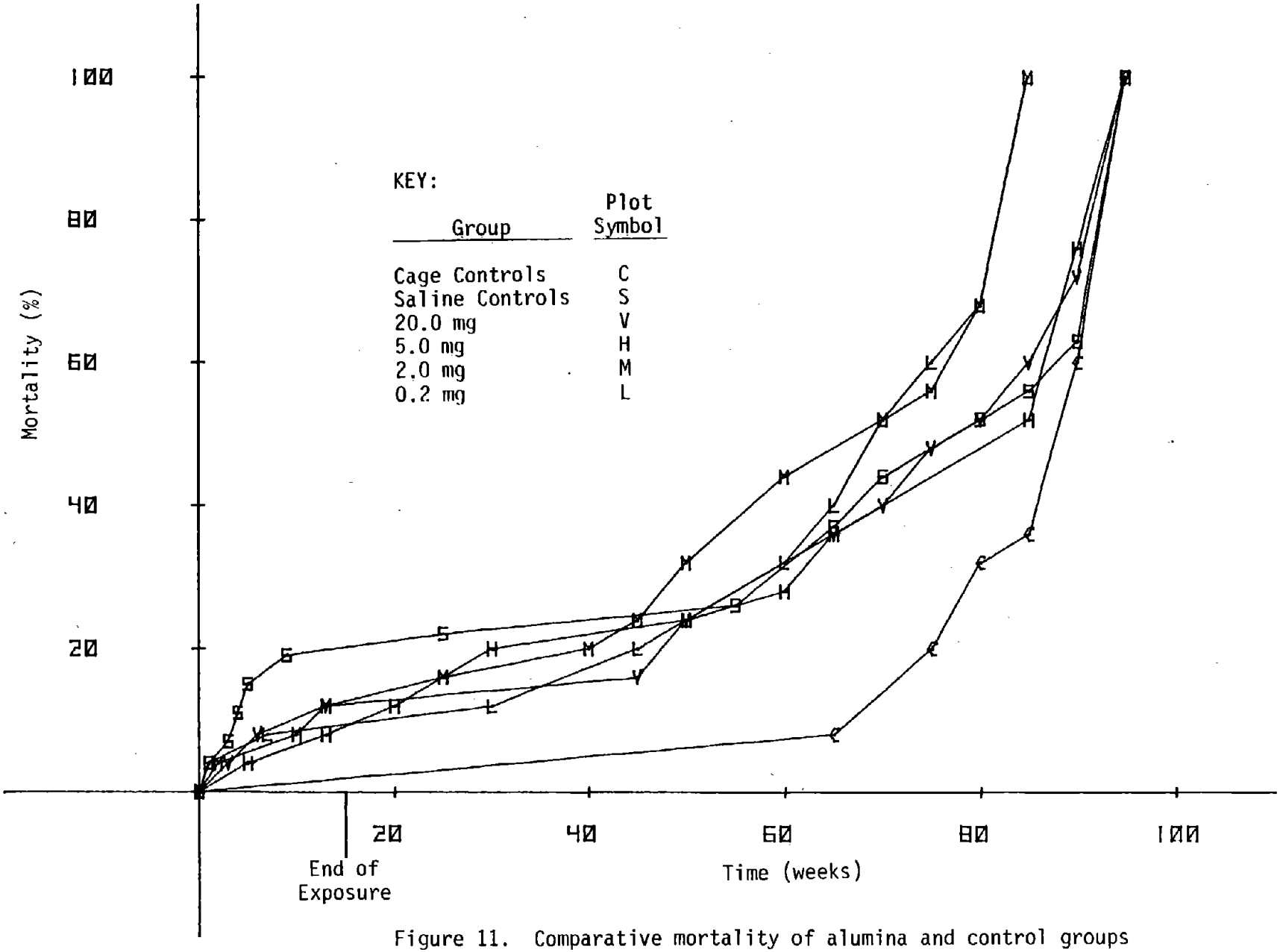

Figure 11. Comparative mortality of alumina and control groups

Table 6. Summary of life table analysis data

Group	Mean Survival Time* (± SE)	Terminal Sacrifice*	75th Percentile*	50th Percentile*	Prob. = Saline Control Breslow	Mantel
Quartz						
6.0 mg	348 ± 26	482	299	383	0.0003	0.0001
3.3 mg	383 ± 31	517	356	457	0.006	0.003
0.33 mg	506 ± 41	644	481	601	0.69	0.48
0.03 mg	498 ± 44	664	489	583	0.65	0.49
Quartz + Fe_2O_3						
6.0 mg ea	335 ± 32	482	251	363	0.0001	0.0001
3.3 mg ea	379 ± 37	560	268	413	0.004	0.003
0.33 mg ea	578 ± 28	664	561	630	0.48	0.73
0.03 mg ea	558 ± 32	664	530	620	0.64	0.67
Fibrous Glass						
10.0 mg	555 ± 35	663	531	630	0.40	0.35
1.0 mg	517 ± 35	663	416	587	0.57	0.49
0.5 mg	538 ± 34	663	523	582	0.97	0.92
0.05 mg	475 ± 41	663	385	598	0.40	0.43
Hydrated Alumina						
20.0 mg	500 ± 37	655	440	566	0.33	0.31
5.0 mg	481 ± 41	635	392	589	0.34	0.22
2.0 mg	440 ± 34	592	323	489	0.06	0.04
0.2 mg	444 ± 34	592	395	486	0.05	0.03
Saline Controls	534 ± 35	663	446	602	--	--
Cage Controls	595 ± 14	663	558	616	0.48	0.93

*Number of days from first instillation to death.

Table 7. Summary of pertinent gross lesions

Group	Total No. Animals	Lungs Mottled	Lungs-Mass	Tracheobronchial Lymph nodes Enlarged	Kidneys roughened and/or Granular	Kidneys Pale	Generalized Edema	Right Heart Dilatation	Left Atrium Mottled	Hydro-thorax
Quartz, 6.0 mg	25	22 (88)*	0 (0)	19 (76)	9 (36)	10 (40)	10 (40)	3 (12)	0 (0)	0 (0)
3.3 mg	26	20 (77)	0 (0)	16 (62)	7 (27)	13 (50)	4 (15)	5 (19)	0 (0)	0 (0)
0.33 mg	27	8 (30)	0 (0)	4 (15)	9 (33)	8 (30)	3 (11)	11 (41)	2 (7)	0 (0)
0.03 mg	25	9 (36)	0 (0)	0 (0)	5 (20)	5 (20)	2 (8)	5 (20)	3 (12)	2 (8)
Quartz + Fe_2O_3, 6.0 mg ea	26	25 (96)	0 (0)	22 (85)	9 (35)	9 (35)	7 (27)	4 (15)	0 (0)	0 (0)
3.3 mg ea	28	23 (82)	1 (4)	16 (57)	8 (29)	10 (36)	6 (21)	3 (11)	0 (0)	0 (0)
0.33 mg ea	25	17 (68)	0 (0)	2 (8)	13 (52)	5 (20)	3 (12)	6 (24)	2 (8)	0 (0)
0.03 mg ea	25	12 (48)	1 (4)	0 (0)	8 (32)	5 (20)	2 (8)	2 (8)	0 (0)	0 (0)
Glass, 10.0 mg	25	11 (44)	1 (4)	3 (12)	9 (36)	4 (16)	1 (4)	5 (20)	3 (12)	2 (8)
1.0 mg	26	8 (31)	1 (4)	3 (12)	14 (54)	7 (27)	6 (23)	8 (31)	2 (8)	1 (4)
0.5 mg	25	9 (36)	2 (8)	2 (8)	9 (36)	5 (20)	4 (16)	10 (40)	2 (8)	1 (4)
0.05 mg	26	6 (23)	0 (0)	0 (0)	6 (23)	4 (15)	1 (4)	3 (12)	3 (12)	0 (0)
Alumina, 20.0 mg	25	11 (44)	1 (4)	1 (4)	8 (32)	5 (20)	2 (8)	4 (16)	2 (8)	0 (0)
5.0 mg	25	8 (32)	1 (4)	2 (8)	17 (68)	8 (32)	5 (20)	7 (28)	5 (20)	4 (16)
2.0 mg	25	4 (16)	2 (8)	1 (4)	16 (64)	11 (44)	1 (4)	7 (28)	2 (8)	0 (0)
0.2 mg	25	5 (20)	0 (0)	3 (12)	7 (28)	9 (36)	2 (8)	2 (8)	2 (8)	1 (4)
Saline Controls	27	6 (22)	1 (4)	0 (0)	7 (26)	5 (19)	1 (4)	5 (19)	1 (4)	0 (0)
Cage Controls	25	11 (44)	0 (0)	2 (8)	12 (48)	7 (28)	5 (20)	9 (36)	6 (24)	1 (4)

*Percentage of total in parentheses

All of the instilled particulate materials were visible microscopically in the lung at higher magnification (Figures 12-15). The materials were evenly distributed throughout the lobes of the lungs in most animals. Within individual lobes, there was often a patchy distribution of material, with some areas of the lobe having relatively high concentrations of material. However, these areas were located both centrally and peripherally in the lobes. In lungs from animals necropsied toward the end of the postexposure period, the instilled material was frequently more concentrated in the central portions of the lobes.

The instilled materials were present within alveolar macrophages and free in alveolar lumens; the amount of material observed free in alveoli was greatest in groups instilled with the highest doses, and gradually decreased with time postexposure. Materials were also observed free and within macrophages in the sinuses of tracheobronchial lymph nodes.

Quartz particles were visible with polarized light, varying in size and shape from spicules barely visible at 400X magnification to larger polyhedral particles identifiable at 25X. Ferric oxide was readily visible without polarized light as dark brown, finely granular material, usually within macrophages. With polarized light, ferric oxide was a brilliant orange-brown color. Hydrated alumina was present as relatively large aggregates of large pleomorphic crystals, somewhat more rounded and with fewer sharp spicules than quartz, and brightly refractile with polarized light. Fibrous glass, the most difficult of the instilled materials to observe microscopically, appeared as small, translucent or brownish, nonbirefringent curved fibers with rounded, clubbed ends. There were smaller amounts of fibrous glass visible in the lung than the other materials, even in those animals receiving the highest dose and dying relatively early in the study.

The incidence and severity of pertinent microscopic lesions for individual animals are tabulated in Appendix I; the incidence and mean severity of these lesions in each group are also indicated in these tables. The mean severity was calculated by dividing the total of all grades of each lesion by the number of animals in which that lesion was diagnosed. In evaluating these data it must be kept in mind that the grades assigned to lesions are based on a subjective evaluation rather than a quantitative assay of the tissue.

The incidence and severity of alveolar septal fibrosis is summarized by group in Table 8, using three different sets of criteria for inclusion of data. Column A summarizes the data from all animals examined microscopically; column B omits all animals in which tissues were noted at histopathologic examination to be autolyzed; column C omits these autolyzed animals and all animals necropsied more than 90 days prior to terminal sacrifice for that group. These data are illustrated graphically in Figures 21-23.

Diagnosis and grading the severity of septal fibrosis was complicated by the fact that two principal causes of septal fibrosis are evident in animals on this study. The most frequent and important cause, since it is the lesion upon which this entire study was predicated, was the instilled material. Septal fibrosis was observed to some degree in all exposed groups, and in the quartz and quartz plus ferric oxide groups its incidence and severity were somewhat dose-related. In most animals affected this lesion was multifocal,

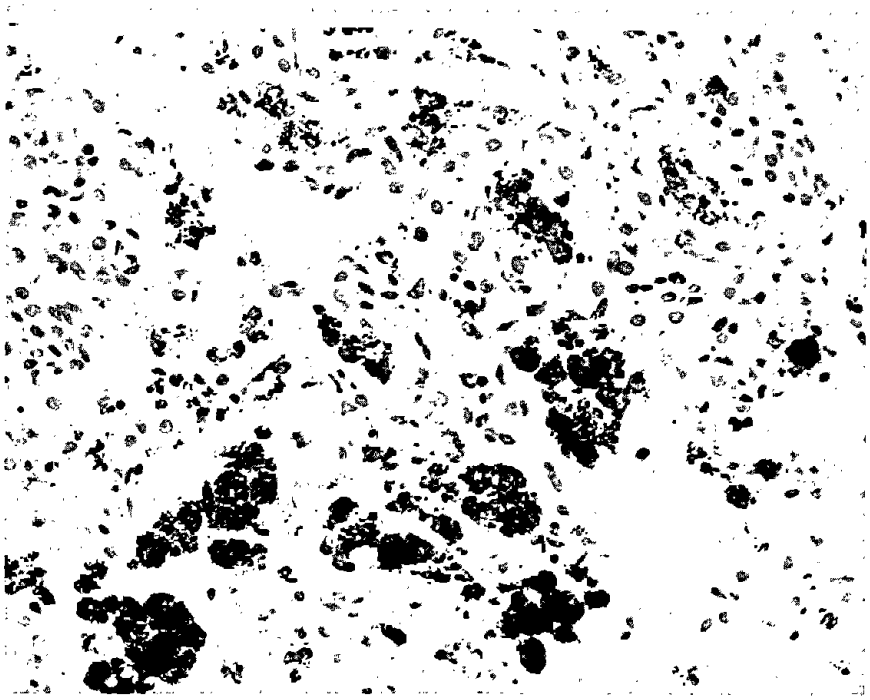

FIGURE 12. Grade 3 septal fibrosis and granulomatous inflammation around instilled quartz and ferric oxide. The ferric oxide is visible as dark, finely granular material in alveoli. This hamster was sacrificed 482 days after initial instillation of 6.0 mg quartz and 6.0 mg ferric oxide. Hematoxylin and eosin, 250X.

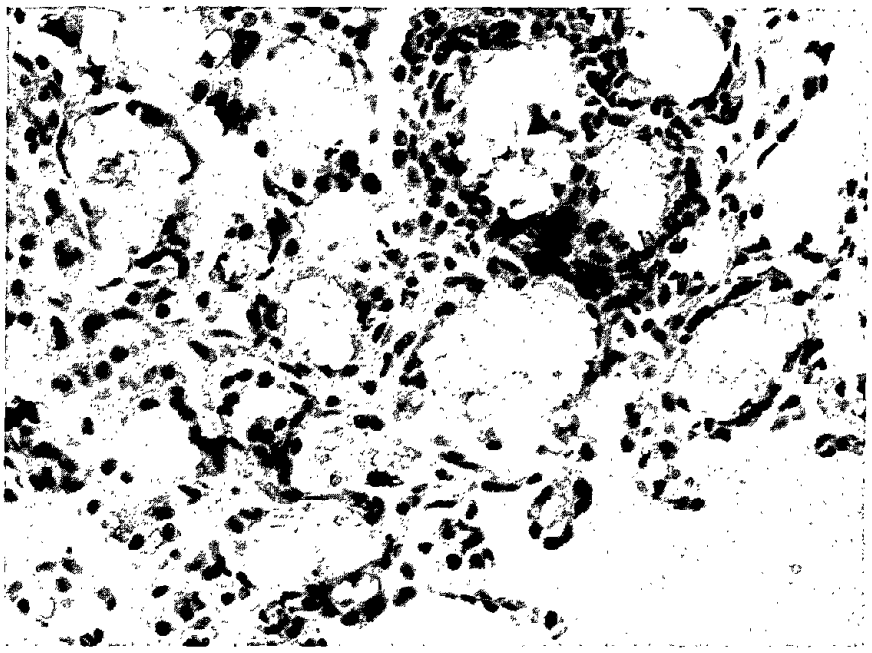

FIGURE 13. Grade 3 fibrosis and granulomatous inflammation around instilled hydrated alumina, visible with polarized light as brightly birefringent crystalline material in large clumps. This hamster was sacrificed 655 days after initial instillation of 20.0 mg of hydrated alumina. Hematoxylin and eosin, 250X.

