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FIBROGENIC POTENTIALS OF COAL SLAGS USED AS ABRASIVE BLASTING SUBSTITUTES

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This study was designed to examine the fibrogenic potentials of four coal slags that are being used as substitutes for silica sand in abrasive blasting. Six groups of 100 male Sprague-Dawley rats, including four coal slag groups, a vehicle control, and a positive control for fibrosis (Minusil quartz), were used. Each dust treatment group was given a single 40-mg dose of test agent via intratracheal instillation. Interim sacrifices of 15 animals per group were performed at 2 d, 3 mo, and 6 mo posttreatment, with the terminal sacrifice conducted at 12 mo. Hematoxylin and eosin stained histologic sections were prepared from designated formalin-fixed tissues collected at each necropsy and examined microscopically. Pulmonary silicon analyses were performed for each group at the 2-d and 12-mo sacrifices. Pulmonary function analyses were conducted for each group at the 3-, 6-, and 12-mo sacrifices. Lung hydroxyproline analyses were conducted for 15 animals in each group at the terminal sacrifice. The pulmonary fibrogenic potentials of the four coal slag groups were compared histologically with the Minusil and vehicle controls. A mild to moderate interstitial fibrosis, which was progressive with time, was noted in each of the coal slag groups. However, the coal slag-induced lung fibrosis was much less than that produced by Minusil. Differences in fibrosis among the individual coal slags were relatively minor and certainly not as striking as those between the slags and Minusil. Other data derived from this study, such as lung hydroxyproline content, pulmonary particulate burdens, pulmonary function, and animal body weights, provided further evidence of a reduced toxicity for the coal slags compared to Minusil.

Because of the well-known hazards of exposure to crystalline silica (e.g., Ziskind et al., 1976), substitutes for silica sand have been used in the abrasive blasting industry for many years. Two of the more common abrasive blasting substitutes are coal slags and various smelter slags. The coal slags are waste products formed from the burning of coal in electric power plants. The smelter slags are waste products from the smelting of ores and scrap metal. Both types of slag substitutes have excellent abrasive properties, are relatively cost competitive with sand, and most importantly, contain <1% crystalline silica. However, little information concerning the toxicity of these materials was known prior to their introduction into commerce.

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Our laboratory and another (see Szymczykiewicz et al., 1984) have been involved in the biological testing of these materials. In our initial study (Mackay et al., 1980), the fibrogenic effects of one coal slag and two copper slag samples (blasted and nonblasted) were examined in male Sprague-Dawley rats treated with 20 mg of dust by intratracheal instillation. At 10 mo, pulmonary interstitial fibrosis was seen in the coal slag-treated rats, while no fibrosis was seen in either copper slag treatment group. Granulomas were seen in the lungs of all slag treatment groups. A copper slag used in abrasive blasting in Poland has also been tested via intratracheal instillation (Szymczykiewicz et al., 1984). A 50-mg dose produced lung fibrosis and significant changes in lung hydroxyproline content at 3 and 6 mo postexposure. It was concluded the dust was weakly fibrogenic.

In a second study in our laboratory, we investigated the trace element chemistry of 18 coal and smelter slags (Stettler et al., 1982). Varying amounts of suspect carcinogens such as beryllium, chromium, arsenic, and nickel were found in the analyzed slags, which suggested the slags may be carcinogenic. To investigate this possibility, the carcinogenic and fibrogenic potentials of two copper slags and one nickel slag containing the highest levels of suspect carcinogens were examined in male Fischer 344 rats treated with 20 mg of dust by intratracheal instillation (Stettler et al., 1988). Only a minimal to slight alveolar wall fibrosis was seen in the rats treated with either copper slag, while the response seen with the nickel slag was consistent with a foreign body reaction. No granulomas were seen in any of the slag-treated animals. Large percentages (27–45%) of lung tumors were seen in two silica groups (Minusil and novaculite) included in this study. Statistically significant but lesser percentages (9–13%) of primary lung tumors (adenocarcinomas and adenomas) were seen in the copper slag treatment groups.

The current study was initiated to investigate further the fibrogenic potentials of coal slags. A major point of interest was whether coal slags from different commercial sources (and presumably different coal mines) would give similar results in the test animals.

MATERIALS AND METHODS

Test Material Preparation and Characterization

Four coal slag samples obtained from commercial suppliers were tested for fibrosis in this study. Included was material from the same lot (Slag I) that was used in our initial animal study (Mackay et al., 1980). Slag II was also obtained from the same commercial supplier as Slag I, but was purchased several years after Slag I. Slags III and IV were obtained from two other suppliers. A quartz sample (5 μm Minusil, Pennsylvania Glass Sand Corporation) was used as a positive control. This Minusil was from the same lot used in our earlier smelter slag study (Stettler et al., 1988).

Respirable size fractions were prepared for each of the test agents. Slags II, III, and IV were ball-milled using alundum balls to reduce their particle size. Slag I had previously been ball-milled to reduce its particle size (Mackay et al., 1980). The final size classifications for all four coal slag samples were done by sedimentation in filtered, deionized water using a 0.5% weight suspension of test materials. In this process, the particles of interest remained in suspension and were collected by centrifugation.

