American Journal of Industrial Medicine 4:515-521 (1983)

Endotoxins in Cotton: Washing Effects and Size Distribution

Stephen A.Olenchock, PhD, Judith C. Mull, MS, and William G. Jones, MS

Endotoxin contamination was measured in washed and unwashed cottons from three distinct growing areas, California, Mississippi, and Texas. The data show differences in endotoxin contamination based upon the geographic source of the cotton. It is also shown that washing bulk cotton before the carding process results in lower endotoxin in the cotton dust. Washing conditions can affect the endotoxin levels, and all size fractions of the airborne dust contain quantifiable endotoxin contamination. Endotoxin analyses provide a simple and reliable method for monitoring the cleanliness of cotton or airborne cotton dusts.

Key words: endotoxins, cotton, agriculture, byssinosis

INTRODUCTION

Occupationally derived organic dusts contain gram-negative bacteria and related, biologically active endotoxins [Pernis et al, 1961; Dutkiewicz, 1978; Mattsby and Rylander, 1978; Rylander and Lundholm, 1978; Lundholm and Rylander, 1980; Olenchock et al, 1980, 1982; Thedell et al, 1980] which, upon inhalation at the workplace, may elicit detrimental effects in the worker. The in vivo and in vitro biological actions of endotoxins are reviewed in depth elsewhere [Donham et al, 1977; Snell, 1966]. However, it should be noted that clinical signs and symptoms of endotoxin-exposed workers have been reported to include cough, headache, nausea, eye and nose irritation, phlegm, chest tightness, fatigue, diarrhea, and fever [Mattsby and Rylander, 1978; Donham et al, 1977]. Laboratory investigations of animal models of airborne endotoxin inhalation found functional as well as histologic changes which

Division of Respiratory Disease Studies, National Institute for Occupational Safety and Health, Morgantown, West Virginia.
Mention of company names or products does not constitute endorsement by the National Institute for Occupational Safety and Health.
Address reprint requests to Stephen A. Olenchock, Ph.D., Chief, Immunology Section, National Institute for Occupational Safety and Health, 944 Chestnut Ridge Road, Morgantown, WV 26504.
Accepted for publication October 27, 1982.

correlated well with the medical findings in the workers [Snell, 1966; Hudson et al, 1977; DeMaria and Burrell, 1980; Rylander et al, 1980].

Cotton dust, in particular, has been shown to contain measurable amounts of gram-negative bacteria and their endotoxins [Pernis et al, 1961; Rylander and Lundholm, 1978; Olenchock et al, 1981; Rylander, 1981]. Correlations between decreases in pulmonary function and numbers of gram-negative bacteria have been reported [Rylander et al, 1979], although others [Boehlecke et al, 1981] reported no correlation between endotoxin content of bulk cotton samples with decrement in pulmonary function (FEV_1) when healthy, non-asthmatic subjects were exposed to cotton dust during model carding procedures.

One method for examining the respiratory effects of inhaled endotoxins in cottons would be to remove the endotoxins from the dusts prior to exposure. We examined, therefore, endotoxin levels in cottons and changes in those levels caused by washing cottons before the carding process. Endotoxin contamination of the subsequently generated dusts was quantified, and we report endotoxin analyses of various size-fractioned samples of airborne cotton dusts.

