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# Endotoxin Content in Cottonseed Oil Mill Dust

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The objective of this study was to quantify the amount of endotoxin present in respirable dusts collected in cottonseed oil mills. The level of endotoxin in workplace air exceeded  $0.5 \mu\text{g}/\text{m}^3$  in about 30% of the examined samples. The amount of endotoxin found in the dust varied over two orders of magnitude from  $0.01 \mu\text{g}$  to  $1.3 \mu\text{g}$  per mg of vertically elutriated particulate. The endotoxin contamination of cottonseed oil mill dust varied both between and within mills and was not consistently related to the amount of dust captured by the vertical elutriator cotton dust sampler.

## Introduction

Dust in cottonseed oil mills has been characterized both chemically and botanically. The chemical composition of cottonseed oil mill dust was found to be highly influenced by the process step rather than by mill-to-mill differences.<sup>(1)</sup> Thus, dusts from the delimiting and baling work areas are rich in cellulosic components whereas dust from the hulling room always has the highest protein content. Also, there is a decrease in the content of bract found in the cottonseed oil mill process stream from work areas starting with cleaning and ending with hulling and separating.<sup>(2)</sup> Levels of respirable dust collected by the vertical elutriator cotton dust sampler vary from  $0.3 \text{ mg}/\text{m}^3$  to  $7.6 \text{ mg}/\text{m}^3$  and show little or no concentration differences with regard to mill work area.<sup>(3)</sup>

In studies in U.S. cottonseed oil mills, it was found that dust in the cleaning, delimiting and baling areas caused a decline in lung function which could not be related to the level of respirable dust measured by the vertical elutriator.<sup>(4)</sup> Studies in Egypt showed that cottonseed oil mill dust adversely affected ventilatory function as measured by larger than expected numbers of people with declines in  $\text{FEV}_1 > 10\%$  over the workshift.<sup>(5)</sup> In a study of 723 cottonseed oil mill workers, NIOSH, however, found that there were no significant increases in the prevalence of dyspnea, bronchitis and byssinosis over that observed in controls.<sup>(6)</sup> In recommending a standard of  $1.0 \text{ mg}/\text{m}^3$  vertically elutriated particulate, NIOSH did, however, caution that cottonseed oil mill dust is not nuisance dust, because of the trend toward lower-than-expected  $\text{FEV}_1$  decrements in these workers, especially for smokers.

Regarding specific agents which have been related to disease caused by cotton dust, endotoxins from Gram-negative bacteria have been implicated as disease causatives in both the textile<sup>(7)</sup> and nontextile cotton industries.<sup>(8,9)</sup> The objectives of this study were to see if endotoxins were present in the respirable dusts collected in cottonseed oil mills and to determine if variation in airborne levels of this microbial material occurred within and between mills.

## Materials and Methods

Vertical elutriator cotton dust samplers were operated according to standard procedures<sup>(10)</sup> in nine cottonseed oil mills over the period from early autumn of 1979 through the late winter of 1981. Some of the mills surveyed in the early autumn of 1979 were processing seed from the 1978 crop. The seed examined in these studies, therefore, was derived from 3 crop years, namely 1978, 1979 and 1980.

Polyvinylchloride filters containing cottonseed dust were stored in a cool dry place until the endotoxin content was analyzed in the late autumn of 1981. For Mills J, 13 and 17 both gravimetric and microbial analyses were made from the same filter. In Mills A, B, C, D, G and H, these analyses were made on different filters collected from the same elutriator either immediately before or after the standard elutriated dust samples were obtained.

The amount of endotoxin was determined by the Limulus amoebocyte lysate (LAL) assay.<sup>(11)</sup> Each filter was shaken in 10 mL of pyrogen-free water and serial dilutions were prepared. Limulus lysate (Cape Cod Associates, Inc.)\* was added to the dilutions according to the manufacturer's recommendations. The last dilution giving a stable clot was read as the *Escherichia coli* endotoxin equivalent concentration. Dilutions were also prepared with commercial *E. coli* endotoxin (*E. coli* 026-B26, Difco) to assess the accuracy of the production reference standard. The values were also found to agree closely with the stated values.

## Results

The amount of respirable dust and endotoxin on the same filters from vertical elutriators at different work sites is reported in Table I. Mill J, which was located in the Southwest, received seed (1980 crop) from cotton gins processing both machine-picked and machine-stripped seed cotton. Levels of elutriated dust in Mill J varied from  $0.2 \text{ mg}/\text{m}^3$  to

\*Mention of company names or products does not constitute endorsement by the National Institute for Occupational Safety and Health.

