# ENDOTOXIN CONTAMINATION OF COTTON: AREA OF GROWTH/VARIETIES

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## Abstract

Cottons of three varieties, DPL61, GSA71, and SJ5, were each grown in three different locations in the United States: Mississippi Delta, California San Joaquin Valley, and Texas High Plains. Human subjects were exposed to card-generated cotton dusts in rooms remote to the card, and gram-negative bacterial endotoxin levels in the elutriated dusts were quantified. Endotoxin contamination of the dusts varied markedly as a result of the geographical area in which the cottons were grown. The data suggest that differences in endotoxin contamination may be related additionally to the variety of cotton. Acute pulmonary function changes as measured by the forced expiratory volume in one second were correlated better with concentrations of elutriated endotoxins than with gravimetric dust levels. It can be concluded from this study that area of growth and cotton variety can affect the endotoxin levels in card-generated cotton dust, and acute pulmonary function changes correlate well with airborne endotoxin concentrations.

## Introduction

Elucidation of the potential role of gram-negative bacterial endotoxins in the etiology or exacerbation of the acute pulmonary function reaction to inhaled cotton dusts is critical to cotton dust research. Gram-negative bacteria and their related endotoxins are associated with cotton fibers and card-generated cotton dusts (5, 7, 10-14), and human subjects who were exposed experimentally to airborne cotton dusts react with acute pulmonary function changes (10). Likewise, laboratory animals responded to the inhalation of endotoxin-containing bacteria with functional respiratory impairment as evidenced by changes in arterial oxygen changes (4) and with "dyspnea" after exposure to aerosolized endotoxins (12).

Comparisons of acute pulmonary function changes in humans with elutriated endotoxin levels suggest that quantification of airborne endotoxin levels may be a good biological indicator of the cleanliness or potency of the cotton or airborne cotton dust (9,10). Water washing of baled cottons is an effective method for reducing the airborne endotoxin burden during carding. However, quantification of the endotoxin contamination of the airborne dusts showed marked differences during the carding of unwashed cottons which were of the same grade but which were grown in different geographical areas in the United States (9,10). In addition, baled cottons from diverse sources world-wide showed large variations in the endotoxin contamination of the fibers (8). Thus, the data suggest that the endotoxin content of the airborne dust may be related to the geographical source of the cotton.

It is the purpose of this paper, therefore, to evaluate further the effect that area of growth may have on the resulting endotoxin contamination of carded cotton dusts. Additionally, the potential contribution of the variety of cotton on the endotoxin content of the airborne dusts is examined.

## Materials and Methods

Three varieties of cotton, DPL61, GSA 71 and SJ5 were each grown during crop year 1982 by Cotton Incorporated in the Mississippi Delta, California San Joaquin Valley, and the Texas High Plains. Cottons from Mississippi and California were spindle-picked, and those from Texas were strip-harvested, consistent with general practices in those areas. All cottons were processed at the USDA Cotton Quality Research Station in Clemson, South Carolina, and airborne dusts were collected during carding operations.

Vertical elutriators were operated in two rooms which were remote to the actual card rooms (2). Five micrometer pore size, 37mm filters with collected airborne dust were provided to our laboratories for endotoxin analyses. The filters represent dust which was collected throughout the exposures in the two different remote rooms. All filters were labeled by number, and no reference to variety or area of growth was made until after the endotoxin analyses were completed.

Sterile, non-pyrogenic plastic ware was used throughout the laboratory procedures. Each filter was extracted separately with 10 ml sterile, non-pyrogenic water (Travenol Laboratories, Inc., Deerfield, IL) by rocking for 60 min at room temperature as previously described (9,10). The fluid was centrifuged at 1000g for 10 min to remove particulate debris, and the supernatant fluids were separated. Five milliliter aliquots from 2 to 4 filter extracts which represent dust collected during the same time period in the same remote room were combined for analysis. The pooled extracts were analyzed immediately or frozen at -80°C until tested. Quantification of gram-negative bacterial endotoxin content was performed in duplicate by a spectrophotometric modification of the Limulus amebocyte lysate gel test (Pyrostat; Millipore Corp., Bedford, MA). Results were analyzed by linear regression, compared to a standard curve, and reported in terms of the United States Reference Endotoxin. Filters used during 8-hour samplings of remote room air while no cottons were being carded were used as negative controls and treated similarly with the exception that each filter was tested separately.

