

Differential pulmonary responses in rats inhaling crystalline, colloidal or amorphous silica dusts

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Pulmonary responses in rats were compared after short-term inhalation exposure to polymorphs of silica dust. Groups of CD rats were exposed 6 h a day for 3 d to crystalline silica or amorphous silica. Another group was exposed to Ludox colloidal silica for 6 h a day, 5 d a week for two or four weeks. Thereafter the groups were killed, and the lungs washed at several postexposure times. The crystalline silica produced persistent pulmonary inflammatory responses characterized by neutrophil recruitment and consistently elevated biomarkers of cytotoxicity in bronchoalveolar lavage fluids, and progressive histopathological lesions were observed within one month of the exposure. Amorphous silica produced a transient pulmonary inflammatory response, and Ludox elicited transient pulmonary inflammatory responses at 50 or 150 mg · m⁻³ but not at 10 mg · m⁻³. After three months most of the biochemical values of the Ludox-exposed animals had returned to the control level. These results demonstrate that crystalline silica dust is more potent in producing pulmonary toxicity when compared with amorphous or colloidal silica particles.

Key terms amorphous, colloidal, crystalline, fibrosis, inflammation, persistent effects, pulmonary, silica, transient effects.

Chronic inhalation of crystalline silica dust causes pulmonary inflammation and the development of fibrosis in experimental animals and exposed humans (1, 2). After acute inhalation exposure of rats, a persistent inflammatory response ensued which progressed to alveolar proteinosis and fibrosis. While it is widely accepted that exposure to the crystalline species of silica dust elicits toxic pulmonary effects, there is limited information regarding lung responses to other forms of silica dust. Accordingly, this report is a compilation of four bioassay studies implemented to compare pulmonary responses in rats after short-term inhalation exposure to several polymorphs of silica dust. The dust comprised two forms of crystalline silica (α -quartz and α -cristobalite), one form of amorphous silica, and one form of colloidal silica particles.

Cells and fluids from groups of sham and dust-exposed animals were recovered by bronchoalveolar lavage (BAL). Lactate dehydrogenase (LDH), protein, and N-acetyl glucosaminidase (NAG) were measured in BAL fluids several times postexposure. The cells were identified, counted, and evaluated for viability. The lungs of additional animals were processed for histopathology, and these results have been reported elsewhere (3, 4). The data generated from these studies provide an interesting comparison of differential pulmonary responses in rats inhaling various forms of silica dust.

Materials and methods

General experimental design. This study was designed to evaluate pulmonary responses in rats after short-term inhalation exposure

to two forms of crystalline silica, one form of amorphous silica and one form of colloidal silica particles. Crystalline silica particles, in the form of Berkeley Min-U-Sil-5, were obtained from the Pennsylvania Glass and Sand Corporation (Pittsburgh, Pennsylvania, United States). α -Cristobalite silica was obtained from the C & E Mineral Corporation (King of Prussia, Pennsylvania, United States) and was a generous gift from Dr David Hemenway from the University of Vermont. Ludox colloidal silica was obtained from DuPont Chemicals. Amorphous silica particles (Zeofree 80) were purchased from the JM Huber Co, Havre de Grace, Maryland. These particles are known to be free of crystallinity (Dr David Hemenway, personal communication).

For two of the inhalation toxicity studies, groups of 24 CD rats were exposed for 3 d at aerosol concentrations of either 10 or 100 mg · m⁻³ to either cristobalite or amorphous silica. In another study with Min-U-Sil α -quartz particles, 24 rats were exposed for 6 h a day for 3 d to concentrations of 100 mg · m⁻³. In the fourth study, rats were exposed to Ludox for 6 h a day, 5 d a week for two or four weeks at concentrations of 10, 50, or 150 mg · m⁻³. After the termination of the exposures, groups of animals were sequentially killed and their lungs washed several times postexposure.

Inhalation exposure and biochemical assays. The dust generation methods have been previously reported (3, 4). The measured mass median aerodynamic diameter (MMAD) ranges for the various generated silica polymorph particles were the following: cristobalite crystalline silica 3.4—3.6 μ m, Zeofree 80

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2.4–3.4 μm , Ludox 2.9–3.7 μm , and Min-U-Sil 3.3–3.5 μm . BAL and cellular quantification procedures were conducted according to previously described methods (5).