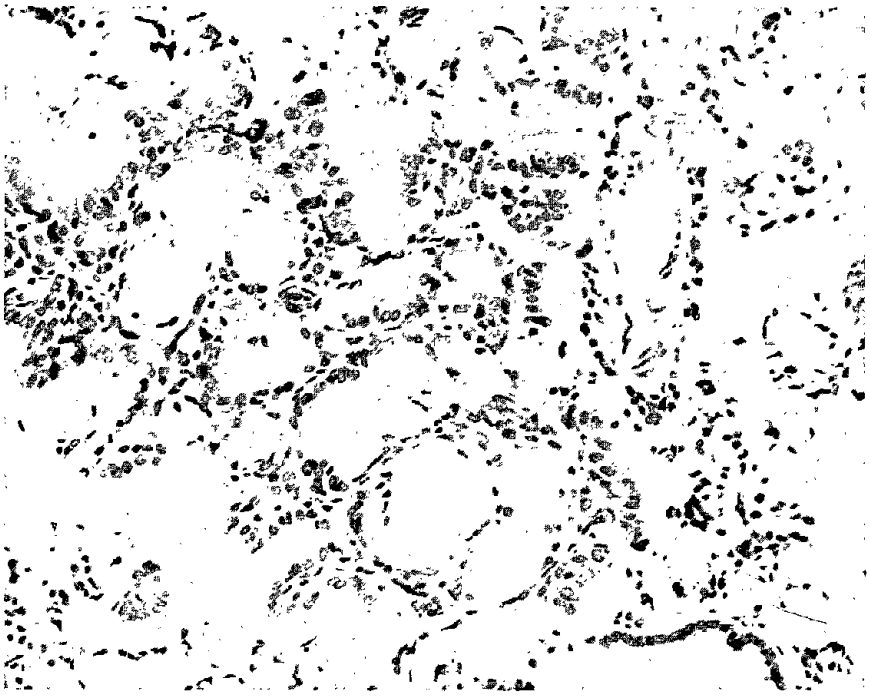

FIGURE 14. Focal pulmonary alveolar lipoproteinosis, septal fibrosis and alveolar epithelial hyperplasia in a hamster sacrificed 655 days after initial instillation of 20 mg of hydrated alumina. Clumps of instilled alumina are visible at top left and bottom center, adjacent to the focal lesion. Hematoxylin and eosin, 160X.

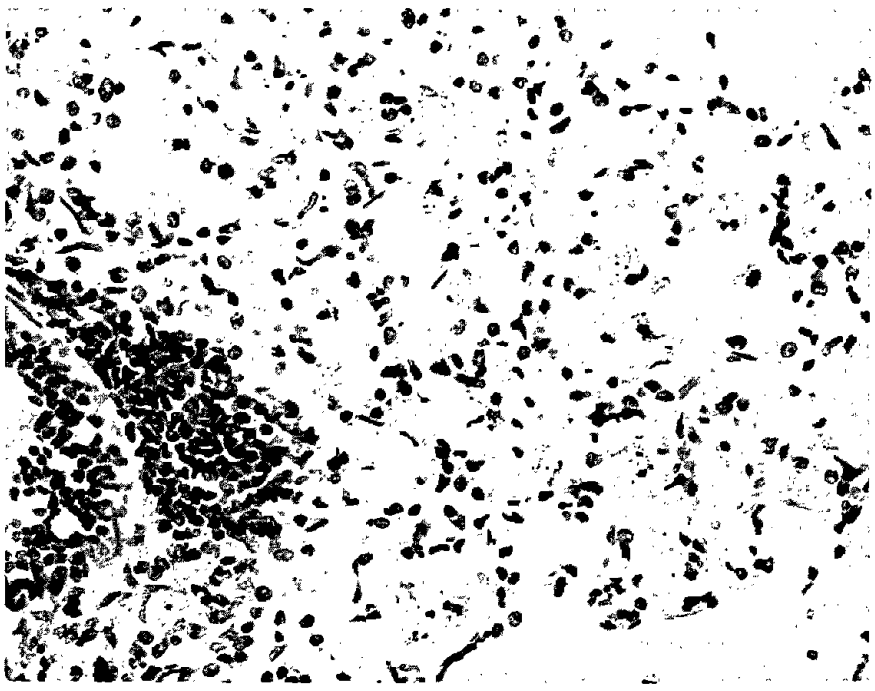

FIGURE 15. Grade 3 septal fibrosis in response to instilled fibrous glass, which is visible within macrophages in alveoli. This hamster was necropsied 520 days after initial instillation of 10 mg of fibrous glass. Hematoxylin and eosin, 250X.

Table 8. Incidence and severity of alveolar septal fibrosis

Group	A*		B*		C*	
	Incidence (%)	Mean Severity	Incidence (%)	Mean Severity	Incidence (%)	Mean Severity
Quartz, 6.0 mg	17/25 (68)	3.3	14/19 (74)	3.4	10/10 (100)	3.1
3.3 mg	19/26 (73)	2.6	19/23 (83)	2.6	12/12 (100)	2.8
0.33 mg	11/27 (41)	2.1	11/24 (42)	2.1	10/16 (63)	2.2
0.03 mg	9/25 (36)	2.1	8/17 (47)	2.1	5/11 (45)	2.2
Quartz + Fe_2O_3						
6.0 mg ea	22/26 (85)	3.2	22/25 (88)	3.2	11/11 (100)	3.3
3.3 mg ea	21/28 (75)	2.8	21/27 (78)	2.8	10/10 (100)	3.0
0.33 mg ea	16/25 (64)	1.9	16/21 (76)	1.9	13/14 (93)	1.9
0.03 mg ea	14/24 (58)	1.9	13/21 (65)	1.9	9/13 (69)	1.9
Glass, 10.0 mg	24/25 (96)	2.2	20/21 (95)	2.1	15/15 (100)	2.4
1.0 mg	10/25 (40)	2.1	9/22 (41)	2.1	5/12 (42)	2.0
0.5 mg	15/25 (63)	1.7	14/19 (78)	1.8	10/12 (83)	1.9
0.05 mg	7/26 (27)	1.4	6/23 (26)	1.5	6/12 (50)	1.5
Alumina, 20.0 mg	21/25 (84)	2.2	19/22 (86)	2.3	12/12 (100)	2.4
5.0 mg	14/25 (56)	1.3	13/21 (62)	1.2	12/15 (80)	1.3
2.0 mg	8/25 (32)	1.6	8/24 (33)	1.6	4/12 (33)	2.0
0.2 mg	8/25 (32)	2.0	8/22 (32)	2.0	6/11 (55)	2.0
Saline Controls	7/27 (26)	1.7	6/23 (26)	1.7	6/12 (50)	1.7
Cage Controls	2/25 (8)	1.0	2/17 (12)	1.0	2/12 (17)	1.0

*Column A represents the incidence and severity of septal fibrosis including all animals examined microscopically; Column B represents the incidence and severity, omitting animals noted to be autolyzed at microscopic examination; Column C represents the incidence and severity omitting autolyzed animals and animals necropsied more than 90 days prior to that group's terminal sacrifice.

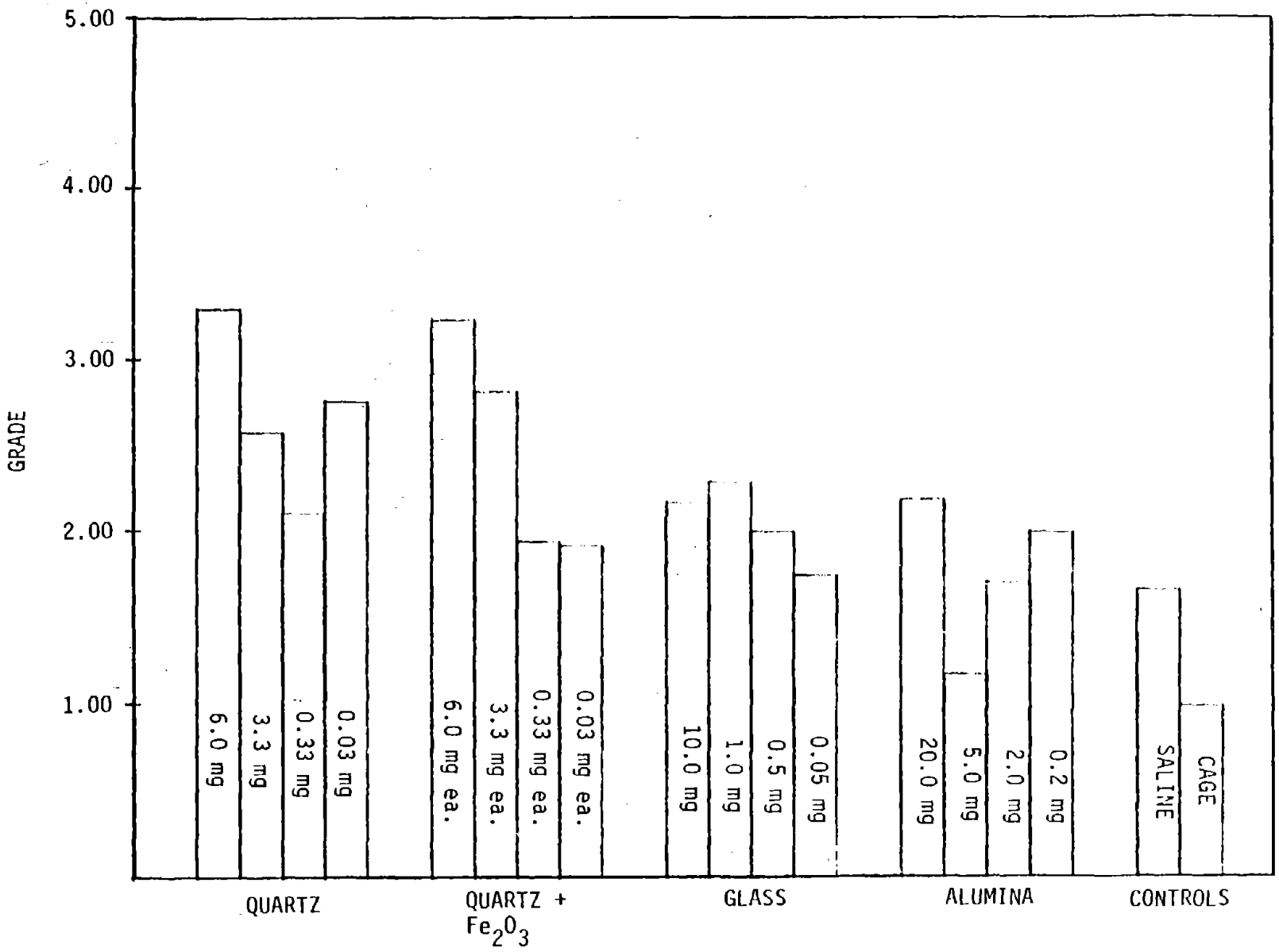

Figure 16. Mean severity of pulmonary fibrosis, including all animals in each group