**TABLE I**  
**Endotoxin and Dust Levels on Single Filters**  
**Collected in Cottonseed Oil Mills<sup>A</sup>**

Mill Code	Workplace	Endotoxin ( $\mu\text{g}/\text{m}^3$ )	Elutriated Dust ( $\text{mg}/\text{m}^3$ )	$\mu\text{g}$ Endotoxin per mg of Elutriated Dust
J	Cleaning	0.09	0.72	0.12
J	Cleaning	0.18	0.56	0.32
J	Delinting	0.37	0.75	0.49
J	Delinting	0.05	0.18	0.28
J	Baling	0.09	0.43	0.21
J	Baling	0.19	0.86	0.22
J	Safety shaker	0.90	1.41	0.64
J	Safety shaker	1.97	1.53	1.29
J	Safety shaker	0.43	1.38	0.31
J	Safety shaker	0.94	1.41	0.67
J	Hulling	0.24	1.49	0.16
J	Hulling	0.12	0.88	0.14
J	Beater-purifier	0.11	0.94	0.12
J	Beater-purifier	0.06	0.86	0.07
J	Beater-purifier	0.06	0.55	0.11
J	Beater-purifier	0.06	0.66	0.09
13	Seed house	0.02	0.97	0.02
13	Baling	0.001	0.20	0.01
17	Hulling	0.05	2.70	0.02
17	Filing <sup>B</sup>	0.02	1.80	0.01

<sup>A</sup>Mill location and crop year. Mill J = Southwest, 1980 crop; Mill 13 = Southeast, 1979 crop; Mill 17 = Southcentral, 1979 crop.

<sup>B</sup>Saw filing area between hulling and delinting work areas.

1.5 mg/m<sup>3</sup>. The highest levels were found at the safety shaker which cleaned seed from the first cut delinters in preparation for subsequent abrasive delinting and hulling.

Concerning airborne endotoxin, three out of four air samples obtained at the safety shaker had airborne endotoxin levels exceeding 0.5  $\mu\text{g}/\text{m}^3$ . Low amounts of endotoxin were consistently found in air in the beater-purifier region of the hulling room. There was a correlation between the amount of elutriated dust and endotoxin simultaneously collected on the 16 filters examined from Mill J ( $r = 0.69$ ;  $P = 0.01$ ).

The amount of endotoxin per mg elutriated cotton dusts collected from Mill J varied from 0.1  $\mu\text{g}$  to 1.3  $\mu\text{g}$  (Table I). Dusts collected in the safety shaker area of the delinter room were generally more contaminated with endotoxin than the dusts from other mill work areas.

Mills 13 and 17 were located in the Southeast and Southcentral regions, respectively, where the crop is entirely harvested by machine. In both mills the seed being processed was from the 1979 season. The elutriated dust levels in three of the four samples were equal to or higher than 1.0 mg/m<sup>3</sup> but the amount of airborne endotoxin was low ( $< 0.1 \mu\text{g}/\text{m}^3$ ). Thus, dusts from Mills 13 and 17 were only minimally contaminated with endotoxin (concentration  $\leq 0.02 \mu\text{g}$  endotoxin per mg dust, Table I). When the levels of elutriated dust and endotoxin simultaneously collected were compared for all filters from Mills 13, 17 and J (Mill 13 = 2 filters, Mill 17 = 2 filters, Mill J = 16 filters) no significant correlation ( $r = 0.31$ ) could be shown between these two parameters.

Filters from vertical elutriator cotton dust samplers operated at six additional cottonseed oil mills were examined for dust and endotoxin content (Table II). Five of these mills (A, B, C, D and H) were located in the Southwest and all processed seed from gins receiving machine-stripped cotton. Mill G, located in the Far West, received seed from gins exclusively processing machine-picked seed cotton.

In three of the mills listed, the levels of airborne endotoxin in one or more samples exceeded 0.5  $\mu\text{g}/\text{m}^3$ . The same value was exceeded in three of the four workplaces examined in Mill D; namely, the cleaning, delinting and baling areas.

In Mill G the amount of endotoxin in the hulling area was 1.7  $\mu\text{g}/\text{m}^3$ , whereas it was the only 0.01  $\mu\text{g}/\text{m}^3$  near the cleaners (Table II). Among the six mills listed in Table II, the elutriated dust in the hulling area of Mill G had the highest estimated endotoxin content (about 1  $\mu\text{g}$  endotoxin per mg of dust or 0.1%).

