

Lung Changes in Rats following Inhalation Exposure to Volcanic Ash for Two Years¹

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Rats were exposed by inhalation to 5 or 50 mg/m³ Mount St. Helens volcanic ash, to 50 mg/m³ quartz (positive controls), or to filtered room air (sham-exposed controls), for 6 hr/day, 5 days/week, for up to 24 months to investigate biological effects of chronic inhalation exposure to volcanic ash under controlled laboratory conditions. Exposure-related lung changes comprised accelerated respiratory frequency; alveolar macrophage accumulation; interstitial reaction; lymphoreticular reaction in peribronchiolar regions and in mediastinal lymph nodes; alveolar proteinosis in the 50- mg/m³ ash- or quartz-exposed groups; increase in fresh lung weights; decreased body weight and increased mortality in the quartz-exposed group; and epidermoid carcinomas especially in the quartz-exposed females and, to a lesser extent, in the 50-mg/m³ ash-exposed females. The observed changes reflect significant dose–response and agent–response relationships. © 1986 Academic Press, Inc.

INTRODUCTION

The violent eruption of the Mount St. Helens volcano on May 18, 1980, deposited volcanic ash on tens of thousands of square kilometers. More than 8 kg/m² of ash were measured in Yakima, Washington, 135 km from the volcano (Findley, 1981), and 2.29 kg/m² of ash were deposited in Moscow, Idaho, more than 400 km from the volcano (Hooper *et al.*, 1980). Human activities, such as logging, and wind are resuspending the ash particles in the air. This results in chronic inhalation exposure of certain population groups. Because more than 1 million people are living in the fallout area, and because of repeated subsequent (and the possibility of future) eruptions of the Mount St. Helens volcano, the potential health hazards presented by inhalation of volcanic ash needed to be determined.

It soon became apparent that acute effects in healthy persons were minor and temporary (Johnson *et al.*, 1982; Washington State Health Services Division, 1980). However, the effects of *chronic* exposure to volcanic ash—as experienced, for example, by loggers in the fallout area—were unknown. This uncertainty emphasized the necessity for evaluating the health hazards of chronic ash inhalation in a suitable animal model under controlled laboratory conditions. The results of our study exceed regional relevance and will be of interest to medical personnel and health authorities in nations experiencing volcanic activity, such as the Pacific rim countries which form “the ring of fire.”

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The objectives of our study were to determine whether chronic inhalation of volcanic ash causes lung changes; type and degree of lung changes as a function of ash aerosol concentration and cumulative exposure time; and regression of ash-induced lung changes as a function of aerosol concentration, cumulative exposure, and recuperation time.

MATERIALS AND METHODS

Volcanic Ash

Mount St. Helens volcanic ash was collected at three undisturbed sites in Yakima, Ritzville, and Spokane in the State of Washington, 5 weeks after the May 18, 1980, eruption. These sites represented major population centers in the areas of heaviest ash deposition. The ash samples were oven-dried at $100 \pm 5^\circ\text{C}$, passed through a No.20 (850- μm) sieve to remove foreign materials, thoroughly mixed in a V-blender, and sterilized by heating in ceramic dishes in an oven at 130°C for 16 hr. After cooling to room temperature, the ash was sealed in polyethylene bags and stored in 5-gal paint buckets for the animal exposures. Ash samples of respirable size, analyzed in our laboratory, contained from 3 to 4% free crystalline silica. Slightly more than half this quantity was quartz and most of the remainder was cristobalite.

Min-U-sil (5 μm) was obtained from Pennsylvania Glass Sand Corporation for exposing a positive-control group to α -quartz, a known potent cytotoxic agent.

Aerosol Generation and Characterization

The ash and quartz aerosols were generated using dual flexible-brush dust-feed mechanisms (Milliman *et al.*, 1981). Before reaching the aerosol exposure chamber, the aerosol was passed through an elutriator in which most larger, "nonrespirable" particles were retained. Total aerosol concentration was measured once per week using open-faced fiber filter samples. Respirable aerosol concentrations were measured hourly, initially using a Simslin II dust monitor (Thompson *et al.*, 1981) and later a GCA real-time aerosol monitor (RAM; Environmental Instruments, Bedford, Mass.). Once a week, the respirable aerosol concentrations were measured using a Casella 113 A gravimetric dust sampler (Casella London, Ltd., London, England) as a standard against which the Simslin and RAM monitors were calibrated. Particle size distributions were initially determined every 2 weeks, using an Andersen cascade impactor (Andersen, 1966). When no substantial particle size differences were noted, subsequent size distributions were determined once every 3 months. The time-weighted average (TWA) respirable dust concentrations for the 24-month exposures and standard deviations of the weekly TWA concentrations were 5.1 ± 0.7 , 50.5 ± 6.3 , and 51.6 ± 7.3 mg/m^3 for the low ash, the high ash, and for the quartz aerosols, respectively. The mass median aerodynamic diameters ranged from 1.6 to 2.1, 1.7 to 2.7, and 1.7 to 2.5 μm , respectively. The geometric standard deviations ranged from 2.1 to 2.5, 2.0 to 2.5, and 1.9 to 2.1, respectively.

After a 3-week quarantine period, which included health evaluation and serologic tests, 576 cesarean-derived, barrier-maintained Fischer-344 (F-344) rats

were randomly divided into four groups of 72 males and 72 females each. To avoid initial mean body weight differences among the groups, the rats were assigned to their groups by stratified random selection. Group 1 served as the sham-exposed control group. Group 2 was exposed to a respirable ash aerosol concentration of 5 mg/m³, Group 3 was exposed to a respirable ash aerosol concentration of 50 mg/m³, and Group 4 was exposed as the positive control to a respirable aerosol concentration of 50 mg/m³ quartz. Exposures started when the rats were approximately 3 months old and continued 6 hr/day, 5 days/week, for up to 24 months in previously described exposure chambers (Brown and Moss, 1981; Moss, 1980a, b; Moss *et al.*, 1982).

The rats were maintained on a 12-hr-light/12-hr-dark cycle in the individual 270-cm² compartments of stainless-steel wire-mesh cages of the aerosol exposure chambers. They had free access to Wayne Rodent Blox laboratory animal feed (Wayne Pet Food Division, Continental Grain Co., Chicago, Ill.) and water when not being exposed, and during exposure to water only. The animals were weighed every 2 weeks during their growth period and monthly thereafter. A permutation test (Lindgren, 1963) was used to analyze the body weight measurements. This test is a nonparametric statistical test which is based on the absolute area between body weight curves (curves not shown). The test allows for correlation of body weight measurements over time.