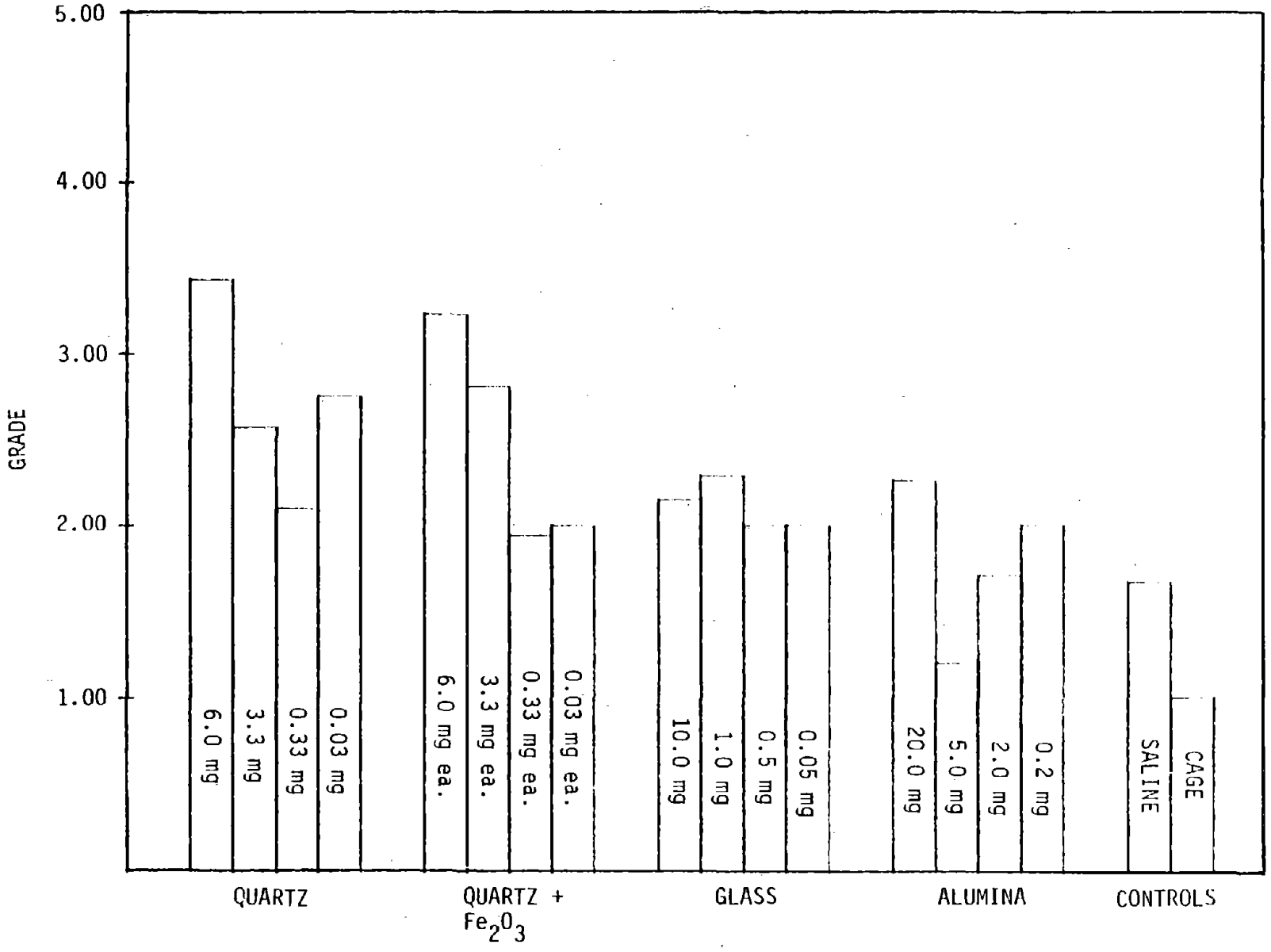

Figure 17. Mean severity of pulmonary fibrosis, omitting autolyzed animals

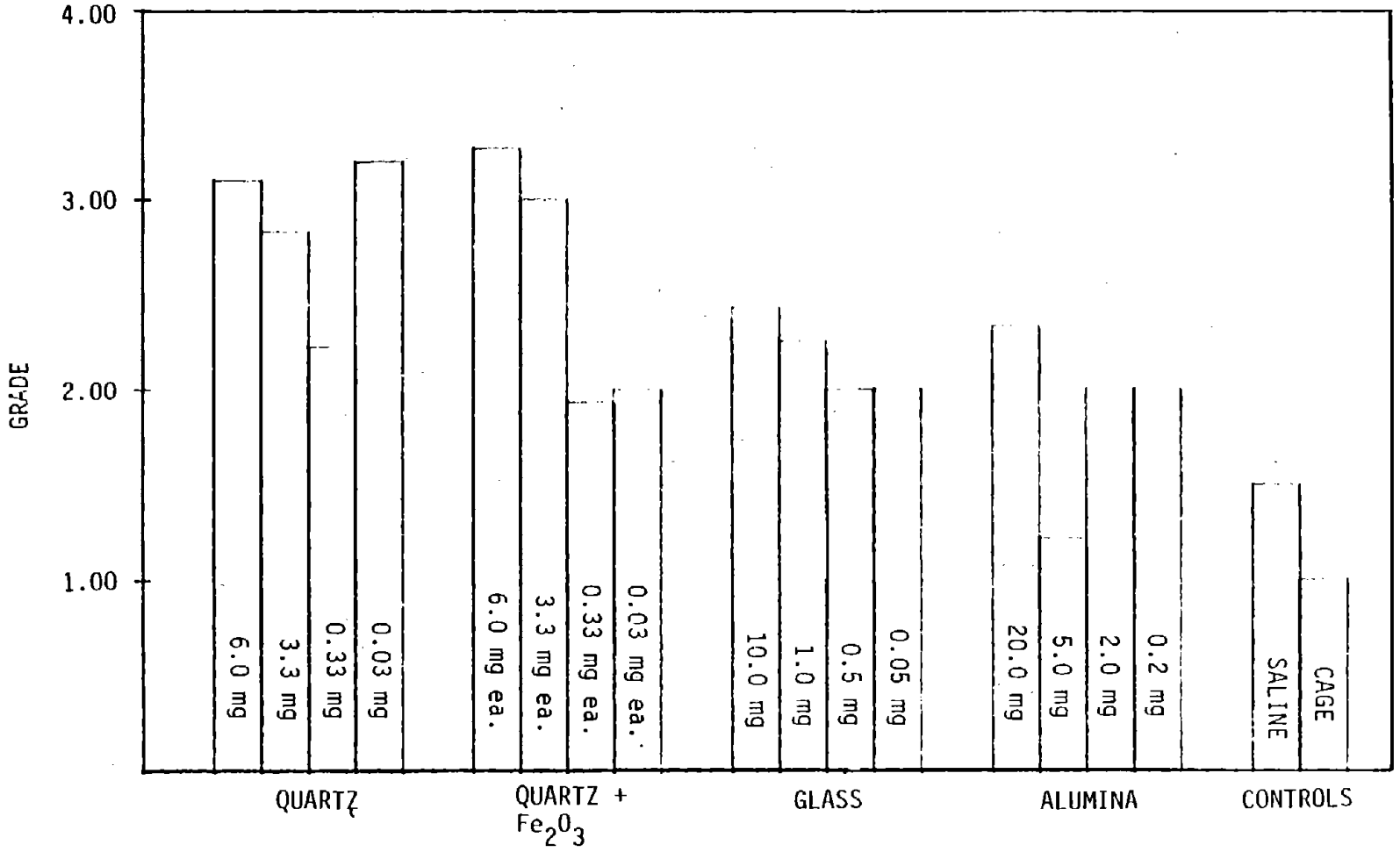

Figure 18. Mean severity of pulmonary fibrosis, omitting all autolyzed animals and all animals necropsied > 90 days before group terminal sacrifice

appearing in association with concentrations of instilled material. In some animals exposed to the higher doses, the fibrosis appeared to be diffusely scattered through all lobes and corresponded to a more uniform distribution of the instilled material throughout the lung.

The septal fibrosis induced by the instilled materials was visible as a variable increase in thickness of alveolar septa in areas around accumulation of instilled material (Figs. 12-15, 19-21). The accompanying inflammatory response to the instilled materials sometimes made it difficult to differentiate septal thickening due to congestion or inflammatory cell infiltration from actual septal fibrosis; however, special stains (Masson's trichrome) for collagen on representative sections confirmed the presence of increased collagen in these thickened septa.

Grading of severity of alveolar septal fibrosis was based on the thickness of alveolar septa in affected areas of the lung sections. In many animals there were small foci of more severe septal thickening within or adjacent to large areas of milder involvement. In tabulating data on severity of septal fibrosis (Appendix I) for purposes of statistical analysis, only the more severe grade of fibrosis recorded for each animal was tabulated; i.e., if an animal had diffuse grade 2 septal fibrosis with foci of grade 3 fibrosis recorded as microscopic findings, this animal's lesions were tabulated in Appendix I and analyzed statistically as grade 3 septal fibrosis.

The second cause of septal fibrosis observed in this study was the presence of chronic pulmonary congestion and edema associated with chronic heart disease (McMartin, 1977) and/or severe chronic renal disease secondary to renal amyloidosis. This septal fibrosis (Fig. 22) was present diffusely with multiple foci of more severe involvement in association with focal aggregates of alveolar macrophages, some of which contain hemosiderin ("heart failure cells"). The septal fibrosis related to chronic pulmonary congestion was recognizable in control animals or in animals exposed to lower doses of materials because of its diffuse, mild nature, the presence of concomitant cardiovascular lesions (atrial thrombosis, dilatation of atria/ventricles, pulmonary congestion/edema, edema of other tissues), and the lack of association of the fibrosis with visible instilled material. However, it was difficult in some cases to differentiate in instilled groups, especially with high doses, between septal fibrosis related to instillation and fibrosis present as part of the chronic cardiovascular or renal disease.

With some exceptions, the incidence and severity of septal fibrosis correlated well with the dose of instilled quartz or quartz and ferric oxide (Table 8). Omitting animals in which autolysis made the visualization of fibrotic lesions more difficult (Table 8, Column B) increased the incidence slightly in most groups but did not noticeably change the severity. The incidence of fibrosis in non-autolyzed animals necropsied less than 90 days from terminal sacrifice (Table 8, Column C) was somewhat higher than the total incidence for each group; again, severity did not change much.

Incidence and severity of septal fibrosis in the groups exposed to fibrous glass or alumina were not clearly dose-related, although the groups exposed to the highest doses of either material had the highest incidence, and there was

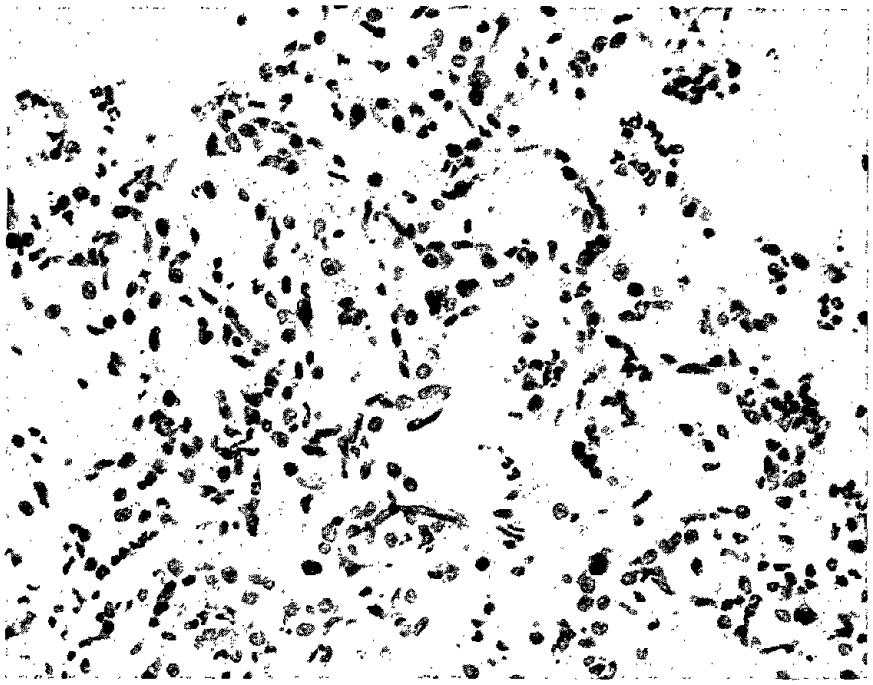

FIGURE 19. Grade 3 septal fibrosis, granulocytes and alveolar macrophages infiltrating alveolar lumens and septa in hamsters sacrificed 482 days after initial instillation of 6.0 mg quartz. Hematoxylin and eosin, 250X.

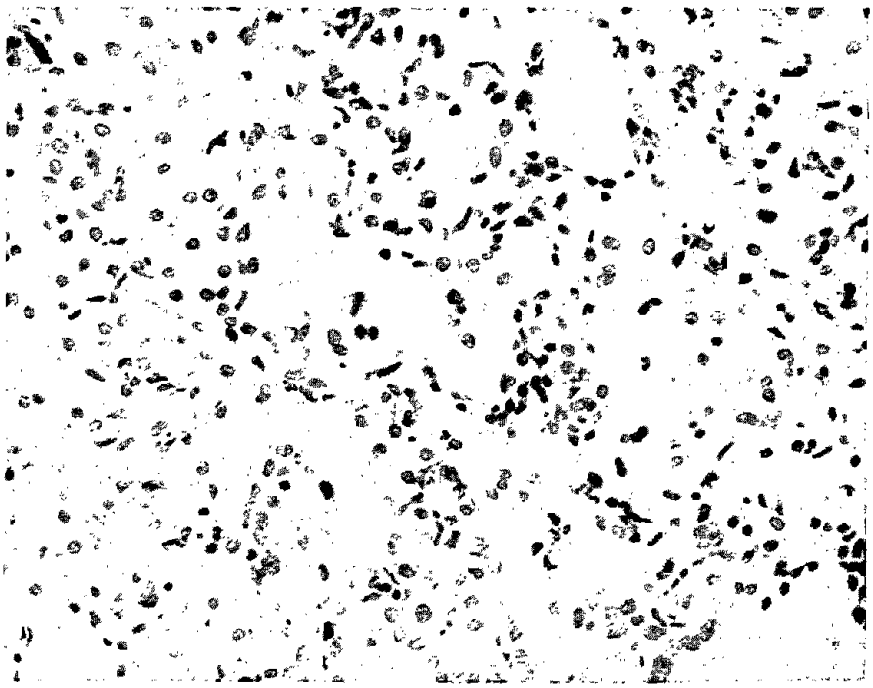

FIGURE 20. Septal fibrosis, granulomatous inflammation, and pulmonary alveolar lipoproteinosis with cholesterol clefts in alveoli in a hamster sacrificed 482 days after initial instillation of 6.0 mg quartz. Hematoxylin and eosin, 250X.

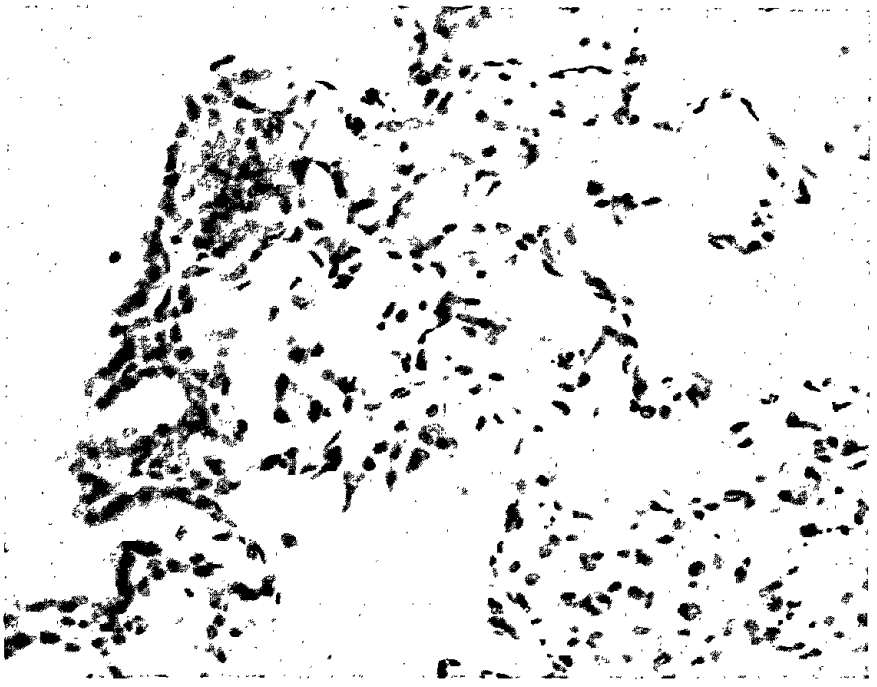

FIGURE 21. Grade 2 septal fibrosis, alveolar macrophages in alveoli, and bronchiolization of alveoli in a hamster sacrificed 644 days after initial instillation of 0.33 mg quartz. Hematoxylin and eosin, 250X.