## Discussion

The study showed that endotoxin was present in the respirable dust collected from all cottonseed mills surveyed. The amounts might be associated with weathering of the cotton plant in the field,<sup>(12)</sup> harvesting variables, geographical origin of the crop, quality of the harvested cotton<sup>(13)</sup> and storage and processing variables at the oil mill. The number of vertically elutriated filter samples examined in this study was too small to assess the reasons for the high levels of endotoxin found in some mills and the low levels found in others. It was interesting to note, however, that the mechanism of harvest of seed cotton appeared unrelated to airborne endotoxin levels in oil mills since high levels of this microbial material were found in facilities processing seed that had been collected by machine-picking (Mills G and J) as well as by machine-stripping (Mills D, H and J).

**TABLE II**  
**Endotoxin and Dust Levels on Different Filters From the**  
**Same Workplace in Cottonseed Oil Mills<sup>A</sup>**

Mill Code	Workplace	Endotoxin ( $\mu\text{g}/\text{m}^3$ )	Elutriated Dust ( $\text{mg}/\text{m}^3$ )	$\mu\text{g}$ Endotoxin Per mg of Elutriated Dust
A	Baling	0.07	0.50	0.14
B <sup>1</sup>	Cleaning	0.29	2.09	0.14
B	Delinting	0.14	0.63	0.22
C	Hulling	0.02	2.50	0.01
D	Cleaning	0.83	5.53	0.15
D	Delinting	0.56	3.55	0.16
D	Delinting	0.58	2.78	0.21
D	Baling	0.54	1.05	0.51
D	Hulling	0.28	3.42	0.08
G	Cleaning	0.01	0.52	0.02
G	Hulling	1.70	1.72	0.99
H	Baling	1.61	6.72	0.24

<sup>A</sup>Mill location and crop year. Mill A = Southwest, 1978 crop; Mill B = Southwest, 1978 crop; Mill C = Southwest, 1979 crop; Mill D = Southwest, 1979 crop; Mill G = Far West, 1979 crop; Mill H = Southwest, 1979 crop.

The results also show that the levels of airborne endotoxin vary in different cottonseed oil mills. As examples, the endotoxin content of dusts from Mills 13 and 17 was consistently lower than that found in any sample from Mill J.

In Mill J the coefficient of determination ( $r^2$ ) between airborne endotoxin and vertically elutriated dust levels is 0.48. Dust levels in this mill can account for almost half of the variability found in airborne endotoxin. However, when all mills in Table I are considered together, the coefficient of determination dropped to 0.09. Thus, on an industry-wide basis the amount of dust collected by a vertical elutriator sampler is probably a poor indicator of the levels of airborne endotoxin in the workplace.

In Mill J the highest levels of airborne endotoxin were found in or around the safety shaker which was located in the delinting work area. In other mills (D, G and H), levels of endotoxin exceeding  $0.5 \mu\text{g}/\text{m}^3$  were also found in the cleaning, baling and hulling areas. Therefore, the occurrence of high levels of airborne endotoxin in a cottonseed oil mill does not appear to be restricted to particular work area or process operation. As the present study is based on a relatively small number of samples, additional studies are required to verify this observation.

The amount of endotoxin found in cotton dust varied over two orders of magnitude from  $0.01 \mu\text{g}$  per mg to  $1.3 \mu\text{g}$  per mg of vertically elutriated particulate. The contamination of cottonseed oil mill dust by endotoxin varies considerably both between and within mills. This variation did not appear to be related to the amount of dust captured by the vertical elutriator sampler.

In this study, the presence of symptoms among workers in the different mills was not investigated. Previous studies among workers in cottonseed oil mills<sup>(3-6)</sup> have not included measurements of endotoxin and the relation between observed changes in respiratory function and the amount of endotoxin exposure thus can not be evaluated. In a study on students and cotton mill workers,<sup>(14)</sup> it was found that the reaction threshold, with regard to a decrease in FEV<sub>1</sub>, over the working shift was about  $0.5 \mu\text{g}$  endotoxin/ $\text{m}^3$ . Among certain smoking workers, a reaction was found even at lower levels.

In the present study the level of endotoxin at various workplaces exceeded  $0.5 \mu\text{g}/\text{m}^3$  on about 30% of all the filters examined. Values below  $0.1 \mu\text{g}/\text{m}^3$  were found on about 40% of the filters. These differences could be the reason behind the variability in lung function responses

found between workers in different mills in previous studies.<sup>(3)</sup> This observation underlines the position that future studies on adverse health effects of cottonseed oil mill dust should take into account the contamination of this dust by endotoxin.

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