# EVALUATION OF HEALTH HAZARD OF AMORPHOUS SILICA-COATED CRISTOBALITE FOLLOWING INTRATRACHEAL INJECTION IN RATS

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## FOREWORD

On May 14, 1971, The National Institute for Occupational Safety and Health (NIOSH) received a request from Mr. James B. Butler, Assistant to the President, Kawecki Berylco Industries, Inc., Reading, Pennsylvania, to evaluate the potential health hazards of samples of silica dusts collected from their National Metallurgical Company plant in Springfield, Oregon. Prior to this request, in March, 1971, a group of concerned citizens living near the Springfield, Oregon, plant contacted Dr. V. Boekelheide, Head, Department of Chemistry, University of Oregon, to determine whether or not the air pollution produced by the plant was a hazard to the health of people living in the valley. In July, 1971, airborne samples of the siliceous material were collected from the plant and sent to NIOSH by Mr. Darrell D. Douglas, P. E., Chief Industrial Hygiene Engineer, Occupational Health Section, Oregon State Board of Health, Portland, Oregon.

SUMMARY

The purpose of this study was to determine the subchronic toxicity of six different samples of silica dusts relative to a standard quartz sample. The samples of silica dusts represented aliquots of material taken from the baghouses of several work areas in the National Metallurgical plant in Springfield, Oregon. The study comprised nine groups of 15-30 rats per group. Each rat in eight of the groups was injected with a single dose, intratracheally, of either 10 mg of one of the silica dusts, 10 mg of the quartz reference dust or isotonic saline. The rats in the ninth group were not injected at all. Sacrifices occurred 3, 6, and 9 months after the injections. Tracheobronchial lymph nodes, lungs (either right or left or both), and spleens were examined microscopically. A large number of the animals contracted pneumonia which was therapeutically treated with addition of an antibiotic in the drinking water. Pneumonia was present in 10-30% of the lungs of most groups. The silica dusts produced granulomatous lesions similar to those seen in early silicosis with the progressive production of collagen up to six months. At that time the amount of collagen appeared to be greater than that produced by the reference quartz sample, but by nine months the collagenization was the same. There was some interstitial fibrosis noted in the lungs which was believed to be related to the combination of superimposed pneumonitis and the particles. There were no pathologic differences

among the animals treated with the silica dusts.

Inhalation of any of the submitted samples would appear to be injurious to the health of workers exposed to these dusts and as such should be avoided. Since these silica dusts samples produced granulomatous lesions with progressive collagenization of the lungs through nine months which was similar to the quartz reference sample, it is recommended that the threshold limits for these siliceous dusts be based on that described for quartz. In addition, it is recommended that engineering controls be installed within the National Metallurgical plant to prevent the dusts from escaping to the outside air and surrounding community. Persons working within the plant should be provided with appropriate preventive respiratory inhalation equipment whenever the atmospheric concentration of the dust exceeds the TLV, such as immediately following the "blowing" of the furnaces.

## Introduction

Representative baghouse samples of certain siliceous dusts were collected from various working areas within the National Metallurgical Company plant in Springfield, Oregon. The materials were received by NIOSH in their original collection tubes.

Dr. Virgil Boekelheide, Department of Chemistry, University of Oregon, analyzed samples of these same siliceous dusts. He describes the material as having a

cristobalite core covered by amorphous silica. To our knowledge, a siliceous material with this type of composition has never been tested toxicologically. A review of chest x-rays of personnel employed in the plant suggest pulmonary impairment in eleven subjects but the diagnosis and causation remain equivocal. Until a relationship is established between the pulmonary impairment and dust exposure, it would appear that the amorphous silica covering protects against fibrosis that the cristobalite core has the potential of producing; however, silicosis may not develop until after exposure of 20 years or so depending upon the intensity of exposure and the particle size and character of the crystalline silica. The employees now in the plant have exposure experience of 5 to 18 years.

## Objective

This study was conducted to evaluate the toxic response and pathologic potential, primarily pulmonary fibrosis, of the silica dusts samples following a single intratracheal injection into laboratory rats.

## Material

Six different "baghouse" samples of the siliceous material were studied. In addition, quartz (glass sand) was used as a positive control sample. The six test materials and the positive control material were measured for particle size and surface area determination by nitrogen adsorption

employing an automatic surface area analyzer and were described as follows:

| Sample | Particle Size Geo. Mean μm | Particle Size Range μm | Surface Area $M^2/g$ |
|---|---|---|---|
| 1 | 0.34 | 0.16-2.28 | 33.7 |
| 2 | 0.35 | 0.16-3.03 | 32.1 |
| 3 | 0.33 | 0.16-2.02 | 27.6 |
| 4 | 0.29 | 0.15-1.41 | 27.4 |
| 5 | 0.31 | 0.15-1.37 | 24.7 |
| 6 | 0.34 | 0.16-1.78 | 27.3 |
| Quartz | 0.25 | -- | -- |

## Methods

### Range-Finding Study

A preliminary range-finding study was conducted to determine the effectiveness of the intratracheal injection technique and to assess the possible potentiation of pulmonary disease and onset of fibrogenesis.

