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COMPARATIVE PULMONARY TOXICITY OF BLASTING SAND AND FIVE SUBSTITUTE ABRASIVE BLASTING AGENTS

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Blasting sand is used for abrasive blasting, but its inhalation is associated with pulmonary inflammation and fibrosis. Consequently, safer substitute materials for blasting sand are needed. In a previous study from this laboratory, the comparative pulmonary toxicity of five abrasive blasting substitutes and blasting sand was reported. In this study, the pulmonary toxicity of blasting sand was compared to five additional abrasive blasting substitutes: steel grit, copper slag, nickel slag, crushed glass, and olivine. Exposed rats received by intratracheal instillation 10 mg of respirable-size particles of blasting sand or an abrasive blasting substitute, while controls were instilled with vehicle. Pulmonary inflammation, damage, and fibrosis were examined 28 d postexposure. Pulmonary inflammation was monitored by determining bronchoalveolar lavage polymorphonuclear cell counts and alveolar macrophage activation by chemiluminescence. Pulmonary damage was assessed by acellular bronchoalveolar (BAL) fluid serum albumin concentrations and

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lactate dehydrogenase activities. Histological examination of lung tissue samples was made to assess the severity and distribution of pulmonary fibrosis, alveolitis, and alveolar epithelial cell hypertrophy and hyperplasia. In comparison to blasting sand, olivine-exposed rats had higher levels of pulmonary inflammation and damage with a similar level of fibrosis. Steel grit-exposed rats had lower levels of pulmonary inflammation and damage, and did not develop fibrosis. However, steel grit-exposed rats had a level of epithelial cell hypertrophy and hyperplasia similar to blasting sand. The other abrasive blasting substitutes gave a mixed profile of toxicity. The data demonstrate that steel grit produced less acute pulmonary toxicity than blasting sand or any of the other abrasive blasting substitutes. Notwithstanding, the data also suggest that chronic exposure to steel grit may pose a health risk due to its effects on epithelial cell proliferation in the lung.

Abrasive blasting is a process whereby a surface is impacted by small abrasive particles that have been accelerated to high speeds. Sand is the most commonly used abrasive blasting agent, but its use is problematic because it contains high levels of crystalline silica, which when inhaled can cause the development of the pulmonary disease silicosis (Shi et al., 1998; Lapp & Castranova, 1993). One approach to eliminating this health risk to workers employed in abrasive blasting operations has been to substitute other abrasive blasting materials for sand.

Numerous abrasive blasting substitute materials have been introduced for commercial use, but their potential pulmonary toxicity has not been examined. In a previous study, blasting sand and five abrasive blasting substitutes were examined as to their ability to induce pulmonary alterations after intratracheal instillation (Hubbs et al., 2001). The results of this study indicated that using bronchoalveolar lavage fluid parameters, four of the five substitutes—coal slag, garnet, staurolite, and treated sand—produced pulmonary inflammation and damage equal to or more extensive than that caused by blasting sand, while specular hematite (iron oxide) was significantly less toxic than blasting sand (Hubbs et al., 2001). These data indicated that several abrasive blasting substitutes may pose a health risk to exposed workers, and investigations of other abrasive blasting substitutes are warranted due to their potential pulmonary toxicity.

Studies were thus undertaken to investigate whether adverse pulmonary alterations occurred in rats 28 d after intratracheal instillation of respirable size particles of the following additional substitute abrasive blasting agents: steel grit, copper slag, nickel slag, crushed glass, and olivine. Pulmonary inflammation, damage, and fibrosis after exposure to these abrasive blasting substitutes were compared to rats that had received an equivalent dose of vehicle (negative control) or blasting sand (positive control).

METHODS

Experimental Design

In a previous report from this laboratory (Hubbs et al., 2001), six abrasive blasting agents were screened to identify possible toxic pulmonary out-

comes after exposure. The purpose of this study is to continue this screening process by examining another five abrasive blasting substitutes to identify possible toxic pulmonary outcomes and determine differential responses to these abrasive blasting substitutes after exposure. The approach used in this study was to expose rats by intratracheal instillation to an abrasive blasting substitute and measure pulmonary inflammation, damage, and fibrosis at 28 d postexposure.

Test Materials

Crushed Glass Crushed glass was VitroGrit VG 3050 from TriVitro Corporation (Kent, WA). The manufacturer reports that in a typical analysis the most abundant compounds are silicon dioxide, sodium oxide, calcium oxide, magnesium oxide, aluminum oxide, and sulfur trioxide, which respectively account for 73%, 14%, 10%, <1%, <1%, and <1% of the material by mass (TriVitro Corporation, 1998). The manufacturer also reports that VitroGrit VG 3050 typically contains no crystalline silica.

Steel Grit Amasteel 40/50 steel grit was purchased from Ervin Industries, Inc. (Ann Arbor, MI). The manufacturer reports that greater than 96% of the material consists of iron, along with less than 1.2% carbon, less than 1.3% manganese, less than 1.2% silicon (with no crystalline silica), less than 0.25% chromium, and less than 0.20% nickel (Ervin Industries, Inc., 2000).

Copper Slag Kleen Blast KB 1640 copper slag was purchased from Kleen Blast Abrasives (Portland, OR). The manufacturer reports that in a typical analysis the most abundant compounds are silicon dioxide, iron oxide, calcium oxide, aluminum oxide, magnesium oxide, and other fused oxides, which respectively account for 38.1%, 27.4%, 22.8%, 5.7%, 3.9%, and less than 1% of the material by mass (Kleen Blast Abrasives, 1998). The typical crystalline silica content is stated to be less than 0.1%.

Nickel Slag Green Diamond 2050 nickel slag was purchased from Green Diamond Products (Riddle, OR). The manufacturer reports that in a typical analysis the most abundant compounds are silicon dioxide, magnesium oxide, iron oxide, aluminum oxide, calcium oxide, chromium oxide, nickel and nickel oxide, and other trace elements and compounds, which respectively account for 50.2%, 31.4%, 15.9%, 1.6%, 0.7%, 0.1%, less than 0.1%, and 1.5% of the material by mass (Green Diamond Products, 2000). The typical respirable crystalline silica content is stated to be less than 0.01%.

Olivine Green Lightning GL 40 blasting abrasive was purchased from Unimin Corporation (New Canaan, CT). The material safety data sheet lists Green Lightning GL 40 as containing greater than 99.3% olivine, 0.1 to 0.3% nickel compounds (as nickel), and 0.1 to 0.4% chromium compounds. The technical data sheet provided by the manufacturer indicates that in a typical analysis the most abundant compounds are magnesium oxide, silicon dioxide, iron oxide, calcium oxide, chromium oxide, nickel, aluminum oxide, and other compounds lost on ignition, which respectively

account for 47.7%, 41%, 8%, 0.35%, 0.25%, 0.35%, 0.8%, and 1.6% of the material by mass (Unimin Corporation, 2000). The typical respirable crystalline silica content is stated to be less than 0.1%.

