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Comparative Pulmonary Toxicity of 6 Abrasive Blasting Agents

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Inhalation of silica dust is associated with pulmonary fibrosis. Therefore, substitute abrasive materials have been suggested for use in abrasive blasting operations. To date, toxicological evaluation of most substitute abrasives has been incomplete. Therefore, the objective of this study was to compare the pulmonary toxicity of a set of substitute abrasives (garnet, staurolite, coal slag, specular hematite, and treated sand) to that of blasting sand. Rats were exposed to blasting sand or an abrasive substitute by intratracheal instillation and pulmonary responses to exposure were monitored 4 weeks postexposure. Pulmonary damage was monitored as lactate dehydrogenase (LDH) in the acellular lavage fluid. Pulmonary inflammation was evaluated from the yield of polymorphonuclear leukocytes (PMN) obtained by bronchoalveolar lavage. The activity of alveolar macrophages was determined by measuring zymosan-stimulated chemiluminescence. Blasting sand caused lung damage and showed histologic evidence for inflammation and fibrosis. Garnet, staurolite, and treated sand exhibited toxicity and inflammation that were similar to blasting sand, while coal slag caused greater pulmonary damage and inflammation than blasting sand. In contrast, specular hematite did not significantly elevate LDH or PMN levels and did not stimulate macrophage activity 4 weeks postexposure.

Key Words: **silica; sand; quartz; coal slag; garnet; specular hematite; iron oxide; staurolite; lung; inflammation; fibrosis.**

Abrasive blasting is the process of cleaning surfaces by bombarding them with small abrasive particles. Sand with a high crystalline silica content has been used for abrasive blasting (sandblasting) for many years. Unfortunately, during the blasting process, silica sand fractures into small respirable particles that are associated with worker morbidity and mortality due to a progressive lung disease known as silicosis.

One approach to the problem of lung disease in the abrasive blasting industry is the replacement of sand with safer abrasive blasting agents. Although sand is the most popular abrasive blasting agent, a number of substitutes for sand are in current use. A 1992 survey reported that coal slag was the most frequently used of these substitutes (Paumanok Publications, Inc., 1992). Unfortunately, the toxicity of most commercially available substitutes is incompletely investigated. In this study, we examined pulmonary alterations in rats 4 weeks after intratracheal instillation of respirable fractions of blasting sand or 5 abrasive blasting substitutes: coal slag, garnet, specular hematite, staurolite, or treated sand (blasting sand coated with a dust suppressant). The toxicity of these abrasive blasting substitutes was compared to the toxicity of blasting sand and to the pulmonary response to the instillation of the vehicle, phosphate-buffered saline.

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MATERIALS AND METHODS

Animals and Husbandry

Male Sprague-Dawley rats, Hla:(SD)CVF, from Hilltop Laboratories (Scottdale, PA), monitored free of endogenous viral pathogens, parasites, mycoplasmas, Helicobacter and CAR Bacillus, 6–8 weeks old at arrival, were used for all experiments. The rats were acclimated for 6–9 days before use and were housed in shoebox cages in HEPA-filtered laminar airflow racks, on Alpha-Dri virgin cellulose chips and hardwood Beta-chips as bedding, provided autoclaved Prolab 3500 diet and tap water *ad libitum*. Facilities are fully accredited by the Association for Assessment and Accreditation of Laboartory Animal Care International, specific pathogen-free, and environmentally controlled.

Test Materials

Coal slag. "Black Beauty®" coal slag was purchased from Reed Minerals (Highland, IN). The manufacturer reports that in a typical analysis, the most abundant compounds are silicon dioxide, aluminum oxide, ferric oxide, calcium oxide, potassium oxide, magnesium oxide, and titanium dioxide, which respectively account for 47.2%, 21.39%, 19.23%, 6.80%, 1.60%, 1.47%, and 1.01% of the material (Reed Minerals, 1992). The typical free-silica content is stated to be less than 0.1% (Reed Minerals, 1992).

Garnet. Garnet was purchased from Emerald Creek Garnet Co. (Fernwood, ID). This abrasive blasting agent is a naturally occurring mixture of almandite garnet ($Fe_3Al_2(SiO_4)_3$) with some magnesium and manganese substitution for iron. Other components include mica and less than 0.1% crystalline silica (Gorrill, 1996). The supplier's typical chemical analysis noted that the major components were silicon dioxide, ferric oxide, aluminum oxide, magnesium oxide, calcium oxide, and manganese oxide, which respectively comprised 36.79%, 32.70%, 25.51%, 3.08%, 1.15%, and 1.01% of the material.

Sand. Blasting sand (precision fractionated sand 2340) was obtained from Waupaca Materials (Waupaca, WI) and was identified as containing crystalline

TABLE 1
Potentially Toxic Elements in Abrasive Blasting Agents

	Coal slag	Staurolite	Specular hematite	Sand	Treated sand	Garnet
Aluminum	2600	220	270	2,200	110	5,700
Barium	13	ND	7	11	1	1
Calcium	650	38	210	4,900	ND	630
Chromium	ND	3	ND	4	ND	5
Cobalt	ND	ND	7	2	ND	1
Copper	ND	ND	4	4	ND	ND
Iron	4200	300	230,000	5,300	360	15,000
Lead	ND	5	ND	ND	ND	ND
Lithium	ND	ND	ND	2	ND	ND
Magnesium	100	5	310	3,000	ND	ND
Manganese	6	13	190	100	3	700
Phosphorus	ND	40	ND	100	ND	93
Platinum	ND	ND	280	ND	ND	ND
Quartz (%)	ND	ND	ND	49	84	1
Sodium	80	90	58	99	16	25
Titanium	88	520	66	230	2	94
Vanadium	5	4	20	9	ND	2
Yttrium	ND	4	ND	3	ND	31
Zinc	ND	2	19	8	1	3
Zirconium	ND	8	13	5	ND	2

Note. Arsenic, beryllium, cadmium, nickel, selenium, silver, tellurium, and thallium were not detected in any of the blasting abrasives. For all elements except quartz (given as %), values are μg/g. ND indicates that levels were below the limit of detection.

silica. This blasting sand contained 49% quartz as measured by x-ray diffraction.