From each of the four groups, randomly selected subgroups of 10 rats, equally divided by sex, were sacrificed after 4, 8, 12, 16, and 20 months of exposure. All surviving animals were sacrificed after 24 months of exposure. Survival was evaluated using the product-limit estimate which is an estimate of the cumulative survival distribution. Survival curves (not shown) were compared using an exponential scores test (Mantel, 1966). The limited objective of the serial sacrifices was to monitor lesion development as a function of dose level and cumulative exposure time. In similar fashion, randomly selected groups of 10 rats were withdrawn from exposure at the same time intervals and maintained for observation for the remainder of their life span up to the 24-month exposure point of their exposed cohorts. The limited objective of the serial withdrawals was to determine whether regression of exposure-induced changes might occur as a function of dose level, cumulative exposure, and recuperation (observation) time.

When the rats were 16½ and 20½ months old, respiratory frequency was recorded by counting respiratory chest/abdominal excursions per minute. Three observations within 1 hr were recorded for each of four randomly selected males per subgroup. The data were then subjected to analysis of variance.

A necropsy was performed on each rat. Fresh (wet) lung weights from serially sacrificed animals were recorded. The lungs were fixed in glutaraldehyde at 25 cm hydrostatic pressure and volumes measured, using the water displacement technique. Routinely processed, paraffin-embedded, hematoxylin and eosin-stained sections of each lobe of lung, trachea, and mediastinal tissue (usually including lymph nodes) were histopathologically examined at random and "blind"; i.e., the pathologist was not aware of the exposure group or of the duration of exposure while examining the tissues. Additional gross lesions of an uncertain nature were also examined histopathologically. The nonneoplastic

changes were graded as follows: 1 = very slight or very small amount; 2 = slight or small amount; 3 = moderate; 4 = marked; and 5 = extreme. The acquisition and management of the pathology data were performed with a PATH/TOX System (Xybion Medical Systems, Cedar Knolls, N.J.). A Jonckheere (1954)/Terpstra (1952) test was used to check whether the lesions increased in severity with time or exposure level. The test for increase as a function of time was made for each exposure group separately, and the test for increase as a function of exposure level was carried out for each time separately. Ultrastructural studies were performed on selected lung specimens embedded in epoxy and examined with a Philips 300 transmission electron microscope.

Lung tissues from five rats per sex per group at the 12-month sacrifice, and from 10 males per group at the 24-month sacrifice, were analyzed for silicon to estimate total pulmonary ash or quartz burdens. The samples were lyophilized, pulverized, and then homogenized in a Spex ball mill, which is constructed entirely of silicon-free polymeric materials. Portions of each pulverized lung sample were then pressed into wafers and analyzed for silicon by X-ray fluorescence (XRF) spectrometry, using both titanium and zirconium sources on a Kevex-ray XRF system (R. Sanders *et al.*, 1983).

RESULTS

Body Weights

The mean body weights show two markedly different effect levels, namely, none for the controls and the 5-mg/m³ ash-exposed animals, and significant weight depression for the 50-mg/m³ quartz-exposed animals (Table 1). This phenomenon was observed first in males and apparently is less dependent on cumulative exposure than on time to develop. More specifically, significantly lower weight was observed in the males having received as little as 4 months of quartz exposure, followed by 20 months of observation, but in the continually exposed males significantly lower weight was observed first only at 12 months of exposure followed by sacrifice. Sixteen months of quartz exposure resulted in significantly lower weights in both sexes of Subgroups A and B (Table 1). The mean body weight values of the 50-mg/m³ ash-exposed animals were more ambiguous. They approached significant difference from the controls in the females sacrificed after 20 months of exposure to 50 mg/m³ ash, and had reached significance in the females sacrificed at 24 months of exposure. This trend was not observed in the males of the serially sacrificed subgroups, although the weight of the males exposed for 12 months, followed by 12 months of observation, reached borderline significance.

Respiratory Frequency

Respiratory frequencies at 16½ and 20½ months of exposure are similar (Table 2). Among the subgroups having received 4 months of exposure, only the quartz-exposed rats had a significantly higher respiratory frequency than the control rats. The subgroups exposed to 50 mg/m³ volcanic ash for 8 months or longer had respiratory rates that were significantly lower than those of the quartz-exposed subgroups, but higher than those of the 5-mg/m³ ash or control subgroups. There were no differences in respiratory rates between the 5-mg/m³ ash subgroups and

TABLE 1
MEAN BODY WEIGHT COMPARISONS OF F-344 RATS EXPOSED TO VOLCANIC ASH OR QUARTZ FOR VARYING PERIODS OF TIME^a

Exposure subgroups			Results
A	4	No significant differences	
B	4 + 20	Quartz-exposed males significantly lighter than controls	
A	8	No significant differences	
B	8 + 16	Quartz-exposed males significantly lighter than controls	
A	12	Quartz-exposed males significantly lighter than controls	
B	12 + 12	Quartz-exposed males significantly ($P = 0.02$) lighter than controls; 50-mg/m ³ ash-exposed males vs controls borderline significance ($P = 0.052$)	
A	16	Quartz-exposed males and females significantly lighter than controls	
B	16 + 8	Quartz-exposed males and females significantly lighter than controls	
A	20	No quartz-exposed survivors; no significant differences, but indication of lighter weight of 50-mg/m ³ ash-exposed females vs controls	
B	20 + 4	No quartz-exposed survivors; no significant differences among remaining groups	
A	24	50-mg/m ³ ash-exposed females significantly lighter than controls	

^a Permutation test (Lindgren, 1963), $P < 0.05$; $N = 5$ rats/sex/group.

Note. A = Subgroups exposed for stated number of months, followed by sacrifice. B = Subgroups exposed for stated number of months, followed by stated number of months of observation.

TABLE 2
MEAN RESPIRATORY RATES (BREATHS/MINUTE \pm SD) OF MALE F-344 RATS EXPOSED TO VOLCANIC ASH OR QUARTZ FOR VARYING PERIODS OF TIME^a

Exposure group		A	B	C	D	E
Controls	(1)	77 \pm 9	69 \pm 9	77 \pm 7	65 \pm 8	69 \pm 4
	(2)	86 \pm 6	72 \pm 5	84 \pm 3		
5 mg/m ³ ash	(1)	76 \pm 8	74 \pm 7	77 \pm 8	76 \pm 7	66 \pm 6
	(2)	74 \pm 3	85 \pm 9	82 \pm 7		
50 mg/m ³ ash	(1)	77 \pm 9	104 \pm 13 ^b	127 \pm 9 ^b	119 \pm 9 ^b	108 \pm 22 ^b
	(2)	82 \pm 1	137 \pm 3 ^b	123 \pm 11 ^b		
50 mg/m ³ quartz	(1)	188 \pm 14 ^c	182 \pm 3 ^c	194 \pm 3 ^{c,d}	182 \pm 6 ^c	109 \pm 31 ^b
	(2)	172 \pm 3 ^c	192 \pm 5 ^c	193 \pm 8 ^c		

Note. (1) = Observations made in 20½-month-old rats as follows: A = 4 months exposure + 13½ months recuperation. B = 8 months exposure + 9½ months recuperation. C = 12 months exposure + 5½ months recuperation. D = 16 months exposure + 1½ months recuperation. E = 17½ months of continual exposure.