MATERIALS AND METHODS

Cottons used in this study were grown in California (CA), Mississippi (MS), and Texas (TX) and processed through a carding machine in a model cardroom at the USDA Cotton Quality Research Station in Clemson, South Carolina. Personal dust samples were obtained during the exposure of healthy, non-asthmatic human subjects by hanging personal air samplers on the subjects near their breathing zones. Five μm pore size, 37 mm polyvinyl chloride filters (VM-1, Gelman Sciences, Inc., Ann Arbor, MI) were used in open face cassettes attached to calibrated model G pumps (Mine Safety Appliances Co., Pittsburgh, PA) to collect the dust during a 6-hr exposure. Washed cottons were obtained by treating the bulk cottons with water (66°C) at a ratio of 50:1 (wt/wt) in a rayon wash line. Mississippi cotton was additionally treated wtih varied temperatures, water to fiber ratios, and wool scouring batch washing in order to assess the effects of washing conditions on endotoxin levels in the dust. In addition, elutriated cotton dust which was generated from the carding of MS cotton was fractionated by aerodynamic size using the Sierra 260 cascade impactor (Sierra Instruments, Inc., Carmel Valley, CA). The impactor was preceded by a vertical elutriator during sampling, and Parafilm "M" laboratory film (American Can Co., Greenwich, CT) was used as the collection substrate. The last stage of the impactor was proceded by a 47 mm glass fiber filter (Type A/E; Gelman Instruments Co, Ann Arbor, MI) with a collection efficiency of 99.97% for particulates $\geqslant$ 0.3 μm in order to capture the dust particles which penetrated the final impaction plate. Pre- and post-sampling weights were obtained for all filters and impaction media, and the collected dust samples were refrigerated (4°C) until analyzed for gram-negative bacterial endotoxin content.

Filters from the personal air samplers were extracted with 10 ml sterile, non-pyrogenic water (Travenol Laboratories, Inc., Deerfield, IL) by rocking at room temperature for 60 min. Sterile, non-pyrogenic plastic ware was used throughout this assay. The fluid was centrifuged at 1000g for 10 min, and the gram-negative bacterial endotoxin content of the supernatant fluid was quantified in duplicate by a spectrophotometric modification of the Limulus amebocyte lysate gel test (Pyrostat; Millipore

Corp, Bedford, MA). The lysate of the amebocytes from the horseshoe crab, Limulus polyphemus clots in the presence of endotoxin. The resulting turbidity is quantified spectrophotometrically as an apparent increase in absorbance at 360 nm. Sample results were analyzed by linear regression, compared to a standard curve, and reported as nanograms of U.S. Reference Endotoxin. Samples from the aerodynamic sizing experiments were extracted with 5 ml water and otherwise treated similarly. Unused, blank filters and Parafilm "M" were used as negative controls in all assays.

RESULTS

Treatment of cottons with water (66°C, approximately 50:1 water to fiber ratio by weight) removed considerable amounts of endotoxins as measured on a cotton dust weight basis. Dust captured in the personal sampler filters showed reductions of endotoxin content of 65%, 96%, and 95% from the unwashed levels for carded cottons from CA, MS, and TX, respectively (Table I). MS and TX cottons showed similar and distinctly higher levels of endotoxin reductions than did the cotton from CA. The unwashed cotton levels of endotoxin contamination were highest in TX cotton (390 ng/mg) and lowest in CA cotton (40.3 ng/mg) while washed levels were similar in the cottons from all 3 geographical areas.

Washing conditions were varied for the MS cottons and examinations of effects on endotoxin content were pursued. Table II shows that washing conditions affected the endotoxin activity in the carded cotton dust. The rayon wash line hot water (66°C) at a ratio of 50:1 (water to fiber by weight) was the most effective, removing 95% of the endotoxins. Cold water (28°C), at the higher water to fiber ratio of 65:1 was the least effective and removed 83% of the endotoxin contamination. Use of the wool scouring batch washing system with hot water removed 87% of the endotoxins.

TABLE I. Effect of Washing on Endotoxin Content of Airborne Cotton Dusts

	Endotoxin		
Cotton	Unwashed (ng/mg)	Washed[a] (ng/mg)	Decrease (%)
CA	40.3 ± 4.1[b]	14.3 ± 2.2[b]	64.5
MS	167.6 ± 7.6	7.4 ± 1.0	95.6
TX	390.2 ± 22.6	21.6 ± 4.0	94.5

[a]Washed: rayon line, 66°C, 50:1 water to fiber.
[b]Mean ± SEM; N = 17 for CA and TX unwashed; all others, N = 16.