Specular hematite. "Bar Shot 50" specular hematite or iron oxide was purchased from Barnes Environmental, Inc. (Waterdown, Ontario, Canada). The manufacturer's material safety data sheet lists the components of this blasting agent as 98–99% Fe_2O_3 with less than 0.1% crystalline silica (Barnes Environmental, Inc., 1996).

Staurolite. "Starblast" staurolite was purchased from DuPont Chemical Company (Wilmington, DE). According to the typical analysis sheet, "Starblast" is about 86% staurolite minerals ($FeAl_5Si_2O_{12}OH$), 6% titanium minerals, 3% zircon, 2% kyanite and less than 5% quartz (DuPont Chemicals, 1993).

Treated sand. Magnum Blast 3.0 treated sand (blasting sand coated with a dust suppressant) was obtained from Fairmont Minerals (Wedron, IL). The supplier's material safety data sheet lists the major ingredient in their "prevent coated silica" as quartz, which comprises more than 90% of the ingredients in a typical analysis (Fairmont Minerals, 1999).

Elemental Analysis

Each test agent was subjected to elemental analysis for aluminum, arsenic, barium, beryllium, cadmium, calcium, chromium, cobalt, copper, iron, lead, lithium, magnesium, manganese, molybdenum, nickel, phosphorus, platinum, quartz, selenium, silver, sodium, tellurium, thallium, titanium, vanadium, yttrium, zinc, and zirconium. The percent quartz in the samples was determined by x-ray diffraction (NIOSH Method NMAM 7500); arsenic, beryllium, cadmium and lead were analyzed by graphite furnace (NIOSH Methods NMAM 7901, 7102, 7048 and 7105, respectively); and the remaining elements were analyzed by atomic emission spectroscopy (NIOSH Method NMAM 7300) as previously described (NIOSH, 1994). Analyses were performed by Data Chem Laboratories, Inc. (Salt Lake City, UT). Results of this analysis are given in Table 1.

Respirable Particle Preparation and Analysis

The 6 bulk abrasive samples were initially examined by stereomicroscopy to determine size and general petrographic features. Garnet, staurolite, and sand samples were generally transparent and exhibited gem-like characteristics. The coal slag and specular hematite samples were predominantly opaque. All samples were polydisperse in size with average diameters visually estimated to be on the order of hundreds of micrometers. Scanning electron microscopy observation of the bulk abrasive samples revealed unusually clean surfaces, often exhibiting concoidal fracture. Under the polarized light microscope, one could occasionally see evidence of inclusions.

Clearly, the bulk samples, as obtained by the manufacturers, were too large for direct use in pulmonary experiments. The absence of a significant small-particle component, made direct sieving or sedimentation inappropriate. Further, given the observation of inclusions (which would certainly be released in the blasting process), size reduction was required. Samples were initially ball milled and the respirable fraction was enriched by liquid sedimentation of the milled particles.

Figure 1 shows scanning electron microscope images of the bulk garnet sample (Figs. 1A and 1B) and the size-classified garnet sample (Figs. 1C and 1D). Although there is roughly a 3 log order size difference between the bulk and size-classified sample, similarities in cleavage characteristics can be observed.

To compare the size-classified dust samples with particles released under actual field blasting conditions, respirable dust samples were collected during abrasive blasting using the same six abrasives. Figures 1E and 1F contain scanning electron microscopy images of an air sample collected near the blasting of a steel surface with garnet abrasive. The magnifications are matched with those of the sized sample prints, allowing for direct comparison. The samples were similar in size and geometry and, although both were polydisperse, the spectrum of size for each falls well within the respirable range. Using scanning electron microscopy, particle diameter measurements were also made on each lab prepared and field blasted sample. Figure 2 provides results comparing the 2 garnet samples. Although the lab prepared sample dust distribution was shifted slightly toward smaller particles, overall it was similar to the size distribution of the field blasted material, i.e., 99% of the particles were less than approximately 4 μm in diameter. Figure 3 compares the size measurements for all six of the size-classified dust samples. Average diameters for each were in the 1 μm range. Particle count/mass estimates for both the laboratory prepared and the field air samples fell within the 10^{11}–10^{12} particles/g range.

As an additional check of the integrity of the laboratory prepared samples, energy dispersive X-ray analysis was applied to these dusts along with the corresponding bulk and field collected air samples. Figure 4 provides results for the 3 garnet samples. The elemental signature of all three dusts was similar. The rise in the iron peak and the trace of potassium seen in the field sample is likely due to the release of particles from the blasted surface.

To investigate the possibility of contamination from the milling process, fragments of the mill (agate) were initially examined under polarized light microscopy. The agate fragments were found to have crystal features distinct from the abrasive blasting particles that allowed for contamination estimates based on visual counts of milled material. Although the agate has a refractive index similar to quartz, surface and extinction characteristics allow one to distinguish between them. Estimates of contamination on replicate samples averaged less than 0.01%.

The garnet samples have been used here to illustrate the comparison of features between the bulk, air, and laboratory prepared samples. Similar relationships were seen with each of the other abrasives.