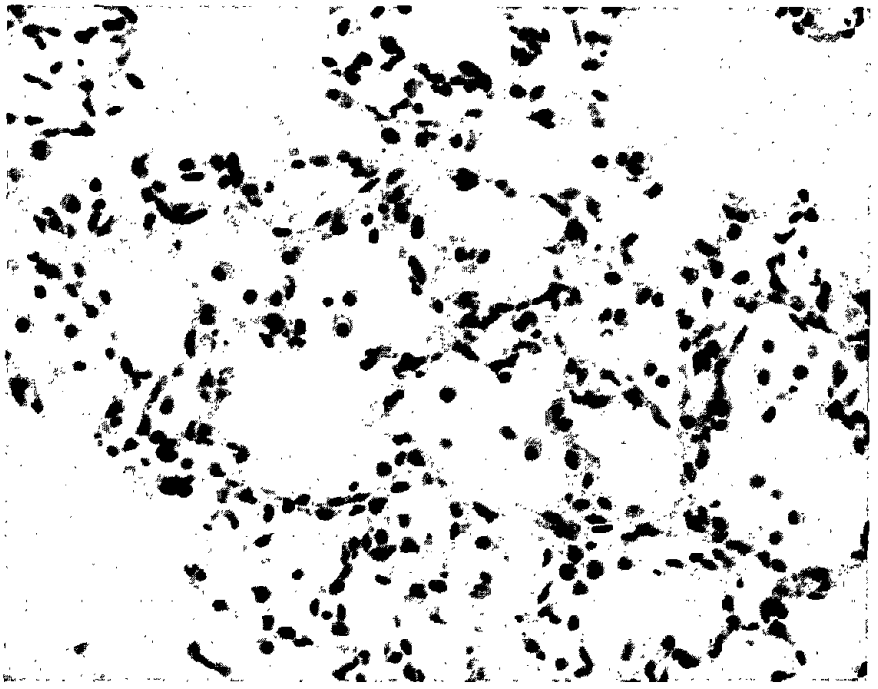

FIGURE 22. Grade 2 alveolar septal fibrosis and alveolar macrophage aggregates in a saline control hamster with chronic cardiac disease which was sacrificed 683 days after initial instillation. Hematoxylin and eosin, 250X.

a clear difference in incidence of fibrosis between the highest dose group of either material and the saline control group. The pulmonary fibrotic response to fibrous glass or alumina was clearly much less striking than the response to quartz and ferric oxide. The presence of septal fibrosis in saline or cage control groups appeared in most cases to be associated with chronic pulmonary congestion secondary to cardiovascular or renal disease. However the incidence and severity of fibrosis was slightly higher in the saline control group than in the untreated controls.

The Mantel chi-square statistic was highly significant ($P < 0.001$) for pulmonary fibrosis for all four compounds. This indicates that a trend toward increased prevalence and/or higher grades with increased dose exists for all four compounds. The two-way analysis of variance on the scaled grades showed significant dose and time effects and nonsignificant interactions in every case.

The two-way analysis of variance can be viewed as using least squares to fit the mathematical model

$$y_{ijk} = M + t_i + d_j + e_{ijk}$$

to the scaled grades. In the model y_{ijk} is the scaled grade for the k^{th} animal in the i^{th} time interval at the j^{th} dose level; M is the overall group mean scaled grade, t_i is the additive effect of the i^{th} time interval; d_j is the additive effect of the j^{th} dose level; and e_{ijk} is a random deviation. The effects t_i and d_j are expressed as deviations from the overall mean.

A progressive increase in fibrosis with time or dose level would be reflected in a progressive increase in the respective effects. The scaled dose level effects d_j are given in Table 9a. Since the scaling was done separately, the effects are not comparable across materials. This is reflected in the same saline controls receiving a different effect for each material. Table 9b shows the dose-adjusted time effects for each material, and demonstrates the increase of fibrosis with time. Both the quartz- and quartz plus ferric oxide-instilled groups exhibited a progressive increase with increasing dose. For hydrated alumina and fibrous glass animals, the very high-dose groups and the saline controls were the high and low extremes, respectively. However the ordering of the intermediate groups did not correspond to the dose level ordering. The results of the second analyses using the same scaling for all materials is given in Table 9c. These time adjusted mean values could be used to rank the various treatment groups; however, no attempt was made to attach statistical significance to differences among groups exposed to different materials.

Pleural fibrosis was observed infrequently in instilled groups, occurring in greatest incidence and severity in the groups exposed to quartz or quartz and ferric oxide (Table 10). The incidence of fibrosis in the tracheobronchial lymph nodes (Fig. 23) was high in the groups instilled with the highest doses of quartz or quartz and ferric oxide (Table 10) and appeared to be dose-related in the groups exposed to these two materials. Incidence and severity of tracheobronchial lymph node fibrosis were lower in the groups instilled with fibrous glass or alumina with little evidence of a dose-response in incidence or severity in these groups. Fibrosis of the tracheobronchial lymph nodes was not observed in either control group.

TABLE 9. Dose and time effects and time adjusted mean scaled grades of septal fibrosis[A]

a) Dose effects

Dose Level	Quartz	Quartz + Ferric Oxide	Hydrated Alumina	Fibrous Glass
Very High	2.60	2.39	2.11	1.83
High	1.50	1.12	-0.44	-0.23
Medium	-0.99	-0.93	-0.44	0.46
Low	-1.18	-1.00	-0.13	-0.90
Saline	-1.74	-2.15	-1.03	-0.93

b) Time Effects

Time Stratum	Quartz	Quartz + Ferric Oxide	Hydrated Alumina	Fibrous Glass
0-440	-1.09	-0.91	-0.71	-0.90
441-600	0.45	0.29	0.09	0.05
601 +	0.75	0.79	0.62	0.33

c) Time-Adjusted Mean Scaled Grades

Dose Level	Quartz	Quartz + Ferric Oxide	Hydrated Alumina	Fibrous Glass
Very High	4.86	5.50	3.23	3.18
High	3.53	4.19	1.14	1.28
Medium	1.41	2.00	1.12	1.83
Low	1.27	1.77	1.30	0.62
Saline	0.57			

[A]Scaled according to Snell (1964). Effects are deviations from group means, and are scaled differently for each material. The time-adjusted means in (c) have a common basis.

Table 10. Incidence and severity of fibrosis of pleura and tracheobronchial lymph nodes

Group	Fibrosis, Tracheobronchial Lymph Node			Fibrosis, Pleura		
	#	(%)	Mean Severity	#	(%)	Mean Severity
Quartz						
6.0 mg	21/22	(95)	2.1	4/25	(16)	3.3
3.3 mg	19/23	(83)	2.7	2/26	(8)	3.0
0.33 mg	13/22	(59)	2.1	2/27	(7)	2.0
0.03 mg	2/12	(17)	1.5	1/25	(4)	5.0
Quartz + Fe_2O_3						
6.0 mg	22/26	(85)	3.4	3/26	(12)	3.3
3.3 mg	18/22	(82)	3.2	1/28	(4)	5.0
0.33 mg	13/19	(68)	3.2	1/25	(4)	2.0
0.03 mg	4/12	(33)	2.5	0/24		
Glass						
10.0 mg	6/16	(38)	1.3	0/25		
1.0 mg	1/18	(6)	2.0	2/25	(8)	2.5
0.5 mg	2/14	(15)	1.0	1/25	(4)	2.0
0.05 mg	3/17	(18)	1.3	1/26	(4)	3.0
Alumina						
20.0 mg	2/15	(13)	1.0	0/25		
5.0 mg	2/16	(13)	1.0	0/25		
2.0 mg	2/16	(13)	1.5	1/25	(4)	2.0
0.2 mg	0/15			0/25		
Saline Controls	0/20			1/27	(4)	2.0
Cage Controls	0/14			0/25		

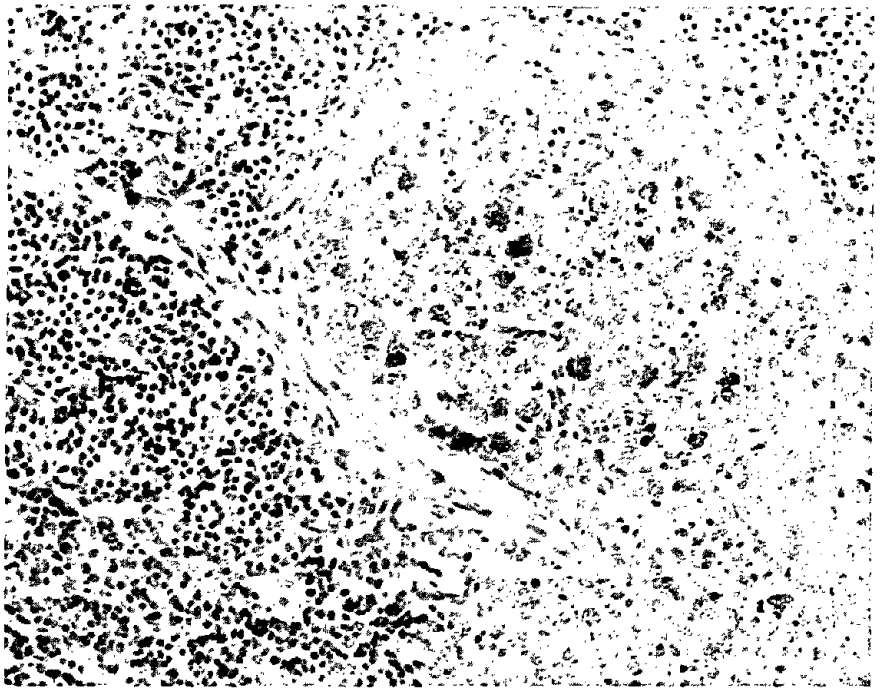

FIGURE 23. Grade 3 fibrosis and accumulation of macrophages in tracheobronchial lymph node of hamster sacrificed 321 days after instillation of 6.0 mg quartz and ferric oxide. Ferric oxide is visible in center of field. Hematoxylin and eosin, 160X.

The term granulomatous inflammation was used to indicate the presence of aggregates of alveolar macrophages, often accompanied by lesser numbers of granulocytic or mononuclear inflammatory cells (Fig. 12-13), in the lung parenchyma in response to instilled material. The determination that these cells were present in response to instilled material was based on the presence of the instilled particulate material in association with these macrophages, usually within the cytoplasm. In some cases, especially in the lower dose groups, macrophages far outnumbered the visible particles of instilled material but were located in small focal aggregates in association with varying degrees of focal septal fibrosis. This lesion is thus differentiated from alveolar macrophage accumulations observed in those animals with chronic pulmonary congestion (see below). The granulomatous inflammation induced by instilled material must also be differentiated from occasional macrophage aggregates observed in otherwise normal lungs in response to inhaled foreign bodies such as food.

Granulomatous inflammation in response to instilled material was by far the most striking pulmonary lesion observed in the study, and its incidence and severity were in most cases related to dose of material received (Table 11). As in the case of induced septal fibrosis, the granulomatous response was much more severe in the groups exposed to quartz or quartz plus ferric oxide than in the groups exposed to fibrous glass or hydrated alumina. The granulomatous inflammation was diffuse in most animals exposed to the higher doses of quartz or quartz and ferric oxide. Grading severity of granulomatous inflammation was based on the numbers of alveolar macrophages and inflammatory cells present in alveolar lumens and septa.

Alveolar macrophage aggregation was diagnosed in animals instilled with the two lower doses of quartz, quartz and ferric oxide, or fibrous glass, in both control groups, and in three of four groups instilled with alumina (Table 11). This diagnostic term was used to identify the accumulation of alveolar macrophages in alveolar lumens observed in conjunction with chronic pulmonary congestion and edema due to cardiovascular disease, described earlier (see above) as one cause of diffuse septal fibrosis. This lesion, although usually diffuse, was sometimes more severe multifocally with small aggregates of macrophages containing hemosiderin. Obviously, these lesions are difficult to differentiate from alveolar macrophages present in alveoli in response to instilled material, and in many cases (e.g., animals exposed to the highest doses) this lesion may have been masked by the intense macrophage accumulation in response to the instilled material. Nevertheless, this lesion was recognized microscopically in many animals as a separate entity from the response to instilled materials, mainly by its usually diffuse distribution and accompanying evidence of chronic cardiovascular disease, i.e., diffuse pulmonary congestion/edema, hemosiderin-filled macrophages ("heart failure cells") in the lung, subcutaneous or generalized edema, atrial thrombosis, and/or cardiac dilatation. Another possible contributing factor in the development of this lesion was the presence of severe renal disease secondary to glomerular amyloidosis. This disease can also lead to "cardiac edema" and may have contributed to the development of chronic pulmonary congestion.

Pulmonary alveolar lipoproteinosis (PAL) is an accumulation of pulmonary surfactant material within alveoli, usually in response to some irritant but in some cases of idiopathic origin. This phospholipid protein material,

Table 11. Incidence and severity of granulomatous inflammation and alveolar macrophage aggregates

Group	Multifocal/Diffuse Granulomatous Inflammation		Multifocal/Diffuse Macrophage Aggregates	
	Incidence (%)	Mean Severity	Incidence (%)	Mean Severity
Quartz, 6.0 mg	25/25 (100)	4.1	--	--
3.3 mg	25/26 (96)	3.4	--	--
0.33 mg	13/27 (48)	2.5	8/27 (30)	2.0
0.03 mg	3/25 (12)	2.7	10/25 (40)	2.2
Quartz + Fe_2O_3, 6.0 mg ea	24/26 (92)	3.9	--	--
3.3 mg ea	24/28 (86)	3.5	--	--
0.33 mg ea	22/25 (88)	1.6	1/25 (4)	2.0
0.03 mg ea	18/24 (75)	1.3	1/24 (4)	2.0
Glass, 10.0 mg	25/25 (100)	2.3	--	--
1.0 mg	12/25 (48)	1.4	--	--
0.5 mg	9/25 (36)	1.4	4/25 (16)	1.3
0.05 mg	2/26 (8)	1.0	4/26 (15)	1.5
Alumina, 20.0 mg	24/25 (96)	2.4	2/25 (8)	2.5
5.0 mg	24/25 (96)	1.0	8/25 (32)	1.3
2.0 mg	22/25 (88)	1.1	--	--
0.2 mg	13/25 (52)	1.0	5/25 (20)	2.0
Saline Controls	--	--	8/27 (30)	1.9
Cage Controls	--	--	11/25 (44)	1.6

secreted by alveolar type II cells and removed through phagocytosis by macrophages, accumulates in alveoli and surrounds irritant materials, in effect walling them off from the lung parenchyma. This lesion was observed in two different histologic patterns in this study. Multifocal PAL was often observed in animals exposed to the highest doses of quartz or quartz and ferric oxide (Table 12). In these animals, PAL was part of the intense granulomatous inflammatory response to the instilled material. Many inflammatory cells were lying within lipoprotein in alveolar lumens, and cholesterol clefts were often present within the lipoprotein (Fig. 17). This lesion was clearly related to instillation of material. The other situation in which lipoprotein was observed was in isolated foci of alveolar epithelial hyperplasia and septal fibrosis (Fig. 14). These were usually discrete and quite large, sometimes observed grossly at necropsy. In some, but not all cases instilled material was visible within these foci. They were also observed in a few saline control hamsters, so their relationship to instillation of materials was not clear. Of the eleven animals observed to have pulmonary "masses" at necropsy (Table 7), six were found at microscopic examination to have large, discrete foci of PAL, with accompanying septal fibrosis and alveolar epithelial hyperplasia. Three of the remaining masses were found to be abscesses, one was a focus of squamous metaplasia with cyst formation, and in one case no discrete mass was found at microscopic examination.