(2) = Observations made in 16½-month-old rats as follows: A = 4 months exposure + 9½ months recuperation. B = 8 months exposure + 5½ months recuperation. C = 12 months exposure + 1½ months recuperation.

^a Three observations within 1 hr in each of four rats/group.

^b Significantly higher than controls and 5 mg/m³ ash values (analysis of variance, $P < 0.05$).

^c Significantly higher than 50 mg/m³ ash values.

^d Four observations within 1 hr in one rat.

the control groups. Breathing rates and their relationships among the subgroups exposed for 12, 16, and 17½ months were similar to those observed in the subgroup exposed for 8 months, with the exception of the subgroup exposed to quartz for 17½ months. The mean respiratory frequency of the latter subgroup (109 ± 31.4 breaths/min) was significantly lower than those of all other quartz-exposed subgroups and in the range of the 50-mg/m³ ash-exposed subgroups, perhaps due to the terminal condition of this subgroup.

The increase in respiratory frequency, concomitant with shallower breathing, indicates respiratory distress. The degree of this distress generally reflected the degree of the causative lung changes, as subsequently confirmed by our pathologic findings. The respiratory frequency observations clearly and consistently distinguish between three effect levels, namely, none for the controls and the 5-mg/m³ ash-exposed animals; moderate for the 50-mg/m³ ash-exposed animals; and severe for the 50-mg/m³ quartz-exposed animals.

Survival

There were no significant differences in survival between the sexes and none among the controls, the 5-mg/m³ or the 50-mg/m³ ash-exposed animals, but survival in the quartz-exposed group was significantly lower than in the other three groups (Table 3). Mean survival times of the rats which were serially withdrawn from exposure showed neither significant differences among the controls or the ash-exposed groups for any exposure period, nor were there differences within each of these groups as a function of cumulative exposure. However, the quartz-exposed animals again had dramatically reduced survival times compared to the other three groups. In addition, the quartz data indicate a cumulative dose-effect relationship, although a statistically significant ($P < 0.05$) difference in survival exists only between the subgroup withdrawn at 4 months of exposure and the other subgroups.

Lung Weights and Volumes

An initial analysis of the lung weights used two-way analysis of covariance with the body weight as a covariate and exposure level and sacrifice time as fixed effects. Male and female data were analyzed separately. Because one would ex-

TABLE 3
MEAN SURVIVAL TIMES (IN DAYS) OF F-344 RATS EXPOSED TO VOLCANIC ASH OR QUARTZ FOR VARYING PERIODS OF TIME^a

Exposure group	Months of exposure					
	4	8	12	16	20	24
Control	705 \pm 36	726 \pm 18	710 \pm 34	714 \pm 23	735 \pm 7	688 \pm 13
5 mg/m ³ ash	700 \pm 25	719 \pm 23	633 \pm 51	703 \pm 25	737 \pm 7	681 \pm 13
50 mg/m ³ ash	720 \pm 16	703 \pm 24	725 \pm 11	710 \pm 27	712 \pm 20	710 \pm 8
50 mg/m ³ quartz	653 \pm 24 ^{b,c}	585 \pm 21 ^b	556 \pm 19 ^b	554 \pm 11 ^b	—	539 \pm 6 ^b

^a The animals were 93 days old at the start of exposure.

^b Significantly lower than the other groups [$P < 0.05$, exponential scores test (Mantel, 1966)].

^c Significantly different from the other quartz values.

pect the lung weight to increase with the body weight, the adjustment for body weight should tend to make the groups more comparable across time. However, the lung weights did not show a consistent relationship to body weights. To eliminate possible biases from comparing across time, the lung weights were analyzed separately for each sacrifice time by one-way analysis of variance (Table 4).

Results of the analyses within each time period and sex show a significant treatment effect ($P < 0.05$) with the lung weights generally increasing with exposure level. Although there were differences in lung weights over time within a dose level, the pattern of the differences makes interpretation difficult. The lung weights for the 50-mg/m³ ash- and quartz-exposed groups increased with time for both males and females.

The lung volumes of the quartz-exposed group were significantly larger than those of the control group at 8 and 12 months. The only other significant differences were at 24 months, when lung volumes for both the 5-mg/m³ and the 50-mg/m³ ash-exposed groups were significantly larger than those of the control group. The lung volume data were not consistent over time, not even for the controls. Therefore, the data may not be comparable over time and treatment-related trends might be masked.

Lung Analysis for Deposited Dust

Background levels of silicon for the lungs of the control animals were below detection limits (Table 5). The pulmonary dust burdens per gram of lung in males and females both from the 5-mg/m³ ash-exposed group and from the 50-mg/m³ ash- or quartz-exposed groups are similar. Sex differences in pulmonary dust burdens retained in the *entire* lung reflect the differences in fresh lung weights between males and females.

Quantities of ash retained at 12 months of exposure in the lungs of the animals of the 5-mg/m³ and the 50-mg/m³ ash-exposed groups are 6.7-fold higher for the males and 6.2-fold higher for the females in the 50-mg/m³ group than for their counterparts in the 5-mg/m³ group. The analogous factor for the males at 24 months (no lungs from females were analyzed at 24 months) is 6.4. These values relate reasonably well to the 10-fold difference in aerosol concentrations, given the complex factors interacting with and affecting pulmonary deposition, retention, and clearance of inhaled materials. Among these factors are breathing patterns and pathologic changes in the respiratory tract. The more rapid and shallower breathing of the 50-mg/m³ ash-exposed animals and their higher incidence and degree of lung changes might explain, at least to some extent, why the 10-fold higher ash aerosol concentration did not result in a 10-fold higher lung burden.

Comparison of the estimated quantities of ash retained in the deep lung at 12 and 24 months of exposure shows a 26% higher 24-month value for the 5-mg/m³ group and a 20% higher value for the 50-mg/m³ group. These increases are within expected magnitudes. It is well known that—at least for chronic exposures—pulmonary deposition and retention of inhaled “insoluble” materials is not a linear function of aerosol concentration. Instead, the lung generally appears to achieve a dynamic equilibrium between deposition and clearance at a lower level than that which would result from a linear increase, barring aerosol concentrations high enough to overwhelm the clearance mechanism.

