



NTP
National Toxicology Program
U.S. Department of Health and Human Services

NTP TECHNICAL REPORT ON THE TOXICITY STUDIES OF

ABRASIVE BLASTING AGENTS
ADMINISTERED BY INHALATION TO
F344/NTAC RATS AND SPRAGUE DAWLEY
(HSD:SPRAGUE DAWLEY[®] SD[®]) RATS

NTP TOX 91

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**NTP Technical Report on
the Toxicity Studies of Abrasive Blasting Agents
Administered by Inhalation to F344/NTac Rats
and Sprague Dawley
(Hsd:Sprague Dawley[®] SD[®]) Rats**

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Errata

Errors were identified in the NTP Toxicity Report on Abrasive Blasting Agents (TOX 91). Incorrect supplier information was reported for the five test materials, and detailed information about trade names and bulk material preparation was inadvertently omitted.

In the Materials and Methods on page 8 and in Appendix C on page C-2, the text was revised to identify the correct suppliers and additional information on trade names was included for each blasting agent in the HTML and PDF versions of this report. [September 1, 2022]

In the Materials and Methods and in Appendix C, additional information describing the bulk material preparation of blasting sand was added to the text, including a summary sentence on page 8 and information regarding the bulk material preparation for blasting sand (page C-2), coal slag (page C-4), crushed glass (page C-5), garnet (page C-6), and specular hematite (page C-6). This information was added to the HTML and PDF versions of this report. [September 1, 2022]

Foreword

The National Toxicology Program (NTP), established in 1978, is an interagency program within the Public Health Service of the U.S. Department of Health and Human Services. Its activities are executed through a partnership of the National Institute for Occupational Safety and Health (part of the Centers for Disease Control and Prevention), the Food and Drug Administration (primarily at the National Center for Toxicological Research), and the National Institute of Environmental Health Sciences (part of the National Institutes of Health), where the program is administratively located. NTP offers a unique venue for the testing, research, and analysis of agents of concern to identify toxic and biological effects, provide information that strengthens the science base, and inform decisions by health regulatory and research agencies to safeguard public health. NTP also works to develop and apply new and improved methods and approaches that advance toxicology and better assess health effects from environmental exposures.

The Toxicity Report series began in 1991. The studies described in the NTP Toxicity Report series are designed and conducted to characterize and evaluate the toxicological potential of selected substances in laboratory animals (usually two species, rats and mice). Substances (e.g., chemicals, physical agents, and mixtures) selected for NTP toxicity studies are chosen primarily on the basis of human exposure, level of commercial production, and chemical structure. The interpretive conclusions presented in the toxicity reports are derived solely from the results of these NTP studies, and extrapolation of these results to other species, including characterization of hazards and risks to humans, requires analyses beyond the intent of these reports. Selection for study per se is not an indicator of a substance's toxic potential.

NTP conducts its studies in compliance with its laboratory health and safety guidelines and the Food and Drug Administration [Good Laboratory Practice Regulations](#) and meets or exceeds all applicable federal, state, and local health and safety regulations. Animal care and use are in accordance with the [Public Health Service Policy on Humane Care and Use of Laboratory Animals](#). Studies are subjected to retrospective quality assurance audits before they are presented for public review. Draft reports undergo external peer review before they are finalized and published.

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For questions about the reports and studies, please email [NTP](#) or call 984-287-3211.

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Peer Review

The draft *NTP Technical Report on the Toxicity Studies of Abrasive Blasting Agents Administered by Inhalation to F344/NTac Rats and Sprague Dawley (Hsd:Sprague Dawley® SD®) Rats* was evaluated by the reviewers listed below. These reviewers served as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, reviewers determined if the design and conditions of these NTP studies were appropriate and ensured that this Toxicity Report presents the experimental results and conclusions fully and clearly.

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Abstract

Abrasive blasting, commonly known as sandblasting, involves forcibly projecting a stream of abrasive particles through compressed air or steam against a surface to change its quality or to remove contaminants. Silica blasting sand contains high levels of crystalline silica—which can cause pulmonary fibrosis (silicosis) after exposure through inhalation and is considered a lung carcinogen—and constitutes approximately 63% of all abrasives used in abrasive blasting. Other abrasives, including specular hematite, are recommended as alternative blasting agents. Due to the health risks associated with using blasting sand in the abrasive blasting process and the lack of toxicity data on alternatives to blasting sand, the National Institute for Occupational Safety and Health (NIOSH) proposed testing blasting sand and alternative abrasives to characterize their associated toxicity.

Using inhalation (whole-body) exposure, male F344/NTac rats were first exposed to blasting sand, coal slag, crushed glass, garnet, or specular hematite in 2-week studies. In subsequent studies, male and female Sprague Dawley (Hsd:Sprague Dawley® SD®) rats were exposed to blasting sand or specular hematite for up to 27 weeks (females) or 39 weeks (males). In the 2-week studies, groups of five male F344/NTac rats were exposed by whole-body inhalation to blasting sand, coal slag, crushed glass, or garnet aerosol at concentrations of 0, 3, 15, or 30 mg/m³ or specular hematite aerosol at concentrations of 0, 3, 15, 30, or 60 mg/m³ for 6 hours plus T₉₀ (theoretical value for the time to achieve 90% of the target concentration after the beginning of aerosol generation; 12 minutes) per day, 5 days per week for 2 weeks, plus 2 days for 12 exposures (day 16). Additional groups of 35 male F344/NTac rats were exposed to the same concentrations of blasting sand, coal slag, crushed glass, garnet, or specular hematite for tissue burden analysis through day 16.

In the 2-week studies of blasting sand, coal slag, crushed glass, garnet, and specular hematite, all core study rats survived to the end of the study; there were no significant differences between exposed groups and the chamber control group in mean body weights for core rats. Except for one coal slag-exposed rat in the 30 mg/m³ group that had an ocular discharge on days 5 and 8, no clinical observations were associated with exposure to blasting sand, coal slag, crushed glass, garnet, or specular hematite. The absolute lung weights of core study rats exposed to 15 or 30 mg/m³ crushed glass in the 2-week study were significantly increased compared to the chamber control group. Lung burdens continued to increase through the last exposure day (day 16) for all five test articles indicating that steady-state lung burdens were not achieved during the 2-week studies. Crushed glass exhibited the shortest clearance half-life.

The incidence of minimal histiocytic cellular infiltration was significantly increased in the lungs of 15 and 30 mg/m³ coal slag-exposed rats compared to the chamber control group in the 2-week study. The incidence of minimal to mild histiocytic cellular infiltration was significantly increased in the lungs of 30 and 60 mg/m³ specular hematite-exposed rats compared to the chamber control group.

The incidence of minimal goblet cell hypertrophy in the nasopharyngeal duct of the nose was significantly increased in 60 mg/m³ specular hematite-exposed rats compared to the chamber control group in the 2-week study.

All crushed glass-exposed rats, except for one in the 3 mg/m³ group, exhibited minimal to mild goblet cell hypertrophy of the respiratory epithelium in the nose, a significant increase compared

to the chamber control groups. The severity of goblet cell hypertrophy increased with increasing crushed glass exposure concentration.

In the larynx, the incidences of minimal to mild hyperplasia, squamous hyperplasia, and inflammation of the epiglottis were significantly increased in the 15 and 30 mg/m³ crushed glass-exposed groups in the 2-week study compared to the chamber control groups.

In the lungs of garnet-exposed rats, the incidence of minimal to mild chronic active inflammation was significantly increased in the 15 and 30 mg/m³ groups compared to the chamber control group in the 2-week study.

Specular hematite and crushed glass appeared to be the least toxic of the four alternative abrasives tested in the 2-week studies on the basis of lung histopathology, but specular hematite was selected for further testing because crushed glass exhibited a relatively short clearance half-life.

In the 39-week studies, groups of 62 male Sprague Dawley rats were exposed by whole-body inhalation to blasting sand or specular hematite aerosol at concentrations of 0, 15, 30, or 60 mg/m³ for 6 hours plus T₉₀ (12 minutes) per day, 5 days per week for up to 39 weeks. Groups of 32 female Sprague Dawley rats were exposed to the same concentrations of blasting sand or specular hematite for up to 27 weeks for immunotoxicity studies.

In the 39-week study of blasting sand, all male rats survived to interim sacrifice or to the end of the study; mean body weights of all exposed groups were similar to the chamber control group. There were no clinical observations associated with exposure to blasting sand. The absolute and relative lung weights in the 30 and 60 mg/m³ groups were significantly increased compared to the chamber control group beginning at week 16 or 8, respectively. The absolute and relative bronchial lymph node weights in the 60 mg/m³ group at all time points and in the 30 mg/m³ group at week 26 were significantly increased compared to the chamber control group. The absolute mediastinal lymph node weights were significantly increased at weeks 16, 26, and 39 in the 60 mg/m³ group, and the relative mediastinal lymph node weights were increased in this group at weeks 16 and 39. Absolute numbers of macrophages, neutrophils, and lymphocytes, and lactate dehydrogenase activity and MCP-1 levels in bronchoalveolar lavage fluid generally increased in magnitude with increasing exposure concentration and time, with the 60 mg/m³ groups often most severely affected. Blasting sand lung burdens continued to increase through the last exposure week indicating that steady-state lung burdens were not achieved during the study. By the end of the study, lung overload conditions were achieved at all exposure concentrations. Time of onset of lung overload was calculated to be 137, 55, and 30 days for 15, 30, and 60 mg/m³ blasting sand groups, respectively. Treatment-related nonneoplastic lesions occurred in the nose, lung (including chronic inflammation, alveolar proteinosis, and interstitial fibrosis), and bronchial and mediastinal lymph nodes. The incidences of these lesions generally increased with increasing exposure concentration and time.

In the 39-week study of specular hematite, two male rats, one chamber control and one exposed to 60 mg/m³, were removed during week 37 for reasons unrelated to exposure to the test article; mean body weights of all exposed groups were similar to those of the chamber control group. There were no clinical observations associated with exposure to specular hematite. Compared to those in the chamber control group, the absolute and relative lung weights were significantly increased in the 30 and 60 mg/m³ groups at weeks 16, 26, and 39. Absolute and relative bronchial lymph node weights were significantly increased at weeks 16, 26, and 39 in the

60 mg/m³ group and at week 26 in the 30 mg/m³ group. The absolute and relative mediastinal lymph node weights were significantly increased at weeks 16 and 26 in the 60 mg/m³ group but not at week 39. Absolute numbers of neutrophils and lymphocytes, and lactate dehydrogenase activity and MCP-1 levels in bronchoalveolar lavage fluid generally increased in magnitude with increasing exposure concentration and time, with the 60 mg/m³ groups often severely affected. Specular hematite lung burdens continued to increase through the last exposure week indicating that steady-state lung burdens were not achieved during the study. By the end of the study, lung overload conditions were achieved in the 30 and 60 mg/m³ groups; the specular hematite volume was at 90% of the overload threshold in the 15 mg/m³ group. Time of onset of lung overload was calculated to be 344 (i.e., after 39 weeks), 118, and 55 days for 15, 30, and 60 mg/m³ specular hematite, respectively. Treatment-related nonneoplastic lesions occurred in the nose, lung (including chronic inflammation, alveolar epithelial hyperplasia, and interstitial fibrosis), larynx (squamous metaplasia of epiglottis), and bronchial and mediastinal lymph nodes. The incidences of these lesions generally increased with increasing exposure concentration and time.

Under the conditions of these 39-week inhalation studies, the lung was the major target tissue in male Sprague Dawley rats exposed to blasting sand or specular hematite. The incidences of chronic active inflammation and interstitial fibrosis were significantly lower in rats exposed to specular hematite (compared to blasting sand) at some time points under some exposure conditions. After 39 weeks of exposure to specular hematite, the lowest-observed-effect level was 15 mg/m³ for chronic active inflammation and interstitial fibrosis within the lung. Alveolar proteinosis was present at week 39 in the lungs of rats exposed to the highest concentration (60 mg/m³) of blasting sand but was notably absent in the lungs of rats exposed to specular hematite. Alveolar epithelial hyperplasia was present at week 16 in the lungs of rats exposed to the two highest concentrations (30 or 60 mg/m³) of specular hematite but not blasting sand. Specular hematite exhibited potential to be an inhalation toxicant in exposed workers who perform abrasive blasting operations but to a lesser degree than blasting sand because the lungs of rats exposed to specular hematite showed a lower incidence of interstitial fibrosis and an absence of alveolar proteinosis.

Summary of Key Findings Considered Toxicologically Relevant in Male Sprague Dawley Rats Exposed to Abrasive Blasting Agents by Inhalation for 39 Weeks

	Blasting Sand Study	Specular Hematite Study
Concentrations in Air	0, 15, 30, or 60 mg/m ³	0, 15, 30, or 60 mg/m ³
Body Weights	Exposed groups similar to the chamber control group	Exposed groups similar to the chamber control group
Survival Rates	13/13, 13/13, 13/13, 13/13	12/13, 13/13, 13/13, 12/13
Clinical Observations	None	None
Organ Weights	↑Absolute and relative lung weights; ↑Absolute and relative bronchial lymph node weights; ↑Absolute and relative mediastinal lymph node weights	↑Absolute and relative lung weights; ↑Absolute and relative bronchial lymph node weights; ↑Absolute and relative mediastinal lymph node weights
Tissue Burden	↑ lung overload time of onset at 137, 55, and 30 days and extent exceeding overload at end of study 1.6, 3.8, 8.4-fold (15, 30, and 60 mg/m ³ , respectively)	↑ lung overload time of onset at 334, 118, and 55 days and extent exceeding overload at end of study 0.9, 2.3, 5.5-fold (15, 30, and 60 mg/m ³ , respectively)
Select Nonneoplastic Effects	Lung: chronic active inflammation (week 4: 0/8, 2/8, 2/8, 3/8; week 16: 1/8, 3/8, 3/8, 8/8; week 26: 2/8, 7/8, 7/8, 8/8; week 39: 2/8, 8/8, 8/8, 8/8); interstitium, fibrosis (week 26: 0/8, 8/8, 7/8, 8/8; week 39: 0/8, 8/8, 8/8, 8/8); proteinosis (week 26: 0/8, 0/8, 0/8, 1/8; week 39: 0/8, 0/8, 0/8, 6/8); alveolar epithelium, hyperplasia (week 26: 0/8, 8/8, 8/8, 8/8; week 39: 0/8, 8/8, 8/8, 8/8) Larynx: epiglottis, metaplasia, squamous (week 39: 0/8, 1/8, 1/8, 1/8) Nose: respiratory epithelium, accumulation, hyaline droplet (week 16:0/8, 8/8, 8/8, 7/8)	Lung: chronic active inflammation (week 16: 1/8, 0/8, 0/8, 3/8; week 26: 0/8, 2/8, 0/8, 4/8; week 39: 0/9, 3/8, 2/8, 7/9); interstitium, fibrosis (week 26: 0/8, 0/8, 0/8, 3/8; week 39: 1/9, 2/8, 8/8, 9/9); alveolar epithelium, hyperplasia (week 16: 0/8, 3/8, 8/8, 8/8; week 26: 0/8, 8/8, 8/8, 8/8; week 39: 2/9, 8/8, 8/8, 9/9) Larynx: epiglottis, metaplasia, squamous (week 4: 0/8, 1/8, 4/8, 5/8; week 16: 0/8, 3/8, 3/8, 4/8; week 26: 0/8, 5/8, 5/8, 7/8; week 39: 0/9, 5/8, 7/8, 7/9) Nose: respiratory epithelium, accumulation, hyaline droplet (week 16:1/8, 4/8, 5/8, 2/8)
Bronchoalveolar Lavage	↑ Total cells counted ↑ Absolute macrophages ↑ Absolute lymphocytes ↑ Absolute neutrophils ↑ Lactose dehydrogenase ↑ MCP-1	↑ Total cells counted ↑ Absolute macrophages ↑ Absolute lymphocytes ↑ Absolute neutrophils ↑ Lactose dehydrogenase ↑ MCP-1

Overview

Abrasive blasting, commonly known as sandblasting, involves forcibly projecting a stream of abrasive particles through compressed air or steam against a surface to change its quality or to remove contaminants. Blasting sand, most often used in abrasive blasting, contains high levels of crystalline silica, which can cause pulmonary fibrosis (silicosis) after exposure through inhalation and is considered a lung carcinogen. Alternatives to blasting sand with lower crystalline silica content exist, including specular hematite, which is mostly iron oxide (Fe_2O_3). Specular hematite has previously been shown to induce less lung injury, inflammation, and fibrosis *in vivo* than blasting sand.¹ Other alternatives to blasting sand include coal slag, crushed glass, and garnet; however, no comprehensive chronic inhalation studies have been performed to evaluate the health effects, including pulmonary toxicity, of these alternative compounds. Testing data are needed due to the high production volume of these compounds, the number of exposed workers, and the inadequacy of available toxicity data to inform safe exposure levels.

In 1974, the National Institute for Occupational Safety and Health (NIOSH) recommended banning the use of silica sand (or other substances containing more than 1% free silica) as an abrasive blasting material, suggesting that less hazardous materials be used in blasting operations.² NIOSH has recommended alternatives to silica sand,³ but they are based on the presence or absence of known hazards—such as respirable quartz, arsenic, beryllium, cadmium, lead, chromium, manganese, nickel, titanium, silver, and vanadium.⁴ They do not consider the potential risks of exposure to these specific blasting agents, as comprehensive evaluations have not been conducted and the health effects of chronic inhalation have not yet been studied. Without adequate testing and evaluation of alternatives, it is difficult to make explicit recommendations of less hazardous alternatives to silica sand.

To begin the process of obtaining health effects data on silica sand substitutes, NIOSH assessed acute pulmonary toxicity in rats following intratracheal instillation of nine commercially available substitutes.^{1;5} The blasting materials tested were coal slag, specular hematite, steel grit, copper slag, nickel slag, crushed glass, staurolite, garnet, and olivine. Of these nine, NIOSH nominated five substitutes (coal slag, crushed glass, garnet, specular hematite, and steel grit) for further testing to determine the potential for these agents to induce lung fibrosis after whole-body inhalation exposure (during which animals in their respective cages are placed into exposure chambers and exposed to test article [particulate] aerosols). Specular hematite is a relatively new alternative abrasive blasting material that appears to have low pulmonary toxicity potential, as reported in the study by Hubbs et al.,¹ and confirmation of this might provide a basis for recommending it as an alternative to silica sand.

To address these data gaps, the National Toxicology Program (NTP) performed 2-week inhalation toxicity studies to compare blasting sand with the alternative abrasive blasting agents coal slag, crushed glass, garnet, and specular hematite. Pulmonary toxicity and tissue burden were assessed in male F344/NTac rats after inhalation (whole-body) exposure to target aerosol concentrations of 0, 3, 15, or 30 mg/m^3 for blasting sand, coal slag, crushed glass, and garnet or 0, 3, 15, 30, or 60 mg/m^3 for specular hematite. The objectives of the 2-week studies were to determine acute toxicity, identify target organs, evaluate lung and lymph node burdens, establish no-effect levels for pathological changes, if any, associated with 2 weeks of exposure, and provide the basis for the selection of test article and exposure concentrations to be used in subsequent 39-week studies.

The objectives of the 39-week inhalation toxicity studies were to compare blasting sand with specular hematite. Because specular hematite also exhibited low pulmonary toxicity in the 2-week study, pulmonary toxicity, as well as fibrogenicity, tissue (lung and lymph node) burden, and immunotoxicity, were assessed in male and female Sprague Dawley (Hsd:Sprague Dawley® SD®) rats after whole-body inhalation exposure to target aerosol concentrations of 0, 15, 30, or 60 mg/m³ blasting sand or specular hematite. Male rats were used for the core study, whereas female rats were used for the immunotoxicity study, although some of the immunotoxicity end points were also evaluated in the core male rats. Data generated from these studies will provide a framework upon which NIOSH can base recommendations for the use of alternatives to crystalline silica and could provide dose-response toxicity data to inform occupational exposure limit recommendations.

Introduction

Characteristics of Abrasive Blasting Agents

Blasting sand contains varying levels of crystalline silica (SiO_2), also known as quartz, which can range widely (39% to 100%) between commercially available samples.¹ Specular hematite primarily consists of iron oxide (Fe_2O_3). Garnet refers to a group of complex silicate materials with isometric crystal structures and similar properties and chemical compositions.⁶ Coal slag is a waste product from the burning of coal in power plants^{4; 7; 8} and contains very low levels of crystalline silica (less than 1%). Crushed glass is generated from recycled glass and contains no crystalline silica.

Production, Use, and Human Exposure

Abrasive blasting involves forcefully projecting a stream of abrasive particles onto a surface, typically using compressed air or steam. Because sand (composed primarily of crystalline silica) is commonly used in the abrasive blasting process, workers who perform this work are commonly known as sandblasters. Tasks performed by sandblasters include removing irregularities from foundry castings; cleaning and removing paint from ship hulls, stone buildings, metal bridges, and other metal surfaces; finishing tombstones; etching or frosting glass; and performing certain artistic endeavors. Total use of blasting sand in 1992 was 1.75 million tons.⁹ When workers inhale the crystalline silica from the sand used in abrasive blasting, their lung tissue reacts by developing fibrotic nodules and scarring around the trapped silica particles.¹⁰ This fibrotic condition of the lung is called silicosis. If the nodules grow too large, breathing becomes difficult and death can result. Silicosis victims are also at high risk of developing active tuberculosis.¹¹⁻¹³

Coal slag is the most commonly used abrasive blasting alternative to silica sand.^{1; 9} It is used in approximately 42% of all blasting operations that use alternative agents. Coal slag is a waste product of burning coal in power plants.^{4; 7; 8} The slag is formed during the burning of powdered coal, which produces an ash with a relatively low melting point. During the burning process, this molten ash falls to the bottom of the furnace as a viscous liquid before then dropping into water where it solidifies. The resulting solid is distributed under the trademark Black Beauty®. Compared with other mineral slags (copper and nickel), coal slags as a class are reported to contain the lowest quantities ($\mu\text{g/g}$) of suspected carcinogens: beryllium (7 to 48 $\mu\text{g/g}$), chromium (110 to 200 $\mu\text{g/g}$), nickel (18 to 70 $\mu\text{g/g}$), and arsenic (4 to 18 $\mu\text{g/g}$).⁸ Crushed glass is a relatively new abrasive blasting agent used in shipbuilding and repair with projected annual usage of 2,725 tons.⁴ As crushed glass contains no free crystalline silica, it is considered a potentially safer alternative blasting material.^{4; 5} In addition, the vast supply of recycled glass could be a favorable economic factor in considering the increased use of crushed glass. In 2004, abrasive blasting accounted for 35% of the estimated 58,600 tons of garnet consumed in the United States.⁶ Recent annual consumption of specular hematite was estimated to be about 25,000 to 30,000 tons.¹⁴

NIOSH estimates that approximately 150,000 workers are employed as abrasive blasters.¹⁵ The National Occupational Exposure Survey indicates that the construction industry employs the largest number of sandblasters, with the highest proportion in the special trades industries.^{3; 16; 17}

Silica sand constitutes approximately 63% of all blasting abrasives used industrially. Overexposure to crystalline silica and silicosis are still prevalent among sandblasters¹⁸ (unpublished SENSOR data). For many abrasive blasting operations, nearby workers (pot tenders, blaster helpers, shipyard workers, painters, welders, and laborers) are without any respiratory protection, so the total number of workers exposed to abrasive blasting materials is likely a multiple of the estimated number of sandblasters.

Regulatory Status

The current Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL) for respirable crystalline silica is 100 $\mu\text{g}/\text{m}^3$ as an 8-hour time-weighted average.¹⁹ The NIOSH recommended exposure limit (REL) for respirable crystalline silica is 50 $\mu\text{g}/\text{m}^3$ as a time-weighted average for up to 10 hours per day during a 40-hour work week.² This REL is intended to prevent silicosis. However, evidence indicates that crystalline silica is a potential occupational carcinogen^{16; 20; 21} and NIOSH is reviewing the data on carcinogenicity. NIOSH, OSHA, and the American Conference of Governmental Industrial Hygienists (ACGIH) do not have specific RELs, PELs, or threshold limit values (TLVs) for any abrasive blasting alternatives to silica sand. These substitutes are currently treated as nuisance dusts with exposure limits of 3 mg/m^3 (respirable particles) and 10 mg/m^3 (inhalable particles) according to ACGIH.²² Specular hematite primarily consists of iron oxide; the NIOSH REL and ACGIH TLV for iron oxide dust and fumes is 5 mg/m^3 , and the OSHA PEL is 10 mg/m^3 .

Acute silicosis is less common today than it was in the 1930s because engineering controls are available to reduce exposure to respirable crystalline silica and the use of alternative abrasives is increasing. However, data indicate that many abrasive blasters continue to work without adequate respiratory protection,²³ and ventilation controls for reducing crystalline silica exposures are not used in most industries.¹⁷ Samimi et al.²⁴ found that, even in short-term sandblasting operations (less than 2.5 hours of blasting during an 8-hour work day), the average concentration of dust was 764 $\mu\text{g}/\text{m}^3$, with an average crystalline silica content of 25.5%. This average crystalline silica concentration was twice the 1974 OSHA standard. In a 1974 study of respiratory protection practices during abrasive blasting,²³ the protection factors for supplied-air respirators with helmets ranged from 1.9 to 3,750. This wide range was attributed to the varied conditions of the equipment rather than to the superiority of any brand. Maintenance was universally poor or nonexistent, and those responsible for selecting respiratory protection for abrasive blasting were inadequately informed about the proper use and maintenance of the equipment. The higher protection factors were associated with high rates of helmet air flow, but these high flow rates increased noise levels due to air turbulence. The study also indicated that the blasters' helmets tended to fall from their shoulders when they stooped.

Because of the high risk for silicosis in sandblasters and the difficulty in controlling exposures, the use of crystalline silica for blast cleaning operations was restricted in Great Britain in 1950²⁵ and in other European countries in 1966.²⁶ In 1974, NIOSH recommended silica sand (or other substances containing more than 1% crystalline silica) be prohibited as an abrasive blasting material and less hazardous materials be used in blasting operations,² due to the silicosis hazard and the difficulty controlling crystalline silica exposure associated with sandblasting. Alternatives to silica sand are recommended by NIOSH³; however, few comprehensive studies have been conducted to evaluate the health effects of most of the alternative blasting abrasives.

A variety of materials (coal and other slags, crushed glass, garnet, specular hematite olivine, staurolite, and steel grit) are available as alternative blasting media.^{23; 27; 28} Engineering controls and personal protective equipment should be used with any of the alternative abrasives to reduce inherent hazards associated with use of blasting abrasives and substrates. In addition to the health hazards of abrasive blasting materials, the finely fractured particles of material being removed (lead paint, for example) likely pose other health risks for workers.²⁹

Toxicity

Experimental Animals

Crystalline Silica

Studies show that exposure of animals to crystalline silica by inhalation, oropharyngeal aspiration, or intratracheal instillation causes lung injury, inflammation, and pulmonary fibrosis. Other evidence suggests that the crystalline silica content of blasting sand correlates with pulmonary toxicity.¹

Coal Slag

In studies conducted by MacKay et al.,²⁷ male rats received single intralobular instillations of distilled water (control), coal slag (20 mg), or quartz (20 mg). All surviving rats were sacrificed 10 months later. Microscopic evaluation of the lungs indicated mean fibrotic scores of 4 for the coal slag lungs compared with 10 for the quartz-treated group. Stettler et al.⁷ dosed male Sprague Dawley rats with single intratracheal instillations of 40 mg coal slag, 40 mg quartz, or vehicle. Interim sacrifices were conducted at 2 days, 3 months, and 6 months, with a terminal sacrifice at 12 months. Mild to moderate interstitial fibrosis that progressed with time was noted in each of the coal slag-exposed rats. Histiocytic infiltrates containing particulate material were seen in tracheobronchial lymph nodes and increased with time; fibrosis was a prominent feature at 12 months. These changes were much less severe than those resulting from exposure to quartz. Massive fibrosis and inflammation developed in much of the entire lobe of the lungs at 3, 6, and 12 months in the quartz group, but these lesions were not evident at any time in any animal in the coal slag groups. Pulmonary function data for the coal slag groups showed less adverse lung effects for coal slag compared with quartz exposure, generally falling between the silica quartz and the vehicle control groups in terms of impact to lung function at both doses and elevated neutrophil levels at the 10 mg level. In addition, these parameters exceeded those in the quartz-dosed group. Lung hydroxyproline levels were significantly elevated only in rats receiving 10 mg of coal slag. By contrast, histopathological fibrosis scores, based on severity and distribution, were significantly higher than vehicle controls only in the rats receiving 10 mg of quartz.

Crushed Glass

In a study by Porter et al.,⁵ male Sprague Dawley rats were dosed by intratracheal instillation with 10 mg per rat of respirable vehicle, crushed glass, or blasting sand (silica quartz). Bronchoalveolar lavage fluid was evaluated 4 weeks later for pulmonary inflammation (including neutrophil counts) and damage (serum albumin concentration and lactate dehydrogenase activity). Lungs were evaluated microscopically for fibrosis, alveolitis, and alveolar hypertrophy and hyperplasia. The results from this study showed that crushed glass significantly increased polymorphonuclear neutrophil yields and lactate dehydrogenase activity

in bronchoalveolar lavage fluid above vehicle control levels to levels comparable to those seen following silica quartz treatment. Bronchoalveolar lavage fluid albumin levels were comparable to vehicle controls and significantly lower than levels noted with silica quartz treatment. Histopathology scores (severity and distribution) of microscopic changes (fibrosis, alveolitis, and alveolar hypertrophy and hyperplasia) were all significantly greater than those of the vehicle control group and comparable to those of silica quartz group.

Garnet

Studies examining the toxicological effects of garnet exposures are limited. In the study by Hubbs et al.,¹ male Sprague Dawley rats were dosed with garnet via a single intratracheal instillation (2.5 or 10 mg/rat) and necropsied 4 weeks later. In garnet-dosed rats, significant treatment-related responses were restricted to the 10 mg group. Toxicity due to garnet treatment included increased lactate dehydrogenase activity, and increased neutrophil and activated macrophage counts in lung bronchoalveolar lavage fluid. These results suggested that garnet caused pulmonary cytotoxicity and inflammation that persisted for 4 weeks after exposure.

Specular Hematite

Specular hematite appears to exhibit low toxicity in vivo compared with crystalline silica. In one study, intratracheal instillation of 10 mg crystalline silica per hamster resulted in recruitment of polymorphonuclear leukocytes and elevation of red blood cell counts, lactate dehydrogenase activity, and albumin levels in lavage fluid, which were significant 1 day postexposure.³⁰ By contrast, Beck and colleagues found in a separate study that specular hematite caused much less inflammation and damage than crystalline silica, and these pulmonary reactions were quickly resolved. The results agree with those of a NIOSH study¹ that examined rats 30 days after intratracheal instillation of 10 mg specular hematite per rat; bronchoalveolar lavage markers of inflammation and damage were not significantly different from vehicle control levels and were significantly lower than marker values observed after blasting sand (crystalline silica) instillation. Histopathologically, the incidence of lung fibrosis was also lower following exposure to specular hematite compared with levels from blasting sand exposure. Mild, transient inflammation has also been reported after short-term (3 hours) inhalation of high levels (274 mg/m³) of specular hematite by hamsters.³¹ Specular hematite, in contrast to crystalline silica, failed to induce significant secretion of inflammatory cytokines (tumor necrosis factor alpha and interleukin 1) in rat lungs after intratracheal exposure.³² The lack of sustained inflammation and lung damage after exposure of animal models to iron oxide is consistent with the absence of pulmonary fibrosis reported in rats after intratracheal instillation of specular hematite.^{1, 33} In summary, the in vivo animal data for lung fibrosis from exposure to specular hematite consistently indicated fewer adverse effects from exposure to iron oxide than to crystalline silica.

Humans

Crystalline Silica

A 1980 Department of Labor report to Congress on occupational disease estimated that one million workers were exposed to crystalline silica. Regardless of the use of respiratory protection, sporadic clusters of silicosis continue to occur in the sandblasting industry.^{30, 31} Overexposures to crystalline silica and silicosis are still prevalent among abrasive blasting workers¹⁸(unpublished SENSOR data). Blasting with silica is restricted in several other

industrialized nations, but it continues to be widely practiced in the United States despite the recommendation by NIOSH to ban it.²³

When workers inhale the crystalline silica used in abrasive blasting, the lung tissue can react by developing either acute or chronic silicosis, depending on the exposure level. Acute silicosis is characterized by alveolar lipoproteinosis and a decrease in gas exchange. Chronic silicosis is characterized by fibrotic nodules and scarring around the trapped silica particles.¹⁰ If the nodules grow too large, breathing becomes difficult and death can result. Silicosis victims are also at high risk for developing active tuberculosis.¹¹⁻¹³ The silica sand used in abrasive blasting typically fractures into fine particles and becomes airborne. Inhalation of freshly fractured silica appears to produce a more severe lung reaction than aged silica that is not freshly fractured.³⁴⁻³⁶ This factor might contribute to the development of acute and accelerated forms of silicosis among sandblasters.

Chronic exposure to low concentrations of crystalline silica promotes the formation of fibrotic nodules in the lung parenchyma with a typical morphological appearance of discrete, rounded, whorled, hyalinized lesions.³⁷ These fibrotic nodules are usually less than a centimeter in diameter and are sharply demarcated from the surrounding lung tissue. This simple silicosis rarely results in any clinically apparent disease. With continued exposure, simple silicosis will lead to increased nodular density and decremental changes in pulmonary function. Progressive massive fibrosis is a common feature of such complicated silicosis resulting in compromised lung function.

Acute silicosis is associated with alveolar lipoproteinosis caused by highly reactive fractured silica and/or intense silica dust exposure and is associated with a high mortality rate. Acute silicosis is known to result from sandblasting, silica flour mill operations, rock drilling, tunneling, and other occupations in which crystalline silica is fractured then inhaled.^{38; 39} Clinically, it is characterized by severe shortness of breath and hypoxemia. Secondary infection is a common terminal complication. Accelerated silicosis with lipoproteinosis is linked to sandblasting and other aforementioned similar operations.^{31; 40} Accelerated silicosis progresses rapidly to classic nodular silicosis at an accelerated rate of 5 to 10 years with no abnormalities except increasing breathlessness. The patient often dies from respiratory failure within 7 to 12 years of diagnosis.

Specular Hematite

The relatively low lung fibrogenicity rates from iron oxide exposure in animal studies are consistent with epidemiology studies of exposed workers. For example, Teculescu and Albu⁴¹ studied 14 workers exposed for an average of 10 years to pure iron oxide dust and found no pulmonary function changes consistent with pulmonary fibrosis. Furthermore, Lay et al.⁴² reported that the presence of inflammation following intrapulmonary instillation of iron oxide in human subjects resolved rapidly after the exposure ended.

Carcinogenicity

Experimental Animals

Crystalline Silica

Exposure to crystalline silica has been shown to be carcinogenic to the lungs of rats but not those of mice and hamsters⁴³; however, rats are relatively more sensitive to the development of lung tumors.^{21; 44} Therefore, the biological unresponsiveness of some animal species or strains to a test article does not necessarily negate evidence of carcinogenicity.

Specular Hematite

Animal investigations of the carcinogenicity of specular hematite have been consistently negative. Intratracheal instillation (380 mg iron oxide by multiple injections) resulted in no lung tumors in a 2.5-year study with rats.⁴⁰ Similar results were reported by Saffiotti et al.³⁸ after 15 weekly instillations of 3 mg iron oxide. Inhalation of iron oxide (40 mg/m³ for 2 years) also produced negative results in hamsters.³⁹ With this evidence, the International Agency for Research on Cancer (IARC)⁴⁵ views the lack of carcinogenicity in animals from iron oxide exposure as convincing.

There are no carcinogenicity data from animal studies for coal slag, crushed glass, or garnet exposures.

Humans

Crystalline Silica

IARC comprehensively reviewed the human and animal experimental studies and concluded that there is sufficient evidence for the carcinogenicity of inhaled crystalline silica in the forms of quartz or cristobalite from occupational sources.^{21; 44; 46} IARC classified crystalline silica under category 1A (i.e., a carcinogen to humans).^{21; 44} However, the conclusions from human epidemiological studies are debatable, mainly because of the lack of well-matched control studies for smoking and other variables known to be carcinogens. A supporting quantitative relationship between crystalline silica exposure and bronchogenic cancer was also demonstrated in workers without radon exposure such as Vermont granite workers, German slate workers, North Carolina Dusty Trade Workers, and ceramic and pottery workers in Sweden and Italy. Although information is not available for people with silicosis who have never smoked, it can be concluded from these studies that silicosis is correlated with an increased risk for bronchogenic carcinoma. NTP⁴⁷ lists respirable crystalline silica as a Known Human Carcinogen in its 14th Report on Carcinogens.

Specular Hematite

Early epidemiology studies reported an increased incidence of lung cancer in workers exposed to iron oxide. However, most of these studies are plagued with confounding exposures.⁴⁸ For example, Boyd et al.⁴⁹ reported a 70% higher than normal lung cancer mortality in underground iron ore miners, whereas surface iron mine workers did not exhibit higher lung cancer mortality. Unaccounted for in this study was the fact that the underground miners were also exposed to high radon levels (100 pCi/L), which could affect lung cancer rates. In addition, a case-control study of factory workers exposed to high amounts of iron oxide dust found no increased

incidence of cancer in the respiratory system or other sites.³⁷ Therefore, the human data for iron oxide-induced cancer is viewed as negative.⁴⁸ In summary, lung cancer data are consistently negative for in vivo animal exposure studies of specular hematite, and when confounding exposures are accounted for, human data are consistent with this conclusion.

There are no carcinogenicity data from human epidemiological studies for coal slag, crushed glass, or garnet exposures.

Study Rationale

Acute and chronic inhalation toxicity testing data are needed due to the high production volume of these abrasive blasting compounds, the number of workers exposed, and the inadequacy of available toxicity data to inform safe exposure levels. Because potential alternatives have not been adequately evaluated using comparative methods, making explicit recommendations for less hazardous blasting agent alternatives to silica sand is difficult.¹ Data generated from inhalation toxicity studies could provide a framework for NIOSH to use in recommending alternative blasting agents to crystalline silica and could provide dose-response toxicity data to recommend occupational exposure limits. Furthermore, an immunotoxicity study was called for to examine the effects of abrasive blasting agents on pulmonary and systemic immune function because of the evidence of a close association between silica exposure and autoimmunity in humans, the reported immunological effects of silicosis in animal models, and systemic effects in animals exposed to particulates.

Materials and Methods

Procurement and Characterization

The blasting sand (*coarse silica sand #2340*) used in the 2-week and 39-week studies was obtained from *Waupaca Sand and Solutions (Division of Faulks Brothers Construction Inc.; Waupaca, WI)* ~~Midwest Research Institute (MRI; Kansas City, MO)~~ in one lot by *Midwest Research Institute (MRI; Kansas City, MO)* and was assigned the lot number W100604JB. The micronized specular hematite (Barshot 50) used in the 2-week and 39-week studies was obtained from *Opta Minerals, Inc. (Waterdown, ON, Canada)* in one lot (0101005CJ) by *MRI (Kansas City, MO)*. The coal slag used in the 2-week study was obtained from *Reed Minerals-Harsco Corporation (LaCygne, KS)* ~~MRI~~ in one lot (R042805KA). The crushed glass (*VitroGrit™ #30/50*) used in the 2-week study was obtained from *TriVitro Corporation (Kent, WA)* ~~MRI~~ in one lot (T092205KA). The garnet used in the 2-week study was obtained from *Emerald Creek Garnet Ltd. (Fernwood, ID)* ~~MRI~~ in one lot (031605).^a *Aveka, Inc. (Woodbury, MN)* reduced the particle size of all five test articles using aqueous ball milling followed by aqueous bead milling.^b Identity and purity analyses were conducted by multiple analytical chemistry laboratories and the study laboratory at Battelle Toxicology Northwest (Richland, WA) (Appendix C). Reports on analyses performed in support of the abrasive blasting agent studies are on file at the National Institute of Environmental Health Sciences (NIEHS).

Blasting Sand

The character and composition of the blasting sand (lot W100604JB), a finely ground, gray powder, was established using analyses that included weight loss on drying, density, Brunauer-Emmett-Teller (BET) surface area, Fourier transform infrared (FTIR) spectroscopy, inductively coupled plasma/atomic emission spectroscopy (ICP/AES) for the identification and quantitation of total silicon (Si), proton induced X-ray emission (PIXE) spectroscopy to quantitate concentrations of major and minor elements, X-ray diffraction (XRD) to identify and quantitate crystalline phases present at greater than 1%, and X-ray fluorescence (XRF) analyses for qualitative measurements.

Lot W100604JB had a density of 2.78 g/cc and a BET surface area of approximately 20 m²/g; weight loss on drying indicated a water content of less than 1% for the bulk material. FTIR, ICP/AES, PIXE, and XRD analyses indicated 39.0%, 39.4%, 37.7%, and 40.7% Si, respectively; the National Institute for Occupational Safety and Health (NIOSH) method 7500 silicon result (29.5%) was inconsistent with the other Si content data. PIXE identified other elements above 1% as aluminum (Al, 2.0% to 2.2%), calcium (Ca, 1.3% to 1.4%), and iron (Fe, 1.1% to 1.3%) and elements approaching concentrations near 1% as magnesium (Mg, 0.6% to 0.8%), potassium (K, 0.8% to 0.9%), and zirconium (Zr, 0.7% to 0.8%). The test material phases were mostly crystalline quartz (SiO₂, 76.4% to 78.5%) with cristobalite (0.6% to 0.8%) and crystalline

^aERRATUM: An error was identified in the NTP Toxicity Report on Abrasive Blasting Agents (TOX 91). The reported supplier information for each blasting agent has been corrected and trade names were added to the text in the HTML and PDF versions of this report; the new information is italicized. [September 1, 2022]

^bERRATUM: An error was identified in the NTP Toxicity Report on Abrasive Blasting Agents (TOX 91). This sentence was added to summarize the bulk material preparation in the HTML and PDF versions of this report; the new information is italicized. [September 1, 2022]

impurities of calcite (CaCO_3 , 0.3% to 0.8%), dolomite [$\text{CaMg}(\text{CO}_3)_2$, 7.0% to 7.6%], and one phase tentatively identified as sanidine [$\text{K}(\text{AlSi}_3)\text{O}_8$, 12.7% to 15.1%] through XRD Rietveld analysis.

Coal Slag

The character and composition of the coal slag (lot R042805KA), a black, mostly amorphous powder, was established using analyses that included weight loss on drying; Karl Fischer titration for water content; density; BET surface area; ICP/AES for identification and quantitation of Si, Al, Fe, and Ca; PIXE spectroscopy; powder XRD; XRF; and coulometric titration for carbon content.

Lot R042805KA had a density of 2.77 g/cc and a BET surface area of approximately 9.26 m²/g. Weight loss on drying experiments yielded average weight losses of 15.3% and 13.2% for the bulk test article. The average weight loss was 17.4% for additional samples dried to constant weight. Karl Fischer titration indicated 11.7% water for the undried test article and less than 1% water for the dried test article. ICP/AES and PIXE analyses showed 21.7% and 21.5% Si, 9.9% and 10.0% Al, 8.1% and 7.2% Fe, and 10.7% and 9.1% Ca, respectively. PIXE also revealed 1.6% Mg and 0.71% K. XRD Rietveld analysis detected CaCO_3 as the only crystalline phase at 1.2% weight fraction. Coulometric analysis indicated 0.127% carbon. No crystalline silica was detected (detection limit approximately 0.5%).

Crushed Glass

The character and composition of the crushed glass (lot T092205KA), a mostly amorphous powder, was established using analyses that included weight loss on drying; Karl Fischer titration; density; BET surface area; ICP/AES for identification and quantitation of Si, Al, Fe, Ca, sodium (Na), Mg, and K; PIXE spectroscopy; and powder XRD. The Reference Intensity Ratio method was used to quantify the weight fractions of the XRD phases detected.

Lot T092205KA had a density of 2.39 g/cc and a BET surface area of approximately 22.4 m²/g. Weight loss on drying averaged 3.1% water, and Karl Fischer titration averaged 3.5% water. ICP/AES and PIXE analyses indicated 31.2% and 28.9% Si, 5.5% and 5.6% Ca, 9.3% and 8.9% Na, 1.9% and 1.9% Mg, 0.3% and 0.4% Al, 0.1% and 0.1% Fe, and 0.2% and 0.2% K, respectively. Crystalline phases detected included natrite (Na_2CO_3 , 0.4% to 1.6%), aragonite (orthorhombic CaCO_3 , 0.3% to 0.5%), calcite (hexagonal CaCO_3 , 0.7% to 1.5%), and quartz (SiO_2 , 0.2% to 0.8%).

Garnet

The character and composition of garnet (lot 031605), a red, mostly amorphous powder, was established using analyses that included weight loss on drying; Karl Fischer titration; density; BET surface area; ICP/AES for identification and quantitation of Si, Al, Fe, and Mg; PIXE spectroscopy; and powder XRD.

Lot 031605 had a density of approximately 4.0 g/cc and a BET surface area of approximately 22.2 m²/g. Weight loss on drying and Karl Fischer titration indicated 1.7% and 1.0% water, respectively, for the bulk material. ICP/AES, PIXE, and XRD analyses indicated 16.7%, 17.5%, and 18.8% Si, respectively. ICP/AES and PIXE indicated 10.6% and 11.3% Al, 22.5% and

23.3% Fe, and 1.4% and 1.5% mg, respectively. The XRD Rietveld method showed that the test material was mostly crystalline garnet [$A_3B_2(SiO_4)_3$, in which A is Fe and/or Mg and B is Al and/or Fe; approximately 89%] with crystalline components of quartz (SiO_2 , 6%) and staurolite ($HFe_2Al_9Si_4O_{24}$, 5% to 6%, by weight).

Specular Hematite

The character and composition of specular hematite (lot O101005CJ), a finely ground, red powder, was established using analyses that included weight loss on drying; Karl Fischer titration; density; BET surface area; ICP/AES for the identification and quantitation of total Si, Al, Fe, and Zr; PIXE spectroscopy to quantitate concentrations of major and minor elements; and powder XRD to identify and quantitate crystalline phases present at greater than 1%.

Lot O101005CJ had a density of approximately 5 g/cc and a BET surface area of approximately 18 m²/g. Karl Fischer titration and weight loss on drying indicated a water content less than 1% for the bulk material. ICP/AES, PIXE, and XRD analyses indicated the presence of Fe at approximately 69.4%, 63.4%, and 69.1%, respectively. ICP/AES values for Si, Al, and Zr were 1.2%, 0.4%, and 0.6%, and PIXE values were 1.2%, 0.4%, and 0.5%, respectively. The XRD Rietveld method showed that the test material was crystalline with phases of specular hematite (Fe_2O_3 , ~96%), magnetite (Fe_3O_4 , 2% to 3%), and quartz (SiO_2 , 1% to 2%).

Aerosol Generation and Exposure Systems

For the 2-week studies of abrasive blasting agents, the aerosol generation system consisted of a linear feed dust-metering device designed and built by Battelle to meter the abrasive blasting agent from a reservoir into an air stream for aerosolization. Within the metering device, periodic blasts of compressed air suspended small volumes of blasting sand, coal slag, crushed glass, garnet, or specular hematite in the air stream for transport to the metering device exhaust tube. For blasting sand, coal slag, and garnet, a jet disperser was positioned immediately downstream from the metering device exhaust tube (Figure C-6, Figure C-7). Coal slag and garnet were moved from the jet disperser to a particle attrition chamber (PAC) to enhance the aerosolization of the test material. Crushed glass and specular hematite were processed in a Trost jet mill (Garlock, Inc., Newtown, PA), used downstream from the metering device exhaust tube, to perform initial particle size reduction; opposing compressed air gas streams drove the jet mill (Figure C-8, Figure C-9).

All generation system components were housed in a glove box in the control center room. From the jet disperser (blasting sand, coal slag, garnet) and jet mill (crushed glass, specular hematite), aerosolized blasting agents were blended with filtered, compressed air before being conveyed down the distribution line from the control center room to the exposure room. For crushed glass, as the air stream entered the exposure room, an in-line cyclone separator further decreased particle size and extracted nonrespirable aerosol. For blasting sand (2-week and 39-week studies), coal slag, crushed glass, garnet, and the 39-week study of specular hematite (Figure C-10), all chambers in the exposure room except for the control were fed aerosol from a single distribution line constructed of stainless steel, bonded and grounded to prevent electrostatic charge buildup. For the 2-week study of specular hematite, the distribution line was split into north and south branches. Aerosol was supplied to the 60 mg/m³ chamber from the south distribution line branch; all remaining chambers in the exposure room except the control

chamber were fed aerosol from the north distribution line branch. During exposures to abrasive blasting agents, the airflow through the distribution line was controlled using a house vacuum regulated by a filter-protected flow meter. A second distribution line flow control system was available during off-exposure periods. This system consisted of a vacuum transducer pump (Air-Vac Engineering Company, Inc., Seymour, CT) of higher flow capacity, positioned in parallel with the flow meter control assembly, and was operational only during critical shutdown periods. At each exposure chamber, aerosol was delivered from the distribution line by a sampling tube. The flow through each sampling tube was induced by a stainless-steel air ejector pump designed and fabricated by Battelle. The flow rate and configuration of the ejector pump and sampling tube combination were chosen to optimize the efficiency of the delivery system. The aerosol then entered the chamber inlet duct where it was further diluted with humidified, Parafil-, charcoal-, and high-efficiency particulate air (HEPA)-filtered air to achieve the desired exposure concentration.

The 39-week study of blasting sand used the same aerosol generation system described for the 2-week study of this test material. For the 39-week study of specular hematite, the aerosol generation system was similar to that described for the 2-week study of blasting sand except for the additions of an in-line settling jar within the glove box and an in-line cyclone separator in the distribution line to the exposure room as described for the 2-week study of crushed glass.

The study laboratory designed the inhalation exposure chamber (Harford Systems Division of Lab Products, Inc., Aberdeen, MD) so that uniform aerosol concentrations could be maintained throughout the chamber with the catch pans in place. The total active mixing volume of each chamber was 1.7 m³.

Aerosol Concentration Monitoring

Summaries of the chamber aerosol concentrations are given in Table C-1 and Table C-2. The concentration of the abrasive blasting agent in the exposure chambers and room air was monitored using two real-time aerosol monitors (RAMs) (Model RAM-1; MIE, Inc., Bedford, MA). The monitors were connected to the chambers by a sampling system designed by Battelle incorporating a valve that multiplexed each RAM to a 0 mg/m³ chamber or the room, a HEPA-filtered room air blank, and two exposure chambers. The output (voltage) of the RAM was recorded by a program designed by Battelle (Battelle Exposure Data Acquisition and Control) to select the correct sample stream and acquire a raw voltage signal from each RAM. Equations for the calibration curves resided within the program and were used to convert the measured RAM voltages to exposure chamber concentrations.

Each RAM was calibrated by constructing a response curve using the measured RAM voltages (voltage readings were corrected by subtracting the RAM zero-offset voltage from measured RAM voltages) and chamber concentrations of the abrasive blasting agents measured gravimetrically or specific to the test article on exposure chamber filters. Developmental studies demonstrated that gravimetric and test article-specific measurements of chamber concentrations were comparable. For all abrasive blasting agents, exposure chamber atmosphere samples were collected each day on 25 mm Pallflex[®] Emfab[™] TX40H120WW Teflon[®]-coated, glass-fiber filters and on 25 mm, 0.45 µm GH Polypro polypropylene filters (both obtained from Pall Corporation, Ann Arbor, MI). Test article-specific assays of blasting sand, coal slag, crushed glass, and garnet measured the amount of Si captured on filters extracted with 1:3 HNO₃:HF, and

used an ICP/AES method. Test article-specific assays of specular hematite measured the amount of Fe captured on filters extracted with HCl, using an ICP/AES system.

The ICP/AES instrument was calibrated against serially diluted NIST-traceable spectrometric standards Si (for blasting sand, coal slag, crushed glass, and garnet) or Fe (for specular hematite) and the internal standard Co. Quality control standards and a reagent blank were analyzed after calibration, after approximately every 10th sample, and at the end of the analysis to determine accuracy and calibration drift during analysis.

Chamber Atmosphere Characterization

Particle size distribution was determined once before the 2-week and 39-week studies began, once during the 2-week studies, and once a month during the 39-week studies. Samples were taken from each exposure chamber using a Mercer-style seven-stage cascade impactor (In-Tox Products, Moriarty, NM). For the 2-week studies of blasting sand, coal slag, crushed glass, and garnet, impactor samples were collected on polypropylene filters (GH Polypro, Pall Corporation), dissolved using HNO₃, HF, and H₃BO₃, and hydroxylamine hydrochloride (NH₂OH·HCl), and assayed for Si using ICP/AES. For the 2-week study of specular hematite, impactor samples were collected on glass slides lightly coated with silicone to reduce particle bounce or on glass-fiber filters (Pallflex Emfab, Pall Corporation), dissolved using HCl and sonication, and analyzed for Fe using ICP/AES. For the 39-week studies, impactor samples of blasting sand and specular hematite were collected on stainless-steel slides or glass-fiber filters (Pallflex Emfab, Pall Corporation) and then measured gravimetrically to determine the amount of test article deposited on each stage. The relative mass of each abrasive blasting agent collected on each stage was analyzed by the NEWCAS impactor analysis program developed at Battelle and was based on probit analysis.⁵⁰ The mass median aerodynamic particle diameter and the geometric standard deviation estimates of each set of samples are given in Table C-3, Table C-4, and Table C-5. All values of mass median aerodynamic diameter were less than 3 μm as required by the protocol (Table 1).

Table 1. Particle Size Distribution in Chambers During the Inhalation Studies of Abrasive Blasting Agents

Abrasive Blasting Agent	MMAD (μm) Range for Two-week Study	MMAD (μm) Range for 39-week Study
Blasting Sand	1.1–1.3	1.0–1.8
Coal Slag	1.1–1.2	–
Crushed Glass	1.0–1.1	–
Garnet	1.0–1.1	–
Specular Hematite	0.7–0.8	0.8–1.1

MMAD = mass median aerodynamic diameter.

Buildup and decay rates for chamber aerosol concentrations were determined with and without animals present in the chambers. At a chamber airflow rate of 15 air changes per hour, the theoretical value for the time to achieve 90% of the target concentration after the beginning of aerosol generation (T₉₀) and the time for the chamber concentration to decay to 10% of the target concentration after conclusion of aerosol generation (T₁₀) was approximately 9.4 minutes. For

the 2-week study of blasting sand, T₉₀ and T₁₀ values ranged from 9 to 10 minutes with animals present. For the 2-week studies of coal slag, crushed glass, garnet, and specular hematite, T₉₀ values ranged from 10 to 13, 12 to 14, 13 to 15, and 9 to 22 minutes, respectively, with animals present; T₁₀ values ranged from 9 to 10, 9 to 10, 10 to 11, and 10 to 11 minutes, respectively. For the 39-week study of blasting sand, T₉₀ values ranged from 12 to 13 minutes without animals present and from 13 to 14 minutes with animals; T₁₀ values ranged from 8 to 9 minutes without animals present and from 10 to 11 minutes with animals. For the 39-week study of specular hematite, T₉₀ values ranged from 12 to 13 minutes without animals present and from 10 to 12 minutes with animals; T₁₀ values were 9 minutes without animals present and ranged from 10 to 11 minutes with animals. A T₉₀ value of 12 minutes was selected for all studies.

The uniformity of aerosol concentration in the inhalation exposure chambers without animals present was evaluated before the 39-week studies began; in addition, concentration uniformity with animals present in the chambers was measured once during the 2-week studies and three times during the 39-week studies. Chamber concentration uniformity was maintained throughout the studies.

The persistence of the abrasive blasting agents in the chambers after aerosol delivery ended was determined by monitoring the concentration overnight in the 30 mg/m³ chambers, except for the 2-week and 39-week studies of specular hematite that monitored concentrations in the 60 mg/m³ chamber, with (all studies) and without (39-week studies only) animals present in the chambers. In the 2-week studies of blasting sand, coal slag, crushed glass, garnet, and specular hematite, the concentration decreased to 1% of the starting concentration within 19, 19, 21, 20, and 21 minutes, respectively. In the 39-week study of blasting sand, the concentration decreased to 1% of the starting concentration within 20 minutes with animals present and within 19 minutes without animals. In the 39-week study of specular hematite, the concentration decreased to less than 1% of the starting concentration within 21 minutes with animals present and within 18 minutes without animals.

Stability studies of the test materials in the generation and exposure systems were performed by the analytical chemistry and study laboratories. During the 2-week studies, before the start of the 39-week studies, and twice during the 39-week studies, blasting sand, coal slag, crushed glass, garnet, or specular hematite powder samples were taken from the low and high exposure concentration chambers and the aerosol distribution lines by collection on 25 mm A/E glass-fiber or polypropylene (GH Polypro) filters (Pall Corporation). On each sample collection day, samples of the bulk test material were collected before filling the generator reservoir and from the reservoir at the end of the generation day; additional test material was added to the generator each day. Samples were analyzed by XRD to identify and quantitate crystalline phases present in each abrasive blasting agent and by ICP/AES and PIXE (2-week studies of blasting sand, coal slag, crushed glass, and garnet) to determine elemental content, and carbon content was assayed by combustion (coal slag only). Results of these stability assays showed that the composition of each abrasive blasting agent in the exposure chambers and distribution lines was stable in the presence and absence of animals, reflected the composition of the bulk test material in the generator reservoir, and was generally comparable to that found during the initial characterization assays of each test article.

Animal Source

Male F344/NTac rats were obtained from the commercial colony at Taconic Farms, Inc. (Germantown, NY) for use in the 2-week studies, and male and female Sprague Dawley (Hsd:Sprague Dawley® SD®) rats were obtained from Harlan Laboratories, Inc. (Livermore, CA) for use in the 39-week studies. For many years, the National Toxicology Program (NTP) used the inbred F344/N rat for its toxicity and carcinogenicity studies. Over time, the F344/N rat strain began exhibiting sporadic seizures and idiopathic chylothorax and consistently disproportionate high rates of mononuclear cell leukemia and testicular neoplasia. Because of these issues in the F344/N rat and NTP's desire to find a more fecund rat model that could be used in both reproductive and carcinogenesis studies for comparative purposes, an alternative rat model for use in these studies was explored. Following a workshop in 2005, the F344 rat from the Taconic commercial colony (F344/NTac) was used for a few NTP studies between 2005 and 2006 to allow NTP time to evaluate different rat models.⁵¹ NTP now uses the Sprague Dawley (Hsd:Sprague Dawley® SD®) rat, which it obtains from Envigo (Indianapolis, IN).

Animal Welfare

Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals (Appendix E). All animal studies were conducted in an animal facility accredited by AAALAC. Studies were approved by the Battelle Toxicology Northwest Animal Care and Use Committee and conducted in accordance with all relevant NIH and NTP animal care and use policies and applicable federal, state, and local regulations and guidelines.

Two-week Studies

On receipt, rats were 3 weeks old. Animals were quarantined for 11 or 12 days and were 5 weeks old on the first day of the studies. Before the studies began, five male rats were randomly selected for parasite evaluation (pinworms: *Syphacia obvalata* and *muris*) and gross observation for evidence of disease. Serology testing was not conducted at the laboratory for the 2-week studies, but rats were obtained from a commercial colony free of the following rat pathogens: Sendai virus, pneumonia virus of mice, sialodacryoadenitis virus, Kilham rat virus, Toolan's H1 virus, *Mycoplasma pulmonis* and *Pneumocystis carinii*.

Groups of five male F344/NTac rats were exposed by whole-body inhalation to blasting sand, coal slag, crushed glass, or garnet aerosol at concentrations of 0, 3, 15, or 30 mg/m³ or specular hematite aerosol at concentrations of 0, 3, 15, 30, or 60 mg/m³ for 6 hours plus T₉₀ (12 minutes) per day, 5 days per week for 2 weeks, plus 2 days for 12 exposures (day 16) (the term "2-week studies" specifically refers to those during which animals were exposed for 2 weeks plus 2 days). Additional groups of 35 male F344/NTac rats were exposed to the same concentrations of blasting sand, coal slag, crushed glass, garnet, or specular hematite for tissue burden analysis through day 16. These test articles were not exposed simultaneously but in separate studies. Feed was available ad libitum except during exposure periods; water was available ad libitum. Rats were housed individually. Clinical observations were recorded daily. Core-study animals were weighed initially, on days 6 and 13, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 2.

Exposure concentrations were informed by inhalation studies of silica quartz by NIOSH, which exposed male F344 rats at 15 or 20 mg/m³. In these studies, rats developed lung fibrosis after exposure to 15 mg/m³ silica over 16 calendar weeks.^{1:5} The selected concentrations also accounted for the estimated lung deposited doses for a 45-year working lifetime at the Occupational Safety and Health Administration permissible exposure limit (PEL) and American Conference of Governmental Industrial Hygienists threshold limit value (TLV). Concentrations of 30 and 60 mg/m³ were expected to produce lung overload in rats, which is necessary to compare effects of overload conditions with the effects seen in nonoverloaded lungs and to obtain deposited lung doses in rats comparable with those estimated for humans over a full working lifetime.

Five pre-assigned tissue burden rats per exposure group were wiped clean, weighed, and anesthetized using 70% carbon dioxide on days 1, 5, 12, and 16 after the 6-hour exposure; days 8 and 15 before the 6-hour exposure; and on day 37 after 21 days of recovery. Paired lung and lymph nodes (bronchial and mediastinal) were removed, weighed, and stored separately in plastic containers at approximately -70°C until analysis. For determination of tissue concentrations, lung samples were acid digested using microwave sample preparation systems and analyzed using ICP/AES for either Si (blasting sand, coal slag, crushed glass, or garnet studies) or Fe (specular hematite study). Total test article burden was calculated using the percent Si (39.4%, blasting sand; 21.8%, coal slag; 31.2%, crushed glass; or 16.7%, garnet) or Fe (69.4%, specular hematite) found during preliminary bulk analysis of the test articles.

Necropsies were performed on all core study rats on day 16. Tissues for microscopic examination were harvested, fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 4–6 µm, and stained with hematoxylin and eosin (H&E). The lung and mediastinal and bronchial lymph nodes were weighed, and histopathological examinations were performed on selected tissues. Table 2 lists the tissues and organs examined.

Thirty-nine-week Studies

On receipt, the rats were 4 to 5 (blasting sand) or 4 (specular hematite) weeks old. Animals were quarantined for 11 or 12 days and were 5 to 7 weeks old on the first day of the studies. Before the studies began, five male and five female rats were randomly selected for parasite evaluation and gross observation for evidence of disease. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix E). All results were negative.

Test article and exposure concentrations selected for the 39-week studies were informed by the results of the 2-week studies. Groups of 32 male Sprague Dawley rats were exposed by whole-body inhalation to blasting sand or specular hematite aerosols at concentrations of 0, 15, 30, or 60 mg/m³ for 6 hours plus T₉₀ (12 minutes) per day, 5 days per week for up to 39 weeks (the term “39-week studies” refers specifically to those during which animals were exposed for up to 39 weeks). Additional groups of 30 male Sprague Dawley rats were exposed to the same concentrations of blasting sand or specular hematite for up to 39 weeks for tissue burden studies. Groups of 32 female Sprague Dawley rats were exposed to the same concentrations of blasting sand or specular hematite for up to 27 weeks for immunotoxicity studies. These test articles were not dosed simultaneously but in separate studies. Feed was available ad libitum except during

exposure periods; water was available ad libitum. Rats were housed individually. For males, body weights were recorded initially, then body weights and clinical observations were recorded weekly beginning on day 8 for 16 weeks, monthly thereafter, and at the end of the studies. Rats were euthanized at interim time points or at the end of the study (39 weeks) by intraperitoneal injection of pentobarbital. Details of the study design and animal maintenance are summarized in Table 2. Information on feed composition and contaminants is presented in Appendix D.

For bronchoalveolar lavage (BAL) fluid studies, two BAL fluid washes were collected in succession from the right lung lobes of eight core study male rats pre-assigned from each exposure group at 4, 16, 26, and 39 weeks (end of studies) and from the whole lung of eight special study female rats from each exposure group at 4 or 26 weeks. Each wash sample was centrifuged separately. Lactate dehydrogenase activity and albumin concentration were measured in the supernatant of the first lavage by the study laboratory, and then the lavage supernatants were combined for each animal and frozen at -70°C until shipment to Virginia Commonwealth University (VCU) for analysis. The cells from both lavages were combined, washed in Hank's balanced salt solution (HBSS), recentrifuged, and resuspended in approximately 1 mL HBSS for cell count, viability, and differential cell count determinations.

For lung and lymph node burden analysis, four or five tissue burden study male rats were pre-assigned from their cages at 1, 4, 8, 16, 26, and 39 weeks and wiped to remove excess test material. Rats were weighed, and the lung and lymph nodes (bronchial and mediastinal) were removed, weighed, processed, and analyzed for Si (blasting sand study) or Fe (specular hematite study) concentrations as described in the methods for the 2-week studies.