Blasting Sand Precision Fractionated Sand 2340 was purchased from Waupaca Materials (Waupaca, WI) and was identified as containing 55% crystalline silica by x-ray diffraction.

Elemental Analysis

Each substitute abrasive blasting agent tested was subjected to elemental analysis for the following elements: aluminum, arsenic, beryllium, cadmium, calcium, chromium, cobalt, copper, iron, lead, lithium, magnesium, manganese, molybdenum, nickel, phosphorus, platinum, selenium, silver, sodium, tellurium, thallium, titanium, vanadium, yttrium, zinc, and zirconium. The percent α-quartz in the samples was determined by x-ray diffraction using NIOSH method NMAM 7500 (NIOSH, 1994a). Arsenic, beryllium, cadmium, and lead were analyzed by graphite furnace using NIOSH methods NMAM 7901, 7102, 7048, and 7105, respectively (NIOSH, 1994a). The remaining elements were analyzed by atomic emission spectroscopy using NIOSH method NMAM 7300 (NIOSH, 1994a). Analyses were performed by DataChem Laboratories (Salt Lake City, UT). Results of these analyses are given in Table 1.

Respirable Particle Preparation

Respirable-size particles of the abrasive blasting substitutes were prepared as previously described (Hubbs et al., 2001). Briefly, the bulk abrasives were milled and the respirable fraction was enriched by liquid sedimentation. The steel grit sample could not be milled, so for this material, respirable samples were obtained by collecting respirable air samples during blasting operations using the steel grit abrasive on a steel surface. Prior to use, each sample was examined by electron microscopy to ensure that the particles were within the respirable range, that is, approximately 1 μm in diameter. As reported previously, no differences in mean particle diameter or particle count/mass were noted among the various abrasive blasting substitute samples isolated by liquid sedimentation (Hubbs et al., 2001).

Animals

The animals used in these experiments were male Sprague-Dawley [Hla:(SD) CVF] rats weighing 200–300 g (approximately 10 wk old at arrival) obtained from Hilltop Lab Animals (Scottsdale, PA). The animals were housed in a AAALAC-accredited, specific-pathogen-free, environmentally controlled facility. The rats were monitored to be free of endogenous viral pathogens, parasites, mycoplasms, *Helicobacter,* and CAR *Bacillus.* Rats were acclimated for at least 5 d before use, and were housed in ventilated cages that were provided with HEPA-filtered air, with Alpha-Dri virgin cellu-

TABLE 1. Elemental Analyses of Abrasive Blasting Agents

	Abrasive blasting agent					
Element	Blasting sand	Steel grit	Copper slag	Nickel slag	Crushed glass	Olivine
Aluminum	1200	300	41,000	1100	120	330
Arsenic	ND	30	23	ND	ND	ND
Beryllium	(0.03)	ND	0.91	(0.01)	ND	(0.01)
Cadmium	ND	ND	ND	ND	(0. 02)	ND
Calcium	2800	ND	170,000	1200	110	44
Chromium	(2.7)	1200	810	550	ND	51
Cobalt	(0.73)	39	32	14	ND	74
Copper	4	870	1500	6	(1)	(1)
Iron	2900	560,000	140,000	26,000	170	42,000
Lead	(0.47)	7.0	1.5	(0.3)	0.7	(0.3)
Lithium	1.8	ND	27.0	0.5	(0.1)	1.7
Magnesium	1900	21	24,000	42,000	35	220,000
Manganese	88	6,400	3400	240	3	470
Molybdenum	ND	300	ND	ND	ND	ND
Nickel	ND	700	22	570	ND	1700
Phosphorus	85	130	1200	56	(8)	ND
Platinum	ND	92	ND	ND	ND	(20)
Quartz (%)	55	ND	ND	ND	ND	ND
Selenium	ND	ND	1500	260	ND	530
Silver	ND	ND	1	ND	ND	ND
Sodium	(22)	ND	880	44	230	ND
Tellurium	ND	28	ND	ND	ND	ND
Thallium	ND	ND	80	ND	ND	ND
Titanium	82	5.3	1200	20	6	8
Vanadium	4	76	96	7	ND	(0.5)
Yttrium	3.2	0.43	11.0	(0.2)	ND	ND
Zinc	(5.5)	12	91	10	70	17
Zirconium	3	ND	30	(0.6)	4	ND

Note. Concentrations are μg element/g bulk material except quartz, which is expressed as a percent. ND indicates that the value was below the limit of detection. Values in parentheses were between the limit of detection and limit of quantitation.

lose chips and hardwood Beta-chips used as bedding. The rats were maintained on ProLaB 3500 diet and tap water, both of which were provided ad libitum.

Intratracheal Instillation

Particles were suspended in sterile, endotoxin- and Ca^{2+},Mg^{2+}-free phosphate-buffered saline (PBS), pH 7.4, at a concentration of 33.3 mg/ml and dispersed by brief sonication. The rats were anesthetized with an intraperitoneal (ip) injection (28 mg/kg body weight) of sodium methohexital (Brevital, Eli Lilly and Company, Indianapolis, IN) and were intratracheally (IT) instilled using a 20-gauge, 4-inch ball-tipped animal feeding needle.

Rats received either a 10 mg/rat dose of particles or an equivalent dose of PBS (vehicle).

Bronchoalveolar Lavage and Cell Differentials

Rats were euthanized 28 d post IT exposure with an ip injection of sodium pentobarbital (≥100 mg/kg body weight) and exsanguinated by cutting the vena cava and descending aorta. A tracheal cannula was inserted and the left lung lobe was clamped off with a hemostat. Bronchoalveolar lavage (BAL) of the right lung was performed through the cannula using ice-cold PBS (pH 7.4) containing 5.5 m*M* D-glucose. The first lavage was 3 ml and subsequent lavages used 4 ml until a total of 40 ml of lavage fluid was collected. The first BAL was kept separate from the rest of the lavage fluid. BAL cells were isolated by centrifugation (650 × g, 10 min, 4°C). An aliquot of the acellular supernatant from the first BAL (BAL fluid) was decanted, transferred to tubes, and frozen at –20°C; the remaining BAL fluid was kept at 4°C for analysis of lactate dehydrogenase activity and serum albumin levels. The acellular supernatants from the remaining lavage samples were decanted and discarded. BAL cells were resuspended in HEPES-buffered medium (10 m*M* *N*-[2-hydroxyethylpiperazine- *N*′-2-ethanesulfonic acid], 145 m*M* NaCl, 5.0 m*M* KCl, 1.0 m*M* $CaCl_2$, 5.5 m*M* D-glucose; pH 7.4), centrifuged a second time (650 × g, 10 min, 4°C), and the supernatant was decanted and discarded. The BAL cell pellet was then resuspended in HEPES-buffered medium and placed on ice.