Three of the six test materials, samples 1, 3, and 5, and the positive control sample, quartz, were each administered as a single dose, intratracheally, to Caesarean-derived, male, albino rats weighing between 274 and 312 grams. The animals were observed for 3 months. Twenty animals, 5 for each test and control material, were used. The control and test materials, 10 mg of each, were suspended in 0.4 cc saline and administered as a single intratracheal injection to the animals within their respective

groups followed by a 0.4 cc saline rinse. The total volume of the dose was 0.8 cc/rat. The saline solution was autoclaved prior to administration to the animals. An effort was made to administer the materials so that it would be dispersed to all lobes of the lungs. The animals were housed in their respective groups, 5 per cage, with standard laboratory rat chow and water available at all times. Individual body weights were recorded initially, daily for the first 3 days, and biweekly thereafter for 3 months. Observations for appearance, behavior, and mortality were made daily. Two animals from each sample and positive control group were sacrificed, (pentobarbital sodium overdose) on the third day; all remaining animals were sacrificed (pentobarbital sodium overdose) at the end of 3 months. A gross evaluation of all major tissues and organs was made at the time of sacrifice. The lungs of the animals sacrificed were preserved in neutral buffered formalin, but were not evaluated histopathologically.

Nine-Month Study

One hundred and twenty-five male (250-280 g) and ninety-five female (200-225 g), Caesarean-derived, albino rats, were selected at random and divided into the following groups:

| Group | No. of Animals | | Treatment |
|---|---|---|---|
| | M | F | |
| 1 | 15 | (0) | Placebo (negative) control |
| 2 | 15 | (0) | Quartz (positive) control |
| 3 | 15 | 15 | Baghouse sample 1 |
| 4 | 15 | 15 | Baghouse sample 2 |
| 5 | 15 | 15 | Baghouse sample 3 |
| 6 | 15 | 15 | Baghouse sample 4 |
| 7 | 15 | 15 | Baghouse sample 5 |
| 8 | 15 | 15 | Baghouse sample 6 |
| 9 | 5 | 5 | Untreated control |

The materials, 10 mg of each, were suspended in 0.4 cc saline and administered as a single dose, intratracheally, to animals within the respective groups, followed by a 0.4 cc saline rinse. The total volume of the dose was 0.8 cc/rat. The placebo control animals received 0.8 cc saline. The saline solution was autoclaved prior to administration. An effort was made to administer the material so that it was dispersed to all lobes of the lungs. The animals were housed within their respective treatment groups, 5 per cage, with standard lab chow and water available at all times. Individual body weights were recorded initially and at frequent intervals thereafter during the course of the study. All animals were observed daily for appearance, behavior, and mortality. At 3-, 6-, and 9-month intervals a

representative number of animals from each control (except the untreated control) and experimental group were sacrificed. Efforts were made to sacrifice any animal that was considered moribund. A gross evaluation of all tissues or organs was made on each animal sacrificed or found dead. Organ weight and organ/body weight ratios were determined for the lungs and spleen. The spleen, lungs, and tracheobronchial lymph nodes were preserved in neutral buffered formalin and subsequently examined histopathologically.

## Results

### Range-Finding Study

Most of the animals from each group lost weight during the first 2 weeks following intratracheal administration. After the second week all animals gained weight steadily through termination of the study. A few animals, scattered among all groups, exhibited signs of respiratory complications characterized by rales and/or a nasal exudate. Otherwise, all animals appeared normal in appearance and behavior throughout the study.

Upon sacrifice of the animals at the 3-day and 3-month intervals, the test materials were evident in the lungs and appeared evenly distributed throughout all lobes. Signs of pneumonia were noted in the lungs of some animals sacrificed at 3 months. Other than these observations, all other tissues and organs appeared normal.