The Minusil was treated with 1 *N* HCl to remove any iron oxide, since its presence has been reported to affect the biological activity of quartz (Allison, 1976; Dauber et al., 1980). After removal of the iron oxide, the Minusil was washed with filtered, deionized water until the wash water was free of chloride as determined by the addition of silver nitrate. The Minusil was then classified using the same sedimentation process used for the coal slags. All five dust samples were then sized at a magnification of 1000x using a scanning electron microscope (model Super IIIA, International Scientific Instruments) equipped with a computer-based image analyzer (model DA 10, Lemont Scientific). At a minimum, 1800 particles of each dust sample were sized. Chemical characterizations were performed for each dust sample using proton-induced x-ray emission and atomic absorption and atomic emission spectrophotometry. Quartz determinations were performed by x-ray diffraction. The surface area of each of the dust samples was determined by nitrogen adsorption using an automatic surface area analyzer (model 2200, Micromeritics).

Animal Study

The test animals for the study were male, cesarean-derived, Sprague-Dawley rats (Charles River Breeding Laboratories, Inc.). These rats, as received, had body weights ranging from 151 to 175 g. After a 2-wk quarantine period, the rats were randomly divided into six treatment groups as shown in Table 1. The rats were housed at a temperature of 72 ± 5°F and 50 ± 5% relative humidity. A 12-h light and dark cycle was used in the housing rooms. Rats were housed three per cage and were fed a commercial pelleted diet (Purina rat chow) with tap water given ad libitum. The rats were weighed monthly during the 12 mo of the study.

The rats in the five dust treatment groups were given a single intratracheal instillation of the appropriate test agent using a procedure described previously (Mackay et al., 1980). The nominal dose of test material was 40 mg to enhance the chances of detecting differences among the four coal slags. To better assess the true dose of test agent and to test the variation that occurred when delivering the dose, after every 10th instillation, the next suspension (a total of 10 for each dust) was injected into a preweighed container and the net (dry) weight of the delivered doses determined. The rats in the remaining treatment group were given an instillation of the vehicle used to suspend the treatment dusts (sterile, filtered, deionized water).

Rat sacrifice intervals and on-study mortality are shown in Table 1. The

TABLE 1. Rat Treatment Groups and Disposition

Treatment group	2 d sacrifice	Died 0–3 mo	3-mo sacrifice	Died 3–6 mo	6-mo sacrifice	Died 6–12 mo	12-mo Sacrifice		
							Hydroxyproline	Lung silicon	Histopathology
Vehicle	15	2	15	0	15	1	15	15	22
Slag I	14[a]	3	15	0	15	4	15	15	19
Slag II	16[a]	1	15	0	15	2	15	15	21
Slag III	15	0	15	1	15	2	15	15	22
Slag IV	15	0	15	0	15	2	15	15	23
Minusil	15	1	15	2	15	2	15	15	20

[a]Because of an error in reading an ear tag, one animal selected for sacrifice from the Slag I group actually belonged to the Slag II group.

rats, which were randomly selected for sacrifice, were killed with an overdose of sodium pentobarbital administered by intraperitoneal injection.

Sacrifices were conducted at 3, 6, and 12 mo to study the time progression of any treatment-related histopathological lesions and pulmonary function deficits. The tissues taken at each sacrifice included the lungs, tracheobronchial lymph nodes (TBLN), liver, kidney, spleen, mesenteric lymph nodes, pancreas, adrenals, thyroid, brain, pituitary, heart, and all gross lesions. All of these tissues were fixed in 10% neutral, phosphate-buffered formalin. The lungs were perfused with fixative in situ. For the 12-mo sacrifice, hematoxylin and eosin stained sections of each of these tissues were prepared for and examined by light microscopy. At 3 and 6 mo, only the lungs, tracheobronchial and mesenteric lymph nodes, and gross lesions were evaluated histologically.

Pulmonary Dust Burdens and Hydroxyproline Analyses

Fifteen rats per group were sacrificed at 2 d (48 ± 1 h of initial dosing) and at 12 mo for silicon analyses to determine pulmonary dust burdens. The lungs (and tracheobronchial lymph nodes at 12 mo) were trimmed, weighed, and then freeze-dried to constant weight. The freeze-dried tissues were analyzed for silicon by atomic absorption spectroscopy.

Fifteen rats per group also were sacrificed at 12 mo for hydroxyproline determinations. The lungs were trimmed, weighed to determine wet weight, and then frozen. At the time of analysis, the whole lungs were thawed and then homogenized in cold distilled water. The homogenates were hydrolyzed overnight using 6 *N* HCl at 120°C. Hydroxyproline was determined spectrophotometrically (Bergman & Loxley, 1969).