Forty-three subjects were present for all dust exposures, and only their results were analyzed. Details of the selection of healthy human subjects and the measurement of acute changes in pulmonary function are presented elsewhere (3). Because of space limitations, each subject was assigned to one of two subgroups. Each subgroup with approximately equal number of subjects was assigned to one of two identical rooms for 6-hour exposures to dust from the various cottons and to clean air for control exposures. At least two days without dust exposure intervened between exposures to dust. Forced expiratory volume ($FEV_1$) was measured immediately before and after each exposure period, and each individual's change in pulmonary function was expressed as $\pm$ $FEV_1$ (%) $=[(FEV_1$ before exposure $-FEV_1$ after exposure$/FEV_1$ before exposure$]$ X 100.

Least squares linear regression was used to determine dose response relationships between $\pm$ $FEV_1$ (%) and either gravimetric quantity of vertical elutriated cotton dust or quantitative exposure to elutriated endotoxins. For the latter, a mean value of endotoxin per milligram of vertically elutriated dust was determined for each type of cotton dust on the basis of endotoxin analysis of all available filters. To determine exposure values in terms of endotoxin per cubic meter, the ng/mg value for dust from each particular cotton was multiplied by the appropriate time-weighted averages of elutriated dust ($mg/m^3$) for each exposure.

## Results and Discussion

Results of airborne elutriated dust levels and elutriated endotoxin levels during the carding of the area of growth/variety cottons are presented in Table 1. For each area of growth, the gravimetric dust levels were similar during the exposures. Subjects were exposed to the three varieties which were grown in the Mississippi Delta region at levels of 0.43, 0.50, and 0.41 $mg/m^3$ for DPL61, GSA71, and SJ5, respectively. The cottons grown in the Texas High Plains region resulted in airborne concentrations of 0.50, 0.32, and 0.42 $mg/m^3$ for the same varieties. Exposures to the California San Joaquin Valley cottons were at the lower dust levels of 0.27, 0.28, and 0.28 $mg/m^3$. Examination of the airborne gram-negative bacterial endotoxin levels, when the variety of cottons were at the same dust levels, shows different concentrations while the Mississippi grown cottons were carded. Variety DPL61 produced an airborne endotoxin concentration of 87.60 $ng/m^3$ while GSA71 produced 112.62 $ng/m^3$ and SJ5 produced 164.63 $ng/m^3$. These results suggest that differences in endotoxin contamination may be related to the variety of cotton which was grown. Less markedly different are the airborne endotoxin concentrations during the carding of the same

varieties grown in California (13.86, 31.36, and 16.37 ng/m$^3$) or in Texas (14.05, 22.05, and 10.63 ng/m$^3$), although some variation in the levels may be based on the variety of cotton. Perhaps airborne exposure concentrations in ng/m$^3$ are not the most appropriate values for comparison. Endotoxin levels which are based upon an elutriated dust weight basis (ng/mg) may be more representative of endotoxin contamination of dust.

Table 2 depicts the endotoxin content of the elutriated dusts upon replicate exposures. The results are expressed in terms of dust weight (ng/mg), and some differences in endotoxin contamination because of variety are now more pronounced. Notably, the three varieties, when grown in California and Texas, show greater variation in their endotoxin contents than was apparent in the airborne levels. As noted when airborne concentrations were compared, endotoxin levels in the dusts from cottons from Mississippi suggest variety-related differences.

Readily observed from Table 2, however, is the marked variation in endotoxin contamination due to the area in which the cottons were grown. For each variety, the most highly contaminated dust was generated from cotton which was grown in the Mississippi Delta region. California grown cottons generated less endotoxins per milligram of elutriated dust, and carding of the cottons from Texas resulted in the least endotoxins for each of the three varieties. These data corroborate and reinforce our previous observations that endotoxin contents of airborne dusts can vary markedly based upon the geographic source of the carded cotton as evidenced from cottons grown within the United States (9) and in various other countries world-wide (8). It was suggested previously that area differences in endotoxin contamination may be a combined result of differences in variety of cotton, grade, temperatures, soils, or growth conditions which select for different species or quantities of microbial organisms (9,10). The contribution of the variety of cotton to the area differences has been lessened by this study, because the same varieties were grown in all three locations. Overall growth conditions may have greater impact on the microbial flora. Further, harvest conditions may provide another variable because Texas grown cottons were strip-harvested while California and Mississippi cottons were spindle-picked. It should be noted also that dusts from California grown cottons were the least contaminated with endotoxin in previous area of growth studies (9,10). The results of this study show that Texas grown cottons produced the least contaminated dusts. Perhaps growth conditions in the areas during the 1982 crop year had been different from other crop years. One additional consideration should be the possibility of differential toxicities of the endotoxins which contaminate the cotton dusts (1,6). However, this study does not address the idea that the different organisms which colonize the cotton plant may produce endotoxins of varied toxicities.