TABLE 4
 MEAN BODY WEIGHTS, FRESH LUNG WEIGHTS, AND LUNG VOLUMES (\pm SE) OF SERIALLY SACRIFICED F-344 RATS EXPOSED TO VOLCANIC ASH
 OR QUARTZ FOR VARYING PERIODS OF TIME

Months of exposure	Group	Males			Females		
		Body weight (g)	Lung weight (g)	Lung volume (ml)	Body weight (g)	Lung weight (g)	Lung volume (ml)
4	Control	385 \pm 9	2.14 \pm 0.02	11.9 \pm 0.5	197 \pm 3	1.31 \pm 0.04	8.2 \pm 0.4
	5 mg/m ³ ash	358 \pm 5 ^a	2.22 \pm 0.12	12.0 \pm 0.3	201 \pm 2	1.44 \pm 0.04 ^a	8.2 \pm 0.5
	50 mg/m ³ ash	365 \pm 9	2.63 \pm 0.15 ^a	10.2 \pm 0.6	204 \pm 10	1.88 \pm 0.11 ^a	7.4 \pm 1.0
	50 mg/m ³ quartz	346 \pm 11	5.10 \pm 0.17 ^a	12.9 \pm 0.7	188 \pm 5	3.28 \pm 0.14 ^a	8.9 \pm 1.1
8	Control	401 \pm 7	1.48 \pm 0.05	11.1 \pm 1.0	214 \pm 5	1.15 \pm 0.08	6.6 \pm 1.0
	5 mg/m ³ ash	419 \pm 6	1.89 \pm 0.06 ^a	11.5 \pm 0.7	212 \pm 11	1.05 \pm 0.04	4.7 \pm 0.7
	50 mg/m ³ ash	392 \pm 8	3.15 \pm 0.30 ^a	10.6 \pm 1.8	217 \pm 2	1.78 \pm 0.05 ^a	8.1 \pm 0.6
	50 mg/m ³ quartz	397 \pm 10	7.18 \pm 0.83 ^a	14.9 \pm 1.5	218 \pm 5	4.35 \pm 0.30 ^a	12.5 \pm 0.5 ^a
12	Control	442 \pm 7	1.90 \pm 0.11	7.3 \pm 0.7	244 \pm 2	1.36 \pm 0.05	4.1 \pm 1.0
	5 mg/m ³ ash	430 \pm 6	2.26 \pm 0.34	6.4 \pm 1.3	228 \pm 14	1.72 \pm 0.32	6.1 \pm 1.1
	50 mg/m ³ ash	422 \pm 14	3.72 \pm 0.38 ^a	8.8 \pm 1.0	223 \pm 9	2.32 \pm 0.15 ^a	6.0 \pm 0.9
	50 mg/m ³ quartz	342 \pm 12 ^a	9.22 \pm 0.24 ^a	12.0 \pm 1.3 ^a	218 \pm 4 ^a	5.08 \pm 0.34 ^a	8.3 \pm 0.7 ^a
16	Control	433 \pm 7	1.84 \pm 0.16	4.9 \pm 2.0	268 \pm 8	1.56 \pm 0.09	6.2 \pm 1.4
	5 mg/m ³ ash	431 \pm 10	2.12 \pm 0.05	8.5 \pm 1.0	265 \pm 13	1.51 \pm 0.06	8.1 \pm 0.3
	50 mg/m ³ ash	435 \pm 11	3.91 \pm 0.05 ^a	11.7 \pm 0.4 ^a	234 \pm 14	2.55 \pm 0.19 ^a	8.8 \pm 0.7
	50 mg/m ³ quartz	282 \pm 14 ^a	11.70 \pm 0.60 ^a	17.4 \pm 1.0 ^a	203 \pm 8 ^a	5.73 \pm 0.62 ^a	7.5 \pm 1.7
20	Control	403 \pm 25	1.97 \pm 0.08	9.1 \pm 1.4	295 \pm 10	1.78 \pm 0.16	6.2 \pm 0.9
	5 mg/m ³ ash	441 \pm 14	2.48 \pm 0.18 ^a	10.8 \pm 0.5	294 \pm 5	2.12 \pm 0.15	7.6 \pm 0.5
	50 mg/m ³ ash	387 \pm 32	4.57 \pm 0.19 ^a	12.5 \pm 0.9	244 \pm 12 ^a	4.06 \pm 0.30 ^a	8.1 \pm 1.2
	50 mg/m ³ quartz	358 \pm 18	2.88 \pm 0.58	5.9 \pm 1.8	277 \pm 10	1.40 \pm 0.05	5.7 \pm 0.3
24	Control	390 \pm 16	2.54 \pm 0.16	5.9 \pm 1.0	263 \pm 11	1.91 \pm 0.07 ^a	6.8 \pm 0.5 ^a
	50 mg/m ³ ash	374 \pm 13	5.14 \pm 0.19 ^a	8.9 \pm 1.1	243 \pm 7 ^a	3.48 \pm 0.23 ^a	8.4 \pm 0.4 ^a

Note. The rats were 93 days old at start of exposure.

^a Significantly different from controls (analysis of variance, $P < 0.05$).

TABLE 5
ESTIMATED QUANTITIES OF SILICON (mg/g LUNG), VOLCANIC ASH, AND QUARTZ IN RAT LUNGS (mg/g LUNG; mg/LUNG)
AT 12 AND 24 MONTHS OF EXPOSURE

	Controls			5 mg/m ³ Ash			50 mg/m ³ Ash			50 mg/m ³ Quartz		
	Male	Female	Male	Male	Female	Male	Male	Female	Male	Male	Female	Male
Exposure months	12	12	24	12	12	24	12	12	24	12	12	24
Silicon												
\bar{x}	<0.26	<0.25	<0.25	3.480	3.124	1.634	14.228	14.408	7.632	5.638	6.018	0
SD	—	—	—	0.646	0.571	0.419	1.103	0.892	0.832	0.361	0.246	—
N	5	5	10	5	5	10	5	5	10	5	5	0
Ash												
mg/g lung	0	0	0	11.345	10.184	5.327	46.383	46.970	24.880	NA ^a	NA	NA
mg/lung	0	0	0	25.640	17.516	32.28	172.461	108.999	206.85	NA	NA	NA
Quartz												
mg/g lung	—	—	—	—	—	—	—	—	—	12.065	12.879	—
mg/lung	—	—	—	—	—	—	—	—	—	111.191	65.935	—

^a NA = Not applicable.