For the immunotoxicity studies, BAL fluid and blood were collected from eight core study males at 4, 16, 26, and 39 weeks and eight unimmunized special study females at 4 and 26 weeks. Serum was prepared at the study laboratory, and the BAL fluid and serum samples were frozen at -70°C and shipped on dry ice to VCU for analyses. In addition, spleens from the unimmunized special study females were collected and weighed, placed into tubes containing medium, and shipped on ice to VCU for next-day cell preparation. Serum and spleens were similarly collected from additional groups of eight immunized special study females at weeks 5 and 27 (females at 5 and 27 weeks had received tail vein injections of sheep red blood cells 4 days earlier) and shipped to VCU for analyses. Details of the immunotoxicity studies are presented in Appendix F. The parameters evaluated are listed in Appendix F.

Necropsies were performed on the male rats used for BAL studies at 4, 16, 26, and 39 weeks. Tissues for microscopic examination were harvested, fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 μm , and stained with hematoxylin and eosin. The lung as well as mediastinal and bronchial lymph nodes were weighed, and histopathological examinations were performed on selected tissues. Table 2 lists the tissues and organs examined.

After a review of the laboratory reports and selected histopathology slides by a quality assessment (QA) pathologist, the findings and reviewed slides were submitted to an NTP Pathology Working Group (PWG) coordinator for a second independent review. Any inconsistencies in the diagnoses made by the study laboratory and QA pathologists were resolved by the NTP pathology peer review process. Final diagnoses for reviewed lesions represent a consensus of the PWG or a consensus between the study laboratory pathologist, NTP

pathologist, QA pathologist(s), and the PWG coordinator. Details of these review procedures have been described, in part, by Maronpot and Boorman⁵² and Boorman et al.⁵³

Table 2. Experimental Design and Materials and Methods in the Inhalation Studies of Abrasive Blasting Agents

Two-week Studies	Thirty-nine-week Studies
Study Laboratory	
Battelle Toxicology Northwest (Richland, WA)	Battelle Toxicology Northwest (Richland, WA)
Strain and Species	
F344/NTac rats	Hsd: Sprague Dawley® SD® rats
Animal Source	
Taconic Farms, Inc. (Germantown, NY)	Harlan Laboratories, Inc. (Livermore, CA)
Time Held Before Studies	
Blasting sand: 11 days Coal slag: 12 days Crushed glass: 11 days Garnet: 12 days Specular hematite: 12 days	Blasting sand: 11 days Specular hematite: 12 days
Average Age When Studies Began	
5 weeks	Blasting sand: 6 to 7 weeks Specular hematite: 5 to 6 weeks
Date of First Exposure	
Blasting sand: October 10, 2005 Coal slag: December 5, 2005 Crushed glass: June 12, 2006 Garnet: February 27, 2006 Specular hematite: August 14, 2006	Blasting sand: April 20, 2009 Specular hematite: July 27, 2009
Duration of Exposure	
6 hours plus T ₉₀ (12 minutes) per day, 5 days per week, for 16 days	6 hours plus T ₉₀ (12 minutes) per day, 5 days per week, for up to 27 (females) or 39 (males) weeks
Date of Last Exposure	
Blasting sand: October 25, 2005 Coal slag: December 20, 2005 Crushed glass: June 27, 2006 Garnet: March 14, 2006 Specular hematite: August 29, 2006	Blasting sand: January 15, 2010 Specular hematite: April 23, 2010
Necropsy Dates	
Blasting sand: October 25, 2005 Coal slag: December 20, 2005 Crushed glass: June 27, 2006 Garnet: March 14, 2006 Specular hematite: August 29, 2006	Blasting sand: January 15, 2010 Specular hematite: April 23, 2010
Average Age at Necropsy	
7 weeks	45 weeks

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Two-week Studies	Thirty-nine-week Studies
Size of Study Groups	
5 males (core study)	32 males (core study)
35 males (tissue burden study)	30 males (tissue burden study)
	32 females (special studies)
Method of Distribution	
Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 2-week studies
Animals per Cage	
1	1
Method of Animal Identification	
Tail tattoo	Tail tattoo
Diet	
Irradiated NTP-2000 open formula pelleted diet (Zeigler Brothers, Inc., Gardners, PA), available ad libitum, except during exposure periods, changed daily (crushed glass, specular hematite) or weekly (blasting sand, coal slag, garnet)	Same as 2-week studies, except changed daily
Water	
Tap water (Richland municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI), available ad libitum	Same as 2-week studies
Cages	
Stainless-steel wire bottom (Lab Products, Inc., Seaford, DE), changed weekly, rotated on exposure days	Same as 2-week studies, except rotated weekly
Cageboard	
Untreated paper (Shepherd Specialty Papers, Kalamazoo, MI), changed daily	Untreated paper (Shepherd Specialty Papers, Watertown, TN), changed daily
Chamber Air Supply Filters	
Single HEPA (open stock); charcoal (RSE, Inc., New Baltimore, MI); Purafil (Environmental Systems, Linwood, WA), new at study start	Same as 2-week studies
Chambers	
Stainless-steel, excreta pan at each of six levels (Lab Products, Inc., Seaford, DE), chambers changed weekly; excreta pans changed daily	Same as 2-week studies
Chamber Environment	
Temperature: 72°F ± 3°F	Same as 2-week studies
Relative humidity: 50% ± 15%	
Room fluorescent light: 12 hours/day	
Chamber air changes: 15/hour	

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Two-week Studies	Thirty-nine-week Studies
Exposure Concentrations Blasting sand: 0, 3, 15, or 30 mg/m ³ Coal slag: 0, 3, 15, or 30 mg/m ³ Crushed glass: 0, 3, 15, or 30 mg/m ³ Garnet: 0, 3, 15, or 30 mg/m ³ Specular hematite: 0, 3, 15, 30, or 60 mg/m ³	Blasting sand: 0, 15, 30, or 60 mg/m ³ Specular hematite: 0, 15, 30, or 60 mg/m ³
Type and Frequency of Observation Observed twice daily; core study animals were weighed initially, on days 6 and 13, and at the end of the studies; clinical observations were recorded daily.	Observed twice daily; males were weighed initially, weekly for 16 weeks, monthly thereafter, and at the end of the studies. Clinical observations for males were recorded weekly beginning day 8 for 16 weeks, then monthly and at the end of the studies.
Method of Kill Carbon dioxide asphyxiation	Intraperitoneal injection of pentobarbital
Necropsy Necropsies were performed on core study rats. The lungs and mediastinal and bronchial lymph nodes were weighed.	Necropsies were performed on eight core study male rats per exposure group at 4, 16, 26, and 39 weeks. The lungs and mediastinal and bronchial lymph nodes were weighed.
Histopathology Histopathology was performed on all core study rats. In addition to gross lesions and tissue masses the following tissues were examined: larynx, lung, lymph nodes (bronchial and mediastinal), nose, pharynx, and trachea.	Histopathology was performed on eight male rats per exposure group at 4, 16, 26, and 39 weeks. In addition to gross lesions and tissue masses the following tissues were examined: larynx, left lung lobe, lymph nodes (bronchial and mediastinal), nose, pharynx, and trachea.
Bronchoalveolar Lavage Studies None	Bronchoalveolar lavage fluid was collected from the right lung lobe of eight core study male rats per exposure group at 4, 16, 26, and 39 weeks and whole lung from eight special study female rats per exposure group at 4 and 26 weeks and evaluated for albumin concentration, lactate dehydrogenase activity, and cell viability, counts, and differentials.
Tissue Burden Studies Lungs and bronchial and mediastinal lymph nodes were weighed, and lungs were analyzed for silicon (blasting sand, coal slag, crushed glass, garnet) or iron (specular hematite) concentrations from five male rats per exposure group on days 8 and 15 prior to daily exposure; on days 1, 5, 12, and 16 after daily exposure; and on day 37 after 3 weeks of recovery.	Lungs and bronchial and mediastinal lymph nodes from four or five tissue burden male rats per exposure group at 1, 4, 8, 16, 26, and 39 weeks were weighed and analyzed for concentrations of silicon (blasting sand) or iron (specular hematite).

Two-week Studies	Thirty-nine-week Studies
<p>Immunotoxicity Studies</p> <p>None</p>	<p>For each exposure group, blood was collected from eight core study males at 4, 16, 26 and 39 weeks and eight special study females at 4, 5, 26, and 27 weeks (immunized females at 5 and 27 weeks had received tail vein injections of sheep red blood cells 4 days earlier). Splens from all special study females were collected and weighed. Aliquots of the BAL fluid collected from core study male and unimmunized special study females were evaluated for cytokine levels. See Appendix F for detailed methods and parameters measured.</p>

Statistical Methods

Calculation and Analysis of Lesion Incidences

The incidences of lesions were calculated as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. The Fisher exact test,⁵⁴ a procedure that incorporates the overall proportion of affected animals, was used to determine significance.

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which historically have had approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett⁵⁵ and Williams.^{56; 57} Bronchoalveolar lavage and tissue concentration data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley⁵⁸ (as modified by Williams⁵⁹) and Dunn.⁶⁰ The Jonckheere test⁶¹ was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (the Williams or Shirley test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (the Dunnett or Dunn test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey⁶² were examined by NTP personnel, and implausible values were eliminated from the analysis. P values less than 0.05 were considered statistically significant.

For the immunotoxicity studies, data were evaluated for homogeneity using the Bartlett test.⁶³ Homogeneous data were analyzed using a one-way analysis of variance,⁶⁴ and the Dunnett test⁵⁵ was used to determine differences between the control groups and the experimental groups. For nonhomogenous data, a nonparametric analysis of variance was used,⁶⁵ and differences between the control group and the experimental groups were determined by the Wilcoxon Rank Test.⁶⁶ The Fisher exact test⁶⁷ was used to analyze the incidence data in the antinuclear antibody enzyme-linked immunosorbent assay (ANA ELISA) studies. The Jonckheere test⁶¹ was used to test for exposure-related trends across the control and the blasting sand exposure groups.

Quality Assurance Methods

The 2-week and 39-week studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations.⁶⁸ In addition, the 39-week study reports were audited retrospectively by an independent QA contractor against study records submitted to the NTP archives. Separate audits covered completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Toxicity Report. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed by NTP staff, and all comments were resolved or otherwise addressed during the preparation of this Toxicity Report.

Results

Data Availability

The National Toxicology Program (NTP) evaluated all study data. Data relevant for evaluating toxicological findings are presented here. All study data are available in the NTP Chemical Effects in Biological Systems (CEBS) database: <https://doi.org/10.22427/NTP-DATA-TOX-91>.⁶⁹

Two-week Studies of Abrasive Blasting Agents in Male F344/NTac Rats

Blasting Sand

All core study rats survived to the end of the study; no significant differences were observed in mean body weights or body weight gains between exposed groups and the chamber control group (Table 3). No clinical observations were related to blasting sand exposure.

Absolute and relative lung, bronchial lymph node, and mediastinal lymph node weights of core study and tissue burden rats exposed to blasting sand were similar to those of the chamber control group (Table 4). For all five of the 2-week studies, only the day 16 body weight data for the core and tissue burden rats are shown in Table 4.

Blasting sand lung burdens continued to increase through the last exposure day (day 16) indicating that steady-state lung burdens were not achieved during the study (Table B-1). The calculated clearance rate of blasting sand was slower and the clearance half-life longer in the 3 mg/m³ group compared to the 15 and 30 mg/m³ groups (Table B-3). Clearance half-life values were 93, 35, and 33 days for the 3, 15, and 30 mg/m³ groups, respectively (Table B-3). Blasting sand lung burdens in the total lung decreased approximately 15% in the 3 mg/m³ group and approximately 34% to 36% in the 15 and 30 mg/m³ groups during recovery (Table B-1). Blasting sand lung burdens at the end of the exposure interval (day 16) were approximately 124, 581, and 1,337 µg blasting sand/g lung for rats exposed to 3, 15, and 30 mg/m³, respectively (Table B-1). Steady-state lung burdens for blasting sand were 975, 1,993, and 3,931 µg blasting sand/total lung in the 3, 15, and 30 mg/m³ groups, respectively (Table B-3). Blasting sand burdens for the mediastinal and bronchial lymph nodes were indistinguishable from solvent blanks (Table B-3).

Minimal histiocytic cellular infiltration^{70; 71} occurred in the alveolus of the lung of exposed groups of rats, and the incidence in the 30 mg/m³ group was significantly greater than that in the chamber control group (Table 5). This lesion was characterized by an increase in the number of alveolar macrophages diffusely scattered throughout the lungs. The test article was visible under polarized light in the cytoplasm of some of the macrophages.

Table 3. Survival and Body Weights of Male F344/NTac Rats in the Two-week Inhalation Studies of Abrasive Blasting Agents^a

Concentration (mg/m ³)	Survival ^b	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Control Groups (%)
Blasting Sand					
0	5/5	100 ± 3	159 ± 3	59 ± 2	
3	5/5	100 ± 3	151 ± 5	51 ± 3	95
15	5/5	100 ± 3	154 ± 4	53 ± 2	97
30	5/5	100 ± 3	156 ± 2	55 ± 2	98
Coal Slag					
0	5/5	85 ± 2	141 ± 2	56 ± 3	
3	5/5	86 ± 3	145 ± 5	59 ± 4	103
15	5/5	85 ± 3	143 ± 3	58 ± 1	102
30	5/5	87 ± 3	142 ± 3	55 ± 4	100
Crushed Glass					
0	5/5	87 ± 4	146 ± 5	59 ± 3	
3	5/5	86 ± 4	144 ± 4	58 ± 4	99
15	5/5	87 ± 4	148 ± 5	61 ± 3	101
30	5/5	86 ± 4	148 ± 3	62 ± 2	101
Garnet					
0	5/5	91 ± 1	146 ± 3	55 ± 3	
3	5/5	91 ± 1	154 ± 4	63 ± 3	105
15	5/5	92 ± 1	157 ± 4	64 ± 4	107
30	5/5	91 ± 0	150 ± 4	59 ± 4	103
Specular Hematite					
0	5/5	94 ± 3	146 ± 5	52 ± 4	
3	5/5	92 ± 3	147 ± 3	55 ± 2	101
15	5/5	91 ± 2	148 ± 5	58 ± 3	102
30	5/5	91 ± 4	143 ± 5	53 ± 2	98
60	5/5	91 ± 2	143 ± 4	51 ± 5	98

^aWeights and weight changes are given as mean ± standard error. Differences from the chamber control group are not significant by the Dunnett test.

^bNumber of animals surviving at 16 days/number initially in group.

Table 4. Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male F344/NTac Rats in the Two-week Inhalation Studies of Abrasive Blasting Agents^a

	Chamber Control	3 mg/m ³	15 mg/m ³	30 mg/m ³	60 mg/m ³
n	5	5	5	5	5
Blasting Sand Core Study Rats					
Necropsy Body Wt.	159 ± 3	151 ± 5	154 ± 4	156 ± 2	–
Lung					
Absolute	1.13 ± 0.03	1.09 ± 0.08	1.25 ± 0.07	1.27 ± 0.10	–
Relative	7.10 ± 0.16	7.15 ± 0.28	8.19 ± 0.61	8.18 ± 0.59	–
Blasting Sand Tissue Burden Rats^b					
Day 16 Body Wt.	146 ± 4	153 ± 5	158 ± 3	160 ± 4*	–
Lung					
Absolute	0.94 ± 0.05	0.89 ± 0.07	0.93 ± 0.06	0.86 ± 0.05 ^c	–
Relative	6.44 ± 0.17	5.82 ± 0.33	5.86 ± 0.43	5.37 ± 0.43 ^c	–
Bronchial Lymph Node					
Absolute	0.016 ± 0.004	0.017 ± 0.004	0.017 ± 0.003	0.016 ± 0.004	–
Relative	0.11 ± 0.03	0.11 ± 0.03	0.11 ± 0.02	0.10 ± 0.02	–
Mediastinal Lymph Node					
Absolute	0.045 ± 0.008	0.035 ± 0.003	0.038 ± 0.003	0.041 ± 0.005	–
Relative	0.31 ± 0.05	0.23 ± 0.02	0.24 ± 0.02	0.26 ± 0.03	–
Coal Slag Core Study Rats					
Necropsy Body Wt.	141 ± 2	145 ± 5	143 ± 3	142 ± 3	–
Lung					
Absolute	1.11 ± 0.06	1.36 ± 0.19	1.41 ± 0.18	1.17 ± 0.05	–
Relative	7.87 ± 0.34	9.29 ± 1.24	9.84 ± 1.27	8.25 ± 0.25	–
Coal Slag Tissue Burden Rats					
Day 16 Body Wt.	137 ± 3	141 ± 4	135 ± 2	139 ± 6	–
Lung					
Absolute	0.73 ± 0.02	0.73 ± 0.04	0.79 ± 0.03	0.88 ± 0.06*	–
Relative	5.34 ± 0.08	5.19 ± 0.18	5.87 ± 0.28	6.37 ± 0.48*	–
Bronchial Lymph Node					
Absolute	0.010 ± 0.001	0.011 ± 0.001	0.009 ± 0.002	0.016 ± 0.005	–
Relative	0.07 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.11 ± 0.04	–
Mediastinal Lymph Node					
Absolute	0.042 ± 0.004	0.035 ± 0.003	0.033 ± 0.003	0.039 ± 0.003	–
Relative	0.30 ± 0.03	0.25 ± 0.03	0.25 ± 0.02	0.28 ± 0.01	–

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	Chamber Control	3 mg/m ³	15 mg/m ³	30 mg/m ³	60 mg/m ³
Crushed Glass Core Study Rats					
Necropsy Body Wt.	146 ± 5	144 ± 4	148 ± 5	148 ± 3	–
Lung					
Absolute	1.07 ± 0.05	1.10 ± 0.03	1.27 ± 0.07*	1.22 ± 0.05*	–
Relative	7.33 ± 0.31	7.63 ± 0.30	8.67 ± 0.57	8.31 ± 0.44	–
Crushed Glass Tissue Burden Rats					
Day 16 Body Wt.	140 ± 4	140 ± 4	144 ± 2	146 ± 6	–
Lung					
Absolute	0.88 ± 0.04	0.92 ± 0.08	0.82 ± 0.02	0.86 ± 0.04	–
Relative	6.37 ± 0.46	6.52 ± 0.43	5.68 ± 0.10	5.92 ± 0.36	–
Bronchial Lymph Node					
Absolute	0.018 ± 0.002	0.013 ± 0.002	0.013 ± 0.001	0.016 ± 0.002	–
Relative	0.13 ± 0.02	0.10 ± 0.02	0.09 ± 0.01	0.11 ± 0.02	–
Mediastinal Lymph Node					
Absolute	0.030 ± 0.004	0.028 ± 0.001	0.032 ± 0.004	0.032 ± 0.005	–
Relative	0.21 ± 0.03	0.20 ± 0.01	0.22 ± 0.03	0.22 ± 0.03	–
Garnet Core Study Rats					
Necropsy Body Wt.	146 ± 3	154 ± 4	157 ± 4	150 ± 4	–
Lung					
Absolute	1.14 ± 0.07	1.12 ± 0.07	1.28 ± 0.05	1.29 ± 0.09	–
Relative	7.84 ± 0.62	7.30 ± 0.49	8.18 ± 0.31	8.62 ± 0.52	–
Garnet Tissue Burden Rats					
Day 16 Body Wt.	145 ± 6	155 ± 3	156 ± 4	145 ± 4	–
Lung					
Absolute	0.82 ± 0.05	0.81 ± 0.04	0.83 ± 0.02	0.84 ± 0.02	–
Relative	5.63 ± 0.15	5.26 ± 0.31	5.33 ± 0.18	5.77 ± 0.16	–
Bronchial Lymph Node					
Absolute	0.012 ± 0.001	0.017 ± 0.004	0.021 ± 0.003	0.013 ± 0.002	–
Relative	0.09 ± 0.01	0.11 ± 0.03	0.14 ± 0.02	0.09 ± 0.01	–
Mediastinal Lymph Node					
Absolute	0.052 ± 0.006	0.054 ± 0.006	0.051 ± 0.007	0.041 ± 0.003	–
Relative	0.36 ± 0.05	0.35 ± 0.04	0.32 ± 0.04	0.29 ± 0.02	–
Specular Hematite Core Study Rats					
Necropsy Body Wt.	146 ± 5	147 ± 3	148 ± 5	143 ± 5	143 ± 4
Lung					

Abrasive Blasting Agents, TOX 91

	Chamber Control	3 mg/m³	15 mg/m³	30 mg/m³	60 mg/m³
Absolute	1.19 ± 0.13	1.12 ± 0.06	1.18 ± 0.07	1.15 ± 0.07	1.38 ± 0.17
Relative	8.13 ± 0.73	7.65 ± 0.29	7.98 ± 0.31	8.08 ± 0.54	9.61 ± 1.06
Specular Hematite Tissue Burden Rats					
Day 16 Body Wt.	138 ± 5	138 ± 5	143 ± 2	134 ± 4	139 ± 3
Lung					
Absolute	0.81 ± 0.04	0.78 ± 0.02	0.87 ± 0.04	0.83 ± 0.11	0.79 ± 0.04
Relative	5.89 ± 0.10	5.67 ± 0.21	6.07 ± 0.19	6.21 ± 0.73	5.68 ± 0.25
Bronchial Lymph Node					
Absolute	0.018 ± 0.003	0.017 ± 0.002	0.015 ± 0.002	0.013 ± 0.001	0.012 ± 0.003
Relative	0.13 ± 0.02	0.12 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.09 ± 0.02
Mediastinal Lymph Node					
Absolute	0.029 ± 0.003	0.031 ± 0.002	0.033 ± 0.004	0.034 ± 0.005	0.030 ± 0.003
Relative	0.21 ± 0.02	0.23 ± 0.02	0.23 ± 0.03	0.26 ± 0.04	0.21 ± 0.02

*Significantly different ($p \leq 0.05$) from the chamber control group by the Williams or Dunnett tests.

^aOrgan weights (absolute weights) and body weights are given in grams and were recorded on day 16 after exposure for 5 days per week for 2 weeks plus 2 days (12 exposures); organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error). There was no 60 mg/m³ group for blasting sand, coal slag, crushed glass, or garnet.

^bLung and lymph node tissue burden data are in Appendix B.

^cn = 4.

Table 5. Incidences of Selected Nonneoplastic Lesions in Male F344/NTac Rats in the Two-week Inhalation Studies of Abrasive Blasting Agents

	Chamber Control	3 mg/m ³	15 mg/m ³	30 mg/m ³	60 mg/m ³
Blasting Sand					
Lung ^a	5	5	5	5	–
Alveolus, infiltration cellular, histiocyte ^b	1 (1.0) ^c	4 (1.0)	4 (1.0)	5** (1.0)	–
Coal Slag					
Lung	5	5	5	5	–
Infiltration cellular, histiocyte diffuse	0	0	5** (1.0)	5** (1.0)	–
Inflammation, focal	0	0	1 (1.0)	2 (1.0)	–
Proteinosis	0	1 (1.0)	2 (1.0)	0	–
Crushed Glass					
Nose	5	5	5	5	–
Hypertrophy, goblet cell	1 (1.0)	4 (1.3)	5** (1.6)	5** (2.0)	–
Larynx	5	5	5	5	–
Epiglottis, epithelial hyperplasia	0	1 (1.0)	5** (1.6)	5** (1.2)	–
Epiglottis, metaplasia, squamous	0	0	5** (1.0)	5** (1.0)	–
Epiglottis, inflammation	0	1 (1.0)	5** (1.0)	5** (1.0)	–
Garnet					
Lung	5	5	5	5	–
Inflammation, chronic active	0	2 (2.0)	5** (1.2)	5** (2.4)	–
Pigmentation	0	5** (1.0)	5** (2.0)	5** (2.0)	–
Specular Hematite					
Nose	5	5	5	5	5
Goblet cell, nasopharyngeal duct, hypertrophy	0	0	0	1 (1.0)	5** (1.0)
Lung	5	5	5	5	5
Foreign body material	0	5** (1.0)	5** (2.0)	5** (2.2)	5** (3.0)
Alveolus, infiltration cellular, histiocyte	0	0	2 (1.0)	5** (1.2)	5** (2.0)

**Significantly different ($p \leq 0.01$) from the chamber control group by the Fisher exact test.

^aNumber of animals with tissue examined microscopically; there was no 60 mg/m³ group for blasting sand, coal slag, crushed glass, or garnet.

^bNumber of animals with lesion.

^cAverage severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Coal Slag

All core study rats survived to the end of the study; no significant differences were observed in mean body weights or body weight gains between exposed groups and the chamber control group (Table 3). One rat in the 30 mg/m³ group had an ocular discharge on days 5 and 8; no other clinical observations associated with coal slag exposure were noted.

Sporadic increases (some statistically significant) were observed in the absolute and relative lung and bronchial and mediastinal lymph node weights in animals exposed to coal slag; however, there was no consistent pattern of increasing organ weights over time in the lung burden animals (Table 4). Furthermore, the lung weights from the core study animals were inconsistent with the lung weights from tissue burden animals. Consequently, the organ weight data were determined not to be toxicologically significant.

Coal slag lung burdens continued to increase through the last exposure day (day 16) indicating that steady-state lung burdens were not achieved during the study (Table B-4). The calculated clearance rate of coal slag was faster and the clearance half-life shorter in the 3 mg/m³ group compared to the 15 and 30 mg/m³ groups (Table B-6).

During the 21-day recovery period, coal slag lung burdens decreased approximately 28%, 23%, and 19% in the 3, 15, and 30 mg/m³ groups, respectively (Table B-4). Coal slag lung burdens at the end of the exposure interval (day 16) were approximately 162, 673, 1,104 µg coal slag/g lung for rats exposed to 3, 15, and 30 mg/m³ of coal slag, respectively (Table B-4). Steady-state lung burdens for coal slag were 524, 2,986, and 6,419 µg coal slag/total lung in the 3, 15, and 30 mg/m³ groups, respectively (Table B-6). Coal slag tissue burdens for the mediastinal and bronchial lymph nodes were indistinguishable from solvent blanks (Table B-6).

No gross lesions were associated with coal slag exposure. Microscopic lesions were limited to the lung (Table 5). Histiocytic cellular infiltration occurred in all animals in the 15 and 30 mg/m³ groups and was graded minimal in all cases; the lesion did not occur in any chamber control animals or those exposed to 3 mg/m³. Two rats from the 30 mg/m³ group and one rat from the 15 mg/m³ group had minimal, focal, chronic active inflammation. Two rats from the 15 mg/m³ group and one rat from the 3 mg/m³ group had minimal alveolar proteinosis.

Histiocytic cellular infiltration was characterized by increased numbers of alveolar macrophages within the alveoli. The chronic active inflammation was characterized as small, focal accumulations of macrophages and neutrophils with scant cellular debris within the alveolar spaces and interstitium. The alveolar proteinosis was characterized by small amounts of eosinophilic, proteinaceous material within some alveolar spaces.

Crushed Glass

All core study rats survived to the end of the study; no significant differences were observed in mean body weights or body weight gains between exposed groups and the chamber control group (Table 3). No clinical observations were associated with exposure to crushed glass.

The absolute lung weights of core study rats exposed to 15 or 30 mg/m³ were significantly increased approximately 19% and 14%, respectively, compared to that of the chamber control group (Table 4). Relative lung weights from these groups were approximately 18% and 13% greater than the chamber control group, respectively. The absolute and relative lung weights of tissue burden rats exposed to crushed glass were similar to those of the chamber control group.

Crushed glass lung burdens continued to increase through the last exposure day (day 16), indicating that steady-state lung burdens were not achieved during the study (Table B-7). The calculated clearance rate of crushed glass was faster and the clearance half-life shorter in the 3 mg/m³ group compared to the 15 and 30 mg/m³ groups (Table B-9). Clearance half-life values

were 10, 17, and 16 days for the 3, 15, and 30 mg/m³ groups, respectively (Table B-9). Burdens of crushed glass in the total lung were generally proportional to exposure concentration except when comparing the 3 mg/m³ group to the 15 and 30 mg/m³ groups at days 5 and 37 (Table B-7). During the 21-day recovery period, total lung crushed glass burdens, as measured in day 37 samples, decreased to approximately 22%, 42%, and 41% of the day 16 burdens in the 3, 15, and 30 mg/m³ groups, respectively (Table B-7). Crushed glass lung burdens at the end of the exposure interval (day 16) were approximately 61, 347, and 604 µg crushed glass/g lung for rats exposed to 3, 15, or 30 mg/m³, respectively (Table B-7). Steady-state lung burdens for crushed glass were 80, 586, and 1,035 µg crushed glass/total lung in the 3, 15, and 30 mg/m³ groups, respectively (Table B-9). These values were the lowest for all abrasive blasting agents tested. Comparable values for garnet were 474, 5,089, and 9,089 µg garnet/total lung for rats exposed to 3, 15, or 30 mg/m³, respectively (Table B-12). Crushed glass burdens for the mediastinal and bronchial lymph nodes were indistinguishable from solvent blanks (Table B-8).

No gross lesions were associated with crushed glass exposure. Microscopic lesions occurred in the nose and larynx (Table 5), but no microscopic lesions were seen in the lungs. All exposed rats, except for one in the 3 mg/m³ group, had minimal to mild goblet cell hypertrophy of the respiratory epithelium in the nose. One chamber control rat also had minimal respiratory epithelium goblet cell hypertrophy. The severity of the lesion increased with increasing exposure concentration. In the larynx, all animals in the 15 and 30 mg/m³ groups had respiratory epithelial hyperplasia and squamous metaplasia and chronic inflammation. The squamous metaplasia and inflammation were minimal in all cases, but a few cases of respiratory epithelial hyperplasia were considered mild. Additionally, in the 3 mg/m³ group, one rat had minimal respiratory epithelial hyperplasia, and one rat had minimal inflammation.

Goblet cell hypertrophy of the respiratory epithelium of the nose was characterized by goblet cells that were taller than those in the chamber control animals and contained increased amounts of mucin. The laryngeal lesions were seen mainly at the base of the epiglottis. Epithelial hyperplasia of the laryngeal epithelium was characterized by an increase in the number of epithelial cell layers (two to five layers as opposed to one to three layers in the chamber control groups). Squamous metaplasia was diagnosed when the normally cuboidal epithelial cells were flattened and elongated. Chronic inflammation was characterized most frequently by increased numbers of mononuclear cells in the lamina propria. In some rats in the 15 and 30 mg/m³ groups, superficial, focal, nodular expansions of the lamina propria contained mononuclear cells, fewer neutrophils, and prominent capillaries. These nodular expansions bulged into the laryngeal lumen and were covered by hyperplastic and squamous metaplastic epithelium.

Garnet

All core study rats survived to the end of the study; no significant differences were observed in mean body weights or body weight gains between exposed groups and the chamber control group (Table 3). No clinical observations were associated with garnet exposure.

Sporadic increases (some statistically significant) were observed in the absolute and relative lung and bronchial and mediastinal lymph node weights in animals exposed to garnet (Table 4); however, there was no consistent pattern of increasing organ weights over time in the lung burden animals. Furthermore, the lung weights from the core study animals were inconsistent

with the lung weights from tissue burden animals. Consequently, the organ weight data were determined not to be toxicologically significant.

Garnet lung burdens continued to increase through the last exposure day (day 16), indicating that steady-state lung burdens were not achieved during this study (Table B-10). The calculated clearance rate of garnet was faster and the clearance half-life shorter in the 3 mg/m³ group compared to the 15 and 30 mg/m³ groups (Table B-12). Garnet lung burdens in the total lung decreased approximately 34%, 15%, and 15% in the 3, 15, and 30 mg/m³ groups, respectively, between the last day of exposure (day 16) and the last day of recovery (day 37) (Table B-10). Garnet lung burdens at the end of the exposure interval (day 16) were approximately 160, 714, and 1,280 µg garnet/g lung for rats exposed to 3, 15, or 30 mg/m³, respectively (Table B-10). Garnet burdens for the mediastinal and bronchial lymph nodes were indistinguishable from solvent blanks (Table B-11).

No gross lesions were associated with garnet exposure. Microscopic lesions were observed in the lung only (Table 5). A treatment-related increase in the incidence of chronic active inflammation was observed, as the lesion occurred in two 3 mg/m³ rats and all 15 and 30 mg/m³ rats. The severity ranged from minimal to mild in the 3 and 15 mg/m³ groups and was mild to moderate in the 30 mg/m³ group. Minimal to mild pigmentation occurred in all exposed animals and was generally more severe in the 15 and 30 mg/m³ groups than in the 3 mg/m³ group.

Chronic active inflammation of the lung was characterized by a diffuse increase in alveolar macrophages with occasional neutrophils within alveolar spaces. Scattered foci of more intense inflammation with accumulations of lymphocytes, macrophages, neutrophils, fibrin, and cellular debris obscured the alveolar architecture. Numerous lymphocytes were also observed surrounding pulmonary vessels. Pigmentation was characterized by brown to black foreign bodies (granular material) within the cytoplasm of alveolar macrophages. The pigmented material is likely the test agent because this material was not seen in chamber control animals, it was present within macrophages, and it was more severe in animals exposed to higher concentrations of garnet.

Specular Hematite

All core study rats survived to the end of the study, and final mean body weights and body weight gains of all exposed groups were similar to those of the chamber control group (Table 3). No clinical observations were related to specular hematite exposure.

Differences between the exposed and chamber control core study groups in absolute and relative lung weights were not significant (Table 4). Sporadic increases (some statistically significant) were observed in the absolute and relative lung and bronchial and mediastinal lymph node weights in animals exposed to specular hematite. No consistent pattern was observed, however, of increasing organ weights over time in the lung burden animals (in fact, there were some statistically significant decreases in lung weight). Furthermore, the lung weights from the core study animals were inconsistent with the lung weights from tissue burden animals. Consequently, the organ weight data were determined not to be toxicologically significant.

Specular hematite lung burdens continued to increase through the last exposure day (day 16), indicating that steady-state lung burdens were not achieved during this study (Table B-13). The calculated clearance half-lives were 48, 30, 50, and 44 days for the 3, 15, 30, and 60 mg/m³

groups, respectively (Table B-15). During the 21-day recovery period, 26%, 38%, 25%, and 28% of the specular hematite deposited in the lungs was eliminated in the 3, 15, 30, and 60 mg/m³ groups, respectively (Table B-13). Specular hematite lung burdens at the end of the exposure interval (day 16) were approximately 149, 662, 1,126, and 2,282 µg specular hematite/g lung for rats exposed to 3, 15, 30, or 60 mg/m³, respectively (Table B-13). Steady-state lung burdens for specular hematite were 559, 1,868, 4,557, and 8,057 µg specular hematite/total lung in the 3, 15, 30, and 60 mg/m³ groups, respectively (Table B-15). Specular hematite burdens for the mediastinal and bronchial lymph nodes were indistinguishable from solvent blanks (Table B-14).

At necropsy, the lungs of all rats exposed to 60 mg/m³ and most rats exposed to 30 mg/m³ were tan in color. In the lung, the presence of foreign body material in all exposed groups and histiocytic cellular infiltration in the 30 and 60 mg/m³ groups were significantly greater than those in the chamber control group (Table 5). The severity of foreign body accumulation increased with increasing exposure concentration. This material was determined to be the test article because foreign body material was not seen in chamber control animals, it was present within macrophages, and it was more severe in animals exposed to higher concentrations of specular hematite. The incidence of goblet cell hypertrophy in the nasopharyngeal duct of the nose was significantly increased in 60 mg/m³ rats. The histiocytic cellular infiltration was characterized by an increase in the number of alveolar macrophages (histiocytes) within the lung. Many of these macrophages were enlarged and contained brown to golden-brown, refractile, granular material, determined to be foreign bodies, within the cytoplasm. Some of this material was also free in the alveoli. Goblet cell hypertrophy in the nasopharyngeal duct was characterized by an increase in the height of affected goblet cells, which were distended with mucus.

Rationale for Selection of Test Articles, Exposure Concentrations, and Exposure Duration for the 39-week Studies: Specular hematite and crushed glass appeared to be the least toxic of the four alternatives to blasting sand tested in the 2-week studies because of the absence of induced lung inflammation (observed for coal slag and garnet) and proteinosis (observed for coal slag), but crushed glass had relatively faster clearance rates (shorter clearance half-lives) compared to the other test articles. Crushed glass also appeared to be more reactive to the upper respiratory tract (larynx) than the other blasting agents. Therefore, specular hematite (instead of crushed glass) was selected as the alternative blasting agent to compare to blasting sand in separate 39-week studies in Sprague Dawley rats. Informed by the low lung toxicity and burdens in F344/NTac rats at the exposure concentrations tested in the 2-week inhalation studies, exposure concentrations of 0, 15, 30, and 60 mg/m³ were selected (3 mg/m³ was removed) for the 39-week studies. The 39-week time point was selected to allow adequate time for the development of an adverse histopathological response (including interstitial fibrosis) in the lung. Comparisons between the two agents could then be made after evaluating both the time for the development of fibrosis and the severity of fibrosis over time.

Thirty-nine-week Studies of Abrasive Blasting Agents in Sprague Dawley Rats

Blasting Sand

All male core study rats survived to the end of the study; mean body weights and body weight gains of all exposed groups were similar to those of the chamber control group throughout the study (Table 6 and Figure 1). No clinical observations were associated with exposure to blasting sand.

Table 6. Mean Body Weights and Survival of Male Sprague Dawley Rats in the 39-week Inhalation Study of Blasting Sand

Day	0 mg/m ³		15 mg/m ³		30 mg/m ³		60 mg/m ³				
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors
1	142	62	141	99	62	141	99	62	141	99	62
8 ^a	192	57	191	100	57	190	99	57	191	100	57
15	237	57	236	100	57	235	99	57	239	101	57
22	274	57	273	100	57	272	99	57	277	101	57
29 ^a	299	44	301	101	44	298	100	44	305	102	44
36	320	44	323	101	44	319	100	44	329	103	44
43	338	44	341	101	44	338	100	44	348	103	44
50	354	44	356	101	44	353	100	44	363	103	44
57 ^a	365	39	369	101	39	368	101	39	377	103	39
64	376	39	382	101	39	381	101	39	389	103	39
71	386	39	392	102	39	391	101	39	398	103	39
78	397	39	403	102	39	401	101	39	409	103	39
85	404	39	411	102	39	410	101	39	416	103	39
92	412	39	419	102	39	417	101	39	423	103	39
99	418	39	426	102	39	424	102	39	430	103	39
106	423	39	432	102	39	431	102	39	438	103	39
134 ^a	444	26	444	100	26	449	101	26	453	102	26
162	461	26	460	100	26	466	101	26	470	102	26
190 ^a	477	13	470	99	13	485	102	13	485	102	13
218	489	13	487	100	13	499	102	13	498	102	13
246	497	13	500	101	13	512	103	13	510	103	13

^aInterim evaluations occurred during weeks 1, 4, 8, 16, and 26.

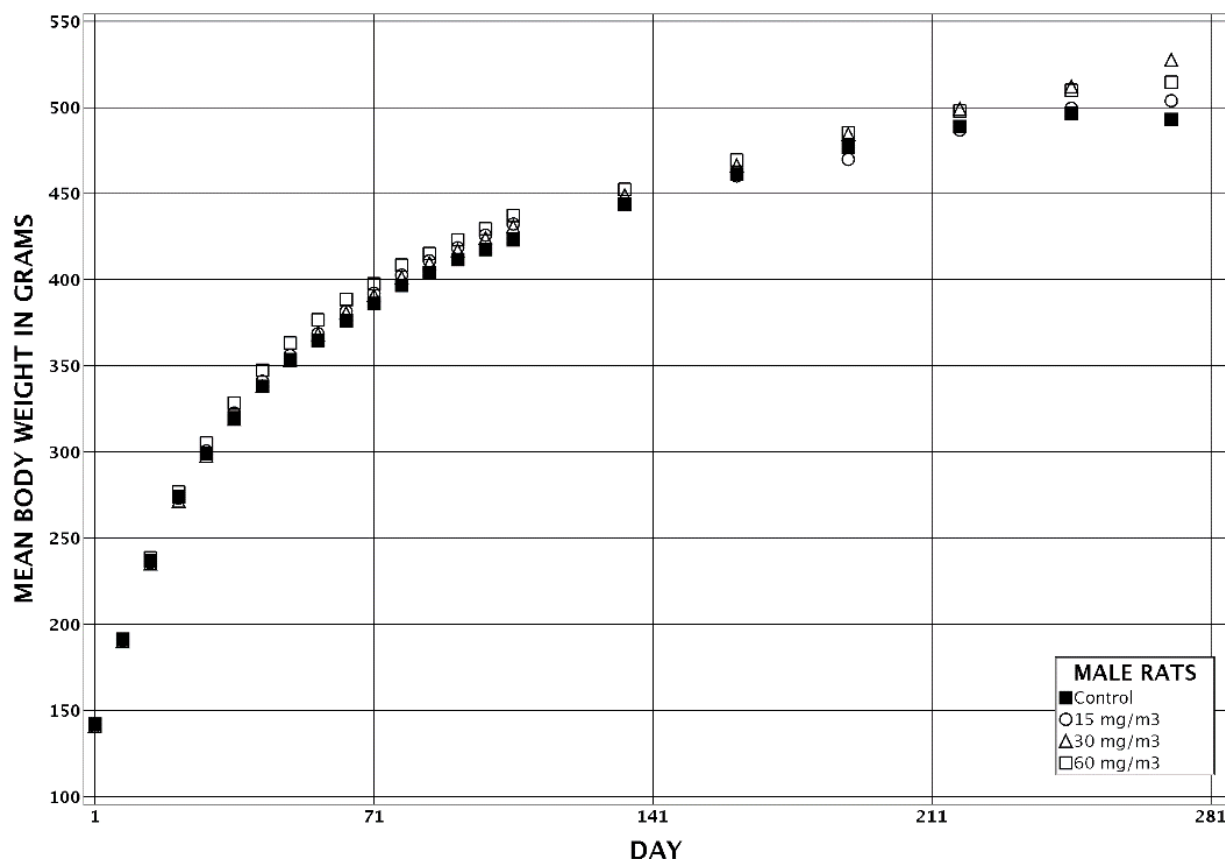


Figure 1. Growth Curves for Male Sprague Dawley Rats Exposed to Blasting Sand by Inhalation for 39 Weeks

Lungs and bronchial and mediastinal lymph nodes were weighed in male rats used for tissue burden analyses (Table 7). The absolute and relative lung weights in the 30 and 60 mg/m³ groups were significantly increased compared to those in the chamber control group beginning at week 16 or 8, respectively. The absolute and relative bronchial lymph node weights in the 60 mg/m³ group at all time points and in the 30 mg/m³ group at week 26 were significantly increased compared to those in the chamber control group. The absolute mediastinal lymph node weights were significantly increased at weeks 16, 26, and 39 in the 60 mg/m³ group, and the relative mediastinal lymph node weights were increased in this group at weeks 16 and 39.

Table 7. Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male Sprague Dawley Tissue Burden Rats in the 39-week Inhalation Study of Blasting Sand^a

	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³
Week 1				
n	5	5	5	5
Body Wt.	156 ± 3	167 ± 3	161 ± 6	161 ± 2
Lung				
Absolute	0.93 ± 0.03	0.98 ± 0.03	0.99 ± 0.05	0.98 ± 0.02

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	Chamber Control	15 mg/m³	30 mg/m³	60 mg/m³
Relative	5.95 ± 0.08	5.90 ± 0.12	6.17 ± 0.06	6.11 ± 0.09
Bronchial Lymph Node				
Absolute	0.012 ± 0.002	0.016 ± 0.005	0.016 ± 0.003	0.025 ± 0.003*
Relative	0.08 ± 0.01	0.09 ± 0.03	0.10 ± 0.02	0.16 ± 0.02*
Mediastinal Lymph Node				
Absolute	0.027 ± 0.003	0.034 ± 0.005	0.032 ± 0.005	0.032 ± 0.005
Relative	0.18 ± 0.02	0.20 ± 0.03	0.20 ± 0.03	0.20 ± 0.03
Week 4				
n	5	5	5	5
Body Wt.	277 ± 7	283 ± 8	283 ± 6	282 ± 7
Lung				
Absolute	1.18 ± 0.04	1.27 ± 0.03	1.28 ± 0.04	1.30 ± 0.04
Relative	4.27 ± 0.18	4.50 ± 0.18	4.55 ± 0.10	4.63 ± 0.10
Bronchial Lymph Node				
Absolute	0.020 ± 0.002	0.025 ± 0.003	0.022 ± 0.001	0.037 ± 0.004**
Relative	0.07 ± 0.01	0.09 ± 0.01	0.08 ± 0.00	0.13 ± 0.01**
Mediastinal Lymph Node				
Absolute	0.024 ± 0.004	0.032 ± 0.007	0.035 ± 0.005	0.033 ± 0.010
Relative	0.08 ± 0.01	0.11 ± 0.03	0.12 ± 0.02	0.12 ± 0.04
Week 8				
n	5	5	5	5
Body Wt.	363 ± 8	359 ± 13	346 ± 9	363 ± 10
Lung				
Absolute	1.34 ± 0.04	1.39 ± 0.04	1.39 ± 0.02	1.71 ± 0.05**
Relative	3.69 ± 0.08	3.86 ± 0.05	4.02 ± 0.07**	4.69 ± 0.07**
Bronchial Lymph Node				
Absolute	0.023 ± 0.002	0.022 ± 0.004	0.026 ± 0.007	0.043 ± 0.007*
Relative	0.06 ± 0.01	0.06 ± 0.01	0.08 ± 0.02	0.12 ± 0.02*
Mediastinal Lymph Node				
Absolute	0.032 ± 0.006	0.040 ± 0.007	0.043 ± 0.008	0.053 ± 0.008
Relative	0.09 ± 0.02	0.11 ± 0.02	0.12 ± 0.02	0.15 ± 0.02
Week 16				
n	5	5	5	5
Body Wt.	406 ± 21	427 ± 14	406 ± 11	438 ± 18
Lung				
Absolute	1.40 ± 0.05	1.55 ± 0.05	1.66 ± 0.05**	1.97 ± 0.07**

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	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³
Relative	3.45 ± 0.09	3.63 ± 0.05	4.09 ± 0.09**	4.51 ± 0.11**
Bronchial Lymph Node				
Absolute	0.017 ± 0.002	0.029 ± 0.006	0.045 ± 0.006	0.101 ± 0.018**
Relative	0.04 ± 0.01	0.07 ± 0.01	0.11 ± 0.01*	0.23 ± 0.03**
Mediastinal Lymph Node				
Absolute	0.024 ± 0.004	0.031 ± 0.003	0.038 ± 0.007	0.046 ± 0.006*
Relative	0.06 ± 0.01	0.07 ± 0.01	0.10 ± 0.02	0.11 ± 0.02*
Week 26				
n	4	5	5	5
Body Wt.	466 ± 22	472 ± 14	463 ± 9	475 ± 5
Lung				
Absolute	1.48 ± 0.02 ^b	1.67 ± 0.05	1.92 ± 0.04**	2.43 ± 0.15**
Relative	3.24 ± 0.15	3.54 ± 0.06	4.16 ± 0.13**	5.10 ± 0.28**
Bronchial Lymph Node				
Absolute	0.018 ± 0.002 ^b	0.039 ± 0.005	0.077 ± 0.018**	0.079 ± 0.008**
Relative	0.04 ± 0.00	0.08 ± 0.01	0.16 ± 0.04**	0.17 ± 0.02**
Mediastinal Lymph Node				
Absolute	0.030 ± 0.005 ^b	0.052 ± 0.007	0.075 ± 0.009	0.101 ± 0.037*
Relative	0.07 ± 0.01	0.11 ± 0.01	0.16 ± 0.02	0.21 ± 0.08
Week 39				
n	5	5	5	5
Body Wt.	507 ± 22	499 ± 17	495 ± 13	504 ± 4
Lung				
Absolute	1.63 ± 0.02	1.70 ± 0.04	1.96 ± 0.09*	2.73 ± 0.16**
Relative	3.24 ± 0.09	3.43 ± 0.14	3.96 ± 0.15*	5.41 ± 0.32**
Bronchial Lymph Node				
Absolute	0.015 ± 0.002	0.115 ± 0.013	0.089 ± 0.019	0.193 ± 0.063**
Relative	0.03 ± 0.01	0.23 ± 0.03	0.18 ± 0.04	0.38 ± 0.12**
Mediastinal Lymph Node				
Absolute	0.020 ± 0.001	0.034 ± 0.013	0.062 ± 0.017	0.072 ± 0.017*
Relative	0.04 ± 0.00	0.07 ± 0.02	0.12 ± 0.03	0.14 ± 0.03*

*Significantly different ($p \leq 0.05$) from the chamber control group by the Dunnett or Williams tests.

** $p \leq 0.01$.

^aOrgan weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

^bn = 5.

The greatest changes in measured bronchoalveolar lavage (BAL) fluid parameters for blasting sand occurred in pulmonary alveolar macrophages, neutrophils, lymphocytes, and lactate dehydrogenase (LDH) activity (Table 8 and Table A-1). Absolute numbers of macrophages, neutrophils, and lymphocytes and LDH activity were significantly increased in BAL fluid of core male rats, most notably at week 39 at the 60 mg/m³ blasting sand exposure concentration compared to the chamber control group. The changes in the exposed male rats generally increased in magnitude with exposure concentration and time, with the 60 mg/m³ group often most severely affected. Compared to those of the chamber control groups, total BAL fluid cell counts first increased at week 16 in all exposed groups and reached statistical significance at week 26 in the 60 mg/m³ group and at week 39 in the remaining exposed groups. The percentage of pulmonary alveolar macrophages in BAL fluid decreased in groups exposed to 30 or 60 mg/m³ starting at week 4 and decreased in all exposed groups starting at week 16. This decrease in the percent pulmonary alveolar macrophages resulted from cells, primarily neutrophils and a small number of lymphocytes, infiltrating the lung. Cytotoxic lung injury resulted from blasting sand exposures, as evident from elevated BAL fluid LDH concentrations as early as week 4 of exposure in the 60 mg/m³ group. Beginning at week 16, all exposed groups had increased BAL fluid LDH concentrations. The lung injury was not accompanied by an increase in protein exudation from the lung vasculature, as evident from the lack of increases in BAL fluid albumin. Trends in BAL fluid measurements in the female rats were generally similar to those in males.

Lung burdens of blasting sand were approximately 5, 10, and 15 mg blasting sand/g lung in the 15, 30, and 60 mg/m³ male rats, respectively, at 39 weeks and did not reach steady-state levels (Table B-16). Normalized lung burdens and calculated deposition rates for blasting sand were proportional to exposure concentrations (Table B-16 and Table B-19). The calculated lung deposition rates were 46, 98, and 172 µg/blasting sand/total lung per day, and the calculated lung clearance half-lives were 207, 271, and 848 days in groups exposed to 15, 30, and 60 mg/m³ blasting sand, respectively (Table B-19). Generally, blasting sand burdens in bronchial lymph nodes were greater than in mediastinal lymph nodes, however, they were both quite variable (Table B-17 and Table B-18). Due to the large blasting sand lung burdens, high deposition rates, and long lung clearance half-lives, the lung burdens were evaluated for possible lung overload (Table B-20). Lung overload is assumed to begin when the volume of blasting sand in the lung reaches a threshold volume equal to approximately 6% of the volume of the total lung alveolar macrophage pool.⁷² This condition was first reached at 137, 55, and 30 days in the 15, 30, and 60 mg/m³ groups, respectively. By the end of the study, lung blasting sand volumes exceeded the threshold for overload by factors of 1.6, 3.8, and 8.4 for the 15, 30, and 60 mg/m³ groups, respectively (Table B-20). Tissue burdens of blasting sand, although quite large, were proportional to exposure concentration in the lungs but were quite variable in the lymph nodes. The large lung burdens, the long lung half-lives, and the magnitude by which 6% of the alveolar macrophage lung volume was exceeded by the end of the study collectively indicated that lung overload conditions were reached for blasting sand at all exposure concentrations tested.

Table 8. Bronchoalveolar Lavage Data for Sprague Dawley Rats in the 39-week Inhalation Study of Blasting Sand^a

	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³
n	8	8	8	8
Male				
Viable Cells (%)				
Week 4	90.53 ± 1.32	90.45 ± 1.89	94.75 ± 1.26*	94.76 ± 0.70*
Week 16	70.20 ± 4.96	79.11 ± 4.31	77.96 ± 3.26	84.70 ± 2.26
Week 26	77.31 ± 2.77	87.55 ± 1.97*	87.26 ± 2.08	87.00 ± 1.91
Week 39	93.44 ± 2.17	92.21 ± 2.94	95.85 ± 1.06	98.19 ± 0.27
Total Cells Counted				
Week 4	248.8 ± 24.4	249.4 ± 23.2	280.6 ± 32.6	301.3 ± 30.6
Week 16	308 ± 67.0	413 ± 8.0	439 ± 73.0	502 ± 86.0
Week 26	186.3 ± 22.4	184.4 ± 28.7	262.5 ± 32.0	466.9 ± 52.4**
Week 39	128 ± 17.0	339 ± 65.0**	450 ± 64.0**	1,849 ± 301**
Macrophages (%)				
Week 4	99.6 ± 0.2	99.6 ± 0.2	92.8 ± 2.1**	61.9 ± 3.4**
Week 16	99.9 ± 0.1	75.5 ± 3.4**	49.7 ± 2.9**	51.2 ± 4.6**
Week 26	99.8 ± 0.1	69.9 ± 3.2**	48.7 ± 4.9**	33.3 ± 3.5**
Week 39	99.6 ± 0.2	57.0 ± 4.3**	53.4 ± 4.8**	43.6 ± 5.0**
Absolute Macrophages (#/μL)				
Week 4	247.8 ± 24.5	248.5 ± 23.2	260.1 ± 29.1	182.5 ± 13.5
Week 16	307.8 ± 67.5	308.6 ± 60.7	216.5 ± 35.5	270.2 ± 56.9
Week 26	185.9 ± 22.4	131.0 ± 23.4	118.5 ± 7.5	148.9 ± 12.8
Week 39	127.6 ± 17.1	182.7 ± 29.0	238.0 ± 39.8*	755.0 ± 88.4**
Lymphocytes (%)				
Week 4	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.163 ± 0.125
Week 16	0.038 ± 0.038	0.238 ± 0.121	0.213 ± 0.113	1.125 ± 0.397**
Week 26	0.000 ± 0.000	0.513 ± 0.205	0.588 ± 0.163**	1.138 ± 0.235**
Week 39	0.075 ± 0.049	0.613 ± 0.288	1.300 ± 0.306**	0.713 ± 0.215
Absolute Lymphocytes (#/μL)				
Week 4	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0388 ± 0.282
Week 16	0.100 ± 0.100	0.613 ± 0.361	1.013 ± 0.611	5.850 ± 2.092**
Week 26	0.000 ± 0.000	0.775 ± 0.291*	1.625 ± 0.448**	5.425 ± 1.350**
Week 39	0.113 ± 0.074	3.013 ± 1.943	5.663 ± 1.557**	11.663 ± 3.723**

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	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³
Neutrophils (%)				
Week 4	0.450 ± 0.154	0.375 ± 0.209	7.250 ± 2.069**	37.913 ± 3.405**
Week 16	0.075 ± 0.049	24.213 ± 3.419**	50.125 ± 2.935**	47.700 ± 4.788**
Week 26	0.200 ± 0.089	29.625 ± 3.159**	50.750 ± 4.756**	65.538 ± 3.461**
Week 39	0.325 ± 0.164	42.375 ± 4.191**	45.288 ± 4.850**	55.700 ± 4.975**
Absolute Neutrophils (#/μL)				
Week 4	0.950 ± 0.271	0.850 ± 0.385	20.563 ± 7.392**	118.463 ± 20.735**
Week 16	0.188 ± 0.123	102.963 ± 27.692**	221.288 ± 42.081**	255.875 ± 37.469**
Week 26	0.338 ± 0.129	52.588 ± 7.867**	142.350 ± 27.283**	312.600 ± 45.920**
Week 39	0.463 ± 0.221	153.113 ± 39.785**	206.413 ± 38.182**	1,082.09 ± 237.138**
Eosinophils (%)				
Week 4	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 16	0.000 ± 0.000	0.075 ± 0.049	0.000 ± 0.000	0.000 ± 0.000
Week 26	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 39	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Absolute Eosinophils (#/μL)				
Week 4	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 16	0.000 ± 0.000	0.300 ± 0.237	0.000 ± 0.000	0.000 ± 0.000
Week 26	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 39	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Lactate Dehydrogenase (IU/L)				
Week 4	34 ± 8	36 ± 4	40 ± 5	93 ± 7**
Week 16	31 ± 7	63 ± 6*	104 ± 7**	162 ± 16**
Week 26	36 ± 6	87 ± 7**	160 ± 21**	322 ± 26**
Week 39	19 ± 6	104 ± 29**	144 ± 26**	338 ± 24**
Albumin (g/dL)				
Week 4	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
Week 16	0.02 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 26	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.02
Week 39	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Female				
Viable Cells (%)				
Week 4	89.31 ± 1.66	90.10 ± 1.90	90.46 ± 0.66	91.35 ± 1.99
Week 26	86.49 ± 2.93	89.69 ± 2.20	90.00 ± 1.79	90.90 ± 1.83

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	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³
Total Cells Counted				
Week 4	446.9 ± 30.9	398.8 ± 24.2	403.1 ± 38.5	311.3 ± 24.6**
Week 26	294.4 ± 21.6	298.8 ± 31.4	592.5 ± 49.1**	768.1 ± 30.3**
Macrophages (%)				
Week 4	99.7 ± 0.1	99.8 ± 0.1	98.3 ± 0.5*	72.2 ± 5.9**
Week 26	99.4 ± 0.3	53.1 ± 4.0**	34.2 ± 3.5**	27.3 ± 2.8**
Absolute Macrophages (#/μL)				
Week 4	445.6 ± 31.1	397.9 ± 24.5	396.2 ± 37.5	222.1 ± 24.4**
Week 26	292.4 ± 21.0	158.1 ± 20.0**	196.8 ± 17.9*	207.7 ± 21.2
Lymphocytes (%)				
Week 4	0.163 ± 0.091	0.038 ± 0.038	0.363 ± 0.082	0.650 ± 0.155**
Week 26	0.038 ± 0.038	0.250 ± 0.109	0.413 ± 0.091**	0.200 ± 0.089
Absolute Lymphocytes (#/μL)				
Week 4	0.650 ± 0.358	0.125 ± 0.125	1.575 ± 0.296*	2.138 ± 0.523*
Week 26	0.138 ± 0.138	0.688 ± 0.271	2.538 ± 0.554**	1.675 ± 0.734*
Neutrophils (%)				
Week 4	0.150 ± 0.057	0.075 ± 0.049	0.963 ± 0.364	30.913 ± 5.685**
Week 26	0.538 ± 0.285	46.663 ± 4.075**	65.363 ± 3.427**	72.538 ± 2.769**
Absolute Neutrophils (#/μL)				
Week 4	0.663 ± 0.256	0.325 ± 0.220	3.938 ± 1.834	92.838 ± 17.475**
Week 26	1.800 ± 0.962	139.988 ± 21.328**	393.200 ± 46.620**	558.775 ± 34.693**
Eosinophils (%)				
Week 4	0.000 ± 0.000	0.125 ± 0.090	0.363 ± 0.240	0.375 ± 0.209
Week 26	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Absolute Eosinophils (#/μL)				
Week 4	0.000 ± 0.000	0.413 ± 0.297	1.413 ± 0.964	1.250 ± 0.715
Week 26	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Lactate Dehydrogenase (IU/L)				
Week 4	34 ± 4	30 ± 2	37 ± 4	67 ± 11**
Week 26	26 ± 2	87 ± 12**	235 ± 27**	420 ± 20**
Albumin (g/dL)				
Week 4	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01
Week 26	0.06 ± 0.02	0.10 ± 0.01	0.07 ± 0.01	0.05 ± 0.01

*Significantly different ($p \leq 0.05$) from the chamber control group by Dunn's or Shirley's test.

** $p \leq 0.01$.

^aData are presented as mean ± standard error.

No test article-related gross lesions were observed at necropsy in any of the exposed groups. Test article-related nonneoplastic lesions occurred in the lung, larynx, nose, and bronchial and mediastinal lymph nodes (Table 9).

Exposure-related lesions in the lung included histiocytic cellular infiltration, chronic active inflammation, interstitial fibrosis, foreign bodies (presumably the test article), proteinosis, and alveolar epithelial hyperplasia (Table 9).^{70; 71} The incidence of histiocytic cellular infiltration was significantly increased beginning at week 4 in the 60 mg/m³ group and at week 16 in the 15 and 30 mg/m³ groups compared to the chamber control incidence; severity of the lesion tended to increase with exposure concentration and duration. The incidence of chronic active inflammation was significantly increased in the 60 mg/m³ group beginning at week 16 and in the 15 and 30 mg/m³ groups beginning at week 26. Histiocytic cellular infiltration was characterized by increased numbers of alveolar macrophages within the alveoli. Many of the macrophages, particularly at the higher exposure concentrations, contained fine intracytoplasmic foreign bodies (granular material), presumably the test material. Chronic active inflammation was diagnosed when the increased numbers of macrophages were accompanied by scattered neutrophils and/or lymphocytes. With longer exposure duration and greater exposure concentration, the alveolar septa were often thickened by mononuclear inflammatory cells, fibrosis, and alveolar epithelial hyperplasia. Most of these lesions were graded according to the following scale: minimal: ≤5%; mild: greater than 5% but ≤35%; moderate: greater than 35% but ≤65%; and marked: greater than 65% of alveoli affected. Alveolar epithelial hyperplasia was considered minimal when less than 10% of alveolar spaces were lined by hyperplastic pneumocytes and mild when 10–30% of alveolar spaces were lined by hyperplastic pneumocytes.

The incidence of minimal to mild interstitial fibrosis was significantly increased in all exposed groups at weeks 26 and 39 compared to the chamber control group incidence (Table 9). Fibrosis was typically seen in areas also affected by chronic active inflammation, histiocytic cellular infiltration, or alveolar epithelium hyperplasia. Interstitium fibrosis was characterized by an increase in lacey collagen fibers in the alveolar septa. Masson's Trichrome staining was used to confirm the presence of interstitial fibrosis (Figure 2**Error! Reference source not found.**).

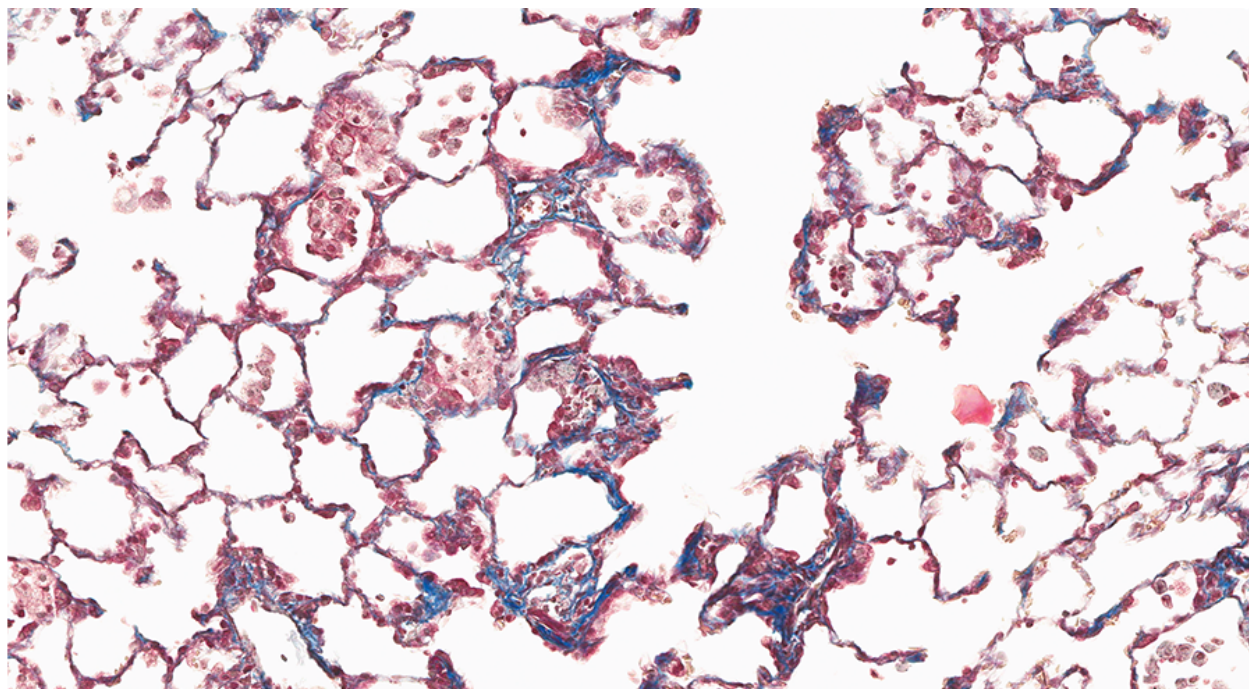


Figure 2. Pulmonary Interstitial Fibrosis in a Male Rat Administered 60 mg/m³ Blasting Sand by Whole Body Inhalation for 39 Weeks (Masson's Trichrome Stain)

The blue staining in the interalveolar septa is collagen, consistent with interstitial fibrosis.

