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# NOTE

## Short term inhalation exposure to silica aerosol on respiratory parameters monitored by online computer programme in mice

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Silicosis or pulmonary fibrosis is caused by inhaling dusty air polluted with silica. It is an occupational hazard for workers in construction industry as well as mining. While there are studies available on long term exposure to such dust, reports on short term exposure are scarce. Hence, in the present study, we exposed mice to silica aerosol repeatedly and monitored the respiratory parameters during inhalation by online computer programme for short term. Mice were exposed in a head-out-body-plethysmograph to 150 mg $m^{-3}$ of silica aerosol generated by an airblast nebulizer for 4 h and for 5 days continuously. The respiratory changes were monitored by a volumetric pressure transducer and analyzed by an online computer programme capable of quantifying the breathing pattern and the respiratory variables. Immediately after the start of silica inhalation the normal breathing pattern decreased and airway obstruction pattern increased. There was no sensory or pulmonary irritation pattern. Correspondingly, the frequency increased and the tidal volume decreased. After the end of inhalation exposure, only partial recovery was observed. On the next day, the respiratory frequency was increased and the tidal volume decreased before the start of silica inhalation compared to the previous day. Following exposure to silica on subsequent days the respiratory frequency further increased and the tidal volume decreased. This study shows that silica aerosol inhalation can cause an immediate change in the respiratory variables with an increase in the respiratory frequency and decrease in tidal volume (rapid shallow breathing), and a breathing pattern of airway obstruction.

**Keywords:** Air pollution, Airway obstruction, Mining, Pulmonary fibrosis, Short term exposure, Silicosis

Silica ($SiO_2$) is a fibrogenic material present in sand, quartz and rocks. Exposure to silica particles causes pulmonary inflammation and damage to the lung tissues known as silicosis[1]. It is an occupational disease and workers involved in mining and quarrying of silica are highly susceptible. Exposure to silica is more injurious than other nuisance dusts like titanium dioxide or iron particles[2,3]. Silica exposure causes increased damage with other lung damaging diseases[4]. Evidences show that even after stopping exposure, silicosis may develop. Animal experimentation also showed that pulmonary fibrosis can develop even after stopping the exposure[5].

Ninety day exposure to silica aerosols showed an increase in lung and tracheobronchial lymph node weight, and histopathological changes in the lung. Similar effects were observed in short term exposure for 6 h and 5 days[6]. Exposure for 1 hr for 6 days also showed histopathological changes in the lungs and lymphatic tissues[7]. The minimum time required for the development of inflammatory response has been shown to be 2 h[8]. Reports are available that short and long term exposure to silica can cause similar effects[9].

Several reports are available on long term exposure to silica, but less information is available on short term exposures. We have earlier designed to quantify the breathing pattern and respiratory variables of unanaesthetised mice while exposure to the aerosols of various chemicals[10,11]. This method can characterize chemicals as sensory irritants, pulmonary irritants and chemicals causing airway obstruction. In the present study we to used the above method to observe the breathing pattern of mice during and after short term exposure to silica.

## Materials and Methods

### Animals

Male Swiss mice weighing 25-30 g bred and maintained at the Animal Facility of Defence Research and Development Establishment, Gwalior were used. They were housed in polypropylene cages, four per cage, with sterilized paddy husk as the bedding material. The animals were kept at 25±2ºC, relative humidity 40-70% and natural light/dark cycle. The mice were provided with pellet diet and water *ad libitum*. The care and maintenance of the animals were as per the approved guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, India). This study was approved by the Institutional Animal Ethics Committee.

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**Chemicals**

Amorphous silica was purchased from M/s Sigma-Aldrich (USA) and the purity was about 99 %. The particle size varied between 0.5-10 μm of which about 80% were between 1-5 μm.

**Inhalation exposure**

The silica was suspended in water (6 %; 200 mL) in a 250 mL conical flask and continuously stirred by keeping on a magnetic plate. The conical flask was connected to an air blast nebuliser. Pressurised air at 10 psi (1 psi = 0.075 kg/cm²) was passed through the nebulizer and the negative pressure created sucked the liquid from the flask. The excess liquid was made to return to the flask. After 2 h, the contents of the conical flask were changed with fresh silica suspension (6 %; 200 mL) to make the concentration uniform. The nebulized silica aerosol was diluted with filtered air (total 20 L) in a glass helical assembly and passed into the dynamically operated glass animal exposure chamber. The capacity of the exposure chamber was 4 L (Fig. 1).