"Bronchiolization" of alveoli indicates the presence of cuboidal to low columnar epithelial cells replacing the normal flattened epithelial cells of alveoli and alveolar ducts adjacent to respiratory bronchioles. This is thought to occur through hyperplasia of bronchiolar epithelium and metaplasia of the epithelium of alveolar ducts and alveoli (Stewart, et al., 1979; Nettesheim and Szalkal, 1972). Bronchiolization of alveoli was observed in high incidence in both exposed and control groups (Table 12), especially in animals dying or sacrificed after 400 days on study (Fig. 18). This lesion appeared to be more severe and occurred more frequently in groups exposed to the two highest doses of quartz or quartz and ferric oxide. Mean severity of this lesion was somewhat higher in all groups exposed to quartz or quartz and ferric oxide, when compared with control groups.

Alveolar epithelial hyperplasia (Fig. 14) is a term used to indicate the proliferation of type 2 epithelial cells lining alveoli; synonyms used to denote this lesion in rodent lungs include adenomatosis, fetalization, columnar or cuboidal cell metaplasia, and epithelialization. This lesion was observed infrequently in both exposed and control groups (Table 12); the only group in which it appeared to be much different from saline controls in incidence/severity was the group instilled with the highest dose of fibrous glass. A single large focus of severe squamous metaplasia, apparently of bronchiolar epithelium, with cyst formation was observed in the lung of one hamster instilled with 1.0 mg fibrous glass.

Chronic glomerulonephritis was observed in virtually all animals in which the kidneys were examined microscopically. The majority of these lesions were severe and appeared to be the cause of death in many cases. Congo red stains of representative kidney sections demonstrated the presence of large amounts of amyloid in glomeruli of these animals. Chronic degenerative myocardial lesions, hypertrophy of myocardium, and degeneration of coronary arteries, accompanied by mural thrombi of the atrium in various stages of organization were observed in heart sections examined microscopically from instilled and control groups.

Table 12. Incidence and severity of pulmonary lesions in hamsters

Group	Bronchiolization Incidence (%)	Bronchiolization Severity	Alveolar Lipoproteinosis Incidence (%)	Alveolar Lipoproteinosis Severity	Alveolar Epithelial Hyperplasia Incidence (%)	Alveolar Epithelial Hyperplasia Severity
Quartz						
6.0 mg	17/25 (68)	2.8	14/25 (56)	3.1	3/25 (12)	2.7
3.3 mg	20/26 (77)	2.9	14/26 (54)	2.4	0/26	--
0.33 mg	16/27 (59)	2.3	2/27 (7)	2.5	0/27	--
0.03 mg	13/25 (52)	2.5	4/25 (16)	2.3	3/25 (12)	2.7
Quartz + Fe_2O_3						
6.0 mg ea	21/26 (81)	3.6	14/26 (54)	3.2	1/26 (04)	5.0
3.3 mg ea	18/28 (64)	3.3	10/28 (36)	2.4	0/28	--
3.33 mg ea	18/25 (72)	2.5	5/25 (20)	1.8	2/25 (08)	3.0
0.03 mg ea	9/24 (38)	2.0	4/24 (17)	3.8	2/24 (08)	3.5
Glass						
10.0 mg	14/25 (56)	2.8	12/25 (48)	2.4	11/25 (44)	2.5
1.0 mg	12/25 (48)	1.5	4/25 (16)	2.0	2/25 (08)	2.5
0.5 mg	12/25 (48)	2.2	7/25 (28)	1.9	5/25 (20)	2.4
0.05 mg	10/26 (38)	1.6	2/26 (08)	2.0	2/26 (08)	2.0
Alumina						
20.0 mg	11/25 (44)	2.4	6/25 (24)	2.0	5/25 (20)	2.2
5.0 mg	10/25 (40)	1.7	2/25 (08)	2.0	2/25 (08)	2.0
2.0 mg	9/25 (36)	1.4	6/25 (24)	2.2	5/25 (20)	1.8
0.2 mg	11/25 (44)	2.0	8/25 (32)	2.1	10/25 (40)	2.1
Saline Controls	8/27 (30)	1.6	3/27 (11)	1.3	7/27 (26)	1.4
Cage Controls	13/25 (52)	1.5	0/25		0/25	

DISCUSSION

Extensive efforts were made to obtain particulate materials with the specific chemical composition and particle size described in published studies in which pulmonary fibrosis was induced. The two references by investigators from the Postgraduate Medical School of London (Engelbrecht et al., 1959; Stacy et al., 1959) cited a sample of hydrated alumina designated as HX-1010, an alumina gel produced in the late 1940's and early 1950's by the Aluminum Company of America (ALCOA) as an experimental product. NIOSH contracted with ALCOA in October, 1979 to provide a similar material. Two pilot plant efforts in November, 1979 failed to produce a hydrated alumina with the same form (amorphous, and boehmite or pseudoboehmite) or surface area (300 m^2/g) as described in the references by Engelbrecht et al. and Stacy et al. Following three additional bench trials, ALCOA delivered 60 grams of material comparable in physical characteristics to HX-100, i.e., a non-crystalline (amorphous) material with primary particles in the range of 50 to 200A and a surface area of 250 m^2/g. However, the delivery occurred too late for inclusion in the study and a commercial hydrated alumina was used (Fisher Scientific Co. A-582, lot 791077) with the specifications reported in Table 2.

A similar problem was encountered when an attempt was made to obtain fibrous glass with fibers 1-2 µm diameter and 10-20 µm length, dimensions demonstrated to be a key factor in the pulmonary toxicity of fibrous glass (Wright and Kuschner, 1975). Owens-Corning Inc., Brookhaven Laboratories and the Lovelace Inhalation Toxicology Research Institute were contacted as potential suppliers of fibrous glass with these specifications. These institutions could not provide such material and related that costs to obtain the approximately one gram of material required for fifteen instillations of 100 animals would be prohibitive. A concurrent NIOSH-sponsored inhalation study on fibrous glass at another contract laboratory included as one of the test atmospheres a fibrous glass sample with fiber dimension specifications of less than 3.5 µm diameter and greater than 10 µm length. A sample of this fibrous glass was obtained and used in this intratracheal instillation study. This fibrous glass sample had approximately twelve percent of the fibers characterized as greater than 10 µm in length and 2.0 µm or less in diameter (Table 2). On a weight basis, this would be in excess of 90 percent of the sample.

Clinical observations of subcutaneous edema and cardiac and renal lesions observed at necropsy reflect a relatively high incidence of serious cardiovascular and renal disease, which had an impact not only on survival rates but on the evaluation and interpretation of histologic findings in the lung. Several lesions present in the animals in this study could have contributed to the relatively high incidence of subcutaneous or generalized edema and cardiac dilatation observed clinically and at necropsy. The most obvious is the cardiac lesions observed grossly and microscopically. Atrial thrombosis and myocardial degeneration frequently occur spontaneously in hamsters and have been well described previously (McMartin, 1977). Severe glomerulonephritis

secondary to amyloid deposition in glomeruli (another disease which frequently occurs spontaneously in hamsters), was very prevalent in animals in this study and may have been responsible for some of the edema and cardiac dilatation through the osmotic effect of protein and electrolyte loss in the urine. Another factor is the potential vascular effect of pulmonary fibrosis, congestion and edema caused by the instilled materials. The pulmonary fibrosis observed in the animals in this study did not appear to be severe enough to cause problems with pulmonary blood flow. However, the combined fibrotic and inflammatory effects of the instilled materials may have contributed to a decrease in pulmonary vascular flow, which in combination with the other factors mentioned above resulted in death from congestive heart failure. The highest incidence of edema was observed in the groups instilled with the largest doses of quartz or quartz plus ferric oxide (Tables 3 and 7).

It is difficult to assess the impact of the apparent Sendai virus infection early in the study on mortality or the incidence and severity of pulmonary lesions. Lesions of Sendai virus infection normally regress within two months after onset in mice (Ward, 1974), and should not be expected to persist in hamsters (Profeta, et al., 1969). However, the effect of the instillation of the test materials on the pathogenesis of Sendai virus in the lung is not known. The high incidence of bronchiolization in test and control groups may be due in part to the Sendai virus infection.

The diarrhea frequently observed in instilled and control groups (Table 3) was apparently not of infectious origin. These animals had a high incidence of severe glomerulonephritis and the diarrhea was probably secondary to uremia resulting from breakdown of urea by gut microflora to form ammonia, which irritates the gut wall.

Comparison of data from the various groups on body weight (Figs. 1-5) and lung weight (Table 4 and Fig. 6) indicates that groups exposed to the two highest doses of quartz or quartz and ferric oxide were the most profoundly affected. Lung weight increases in these groups undoubtedly reflect the intense inflammatory responses in the lungs of these animals.

The survival of the quartz- and quartz plus ferric oxide-instilled animals was clearly related to dose level. A consistent trend towards shorter survival with increased dose is exhibited by both the mean survival time and the median survival time for animals exposed to these two compounds (Table 6). In interpreting the survival data on these groups of animals, one must consider the possibility that instillation of large doses of quartz and the response to it aggravated or shortened the latency period for the cardiovascular and/or renal disease observed in all groups, and that the decreased survival rate observed in these groups may be due to the combined effect of several disease processes.

Survival of the groups instilled with fibrous glass was not significantly different from the saline controls, nor was there an evident trend in the mean or median survival times. It can be concluded that fibrous glass instillation did not significantly affect survival.

The animals in the groups instilled with the two lower doses of hydrated alumina had survival times significantly shorter than that of the saline controls. The survival of the groups instilled with the two higher doses was similar to the saline controls. The hypothesis that hydrated alumina instillation affects survival can neither be affirmed nor refuted.

Both methods used to analyze the pulmonary fibrosis data indicated a dose-response effect for all materials. The Mantel test statistic shows that the response to higher dose levels tends to be greater in both incidence and severity.

All instilled materials caused significant dose and time effects in the analysis of variance and multiple classification analysis. The instillation of quartz or quartz and ferric oxide produced consistent increases in fibrosis with time post-instillation and with increasing dose. For fibrous glass, the 1.0 mg dose level appeared low, but otherwise the trend was consistent.

Although both statistical tests indicated a significant positive dose effect on pulmonary fibrosis for instillation of hydrated alumina, the results are somewhat ambiguous. When the analysis of variance was repeated without the highest dose group, both main effects (dose and time) were not significant. This result, coupled with the anomalous survival data for animals instilled with this material, suggests either a statistical anomaly or the presence of some unobserved factor.

CONCLUSIONS AND RECOMMENDATIONS

Based on the results of this study, we conclude that each of the materials instilled will induce some fibrotic response in hamsters at the dose levels used. The instillation of quartz or quartz and ferric oxide induced a much greater response than did the other materials, and the dose response was much clearer with these materials. Unfortunately, the mean life span was significantly decreased (Table 6) in those groups instilled with the two highest doses of quartz or quartz and ferric oxide. Of the remaining groups, the mean severity of septal fibrosis observed (Table 9) was the greatest in the group instilled with 20 mg of hydrated alumina, followed by the group instilled with 10 mg of fibrous glass, then the group instilled with 0.33 mg each of quartz and ferric oxide. However, the fibrotic response in any of these three groups was not severe enough to be regarded by us as "pronounced" fibrosis; therefore, our recommendation for a material to induce a pronounced septal fibrosis in an animal model for the study of the effect of fibrosis on pulmonary neoplasia is the quartz material we utilized. Recommending a dose of this material for use in the hamster is difficult based on our results. We did not succeed in inducing a pronounced fibrosis without decreasing the life span of the animal. However, as mentioned above, there appeared to be several factors responsible for the shortened life span of the hamsters instilled with the highest doses of quartz or quartz and ferric oxide. If one used an animal species or strain which was not subject to the renal and cardiac disease observed in the hamsters in this study, decreased survival might not be a problem at the higher dose levels we used.

Another factor is the question of the degree of severity of fibrosis and the amount of mature collagenous fibrous tissue required to have an effect on chemically induced pulmonary neoplasia. The most severe fibrosis observed in our study was much less severe than that induced in rats with quartz (Renne et al., 1980), and the amount of mature fibrous tissue in the lung was relatively small. No animal in our study had large, discrete foci of dense fibrous tissue containing solid bands of mature collagen, as is present in classical silicosis of man and in experimental silicosis in rats. One must consider the possibility that the hamster, like the mouse or guinea pig (Engelbrecht, 1959), is simply not the best choice to use for studies requir-requiring induction of intense pulmonary fibrosis. Also to be considered in planning studies of the effects of pulmonary fibrosis on induction or pathogenesis of chemically induced pulmonary neoplasia is the relationship between the most likely site of neoplastic response and the site of fibrotic response. The hamster, although it has been the animal of choice for induction of pulmonary carcinogenesis by intratracheal instillation of benzo(a)pyrene (BaP) or other polycyclic aromatic hydrocarbon carcinogens, has an important disadvantage when compared with the rat as a model for studying the role of pulmonary fibrosis in the etiology or pathogenesis of lung cancer. Tumors induced in the respiratory tract in hamsters are more frequently located in the upper airways (larnyx, trachea, mainstem bronchi) than in the smaller

bronchioles or alveoli (Saffiotti et al., 1968; Henry et al., 1975), whereas pulmonary tumors induced in rats by intratracheal instillation are most often located in the distal airways or alveoli (Blair, 1974). This is an important point to consider because the quartz-induced pulmonary fibrosis will occur in the lung parenchyma at a site relatively distant from the most frequent site of induction of respiratory tract tumors in hamsters.

Our recommendation for the development of an animal model to study the role of pulmonary fibrosis in lung cancer is the use of multiple instillations of quartz in laboratory rats. Several protocols seem feasible; one could instill quartz and ferric oxide (as we did in hamsters), and by attaching benzo(a)pyrene to the ferric oxide, simultaneous development of fibrosis and neoplasia should be obtained. An alternative would be initial instillations of quartz, followed by instillation of benzo(a)pyrene and ferric oxide (after waiting a suitable interval of time to allow fibrotic lesions to develop). This would have the disadvantage of exposure of pulmonary tissues to benzo(a)pyrene for a shorter duration. Pylev's work (1979) indicates that simultaneous instillation of benzo(a)pyrene and quartz induces a higher rate of lung tumor response than does instillation of benzo(a)pyrene 4 months after a single instillation of 50 mg of quartz.

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APPENDIX I

Individual Animal Data

Key to histopathology data:

X = Tissue was examined microscopically; none of the lesions listed in the table were present.