Cell counts of alveolar macrophages (AMs) and polymorphonuclear leukocytes (PMNs) were obtained using a Coulter Multisizer II (Coulter Electronics, Hialeah, FL). Cytospin preparations of BAL cells were made using 0.1×10^6 total phagocytes (AM + PMN) in 200 µl HEPES-buffered solution with a Shandon Elliot cytocentrifuge (speed control = 80, 5 min). The cytospin preparations were stained with modified Wright–Giemsa stain, and cell differentials were determined by light microscopy. Differential cell counts were calculated by multiplying the total cell counts (AM + PMN) obtained from the Coulter Counter by the cell differential percentage obtained from the cytospin preparations.

Lung Necropsy and Histopathology

After the lung lavage was completed, the hemostats were removed and the lungs were inflated with 6 ml of 10% neutral buffered formalin. The left lung lobes, which were not lavaged, were routinely processed for histology, embedded in paraffin, sectioned at 4 to 5 µm and stained with hematoxylin and eosin and Masson's trichrome. The slides were evaluated for the severity and distribution of fibrosis, alveolitis, and alveolar epithelial cell hypertrophy and hyperplasia, by a board-certified veterinary pathologist blinded to exposure status. Because the study was only 28 d in length, loose fibrosis was considered to be treatment associated and the occasional background foci of mature dense fibrosis were not scored as treatment associated. Tissue

alterations were scored for severity (0 = none, 1 = minimal, 2 = mild, 3 = moderate, 4 = marked, and 5 = severe) and distribution (0 = none, 1 = focal, 2 = locally extensive, 3 = multifocal, 4 = multifocal and coalescent, 5 = diffuse) as previously described (Hubbs et al., 1997). The histopathology score was the sum of lesion severity and distribution scores.

Alveolar Macrophage Chemiluminescence

Alveolar macrophage (AM) chemiluminescence was determined in a total volume of 0.25 ml HEPES-buffered medium. Resting AM chemiluminescence was determined by incubating 1×10^6 AM/ml at 37°C for 10 min, followed by the addition of 5-amino-2,3-dihydro-1,4-phthalazinedione (luminol) to a final concentration of 0.08 μg/ml, followed by the measurement of chemiluminescence. To determine zymosan-stimulated chemiluminescence, 2 mg/ml unopsonized zymosan was added immediately prior to the measurement of chemiluminescence. All chemiluminescence measurements were made with an automated luminometer (Berthold Autolumat LB 953, Gaithersburg, MD) at 390–620 nm for 15 min. The integral of counts per minute (cpm) versus time was calculated. Zymosan-stimulated chemiluminescence was calculated as the cpm in the zymosan-stimulated assay minus the cpm in the resting assay.

BAL Fluid Serum Albumin Concentration

BAL fluid serum albumin concentrations were determined colorimetrically at 628 nm based on albumin binding to bromcresol green (albumin BCG diagnostic kit, Sigma Chemical Company, St. Louis, MO) using a Cobas Fara II analyzer (Roche Diagnostic Systems, Montclair, NJ).

BAL Fluid Lactate Dehydrogenase Activity

Prior to freezing at –20°C, BAL fluid lactate dehydrogenase (LDH) activities were determined by monitoring the LDH-catalyzed oxidation of lactate coupled with the reduction of NAD at 340 nm using a commercial assay kit (Roche Diagnostics Systems, Montclair, NJ) using a Cobas Fara II analyzer (Roche Diagnostic Systems, Montclair, NJ).

Statistics

To test for differences in pulmonary toxicity, the Wilcoxon rank-sum test, which is the nonparametric equivalent of the *t*-test, was conducted between vehicle control and exposed animals. Differences were also tested between blasting sand-exposed animals and animals exposed to each of the abrasive blasting substitutes. Nonparametric methods were implemented because of the highly skewed nature of some of these measurements. To control the overall type I error of the experiments, the Bonferroni adjustment was used to assess statistical significance for each set of comparisons. Results where $p \leq .05$, divided by the number of comparisons made, were considered statistically significant.

RESULTS

BAL Cell Differentials

Olivine-exposed rats exhibited a significantly higher yield of AMs than either vehicle or blasting sand-exposed rats (Figure 1). No other significant differences in AM yields were detected for any of the substitute abrasive blasting agents in comparison to vehicle or blasting sand-exposed rats (Figure 1).

PMN yields from rats exposed to all abrasive blasting agents tested were significantly higher in comparison to vehicle-exposed rats (Figure 2). In comparison to blasting sand-exposed animals, rats exposed to steel grit, copper slag, and nickel slag had significantly lower PMN yields, whereas olivine-