The animals were exposed to silica aerosols head only in a glass body plethysmograph (head-out-body-plethysmograph). The body portion was also ventilated by continuously drawing air at the rate of 170 mL/min using a critical orifice (27 gauge needle). The plethysmograph was connected to a volumetric pressure transducer (model PT5, Grass Instrument, USA) for recording the respiratory flow signals. The signals from the individual transducers were amplified using universal amplifiers (Gould, USA). The amplified signals were digitised using an analog to digital converter (Metrabyte, Taunton, USA) and stored and analysed in a personal computer. The amplified signals from the amplifiers were also fed into an oscillograph for recording of breathing pattern (WindoGraf, Gould, USA). A computer programme (developed in the University of Pittsburgh, USA) for monitoring (i) breathing pattern and (ii) respiratory variables was used[11]. The inhalation chamber was equipped with ports for monitoring temperature, humidity and also for sampling chamber air for estimation of concentration of test chemical. The chamber was operated dynamically and the exit aerosol was passed through a series of scrubbing units containing alkali solution and cotton wool to a suction pump, and out of the room[12]. The whole inhalation exposure assembly was housed in a fume hood.

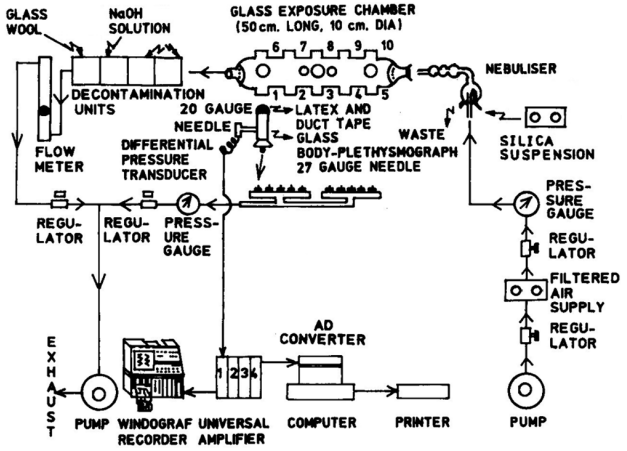


Fig. 1 — Schematic representation of inhalation exposure assembly.

**Measurement of respiratory variables**

The glass inhalation exposure chamber could accommodate 10 animals at one time and out of that 4 animals respiratory signals can be monitored by the online computer programme and analyzed. The mice were gently guided inside the head-out-body-plethysmograph and closed with a rubber bung[10,11]. They were acclimatised inside the plethysmograph for 30 min, followed by 30 min base line control data recording. Thereafter, the animals were exposed for 4 h continuously with a post exposure 30 min recovery period. The same animals were exposed for 5 days continuously with the same protocol (repeated exposure). The time of exposure was kept constant on all the 5 days. The breathing pattern was characterized as normal, sensory irritation, pulmonary irritation, airflow limitation (airway obstruction) and their combinations. Among the respiratory variables recorded are, tidal volume (VT), respiratory frequency (f), airflow at middle of expiration (VD), time of inspiration (TI), time of expiration (TE), time of brake at the end of inspiration (TB) and time of pause at the end of expiration (TP).

**Analysis of chamber air**

The exposure chamber air was analyzed gravimetrically using Whatman 1 filter paper for determining of concentration of silica in the chamber air. The chamber air was sucked out through a Millipore double-cone filter disc holder (Millipore, USA) fitted with a pre weighed Whatman 1 filter paper, at a flow rate of 2.0 LPM for 5 min. The filter paper was removed and weighed again. The difference in weight multiplied by 100 gives the concentration of silica aerosol inside the chamber per cubic meter.

**Statistical analysis**

The experiment was carried out twice and the pooled data was analyzed for respiratory parameters by (i) one way ANOVA with Dunnett's multiple comparison test for comparison with control; and (ii) two way repeated measures ANOVA followed by Holm-Sidak test for comparison with pre-exposure control values. A probability of 0.05 and less was taken as statistically significant. The analysis and plotting of graphs were carried out using SigmaPlot 13 (Systat Inc, USA).

