

## COTTON AND OTHER ORGANIC DUSTS

### Time Course of Pulmonary Responses to Inhalation of Cotton Dust in the Guinea Pig Animal Model

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#### INTERPRETIVE SUMMARY

Currently, the permissible exposure limit (PEL) set by Occupational Safety and Health Administration (OSHA) is 0.2 mg/m<sup>3</sup> for yarn manufacturing and cotton washing. This PEL is for an 8 h workday. It is not uncommon for cotton mill workers to have extended workshifts of 10 h or more. Therefore, the question has been raised as to how one would set an appropriate exposure standard for extended workdays. The simplest approach would be to assume that pulmonary reactions are directly or linearly proportional to the product of exposure duration and concentration. According to this approach, it is total daily exposure that must be controlled, with an 8 h exposure to 0.2 mg/m<sup>3</sup> giving a total exposure of 1.6 mg h/m<sup>3</sup>. Therefore, the dust level for a 10 h workday should be limited to 0.16 mg/m<sup>3</sup>. However, since the magnitude of pulmonary reactions to cotton dust exposure is not as great on the second exposure day as after an initial exposure, there is reason to suspect that negative feedback mechanisms could be involved that limit the pulmonary reaction to cotton dust. If these accommodation mechanisms are initiated during an extended workshift, the exposure-response relationship could deviate from linearity so that levels of cotton dust higher than 0.16 mg/m<sup>3</sup> would be safe for a 10 h workday.

As an initial attempt to address this issue, we employed a guinea pig model of the acute pulmonary reactions to inhalation of cotton dust. Guinea pigs were exposed to 1.5 mg/m<sup>3</sup> of cotton dust for 4 to 16 h and acute inflammatory reactions monitored 0 and 18 h post-exposure. Total bronchoalveolar lavage cells, leukocytes, and red blood cells increased linearly with total exposure, that is, the concentration-duration product, both

immediately and 18 h after exposure. Macrophage activity, measured as zymosan-stimulated chemiluminescence, exhibited a similar linear relationship to total exposure.

In conclusion, the results employing a guinea pig animal model suggest that at a relatively low exposure concentration of cotton dust acute pulmonary inflammatory reactions increase linearly with exposure duration. Although caution should be used when extrapolating animal results to humans, the data are consistent with the simple linear extrapolation of the concentration-time product as a reasonable estimation of total exposure over extended workdays. The data do not support the hypothesis that down regulation of responsiveness occurs within an extended workshift.

#### ABSTRACT

**Exposure to cotton (*Gossypium hirsutum* L.) dust can result in acute pulmonary inflammation marked by elevation of neutrophils in nasal and bronchoalveolar lavage fluids and activation of alveolar macrophages. Such exposure may also result in chest tightness and airway constriction. For this reason, OSHA has established a PEL of 0.2 mg/m<sup>3</sup> for yarn manufacturing and cotton washing. This PEL is for an 8 h workday. Questions concerning an appropriate standard for extended workdays (longer than 8 h) have been raised. As an initial attempt to address this issue, we employed a guinea pig (*Cavia porcellus*) model exposed to 1.5 mg/m<sup>3</sup> of cotton dust for 4 to 16 h and monitored pulmonary inflammation 0 and 18 h post-exposure. Total bronchoalveolar lavage cells, leukocytes, and red blood cells increased**

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**Abbreviations:** OSHA, Occupational Safety and Health Administration; NIOSH, National Institute for Occupational Safety and Health; REL, recommended exposure limit; PEL, permissible exposure limit; AAALAC, Association for Assessment and Accreditation of Laboratory Animal Care International; FEV<sub>1</sub>, forced expiratory volume in 1 second; SE, standard error of the mean; AM, alveolar macrophages; HEPES, N-2-hydroxyethylpiperazine-N<sup>1</sup>-2-ethane sulfonic acid; TNF, tumor necrosis factor.