N = Tissue not examined microscopically

NA = Tissue not interpretable microscopically due to autolysis

1 = Minimal lesion

2 = Mild lesion

3 = Moderate lesion

4 = Moderate to severe lesion

5 = Severe lesion

Individual Animal Data - Hamsters Instilled Intratracheally with 6.0 mg Quartz

Day of Death:	293	107	482	83	457	377	448	401	475	391	79	120	410	482	343	356	383	482	355	415	299	314	240	482	438	Total (%)	Mean Severity
Animal No:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25		
LUNG:																											
Alveolar septal fibrosis	3	2	2		3	4	3	3	5	4			4	3	4			3		3	4			3	3	17/25 (68)	56/17 = 3.3
Granulomatous inflammation	5	4	5	5	4	5	4	4	5	4	5	3	4	5	4	3	4	5	4	3	4	3	4	4	3	25/25 (100)	103/25 = 4.1
Bronchiolization	2	3	2		3		3	3	4	3		2	1	4	4			3		2	3			3	3	17/25 (68)	48/17 = 2.8
Alveolar lipoproteinosis			4			3		3	5	3			3	3	2			4	2	3			2	3	3	14/25 (56)	43/14 = 3.1
Alveolar epithelial hyperplasia	2		2						4																	3/25 (12)	8/3 = 2.7
Alveolar macrophage aggregates																											
Pulmonary congestion																3						2				2/25 (8)	5/2 = 2.5
Pulmonary edema																											
Pulmonary hemorrhage																											
Interstitial pneumonitis		3																								1/25 (4)	3/1 = 3.0
Suppurative alveolitis			4																							1/25 (4)	4/1 = 4.0
Suppurative bronchiolitis																											
Pulmonary calcification																											
Suppurative pleuritis																											
Pleural fibrosis						3	3											4						3		4/25 (16)	13/4 = 3.3
TRACHEOBRONCHIAL LYMPH NODE:		N			N										N												
Macrophage accumulation			4	4		4	4	4	4	4	4	4	4	3		3	3	3	3	2	4	3	3	4	4	21/22 (95)	75/21 = 3.6
Fibrosis	3		2			3	4	4	4	3	2	2	3	3		3	2	2	3	1	2	1	1	3	3	21/22 (95)	44/21 = 2.1
Suppurative inflammation			3	4		4	3	4	4	4	4	3	4	3		3	3	3	3	2	4	3	3	4	4	21/22 (95)	72/21 = 3.4
FINAL BODY WEIGHT (g)	165	107	126	--	116	149	145	126	137	143	126	122	98	150	170	69	112	139	125	114	78	77	139	141	70		
LUNG WEIGHT (g)	3.7	--	3.2	--	3.5	3.1	4.4	2.8	6.2	2.9	--	--	3.6	3.9	2.8	2.2	4.0	4.0	3.1	2.3	3.0	3.4	--	4.4	2.5		

Individual Animal Data - Hamsters Instilled Intratracheally with 3.3 mg Quartz

Day of Death:	83	517	278	484	457	438	163	14	470	487	517	42	81	517	517	415	474	458	312	359	356	29	390	517	491	377	Total (%)	Mean Severity
Animal No:	26	27	28	29	30	31	32	33	33A	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50		
LUNG:																												
Alveolar septal fibrosis	2	3	2	2	3	2			2	2	3			4	3	2	4	3	3	2			2	3		2	19/26 (73)	49/19 = 2.6
Granulomatous inflammation	4	4	3	3	4	4	3		2	3	4	3	4	4	4	3	3	3	3	2	3	5	4	4	3	4	25/26 (96)	86/25 = 3.4
Bronchiolization	3	3	3	2	3	3			3	3	4	3		3		3	3		3	2		2	3	4	2	3	20/26 (77)	58/20 = 2.9
Alveolar lipoproteinosis		3	2	3	3	2				3	3			3			2	2		1	2		2	3			14/26 (54)	34/14 = 2.4
Alveolar epithelial hyperplasia																												
Alveolar macrophage aggregates																												
Pulmonary congestion							3						1														2/26 (8)	4/2 = 2.0
Pulmonary edema																												
Pulmonary hemorrhage						3																					2/25 1/26 (4)	3/1 = 3.0
Interstitial pneumonitis																												
Suppurative alveolitis								5																			1/26 (4)	5/1 = 5.0
Suppurative bronchiolitis								5																			1/26 (8)	5/1 = 5.0
Pulmonary calcification																												
Suppurative pleuritis																												
Pleural fibrosis															3						3						2/26 (8)	6/2 = 3.0
TRACHEOBRONCHIAL LYMPH NODE:	N							N	N																			
Macrophage accumulation		3	3	3	3	3	4			2	3	1	1	3	3	3	3	3	3	3	3	2	3	3	3	3	23/23 (100)	64/23 = 2.8
Fibrosis		3		4	4	2	2			1	4			3	3	3	2	3	3	2	2		3	3	2	2	19/23 (83)	51/19 = 2.7
Suppurative inflammation		3		3	3	2	2			2	3			3	3	3	3	3	3	2	3	3	3	3	2	3	20/23 (87)	55/20 = 2.8
FINAL BODY WEIGHT (g)	--	147	106	92	104	130	70	49	85	108	131	108	129	147	120	209	138	141	92	84	123	88	--	145	84	163		
LUNG WEIGHT (g)	--	3.0	1.3	2.3	3.2	5.4	--	--	1.8	2.4	--	--	--	3.7	2.7	2.9	2.7	2.5	2.5	2.6	2.8	--	3.1	3.1	--	2.7		

Individual Animal Data - Hamsters Instilled Intratracheally with 0.33 mg Quartz

Day of Death:	16	542	643	643	607	18	377	637	635	583	618	601	552	643	644	130	644	644	573	65	625	644	585	15	391	481	91	Total (%)	Mean Severity
Animal No:	51	51A	52	53	54	55	55A	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75		
LUNG:							X																						
Alveolar septal fibrosis		1		2				2		2		2		3	3		2	2			2	2						11/27 (41)	23/11 = 2.1
Granulomatous inflammation		1		2		5				4		2		2	3	5		2		1	2	2	1			1		13/27 (48)	33/13 = 2.5
Bronchiolization		1	1	1	2			1		3	3	3		3	3		3	2			3	4	2			1		16/27 (59)	36/16 = 2.3
Alveolar lipoproteinosis											3												2					2/27 (7)	5/2 = 2.5
Alveolar epithelial hyperplasia																													
Alveolar macrophage aggregates					2				2				2	3			3		1	2					1			8/27 (30)	16/8 = 2.0
Pulmonary congestion													2						2				2	3		1	2	6/27 (22)	12/6 = 2.0
Pulmonary edema																								3				1/27 (4)	3/1 = 3.0
Pulmonary hemorrhage												2												3				2/27 (7)	5/2 = 2.5
Interstitial pneumonitis																													
Suppurative alveolitis	4							2	5																			3/27 (11)	11/3 = 3.7
Suppurative bronchiolitis	4								5																			2/27 (7)	9/2 = 4.5
Pulmonary calcification																													
Suppurative pleuritis																													
Pleural fibrosis											1								3									2/27 (7)	4/2 = 2.0
TRACHEOBRONCHIAL LYMPH NODE:	N												N		N				N				N						
Macrophage accumulation		1	1	2	3	2	1	2	2	3	3	2		3			2	2		2	2	3			2	3	1	20/22 (91)	42/20 = 2.1
Fibrosis				2	3			1	2	3	3			3		2	2	1			1	3				2		13/23 (59)	28/13 = 2.1
Suppurative inflammation			2	2	3	4			3	2	2	3		3		3	2	1			1	2		3		2		16/22 (73)	38/16 = 2.4
FINAL BODY WEIGHT (g)	93	97	104	98	116	--	115	135	92	134	74	238	142	172	116	--	146	151	71	104	151	136	92	86	99	118	154		
LUNG WEIGHT (g)	--	--	1.3	1.5	1.5	--	1.2	1.8	2.4	3.6	1.0	3.2	2.2	2.5	1.3	--	2.3	1.5	1.6	--	3.1	1.2	3.1	--	1.8	2.6	--		

Individual Animal Data - Hamsters Instilled Intratracheally with 0.03 mg Quartz

Day of Death:	96	650	664	45	30	664	664	629	506	657	643	650	506	664	489	285	588	469	583	560	46	575	650	91	559	Total (%)	Mean Severity
Animal No:	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100		
LUNG:																	X										
Alveolar septal fibrosis		2						2		2	2	3							2	2				2	2	9/25 (36)	19/9 = 2.1
Granulomatous inflammation	2	2			4																					3/25 (12)	8/3 = 2.7
Bronchiolization		3	3			2	2		2	2	5	2	2	1					3				3	3		13/25 (52)	33/13 = 2.5
Alveolar lipoproteinosis								2		2		3								2						4/25 (16)	9/4 = 2.3
Alveolar epithelial hyperplasia		4						2		2																3/25 (12)	8/3 = 2.7
Alveolar macrophage aggregates						1		1	1		5	2			1				2	3				3	3	10/25 (40)	22/10 = 2.2
Pulmonary congestion				2														1			3	3			3	5/25 (20)	12/5 = 2.4
Pulmonary edema																											
Pulmonary hemorrhage						1										2							2			3/25 (12)	5/3 = 1.7
Interstitial pneumonitis																		1			3				2	3/25 (12)	6/3 = 2.0
Suppurative alveolitis											5										3					2/25 (8)	8/2 = 4.0
Suppurative bronchiolitis																											
Pulmonary calcification																											
Suppurative pleuritis																								5		1/25 (4)	5/1 = 5.0
Pleural fibrosis											5															1/25 (4)	5/1 = 5.0
TRACHEOBRONCHIAL LYMPH NODE:	N	N	N	N	N	N			X				N	X		N				N	N	X	N	N	N		
Macrophage accumulation							1	1		1	1	1			2		1	1	2							9/12 (75)	11/9 = 1.2
Fibrosis															1				2							2/12 (17)	3/2 = 1.5
Suppurative inflammation																										0/12	
FINAL BODY WEIGHT (g)	114	113	130	111	107	163	169	173	75	116	90	180	77	131	88	109	64	100	85	146	74	135	109	142	91		
LUNG WEIGHT (g)	--	1.2	1.3	--	--	2.0	1.9	1.9	1.0	1.3	1.6	2.1	1.1	1.4	1.2	1.6	1.2	2.2	3.3	3.7	--	2.3	1.4	--	2.7		

Individual Animal Data - Hamsters Instilled Intratracheally with 6.0 mg ea Quartz plus Ferric Oxide

Day of Death:	482	321	265	321	482	372	255	449	412	475	322	251	465	363	436	83	482	222	15	338	457	420	28	151	419	137	Total (%)	Mean Severity
Animal No:	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	119A	120	121	122	123	124	125		
LUNG:																												
Alveolar septal fibrosis	4	3	3	4	4	3	4	3	3	3	4	3	3	4	4	3	3	3			3	2			4	1	22/26 (85)	71/22 = 3.2
Granulomatous inflammation	5	4	4	4	4	4	4	4	4	4	4	4	4	4	4	5	4	4		2	3	3		3	4	4	24/26 (92)	93/24 = 3.9
Bronchiolization	3	4	3		4	4	4	3	4	4	4	4	3	3	4	3	4	4		3	3	3			4		21/26 (81)	75/21 = 3.6
Alveolar lipoproteinosis	3				3	3				4	3	3	3	3	4		3	3			2	4			4		14/26 (54)	45/14 = 3.2
Alveolar epithelial hyperplasia				5																							1/26 (4)	5/1 = 5.0
Alveolar macrophage aggregates																												
Pulmonary congestion																				4							1/26 (4)	4/1 = 4.0
Pulmonary edema																												
Pulmonary hemorrhage																												
Interstitial pneumonitis																			4				5				2/26 (8)	9/2 = 4.5
Suppurative alveolitis																							5				1/26 (4)	5/1 = 5.0
Suppurative bronchiolitis																												
Pulmonary calcification																												
Suppurative pleuritis																												
Pleural fibrosis							3							3				4									3/26 (12)	10/3 = 3.3
TRACHEOBRONCHIAL LYMPH NODE:																			X									
Macrophage accumulation	3	4			4	4	3	4	4	4	4	4	4	4	4	3	4	4		3	3	3	2	3	3	3	23/26 (88)	81/23 = 3.5
Fibrosis	3	4	4	4	4	3	4	4	4	3	4	4	4	3	4	2	3	2			2	4		3	3		22/26 (85)	75/22 = 3.4
Suppurative inflammation	1	1				2			1						1	1		2								3	8/26 (31)	12/8 = 1.5
FINAL BODY WEIGHT (g)	103	217	101	155	128	157	101	98	89	129	131	102	75	75	141	--	114	90	92	106	80	101	139	96	88	79		
LUNG WEIGHT (g)	3.8	4.5	3.6	3.1	3.7	3.7	3.4	4.1	3.0	3.5	3.9	--	3.8	5.0	4.2	--	3.7	--	--	4.4	2.5	3.9	--	--	3.2	--		

Individual Animal Data - Hamsters Instilled Intratracheally with 3.3 mg ea Quartz plus Ferric Oxide

Day of Death:	141	137	552	400	413	14	510	27	517	392	560	560	529	21	517	377	349	49	448	346	212	16	429	19	560	533	543	268	Total (%)	Mean Severity
Animal No:	126	127	128	129	130	131	131A	132	132A	133	134	135	136	137	137A	138	139	140	141	142	143	144	145	146	147	148	149	150		
LUNG:																														
Alveolar septal fibrosis			2	3	4		3		3	3	4	3	3		3	3	3	1	2	3	2		2		3	4	2	3	21/28 (75)	59/21 = 2.8
Granulomatous inflammation	4	3	3	4	4			3	3	5	3	4	3	4	3	4	2	4	3	4	3		4		3	4	3	4	24/28 (86)	84/24 = 3.5
Bronchiolization	2		4	4			4		3	3	5	5	3		3	4	3	2	3				3		3	3	3		18/28 (64)	60/18 = 3.3
Alveolar lipoproteinosis			2	3					2		3		2			2	2			3					3		2		10/28 (36)	24/10 = 2.4
Alveolar epithelial hyperplasia																														
Alveolar macrophage aggregates																														
Pulmonary congestion																											2		1/28 (4)	2/1 = 2.0
Pulmonary edema																														
Pulmonary hemorrhage																														
Interstitial pneumonitis						4																2		3					3/28 (11)	9/3 = 3.0
Suppurative alveolitis										4										4				4					3/28 (11)	12/3 = 4.0
Suppurative bronchiolitis										4										4				4					3/28 (11)	12/3 = 4.0
Pulmonary calcification																														
Suppurative pleuritis																														
Pleural fibrosis																												5	1/28 (4)	5/1 = 5.0
TRACHEOBRONCHIAL LYMPH NODE:		N				N	N	N						N								X		N						
Macrophage accumulation	3		3	4	3				3	3	3	3	2		3	4	4	2	3	3	3		5		3	4	2	4	21/22 (95)	67/21 = 3.2
Fibrosis	3		4	3	4				4	3	2	3			3	4	4		3	2	3		3		3	3		4	18/22 (82)	58/18 = 3.2
Suppurative inflammation													1		2														2/22 (9)	3/2 = 1.5
FINAL BODY WEIGHT (g)	100	124	81	91	171	--	120	72	146	127	126	101	82	121	131	189	78	108	92	87	72	48	88	70	95	108	119	86		
LUNG WEIGHT (g)	--	--	2.5	3.0	3.3	--	2.1	--	2.2	4.5	2.6	3.2	3.2	--	3.3	2.3	2.9	--	2.3	4.3	--	--	3.1	--	2.1	3.4	3.6	3.1		