Intratracheal Instillations

For intratracheal administration, rats were briefly anesthetized with methohexital (Brevital, 37.5 mg/kg). The test agents were suspended at a concentration of 33.3 or 8.33 mg/ml in sterile Dulbecco's phosphate-buffered saline (Sigma Chemical Company, St. Louis, MO) and sonicated for 1–2 min. Rats

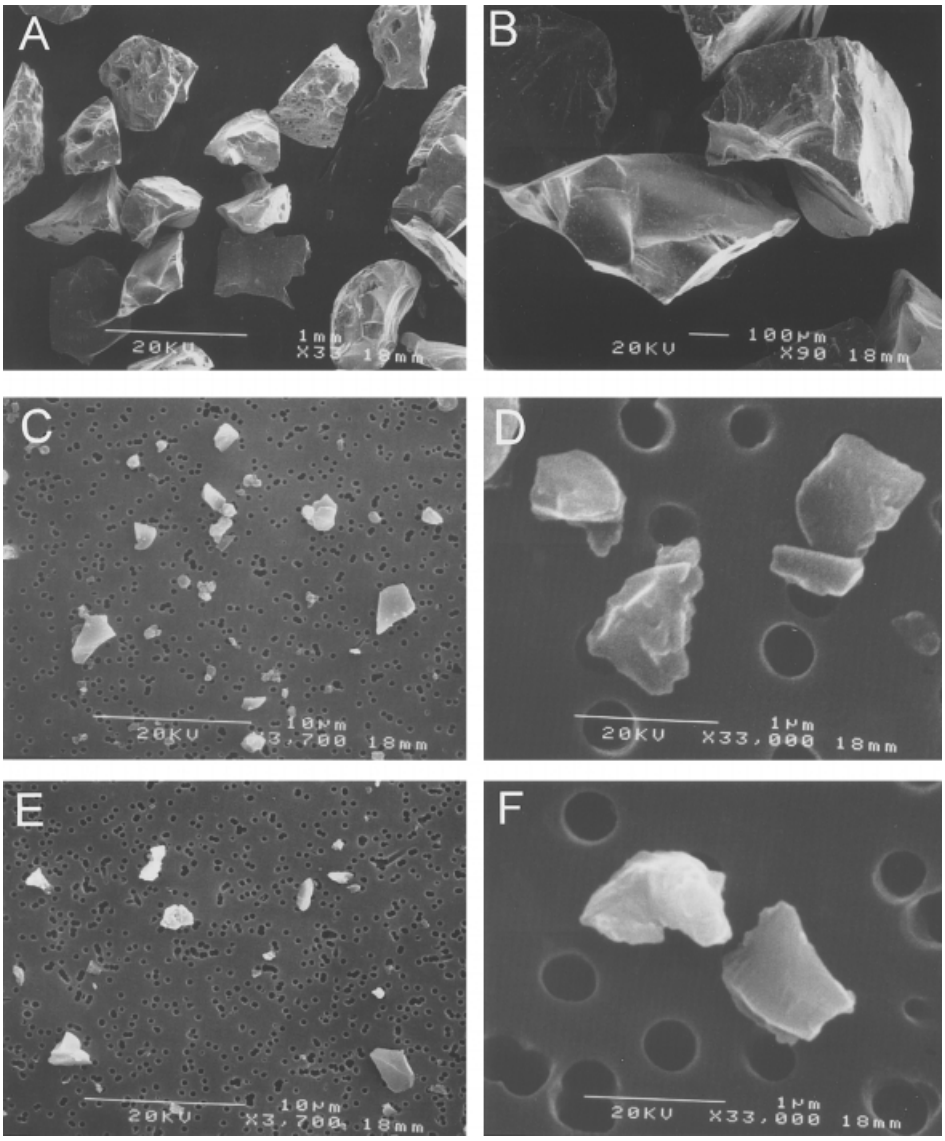

FIG. 1. (A) and (B): Scanning electron microscope (SEM) images of the bulk garnet sample. (C) and (D): SEM images of the size-selected garnet sample. (E) and (F): SEM images of air samples collected during blasting with garnet abrasive. Although 3 log orders smaller in size, the size-selected particles retain cleavage characteristics of parent material. Note also the similarity between the laboratory prepared and air samples.

were exposed to 10 or 2.5 mg blasting agent/rat by instillation of 0.3 ml of the concentrated or diluted suspension, respectively. The earliest timepoint for significant fibrosis in silicosis is at 4 weeks after instillation (Driscoll *et al.*, 1990b), the exposure duration selected for our study. The 10 mg exposure was based upon previous studies which showed that 50 mg/kg of crystalline silica (Min-U-Sil) in 180–200 mg rats (~10 mg/rat) was the minimum dose producing histopathologic evidence of pulmonary fibrosis in rats 4 weeks after instillation (Driscoll *et al.*, 1990b). The 2.5 mg exposure was designed to provide dose-response data. A 0.3 ml volume of the particle suspension or vehicle (phosphate-buffered saline) was administered via the trachea using a 20-gauge 4-inch ball-tipped animal feeding needle (Perfectum, New Hyde Park, NY). The test agents were administered once. Six rats were exposed to each concentration of each blasting agent except for specular hematite. Due to the limited amount of available specular hematite, 4 rats were each exposed to 0.3 ml of the 33.3 mg/ml suspension of specular hematite (10 mg/rat).

Collection of Biological Samples

Rats were deeply anesthetized using an overdose of intraperitoneal pentobarbital 4 weeks after exposure. After assuring deep anesthesia, the abdominal aorta was transected. The left lung lobe was then briefly ligated.

With the left lobe ligated, a tracheal cannula was inserted and the right lung inflated with 3 ml of chilled Ca^{2+}/Mg^{2+}-free phosphate-buffered saline (PBS; BioWhittaker, Walkersville, MD). This procedure was repeated using 4 ml volumes of PBS until a 40 ml total volume of bronchoalveolar fluid (BALF) was obtained. The BALF was centrifuged at 500 × g at 4°C for 10 min. The first lavage sample was centrifuged separately from the subsequent lavage samples with the supernatant collected for later analyses. After removal of supernatant, lavage cells from each rat were combined and resuspended in HEPES-buffered solution (145 mM NaCl, 5 mM KCl, 10 mM HEPES, 1 mM $CaCl_2$, 5.5 mM dextrose, pH 7.4).