**linearly with total exposure (concentration x exposure time) both immediately and 18 h post-exposure. Macrophage activity, measured as zymosan-stimulated chemiluminescence, exhibited a similar linear relationship to total exposure. These animal results suggest that the concentration-time product may be an appropriate exposure metric when evaluating the risk of acute inflammation in response to cotton dust.**

The byssinotic syndrome in cotton mill workers was first characterized by Schilling et al. (1955) as chest tightness, which is most pronounced on the first day of the workweek (Monday accentuation). In addition to the classic symptom of chest tightness, inhalation of cotton dust results in several other pulmonary responses (Rylander et al., 1987). Cotton mill workers may experience increased airway resistance that results in a cross-shift decline in forced expiratory volume in 1 s ( $FEV_1$ ) (McKerrow et al., 1958). As with chest tightness, this cross-shift decline in  $FEV_1$  exhibits Monday accentuation (Merchant et al., 1972). Cotton dust exposure is also associated with an inflammatory response characterized by elevations of neutrophils in the blood (Bouhuys et al., 1961), nasal fluids (Merchant et al., 1975), and bronchoalveolar lavage fluid (Baur et al., 1993).

National Institute for Occupational Safety and Health (NIOSH) has published a recommended exposure limit (REL) of  $<0.2 \text{ mg/m}^3$  for lint-free cotton dust (NIOSH, 1994). OSHA's current PEL is  $0.2 \text{ mg/m}^3$  for yarn manufacturing and cotton washing;  $0.5 \text{ mg/m}^3$  for textile mill house operations or for yarn manufacturing with washed cotton;  $0.75 \text{ mg/m}^3$  for textile slashing and weaving; and  $1 \text{ mg/m}^3$  for cotton waste processing and garnetting. All these PEL's are for an 8 h workday (OSHA, 1984).

Recently, the issue of longer than 8 h workdays has been raised in regard to the cotton dust PEL. For example, an 8 h exposure to  $0.2 \text{ mg/m}^3$  of cotton dust would result in a total daily exposure of  $1.6 \text{ mg h/m}^3$ , that is, the concentration-time product. If a worker were exposed at  $0.18 \text{ mg/m}^3$  for 10 h ( $1.8 \text{ mg h/m}^3$  daily exposure), would the individual be at risk of acute respiratory inflammation? To date, no information is available to address this issue. The simplest approach would be that pulmonary reactions are directly related to the time-concentration product. However, it is known that pulmonary responses to cotton dust are not as great

on subsequent exposure days as they are after the initial exposure (Monday accentuation). This indicates that negative feedback mechanisms are in place to limit the reaction to cotton dust. The question is: would these limiting factors occur within an extended workday? If so, the exposure-response relationship would deviate from linearity and a 10 h exposure to  $0.18 \text{ mg/m}^3$  might not be more inflammatory than an 8 h exposure to  $0.2 \text{ mg/m}^3$ .

Extensive effort from several laboratories has resulted in the development of a guinea pig animal model for cotton dust exposure (Castranova et al., 1996). This model exhibits concentration-dependent increases in breathing rate (Ellakkani et al., 1985), airway constriction (Smith et al., 1993), neutrophil infiltration (Robinson et al., 1988), and alveolar macrophage activation (Robinson et al., 1988). As in workers, these responses exhibit Monday accentuation (Castranova et al., 1990) and a dependence on the endotoxin content of the cotton dust (Castellan et al., 1987; Robinson et al., 1995). The objective of the present investigation was to employ this animal model to investigate whether a linear relationship exists between the concentration-time product for cotton dust inhalation and pulmonary response in guinea pigs. This was accomplished by exposing guinea pigs to an average of  $1.5 \text{ mg/m}^3$  cotton dust for 4 to 16 h and monitoring pulmonary reactions immediately, as well as 18 h post-exposure.

## MATERIAL AND METHODS

### Experimental Design

The objective of this study was to determine the role that exposure time plays in the inflammatory response observed following cotton dust exposure in guinea pigs. Guinea pigs were housed in the exposure chambers for the duration of the exposure, during which time, they were exposed to a computer controlled aerosol of approximately  $1.5 \text{ mg/m}^3$  cotton dust. At set intervals throughout the exposure, 10 animals were removed from the chamber. The breathing rates of all 10 animals were measured in 10%  $\text{CO}_2$  in air. Half of each group, that is, five animals, were sacrificed immediately (0 h post exposure) for bronchoalveolar lavage and cellular measurements. The remaining five guinea

pigs of each group were sacrificed at 18 h post-exposure.