Even though the characteristics of the 50-mg/m³ quartz aerosol were quite similar to those of the 50-mg/m³ ash aerosol, at 12 months of exposure the pulmonary quartz burden was 36 and 40% lower than the pulmonary ash burden for males and females, respectively (no quartz-exposed animals survived at 24 months of exposure). These differences might be due to the observed significant differences in breathing patterns and lung pathology.

Pathology

The lungs of rats exposed to 5 mg/m³ volcanic ash appeared normal on gross examination after 4, 8, and 12 months of exposure. At longer time intervals, however, there was an increased tendency for the lungs to have a mottled appearance. Histopathologic examination as early as the first sacrifice time at 4 months showed very small aggregates of alveolar macrophages (AM) with intracellular dust deposition (Table 6). The aggregates of AM were randomly distributed or accumulated in peribronchiolar areas. The adjacent alveolar septa were generally thickened, with a few prominent alveolar epithelial cells. These changes progressed in severity in the rats sacrificed after 8 months of exposure, but did not appear to progress after longer periods of exposure. After 8 months of exposure, the majority of the rats had very small amounts of peribronchiolar lymphoreticular reaction which generally progressed in time up to the final sacrifice. The lymphoreticular reaction, composed of prominent histiocytic macrophages containing phagocytosed dust surrounded by lymphocytes, also occurred in pleural or subpleural areas (Fig. 1).

The lung lesions were more severe in the rats exposed to 50 mg/m³ volcanic ash than in those exposed to 5 mg/m³. At gross examination, the lungs showed a brownish discoloration after 4 months of exposure, with gray to white subpleural focal mottling after 8 to 24 months of exposure. After 4 months of exposure, there were small-to-moderate amounts of AM accumulation with small-to-moderate degrees of associated interstitial reaction. The AM accumulation and interstitial reaction, with associated neutrophils, early septal fibrosis, and glandular metaplasia of alveolar epithelium, progressed in severity from 4 to 8 months of exposure, but then had a tendency to become stable. Fig. 2 is an electron micrograph of particle-laden AM. Lymphoreticular reactions, composed primarily of peribronchiolar accumulations of macrophages and lymphocytes as described for the 5-mg/m³ ash-exposed lungs, were prominent as early as 4 months of exposure and progressed in severity for the duration of the exposures. The alveoli first started accumulating amorphous eosinophilic PAS-positive material, considered as alveolar proteinosis, after 8 months of exposure (Fig. 3). The alveolar proteinosis progressed in severity up to approximately 12 months and then leveled off. Occasionally, rats developed small areas of pulmonary adenomatosis (Fig. 4).

The lungs of the quartz-exposed rats were grossly heavy, spongy to firm in texture, diffusely mottled with brown to purple discoloration, and had gray-to-white subpleural foci after as early as 4 months of exposure and progressed in severity with time. Histopathologically, alveolar proteinosis, accumulations of AM, interstitial reactions associated with AM, and peribronchiolar lympho-

reticular reaction were prominent after 4 months of exposure. The AM and interstitial reactions were also associated with necrotic debris, characterized primarily by pyknotic and karyorrhectic nuclei, and neutrophilic infiltration. After 16 months of exposure, there were foci of macrophages associated with cholesterol clefts. The interstitial reaction tended to progress to focal areas of interstitial fibrosis and pronounced alveolar epithelial metaplasia. Nodules of pulmonary adenomatosis occurred in rats after 12 months of exposure. Alveolar proteinosis became progressively more severe so that essentially all alveoli were distended with eosinophilic material by 16 months of exposure, causing sharply increased mortality after 16 months of exposure. Lymphoreticular reactions, as described in the ash-exposed rats, progressed from 4 to 12 months of exposure, and then decreased.

Epidermoid carcinomas were the only lung tumors considered related to the experimental procedures. The first epidermoid tumor was found in a quartz-exposed rat 494 days (minimal latency period) after the beginning of exposure. Ten of 53 (19%) female and 1 of 47 (2%) male rats exposed to quartz for this period of time or longer, and 3 of 48 (6%) female and 1 of 45 (2%) male rats exposed to 50 mg/m³ volcanic ash developed epidermoid tumors. The 19% incidence in the quartz-exposed females is significantly different from the controls ($P < 0.05$, Fisher's exact test). The epidermoid tumors consisted of stratified squamous epithelium replacing large areas of parenchyma with cavities in the center (Fig. 5). The stratified squamous epithelium extended into adjacent alveolar parenchyma and, in one rat, metastasized to a mediastinal lymph node.

There was no regression in lung changes with the exception of lymphoreticular proliferation which decreased in those quartz-exposed animals which were withdrawn from exposure, and diminished alveolar proteinosis in the rats exposed to 50 mg/m³ volcanic ash.

The only extrapulmonary lesions attributed to experimental procedures were in the mediastinal lymph nodes draining the lungs. In the rats exposed to 5 mg/m³ volcanic ash, a lymphoreticular reaction was found in two rats after 8 months of exposure and six rats after 12 months of exposure. The lymph nodes were grossly enlarged with fairly consistent lymphoreticular histopathologic reactions at 12 to 24 months of exposure. In the rats exposed to 50 mg/m³ ash, the mediastinal lymph nodes became progressively larger on gross examination between 4 and 24 months of exposure. Histopathologically, they had a lymphoreticular reaction with histiocytic macrophages containing numerous phagocytosed dust particles associated with an increased number of lymphocytes. In the rats exposed to quartz, the mediastinal lymph nodes were grossly larger than those found in the ash-exposed rats. In addition to lymphoreticular reaction, histopathologic examination showed necrotic debris and fibroplasia at longer durations of exposure.

DISCUSSION

Several studies with Mount St. Helens volcanic ash have been published (Ake-matsu *et al.*, 1982; Beck *et al.*, 1981; Fedan *et al.*, 1981; Green *et al.*, 1981; Martin