Immediately after completion of bronchoalveolar lavage, the right lung lobe was ligated and removed below the ligature for hydroxyproline analysis. The temporary ligature on the left lung lobe was then removed, and the left lung lobe was perfused via the trachea with 3 ml Carnoys solution.

Cellular and Biochemical Assays of BALF

The BALF cell counts and differentials were performed on a Coulter Multisizer II cell counter (Coulter Electronics, Hialeah, FL) with a cell-sizing attachment to distinguish between rat lavage cell types as previously described

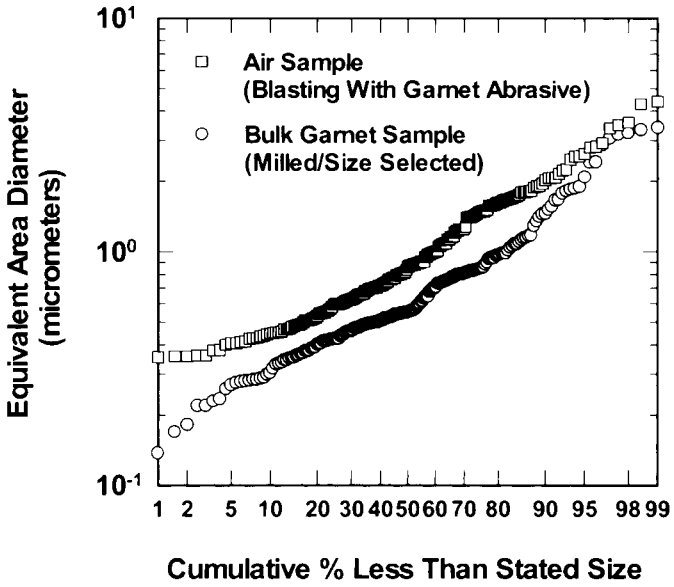

FIG. 2. Log probability plot of size measurements of milled/size-classified garnet and an air sample collected during abrasive blasting on mild steel using garnet abrasive. For each sample, equivalent area diameters of 200 randomly selected particles were measured under scanning electron microscopy. For each distribution 99% of particles were less than approximately 4 μm in diameter.

(Castranova *et al.,* 1979). The polymorphonuclear leukocyte (PMN) designation excludes alveolar macrophages but includes some lymphocytes.

The zymosan-stimulated chemiluminescence assay was conducted using 5×10^5 alveolar macrophages in a total volume of 0.25 ml HEPES buffer (145 mM NaCl, 5 mM KCl, 10 mM HEPES, 1 mM $CaCl_2$, 5.5 mM dextrose, pH 7.4) as previously described (Porter *et al.,* 1999). Zymosan-stimulated CL was calculated as the counts per minute (c.p.m.) in the assay of zymosan-stimulated cells minus the c.p.m. in the assay of resting cells.

Before storage at –30°C, an aliquot of first BAL fluid was removed for analysis of lactate dehydrogenase (LDH) activity. LDH activity was determined spectrophotometrically using a Cobas Fara II analyzer (Roche Diagnostic Systems, Montclair, NJ) and a previously described technique (Ma *et al.,* 1999).

BALF albumin concentration was assessed colorimetrically at 628 nm based upon bromcresol green binding (albumin BCG diagnostic kit, Sigma Chemical Company, St. Louis, MO) using a Cobas Fara II Analyzer (Roche Diagnostic Systems, Montclair, NJ).

For determination of the hydroxyproline content of the lung, the lavaged right lungs of the rats were liquified by mincing and then hydrolyzed in 6 N HCL for 48–72 h at 110°C. Hydroxyproline was measured by a previously published method (Kivirikko, 1967).

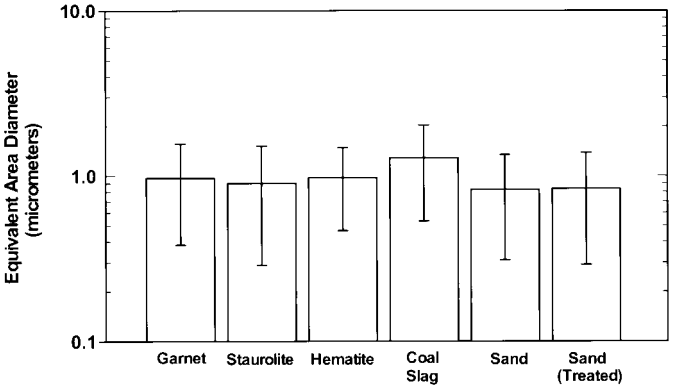

FIG. 3. Mean size for the milled/size-classified abrasive blasting agents (means ± SD). For each sample, equivalent area diameters of 200 randomly selected particles were measured under scanning electron microscopy.