Forty animals were housed in the exposure chamber at the beginning of each of three-exposure runs. The durations of exposure for the different groups was 4, 5, 6, 7, 8, 10, 12, 14, and 16 h. Three separate runs were made to expose all of the groups. The mean concentration of the exposures was  $1.47 \pm 0.10 \text{ mg/m}^3$  (mean  $\pm$  standard error), measured gravimetrically with Gelman VM-1 filters.

### Cotton Dust and Animals

Bulk cotton dust (DB 5/89) was obtained from the National Cotton Council, Memphis, TN. Male Dunkin Hartley guinea pigs were purchased from Sprague Dawley (Indianapolis, IN). The animals were specific pathogen free and acclimated for 7 d at the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) approved NIOSH animal facility prior to the cotton dust exposures. The body weight of

the guinea pigs was  $305.1 \pm 2.6 \text{ g}$  (mean  $\pm$  SE) at the time of exposure.

### Cotton Dust Generator and Exposure System

The system used to expose guinea pigs to cotton dust is shown in Figure 1. Two modified Pitt-3 acoustical generators similar in design to the one described by Weyel et al. (1984) were used in parallel to generate a cotton dust aerosol. Details of the generator design have been described previously (Frazer et al., 1991). Filtered air from a compressed air source passed through each generator at a constant rate of 15 L/min. During an exposure period, animals were housed in individual cages within a large stainless steel chamber (Hazlton 2000) that was comparable in design to the one that Moss et al. (1982) described. The mass concentration of cotton dust inside the chamber was held constant by adjusting the amount of diluent air added to the elutriated aerosol produced by the