Results

The cell differential analyses of rats exposed to Min-U-Sil and cristobalite indicated that inflammation was a prominent feature in the lungs of rats exposed to crystalline silica. Pulmonary inflammatory responses, characterized by the presence of granulocytes (primarily neutrophils) in BAL fluids, persisted throughout a three-month postexposure period (figure 1). In contrast, exposure to amorphous silica produced a transient inflammatory response which was present 24 h postexposure but was abrogated within 8 d postexposure (figure 1). Similarly, exposures to Ludox for two or four weeks at 50 and 150 $\text{mg} \cdot \text{m}^{-3}$, but not at 10 $\text{mg} \cdot \text{m}^{-3}$, produced increased numbers of lavaged granulocytes ($P < 0.05$), but these numbers were significantly reduced following a three-month recovery period. Extracellular LDH in the BAL fluids was considered to be a sensitive indicator of pulmonary cytotoxicity. Transient increases in LDH were measured in rats exposed to amorphous silica for 3 d. The BAL fluid LDH values increased within 24 h after exposure but returned to control levels by 8 d postexposure (figure 2). In rats exposed to 150 $\text{mg} \cdot \text{m}^{-3}$ of Ludox, the LDH was significantly increased above the control value at two or four weeks, but was reduced after the recovery period (table 1). No significant differences were measured for LDH at any time post-exposure between the rats exposed to 10 or 50 $\text{mg} \cdot \text{m}^{-3}$ concentrations of Ludox and the controls (table 1). In contrast, the LDH was

increased within 24 h after Min-U-Sil and cristobalite exposure, and it remained elevated throughout a three-month postexposure period. Rats exposed to cristobalite for 3 d demonstrated a 12-fold increase in LDH three months after the exposure (figure 2).

The assessments of protein in BAL fluids showed a trend similar to that described for LDH activity. Exposures to Ludox for two or four weeks or to amorphous silica for 3 d produced transient increases in BAL fluid protein (table 1, figure 3). In contrast, exposure to cristobalite or Min-U-Sil produced sustained effects which were substantially increased over those of the controls ($P < 0.05$) three months postexposure (figure 3). Likewise, BAL fluid protein levels were transiently increased in rats exposed to amorphous silica and were sustained in all of the groups of animals exposed to crystalline silica (figure 3).

NAG was significantly increased in BAL fluids ($P < 0.05$) after a 3-d exposure to cristobalite, while the NAG values of rats exposed to amorphous silica did not significantly differ from those of controls by 8 d postexposure (figure 4). Similarly, the NAG values of rats exposed to Min-U-Sil were significantly increased after the 3-d exposure. This increase was sustained throughout the postexposure period (figure 4).

Discussion and concluding remarks

Chronic inhalation of crystalline silica produces pulmonary inflammation and the consequent development of silica-induced pulmonary fibrosis (1, 2). During the acute phase, a pulmonary inflammatory response develops and progresses to alveolar proteinosis and a granulomatous-type pattern of disease in animals.

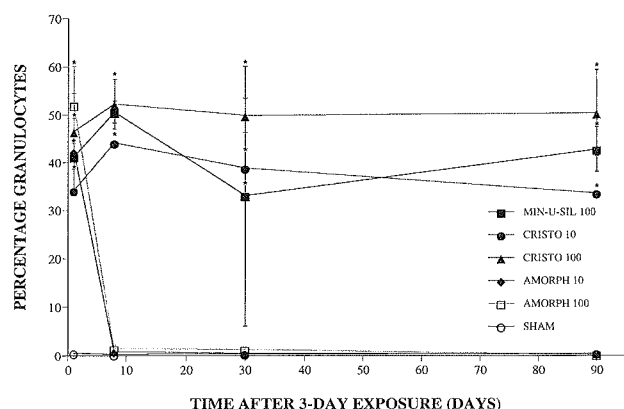


Figure 1. Granulocyte differential percentages for bronchoalveolar lavage fluids of rats exposed to amorphous silica (AMORPH), Min-U-Sil, or cristobalite (CRISTO) ($P < 0.05$).

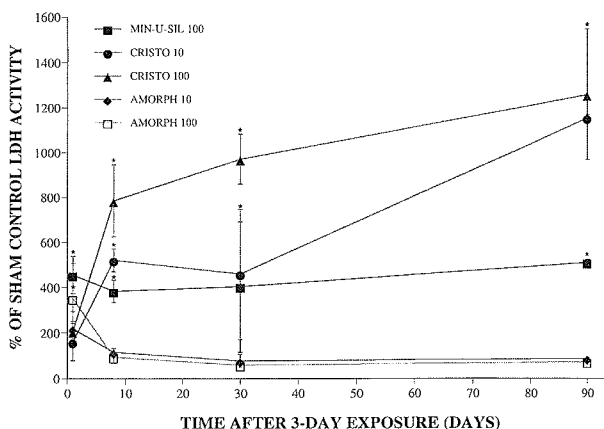


Figure 2. Lactate dehydrogenase (LDH) values for bronchoalveolar lavage fluids of rats exposed to cristobalite (CRISTO), Min-U-Sil, or amorphous (AMORPH) silica ($P < 0.05$).

Table 1. Lactate dehydrogenase and protein in rats exposed to Ludox colloidal silica – percentage of sham control responses.