Pulmonary Function Analyses

Fibrogenic responses produce a stiffer lung, which results in restrictive (reduced lung volumes) and obstructive (reduced airflows) defects. To detect these functional responses during life, pulmonary function analyses were performed on 15 rats from each treatment group on the day of the 3-, 6-, and 12-mo sacrifices. Pulmonary function parameters evaluated included mechanical properties (compliance, CL; resistance, RL), lung volumes, and flow rates. The lung volume parameter evaluated for restrictive impairment was the forced vital capacity (FVC), while the primary flow parameter studied for obstructive impairment was the forced expiratory flow rate at 50% of the FVC (FEF_{50}).

The rats were anesthetized via an intramuscular injection of ketamine and xylazine (8.0 mg/kg of ketamine and 12 mg/kg of xylazine) and transorally intubated. The intubated rat was connected to a custom-designed pneumotachograph (Hans Rudolph, St. Louis, Mo.). Flow, volume, and transpulmonary pressures were measured during normal quiet breathing to calculate CL and RL (Frank et al., 1957). Ventilatory performance was assessed using a variable pressure plethysmograph (Moorman et al., 1975). Briefly, breathing

maneuvers were produced by application of ±60 cm H_2O extrathoracically. The forced vital capacity was produced by forced expiration from total lung capacity (as defined by inspiration to plateau resulting from 60 cm H_2O external negative pressure). During the forced expiration (as produced from 60 cm H_2O positive pressure), flow rates were measured at 50% and 25% of the FVC.

Statistical Analyses

t-Tests were used to compare the average dose delivered to each group to the nominal dose. One-factor analyses of variance (ANOVAs) were used to compare the mean body weights of the groups at each month. If an ANOVA was significant, contrasts were done to compare each group to the control group, and the Minusil group to each of the coal slag groups. One-factor ANOVAs followed by Fisher's least significant difference (LSD) test were used to analyze the differences in mean lung weights, mean amounts of hydroxyproline in the lungs, and mean silicon levels in the lungs and tracheobronchial lymph nodes. The means of each pulmonary function parameter were compared at each sacrifice period using Tukey's studentized range test.

RESULTS

Test Material Characterization

The particle size distribution for each of the test dusts was lognormal. The geometric median diameters (and geometric standard deviations, σ_g) were as follows: Slag I, 0.47 μm (σ_g = 1.96); Slag II, 0.62 μm (σ_g = 2.18); Slag III, 0.60 μm (σ_g = 2.30); Slag IV, 0.56 μm (σ_g = 1.96); and Minusil, 0.41 μm (σ_g = 2.51). The surface areas for each dust were Slag I, 5.7 m^2/g; Slag II, 5.5 m^2/g; Slag III, 4.9 m^2/g; Slag IV, 5. 2 m^2/g; and Minusil, 5.6 m^2/g.

The results of the chemical analyses for each dust are summarized in Table 2, and show the four coal slags to be very similar chemically. Each coal slag had the same major elemental components: silicon, aluminum, iron, calcium, chlorine, sodium, magnesium, and potassium. The largest variations in major elemental components were seen for chlorine (1.2–8.4%), iron (11–16%), and calcium (1.1–5.7%). The minor elemental components of each coal slag were also relatively similar, with titanium, barium, strontium, vanadium, and zirconium being the most prominent for each slag. Each coal slag did contain a small but measurable concentration of beryllium. Quartz was not detected in any of the coal slag samples (limit of detection = 0.5% by weight).

Animal Dosing Proficiency

The results of instillation proficiency testing for determining delivered dose (average net weights ± standard deviation for 10 instillations) for the dust groups were as follows: Slag I, 39.4 ± 1.8 mg; Slag II, 40.2 ± 1.3 mg; Slag III, 36.1 ± 0.6 mg; Slag IV, 40.3 ± 1.8 mg; and Minusil, 39.4 ± 1.2

TABLE 2. Chemical Characterization of Test Materials

Major elements (wt %)	Slag I	Slag II	Slag III	Slag IV	Minusil
Si	22	23	23	24	44
Na	0.9	0.7	0.6	0.8	0.01
Mg	0.8	0.4	0.6	0.6	0.02
Al	9.5	11	11	9.7	0.1
K	1.3	1.3	1.3	1.4	0.03
Ca	5.7	2.4	1.1	4.5	0.01
Fe	11	14	16	12	0.01
Cl	4.6	8.4	3.1	1.2	0.3
Minor elements (μg/g)	**Slag I**	**Slag II**	**Slag III**	**Slag IV**	**Minusil**
Ti	3800 ± 300	4500 ± 200	4930 ± 80	2960 ± 100	90 ± 5
V	230 ± 50	320 ± 40	240 ± 20	230 ± 70	<10
Cr	<180	140 ± 30	80 ± 20	<130	<8
Ni	25 ± 10	<20	14 ± 5	31 ± 10	<2
Cu	50 ± 5	72 ± 3	63 ± 3	<5	<2
Zn	102 ± 6	30 ± 3	63 ± 3	81 ± 8	4.3 ± 0.4
Ga	11 ± 4	12 ± 1	7 ± 2	<3	<0.7
As	4 ± 1	4 ± 2	<3	7.5 ± 1.0	0.8 ± 0.2
Br	27 ± 2	23 ± 3	33 ± 3	30 ± 7	20 ± 1
Rb	86 ± 4	70 ± 3	86 ± 4	68 ± 8	<1.1
Sr	760 ± 20	569 ± 10	400 ± 9	196 ± 7	0.7 ± 0.3
Zr	210 ± 10	280 ± 20	240 ± 10	196 ± 7	11 ± 1
Pb	<11	<10	39 ± 4	<12	<1.1
Y	38 ± 4	38 ± 2	36 ± 2	40 ± 4	<0.6
Nb	14 ± 5	21 ± 4	14 ± 5	20 ± 3	<0.9
Ba	1800 ± 100	900 ± 200	900 ± 400	1100 ± 100	<9
Be	8.8 ± 0.6	5.8 ± 0.3	7.8 ± 0.5	7.1 ± 0.5	<0.08