TABLE II. Effect of Wash Conditions on Endotoxin Content of Airborne Cotton Dust

Wash line[a]	Water temp. (°C)	Water to fiber ratio (wt/wt)	Endotoxin (ng/mg)	Decrease (%)
Unwashed	—	—	167.6 ± 7.6[b]	—
Rayon	28	65:1	28.5 ± 1.6	83.0
Rayon	66	65:1	17.9 ± 2.0	89.3
Rayon	66	50:1	7.4 ± 1.0	95.6
Wool scouring	66	—	22.0 ± 1.8	86.9

[a]Mississippi cotton.
[b]Mean ± SEM; N = 16.

Quantification (ng/m^3) of the airborne gram-negative bacterial endotoxin showed marked reductions in airborne levels when washed cottons were carded as compared to their respective, unwashed, cottons (Table III). When the airborne dust levels were purposefully maintained at similar concentrations in the room, unwashed TX cotton showed the highest endotoxin contamination in the air (202 ng/m^3). Analysis of the effects of washing conditions on the airborne concentration of endotoxins in MS cotton showed that treatment with hot water (66°C) at a 50:1 water to fiber ratio resulted in the least airborne endotoxins during carding (Table IV) when the dust level was purposefully maintained at a relatively constant level. Extraction of blank filters resulted in a mean endotoxin level of 0.4 ± 0.01 ng/ml (N = 4).

Airborne dust which was collected on various stages of the cascade impactor was analyzed for endotoxin content, and these data are presented in Table V. Higher endotoxin levels were observed in fractions 4 and 3 (2.9–5.9 μm) while lesser amounts were detected in size ranges both above and below those fractions. As the stages progressed from the 16.5 μm, 50% cut point to the smallest, 0.7 μm, the amount of dust which was trapped increased (0.06–0.16 mg, respectively). The back-up filter collected the most dust (0.24 mg) and the endotoxin content of that dust was higher than any stage in the impactor. Extraction of blank Parafilm "M" impaction discs and blank back-up filters resulted in mean endotoxin levels of < 0.2 ng/ml (n = 4) and 0.5 ± 0.2 ng/ml (N = 2), respectively.

DISCUSSION

Neal et al [1942] showed by light microscopy that gram-negative bacteria were within the lumen of the cotton fiber. Further detail concerning the close association

TABLE III. Comparison of Airborne Endotoxin Contamination at Similar Dust Levels During Carding

Cotton	Dust level (mg/m^3)	Endotoxin (ng/m^3)
CA, unwashed	0.61 ± 0.02[a]	23.7 ± 1.9[a]
CA, washed	0.52 ± 0.02	7.3 ± 1.0
MS, unwashed	0.52 ± 0.01	87.6 ± 4.5
MS, washed	0.51 ± 0.02	3.8 ± 0.5
TX, unwashed	0.52 ± 0.01	202.2 ± 13.2
TX, washed	0.50 ± 0.02	10.8 ± 2.1

[a]Mean ± SEM; N = 17 for CA and TX unwashed; all others, N = 16.

TABLE IV. Effect of Wash Conditions on Airborne Endotoxin Levels During Carding

Wash conditions[a]	Dust level (mg/m^3)	Endotoxin (ng/m^3)
Unwashed	0.52 ± 0.01[b]	87.6 ± 4.5[b]
28°C, 65:1	0.44 ± 0.02	12.5 ± 0.8
66°C, 65:1	0.60 ± 0.02	10.6 ± 1.1
66°C, 50:1	0.51 ± 0.02	3.8 ± 0.5
66°C, Wool scouring	0.58 ± 0.01	12.9 ± 1.1

[a]Mississippi cotton, rayon line; temperature (°C), water to fiber ratio.
[b]Mean ± SEM; N = 16.