TABLE 6
LUNG CHANGES IN F-344 RATS EXPOSED TO VOLCANIC ASH OR QUARTZ FOR VARYING PERIODS OF TIME^a

Months of exposure	Exposure group	Subgroup	Alveolar proteinosis	Alveolar macrophages	Interstitial reaction	Lympho-reticular reaction	Adenomatosis	Epidermoid carcinoma
4	Control	A	0/0 ^a	0.1/1	0.1/1	0/0	0/0	0
		B	0/0	0.2/2	0.3/3	0/0	0/0	0
	5 mg/m ³ ash	A	0/0	0.9/9	0.6/6	0.1/1	0/0	0
		B	0/0	0.9/7	0.9/7	1.4/8	0/0	0
	50 mg/m ³ ash	A	0.2/1	2.6/10	2.4/10	1.6/10	0/0	0
		B	0.1/1	2.1/10	2.5/10	2.6/10	0.3/1	0
	50 mg/m ³ quartz	A	3.1/10 ^b	2.9/10 ^b	3.0/10 ^b	2.4/10 ^b	0/0	0
		B	3.7/10 ^b	3.0/10 ^b	3.1/9 ^b	1.9/9 ^b	2.0/6 ^b	3
8	Control	A	0/0	0/0	0/0	0/0	0/0	0
		B	0/0	0/0	0/0	0/0	0/0	0
	5 mg/m ³ ash	A	0/0	1.9/10	1.9/10	1.2/7	0/0	0
		B	0/0	1.2/9	1.2/9	2.1/10	0/0	0
	50 mg/m ³ ash	A	2.0/10	3.1/10	2.9/10	2.7/10	0/0	0
		B	0.9/6	2.8/10	3.1/10	2.9/9 ^b	0.3/2	2
	50 mg/m ³ quartz	A	3.5/10 ^b	2.9/10 ^b	3.1/10 ^b	2.9/10 ^b	0/0	0
		B	3.8/10 ^b	3.0/10 ^b	3.4/10 ^b	1.4/7	1.4/5 ^b	1
12	Control	A	0/0	0.2/2	0.1/1	0/0	0/0	0
		B	0/0	0.1/1	0.1/1	0/0	0/0	0
	5 mg/m ³ ash	A	0/0	1.7/10	1.6/10	2.3/10	0/0	0
		B	0/0	1.4/9	1.2/9	2.0/8	0/0	0

16	50 mg/m ³ ash	A	2.9/10	2.9/10	3.0/10	2.8/10	0/0	0
		B	1.1/7	2.9/10	3.1/10	3.0/9	0.9/5	0
	50 mg/m ³ quartz	A	3.8/10 ^b	2.8/10 ^b	3.6/10 ^b	3.0/10 ^b	0.2/2	0
		B	4.0/10 ^b	2.7/9 ^b	3.1/10 ^b	1.1/7 ^b	1.8/5 ^b	1
20	Control	A	0/0	0/0	0/0	0/0	0/0	0
		B	0.1/1	0.2/2	0.2/2	0/0	0/0	0
	5 mg/m ³ ash	A	0/0	1.9/10	1.9/10	2.5/10	0/0	0
		B	0/0	1.7/10	1.6/10	2.6/10	0/0	0
	50 mg/m ³ ash	A	2.6/10	3.0/10	3.0/10	3.1/10	0/0	0
		B	1.5/9	3.0/10	2.9/9	3.5/10	0.2/2	0
	50 mg/m ³ quartz	A	4.3/10 ^{b,c}	3.0/10 ^b	3.4/10 ^{b,c}	2.3/10 ^b	0.7/5 ^c	1
		B	4.0/10 ^b	3.1/10 ^b	3.3/10 ^b	0.9/6 ^{b,c}	1.6/6 ^b	1
	Control	A	0/0	0.1/1	0.1/1	0/0	0/0	0
		B	0/0	0.1/1	0.2/2	0/0	0/0	0
	5 mg/m ³ ash	A	0.1/1	1.8/10	1.8/10	2.1/10	0/0	0
		B	0.2/2	1.6/10 ^c	1.6/10 ^c	2.8/10 ^c	0/0	0
24	50 mg/m ³ ash	A	1.7/10 ^b	3.0/10 ^b	3.1/10 ^b	3.1/10 ^b	0.2/2	0
		B	1.3/9 ^{b,c}	2.9/10 ^b	3.0/10 ^{b,c}	3.2/10 ^{b,c}	0.6/4	1
	Control		0/0	0.05/1	0/0	0/0	0/0	0
	5 mg/m ³ ash		0.05/1	1.8/19	2/19	2.8/19 ^c	0/0	0
	50 mg/m ³ ash		2.0/22 ^b	3.0/23 ^b	3.0/23 ^b	3.65/23 ^{b,c}	1/11	0

Note. The rats were 93 days old at start of exposure. A = subgroups exposed for stated number of months, followed by sacrifice. B = subgroups exposed for stated number of months, followed by observation and sacrifice at the 24-month exposure point of their continually exposed cohorts.

^a Mean grade/number of animals with lesion.

^b Indicating significant trends over dose [$P < 0.05$, Jonckheere (1954)/Terpstra (1952)].

^c Indicating significant trends over time [$P < 0.05$, Jonckheere (1954)/Terpstra (1952)].

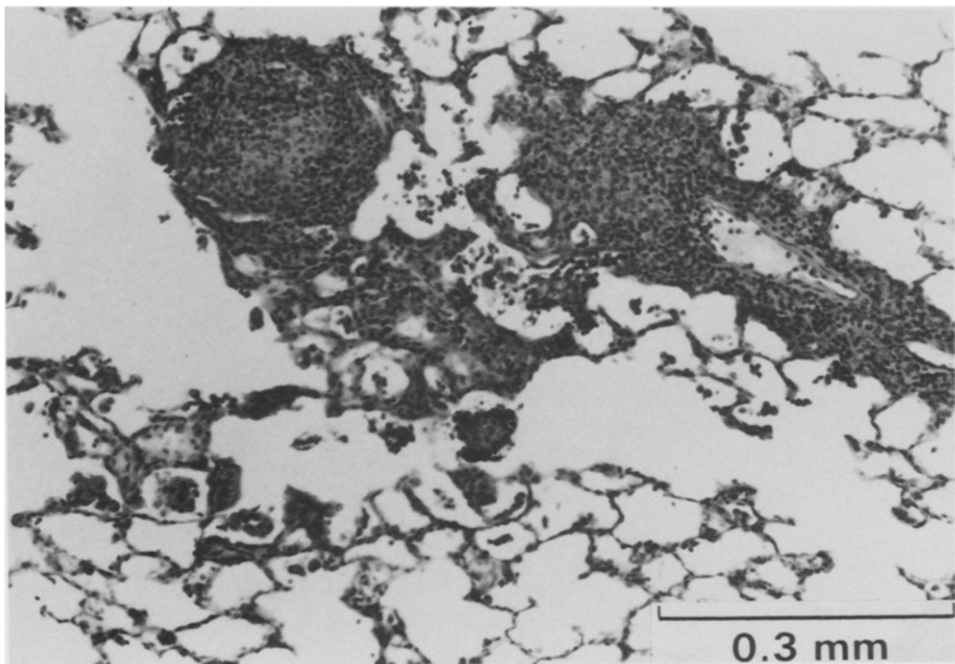


FIG. 1. Lung section from rat exposed to 5 mg/m³ volcanic ash for 24 months, showing interstitial and lymphoreticular reactions (H&E Stain).