mg. Only the average dose for Slag III was statistically different from the nominal target dose.

Animal Body Weights

Body weight curves for the six test groups are shown in Figure 1. The mean body weights for the Minusil group were consistently lower than those for the other test groups. These lower mean weights were statistically significant compared to the vehicle, Slag I, Slag II, and Slag IV group means at mo 1–12, and to the Slag III group means at mo 2–5. While the mean monthly body weights for the slag groups were typically less than the corresponding mean weights for the vehicle group, these differences generally were not significant.

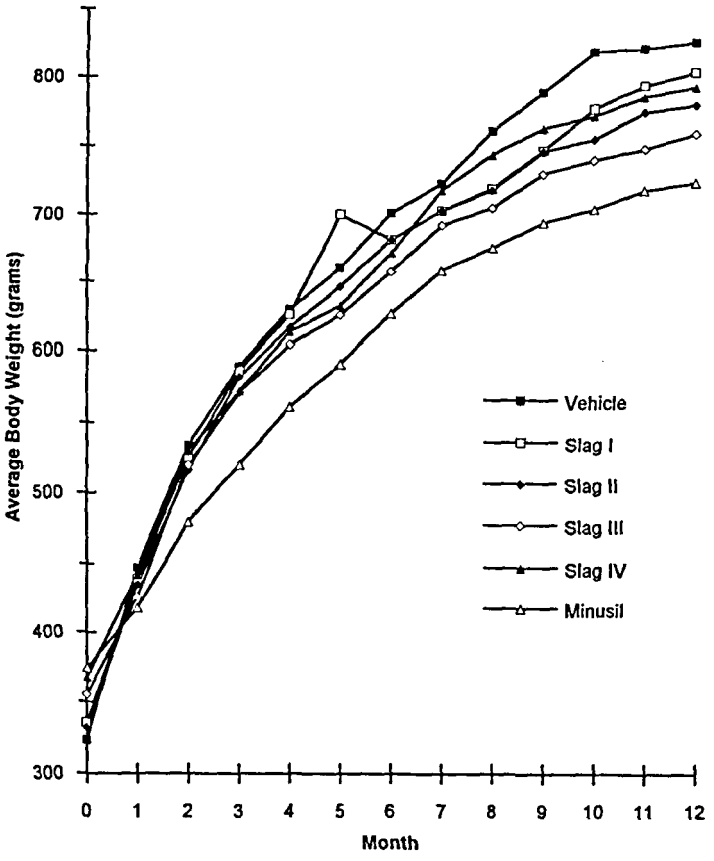

FIGURE 1. Average body weights for the vehicle control, coal slag, and Minusil exposure groups.

Lung Weights and Hydroxyproline Analyses

Data for wet lung weights and lung hydroxyproline contents at the terminal sacrifice are given in Table 3. The ANOVAs for lung weights and hydroxyproline content were both significant. The average lung weight and hydroxyproline content of the Minusil group were significantly greater than the other five groups. In addition, the mean lung weights of the Slag II and Slag III groups were significantly greater than the vehicle control group.

Pulmonary Dust Burdens

Data summarizing pulmonary dust burdens at 48 h and 12 mo postinstillation are shown in Table 4. The average dust burdens for each test material were calculated from the average lung (or tracheobronchial lymph node, TBLN) silicon contents and the percentage of silicon in the test material (Table 2). Dust retention was calculated using the average lung (lung +

TABLE 3. Terminal Sacrifice Wet Lung Weights and Hydroxyproline Contents

Test group	*n*	Average wet lung weight (g ± SD)	*n*	Average hydroxyproline (μg/lung ± SD)
Slag I	30	2.71 ± 0.34	15	91 ± 37
Slag II	29[a]	2.91 ± 0.71[b]	14[c]	105 ± 30
Slag III	30	2.91 ± 0.48[b]	15	97 ± 31
Slag IV	30	2.77 ± 0.29	15	94 ± 26
Minusil	30	6.54 ± 1.47[d]	15	375 ± 184[d]
Vehicle	30	2.38 ± 0.25	15	73 ± 28

[a]Terminal weight for one lung was not recorded.
[b]Significantly greater than the vehicle control group ($p < .05$).
[c]One sample was lost.
[d]Significantly greater than all other test groups ($p < .05$).