Nine-Month Study

A large number of the animals, both male and female, in each control and test group developed pneumonia during the course of this study. Some animals developed pnemonia during the third week following compound administration which persisted and became progressively worse through termination of the study. On several occasions all animals received Polyotic, an antibiotic, in their drinking water which kept the disease controlled for short periods of time. The disease was more prevalent among the male animals. Several animals died or were sacrificed in a moribund condition as a result of the pneumonia. The following is a summary of these deaths:

| | Group | Male | Female |
|---|---|---|---|
| 1. | Negative (placebo control) | 0 | - * |
| 2. | Quartz (positive control) | 5 | - * |
| 3. | Baghouse sample 1 | 1 | 1 |
| 4. | Baghouse sample 2 | 5 | 3 |
| 5. | Baghouse sample 3 | 3 | 0 |
| 6. | Baghouse sample 4 | 2 | 0 |
| 7. | Baghouse sample 5 | 1 | 2 |
| 8. | Baghouse sample 6 | 1 | 1 |
| 9. | Untreated control | 2 | 2 |

* - (not applicable)

The pneumonia was not believed to be related to the test materials since the disease was noted in all groups including the controls. However, it is possible that the stress caused by the intratracheal injection technique and/or the particulate made the animals more susceptible to pneumonia.

One female rat that received sample 2 developed a subcutaneous tumor in the right axilla which was first observed during the fourth month on study. The tumor grew in size and the animal died during the eighth month. The tumor was not considered to be related to the test material.

Aside from the respiratory complications and pneumonia symptoms noted among some animals in all groups and the one female animal with the subcutaneous tumor, no other significant clinical symptoms were noted.

Body weight gains among both males and females in all groups were erratic due to the health condition of the animals. Likewise, the lung weight/body weight ratios were inconclusive due to the presence of pneumonia in the lungs. The spleen weight/body weight ratios were comparable among the animals, both males and females, in the test and control groups which with the pathologic findings described below, indicates that the material is not toxic to the spleen.

Gross necropsy observations of the dead animals and those animals sacrificed at 3, 6, 9 months included pneumonia in the lungs of some animals in all groups and enlarged and dark gray tracheobronchial lymph nodes among the

animals that received the silica dusts samples. The lungs of the silica dusts treated animals were also grayish in appearance which indicated that the materials were dispersed to all lobes of the lungs. These materials were still present in the lungs after 9 months of study.

Microscopic examination of the lungs injected with the six samples revealed lesions similar to those seen with the quartz reference sample. There was no difference between the samples.

Pulmonary lesions in the silica dusts treated animals at the 3-month sacrifice were primarily cellular, consisting of lymphocytes, macrophages, and fibroblasts. Very small (0.5μm) birefringent crystals, aggregates of these crystals, and black particles were scattered throughout the granulomas. Collagen fibers were arranged in a random fashion in the same areas. The lymph nodes, even at this early stage, contained the dust and moderate amount of excess collagen. The amount of collagen in both the lungs and lymph nodes of the silica dust animals appeared to be greater than that in the quartz reference animals.

By 6 months the numbers of cells had decreased and the amount of collagen had increased in the pulmonary granulomas. In addition, the collagen radiated from the granulomas into the alveolar walls giving the appearance

of interstitial fibrosis. The collagen was more abundant in the lymph nodes. The lungs and lymph nodes of the quartz reference animals still contained less collagen than the silica dust animals.

In the 9-month rats there was no apparent increase in the amount of collagen, but a reduction in the number of cells in the granulomas. The quartz reference and silica dust lungs appeared to contain about the same amount of collagen at this sacrifice. The collagen was still arranged in a haphazard fashion in the granulomas of all groups. The failure of these lesions to progress to classical, hyalinized, silicotic nodules is probably related to the size of the particles. In this laboratory, as well as others, classical silicosis is best produced with quartz particles ranging in size from one to three micrometers in diameter.

Pneumonia was present in 10-30% of the lungs of most groups and probably did contribute to the production of the interstitial fibrosis.

No significant abnormalities or particles were seen in the spleens in any of the groups.

The conclusions from the histopathology are as follows: The silica dusts produced granulomatous lesions similar to those seen in early silicosis

with the progressive production of collagen up to 6 months. At that time the amount of collagen appeared to be greater than that produced by the reference quartz sample, but by 9 months the extent of collagenization was the same. In addition, there was some interstitial fibrosis radiating from the granulomas in all groups. This phenomenon could be explained by the combination of superimposed pneumonitis and the silica dusts, since it usually does not occur with particle size greater than one micrometer in diameter.

The results of this study indicate that the silica dusts samples collected from the National Metallurgical Corporation plant in Springfield, Oregon, do possess the potential for inducing a fibrogenic pulmonary response following inhalation of sufficient quantity of the material. Since these silica dusts samples produced granulomatous lesions with progressive collagenization of the lungs through nine months which was similar to the quartz reference sample, it is recommended that the threshold limits for these siliceous dusts be based on that described for quartz. In addition, it is recommended that engineering controls be installed within The National Metallurgical plant to prevent the dusts from escaping to the outside air and surrounding community. Persons working within the plant should be provided with appropriate preventive respiratory inhalation equipment whenever the atmospheric concentration of the dusts exceeds the TLV, such as immediately following the "blowing" of the furnaces.