## Results

The concentration of silica inside the exposure chamber, near the breathing zone of the mice was found to be 150±20 mg/m³, by gravimetric analysis. In the present set up, the respiratory variables of mice could be recorded continuously for a long time to fresh air, without any change in the variables as shown in Fig. 2. During the baseline control data collection there was good consistency in the breathing pattern of the mice. Table 1 shows the control values of the mice before the silica aerosol exposure. Immediately after the start of silica inhalation, the normal pattern decreased and airflow limitation (airway obstruction) increased. There was no sensory or pulmonary irritation pattern. After the end of inhalation exposure, partial recovery was observed. Correspondingly, the frequency increased and the

Table 1 — Respiratory variables of male mice – base line data before exposure

| Variable | Day 0 (Control) | Day 1 (Before Exposure) | Day 2 (After 1 Exposure) | Day 3 (After 2 Exposures) | Day 4 (After 3 Exposures) | Day 5 (After 4 Exposures) | F | P |
|---|---|---|---|---|---|---|---|---|
| VT(mL) | 0.04±0.005 | 0.04±0.002 | 0.028±0.007 | 0.038±0.010 | 0.062±0.026* | 0.064±0.007* | 12.3 | <0.001 |
| F (breaths/minute) | 211±11 | 226±28 | 267±26* | 259*±28 | 254±17* | 237±17* | 20.66 | <0.001 |
| VD (mL/s) | 0.351±0.071 | 0.393±0.062 | 0.323±0.113 | 0.376±0.065 | 0.643±0.234* | 0.624±0.152* | 10.13 | <0.001 |
| TI (s) | 0.117±0.008 | 0.113±0.012 | 0.112±0.033 | 0.097±0.009 | 0.101±0.006 | 0.105±0.004 | 2.46 | NS |
| TE (s) | 0.175±0.011 | 0.169±0.021 | 0.155±0.025* | 0.153*±0.021 | 0.147±0.014* | 0.163±0.020 | 7.54 | <0.001 |
| TB (as % of TI+TE) | 16±3.4 | 17.1±2.0 | 17.4±1.8 | 14.1±1.6 | 17.7±2.8 | 16.5±2.1 | 3.25 | <0.01 |
| TP (as % of TI+TE) | 12.5±0.4 | 13.6±1.6 | 14.5±1.6* | 13.8±2.0 | 12.4±1.2 | 13.1±1.2 | 2.79 | <0.05 |

[Values are mean ± SEM (n =8). *Statistically significant from Day 1(control) by one way ANOVA with Dunnett's test]