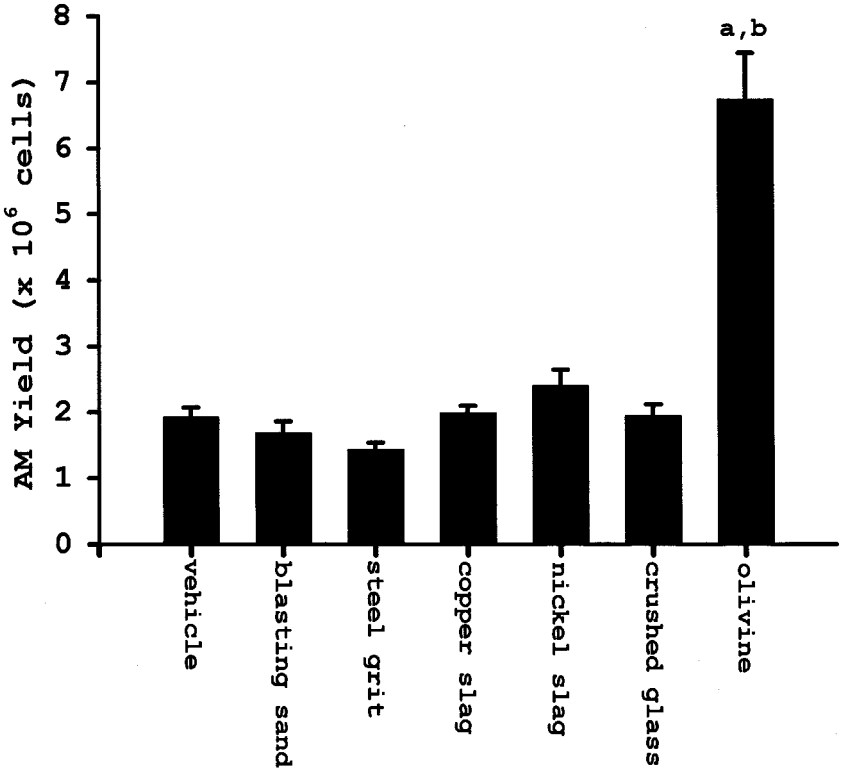

FIGURE 1. BAL AM yield. Rats were IT instilled with vehicle (PBS) or abrasive blasting substitute material (10 mg/rat). Bronchoalveolar lavage was conducted on the right lung and AM yield was determined as described in the Methods. An *a* above a bar indicates that the AM yield from rats exposed to an abrasive blasting agent was significantly greater than from vehicle-exposed rats. A *b* above a bar indicates that the AM yield from rats exposed to an abrasive blasting agent was significantly greater than from blasting sand-exposed rats. Values are means ± SE; $n = 16$ rats exposed to vehicle, $n = 8$ rats exposed to crushed glass, olivine, steel grit, or nickel slag, and $n = 7$ rats exposed to copper slag.

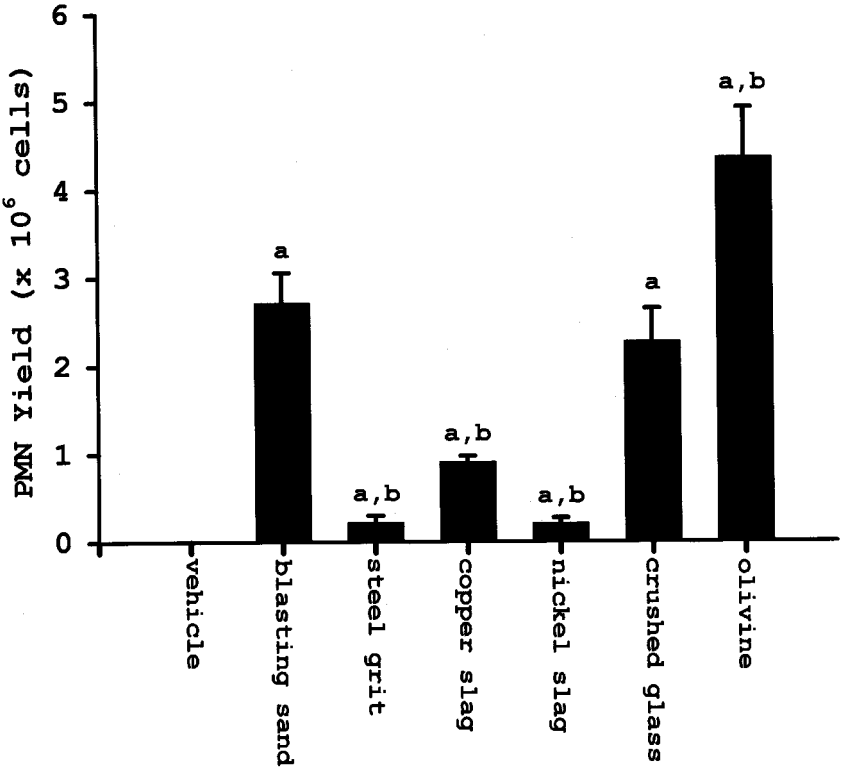

FIGURE 2. BAL PMN yield. Rats were IT instilled with vehicle (PBS) or abrasive blasting substitute material (10 mg/rat). Bronchoalveolar lavage was conducted on the right lung and PMN yield was determined as described in the Methods. An *a* above a bar indicates that the PMN yield from rats exposed to an abrasive blasting agent was significantly greater than that from vehicle-exposed rats. A *b* above a bar indicates that the PMN yield from rats exposed to an abrasive blasting agent was significantly different from that of blasting sand-exposed rats. Values are means ± SE; $n = 16$ rats exposed to vehicle, $n = 8$ rats exposed to crushed glass, olivine, steel grit, or nickel slag, and $n = 7$ rats exposed to copper slag.

exposed rats were significantly higher. There was no significant difference between the PMN yield of blasting sand- and crushed glass-exposed rats (Figure 2).

First BAL Fluid LDH

The LDH activity in first BAL fluid isolated from vehicle-exposed rats was significantly lower than that after exposure to the abrasive blasting substitutes examined, except for steel grit (Figure 3). In comparison to blasting sand-exposed rats, olivine-exposed rats had significantly higher BAL fluid LDH activity, while levels in steel grit- and nickel slag-exposed rats were significantly lower. There was no statistical difference between blasting sand-exposed rat BAL fluid LDH activity and that from copper slag- or crushed glass-exposed rats (Figure 3).