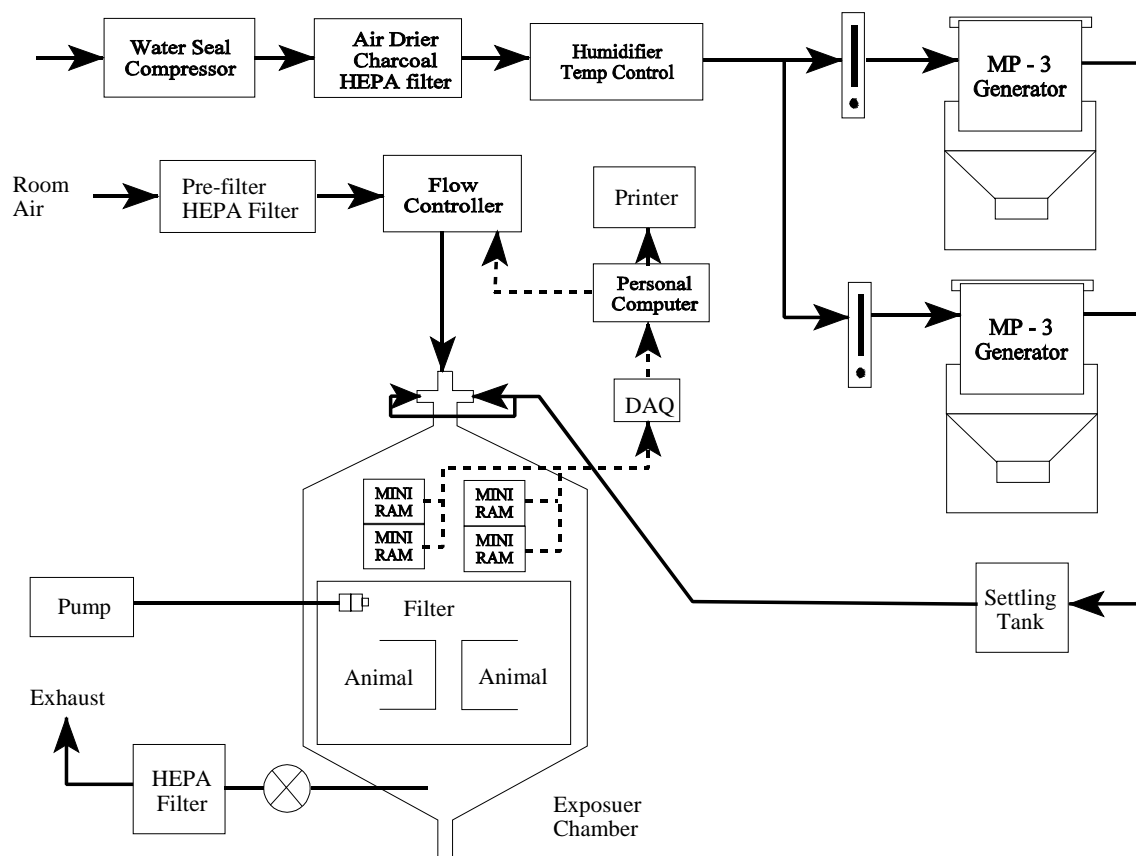


Fig.1. Schematic of the cotton dust generator and the exposure system.

generation system. The diluent air was conditioned with an air-handling system that continuously monitored and recorded the temperature and humidity of the room air being pulled through a pre-filter and HEPA filtering system. Four minirams (Miniature Real Time Aerosol Monitor, MIE, model PDM-3) were spaced at equal intervals near the top of the chamber. Analog outputs of the minirams were sampled, digitized (DATAQ Model DI220) and averaged with a computer to estimate the average mass concentration of the cotton dust aerosol at 5-s intervals. The estimated mass concentration was used by a computer controlled geared stepper motor to regulate the amount of diluent air that was mixed with the aerosol. Exhaust aerosols and gases were removed from the exposure chamber and cleaned by HEPA filtration. In addition, the mass concentration of the respirable dust in the exposure chamber was measured gravimetrically by pulling aerosol samples through a VM-1 filter at a flow rate of 2 L/min. Filters were changed at one-half hour intervals.

#### Bronchoalveolar Lavage Cell Assays

Lung cells were obtained by bronchoalveolar lavage after the method of Castranova et al (1990). In short, guinea pigs were anesthetized with sodium pentobarbital and exsanguinated. Calcium and magnesium-free phosphate buffered saline (145 mM NaCl, 5mM KCl, 1.9 mM NaH<sub>2</sub>PO<sub>4</sub>, 9.35 mM Na<sub>2</sub>HPO<sub>4</sub>, and 5.5 mM dextrose; pH = 7.4) was instilled into the cannulated trachea of the guinea pigs. This solution was then aspirated from the lung and collected. A 6 mL aliquot of buffer was used for the initial instillation and 8 mL thereafter, until 80 mL of lavage fluid were collected per guinea pig. The harvested cells were concentrated by centrifugation at 1500 x G, washed in HEPES buffered medium, (145 mM NaCl, 5 mM KCl, 10 mM Na HEPES, 10 mM CaCl<sub>2</sub>, and 5.5 mM dextrose; pH = 7.4) and reconstituted. The final cell pellet was resuspended in HEPES buffered medium.

Total and differential cell counts were determined with an electronic cell counter equipped with a cell-sizing attachment. Red blood cells, alveolar macrophages, and leukocytes (lymphocytes and polymorphonuclear leukocytes) were identified by their characteristic cell volumes (Castranova et al., 1990).

Macrophage activation was determined by the measurement of luminol-enhanced chemiluminescence. Alveolar macrophages ( $5 \times 10^5/0.5$  mL of HEPES buffered medium) were preincubated at 37°C for 15 min. After preincubation, luminol was added (0.08 µg/mL) along with an aliquot of medium with or without unopsonized zymosan (2 mg/mL) for resting and zymosan samples, respectively. Chemiluminescence, an indicator of production of reactive species, was then measured at 37°C for 10 min using a Berthold luminometer. Chemiluminescence was determined as cpm/ $5 \times 10^5$  AM/10 min. Zymosan-stimulated chemiluminescence was determined by the subtraction of the resting rate (without zymosan) from the rate with zymosan. Evidence indicates that unopsonized zymosan stimulates alveolar macrophages but not polymorphonuclear leukocytes (Castranova et al, 1990); therefore, zymosan-stimulated chemiluminescence is an indicator of alveolar macrophage activity.