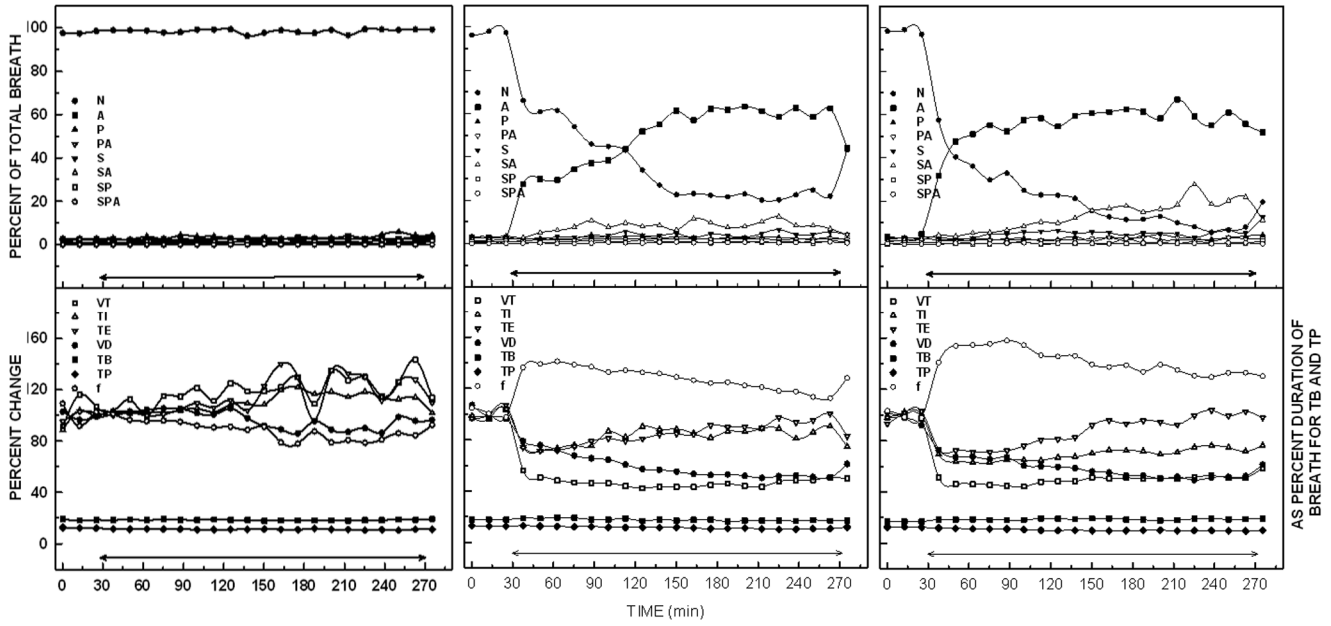


Fig. 2 — Time response analysis of breath classification and measured variables — before, during and after exposure to water vapour only (mean of 4 mice), silica - day 1 exposure (150 mg/m$^3$, mean of 8 mice) and silica - day 5 exposure (150 mg/m$^3$, mean of 8 mice). [The arrow indicates the duration of exposure. N normal, S sensory irritation, A airflow limitation, P pulmonary irritation, and SA, SP, PA and SPA are combinations of breath classification. VT tidal volume, TI time of inspiration, TE time of expiration, VD mid expiratory flow, TB time of brake, TP time of pause and f respiratory frequency]

tidal volume decreased. There was no increase in the time of brake and time of pause, but the mid airflow during expiration was decreased (Fig. 2).

Exposure to silica aerosol on the second day increased the respiratory frequency further and the tidal volume decreased (Table 1). Following exposure to silica on the subsequent days, the same changes were observed viz. decrease in normal breathing pattern, increase in airway obstruction pattern, increase in respiratory frequency and decrease in tidal volume (Fig. 2).

Fig. 3 shows the analyzed data on normal respiration, airflow limitation (airway obstruction), pulmonary irritation and sensory irritation of eight mice exposed to silica aerosols daily. In this figure, the daily baseline recording was taken as control and the effects of silica aerosol was evaluated based on the control recording of that day. On the first day following silica aerosol exposure, the normal breathing was about 20 to 30 % and airway obstruction was about 50 to 60%. The second and third day effect (additional effect) was less, but on the fourth and fifth day the normal respiration was about 10 % only. Exposure to silica aerosol did not cause any appreciable pulmonary or sensory irritation. One of the significant findings was an increase in respiration immediately after silica exposure. The increase in respiration was observed on all the days from the baseline respiration. About 120-150% increase in respiration was observed following silica aerosol exposure (Fig. 4). Corresponding to the increase in respiration there was a decrease in the tidal volume on all the days of exposure. About 40-50% decrease in tidal volume was observed following