TBLN at 12 mo) dust burdens and the average delivered dose of test agent. At the 48-h sacrifice, the ANOVA for dust retention (the percentage of delivered dose remaining in the pulmonary system) was not significant. At 12 mo, the ANOVAs for the average lung and TBLN dust burdens and dust retention were all significant. The average lung and tracheobronchial lymph node dust burdens and dust retention for the Minusil group were significantly greater than the corresponding means of all the slag groups. The average tracheobronchial lymph node dust burden and dust retention for Slag IV were significantly greater than the corresponding levels for Slags II and III.

TABLE 4. Pulmonary Dust Burdens at 48 h and 12 mo Postinstillation

	48 h		12 mo		
Test agent	Average lung dust burden (mg ± SD)	Dust retention (percent of delivered dose remaining, mean ± SD)	Average lung dust burden (mg ± SD)	Average TBLN dust burden (mg ± SD)	Dust retention (percent of delivered dose remaining, mean ± SD)
Slag I	31 ± 8.8	78 ± 22	10 ± 4.7	4.9 ± 2.2	38 ± 16
Slag II	29 ± 6.5	73 ± 16	10 ± 2.9	3.9 ± 1.7	35 ± 9.7
Slag III	30 ± 5.2	84 ± 14	8.9 ± 2.6	3.4 ± 1.2	34 ± 8.7
Slag IV	31 ± 6.7	76 ± 17	12 ± 4.9	5.7 ± 2.4[a]	44 ± 16[a]
Minusil	35 ± 4.8	88 ± 12	15 ± 2.8[b]	14 ± 3.7[b]	74 ± 10[b]

[a]Significantly greater than means for Slags II and III ($p < .05$).
[b]Significantly greater than means for all other test groups ($p < .05$).

Pulmonary Function Analyses

The results of the pulmonary function analyses are summarized in Table 5, and represent the temporal changes noted for lung volumes (FVC), flow rates (FEF_{50}), compliance, and resistance. The Minusil-treated rats had significantly lowered FVC (noting volume restrictive disease) and FEF_{50} (noting flow obstructive disease) at all sacrifice periods compared to the vehicle controls. The restrictive impairment in the Minusil group increased over time from a 15.9% reduction at 3 mo to a 23.2% reduction at 12 mo compared to the vehicle controls. The obstructive impairment for the Minusil group also increased over time, from 31.2% to 38.8% of control value when comparing FEF_{50} at 3 and 12 mo. The FVC and FEF_{50} data for the slag treatment groups generally ranked between the vehicle controls and the Minusil test group; however, these differences were not statistically significant. No statistically significant differences were seen for the mechanical properties, compliance and resistance, although the Minusil group compliance values were generally smaller while the resistance values were higher than the vehicle controls.

Pathology

For all treatment groups, the target organs were the lung and tracheobronchial lymph nodes. The major histopathological findings are summarized next by treatment group. The severity of the lesions noted were graded as follows: minimal (1), mild (2), moderate (3), marked (4), and severe (5). The average severities of the primary treatment-related lesions are summarized in Table 6. No primary lung neoplasia was evident in this study.

Vehicle Controls Minimal perivascular and peribronchial mononuclear cell infiltrates were present in the lungs at 3 mo (4/15 animals) and 6 mo (12/15 animals). At 6 mo, a minimal to mild interstitial pneumonia was present in 6/15 animals. These lesions were suggestive of exposure to murine respiratory pathogens. A slight clearing effect was noted at 12 mo, with minimal perivascular and peribronchial mononuclear cell infiltrates present in the lungs of 12/22 rats and minimal, subacute pneumonia noted in only 3 rats. In the tracheobronchial lymph nodes, hemosiderin-like pigment was noted in five, two, and three animals at 3, 6, and 12 mo, respectively. Hemorrhage (4/15 animals) was noted at 3 mo and in 2 animals at 6 and 12 mo.

Coal Slags The primary treatment-related changes noted in all coal slag animals were pigment-containing macrophages located within the alveoli, foci of alveolar interstitial reaction, and hyperplasia of type II pneumocytes associated with the macrophages. Typically, the interstitial reaction was characterized by interstitial thickening due primarily to haphazardly arranged collagen fibers with fibroblasts, and a few other mononuclear inflammatory cells. In general, the interstitial fibrosis increased in severity with time, with areas of mature fibrosis noted around alveolar ducts (Figure 2). A more extensive nodular fibrotic reaction was noted in a few animals at 6 and 12 mo.