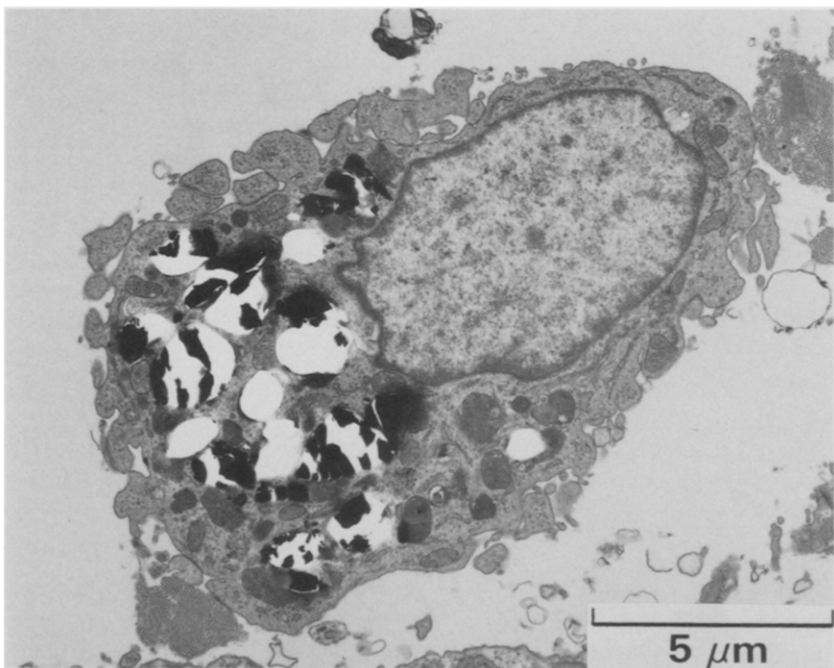


FIG. 2. Electron micrograph of phagocytosed volcanic ash particles in alveolar macrophage.

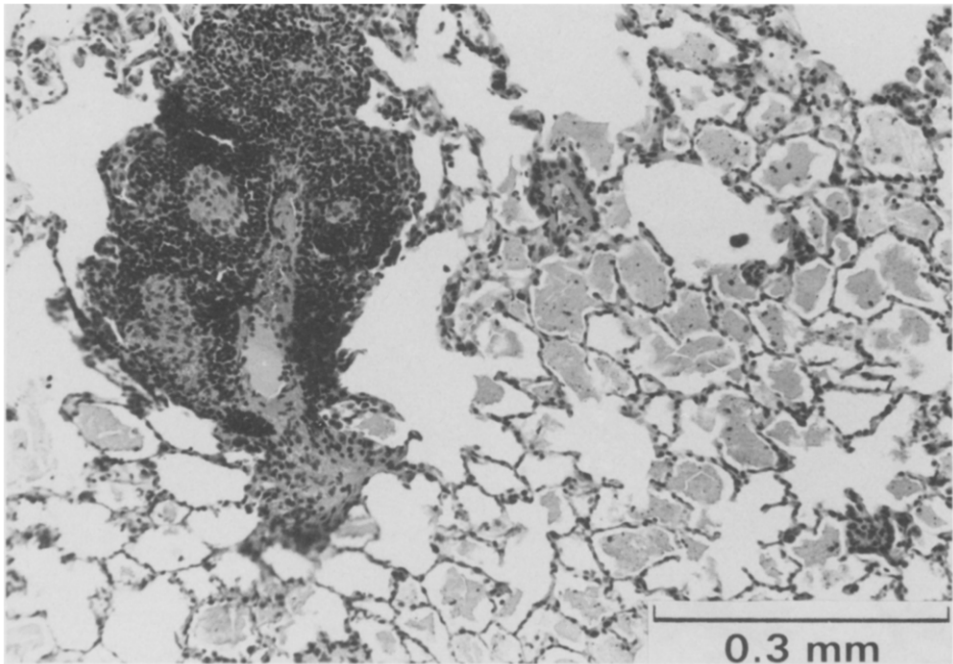


FIG. 3. Lung section from rat exposed to 50 mg/m³ volcanic ash for 24 months, showing lympho-reticular reaction and alveolar proteinosis (H&E Stain).

et al., 1983a; C. Sanders *et al.*, 1982, 1983; Vallayathan *et al.*, 1981, 1984; Wehner *et al.*, 1983b) and reviewed elsewhere (Craighead *et al.*, 1983; Martin *et al.*, 1983b). Ours appears to be the only investigation to provide information on the effects of *chronic inhalation* exposure to volcanic ash. Exposure of laboratory animals to volcanic ash by inhalation simulates more closely human exposure conditions than *in vitro* or intratracheal instillation studies. Therefore, inhalation exposure is the method of choice if the potential risk to humans warrants the effort.

Our results show general trends as well as dose-response and agent-response relationships. Most of the changes in most of the dose groups did not increase appreciably in severity after 4 to 8 months of exposure even though the exposures continued. Notable exceptions are the precipitous increase in mortality in the quartz-exposed group following 16 months of exposure, and the significantly increased incidence of lung cancer in the quartz-exposed female rats and—to a lesser degree—in the 50-mg/m³ ash-exposed female rats.

As to the rationale for our dose selection, our low-dose group was exposed to 5 mg/m³ volcanic ash, containing approximately 3.5% or 175 µg/m³ free silica. This concentration equals the recommended threshold limit value (TLV) for nuisance particulates containing less than 1% quartz (ACGIH, 1982), and is between three- and fourfold higher than the limit for the time-weighted average concentration of 50 µg/m³, recommended by the National Institute of Occupational Safety and Health (NIOSH) for occupational exposures to free silica (U.S. HEW, 1974).