All exposed male rats had foreign body material in the lung; this lesion was not seen in any chamber control rat (Table 9). Foreign bodies were characterized by a fine, golden-brown, granular material within the cytoplasm of alveolar macrophages or free within the alveolar spaces. The number of macrophages containing the material increased with increasing exposure concentration. The absence of the material in chamber control rats, the positive correlation between exposure concentration and the amount of the material present, and the morphological appearance of the material were consistent with the material being blasting sand.

Alveolar proteinosis was observed in one 60 mg/m³ rat at week 26 and six 60 mg/m³ rats at week 39, and the severity was slightly increased at week 39 (Table 9). Proteinosis was characterized by the presence of amorphous eosinophilic material within the alveolar spaces, often with fine, granular, golden-brown material, assumed to be foreign bodies.

Table 9. Incidences of Selected Nonneoplastic Lesions in Male Sprague Dawley Rats in the 39-week Inhalation Study of Blasting Sand

	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³
Four-week Interim Evaluation				
Nose ^a	8	8	8	8
Olfactory epithelium, accumulation, hyaline droplet ^b	0	2 (1.0) ^c	3 (1.1)	2 (1.0)
Lung	8	8	8	8
Infiltration cellular, histiocyte	1 (1.0)	1 (1.0)	4 (1.0)	8** (1.0)
Inflammation, chronic active	0	2 (1.0)	2 (1.0)	3 (1.0)
Foreign body material	0	8**	8**	8**
Alveolar epithelium, hyperplasia, focal	0	0	0	1 (3.0)
16-week Interim Evaluation				
Nose	8	8	8	8
Olfactory epithelium, accumulation, hyaline droplet	8 (1.0)	8 (1.6)	8 (1.4)	8 (2.4)
Respiratory epithelium, accumulation, hyaline droplet	0	8** (1.0)	8** (1.3)	7** (1.1)
Lung	8	8	8	8
Infiltration cellular, histiocyte	2 (1.0)	8** (1.4)	8** (2.0)	8** (2.0)
Inflammation, chronic active	1 (1.0)	3 (1.0)	3 (1.0)	8** (1.0)
Foreign body material	0	8**	8**	8**
Lymph Node, Bronchial	5	7	8	8
Foreign body material	0	7**	8**	7**
Infiltration cellular, histiocyte	0	7** (1.0)	8** (1.5)	7** (1.4)
Lymph Node, Mediastinal	8	6	8	8
Foreign body material	0	5**	5*	8**
Infiltration cellular, histiocyte	0	5** (2.2)	5* (2.4)	8** (3.0)
26-week Interim Evaluation				
Nose	8	8	8	8
Olfactory epithelium, accumulation, hyaline droplet	8 (1.9)	8 (2.8)	8 (2.9)	8 (2.6)
Respiratory epithelium, accumulation, hyaline droplet	6 (1.0)	8 (1.0)	8 (1.1)	8 (1.0)
Lung	8	8	8	8
Infiltration cellular, histiocyte	4 (1.0)	8* (1.5)	8* (1.8)	8* (2.1)
Inflammation, chronic active	2 (1.0)	7* (1.0)	7* (1.0)	8** (1.0)
Interstitial fibrosis	0	8** (1.0)	7** (1.0)	8** (1.6)
Foreign body material	0	8**	8**	8**
Proteinosis	0	0	0	1 (1.0)
Alveolar epithelium, hyperplasia	0	8** (1.0)	8** (1.0)	8** (1.0)

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	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³
Lymph Node, Bronchial	7	8	8	8
Foreign body material	0	6**	5*	8**
Infiltration cellular, histiocyte	0	6** (1.3)	5* (2.0)	8** (2.1)
Fibrosis	0	0	1 (1.0)	1 (1.0)
Lymph Node, Mediastinal	8	8	7	7
Foreign body material	0	6**	7**	7**
Infiltration cellular, histiocyte	0	6** (2.7)	7** (3.0)	7** (3.1)
Fibrosis	0	0	0	2 (1.5)
39-week Evaluation				
Nose	8	8	8	8
Olfactory epithelium, accumulation, hyaline droplet	8 (1.6)	8 (3.1)	8 (3.5)	8 (3.4)
Respiratory epithelium, accumulation, hyaline droplet	6 (1.0)	8 (1.0)	8 (1.3)	8 (1.0)
Larynx	8	8	8	8
Epiglottis, metaplasia, squamous	0	1 (1.0)	1 (1.0)	1 (1.0)
Lung	8	8	8	8
Infiltration cellular, histiocyte	4 (1.0)	8* (1.5)	8* (2.4)	8** (3.0)
Inflammation, chronic active	2 (1.0)	8** (1.0)	8** (1.1)	8** (1.6)
Interstitial, fibrosis	0	8** (1.0)	8** (1.0)	8** (2.0)
Foreign body material	0	8**	8**	8**
Proteinosis	0	0	0	6** (1.3)
Alveolar epithelial, hyperplasia	0	8** (1.0)	8** (2.0)	8** (2.0)
Alveolar epithelial, hyperplasia, focal	0	0	0	1 (4.0)
Lymph node, bronchial	7	5	7	8
Foreign body material	0	3*	6**	7**
Infiltration cellular, histiocyte	0	3* (1.7)	6** (1.3)	7** (2.1)
Fibrosis	0	0	0	2 (1.0)
Lymph node, mediastinal	7	8	8	8
Foreign body material	0	8**	8**	8**
Infiltration cellular, histiocyte	0	8** (3.0)	8** (4.0)	8** (4.0)
Fibrosis	0	1 (1.0)	1 (2.0)	2 (2.0)
Necrosis	0	0	0	1 (1.0)

*Significantly different ($p \leq 0.05$) from the chamber control group by the Fisher exact test.

** $p \leq 0.01$.

^aNumber of animals with tissue examined microscopically.

^bNumber of animals with lesion.

^cAverage severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Alveolar epithelial hyperplasia occurred in the lung of all exposed rats beginning at week 26 (Table 9). Most of these lesions were associated with chronic active inflammation and were indicative of a regenerative response of alveolar epithelium cells (Figure 3, Figure 4). Two animals in the 60 mg/m³ group (one at 4 weeks and one at the end of the study) had focal alveolar epithelial hyperplasia that was not associated with inflammation (Figure 5). Alveolar epithelial hyperplasia was characterized by cuboidal cells lining the alveolar septa in areas with inflammation. With more severe inflammation, the cuboidal alveolar epithelium cells were closely packed, and they were more sparsely distributed in areas with less severe inflammation. Focal alveolar epithelial hyperplasia had a similar appearance but occurred in areas with no inflammation, and the lesion was better demarcated than alveolar epithelial hyperplasia. Neither form of the lesion caused compression of adjacent tissue.

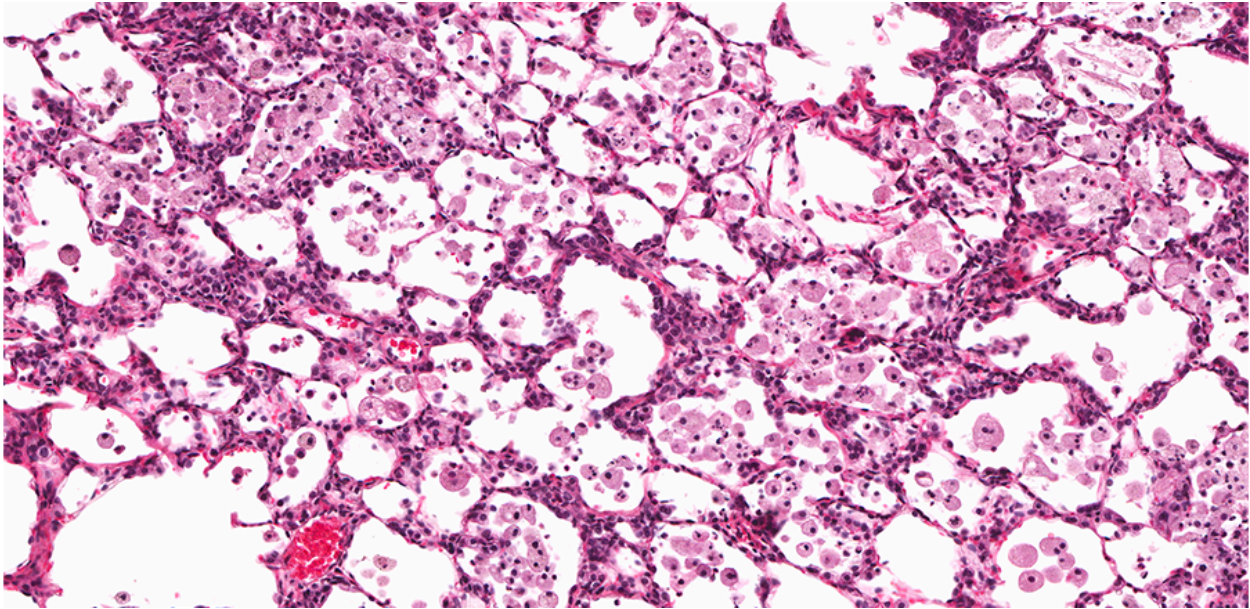


Figure 3. Alveolar Epithelial Hyperplasia in a Male Rat Administered 60 mg/m³ Blasting Sand by Whole Body Inhalation for 39 Weeks (H&E)

This form of alveolar epithelial hyperplasia, which accompanies inflammation in the lung, is thought to be secondary to inflammation and alveolar epithelial damage.

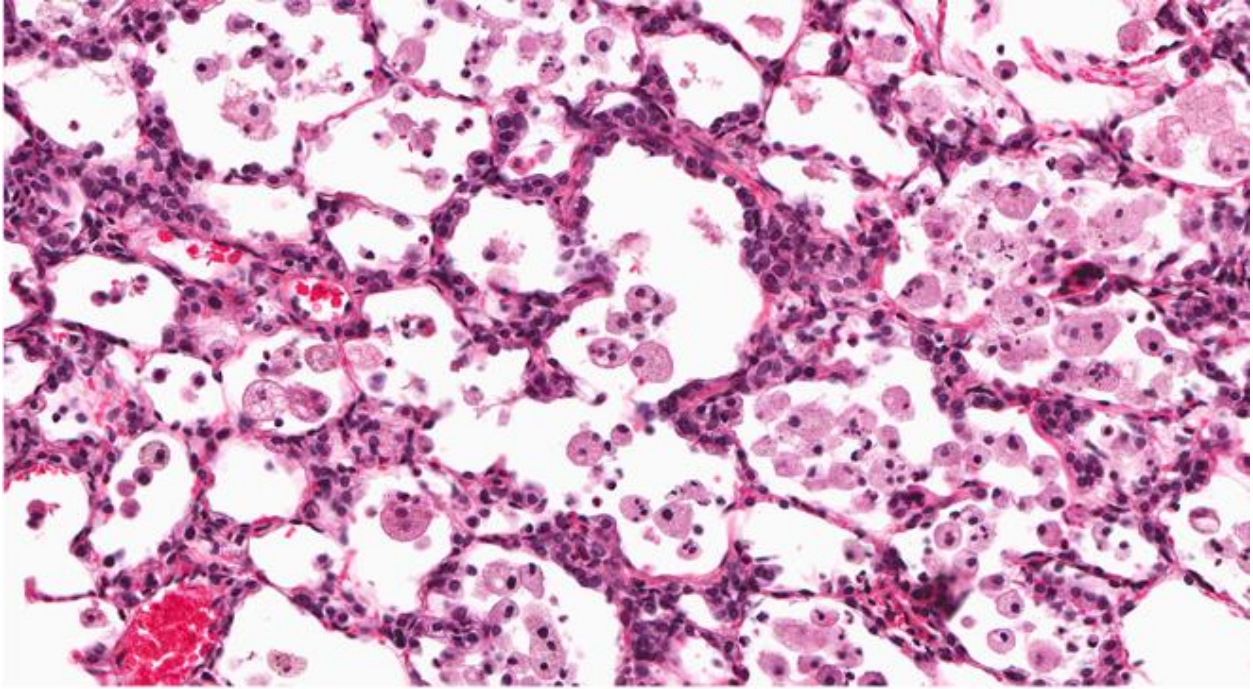


Figure 4. Alveolar Epithelial Hyperplasia in a Male Rat Administered 60 mg/m³ Blasting Sand by Whole Body Inhalation for 39 Weeks (H&E)

This is a higher magnification image of Figure 3.

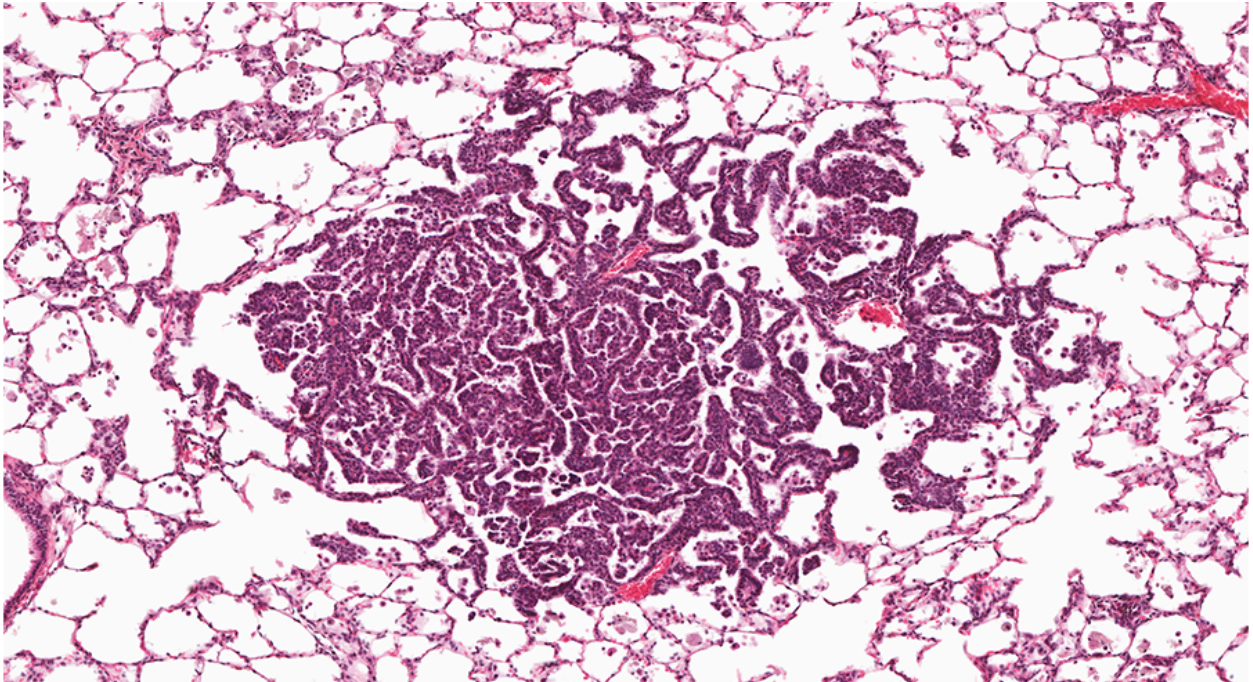


Figure 5. Focal Alveolar Epithelial Hyperplasia in a Male Rat Administered 60 mg/m³ Blasting Sand by Whole Body Inhalation for 39 Weeks (H&E)

This well-circumscribed area of alveolar epithelial hyperplasia is thought to be on a continuum with alveolar/bronchiolar neoplasia. Note the lack of accompanying inflammation.

In the larynx at 39 weeks, squamous metaplasia of the epithelium at the base of the epiglottis occurred in one rat from each exposed group (Table 9). This lesion rarely occurs as a background lesion and is the most common induced laryngeal lesion seen in inhalation studies. Therefore, although the incidence in any of the exposed groups was not significantly increased compared to that in the chamber control group, the occurrences of the lesion were considered exposure related. Squamous metaplasia of the laryngeal epithelium was characterized by replacement of the normally cuboidal epithelium by flattened and elongated epithelial cells.

Accumulations of hyaline droplets in the olfactory and respiratory epithelium were the only exposure-related lesions that occurred in the nose (Table 9). Although hyaline droplets are commonly seen in control animals, they can increase in size and have a greater distribution with exposure to various chemicals or particulates in inhalation studies. Hyaline droplet accumulation in the olfactory epithelium occurred in all rats, including chamber control animals, beginning at week 16, and the severity of the lesions increased with increasing exposure duration. The incidence of hyaline droplet accumulation in the respiratory epithelium was significantly increased in all exposed groups at 16 weeks, compared to the chamber control group incidence. Hyaline droplet accumulation in the olfactory and respiratory epithelium was noted primarily at Levels II and III of the nose and consisted of brightly eosinophilic intracytoplasmic globules that occasionally distorted cells and displaced nuclei. Locations commonly affected were the septum and dorsal meatus of Level II at the junction of the respiratory and olfactory epithelium and the ventral ethmoturbinates and, less often, the dorsal ethmoturbinates of Level III.

Exposure-related lesions in the bronchial and mediastinal lymph nodes included foreign bodies, histiocytic cellular infiltration, and fibrosis (Table 9). There was also necrosis in the mediastinal lymph node of one 60 mg/m³ rat at the end of the study.

The presence of foreign body material and histiocytic cellular infiltration in the bronchial and mediastinal lymph nodes of all exposed groups were significantly greater than those of the chamber control group beginning at week 16 (Table 9). In general, the severity of histiocytic cellular infiltration increased with increasing exposure concentration. Foreign body material and histiocytic cellular infiltration in the lymph nodes were morphologically similar to those lesions seen in the lung. Histiocytic cellular infiltration was characterized by increased numbers of macrophages within the sinuses and, scattered amid the lymphocytes, in the cortex and paracortex. Nearly all these macrophages contained fine, golden-brown to brown foreign bodies (granular material) within the cytoplasm. All the macrophages and foreign body material were presumed to have migrated to the lymph nodes from the lungs.

Minimal to mild fibrosis occurred in the bronchial and mediastinal lymph nodes of a few exposed rats beginning at week 26 (Table 9). The severity of the lesion in the mediastinal lymph node generally increased with increasing exposure concentration. Fibrosis was characterized by an increase in eosinophilic material (consistent with collagen) in the lymph node. Additionally, minimal necrosis, characterized by loss of cells and the presence of cellular debris, was present in an area of fibrosis in one rat from the 60 mg/m³ group at 39 weeks.

Specular Hematite

Two male rats, one chamber control and one exposed to 60 mg/m³, were removed from the study during week 37 for reasons unrelated to exposure to the test article; mean body weights of all exposed groups were similar to those of the chamber control group throughout the study (Table 10 and Figure 6. No clinical observations were associated with exposure to specular hematite.

Table 10. Mean Body Weights and Survival of Male Sprague Dawley Rats in the 39-week Inhalation Study of Specular Hematite

Day	0 mg/m ³		15 mg/m ³		30 mg/m ³		60 mg/m ³				
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors
1	123	62	122	99	62	123	100	62	121	98	62
8 ^a	169	57	169	100	57	170	100	57	168	99	57
16	220	57	220	100	57	222	101	57	219	100	57
23	257	57	258	100	57	263	102	57	259	101	57
30 ^a	282	44	287	102	44	292	103	44	288	102	44
37	301	44	306	102	44	313	104	44	308	102	44
44	320	44	326	102	44	331	103	44	328	103	44
51	337	44	342	101	44	347	103	44	345	102	44
58 ^a	350	39	352	101	39	361	103	39	359	103	39
65	361	39	365	101	39	373	103	39	370	103	39
72	373	39	376	101	39	384	103	39	380	102	39
79	381	39	385	101	39	394	103	39	392	103	39
86	391	39	394	101	39	403	103	39	401	103	39
93	398	39	401	101	39	411	103	39	409	103	39
100	406	39	410	101	39	418	103	39	416	103	39
107	413	39	414	100	39	424	103	39	423	102	39
135 ^a	439	26	435	99	26	442	101	26	445	101	26
163	457	26	452	99	26	461	101	26	465	102	26
191 ^a	470	13	473	101	13	488	104	13	466	99	13
219	488	13	492	101	13	501	103	13	481	99	13
248	501	13	507	101	13	512	102	13	496	99	13

^aInterim evaluations occurred during weeks 1, 4, 8, 16, and 26.

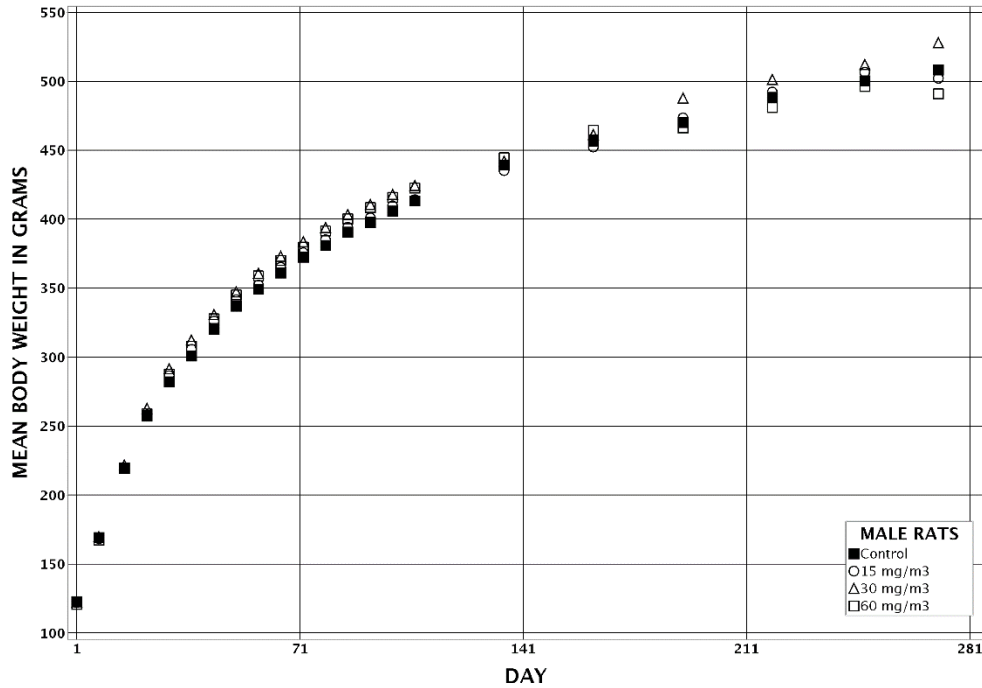


Figure 6. Growth Curves for Male Sprague Dawley Rats Exposed to Specular Hematite by Inhalation for 39 Weeks

Lungs and bronchial and mediastinal lymph nodes were weighed in male rats used for tissue burden analyses. Compared to those in the chamber control group, the absolute and relative lung weights were significantly increased in the 30 and 60 mg/m³ groups at weeks 16, 26, and 39 (Table 11). Absolute and relative bronchial lymph node weights were significantly increased at weeks 16, 26, and 39 in the 60 mg/m³ group and at week 26 in the 30 mg/m³ group. The absolute and relative mediastinal lymph node weights were significantly increased at weeks 16 and 26 in the 60 mg/m³ group but not at week 39.

Table 11. Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male Sprague Dawley Tissue Burden Rats in the 39-week Inhalation Study of Specular Hematite^a

	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³
Week 1				
n	5	5	5	5
Body Wt.	146 ± 5	140 ± 4	144 ± 4	144 ± 7
Lung				
Absolute	0.93 ± 0.02	0.87 ± 0.01	0.91 ± 0.03	0.87 ± 0.03
Relative	6.34 ± 0.09	6.24 ± 0.20	6.34 ± 0.17	6.08 ± 0.15
Bronchial Lymph Node				
Absolute	0.010 ± 0.002	0.011 ± 0.003	0.016 ± 0.003	0.011 ± 0.002
Relative	0.07 ± 0.01	0.08 ± 0.02	0.11 ± 0.02	0.08 ± 0.01
Mediastinal Lymph Node				
Absolute	0.018 ± 0.005	0.021 ± 0.003	0.026 ± 0.003	0.019 ± 0.001
Relative	0.13 ± 0.03	0.15 ± 0.02	0.18 ± 0.02	0.13 ± 0.00
Week 4				
n	5	5	5	5
Body Wt.	263 ± 8	273 ± 9	270 ± 8	266 ± 6
Lung				
Absolute	1.15 ± 0.03	1.16 ± 0.03	1.18 ± 0.04	1.17 ± 0.03
Relative	4.37 ± 0.05	4.27 ± 0.06	4.37 ± 0.08	4.41 ± 0.05
Bronchial Lymph Node				
Absolute	0.026 ± 0.005	0.010 ± 0.003	0.020 ± 0.006	0.021 ± 0.004
Relative	0.10 ± 0.02	0.04 ± 0.01*	0.07 ± 0.02	0.08 ± 0.01
Mediastinal Lymph Node				
Absolute	0.025 ± 0.003	0.024 ± 0.005	0.027 ± 0.005	0.023 ± 0.003
Relative	0.10 ± 0.01	0.09 ± 0.02	0.10 ± 0.02	0.08 ± 0.01
Week 8				
n	5	5	5	5
Body Wt.	339 ± 13	365 ± 8	357 ± 12	344 ± 11
Lung				
Absolute	1.30 ± 0.05	1.35 ± 0.06	1.36 ± 0.06	1.40 ± 0.03
Relative	3.85 ± 0.12	3.68 ± 0.09	3.81 ± 0.06	4.09 ± 0.09
Bronchial Lymph Node				
Absolute	0.019 ± 0.003	0.020 ± 0.003	0.031 ± 0.004	0.028 ± 0.006
Relative	0.06 ± 0.01	0.05 ± 0.01	0.09 ± 0.01	0.08 ± 0.02
Mediastinal Lymph Node				
Absolute	0.025 ± 0.004	0.025 ± 0.002	0.026 ± 0.005	0.035 ± 0.008
Relative	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.10 ± 0.02

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	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³
Week 16				
n	5	5	5	5
Body Wt.	406 ± 8	418 ± 4	436 ± 13	412 ± 11
Lung				
Absolute	1.31 ± 0.04	1.44 ± 0.03*	1.55 ± 0.04**	1.56 ± 0.04**
Relative	3.24 ± 0.10	3.44 ± 0.07	3.56 ± 0.03**	3.80 ± 0.06**
Bronchial Lymph Node				
Absolute	0.014 ± 0.003	0.015 ± 0.003	0.027 ± 0.004	0.036 ± 0.007**
Relative	0.04 ± 0.01	0.04 ± 0.01	0.06 ± 0.01	0.09 ± 0.02**
Mediastinal Lymph Node				
Absolute	0.019 ± 0.002	0.020 ± 0.003	0.029 ± 0.004	0.034 ± 0.005*
Relative	0.05 ± 0.01	0.05 ± 0.01	0.07 ± 0.01	0.08 ± 0.01*
Week 26				
n	5	5	5	5
Body Wt.	453 ± 13	456 ± 15	451 ± 7	492 ± 11
Lung				
Absolute	1.48 ± 0.04	1.52 ± 0.05	1.67 ± 0.04*	1.96 ± 0.05**
Relative	3.28 ± 0.12	3.34 ± 0.06	3.70 ± 0.10*	3.99 ± 0.15**
Bronchial Lymph Node				
Absolute	0.013 ± 0.002	0.023 ± 0.005	0.040 ± 0.004**	0.050 ± 0.006**
Relative	0.03 ± 0.01	0.05 ± 0.01	0.09 ± 0.01**	0.10 ± 0.01**
Mediastinal Lymph Node				
Absolute	0.019 ± 0.004	0.032 ± 0.007	0.030 ± 0.002	0.046 ± 0.002**
Relative	0.04 ± 0.01	0.07 ± 0.01	0.07 ± 0.00	0.09 ± 0.01**
Week 39				
n	4	5	5	4
Body Wt.	499 ± 13	520 ± 26	501 ± 12	485 ± 8
Lung				
Absolute	1.51 ± 0.07	1.62 ± 0.03	1.76 ± 0.05**	2.11 ± 0.05**
Relative	3.03 ± 0.17	3.15 ± 0.14	3.52 ± 0.04**	4.35 ± 0.03**
Bronchial Lymph Node				
Absolute	0.017 ± 0.006	0.025 ± 0.005	0.041 ± 0.005	0.051 ± 0.014*
Relative	0.03 ± 0.01	0.05 ± 0.01	0.08 ± 0.01	0.11 ± 0.03*
Mediastinal Lymph Node				
Absolute	0.033 ± 0.009	0.031 ± 0.011	0.031 ± 0.006	0.060 ± 0.011
Relative	0.07 ± 0.02	0.06 ± 0.02	0.06 ± 0.01	0.12 ± 0.02

*Significantly different ($p \leq 0.05$) from the chamber control group by the Dunnett or Williams tests.

** $p \leq 0.01$.

*Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

The greatest changes in measured BAL fluid parameters after specular hematite exposure occurred in neutrophils, lymphocytes, and lactate dehydrogenase (LDH) activity (Table 12 and Table A-2). Absolute numbers of neutrophils and lymphocytes and LDH activity were significantly increased in BAL fluid of core male rats, mostly at week 39 at the 60 mg/m³ specular hematite exposure concentration compared to the chamber control group. The changes in the exposed groups generally increased in magnitude with exposure concentration and time, with the 60 mg/m³ group often being most severely affected. Numerous statistical differences in BAL fluid composition occurred between exposed and chamber control groups (increased percentages of lymphocytes and neutrophils, decreased percentage of alveolar macrophages) that were consistent with cells (mainly neutrophils and a small number of lymphocytes) infiltrating the lung in response to specular hematite exposure. For males, all these changes were present in all exposed groups at weeks 16, 26, and 39 (except increased lymphocytes at week 26); for females, these changes were present in all exposed groups at week 26. In addition, at week 4, the 60 mg/m³ groups exhibited increased lymphocytes (females), decreased macrophage percentage (males), and increased neutrophil percentage (males). BAL fluid composition assessments also revealed exposure-induced cytotoxic lung injury with increased lactate dehydrogenase activities in all exposed groups of males beginning at week 16, and in all exposed groups of females at week 26.

Specular hematite lung burdens rose steadily with time and did not reach steady-state levels; at week 39, lung burdens were approximately 5, 12, and 23 mg specular hematite/g lung in the 15, 30, and 60 mg/m³ male rats, respectively (Table B-21). Normalized lung burdens and calculated deposition rates were proportional to exposure concentrations (Table B-21 and Table B-24). The calculated lung deposition rates were 44, 76, and 160 µg specular hematite/total lung per day for the 15, 30, and 60 mg/m³ exposure concentrations, respectively (Table B-24). Specular hematite burdens in bronchial lymph nodes were generally greater than those of mediastinal lymph nodes, however, burdens in both types of lymph nodes were quite variable (Table B-22 and Table B-23). Specular hematite lung clearance rates were slow following specular hematite exposures. In the 15 mg/m³ group, approximately 0.3% of the daily deposited lung burden was cleared each day and the clearance half-life was estimated to be 212 days (Table B-24). Model-fitting the 30 and 60 mg/m³ concentrations resulted in non-meaningful (negative) clearance rates in which 95% confidence intervals each included zero, indicating the calculated clearance rates were indistinguishable from zero. Actual clearance rates for these exposure groups were likely relatively small in magnitude. By week 39, the lung specular hematite volumes for the 30 and 60 mg/m³ groups exceeded the overload threshold by factors of 2.3 and 5.5, respectively; overload began in these groups at 118 and 55 days, respectively (Table B-25). For the 15 mg/m³ group at week 39, specular hematite volume was 90% of the overload threshold and overload was estimated to begin at 344 days (Table B-25). Tissue burdens of specular hematite, although quite large, were proportional to exposure concentration in the lungs but were quite variable in the lymph nodes. The large lung burdens, the very small clearance rates (indistinguishable from zero in some groups), and the magnitude by which 6% of the alveolar macrophage lung volume was exceeded (or nearly exceeded) by the end of the study, indicated that lung overload conditions were achieved for specular hematite at all exposure concentrations tested in this study.

Table 12. Bronchoalveolar Lavage Data for Sprague Dawley Rats in the 39-week Inhalation Study of Specular Hematite^a

	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³
Male				
Week 4	8	8	8	8
Week 16	7	8	7	6
Week 26	8	8	8	8
Week 39	8	8	8	8
Viable Cells (%)				
Week 4	81.76 ± 3.80	86.33 ± 3.33	87.74 ± 2.17	91.41 ± 1.67
Week 16	88.67 ± 0.52	91.03 ± 1.74	87.97 ± 1.91	92.95 ± 2.40
Week 26	93.03 ± 1.20	94.41 ± 1.26	98.19 ± 0.74**	98.35 ± 0.60**
Week 39	89.75 ± 2.29	94.53 ± 1.82	97.35 ± 0.90*	98.96 ± 0.39**
Total Cells Counted				
Week 4	153.1 ± 22.1	198.1 ± 23.0	186.9 ± 24.0	155.6 ± 18.1
Week 16	554 ± 61.0	371 ± 42.0	313 ± 36.0**	391 ± 28.0
Week 26	204.4 ± 18.9	192.5 ± 17.2	280.6 ± 27.6*	608.1 ± 83.4**
Week 39	173 ± 11.0	273 ± 27.0*	394 ± 51.0**	749 ± 46.0**
Macrophages (%)				
Week 4	99.7 ± 0.1	99.7 ± 0.2	99.2 ± 0.2	96.1 ± 0.8**
Week 16	99.7 ± 0.1	90.7 ± 2.1**	78.6 ± 3.0**	48.0 ± 3.6**
Week 26	99.9 ± 0.1	91.0 ± 1.8**	73.7 ± 3.7**	44.4 ± 2.4**
Week 39	99.8 ± 0.1	87.2 ± 2.4**	65.1 ± 3.7**	34.9 ± 4.3**
Absolute Macrophages (#/μL)				
Week 4	152.6 ± 22.1	197.8 ± 23.2	185.3 ± 23.6	150.2 ± 18.6
Week 16	552.0 ± 60.9	334.8 ± 37.1*	244.7 ± 30.4**	188.5 ± 21.4**
Week 26	204.2 ± 19.0	173.7 ± 13.8	206.2 ± 22.5	273.4 ± 45.1
Week 39	172.8 ± 11.4	233.7 ± 18.2	252.0 ± 31.7	270.7 ± 44.3
Lymphocytes (%)				
Week 4	0.200 ± 0.089	0.125 ± 0.125	0.413 ± 0.126	0.413 ± 0.126
Week 16	0.043 ± 0.043	0.838 ± 0.191**	0.671 ± 0.148**	1.733 ± 0.429**
Week 26	0.038 ± 0.038	0.000 ± 0.000	0.038 ± 0.038	0.038 ± 0.038
Week 39	0.000 ± 0.000	0.838 ± 0.328**	1.463 ± 0.272**	1.675 ± 0.351**
Absolute Lymphocytes (#/μL)				
Week 4	0.288 ± 0.109	0.138 ± 0.138	0.838 ± 0.345	0.563 ± 0.148
Week 16	0.314 ± 0.314	3.150 ± 0.840*	2.329 ± 0.637*	6.350 ± 1.358**
Week 26	0.063 ± 0.063	0.000 ± 0.000	0.188 ± 0.188	0.263 ± 0.263
Week 39	0.000 ± 0.000	2.675 ± 1.281**	5.500 ± 1.104**	11.850 ± 2.142**

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	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³
Neutrophils (%)				
Week 4	0.125 ± 0.090	0.163 ± 0.091	0.375 ± 0.171	3.500 ± 0.721**
Week 16	0.286 ± 0.155	8.450 ± 1.943**	20.743 ± 2.993**	50.217 ± 3.741**
Week 26	0.088 ± 0.088	9.050 ± 1.772**	26.313 ± 3.735**	55.613 ± 2.385**
Week 39	0.188 ± 0.055	11.925 ± 2.173**	33.550 ± 3.733**	63.425 ± 4.085**
Absolute Neutrophils (#/μL)				
Week 4	0.238 ± 0.169	0.200 ± 0.102	0.750 ± 0.351	4.900 ± 0.992**
Week 16	1.257 ± 0.647	32.700 ± 8.403**	65.786 ± 13.558**	195.767 ± 19.181**
Week 26	0.113 ± 0.113	18.788 ± 4.945**	74.250 ± 12.106**	334.488 ± 43.107**
Week 39	0.363 ± 0.112	36.175 ± 9.806**	137.063 ± 32.091**	466.263 ± 26.946**
Eosinophils (%)				
Week 4	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 16	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.050 ± 0.050
Week 26	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 39	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Absolute Eosinophils (#/μL)				
Week 4	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 16	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.250 ± 0.250
Week 26	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 39	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Lactate Dehydrogenase (IU/L)				
Week 4	22 ± 4	27 ± 5 ^b	25 ± 7	30 ± 4
Week 16	38 ± 4 ^c	64 ± 9*	77 ± 8** ^c	263 ± 43** ^c
Week 26	14 ± 4 ^b	31 ± 3**	45 ± 3**	126 ± 14**
Week 39	25 ± 2	43 ± 4**	93 ± 6**	219 ± 23**
Albumin (g/dL)				
Week 4	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.00
Week 16	0.02 ± 0.01 ^c	0.03 ± 0.01	0.01 ± 0.00 ^c	0.02 ± 0.01 ^c
Week 26	0.02 ± 0.01	0.03 ± 0.01	0.01 ± 0.00	0.02 ± 0.01
Week 39	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01
Female				
n	8	8	8	8
Viable Cells (%)				
Week 4	84.15 ± 3.63	82.81 ± 4.89	88.60 ± 1.84	90.39 ± 1.38
Week 26	93.45 ± 0.71	93.95 ± 1.38	94.66 ± 1.67	98.43 ± 0.36**

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	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³
Total Cells Counted				
Week 4	353.8 ± 32.0	337.5 ± 22.4	341.3 ± 16.6	268.8 ± 20.2
Week 26	248.1 ± 22.2	288.1 ± 15.2	490.0 ± 51.6**	1,766.9 ± 95.1**
Macrophages (%)				
Week 4	99.8 ± 0.1	99.4 ± 0.1	99.8 ± 0.1	96.0 ± 1.9*
Week 26	99.5 ± 0.2	92.2 ± 2.4**	71.0 ± 2.6**	30.0 ± 2.0**
Absolute Macrophages (#/μL)				
Week 4	353.0 ± 31.8	335.6 ± 22.3	340.7 ± 16.6	257.6 ± 19.6*
Week 26	246.9 ± 22.1	264.1 ± 10.7	351.2 ± 42.3*	525.6 ± 40.0**
Lymphocytes (%)				
Week 4	0.000 ± 0.000	0.338 ± 0.115*	0.163 ± 0.091	0.363 ± 0.158*
Week 26	0.038 ± 0.038	0.650 ± 0.197*	1.575 ± 0.184**	2.088 ± 0.391**
Absolute Lymphocytes (#/μL)				
Week 4	0.000 ± 0.000	0.013 ± 0.339*	0.588 ± 0.325	1.038 ± 0.499
Week 26	0.113 ± 0.113	1.975 ± 0.641*	7.638 ± 1.125**	38.475 ± 9.136**
Neutrophils (%)				
Week 4	0.213 ± 0.113	0.250 ± 0.139	0.000 ± 0.000	3.650 ± 1.713
Week 26	0.450 ± 0.154	7.163 ± 2.181**	27.463 ± 2.634**	67.950 ± 1.916**
Absolute Neutrophils (#/μL)				
Week 4	0.800 ± 0.467	0.888 ± 0.534	0.000 ± 0.000	10.138 ± 5.284
Week 26	1.163 ± 0.362	22.000 ± 7.745**	131.175 ± 14.546**	1,202.813 ± 77.715**
Eosinophils (%)				
Week 4	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 26	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Absolute Eosinophils (#/μL)				
Week 4	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 26	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Lactate Dehydrogenase (IU/L)				
Week 4	24 ± 2	24 ± 2	26 ± 4	35 ± 5
Week 26	14 ± 4	29 ± 4*	71 ± 4**	212 ± 17**
Albumin (g/dL)				
Week 4	0.01 ± 0.00	0.02 ± 0.01	0.03 ± 0.01	0.06 ± 0.01**
Week 26	0.03 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.05 ± 0.01

*Significantly different ($p \leq 0.05$) from the chamber control group by Dunn's or Shirley's test.

** $p \leq 0.01$.

^aData are presented as mean ± standard error.

^bn = 7.

^cn = 8.

Lung burden modeling in both the blasting sand and specular hematite studies demonstrated very small (in some cases indistinguishable from zero) clearance rates that resulted in corresponding clearance half-lives that were much longer than those predicted from the respective 2-week studies. Because the density of blasting sand is less than that of specular hematite, lung overload was calculated (by volume) to begin much earlier for all blasting sand exposure groups.

Test article-related gross lesions at necropsy included enlarged and mottled lymph nodes. Test article-related nonneoplastic lesions occurred in the lung, larynx, nose, trachea, and bronchial and mediastinal lymph nodes (Table 13). One 30 mg/m³ male rat had an alveolar/bronchiolar adenoma at the 26-week interim evaluation.

Exposure-related lesions in the lung included histiocytic cellular infiltration, chronic active inflammation, interstitial fibrosis, alveolar epithelial hyperplasia, and foreign bodies (presumably the test article) (Table 13). As with blasting sand, the majority of these lesions were graded according to the following scale: minimal: ≤5%; mild: greater than 5% but ≤35%; moderate: greater than 35% but ≤65%; and marked: greater than 65% of alveoli affected. Alveolar epithelial hyperplasia was considered minimal when less than 10% of alveolar spaces were lined by hyperplastic pneumocytes, and mild when 10–30% of alveolar spaces were lined by hyperplastic pneumocytes.

Compared to the incidence in the chamber control group, significant increases in the incidence of histiocytic cellular infiltration occurred in all exposed groups at weeks 16 and 26, in the 60 mg/m³ group at week 4, and in the 15 and 30 mg/m³ groups at 39 weeks (Table 13). Except at 4 weeks, the severity of this lesion increased with increasing exposure concentration. The incidence of chronic active inflammation was significantly increased in the 60 mg/m³ group at weeks 26 and 39, and the severity of this lesion was slightly increased in this group at 39 weeks. Histiocytic cellular infiltration was characterized by increased numbers of alveolar macrophages.

Table 13. Incidences of Selected Nonneoplastic Lesions in Male Sprague Dawley Rats in the 39-week Inhalation Study of Specular Hematite

	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³
Four-week Interim Evaluation				
Larynx ^a	8	8	8	8
Epiglottis, metaplasia, squamous ^b	0	1 (1.0) ^c	4* (1.0)	5* (1.0)
Foreign body material	0	7**	8**	8**
Lung	8	8	8	8
Infiltration cellular, histiocyte	1 (1.0)	1 (1.0)	1 (1.0)	8** (1.0)
Foreign body material	0	8**	8**	8**
Lymph Node, Bronchial	8	8	7	8
Foreign body material	0	0	2	5*
Lymph Node, Mediastinal	5	6	6	5
Foreign body material	0	0	0	1

Abrasive Blasting Agents, TOX 91

	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³
16-week Interim Evaluation				
Nose	8	8	8	8
Olfactory epithelium, accumulation, hyaline droplet	7 (1.1)	8 (1.1)	7 (1.1)	8 (1.0)
Respiratory epithelium, accumulation, hyaline droplet	1 (1.0)	4 (1.0)	5 (1.0)	2 (1.0)
Larynx	8	8	8	8
Epiglottis, metaplasia, squamous	0	3 (1.0)	3 (1.0)	4* (1.0)
Foreign body material	0	8**	8**	8**
Lung	8	8	8	8
Infiltration cellular, histiocyte	2 (1.0)	8** (1.0)	8** (1.1)	8** (2.3)
Inflammation, chronic active	1 (1.0)	0	0	3 (1.0)
Alveolar epithelial, hyperplasia	0	3 (1.0)	8** (1.0)	8** (1.0)
Foreign body material	0	8**	8**	8**
Lymph Node, Bronchial	8	7	8	8
Foreign body	0	7**	6**	7**
Infiltration cellular, histiocyte	0	2 (2.0)	6** (2.3)	7** (2.7)
Hyperplasia	0	1 (2.0)	2 (1.5)	3 (1.7)
Lymph Node, Mediastinal	6	8	8	8
Foreign body material	0	4	6**	8**
Infiltration cellular, histiocyte	0	3 (2.0)	5* (2.6)	8** (2.9)
Hyperplasia	0	3 (2.0)	6** (2.5)	8** (2.6)
26-week Interim Evaluation				
Nose	8	8	8	8
Olfactory epithelium, accumulation, hyaline droplet	8 (1.3)	8 (1.8)	8 (1.3)	8 (1.9)
Respiratory epithelium, accumulation, hyaline droplet	2 (1.0)	2 (1.0)	3 (1.0)	5 (1.0)
Larynx	8	8	8	8
Epiglottis, metaplasia, squamous	0	5* (1.0)	5* (1.0)	7** (1.0)
Foreign body material	0	8**	8**	8**
Trachea	8	8	8	8
Foreign body material	0	1	3	1
Lung	8	8	8	8
Infiltration cellular, histiocyte	2 (1.0)	8** (1.0)	8** (1.5)	8** (2.6)
Inflammation, chronic active	0	2 (1.0)	0	4* (1.0)
Interstitium, fibrosis	0	0	0	3 (1.3)
Alveolar epithelial, hyperplasia	0	8** (1.0)	8** (1.0)	8** (2.0)
Foreign body material	0	8**	8**	8**

Abrasive Blasting Agents, TOX 91

	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³
Lymph Node, Bronchial	7	5	7	7
Foreign body material	0	5**	7**	5**
Infiltration cellular, histiocyte	0	3* (2.3)	7** (2.7)	5** (2.8)
Lymph Node, Mediastinal	8	7	8	8
Foreign body material	0	6**	7**	7**
Infiltration cellular, histiocyte	0	5** (2.4)	7** (2.6)	7** (2.9)
Hyperplasia	0	3 (1.0)	3 (1.0)	6** (2.5)
39-week Evaluation				
Nose	9	8	8	9
Olfactory epithelium, accumulation, hyaline droplet	8 (2.1)	8 (2.8)	8 (3.0)	8 (3.5)
Larynx	9	8	8	9
Epiglottis, metaplasia, squamous	0	5** (1.0)	7** (1.0)	7** (1.0)
Foreign body material	0	8**	8**	9**
Lung	9	8	8	9
Infiltration cellular, histiocyte	4 (1.0)	8* (1.4)	8* (2.0)	8 (3.0)
Inflammation, chronic active	0	3 (1.0)	2 (1.0)	7** (1.4)
Interstitial, fibrosis	1 (1.0)	2 (1.0)	8** (1.0)	9** (2.0)
Alveolar epithelial, hyperplasia	2 (1.5)	8** (1.0)	8** (1.0)	9** (2.0)
Foreign body material	0	8**	8**	9**
Lymph Node, Bronchial	5	7	8	8
Foreign body material	0	5*	7**	7**
Infiltration cellular, histiocyte	0	5* (2.4)	7** (2.7)	7** (3.0)
Hyperplasia	0	1 (1.0)	3 (1.3)	4 (1.3)
Lymph Node, Mediastinal	9	8	7	9
Foreign body material	0	7**	6**	9**
Infiltration cellular, histiocyte	0	7** (2.6)	6** (3.0)	9** (3.0)
Hyperplasia	0	3 (1.7)	4* (1.8)	7** (3.0)

*Significantly different ($p \leq 0.05$) from the chamber control group by the Fisher exact test.

** $p \leq 0.01$.

^aNumber of animals with tissue examined microscopically.

^bNumber of animals with lesion.

^cAverage severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Many of the macrophages, particularly at higher exposure concentrations, contained fine intracytoplasmic foreign bodies (granular material), presumed to be test material. Chronic active inflammation was diagnosed when the increased numbers of macrophages were accompanied by scattered neutrophils and/or lymphocytes. With longer exposure duration and greater exposure concentrations, the alveolar septa were often thickened by mononuclear inflammatory cells, fibrosis, and alveolar epithelial hyperplasia.

The incidence of minimal to mild interstitial fibrosis in the 30 and 60 mg/m³ groups at 39 weeks was significantly greater than that in the chamber control group, and the severity was increased in the 60 mg/m³ group (Table 13). Fibrosis was typically seen in areas also affected by chronic active inflammation, histiocytic cellular infiltration, or alveolar epithelial hyperplasia. Interstitial fibrosis was characterized by an increase in lacey-collagen fibers in the alveolar septa.

The incidence of alveolar epithelial hyperplasia was significantly increased in the 30 and 60 mg/m³ groups at week 16 and in all exposed groups at weeks 26 and 39, compared to the chamber control group incidence (Table 13). The lesions were associated with chronic active inflammation, and they were indicative of a regenerative response of alveolar epithelial cells. Alveolar epithelial hyperplasia was characterized by cuboidal cells lining the alveolar septa in areas with inflammation. The cuboidal cells were closely packed with severe inflammation and sparsely distributed with less severe inflammation. The lesion did not cause compression of adjacent tissue.

Foreign body accumulation was noted in the lungs of all exposed rats at all time points and in none of the chamber control rats (Table 13). The lesion was characterized by fine, dark brown to black granular material within the cytoplasm of alveolar macrophages or free within the alveolar spaces. The number of macrophages containing the material increased with increasing exposure concentration. The absence of the material in the lungs of chamber control rats, the correlation between exposure concentration and the amount of the material, and the morphology of the material are consistent with specular hematite.

In the larynx, exposure-related lesions included squamous metaplasia of the epiglottis and foreign body material (Table 13). The incidence of minimal squamous metaplasia of the epithelium at the base of the epiglottis was significantly increased in the 60 mg/m³ group at all time points, in the 30 mg/m³ group at weeks 4, 26, and 39, and in the 15 mg/m³ group at weeks 26 and 39. Squamous metaplasia was characterized by replacement of the normally cuboidal epithelium by flattened and elongated epithelial cells. Foreign body accumulation occurred in all exposed male rats at all time points except at 4 weeks in the 15 mg/m³ group; foreign body material did not occur in any of the chamber control rats. Morphologically, foreign body material in the larynx was identical to that seen in the lung and was characterized by fine, dark brown to black granular material. The material was present in the cytoplasm of epithelial cells or extracellularly, or in the cytoplasm of macrophages in the mucosa, submucosa, or on the luminal surface at the base of the epiglottis. As in the lung, the morphological appearance and association with exposure were consistent with the material being specular hematite. Two rats, one in the 15 mg/m³ group at 26 weeks and one in the 60 mg/m³ group at 39 weeks, had minimal or mild ulceration of the laryngeal epithelium. It was unclear whether this lesion was related to exposure to specular hematite.

In the nose, accumulation of hyaline droplets in the olfactory epithelium occurred in most male rats, including chamber control animals beginning at week 16; the severity of the lesion generally increased with increasing exposure concentration at 39 weeks (Table 13). Hyaline droplet accumulation in the respiratory epithelium also occurred beginning at week 16; although the incidence in the exposed groups was greater than those in the chamber control group, the differences were not statistically significant. Hyaline droplets are normally seen in control animals but may be increased in size and have a greater distribution with exposure to various chemicals or particulates in inhalation studies. Hyaline droplet accumulation in the olfactory and

respiratory epithelia was noted primarily at Levels II and III of the nose and consisted of brightly eosinophilic intracytoplasmic globules that occasionally distorted cells and displaced nuclei. Locations commonly affected were the septum and medial nasoturbinates at the junction of the respiratory and olfactory epithelium in Level II and the olfactory epithelium in the ventral half of Level III.

In the trachea, foreign body accumulation occurred in all exposed groups and chronic active inflammation occurred in all groups at 26 weeks; differences from the chamber control group incidence were not significant (Table 13). Foreign body material was characterized by small amounts of dark brown to black, finely granular material present in the cytoplasm of epithelial cells, or extracellularly, or in the cytoplasm of macrophages in the mucosa, submucosa, or on the luminal surface of the trachea. As in the lung and larynx, the morphological appearance and association with exposure were consistent with the material being specular hematite.

Exposure-related increases in the incidences of foreign bodies, histiocytic cellular infiltration, and hyperplasia occurred in the bronchial and mediastinal lymph nodes (Table 13). The presence of foreign body material in the bronchial lymph nodes was significantly increased in the 60 mg/m³ group beginning at week 4 and in the 15 and 30 mg/m³ groups beginning at week 16, compared to the chamber control group incidence. The presence of foreign body material in the mediastinal lymph node was significantly increased beginning at week 16 in the 30 and 60 mg/m³ groups and beginning at week 26 in the 15 mg/m³ group. The morphological appearance of the foreign body material was identical to that in the respiratory system. It was characterized by fine, dark brown to black granular material in the cytoplasm of macrophages.

The incidence of minimal to mild histiocytic cellular infiltration in the bronchial and mediastinal lymph nodes was significantly increased beginning at week 16 in the 30 and 60 mg/m³ groups and at week 26 in the 15 mg/m³ group (Table 13). The severity of the lesion generally increased with exposure concentration and was most severe at 39 weeks. Histiocyte cellular infiltration was characterized by the presence of excessive numbers or aggregates of macrophages containing intracytoplasmic foreign body material that was presumed to be specular hematite. The macrophages were seen primarily in subcortical, paracortical, and medullary sinuses and were similar in appearance to the foreign body-laden macrophages in the lungs. Often the deposits of foreign bodies were so large that cytoplasmic and nuclear detail were obscured. The histiocytic cellular infiltrates often contributed to the overall enlargement of the nodes, and many nodes were noted as trackable gross lesions due to enlargement or dark red mottling. All the macrophages and foreign body material were presumed to have migrated to the lymph nodes from the lungs.

Hyperplasia occurred in the bronchial and mediastinal lymph nodes in some exposed animals beginning at 16 weeks, and the incidence of hyperplasia in the mediastinal lymph node in 30 and 60 mg/m³ rats was generally significantly greater than that of the chamber control group (Table 13). The incidence of hyperplasia was greater in the mediastinal lymph node than in the bronchial lymph node. The severity of this lesion generally increased with time and exposure concentration and was slightly greater in the mediastinal lymph nodes compared to the bronchial lymph nodes. Hyperplasia was characterized by an increase in the number of primarily small and intermediate sized lymphocytes in the paracortex, resulting in increased paracortical area.

Immunotoxicity Studies

The predominant effect on measures of immunity and inflammation was an exposure concentration-related increase in MCP-1 levels in BAL fluid after exposure to blasting sand and specular hematite. Changes in MCP-1 were the most significant effect; however, data for the other immunotoxicity endpoints that were tested are in Appendix F. Male rats exposed to 60 mg/m³ blasting sand for 4 weeks had significantly increased levels of MCP-1 in BAL fluid; MCP-1 levels were significantly increased at all exposure levels of blasting sand at the 16-, 26-, and 39-week evaluations (Table F-14 and Figure 7). Similar increases in MCP-1 levels were observed at 26 weeks in the BAL fluid from female rats exposed to blasting sand (Table F-13). The exposure concentration and duration of exposure to blasting sand appeared to be important factors contributing to the changes in MCP-1 levels. There were no significant alterations in any of the other cytokines or chemokines measured at any of the time points evaluated.

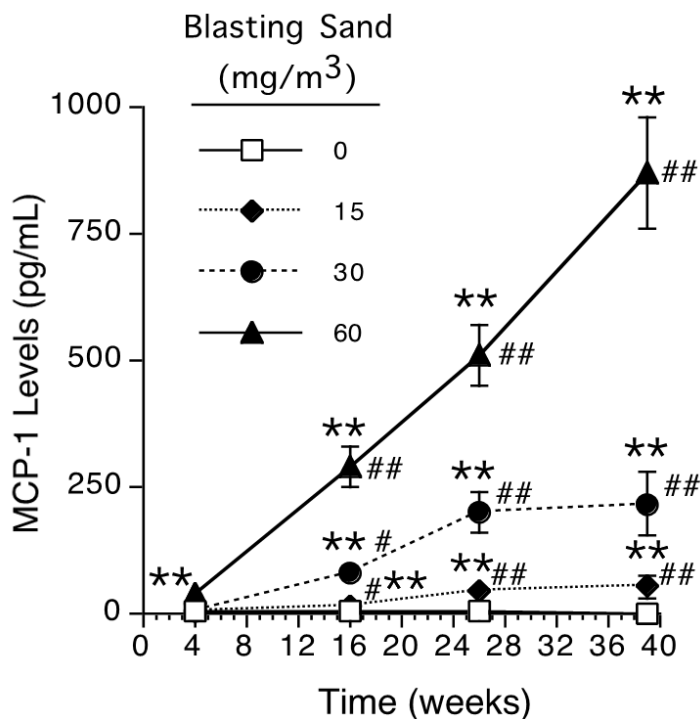


Figure 7. MCP-1 Levels in the Bronchoalveolar Lavage Fluid of Male Sprague Dawley Rats Exposed to Blasting Sand by Inhalation for 4, 16, 26, or 39 Weeks

**Significantly different ($p \leq 0.01$) from the chamber control group at the same time point.

#Significantly different ($p \leq 0.05$) from the week 4 time point within the same exposure concentration.

$p \leq 0.01$.

MCP-1 levels were also significantly increased in an exposure concentration-related manner in male rats exposed to specular hematite at doses of 30 or 60 mg/m³, beginning at the 16-week time point (Table F-29). Exposure to specular hematite for 26 weeks also resulted in a significant increase in MCP-1 levels in males exposed to 15 mg/m³. After 39 weeks of exposure to specular hematite, MCP-1 levels were significantly increased in BAL fluid from the 30 and 60 mg/m³ groups (Table F-29 and Figure 8). Similar increases in MCP-1 levels were observed in female rats exposed to 30 or 60 mg/m³ at the 26-week time point (Table F-28).

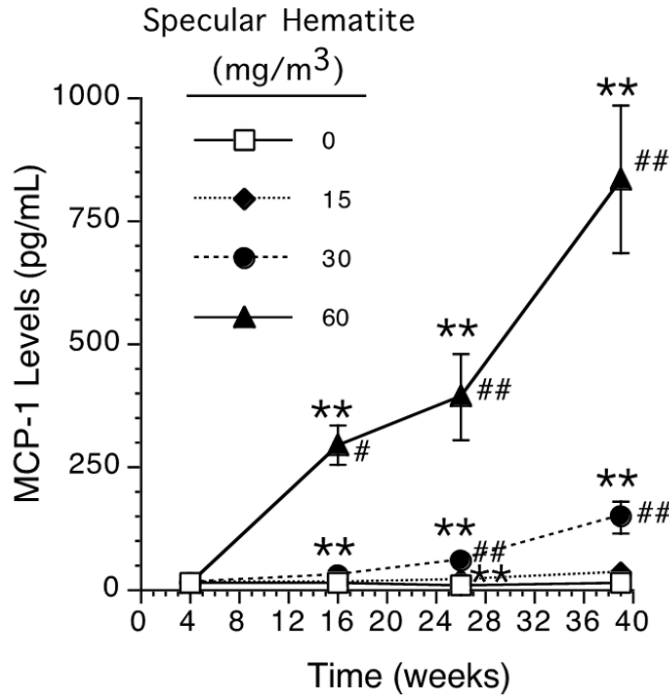


Figure 8. MCP-1 Levels in Bronchoalveolar Lavage Fluid of Male Sprague Dawley Rats Exposed to Specular Hematite by Inhalation for 4, 16, 26, or 39 Weeks

**Significantly different ($p \leq 0.01$) from the chamber control group at the same time point.

#Significantly different ($p \leq 0.05$) from the week 4 time point within the same exposure concentration.

$p \leq 0.01$.

Limited effects were observed on measures of immunity in female rats exposed to either blasting sand or specular hematite (detailed results are provided in Appendix F). No significant effects were observed on the antibody-forming cell (AFC) response, serum anti-sheep red blood cell (sRBC) IgM antibody levels, or natural killer (NK) cell activity in female rats exposed to blasting sand at either the 4-week or 26-week time point. Minimal changes in leukocyte subpopulations in the spleen were observed in female rats exposed to 60 mg/m³ blasting sand for 26 weeks. Anti-CD3 mediated spleen cell proliferation was unaffected at 4 weeks but was increased at 26 weeks in rats exposed to 60 mg/m³ blasting sand.

No significant effects were observed in any of the immunological assays, including the AFC response, serum IgM antibody levels against sRBCs, spleen cell numbers, spleen cell phenotypes, anti-CD3 mediated proliferation, or NK cell activity following exposure to specular hematite (Appendix F).

Discussion

Abrasive blasting involves forcibly projecting a stream of abrasive particles through compressed air or steam against a surface to change its quality or to remove contaminants. Blasting sand, the most commonly used abrasive blasting agent, contains high levels of crystalline silica (SiO_2) and can cause pulmonary fibrosis (silicosis) following inhalation exposure. Crystalline silica is also considered to be a lung carcinogen. Acute silicosis is characterized by alveolar proteinosis with reduced gas exchange, whereas chronic silicosis is characterized by scarring and formation of fibrotic nodules around the trapped silica particles. Alternatives to blasting sand with lower crystalline silica content exist, including specular hematite, which is mostly iron oxide (Fe_2O_3). Exposure to specular hematite via in vivo intratracheal instillation has been shown to induce less lung injury, inflammation, and fibrosis than blasting sand.¹ Other alternatives to blasting sand include coal slag, crushed glass, and garnet; however, no comprehensive acute or chronic inhalation studies have been performed to evaluate the health effects, including pulmonary toxicity, of these alternative compounds.

Studies evaluating exposure risks are needed due to the high production volume of these compounds, the number of workers exposed, and the inadequacy of available toxicity data to determine safe exposure concentrations. Acute inhalation toxicity testing to compare blasting sand to coal slag, crushed glass, garnet, and specular hematite was initially performed in male rats via whole-body exposure in separate, short-term 2-week studies. The main objectives of the 2-week studies were to determine acute toxicity, identify target organs, evaluate tissue burden, and provide a basis for selection of test article and exposure concentrations to be used in subsequent 39-week inhalation studies. Data generated from these studies might inform subsequent recommendations from the National Institute for Occupational Safety and Health for alternative abrasive compounds to blasting sand.

The lung toxicity of blasting sand, which should have served as a positive control in the 2-week studies due to the known adverse effects of silica sand, was low after the 2-week exposure window, a result likely attributable to low lung burden (steady-state lung burden was not achieved by the end of the study). In fact, all abrasive compounds (blasting sand and alternatives) tested in the 2-week studies exhibited low lung toxicity, perhaps due to low lung burdens.

The lung histopathology from the 2-week studies indicated that specular hematite and crushed glass appeared to be the least toxic in causing lung inflammation of the four alternative blasting agents tested; however, crushed glass exhibited the shortest clearance half-lives (10, 17, and 16 days for the 3, 15, and 30 mg/m^3 groups, respectively), resulting in the lowest lung burdens. Thus, due to the relatively fast clearance rate of crushed glass, the 39-week study design favored comparison of blasting sand with specular hematite, which had more similar clearance half-lives (95, 35, and 33 days for blasting sand and 48, 30, and 50 days for specular hematite for the 3, 15, and 30 mg/m^3 groups, respectively). Garnet, which contained approximately 6% crystalline silica and exhibited the longest clearance half-lives (90 and 89 days for the 15 and 30 mg/m^3 groups, respectively)—resulting in the highest steady-state lung burdens (5,089 and 9,089 μg garnet/total lung for the 15 and 30 mg/m^3 groups, respectively)—was the most toxic of the alternative blasting agents in regard to the incidence of chronic active inflammation in the lung (five of five animals at the 15 and 30 mg/m^3 exposure concentrations), followed by coal slag (despite the

absence of crystalline silica). This finding suggests that the presence of crystalline silica was not the only determinant of lung toxicity in the 2-week studies because the coal slag tested contained no crystalline silica, yet there was some incidence of focal inflammation (two of five animals at the 30 mg/m³ exposure concentration) and proteinosis (two of five animals at the 15 mg/m³ exposure concentration) in the lungs of coal slag-exposed rats. Garnet was the most toxic of all the alternative blasting agents tested in the 2-week studies, perhaps because it contained the most crystalline silica of all the alternatives or had the longest clearance half-lives (for 15 and 20 mg/m³ exposure concentrations) relative to the other abrasive blasting agents. However, in terms of lung inflammation, garnet was more toxic than blasting sand (which contained the highest levels of crystalline silica of all the blasting agents), suggesting that the toxicity of garnet was more likely due to longer clearance half-lives (90 and 89 days for garnet versus 35 and 33 days for blasting sand for the 15 and 30 mg/m³ groups, respectively). Crushed glass also appeared to be more reactive than the other blasting agents to the upper respiratory tract (larynx) in causing hyperplasia, squamous metaplasia, and inflammation of the epiglottis. Informed by upper airway and lung histopathology, as well as clearance data from the 2-week studies, specular hematite was chosen as the alternative test article (instead of crushed glass) to compare with blasting sand in separate 39-week studies.