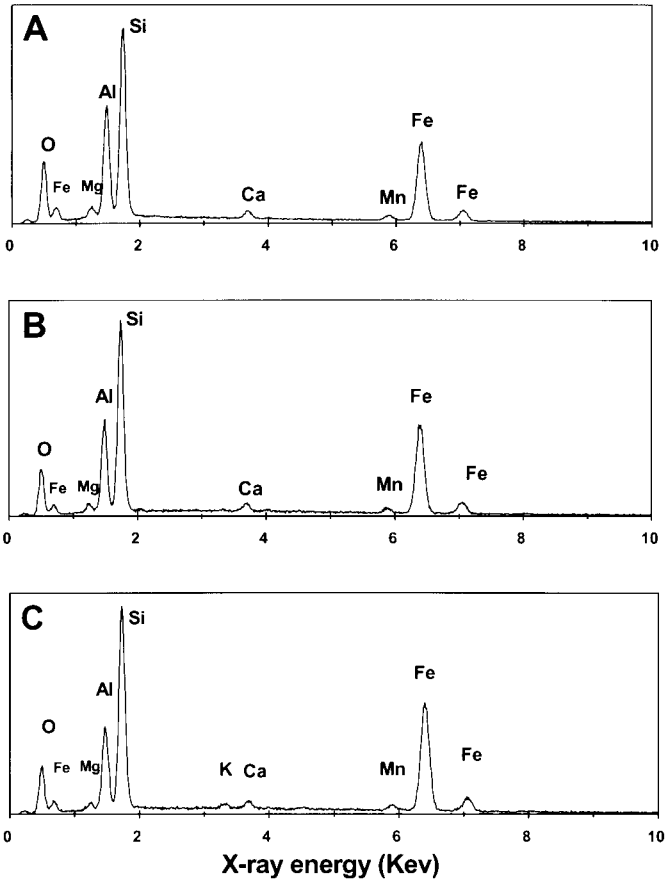

FIG. 4. Elemental analysis of (A) bulk sample of garnet abrasive blasting material as received from vendor; (B) milled and size-classified garnet abrasive blasting sample; and (C) air sample collected during abrasive blasting using garnet abrasive material.

Histopathology

Carnoy's-fixed left lungs were embedded in paraffin and stained with Masson's trichrome stain for examination of collagen formation by light microscopy. The slides were scored for the severity and distribution of fibrosis by a board-certified veterinary pathologist blinded to exposure status. Semiquantitative numeric values were assigned to these scores so that severity was scored as: 0 = none; 1 = minimal; 2 = mild; 3 = moderate; 4 = marked; or 5 = severe. Tissue distribution was converted to numeric values with: 0 = none; 1 = focal; 2 = locally extensive; 3 = multifocal; 4 = multifocal and coalescent; and 5 = diffuse. The total fibrosis score was the sum of the severity and distribution scores.

Statistics

In general, data are presented as means ± SE of 4–6 separate experiments. Findings from rats exposed to different abrasives were compared to results in

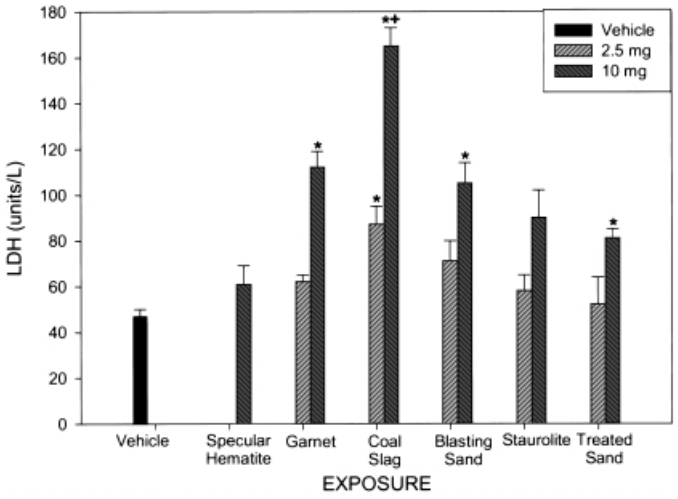

FIG. 5. LDH in the acellular BALF of rats exposed to abrasive blasting agents or vehicle. Values are means ± SE. *Statistically different from the vehicle control. A plus sign indicates statistically greater than blasting sand.

the control group or to the blasting sand group, respectively, using the Wilcoxon rank sum test. The Bonferroni adjustment was used to account for multiple comparisons; *p*-values without the Bonferroni adjustment were also noted in cases where the individual result was statistically significant before adjustment. Nonparametric methods were necessary to account for data which were not normally or log-normally distributed and for heterogeneity of variance among groups, which violates the assumptions of relevant parametric tests such as Dunnett's test. Significance was set at $p \leq 0.05$.

RESULTS

Biochemical and Cytological Changes in BAL Fluid

BAL fluid LDH activity was significantly increased above control after exposure to 10 mg of blasting sand, treated sand, garnet, coal slag, and staurolite. (Note that $p = 0.037$ for staurolite without the Bonferroni adjustment, but not after adjustment for multiple comparisons as indicated in Figure 5.) In contrast, specular hematite, which resulted in substantially lower LDH activity than blasting sand, did not differ from control. LDH concentrations were also increased above control after exposure to 2.5 mg of coal slag. Furthermore, 10 mg of coal slag caused significantly greater LDH activity in BAL fluid than 10 mg of blasting sand. These findings, except for specular hematite, are consistent with persistent cytotoxicity (Fig. 5). Albumin concentrations in the acellular lavage fluid were not significantly increased in BAL fluid 4 weeks after exposure to any abrasive blasting agent (data not shown).

The total cell yield in the BAL was not significantly affected by exposure to abrasive blasting agents (data not shown). However, PMN were significantly increased in the BAL 4 weeks after exposure to 10 mg of garnet, coal slag, staurolite, or treated sand. Lavage PMN were 2.4 times higher in blasting sand-exposed rats (10 mg) than controls ($p = 0.017$ without adjustment for multiple comparisons). No increase from control was noted after specular hematite exposure, which was substantially less potent than any of the other blasting agents. In addition, PMN infiltration in the 10 mg coal slag group was significantly higher than blasting sand. No significant alterations were seen in PMN from rats in the 2.5 mg exposure groups (Fig. 6).

The activity of alveolar macrophages harvested from exposed rats was determined by measuring the *ex vivo* release of reactive species in response to unopsonized zymosan particles. Zymosan-stimulated chemiluminescence generated by alveolar macrophages was enhanced by exposure to 10 mg coal slag, staurolite or garnet or 2.5 mg coal slag. Chemiluminescence was 8.8 or 6 times higher in rats exposed to 10 mg of blasting sand or treated sand, respectively, compared to controls (statistically significant without comparisons). No significant alterations were seen in the chemiluminescence of alveolar macrophages of rats in the other exposure groups (Fig. 7).