Individual Animal Data - Hamsters Instilled Intratracheally with 0.33 mg ea Quartz plus Ferric Oxide

Day of Death:	630	624	518	567	511	32	650	650	603	664	664	636	438	664	664	620	490	664	650	664	650	664	663	565	561	Total (%)	Mean Severity
Animal No:	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175		
LUNG:						X																					
Alveolar septal fibrosis	1	1					2	2		2	1	2		3		2	2		1	3	2	3	1		3	16/25 (64)	31/16 = 1.9
Granulomatous inflammation	1	1	1	2	2			1	2	2	1	2	1	2	1	1	1	1	2	2	3	2	2	1		22/25 (88)	34/22 = 1.6
Bronchiolization	2	3		2	3			2	2	2	2	3		3	3	2	3		2	3	2	4			2	18/25 (72)	45/18 = 2.5
Alveolar lipoproteinosis					1			1		1				3								3				5/25 (20)	9/5 = 1.8
Alveolar epithelial hyperplasia														4											2	2/25 (8)	6/2 = 3.0
Alveolar macrophage aggregates							2																			1/25 (4)	2/1 = 2.0
Pulmonary congestion	2				2		3		2															1	2	6/25 (24)	12/6 = 2.0
Pulmonary edema																											
Pulmonary hemorrhage							2																			1/25 (4)	2/1 = 2.0
Interstitial pneumonitis																											
Suppurative alveolitis																							3			1/25 (4)	3/1 = 3.0
Suppurative bronchiolitis																							3			1/25 (4)	3/1 = 3.0
Pulmonary calcification																											
Suppurative pleuritis																											
Pleural fibrosis									2																	1/25 (4)	2/1 = 2.0
TRACHEOBRONCHIAL LYMPH NODE:	X	N		N	N	X	X										N		N			N					
Macrophage accumulation			4					3	3	3	3	3	3		3			3		3	2		2	2	2	14/19 (74)	39/14 = 2.8
Fibrosis			4					4	3	2	3	3	3	2	4	3		4		3	3					13/19 (68)	41/13 = 3.2
Suppurative inflammation																										0/19	
FINAL BODY WEIGHT (g)	160	87	99	87	98	79	124	141	104	130	135	194	115	150	158	130	132	69	83	129	113	161	118	131	138		
LUNG WEIGHT (g)	3.1	1.3	1.9	2.2	1.7	--	4.3	2.1	1.8	2.0	2.1	1.7	1.7	2.0	4.3	1.4	1.7	1.4	1.1	1.9	1.9	1.5	2.0	2.4	2.1		

Individual Animal Data - Hamsters Instilled Intratracheally with 0.03 mg ea Quartz plus Ferric Oxide

Day of Death:	439	664	362	530	664	650	664	538	44	643	537	664	572	501	28	620	650	664	620	664	664	607	342	21	612	255	Total (%)	Mean Severity
Animal No:	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	190A	191	192	193	194	195	196	197	198	199	200		
LUNG:	N																					NA						
Alveolar septal fibrosis		1		1	1	1	1	2		4	3	4		1			3			1	1					2	14/24 (58)	26/14 = 1.9
Granulomatous inflammation			1	1	1	1	1	1		1	1	1		1				1	1	1	1		1	5	1	2	18/24 (75)	23/18 = 1.3
Bronchiolization			2		2	3	2						1			1	2		3	2							9/24 (38)	18/9 = 2.0
Alveolar lipoproteinosis										4	3	4					4									2	4/24 (17)	15/4 = 3.8
Alveolar epithelial hyperplasia										4							3										2/24 (8)	7/2 = 3.5
Alveolar macrophage aggregates		2																									1/24 (4)	2/1 = 2.0
Pulmonary congestion				3				3	2																2		4/24 (17)	10/4 = 2.5
Pulmonary edema																												
Pulmonary hemorrhage						1				2							3		3								4/24 (17)	9/4 = 2.3
Interstitial pneumonitis															2												1/24 (4)	2/1 = 2.0
Suppurative alveolitis											3																1/24 (4)	3/1 = 3.0
Suppurative bronchiolitis											3																1/24 (4)	3/1 = 3.0
Pulmonary calcification																												
Suppurative pleuritis																												
Pleural fibrosis																												
TRACHEOBRONCHIAL LYMPH NODE:	N			N	N	N	N	N	N		N			N		X	N					N		N			
Macrophage accumulation		2	2							3		1	3					1	2	2		2		2	2	2	12/12 (100)	24/12 = 2.0
Fibrosis		3								3			3													1	4/12 (33)	10/4 = 2.5
Suppurative inflammation										3					1												2/12 (17)	4/2 = 2.0
FINAL BODY WEIGHT (g)	--	130	57	105	134	140	153	239	--	101	106	152	62	80	138	157	108	119	120	157	152	86	77	84	110	126		
LUNG WEIGHT (g)	--	4.0	1.1	1.7	--	1.6	1.5	2.9	--	1.5	1.5	1.7	1.5	1.2	--	1.9	1.7	1.3	2.3	1.9	1.5	1.1	1.2	--	2.4	2.5		

Individual Animal Data - Hamsters Instilled Intratracheally with 10.0 mg Fibrous Glass

Day of Death: / Animal No:	663 201	663 202	663 203	531 204	605 205	663 206	663 207	520 208	630 209	649 210	96 211	663 212	119 213	663 214	663 215	663 216	602 217	614 218	356 219	663 220	622 221	409 222	263 223	556 224	663 225	Total (%)	Mean Severity
LUNG:																											
Alveolar septal fibrosis	1	2	3		3	3	3	3	2	2	1	2	1	2	3	2	2	3	1	3	3	1	2	1	3	24/25 (96)	52/24 = 2.2
Granulomatous inflammation	3	2	3	1	1	3	3	3	2	2	2	3	2	3	3	2	3	3	2	2	2	1	2	2	2	25/25 (100)	57/25 = 2.3
Bronchiolization		1	3					3	3			4		3	3	3	3	3		3	1			3	3	14/25 (56)	39/14 = 2.8
Alveolar lipoproteinosis			2		2	3	2	2				2			3			3		2	3		2		3	12/25 (48)	29/12 = 2.4
Alveolar epithelial hyperplasia					2	3	3	2				2			3			3		2	3		2		2	11/25 (44)	27/11 = 2.5
Alveolar macrophage aggregates																											
Pulmonary congestion																			2							1/25 (4)	2/1 = 2.0
Pulmonary edema																											
Pulmonary hemorrhage																											
Interstitial pneumonitis																											
Suppurative alveolitis												3														1/25 (4)	3/1 = 3.0
Suppurative bronchiolitis							2																			1/25 (4)	2/1 = 2.0
Pulmonary calcification																											
Suppurative pleuritis																											
Pleural fibrosis																											
TRACHEOBRONCHIAL LYMPH NODE:	X	N			N			N				N		N			N			X		N	N	N			
Macrophage accumulation			2	2		2	1		3	1	1		2		1			3	1		2				3	13/16 (81)	24/13 = 1.9
Fibrosis							2		1		1				1	2									1	6/16 (38)	8/6 = 1.3
Suppurative inflammation																										0/16	
FINAL BODY WEIGHT (g)	113	117	146	124	112	149	147	61	106	114	108	158	88	150	137	168	77	130	110	118	193	121	75	117	147		
LUNG WEIGHT (g)	1.8	1.8	2.0	1.6	3.3	2.1	3.6	1.5	2.3	3.4	--	1.9	--	2.1	1.8	2.3	1.8	3.8	2.3	1.5	3.2	2.1	1.3	2.0	2.1		

Individual Animal Data - Hamsters Instilled Intratracheally with 1.0 mg Fibrous Glass

Day of Death:	663	663	460	656	94	416	628	638	415	509	490	28	368	655	525	639	413	587	277	34	663	642	553	663	663	604	Total (%)	Mean Severity
Animal No:	226	227	228	229	230	231	232	233	234	235	236	237	237A	238	239	240	241	242	243	244	245	246	247	248	249	250		
LUNG:																			NA									
Alveolar septal fibrosis		3								1	4			1	2	2	2	2			1					3	10/25 (40)	21/10 = 2.1
Granulomatous inflammation		1		1		1			1	1				2	2	2		2			1				1	2	12/25 (48)	17/12 = 1.4
Bronchiolization	1	1	1	1							3		2		2		2	2			1	1		1			12/25 (48)	18/12 = 1.5
Alveolar lipoproteinosis											4						1	1								2	4/25 (16)	8/4 = 2.0
Alveolar epithelial hyperplasia		3																								2	2/25 (8)	5/2 = 2.5
Alveolar macrophage aggregates																												
Pulmonary congestion			1		1	2		2				4	1	2	2	1							2			3	11/25 (44)	21/11 = 1.9
Pulmonary edema																												
Pulmonary hemorrhage				3																							1/25 (4)	3/1 = 3.0
Interstitial pneumonitis												4															1/25 (4)	4/1 = 4.0
Suppurative alveolitis																				4							1/25 (4)	4/1 = 4.0
Suppurative bronchiolitis																												
Pulmonary calcification											4							2									2/25 (8)	6/2 = 3.0
Suppurative pleuritis																												
Pleural fibrosis											4										1						2/25 (8)	5/2 = 2.5
Squamous metaplasia							5																					
TRACHEOBRONCHIAL LYMPH NODE:	N	N		X		N		N	N					X	X				N						N			
Macrophage accumulation			1		1		2			2	1		2			1	2	2			1	1	1	1		1	14/18 (78)	19/14 = 1.4
Fibrosis																		2									1/18 (6)	2/1 = 2.0
Suppurative inflammation			1									2						1		1							4/18 (22)	5/4 = 1.3
FINAL BODY WEIGHT (g)	135	170	131	93	140	156	102	113	116	104	67	86	71	156	142	119	106	102	115	98	125	109	120	173	135	149		
LUNG WEIGHT (g)	1.4	1.5	2.5	1.1	--	2.5	1.6	1.8	1.8	1.2	1.5	--	1.6	2.6	2.9	2.3	2.6	2.3	1.6	--	1.5	1.5	2.5	1.9	1.6	3.6		

Individual Animal Data - Hamsters Instilled Intratracheally with 0.5 mg Fibrous Glass

Day of Death: / Animal No:	663 251	663 252	625 253	649 254	507 255	663 256	558 257	542 258	523 259	663 260	208 261	619 262	228 263	523 264	663 265	613 266	525 267	377 268	635 269	582 270	552 271	663 272	500 273	31 274	603 275	Total (%)	Mean Severity
LUNG:																						2					
Alveolar septal fibrosis	2		2	3	2	1		2		2		1		1	1	2		1	3	1		2				15/25 (60)	26/15 = 1.7
Granulomatous inflammation	2			2	2	1				2		1		1	1				1							9/25 (36)	13/9 = 1.4
Bronchiolization		1	1	4							2	2	2	2	2	3			2			2			3	12/25 (48)	26/12 = 2.2
Alveolar lipoproteinosis			2	2				2		1						2			3			1				7/25 (28)	13/7 = 1.9
Alveolar epithelial hyperplasia			2					2								2			4			2				5/25 (20)	12/5 = 2.4
Alveolar macrophage aggregates												1		1				1		2						4/25 (16)	5/4 = 1.3
Pulmonary congestion	2				2		1					2					2				4		2	2		8/25 (32)	17/8 = 2.1
Pulmonary edema									1																		
Pulmonary hemorrhage																											
Interstitial pneumonitis																											
Suppurative alveolitis																				2			4			2/25 (8)	6/2 = 3.0
Suppurative bronchiolitis																											
Pulmonary calcification							1		1				2												2	3/25 (12)	5/3 = 1.7
Suppurative pleuritis																											
Pleural fibrosis																	2									1/25 (4)	2/1 = 2.0
TRACHEOBRONCHIAL LYMPH NODE:		N	N	N	X	N	N	N	N	N		X				N		N			N						
Macrophage accumulation	1										1		1	1	1		1		2	1		1	2	1	1	12/14 (86)	14/12 = 1.2
Fibrosis	1																								1	2/14 (14)	1/1 = 1.0
Suppurative inflammation														1	1											2/14 (14)	2/2 = 1.0
FINAL BODY WEIGHT (g)	176	119	142	113	139	141	97	136	78	142	102	210	144	233	141	75	120	136	190	139	84	159	127	110	152		
LUNG WEIGHT (g)	3.4	1.3	3.3	0.8	2.2	--	1.5	1.7	1.3	1.9	--	4.2	--	2.4	1.7	0.9	1.6	1.8	1.8	3.5	2.0	1.6	4.9	--	1.6		

Individual Animal Data - Hamsters Instilled Intratracheally with 0.05 mg Fibrous Glass

Day of Death:	598	160	472	15	620	663	623	601	631	264	466	147	150	663	642	163	663	449	35	385	663	622	460	598	663	453	Total (%)	Mean Severity
Animal No:	276	277	278	279	279A	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300		
LUNG:																												
Alveolar septal fibrosis	3				1		1	1	1						2									1			7/26 (27)	10/7 = 1.4
Granulomatous inflammation								1							1												2/26 (8)	2/2 = 1.0
Bronchiolization	2											2		2	1		1			2	2		1		2	1	10/26 (38)	16/10 = 1.6
Alveolar lipoproteinosis							2								2												2/26 (8)	4/2 = 2.0
Alveolar epithelial hyperplasia							2								2												2/26 (8)	4/2 = 2.0
Alveolar macrophage aggregates	1				2				2															1			4/26 (15)	6/4 = 1.5
Pulmonary congestion	2	3		1			3		2			2				1		1			1			2			10/26 (38)	18/10 = 1.8
Pulmonary edema	2	3																									2/26 (8)	5/2 = 2.5
Pulmonary hemorrhage					2																						1/26 (4)	2/1 = 2.0
Interstitial pneumonitis					2					2	1		2						2	1		1					7/26 (27)	11/7 = 1.6
Suppurative alveolitis					2																						1/26 (4)	2/1 = 2.0
Suppurative bronchiolitis																												
Pulmonary calcification														1	1					1				1			4/26 (15)	4/4 = 1.0
Suppurative pleuritis	3																										1/26 (4)	3/1 = 3.0
Pleural fibrosis	3																										1/26 (4)	3/1 = 3.0
TRACHEOBRONCHIAL LYMPH NODE:		X	N					X	N		N					X	N	N	X	N	N			N	N			
Macrophage accumulation				2		1	1			2		1	1	1	1							1	1			1	11/17 (65)	13/11 = 1.2
Fibrosis														1									1			2	3/17 (18)	4/3 = 1.3
Suppurative inflammation	1			2	2																						3/17 (18)	5/3 = 1.7
FINAL BODY WEIGHT (g)	112	152	96	102	122	114	96	106	129	80	106	131	110	151	104	106	133	95	135	76	127	71	103	111	140	95		
LUNG WEIGHT (g)	3.0	--	1.0	--	2.8	1.2	1.6	1.7	3.6	1.2	1.8	--	--	1.4	1.6	--	1.4	1.6	--	1.3	1.6	1.0	1.3	2.1	1.4	1.3		