TABLE V. Endotoxin Content of Aerodynamically Fractionated Cotton Dust*

Stage	50% Cut point (μm)	Dust weight (mg)	Endotoxin content (ng/mg)
1	16.5	0.06 ± 0.01 (3)[a]	674.8 ± 212.2 (5)[a]
2	10.7	0.06 ± 0.01 (3)	869.8 ± 116.5 (6)
3	5.9	0.09 ± 0.02 (3)	1070.3 ± 166.3 (6)
4	2.9	0.13 ± 0.03 (3)	1072.3 ± 106.6 (6)
5	1.3	0.15 ± 0.01 (3)	822.4 ± 55.1 (6)
6	0.7	0.16 ± 0.02 (3)	748.7 ± 72.1 (6)
BF[b]	—	0.24 ± 0.07 (3)	1587.6 ± 208.9 (6)

*Mississippi cotton, unwashed.
[a]Mean ± SEM (N).
[b]Back-up filter.

of gram-negative bacteria with cotton fibers was provided by electron microscopic analyses [Helander and Lounatmaa, 1981]. Free and liberating outer membrane fragments from bacteria were observed in that study and attest to the ease with which bacteria (and endotoxins) may contaminate airborne dusts during the carding operation. In addition, viable gram-negative bacterial counts were related to the development of airway constriction following cotton dust exposures [Rylander et al, 1979]. As part of a larger study which evaluated the respiratory effects of dusts from washed and unwashed cottons, we studied the endotoxin contamination as assayed by the Limulus amebocyte lysate gel test (LAL). These data showed that prior washing of cottons is effective in removing endotoxin activity. Airborne dusts which were generated during the carding process were also affected by prior washing of the cottons. At similar dust levels, the endotoxin contaminations of the airborne dusts were markedly lower when washed cottons were carded than when their unwashed counterparts were used. Washing conditions vary in efficacy in removing endotoxin contamination. Hot water (66°C) at a ratio of 50:1 (water:fiber) appeared to be the most efficient of the methods which we tested. Why hot water at the higher ratio of 65:1 was not more efficient remains unexplained. However, it should be noted that the most efficient washing combination may not have been tested yet.

Of distinct interest to this study was the observation that cotton which was grown in California had notably less endotoxin contamination than cottons which were grown in Mississippi (4-fold) or Texas (9-fold). In fact, there was greater than a 2-fold difference in quantifiable endotoxins between MS and TX cottons as well. One might speculate that the combination of different strains of cotton and different temperatures, soils and growth conditions may have resulted in a) varied quantities of bacterial contamination and/or b) different species of organisms which colonize the cotton. This study does not address the reasons for the differences in endotoxin contamination with geographically distinct cottons. Others have shown that endotoxins from different gram-negative bacteria differ in their relative toxicities as measured by such parameters as the amount of lactic dehydrogenase in pulmonary lavage fluid [Burrell and Rylander, 1982] and measurement of free lung cells (Helander et al, 1980]. Differential toxicities may depend upon the chemical composition of the lipopolysaccharides [Helander et al, 1980] and the outer membrane shedding characteristics of the organism [Lounatmaa and Helander, 1982].

Size-fractionation of the airborne cotton dust with subsequent endotoxin quantification showed that endotoxins are present in every size of particle collected, with a trend for higher concentration in particles 2.9 and 5.9 μm in size. The universal association between endotoxins and cotton dusts, regardless of size, would be expected in light of previous studies which showed the intimate association between gram-negative bacteria and the cotton fiber [Neal et al, 1942); Helander and Lounatmaa, 1981] as well as between bacteria and various other cotton plant parts [Rylander and Lundholm, 1978]. The higher concentration of endotoxins in the dusts at 2.9 and 5.9 μm size as well as the smallest (<0.7 μm) particle size suggests that airborne endotoxins are readily inhaled deeply into the lung. Cotton plant parts, especially bract, are friable and easily produce small particles of dust [Morey, 1981]. Bacteria and bacterial components would be expected in association with these cotton-related materials. However, it should be noted that the greater amounts of endotoxins found in the smallest dust particles (< 0.7 μm) may reflect a more efficient extraction from the larger surface area of the smaller particles rather than an actual difference in endotoxin contamination. As a technical note, we report that Parafilm "M" laboratory film provided a good medium when the Sierra 260 impactor is used to sample cotton dust which will be analyzed subsequently for endotoxin contamination. Analysis of unused Parafilm "M" laboratory film showed negligible background contamination with endotoxins as measured by LAL.