#### Breathing Rate Measurements

Guinea pigs were placed in a flow plethysmograph that has been previously described in detail (Frazer et al., 1997). The plethysmograph chamber was constructed of an acrylic tube enclosed at each end. One end of the chamber had a circular port that contained three 400 mesh stainless steel screens. Pressure variations across the screen generated by flow into and out of the chamber were measured with a pressure transducer (Setra, model 239). A digital oscilloscope (Tektronix, model 222) was used to record flow signals that were transferred to a digital computer for analysis. After the guinea pigs were equilibrated with 10% CO<sub>2</sub> in air for 3 min, their average breathing rates were calculated based on the time between zero crossings of the flow signal at the beginning and end of an inhalation-exhalation cycle.

## RESULTS

#### Breathing Frequency

Although at 0 h post-exposure there was a tendency for the breathing rate of guinea pigs in 10% CO<sub>2</sub> to increase as exposure duration increased, these breathing frequencies were not significantly different from the control (unexposed)

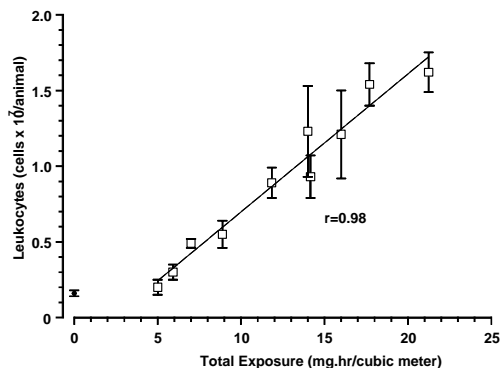


Fig. 2. Leukocytes (lymphocytes and polymorphonuclear leukocytes) harvested by bronchoalveolar lavage of guinea pigs immediately after (0 h post-exposure) inhalation of cotton dust for 4 to 16 h. Exposure is given as the concentration-time product. Values are means  $\pm$  standard errors of three experiments. The control and exposure values are noted as  $\bullet$  or  $\square$ , respectively. Exposure values are fitted by linear regression with  $r = 0.98$ .

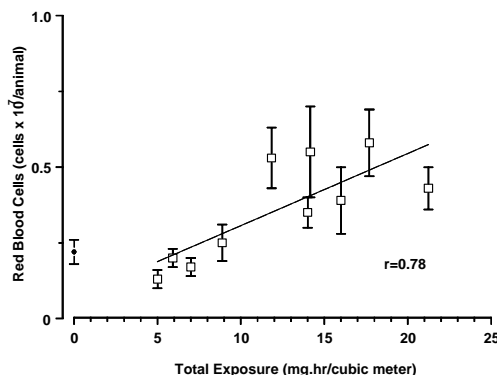


Fig. 3. Red blood cells harvested by bronchoalveolar lavage of guinea pigs immediately after (0 h post-exposure) inhalation of cotton dust for 4 to 16 h. Exposure is given as the concentration-time product. Values are means  $\pm$  standard errors of three experiments. The control and exposure values are noted as  $\bullet$  or  $\square$ , respectively. Exposure values are fitted by linear regression with  $r = 0.78$ .

rate (data not shown). At 18 h post-exposure, even these slight elevations were absent.

### Bronchoalveolar Cell Yield

There was a significant increase in the total number of cells harvested by bronchoalveolar lavage from exposed guinea pigs compared to controls. This increase was linearly related to the total exposure, that is, the concentration-time product, over the exposure duration range of 4 to 16 h. This linear elevation in total lavaged cells was noted both at 0 and 18 h post-exposure and was due to significant, total exposure-dependent increases in both leukocytes (Figure 2) and red blood cell counts (Figure 3). Alveolar macrophage yield did not increase as a result of this cotton dust exposure.

### Macrophage Activation

Zymosan-stimulated chemiluminescence was significantly increased both 0 and 18 h after cotton dust exposure. This increase was linearly related to the concentration-time product of exposure (Figure 4). Chemiluminescence, that is, the production of reactive species by alveolar macrophages, tended to be higher 18 h post-exposure than 0 h post-exposure.