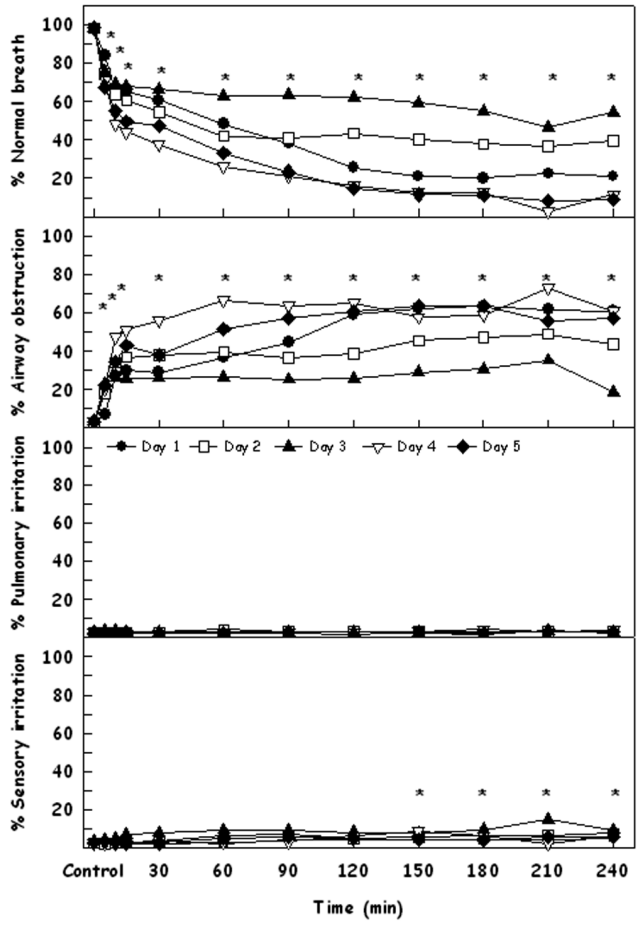


Fig. 3 — Time response of normal respiration, airway obstruction, pulmonary irritation and sensory irritation during exposure to 150 mg/m$^3$ silica on day 1 to 5. [Values are mean (n = 8). *Statistically significant from day 1 control baseline data by repeated measures two way ANOVA with Holm-Sidak multiple comparison test]

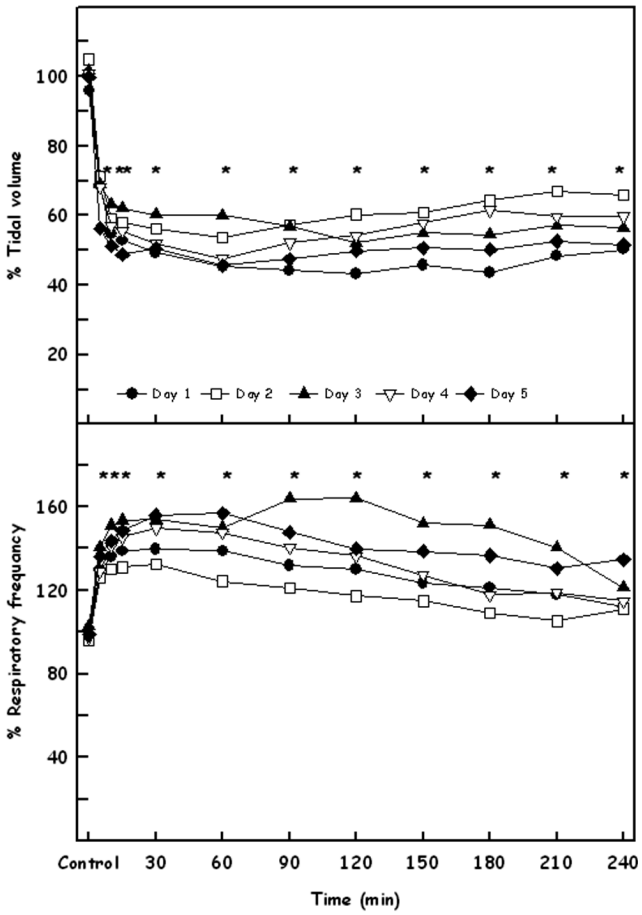


Fig. 4 — Time response of tidal volume and respiratory frequency during exposure to 150 mg/m$^3$ silica on day 1 to 5. [Values are mean (n = 8). *Statistically significant from day 1 control baseline data by repeated measures two way ANOVA with Holm-Sidak multiple comparison test]