TABLE 5. Pulmonary Function Analyses

Parameter (mean ± SD)	Vehicle	Slag I	Slag II	Slag III	Slag IV	Minusil
FVC (ml)						
3 mo	17.0 ± 2.3	16.8 ± 2.1	16.4 ± 2.4	16.4 ± 1.8	16.7 ± 2.8	14.3 ± 1.9[a]
6 mo	16.8 ± 2.4	14.9 ± 2.3	15.9 ± 3.3	14.5 ± 2.2	15.4 ± 2.3	13.4 ± 2.5[a]
12 mo	17.7 ± 2.1	16.4 ± 1.6	14.9 ± 1.5	15.1 ± 3.7	16.9 ± 3.3	13.6 ± 2.1[a]
FEF_{50} (ml/s)						
3 mo	75.3 ± 16.1	63.1 ± 17.6	62.8 ± 22.6	66.9 ± 22.3	68.9 ± 21.1	51. 8 ± 17.7[a]
6 mo	72.5 ± 18.4	76.3 ± 27.8	67.1 ± 14.9	74.9 ± 24.3	80.5 ± 16.4	54.1 ± 20.7[a]
12 mo	86.0 ± 28.2	75.0 ± 21.0	80.3 ± 23.2	75.6 ± 22.2	68.7 ± 25.8	52.6 ± 16.1[a]
Compliance (ml/cm H_2O)						
3 mo	0.48 ± 0.13	0.50 ± 0.20	0.52 ± 0.29	0.47 ± 0.20	0.51 ± 0.15	0.38 ± 0.26
6 mo	0.58 ± 0.17	0.56 ± 0.22	0.56 ± 0.25	0.58 ± 0.17	0.46 ± 0.16	0.45 ± 0.12
12 mo	0.40 ± 0.13	0.48 ± 0.30	0.34 ± 0.11	0.39 ± 0.16	0.42 ± 0.22	0.34 ± 0.12
Resistance (cm H_2O/L/s)						
3 mo	0.039 ± 0.054	0.035 ± 0.036	0.028 ± 0.018	0.048 ± 0.065	0.055 ± 0.080	0.044 ± 0.038
6 mo	0.033 ± 0.023	0.036 ± 0.026	0.030 ± 0.022	0.042 ± 0.026	0.041 ± 0.030	0.065 ± 0.063
12 mo	0.076 ± 0.046	0.096 ± 0.074	0.078 ± 0.067	0.095 ± 0.090	0.110 ± 0.083	0.081 ± 0.056

[a]Significantly less than means for the vehicle ($p < .05$).

TABLE 6. Lung and Tracheobronchial Lymph Node Histopathology Summary

Months postexposure	Exposure group	*n*	Lung			Tracheobronchial lymph nodes	
			Alveolar macrophages	Interstitial fibrosis	Nodular fibrosis	Fibrosis	Histiocytosis
3	Vehicle	15	0/0	0/0	0/0	0/0	0/0
6		15	0.07/1	0/0	0/0	0/0	0/0
12		22	0.05/1	0/0	0.05/1	0/0	0/0
3	Slag I	15	1.5/15	0.9/10	0/0	0.7/9	1.7/15
6		15	2.0/15	1.2/12	0/0	1.6/12	2.1/15
12		19	1.6/19	1.3/17	0.1/1	3.2/18	2.1/19
3	Slag II	15	1.4/15	1.4/12	0/0	0.5/5	1.5/15
6		15	1.9/15	1.1/8	0.9/6	2.0/13	2.3/15
12		21	1.9/21	2.3/20	0.1/1	2.8/21	2.1/21
3	Slag III	15	1.5/15	1.3/13	0/0	0.5/6	1.5/15
6		15	1.9/15	1.1/12	0.3/2	1.5/11	2.2/15
12		22	1.5/22	1.7/22	0/0	2.8/21	1.9/21
3	Slag IV	15	1.1/15	0.8/11	0.07/1	0.3/4	1.6/15
6		15	1.7/15	1.2/11	0.1/1	1.4/10	2.0/15
12		23	1.7/23	1.9/23	0/0	2.7/22	2.3/22
3	Minusil	15	1.9/15	0/0	4.2/15	1.1/10	3.3/15
6		15	2.2/15	0/0	4.3/15	1.0/11	3.9/15
12		20	1.2/20	0/0	3.8/20	0.6/9	4.8/20

Note. Values are mean grade/number of animals with lesion.