Pulmonary Fibrosis

Hydroxyproline was significantly elevated above control only in the lungs of rats receiving 10 mg of coal slag (Fig. 8). Histopathologic evidence of minimal, focal to multifocal, pulmonary fibrosis was seen in the lungs of 50–100% of the rats receiving 10 mg coal slag, blasting sand, and staurolite and 2.5 mg coal slag. Histopathologic fibrosis scores were significantly elevated from control only in rats exposed to 10 mg blasting sand (Fig. 9). Foci of fibrosis were often associated with histologic evidence of pulmonary inflammation (Figs. 10A and 10B).

DISCUSSION

In this study, we have examined the subchronic toxicity of the commercial blasting agents: specular hematite, garnet, coal

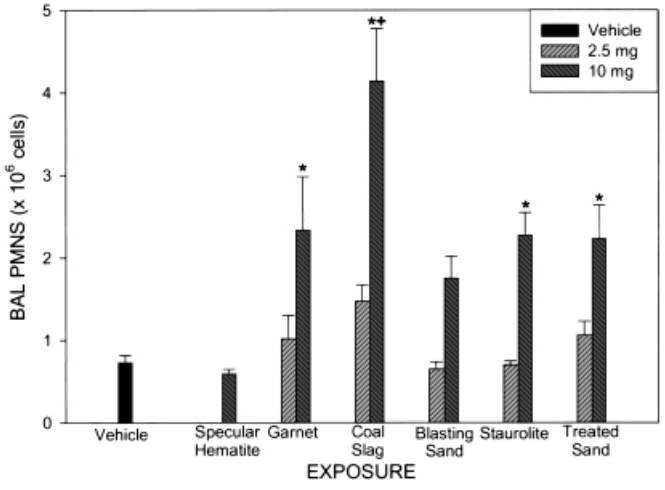

FIG. 6. PMN in the BALF of rats exposed to abrasive blasting agents or vehicle. Values are means ± SE. *Statistically different from the vehicle control. A plus sign indicates statistically greater than blasting sand.

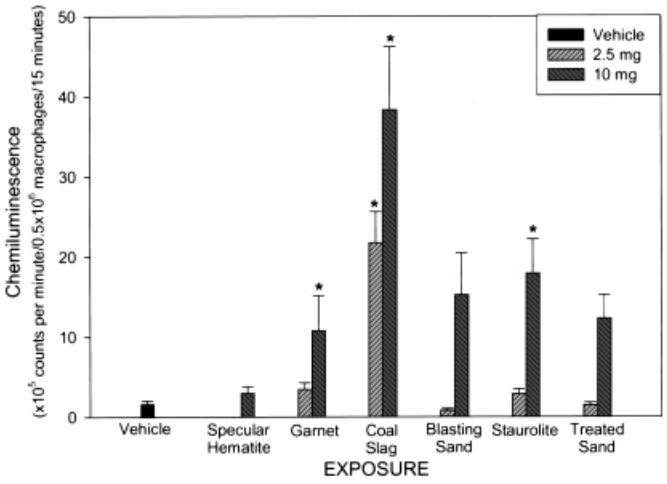

FIG. 7. Chemiluminescence in alveolar macrophages of rats exposed to abrasive blasting agents or vehicle. Values are means ± SE. *Statistically different from the vehicle control.

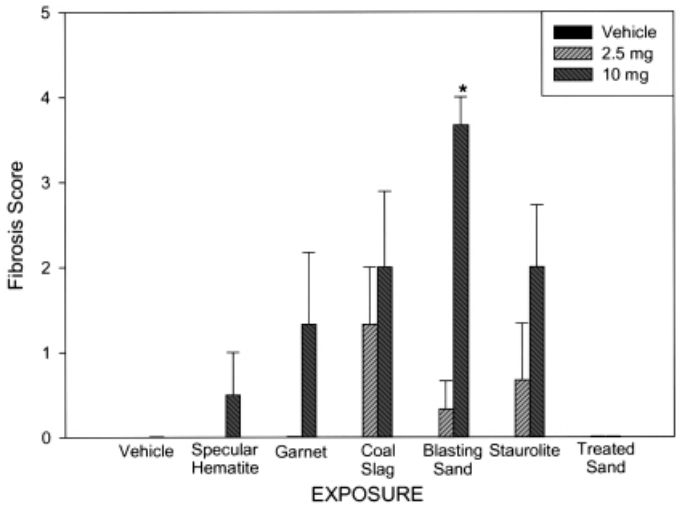

FIG. 9. Fibrosis scores from the histopathologic assessment of fibrosis in the lung. The scores are the sum of the numerical scores from the severity and distribution of fibrosis in the left lung lobes of rats exposed to abrasive blasting agents or vehicle. Values are means ± SE. *Statistically different from the vehicle control.

slag, sand, staurolite, and treated sand. An unexpected finding was that 10 mg of garnet, coal slag, staurolite, or treated sand each produced statistically significant elevations in BAL PMN 4 weeks after exposure. At this timepoint, rats receiving 10 mg of a commercial blasting sand exhibited BAL neutrophil counts that were 2.4 times the control level. Thus, under these experimental conditions, garnet, coal slag, staurolite, and treated sand did not demonstrate less inflammatory potential than the blasting sand used in this study. With the apparent role of inflammatory cell influx in initiating pulmonary fibrosis (Marshall *et al.,* 1997), our finding of persistent inflammation with 4 currently used substitutes for blasting sand suggests the possibility that these agents may cause pulmonary fibrosis in exposed workers. In humans, increased BAL neutrophils are seen in patients with interstitial lung involvement in idiopathic pulmonary fibrosis and connective tissue disease (Cherniack *et al.,* 1990).