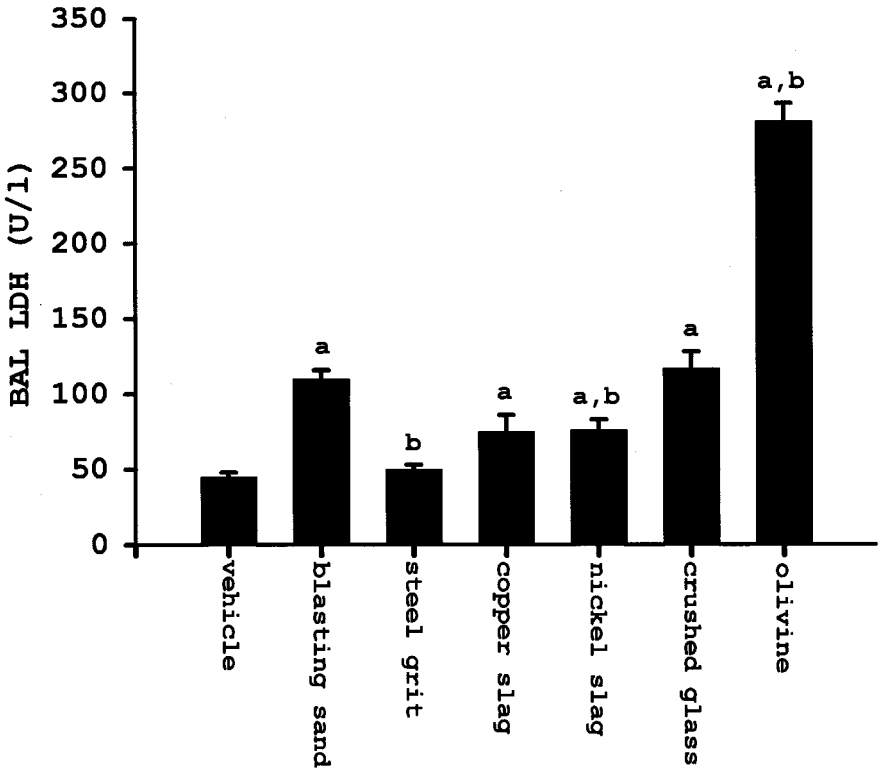

FIGURE 3. BAL fluid LDH activities. Rats were IT instilled with vehicle (PBS) or abrasive blasting substitute material (10 mg/rat). Bronchoalveolar lavage was conducted on the right lung and BAL fluid LDH activity was determined as described in the Methods. An *a* above a bar indicates that the BAL fluid LDH activity from rats exposed to an abrasive blasting agent was significantly greater than that from vehicle-exposed rats. A *b* above a bar indicates that the BAL fluid LDH activity from rats exposed to an abrasive blasting agent was significantly different from that of blasting sand-exposed rats. Values are means ± SE; $n = 16$ rats exposed to vehicle, $n = 8$ rats exposed to crushed glass, olivine, steel grit, or nickel slag, and $n = 7$ rats exposed to copper slag.

First BAL Fluid Serum Albumin Concentration

Of the abrasive blasting substitutes tested, only blasting sand- and olivine-exposed rats had significantly higher BAL serum albumin concentrations when compared to vehicle-exposed rats (Figure 4). In comparison to blasting sand-exposed rats, olivine-exposed rats had a significantly higher BAL fluid serum albumin concentration, while the other four abrasive blasting substitutes yielded significantly lower concentrations of BAL fluid serum albumin.

AM Chemiluminescence

Every abrasive blasting substitute and blasting sand produced a significant increase in zymosan-stimulated AM chemiluminescence in comparison to vehicle-exposed rats (Figure 5). AM chemiluminescence from rats

exposed to steel grit, copper slag, or nickel slag was significantly lower than for blasting sand-exposed rats, while no differences were detected between blasting sand-, crushed glass-, and olivine-exposed rats (Figure 5).

Pulmonary Histopathology

The following histopathological endpoints were evaluated at 28 d postexposure: fibrosis (Figure 6), alveolitis, and alveolar epithelial cell hypertrophy and hyperplasia (Figure 7). The results of the histopathology scores are summarized in Table 2. All of the abrasive blasting substitutes had levels of pulmonary fibrosis that were similar to blasting sand-exposed rats except for steel grit, which was significantly less potent than blasting sand and was not different from vehicle control. All of the abrasive blasting substitutes caused a significant increase in alveolitis (Table 2) when compared to vehicle-exposed

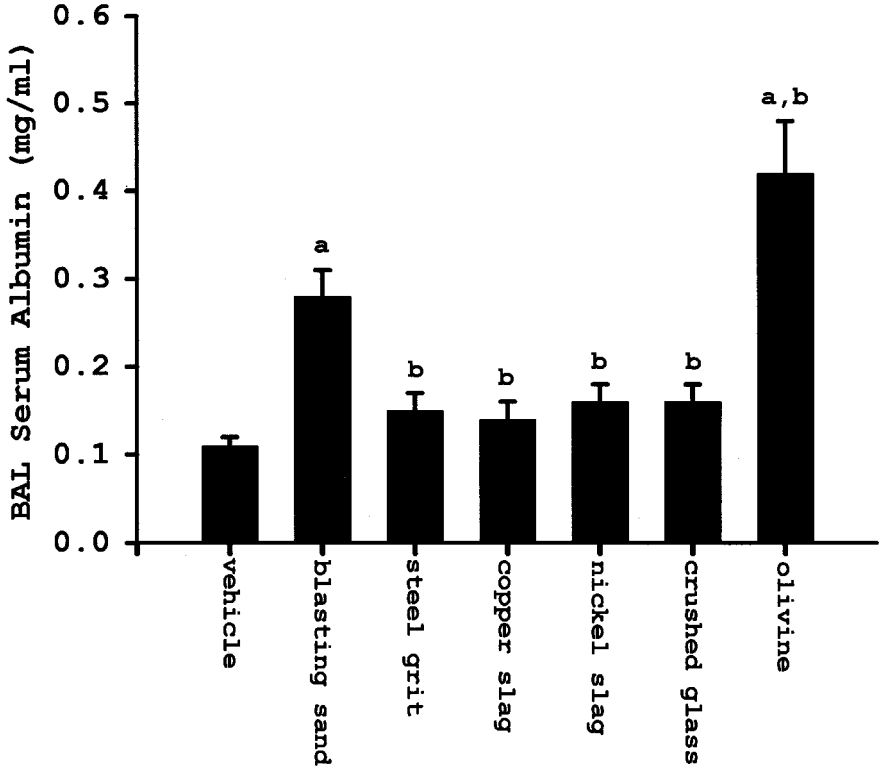

FIGURE 4. BAL fluid serum albumin concentration. Rats were IT instilled with vehicle (PBS) or abrasive blasting substitute material (10 mg/rat). Bronchoalveolar lavage was conducted on the right lung and BAL fluid serum albumin concentration was determined as described in the Methods. An *a* above a bar indicates that the BAL fluid serum albumin concentration from rats exposed to an abrasive blasting agent was significantly greater than that from vehicle-exposed rats. A *b* above a bar indicates that the BAL fluid serum albumin concentration from rats exposed to an abrasive blasting agent was significantly different from that of blasting sand-exposed rats. Values are means ± SE; $n = 16$ rats exposed to vehicle, $n = 8$ rats exposed to crushed glass, olivine, steel grit or nickel slag, and $n = 7$ rats exposed to copper slag.