These studies were not intended to define the etiologic agent of byssinosis. Rather, they offer a simple and reliable test to monitor the efficacy of washing cotton, should washing be used as a method to reduce the presence of any byssinotic agent in the cotton mills. Alternatively, endotoxin levels may provide a way to monitor the "cleanliness" of cottons and their airborne dusts.

ACKNOWLEDGMENTS

We thank the employees of the USDA Cotton Quality Research Station, Clemson, South Carolina for providing us with the dust samples; and we thank Ms Beverly J. Wilhelm for helping us to prepare this manuscript.

REFERENCES

Boehlecke B, Cocke J, Bragg K, Hancock J, Petsonk E, Piccirillo R, Merchant J (1981): Pulmonary function response to dust from standard and closed boll harvested cotton. Chest 79S:77S-81S.

Burrell R, Rylander R (1982): Further studies on inhaled endotoxin-containing bacteria. Environ Res 27:325–336.

DeMaria TF, Burrell R (1980): Effects of inhaled endotoxin-containing bacteria. Environ Res 23:87–97.

Donham KJ, Rubino M, Thedell TD, Kammermeyer J (1977): Potential health hazards to agricultural workers in swine confinement buildings. J Occup Med 19:383–387.

Dutkiewicz J (1978): Exposure to dust-borne bacteria in agriculture. I. Environmental studies. Arch Environ Health 33:250–259.

Helander I, Lounatmaa K (1981): Cotton bacterial endotoxin assessed by electron microscopy. Br J Indust Med 38:394–396.

Helander I, Salkinoja-Salonen M, Rylander R (1980): Chemical structure and inhalation toxicity of lipopolysaccharides from bacteria on cotton. Infect Immunity 29:859–862.

Hudson AR, Kilburn KH, Halprin GM, McKenzie WN (1977): Granulocyte recruitment to airways exposed to endotoxin aerosols. Am Rev Respir Dis 115:89–95.

Lounatmaa K, Helander I (1982): Ultrastructure of gram-negative cotton bacteria with different pulmonary toxicities. Infect Immunity 35:359–362.

Lundholm M, Rylander R (1980): Occupational symptoms among compost workers. J Occup Med 22:256–257.

Mattsby I, Rylander R (1978): Clinical and immunological findings in workers exposed to sewage dust. J Occup Med 20:690–692.

Morey PR (1981): Vegetable ingredients of dusts in textile and nontextile cotton industries. Chest 79S:97S–101S.

Neal PA, Schneiter R, Caminita BH (1942): Report on acute illness among rural mattress makers using low grade, stained cotton. JAMA 119:1074–1082.

Olenchock SA, Lenhart SW, Mull JC (1982): Occupational exposure to airborne endotoxins during poultry processing. J Toxicol Environ Health 9:339–349.

Olenchock SA, Mull JC, Boehlecke BA, Major PC (1981): Cotton dust and complement in vivo. Chest 79S:53S–55S.

Olenchock SA, Mull JC, Major PC (1980): Extracts of airborne grain dusts activate alternative and classical complement pathways. Ann Allergy 44:23–28.

Pernis B, Vigliani EC, Cavagna C, Finulli M (1961): The role of bacterial endotoxins in occupational diseases caused by inhaling vegetable dusts. Br J Indust Med 18:120–129.

Rylander R, Lundholm M (1978): Bacterial contamination of cotton and cotton dust and effects on the lung. Br J Indust Med 35:204–207.

Rylander R, Mattsby I, Snella MC (1980): Airway immune response after exposure to inhaled endotoxin. Bull Eur Physiopath Resp 16:501–509.

Rylander R (1981): Bacterial toxins and etiology of byssinosis. Chest 79S:34S–38S.

Rylander R, Imbus HR, Suh MW (1979): Bacterial contamination of cotton as an indicator of respiratory effects among card room workers. Br J Indust Med 36:299–304.

Snell JD (1966): Effects of inhaled endotoxins. J Lab Clin Med 67:624–632.

Thedell TD, Mull JC, Olenchock SA (1980): A brief report of gram-negative bacterial endotoxin levels in airborne and settled dusts in animal confinement buildings. Am J Indust Med 1:3–7.