The objectives of the long-term inhalation studies were to assess the pulmonary toxicity, fibrogenicity, tissue (lung and lymph node) burden, and immunotoxicity in rats after chronic exposure to blasting sand or specular hematite for up to 39 weeks. The crystalline silica content of these two abrasive blasting agents differ greatly, with blasting sand consisting of greater than 75% crystalline silica and specular hematite consisting of only 1% to 2% crystalline silica (greater than 95% iron oxide). Thus, these studies may help to further address the chronic lung effects from inhalation exposure to crystalline silica. There were no treatment-related changes in survival, body weights, or clinical observations for either test article. There was a significant exposure concentration-dependent increase in relative lung and bronchial lymph node weights for both blasting sand and specular hematite compared to the chamber control groups at week 39. When comparing the two test articles, relative lung and bronchial lymph node weights at week 39 (60 mg/m³) were increased for blasting sand compared to specular hematite, but the differences were not statistically significant.

Significant exposure concentration-dependent increases were observed in total cells counted, absolute neutrophils and lymphocytes, LDH activity, and MCP-1 protein levels in bronchoalveolar lavage (BAL) fluid at week 39 compared to chamber control groups for both blasting sand and specular hematite. Total cells counted and absolute macrophages in BAL fluid at week 39 (60 mg/m³) were significantly increased for blasting sand compared to specular hematite (Table A-3). Absolute neutrophils in BAL fluid at week 39 (60 mg/m³) were also greater for blasting sand compared to specular hematite, but the difference was not statistically significant. Neutrophils and lymphocytes, however, were significantly increased for blasting sand compared to specular hematite at earlier time points and for other exposure concentrations (Table A-3).

Furthermore, LDH activity in BAL fluid at week 26 was significantly increased for blasting sand compared to specular hematite at all exposure concentrations (Table A-3). These data suggest that markers of airway/lung injury and inflammation were increased in animals exposed to blasting sand compared to specular hematite. Except for significantly increased MCP-1 in BAL fluid for both blasting sand and specular hematite, there were minimal immunotoxic effects.

MCP-1 is a potent pro-inflammatory chemokine that recruits leukocytes (including macrophages) from the circulation into the lung.⁷³ Resident and recruited macrophages are critical phagocytic cells within the lung, acting in response to particle inhalation.

Regarding histopathological changes in the airways and lung, respiratory epithelial hyaline droplet accumulation in the nose was less apparent in rats exposed to specular hematite compared to those exposed to blasting sand. The incidence of respiratory epithelial hyaline droplet accumulation in the nose at week 16 (15 and 60 mg/m³ groups) was significantly lower for specular hematite compared to blasting sand (Table 14). Lung histopathology showed that the incidences of chronic active inflammation and interstitial fibrosis were both less in rats exposed to specular hematite compared to those exposed to blasting sand for several exposure concentrations and time points. The incidence of chronic active inflammation in the lung at weeks 16 (60 mg/m³), 26 (all exposure concentrations), and 39 (15 and 30 mg/m³) was significantly lower for specular hematite compared to blasting sand (Table 14). The incidence of interstitial (alveolar) fibrosis in the lung at weeks 26 (all exposure concentrations) and 39 (15 mg/m³) was significantly lower for specular hematite compared to blasting sand. In addition, alveolar proteinosis occurred in the lungs of rats exposed to 60 mg/m³ blasting sand but not specular hematite at week 39 (Table 14). This important finding should be emphasized because acute silicosis includes alveolar lipoproteinosis, which contributes to reduced gas exchange, and specular hematite did not induce alveolar proteinosis in this 39-week study. Crystalline silica has been shown to be cytotoxic to alveolar macrophages.⁷⁴ A crystalline silica-induced decrease in resident alveolar macrophages can impair the ability of the lung to clear surfactant lipoproteins resulting in alveolar proteinosis.⁷⁵ However, alveolar epithelial hyperplasia occurred in the lung of rats exposed to 30 and 60 mg/m³ specular hematite but not blasting sand at week 16. Also, in the larynx, the incidence of squamous metaplasia of the epiglottis at weeks 4 (30 and 60 mg/m³) and 16 (60 mg/m³) and at weeks 26 and 39 (all exposure concentrations) was greater in rats exposed to specular hematite but not blasting sand. Exposure-related histopathological effects in the airways and lung were mostly observed in terms of incidence because the overall severity grades for changes in histopathological parameters were low (minimal to mild) in the 39-week studies. Blasting sand should have acted as a positive control in the 39-week studies (as in the 2-week studies), and thus we expected greater severity scores for lung histopathological parameters (in particular, interstitial fibrosis) in the 39-week blasting sand study.

Table 14. Comparisons of the Incidences of Selected Nonneoplastic Lesions in Male Sprague Dawley Rats in the 39-week Inhalation Studies of Blasting Sand and Specular Hematite^a

	Study	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³
16-week Interim Evaluation					
Lung					
Inflammation, chronic active	Blasting sand	1/8	3/8	3/8	8/8*
	Specular hematite	1/8	0/8	0/8	3/8
Nose					
Respiratory epithelium, accumulation, hyaline droplet	Blasting sand	0/8	8/8*	8/8	7/8*
	Specular hematite	1/8	4/8	5/8	2/8
26-week Interim Evaluation					
Lung					
Inflammation, chronic active	Blasting sand	2/8	7/8*	7/8**	8/8*
	Specular hematite	0/8	2/8	0/8	4/8
Interstitialium, fibrosis	Blasting sand	0/8	8/8**	7/8**	8/8*
	Specular hematite	0/8	0/8	0/8	3/8
Nose					
Respiratory epithelium, accumulation, hyaline droplet	Blasting sand	6/8	8/8**	8/8*	8/8
	Specular hematite	2/8	2/8	3/8	5/8
39-week Evaluation					
Larynx					
Epiglottis, metaplasia, squamous	Blasting sand	0/8	1/8	1/8	1/8
	Specular hematite	0/9	5/8	7/8	7/9*
Lung					
Inflammation, chronic active	Blasting sand	2/8	8/8*	8/8**	8/8
	Specular hematite	0/9	3/8	2/8	7/9
Interstitialium, fibrosis	Blasting sand	0/8	8/8**	8/8	8/8
	Specular hematite	1/9	2/8	8/8	9/9
Nose					
Respiratory epithelium, accumulation, hyaline droplet	Blasting sand	6/8**	8/8**	8/8*	8/8**
	Specular hematite	0/9	0/8	3/8	3/9

*Significantly different ($p \leq 0.05$) from the group exposed to the same concentration of specular hematite by the one-sided Fisher exact test.

** $p \leq 0.01$.

^aNumber of animals with lesion per number of animals with tissue examined microscopically.

Except for 15 mg/m³ specular hematite, lung overload was achieved by week 39 for both blasting sand and specular hematite at all exposure concentrations; however, blasting sand was calculated to achieve lung overload conditions in less time compared to specular hematite (55 and 30 days for blasting sand versus 118 and 55 days for specular hematite for the 30 and 60 mg/m³ groups, respectively). Marginal differences between blasting sand and specular hematite relating to lung toxicity and fibrogenicity might have been attributable to the differences in the time of onset of lung overload; however, specular hematite is denser than blasting sand, which may have affected its volume occupied in the lung tissue and/or its solubility within the lung and thus toxicity/fibrogenicity. Future studies are needed to evaluate the relative solubility of blasting sand and specular hematite within the lung.

The data from the 39-week studies suggest that specular hematite could be, to some extent, less toxic and fibrogenic to the lower respiratory tract, especially concerning the induction of alveolar proteinosis within the lung, which was absent in all rats exposed to specular hematite. These adverse lung effects might have been due to the lower crystalline silica content, lower lung burden (increased time of onset of overload), or reduced solubility of specular hematite compared with blasting sand. Specular hematite was also more reactive than blasting sand to the upper respiratory tract (larynx) in causing squamous metaplasia of the epiglottis. The 39-week studies suggest that specular hematite could be a slightly safer alternative to blasting sand for abrasive sandblasting endeavors because of blasting sand's potential to induce lung injury, chronic inflammation, alveolar proteinosis, and interstitial fibrosis. However, both test articles exhibited some degree of lung toxicity and fibrogenicity over 39 weeks of exposure and, unlike blasting sand, specular hematite also exhibited toxic effects on the larynx.

Under the conditions of these 39-week inhalation studies, the major target tissue in male Sprague Dawley rats exposed to blasting sand or specular hematite was the lung. The incidences of chronic active inflammation and interstitial fibrosis were significantly lower in rats exposed to specular hematite (compared with blasting sand) at some time points under some exposure conditions. After 39 weeks of exposure to specular hematite, the lowest-observed-effect level was 15 mg/m³ for chronic active inflammation and interstitial fibrosis within the lung. Alveolar proteinosis was present at week 39 in the lungs of rats exposed to the highest concentration (60 mg/m³) of blasting sand but was notably absent in the lungs of rats exposed to specular hematite. Alveolar epithelial hyperplasia was present at week 16 in the lungs of rats exposed to the two highest concentrations (30 or 60 mg/m³) of specular hematite but not blasting sand. Specular hematite exhibited the potential to be an inhalation toxicant in workers exposed via abrasive blasting operations but to a lesser degree than blasting sand because the lungs of rats exposed to specular hematite showed a lower incidence of interstitial fibrosis and an absence of alveolar proteinosis.

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Appendix A. Bronchoalveolar Lavage Results

Tables

Table A-1. Bronchoalveolar Lavage Data for Sprague Dawley Rats in the 39-week Inhalation Study of Blasting Sand	A-2
Table A-2. Bronchoalveolar Lavage Data for Sprague Dawley Rats in the 39-week Inhalation Study of Specular Hematite	A-6
Table A-3. Comparisons of Selected Bronchoalveolar Lavage Data for Male Sprague Dawley Rats in the 39-week Inhalation Studies of Blasting Sand and Specular Hematite	A-10

Table A-1. Bronchoalveolar Lavage Data for Sprague Dawley Rats in the 39-week Inhalation Study of Blasting Sand

	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³
n	8	8	8	8
Male				
Viable Cells (%)				
Week 4	90.53 ± 1.32	90.45 ± 1.89	94.75 ± 1.26*	94.76 ± 0.70*
Week 16	70.20 ± 4.96	79.11 ± 4.31	77.96 ± 3.26	84.70 ± 2.26
Week 26	77.31 ± 2.77	87.55 ± 1.97*	87.26 ± 2.08	87.00 ± 1.91
Week 39	93.44 ± 2.17	92.21 ± 2.94	95.85 ± 1.06	98.19 ± 0.27
Total Cells Counted				
Week 4	248.8 ± 24.4	249.4 ± 23.2	280.6 ± 32.6	301.3 ± 30.6
Week 16	308 ± 67	413 ± 81	439 ± 73	502 ± 86
Week 26	186.3 ± 22.4	184.4 ± 28.7	262.5 ± 32.0	466.9 ± 52.4**
Week 39	128 ± 17	339 ± 65**	450 ± 64**	1,849 ± 301**
Macrophages (%)				
Week 4	99.6 ± 0.2	99.6 ± 0.2	92.8 ± 2.1**	61.9 ± 3.4**
Week 16	99.9 ± 0.1	75.5 ± 3.4**	49.7 ± 2.9**	51.2 ± 4.6**
Week 26	99.8 ± 0.1	69.9 ± 3.2**	48.7 ± 4.9**	33.3 ± 3.5**
Week 39	99.6 ± 0.2	57.0 ± 4.3**	53.4 ± 4.8**	43.6 ± 5.0**
Absolute Macrophages (#/μL)				
Week 4	247.8 ± 24.5	248.5 ± 23.2	260.1 ± 29.1	182.5 ± 13.5
Week 16	307.8 ± 67.5	308.6 ± 60.7	216.5 ± 35.5	270.2 ± 56.9
Week 26	185.9 ± 22.4	131.0 ± 23.4	118.5 ± 7.5	148.9 ± 12.8
Week 39	127.6 ± 17.1	182.7 ± 29.0	238.0 ± 39.8*	755.0 ± 88.4**
Lymphocytes (%)				
Week 4	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.163 ± 0.125
Week 16	0.038 ± 0.038	0.238 ± 0.121	0.213 ± 0.113	1.125 ± 0.397**
Week 26	0.000 ± 0.000	0.513 ± 0.205	0.588 ± 0.163**	1.138 ± 0.235**
Week 39	0.075 ± 0.049	0.613 ± 0.288	1.300 ± 0.306**	0.713 ± 0.215
Absolute Lymphocytes (#/μL)				
Week 4	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0388 ± 0.282
Week 16	0.100 ± 0.100	0.613 ± 0.361	1.013 ± 0.611	5.850 ± 2.092**
Week 26	0.000 ± 0.000	0.775 ± 0.291*	1.625 ± 0.448**	5.425 ± 1.350**
Week 39	0.113 ± 0.074	3.013 ± 1.943	5.663 ± 1.557**	11.663 ± 3.723**

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	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³
Neutrophils (%)				
Week 4	0.450 ± 0.154	0.375 ± 0.209	7.250 ± 2.069**	37.913 ± 3.405**
Week 16	0.075 ± 0.049	24.213 ± 3.419**	50.125 ± 2.935**	47.700 ± 4.788**
Week 26	0.200 ± 0.089	29.625 ± 3.159**	50.750 ± 4.756**	65.538 ± 3.461**
Week 39	0.325 ± 0.164	42.375 ± 4.191**	45.288 ± 4.850**	55.700 ± 4.975**
Absolute Neutrophils (#/μL)				
Week 4	0.950 ± 0.271	0.850 ± 0.385	20.563 ± 7.392**	118.463 ± 20.735**
Week 16	0.188 ± 0.123	102.963 ± 27.692**	221.288 ± 42.081**	255.875 ± 37.469**
Week 26	0.338 ± 0.129	52.588 ± 7.867**	142.350 ± 27.283**	312.600 ± 45.920**
Week 39	0.463 ± 0.221	153.113 ± 39.785**	206.413 ± 38.182**	1,082.09 ± 237.138**
Eosinophils (%)				
Week 4	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 16	0.000 ± 0.000	0.075 ± 0.049	0.000 ± 0.000	0.000 ± 0.000
Week 26	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 39	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Absolute Eosinophils (#/μL)				
Week 4	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 16	0.000 ± 0.000	0.300 ± 0.237	0.000 ± 0.000	0.000 ± 0.000
Week 26	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 39	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Basophils (%)				
Week 4	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 16	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 26	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 39	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Absolute Basophils (#/μL)				
Week 4	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 16	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 26	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 39	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Lactate Dehydrogenase (IU/L)				
Week 4	34 ± 8	36 ± 4	40 ± 5	93 ± 7**
Week 16	31 ± 7	63 ± 6*	104 ± 7**	162 ± 16**
Week 26	36 ± 6	87 ± 7**	160 ± 21**	322 ± 26**
Week 39	19 ± 6	104 ± 29**	144 ± 26**	338 ± 24**
Albumin (g/dL)				

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	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³
Week 4	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
Week 16	0.02 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 26	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.02
Week 39	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Female				
Viable Cells (%)				
Week 4	89.31 ± 1.66	90.10 ± 1.90	90.46 ± 0.66	91.35 ± 1.99
Week 26	86.49 ± 2.93	89.69 ± 2.20	90.00 ± 1.79	90.90 ± 1.83
Total Cells Counted				
Week 4	446.9 ± 30.9	398.8 ± 24.2	403.1 ± 38.5	311.3 ± 24.6**
Week 26	294.4 ± 21.6	298.8 ± 31.4	592.5 ± 49.1**	768.1 ± 30.3**
Macrophages (%)				
Week 4	99.7 ± 0.1	99.8 ± 0.1	98.3 ± 0.5*	72.2 ± 5.9**
Week 26	99.4 ± 0.3	53.1 ± 4.0**	34.2 ± 3.5**	27.3 ± 2.8**
Absolute Macrophages (#/μL)				
Week 4	445.6 ± 31.1	397.9 ± 24.5	396.2 ± 37.5	222.1 ± 24.4**
Week 26	292.4 ± 21.0	158.1 ± 20.0**	196.8 ± 17.9*	207.7 ± 21.2
Lymphocytes (%)				
Week 4	0.163 ± 0.091	0.038 ± 0.038	0.363 ± 0.082	0.650 ± 0.155**
Week 26	0.038 ± 0.038	0.250 ± 0.109	0.413 ± 0.091**	0.200 ± 0.089
Absolute Lymphocytes (#/μL)				
Week 4	0.650 ± 0.358	0.125 ± 0.125	1.575 ± 0.296*	2.138 ± 0.523*
Week 26	0.138 ± 0.138	0.688 ± 0.271	2.538 ± 0.554**	1.675 ± 0.734*
Neutrophils (%)				
Week 4	0.150 ± 0.057	0.075 ± 0.049	0.963 ± 0.364	30.913 ± 5.685**
Week 26	0.538 ± 0.285	46.663 ± 4.075**	65.363 ± 3.427**	72.538 ± 2.769**
Absolute Neutrophils (#/μL)				
Week 4	0.663 ± 0.256	0.325 ± 0.220	3.938 ± 1.834	92.838 ± 17.475**
Week 26	1.800 ± 0.962	139.988 ± 21.328**	393.200 ± 46.620**	558.775 ± 34.693**
Eosinophils (%)				
Week 4	0.000 ± 0.000	0.125 ± 0.090	0.363 ± 0.240	0.375 ± 0.209
Week 26	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Absolute Eosinophils (#/μL)				
Week 4	0.000 ± 0.000	0.413 ± 0.297	1.413 ± 0.964	1.250 ± 0.715
Week 26	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Basophils (%)				

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	Chamber Control	15 mg/m³	30 mg/m³	60 mg/m³
Week 4	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 26	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Absolute Basophils (#/μL)				
Week 4	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 26	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Lactate Dehydrogenase (IU/L)				
Week 4	34 ± 4	30 ± 2	37 ± 4	67 ± 11**
Week 26	26 ± 2	87 ± 12**	235 ± 27**	420 ± 20**
Albumin (g/dL)				
Week 4	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01
Week 26	0.06 ± 0.02	0.10 ± 0.01	0.07 ± 0.01	0.05 ± 0.01

*Significantly different ($p \leq 0.05$) from the chamber control group by Dunn's or Shirley's test.

** $p \leq 0.01$.

Data are presented as mean ± standard error.

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Table A-2. Bronchoalveolar Lavage Data for Sprague Dawley Rats in the 39-week Inhalation Study of Specular Hematite

	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³
Male				
Week 4	8	8	8	8
Week 16	7	8	7	6
Week 26	8	8	8	8
Week 39	8	8	8	8
Viable Cells (%)				
Week 4	81.76 ± 3.80	86.33 ± 3.33	87.74 ± 2.17	91.41 ± 1.67
Week 16	88.67 ± 0.52	91.03 ± 1.74	87.97 ± 1.91	92.95 ± 2.40
Week 26	93.03 ± 1.20	94.41 ± 1.26	98.19 ± 0.74**	98.35 ± 0.60**
Week 39	89.75 ± 2.29	94.53 ± 1.82	97.35 ± 0.90*	98.96 ± 0.39**
Total Cells Counted				
Week 4	153.1 ± 22.1	198.1 ± 23.0	186.9 ± 24.0	155.6 ± 18.1
Week 16	554 ± 61	371 ± 42	313 ± 36**	391 ± 28
Week 26	204.4 ± 18.9	192.5 ± 17.2	280.6 ± 27.6*	608.1 ± 83.4**
Week 39	173 ± 11	273 ± 27*	394 ± 51**	749 ± 46**
Macrophages (%)				
Week 4	99.7 ± 0.1	99.7 ± 0.2	99.2 ± 0.2	96.1 ± 0.8**
Week 16	99.7 ± 0.1	90.7 ± 2.1**	78.6 ± 3.0**	48.0 ± 3.6**
Week 26	99.9 ± 0.1	91.0 ± 1.8**	73.7 ± 3.7**	44.4 ± 2.4**
Week 39	99.8 ± 0.1	87.2 ± 2.4**	65.1 ± 3.7**	34.9 ± 4.3**
Absolute Macrophages (#/μL)				
Week 4	152.6 ± 22.1	197.8 ± 23.2	185.3 ± 23.6	150.2 ± 18.6
Week 16	552.0 ± 60.9	334.8 ± 37.1*	244.7 ± 30.4**	188.5 ± 21.4**
Week 26	204.2 ± 19.0	173.7 ± 13.8	206.2 ± 22.5	273.4 ± 45.1
Week 39	172.8 ± 11.4	233.7 ± 18.2	252.0 ± 31.7	270.7 ± 44.3
Lymphocytes (%)				
Week 4	0.200 ± 0.089	0.125 ± 0.125	0.413 ± 0.126	0.413 ± 0.126
Week 16	0.043 ± 0.043	0.838 ± 0.191**	0.671 ± 0.148**	1.733 ± 0.429**
Week 26	0.038 ± 0.038	0.000 ± 0.000	0.038 ± 0.038	0.038 ± 0.038
Week 39	0.000 ± 0.000	0.838 ± 0.328**	1.463 ± 0.272**	1.675 ± 0.351**
Absolute Lymphocytes (#/μL)				
Week 4	0.288 ± 0.109	0.138 ± 0.138	0.838 ± 0.345	0.563 ± 0.148
Week 16	0.314 ± 0.314	3.150 ± 0.840*	2.329 ± 0.637*	6.350 ± 1.358**
Week 26	0.063 ± 0.063	0.000 ± 0.000	0.188 ± 0.188	0.263 ± 0.263

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	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³
Week 39	0.000 ± 0.000	2.675 ± 1.281**	5.500 ± 1.104**	11.850 ± 2.142**
Neutrophils (%)				
Week 4	0.125 ± 0.090	0.163 ± 0.091	0.375 ± 0.171	3.500 ± 0.721**
Week 16	0.286 ± 0.155	8.450 ± 1.943**	20.743 ± 2.993**	50.217 ± 3.741**
Week 26	0.088 ± 0.088	9.050 ± 1.772**	26.313 ± 3.735**	55.613 ± 2.385**
Week 39	0.188 ± 0.055	11.925 ± 2.173**	33.550 ± 3.733**	63.425 ± 4.085**
Absolute Neutrophils (#/μL)				
Week 4	0.238 ± 0.169	0.200 ± 0.102	0.750 ± 0.351	4.900 ± 0.992**
Week 16	1.257 ± 0.647	32.700 ± 8.403**	65.786 ± 13.558**	195.767 ± 19.181**
Week 26	0.113 ± 0.113	18.788 ± 4.945**	74.250 ± 12.106**	334.488 ± 43.107**
Week 39	0.363 ± 0.112	36.175 ± 9.806**	137.063 ± 32.091**	466.263 ± 26.946**
Eosinophils (%)				
Week 4	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 16	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.050 ± 0.050
Week 26	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 39	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Absolute Eosinophils (#/μL)				
Week 4	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 16	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.250 ± 0.250
Week 26	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 39	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Basophils (%)				
Week 4	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 16	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 26	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 39	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Absolute Basophils (#/μL)				
Week 4	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 16	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 26	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 39	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Lactate Dehydrogenase (IU/L)				
Week 4	22 ± 4	27 ± 5 ^a	25 ± 7	30 ± 4
Week 16	38 ± 4 ^b	64 ± 9*	77 ± 8 ^{**b}	263 ± 43 ^{**b}
Week 26	14 ± 4 ^a	31 ± 3 ^{**}	45 ± 3 ^{**}	126 ± 14 ^{**}
Week 39	25 ± 2	43 ± 4 ^{**}	93 ± 6 ^{**}	219 ± 23 ^{**}

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	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³
Albumin (g/dL)				
Week 4	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.00
Week 16	0.02 ± 0.01 ^b	0.03 ± 0.01	0.01 ± 0.00 ^b	0.02 ± 0.01 ^b
Week 26	0.02 ± 0.01	0.03 ± 0.01	0.01 ± 0.00	0.02 ± 0.01
Week 39	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01
Female				
n	8	8	8	8
Viable Cells (%)				
Week 4	84.15 ± 3.63	82.81 ± 4.89	88.60 ± 1.84	90.39 ± 1.38
Week 26	93.45 ± 0.71	93.95 ± 1.38	94.66 ± 1.67	98.43 ± 0.36**
Total Cells Counted				
Week 4	353.8 ± 32.0	337.5 ± 22.4	341.3 ± 16.6	268.8 ± 20.2
Week 26	248.1 ± 22.2	288.1 ± 15.2	490.0 ± 51.6**	1,766.9 ± 95.1**
Macrophages (%)				
Week 4	99.8 ± 0.1	99.4 ± 0.1	99.8 ± 0.1	96.0 ± 1.9*
Week 26	99.5 ± 0.2	92.2 ± 2.4**	71.0 ± 2.6**	30.0 ± 2.0**
Absolute Macrophages (#/μL)				
Week 4	353.0 ± 31.8	335.6 ± 22.3	340.7 ± 16.6	257.6 ± 19.6*
Week 26	246.9 ± 22.1	264.1 ± 10.7	351.2 ± 42.3*	525.6 ± 40.0**
Lymphocytes (%)				
Week 4	0.000 ± 0.000	0.338 ± 0.115*	0.163 ± 0.091	0.363 ± 0.158*
Week 26	0.038 ± 0.038	0.650 ± 0.197*	1.575 ± 0.184**	2.088 ± 0.391**
Absolute Lymphocytes (#/μL)				
Week 4	0.000 ± 0.000	0.013 ± 0.339*	0.588 ± 0.325	1.038 ± 0.499
Week 26	0.113 ± 0.113	1.975 ± 0.641*	7.638 ± 1.125**	38.475 ± 9.136**
Neutrophils (%)				
Week 4	0.213 ± 0.113	0.250 ± 0.139	0.000 ± 0.000	3.650 ± 1.713
Week 26	0.450 ± 0.154	7.163 ± 2.181**	27.463 ± 2.634**	67.950 ± 1.916**
Absolute Neutrophils (#/μL)				
Week 4	0.800 ± 0.467	0.888 ± 0.534	0.000 ± 0.000	10.138 ± 5.284
Week 26	1.163 ± 0.362	22.000 ± 7.745**	131.175 ± 14.546**	1,202.813 ± 77.715**
Eosinophils (%)				
Week 4	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 26	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Absolute Eosinophils (#/μL)				
Week 4	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000

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	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³
Week 26	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Basophils (%)				
Week 4	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 26	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Absolute Basophils (#/μL)				
Week 4	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 26	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Lactate Dehydrogenase (IU/L)				
Week 4	24 ± 2	24 ± 2	26 ± 4	35 ± 5
Week 26	14 ± 4	29 ± 4 ^a	71 ± 4 ^{**}	212 ± 17 ^{**}
Albumin (g/dL)				
Week 4	0.01 ± 0.00	0.02 ± 0.01	0.03 ± 0.01	0.06 ± 0.01 ^{**}
Week 26	0.03 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.05 ± 0.01

*Significantly different ($p \leq 0.05$) from the chamber control group by Dunn's or Shirley's test.

** $p \leq 0.01$.

Data are presented as mean ± standard error.

^an = 7.

^bn = 8.

Table A-3. Comparisons of Selected Bronchoalveolar Lavage Data for Male Sprague Dawley Rats in the 39-week Inhalation Studies of Blasting Sand and Specular Hematite

	Concentration (mg/m ³)	Blasting Sand ^a	Specular Hematite	P Value ^a
Total Cells Counted				
Week 4	0	248.75 ± 24.38 (8)	153.13 ± 22.12 (8)	0.173
	15	249.38 ± 23.15 (8)	198.13 ± 23.03 (8)	0.645
	30	280.63 ± 32.63 (8)	186.88 ± 24.00 (8)	0.234
	60	301.25 ± 30.60 (8)	155.63 ± 18.11 (8)	0.029
Week 16	0	308.13 ± 67.41 (8)	553.57 ± 60.62 (7)	0.104
	15	412.50 ± 81.10 (8)	370.63 ± 41.63 (8)	1.000
	30	438.75 ± 72.76 (8)	312.86 ± 36.35 (7)	1.000
	60	501.88 ± 85.76 (8)	390.83 ± 28.03 (6)	1.000
Week 26	0	186.25 ± 22.36 (8)	204.38 ± 18.88 (8)	1.000
	15	184.38 ± 28.65 (8)	192.50 ± 17.19 (8)	1.000
	30	262.50 ± 32.00 (8)	280.63 ± 27.56 (8)	1.000
	60	466.88 ± 52.39 (8)	608.13 ± 83.86 (8)	0.983
Week 39	0	128.13 ± 17.14 (8)	173.13 ± 11.41 (8)	0.283
	15	338.75 ± 64.98 (8)	272.50 ± 27.37 (8)	1.000
	30	450.00 ± 63.84 (8)	393.75 ± 51.33 (8)	1.000
	60	1,848.75 ± 300.88 (8)	748.75 ± 45.84 (8)	0.019
Lactate Dehydrogenase (IU/L)				
Week 4	0	33.88 ± 8.26 (8)	22.38 ± 3.71 (8)	1.000
	15	36.13 ± 4.42 (8)	27.00 ± 4.50 (7)	0.745
	30	40.25 ± 5.03 (8)	25.25 ± 7.13 (8)	0.647
	60	93.38 ± 6.69 (8)	29.50 ± 3.86 (8)	0.019
Week 16	0	30.75 ± 7.42 (8)	39.71 ± 4.56 (7)	1.000
	15	63.25 ± 6.49 (8)	64.13 ± 9.06 (8)	1.000
	30	104.00 ± 7.31 (8)	78.43 ± 9.70 (7)	0.406
	60	162.00 ± 15.60 (8)	250.33 ± 55.87 (6)	1.000
Week 26	0	35.88 ± 5.94 (8)	14.14 ± 3.78 (7)	0.074
	15	87.38 ± 7.24 (8)	30.50 ± 2.88 (8)	0.019
	30	160.00 ± 20.95 (8)	45.25 ± 3.02 (8)	0.019
	60	321.75 ± 26.34 (8)	125.88 ± 14.30 (8)	0.019
Week 39	0	18.88 ± 5.50 (8)	25.00 ± 2.31 (8)	0.590
	15	103.50 ± 29.14 (8)	42.50 ± 3.91 (8)	0.646
	30	143.63 ± 26.01 (8)	92.75 ± 5.95 (8)	0.707
	60	338.25 ± 24.39 (8)	219.25 ± 23.26 (8)	0.069

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	Concentration (mg/m ³)	Blasting Sand ^a	Specular Hematite	P Value ^a
Absolute Macrophages (#/μL)				
Week 4	0	247.80 ± 24.48 (8)	152.61 ± 22.08 (8)	0.192
	15	248.54 ± 23.22 (8)	197.79 ± 23.19 (8)	0.768
	30	260.08 ± 29.13 (8)	185.31 ± 23.61 (8)	0.415
	60	182.45 ± 13.53 (8)	150.18 ± 18.55 (8)	0.707
Week 16	0	307.84 ± 67.45 (8)	552.01 ± 60.90 (7)	0.104
	15	308.64 ± 60.67 (8)	334.83 ± 37.14 (8)	1.000
	30	216.46 ± 35.54 (8)	244.74 ± 30.36 (7)	1.000
	60	270.16 ± 56.87 (8)	188.45 ± 21.42 (6)	1.000
Week 26	0	185.92 ± 22.40 (8)	204.21 ± 18.97 (8)	1.000
	15	131.02 ± 23.42 (8)	173.73 ± 13.84 (8)	0.284
	30	118.54 ± 7.50 (8)	206.21 ± 22.49 (8)	0.029
	60	148.86 ± 12.81 (8)	273.40 ± 45.12 (8)	0.069
Week 39	0	127.55 ± 17.06 (8)	172.76 ± 11.38 (8)	0.283
	15	182.66 ± 29.01 (8)	233.69 ± 18.19 (8)	0.836
	30	237.96 ± 39.77 (8)	251.98 ± 31.65 (8)	1.000
	60	755.00 ± 88.39 (8)	270.66 ± 44.33 (8)	0.024
Absolute Neutrophils (#/μL)				
Week 4	0	0.95 ± 0.27 (8)	0.24 ± 0.17 (8)	0.273
	15	0.85 ± 0.39 (8)	0.20 ± 0.10 (8)	1.000
	30	20.56 ± 7.39 (8)	0.75 ± 0.35 (8)	0.023
	60	118.46 ± 20.74 (8)	4.90 ± 0.99 (8)	0.019
Week 16	0	0.19 ± 0.12 (8)	1.26 ± 0.65 (7)	1.000
	15	102.95 ± 27.69 (8)	32.70 ± 8.40 (8)	0.157
	30	221.28 ± 42.08 (8)	65.79 ± 13.56 (7)	0.042
	60	225.87 ± 37.47 (8)	195.77 ± 19.18 (6)	1.000
Week 26	0	0.33 ± 0.13 (8)	0.11 ± 0.11 (8)	0.974
	15	52.58 ± 7.86 (8)	18.79 ± 4.95 (8)	0.056
	30	142.34 ± 27.28 (8)	74.25 ± 12.11 (8)	0.284
	60	312.59 ± 45.91 (8)	334.49 ± 43.02 (8)	1.000
Week 39	0	0.47 ± 0.22 (8)	0.36 ± 0.11 (8)	1.000
	15	153.10 ± 39.78 (8)	36.18 ± 9.81 (8)	0.069
	30	206.39 ± 38.18 (8)	137.06 ± 32.09 (8)	0.415
	60	1,082.08 ± 237.14 (8)	466.26 ± 26.95 (8)	0.128

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	Concentration (mg/m ³)	Blasting Sand ^a	Specular Hematite	P Value ^a
Absolute Lymphocytes (#/μL)				
Week 4	0	0.00 ± 0.00 (8)	0.29 ± 0.11 (8)	0.193
	15	0.00 ± 0.00 (8)	0.14 ± 0.14 (8)	1.000
	30	0.00 ± 0.00 (8)	0.83 ± 0.34 (8)	0.049
	60	0.39 ± 0.28 (8)	0.56 ± 0.15 (8)	0.866
Week 16	0	0.10 ± 0.10 (8)	0.31 ± 0.31 (7)	1.000
	15	0.61 ± 0.36 (8)	3.15 ± 0.84 (8)	0.120
	30	1.01 ± 0.61 (8)	2.33 ± 0.64 (7)	0.466
	60	5.84 ± 2.09 (8)	6.35 ± 1.36 (6)	1.000
Week 26	0	0.00 ± 0.00 (8)	0.06 ± 0.06 (8)	1.000
	15	0.78 ± 0.29 (8)	0.00 ± 0.00 (8)	0.100
	30	1.63 ± 0.45 (8)	0.19 ± 0.19 (8)	0.126
	60	5.42 ± 1.35 (8)	0.26 ± 0.26 (8)	0.022
Week 39	0	0.11 ± 0.07 (8)	0.00 ± 0.00 (8)	0.764
	15	2.99 ± 1.94 (8)	2.68 ± 1.28 (8)	1.000
	30	5.65 ± 1.56 (8)	5.50 ± 1.10 (8)	1.000
	60	11.67 ± 3.72 (8)	11.85 ± 2.14 (8)	1.000

Data are presented as mean ± standard error (n).

^aP values determined by the two-sided Wilcoxon rank-sum test with Bonferroni correction.

Appendix B. Tissue Burden Results

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B.1. Lung Deposition and Clearance Equations Used in the Two-week Inhalation Studies of Abrasive Blasting Agents

Lung clearance rates for the abrasive blasting agents blasting sand, coal slag, crushed glass, garnet, or specular hematite were calculated using Equation (1):

$$\text{Equation (1): } A(t) = A_0(e^{-kt})$$

where $A(t)$ is the lung burden (μg abrasive blasting agents) at time t ($t = 21$ days postexposure), A_0 is the lung burden at $t = 0$ days postexposure (day 16), and k is the lung clearance rate constant (fraction cleared per day). Lung burdens for the specular hematite study were all control corrected.

Lung clearance half-lives in days ($t_{1/2}$) were calculated from Equation (2), where $\ln 2$ is the Napierian logarithm of 2:

$$\text{Equation (2): } t_{1/2} = \ln 2/k$$

Deposition rates were calculated from lung abrasive blasting agent burdens using Equation (3). The lung burden and time at terminal euthanasia and the calculated lung clearance rate constant were used to solve for the deposition rate α ($\mu\text{g}/\text{day}$).

$$\text{Equation (3): } A(t) = (\alpha/k)(1 - e^{-kt})$$

In Equation (3), $A(t)$ is the lung burden (μg abrasive blasting agents) at time t [$t = 16$ days on study]; α is the amount of abrasive blasting agents deposited ($\mu\text{g}/\text{day}$); and k is the first-order clearance rate constant derived from Equation (1). Steady-state or equilibrium lung burdens (A_e , μg abrasive blasting agents) were calculated according to Equation (4):

$$\text{Equation (4): } A_e = \alpha/k$$

B.2. Lung Deposition and Clearance Equations Used in the 39-week Inhalation Studies of Abrasive Blasting Agents

The lung burden model used for these studies assumed a zero-order (constant) deposition rate and a first-order (with respect to lung burden) clearance rate as shown in Equation (5):

$$\text{Equation (5): } L(t) = (D/k)(1 - e^{-kt})$$

In Equation (5), $L(t)$ is the retained abrasive blasting agents lung burden ($\mu\text{g}/\text{lung}$) at any time t (days on study); D is the deposition rate ($\mu\text{g}/\text{day}$); and k is the lung clearance rate constant (days^{-1}). Equation (5) also contains a boundary condition that at $t = 0$, the mass of abrasive blasting agents in the lung is zero. Lung burdens for the specular hematite study were all control corrected.

This model was fit to the abrasive blasting agents' lung burden data collected during the in-life part of the 39-week studies. The model was fit to the individual animal data from each exposure group using SAS PROCNLIN (SAS Institute Inc., Cary, NC). Due to the potential for more uncertainty with relatively higher lung burdens, several weighting schemes were investigated, including unweighted, $1/\text{variance}$, $1/\text{square root of the variance}$, and bounding permissible

estimate of k to greater than zero. Review of the results suggested that 1/square root of the variance was the best choice, so all results are presented from model fits using this weighting.

This model fit provided direct estimates of D and k along with their standard errors for each exposure group. Parameters estimated by the model fit were used to calculate the lung clearance half-time ($t_{1/2}$, days) and the theoretical steady-state lung burden (L_{ss} , μg abrasive blasting agents) for each exposure group along with their standard errors using propagation of error techniques and Equations (6) and (7), respectively:

$$\text{Equation (6): } t_{1/2} = \ln 2/k$$

$$\text{Equation (7): } L_{ss} = D/k$$

The total lung dose (mg/lung) administered in each exposure group was calculated using Equation (8):

$$\text{Equation (8): Total dose} = 271 \times D/1,000$$

In Equation (8), 271 is the total number of study days; D is the deposition rate ($\mu\text{g}/\text{day}$); and dividing by 1,000 converts units from μg to mg. Also determined for each exposure group was the total amount of abrasive blasting agents cleared from the lungs during the studies, calculated as the difference between the calculated dose and the calculated lung burden at study day 271. This was expressed as both an absolute value and as the percentage relative to the total dose.

B.3. Lung Overload Evaluation Used in the 39-week Inhalation Studies of Abrasive Blasting Agents

Calculations were undertaken to investigate the possibility that lung overload may have occurred in one or more of the exposure groups during the 39-week studies. Calculated lung burdens were converted to the equivalent volume of the abrasive blasting agents and compared to the respective threshold volume required to reach overload.

Using Equation (9), the total volume (μm^3) of abrasive blasting agents in the lungs for each day on study was calculated:

$$\text{Equation (9): } V_p(t) = [10^{-3} \times L(t)/p] \times (10^{12})$$

In Equation (9), $V_p(t)$ is the volume of the retained lung burden in μm^3 at any time t (days on study), L(t) is the calculated lung burden (converted to mg by multiplying by 10^{-3} mg/ μg) at time t, p is the density of the abrasive blasting agents (4,990 mg/ cm^3 or 2,780 mg/ cm^3 for specular hematite or blasting sand, respectively), and the factor 10^{12} converts cm^3 to μm^3 .

Through an iterative calculation using Equation (10), the ratios of the volume of retained abrasive blasting agents' lung burden at time t [$V_p(t)$] to the threshold volume required for the onset of lung overload (V^*) were determined:

$$\text{Equation (10): } R(t) = V_p(t)/V^*$$

The calculated ratios were used to determine the time of onset of lung overload and the extent of overload achieved over the course of the studies. The time of onset of the overload condition (t^* ,

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days) was determined as the earliest time (days on study) for which $R(t) > 1.0$. The maximum value of the ratio (R_{max}) was used to determine the extent of overload that occurred by the end of the study (day 271).

Table B-1. Lung Weights and Lung Burdens for Male F344/NTac Rats in the Two-week Inhalation Study of Blasting Sand

	Chamber Control	3 mg/m ³	15 mg/m ³	30 mg/m ³
n	5	5	5	5
Absolute Lung Wt. (g)				
Day 1	0.59 ± 0.04	0.54 ± 0.01	0.55 ± 0.01	0.56 ± 0.02
Day 5	0.70 ± 0.06	0.64 ± 0.03	0.58 ± 0.01	0.66 ± 0.02
Day 8	0.78 ± 0.04	0.73 ± 0.01	0.68 ± 0.03	0.71 ± 0.04
Day 12	0.77 ± 0.03	0.77 ± 0.01	0.91 ± 0.07	0.82 ± 0.05
Day 15	0.76 ± 0.04	0.85 ± 0.04	0.81 ± 0.07	0.82 ± 0.06
Day 16	0.94 ± 0.05	0.89 ± 0.07	0.93 ± 0.06	0.86 ± 0.05 ^a
Day 37	0.99 ± 0.04	1.12 ± 0.06	1.00 ± 0.04	1.03 ± 0.04
µg Si/g Lung				
Day 1	0.6 ± 0.0	6.9 ± 0.6**	32.9 ± 0.8**	68.2 ± 3.1**
Day 5	0.6 ± 0.0	33.4 ± 1.1**	156.5 ± 3.7**	277.4 ± 10.0**
Day 8	1.0 ± 0.3	28.0 ± 1.4**	124.0 ± 8.7**	245.3 ± 18.0**
Day 12	0.7 ± 0.1	52.1 ± 3.0**	210.8 ± 13.4**	464.0 ± 27.6**
Day 15	0.6 ± 0.0	43.1 ± 3.0**	203.0 ± 19.6**	401.9 ± 28.7**
Day 16	0.7 ± 0.1	48.9 ± 2.8**	228.9 ± 13.4**	526.7 ± 24.9** ^a
Day 37	0.8 ± 0.2	33.3 ± 4.0**	136.9 ± 5.6**	280.6 ± 23.1**
µg Si/Total Lung				
Day 1	0.4 ± 0.0	3.7 ± 0.3**	18.2 ± 0.6**	38.3 ± 1.7**
Day 5	0.4 ± 0.0	21.4 ± 1.0**	90.8 ± 3.4**	183.9 ± 7.6**
Day 8	0.8 ± 0.2	20.5 ± 0.8**	83.1 ± 3.6**	170.4 ± 5.2**
Day 12	0.6 ± 0.1	40.2 ± 2.0**	188.8 ± 5.0**	377.0 ± 13.8**
Day 15	0.5 ± 0.0	36.1 ± 1.4**	159.3 ± 5.8**	322.8 ± 5.7**
Day 16	0.7 ± 0.1	43.3 ± 2.7**	208.9 ± 5.8**	444.8 ± 8.0** ^a
Day 37	0.8 ± 0.2	36.9 ± 3.5**	137.1 ± 6.2**	285.2 ± 20.4**
µg Blasting Sand/g Lung				
Day 1	1.5 ± 0.0	17.5 ± 1.5**	83.6 ± 2.1**	173.0 ± 7.9**
Day 5	1.5 ± 0.0	84.8 ± 2.7**	397.3 ± 9.5**	704.2 ± 25.3**
Day 8	2.6 ± 0.6	71.2 ± 3.7**	314.7 ± 22.2**	622.6 ± 45.6**
Day 12	1.8 ± 0.3	132.2 ± 7.6**	535.1 ± 34.0**	1,177.8 ± 70.1**

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	Chamber Control	3 mg/m ³	15 mg/m ³	30 mg/m ³
Day 15	1.5 ± 0.0	109.5 ± 7.5**	515.4 ± 49.7**	1,020.1 ± 72.9**
Day 16	1.8 ± 0.3	124.1 ± 7.1**	581.0 ± 34.0**	1,336.9 ± 63.1** ^a
Day 37	2.0 ± 0.5	84.6 ± 10.1**	347.5 ± 14.2**	712.1 ± 58.6**
µg Blasting Sand/Total Lung				
Day 1	0.9 ± 0.1	9.4 ± 0.7**	46.2 ± 1.6**	97.1 ± 4.3**
Day 5	1.0 ± 0.1	54.4 ± 2.5**	230.4 ± 8.6**	466.6 ± 19.3**
Day 8	2.0 ± 0.6	52.0 ± 1.9**	210.9 ± 9.2**	432.6 ± 13.3**
Day 12	1.4 ± 0.2	102.1 ± 5.2**	479.1 ± 12.6**	956.9 ± 35.0**
Day 15	1.1 ± 0.1	91.7 ± 3.5**	404.2 ± 14.7**	819.3 ± 14.4**
Day 16	1.7 ± 0.2	109.8 ± 7.0**	530.2 ± 14.8**	1,128.8 ± 20.4** ^a
Day 37	2.0 ± 0.6	93.8 ± 8.9**	347.9 ± 15.8**	723.9 ± 51.8**
µg Blasting Sand/g Lung per mg Blasting Sand/m³				
Day 1	NA	5.8 ± 0.5	5.6 ± 0.1	5.8 ± 0.3
Day 5	NA	28.3 ± 0.9	26.5 ± 0.6	23.5 ± 0.8
Day 8	NA	23.7 ± 1.2	21.0 ± 1.5	20.8 ± 1.5
Day 12	NA	44.1 ± 2.5	35.7 ± 2.3	39.3 ± 2.3
Day 15	NA	36.5 ± 2.5	34.4 ± 3.3	34.0 ± 2.4
Day 16	NA	41.4 ± 2.4	38.7 ± 2.3	44.6 ± 2.1 ^a
Day 37	NA	28.2 ± 3.4	23.2 ± 0.9	23.7 ± 2.0
µg Blasting Sand/Total Lung per mg Blasting Sand/m³				
Day 1	NA	3.1 ± 0.2	3.1 ± 0.1	3.2 ± 0.1
Day 5	NA	18.1 ± 0.8	15.4 ± 0.6	15.6 ± 0.6
Day 8	NA	17.3 ± 0.6	14.1 ± 0.6	14.4 ± 0.4
Day 12	NA	34.0 ± 1.7	31.9 ± 0.8	31.9 ± 1.2
Day 15	NA	30.6 ± 1.2	26.9 ± 1.0	27.3 ± 0.5
Day 16	NA	36.6 ± 2.3	35.3 ± 1.0	37.6 ± 0.7 ^a
Day 37	NA	31.3 ± 3.0	23.2 ± 1.1	24.1 ± 1.7

**Significantly different (p ≤ 0.01) from the chamber control group by Shirley's test.

Data are presented as mean ± standard error. Days 1, 5, 12, and 16 were after the daily exposure, days 8 and 15 were before the daily exposure, and day 37 was after 21 days of recovery. For values reported as below the limit of quantification, the values shown are the constant of half the limit of quantification. Statistical tests were performed only on data that were not exposure concentration normalized.

NA = not applicable.

^an = 4.

Table B-2. Lymph Node Weights for Male F344/NTac Rats in the Two-week Inhalation Study of Blasting Sand

	Chamber Control	3 mg/m ³	15 mg/m ³	30 mg/m ³
n	5	5	5	5
Bronchial Lymph Node (g)				
Day 1	0.010 ± 0.002	0.011 ± 0.003	0.013 ± 0.002	0.010 ± 0.001
Day 5	0.017 ± 0.009	0.013 ± 0.001	0.007 ± 0.000	0.015 ± 0.002
Day 8	0.012 ± 0.002	0.015 ± 0.003	0.012 ± 0.003	0.011 ± 0.002
Day 12	0.011 ± 0.001	0.011 ± 0.001	0.017 ± 0.002	0.015 ± 0.004
Day 15	0.018 ± 0.003	0.011 ± 0.001	0.011 ± 0.001	0.016 ± 0.005
Day 16	0.016 ± 0.004	0.017 ± 0.004	0.017 ± 0.003	0.016 ± 0.004
Day 37	0.017 ± 0.003	0.028 ± 0.004	0.018 ± 0.004	0.025 ± 0.005
Mediastinal Lymph Node (g)				
Day 1	0.037 ± 0.020	0.017 ± 0.002	0.019 ± 0.003	0.015 ± 0.003
Day 5	0.022 ± 0.004	0.023 ± 0.004	0.024 ± 0.003	0.025 ± 0.005
Day 8	0.026 ± 0.003	0.027 ± 0.003	0.025 ± 0.006	0.023 ± 0.003
Day 12	0.039 ± 0.011	0.035 ± 0.005	0.032 ± 0.002	0.035 ± 0.006
Day 15	0.035 ± 0.006	0.026 ± 0.006	0.029 ± 0.003	0.029 ± 0.004
Day 16	0.045 ± 0.008	0.035 ± 0.003	0.038 ± 0.003	0.041 ± 0.005
Day 37	0.052 ± 0.003	0.067 ± 0.009	0.057 ± 0.002	0.066 ± 0.004

Data are presented as mean ± standard error. Days 1, 5, 12, and 16 were after the daily exposure, days 8 and 15 were before the daily exposure, and day 37 was after 21 days of recovery. Differences from the chamber control group are not significant by Dunnett's test.

Table B-3. Lung Deposition and Clearance Parameter Estimates for Male F344/NTac Rats in the Two-week Inhalation Study of Blasting Sand

Parameter	3 mg/m ³	15 mg/m ³	30 mg/m ³
k (days ⁻¹)	0.007	0.020	0.021
t _{1/2} (days)	93	35	33
α (μg blasting sand/total lung per day)	7	39	83
A _e (μg blasting sand/total lung)	975	1,933	3,931

Data are presented as group mean values.

k = first-order lung clearance rate constant; t_{1/2} = clearance half-life; α = deposition rate; A_e = steady-state lung burden.

Table B-4. Lung Weights and Lung Burdens for Male F344/NTac Rats in the Two-week Inhalation Study of Coal Slag

	Chamber Control	3 mg/m ³	15 mg/m ³	30 mg/m ³
n	5	5	5	5
Absolute Lung Wt. (g)				
Day 1	0.56 ± 0.11	0.50 ± 0.02	0.56 ± 0.04	0.54 ± 0.04
Day 5	0.64 ± 0.06	0.64 ± 0.04	0.68 ± 0.04	0.65 ± 0.04
Day 8	0.84 ± 0.01	0.71 ± 0.05	0.75 ± 0.07	0.80 ± 0.10
Day 12	0.77 ± 0.10	0.82 ± 0.05	0.85 ± 0.03	0.83 ± 0.08
Day 15	0.89 ± 0.09	0.84 ± 0.04	0.94 ± 0.06	0.88 ± 0.04
Day 16	0.73 ± 0.02	0.73 ± 0.04	0.79 ± 0.03	0.88 ± 0.06*
Day 37	1.03 ± 0.04	1.01 ± 0.08	1.16 ± 0.05	1.20 ± 0.06
µg Si/g Lung				
Day 1	0.6 ± 0.0	0.9 ± 0.3	13.8 ± 1.5**	30.9 ± 2.0**
Day 5	0.6 ± 0.0	16.4 ± 0.7**	69.5 ± 3.2**	115.3 ± 5.6**
Day 8	0.6 ± 0.0	13.8 ± 1.1**	60.1 ± 5.0**	91.4 ± 11.2**
Day 12	0.6 ± 0.0	25.0 ± 1.5**	112.4 ± 2.2**	200.1 ± 15.2**
Day 15	0.6 ± 0.0	21.8 ± 1.7**	93.9 ± 7.9**	176.0 ± 4.6**
Day 16	0.6 ± 0.0	35.2 ± 2.1**	146.7 ± 5.6**	240.6 ± 14.4**
Day 37	0.6 ± 0.0	18.5 ± 1.7**	77.7 ± 4.2**	142.4 ± 8.1**
µg Si/Total Lung				
Day 1	0.3 ± 0.1	0.4 ± 0.1	7.6 ± 0.8**	16.3 ± 0.7**
Day 5	0.4 ± 0.0	10.3 ± 0.2**	46.9 ± 1.8**	74.6 ± 2.1**
Day 8	0.5 ± 0.1	9.5 ± 0.1**	43.6 ± 0.9**	69.7 ± 5.0**
Day 12	0.5 ± 0.1	20.1 ± 0.4**	94.9 ± 1.6**	160.8 ± 3.3**
Day 15	0.5 ± 0.1	18.2 ± 1.2**	86.8 ± 2.2**	155.0 ± 3.3**
Day 16	0.4 ± 0.0	25.5 ± 0.8**	115.1 ± 3.6**	209.9 ± 9.3**
Day 37	0.6 ± 0.0	18.3 ± 1.0**	89.2 ± 1.3**	169.5 ± 5.4**
µg Coal Slag/g Lung				
Day 1	2.8 ± 0.0	4.0 ± 1.2	63.4 ± 6.9**	141.5 ± 9.0**
Day 5	2.8 ± 0.0	75.0 ± 3.2**	318.8 ± 14.8**	529.0 ± 25.6**
Day 8	2.8 ± 0.0	63.1 ± 5.0**	275.7 ± 22.9**	419.1 ± 51.3**
Day 12	2.8 ± 0.0	114.7 ± 6.9**	515.8 ± 10.0**	917.9 ± 69.7**
Day 15	2.8 ± 0.0	100.1 ± 7.6**	430.7 ± 36.0**	807.2 ± 21.0**
Day 16	2.8 ± 0.0	161.7 ± 9.6**	672.8 ± 25.9**	1,103.6 ± 65.9**
Day 37	2.8 ± 0.0	84.7 ± 7.8**	356.6 ± 19.1**	653.3 ± 37.1**

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	Chamber Control	3 mg/m ³	15 mg/m ³	30 mg/m ³
µg Coal Slag/Total Lung				
Day 1	1.5 ± 0.3	1.9 ± 0.6	34.9 ± 3.7**	74.7 ± 3.3**
Day 5	1.8 ± 0.2	47.4 ± 1.1**	215.3 ± 8.2**	342.2 ± 9.7**
Day 8	2.3 ± 0.3	43.6 ± 0.5**	199.9 ± 3.9**	319.8 ± 22.7**
Day 12	2.1 ± 0.3	92.2 ± 1.6**	435.2 ± 7.5**	737.6 ± 15.3**
Day 15	2.5 ± 0.3	83.6 ± 5.5**	398.0 ± 10.1**	710.9 ± 15.1**
Day 16	2.0 ± 0.1	116.9 ± 3.9**	528.2 ± 16.4**	962.8 ± 42.8**
Day 37	2.8 ± 0.1	83.7 ± 4.8**	409.4 ± 6.1**	777.5 ± 24.7**
µg Coal Slag/g Lung per mg Coal Slag/m³				
Day 1	NA	1.3 ± 0.4	4.2 ± 0.5	4.7 ± 0.3
Day 5	NA	25.0 ± 1.1	21.3 ± 1.0	17.6 ± 0.9
Day 8	NA	21.0 ± 1.7	18.4 ± 1.5	14.0 ± 1.7
Day 12	NA	38.2 ± 2.3	34.4 ± 0.7	30.6 ± 2.3
Day 15	NA	33.4 ± 2.5	28.7 ± 2.4	26.9 ± 0.7
Day 16	NA	53.9 ± 3.2	44.9 ± 1.7	36.8 ± 2.2
Day 37	NA	28.2 ± 2.6	23.8 ± 1.3	21.8 ± 1.2
µg Coal Slag/Total Lung per mg Coal Slag/m³				
Day 1	NA	0.6 ± 0.2	2.3 ± 0.3	2.5 ± 0.1
Day 5	NA	15.8 ± 0.4	14.4 ± 0.6	11.4 ± 0.3
Day 8	NA	14.6 ± 0.2	13.3 ± 0.3	10.7 ± 0.8
Day 12	NA	30.7 ± 0.5	29.0 ± 0.5	24.6 ± 0.5
Day 15	NA	27.9 ± 1.8	26.5 ± 0.7	23.7 ± 0.5
Day 16	NA	39.0 ± 1.3	35.2 ± 1.1	32.1 ± 1.4
Day 37	NA	27.9 ± 1.6	27.3 ± 0.4	25.9 ± 0.8

**Significantly different ($p \leq 0.01$) from the chamber control group by Shirley's test.

Data are presented as mean ± standard error. Days 1, 5, 12, and 16 were after the daily exposure, days 8 and 15 were before the daily exposure, and day 37 was after 21 days of recovery. For values reported as below the limit of quantification, the values shown are the constant of half the limit of quantification. Statistical tests were performed only on data that were not exposure concentration normalized.

NA = not applicable.

Table B-5. Lymph Node Weights for Male F344/NTac Rats in the Two-week Inhalation Study of Coal Slag

	Chamber Control	3 mg/m ³	15 mg/m ³	30 mg/m ³
n	5	5	5	5
Bronchial Lymph Node (g)				
Day 1	0.006 ± 0.002	0.004 ± 0.001	0.006 ± 0.001	0.004 ± 0.002
Day 5	0.005 ± 0.002	0.006 ± 0.003	0.006 ± 0.003	0.006 ± 0.003
Day 8	0.006 ± 0.003	0.006 ± 0.002	0.009 ± 0.004	0.006 ± 0.004
Day 12	0.007 ± 0.002	0.009 ± 0.003	0.017 ± 0.011	0.009 ± 0.006
Day 15	0.012 ± 0.003	0.013 ± 0.009	0.012 ± 0.005	0.010 ± 0.003
Day 16	0.010 ± 0.003	0.012 ± 0.003	0.010 ± 0.004	0.016 ± 0.012
Day 37	0.023 ± 0.002	0.025 ± 0.032	0.032 ± 0.019	0.035 ± 0.024
Mediastinal Lymph Node (g)				
Day 1	0.027 ± 0.009	0.029 ± 0.011	0.030 ± 0.006	0.031 ± 0.010
Day 5	0.031 ± 0.004	0.032 ± 0.012	0.033 ± 0.007	0.031 ± 0.007
Day 8	0.025 ± 0.007	0.038 ± 0.004	0.037 ± 0.003	0.039 ± 0.021
Day 12	0.038 ± 0.002	0.041 ± 0.011	0.046 ± 0.010	0.051 ± 0.010
Day 15	0.048 ± 0.010	0.046 ± 0.011	0.050 ± 0.018	0.045 ± 0.017
Day 16	0.042 ± 0.009	0.035 ± 0.007	0.033 ± 0.006	0.039 ± 0.006
Day 37	0.065 ± 0.022	0.065 ± 0.026	0.068 ± 0.026	0.076 ± 0.013

Data are presented as mean ± standard error. Days 1, 5, 12, and 16 were after the daily exposure, days 8 and 15 were before the daily exposure, and day 37 was after 21 days of recovery. Differences from the chamber control group are not significant by Dunnett's test.

Table B-6. Lung Deposition and Clearance Parameter Estimates for Male F344/NTac Rats in the Two-week Inhalation Study of Coal Slag

Parameter	3 mg/m ³	15 mg/m ³	30 mg/m ³
k (days ⁻¹)	0.016	0.012	0.010
t _{1/2} (days)	44	57	68
α (μg coal slag/total lung per day)	8	36	65
A _e (μg coal slag/total lung)	524	2,986	6,419

Data are presented as group mean values.

k = first-order lung clearance rate constant; t_{1/2} = clearance half-life; α = deposition rate; A_e = steady-state lung burden.

Table B-7. Lung Weights and Lung Burdens for Male F344/NTac Rats in the Two-week Inhalation Study of Crushed Glass

	Chamber Control	3 mg/m ³	15 mg/m ³	30 mg/m ³
n	5	5	5	5
Absolute Lung Wt. (g)				
Day 1	0.63 ± 0.06	0.52 ± 0.03	0.54 ± 0.03	0.50 ± 0.01
Day 5	0.62 ± 0.02	0.64 ± 0.02	0.61 ± 0.01	0.61 ± 0.02
Day 8	0.83 ± 0.04	0.72 ± 0.05	0.79 ± 0.06	0.70 ± 0.03
Day 12	0.80 ± 0.04	0.81 ± 0.04	0.79 ± 0.02	0.80 ± 0.06
Day 15	0.90 ± 0.08	0.89 ± 0.04	0.91 ± 0.06	0.92 ± 0.06
Day 16	0.88 ± 0.04	0.92 ± 0.08	0.82 ± 0.02	0.86 ± 0.04
Day 37	1.19 ± 0.07	1.20 ± 0.05	1.12 ± 0.06	1.04 ± 0.03
µg Si/g Lung				
Day 1	0.6 ± 0.0	1.6 ± 0.8	15.1 ± 1.0**	34.7 ± 2.0**
Day 5	0.6 ± 0.0	6.8 ± 0.4**	54.1 ± 2.5**	95.9 ± 3.2**
Day 8	0.6 ± 0.0	8.7 ± 0.6**	38.3 ± 3.4**	82.6 ± 5.3**
Day 12	0.6 ± 0.0	18.9 ± 0.8**	90.2 ± 3.1**	172.9 ± 9.3**
Day 15	0.6 ± 0.0	13.2 ± 1.1**	70.4 ± 2.5**	126.4 ± 7.7**
Day 16	0.6 ± 0.0	19.2 ± 1.9**	108.2 ± 5.5**	188.3 ± 14.5**
Day 37	0.6 ± 0.0	3.1 ± 0.4**	32.7 ± 1.9**	62.8 ± 1.0**
µg Si/Total Lung				
Day 1	0.4 ± 0.0	0.9 ± 0.4	8.1 ± 0.5**	17.5 ± 1.2**
Day 5	0.4 ± 0.0	4.3 ± 0.3**	33.0 ± 1.8**	58.8 ± 3.1**
Day 8	0.5 ± 0.0	6.2 ± 0.2**	29.3 ± 0.7**	57.3 ± 2.8**
Day 12	0.5 ± 0.0	15.3 ± 0.8**	71.3 ± 2.5**	136.1 ± 3.8**
Day 15	0.5 ± 0.1	11.6 ± 0.4**	63.2 ± 2.7**	114.4 ± 2.5**
Day 16	0.5 ± 0.0	17.0 ± 0.9**	88.5 ± 4.7**	159.9 ± 7.5**
Day 37	0.7 ± 0.0	3.7 ± 0.6**	37.1 ± 4.0**	65.3 ± 2.6**
µg Crushed Glass/g Lung				
Day 1	1.9 ± 0.0	5.1 ± 2.5	48.3 ± 3.2**	111.2 ± 6.4**
Day 5	1.9 ± 0.0	21.7 ± 1.3**	173.3 ± 8.0**	307.3 ± 10.3**
Day 8	1.9 ± 0.0	27.7 ± 1.8**	122.6 ± 10.8**	264.9 ± 17.0**
Day 12	1.9 ± 0.0	60.5 ± 2.7**	289.0 ± 9.8**	554.1 ± 29.8**
Day 15	1.9 ± 0.0	42.4 ± 3.6**	225.6 ± 8.1**	405.2 ± 24.8**
Day 16	1.9 ± 0.0	61.4 ± 6.2**	346.9 ± 17.6**	603.6 ± 46.3**
Day 37	1.9 ± 0.0	9.9 ± 1.4**	104.7 ± 6.1**	201.3 ± 3.2**

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	Chamber Control	3 mg/m ³	15 mg/m ³	30 mg/m ³
µg Crushed Glass/Total Lung				
Day 1	1.2 ± 0.1	2.7 ± 1.4	26.0 ± 1.6**	56.1 ± 4.0**
Day 5	1.2 ± 0.0	13.8 ± 1.0**	105.9 ± 5.6**	188.6 ± 10.0**
Day 8	1.6 ± 0.1	19.7 ± 0.6**	93.9 ± 2.1**	183.6 ± 9.0**
Day 12	1.5 ± 0.1	49.0 ± 2.7**	228.6 ± 7.9**	436.2 ± 12.3**
Day 15	1.7 ± 0.2	37.1 ± 1.2**	202.6 ± 8.5**	366.7 ± 8.1**
Day 16	1.7 ± 0.1	54.6 ± 2.7**	283.5 ± 15.1**	512.5 ± 23.9**
Day 37	2.3 ± 0.1	11.9 ± 1.9**	118.8 ± 12.9**	209.4 ± 8.4**
µg Crushed Glass/g Lung per mg Crushed Glass/m³				
Day 1	NA	1.7 ± 0.8	3.2 ± 0.2	3.7 ± 0.2
Day 5	NA	7.2 ± 0.4	11.6 ± 0.5	10.2 ± 0.3
Day 8	NA	9.3 ± 0.6	8.2 ± 0.7	8.8 ± 0.6
Day 12	NA	20.2 ± 0.9	19.3 ± 0.7	18.5 ± 1.0
Day 15	NA	14.1 ± 1.2	15.0 ± 0.5	13.5 ± 0.8
Day 16	NA	20.5 ± 2.1	23.1 ± 1.2	20.1 ± 1.5
Day 37	NA	3.3 ± 0.5	7.0 ± 0.4	6.7 ± 0.1
µg Crushed Glass/Total Lung per mg Crushed Glass/m³				
Day 1	NA	0.9 ± 0.5	1.7 ± 0.1	1.9 ± 0.1
Day 5	NA	4.6 ± 0.3	7.1 ± 0.4	6.3 ± 0.3
Day 8	NA	6.6 ± 0.2	6.3 ± 0.1	6.1 ± 0.3
Day 12	NA	16.3 ± 0.9	15.2 ± 0.5	14.5 ± 0.4
Day 15	NA	12.4 ± 0.4	13.5 ± 0.6	12.2 ± 0.3
Day 16	NA	18.2 ± 0.9	18.9 ± 1.0	17.1 ± 0.8
Day 37	NA	4.0 ± 0.7	7.9 ± 0.9	7.0 ± 0.3

**Significantly different ($p \leq 0.01$) from the chamber control group by Shirley's test.

Data are presented as mean ± standard error. Days 1, 5, 12, and 16 were after the daily exposure, days 8 and 15 were before the daily exposure, and day 37 was after 21 days of recovery. For values reported as below the limit of quantification, the values shown are the constant of half the limit of quantification. Statistical tests were performed only on data that were not exposure concentration normalized.