Replicate exposure trial 2 was performed 1 to 2 months after the first exposure. Comparison of the data from Table 2 shows, with perhaps the singular exception of the exposures of DPL61-CA, striking consistency in endotoxin contamination of the airborne dusts which were collected during trials 1 and 2. The results, therefore, were combined and presented in Table 3 which compares the mean endotoxin content of the elutriated dusts (ng/mg) with the mean change in acute pulmonary function as measured by the forced expiratory volume in one second ($FEV_1$) and expressed as (% per mg/m$^3$). For each variety, dusts from cottons grown in the Mississippi Delta region contained the highest amounts of endotoxins. Dusts from California grown cottons had markedly less, and dusts from the Texas grown cottons had the least endotoxin contamination. Acute pulmonary function changes in the human subjects exposed to these dusts showed a similar pattern. For each variety, the greatest effect on $FEV_1$ occurred with exposure to dust from the Mississippi grown cottons (-9.4, -13.1, and -11.2 % per mg/m$^3$). Lesser effects on $FEV_1$ were observed in response to the dusts from California grown cottons (-4.1, -7.4, and -5.3% per mg/m$^3$), and the least effect was after exposure to the dusts from carded Texas cottons (-1.4, -1.9, -2.6% per mg/m$^3$). Changes in the acute pulmonary functions following exposures to TX-DPL61 and TX-GSA71 were not statistically different from zero change. All other changes in $FEV_1$ were highly significant ($p<0.025$ to $p<0.00001$). Differences in acute pulmonary function were related, therefore, to the area of growth of the cotton. Statistical analyses of $FEV_1$ changes based on the variety of cotton carded suggested some variety-related differences when the cottons were grown in Mississippi, but no statistical variety differences were found with California or Texas grown cottons.

Vertical elutriators collected air during 8 hours of exposure to clean air when no cotton was being carded. The mean gravimetric dust concentration from all the clean air exposures was 0.03 mg/m$^3$. Extraction of the filters and analysis for endotoxin contamination resulted in mean endotoxin content in the airborne dust of less than 6 ng/mg. Mean pulmonary function change in response to the same clean air exposures was 0.0%. One may conclude from these data that exposures to dust and endotoxins as well as changes in acute pulmonary function were negligible on clean air days.

The relationship between elutriated dust concentrations or elutriated endotoxin concentrations and change in acute pulmonary function is depicted in Figure 1A and 1B. In both plots, each point represents the mean and standard error for each subgroup of subjects at each dose to which each subgroup was exposed. Figure 1A displays the relationship between mean $FEV_1$ change in percent and the elutriated dust concentration expressed as mg/m$^3$ (correlation coefficient: -0.53). By comparison, Figure 1B depicts the relationship between $FEV_1$ change and elutriated endotoxin concentration (ng/m$^3$). The correlation coefficient for that relationship is -0.77, an improved correlation. We reported previously that levels of elutriated endotoxins were correlated better with acute pulmonary function changes than were elutriated dust levels (10). The results of the current study show again that airborne concentrations of endotoxins demonstrate a better correlation with acute pulmonary function change than do gravimetric dust levels.

It can be concluded from this study that endotoxin contamination of card-generated dust can vary markedly as a result of the geographical area in which the carded cotton was grown. The data suggest that differences in endotoxin content of the dust may be related to the variety of cotton, although confirmation of these observations should be pursued. Finally, as shown previously, acute pulmonary function effects of exposure to carded cotton dust correlate better with elutriated endotoxin levels than with gravimetric dust concentrations.

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Table I. Airborne Dust and Endotoxin Levels During Cotton Dust Exposures

| Variety | Growth Area | Dust Level ($mg/m^3$) | Endotoxin ($ng/m^3$) |
|---|---|---|---|
| DPL61 | MS | 0.43 ± 0.01[1] | 87.60 ± 5.35[1] |
| | CA | 0.27 ± 0.01 | 13.86 ± 2.08 |
| | TX | 0.50 ± 0.02 | 14.05 ± 0.71 |
| GSA71 | MS | 0.50 ± 0.02 | 112.62 ± 9.28 |
| | CA | 0.28 ± 0.01 | 31.26 ± 3.90 |
| | TX | 0.32 ± 0.02 | 22.05 ± 1.91 |
| SJ5 | MS | 0.41 ± 0.02 | 164.89 ± 10.41 |
| | CA | 0.28 ± 0.01 | 16.37 ± 2.48 |
| | TX | 0.42 ± 0.02 | 10.63 ± 0.72 |

[1] Mean ± SEM.