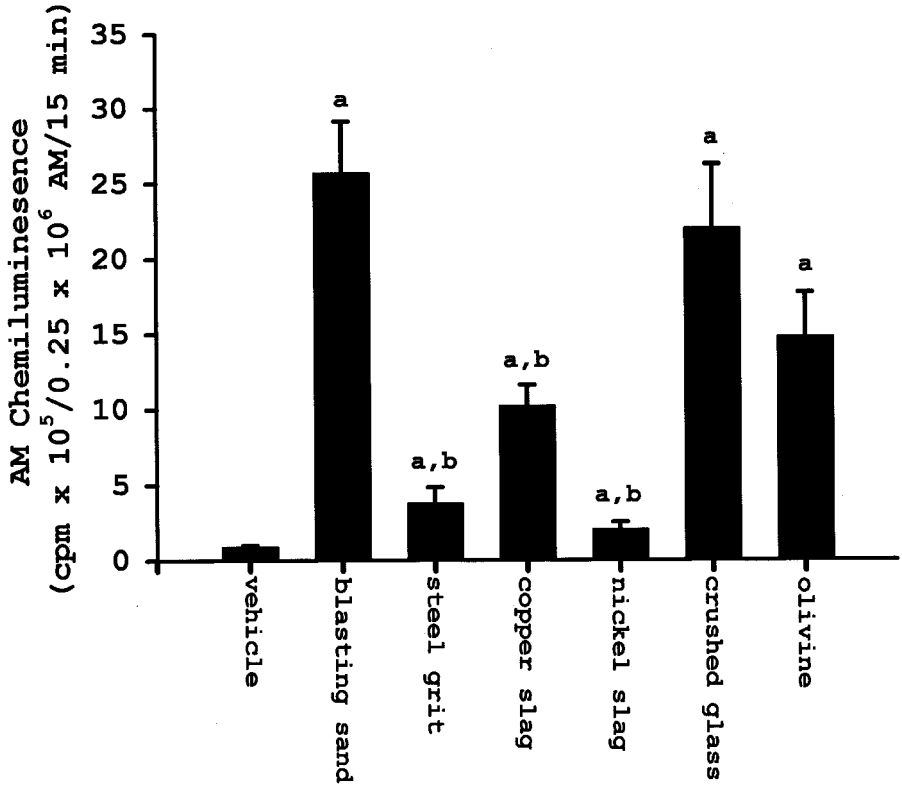

FIGURE 5. Zymosan-stimulated AM chemiluminescence. Rats were IT instilled with vehicle (PBS) or abrasive blasting substitute material (10 mg/rat). Bronchoalveolar lavage was conducted on the right lung and zymosan-stimulated AM chemiluminescence was determined as described in the Methods. An *a* above a bar indicates that the AM chemiluminescence activity from rats exposed to an abrasive blasting agent was significantly greater than that from vehicle-exposed rats. A *b* above a bar indicates that the AM chemiluminescence from rats exposed to an abrasive blasting agent was significantly less than that from blasting sand-exposed rats. Values are means ± SE; $n = 16$ rats exposed to vehicle, $n = 8$ rats exposed to crushed glass, olivine, steel grit, or nickel slag, and $n = 7$ rats exposed to copper slag.

rats, but only olivine caused significantly more than in blasting sand-exposed rats (Table 2). All of the abrasive blasting substitutes caused a significant increase in alveolar epithelial cell hypertrophy and hyperplasia in comparison to vehicle-exposed rats and were similar in magnitude to that determined for blasting sand-exposed rats. Alveolar lipoproteinosis was observed in one of the blasting sand-exposed rats and in all of the olivine-exposed rats.

DISCUSSION

The primary objective of this investigation was to evaluate the comparative pulmonary toxicity of blasting sand and several abrasive blasting substitutes for sand that are currently in commercial use. The experimental design

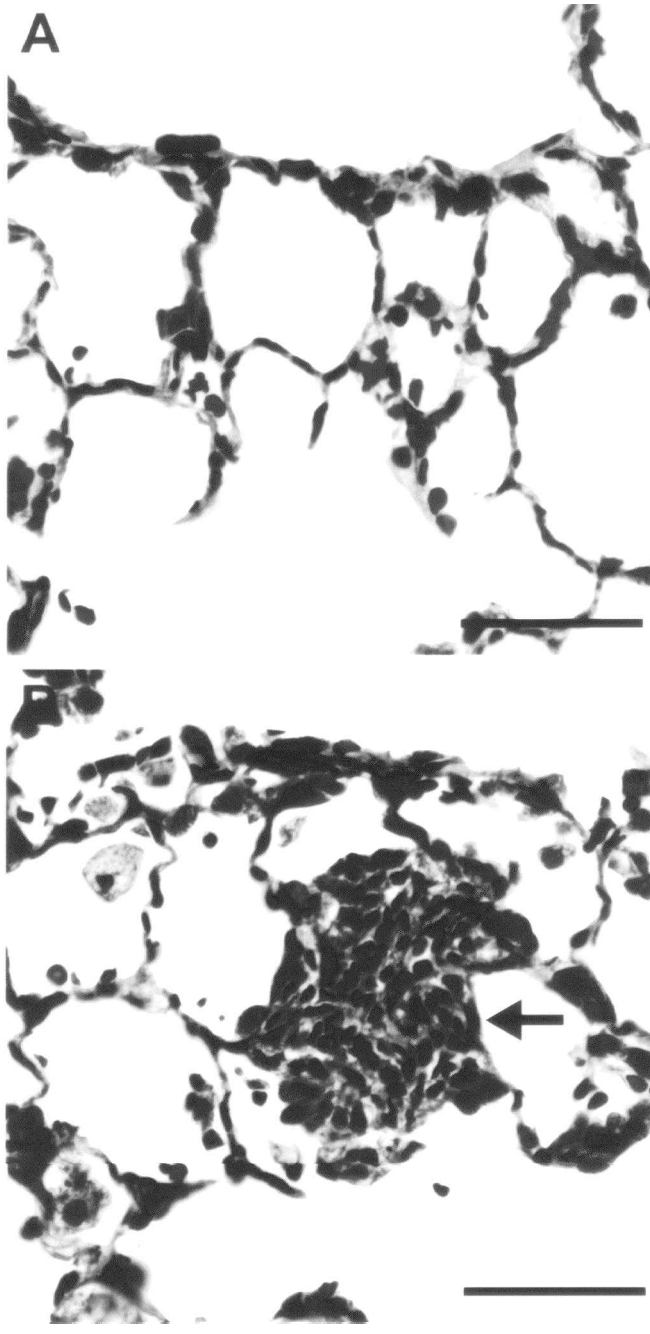

FIGURE 6. Representative photomicrograph of a trichrome-stained section from the alveolar region from (A) a vehicle-exposed rat and (B) a rat exposed to an abrasive blasting substitute, specifically crushed glass. Increased collagen deposition (arrow) stains blue and is localized to foci of interstitial inflammation. Bar = 50 μm.