NA = not applicable.

Table B-8. Lymph Node Weights for Male F344/NTac Rats in the Two-week Inhalation Study of Crushed Glass

	Chamber Control	3 mg/m ³	15 mg/m ³	30 mg/m ³
n	5	5	5	5
Bronchial Lymph Node (g)				
Day 1	0.015 ± 0.004	0.012 ± 0.005	0.010 ± 0.004	0.011 ± 0.004
Day 5	0.011 ± 0.004	0.011 ± 0.002	0.012 ± 0.005	0.012 ± 0.006
Day 8	0.016 ± 0.005	0.015 ± 0.005	0.014 ± 0.008	0.016 ± 0.006
Day 12	0.014 ± 0.001	0.012 ± 0.007	0.019 ± 0.015	0.011 ± 0.002
Day 15	0.012 ± 0.004	0.010 ± 0.006	0.014 ± 0.002	0.015 ± 0.006
Day 16	0.018 ± 0.005	0.013 ± 0.005	0.013 ± 0.003	0.016 ± 0.005
Day 37	0.023 ± 0.005	0.023 ± 0.004	0.018 ± 0.009	0.020 ± 0.005
Mediastinal Lymph Node (g)				
Day 1	0.023 ± 0.004	0.025 ± 0.003	0.021 ± 0.003	0.024 ± 0.005
Day 5	0.023 ± 0.005	0.028 ± 0.003	0.027 ± 0.007	0.020 ± 0.004
Day 8	0.025 ± 0.001	0.034 ± 0.004	0.029 ± 0.009	0.032 ± 0.009
Day 12	0.030 ± 0.005	0.031 ± 0.008	0.026 ± 0.007	0.032 ± 0.005
Day 15	0.037 ± 0.006	0.040 ± 0.009	0.039 ± 0.003	0.037 ± 0.007
Day 16	0.030 ± 0.008	0.028 ± 0.002	0.032 ± 0.009	0.032 ± 0.010
Day 37	0.056 ± 0.013	0.049 ± 0.006	0.061 ± 0.020	0.059 ± 0.008

Data are presented as mean ± standard error. Days 1, 5, 12, and 16 were after the daily exposure, days 8 and 15 were before the daily exposure, and day 37 was after 21 days of recovery. Differences from the chamber control group are not significant by the Dunnett test.

Table B-9. Lung Deposition and Clearance Parameter Estimates for Male F344/NTac Rats in the Two-week Inhalation Study of Crushed Glass

Parameter	3 mg/m ³	15 mg/m ³	30 mg/m ³
k (days ⁻¹)	0.072	0.041	0.043
t _{1/2} (days)	10	17	16
α (μg crushed glass/total lung per day)	6	24	44
A _e (μg crushed glass/total lung)	80	586	1,035

Data are presented as group mean values.

k = first-order lung clearance rate constant; t_{1/2} = clearance half-life; α = deposition rate; A_e = steady-state lung burden.

Table B-10. Lung Weights and Lung Burdens for Male F344/NTac Rats in the Two-week Inhalation Study of Garnet

	Chamber Control	3 mg/m ³	15 mg/m ³	30 mg/m ³
n	5	5	5	5
Absolute Lung Wt. (g)				
Day 1	0.53 ± 0.02	0.53 ± 0.02	0.56 ± 0.04	0.60 ± 0.02
Day 5	0.63 ± 0.03	0.59 ± 0.01	0.59 ± 0.02	0.63 ± 0.05
Day 8	0.72 ± 0.03	0.71 ± 0.04	0.78 ± 0.05	0.69 ± 0.04
Day 12	0.67 ± 0.03	0.74 ± 0.03	0.80 ± 0.06	0.77 ± 0.03
Day 15	0.75 ± 0.03	0.75 ± 0.04	0.82 ± 0.05	0.84 ± 0.03
Day 16	0.82 ± 0.05	0.81 ± 0.04	0.83 ± 0.02	0.84 ± 0.02
Day 37	1.42 ± 0.28	1.14 ± 0.07	1.31 ± 0.10	1.35 ± 0.07
µg Si/g Lung				
Day 1	0.6 ± 0.0	2.6 ± 0.3**	15.2 ± 1.2**	26.2 ± 1.0**
Day 5	0.6 ± 0.0	15.0 ± 0.6**	68.0 ± 3.5**	115.7 ± 4.9**
Day 8	0.6 ± 0.0	10.5 ± 0.4**	50.2 ± 2.6**	100.9 ± 5.1**
Day 12	0.6 ± 0.0	25.3 ± 0.8**	110.4 ± 8.0**	204.7 ± 11.8**
Day 15	0.6 ± 0.0	20.1 ± 1.5**	96.7 ± 2.6**	168.2 ± 4.3**
Day 16	1.1 ± 0.5	26.7 ± 0.6**	119.2 ± 7.7**	213.8 ± 12.6**
Day 37	0.6 ± 0.0	12.6 ± 0.9**	65.5 ± 5.7**	113.6 ± 10.1**
µg Si/Total Lung				
Day 1	0.3 ± 0.0	1.4 ± 0.1**	8.3 ± 0.4**	15.6 ± 0.5**
Day 5	0.4 ± 0.0	8.9 ± 0.4**	40.1 ± 2.9**	72.3 ± 3.7**
Day 8	0.4 ± 0.0	7.4 ± 0.3**	38.5 ± 1.5**	68.6 ± 2.6**
Day 12	0.4 ± 0.0	18.7 ± 0.2**	86.9 ± 3.4**	155.9 ± 6.8**
Day 15	0.5 ± 0.0	15.2 ± 1.6**	78.3 ± 2.8**	140.3 ± 5.6**
Day 16	0.8 ± 0.3	21.6 ± 0.9**	99.1 ± 7.1**	178.0 ± 8.5**
Day 37	0.9 ± 0.2	14.2 ± 0.6**	84.2 ± 4.9**	151.2 ± 9.8**
µg Garnet/g Lung				
Day 1	3.6 ± 0.0	15.3 ± 1.6**	91.0 ± 7.2**	157.0 ± 5.7**
Day 5	3.6 ± 0.0	90.0 ± 3.6**	406.9 ± 21.0**	692.7 ± 29.4**
Day 8	3.6 ± 0.0	62.6 ± 2.3**	300.3 ± 15.6**	604.4 ± 30.3**
Day 12	3.6 ± 0.0	151.4 ± 4.7**	660.8 ± 47.7**	1,225.6 ± 70.4**
Day 15	3.6 ± 0.0	120.1 ± 8.9**	579.0 ± 15.5**	1,007.1 ± 25.7**
Day 16	6.6 ± 3.0	160.0 ± 3.3**	713.9 ± 46.3**	1,280.2 ± 75.6**
Day 37	3.6 ± 0.0	75.5 ± 5.2**	392.5 ± 34.1**	680.4 ± 60.4**

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	Chamber Control	3 mg/m ³	15 mg/m ³	30 mg/m ³
µg Garnet/Total Lung				
Day 1	1.9 ± 0.1	8.1 ± 0.8**	49.6 ± 2.4**	93.4 ± 2.9**
Day 5	2.3 ± 0.1	53.4 ± 2.2**	240.0 ± 17.5**	432.8 ± 22.1**
Day 8	2.6 ± 0.1	44.0 ± 1.5**	230.5 ± 8.8**	411.0 ± 15.7**
Day 12	2.4 ± 0.1	111.7 ± 1.0**	520.1 ± 20.4**	933.5 ± 40.7**
Day 15	2.7 ± 0.1	90.8 ± 9.4**	469.0 ± 16.9**	840.4 ± 33.5**
Day 16	5.0 ± 2.0	129.5 ± 5.4**	593.5 ± 42.6**	1,065.8 ± 51.2**
Day 37	5.1 ± 1.0	85.0 ± 3.4**	504.3 ± 29.5**	905.3 ± 58.4**
µg Garnet/g Lung per mg Garnet/m³				
Day 1	NA	5.1 ± 0.5	6.1 ± 0.5	5.2 ± 0.2
Day 5	NA	30.0 ± 1.2	27.1 ± 1.4	23.1 ± 1.0
Day 8	NA	20.9 ± 0.8	20.0 ± 1.0	20.2 ± 1.0
Day 12	NA	50.5 ± 1.6	44.1 ± 3.2	40.9 ± 2.4
Day 15	NA	40.0 ± 3.0	38.6 ± 1.0	33.6 ± 0.9
Day 16	NA	53.3 ± 1.1	47.6 ± 3.1	42.7 ± 2.5
Day 37	NA	25.2 ± 1.8	26.2 ± 2.3	22.7 ± 2.0
µg Garnet/Total Lung per mg Garnet/m³				
Day 1	NA	2.7 ± 0.3	3.3 ± 0.2	3.1 ± 0.1
Day 5	NA	17.8 ± 0.8	16.0 ± 1.2	14.4 ± 0.7
Day 8	NA	14.7 ± 0.5	15.4 ± 0.6	13.7 ± 0.5
Day 12	NA	37.2 ± 0.3	34.7 ± 1.4	31.1 ± 1.4
Day 15	NA	30.3 ± 3.1	31.3 ± 1.1	28.0 ± 1.1
Day 16	NA	43.2 ± 1.8	39.6 ± 2.8	35.5 ± 1.7
Day 37	NA	28.3 ± 1.1	33.6 ± 2.0	30.2 ± 2.0

**Significantly different ($p \leq 0.01$) from the chamber control group by Shirley's test.

Data are presented as mean ± standard error. Days 1, 5, 12, and 16 were after the daily exposure, days 8 and 15 were before the daily exposure, and day 37 was after 21 days of recovery. For values reported as below the limit of quantification, the values shown are the constant of half the limit of quantification. Statistical tests were performed only on data that were not exposure concentration normalized.

NA = not applicable.

Table B-11. Lymph Node Weights for Male F344/NTac Rats in the Two-week Inhalation Study of Garnet

	Chamber Control	3 mg/m ³	15 mg/m ³	30 mg/m ³
n	5	5	5	5
Bronchial Lymph Node (g)				
Day 1	0.009 ± 0.002	0.013 ± 0.002	0.011 ± 0.004	0.014 ± 0.005
Day 5	0.012 ± 0.004	0.007 ± 0.003	0.007 ± 0.002	0.010 ± 0.002
Day 8	0.013 ± 0.005	0.013 ± 0.004	0.010 ± 0.003	0.015 ± 0.002
Day 12	0.008 ± 0.002	0.013 ± 0.005*	0.011 ± 0.002	0.014 ± 0.002*
Day 15	0.011 ± 0.005	0.016 ± 0.004	0.013 ± 0.002	0.016 ± 0.003
Day 16	0.012 ± 0.003	0.017 ± 0.009	0.021 ± 0.008	0.013 ± 0.004
Day 37	0.015 ± 0.006	0.016 ± 0.006	0.024 ± 0.012	0.025 ± 0.014
Mediastinal Lymph Node (g)				
Day 1	0.015 ± 0.006	0.017 ± 0.006	0.018 ± 0.006	0.017 ± 0.002
Day 5	0.020 ± 0.009	0.019 ± 0.013	0.015 ± 0.007	0.017 ± 0.005
Day 8	0.030 ± 0.007	0.024 ± 0.007	0.028 ± 0.008	0.028 ± 0.006
Day 12	0.037 ± 0.004	0.031 ± 0.005	0.034 ± 0.008	0.029 ± 0.007
Day 15	0.038 ± 0.006	0.043 ± 0.007	0.044 ± 0.004	0.044 ± 0.004
Day 16	0.052 ± 0.014	0.056 ± 0.013	0.051 ± 0.015	0.042 ± 0.017
Day 37	0.055 ± 0.008	0.053 ± 0.003	0.062 ± 0.014	0.063 ± 0.006

*Significantly different ($p \leq .05$) from the chamber control group by Dunnett's test.

Data are presented as mean ± standard error. Days 1, 5, 12, and 16 were after the daily exposure, days 8 and 15 were before the daily exposure, and day 37 was after 21 days of recovery.

Table B-12. Lung Deposition and Clearance Parameter Estimates for Male F344/NTac Rats in the Two-week Inhalation Study of Garnet

Parameter	3 mg/m ³	15 mg/m ³	30 mg/m ³
k (days ⁻¹)	0.020	0.008	0.008
t _{1/2} (days)	35	90	89
α (μg garnet/total lung per day)	9	39	71
A _e (μg garnet/total lung)	474	5,089	9,089

Data are presented as group mean values.

k = first-order lung clearance rate constant; t_{1/2} = clearance half-life; α = deposition rate; A_e = steady-state lung burden.

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Table B-13. Lung Weights and Lung Burdens for Male F344/NTac Rats in the Two-week Inhalation Study of Specular Hematite

	Chamber Control	3 mg/m ³	15 mg/m ³	30 mg/m ³	60 mg/m ³
n	5	5	5	5	5
Absolute Lung Wt. (g)					
Day 1	0.57 ± 0.03	0.56 ± 0.03	0.58 ± 0.02	0.54 ± 0.02	0.54 ± 0.02
Day 5	0.66 ± 0.05	0.63 ± 0.02	0.64 ± 0.06	0.68 ± 0.04	0.60 ± 0.03
Day 8	0.70 ± 0.03	0.74 ± 0.04	0.69 ± 0.05	0.73 ± 0.05	0.74 ± 0.07
Day 12	0.83 ± 0.06	0.85 ± 0.09	0.72 ± 0.02	0.80 ± 0.05	0.81 ± 0.06
Day 15	0.79 ± 0.03	0.74 ± 0.03	0.81 ± 0.04	0.75 ± 0.04	0.87 ± 0.06
Day 16	0.81 ± 0.04	0.78 ± 0.02	0.87 ± 0.04	0.83 ± 0.11	0.79 ± 0.04
Day 37	0.95 ± 0.04	1.03 ± 0.07	1.01 ± 0.03	1.08 ± 0.02	1.52 ± 0.40*
µg Fe/g Lung					
Day 1	48.82 ± 1.32	67.13 ± 3.62**	134.02 ± 8.21**	144.88 ± 4.69**	238.41 ± 7.83**
Day 5	55.22 ± 2.45	124.91 ± 4.50**	362.65 ± 35.17**	504.46 ± 28.72**	916.49 ± 43.84**
Day 8	54.50 ± 4.26	106.30 ± 4.83**	326.25 ± 17.90**	423.97 ± 27.71**	709.69 ± 76.78**
Day 12	54.37 ± 0.59	141.68 ± 9.91**	503.76 ± 14.31**	797.62 ± 68.57**	1,353.0 ± 68.74**
Day 15	64.17 ± 5.51	140.80 ± 4.96**	432.67 ± 27.70**	745.63 ± 28.70**	1,264.5 ± 75.81**
Day 16	54.12 ± 2.99	157.68 ± 4.34**	513.81 ± 17.19**	835.34 ± 50.01**	1,637.9 ± 87.70**
Day 37	68.66 ± 2.24	127.31 ± 4.04**	309.93 ± 13.55**	506.94 ± 19.01**	797.55 ± 142.71**
µg Fe/g Lung (Control Corrected)					
Day 1	NA	18.313 ± 3.619	85.200 ± 8.215	96.058 ± 4.693	189.589 ± 7.828
Day 5	NA	69.682 ± 4.497	307.429 ± 35.173	449.239 ± 28.720	861.263 ± 43.842
Day 8	NA	51.801 ± 4.826	271.749 ± 17.904	369.476 ± 27.714	655.194 ± 76.776
Day 12	NA	87.312 ± 9.908	449.385 ± 14.308	743.244 ± 68.574	1,298.65 ± 68.741
Day 15	NA	76.628 ± 4.960	368.499 ± 27.700	681.460 ± 28.705	1,200.33 ± 75.805
Day 16	NA	103.558 ± 4.339	459.688 ± 17.189	781.225 ± 50.009	1,583.73 ± 87.702
Day 37	NA	58.654 ± 4.041	241.275 ± 13.552	438.280 ± 19.014	728.890 ± 142.711
µg Fe/Total Lung (Control Corrected)					
Day 1	NA	9.951 ± 1.714	48.450 ± 2.935	51.263 ± 2.529	101.681 ± 3.393
Day 5	NA	43.272 ± 1.878	189.985 ± 9.708	300.703 ± 6.666	514.595 ± 14.909
Day 8	NA	37.465 ± 2.154	184.982 ± 5.977	263.676 ± 9.185	465.181 ± 36.399
Day 12	NA	70.293 ± 1.164	322.883 ± 16.120	582.229 ± 28.068	1,040.18 ± 48.300
Day 15	NA	56.842 ± 3.567	294.985 ± 17.426	508.902 ± 7.167	1,030.47 ± 53.236
Day 16	NA	80.644 ± 3.755	396.829 ± 12.272	630.258 ± 33.507	1,235.45 ± 36.812
Day 37	NA	59.401 ± 3.259	245.654 ± 19.602	470.798 ± 16.954	890.235 ± 115.848

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	Chamber Control	3 mg/m ³	15 mg/m ³	30 mg/m ³	60 mg/m ³
µg Specular Hematite/g Lung (Control Corrected)					
Day 1	NA	26.388 ± 5.215	122.767 ± 11.837	138.412 ± 6.762	273.183 ± 11.280
Day 5	NA	100.406 ± 6.479	442.981 ± 50.681	647.319 ± 41.383	1,241.01 ± 63.173
Day 8	NA	74.642 ± 6.954	391.569 ± 25.799	532.386 ± 39.933	944.083 ± 110.628
Day 12	NA	125.810 ± 14.277	647.529 ± 20.617	1,070.96 ± 98.810	1,871.26 ± 99.051
Day 15	NA	110.415 ± 7.147	530.978 ± 39.913	981.930 ± 41.361	1,729.58 ± 109.230
Day 16	NA	149.219 ± 6.252	662.375 ± 24.767	1,125.68 ± 72.059	2,282.04 ± 126.372
Day 37	NA	84.516 ± 5.823	347.659 ± 19.527	631.528 ± 27.398	1,050.27 ± 205.635
µg Specular Hematite/Total Lung (Control Corrected)					
Day 1	NA	14.339 ± 2.470	69.813 ± 4.228	73.866 ± 3.644	146.515 ± 4.889
Day 5	NA	62.352 ± 2.706	273.753 ± 13.989	433.290 ± 9.605	741.491 ± 21.482
Day 8	NA	53.984 ± 3.103	266.545 ± 8.612	379.937 ± 13.235	670.290 ± 52.448
Day 12	NA	101.287 ± 1.677	465.250 ± 23.229	838.947 ± 40.443	1,498.82 ± 69.597
Day 15	NA	81.905 ± 5.139	425.051 ± 25.110	733.288 ± 10.326	1,484.83 ± 76.708
Day 16	NA	116.202 ± 5.411	571.799 ± 17.683	908.153 ± 48.281	1,780.18 ± 53.043
Day 37	NA	85.591 ± 4.696	353.968 ± 28.245	678.383 ± 24.429	1,282.76 ± 166.928
µg Specular Hematite/Total Lung per mg Specular Hematite/m³ (Control Corrected)					
Day 1	NA	4.780 ± 0.824	4.654 ± 0.282	2.462 ± 0.122	2.442 ± 0.081
Day 5	NA	20.784 ± 0.902	18.250 ± 0.933	14.443 ± 0.320	12.358 ± 0.358
Day 8	NA	17.995 ± 1.034	17.770 ± 0.574	12.665 ± 0.441	11.171 ± 0.874
Day 12	NA	33.763 ± 0.559	31.017 ± 1.549	27.965 ± 1.348	24.980 ± 1.160
Day 15	NA	27.302 ± 1.713	28.337 ± 1.674	24.443 ± 0.344	24.747 ± 1.279
Day 16	NA	38.734 ± 1.804	38.120 ± 1.179	30.272 ± 1.609	29.670 ± 0.884
Day 37	NA	28.531 ± 1.565	23.598 ± 1.883	22.613 ± 0.814	21.379 ± 2.782

*Significantly different ($p \leq 0.05$) from the chamber control group by the Williams or Shirley tests.

** $p \leq 0.01$.

Data are presented as mean ± standard error. Days 1, 5, 12, and 16 were after the daily exposure, days 8 and 15 were before the daily exposure, and day 37 was after 21 days of recovery. Statistical tests were performed only on data that were not control corrected.

NA = not applicable.

Table B-14. Lymph Node Weights for Male F344/NTac Rats in the Two-week Inhalation Study of Specular Hematite

	Chamber Control	3 mg/m ³	15 mg/m ³	30 mg/m ³	60 mg/m ³
n	5	5	5	5	5
Bronchial Lymph Node (g)					
Day 1	0.017 ± 0.001	0.016 ± 0.003	0.018 ± 0.002	0.024 ± 0.003	0.021 ± 0.004
Day 5	0.010 ± 0.001	0.008 ± 0.001	0.009 ± 0.002	0.007 ± 0.002	0.008 ± 0.001
Day 8	0.020 ± 0.003	0.023 ± 0.003	0.022 ± 0.003	0.018 ± 0.002	0.025 ± 0.002
Day 12	0.031 ± 0.004	0.015 ± 0.003*	0.023 ± 0.005	0.024 ± 0.003	0.022 ± 0.005
Day 15	0.015 ± 0.004	0.013 ± 0.002	0.012 ± 0.002	0.011 ± 0.001	0.016 ± 0.003
Day 16	0.018 ± 0.003	0.017 ± 0.002	0.015 ± 0.002	0.013 ± 0.001	0.012 ± 0.003
Day 37	0.019 ± 0.003	0.022 ± 0.010	0.033 ± 0.008	0.027 ± 0.003	0.036 ± 0.008
Mediastinal Lymph Node (g)					
Day 1	0.025 ± 0.002	0.027 ± 0.002	0.029 ± 0.006	0.030 ± 0.002	0.031 ± 0.003
Day 5	0.026 ± 0.004	0.036 ± 0.003	0.028 ± 0.003	0.026 ± 0.005	0.032 ± 0.004
Day 8	0.038 ± 0.005	0.035 ± 0.002	0.038 ± 0.003	0.035 ± 0.004	0.035 ± 0.002
Day 12	0.027 ± 0.001	0.035 ± 0.002	0.030 ± 0.003	0.039 ± 0.003*	0.028 ± 0.004
Day 15	0.037 ± 0.003	0.040 ± 0.007	0.042 ± 0.004	0.042 ± 0.004	0.045 ± 0.004
Day 16	0.029 ± 0.003	0.031 ± 0.002	0.033 ± 0.004	0.034 ± 0.005	0.030 ± 0.003
Day 37	0.056 ± 0.006	0.062 ± 0.008	0.057 ± 0.005	0.050 ± 0.003	0.063 ± 0.007

*Significantly different ($p \leq 0.05$) from the chamber control group by Dunnett's test.

Data are presented as mean ± standard error. Days 1, 5, 12, and 16 were after the daily exposure, days 8 and 15 were before the daily exposure, and day 37 was after 21 days of recovery.

Table B-15. Lung Deposition and Clearance Parameter Estimates for Male F344/NTac Rats in the Two-week Inhalation Study of Specular Hematite

Parameter	3 mg/m ³	15 mg/m ³	30 mg/m ³	60 mg/m ³
k (days ⁻¹)	0.015	0.023	0.014	0.016
t _{1/2} (days)	48	30	50	44
α (µg specular hematite/total lung per day)	8	43	63	126
A _e (µg specular hematite/total lung)	559	1,868	4,557	8,057

Data are presented as group mean values.

k = first-order lung clearance rate constant; t_{1/2} = clearance half-life; α = deposition rate; A_e = steady-state lung burden.

Table B-16. Lung Weights and Lung Burdens for Male Sprague Dawley Rats in the 39-week Inhalation Study of Blasting Sand

	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³
n	5	5	5	5
Absolute Lung Wt. (g)				
Week 1	0.93 ± 0.03	0.98 ± 0.03	0.99 ± 0.05	0.98 ± 0.02
Week 4	1.18 ± 0.04	1.27 ± 0.03	1.28 ± 0.04	1.30 ± 0.04
Week 8	1.34 ± 0.04	1.39 ± 0.04	1.39 ± 0.02	1.71 ± 0.05**
Week 16	1.40 ± 0.05	1.55 ± 0.05	1.66 ± 0.05**	1.97 ± 0.07**
Week 26	1.48 ± 0.02	1.67 ± 0.05	1.92 ± 0.04**	2.43 ± 0.15**
Week 39	1.63 ± 0.02	1.70 ± 0.04	1.96 ± 0.09*	2.73 ± 0.16**
µg Si/g Lung				
Week 1	0.6 ± 0.0	126.2 ± 6.3**	237.4 ± 8.0**	393.2 ± 24.5**
Week 4	0.6 ± 0.0	338.1 ± 6.4**	697.9 ± 36.8**	1,209.6 ± 54.3**
Week 8	0.6 ± 0.0	660.7 ± 25.1**	1,182.3 ± 103.3**	2,208.1 ± 130.6**
Week 16	0.6 ± 0.0	938.6 ± 74.2**	2,093.6 ± 126.2**	3,803.2 ± 141.8**
Week 26	0.6 ± 0.0	1,415.2 ± 96.3**	3,023.4 ± 78.6**	5,039.9 ± 105.6**
Week 39	0.6 ± 0.0	1,978.5 ± 81.9**	3,773.5 ± 163.8**	5,753.7 ± 224.2**
µg Si/Total Lung				
Week 1	0.6 ± 0.0	124.5 ± 8.6**	235.1 ± 13.0**	387.8 ± 30.1**
Week 4	0.7 ± 0.0	428.7 ± 14.0**	896.3 ± 55.2**	1,575.5 ± 77.5**
Week 8	0.8 ± 0.0	916.2 ± 46.2**	1,634.0 ± 132.2**	3,766.0 ± 261.9**
Week 16	0.8 ± 0.0	1,461.0 ± 144.2**	3,484.5 ± 276.7**	7,486.0 ± 339.5**
Week 26	0.9 ± 0.0	2,356.7 ± 155.6**	5,822.0 ± 247.5**	12,230.5 ± 809.5**
Week 39	1.0 ± 0.0	3,376.5 ± 201.4**	7,450.0 ± 663.3**	15,643.5 ± 894.5**
µg Blasting Sand/g Lung				
Week 1	1.5 ± 0.0	320.3 ± 15.9**	602.5 ± 20.2**	998.0 ± 62.3**
Week 4	1.5 ± 0.0	858.0 ± 16.3**	1,771.2 ± 93.5**	3,069.9 ± 137.8**
Week 8	1.5 ± 0.0	1,676.8 ± 63.6**	3,000.9 ± 262.2**	5,604.4 ± 331.4**
Week 16	1.5 ± 0.0	2,382.1 ± 188.4**	5,313.7 ± 320.3**	9,652.9 ± 359.8**
Week 26	1.5 ± 0.0	3,591.8 ± 244.4**	7,673.6 ± 199.4**	12,791.7 ± 268.0**
Week 39	1.5 ± 0.0	5,021.7 ± 207.9**	9,577.4 ± 415.7**	14,603.2 ± 569.1**
µg Blasting Sand/Total Lung				
Week 1	1.4 ± 0.0	316.1 ± 21.9**	596.8 ± 33.0**	984.1 ± 76.5**
Week 4	1.8 ± 0.1	1,087.9 ± 35.5**	2,274.9 ± 140.0**	3,998.7 ± 196.7**
Week 8	2.0 ± 0.1	2,325.4 ± 117.4**	4,147.1 ± 335.5**	9,558.4 ± 664.7**
Week 16	2.1 ± 0.1	3,708.0 ± 366.0**	8,843.9 ± 702.2**	19,000.0 ± 861.8**

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	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³
Week 26	2.2 ± 0.0	5,981.5 ± 395.0**	14,776.6 ± 628.1**	31,041.9 ± 2,054.5**
Week 39	2.5 ± 0.0	8,569.8 ± 511.2**	18,908.6 ± 1,683.4**	39,704.3 ± 2,270.3**
<hr/>				
μg Blasting Sand/Total Lung per mg Blasting Sand/m ³				
Week 1	NA	21.1 ± 1.5	19.9 ± 1.1	16.4 ± 1.3
Week 4	NA	72.5 ± 2.4	75.8 ± 4.7	66.6 ± 3.3
Week 8	NA	155.0 ± 7.8	138.2 ± 11.2	159.3 ± 11.1
Week 16	NA	247.2 ± 24.4	294.8 ± 23.4	316.7 ± 14.4
Week 26	NA	398.8 ± 26.3	492.6 ± 20.9	517.4 ± 34.2
Week 39	NA	571.3 ± 34.1	630.3 ± 56.1	661.7 ± 37.8

*Significantly different ($p \leq 0.05$) from the chamber control group by the Williams or Shirley tests.

** $p \leq 0.01$.

Data are presented as mean \pm standard error. For values reported as below the limit of quantification, the values shown are the constant of half the limit of quantification. Statistical tests were performed only on data that were not exposure concentration normalized.

NA = not applicable.

Table B-17. Bronchial Lymph Node Weights and Bronchial Lymph Node Burdens for Male Sprague Dawley Rats in the 39-week Inhalation Study of Blasting Sand

	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³
n	5	5	5	5
Absolute Bronchial Lymph Node Wt. (g)				
Week 1	0.012 ± 0.002	0.016 ± 0.005	0.016 ± 0.003	0.025 ± 0.003*
Week 4	0.020 ± 0.002	0.025 ± 0.003	0.022 ± 0.001	0.037 ± 0.004**
Week 8	0.023 ± 0.002	0.022 ± 0.004	0.026 ± 0.007	0.043 ± 0.007*
Week 16	0.017 ± 0.002	0.029 ± 0.006	0.045 ± 0.006	0.101 ± 0.018**
Week 26	0.018 ± 0.002	0.039 ± 0.005	0.077 ± 0.018**	0.079 ± 0.008**
Week 39	0.015 ± 0.002	0.115 ± 0.013	0.089 ± 0.019	0.193 ± 0.063**
µg Si/g Bronchial Lymph Node				
Week 1	0.6 ± 0.0	0.6 ± 0.0	20.4 ± 19.9	0.6 ± 0.0
Week 4	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	156.9 ± 63.0**
Week 8	0.6 ± 0.0	250.0 ± 69.6*	1,648.7 ± 462.4**	4,023.1 ± 1,064.6**
Week 16	0.6 ± 0.0	4,328.7 ± 1,340.9*	12,851.2 ± 744.0**	12,355.6 ± 863.2**
Week 26	0.6 ± 0.0	10,247.9 ± 834.9**	14,973.8 ± 1,845.7**	17,755.9 ± 4,395.9**
Week 39	0.6 ± 0.0	12,679.7 ± 1,598.5**	21,846.3 ± 2,886.6**	24,475.2 ± 4,539.0**
µg Si/Total Bronchial Lymph Node				
Week 1	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.4	0.0 ± 0.0
Week 4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	6.4 ± 2.7*
Week 8	0.0 ± 0.0	5.3 ± 1.9**	51.2 ± 22.0**	187.9 ± 58.0**
Week 16	0.0 ± 0.0	157.3 ± 55.8	580.0 ± 85.3**	1,245.9 ± 252.3**
Week 26	0.0 ± 0.0	401.3 ± 54.2**	1,022.3 ± 84.7**	1,481.4 ± 516.0**
Week 39	0.0 ± 0.0	1,460.7 ± 236.8**	1,767.6 ± 219.1**	3,773.6 ± 709.4**
µg Blasting Sand/g Bronchial Lymph Node				
Week 1	1.4 ± 0.0	1.4 ± 0.0	51.9 ± 50.5	1.4 ± 0.0
Week 4	1.4 ± 0.0	1.4 ± 0.0	1.4 ± 0.0	398.1 ± 159.9**
Week 8	1.4 ± 0.0	634.5 ± 176.7*	4,184.4 ± 1,173.5**	10,210.9 ± 2,702.0**
Week 16	1.4 ± 0.0	10,986.6 ± 3,403.4*	32,617.2 ± 1,888.4**	31,359.3 ± 2,190.9**
Week 26	1.4 ± 0.0	26,009.9 ± 2,119.1**	38,004.5 ± 4,684.4**	45,065.9 ± 11,157.2**
Week 39	1.4 ± 0.0	32,181.9 ± 4,057.1**	55,447.4 ± 7,326.4**	62,119.8 ± 11,520.4**
µg Blasting Sand/Total Bronchial Lymph Node				
Week 1	0.0 ± 0.0	0.0 ± 0.0	1.1 ± 1.1	0.0 ± 0.0
Week 4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	16.1 ± 6.8*
Week 8	0.0 ± 0.0	13.3 ± 4.9**	130.1 ± 55.8**	476.8 ± 147.1**
Week 16	0.0 ± 0.0	399.3 ± 141.6	1,472.0 ± 216.6**	3,162.1 ± 640.3**

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	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³
Week 26	0.0 ± 0.0	1,018.5 ± 137.5**	2,594.5 ± 214.9**	3,759.8 ± 1,309.6**
Week 39	0.0 ± 0.0	3,707.4 ± 601.0**	4,486.3 ± 556.0**	9,577.7 ± 1,800.5**
μg Blasting Sand/Total Bronchial Lymph Node per mg Blasting Sand/m ³				
Week 1	NA	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Week 4	NA	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.1
Week 8	NA	0.9 ± 0.3	4.3 ± 1.9	7.9 ± 2.5
Week 16	NA	26.6 ± 9.4	49.1 ± 7.2	52.7 ± 10.7
Week 26	NA	67.9 ± 9.2	86.5 ± 7.2	62.7 ± 21.8
Week 39	NA	247.2 ± 40.1	149.5 ± 18.5	159.6 ± 30.0

*Significantly different ($p \leq 0.05$) from the chamber control group by the Dunnett, Williams, Shirley, or Dunn tests.

** $p \leq 0.01$.

Data are presented as mean \pm standard error. For values reported as below the limit of quantification, the values shown are the constant of half the limit of quantification. Statistical tests were performed only on data that were not exposure concentration normalized.

NA = not applicable.

Table B-18. Mediastinal Lymph Node Weights and Mediastinal Lymph Node Burdens for Male Sprague Dawley Rats in the 39-week Inhalation Study of Blasting Sand

	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³
n	5	5	5	5
Absolute Mediastinal Lymph Node Wt. (g)				
Week 1	0.027 ± 0.003	0.034 ± 0.005	0.032 ± 0.005	0.032 ± 0.005
Week 4	0.024 ± 0.004	0.032 ± 0.007	0.035 ± 0.005	0.033 ± 0.010
Week 8	0.032 ± 0.006	0.040 ± 0.007	0.043 ± 0.008	0.053 ± 0.008
Week 16	0.024 ± 0.004	0.031 ± 0.003	0.038 ± 0.007	0.046 ± 0.006*
Week 26	0.030 ± 0.005	0.052 ± 0.007	0.075 ± 0.009	0.101 ± 0.037*
Week 39	0.020 ± 0.001	0.034 ± 0.013	0.062 ± 0.017	0.072 ± 0.017*
µg Si/g Mediastinal Lymph Node				
Week 1	10.4 ± 9.9	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0
Week 4	7.8 ± 7.2	0.6 ± 0.0	0.6 ± 0.0	114.3 ± 72.8
Week 8	0.6 ± 0.0	130.7 ± 71.6	1,317.6 ± 616.8**	2,728.4 ± 901.2**
Week 16	59.8 ± 31.7	2,200.3 ± 834.3	4,117.0 ± 1,746.9*	4,445.8 ± 1,964.6*
Week 26	0.6 ± 0.0	9,272.0 ± 1,665.8**	11,802.4 ± 1,061.5**	12,320.0 ± 1,721.2**
Week 39	13.2 ± 12.7	1,573.9 ± 1,124.5	14,494.7 ± 4,769.8*	20,352.0 ± 6,432.3**
µg Si/total Mediastinal Lymph Node				
Week 1	0.3 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Week 4	0.3 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	4.9 ± 3.2
Week 8	0.0 ± 0.0	4.1 ± 1.8*	54.8 ± 19.5*	171.0 ± 79.1**
Week 16	1.4 ± 0.6	73.1 ± 27.3	170.8 ± 91.2*	218.7 ± 121.0**
Week 26	0.0 ± 0.0	450.6 ± 72.0**	888.4 ± 139.1**	1,266.2 ± 461.2**
Week 39	0.2 ± 0.2	105.8 ± 96.4	1,182.6 ± 456.9	1,767.0 ± 712.2**
µg Blasting Sand/g Mediastinal Lymph Node				
Week 1	26.5 ± 25.1	1.4 ± 0.0	1.4 ± 0.0	1.4 ± 0.0
Week 4	19.8 ± 18.4	1.4 ± 0.0	1.4 ± 0.0	290.2 ± 184.8
Week 8	1.4 ± 0.0	331.8 ± 181.7	3,344.2 ± 1,565.4**	6,924.8 ± 2,287.3**
Week 16	151.9 ± 80.5	5,584.4 ± 2,117.5	10,449.4 ± 4,433.7*	11,283.8 ± 4,986.4*
Week 26	1.4 ± 0.0	23,532.9 ± 4,227.8**	29,955.5 ± 2,694.2**	31,268.9 ± 4,368.6**
Week 39	33.6 ± 32.2	3,994.6 ± 2,854.1	36,788.6 ± 12,106.2*	51,654.8 ± 6,325.6**
µg Blasting Sand/Total Mediastinal Lymph Node				
Week 1	0.8 ± 0.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Week 4	0.7 ± 0.7	0.0 ± 0.0	0.0 ± 0.0	12.4 ± 8.1
Week 8	0.0 ± 0.0	10.5 ± 4.6*	139.1 ± 49.6*	434.0 ± 200.7**
Week 16	3.5 ± 1.6	185.5 ± 69.4	433.6 ± 231.5*	555.2 ± 307.0**

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	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³
Week 26	0.0 ± 0.0	1,143.7 ± 182.7**	2,254.7 ± 352.9**	3,213.7 ± 1,170.5**
Week 39	0.6 ± 0.6	268.6 ± 244.7	3,001.6 ± 1,159.7	4,484.8 ± 1,807.7**
µg Blasting Sand/Total Mediastinal Lymph Node per mg Blasting Sand/m ³				
Week 1	NA	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Week 4	NA	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.1
Week 8	NA	0.7 ± 0.3	4.6 ± 1.7	7.2 ± 3.3
Week 16	NA	12.4 ± 4.6	14.5 ± 7.7	9.3 ± 5.1
Week 26	NA	76.2 ± 12.2	75.2 ± 11.8	53.6 ± 19.5
Week 39	NA	17.9 ± 16.3	100.1 ± 38.7	74.7 ± 30.1

*Significantly different ($p \leq 0.05$) from the chamber control group by the Williams or Shirley tests.

** $p \leq 0.01$.

Data are presented as mean ± standard error. For values reported as below the limit of quantification, the values shown are the constant of half the limit of quantification. Statistical tests were performed only on data that were not exposure concentration normalized.

NA = not applicable.

Table B-19. Lung Deposition and Clearance Parameter Estimates for Male Sprague Dawley Rats in the 39-week Inhalation Study of Blasting Sand

Parameter	15 mg/m ³	30 mg/m ³	60 mg/m ³
k (days ⁻¹)	0.003352 ± 0.000761	0.002556 ± 0.000841	0.000817 ± 0.000595
t _{1/2} (days)	207 ± 47	271 ± 89	848 ± 618
D (µg blasting sand/total lung per day)	45.67 ± 1.80	98.04 ± 5.14	171.98 ± 7.17
L _{ss} (µg blasting sand/total lung)	13,624 ± 2,737	38,358 ± 11,090	210,387 ± 146,564

Data are presented as mean ± standard error.

k = first-order lung clearance rate constant; t_{1/2} = clearance half-life; D = deposition rate; L_{ss} = steady-state lung burden.

Table B-20. Lung Burden Overload Parameter Estimates for Male Sprague Dawley Rats in the 39-week Inhalation Study of Blasting Sand

Parameter	15 mg/m ³	30 mg/m ³	60 mg/m ³
t* = time of onset of overload condition [(R(t)>1.0)] (days)	137	55	30
L(t*) = lung burden at t* (mg blasting sand/lung)	5.0	5.0	5.1
R _{max} = maximum R(t) at 271 days (unitless)	1.6	3.8	8.4
Lung burden at R _{max} = L(t) at 271 days (mg blasting sand/lung)	8.1	19.2	41.8
Total dose = deposited blasting sand at 271 days (mg/lung)	12.4	26.6	46.6
Total blasting sand cleared = cleared blasting sand up through 271 days (mg/lung)	4.2	7.4	4.8
% Total blasting sand cleared = (total blasting sand cleared/total dose) × 100 (unitless)	34	28	10

R(t) = ratio of the volume of retained blasting sand lung burden at time t and the threshold volume required for the onset of lung overload.

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Table B-21. Lung Weights and Lung Burdens for Male Sprague Dawley Rats in the 39-week Inhalation Study of Specular Hematite

	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³
n	5	5	5	5
Absolute Lung Wt. (g)				
Week 1	0.93 ± 0.02	0.87 ± 0.01	0.91 ± 0.03	0.87 ± 0.03
Week 4	1.15 ± 0.03	1.16 ± 0.03	1.18 ± 0.04	1.17 ± 0.03
Week 8	1.30 ± 0.05	1.35 ± 0.06	1.36 ± 0.06	1.40 ± 0.03
Week 16	1.31 ± 0.04	1.44 ± 0.03*	1.55 ± 0.04**	1.56 ± 0.04**
Week 26	1.48 ± 0.04	1.52 ± 0.05	1.67 ± 0.04*	1.96 ± 0.05**
Week 39	1.51 ± 0.07 ^a	1.62 ± 0.03	1.76 ± 0.05**	2.11 ± 0.05** ^a
µg Fe/g Lung				
Week 1	49.16 ± 1.85	298.59 ± 20.00**	519.56 ± 29.77**	954.22 ± 35.07**
Week 4	63.41 ± 4.17	743.12 ± 25.15**	1,342.6 ± 71.31**	2,364.6 ± 104.08**
Week 8	70.11 ± 1.13	1,175.6 ± 102.53**	2,044.0 ± 111.09**	4,422.7 ± 113.60**
Week 16	82.04 ± 3.51	1,927.5 ± 197.10**	3,915.1 ± 312.96**	8,494.7 ± 383.33**
Week 26	85.84 ± 2.52	2,559.3 ± 315.11**	5,338.8 ± 382.59**	11,344 ± 467.86**
Week 39	95.50 ± 5.94 ^a	3,501.4 ± 154.31*	8,524.5 ± 567.31**	16,136 ± 1,023.4** ^a
µg Fe/g Lung (Control Corrected)				
Week 1	NA	249.428 ± 20.002	470.403 ± 29.771	905.059 ± 35.074
Week 4	NA	679.703 ± 25.154	1,279.13 ± 71.305	2,301.21 ± 104.078
Week 8	NA	1,105.53 ± 102.530	1,973.86 ± 111.092	4,352.62 ± 113.600
Week 16	NA	1,845.49 ± 197.093	3,833.07 ± 312.958	8,412.68 ± 383.334
Week 26	NA	2,473.47 ± 315.108	5,252.99 ± 382.587	11,257.7 ± 467.856
Week 39	NA	3,405.85 ± 154.306	8,429.03 ± 567.310	16,040.4 ± 1,023.37 ^a
µg Fe/Total Lung (Control Corrected)				
Week 1	NA	217.575 ± 18.963	427.885 ± 25.625	791.542 ± 48.353
Week 4	NA	790.089 ± 36.127	1,502.32 ± 72.776	2,686.73 ± 93.504
Week 8	NA	1,495.88 ± 183.505	2,702.75 ± 249.133	6,114.12 ± 238.881
Week 16	NA	2,663.99 ± 319.488	5,926.81 ± 462.104	13,162.7 ± 732.920
Week 26	NA	3,825.04 ± 581.773	8,811.78 ± 827.017	22,038.5 ± 1,110.46
Week 39	NA	5,521.67 ± 269.869	14,968.5 ± 1,412.83	33,923.6 ± 2,696.84 ^a
µg Specular Hematite/g Lung (Control Corrected)				
Week 1	NA	359.407 ± 28.822	677.814 ± 42.898	1,304.12 ± 50.539
Week 4	NA	979.400 ± 36.244	1,843.13 ± 102.746	3,315.86 ± 149.968
Week 8	NA	1,592.98 ± 147.737	2,844.17 ± 160.075	6,271.78 ± 163.688
Week 16	NA	2,659.20 ± 283.996	5,523.16 ± 450.948	12,122.0 ± 552.355

Abrasive Blasting Agents, TOX 91

	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³
Week 26	NA	3,564.07 ± 454.045	7,569.15 ± 551.278	16,221.5 ± 674.145
Week 39	NA	4,907.56 ± 222.344	12,145.6 ± 817.449	23,113.0 ± 1,474.60 ^a
µg Specular Hematite/Total Lung (Control Corrected)				
Week 1	NA	313.509 ± 27.324	616.549 ± 36.924	1,140.55 ± 69.673
Week 4	NA	1,138.46 ± 52.056	2,164.73 ± 104.865	3,871.37 ± 134.732
Week 8	NA	2,155.44 ± 264.416	3,894.45 ± 358.981	8,809.97 ± 344.208
Week 16	NA	3,838.61 ± 460.357	8,540.07 ± 665.855	18,966.5 ± 1,056.08
Week 26	NA	5,511.59 ± 838.290	12,697.1 ± 1,191.67	31,755.7 ± 1,600.09
Week 39	NA	7,956.30 ± 388.860	21,568.5 ± 2,035.77	48,881.2 ± 3,885.94 ^a
µg Specular Hematite/Total Lung per mg Specular Hematite/m³ (Control Corrected)				
Week 1	NA	20.901 ± 1.822	20.552 ± 1.231	19.009 ± 1.161
Week 4	NA	75.897 ± 3.470	72.158 ± 3.495	64.523 ± 2.246
Week 8	NA	143.696 ± 17.628	129.815 ± 11.966	146.833 ± 5.737
Week 16	NA	255.907 ± 30.690	284.669 ± 22.195	316.108 ± 17.601
Week 26	NA	367.439 ± 55.886	423.236 ± 39.722	529.262 ± 26.668
Week 39	NA	530.420 ± 25.924	718.950 ± 67.859	814.687 ± 64.766 ^a

*Significantly different ($p \leq 0.05$) from the chamber control group by the Williams or Shirley tests.

** $p \leq 0.01$.

Data are presented as mean ± standard error. Statistical tests were performed only on data that were not control corrected.

NA = not applicable.

^an = 4.

Abrasive Blasting Agents, TOX 91

Table B-22. Bronchial Lymph Node Weights and Bronchial Lymph Node Burdens for Male Sprague Dawley Rats in the 39-week Inhalation Study of Specular Hematite

	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³
n	5	5	5	5
Absolute Bronchial Lymph Node Wt. (g)				
Week 1	0.010 ± 0.002	0.011 ± 0.003	0.016 ± 0.003	0.011 ± 0.002
Week 4	0.026 ± 0.005	0.010 ± 0.003	0.020 ± 0.006	0.021 ± 0.004
Week 8	0.019 ± 0.003	0.020 ± 0.003	0.031 ± 0.004	0.028 ± 0.006
Week 16	0.014 ± 0.003	0.015 ± 0.003	0.027 ± 0.004	0.036 ± 0.007**
Week 26	0.013 ± 0.002	0.023 ± 0.005	0.040 ± 0.004**	0.050 ± 0.006**
Week 39	0.017 ± 0.006 ^a	0.025 ± 0.005	0.041 ± 0.005	0.051 ± 0.014 ^{*,a}
µg Fe/Total Bronchial Lymph Node				
Week 1	0.245 ± 0.245	0.322 ± 0.171	0.405 ± 0.162	0.198 ± 0.112
Week 4	0.943 ± 0.173	0.206 ± 0.206	1.255 ± 0.641	2.542 ± 0.641
Week 8	0.954 ± 0.165	1.272 ± 0.417	17.879 ± 4.765**	131.660 ± 35.090**
Week 16	1.498 ± 0.320	46.721 ± 18.599**	274.478 ± 118.208**	737.400 ± 103.996**
Week 26	1.713 ± 0.320	209.339 ± 112.024**	1,532.45 ± 341.267**	2,273.60 ± 397.628**
Week 39	3.360 ± 1.198 ^a	417.725 ± 102.586*	2,227.05 ± 359.973**	2,819.81 ± 1,232.548 ^{*,a}
µg Fe/Total Bronchial Lymph Node (Control Corrected)				
Week 1	NA	0.003 ± 0.001	0.146 ± 0.141	0.003 ± 0.001
Week 4	NA	0.003 ± 0.001	0.541 ± 0.536	1.610 ± 0.634
Week 8	NA	0.471 ± 0.305	16.925 ± 4.765	130.706 ± 35.090
Week 16	NA	45.223 ± 18.599	272.980 ± 118.208	735.902 ± 103.996
Week 26	NA	207.627 ± 112.024	1,530.74 ± 341.267	2,271.89 ± 397.628
Week 39	NA	414.365 ± 102.586	2,223.69 ± 359.973	2,816.45 ± 1,232.54 ^a
µg Fe/g Bronchial Lymph Node (Control Corrected)				
Week 1	NA	0.305 ± 0.000	7.109 ± 6.804	0.305 ± 0.000
Week 4	NA	0.305 ± 0.000	13.736 ± 13.431	66.952 ± 23.086
Week 8	NA	19.536 ± 12.140	512.937 ± 126.805	4,632.85 ± 1,399.06
Week 16	NA	3,069.96 ± 1,345.72	9,608.73 ± 3,610.05	24,942.6 ± 5,803.36
Week 26	NA	6,613.07 ± 2,849.64	3,7020.1 ± 6,164.50	44,022.6 ± 3,908.51
Week 39	NA	19,295.5 ± 5,805.73	54,700.0 ± 6,555.39	48,099.2 ± 10,754.6 ^a
µg Specular hematite/g bronchial lymph node (Control Corrected)				
Week 1	NA	0.440 ± 0.000	10.244 ± 9.804	0.440 ± 0.000
Week 4	NA	0.440 ± 0.000	19.794 ± 19.354	96.473 ± 33.266
Week 8	NA	28.151 ± 17.492	739.103 ± 182.716	6,675.57 ± 2,015.94
Week 16	NA	4,423.58 ± 1,939.07	13,845.4 ± 5,201.81	35,940.3 ± 8,362.19

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	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³
Week 26	NA	9,528.92 ± 4,106.11	53,343.1 ± 8,882.56	63,433.2 ± 5,631.86
Week 39	NA	27,803.3 ± 8,365.61	78,818.4 ± 9,445.80	69,307.2 ± 15,496.6 ^a
µg Specular Hematite/Total Bronchial Lymph Node (Control Corrected)				
Week 1	NA	0.005 ± 0.001	0.210 ± 0.203	0.005 ± 0.001
Week 4	NA	0.004 ± 0.002	0.779 ± 0.772	2.320 ± 0.913
Week 8	NA	0.679 ± 0.439	24.387 ± 6.867	188.337 ± 50.562
Week 16	NA	65.163 ± 26.800	393.343 ± 170.329	1,060.38 ± 149.850
Week 26	NA	299.174 ± 161.418	2,205.67 ± 491.739	3,273.61 ± 572.951
Week 39	NA	597.068 ± 147.818	3,204.16 ± 518.694	4,058.29 ± 1,775.99 ^a
µg Specular hematite/total bronchial lymph node per mg specular hematite/m ³ (Control Corrected)				
Week 1	NA	0.000 ± 0.000	0.007 ± 0.007	0.000 ± 0.000
Week 4	NA	0.000 ± 0.000	0.026 ± 0.026	0.039 ± 0.015
Week 8	NA	0.045 ± 0.029	0.813 ± 0.229	3.139 ± 0.843
Week 16	NA	4.344 ± 1.787	13.111 ± 5.678	17.673 ± 2.498
Week 26	NA	19.945 ± 10.761	73.522 ± 16.391	54.560 ± 9.549
Week 39	NA	39.805 ± 9.855	106.805 ± 17.290	67.638 ± 29.600 ^a

*Significantly different ($p \leq 0.05$) from the chamber control group by the Williams or Shirley tests.

** $p \leq 0.01$.

Data are presented as mean ± standard error. Statistical tests were performed only on data that were not control corrected. For values reported as below the limit of quantification, the values shown are the constant of half the limit of quantification.

NA = not applicable.

^an = 4.

Table B-23. Mediastinal Lymph Node Weights and Mediastinal Lymph Node Burdens for Male Sprague Dawley Rats in the 39-week Inhalation Study of Specular Hematite

	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³
n	5	5	5	5
Absolute Mediastinal Lymph Node Wt. (g)				
Week 1	0.018 ± 0.005	0.021 ± 0.003	0.026 ± 0.003	0.019 ± 0.001
Week 4	0.025 ± 0.003	0.024 ± 0.005	0.027 ± 0.005	0.023 ± 0.003
Week 8	0.025 ± 0.004	0.025 ± 0.002	0.026 ± 0.005	0.035 ± 0.008
Week 16	0.019 ± 0.002	0.020 ± 0.003	0.029 ± 0.004	0.034 ± 0.005*
Week 26	0.019 ± 0.004	0.032 ± 0.007	0.030 ± 0.002	0.046 ± 0.002**
Week 39	0.033 ± 0.009 ^a	0.031 ± 0.011	0.031 ± 0.006	0.060 ± 0.011 ^a
µg Fe/Total Mediastinal Lymph Node				
Week 1	0.473 ± 0.473	0.893 ± 0.865	0.000 ± 0.000	0.128 ± 0.128
Week 4	0.194 ± 0.194	0.489 ± 0.489	0.139 ± 0.087	1.181 ± 0.490
Week 8	0.305 ± 0.222	0.041 ± 0.041	5.433 ± 3.848	193.108 ± 61.060**
Week 16	0.293 ± 0.265	41.371 ± 36.088	218.454 ± 75.099**	925.510 ± 368.088**
Week 26	10.06 ± 9.11	155.20 ± 88.09	270.45 ± 145.34*	952.30 ± 239.99**
Week 39	17.35 ± 9.88 ^a	277.40 ± 119.71*	554.53 ± 205.17**	2,836.7 ± 873.64*** ^a
µg Fe/Total Mediastinal Lymph Node (Control Corrected)				
Week 1	NA	0.176 ± 0.028	0.219 ± 0.023	0.162 ± 0.008
Week 4	NA	0.207 ± 0.040	0.227 ± 0.046	0.192 ± 0.025
Week 8	NA	0.208 ± 0.021	4.186 ± 4.000	190.247 ± 62.967
Week 16	NA	41.337 ± 36.014	218.161 ± 75.099	925.040 ± 368.199
Week 26	NA	148.829 ± 86.492	260.829 ± 145.148	942.243 ± 239.989
Week 39	NA	260.049 ± 119.710	537.184 ± 205.170	2,819.34 ± 873.636 ^a
µg Fe/g Mediastinal Lymph Node (Control Corrected)				
Week 1	NA	8.500 ± 0.000	8.500 ± 0.000	8.500 ± 0.000
Week 4	NA	8.500 ± 0.000	8.500 ± 0.000	8.500 ± 0.000
Week 8	NA	8.500 ± 0.000	106.23 ± 97.736	5,156.3 ± 1,494.29
Week 16	NA	1,392.47 ± 1,150.63	6,673.66 ± 1,634.18	25,051.1 ± 11,041.0
Week 26	NA	5,032.76 ± 3,039.27	9,028.06 ± 5,037.73	20,818.0 ± 5,546.83
Week 39	NA	10,365.0 ± 4,741.15	15,503.4 ± 4,040.53	46,919.3 ± 14,649.1 ^a
µg Specular Hematite/g Mediastinal Lymph Node (Control Corrected)				
Week 1	NA	12.250 ± 0.000	12.250 ± 0.000	12.250 ± 0.000
Week 4	NA	12.250 ± 0.000	12.250 ± 0.000	12.250 ± 0.000
Week 8	NA	12.250 ± 0.000	153.079 ± 140.829	7,429.96 ± 2,153.16
Week 16	NA	2,006.44 ± 1,657.97	9,616.22 ± 2,354.72	36,096.7 ± 15,909.2

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	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³
Week 26	NA	7,251.82 ± 4,379.35	13,008.7 ± 7,258.98	29,997.1 ± 7,992.56
Week 39	NA	14,935.1 ± 6,831.62	22,339.2 ± 5,822.09	67,607.0 ± 21,108.3 ^a
µg Specular Hematite/Total Mediastinal Lymph Node (Control Corrected)				
Week 1	NA	0.254 ± 0.040	0.316 ± 0.033	0.234 ± 0.011
Week 4	NA	0.298 ± 0.058	0.327 ± 0.067	0.276 ± 0.036
Week 8	NA	0.300 ± 0.030	6.032 ± 5.764	274.131 ± 90.730
Week 16	NA	59.564 ± 51.893	314.352 ± 108.212	1,332.91 ± 530.546
Week 26	NA	214.451 ± 124.628	375.834 ± 209.147	1,357.70 ± 345.805
Week 39	NA	374.711 ± 172.493	774.041 ± 295.633	4,062.45 ± 1,258.84 ^a
µg Specular Hematite/Total Mediastinal Lymph node per mg Specular Hematite/m ³ (Control Corrected)				
Week 1	NA	0.017 ± 0.003	0.011 ± 0.001	0.004 ± 0.000
Week 4	NA	0.020 ± 0.004	0.011 ± 0.002	0.005 ± 0.001
Week 8	NA	0.020 ± 0.002	0.201 ± 0.192	4.569 ± 1.512
Week 16	NA	3.971 ± 3.460	10.478 ± 3.607	22.215 ± 8.842
Week 26	NA	14.297 ± 8.309	12.528 ± 6.972	22.628 ± 5.763
Week 39	NA	24.981 ± 11.500	25.801 ± 9.854	67.708 ± 20.981 ^a

*Significantly different ($p \leq 0.05$) from the chamber control group by the Dunnett, Williams, or Shirley tests.

** $p \leq 0.01$.

Data are presented as mean ± standard error. Statistical tests were performed only on data that were not control corrected. For values reported as below the limit of quantification, the values shown are the constant of half the limit of quantification.

NA = not applicable.

^an = 4.

Table B-24. Lung Deposition and Clearance Parameter Estimates for Male Sprague Dawley Rats in the 39-week Inhalation Study of Specular Hematite

Parameter	15 mg/m ³	30 mg/m ³	60 mg/m ³
k (days ⁻¹)	0.00327 ± 0.00092	-0.00004 ± 0.00086	-0.00095 ± 0.00052
t _{1/2} (days)	212 ± 59	- ^a	-
D (µg specular hematite/total lung per day)	43.52 ± 3.79	76.34 ± 6.48	159.9 ± 8.1
L _{ss} (µg specular hematite/total lung)	13,325 ± 2,705	-	-

Data are presented as mean ± standard error.

k = first-order lung clearance rate constant; t_{1/2} = clearance half-life; D = deposition rate; L_{ss} = steady-state lung burden.

^aResulting estimates are negative and so are not presented.

Table B-25. Lung Burden Overload Parameter Estimates for Male Sprague Dawley Rats in the 39-week Inhalation Study of Specular Hematite

Parameter	15 mg/m ³	30 mg/m ³	60 mg/m ³
t* = time of onset of overload condition [(R(t)>1.0)] (days)	344	118	55
L(t*) = lung burden at t* (mg specular hematite/lung)	9.0	9.0	9.0
R _{max} = maximum R(t) at 271 days (unitless)	0.9	2.3	5.5
Lung burden at R _{max} = L(t) at 271 days (mg specular hematite/lung)	7.8	20.8	49.4
Total dose = deposited specular hematite at 271 days (mg/lung)	11.8	20.7	43.3
Total specular hematite cleared = cleared specular hematite up through 271 days (mg/lung)	4.0	–	–
% Total specular hematite cleared = (total specular hematite cleared/total dose) × 100 (unitless)	34	–	–

R(t) = ratio of the volume of retained specular hematite lung burden at time t and the threshold volume required for the onset of lung overload. Resulting estimates are negative and so are not presented.

Appendix C. Chemical Characterization and Generation of Chamber Concentrations

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C.1. Procurement and Characterization

The blasting sand (*coarse silica sand #2340*) used in the 2-week and 39-week studies was obtained from *Waupaca Sand and Solutions (Division of Faulks Brothers Construction Inc.; Waupaca, WI)* ~~Midwest Research Institute (MRI; Kansas City, MO)~~ in one lot by *Midwest Research Institute (MRI; Kansas City, MO)* and was assigned the lot number W100604JB. The micronized specular hematite (Barshot 50) used in the 2-week and 39-week studies was obtained from *Opta Minerals (Waterdown, ON, Canada)* ~~MRI~~ in one lot (0101005CJ) by *MRI (Kansas City, MO)*. The coal slag used in the 2-week study was obtained from *Reed Minerals-Harsco Corporation (LaCygne, KS)* ~~MRI~~ in one lot (R042805KA). The crushed glass (*VitroGrit™ #30/50*) used in the 2-week study was obtained from *TriVitro Corporation (Kent, WA)* ~~MRI~~ in one lot (T092205KA). The garnet used in the 2-week study was obtained from *Emerald Creek Garnet Ltd. (Fernwood, ID)* ~~MRI~~ in one lot (031605).^c Identity and purity analyses were conducted by multiple analytical chemistry laboratories and the study laboratory at Battelle Toxicology Northwest (Richland, WA). Reports on analyses performed in support of the abrasive blasting agent studies are on file at the National Institute of Environmental Health Sciences (NIEHS).

C.1.1. Blasting Sand

Prior to use in the studies, the particle size of blasting sand (lot W100604JB) was reduced by aqueous ball milling for 24 hours, followed by 5 hours of aqueous bead milling of 40/57/3 test material/water/ethanol (weight/weight/weight) by Aveka, Inc. (Woodbury, MN). The milled material was mixed with water and the mixture was dispersed under strong agitation and then spray dried.^d Lot W100604JB of blasting sand, a finely ground, gray powder, was expected to contain 90% to 100% silicon dioxide, with 49% to 90% of it being crystalline silica (SiO₂).^{1;5} The test article was characterized and the composition was determined using analyses that included weight loss on drying (study laboratory); density (Quantachrome Instruments, Boynton Beach, FL); surface area by the Brunauer-Emmett-Teller (BET) surface area method using method A (Clear Science, Inc., Minneapolis, MN); Fourier transform infrared (FTIR) spectroscopy (EMSL Analytical, Inc., Westmont, NJ); inductively coupled plasma/atomic emission spectroscopy (ICP/AES) by method B for the identification and quantitation of silicon (Si) (study laboratory); proton induced X-ray emission (PIXE) spectroscopy to quantitate concentrations of major and minor elements (Elemental Analysis, Inc., Lexington, KY); powder X-ray diffraction (XRD) by methods C and D (EMSL Analytical, Inc., and H & M Analytical Services, Inc., Allentown, NJ, respectively), to identify and quantitate crystalline phases present at greater than 1%; and X-ray fluorescence (XRF) analyses for qualitative phase identification measurements using a SPECTRO iQ II spectrometer (SPECTRO Analytical Instruments GmbH, Kleve, Germany) with palladium radiation (H & M Analytical Services, Inc.). All spectra were

^cERRATUM: An error was identified in the NTP Toxicity Report on Abrasive Blasting Agents (TOX 91). The reported supplier information for each blasting agent has been corrected and trade names were added to the text in the HTML and PDF versions of this report; new information is italicized. [September 1, 2022]

^dERRATUM: An error was identified in the NTP Toxicity Report on Abrasive Blasting Agents (TOX 91). Text describing the bulk material preparation of blasting sand was added in the HTML and PDF versions of this report; new information is italicized. [September 1, 2022]

consistent with the expected composition of blasting sand. A representative XRD spectrum is presented in Figure C-1.

(A) For BET surface area analysis including an adsorption/desorption isotherm, the samples were degassed at 90°C for 1 hour and 150°C (specular hematite) or 250°C (blasting sand, coal slag, crushed glass, and garnet) for 13 hours and then analyzed using a system that included a Micromeritics Gemini 2375 Surface Area Analyzer (Micromeritics Instrument Corporation, Norcross, GA), UHP nitrogen (adsorbate gas), liquid nitrogen (cryogen), a pressure sequence of 16 adsorption points from 0.01 P/P_0 to 0.995 P/P_0 and 11 desorption points from 0.995 P/P_0 to 0.5 P/P_0 , and an evacuation time of 3 minutes. A kaolinite standard reference material was included in the analysis.