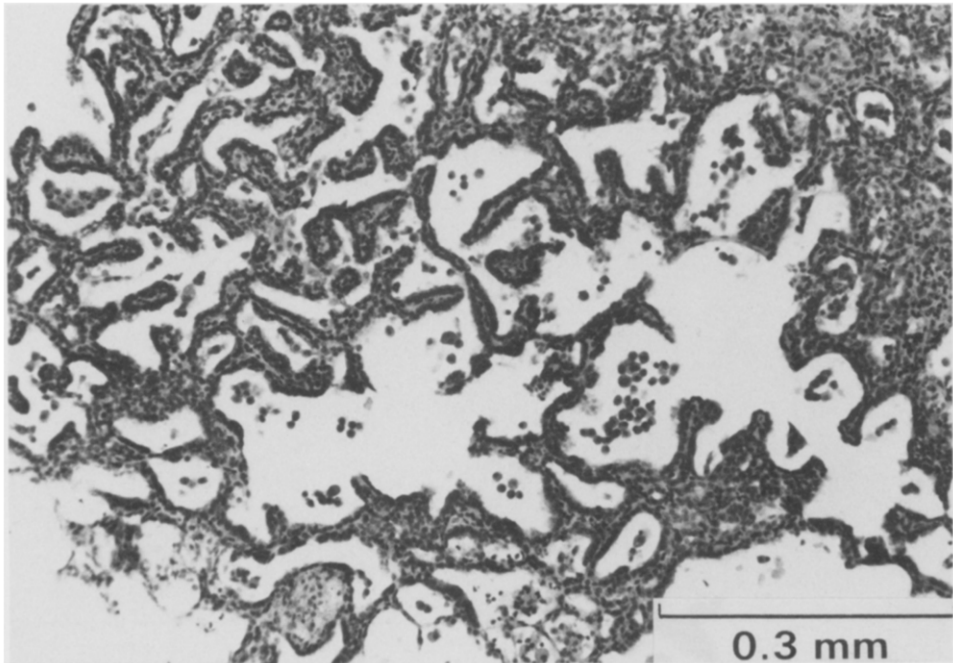


FIG. 4. Lung section from rat exposed to 50 mg/m³ volcanic ash for 24 months, showing alveolar adenomatosis (H&E Stain).

Thus, at our exposure levels of 5 and 50 mg/m³ volcanic ash, the concentrations of free silica were about 3.5 and 35 times, respectively, the NIOSH-recommended limits. The two dose levels bracket peak concentrations of 30 to 35 mg/m³ total particulate matter measured near ground level in the area most heavily affected by the eruption of Mount St. Helens (CDC, 1980).

Based on a minute volume of 8000 ml for humans and 200 ml for the male Fischer rat (Loscutt *et al.*, 1982), and a 6% alveolar retention of inhaled volcanic ash (Wehner *et al.*, 1984), a 6-hr exposure to 50 mg/m³ ash would theoretically result in an alveolar ash burden of about 8.64 mg for humans and 0.216 mg for rats. Based on a total alveolar surface area of 43 to 80 m² for humans (Weibel, 1963) and about 7000 cm² for rats (Burri *et al.*, 1973), the alveolar ash burden per square centimeter of alveolar surface area would be 11 to 20 ng/cm² for humans and about 31 ng/cm² for rats, representing an estimated dose ratio of about 1:2 per square centimeter of target tissue.

Our results demonstrate that, under chronic exposure conditions, volcanic ash can be more pathogenic than a nuisance dust because exposure to 5 mg/m³ of a nuisance dust—its TLV—should not result in the lesions observed in the 5-mg/m³ ash-exposed rats. This observation is supported by the results of an intratracheal instillation study conducted by C. Sanders *et al.* (1983). The increased pathogenicity of volcanic ash as compared to a nuisance dust may be due, at least in part, to the 3 to 4% of free crystalline silica present in the volcanic ash. Ash-in-

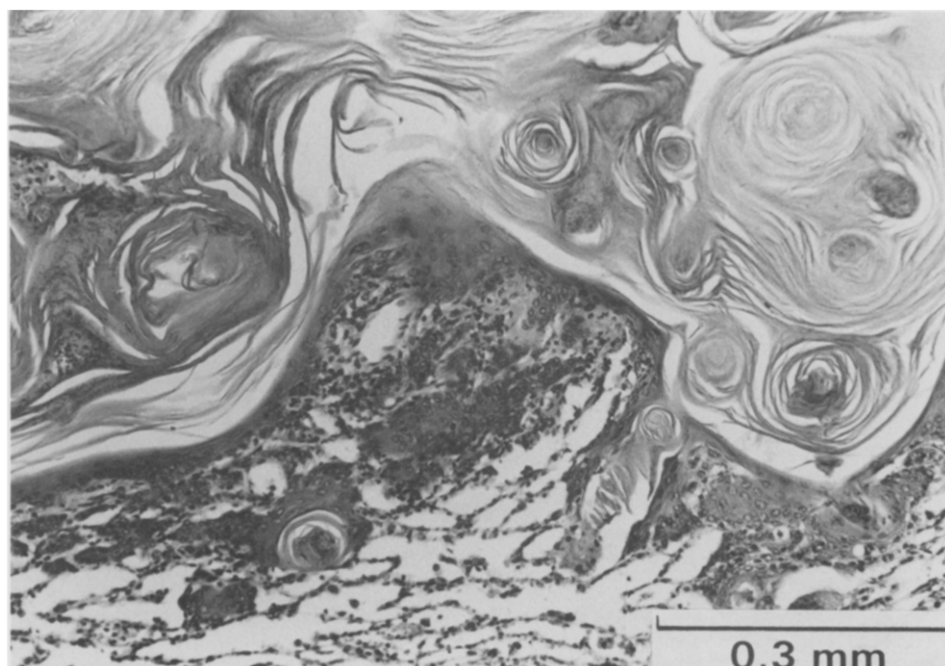


FIG. 5. Lung section from female rat exposed to 50 mg/m³ volcanic ash for 24 months, showing epidermoid carcinoma (H&E Stain).

duced lesions in the 50-mg/m³ ash lungs were generally more similar to those in the 50-mg/m³ quartz lungs than to those in the 5-mg/m³ ash lungs.

The observation of a significant number of epidermoid carcinomas in the lungs of quartz-exposed female rats (Dagle *et al.*, 1986) was the most noteworthy aspect of the quartz exposures. Our results provide additional experimental animal data to support epidemiological evidence linking silica exposure with lung cancer in man, described by Goldsmith *et al.* (1982), and reported by others at the International Symposium on Silica, Silicosis, and Cancer, at the University of North Carolina in Chapel Hill, April 3–5, 1984 (Goldsmith *et al.*, 1986).

The results of this 24-month exposure study support our previously reported findings at 12 months of exposure (Wehner *et al.*, 1983a). They suggest the following, within the limitations inherent to extrapolating from laboratory animal studies to human field conditions: (1) Inhalation exposure to volcanic ash under conditions normally encountered in fallout areas probably does not present a significant health risk to healthy persons in the general population. This statement is based on the observation that general populations exposed to volcanic ash after a volcanic eruption might encounter excessive concentrations only hours at a time, not longer than a few days at most. These acute exposures apparently are inconsequential for healthy persons (Johnson *et al.*, 1982; Washington State Health Services Division, 1980). (2) Repeated and prolonged exposure to high concen-

trations should be avoided if possible, especially by those with preexisting lung disease, because these persons generally tend to be more susceptible to additional pulmonary insults. (3) If repeated and prolonged exposure to high concentrations of volcanic ash cannot be avoided, appropriate protective measures may be indicated.

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