Table 2. Comparison of endotoxin content in airborne cotton dusts during replicate trials

| Variety | Growth Area | Endotoxin Trial 1 (ng/mg) | Endotoxin Trial 2 (ng/mg) |
|---|---|---|---|
| DPL61 | MS | 206.55 ± 18.52[1] | 200.68 ± 10.41[1] |
| | CA | 65.86 ± 4.19 | 29.35 ± 3.22 |
| | TX | 28.40 ± 1.22 | 31.20 ± 2.49 |
| GSA71 | MS | 207.07 ± 24.38 | 236.11 ± 27.00 |
| | CA | 117.89 ± 8.73 | 96.66 ± 13.84 |
| | TX | 56.86 ± 5.36 | 81.91 ± 5.78 |
| SJ5 | MS | 459.86 ± 43.63 | 382.72 ± 24.08 |
| | CA | 69.03 ± 9.11 | 43.15 ± 3.79 |
| | TX | 25.51 ± 1.53 | 26.25 ± 2.31 |

[1] Mean ± SEM.

Table 3. Endotoxin content and pulmonary function effect of airborne dust from cottons of different variety/growth area

| Variety | Growth area | Endotoxin (ng/mg) | Delta $FEV_1$ (% per $mg/m^3$) |
|---|---|---|---|
| DPL61 | MS | 203.94 ± 11.01[1] | -9.4[2] |
| | CA | 50.21 ± 5.68 | -4.1 |
| | TX | 29.45 ± 1.21 | -1.4 |
| GSA71 | MS | 219.98 ± 17.91 | -13.1 |
| | CA | 107.27 ± 8.44 | -7.4 |
| | TX | 69.39 ± 5.34 | -1.9 |
| SJ5 | MS | 426.80 ± 28.19 | -11.2 |
| | CA | 57.94 ± 6.37 | -5.3 |
| | TX | 25.28 ± 1.27 | -2.6 |

[1] Mean ± SEM.

[2] Slope of dose-response using linear regression model: Mean delta $FEV_1(\%)$ = intercept + $K_{dust}$ X elutriated dust concentration.

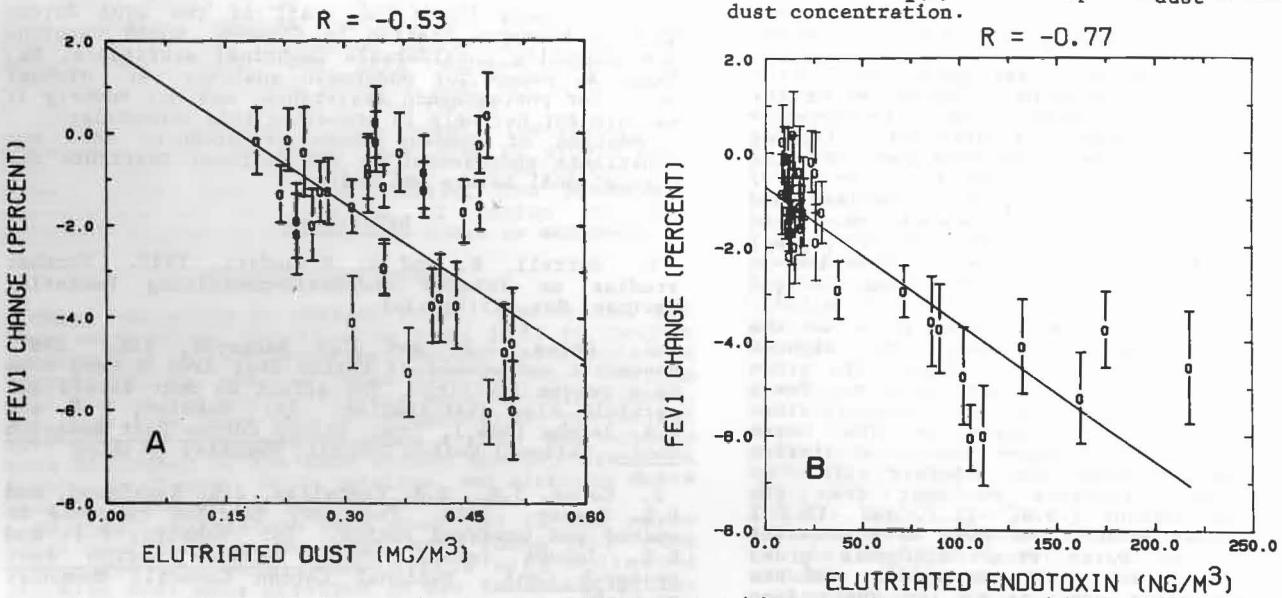


Figure 1. Relationship between $FEV_1$ change and elutriated dust (A) or elutriated endotoxins (B). Each point represents the mean ± standard error for each subgroup of subjects at each dose of cotton dust to which the subgroup was exposed. R = correlation coefficient.

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