(B) For ICP/AES analysis, standards obtained from the National Institute of Standards and Technology (NIST) were added to samples of the milled bulk material and the samples were subjected to room temperature and heated 55°C to 80°C acid digestions in a matrix of nitric acid (HNO₃)/hydrofluoric acid (HF). After an additional heated 55°C to 80°C digestion in boric acid (H₃BO₃), hydroxylamine hydrochloride (NH₂OH·HCl) was added to dissolve any metal oxides that may have remained, and the samples were analyzed on a Thermo Elemental IRIS Intrepid II instrument (Thermo Electron Corporation, Waltham, MA). Total Si content was quantitated by measurement of atomic emission at 212.412 nm; the internal standard cobalt (Co) was quantitated at 228.16 nm.

(C) XRD analysis conducted by EMSL Analytical, Inc., included a Rigaku D/Max-B diffractometer (Rigaku Americas Corporation, Lake Oswego, OR) equipped with a copper (Cu) X-ray tube and utilized National Institute for Occupational Safety and Health (NIOSH) Method 7500 (modified for bulk samples) to determine the concentration of crystalline silica.

(D) XRD analysis conducted by H & M Analytical Services, Inc., included a Philips PW1830 (Philips Analytical, San Francisco, CA), Siemens D5000 (Siemens, Munich, Germany), or PANalytical X'Pert Pro MPD (PANalytical B.V., Almelo, Netherlands) diffractometer with Cu radiation at 40 KV/30 mA or 45 KV/40 mA over the angular range of 10° to 70° with step sizes of 0.02° to 0.032° and counting times of 5 to 1,500 seconds per step or 8 hours per sample with a detection limit of 0.5% for the trace phases. The crystalline phases were identified using the Powder Diffraction File (International Centre for Diffraction Data) and quantitated using Rietveld refinement.

Lot W100604JB was determined to have a density of 2.78 g/cc and a BET surface area of approximately 20 m²/g; weight loss on drying indicated a water content of less than 1% for the bulk material. FTIR, ICP/AES, PIXE, and XRD Rietveld analyses indicated 39.0%, 39.4%, 37.7%, and 40.7% Si, respectively; the NIOSH method 7500 silicon result (29.5%) was inconsistent with the other Si content data. PIXE identified other elements above 1% as aluminum (Al, 2.0% to 2.2%), calcium (Ca, 1.3% to 1.4%), and iron (Fe, 1.1% to 1.3%), and elements approaching concentrations near 1% as magnesium (Mg, 0.6% to 0.8%), potassium (K, 0.8% to 0.9%), and zirconium (Zr, 0.7% to 0.8%). The test material phases were mostly crystalline quartz (SiO₂, 76.4% to 78.5%) with cristobalite (0.6% to 0.8%) and crystalline impurities of calcite (CaCO₃, 0.3% to 0.8%), dolomite [CaMg (CO₃)₂, 7.0% to 7.6%], and one

phase tentatively identified as sanidine [$K(AlSi_3)O_8$, 12.7% to 15.1%] as determined through XRD Rietveld analysis.

To ensure stability, the bulk test material was stored at room temperature in safety-coated amber glass containers with Teflon[®]-lined caps. Periodic reanalyses of the test material were performed by the analytical chemistry and study laboratories during the 2-week and 39-week studies with ICP/AES, PIXE (2-week study only), and XRD, and no degradation of the test material was detected.

C.1.2. Coal Slag

Prior to use in the studies, the particle size of coal slag (lot R042805KA) was reduced by aqueous ball milling for up to 27 hours, followed by 16 hours of aqueous bead milling of 50/50 test material/water (weight/weight) by Aveka, Inc. (Woodbury, MN). The milled material was mixed with water and the mixture was dispersed under strong agitation and then spray dried.^e Lot R042805KA of coal slag, a black, mostly amorphous powder was expected to contain 45% to 51% SiO₂ with less than 1% of it being crystalline silica.⁹ The test article was characterized and the composition was determined using analyses that included weight loss on drying (study laboratory and Galbraith Laboratories, Inc., Knoxville, TN); Karl Fischer titration for water content (Galbraith Laboratories, Inc.); density (Quantachrome Instruments); BET surface area by method A (Clear Science, Inc.); ICP/AES by method B for the identification and quantitation of total Si, and also total Al, Fe, and Ca by measurement of atomic emission at 309.271, 259.940, and 317.933 nm, respectively (study laboratory); PIXE spectroscopy to quantitate concentrations of major and minor elements (Elemental Analysis, Inc.); powder XRD by a method similar to method D to identify and quantitate crystalline phases present at greater than 1% (H & M Analytical Services, Inc.); XRF for qualitative phase identification (H & M Analytical Services, Inc.); and carbon content using coulometric titration [samples were combusted using a LECO induction furnace (LECO Corporation, St. Joseph, MI) at greater than 1,400°C in an oxygen atmosphere, acidified, and automatically titrated with a Model 5010 carbon dioxide coulometer (Coulometrics, Inc., Joliet, IL)] (Galbraith Laboratories, Inc.). Weight loss on drying and Karl Fischer titration analyses were performed on both dried (to constant weight) and undried samples of the test article. Samples for density, ICP/AES, PIXE, XRD, and XRF analyses were dried to a constant weight before their submission to the analytical chemistry laboratories for analysis. The samples for BET analysis were dried at the analytical chemistry laboratory before analysis, and the samples for carbon analysis were not dried. All spectra were consistent with the expected composition of coal slag. A representative XRD spectrum is presented in Figure C-2.

Lot R042805KA was determined to have a density of 2.77 g/cc and a BET surface area of approximately 9.26 m²/g. An initial weight loss on drying experiment yielded an average weight loss of 15.3% for the bulk test article; additional samples of the test article were dried to constant weight, and the average weight loss was 17.4%. Karl Fischer titration of the undried test article (11.7% water) compared favorably with a concurrent weight loss on drying measurement (13.2% water). Samples of the dried test article underwent an additional weight loss on drying assessment and Karl Fischer analysis; the mean percent weight loss and water content were both less than or equal to 1%. ICP/AES and PIXE analyses indicated 21.7% and 21.5% Si,

^eERRATUM: An error was identified in the NTP Toxicity Report on Abrasive Blasting Agents (TOX 91). Text describing the bulk material preparation of coal slag was added in the HTML and PDF versions of this report; new information is italicized. [September 1, 2022]

respectively. Other elements identified at values greater than 1% were Al, Fe, and Ca by ICP/AES at 9.9%, 8.1%, and 10.7%, respectively, and by PIXE at 10.0%, 7.2%, and 9.1%, respectively. PIXE values for other elements near 1% were Mg (1.6 %) and K (0.71 %). No crystalline silica was detected; the only crystalline phase detected was CaCO₃ at a 1.2% weight fraction as determined through the XRD Rietveld analysis. Coulometric analysis indicated 0.127% carbon.

To ensure stability, the bulk test material (dried to constant weight and sieved) was stored at room temperature in safety-coated amber glass containers with Teflon-lined caps. Periodic reanalyses of the test material were performed by the analytical chemistry and study laboratories during the 2-week study with ICP/AES, PIXE, and XRD, and no degradation of the test material was detected.

C.1.3. Crushed Glass

Prior to use in the studies, the particle size of crushed glass (lot T092205KA) was reduced by aqueous ball milling for up to 27 hours, followed by 35 hours of aqueous bead milling of 50/50 test material/water (weight/weight) by Aveka, Inc. (Woodbury, MN). The milled material was mixed with water and the mixture was dispersed under strong agitation and then spray dried. The final material was oven dried at 200 °C for 8 hours.^f Lot T092205KA of crushed glass, a mostly amorphous powder, was expected to contain approximately 72.5% SiO₂, with none of it being crystalline silica.⁹ The test article was characterized and the composition was determined by weight loss on drying (study laboratory and Galbraith Laboratories, Inc.); using Karl Fischer titration for water content (Galbraith Laboratories, Inc.); density (Quantachrome Instruments); BET surface area by method A (Clear Science, Inc.); ICP/AES by method B for the identification and quantitation of total Si, Al, Fe, and Ca as described for coal slag and also total sodium (Na), Mg, and K by measurement of atomic emission at 589.592, 279.553, and 766.491 nm, respectively (study laboratory); PIXE spectroscopy to quantitate concentrations of major and minor elements (Elemental Analysis, Inc.); and powder XRD by a method similar to method D to identify and quantitate crystalline phases (H & M Analytical Services, Inc.). Due to the presence of unidentified phases at less than 1%, the Reference Intensity Ratio (RIR) method was used to quantify the weight fractions of the XRD phases detected. All spectra were consistent with the expected composition of crushed glass; a representative XRD spectrum is presented in Figure C-3.

Lot T092205KA was determined to have a density of 2.39 g/cc and a BET surface area of approximately 22.4 m²/g. Weight loss on drying indicated a water content of less than 1%, but Karl Fischer titration averaged 3.5% water. Additional weight loss on drying analysis at an elevated oven temperature averaged 3.1% water, confirming the presence of tightly bound water in the bulk test article. ICP/AES and PIXE analyses indicated 31.2% and 28.9% Si, respectively; the percent values for Ca, Na, Mg, Al, Fe, and K by ICP/AES were 5.5%, 9.3%, 1.9%, 0.3%, 0.1%, and 0.2%, respectively; PIXE values for these elements were 5.6%, 8.9%, 1.9%, 0.4%, 0.1%, and 0.2%, respectively. The test material appeared to be mostly amorphous (93% to 98%) with crystalline phases of natrite (Na₂CO₃, 0.4% to 1.6%), aragonite (orthorhombic CaCO₃, 0.3%

^fERRATUM: An error was identified in the NTP Toxicity Report on Abrasive Blasting Agents (TOX 91). Text describing the bulk material preparation of crushed glass was added in the HTML and PDF versions of this report; new information is italicized. [September 1, 2022]

to 0.5%), calcite (hexagonal CaCO_3 , 0.7% to 1.5%), and quartz (SiO_2 , 0.2% to 0.8%) as determined through the XRD RIR method.

To ensure stability, the bulk test material was stored at room temperature in safety-coated amber glass containers with Teflon-lined caps. Periodic reanalyses of the test material were performed by the analytical chemistry and study laboratories during the 2-week study with ICP/AES, PIXE, and XRD, and no degradation of the test material was detected.

C.1.4. Garnet

Prior to use in the studies, the particle size of garnet (lot 031605) was reduced by aqueous ball milling for up to 27 hours, followed by 20 hours of aqueous bead milling of 56/44 test material/water (weight/weight) by Aveka, Inc. (Woodbury, MN). The milled material was mixed with water and the mixture was dispersed under strong agitation and then spray dried.⁸ Lot 031605 of garnet, a red, mostly amorphous powder, was expected to contain 36% to 38% SiO_2 with less than 0.8% of it being crystalline silica.⁹ The test article was characterized and the composition was determined using analyses that included weight loss on drying (study laboratory); Karl Fischer titration for water content (Galbraith Laboratories, Inc.), density (Quantachrome Instruments); BET surface area by method A (Clear Science, Inc.); ICP/AES by method B for the identification and quantitation of total Si, Al, Fe, and Mg as described for the analysis of crushed glass except that total mg was determined by measurement of atomic emission at 280.271 nm (study laboratory); PIXE spectroscopy to quantitate concentrations of major and minor elements (Elemental Analysis, Inc.); and powder XRD by a method similar to method D to identify and quantitate crystalline phases present at greater than 1% (H & M Analytical Services, Inc.). All spectra were consistent with the composition of garnet; a representative XRD spectrum is presented in Figure C-4.

Lot 031605 was determined to have a density of approximately 4.0 g/cc and a BET surface area of approximately 22.2 m^2/g . Weight loss on drying and Karl Fischer titration indicated 1.7% and 1.0% water, respectively, for the bulk material. ICP/AES, PIXE, and XRD analyses indicated 16.7%, 17.5%, and 18.8% Si, respectively; ICP/AES values for Al, Fe, and mg were 10.6%, 22.5%, and 1.4%, respectively; PIXE values for these elements were 11.3%, 23.3%, and 1.5%, respectively. The test material was mostly crystalline garnet [general formula $\text{A}_3\text{B}_2(\text{SiO}_4)_3$, in which A is Fe and/or Mg and B is Al and/or Fe (note: compare with almandine in Figure C-4); 89%] with crystalline components of quartz (SiO_2 , 6%) and staurolite ($\text{HFe}_2\text{Al}_9\text{Si}_4\text{O}_{24}$, 5% to 6%) as determined through the XRD Rietveld method.

To ensure stability, the bulk test material was stored at room temperature in safety-coated amber glass containers with Teflon-lined caps. Periodic reanalyses of the test material were performed by the analytical chemistry and study laboratories during the 2-week study with ICP/AES, PIXE, and XRD, and no degradation of the test material was detected.

⁸ERRATUM: An error was identified in the NTP Toxicity Report on Abrasive Blasting Agents (TOX 91). Text describing the bulk material preparation of garnet was added in the HTML and PDF versions of this report; new information is italicized. [September 1, 2022]

C.1.5. Specular Hematite

Prior to use in the studies, the particle size of specular hematite (lot O101005CJ) was reduced by aqueous ball milling for up to 27 hours, followed by 30 hours of aqueous bead milling of 60/40 test material/water (weight/weight) by Aveka, Inc. (Woodbury, MN). The milled material was mixed with water and the mixture was dispersed under strong agitation and then spray dried. The spray-dried material was oven dried at 200 °C for 8 hours.^h Lot O101005CJ of specular hematite, a finely divided, red powder, was expected to contain less than 1% of crystalline silica Paumanok, 1992. The test article was characterized and the composition was determined using analyses that included weight loss on drying (study laboratory); Karl Fischer titration for water content (Galbraith Laboratories, Inc.); density (Quantachrome Instruments); BET surface area by method A (Clear Science, Inc.); ICP/AES by a method similar to method B [except that the initial room temperature digestion used hydrochloric acid (HCl), subsequent digestions in HNO₃/HF and H₃BO₃ were conducted in a microwave (200°C and 150°C, respectively), and NH₂OH·HCL was not used to dissolve remaining metal oxides]. The samples were then analyzed for the identification and quantitation of total Si, Al, and Fe as described for the analysis of coal slag, and also total Zr by measurement of atomic emission at 339.198 nm (study laboratory); PIXE spectroscopy to quantitate concentrations of major and minor elements (Elemental Analysis, Inc.); and powder XRD by method E (H & M Analytical Services, Inc.) to identify and quantitate crystalline phases present at greater than 1%. All spectra were consistent with the expected composition of specular hematite; a representative XRD spectrum is presented in Figure C-5.

(E) For XRD analysis, a Philips PW3020 diffractometer (Philips Analytical, San Francisco, CA) or PANalytical X'Pert Pro MPD diffractometer (PANalytical B.V.) was used, with Cu radiation at 40 KV/30 mA or 45 KV/40 mA over angular ranges of 10° or 20° to 70° with step sizes of 0.0156° or 0.05° and counting times of 40 seconds per step or 3.5 to 6.5 hours per sample with a detection limit of approximately 0.5% for the trace phases. The crystalline phases were identified using the Powder Diffraction File (International Centre for Diffraction Data) and quantitated using Rietveld refinement.

Lot O101005CJ was determined to have a density of approximately 5 g/cc and a BET surface area of approximately 18.2 m²/g. Weight loss on drying and Karl Fischer titration both indicated less than 1% water for the bulk test material. ICP/AES, PIXE, and XRD analyses indicated the presence of Fe at approximately 69.4%, 63.4%, and 69.1%, respectively. ICP/AES values for Si, Al, and Zr were 1.2%, 0.4%, and 0.6%, and PIXE values for these elements were 1.2%, 0.4%, and 0.5%, respectively. The test material was crystalline with phases of specular hematite (Fe₂O₃, approximately 96%), magnetite (Fe₃O₄, 2% to 3%), and quartz (SiO₂, 1% to 2%), as determined through the XRD Rietveld method.

To ensure stability, the bulk test material was stored at room temperature in safety-coated amber glass containers with Teflon-lined caps. Periodic reanalyses of the test material were performed by the analytical chemistry and study laboratories during the 2-week and 39-week studies with ICP/AES and XRD, and no degradation of the test material was detected.

^hERRATUM: An error was identified in the NTP Toxicity Report on Abrasive Blasting Agents (TOX 91). Text describing the bulk material preparation of specular hematite was added in the HTML and PDF versions of this report; new information is italicized. [September 1, 2022]

C.2. Aerosol Generation and Exposure Systems

Schematic diagrams of the aerosol generation and distribution systems used in the abrasive blasting agent studies are shown in Figure C-6 through Figure C-10.

For the 2-week studies of abrasive blasting agents, the aerosol generation system consisted of a linear feed dust-metering device designed and built by Battelle to meter the abrasive blasting agent from a reservoir into an air stream for aerosolization. Within the metering device, periodic blasts of compressed air suspended small volumes of blasting sand, coal slag, crushed glass, garnet, or specular hematite in the air stream for transport to the metering device exhaust tube. For blasting sand (Figure C-6), coal slag, and garnet (Figure C-7), a jet disperser was positioned immediately downstream from the metering device exhaust tube. For coal slag and garnet, the jet disperser was followed by a particle attrition chamber (PAC) to enhance the aerosolization of the test material. For crushed glass (Figure C-8) and specular hematite (Figure C-9), a Trost jet mill (Garlock, Inc., Newtown, PA), was used downstream from the metering device exhaust tube to perform initial particle size reduction; opposing compressed air gas streams drove the jet mill.

All generation system components were housed in a glove box in the control center room. From the jet disperser (blasting sand, coal slag, garnet), jet mill (crushed glass, specular hematite) aerosol was blended with filtered, compressed air before it was conveyed down the distribution line from the control center room to the exposure room. For crushed glass, as the air stream entered the exposure room, an in-line cyclone separator was used to further decrease particle size and extract nonrespirable aerosol. For blasting sand (2-week and 39-week studies), coal slag, crushed glass, garnet, and the 39-week study of specular hematite, all chambers in the exposure room except for the control were fed aerosol from a single distribution line constructed of stainless steel, bonded and grounded to prevent electrostatic charge buildup. For the 2-week study of specular hematite, the distribution line was split into north and south branches. Aerosol was supplied to the 60 mg/m³ chamber from the south distribution line branch; all remaining chambers in the exposure room except the control chamber were fed aerosol from the north distribution line branch. During exposures to all the abrasive blasting agents, the airflow through the distribution line was controlled using house vacuum regulated by a filter-protected flow meter. A second distribution line flow control system was available during off-exposure periods. This system consisted of a vacuum transducer pump (Air-Vac Engineering Company, Inc., Seymour, CT) of higher flow capacity, positioned in parallel with the flow meter control assembly and was operational only during critical shutdown periods. At each exposure chamber, aerosol was delivered from the distribution line by a sampling tube. The flow through each sampling tube was induced by a stainless-steel air ejector pump designed and fabricated by Battelle. The flow rate and configuration of the ejector pump and sampling tube combination were chosen to optimize the efficiency of the delivery system. The aerosol then entered the chamber inlet duct where it was further diluted with humidified, Parafil-, charcoal-, and high-efficiency particulate air (HEPA)-filtered air to achieve the desired exposure concentration.

The 39-week study of blasting sand used the same aerosol generation system described for the 2-week study of this test material (Figure C-6). For the 39-week study of specular hematite, the aerosol generation system (Figure C-10) was similar to that described for the 2-week study of blasting sand (Figure C-6) except for the additions of an in-line settling jar within the glove box

and an in-line cyclone separator in the distribution line entering the exposure room as described for the 2-week study of crushed glass (Figure C-8).

The study laboratory designed the inhalation exposure chamber (Harford Systems Division of Lab Products, Inc., Aberdeen, MD) so that uniform aerosol concentrations could be maintained throughout the chamber with the catch pans in place. The total active mixing volume of each chamber was 1.7 m³.

C.3. Aerosol Concentration Monitoring

Summaries of the chamber aerosol concentrations are given in Table C-1 and Table C-2. The concentration of the abrasive blasting agent in the exposure chambers and room air was monitored using two real-time aerosol monitors (RAMs) (Model RAM-1; MIE, Inc., Bedford, MA). The monitors were connected to the chambers by a sampling system designed by Battelle incorporating a valve that multiplexed each RAM to a 0 mg/m³ chamber or the room, a HEPA-filtered room air blank, and two exposure chambers. The output (voltage) of the RAM was recorded by a program designed by Battelle (Battelle Exposure Data Acquisition and Control) to select the correct sample stream and acquire a raw voltage signal from each RAM. Equations for the calibration curves resided within the program and were used to convert the measured RAM voltages to exposure chamber concentrations. Concentration control limits within the program were compared to each measured concentration and, if limits were exceeded, an audible alarm was triggered or, in extreme cases, exposure was terminated.

Each RAM was calibrated by constructing a response curve using the measured RAM voltages (voltage readings were corrected by subtracting the RAM zero-offset voltage from measured RAM voltages) and chamber concentrations of the abrasive blasting agents measured gravimetrically or specific to the test article on exposure chamber filters. Developmental studies demonstrated that gravimetric and test article-specific measurements of chamber concentrations were comparable. For all abrasive blasting agents, exposure chamber atmosphere samples were collected each day on 25 mm Pallflex Emfab TX40H120WW Teflon-coated, glass-fiber filters and on 25 mm, 0.45 µm GH Polypro polypropylene filters (both obtained from Pall Corporation, Ann Arbor, MI). Test article-specific assays of blasting sand, coal slag, crushed glass, and garnet measured the amount of Si captured on filters extracted with 1:3 HNO₃:HF, and used an ICP/AES method otherwise similar to method B. Test article-specific assays of specular hematite measured the amount of Fe captured on filters extracted with HCl, also using an ICP/AES method similar to method B.

The ICP/AES instrument was calibrated against serially diluted NIST-traceable spectrometric standards of Si (for blasting sand, coal slag, crushed glass, and garnet) or Fe (for specular hematite) and the internal standard Co. Quality control standards and a reagent blank were analyzed after calibration, after approximately every 10th sample, and at the end of the analysis to determine accuracy and calibration drift during analysis.

C.4. Chamber Atmosphere Characterization

Particle size distribution was determined once before the 2-week and 39-week studies began, once during the 2-week studies, and once a month during the 39-week studies. Samples were

taken from each exposure chamber using a Mercer-style seven-stage cascade impactor (In-Tox Products, Moriarty, NM). For the 2-week studies of blasting sand, coal slag, crushed glass, and garnet, impactor samples were collected on polypropylene filters (GH Polypro, Pall Corporation), dissolved using HNO₃, HF, H₃BO₃ and NH₂OH·HCl, and assayed for Si using ICP/AES by a method otherwise similar to method B. For the 2-week study of specular hematite, impactor samples were collected on glass slides lightly coated with silicone to reduce particle bounce or on glass-fiber filters (Pallflex[®] Emfab[™], Pall Corporation), dissolved using HCl and sonication, and analyzed for Fe using ICP/AES by a method otherwise similar to method B. For the 39-week studies, impactor samples of blasting sand and specular hematite were collected on stainless-steel slides or glass-fiber filters (Pallflex Emfab, Pall Corporation) and then measured gravimetrically to determine the amount of test article deposited on each stage. The relative mass of each abrasive blasting agent collected on each stage was analyzed by the NEWCAS impactor analysis program developed at Battelle and was based on probit analysis.⁵⁰ The resulting estimates of the mass median aerodynamic particle diameter and the geometric standard deviation of each set of samples collected during the studies are given in Table C-3 through Table C-5. All values of mass median aerodynamic diameter were less than 3 μm as required by the protocol.

Buildup and decay rates for chamber aerosol concentrations were determined with and without animals present in the chambers. At a chamber airflow rate of 15 air changes per hour, the theoretical value for the time to achieve 90% of the target concentration after the beginning of aerosol generation (T₉₀) and the time for the chamber concentration to decay to 10% of the target concentration after aerosol generation was terminated (T₁₀) was approximately 9.4 minutes. For the 2-week study of blasting sand, T₉₀ and T₁₀ values ranged from 9 to 10 minutes with animals present. For the 2-week studies of coal slag, crushed glass, garnet, and specular hematite, T₉₀ values ranged from 10 to 13, 12 to 14, 13 to 15, and 9 to 22 minutes, respectively, with animals present; T₁₀ values ranged from 9 to 10, 9 to 10, 10 to 11, and 10 to 11 minutes, respectively. For the 39-week study of blasting sand, T₉₀ values ranged from 12 to 13 minutes without animals present and from 13 to 14 minutes with animals; T₁₀ values ranged from 8 to 9 minutes without animals present and from 10 to 11 minutes with animals. For the 39-week study of specular hematite, T₉₀ values ranged from 12 to 13 minutes without animals present and from 10 to 12 minutes with animals; T₁₀ values were 9 minutes without animals present and ranged from 10 to 11 minutes with animals. A T₉₀ value of 12 minutes was selected for all studies.

The uniformity of aerosol concentration in the inhalation exposure chambers without animals present was evaluated before the 39-week studies began; in addition, concentration uniformity with animals present in the chambers was measured once during the 2-week studies and three times during the 39-week studies. Aerosol concentrations were measured using the on-line monitor with the stream-selection valve fixed in one position to allow continuous monitoring from a single input line. Concentrations were measured at 12 chamber sample ports, one in front and one in back for each of six possible cage unit positions per chamber. Chamber concentration uniformity was maintained throughout the studies.

The persistence of the abrasive blasting agents in the chambers after aerosol delivery ended was determined by monitoring the concentration overnight in the 30 mg/m³ chambers (except for the 2-week and 39-week studies of specular hematite that monitored concentrations in the 60 mg/m³ chamber), with (all studies) and without (39-week studies only) animals present in the chambers. In the 2-week studies of blasting sand, coal slag, crushed glass, garnet, and specular hematite, the

concentration decreased to less than 1% of the starting concentration within 19, 19, 21, 20, and 21 minutes, respectively. In the 39-week study of blasting sand, the concentration decreased to less than 1% of the starting concentration within 20 minutes with animals present and within 19 minutes without animals. In the 39-week study of specular hematite, the concentration decreased to less than 1% of the starting concentration within 21 minutes with animals present and within 18 minutes without animals.

Stability studies of the test materials in the generation and exposure systems were performed by the analytical chemistry and study laboratories. During the 2-week studies, before the start of the 39-week studies, and twice during the 39-week studies, blasting sand, coal slag, crushed glass, garnet, or specular hematite powder samples were taken from the low and high exposure concentration chambers and the aerosol distribution lines by collection on 25 mm A/E glass-fiber or polypropylene (GH Polypro) filters (Pall Corporation). On each sample collection day, samples of the bulk test material were collected before filling the generator reservoir and from the reservoir at the end of the generation day; additional test material was added to the generator each day. Samples were analyzed by XRD to identify and quantitate crystalline phases present in each abrasive blasting agent and by ICP/AES and PIXE (2-week studies of blasting sand, coal slag, crushed glass, and garnet) to determine elemental content, and carbon content was assayed by combustion (coal slag only). The analytical methods for these in-system stability assays were generally similar to those described earlier for initial characterization of each bulk test article. After accounting for minor inconsistencies introduced by changes in instrumentation and techniques, results of the XRD, ICP/AES, PIXE, and carbon in-system stability assays showed that the composition of each abrasive blasting agent in the exposure chambers and distribution lines was stable in the presence and absence of animals, reflected the composition of the bulk test material in the generator reservoir, and was generally comparable to that found during the initial characterization assays of each test article. These assays also indicated that contaminations from metal materials in the exposure generation systems did not occur.

Table C-1. Summary of Chamber Concentrations in the Two-week Inhalation Studies of Abrasive Blasting Agents in F344/NTac Rats

	Total Concentration (mg/m ³)	Total Number of Readings	Average Concentration ^a (mg/m ³)
Blasting Sand	3	127	3.0 ± 0.2
	15	126	14.6 ± 0.6
	30	127	30.5 ± 1.3
Coal Slag	3	128	2.92 ± 0.21
	15	126	14.9 ± 0.8
	30	128	30.4 ± 1.8
Crushed Glass	3	118	2.94 ± 0.12
	15	119	15.0 ± 0.6
	30	118	30.4 ± 0.9
Garnet	3	119	2.87 ± 0.18
	15	118	14.8 ± 0.9

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	Total Concentration (mg/m³)	Total Number of Readings	Average Concentration^a (mg/m³)
	30	119	29.8 ± 1.6
Specular Hematite	3	117	2.92 ± 0.19
	15	118	14.8 ± 1.0
	30	118	29.5 ± 1.9
	60	118	58.1 ± 3.5

^aMean ± standard deviation.

Table C-2. Summary of Chamber Concentrations in the 39-week Inhalation Studies of Blasting Sand and Specular Hematite in Sprague Dawley Rats

	Total Concentration (mg/m³)	Total Number of Readings	Average Concentration^a (mg/m³)
Blasting Sand	15	1,929	14.5 ± 1.0
	30	1,916	29.2 ± 1.7
	60	1,929	59.1 ± 3.3
Specular Hematite	15	1,973	14.9 ± 0.9
	30	1,966	29.5 ± 1.7
	60	1,973	59.4 ± 3.1

^aMean ± standard deviation.

Table C-3. Summary of Aerosol Size Measurements for the F344/NTac Rat Exposure Chambers in the Two-week Inhalation Studies of Abrasive Blasting Agents

	Target Concentration (mg/m ³)	Mass Median Aerodynamic Diameter (µm)	Geometric Standard Deviation
Blasting Sand	3	1.13	2.50
	15	1.24	2.34
	30	1.26	2.32
Coal Slag	3	1.13	2.26
	15	1.14	2.22
	30	1.18	2.31
Crushed Glass	3	1.0	2.6
	15	1.1	2.7
	30	1.1	2.5
Garnet	3	1.1	2.4
	15	1.0	2.4
	30	1.1	2.4
Specular Hematite	3	0.8	2.3
	15	0.7	2.1
	30	0.8	2.1
	60	0.8	2.1

Table C-4. Summary of Aerosol Size Measurements for the Sprague Dawley Rat Exposure Chambers in the 39-week Inhalation Study of Blasting Sand

Date of Test	Target Concentration (mg/m ³)	Mass Median Aerodynamic Diameter (µm)	Geometric Standard Deviation
April 2009	15	1.3	2.2
	30	1.4	2.3
	60	1.5	2.3
May 2009	15	1.3	2.4
	30	1.1	2.6
	60	1.3	2.6
June 2009	15	1.5	2.2
	30	1.4	2.1
	60	1.6	2.2
July 2009	15	1.3	2.5
	30	1.6	2.2
	60	1.6	2.5
August 2009	15	1.5	2.5
	30	1.8	2.6

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Date of Test	Target Concentration (mg/m ³)	Mass Median Aerodynamic Diameter (µm)	Geometric Standard Deviation
September 2009	60	1.5	2.6
	15	1.0	2.1
	30	1.8	2.3
October 2009	60	1.4	2.1
	15	1.4	2.1
	30	1.4	2.3
November 2009	60	1.5	2.2
	15	1.3	2.6
	30	1.6	2.7
December 2009	60	1.5	2.1
	15	1.3	2.2
	30	1.6	2.4
January 2010	60	1.6	2.4
	15	1.4	2.2
	30	1.3	2.4
Range	60	1.8	2.8
	15	1.0–1.5	2.1–2.6
	30	1.1–1.8	2.1–2.7
	60	1.3–1.8	2.1–2.8

Table C-5. Summary of Aerosol Size Measurements for the Sprague Dawley Rat Exposure Chambers in the 39-week Inhalation Study of Specular Hematite

Date of Test	Target Concentration (mg/m ³)	Mass Median Aerodynamic Diameter (µm)	Geometric Standard Deviation
August 2009	15	0.9	2.3
	30	1.0	2.2
	60	1.0	2.1
September 2009	15	0.9	1.9
	30	0.9	2.3
	60	0.9	2.0
October 2009	15	0.8	2.0
	30	1.0	2.1
	60	0.9	1.9
November 2009	15	1.1	2.2
	30	1.1	2.1

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Date of Test	Target Concentration (mg/m ³)	Mass Median Aerodynamic Diameter (µm)	Geometric Standard Deviation
December 2009	60	1.0	2.2
	15	0.9	2.4
	30	1.1	2.3
January 2010	60	0.9	2.0
	15	0.9	1.8
	30	0.8	2.0
February 2010	60	0.9	1.9
	15	0.9	2.1
	30	1.0	2.5
March 2010	60	1.1	2.3
	15	0.9	2.4
	30	0.9	2.7
April 2010	60	0.9	2.2
	15	1.0	2.1
	30	0.9	2.1
Range	60	1.1	2.0
	15	0.8–1.1	1.8–2.4
	30	0.8–1.1	2.0–2.7
	60	0.9–1.1	1.9–2.3

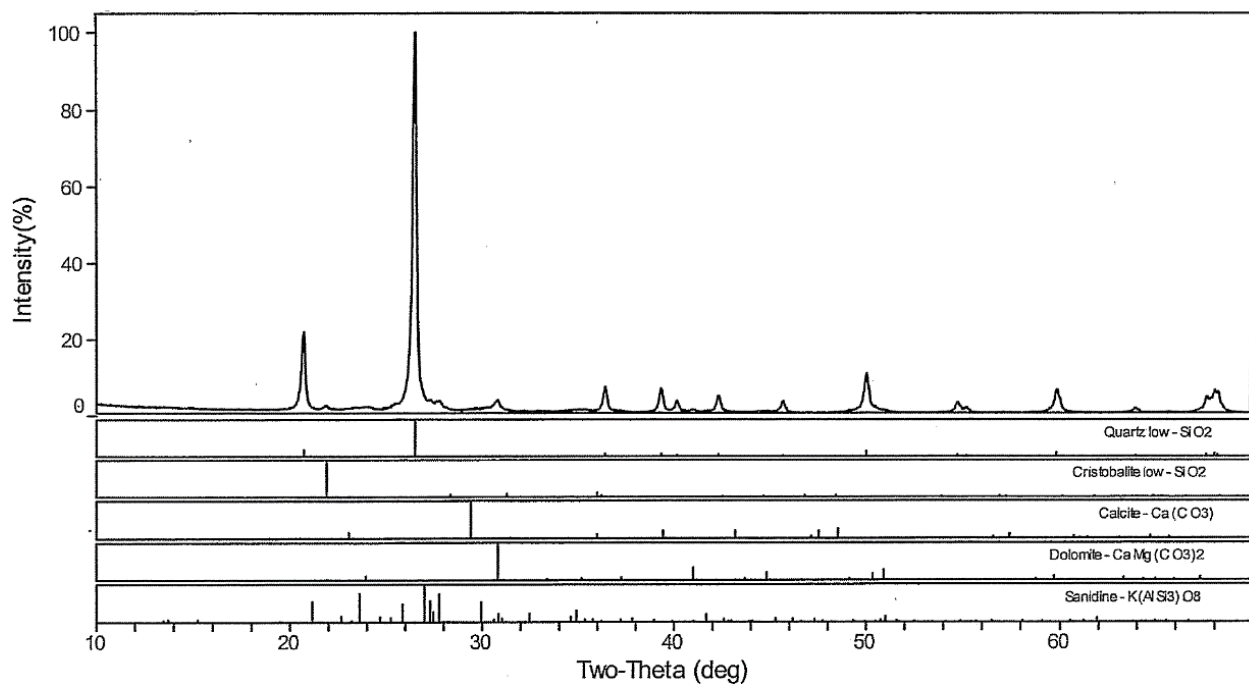


Figure C-1. X-ray Diffraction Pattern of Blasting Sand

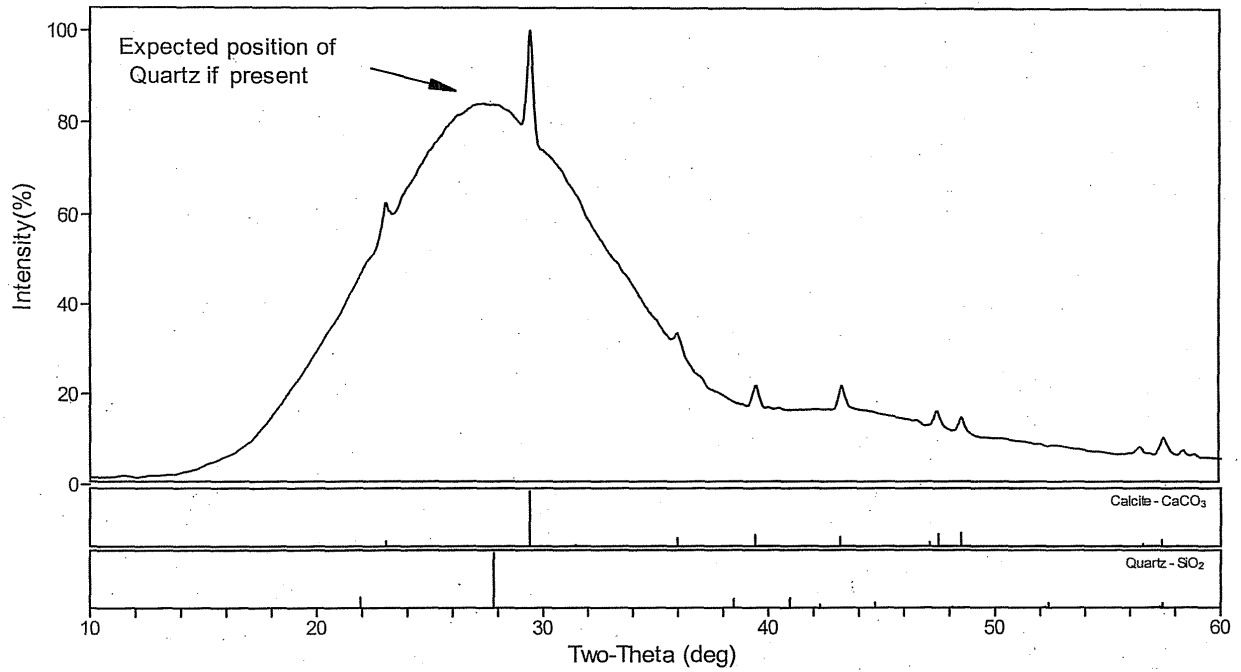


Figure C-2. X-ray Diffraction Pattern of Coal Slag

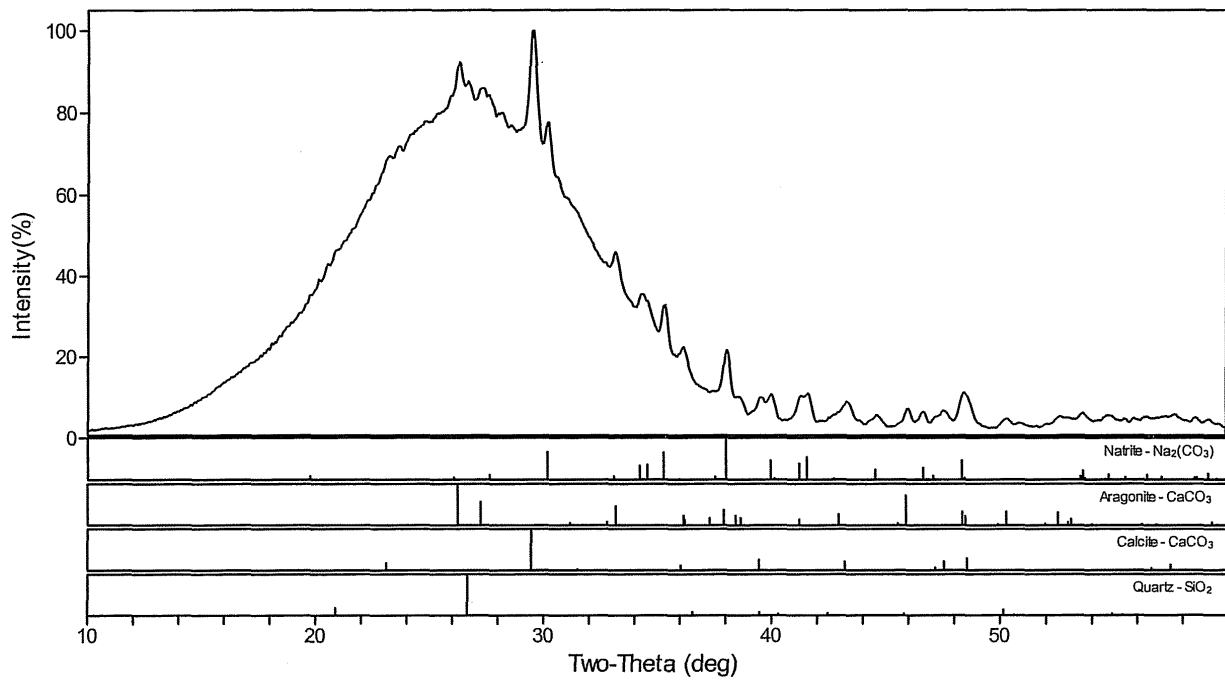


Figure C-3. X-ray Diffraction Pattern of Crushed Glass

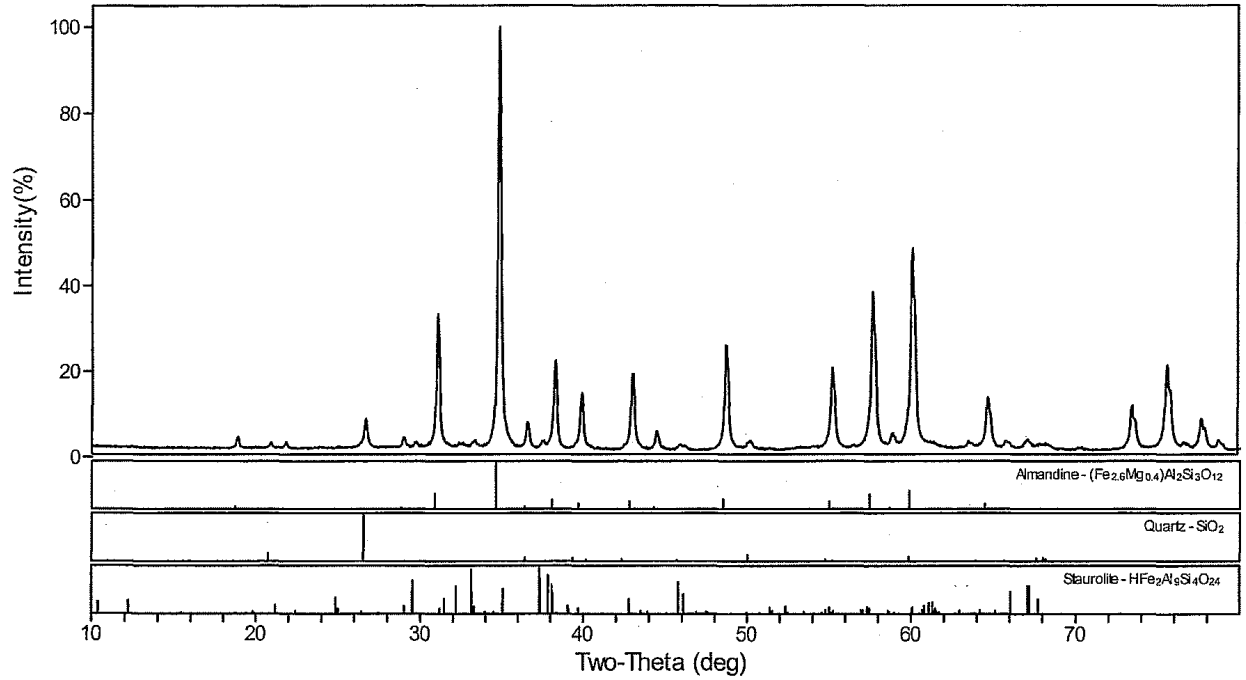


Figure C-4. X-ray Diffraction Pattern of Garnet

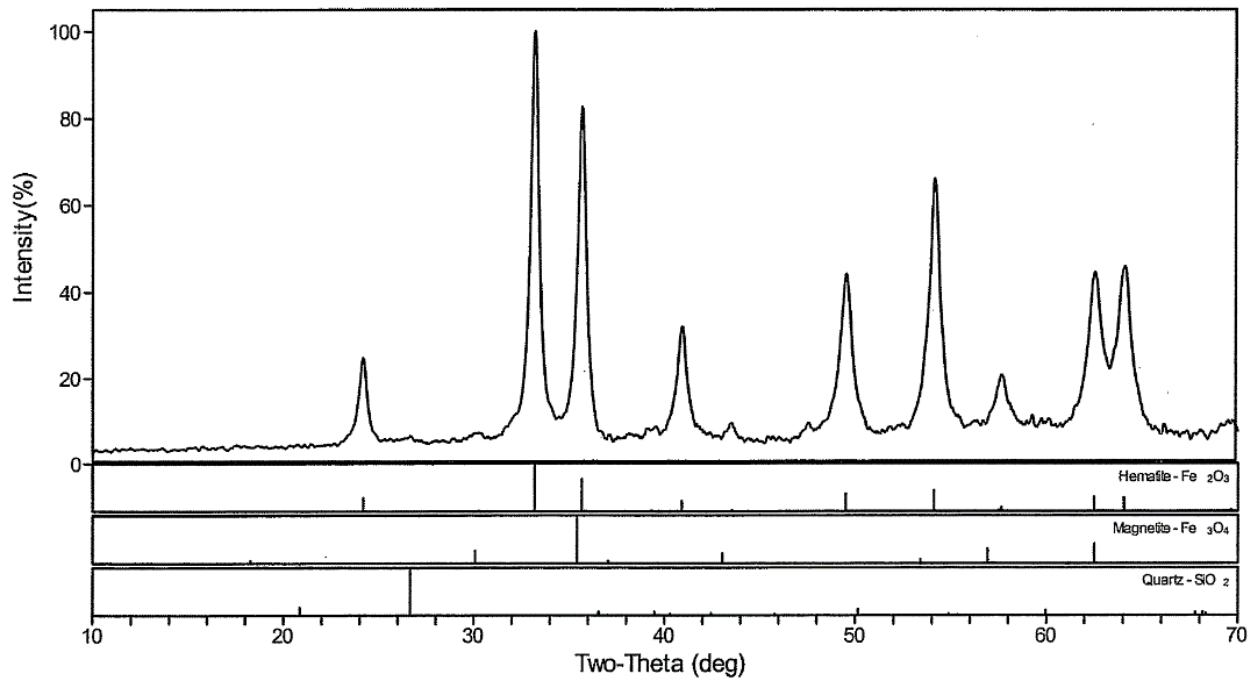


Figure C-5. X-ray Diffraction Pattern of Specular Hematite

Abrasive Blasting Agents, TOX 91

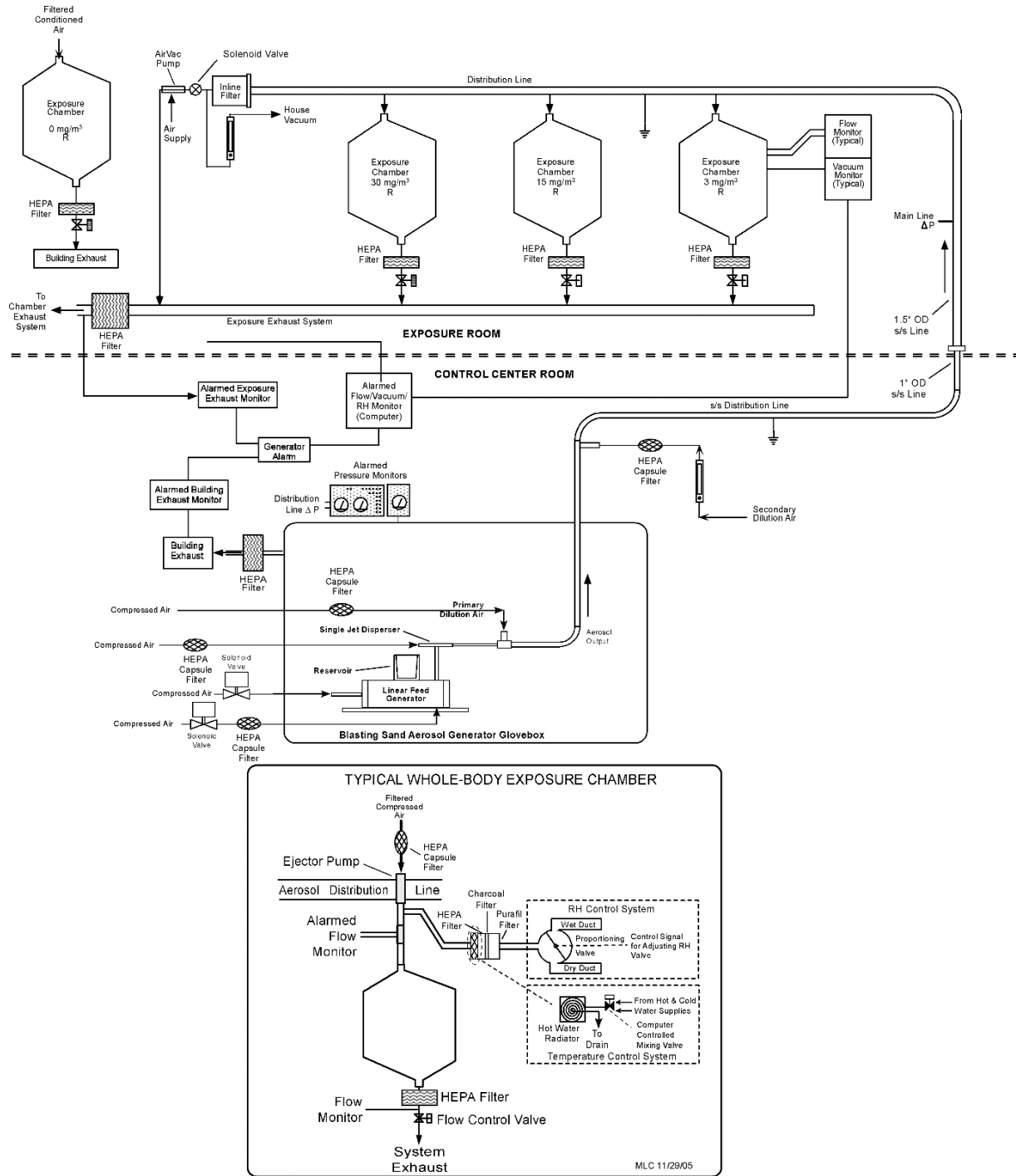


Figure C-6. Schematic of the Aerosol Generation and Delivery System in the Two-week and 39-week Inhalation Studies of Blasting Sand

Chamber concentrations shown are for the 2-week study.

Abrasive Blasting Agents, TOX 91

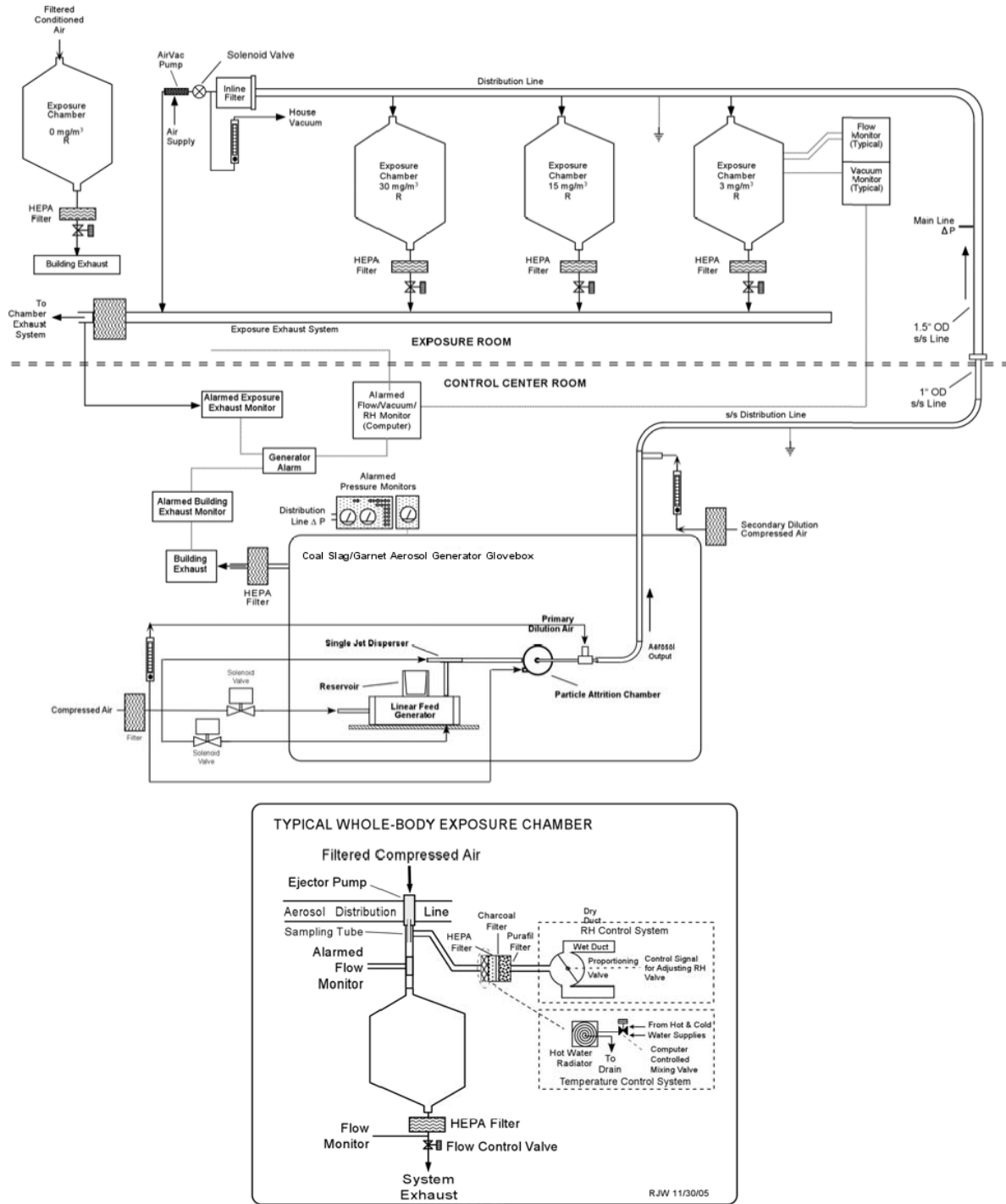


Figure C-7. Schematic of the Aerosol Generation and Delivery System in the Two-week Inhalation Studies of Coal Slag and Garnet

Abrasive Blasting Agents, TOX 91

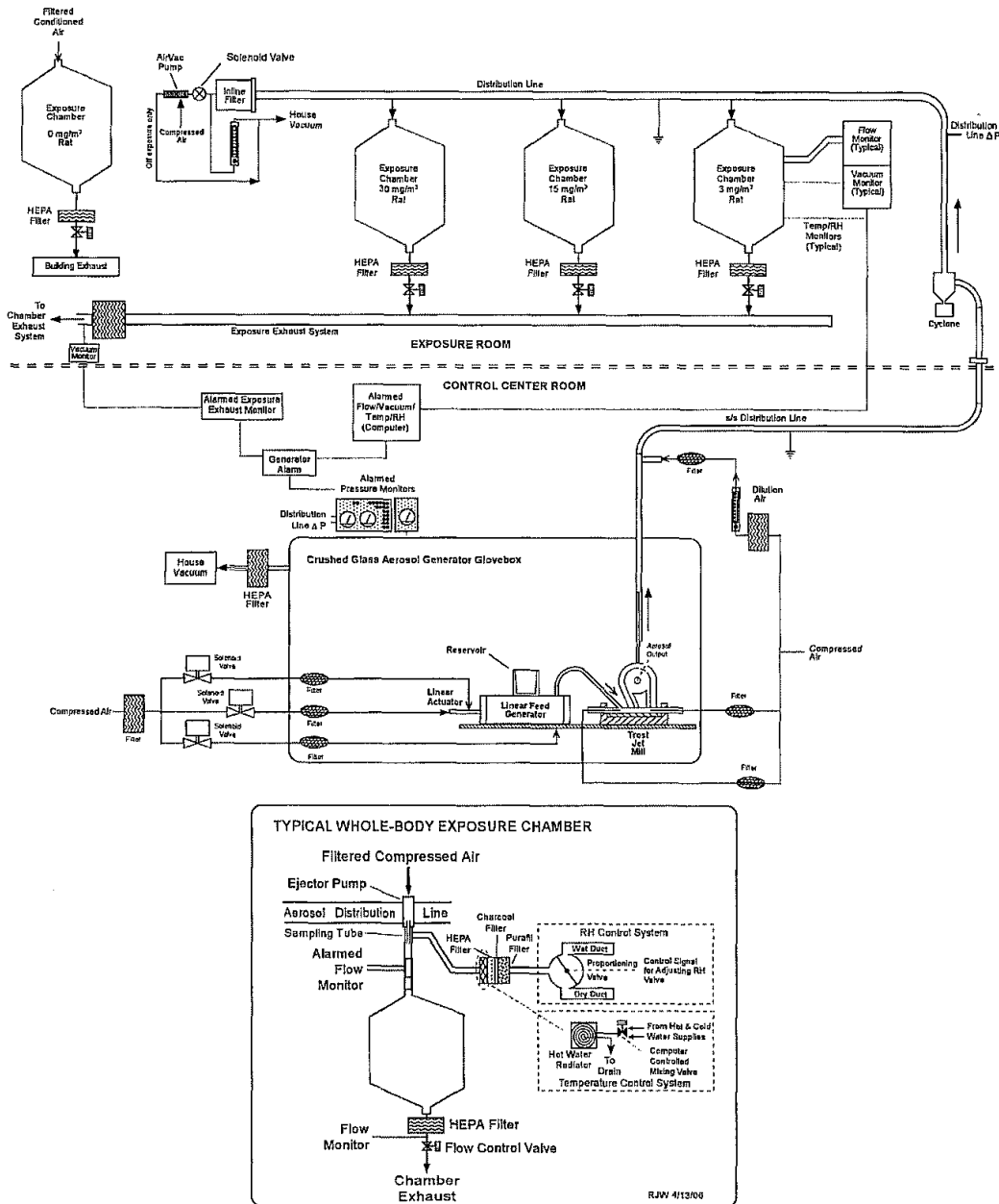


Figure C-8. Schematic of the Aerosol Generation and Delivery System in the Two-week Inhalation Study of Crushed Glass

Abrasive Blasting Agents, TOX 91

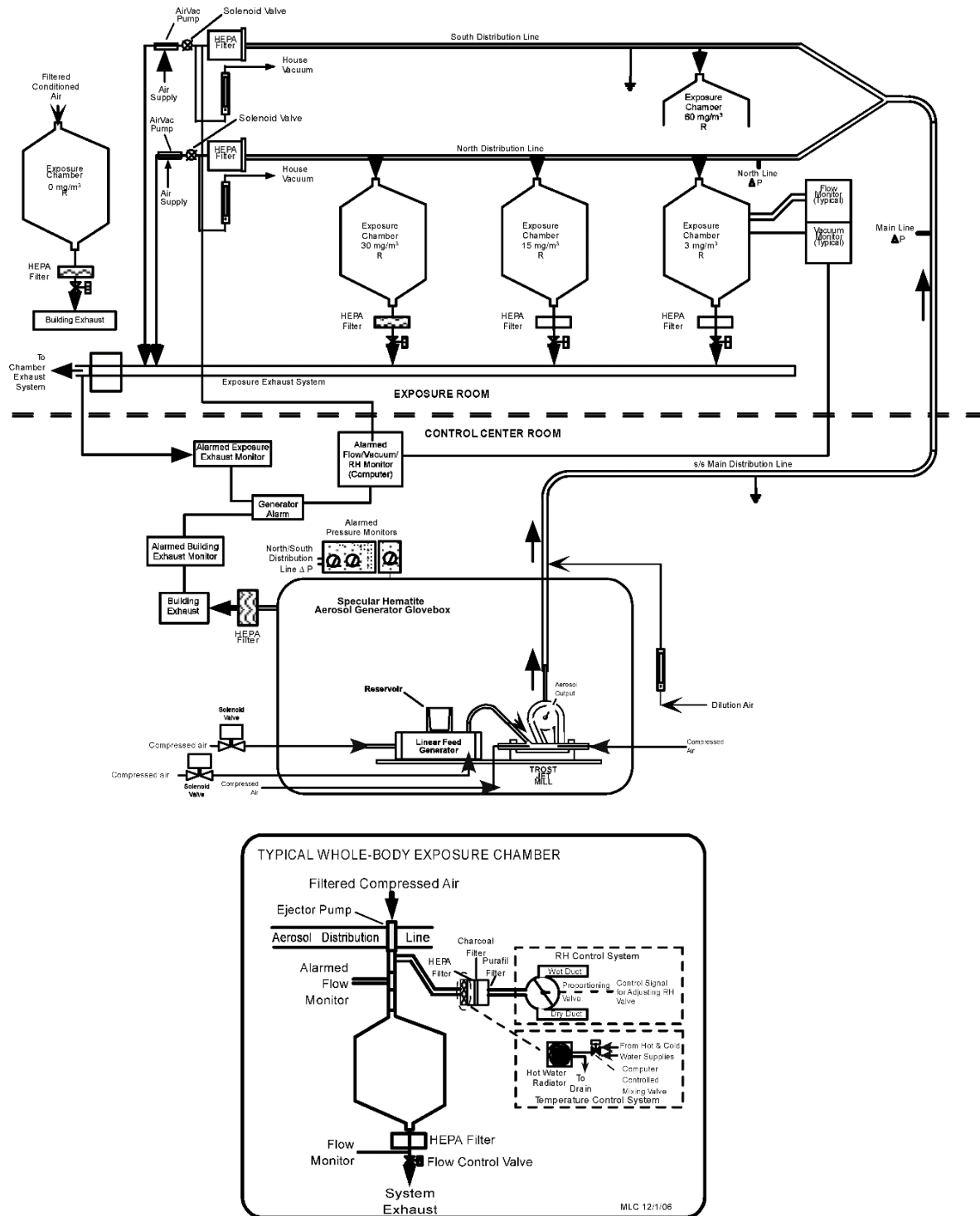


Figure C-9. Schematic of the Aerosol Generation and Delivery System in the Two-week Inhalation Study of Specular Hematite

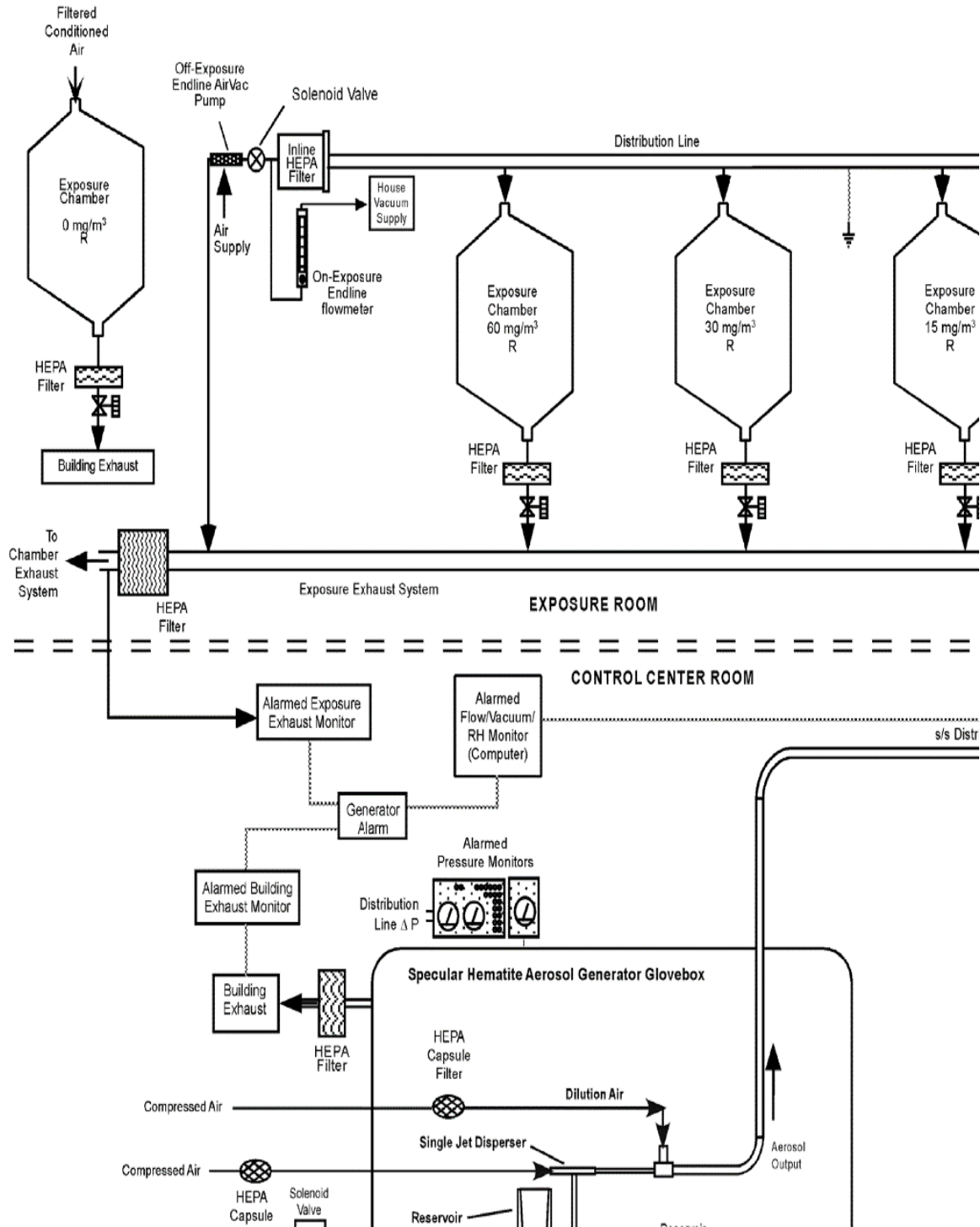


Figure C-10. Schematic of the Aerosol Generation and Delivery System in the 39-week Inhalation Study of Specular Hematite

Appendix D. Ingredients, Nutrient Composition, and Contaminant Levels in NTP-2000 Rat and Mouse Ration

Tables

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Table D-1. Ingredients of NTP-2000 Rat and Mouse Ration

Ingredients	Percentage by Weight
Ground Hard Winter Wheat	22.26
Ground #2 Yellow Shelled Corn	22.18
Wheat Middlings	15.0
Oat Hulls	8.5
Alfalfa Meal (Dehydrated, 17% Protein)	7.5
Purified Cellulose	5.5
Soybean Meal (49% Protein)	5.0
Fish Meal (60% Protein)	4.0
Corn Oil (without Preservatives)	3.0
Soy Oil (without Preservatives)	3.0
Dried Brewer's Yeast	1.0
Calcium Carbonate (USP)	0.9
Vitamin Premix ^a	0.5
Mineral Premix ^b	0.5
Calcium Phosphate, Dibasic (USP)	0.4
Sodium Chloride	0.3
Choline Chloride (70% Choline)	0.26
Methionine	0.2

USP = United States Pharmacopeia.