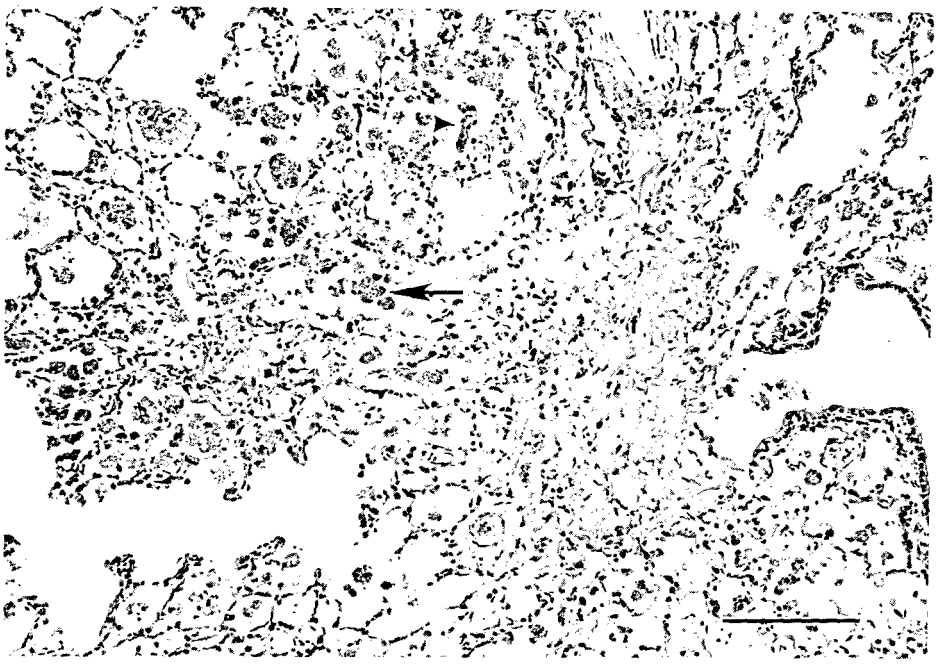

FIGURE 2. Light micrograph of a 5-μm-thick hematoxylin and eosin (H&E) stained section of the lung from a Slag III-treated rat at 12 mo postexposure, showing a typical lesion for coal slag-treated rats at this time. The lesion consists of a mature area of fibrosis (f) in the alveolar duct area with collagen fibers streaming out into the surrounding alveolar septa. The alveoli contain pigment-laden macrophages (arrow) and type II cell hyperplasia (arrow head). Bar equals 100 μm.

This nodular fibrosis was most prominent in animals treated with Slag II, with the nodules ranging in size from 0.5 mm to 1.75 mm. Interstitial pneumonia, similar in severity to that in the vehicle controls, was also seen in a few coal slag-treated rats, but did not compromise any of the lung histopathology.

The reactions seen in the tracheobronchial lymph nodes for all of the coal slag-treated animals were similar, both in type and intensity. At 3 mo, these lymph nodes contained infiltrates of histiocytes, most of which contained particulate material. Areas of fibrosis associated with the histiocytes, generally minimal in severity, were also seen. Accumulations of pigment-containing histiocytes present in the tracheobronchial lymph nodes increased at 6 mo, as did the levels of fibrosis associated with these histiocytes. At 12 mo, fibrosis was a prominent feature in the lymph nodes. Small foci of mineralization were also seen. No treatment-related pathology was seen in the other tissues examined from the rats in the coal slag groups.

Minusil At all sacrifice periods, the lungs of the Minusil-treated rats contained multiple fibrotic nodules to coalescing masses of fibrosis with cellular infiltrates of pigment-containing macrophages, fibroblasts, and mono-

nuclear inflammatory cells (Figure 3). In many instances, the lesions were severe and tended to efface much of the parenchyma of a lung lobe. Cyst-like structures lined by epithelium became trapped in areas of fibrosis, many containing protein-like material. Alveolar proteinosis was also prominent adjacent to fibrotic areas. Additionally, hyperplasia of the bronchial-associated lymphoid tissue was also evident in the lungs of this group. As in other groups where more normal alveolar tissue was remaining, type II cell hyperplasia was noted. The fibrosis noted for the 12-mo animals was more well defined, with collagen fibers assuming a more concentric arrangement.

At 3 mo, the morphological changes in the tracheobronchial lymph nodes were similar to those noted in the coal slag-treated animals. However, the levels of histiocyte infiltrations and fibrosis were sightly higher than in the coal slag animals. At 6 mo, the levels of histiocytes in the lymph nodes of Minusil-treated rats continued to be higher than in the coal slag animals while the levels of fibrosis had decreased below those seen for the coal slags. At 12 mo, lymphoid hyperplasia had become a prominent feature of the tracheobronchial lymph nodes, and in conjunction with moderate to severe accumulations of pigmented histiocytes, resulted in enlargement of many of the nodes. Minimal fibrosis and no mineralization were evident.