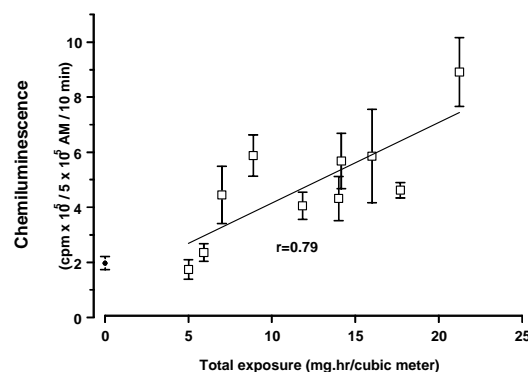


Fig. 4. Zymosan-stimulated chemiluminescence from alveolar macrophage harvested by bronchoalveolar lavage of guinea pigs 18 h after inhalation of cotton dust for 4 to 16 h. Exposure is given as the concentration-time product. Values are means  $\pm$  standard errors of three experiments. The control and exposure values are noted as  $\bullet$  or  $\square$ , respectively. Exposure values are fitted by linear regression with  $r = 0.79$ .

## DISCUSSION

To date, little or no information is available concerning the health effects of a longer than 8 h workday in cotton textile mills. Is it true that a 12 h exposure to a given level of cotton dust is 50%

worse than an 8 h exposure? Although a linear relationship between pulmonary response and total exposure (concentration x exposure duration) is the simplest hypothesis, it need not be correct. Indeed, it is well documented in workers and animal models that the magnitude of pulmonary responses to cotton dust declines on successive exposure days (Schilling et al., 1955; Castranova et al., 1990). These results indicate that negative feedback or down regulation of responsiveness to cotton dust can occur. The question is, will such down regulation occur within a given workday? If it does, a 12 h exposure to cotton dust may result in pulmonary responses that would be <150% of those for an 8 h exposure.

Merchant et al. (1975) have reported responses of human volunteers to inhalation of cotton dust for the exposure times of 2, 4, and 6 h. In general, as exposure time to a given level of cotton dust increased, blood white cell counts increased and FEV<sub>1</sub> values decreased. Although these data exhibit a response-exposure duration relationship, they do not address the issue of down regulation occurring within an extended workday. In the present investigation, we employed the guinea pig animal model exposed to 1.5 mg/m<sup>3</sup> of cotton dust for 4 to 16 h to evaluate this question. Our results indicate that total cells, leukocytes, and red blood cells harvested by bronchoalveolar lavage either 0 or 18 h post-exposure increased in a linear fashion with total exposure determined as the product of concentration and exposure duration. A similar linear relationship was demonstrated between alveolar macrophage activity and total exposure. Therefore, over the course of a 16 h exposure to cotton dust, no evidence of down regulation of the inflammatory response was discernible in this guinea pig model.

In previous studies, an increase in breathing rate has been viewed as a hallmark of the guinea pig animal model (Ellakkani et al., 1985; Castranova et al., 1996). However, breathing rate was not significantly elevated in response to cotton dust inhalation in the present study. This lack of response is due to the relatively low exposure concentration used (1.5 mg/m<sup>3</sup>) in our study. Indeed, Robinson et al. (1988) have reported the concentration dependence of pulmonary responses to cotton dust and have demonstrated that the breathing rate response was not discernible at doses below 2 mg/m<sup>3</sup> while cellular changes (leukocyte

infiltration and macrophage activation) were more sensitive responses.

The present study found no increase in lavageable alveolar macrophages in response to cotton dust inhalation. This agrees with the previous studies where decreases in macrophage yield have been reported (Castranova et al., 1987; Ryan and Karol, 1991). This decreased yield may reflect an increase in the adherence of alveolar macrophages within cotton dust-exposed lungs rather than an actual decrease in pulmonary macrophage number. Activation of alveolar macrophages following inhalation of cotton dust has been demonstrated by enhanced tumor necrosis factor (TNF) release, superoxide production, and chemiluminescence (Ryan and Karol, 1991; Castranova et al., 1987; Figure 4). In fact, histological evaluation of lungs from dust-exposed guinea pigs demonstrates substantial recruitment of alveolar macrophages into the alveolar air spaces (Castranova et al., 1987).

In conclusion, data presented in this investigation using the guinea pig animal model suggest that at a relatively low exposure concentration (1.5 mg/m<sup>3</sup>) pulmonary inflammation increases with exposure duration. It should be noted the results in animals may not always be directly relevant to humans. However, the data are consistent with the simple linear extrapolation of the concentration-time product as a reasonable estimation of total exposure over extended workdays. The data do not support the hypothesis that down regulation of responsiveness occurs within an extended workday.

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