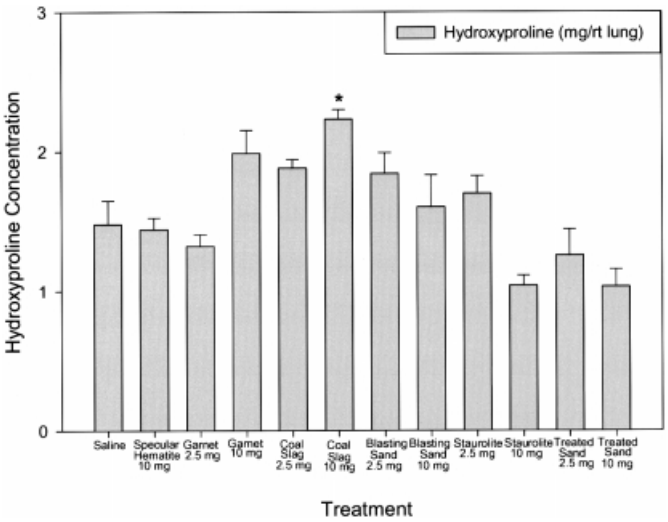

FIG. 8. Hydroxyproline concentration of the right lung lobes in rats exposed to abrasive blasting agents or vehicle. Values are means ± SE. *Statistically different from the vehicle control.

The persistent PMN response associated with pulmonary exposure to the abrasive blasting agents may be associated with persistent pulmonary cytotoxicity. Blasting sand, garnet, treated sand, and coal slag at the 10 mg exposure dose caused significant elevation in LDH, a cytosolic protein which leaks from cells after membrane damage or destruction (Drent *et al.,* 1996). In addition, exposure to 2.5 mg coal slag also caused elevation in LDH. Furthermore, staurolite (10 mg) resulted in a 91% increase in LDH above control. Since serum levels of LDH are higher than LDH levels in the lung, serum leakage into the lung as a source of the elevated LDH was excluded by determining that the serum protein, albumin, was not significantly elevated by exposure to any of the blasting agents studied. These findings are consistent with the hypothesis that garnet, coal slag, sand, staurolite, and treated sand continue to damage lung cells 4 weeks after intratracheal instillation of these abrasive blasting agents. Coal slag is the most frequently used substitute for sand in abrasive blasting (Paumanok Publications, Inc., 1992), and it was more inflammatory and caused greater cell damage than blasting sand.

The significant elevation in zymosan-stimulated macrophage chemiluminescence associated with exposure to coal slag (2.5 and 10 mg), staurolite (10 mg) and garnet (10 mg) is consistent with macrophage activation after exposure to these compounds. Exposure to 10 mg of blasting sand or 10 mg of treated sand resulted in a greater than 8- or 6-fold elevation, respectively, in zymosan-stimulated macrophage chemilumi-

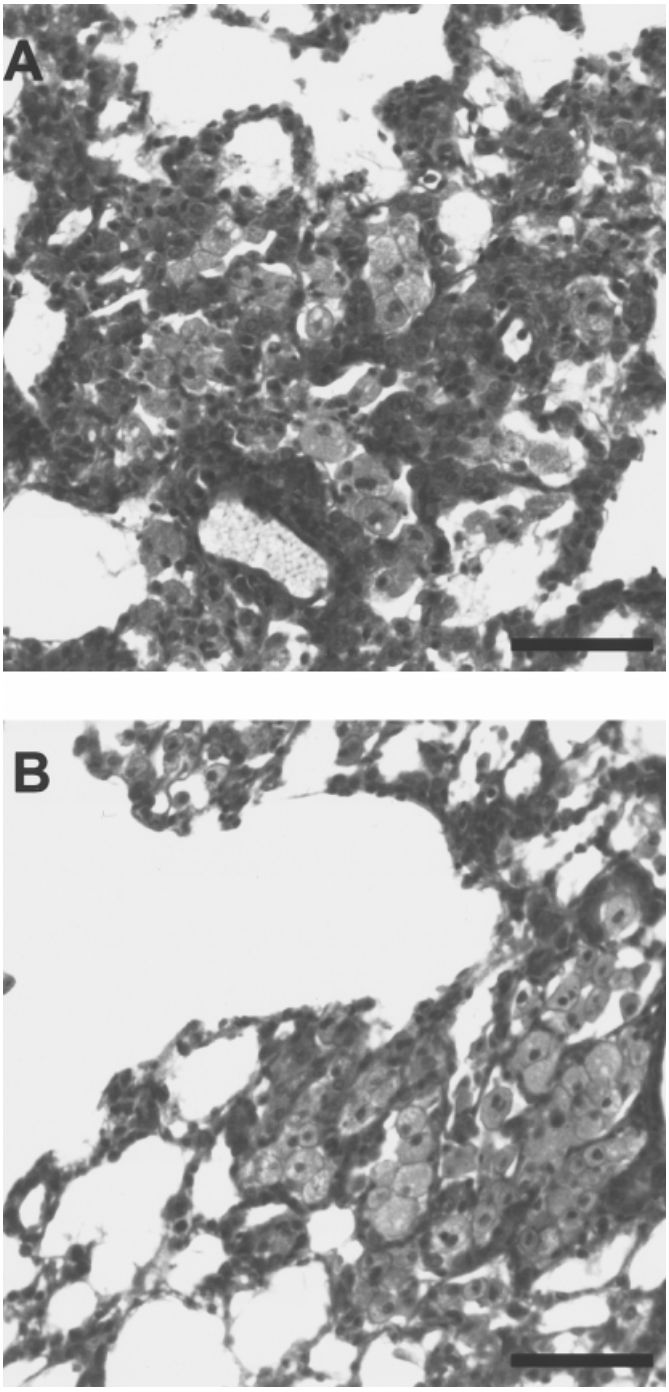

FIG. 10. Photomicrographs of the lung of rats exposed to abrasive blasting agents. (A) Histiocytic and proliferative alveolitis in the lung of a rat exposed to coal slag. (B) Histiocytic and proliferative alveolitis in the lung of a rat exposed to blasting sand. Bar = 100 microns.

nescence (significant when not adjusted for multiple comparisons).