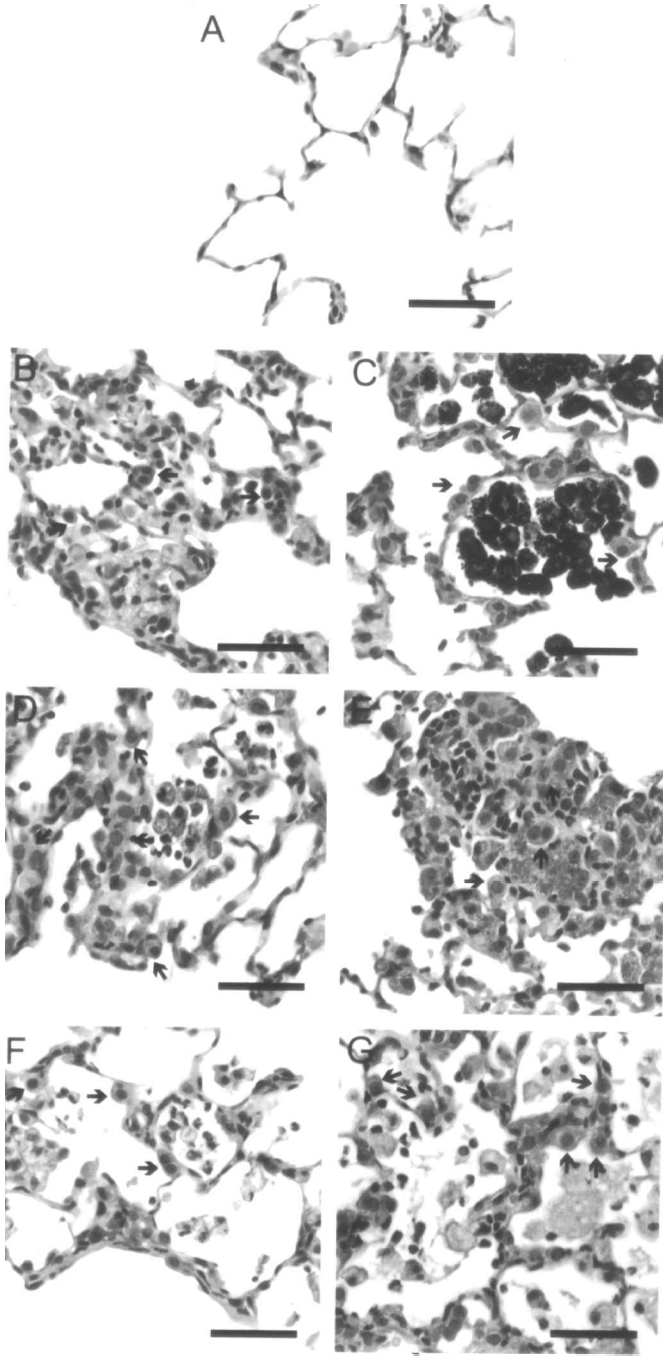

FIGURE 7. Photomicrographs from the alveolar region of rats exposed to abrasive blasting agents. Each of the agents caused alveolar inflammation and alveolar epithelial cell hypertrophy and hyperplasia (arrows). Only olivine consistently produced alveolar lipoproteinosis. Bar = 50 μm. A, vehicle; B, blasting sand; C, steel grit; D, copper slag; E, nickel slag; F, crushed glass; and G, olivine.

of this investigation was to expose rats to a single 10-mg/rat dose of an abrasive blasting agent and conduct toxicological studies at 28 d post IT exposure. This dose was chosen because silica (Driscoll et al., 1990) and some other abrasive blasting agents (Hubbs et al., 2001) have been shown to induce pulmonary toxicity and fibrosis at similar doses. Alterations in the pulmonary toxicity parameters were examined at 28 d postexposure in this study, and thus focused on unresolved inflammation. Unresolved pulmonary inflammation, as opposed to acute inflammation that resolves, has been implicated as an important factor in the development of pulmonary fibrosis (Cotran et al., 1999).

There is considerable debate concerning the use of IT instillation versus inhalation exposures to assess pulmonary toxicity. Carefully conducted IT instillation exposures can yield valuable mechanistic information that correlates well with results of inhalation exposures (Henderson et al., 1995), and multiple IT instillations of silica over time can produce effects similar to those of a single bolus dose (Reasor & Antonini, 2001). Furthermore, a recent review by leading investigators in pulmonary toxicology indicated that IT instillations can be appropriately used in comparative pulmonary toxicology studies, such as those designed to compare the relative pulmonary toxicity of different particles (Driscoll et al., 2000).

Essentially pure crystalline silica (α-quartz) is well established in regard to its ability to stimulate pulmonary inflammation, damage and fibrosis (Lapp & Castranova, 1993; Driscoll et al., 1990). In this study, blasting sand that contained 55% crystalline quartz (Table 1) was used as the positive control. Blasting sand is used in commercial abrasive blasting operations, and thus was considered a more appropriate choice than pure crystalline silica for a positive control in this study. PBS was used as the vehicle (negative control), since previous studies in this laboratory have indicated that it induces minimal to no pulmonary inflammation, damage, or fibrosis when administered by IT instillation (Porter et al., 1999).

TABLE 2. Summary of Histopathological Alterations

Exposure	*n*	Fibrosis	Alveolitis	Alveolar epithelial hypertrophy and hyperplasia
Vehicle	16	ND	1.1 ± 0.4	0.1 ± 0.1
Blasting sand	6	2.0 ± 0.9[a]	5.2 ± 0.2[a]	5.0 ± 0.3[a]
Steel grit	8	ND	5.6 ± 0.2[a]	5.5 ± 0.3[a]
Copper slag	7	2.7 ± 0.8[a]	6.0 ± 0.3[a]	5.7 ± 0.2[a]
Nickel slag	8	1.9 ± 0.8[a]	6.3 ± 0.3[a]	5.4 ± 0.3[a]
Crushed glass	8	2.5 ± 0.7[a]	5.1 ± 0.2[a]	5.3 ± 0.3[a]
Olivine	8	3.4 ± 0.6[a]	6.6 ± 0.2[a,b]	5.3 ± 0.2[a]

Note. Values for the histopathology scores are the sum of the severity and distribution scores as described in the Methods. Values represent means ± SE; ND, nondetectable.

[a]Significant increase versus control.

[b]Significant difference versus blasting sand.

BAL PMN cell yields measured in this study demonstrated that all of the abrasive blasting substitutes caused a significant increase in pulmonary inflammation in comparison to vehicle-exposed rats. Inflammatory cell influxes into the lung (Marshall et al., 1997) and persistent pulmonary inflammation (ILSI Risk Science Institute Workshop Participants, 2000) have been associated with pulmonary fibrosis. Additionally, increased BAL PMNs have been observed in humans with idiopathic pulmonary fibrosis and connective-tissue disease (BAL Cooperative Group Steering Committee, 1990).