^aWheat middlings as carrier.

^bCalcium carbonate as carrier.

Table D-2. Vitamins and Minerals in NTP-2000 Rat and Mouse Ration^a

	Amount	Source
Vitamins		
A	4,000 IU	Stabilized vitamin A palmitate or acetate
D	1,000 IU	D-activated animal sterol
K	1.0 mg	Menadione sodium bisulfite complex
α -Tocopheryl Acetate	100 IU	—
Niacin	23 mg	—
Folic Acid	1.1 mg	—
d-Pantothenic Acid	10 mg	d-Calcium pantothenate
Riboflavin	3.3 mg	—
Thiamine	4 mg	Thiamine mononitrate
B ₁₂	52 μ g	—
Pyridoxine	6.3 mg	Pyridoxine hydrochloride

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	Amount	Source
Biotin	0.2 mg	d-Biotin
Minerals		
Magnesium	514 mg	Magnesium oxide
Iron	35 mg	Iron sulfate
Zinc	12 mg	Zinc oxide
Manganese	10 mg	Manganese oxide
Copper	2.0 mg	Copper sulfate
Iodine	0.2 mg	Calcium iodate
Chromium	0.2 mg	Chromium acetate

^aPer kg of finished product.

Table D-3. Nutrient Composition of NTP-2000 Rat and Mouse Ration (Blasting Sand)

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by Weight)	14.4 ± 0.33	13.7–14.8	11
Crude Fat (% by Weight)	8.2 ± 0.27	7.7–8.5	11
Crude Fiber (% by Weight)	9.4 ± 1.19	7.1–11.1	11
Ash (% by Weight)	5.1 ± 0.10	4.93–5.21	11
Amino Acids (% of Total Diet)			
Arginine	0.783 ± 0.070	0.670–0.970	22
Cystine	0.220 ± 0.024	0.150–0.250	22
Glycine	0.701 ± 0.041	0.620–0.800	22
Histidine	0.352 ± 0.077	0.270–0.680	22
Isoleucine	0.546 ± 0.044	0.430–0.660	22
Leucine	1.095 ± 0.067	0.960–1.240	22
Lysine	0.711 ± 0.114	0.310–0.860	22
Methionine	0.409 ± 0.046	0.260–0.490	22
Phenylalanine	0.628 ± 0.040	0.540–0.720	22
Threonine	0.505 ± 0.043	0.430–0.610	22
Tryptophan	0.150 ± 0.028	0.110–0.200	22
Tyrosine	0.401 ± 0.061	0.280–0.540	22
Valine	0.665 ± 0.043	0.550–0.730	22
Essential Fatty Acids (% of Total Diet)			
Linoleic	3.95 ± 0.259	3.49–4.55	22
Linolenic	0.30 ± 0.032	0.21–0.35	22
Vitamins			
Vitamin A (IU/kg)	3,862 ± 95.8	2,110–5,120	11

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Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Vitamin D (IU/kg)	1,000 ^a	–	–
α-Tocopherol (ppm)	80.6 ± 22.03	27.0–124.0	22
Thiamine (ppm) ^b	7.5 ± 1.35	6.1–11.0	11
Riboflavin (ppm)	7.6 ± 2.89	4.20–17.50	22
Niacin (ppm)	78.9 ± 9.08	66.4–98.2	22
Pantothenic Acid (ppm)	26.9 ± 12.63	17.4–81.0	22
Pyridoxine (ppm) ^b	9.54 ± 1.99	6.44–13.7	22
Folic Acid (ppm)	1.62 ± 0.48	1.15–3.27	22
Biotin (ppm)	0.32 ± 0.10	0.2–0.704	22
Vitamin B ₁₂ (ppb)	53.6 ± 39.6	18.3–174.0	22
Choline (ppm) ^b	2,846 ± 485	1,820–3,790	22
Minerals			
Calcium (%)	0.889 ± 0.038	0.810–0.960	11
Phosphorus (%)	0.542 ± 0.022	0.504–0.587	11
Potassium (%)	0.666 ± 0.030	0.626–0.733	22
Chloride (%)	0.386 ± 0.039	0.300–0.474	22
Sodium (%)	0.189 ± 0.016	0.160–0.222	22
Magnesium (%)	0.216 ± 0.062	0.185–0.490	22
Sulfur (%)	0.170 ± 0.029	0.116–0.209	22
Iron (ppm)	186 ± 39.21	135–311	22
Manganese (ppm)	51.4 ± 10.28	21.0–73.1	22
Zinc (ppm)	53.4 ± 8.46	43.3–78.5	22
Copper (ppm)	7.01 ± 2.562	3.21–16.30	22
Iodine (ppm)	0.503 ± 0.206	0.158–0.972	22
Chromium (ppm)	0.694 ± 0.276	0.330–1.380	21
Cobalt (ppm)	0.26 ± 0.164	0.098–0.864	20

^aFrom formulation.

^bAs hydrochloride (thiamine and pyridoxine) or chloride (choline).

Table D-4. Contaminant Levels in NTP-2000 Rat and Mouse Ration (Blasting Sand)^a

	Mean ± Standard Deviation ^b	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.24 ± 0.046	0.169–0.307	11
Cadmium (ppm)	0.06 ± 0.005	0.053–0.069	11
Lead (ppm)	0.15 ± 0.243	0.064–0.887	11
Mercury (ppm)	<0.02	–	11
Selenium (ppm)	0.23 ± 0.055	0.16–0.342	11
Aflatoxins (ppb)	<5.00	–	11
Nitrate Nitrogen (ppm) ^c	23.7 ± 9.37	10.0–35.9	11
Nitrite Nitrogen (ppm) ^c	<0.61	–	11
BHA (ppm) ^d	<1.0	–	11
BHT (ppm) ^d	<1.0	–	11
Aerobic Plate Count (CFU/g)	10 ± 0.0	10	11
Coliform (MPN/g)	3.0 ± 0.0	3.0	11
<i>Escherichia coli</i> (MPN/g)	<10	–	11
<i>Salmonella</i> (MPN/g)	Negative	–	11
Total Nitrosoamines (ppb) ^e	11.2 ± 3.82	6.1–17.2	11
<i>N</i> -Nitrosodimethylamine (ppb) ^e	4.25 ± 3.424	1.1–11.1	11
<i>N</i> -Nitrosopyrrolidine (ppb) ^e	8.13 ± 2.427	5.0–11.0	11
Pesticides (ppm)			
α-BHC	<0.01	–	11
β-BHC	<0.02	–	11
γ-BHC	<0.01	–	11
δ-BHC	<0.01	–	11
Heptachlor	<0.01	–	11
Aldrin	<0.01	–	11
Heptachlor Epoxide	<0.01	–	11
DDE	<0.01	–	11
DDD	<0.01	–	11
DDT	<0.01	–	11
HCB	<0.01	–	11
Mirex	<0.01	–	11
Methoxychlor	<0.05	–	11
Dieldrin	<0.01	–	11
Endrin	<0.01	–	11

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	Mean ± Standard Deviation ^b	Range	Number of Samples
Telodrin	<0.01	–	11
Chlordane	<0.05	–	11
Toxaphene	<0.10	–	11
Estimated PCBs	<0.20	–	11
Ronnel	<0.01	–	11
Ethion	<0.02	–	11
Trithion	<0.05	–	11
Diazinon	<0.10	–	11
Methyl Chlorpyrifos	0.186 ± 0.144	0.02–0.553	11
Methyl Parathion	<0.02	–	11
Ethyl Parathion	<0.02	–	11
Malathion	0.115 ± 0.119	0.020–0.395	11
Endosulfan I	<0.01	–	11
Endosulfan II	<0.01	–	11
Endosulfan Sulfate	<0.03	–	11

CFU = colony-forming units; MPN = most probable number; BHC = hexachlorocyclohexane or benzene hexachloride; PCB = polychlorinated biphenyl.

^aAll samples were irradiated.

^bFor values less than the limit of detection, the detection limit is given as the mean.

^cSources of contamination: alfalfa, grains, and fish meal.

^dSources of contamination: soy oil and fish meal.

^eAll values were corrected for percent recovery.

Table D-5. Nutrient Composition of NTP-2000 Rat and Mouse Ration (Specular Hematite)

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by Weight)	14.6 ± 0.26	14.1–15.0	11
Crude Fat (% by Weight)	8.3 ± 0.27	7.7–8.6	11
Crude Fiber (% by Weight)	9.5 ± 1.25	7.1–11.1	11
Ash (% by Weight)	5.0 ± 0.10	4.88–5.21	11
Amino Acids (% of Total Diet)			
Arginine	0.783 ± 0.070	0.670–0.970	22
Cystine	0.220 ± 0.024	0.150–0.250	22
Glycine	0.701 ± 0.041	0.620–0.800	22
Histidine	0.352 ± 0.077	0.270–0.680	22
Isoleucine	0.546 ± 0.044	0.430–0.660	22
Leucine	1.095 ± 0.067	0.960–1.240	22
Lysine	0.711 ± 0.114	0.310–0.860	22
Methionine	0.409 ± 0.046	0.260–0.490	22
Phenylalanine	0.628 ± 0.040	0.540–0.720	22

Abrasive Blasting Agents, TOX 91

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Threonine	0.505 ± 0.043	0.430–0.610	22
Tryptophan	0.150 ± 0.028	0.110–0.200	22
Tyrosine	0.401 ± 0.061	0.280–0.540	22
Valine	0.665 ± 0.043	0.550–0.730	22
Essential Fatty Acids (% of Total Diet)			
Linoleic	3.95 ± 0.259	3.49–4.55	22
Linolenic	0.30 ± 0.032	0.21–0.35	22
Vitamins			
Vitamin A (IU/kg)	3,776 ± 93.5	2,110–5,330	11
Vitamin D (IU/kg)	1,000 ^a		
α-Tocopherol (ppm)	80.6 ± 22.03	27.0–124.0	22
Thiamine (ppm) ^b	7.8 ± 1.25	6.4–11.0	11
Riboflavin (ppm)	7.6 ± 2.89	4.20–17.50	22
Niacin (ppm)	78.9 ± 9.08	66.4–98.2	22
Pantothenic Acid (ppm)	26.9 ± 12.63	17.4–81.0	22
Pyridoxine (ppm) ^b	9.54 ± 1.99	6.44–13.7	22
Folic Acid (ppm)	1.62 ± 0.48	1.15–3.27	22
Biotin (ppm)	0.32 ± 0.10	0.2–0.704	22
Vitamin B ₁₂ (ppb)	53.6 ± 39.6	18.3–174.0	22
Choline (ppm) ^b	2,846 ± 485	1,820–3,790	22
Minerals			
Calcium (%)	0.887 ± 0.040	0.810–0.960	11
Phosphorus (%)	0.546 ± 0.022	0.504–0.587	11
Potassium (%)	0.666 ± 0.030	0.626–0.733	22
Chloride (%)	0.386 ± 0.039	0.300–0.474	22
Sodium (%)	0.189 ± 0.066	0.160–0.222	22
Magnesium (%)	0.216 ± 0.062	0.185–0.490	22
Sulfur (%)	0.170 ± 0.029	0.116–0.209	22
Iron (ppm)	186 ± 39.21	135–311	22
Manganese (ppm)	51.4 ± 10.28	21.0–73.1	22
Zinc (ppm)	53.4 ± 8.46	43.3–78.5	22
Copper (ppm)	7.01 ± 2.562	3.21–16.30	22
Iodine (ppm)	0.503 ± 0.206	0.158–0.972	22
Chromium (ppm)	0.694 ± 0.276	0.330–1.380	21
Cobalt (ppm)	0.26 ± 0.164	0.098–0.864	20

^aFrom formulation.

^bAs hydrochloride (thiamine and pyridoxine) or chloride (choline).

Table D-6. Contaminant Levels in NTP-2000 Rat and Mouse Ration (Specular Hematite)^a

	Mean ± Standard Deviation ^b	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.26 ± 0.021	0.240–0.307	11
Cadmium (ppm)	0.06 ± 0.005	0.053–0.069	11
Lead (ppm)	0.15 ± 0.243	0.064–0.887	11
Mercury (ppm)	<0.02	–	11
Selenium (ppm)	0.22 ± 0.054	0.166–0.342	11
Aflatoxins (ppb)	<5.00	–	11
Nitrate Nitrogen (ppm) ^c	22.1 ± 8.53	10.0–35.9	11
Nitrite Nitrogen (ppm) ^c	<0.61	–	11
BHA (ppm) ^d	<1.0	–	11
BHT (ppm) ^d	<1.0	–	11
Aerobic Plate Count (CFU/g)	10 ± 0.0	10	11
Coliform (MPN/g)	3.0 ± 0.0	3.0	11
<i>Escherichia coli</i> (MPN/g)	<10	–	11
<i>Salmonella</i> (MPN/g)	Negative	–	11
Total Nitrosoamines (ppb) ^e	10.7 ± 4.03	5.6–16.2	11
<i>N</i> -Nitrosodimethylamine (ppb) ^e	3.36 ± 2.462	1.0–9.6	11
<i>N</i> -Nitrosopyrrolidine (ppb) ^e	7.68 ± 2.420	4.6–11.2	11
Pesticides (ppm)			
α-BHC	<0.01	–	11
β-BHC	<0.02	–	11
γ-BHC	<0.01	–	11
δ-BHC	<0.01	–	11
Heptachlor	<0.01	–	11
Aldrin	<0.01	–	11
Heptachlor Epoxide	<0.01	–	11
DDE	<0.01	–	11
DDD	<0.01	–	11
DDT	<0.01	–	11
HCB	<0.01	–	11
Mirex	<0.01	–	11
Methoxychlor	<0.05	–	11
Dieldrin	<0.01	–	11
Endrin	<0.01	–	11

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	Mean \pm Standard Deviation ^b	Range	Number of Samples
Telodrin	<0.01	–	11
Chlordane	<0.05	–	11
Toxaphene	<0.10	–	11
Estimated PCBs	<0.20	–	11
Ronnel	<0.01	–	11
Ethion	<0.02	–	11
Trithion	<0.05	–	11
Diazinon	<0.10	–	11
Methyl Chlorpyrifos	0.173 \pm 0.153	0.02–0.553	11
Methyl Parathion	<0.02	–	11
Ethyl Parathion	<0.02	–	11
Malathion	0.147 \pm 0.113	0.020–0.395	11
Endosulfan I	<0.01	–	11
Endosulfan II	<0.01	–	11
Endosulfan Sulfate	<0.03	–	11

CFU = colony-forming units; MPN = most probable number; BHC = hexachlorocyclohexane or benzene hexachloride;
PCB = polychlorinated biphenyl.

^aAll samples were irradiated.

^bFor values less than the limit of detection, the detection limit is given as the mean.

^cSources of contamination: alfalfa, grains, and fish meal.

^dSources of contamination: soy oil and fish meal.

^eAll values were corrected for percent recovery.

Appendix E. Sentinel Animal Program

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E.1. Methods

Rodents used in the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that may affect study results. The Sentinel Animal Program is part of the periodic monitoring of animal health that occurs during the toxicological evaluation of test compounds. Under this program, the disease state of the rodents is monitored via sera or feces from extra (sentinel) or dosed animals in the study rooms. The sentinel animals and the study animals are subject to identical environmental conditions. Furthermore, the sentinel animals come from the same production source and weanling groups as the animals used for the studies of test compounds.

For these toxicity studies, blood samples were collected from each animal and allowed to clot, and the serum was separated. Additionally, fecal samples were collected and tested for *Helicobacter* species. All samples were processed appropriately and evaluated for the presence of pathogens. Samples were tested in-house or sent to Research Animal Diagnostic Laboratory (Columbia, MO). The laboratory methods and agents for which testing was performed are tabulated below; the times at which samples were collected during the studies are also listed.

Blood was collected at the following time points during the 39-week studies:

- Week 3; 5 male and five female extra rats
- Week 16; 5 male control rats
- Week 26; 5 female control rats
- Week 39; 5 male control rats

Table E-1. Blasting Sand

Method and Test	Time of Collection
Multiplex Fluorescent Immunoassay	
Kilham's Rat Virus (KRV)	16, 26, and 39 weeks
<i>Mycoplasma pulmonis</i>	3, 16, 26, and 39 weeks
Parvo NS-1	16, 26, and 39 weeks
Pneumonia Virus of Mice (PVM)	3, 16, 26, and 39 weeks
Rat Coronavirus/Sialodacryoadenitis Virus (RCV/SDA)	3, 16, 26, and 39 weeks
Rat Minute Virus (RMV)	16, 26, and 39 weeks
Rat Parvovirus (RPV)	3, 16, 26, and 39 weeks
Rat Theilovirus (RTV)	16, 26, and 39 weeks
Sendai	3, 16, 26, and 39 weeks
Theiler's Murine Encephalomyelitis Virus-Mouse Poliovirus, Strain GDVII (TMEV GDVII)	16, 26, and 39 weeks
Toolan's H-1	16, 26, and 39 weeks

Table E-2. Specular Hematite

Method and Test	Time of Collection
Multiplex Fluorescent Immunoassay	
KRV	3, 16, 26, and 39 weeks
<i>M. pulmonis</i>	3, 16, 26, and 39 weeks
PVM	3, 16, 26, and 39 weeks
RCV/SDA	3, 16, 26, and 39 weeks
RMV	3, 16, 26, and 39 weeks
RPV	3, 16, 26, and 39 weeks
RTV	3, 16, 26, and 39 weeks
Sendai	3, 16, 26, and 39 weeks
TMEV GDVII	3, 16, 26, and 39 weeks
Toolan's H-1	3, 16, 26, and 39 weeks

E.2. Results

All test results were negative.

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F.1. Methods

Studies evaluating the potential immunotoxicity of blasting sand or specular hematite in core study male and special study female Sprague Dawley rats exposed by inhalation for up to 39 weeks were conducted at Battelle Toxicology Northwest (Richmond, WA). Animals were exposed by whole-body inhalation to blasting sand or specular hematite at concentrations of 0, 15, 30, or 60 mg/m³, 5 days per week for up to 39 weeks. For each compound, groups of eight unimmunized male and eight immunized and eight unimmunized female rats per time point were selected for immunotoxicological evaluations.

Bronchoalveolar lavage (BAL) fluid and blood samples for analyses of antinuclear antibodies were collected from unimmunized male and unimmunized female rats. On the day of study termination (1 day after the last exposure) rats were euthanized by intraperitoneal injection of pentobarbital. The spleens of immunized and unimmunized female rats were aseptically removed from the animals, placed in tubes containing Earle's Balanced Salt Solution (EBSS) with 15 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), supplemented with gentamicin (a bacteriostat), and a "wet" weight was obtained. Tubes containing the whole spleens were placed on crushed ice and shipped overnight to the designated immunotoxicology contract laboratory, Virginia Commonwealth University (VCU) in Richmond, VA. Lavage samples were centrifuged, and the supernatant (i.e., BAL fluid) was removed and frozen at -70°C. Serum was prepared from the whole blood samples and frozen at -70°C. The BAL fluid and serum samples were shipped to VCU on dry ice and stored frozen at -70°C for analysis.

Spleens from immunized and unimmunized female rats were processed for immunotoxicological evaluation. Assessment of immune function in lymphoid tissues up to 24 hours after tissue collection has been shown to produce comparable results to studies conducted on freshly harvested tissues.⁷⁶ Single-cell suspensions were prepared by mashing spleens as previously described.⁷⁷ Spleen cell suspensions from sheep red blood cell (sRBC)-immunized animals were centrifuged and resuspended in 6 mL of EBSS with 15 mM HEPES. Cell suspensions for the unimmunized animals were centrifuged and resuspended in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% fetal bovine serum (FBS).

The primary IgM response to sRBCs was enumerated using a modification of the hemolytic plaque assay as described by White et al.⁷⁸ Rats were immunized with 2×10^8 sRBCs by intravenous injection 4 days before study termination. Single-cell suspensions were prepared from the spleens of immunized rats and resuspended in 6 mL of EBSS. An aliquot of cells was added to the test tube containing guinea pig complement, sRBCs, and warm agar. After thoroughly mixing, the test tube mixture was plated in a petri dish, covered with a microscope slide cover slip, and incubated at 37°C for 3 hours. Cell counts were performed on the 6 mL cell suspensions, and the numbers of cells/spleen, antibody-forming cells (AFCs) /10⁶ spleen cells, and AFCs/spleen were determined.

An enzyme-linked immunosorbent assay (ELISA) system, developed at VCU,⁷⁹ was used to determine serum titers of antigen-specific IgM in blood obtained from the same animals immunized with sRBCs for the AFC assay. One day before the ELISA was conducted, sRBC membrane high-salt release antigens (1 mg/mL) were diluted 1:100 in phosphate buffered saline (PBS) and applied to Immulon[®] 2 microtiter plates (Thermo-Fisher Scientific, Inc., Waltham,

MA) (100 μ L/well) and incubated at 4°C overnight. Before each subsequent step, plates were washed three times with 200 μ L per well per wash of PBS with 0.05% Tween[®] 20 (Thermo-Fisher Scientific, Inc., Waltham, MA) (assay buffer). After the plates were incubated for 60 minutes with assay buffer (175 μ L/well), serum samples diluted with assay buffer were added to wells of the appropriate plates for a final volume of 100 μ L of diluted serum. After 60 minutes of incubation at room temperature, the plates were washed, and the secondary antibody [affinity-purified horseradish peroxidase-conjugated goat anti-rat IgM antibody (Southern BioTech, Birmingham, AL) diluted 1:500 in assay buffer] was added (100 μ L/well) and allowed to incubate for approximately 60 minutes. Plates were subsequently washed, and peroxidase substrate [2,2'-azino-bis(3-ethyl-benzthiazoline-6-sulfonic acid), Sigma-Aldrich, St. Louis, MO] was added (100 μ L/well). The color in each well was read at 405 nm on a Molecular Devices (San Jose, CA) plate reader after a 45-minute incubation period. Results were obtained using SoftMax[®] (v. 2.32, Molecular Devices). The antibody titer was defined to be the reciprocal of the dilution at which the sample absorbance had an optical density (OD) of 0.5 and was obtained by interpolating within the linear portion of the log-log regression curve. If a sample did not reach an OD of 0.5, it was assigned a titer of the starting dilution.

For immunophenotyping studies, single-cell suspensions were prepared from the spleens of unimmunized female rats and resuspended in 6 mL of RPMI 1640 media supplemented with 10% FBS. Red blood cells were removed via ammonium chloride lysis and 1.0×10^6 spleen cells per well were seeded into a 96-well microtiter plate. Individual wells contained 100 μ L of spleen cells and 100 μ L of a surface marker specific monoclonal antibody conjugated to fluorescein isothiocyanate (FITC) or phycoerythrin (PE). The specific monoclonal antibodies used were OX19 conjugated to PE to enumerate T cells (CD5⁺), OX38 conjugated to FITC to enumerate CD4⁺ cells, and OX8 conjugated to FITC to enumerate CD8⁺ cells. For both the CD4⁺ and CD8⁺ cells, a double label with OX19 and OX38 or OX8 was used. OX33 conjugated to FITC was used to enumerate CD45RA⁺ B cells. Splenic natural killer (NK) cells were enumerated using a FITC-conjugated NKR-P1A antibody and OX8 conjugated to PE, and splenic macrophages were enumerated using HIS36 antibody conjugated to PE. The final dilution of each antibody in the well was 1:80.

An appropriate isotype control was run for each of the antibodies used. Following the initial staining with antibody and washing with staining buffer, 100 μ L of propidium iodide (PI) was added to each well to determine viability. Following 5 minutes of incubation with PI, the cells were washed once with staining buffer and then enumerated on a Becton Dickinson FACScan[™] flow cytometer (Beckton, Dickinson and Company San Jose, CA). Five thousand cells were counted for each sample.

T cell proliferation was measured in splenocytes from unimmunized female rats to assess cell-mediated immunity. Flat-bottom 96-well microtiter plates were coated with either rat anti-CD3 monoclonal antibody (diluted to 1 μ g/mL in PBS; 0.1 mL/well) or PBS only (100 μ L/well) and incubated overnight. The plates were then washed with 200 μ L of sterile PBS before the addition of spleen cells. Single-cell suspensions were prepared under aseptic conditions, centrifuged, and resuspended in 6 mL of RPMI 1640 media supplemented with 10% FBS at a concentration of 5×10^6 cells/mL. One hundred μ L of spleen cells and 100 μ L of medium (RPMI 1640 supplemented with 10% FBS and 50 μ M 2-mercaptoethanol) were added to each well. The splenocytes were cultured in both uncoated and anti-CD3 coated wells in quadruplicate for

3 days. Eighteen to 24 hours before harvest on day 3, 1 μCi of ^3H -thymidine was added to each well. Cells were harvested onto Wallac filtermats (Perkin Elmer, Waltham, MA) using a Harvester 96 Mach IIIM cell harvester (Tomtec, Hamden, CT) and counted using a 1450 Microbeta[®] Trilux Liquid Scintillation and Luminescence Counter (Perkin Elmer). The incorporation of ^3H -thymidine into DNA was used as a measure of cell proliferation, and the data were expressed as counts per minute (CPM)/ 5×10^5 cells.

NK cell activity was evaluated in spleen cells from unimmunized female rats as a measure of innate immune function. Spleen cells were prepared under aseptic conditions, resuspended in 6 mL of RPMI 1640 medium supplemented with 10% FBS, and adjusted to six concentrations, 2×10^7 , 10^7 , 5×10^6 , 2.5×10^6 , 1.25×10^6 and 0.625×10^6 cells/mL. YAC-1 murine lymphoma cells, maintained in a stock culture at VCU, were used as the target cells in this assay. Cultured YAC-1 cells were harvested, centrifuged, and resuspended in RPMI 1640 medium supplemented with 10% FBS at a concentration of 10^7 cells/mL. YAC-1 cells were radiolabeled by incubation with 200 μCi of ^{51}Cr for 90 minutes at 37°C . Following the incubation, the cells were washed three times in RPMI 1640 with 10% FBS, counted, and adjusted to 10^5 nucleated cells/mL. The target cells were added to each well in a volume of 0.1 mL (10^5 YAC-1 cells/mL). Spleen cells were added in a volume of 0.1 mL to each of two replicate wells of target cells at each effector concentration to obtain effector-to-target (E:T) ratios of 200:1, 100:1, 50:1, 25:1, 12.5:1, and 6.25:1. Maximum release of ^{51}Cr was determined by adding 0.1 mL of the labeled YAC-1 cells and 0.1 mL 0.1% Triton X-100 to each of 12 replicate wells. Spontaneous release was determined by adding 0.1 mL of medium to each of 12 replicate cultures containing the targets. The plates were incubated for 4 hours at 37°C under 5% CO_2 . Following the incubation, the plates were centrifuged at $250 \times g$ for 10 minutes, and 0.1 mL of the supernatant was removed from each well and counted in a Wallac 1480 Wizard[®] 3" gamma counter Perkin Elmer. The mean percent cytotoxicity at each effector concentration was determined for each exposed group and compared to the values for the chamber control group. The results are expressed as percentage of cytotoxicity as follows:

$$\% \text{ Cytotoxicity} = (\text{CPM}_{\text{exp}} - \text{CPM}_{\text{spon}}) / (\text{CPM}_{\text{total}} - \text{CPM}_{\text{spon}}) \times 100,$$

where CPM_{exp} = counts per minute in experimental wells, CPM_{spon} = spontaneous release, and $\text{CPM}_{\text{total}}$ = total release upon addition of 0.1% Triton X-100.

BAL fluid received at VCU was stored frozen at -70°C until analysis. Samples were thawed overnight in a refrigerator at 2° to 4°C . Cytokine levels were determined on a single 25 μL aliquot of each sample of BAL fluid using FlowCytomix[™] bead array kits (eBioscience, Inc., San Diego, CA) according to the manufacturer's instructions. Cytokines evaluated were: interleukin (IL)- 1α , monocyte chemotactic protein (MCP)-1, tumor necrosis factor (TNF)- α , granulocyte-macrophage colony stimulating factor (GM-CSF), IL-4, and interferon (IFN)- γ . Cytokine concentrations were determined by interpolation against standard curves generated for each cytokine using standards included in the FlowCytomix[™] bead array kits. A value of 0 was assigned to serum samples that had levels below the limit of detection for any given cytokine.

An ELISA was used to analyze serum for autoantibodies against nuclear antigens. The ELISA was conducted using a modification of commercially available human antinuclear antibody (ANA) ELISA kits (INOVA Diagnostics, Inc., San Diego, CA). Modifications included the use of affinity-purified horseradish peroxidase-conjugated goat anti-rat heavy- and light-chain

specific immunoglobulin [Ig(H+L)] (Southern BioTech), diluted 1:1000 in PBS as the secondary antibody, and 3,3',5,5'-tetramethylbenzidine (BD Biosciences, San Jose, CA) as the substrate. ANA-positive serum (for use as a positive control on each plate) was generated in female Brown Norway rats following exposure to 1 mg/kg mercuric chloride by subcutaneous injection three times per week for 2 weeks. According to the manufacturer, the antigens bound to the surface of the wells of the precoated plates included chromatin (dsDNA and histones), Sm/RNP, SS-A, SS-B, Scl-70, centromere, PCNA, Jo-1, mitochondria (M-2), ribosomal-P protein, and highly purified extracts from HEp-2 nuclei and nucleoli.

Serum samples were diluted 1:50 in sample diluent and added in a volume of 100 μ L per well to the precoated plates. Following 30 minutes of incubation at room temperature, the plates were washed three times, and secondary antibody was added to all wells in a volume of 100 μ L/well. After 30 minutes, the plates were washed four times, and 100 μ L of substrate was added to each well. Five minutes later, stop solution (2 N sulfuric acid) was added in a volume of 100 μ L/well. The OD of each well was read at 450 nm on a Molecular Devices plate reader within 30 minutes of the addition of the stop solution. Results were obtained using SoftMax v. 2.32. The mean OD of the background wells (wells containing no sample) was subtracted from the OD obtained for each sample and positive control to obtain corrected OD values. For each time point (4-, 16-, 26-, and 39-week samples), the mean and standard deviation (SD) of the corrected OD values for the chamber control animals was determined. The data were then analyzed to determine whether any sample fell outside of the 99% confidence interval of the mean for the chamber control group. Per the manufacturer's instructions, if a sample had a corrected OD greater than the mean + 3 \times the SD, the sample was classified as "positive," indicating that the sample had a significantly greater level of ANAs than that of the chamber control group. Samples within the limits of the 99% confidence interval were classified as "negative," which indicated that the ANA response of that sample was not significantly different from that of the appropriate chamber control group. Positive control serum was run on each plate to demonstrate the assay was capable of detecting serum ANAs. Results are presented as incidence of ANA-positive samples and as the percentage of positive responders.

F.2. Results

F.2.1. Blasting Sand

The results of the assay measuring the IgM AFC response to sRBCs (T-dependent antigen) using spleen cells from immunized female rats are shown in Table F-1 and Table F-2 at weeks 5 and 27, respectively. No effects were observed on total spleen cell numbers or on the AFC response of female rats following exposure to blasting sand at either time point. Similarly, blasting sand exposure did not result in any differences in serum anti-sRBC IgM antibody titers at either week 5 or week 27 (Table F-3 and Table F-4).

Spleen weights were unaffected in immunized female rats that were exposed to blasting sand for 27 weeks (Table F-2) and unimmunized female rats exposed to blasting sand for 26 weeks (Table F-12). Total spleen cell numbers in unimmunized females were unaffected following 4 weeks of blasting sand exposure (Table F-5). However, after 26 weeks, total spleen cell numbers were significantly lower (27%) in the 60 mg/m³ group of unimmunized females (Table F-7).

Phenotypic analysis was conducted to evaluate multiple spleen cell populations, including: B cells (OX33⁺), T cells (CD5⁺), T_H cells (CD4⁺CD5⁺), T_{CTL} cells (CD8⁺CD5⁺), NK cells (NK⁺CD8⁺), and macrophages (His36⁺). In unimmunized female rats exposed to blasting sand for 4 weeks, the absolute numbers of B cells, T cells, T_{CTL} cells, NK cells, and macrophages were all unaffected, although a positive trend occurred in absolute T cell numbers (Table F-5). T_H cells were increased 19% and 37% at the 15 and 60 mg/m³ exposure levels, respectively. When evaluated as percent values (Table F-6), significant increases occurred in T cells (22%) and T_H cells (32%) at the 30 mg/m³ exposure level. In addition, T_H cells were significantly increased in the 60 mg/m³ group. The percentages of B cells, T_{CTL} cells, NK cells, and macrophages were unaffected, although a negative trend occurred in the percentage of B cells.

Following exposure of unimmunized female rats to blasting sand for 26 weeks, absolute numbers of B cells were lower, in an exposure concentration-related manner, at all exposure levels (Table F-7). In addition, absolute NK cell and macrophage numbers were significantly lower in the 60 mg/m³ group. Negative trends occurred in all cell populations with the exception of T_H cells, when evaluated as absolute values. When evaluated in terms of percent values at both 30 and 60 mg/m³, B cells were significantly decreased and T_H cells were significantly increased (Table F-8). No other significant differences were observed.

As a measure of the effects of blasting sand exposure on cell-mediated immunity, the anti-CD3 antibody-mediated proliferative response was evaluated in unimmunized female rats. After 4 weeks of exposure, no significant effects were observed on either unstimulated or anti-CD3-stimulated proliferation (Table F-9). In contrast, the anti-CD3-stimulated proliferative response of spleen cells from female rats exposed to 60 mg/m³ blasting sand for 26 weeks was significantly increased (68%; Table F-10).

NK cell activity was assessed in unimmunized female rats using six Effector:Target (E:T) ratios in a 4-hour ⁵¹Cr-release assay, and the results were expressed in terms of percent cytotoxicity. No significant differences occurred NK cell activity between the exposed and chamber control groups at any E:T ratio following exposure to blasting sand for either 4 weeks (Table F-11) or 26 weeks (Table F-12).

Cytokine levels in the BAL fluid of unimmunized male and female rats exposed to chamber control air or blasting sand were evaluated in a single 25 µL sample using a commercially available cytometric bead array kit, as described in the methods section. The six cytokines evaluated were: IL-1α, MCP-1, TNF-α, IFN-γ, GM-CSF, and IL-4. IFN-γ was not detected in any of the samples at any time point (Table F-13 and Table F-14). After 4 weeks of exposure to blasting sand, levels of IL-1α, TNF-α, and IL-4 were significantly increased in the 15 mg/m³ group of female rats (Table F-13). MCP-1 and GM-CSF levels were not affected at this time point (Table F-3 and Table F-14). In female rats exposed to blasting sand for 26 weeks, MCP-1 levels were significantly increased in an exposure concentration-dependent manner. MCP-1 was not detected in BAL fluid from any of the chamber control animals, therefore percent increases for this cytokine could not be calculated. GM-CSF and IL-4 levels were each significantly decreased in the 30 and 60 mg/m³ groups, whereas TNF-α was significantly decreased only in the 60 mg/m³ group. IL-1 levels were unaffected. In male rats exposed to 60 mg/m³ blasting sand for 4 weeks, MCP-1 levels were significantly increased (441%) compared to the chamber control group (Table F-14). No other significant differences between the exposed and chamber control groups occurred at this time point. MCP-1 levels were significantly increased in all groups of

male rats exposed to blasting sand at all of the later time points evaluated (16, 26, and 39 weeks; Table F-14 and Figure F-1).

Following exposure to chamber control air or blasting sand for 4 or 26 weeks, serum from unimmunized female rats was analyzed for the presence of autoantibodies. After 4 weeks of exposure, serum ANA levels of one female rat in the 15 mg/m³ group and three female rats in the 60 mg/m³ group were greater than the upper limit of the 99% confidence interval of the chamber control group mean (Table F-15). No female rats in the 30 mg/m³ group had ANA levels exceeding that upper limit. Statistical analysis of these incidences using the Fisher exact chi-square test indicated that none of the groups exposed to blasting sand were significantly different from the chamber control group at this time point. At the 26-week time point, only one female rat in the 15 mg/m³ group had serum ANA levels outside the 99% confidence interval of the chamber control group mean. No other animals exceeded the upper confidence limit. The Fisher exact chi-square test indicated no significant differences in incidence between groups exposed to blasting sand and the chamber control group at this time point. Serum from unimmunized male rats exposed to chamber control air or blasting sand for 4, 16, 26, or 39 weeks was also analyzed for autoantibody levels. After 26 weeks of exposure, one, two, and three male rats in the 15, 30, and 60 mg/m³ groups, respectively, had serum ANA levels outside the upper limit of the 99% confidence interval of the chamber control group mean (Table F-15). Statistical analysis of these incidences using the Fisher exact chi-square test indicated that none of the male groups exposed to blasting sand were significantly different from the chamber control group at this time point. No other male rats in any of the exposed groups at any other time point had serum ANA levels outside the 99% confidence intervals of the chamber control group means.

F.2.2. Specular Hematite

The results of the assay measuring the IgM AFC response to sRBCs (T-dependent antigen) using spleen cells from immunized female rats are shown in Table F-16 and Table F-17 at weeks 5 and 27, respectively. No effects were observed on spleen weights, total spleen cell numbers, or the AFC response in immunized female rats following exposure to specular hematite at either time point. Exposure to specular hematite also did not affect the serum anti-sRBC IgM antibody titers at either week 5 (Table F-18) or week 27 (Table F-19).

The effects of specular hematite exposure on spleen weights in female rats that were not immunized with sRBCs are shown in Table F-26 and Table F-27; no significant differences were observed between the specular hematite-exposed groups and the chamber control groups at either weeks 4 or 26.

Phenotypic analysis of spleen cells from unimmunized female rats was conducted for multiple cell populations, including: B cells (OX33+), T cells (CD5+), TH cells (CD4+CD5+), TCTL cells (CD8+CD5+), NK cells (NK+CD8+), and macrophages (His36+). Total spleen cell numbers were unaffected by specular hematite exposure for 4 or 26 weeks, as shown in Table F-20 and Table F-22, respectively. Following exposure to specular hematite for 4 weeks, no effects were observed on the absolute values of the various spleen cell phenotypes (Table F-20). When evaluated as percent values, a significant decrease (11%) in the percentage of B cells occurred in female rats exposed to 15 mg/m³ specular hematite for 4 weeks (Table F-21); no effects on percent values of any other cell population were observed at week 4.

No effects were observed on the absolute numbers of B cells, T cells, TH cells, TCTL cells, NK cells, or macrophages in unimmunized female rats exposed to specular hematite for 26 weeks (Table F-22). When evaluated as percent values, B cells, TH cells, NK cells, and macrophages were all unaffected (Table F-23). Significant decreases of 20% and 29% occurred in the percentages of T cells and TCTL cells in female rats exposed to 30 mg/m³ specular hematite, and significant negative trends were observed for each of these cell populations. However, no significant changes were observed at the higher exposure concentration of 60 mg/m³.

The anti-CD3 antibody-stimulated proliferative response was evaluated in unimmunized female rats following 4 and 26 weeks of exposure to specular hematite. At 4 weeks, significant increases in the basal (unstimulated) proliferative response occurred in rats exposed to 15 or 30 mg/m³ specular hematite (Table F-24). No effects were observed on the unstimulated response in the 60 mg/m³ group. No significant effects were observed on anti-CD3-stimulated proliferation in any of the groups exposed to specular hematite for 4 weeks. Exposure to specular hematite for 26 weeks did not affect basal or anti-CD3-stimulated proliferation in cells obtained from the spleens of exposed rats (Table F-25).

Following exposure to specular hematite for 4 weeks, NK cell activity in the spleen of unimmunized female rats was unaffected at all E:T ratios examined (Table F-26). In unimmunized female rats exposed to specular hematite for 26 weeks, significant increases in NK cell activity occurred at the 50:1 and the 25:1 E:T ratios for the 30 and 60 mg/m³ exposure groups, respectively (Table F-27). No other significant effects were observed, although positive trends occurred at all E:T ratios in rats exposed to specular hematite for 26 weeks.

Two of eight unimmunized female rats exposed to 60 mg/m³ specular hematite for 4 weeks had detectable levels of IFN- γ in their BAL fluid (Table F-28). However, there were no statistically significant changes in any of the six cytokines measured in female rats exposed for 4 weeks. After 26 weeks of exposure to specular hematite, MCP-1 levels were significantly increased in BAL fluid from 30 and 60 mg/m³ female rats. In the BAL fluid of unimmunized male rats exposed to specular hematite for 4 weeks, no significant changes occurred in any of the six cytokines measured (Table F-29). However, exposure concentration-related increases in MCP-1 levels did occur following 16 weeks of exposure, and the increases were significant in the 30 and 60 mg/m³ groups (Table F-29 and Figure F-2). Exposure to specular hematite for 26 weeks also resulted in exposure concentration-dependent increases in MCP-1 levels in male rats, and the increases were statistically significant at all exposure levels. Exposure to specular hematite for 39 weeks resulted in significant increases in MCP-1 levels in BAL fluid of male rats exposed to 30 or 60 mg/m³. Levels of all other cytokines evaluated in male rats were not detectable or were not significantly different from those in the chamber control animals at the 16-, 26-, or 39-week time points (Table F-29).

After 4 weeks of exposure to specular hematite, one unimmunized 15 mg/m³ female rat had a serum ANA level outside the upper limit of the 99% confidence interval for the chamber control group mean (Table F-30). No other female rats in any exposed group had serum ANA levels outside the upper limit of the 99% confidence interval of the chamber control group mean after 4 weeks of exposure. In unimmunized female rats exposed to specular hematite for 26 weeks, two animals in the 30 mg/m³ group had serum ANA levels greater than the 99% confidence interval upper limit for the chamber control group. One 15 mg/m³ and one 60 mg/m³ unimmunized male rat exposed to specular hematite for 4 and 39 weeks, respectively, had serum

ANA levels outside the 99% confidence interval upper limits for the chamber control groups. The Fisher exact chi-square test indicated that none of the incidences of ANA-positive responses in groups exposed to specular hematite were significantly different from those in the chamber control groups at any of the time points examined for male or female rats.

F.3. Conclusions

Exposure to blasting sand by inhalation for up to 27 weeks produced minimal immunotoxic effects in female Sprague Dawley rats. The functional responses of humoral and innate immunity were unaffected. Total spleen cell numbers were lower, whereas anti-CD3-mediated proliferation was increased in female rats exposed to 60 mg/m³ blasting sand for 26 weeks. Levels of MCP-1 were increased in an exposure concentration-dependent manner in the BAL fluid of both male and female Sprague Dawley rats exposed to blasting sand, beginning at week 4 in males and at week 26 in females. Antinuclear autoantibody levels in serum from male and female Sprague Dawley rats were not affected by exposure to blasting sand.

Similarly, exposure to specular hematite by inhalation for up to 27 weeks in female Sprague Dawley rats and up to 39 weeks in male Sprague Dawley rats produced minimal effects on the immune measures examined. The functional responses of humoral, cell-mediated and innate immunity were generally unaffected in female Sprague Dawley rats exposed to specular hematite. MCP-1 was the only cytokine in the BAL fluid that was modulated by exposure to specular hematite. Levels of MCP-1 were significantly increased in rats exposed to specular hematite, beginning at week 16 in males and at week 26 in females. Exposure to specular hematite did not significantly affect serum levels of antinuclear autoantibodies in male or female Sprague Dawley rats.

Table F-1. Spleen IgM Antibody-Forming Cell Response to the T-Dependent Antigen Sheep Erythrocytes in Female Sprague Dawley Rats Exposed to Blasting Sand for Five Weeks (Day 4 Response)

	Total Spleen Cells ($\times 10^7$)	IgM AFC/ 10^6 Spleen Cells	IgM AFC/Spleen ($\times 10^3$)
n	8	8	8
Chamber Control	88.42 \pm 6.79	623 \pm 116	532 \pm 103
Blasting Sand			
15 mg/m ³	87.65 \pm 4.10	960 \pm 260	875 \pm 250
30 mg/m ³	93.95 \pm 4.99	811 \pm 149	762 \pm 148
60 mg/m ³	91.55 \pm 3.28	1,033 \pm 211	969 \pm 222
H/NH	H	H	H
Trend Analysis	NS	NS	NS

Female Sprague Dawley rats were exposed to chamber control air or blasting sand by inhalation. Four days before euthanasia, the rats were immunized with 2×10^8 sheep red blood cells (sRBCs). On the day of euthanasia, spleens were placed into tubes containing medium and sent to Virginia Commonwealth University (Richmond, VA) on ice for next-day cell preparation. Spleens were prepared into single-cell suspensions and the number of IgM sRBC antibody-forming cells (AFCs) was determined. Values represent the mean \pm standard error derived from the number of animals indicated by n.

H = homogeneous data and NH = nonhomogeneous data using Bartlett's test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance; differences from the chamber control group are not significant by Dunnett's t-test. Jonckheere's test was used to assess the significance of exposure concentration-related trends.

NS = not significant.

Table F-2. Spleen IgM Antibody-Forming Cell Response to the T-Dependent Antigen Sheep Erythrocytes in Female Sprague Dawley Rats Exposed to Blasting Sand for 27 Weeks (Day 4 Response)

	Spleen Weight (mg)	Total Spleen Cells ($\times 10^7$)	IgM AFC/ 10^6 Spleen Cells	IgM AFC/Spleen ($\times 10^3$)
n	8	8	8	8
Chamber Control	758 \pm 45	87.50 \pm 5.08	1,093 \pm 403	1,006 \pm 404
Blasting Sand				
15 mg/m ³	729 \pm 45	76.44 \pm 4.14	1,056 \pm 417	832 \pm 346
30 mg/m ³	789 \pm 54	78.32 \pm 4.28	1,555 \pm 571	1,212 \pm 449
60 mg/m ³	790 \pm 82	78.06 \pm 5.14	688 \pm 178	578 \pm 195
H/NH	H	H	H	H
Trend Analysis	NS	NS	NS	NS

Female Sprague Dawley rats were exposed to chamber control air or blasting sand by inhalation. Four days before euthanasia, the rats were immunized with 2×10^8 sheep red blood cells (sRBCs). On the day of euthanasia, spleens were placed into tubes containing medium and sent to Virginia Commonwealth University (Richmond, VA) on ice for next-day cell preparation. Spleens were prepared into single-cell suspensions and the number of IgM sRBC antibody-forming cells (AFCs) was determined. Values represent the mean \pm standard error derived from the number of animals indicated by n.

H = homogeneous data and NH = nonhomogeneous data using Bartlett's test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance; differences from the chamber control group are not significant by Dunnett's t-test. Jonckheere's test was used to assess the significance of exposure concentration-related trends.

NS = not significant.

Table F-3. Serum IgM Antibody Titers to the T-Dependent Antigen Sheep Erythrocytes in Female Sprague Dawley Rats Exposed to Blasting Sand for Five Weeks

	Number of Animals	Serum Titer Log ₂
Chamber Control	8	6.168 \pm 0.317
Blasting Sand		
15 mg/m ³	8	6.609 \pm 0.388
30 mg/m ³	8	6.272 \pm 0.618
60 mg/m ³	8	6.343 \pm 0.529
H/NH	–	H
Trend Analysis	–	NS

Female Sprague Dawley rats were exposed to chamber control air or blasting sand by inhalation. On the day of euthanasia, serum was obtained via cardiac puncture and frozen. Serum samples were shipped to Virginia Commonwealth University (Richmond, VA) on dry ice for analysis. Values represent the mean \pm standard error derived from the indicated number of animals.

H = homogeneous data and NH = nonhomogeneous data using Bartlett's test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance; differences from the chamber control group are not significant by Dunnett's t-test. Jonckheere's test was used to assess the significance of exposure concentration-related trends.

NS = not significant.

Table F-4. Serum IgM Antibody Titers to the T-Dependent Antigen Sheep Erythrocytes in Female Sprague Dawley Rats Exposed to Blasting Sand for 27 Weeks

	Number of Animals	Serum Titer Log ₂
Chamber Control	8	7.544 ± 0.529
Blasting Sand		
15 mg/m ³	8	7.524 ± 0.817
30 mg/m ³	8	6.298 ± 0.435
60 mg/m ³	8	6.659 ± 0.625
H/HN	–	H
Trend Analysis	–	NS

Female Sprague Dawley rats were exposed to chamber control air or blasting sand by inhalation. On the day of euthanasia, serum was obtained via cardiac puncture and frozen. Serum samples were shipped to Virginia Commonwealth University (Richmond, VA) on dry ice for analysis. Values represent the mean ± standard error derived from the indicated number of animals. H = homogeneous data and NH = nonhomogeneous data using Bartlett's test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance; differences from the chamber control group are not significant by Dunnett's t-test. Jonckheere's test was used to assess the significance of exposure concentration-related trends. NS = not significant.

Table F-5. Splenocyte Surface Marker Differential in Female Sprague Dawley Rats Exposed to Blasting Sand for Four Weeks (Absolute Values)

	Total Spleen Cells (×10 ⁷)	OX33 ^a	CD5 ^b	CD4 ^c CD5 ^c	CD8 ^d CD5 ^d	NK ^e CD8 ^e	HIS36 ^f
n	8	8	8	8	8	8	8
Chamber Control	79.56 ± 1.25	450.7 ± 8.6	161.1 ± 7.3	83.8 ± 3.9	69.5 ± 3.8	29.1 ± 1.8	34.2 ± 3.6
Blasting Sand							
15 mg/m ³	79.09 ± 3.45	449.9 ± 22.6	179.0 ± 9.5	99.7 ± 7.0*	76.9 ± 5.0	24.2 ± 1.8	33.2 ± 2.9
30 mg/m ³	76.41 ± 4.55	435.8 ± 29.5	186.9 ± 10.5	106.2 ± 9.1	71.6 ± 5.7	26.9 ± 2.5	37.0 ± 4.8
60 mg/m ³	80.70 ± 4.08	432.8 ± 26.4	193.1 ± 14.9	115.0 ± 13.9*	76.7 ± 4.4	27.5 ± 2.6 ^g	37.7 ± 3.8
H/NH	NH	NH	H	NH	H	H	H
Trend Analysis	NS	NS	p ≤ 0.05	p ≤ 0.01	NS	NS	NS

*Significantly different (p ≤ 0.05) from the chamber control group by Wilcoxon's rank test.

Female Sprague Dawley rats were exposed to chamber control air or blasting sand by inhalation. On the day of euthanasia, spleens were placed into tubes containing medium and sent to Virginia Commonwealth University (Richmond, VA) on ice for next-day cell preparation. B cells, T cells, T-subsets, NK cells, and macrophages were enumerated. Surface marker values are expressed as the absolute number per spleen × 10⁶ and represented as the mean ± standard error derived from the number of animals indicated by n.

H = homogeneous data and NH = nonhomogeneous data using Bartlett's test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance; differences from the chamber control group are not significant by Dunnett's t-test. Nonhomogeneous data were evaluated using a nonparametric analysis of variance. When significant differences occurred, exposed groups were compared to the chamber control group using Wilcoxon's rank test. Jonckheere's test was used to assess the significance of exposure concentration-related trends.

NS = not significant.

^aB cell.

^bT cell.

^cHelper/DTH – T cell.

^dCytotoxic T cell.

^eNatural killer cell.

^fMacrophage.

^gn = 7; no sample aliquot was available to analyze the NK cell marker for one animal due to a technical error.

Table F-6. Splenocyte Surface Marker Differential in Female Sprague Dawley Rats Exposed to Blasting Sand for Four Weeks (Percent Values)

	OX33 ^{+a}	CD5 ^{+b}	CD4 ⁺ CD5 ^{+c}	CD8 ⁺ CD5 ^{+d}	NK ⁺ CD8 ^{+e}	HIS36 ^{+f}
n	8	8	8	8	8	8
Chamber Control	56.7 ± 1.2	20.3 ± 0.9	10.5 ± 0.5	8.7 ± 0.5	3.7 ± 0.2	4.3 ± 0.4
Blasting Sand						
15 mg/m ³	56.8 ± 0.8	22.8 ± 1.1	12.6 ± 0.7	9.8 ± 0.7	3.1 ± 0.2	4.2 ± 0.4
30 mg/m ³	56.9 ± 1.3	24.8 ± 1.3*	13.9 ± 0.9*	9.6 ± 0.8	3.5 ± 0.2	4.9 ± 0.7
60 mg/m ³	53.6 ± 1.2	23.8 ± 1.0	14.0 ± 1.0*	9.6 ± 0.5	3.4 ± 0.3 ^g	4.7 ± 0.4
H/NH	H	H	H	H	H	H
Trend Analysis	p ≤ 0.05	p ≤ 0.01	p ≤ 0.01	NS	NS	NS

*Significantly different ($p \leq 0.05$) from the chamber control group by Dunnett's t-test.

Female Sprague Dawley rats were exposed to chamber control air or blasting sand by inhalation. On the day of euthanasia, spleens were placed into tubes containing medium and sent to Virginia Commonwealth University (Richmond, VA) on ice for next-day cell preparation. B cells, T cells, T-subsets, NK cells, and macrophages were enumerated. Values are expressed as percent of total spleen cells counted for each animal (Table F-5) and represented as the mean ± standard error derived from the number of animals indicated by n.

H = homogeneous data and NH = nonhomogeneous data using Bartlett's test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the chamber control group using Dunnett's t-test. Jonckheere's test was used to assess the significance of exposure concentration-related trends.

NS = not significant.

^aB cell.

^bT cell.

^cHelper/DTH – T cell.

^dCytotoxic T cell.

^eNatural killer cell.

^fMacrophage.

^gn = 7; there was no sample aliquot available to analyze the NK marker for one animal due to a technical error.

Table F-7. Splenocyte Surface Marker Differential in Female Sprague Dawley Rats Exposed to Blasting Sand for 26 Weeks (Absolute Values)

	Total Spleen Cells ($\times 10^7$)	OX33 ^a	CD5 ^{+b}	CD4 ⁺ CD5 ^{+c}	CD8 ⁺ CD5 ^{+d}	NK ⁺ CD8 ^{+e}	HIS36 ^f
n	8	8	8	8	8	8	8
Chamber Control	76.88 \pm 3.64	434.2 \pm 24.8	148.6 \pm 12.1	73.6 \pm 5.4	53.4 \pm 5.4	26.6 \pm 1.9	46.1 \pm 6.0
Blasting Sand							
15 mg/m ³	64.32 \pm 4.74	345.7 \pm 26.2*	123.6 \pm 12.3	63.9 \pm 5.4	50.8 \pm 6.7	22.9 \pm 2.4	47.3 \pm 6.2
30 mg/m ³	63.31 \pm 4.01	318.3 \pm 21.8**	129.8 \pm 10.3	79.7 \pm 7.3	44.0 \pm 3.4	21.3 \pm 1.8	41.3 \pm 5.2
60 mg/m ³	55.79 \pm 3.08**	275.5 \pm 15.3**	111.1 \pm 3.4	65.5 \pm 3.6	37.8 \pm 2.1	16.1 \pm 1.7**	24.8 \pm 2.0*
H/NH	H	H	NH	H	NH	H	H
Trend Analysis	p \leq 0.01	p \leq 0.01	p \leq 0.05	NS	p \leq 0.01	p \leq 0.01	p \leq 0.01

*Significantly different (p \leq 0.05) from the chamber control group by Dunnett's t-test.

**p \leq 0.01.

Female Sprague Dawley rats were exposed to chamber control air or blasting sand by inhalation. On the day of euthanasia, spleens were placed into tubes containing medium and sent to Virginia Commonwealth University (Richmond, VA) on ice for next-day cell preparation. B cells, T cells, T-subsets, NK cells, and macrophages were enumerated. Surface marker values are expressed as the absolute number per spleen $\times 10^6$ and represented as the mean \pm standard error derived from the number of animals indicated by n.

H = homogeneous data and NH = nonhomogeneous data using Bartlett's test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the chamber control group using Dunnett's t-test. Nonhomogeneous data were evaluated using a nonparametric analysis of variance; differences from the chamber control group are not significant by Wilcoxon's rank test. Jonckheere's test was used to assess the significance of exposure concentration-related trends.

NS = not significant.

^aB cell.

^bT cell.

^cHelper/DTH – T cell.

^dCytotoxic T cell.

^eNatural killer cell.

^fMacrophage.

Table F-8. Splenocyte Surface Marker Differential in Female Sprague Dawley Rats Exposed to Blasting Sand for 26 Weeks (Percent Values)

	OX33 ^a	CD5 ^b	CD4 ^c CD5 ^c	CD8 ^d CD5 ^d	NK ^e CD8 ^e	HIS36 ^f
n	8	8	8	8	8	8
Chamber Control	56.4 ± 1.4	19.3 ± 1.3	9.6 ± 0.5	7.0 ± 0.7	3.5 ± 0.2	6.1 ± 0.8
Blasting Sand						
15 mg/m ³	53.7 ± 1.0	19.1 ± 1.0	9.9 ± 0.4	7.8 ± 0.8	3.6 ± 0.4	7.4 ± 0.9
30 mg/m ³	50.3 ± 1.4**	20.6 ± 1.1	12.5 ± 0.7**	7.1 ± 0.6	3.3 ± 0.1	6.7 ± 0.9
60 mg/m ³	49.4 ± 0.7**	20.2 ± 0.9	11.9 ± 0.7*	6.9 ± 0.4	2.9 ± 0.2	4.5 ± 0.5
H/NH	H	H	H	H	NH	H
Trend Analysis	p ≤ 0.01	NS	p ≤ 0.01	NS	p ≤ 0.05	NS

*Significantly different (p ≤ 0.05) from the chamber control group by Dunnett's t-test.

**p ≤ 0.01.

Female Sprague Dawley rats were exposed to chamber control air or blasting sand by inhalation. On the day of euthanasia, spleens were placed into tubes containing medium and sent to Virginia Commonwealth University (Richmond, VA) on ice for next-day cell preparation. B cells, T cells, T-subsets, NK cells, and macrophages were enumerated. Values are expressed as percent of total spleen cells counted for each animal (Table F-7) and represented as the mean ± standard error derived from the number of animals indicated by n.

H = homogeneous data and NH = nonhomogeneous data using Bartlett's test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the chamber control group using Dunnett's t-test. Nonhomogeneous data were evaluated using a nonparametric analysis of variance; differences from the chamber control group are not significant by Wilcoxon's rank test. Jonckheere's test was used to assess the significance of exposure concentration-related trends.

NS = not significant.

^aB cell.

^bT cell.

^cHelper/DTH – T cell.

^dCytotoxic T cell.

^eNatural killer cell.

^fMacrophage.

Table F-9. Spleen Cell Proliferative Response to Anti-CD3 Stimulation in Female Sprague Dawley Rats Exposed to Blasting Sand for Four Weeks

	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³	H/NH	Trend Analysis
n	8	8	8	8	–	–
Spleen Cell Cultures						
Unstimulated	7,252 ± 870	6,958 ± 853	7,097 ± 1,099	12,667 ± 3,384	NH	NS
Stimulated	74,323 ± 9,547	78,623 ± 8,681	82,460 ± 13,382	80,696 ± 6,730	H	NS
Total Spleen Cells × 10 ⁷	79.56 ± 1.25	79.09 ± 3.45	76.41 ± 4.55	80.70 ± 4.08	NH	NS

Female Sprague Dawley rats were exposed to chamber control air or blasting sand by inhalation. On the day of euthanasia, spleens were placed into tubes containing medium and sent to Virginia Commonwealth University (Richmond, VA) on ice for next-day cell preparation. Spleens were prepared into single-cell suspensions and incubated in flat-bottom microtiter plates. The medium for the proliferative assay was RPMI supplemented with 10% FBS and 50 μM 2-mercaptoethanol. The spleen cells were cultured in either nontreated or treated anti-CD3 wells. Prior to harvest, the cells were pulsed with ³H-thymidine for 18 to 24 hours. The cultured cell data are presented as mean ± standard error derived from four replicate cultures from the number of animals indicated by n.

H = homogeneous data and NH = nonhomogeneous data using Bartlett's test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. Nonhomogeneous data were evaluated using a nonparametric analysis of variance. Differences from the chamber control group are not significant by Dunnett's t-test (homogeneous data) or Wilcoxon's rank test (nonhomogeneous data). Jonckheere's test was used to assess the significance of exposure concentration-related trends. NS = not significant.

Table F-10. Spleen Cell Proliferative Response to Anti-CD3 Stimulation in Female Sprague Dawley Rats Exposed to Blasting Sand for 26 Weeks

	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³	H/NH	Trend Analysis
n	8	8	8	8	–	
Spleen Cell Cultures						
Unstimulated	8,536 ± 868	8,701 ± 999	8,819 ± 1,529	8,934 ± 521	H	NS
Stimulated	167,122 ± 21,667	195,523 ± 27,959	243,652 ± 31,761	281,322 ± 32,010*	H	p ≤ 0.01
Total Spleen Cells × 10 ⁷	76.88 ± 3.64	64.32 ± 4.74	63.31 ± 4.01	55.79 ± 3.08**	H	p ≤ 0.01

*Significantly different (p ≤ 0.05) from the chamber control group by the Dunnett test.

**p ≤ 0.01.

Female Sprague Dawley rats were exposed to chamber control air or blasting sand by inhalation. On the day of euthanasia, spleens were placed into tubes containing medium and sent to Virginia Commonwealth University (Richmond, VA) on ice for next-day cell preparation. Spleens were prepared into single-cell suspensions and incubated in flat-bottom microtiter plates. The medium for the proliferative assay was RPMI supplemented with 10% FBS and 50 μM 2-mercaptoethanol. The spleen cells were cultured in either nontreated or treated anti-CD3 wells. Prior to harvest, the cells were pulsed with ³H-thymidine for 18 to 24 hours. The cultured cell data are presented as mean ± standard error CPM cells derived from four replicate cultures from the number of animals indicated by n.

H = homogeneous data and NH = nonhomogeneous data using Bartlett's test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the chamber control group using Dunnett's t-test. Jonckheere's test was used to assess the significance of exposure concentration-related trends.

NS = not significant.

Table F-11. Natural Killer Cell Activity in Female Sprague Dawley Rats Exposed to Blasting Sand for Four Weeks

	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³	H/NH	Trend Analysis
n	8	8	8	8	–	–
Effector:Target Ratio						
200:1	43.3 ± 3.1	34.9 ± 3.6	35.3 ± 5.3	40.4 ± 3.1	H	NS
100:1	27.3 ± 2.6	20.7 ± 2.6	22.8 ± 3.4	24.4 ± 2.4	H	NS
50:1	15.1 ± 1.4	12.0 ± 1.7	12.5 ± 2.1	13.8 ± 1.3	H	NS
25:1	9.4 ± 1.3	7.2 ± 1.2	7.9 ± 1.5	7.8 ± 0.8	H	NS
12.5:1	6.2 ± 0.8	4.9 ± 0.9	5.1 ± 0.9	6.4 ± 0.9	H	NS
6.25:1	4.3 ± 0.5	3.5 ± 0.6	3.7 ± 0.6	4.2 ± 0.5	H	NS

Female Sprague Dawley rats were exposed to chamber control air or blasting sand by inhalation. On the day of euthanasia, spleens were placed into tubes containing medium and sent to Virginia Commonwealth University (Richmond, VA) on ice for next-day cell preparation. Spleens were prepared into single-cell suspensions and were assayed 4 hours later for natural killer cell activity using ⁵¹Cr-labeled YAC-1 cells as the target. Spontaneous release over the 4-hour incubation period was 9.6% of maximum release. NK cell activity values represent the mean ± standard error percent cytotoxicity derived from the number of animals indicated by n.