Persistently elevated BAL PMN, increased BAL LDH activity, and enhanced zymosan-stimulated chemiluminescence in macrophages each suggest persistent effects from abrasive blasting agents 4 weeks after instillation. However, the most significant disorder observed in the abrasive blasting industry is pulmonary fibrosis. Biochemical confirmation of pulmonary fibrosis, hydroxyproline elevation, was seen only in rats exposed to coal slag. However, hydroxyproline elevation might not be expected in subchronic disease because the normal lung contains substantial concentrations of fibrous connective tissue which undergo daily remodeling (Marshall *et al.*, 1997). In the presence of active ongoing cytotoxicity, preexisting structural collagen may undergo destruction while new fibrous connective tissue forms. Although unresolved inflammation and tissue destruction are important stimulants for fibrosis in any tissue (Cotran *et al.*, 1999), all of the microscopic foci of fibrosis associated with exposure to any of the agents investigated in the present study were considered minimal. This is most likely due to the relatively short exposure time in this study. Indeed, we designed our experiment for the earliest time point producing significant histopathologic evidence of fibrosis induced by a similar concentration of crystalline quartz in rats (Driscoll *et al.*, 1990b). Thus, additional studies will be needed to assess the final result of the unresolved inflammation seen in this subchronic study.

In contrast to the other abrasive blasting agents, specular hematite (10 mg) produced no significant alterations in BAL levels of LDH, numbers of lung PMN, macrophage chemiluminescence, the amount of pulmonary hydroxyproline, or fibrotic score. Since the major component of specular hematite is iron oxide (Barnes Environmental, Inc., 1996), these findings are consistent with the low toxicity of iron oxide in most rat studies (Stokinger, 1984). A recent study in humans also suggests that the initial inflammation associated with intrapulmonary instillation of iron oxide resolves rapidly after exposure (Lay *et al.*, 1999).

The intratracheal instillation technique used in our study can accentuate the pulmonary toxicity of particles with low toxicity, particularly at higher exposures such as the 10 mg dose used in our study, but generally does not affect the relative toxicity of different particles (Driscoll *et al.*, 2000). We selected this method of exposure because it assured control of the dose to the lung in this screening study and allowed prioritization of those agents with the greatest pulmonary toxicity for follow-up studies. Because we have no evidence of pulmonary toxicity under the conditions of this study for one of the abrasives, specular hematite, we believe additional pulmonary toxicity studies may be warranted on coal slag, garnet, staurolite, and treated sand. While only 4 rats were exposed to the specular hematite, responses on all measures of pulmonary inflammation were numerically similar and statistically indistinguishable from the response seen in the 6 saline control rats. Since particle size and particle count/mass were similar for all the abrasive materials studied, the responses seen in rats exposed to 10 mg of the other abrasive blasting agents may not be the simple result of exposure to a very large number of particles or "particle overload." However, other investigators

have seen subtle, persistent pulmonary inflammation after intratracheal instillation of the nuisance dust, titanium dioxide, at concentrations of 50 mg/kg (Driscoll *et al.,* 1990a).

We have used intratracheal instillation at 2 different doses to screen this group of abrasive blasting agents for pulmonary toxicity. The use of intratracheal instillation for such screening studies has recently been reviewed and is accepted (Driscoll *et al.,* 2000). However, it should be noted that pulmonary fibrosis resulting from the instillation of crystalline silica is a progressive disease and can be induced in 90 days by the intratracheal instillation of as little as 5 mg/kg silica or 1 mg per 200 g rat (Driscoll *et al.,* 1990b). Since persistent inflammation plays an important role in pulmonary fibrosis, it is certainly possible that some of the agents that cause persistent inflammation in this 4-week study may produce pulmonary fibrosis in a longer study. Because of the ability of coal slag to induce more cell damage and greater PMN influx than a commercial blasting sand and to elevate the lung hydroxyproline concentration in our study, it is possible that coal slag is as fibrogenic as the commercial blasting sand used in our study. Thus, there is a need for additional studies of longer duration comparing the extent of pulmonary fibrosis produced by exposure to low doses of abrasive blasting agents.

Previous studies of abrasive blasting agents have usually compared a standard crystalline silica sample, e.g. Min-U-Sil, with potential substitutes for blasting sand (Stettler *et al.,* 1988, 1995). In our study, we examined the toxicity of several commercial abrasive blasting agents, including commercial blasting sand, since human disease in the abrasive blasting industry is associated with exposure to blasting sand rather than pure crystalline silica. Because surface properties and surface contaminants of silica are determinants of pulmonary toxicity, crystalline silica toxicity may not reflect the toxicity of commercial blasting sand (Bolsaitis and Wallace, 1996; Le Bouffant *et al.,* 1982). Indeed, data from the present study indicate that the inflammatory and toxic potencies of the blasting sand sample used in our study were substantially lower than that for Min-U-Sil (Blackford *et al.,* 1997). The quartz content of the blasting sand used in this study was 49% while that for Min-U-Sil is 98%. The range for the quartz concentration in 16 samples of commercially available blasting sands is 39–100% (Greskevitch, personal communication, analyses conducted by Data Chem Laboratories, Salt Lake City, UT). Thus the crystalline silica content of blasting sand appears to determine the pulmonary toxicity.

Overall, the pulmonary response 4 weeks after intratracheal instillation of 10 mg respirable blasting sand indicated continued cytotoxicity and very early pulmonary fibrosis consistent with the known role of blasting sand in occupational pulmonary fibrosis. Intratracheal instillation of specular hematite did not cause significant pulmonary fibrosis, cytotoxicity, or persistent inflammation; this is consistent with the general paucity of functional and inflammatory pulmonary changes in workers and research subjects exposed to iron oxide (Lay *et al.,* 1999; Teculescu and Albu, 1973). The persistent cytotoxicity generally observed after exposure to the other commercial substitutes for blasting sand was accompanied by persistent inflammation, and with coal slag by elevated lung hydroxyproline. This suggests the need for additional inhalation studies of abrasive blasting agents.

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