Histological examination of rat lungs stained with Masson's trichrome stain was used to assess pulmonary fibrosis. This study was relatively short term and the severity of exposure-associated fibrosis was never considered to be more than mild. Nevertheless, examinations showed that rats exposed to steel grit and vehicle had no fibrosis, while exposure to blasting sand and the other abrasive blasting substitutes resulted in significantly elevated and similar levels of pulmonary fibrosis. Interestingly, steel-grit-exposed and nickel slag-exposed rats had similar BAL PMN yields, but only the nickel slag-exposed rats developed fibrosis. This suggests that BAL PMN levels alone may not always be predictive of fibrotic outcomes, and other processes may be required for fibrosis to develop after exposure to a particular particle type.

Inflammation in the lung is also associated with AM activation and concomitant production of reactive oxygen species (ROS) by the activated AMs (Castranova et al., 1996). To assess AM activation and ROS production, zymosan-stimulated AM chemiluminescence was measured. Blasting sand and all of the abrasive blasting substitutes caused a significant increase in AM chemiluminescence. The level of AM chemiluminescence in response to exposure to olivine or crushed glass was similar to that caused by blasting sand, whereas the other abrasive blasting substitutes caused less AM chemiluminescence but at levels still above the vehicle control. These data indicate that all of the abrasive blasting substitutes caused, to a variable degree, a persistent AM activation and ROS production, and thus the lungs of these animals were subjected to increased levels of oxidants postexposure. Similarly, alveolar inflammation (alveolitis) was observed in rats exposed to all abrasive blasting agents.

The combination of the persistent BAL PMNs, histopathological alveolitis, and elevated AM activation might be expected to cause some damage to the lungs of exposed rats. Thus, pulmonary damage was assessed by measuring the acellular BAL fluid serum albumin concentrations and LDH activities. Increases in acellular BAL fluid serum albumin concentrations reflect a decrease in the integrity of the pulmonary blood/gas barrier. Blasting sand-exposed and olivine-exposed rats had a significantly higher BAL fluid serum albumin concentration than rats with vehicle control; the other substitute abrasive blasting agents yielded BAL fluid levels similar to those of vehicle-exposed rats. This indicates that damage to the lung blood/gas barrier was significantly greater after exposure to olivine than to blasting sand.

LDH is an intracellular enzyme; thus, its presence in the acellular BAL fluid serves as an indicator of cytotoxicity. In contrast to BAL serum albumin concentrations, all the substitute abrasive blasting agents, except steel grit, produced higher BAL fluid LDH activities versus the vehicle control, with olivine being significantly more potent than blasting sand. Thus, the abrasive blasting agents, except steel grit, did induce some degree of cytotoxic damage, but this damage was not extensive enough to compromise the lung blood/gas barrier, except for olivine and blasting sand. However, LDH is present in plasma (Franken et al., 2000), and thus some of the LDH measured in BAL fluid of rats that had a compromised lung blood/gas barrier may be from LDH that leaked into the lung from the plasma.

In order to further evaluate the inflammatory response, damage, and fibrosis caused by these abrasive blasting substitutes, the results of this study were compared with those obtained in a similar study previously conducted in this laboratory (Hubbs et al., 2001). These data are presented in Table 3. Examination of these data indicate that abrasive blasting substitutes can be divided into three groups. The first group is composed of specular hematite and steel grit. These abrasive blasting substitutes caused no significant inflammation, damage, or detectable fibrosis in comparison to blasting sand. Data for specular hematite previously reported (Hubbs et al., 2001) are consistent with data from other studies in rats (Stokinger, 1984) and occupationally exposed workers (Teculescu & Albu, 1973). Occupational exposure estimates have been made for steel grit (Conroy et al., 1995), but no toxicological data have been reported for this abrasive blasting substitute. Interestingly, similar to specular hematite, the major elemental component of steel grit is iron (Table 1).

The second group of abrasive blasting substitutes is composed of garnet, staurolite, nickel slag, copper slag, crushed glass, and treated sand. These

TABLE 3. Comparison of Substitute Abrasive Blasting Agents to Negative Controls

Group	Exposure	PMN	AM CL	LDH	Albumin	Fibrosis
	Blasting sand	398	1601	235	156	375
1	Specular hematite	97	232	133	106	150
1	Steel grit	95	298	109	94	100
2	Garnet	382	838	243	194	250
2	Staurolite	372	1397	196	119	300
2	Nickel slag	167	161	165	100	290
2	Copper slag	266	801	163	88	370
2	Crushed glass	497	1723	254	100	250
2	Treated sand	366	960	176	138	100
3	Coal slag	679	2995	359	206	300
3	Olivine	1366	1156	611	263	340

Note. Values for control and blasting sand were pooled from two different experiments. Values represent percent of control.

abrasive blasting substitutes caused some degree of inflammation and damage. With the exception of treated sand, they also were associated with increased fibrosis. However, the fibrosis associated with garnet and staurolite did not reach statistical significance compared to the vehicle control (Hubbs et al., 2001). The third group of abrasive blasting substitutes is composed of coal slag and olivine, both of which caused higher levels of inflammation and damage and a similar level of fibrosis in comparison to blasting sand.

The persistent pulmonary inflammation and damage caused by the abrasive blasting substitutes in groups 2 and 3 suggest that they are not nontoxic alternatives to blasting sand. Specular hematite and steel grit produced little pulmonary inflammation, and no damage or fibrosis. However, both of these particles have biological phenomena associated with them that suggest that chronic exposure to them may not be totally benign. A single intrapulmonary instillation of specular hematite particles in humans has been reported to cause an inflammatory response that resolves postexposure (Lay et al., 1999). As shown in Table 1, steel grit contains measurable amounts of arsenic (30 μg/g), nickel (700 μg/g), and chromium (1200 μg/g), which in some compounds are associated with cancer. The use of steel grit in laboratory and field studies for abrasive blasting has generated concentrations of arsenic, nickel, manganese, and chromium above the NIOSH recommended exposure limits for those respective elements (NIOSH, 1994b, 1997; Fairfax, 1995, 1996; NTIS, 1999a, 1999b). However, it is not known if these metals, in the form and concentration found in and generated by these blasting agents, will be carcinogenic. Data from our current study indicate that rats exposed to steel grit had a significantly higher level of epithelial cell hypertrophy and hyperplasia in comparison to vehicle control, suggesting that chronic exposure studies of steel grit are warranted. Until chronic exposure studies to specular hematite and/or steel grit indicate the long-term safety of these abrasive blasting substitutes, it would be prudent to take appropriate measures to reduce the exposure of workers to these particles, as well as to other abrasive blasting materials.

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