H = homogeneous data and NH = nonhomogeneous data using Bartlett's test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance; differences from the chamber control group are not significant by Dunnett's t-test. Jonckheere's test was used to assess the significance of exposure concentration-related trends.

NS = not significant.

Table F-12. Natural Killer Cell Activity in Female Sprague Dawley Rats Exposed to Blasting Sand for 26 Weeks

	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³	H/NH	Trend Analysis
n	8	8	8	8	–	–
Spleen Wt. (mg)	714 ± 41	725 ± 60	817 ± 67	739 ± 33	H	NS
Effector:Target Ratio						
200:1	31.8 ± 2.0	30.6 ± 2.0	39.2 ± 1.5	35.2 ± 3.0	H	p ≤ 0.05
100:1	21.5 ± 1.5	23.0 ± 1.2	27.5 ± 1.9	25.2 ± 3.1	H	p ≤ 0.05
50:1	12.9 ± 1.2	12.4 ± 0.7	16.8 ± 0.7	13.1 ± 1.9	NH	NS
25:1	7.6 ± 0.9	6.9 ± 0.6	9.3 ± 0.5	8.1 ± 1.3	H	NS
12.5:1	4.4 ± 0.7	4.1 ± 0.5	5.6 ± 0.4	4.6 ± 0.9	H	NS
6.25:1	3.1 ± 0.5	3.2 ± 0.3	3.6 ± 0.2	3.2 ± 0.6	NH	NS

Female Sprague Dawley rats were exposed to chamber control air or blasting sand by inhalation. On the day of euthanasia, spleens were placed into tubes containing medium and sent to Virginia Commonwealth University (Richmond, VA) on ice for next-day cell preparation. Spleens were prepared into single-cell suspensions and were assayed 4 hours later for natural killer cell activity using ⁵¹Cr-labeled YAC-1 cells as the target. Spontaneous release over the 4-hour incubation period was 8.2% of maximum release. NK cell activity values represent the mean ± standard error percent cytotoxicity derived from the number of animals indicated by n.

H = homogeneous data and NH = nonhomogeneous data using Bartlett's test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. Nonhomogeneous data were evaluated using a nonparametric analysis of variance. Differences from the chamber control group are not significant by Dunnett's t-test (homogenous data) or Wilcoxon's rank test (nonhomogeneous data). Jonckheere's test was used to assess the significance of exposure concentration-related trends. NS = not significant.

Table F-13. Bronchoalveolar Lavage Fluid Cytokine Levels in Female Sprague Dawley Rats Exposed to Blasting Sand for 4 or 26 Weeks

	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³	H/NH	Trend Analysis
n	8	8	8	8	–	–
Week 4						
IL-1α	17.30 ± 9.67	71.77 ± 15.41**	21.07 ± 13.15	12.58 ± 6.16	H	p ≤ 0.01
MCP-1	ND	ND	ND	26.12 ± 13.49	NH	p ≤ 0.01
TNF-α	25.08 ± 4.38	50.96 ± 5.64**	20.12 ± 6.88	19.12 ± 4.43	H	p ≤ 0.05
IFN-γ	ND	ND	ND	ND	NA	NA
GM-CSF	55.84 ± 9.84	85.46 ± 13.34	49.70 ± 12.05	43.48 ± 9.75	H	NS
IL-4	2.86 ± 0.31	4.64 ± 0.45**	2.71 ± 0.27	2.19 ± 0.34	H	NS
Week 26						
IL-1α	20.00 ± 11.69	26.83 ± 10.06	1.37 ± 1.37	9.67 ± 9.67	NH	p ≤ 0.01
MCP-1	ND	78.52 ± 24.33**	336.77 ± 53.14**	1,094.75 ± 164.11**	NH	p ≤ 0.01
TNF-α	36.60 ± 4.37	26.96 ± 4.59	24.22 ± 4.33	8.80 ± 6.45**	H	p ≤ 0.01
IFN-γ	ND	ND	ND	ND	NA	NA
GM-CSF	67.97 ± 4.40	64.16 ± 3.49	30.18 ± 11.65*	24.50 ± 12.56**	NH	p ≤ 0.01

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	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³	H/NH	Trend Analysis
IL-4	3.22 ± 0.32	2.83 ± 0.18	1.60 ± 0.50*	0.70 ± 0.47**	H	p ≤ 0.01

*Significantly different (p ≤ 0.05) from the chamber control group by Dunnett's test (homogeneous data) or Wilcoxon's test (nonhomogeneous data).

**p ≤ 0.01.

Female Sprague Dawley rats were exposed to chamber control air or blasting sand by inhalation. On the day of euthanasia, bronchoalveolar lavage fluid was collected and frozen. Samples were sent to Virginia Commonwealth University (Richmond, VA) on dry ice for sample analysis. Cytokine levels were analyzed in 25 µL aliquots of sample using FlowCytomix™ bead array kits according to kit instructions. Values represent the mean ± standard error pg/mL of each cytokine derived from the number of animals indicated by n.

H = homogeneous data and NH = nonhomogeneous data using Bartlett's test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the chamber control group using Dunnett's t-test. Nonhomogeneous data were evaluated using a nonparametric analysis of variance. When significant differences occurred, exposed groups were compared to the chamber control group using Wilcoxon's rank test. Jonckheere's test was used to assess the significance of concentration-related trends.

NS = not significant; ND = not detected; NA = not applicable.

Table F-14. Bronchoalveolar Lavage Fluid Cytokine Levels in Male Sprague Dawley Rats Exposed to Blasting Sand for 4, 16, 26, or 39 Weeks

	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³	H/NH	Trend Analysis
n	8	8	8	8	–	–
Week 4						
IL-α	11.95 ± 5.90	9.82 ± 6.13	15.30 ± 8.19	13.20 ± 6.97	H	NS
MCP-1	8.15 ± 1.72	6.75 ± 1.48	9.07 ± 2.11	44.07 ± 5.83**	NH	p ≤ 0.01
TNF-α	8.75 ± 4.38	7.82 ± 3.47	5.18 ± 3.71	2.32 ± 2.19	H	p ≤ 0.01
IFN-γ	ND	ND	ND	ND	NA	NA
GM-CSF	15.80 ± 7.45	16.70 ± 7.44	24.99 ± 11.01	11.71 ± 8.29	H	p ≤ 0.05
IL-4	0.09 ± 0.09	0.03 ± 0.03	0.19 ± 0.15	0.06 ± 0.06	NH	p ≤ 0.01
Week 16						
IL-1	5.20 ± 5.20	2.38 ± 1.70	6.65 ± 5.12	0.73 ± 0.73	NH	p ≤ 0.01
MCP-1α	5.67 ± 2.13	19.67 ± 1.28**	83.96 ± 10.48**	290.69 ± 39.56**	NH	p ≤ 0.01
TNF-α	3.84 ± 3.84	ND	0.12 ± 0.12	ND	NH	p ≤ 0.01
IFN-γ	ND	ND	ND	ND	NA	NA
GM-CSF	6.25 ± 6.25	5.80 ± 5.80	1.76 ± 1.76	ND	NH	p ≤ 0.01
IL-4	0.06 ± 0.06	ND	ND	ND	NH	p ≤ 0.01
Week 26						
IL-1	8.05 ± 4.48	2.79 ± 2.51	7.05 ± 4.32	18.10 ± 9.11	NH	NS
MCP-1α	8.52 ± 1.28	46.50 ± 7.15**	202.67 ± 40.54**	513.65 ± 58.86**	NH	p ≤ 0.01
TNF-α	2.99 ± 2.15	2.34 ± 1.80	22.70 ± 5.10	14.76 ± 5.74	NH	NS
IFN-γ	ND	ND	ND	ND	NA	NA
GM-CSF	5.75 ± 3.23	5.02 ± 4.28	46.16 ± 10.47	47.40 ± 10.76	NH	NS
IL-4	ND	ND	2.28 ± 0.37	2.04 ± 0.49	NH	NS

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	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³	H/NH	Trend Analysis
Week 39						
IL-1 α	ND	ND	ND	ND	NA	NA
MCP-1	2.25 \pm 1.47	55.40 \pm 22.76**	219.68 \pm 63.29**	872.04 \pm 108.00**	NH	p \leq 0.01
TNF- α	ND	ND	ND	ND	NA	NA
IFN- γ	ND	ND	ND	ND	NA	NA
GM-CSF	ND	ND	ND	ND	NA	NA
IL-4	ND	ND	ND	ND	NA	NA

**Significantly different (p \leq 0.01) from the chamber control group by Wilcoxon's rank test.

Male Sprague Dawley rats were exposed to chamber control air or blasting sand by inhalation. On the day of euthanasia, bronchoalveolar lavage fluid was collected and frozen. Samples were sent to Virginia Commonwealth University (Richmond, VA) on dry ice for sample analysis. Cytokine levels were analyzed in 25 μ L aliquots of sample using FlowCytomix™ bead array kits according to kit instructions. Values represent the mean \pm standard error pg/mL for each cytokine derived from the number of animals indicated by n.

H = homogeneous data and NH = nonhomogeneous data using Bartlett's test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance; differences from the chamber control group are not significant by Dunnett's t-test. Nonhomogeneous data were evaluated using a nonparametric analysis of variance. When significant differences occurred, exposed groups were compared to the chamber control group using Wilcoxon's rank test. Jonckheere's test was used to assess the significance of concentration-related trends.

NS = not significant; ND = not detected; NA = not applicable.

Table F-15. Antinuclear Antibody-Positive Responses in Sprague Dawley Rats Exposed to Blasting Sand

	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³
n	8	8	8	8
Male				
Week 4	0 (0)	0 (0)	0 (0)	0 (0)
Week 16	0 (0)	0 (0)	0 (0)	0 (0)
Week 26	0 (0)	1 (13)	2 (25)	3 (38)
Week 39	0 (0)	0 (0)	0 (0)	0 (0)
Female				
Week 4	0 (0)	1 (13)	0 (0)	3 (38)
Week 26	0 (0)	1 (13)	0 (0)	0 (0)

Male and female Sprague Dawley rats were exposed to chamber control air or blasting sand by inhalation. On the day of euthanasia, serum was collected and frozen. Samples were sent to Virginia Commonwealth University (Richmond, VA) on dry ice for sample analysis. Samples were diluted 1:50 and analyzed for the presence of antinuclear antibodies by enzyme-linked immunosorbent assay. Results are presented as the incidence (percent) of positive responses. A positive response was defined as a sample having an optical density that exceeded the upper limit of the 99% confidence interval of the mean of the chamber control group at the same time point. Differences in incidence from the chamber control group are not significant by the Fisher exact chi-square test.

Table F-16. Spleen IgM Antibody-Forming Cell Response to the T-Dependent Antigen Sheep Erythrocytes in Female Sprague Dawley Rats Exposed to Specular Hematite for Five Weeks (Day 4 Response)

	Spleen Weight (mg)	Total Spleen Cells ($\times 10^7$)	IgM AFC/ 10^6 Spleen Cells	IgM AFC/Spleen($\times 10^3$)
n	8	8	8	8
Chamber Control	539 \pm 30	65.09 \pm 4.82	642 \pm 162	431 \pm 109
Specular Hematite				
15 mg/m ³	502 \pm 35	67.27 \pm 4.08	676 \pm 147	468 \pm 113
30 mg/m ³	592 \pm 40	76.05 \pm 6.08	858 \pm 173	659 \pm 149
60 mg/m ³	597 \pm 27	69.02 \pm 3.76	920 \pm 171	650 \pm 137
H/NH	H	H	H	H
Trend Analysis	NS	NS	NS	NS

Female Sprague Dawley rats were exposed to chamber control air or specular hematite by inhalation. Four days before euthanasia, the rats were immunized with 2×10^8 sheep red blood cells (sRBCs). On the day of euthanasia, spleens were placed in tubes containing medium and sent to Virginia Commonwealth University (Richmond, VA) on ice for next-day cell preparation. Spleens were prepared into single-cell suspensions and the number of IgM sRBC antibody-forming cells (AFCs) was determined. Values represent the mean \pm standard error derived from the number of animals indicated by n.

H = homogeneous data and NH = nonhomogeneous data using Bartlett's test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance; differences from the chamber control group are not significant by Dunnett's t-test. Jonckheere's test was used to assess the significance of exposure concentration-related trends.

NS = not significant.

Table F-17. Spleen IgM Antibody-Forming Cell Response to the T-Dependent Antigen Sheep Erythrocytes in Female Sprague Dawley Rats Exposed to Specular Hematite for 27 Weeks (Day 4 Response)

	Spleen Weight (mg)	Total Spleen Cells ($\times 10^7$)	IgM AFC/ 10^6 Spleen Cells	IgM AFC/Spleen ($\times 10^3$)
n	8	8	8	8
Chamber Control	620 \pm 25 ^a	79.88 \pm 3.58 ^a	525 \pm 108	419 \pm 87 ^a
Specular Hematite				
15 mg/m ³	629 \pm 36	85.22 \pm 4.60	520 \pm 136	450 \pm 131
30 mg/m ³	599 \pm 13	70.81 \pm 2.90	669 \pm 113	465 \pm 73
60 mg/m ³	634 \pm 25	71.63 \pm 3.07	822 \pm 207	610 \pm 160
H/NH	H	H	H	H
Trend Analysis	NS	p \leq 0.05	NS	NS

Female Sprague Dawley rats were exposed to chamber control air or specular hematite by inhalation. Four days before euthanasia, the rats were immunized with 2×10^8 sheep red blood cells (sRBCs). On the day of euthanasia, spleens were placed in tubes containing medium and sent to Virginia Commonwealth University (Richmond, VA) on ice for next-day cell preparation. Spleens were prepared into single-cell suspensions and the number of IgM sRBC antibody-forming cells (AFCs) was determined. Values represent the mean \pm standard error derived from the number of animals indicated by n.

H = homogeneous data and NH = nonhomogeneous data using Bartlett's test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance; differences from the chamber control group are not significant by Dunnett's t-test. Jonckheere's test was used to assess the significance of exposure concentration-related trends.

NS = not significant.

^an = 7.

Table F-18. Serum IgM Antibody Titers to the T-Dependent Antigen Sheep Erythrocytes in Female Sprague Dawley Rats Exposed to Specular Hematite for Five Weeks

	Number of Animals	Serum Titer Log ₂
Control	8	5.939 ± 0.208
Specular Hematite		
15 mg/m ³	8	5.370 ± 0.273
30 mg/m ³	8	6.335 ± 0.275
60 mg/m ³	8	6.353 ± 0.252
H/NH	–	H
Trend Analysis	–	NS

Female Sprague Dawley rats were exposed to chamber control air or specular hematite by inhalation. On the day of euthanasia, serum was obtained via cardiac puncture and frozen. Serum samples were shipped to Virginia Commonwealth University (Richmond, VA) on dry ice for analysis. Values represent the mean ± standard error derived from the indicated number of animals.

H = homogeneous data and NH = nonhomogeneous data using Bartlett's test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance; differences from the chamber control group are not significant by Dunnett's t-test. Jonckheere's test was used to assess the significance of exposure concentration-related trends.

NS = not significant.

Table F-19. Serum IgM Antibody Titers to the T-Dependent Antigen Sheep Erythrocytes in Female Sprague Dawley Rats Exposed to Specular Hematite for 27 Weeks

	Number of Animals	Serum Titer Log ₂
Chamber Control	8	6.844 ± 0.505
Specular Hematite		
15 mg/m ³	8	5.756 ± 0.577
30 mg/m ³	8	6.058 ± 0.567
60 mg/m ³	8	6.375 ± 0.322
H/NH	–	H
Trend Analysis	–	NS

Female Sprague Dawley rats were exposed to chamber control air or specular hematite by inhalation. On the day of euthanasia, serum was obtained via cardiac puncture and frozen. Serum samples were shipped to Virginia Commonwealth University (Richmond, VA) on dry ice for analysis. Values represent the mean ± standard error derived from the indicated number of animals.

H = homogeneous data and NH = nonhomogeneous data using Bartlett's test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance; differences from the chamber control group are not significant by Dunnett's t-test. Jonckheere's test was used to assess the significance of exposure concentration-related trends.

NS = not significant.

Table F-20. Splenocyte Surface Marker Differential in Female Sprague Dawley Rats Exposed to Specular Hematite for Four Weeks (Absolute Values)

	Total Spleen Cells ($\times 10^7$)	OX33 ^a	CD5 ^b	CD4 ⁺ CD5 ⁺ ^c	CD8 ⁺ CD5 ⁺ ^d	NK ⁺ CD8 ⁺ ^e	HIS36 ^f
n	8	8	8	8	8	8	8
Chamber Control	59.79 \pm 3.79	346.3 \pm 23.2	133.1 \pm 10.8	76.7 \pm 8.1	49.1 \pm 3.9	24.1 \pm 2.3	25.0 \pm 3.1
Specular Hematite							
15 mg/m ³	60.50 \pm 4.02	309.6 \pm 20.6	158.5 \pm 16.9	82.5 \pm 7.1	62.8 \pm 6.5	20.4 \pm 1.8	29.5 \pm 3.0
30 mg/m ³	60.71 \pm 3.52	352.9 \pm 25.9	130.0 \pm 8.8	71.3 \pm 6.4	52.4 \pm 3.6	22.6 \pm 1.9	33.6 \pm 4.1
60 mg/m ³	61.34 \pm 3.44	341.4 \pm 26.2	137.2 \pm 9.1	72.6 \pm 5.5	55.4 \pm 3.5	22.8 \pm 1.5	25.5 \pm 3.0
H/NH	H	H	H	H	H	H	H
Trend Analysis	NS	NS	NS	NS	NS	NS	NS

Female Sprague Dawley rats were exposed to chamber control air or specular hematite by inhalation. On the day of euthanasia, spleens were placed into tubes containing medium and sent to Virginia Commonwealth University (Richmond, VA) on ice for next-day cell preparation. B cells, T cells, T-subsets, NK cells, and macrophages were enumerated. Surface marker values are expressed as the absolute number per spleen $\times 10^6$ and represented as the mean \pm standard error derived from the number of animals indicated by n.

H = homogeneous data and NH = nonhomogeneous data using Bartlett's test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance; differences from the chamber control group are not significant by Dunnett's t-test. Jonckheere's test was used to assess the significance of exposure concentration-related trends.

NS = not significant.

^aB cell.

^bT cell.

^cHelper/DTH – T cell.

^dCytotoxic T cell.

^eNatural killer cell.

^fMacrophage.

Table F-21. Splenocyte Surface Marker Differential in Female Sprague Dawley Rats Exposed to Specular Hematite for Four Weeks (Percent Values)

	OX33 ^a	CD5 ^b	CD4 ⁺ CD5 ⁺ ^c	CD8 ⁺ CD5 ⁺ ^d	NK ⁺ CD8 ⁺ ^e	HIS36 ^f
n	8	8	8	8	8	8
Chamber Control	58.0 \pm 1.4	22.3 \pm 1.4	12.9 \pm 1.3	8.2 \pm 0.4	4.0 \pm 0.3	4.2 \pm 0.4
Specular Hematite						
15 mg/m ³	51.4 \pm 1.5**	26.1 \pm 1.5	13.7 \pm 0.6	10.3 \pm 0.7	3.4 \pm 0.3	4.9 \pm 0.5
30 mg/m ³	57.8 \pm 1.3	21.7 \pm 1.3	11.9 \pm 0.9	8.7 \pm 0.5	3.7 \pm 0.2	5.5 \pm 0.5
60 mg/m ³	55.3 \pm 1.4	22.5 \pm 1.2	11.9 \pm 0.8	9.1 \pm 0.5	3.9 \pm 0.5	4.1 \pm 0.3
H/NH	H	H	H	H	H	H
Trend Analysis	NS	NS	NS	NS	NS	NS

**Significantly different ($p \leq 0.01$) from the chamber control group by Dunnett's t-test.

Female Sprague Dawley rats were exposed to chamber control air or specular hematite by inhalation. On the day of euthanasia, spleens were placed into tubes containing medium and sent to Virginia Commonwealth University (Richmond, VA) on ice for next-day cell preparation. B cells, T cells, T-subsets, NK cells, and macrophages were enumerated. Values are expressed as percent of total spleen cells counted for each animal (Table F-20) and represented as the mean \pm standard error derived from the number of animals indicated by n.

H = homogeneous data and NH = nonhomogeneous data using Bartlett's test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the chamber control group using Dunnett's t-test. Jonckheere's test was used to assess the significance of exposure concentration-related trends.

NS = not significant.

^aB cell.

^bT cell.

^cHelper/DTH – T cell.

^dCytotoxic T cell.

^eNatural killer cell.

^fMacrophage.

Table F-22. Splenocyte Surface Marker Differential in Female Sprague Dawley Rats Exposed to Specular Hematite for 26 Weeks (Absolute Values)

	Total Spleen Cells ($\times 10^7$)	OX33 ^a	CD5 ^b	CD4 ⁺ CD5 ⁺ ^c	CD8 ⁺ CD5 ⁺ ^d	NK ⁺ CD8 ⁺ ^e	HIS36 ^f
n	8	8	8	8	8	8	8
Chamber Control ^g	65.63 \pm 3.93	299.0 \pm 32.6	206.0 \pm 12.7	97.2 \pm 9.5	95.3 \pm 6.0	14.5 \pm 0.8	30.8 \pm 3.9
Specular Hematite							
15 mg/m ³	73.36 \pm 4.02	330.8 \pm 16.6	231.8 \pm 14.7	124.1 \pm 11.5	106.6 \pm 11.3	18.8 \pm 1.8	30.1 \pm 2.7
30 mg/m ³	64.82 \pm 3.37	305.0 \pm 19.1	164.4 \pm 17.1	97.4 \pm 11.5	66.0 \pm 7.8	16.0 \pm 1.2	31.8 \pm 3.2
60 mg/m ³	66.66 \pm 2.67	305.6 \pm 18.3	191.9 \pm 13.4	105.6 \pm 8.9	77.1 \pm 8.3	17.9 \pm 1.1	24.6 \pm 1.8
H/NH	H	H	H	H	H	H	H
Trend Analysis	NS	NS	p \leq 0.05	NS	p \leq 0.05	NS	NS

Female Sprague Dawley rats were exposed to chamber control air or specular hematite by inhalation. On the day of euthanasia, spleens were placed into tubes containing medium and sent to Virginia Commonwealth University (Richmond, VA) on ice for next-day cell preparation. B cells, T cells, T-subsets, NK cells, and macrophages were enumerated. Surface marker values are expressed as the absolute number per spleen $\times 10^6$ and represented as the mean \pm standard error derived from the number of animals indicated by n.

H = homogeneous data and NH = nonhomogeneous data using Bartlett's test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance; differences from the chamber control group are not significant by Dunnett's t-test. Jonckheere's test was used to assess the significance of exposure concentration-related trends.

NS = not significant.

^aB cell.

^bT cell.

^cHelper/DTH – T cell.

^dCytotoxic T cell.

^eNatural killer cell.

^fMacrophage.

^gn = 7; the values for one chamber control animal were excluded due to very low cell viabilities for all markers.

Table F-23. Splenocyte Surface Marker Differential in Female Sprague Dawley Rats Exposed to Specular Hematite for 26 Weeks (Percent Values)

Exposure	OX33 ⁺ ^a	CD5 ⁺ ^b	CD4 ⁺ CD5 ⁺ ^c	CD8 ⁺ CD5 ⁺ ^d	NK ⁺ CD8 ⁺ ^e	HIS36 ⁺ ^f
n	8	8	8	8	8	8
Chamber Control ^g	44.9 ± 2.8	31.6 ± 1.3	14.6 ± 0.7	14.6 ± 0.6	2.2 ± 0.1	4.6 ± 0.5
Specular Hematite						
15 mg/m ³	45.3 ± 1.4	31.7 ± 1.4	16.8 ± 1.1	14.3 ± 0.9	2.6 ± 0.3	4.1 ± 0.3
30 mg/m ³	46.9 ± 1.1	25.4 ± 2.2*	14.8 ± 1.2	10.3 ± 1.2*	2.5 ± 0.1	4.9 ± 0.3
60 mg/m ³	45.7 ± 1.6	28.8 ± 1.5	15.8 ± 1.1	11.6 ± 1.0	2.7 ± 0.2	3.8 ± 0.3
H/NH	H	H	H	H	NH	H
Trend Analysis	NS	p ≤ 0.05	NS	p ≤ 0.01	NS	NS

*Significantly different ($p \leq 0.05$) from the chamber control group by Dunnett's test.

Female Sprague Dawley rats were exposed to chamber control air or specular hematite by inhalation. On the day of euthanasia, spleens were placed into tubes containing medium and sent to Virginia Commonwealth University (Richmond, VA) on ice for next-day cell preparation. B cells, T cells, T-subsets, NK cells, and macrophages were enumerated. Values are expressed as percent total spleen cells counted for each animal (Table F-22) and represented as the mean ± standard error derived from the number of animals indicated by n.

H = homogeneous data and NH = nonhomogeneous data using Bartlett's test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the chamber control group using Dunnett's t-test. Nonhomogeneous data were evaluated using a nonparametric analysis of variance; differences from the chamber control group are not significant by Wilcoxon's test. Jonckheere's test was used to assess the significance of exposure concentration-related trends.

NS = not significant.

^aB cell.

^bT cell.

^cHelper/DTH – T cell.

^dCytotoxic T cell.

^eNatural killer cell.

^fMacrophage.

^gn = 7; the values for one chamber control animal were excluded due to very low cell viabilities for all markers.

Table F-24. Spleen Cell Proliferative Response to Anti-CD3 Stimulation in Female Sprague Dawley Rats Exposed to Specular Hematite for Four Weeks

	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³	H/NH	Trend Analysis
n	8	8	8	8	–	–
Spleen Cell Cultures						
Unstimulated	12,109 ± 710	15,842 ± 1,271*	15,326 ± 1,226*	13,157 ± 2,271	NH	NS
Stimulated	79,744 ± 7,899	119,966 ± 17,221	95,422 ± 10,893	80,043 ± 11,647	H	NS
Total Spleen Cells × 10 ⁷	59.79 ± 3.79	60.50 ± 4.02	60.71 ± 3.52	61.34 ± 3.44	H	NS

*Significantly different ($p \leq 0.05$) from the chamber control group by Wilcoxon's rank test.

Female Sprague Dawley rats were exposed to chamber control air or specular hematite by inhalation. On the day of euthanasia, spleens were placed into tubes containing medium and sent to Virginia Commonwealth University (Richmond, VA) on ice for next-day cell preparation. Spleens were prepared into single-cell suspensions and incubated in flat-bottom microtiter plates. The medium for the proliferative assay was RPMI supplemented with 10% FBS and 50 μ M 2-mercaptoethanol. The spleen cells were cultured in either nontreated or treated anti-CD3 wells. Prior to harvest, the cells were pulsed with ³H-thymidine for 18 to 24 hours. The cultured cell data are presented as the mean \pm standard error CPM derived from four replicate cultures from the number of animals indicated by n.

H = homogeneous data and NH = nonhomogeneous data using Bartlett's test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance; differences from the chamber control group are not significant by Dunnett's t-test. Nonhomogeneous data were evaluated using a nonparametric analysis of variance. When significant differences occurred, exposed groups were compared to the chamber control group using Wilcoxon's rank test. Jonckheere's test was used to assess the significance of exposure concentration-related trends.

NS = not significant.

Table F-25. Spleen Cell Proliferative Response to Anti-CD3 Stimulation in Female Sprague Dawley Rats Exposed to Specular Hematite for 26 Weeks

	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³	H/NH	Trend Analysis
n	8	8	8	8	–	–
Spleen Cell Cultures						
Unstimulated	2,804 ± 697	3,902 ± 352	3,896 ± 304	3,576 ± 673	H	NS
Stimulated	85,255 ± 20,785	103,305 ± 8,458	135,331 ± 12,196	124,159 ± 8,030	NH	$p \leq 0.05$
Total Spleen Cells × 10 ⁷	64.61 ± 3.56	73.36 ± 4.02	64.82 ± 3.37	66.66 ± 2.67	H	NS

Female Sprague Dawley rats were exposed to chamber control air or specular hematite by inhalation. On the day of euthanasia, spleens were placed into tubes containing medium and sent to Virginia Commonwealth University (Richmond, VA) on ice for next-day cell preparation. Spleens were prepared into single-cell suspensions and incubated in flat-bottom microtiter plates. The medium for the proliferative assay was RPMI supplemented with 10% FBS and 50 μ M 2-mercaptoethanol. The spleen cells were cultured in either nontreated or treated anti-CD3 wells. Prior to harvest, the cells were pulsed with ³H-thymidine for 18 to 24 hours. The cultured cell data are presented as mean \pm standard error CPM derived from four replicate cultures from the number of animals indicated by n.

H = homogeneous data and NH = nonhomogeneous data using Bartlett's test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. Nonhomogeneous data were evaluated using a nonparametric analysis of variance. Differences from the chamber control group are not significant by Dunnett's t-test (homogenous data) or Wilcoxon's rank test (nonhomogeneous data). Jonckheere's test was used to assess the significance of exposure concentration-related trends.

NS = not significant.

Table F-26. Natural Killer Cell Activity in Female Sprague Dawley Rats Exposed to Specular Hematite for Four Weeks

	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³	H/NH	Trend Analysis
n	8	8	8	8	–	–
Spleen Wt. (mg)	564 ± 26	526 ± 28	516 ± 14	514 ± 24	H	p ≤ 0.05
Effector:Target Ratio						
200:1	29.6 ± 1.8	31.6 ± 2.4	28.4 ± 2.7	32.6 ± 3.0	H	NS
100:1	20.4 ± 1.8	22.9 ± 1.4	20.8 ± 2.4	24.2 ± 2.0	H	NS
50:1	14.5 ± 1.2	17.3 ± 1.3	13.6 ± 1.4	17.5 ± 1.9	H	NS
25:1	9.4 ± 0.7	11.2 ± 0.8	9.8 ± 1.1	12.1 ± 1.5	H	NS
12.5:1	6.9 ± 0.9	8.3 ± 0.8	6.8 ± 0.9	8.5 ± 1.2	H	NS
6.25:1	6.7 ± 0.6	7.2 ± 0.4	6.0 ± 0.7	7.3 ± 0.9	H	NS

Female Sprague Dawley rats were exposed to chamber control air or specular hematite by inhalation. On the day of euthanasia, spleens were placed into tubes containing medium and sent to Virginia Commonwealth University (Richmond, VA) on ice for next-day cell preparation. Spleens were prepared into single-cell suspensions and were assayed 4 hours later for natural killer cell activity using ⁵¹Cr-labeled YAC-1 cells as the target. Spontaneous release over the 4-hour incubation period was 12.4% of maximum release. NK cell activity values represent the mean ± standard error percent cytotoxicity derived from the number of animals indicated by n.

H = homogeneous data and NH = nonhomogeneous data using the Bartlett test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance; differences from the chamber control group are not significant by the Dunnett t-test. The Jonckheere test was used to assess the significance of exposure concentration-related trends.

NS = not significant.

Table F-27. Natural Killer Cell Activity in Female Sprague Dawley Rats Exposed to Specular Hematite for 26 Weeks

	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³	H/NH	Trend Analysis
n	8	8	8	8	–	–
Spleen Wt. (mg)	608 ± 21	655 ± 20	599 ± 35	644 ± 25	H	NS
Effector:Target Ratio						
200:1	16.2 ± 3.9	23.3 ± 1.9	24.2 ± 1.7	24.6 ± 1.9	H	p ≤ 0.05
100:1	9.0 ± 2.3	13.1 ± 1.3	14.7 ± 1.1	14.4 ± 1.7	H	p ≤ 0.05
50:1	3.4 ± 1.2	7.1 ± 1.1	7.5 ± 1.0*	7.4 ± 1.2	H	p ≤ 0.05
25:1	1.3 ± 30.7	3.6 ± 0.7	4.1 ± 0.8	4.7 ± 1.0*	H	p ≤ 0.01
12.5:1	<1	1.9 ± 0.3	2.2 ± 0.8	2.2 ± 0.7	H	p ≤ 0.05
6.25:1	<1	<1	<1	1.0 ± 0.6	H	p ≤ 0.05

*Significantly different (p ≤ 0.05) from the chamber control group by Dunnett's t-test.

Female Sprague Dawley rats were exposed to chamber control air or specular hematite by inhalation. On the day of euthanasia, spleens were placed into tubes containing medium and sent to Virginia Commonwealth University (Richmond, VA) on ice for next-day cell preparation. Spleens were prepared into single-cell suspensions and were assayed 4 hours later for natural killer cell activity using ⁵¹Cr-labeled YAC-1 cells as the target. Spontaneous release over the 4-hour incubation period was 14.3% of maximum release. NK cell activity values represent the mean ± standard error percent cytotoxicity derived from the number of animals indicated by n.

H = homogeneous data and NH = nonhomogeneous data using Bartlett's test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance; when significant differences occurred, exposed groups were compared to the chamber control group using Dunnett's t-test. Jonckheere's test was used to assess the significance of exposure concentration-related trends.

NS = not significant.

Table F-28. Bronchoalveolar Lavage Fluid Cytokine Levels in Female Sprague Dawley Rats Exposed to Specular Hematite for 4 or 26 Weeks

	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³	H/NH	Trend Analysis
n	8	8	8	8	–	–
Week 4						
IL-1 α	21.31 \pm 7.12	27.05 \pm 12.49	16.18 \pm 4.51	168.20 \pm 106.45	NH	NS
MCP-1	22.12 \pm 0.89	18.94 \pm 4.43	22.24 \pm 0.77	45.77 \pm 13.12	NH	p \leq 0.05
TNF- α	12.63 \pm 3.99	15.62 \pm 6.69	8.97 \pm 3.02	98.33 \pm 63.37	NH	NS
IFN- γ	ND	ND	ND	27.58 \pm 22.95	NH	p \leq 0.01
GM-CSF	45.96 \pm 7.48	48.08 \pm 15.92	40.75 \pm 7.85	125.33 \pm 49.24	NH	NS
IL-4	0.04 \pm 0.04	0.65 \pm 0.34	ND	5.36 \pm 3.89	NH	p \leq 0.05
Week 26						
IL-1 α	40.49 \pm 18.39	21.89 \pm 5.42	6.96 \pm 4.97	23.38 \pm 15.37	NH	p \leq 0.01
MCP-1	25.37 \pm 2.55	27.06 \pm 2.48	91.31 \pm 8.88**	782.32 \pm 99.06**	NH	p \leq 0.01
TNF- α	22.51 \pm 11.00	10.73 \pm 3.97	3.47 \pm 2.56	11.60 \pm 7.29	NH	p \leq 0.01
IFN- γ	0.25 \pm 0.25	ND	ND	ND	NH	p \leq 0.01
GM-CSF	44.39 \pm 22.24	43.39 \pm 10.57	13.92 \pm 9.68	28.72 \pm 19.09	H	p \leq 0.01
IL-4	1.13 \pm 0.67	ND	ND	0.77 \pm 0.56	NH	p \leq 0.01

**Significantly different (p \leq 0.01) from the chamber control group by Wilcoxon's rank test.

Female Sprague Dawley rats were exposed to chamber control air or specular hematite by inhalation. On the day of euthanasia, bronchoalveolar lavage fluid was collected and frozen. Samples were sent to Virginia Commonwealth University (Richmond, VA) on dry ice for sample analysis. Cytokine levels were analyzed in 25 μ L aliquots of sample using FlowCytomix™ bead array kits according to kit instructions. Values represent the mean \pm standard error pg/mL of each cytokine derived from the number of males indicated by n.

H = homogeneous data and NH = nonhomogeneous data using Bartlett's test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance; differences from the chamber control group are not significant by Dunnett's t-test. Nonhomogeneous data were evaluated using a nonparametric analysis of variance. When significant differences occurred, exposed groups were compared to the chamber control group using Wilcoxon's rank test. Jonckheere's test was used to assess the significance of exposure concentration-related trends.

NS = not significant; ND = not detected.

Table F-29. Bronchoalveolar Lavage Fluid Cytokine Levels in Male Sprague Dawley Rats Exposed to Specular Hematite for 4, 16, 26, or 39 Weeks

	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³	H/NH	Trend Analysis
n	8	8	8	8		
Week 4						
IL-1 α	ND	5.30 \pm 3.82	16.90 \pm 7.58	17.29 \pm 13.16	NH	p \leq 0.05
MCP-1 α	15.04 \pm 0.69	17.06 \pm 1.56	17.67 \pm 0.91	18.40 \pm 2.16	NH	NS
TNF- α	ND	4.65 \pm 2.28	2.56 \pm 2.20	4.45 \pm 2.92	NH	p \leq 0.01
IFN- γ	ND	ND	ND	ND	NA	NA
GM-CSF	4.53 \pm 1.98	32.26 \pm 12.54	39.69 \pm 10.45	25.61 \pm 13.59	NH	NS
IL-4	ND	0.06 \pm 0.06	ND	0.17 \pm 0.17	NH	p \leq 0.01
Week 16						
IL-1 α	13.84 \pm 6.58	18.75 \pm 14.29	10.93 \pm 10.93	2.15 \pm 2.15	NH	p \leq 0.01
MCP-1	17.60 \pm 0.98	18.17 \pm 1.89	33.86 \pm 2.73**	296.39 \pm 40.46**	NH	p \leq 0.01
TNF- α	3.44 \pm 2.72	ND	2.23 \pm 2.23	ND	NH	p \leq 0.01
IFN- γ	ND	ND	ND	ND	NA	NA
GM-CSF	39.93 \pm 11.47	9.84 \pm 7.86	18.47 \pm 12.54	2.80 \pm 2.01	NH	p \leq 0.01
IL-4	0.12 \pm 0.12	ND	0.06 \pm 0.06	ND	NH	p \leq 0.01
Week 26						
IL-1 α	3.70 \pm 3.70	21.82 \pm 10.97	8.44 \pm 4.82	33.00 \pm 9.06	NH	p \leq 0.01
MCP-1	12.92 \pm 0.68	22.49 \pm 3.22**	60.20 \pm 5.78**	394.99 \pm 87.34**	NH	p \leq 0.01
TNF- α	ND	10.14 \pm 7.15	ND	1.04 \pm 1.04	NH	p \leq 0.01
IFN- γ	ND	ND	ND	ND	NA	NA
GM-CSF	4.60 \pm 4.60	33.04 \pm 16.47	23.57 \pm 11.16	16.22 \pm 9.95	NH	p \leq 0.05
IL-4	ND	0.09 \pm 0.09	0.04 \pm 0.04	ND	NH	p \leq 0.01
Week 39						
IL-1 α	39.89 \pm 14.09	0.36 \pm 0.36	15.51 \pm 15.51	9.23 \pm 6.30	NH	p \leq 0.01
MCP-1	19.77 \pm 1.87	35.26 \pm 10.73	149.95 \pm 30.47**	838.08 \pm 150.36**	NH	p \leq 0.01
TNF- α	3.88 \pm 2.58	ND	3.58 \pm 3.58	ND	NH	p \leq 0.01
IFN- γ	ND	ND	ND	ND	NA	NA
GM-CSF	53.22 \pm 14.81	2.73 \pm 2.73	16.35 \pm 16.35	15.72 \pm 10.47	NH	p \leq 0.01
IL-4	0.51 \pm 0.33	ND	0.43 \pm 0.43	ND	NH	p \leq 0.01

**Significantly different (p \leq 0.01) from the chamber control group by Wilcoxon's rank test.

Male Sprague Dawley rats were exposed to chamber control air or specular hematite by inhalation. On the day of euthanasia, bronchoalveolar lavage fluid was collected and frozen. Samples were sent to Virginia Commonwealth University (Richmond, VA) on dry ice for sample analysis. Cytokine levels were analyzed in 25 μ L aliquots of sample using FlowCytomix™ bead array kits according to kit instructions.

H = homogeneous data and NH = nonhomogeneous data using Bartlett's test for homogeneity. Nonhomogeneous data were evaluated using a nonparametric analysis of variance. When significant differences occurred, exposed groups were compared to the chamber control group using Wilcoxon's rank test. Jonckheere's test was used to assess the significance of exposure concentration-related trends. Values represent the mean \pm standard error pg/mL of each cytokine derived from the number of animals indicated by n.

NS = not significant; ND = not detected; NA = not applicable.

Table F-30. Antinuclear Antibody-Positive Responses in Sprague Dawley Rats Exposed to Specular Hematite

	Chamber Control	15 mg/m ³	30 mg/m ³	60 mg/m ³
n	8	8	8	8
Male				
Week 4	0 (0)	1 (13)	0 (0)	0 (0)
Week 16	0 (0)	0 (0)	0 (0)	0 (0)
Week 26	0 (0)	0 (0)	0 (0)	0 (0)
Week 39	0 (0)	0 (0)	0 (0)	1 (13)
Female				
Week 4	0 (0)	1 (13)	0 (0)	0 (0)
Week 26	0 (0)	0 (0)	2 (25)	0 (0)

Male and female Sprague Dawley rats were exposed to chamber control air or specular hematite by inhalation. On the day of euthanasia, serum was collected and frozen. Samples were sent to Virginia Commonwealth University (Richmond, VA) on dry ice for sample analysis. Samples were diluted 1:50 and analyzed for the presence of antinuclear antibodies by enzyme-linked immunosorbent assay. Results are presented as the incidence (percent) of positive responses. A positive response was defined as a sample having an optical density that exceeded the upper limit of the 99% confidence interval of the mean of the chamber control group at the same time point. Differences in incidence from the chamber control group are not significant by the Fisher exact chi-square test.

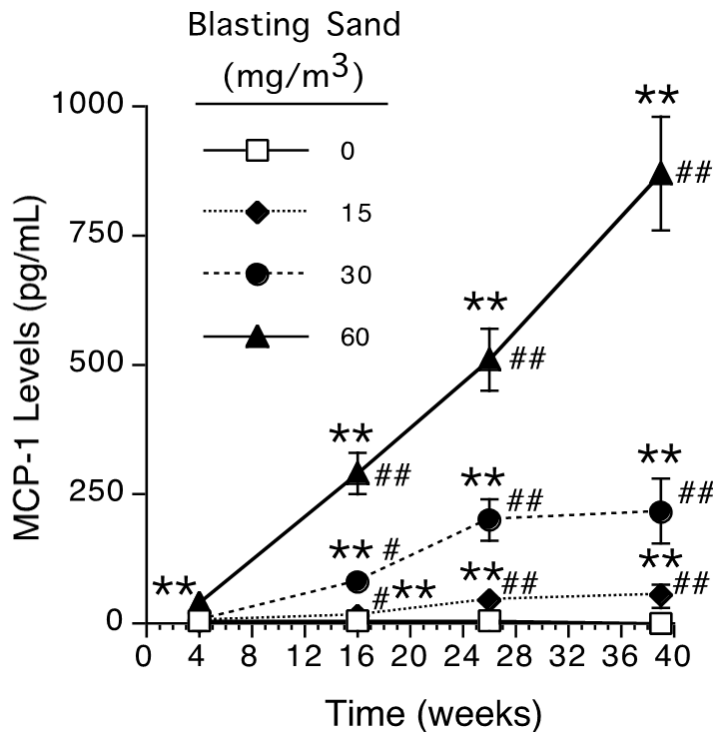


Figure F-1. MCP-1 Levels in the Bronchoalveolar Lavage Fluid of Male Sprague Dawley Rats Exposed to Blasting Sand by Inhalation for 4, 16, 26, or 39 Weeks

**Significantly different ($p \leq 0.01$) from the chamber control group at the same time point.

#Significantly different ($p \leq 0.05$) from the week 4 time point within the same exposure concentration.

$p \leq 0.01$.

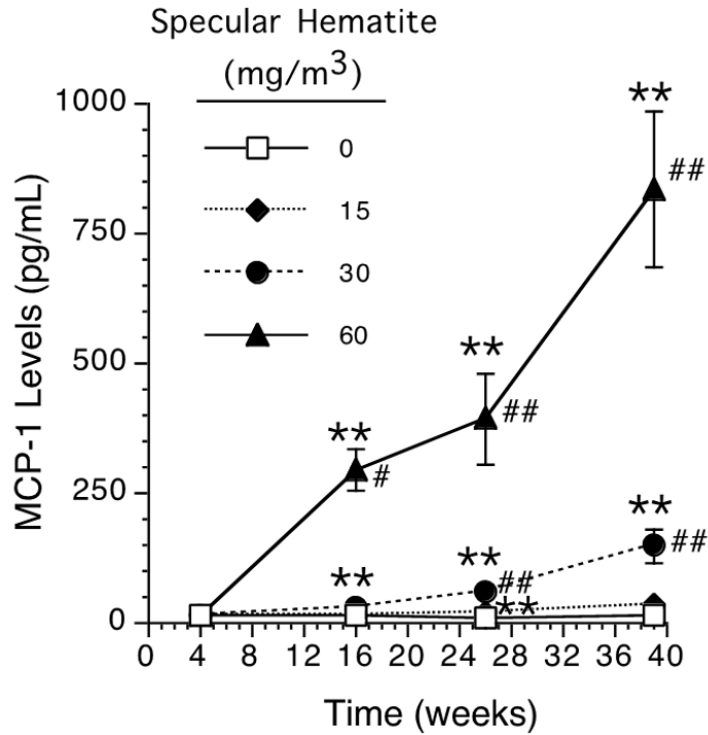


Figure F-2. MCP-1 Levels in Bronchoalveolar Lavage Fluid of Male Sprague Dawley Rats Exposed to Specular Hematite by Inhalation for 4, 16, 26, or 39 Weeks

**Significantly different ($p \leq 0.01$) from the chamber control group at the same time point.

#Significantly different ($p \leq 0.05$) from the week 4 time point within the same exposure concentration.

$p \leq 0.01$.

Appendix G. Supplemental Files

The following supplemental files are available at <https://doi.org/10.22427/NTP-DATA-TOX-91>.

G.1. Two-week Blasting Sand Study Tables – Rats

E03 – Growth Curves

2021301_2_Week_E03_Growth_Curves.pdf

E04 – Mean Body Weights and Survival Table

2021301_2_Week_E04_Mean_Body_Weights_and_Survival_Table.pdf

P03 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site

2021301_2_Week_P03_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site.pdf

P04 – Neoplasms by Individual Animal

2021301_2_Week_P04_Neoplasms_by_Individual_Animal.pdf

P05 – Incidence Rates of Neoplasms by Anatomic Site (Systemic Lesions Abridged)

2021301_2_Week_P05_Incidence_Rates_of_Neoplasms_by_Anatomic_Site_(Systemic_Lesions_Abridged).pdf

P09 – Non-Neoplastic Lesions by Individual Animal

2021301_2_Week_P09_Non-Neoplastic_Lesions_by_Individual_Animal.pdf

P10 – Statistical Analysis of Non-Neoplastic Lesions

2021301_2_Week_P10_Statistical_Analysis_of_Non-Neoplastic_Lesions.pdf

P14 – Individual Animal Pathology Data

2021301_2_Week_P14_Individual_Animal_Pathology_Data.pdf

P18 - Incidence Rates of Non-Neoplastic Lesions by Anatomic Site with Average Severity Grades

2021301_2_Week_P18_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site_with_Average_Severity_Grades.pdf

P40 – Survival Curves

2021301_2_Week_P40_Survival_Curves.pdf

G.2. Two-week Blasting Sand Individual Animal Data – Rats

Male Individual Animal Body Weight Data

2021301_2_Week_Male_Individual_Animal_Body_Weight_Data.xls

Male Individual Animal Non-Neoplastic Pathology Data

2021301_2_Week_Male_Individual_Animal_Non_Neoplastic_Pathology_Data.xls

Male Individual Animal Survival Data

2021301_2_Week_Male_Individual_Animal_Survival_Data.xls

Male Individual Animal Terminal Body Weight Data

2021301_2_Week_Male_Individual_Animal_Terminal_Body_Weight_Data.xls

G.3. Two-week Coal Slag Study Tables – Rats

E03 – Growth Curves

2053201_2_Week_E03_Growth_Curves.pdf

E04 – Mean Body Weights and Survival Table

2053201_2_Week_E04_Mean_Body_Weights_and_Survival_Table.pdf

E05 – Clinical Observations Summary

2053201_2_Week_E05_Clinical_Observations_Summary.pdf

P03 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site

2053201_2_Week_P03_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site.pdf

P04 – Neoplasms by Individual Animal

2053201_2_Week_P04_Neoplasms_by_Individual_Animal.pdf

P05 – Incidence Rates of Neoplasms by Anatomic Site (Systemic Lesions Abridged)

2053201_2_Week_P05_Incidence_Rates_of_Neoplasms_by_Anatomic_Site_(Systemic_Lesions_Abridged).pdf

P09 – Non-Neoplastic Lesions by Individual Animal

2053201_2_Week_P09_Non-Neoplastic_Lesions_by_Individual_Animal.pdf

P10 – Statistical Analysis of Non-Neoplastic Lesions

2053201_2_Week_P10_Statistical_Analysis_of_Non-Neoplastic_Lesions.pdf

P14 – Individual Animal Pathology Data

2053201_2_Week_P14_Individual_Animal_Pathology_Data.pdf

P18 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site with Average Severity Grades

2053201_2_Week_P18_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site_with_Average_Severity_Grades.pdf

P40 – Survival Curves

2053201_2_Week_P40_Survival_Curves.pdf

G.4. Two-week Coal Slag Individual Animal Data – Rats

Male Individual Animal Body Weight Data

2053201_2_Week_Male_Individual_Animal_Body_Weight_Data.xls

Male Individual Animal Non-Neoplastic Pathology Data

2053201_2_Week_Male_Individual_Animal_Non_Neoplastic_Pathology_Data.xls

Male Individual Animal Survival Data

2053201_2_Week_Male_Individual_Animal_Survival_Data.xls

Male Individual Animal Terminal Body Weight Data

2053201_2_Week_Male_Individual_Animal_Terminal_Body_Weight_Data.xls

Male Individual Clinical Observations

2053201_2_Week_Male_Individual_Clinical_Observations.xls

G.5. Two-week Crushed Glass Study Tables – Rats

E03 – Growth Curves

2053401_2_Week_E03_Growth_Curves.pdf

E04 – Mean Body Weights and Survival Table

2053401_2_Week_E04_Mean_Body_Weights_and_Survival_Table.pdf

P03 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site

2053401_2_Week_P03_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site.pdf

P04 – Neoplasms by Individual Animal

2053401_2_Week_P04_Neoplasms_by_Individual_Animal.pdf

P05 – Incidence Rates of Neoplasms by Anatomic Site (Systemic Lesions Abridged)

2053401_2_Week_P05_Incidence_Rates_of_Neoplasms_by_Anatomic_Site_(Systemic_Lesions_Abridged).pdf

P09 – Non-Neoplastic Lesions by Individual Animal

2053401_2_Week_P09_Non-Neoplastic_Lesions_by_Individual_Animal.pdf

P10 – Statistical Analysis of Non-Neoplastic Lesions

2053401_2_Week_P10_Statistical_Analysis_of_Non-Neoplastic_Lesions.pdf

P14 – Individual Animal Pathology Data

2053401_2_Week_P14_Individual_Animal_Pathology_Data.pdf

P18 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site with Average Severity Grades

2053401_2_Week_P18_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site_with_Average_Severity_Grades.pdf

P40 – Survival Curves

2053401_2_Week_P40_Survival_Curves.pdf

G.6. Two-week Crushed Glass Individual Animal Data – Rats

Male Individual Animal Body Weight Data

2053401_2_Week_Male_Individual_Animal_Body_Weight_Data.xls

Male Individual Animal Non-Neoplastic Pathology Data

2053401_2_Week_Male_Individual_Animal_Non_Neoplastic_Pathology_Data.xls

Male Individual Animal Survival Data

2053401_2_Week_Male_Individual_Animal_Survival_Data.xls

Male Individual Animal Terminal Body Weight Data

2053401_2_Week_Male_Individual_Animal_Terminal_Body_Weight_Data.xls

G.7. Two-week Garnet Study Tables – Rats

E03 – Growth Curves

2053301_2_Week_E03_Growth_Curves.pdf

E04 – Mean Body Weights and Survival Table

2053301_2_Week_E04_Mean_Body_Weights_and_Survival_Table.pdf

P03 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site

2053301_2_Week_P03_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site.pdf

P04 – Neoplasms by Individual Animal

2053301_2_Week_P04_Neoplasms_by_Individual_Animal.pdf

P05 – Incidence Rates of Neoplasms by Anatomic Site (Systemic Lesions Abridged)

2053301_2_Week_P05_Incidence_Rates_of_Neoplasms_by_Anatomic_Site_(Systemic_Lesions_Abridged).pdf

P09 – Non-Neoplastic Lesions by Individual Animal

2053301_2_Week_P09_Non-Neoplastic_Lesions_by_Individual_Animal.pdf

P10 – Statistical Analysis of Non-Neoplastic Lesions

2053301_2_Week_P10_Statistical_Analysis_of_Non-Neoplastic_Lesions.pdf

P14 – Individual Animal Pathology Data

2053301_2_Week_P14_Individual_Animal_Pathology_Data.pdf

P18 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site with Average Severity Grades

2053301_2_Week_P18_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site_with_Average_Severity_Grades.pdf

P40 – Survival Curves

2053301_2_Week_P40_Survival_Curves.pdf

G.8. Two-week Garnet Individual Animal Data – Rats

Male Individual Animal Body Weight Data

2053301_2_Week_Male_Individual_Animal_Body_Weight_Data.xls

Male Individual Animal Non-Neoplastic Pathology Data

2053301_2_Week_Male_Individual_Animal_Non_Neoplastic_Pathology_Data.xls

Male Individual Animal Survival Data

2053301_2_Week_Male_Individual_Animal_Survival_Data.xls

Male Individual Animal Terminal Body Weight Data

2053301_2_Week_Male_Individual_Animal_Terminal_Body_Weight_Data.xls

G.9. Two-week Specular Hematite Study Tables – Rats

E03 – Growth Curves

2053601_2_Week_E03_Growth_Curves.pdf

E04 – Mean Body Weights and Survival Table

2053601_2_Week_E04_Mean_Body_Weights_and_Survival_Table.pdf

P03 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site

2053601_2_Week_P03_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site.pdf

P04 – Neoplasms by Individual Animal

2053601_2_Week_P04_Neoplasms_by_Individual_Animal.pdf

P05 – Incidence Rates of Neoplasms by Anatomic Site (Systemic Lesions Abridged)

2053601_2_Week_P05_Incidence_Rates_of_Neoplasms_by_Anatomic_Site_(Systemic_Lesions_Abridged).pdf

P09 – Non-Neoplastic Lesions by Individual Animal

2053601_2_Week_P09_Non-Neoplastic_Lesions_by_Individual_Animal.pdf

P10 – Statistical Analysis of Non-Neoplastic Lesions

2053601_2_Week_P10_Statistical_Analysis_of_Non-Neoplastic_Lesions.pdf

P14 – Individual Animal Pathology Data

2053601_2_Week_P14_Individual_Animal_Pathology_Data.pdf

P18 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site with Average Severity Grades

2053601_2_Week_P18_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site_with_Average_Severity_Grades.pdf

P40 – Survival Curves

2053601_2_Week_P40_Survival_Curves.pdf

G.10. Two-week Specular Hematite Individual Animal Data – Rats

Male Individual Animal Body Weight Data

2053601_2_Week_Male_Individual_Animal_Body_Weight_Data.xls

Male Individual Animal Non-Neoplastic Pathology Data

2053601_2_Week_Male_Individual_Animal_Non_Neoplastic_Pathology_Data.xls

Male Individual Animal Survival Data

2053601_2_Week_Male_Individual_Animal_Survival_Data.xls

Male Individual Animal Terminal Body Weight Data

2053601_2_Week_Male_Individual_Animal_Terminal_Body_Weight_Data.xls

G.11. Interim of 39-week Blasting Sand Study at 4 Weeks – Rats

P03 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site

2021302_4_Week_Interim_P03_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site.pdf

P04 – Neoplasms by Individual Animal

2021302_4_Week_Interim_P04_Neoplasms_by_Individual_Animal.pdf

P05 – Incidence Rates of Neoplasms by Anatomic Sites (Systemic Lesions Abridged)

2021302_4_Week_Interim_P05_Incidence_Rates_of_Neoplasms_by_Anatomic_Sites_(Systemic_Lesions_Abridged).pdf

P09 – Non-Neoplastic Lesions by Individual Animal

2021302_4_Week_Interim_P09_Non-Neoplastic_Lesions_by_Individual_Animal.pdf

P10 – Statistical Analysis of Non-Neoplastic Lesions

2021302_4_Week_Interim_P10_Statistical_Analysis_of_Non-Neoplastic_Lesions.pdf

P18 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site with Average Severity Grades

2021302_4_Week_Interim_P18_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site_with_Average_Severity_Grades.pdf

G.12. Interim of 39-week Blasting Sand Study at 16 Weeks – Rats

P03 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site

2021302_16_Week_Interim_P03_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site.pdf

P04 – Neoplasms by Individual Animal

2021302_16_Week_Interim_P04_Neoplasms_by_Individual_Animal.pdf

P05 – Incidence Rates of Neoplasms by Anatomic Sites (Systemic Lesions Abridged)

2021302_16_Week_Interim_P05_Incidence_Rates_of_Neoplasms_by_Anatomic_Sites_(Systemic_Lesions_Abridged).pdf

P09 – Non-Neoplastic Lesions by Individual Animal

2021302_16_Week_Interim_P09_Non-Neoplastic_Lesions_by_Individual_Animal.pdf

P10 – Statistical Analysis of Non-Neoplastic Lesions

2021302_16_Week_Interim_P10_Statistical_Analysis_of_Non-Neoplastic_Lesions.pdf

P18 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site with Average Severity Grades

2021302_16_Week_Interim_P18_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site_with_Average_Severity_Grades.pdf

G.13. Interim of 39-week Blasting Sand Study at 26 Weeks – Rats

P03 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site

2021302_26_Week_Interim_P03_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site.pdf

P04 – Neoplasms by Individual Animal

2021302_26_Week_Interim_P04_Neoplasms_by_Individual_Animal.pdf

P05 – Incidence Rates of Neoplasms by Anatomic Sites (Systemic Lesions Abridged)

2021302_26_Week_Interim_P05_Incidence_Rates_of_Neoplasms_by_Anatomic_Sites_(Systemic_Lesions_Abridged).pdf

P09 – Non-Neoplastic Lesions by Individual Animal

2021302_26_Week_Interim_P09_Non-Neoplastic_Lesions_by_Individual_Animal.pdf

P10 – Statistical Analysis of Non-Neoplastic Lesions

2021302_26_Week_Interim_P10_Statistical_Analysis_of_Non-Neoplastic_Lesions.pdf

P18 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site with Average Severity Grades

2021302_26_Week_Interim_P18_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site_with_Average_Severity_Grades.pdf

G.14. Thirty-nine-week Blasting Sand Study Tables – Rats

E03 – Growth Curves

2021302_39_Week_E03_Growth_Curves.pdf

E04 – Mean Body Weights and Survival Table

2021302_39_Week_E04_Mean_Body_Weights_and_Survival_Table.pdf

E05 – Clinical Observations Summary

2021302_39_Week_E05_Clinical_Observations_Summary.pdf

P03 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site

2021302_39_Week_P03_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site.pdf

P04 – Neoplasms by Individual Animal

2021302_39_Week_P04_Neoplasms_by_Individual_Animal.pdf

P05 – Incidence Rates of Neoplasms by Anatomic Sites (Systemic Lesions Abridged)

2021302_39_Week_P05_Incidence_Rates_of_Neoplasms_by_Anatomic_Sites_(Systemic_Lesions_Abridged).pdf

P09 – Non-Neoplastic Lesions by Individual Animal

2021302_39_Week_P09_Non-Neoplastic_Lesions_by_Individual_Animal.pdf

P10 – Statistical Analysis of Non-Neoplastic Lesions

2021302_39_Week_P10_Statistical_Analysis_of_Non-Neoplastic_Lesions.pdf

P14 – Individual Animal Pathology Data

2021302_39_Week_P14_Individual_Animal_Pathology_Data.pdf

P18 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site with Average Severity Grades

2021302_39_Week_P18_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site_with_Average_Severity_Grades_.pdf

P40 – Survival Curves

2021302_39_Week_P40_Survival_Curves.pdf

G.15. Thirty-nine-week Blasting Sand Individual Animal Data – Rats

Male Individual Animal Body Weight Data

2053602_39_Week_Male_Individual_Animal_Body_Weight_Data.xls

Male Individual Animal Clinical Observations

2053602_39_Week_Male_Individual_Clinical_Observations.xls

Male Individual Animal Neoplastic Pathology Data

2053602_39_Week_Male_Individual_Animal_Neoplastic_Pathology_Data.xls

Male Individual Animal Non-Neoplastic Pathology Data

2053602_39_Week_Male_Individual_Animal_Non_Neoplastic_Pathology_Data.xls

Male Individual Animal Organ Weight Data

2053602_39_Week_Male_Individual_Animal_Organ_Weight_Data.xls

Male Individual Animal Survival Data

2053602_39_Week_Male_Individual_Animal_Survival_Data.xls

G.16. Interim of 39-week Specular Hematite Study at 4 Weeks – Rats

P03 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site

2053602_4_Week_Interim_P03_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site.pdf

P04 – Neoplasms by Individual Animal

2053602_4_Week_Interim_P04_Neoplasms_by_Individual_Animal.pdf

P05 – Incidence Rates of Neoplasms by Anatomic Sites (Systemic Lesions Abridged)

2053602_4_Week_Interim_P05_Incidence_Rates_of_Neoplasms_by_Anatomic_Sites_(Systemic_Lesions_Abridged).pdf

P09 – Non-Neoplastic Lesions by Individual Animal

2053602_4_Week_Interim_P09_Non-Neoplastic_Lesions_by_Individual_Animal.pdf

P10 – Statistical Analysis of Non-Neoplastic Lesions

2053602_4_Week_Interim_P10_Statistical_Analysis_of_Non-Neoplastic_Lesions.pdf

P18 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site with Average Severity Grades

2053602_4_Week_Interim_P18_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site_with_Average_Severity_Grades.pdf

G.17. Interim of 39-week Specular Hematite Study at 16 Weeks – Rats

P03 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site

2053602_16_Week_Interim_P03_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site.pdf

P04 – Neoplasms by Individual Animal

2053602_16_Week_Interim_P04_Neoplasms_by_Individual_Animal.pdf

P05 – Incidence Rates of Neoplasms by Anatomic Sites (Systemic Lesions Abridged)

2053602_16_Week_Interim_P05_Incidence_Rates_of_Neoplasms_by_Anatomic_Sites_(Systemic_Lesions_Abridged).pdf

P09 – Non-Neoplastic Lesions by Individual Animal

2053602_16_Week_Interim_P09_Non-Neoplastic_Lesions_by_Individual_Animal.pdf

P10 – Statistical Analysis of Non-Neoplastic Lesions

2053602_16_Week_Interim_P10_Statistical_Analysis_of_Non-Neoplastic_Lesions.pdf

P18 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site with Average Severity Grades

2053602_16_Week_Interim_P18_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site_with_Average_Severity_Grades.pdf

G.18. Interim of 39-week Specular Hematite Study at 26 Weeks – Rats

P03 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site

2053602_26_Week_Interim_P03_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site.pdf

P04 – Neoplasms by Individual Animal

2053602_26_Week_Interim_P04_Neoplasms_by_Individual_Animal.pdf

P05 – Incidence Rates of Neoplasms by Anatomic Sites (Systemic Lesions Abridged)

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P09 – Non-Neoplastic Lesions by Individual Animal

2053602_26_Week_Interim_P09_Non-Neoplastic_Lesions_by_Individual_Animal.pdf

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G.19. Thirty-nine-week Specular Hematite Study Tables – Rats

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2053602_39_Week_E03_Growth_Curves.pdf

E04 – Mean Body Weights and Survival Table

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E05 – Clinical Observations Summary

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P05 – Incidence Rates of Neoplasms by Anatomic Sites (Systemic Lesions Abridged)

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P10 – Statistical Analysis of Non-Neoplastic Lesions

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P14 – Individual Animal Pathology Data

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P40 – Survival Curves

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G.20. Thirty-nine-week Specular Hematite Individual Animal Data – Rats

Male Individual Animal Body Weight Data

2053602_39_Week_Male_Individual_Animal_Body_Weight_Data.xls

Male Individual Animal Clinical Observations

2053602_39_Week_Male_Individual_Clinical_Observations.xls

Male Individual Animal Neoplastic Pathology Data

2053602_39_Week_Male_Individual_Animal_Neoplastic_Pathology_Data.xls

Male Individual Animal Non-Neoplastic Pathology Data

2053602_39_Week_Male_Individual_Animal_Non_Neoplastic_Pathology_Data.xls

Male Individual Animal Organ Weight Data

2053602_39_Week_Male_Individual_Animal_Organ_Weight_Data.xls

Male Individual Animal Survival Data

2053602_39_Week_Male_Individual_Animal_Survival_Data.xls



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