Ludox	Lactate dehydrogenase						Protein					
	Two-week exposure, no recovery		Four-week exposure, no recovery		Four-week exposure, three-month recovery		Two-week exposure, no recovery		Four-week exposure, no recovery		Four-week exposure, three-month recovery	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
10 $\text{mg} \cdot \text{m}^{-3}$	99.1	27.9	108.5	26.5	89.8	11.2	144.9	36.1	89.8	.	91.2	.
50 $\text{mg} \cdot \text{m}^{-3}$	110.5		365.9	91.1	159.2	19.8	92.3	19.5	159.2	20.4	75.4	7.5
150 $\text{mg} \cdot \text{m}^{-3}$	437	100*	1033	197*	172	72	224	32*	423	104*	125	15

* $P < 0.05$.

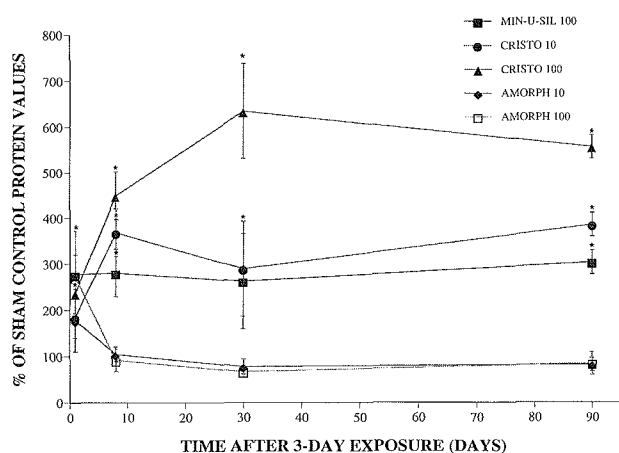


Figure 3. Protein levels of bronchoalveolar lavage fluids in rats exposed to cristobalite (CRISTO), Min-U-Sil, or amorphous (AMORPH) silica ($P < 0.05$).

During the chronic phase, nodular fibrosis occurs in exposed animals and humans. Although the pathological effects of crystalline silica are well known, there is a paucity of information on the pulmonary effects of inhaled amorphous and colloidal forms of silica. The limited toxicologic information available suggests that the pulmonary effects following exposures to amorphous silicates may be reversible in the absence of continuing exposure (6–9). Moreover, in elegant studies presented at the first silica, silicosis and cancer symposium, Hemenway et al (10) exposed rats for 8 d to aerosols of one of three silica species, α -cristobalite, α -quartz or amorphous silica. The greatest measure of lung injury was produced with cristobalite, which caused substantial inflammation and fibrosis. Exposures to α -quartz produced intermediate effects, while amorphous silica produced minimal effects. The authors concluded that amorphous silica particles were less toxic than the different species of crystalline silica polymorphs. In support of the results of Hemenway et al, the results reported in previous studies indicate that Ludox is significantly less active in producing pulmonary effects when compared with Min-U-Sil (3, 4).

In conclusion, brief exposures to two different forms of crystalline silica particles produced a persistent pulmonary inflammatory response, characterized by neutrophil recruitment and consistently elevated biomarkers of cytotoxicity in BAL fluid. Progressive histopathologic lesions were previously observed within one month after a 3-d exposure (3). In contrast, a 3-d exposure to amorphous silica particles produced a transient pulmonary inflammatory response, and two- or four-week exposures to Ludox elicited pulmonary inflammation at 50 or 150 $\text{mg} \cdot \text{m}^{-3}$ but not at 10 $\text{mg} \cdot \text{m}^{-3}$. Most of the biochemical parameters returned to control values after a three-month recovery period. These results demonstrate that the crystalline forms of silica dust are much more

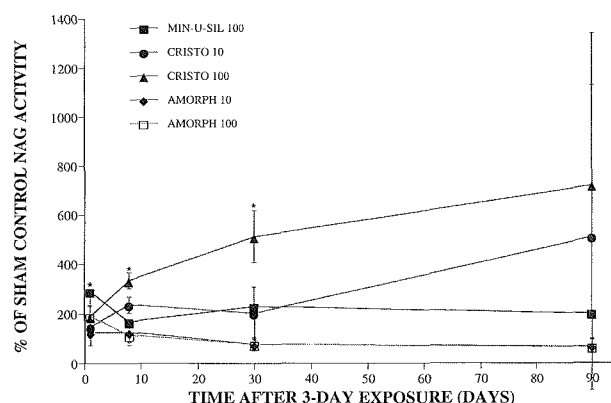


Figure 4. N-acetyl-glucosaminidase (NAG) values of bronchoalveolar lavage fluids in rats exposed to cristobalite (CRISTO), Min-U-Sil, or amorphous (AMORPH) silica ($P < 0.05$).

potent in producing pulmonary toxicity than amorphous or colloidal forms of silica are.

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