Individual Animal Data - Hamsters Instilled Intratracheally with 20.0 mg Hydrated Alumina

Day of Death:	586	655	422	620	510	21	616	333	469	37	510	538	655	627	566	440	441	655	634	90	299	655	655	334	648		
Animal No:	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	Total (%)	Mean Severity
LUNG:																											
Alveolar septal fibrosis	2	3	2	2			2		1	1	2	4	3	2	2	1	2	3	3		3	3	2	2	2	21/25 (84)	47/21 = 2.2
Granulomatous inflammation	3	3	3	3	2		2	2	2	3	3	1	3	3	2	2	3	3	2	1	2	2	3	1	3	24/25 (96)	57/24 = 2.4
Bronchiolization		2	2					2						2	2	2	4		3		2	2	3			11/25 (44)	26/11 = 2.4
Alveolar lipoproteinosis		2												2	2			3					2	1		6/25 (24)	12/6 = 2.0
Alveolar epithelial hyperplasia		2												2				3					2	2		5/25 (20)	11/5 = 2.2
Alveolar macrophage aggregates						3	2																			2/25 (8)	5/2 = 2.5
Pulmonary congestion					2		2																			2/25 (8)	4/2 = 2.0
Pulmonary edema								2																		1/25 (4)	2/1 = 2.0
Pulmonary hemorrhage																											
Interstitial pneumonitis																											
Suppurative alveolitis		2												2				2								3/25 (12)	6/3 = 2.0
Suppurative bronchiolitis																											
Pulmonary calcification																											
Suppurative pleuritis																											
Pleural fibrosis																											
TRACHEOBRONCHIAL LYMPH NODE:	N	N		N	N	N		X	N				N			N	X	N		X			N	X			
Macrophage accumulation			1				1			1	2	2		1	2				1			2			2	10/15 (67)	15/10 = 1.5
Fibrosis												1									1					2/15 (13)	2/2 = 1.0
Suppurative inflammation																										0/15	
FINAL BODY WEIGHT (g)	108	136	116	82	138	115	107	138	110	103	87	86	133	88	91	100	113	129	108	95	127	140	103	145	125		
LUNG WEIGHT (g)	1.7	1.8	1.8	1.5	4.0	--	3.7	2.9	1.8	--	1.3	1.4	2.4	1.8	1.5	1.4	1.6	1.7	2.1	--	1.9	1.8	1.7	2.6	1.9		

Individual Animal Data - Hamsters Instilled Intratracheally with 5.0 mg Hydrated Alumina

Day of Death:	86	32	627	596	627	455	627	635	136	144	620	635	620	392	635	584	635	177	564	589	432	634	589	318	635	Total (%)	Mean Severity
Animal No:	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350		
LUNG:																											
Alveolar septal fibrosis			1	2	1	1		2			1	2	2			1	1		1	1		1	1			14/25 (56)	18/14 = 1.3
Granulomatous inflammation	1		1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	24/25 (96)	25/24 = 1.0
Bronchiolization			2				1			1	2	2			2				1			2	2		2	10/25 (40)	17/10 = 1.7
Alveolar lipoproteinosis																	1								1	2/25 (8)	2/1 = 2.0
Alveolar epithelial hyperplasia																	1		1							2/25 (8)	2/1 = 2.0
Alveolar macrophage aggregates				2		1							2			1			1	1		1	1			8/25 (32)	10/8 = 1.3
Pulmonary congestion	3			2	1	2							2			2				2		1	2			9/25 (36)	17/9 = 1.9
Pulmonary edema						1																				1/25 (4)	1/1 = 1.0
Pulmonary hemorrhage																3						2				2/25 (8)	5/2 = 2.5
Interstitial pneumonitis										2								4			2					3/25 (12)	8/3 = 2.7
Suppurative alveolitis								2											2		2				1	4/25 (16)	7/4 = 1.8
Suppurative bronchiolitis																											
Pulmonary calcification																											
Suppurative pleuritis	4	4																								2/25 (8)	8/2 = 4.0
Pleural fibrosis																											
TRACHEOBRONCHIAL LYMPH NODE:		N	N				N				N	X	N		N		X	N		X	N			X	N		
Macrophage accumulation						2				2				2		2			1							5/16 (31)	9/5 = 1.8
Fibrosis				1																			1			2/16 (13)	2/2 = 1.0
Suppurative inflammation	2				1			2	2							1			1			1				7/16 (44)	10/7 = 1.4
FINAL BODY WEIGHT (g)	174	96	102	144	78	113	108	135	125	96	102	137	175	96	106	133	119	74	126	146	111	192	83	104	115		
LUNG WEIGHT (g)	--	--	2.3	3.2	2.1	4.7	1.3	1.5	--	--	1.5	1.5	2.9	1.4	1.4	3.1	1.3	--	2.7	3.6	3.9	2.2	1.8	1.6	1.5		

Individual Animal Data - Hamsters Instilled Intratracheally with 2.0 mg Hydrated Alumina

Day of Death:	592	592	592	406	266	412	545	516	388	68	592	323	592	544	478	550	86	570	13	172	489	336	592	564	304	Total (%)	Mean Severity
Animal No:	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365	366	367	368	369	370	371	372	373	374	375		
LUNG:																	X										
Alveolar septal fibrosis				2						1			2	2				2		1		1	2			8/25 (32	13/8 = 1.6
Granulomatous inflammation	1		1	1	1	1	1	1	1	1	2	1	1	1	1	1		1		2	1	1	2	1	1	22/25 (88)	25/22 = 1.1
Bronchiolization	1	1		1	2	2					2		2											1	1	9/25 (36)	13/9 = 1.4
Alveolar lipoproteinosis				2				1		1				5				2					2			6/25 (24)	13/6 = 2.2
Alveolar epithelial hyperplasia				2									2					2		1			2			5/25 (20)	9/5 = 1.8
Alveolar macrophage aggregates																											
Pulmonary congestion								2		2		2				1										4/25 (16)	7/4 = 1.8
Pulmonary edema										2																1/25 (4)	2/1 = 2.0
Pulmonary hemorrhage																											
Interstitial pneumonitis					2										2				1							3/25 (12)	5/3 = 1.7
Suppurative alveolitis				2						3			2	2											5	5/25 (20)	14/5 = 2.8
Suppurative bronchiolitis																											
Pulmonary calcification																											
Suppurative pleuritis																											
Pleural fibrosis																									2	1/25 (4)	2/1 = 2.0
TRACHEOBRONCHIAL LYMPH NODE:	N		X	N			N	X	N					N		N		N	N		N						
Macrophage accumulation		1			1	1				2	1	1	1		1		1			1		1		1	1	13/16 (81)	14/13 = 1.1
Fibrosis																							2		1	2/16 (13)	3/2 = 1.5
Suppurative inflammation																	1									1/16 (6)	1/1 = 1.0
FINAL BODY WEIGHT (g)	102	137	154	85	103	96	113	131	94	77	155	165	135	153	80	69	124	77	77	127	109	87	135	140	70		
LUNG WEIGHT (g)	1.4	1.0	1.3	1.3	1.6	1.4	4.8	1.9	1.7	--	1.8	3.6	2.3	2.2	1.2	1.0	--	1.1	--	--	2.1	1.2	1.7	1.7	6.5		

Individual Animal Data - Hamsters Instilled Intratracheally with 0.2 mg Hydrated Alumina

Day of Death:	283	454	347	429	592	592	486	3	527	592	592	484	570	400	508	592	49	592	284	507	208	583	395	464	558	Total (%)	Mean Severity
Animal No:	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394	395	396	397	398	399	400		
LUNG:																											
Alveolar septal fibrosis					2	1			2		2			2		2				3			2			8/25 (32)	16/8 = 2.0
Granulomatous inflammation					1	1	1			1	1	1	1	1					1		1	1		1	1	13/25 (52)	13/13 = 1.0
Bronchiolization	2	2		2	2	1	2			2	3			2							2	2				11/25 (44)	22/11 = 2.0
Alveolar lipoproteinosis					2	1			2					2	3					3			2		2	8/25 (32)	17/8 = 2.1
Alveolar epithelial hyperplasia					2	1			2		2			2		3		2		3			2		2	10/25 (40)	21/10 = 2.1
Alveolar macrophage aggregates									1		3					2				2			2			5/25 (20)	10/5 = 2.0
Pulmonary congestion	2		2					2	3							1	2		1	3						8/25 (32)	16/8 = 2.0
Pulmonary edema																											
Pulmonary hemorrhage				1																						1/25 (4)	1/1 = 1.0
Interstitial pneumonitis				1				1														1				3/25 (12)	3/3 = 1.0
Suppurative alveolitis															2											1/25 (4)	2/1 = 2.0
Suppurative bronchiolitis																											
Pulmonary calcification															2											1/25 (4)	2/1 = 2.0
Suppurative pleuritis																	4									1/25 (4)	4/1 = 4.0
Pleural fibrosis																											
TRACHEOBRONCHIAL LYMPH NODE:	N		N					X	N		N			N	N	N			N	X	N	X	X	N			
Macrophage accumulation		1		1	1	1	1			1		1	1				1	1							2	11/15 (73)	12/11 = 1.1
Fibrosis																										0/15	
Suppurative inflammation																	2									1/15 (7)	2/1 = 2.0
FINAL BODY WEIGHT (g)	66	82	87	117	153	151	89	99	205	162	133	82	99	112	65	141	127	130	101	118	76	96	122	104	100		
LUNG WEIGHT (g)	1.7	1.3	2.5	1.7	1.3	1.4	1.2	--	2.6	1.4	1.3	1.3	1.4	1.7	1.5	2.1	--	1.3	1.5	4.3	--	1.3	3.5	1.8	1.2		

Individual Animal Data - Hamsters Instilled Intratracheally with Saline

Day of Death:	540	31	141	663	358	440	446	602	663	571	15	548	7	458	663	663	663	663	656	478	642	63	442	663	25	613	639	Total (%)	Mean Severity
Animal No:	401	402	402A	403	404	405	406	407	408	409	410	410A	411	412	413	414	415	416	417	418	419	420	421	422	423	424	425		
LUNG:																													
Alveolar septal fibrosis				2				1	1								2			2			2			2		7/27 (26)	12/7 = 1.7
Granulomatous inflammation																													
Bronchiolization	2			2			1								2	1		2					1	2				8/27 (30)	13/8 = 1.6
Alveolar lipoproteinosis								2		1													1					3/27 (11)	4/3 = 1.3
Alveolar epithelial hyperplasia								1		1							2	3					1	1			1	7/27 (26)	10/7 = 1.4
Alveolar macrophage aggregates				1		2				1			3				3								2	1	1	8/27 (30)	13/7 = 1.9
Pulmonary congestion		1	2					2			3	1		1					1		1		1			1	1	11/27 (41)	15/11 = 1.4
Pulmonary edema						2																						1/27 (4)	2/1 = 2.0
Pulmonary hemorrhage		2																										1/27 (4)	2/1 = 2.0
Interstitial pneumonitis			2	2	1	2				1			1									4						7/27 (26)	13/7 = 1.9
Suppurative alveolitis																													
Suppurative bronchiolitis																													
Pulmonary calcification									2																			1/27 (4)	2/1 = 2.0
Suppurative pleuritis																													
Pleural fibrosis					2																							1/27 (4)	2/1 = 2.0
TRACHEOBRONCHIAL LYMPH NODE:	N		X	X		X		N	X	X	X		X			X	X	N	X	N		N	N	N	X	X	X		
Macrophage accumulation		2			2		2					1		1	1						1							7/20 (35)	10/7 = 1.4
Fibrosis																												0/20	
Suppurative inflammation																												0/20	
FINAL BODY WEIGHT (g)	109	107	118	147	95	125	124	106	114	83	87	93	87	106	142	151	135	148	105	121	99	82	105	134	113	84	132		
LUNG WEIGHT (g)	1.3	--	--	1.4	1.5	1.5	1.4	2.0	1.5	1.3	--	1.3	--	2.3	1.7	1.4	3.6	1.8	1.4	2.8	1.5	--	1.4	1.8	--	1.2	2.1		

Individual Animal Data - Cage Control Hamsters

Day of Death:	494	440	491	663	513	663	652	612	663	616	642	602	614	559	627	663	445	558	632	630	663	642	537	579	663	Total (%)	Mean Severity
Animal No:	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445	446	447	448	449	450		
LUNG:																											
Alveolar septal fibrosis															1							1				2/25 (8)	2/2 = 1.0
Granulomatous inflammation																											
Bronchiolization	2	3		2		2			1		1		1	1	1	1					1	2			2	13/25 (52)	20/13 = 1.5
Alveolar lipoproteinosis																											
Alveolar epithelial hyperplasia																											
Alveolar macrophage aggregates		2	1				1	1				3			2	1			1		2	2			2	11/25 (44)	18/11 = 1.6
Pulmonary congestion							2	3		1		4	3	1				2	1	1				1		10/25 (40)	19/10 = 1.9
Pulmonary edema	3																									1/25 (4)	3/1 = 3.0
Pulmonary hemorrhage												3								1			4			3/25 (12)	8/3 = 2.7
Interstitial pneumonitis	2	2													1		1									4/25 (16)	6/4 = 1.5
Suppurative alveolitis			2		2					1								1				1				5/25 (20)	7/5 = 1.4
Suppurative bronchiolitis																											
Pulmonary calcification																											
Suppurative pleuritis																											
Pleural fibrosis																											
TRACHEOBRONCHIAL LYMPH NODE:	X			N		X	X	N	N	N	N		N	X	N	N			N	X		X	X	N	N		
Macrophage accumulation			2		1							2					1	1			2					6/14 (43)	9/6 = 1.5
Fibrosis																										0/14	
Suppurative inflammation		1																								1/14 (7)	1/1 = 1.0
FINAL BODY WEIGHT (g)	138	129	128	127	70	153	146	136	154	111	104	109	81	126	135	128	105	131	149	135	141	189	107	89	121		
LUNG WEIGHT (g)	2.5	1.5	2.5	1.3	1.2	1.7	2.6	3.5	1.2	2.8	1.5	5.2	1.6	2.1	2.4	1.5	1.4	2.3	3.7	2.8	1.6	2.2	4.7	1.5	1.4		