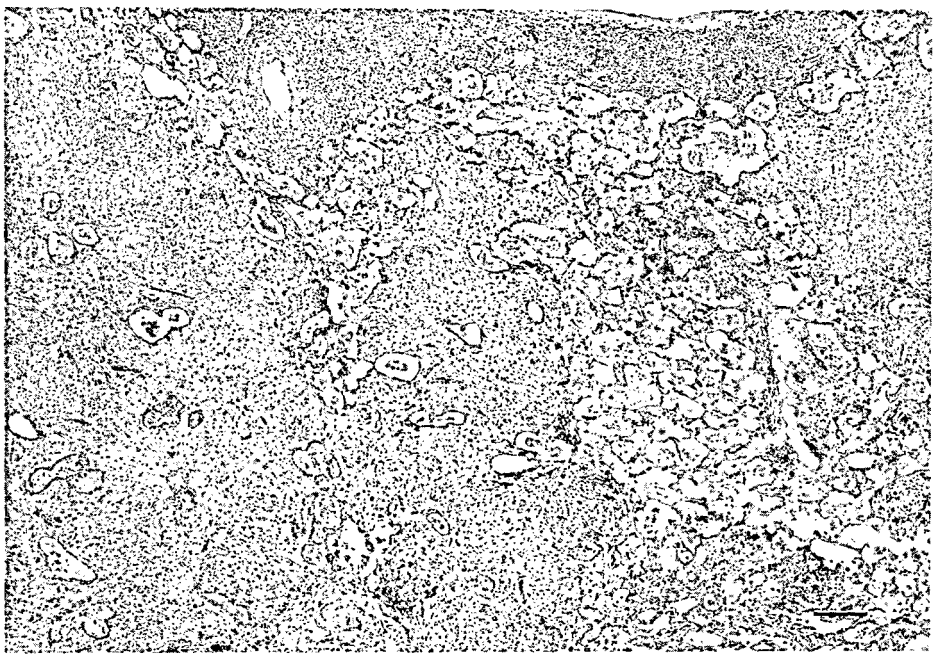

FIGURE 3. Light micrograph of a 5-μm-thick hematoxylin and eosin stained section of the lung from a Minusil-treated rat at 3 mo postexposure, showing the typical diffuse severe fibrotic response. The lesion consists of hypercellular coalescing foci of fibrosis with entrapped alveoli/bronchioles. Alveolar proteinosis and pigment-laden macrophages are present in the alveoli. Bar equals 100 μm.

Histiocytes containing particulate material were also noted in 82% of the pancreatic lymph nodes examined at 12 mo.

DISCUSSION

The National Institute for Occupational Safety and Health has recommended since 1974 that silica sand or other substances containing more than 1% crystalline silica be prohibited as abrasive blasting material (NIOSH, 1974). Although the health hazards of human exposure to crystalline silica have been well documented, preventing silicosis and deaths from sandblasting is still a major problem (NIOSH, 1992). Failure to substitute less toxic abrasive blasting materials for silica sand in abrasive blasting operations has been cited as one of the characteristics of sandblasting worksites where silicosis is a problem (NIOSH, 1992). Hence, efforts for the identification, toxicological evaluation, and utilization of safe substitutes for silica sand in abrasive blasting need to be continued.

This study was designed to evaluate the fibrogenic potential of four different coal slags compared to silica. Our histopathology data indicate coal slag-induced lung fibrosis to be much less severe than that produced by Minusil. A mild to moderate interstitial fibrosis, which was progressive with time, was noted in each of the coal slag treatment groups. A more severe fibrosis with effacement of lung parenchyma was seen in a few animals, primarily those treated with Slag II. However, all of these changes were much less severe than those resulting from exposure to Minusil. The massive fibrosis and inflammation that effaced much of an entire lobe of the lungs in the Minusil group were not evident in any of the coal slag-treated animals.

Other data collected during the study also indicate the reduced pulmonary fibrogenic potentials of coal slags compared to silica. Wet weights and hydroxyproline content of the Minusil lungs were significantly elevated ($p < .05$) compared to all of the slag treatment groups and the vehicle controls. The average lung, tracheobronchial lymph node, and total residual dust burdens at 12 mo were significantly higher for Minusil than for the coal slags, suggesting the slags are more easily cleared from the body than Minusil. The higher tracheobronchial dust burden for Minusil suggests that mucocilliary clearance is more relevant for coal slags, while Minusil is cleared from the lung primarily through the lymphatic system. Other indications of the enhanced toxicity of silica include animal body weights and pulmonary function data. The mean body weights for the Minusil group were significantly lower throughout the study than the mean monthly weights for Slag I, II, and IV groups and the vehicle controls, and significantly lower than the Slag III group at mo 2–5. Statistically significant restrictive and obstructive changes were noted for the Minusil group compared to the vehicle controls. While not statistically significant, pulmonary function data for the slag-treatment groups generally fell between the Minusil and vehicle control data.

Differences in fibrogenic potentials among the slags used in this study were relatively minor and certainly not as striking as those between the slags and Minusil. Only slight variations in the severity of interstitial fibrosis were noted among the four coal slag treatment groups. Furthermore, this interstitial fibrosis was similar in nature and severity to that seen in our previous study (Mackay et al., 1980). Hydroxyproline and pulmonary function data provide further evidence for the similarity of fibrogenic potentials among coal slags, as no statistically significant differences were noted in these parameters for the four coal slag groups in the current study. Perhaps the most significant difference among the coal slag groups from both studies was the nodular fibrosis seen in a few of the animals from the current study, especially those exposed to Slag II. However, these nodular fibrotic reactions seemed to be associated with heavier, focal deposits of particulate material, and hence may be related more to localized dose than to differences in potency among the slags. The only other histopathological difference of note was the lack of granuloma formation. Granulomas were seen in the lungs of both the coal and copper slag groups in our first study (Mackay et al., 1980). However, granulomatous reactions were seen neither in our second smelter slag study (Stettler et al., 1988) nor in the current investigation.

In summary, four coal slags, three copper slags, and one nickel slag have been evaluated in our laboratory. In every instance, the extent of lung fibrosis associated with the slag exposures was much less severe than that seen with quartz (Minusil). Differences in fibrogenic potentials among the coal slags were relatively minor.

While our data clearly show coal slags to be less fibrogenic than quartz, moderate levels of fibrosis were seen for these materials. However, given the artificial nature of the route of exposure (intratracheal instillation), the large doses used in our study, and the potential for dust overload, chronic inhalation studies at multiple dose levels are needed to better define the fibrogenic potential of these materials, as well as their carcinogenic potentials.

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