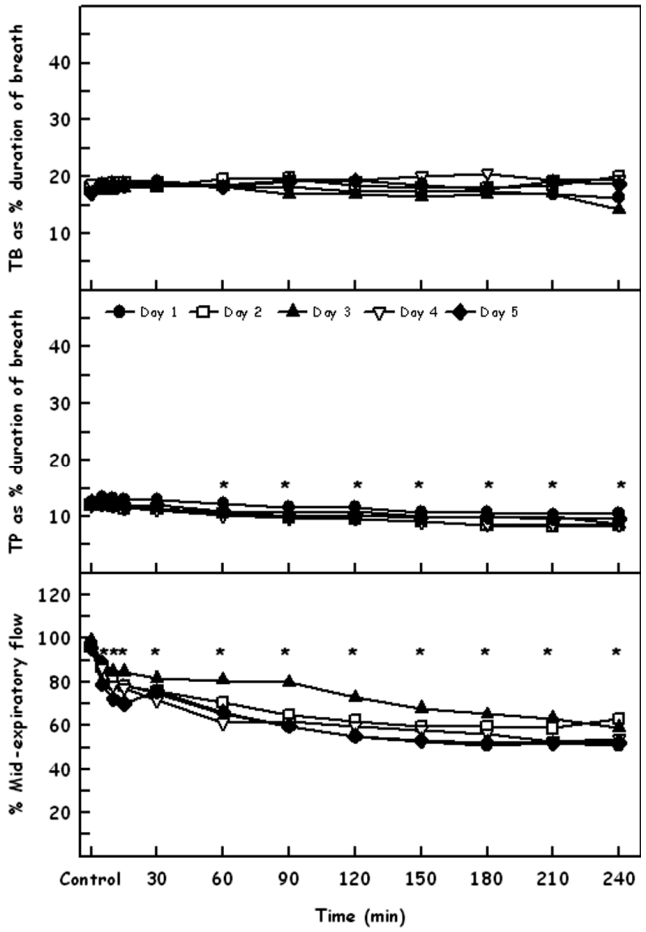


Fig. 5 — Time response of time of brake (TB), time of pause (TP) and mid expiratory flow (VD) during exposure to 150 mg/m$^3$ silica on day 1 to 5. [Values are mean (n = 8). *Statistically significant from day 1 control baseline data by repeated measures two way ANOVA with Holm-Sidak multiple comparison test]

silica aerosol exposure. There was no increase in the time of brake (TB) after the inspiration and time of pause (TP) after the expiration. A significant decrease was observed in the mid expiratory flow (VD) on all the days. About 30 to 40 % decrease was observed in the mid expiratory flow following silica aerosol exposure (Fig. 5).

## Discussion

Several airborne particles are deposited in the alveoli of the lungs, leading to respiratory disorders[13]. Silica particles are poorly soluble in biological fluids. When inhaled the particles get deposited in the alveolar sac and cleared very slowly by mucociliary escalators or by coughing[14]. The macrophages ingest the silica particles and initiate an inflammatory process. Fibroblasts are stimulated and produce collagen around silica particles leading to fibrosis[15]. Though, the development of fibrosis may be delayed, but the inflammatory process can start immediately. In the present study, the first day exposure itself caused an increase in respiratory frequency and a decrease in tidal volume. The normal respiration decreased and respiratory obstruction was shown. This was aggravated on subsequent exposures. Repeated exposure to rats also caused an increase in respiratory frequency and a decrease in tidal volume. The breathing pattern was similar to airway obstruction[16]. Unlike the previous study, here, the respiratory parameters were monitored online during the course of the exposure. Intratracheally instilled silica also caused lung inflammation, alveolar injury and obstruction[17].

Airborne chemicals can cause three different types of response viz. sensory irritation (stimulation of upper respiratory tract and conducting airways), pulmonary irritation (stimulation pulmonary C fibers in the alveolar region) or airway constriction or obstruction (known as airflow limitation) of the conducting airway of the lungs (vagal stimulation). Sensory irritation is characterized by a pause between inspiration and expiration, and pulmonary irritation is characterized by a pause after the expiration. The airway obstruction is characterized by decreased respiratory flow during expiration resulting in longer time of expiration[10,11]. Silica particles are not chemically reactive, and hence did not cause any strong sensory or pulmonary irritation. The earlier report of rat study also did not show any sensory or pulmonary irritation[16].

Silica causes thickening of the alveolar septa with fibrous tissue proliferation and infiltration of mononuclear cells[18,19]. Protein degeneration may also occur due to connective tissue breakdown in the lung with the release of matrix components into the alveolar spaces[20]. This deposition reduces alveolar space, resulting in lower tidal volume. To compensate for the lowering of tidal volume, respiratory frequency was increased in exposed animals as compared to control. Repeated exposure to silica aerosol showed altered biochemical variables, granulomatous inflammation, fibrosis and degeneration[16]. Exposure to silica aerosols resulted in polymorphonuclear leukocyte infiltration and NF-kappa B activation that plays an important role in pulmonary inflammation, cellular damage, and fibrosis[21,22]. The decrease in tidal volume with an increase in respiratory frequency is as a result of these effects.

This study shows that silica aerosol inhalation can cause an immediate change in the respiratory variables with an increase in the respiratory frequency and decrease in tidal volume (rapid shallow breathing), and a breathing pattern of airway obstruction. Hence, appropriate precautions should be taken for occupational exposures.

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