SECOND COLLECTION OF UNIFORM, CARD GENERATED, VERTICALLY ELUTRIATED DUST FOR INTERLABORATORY COMPARISON OF DUST ENDOTOXIN ASSAYS

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Abstract

Previously, an elaborate cotton blending and dust collection protocol was developed and implemented to produce "uniform", vertically elutriated dust samples which were used in a two part interlaboratory endotoxin assay study. The results generated interest in continuing further study. To satisfy this need, a second series of dust samples on two types of filters were produced from 'three sources of cotton'. A description of the cotton blending and dust collection process will be presented as well as a description of the dust samples collected.

Introduction

At last year's Beltwide Cotton Conference, the results of the first phase of an endotoxin assay round robin study were presented. This was followed by a report on the preliminary results of the second phase of the same study at an ACGIH (American Conference of Governmental Industrial Hygienists) workshop held at Chapel Hill, NC (Chun et al. 1998; 1999). A summary of the highlights of the first phase and the complete results of the second phase will be presented at this 1999 Beltwide Cotton Conference. In the first part of the study, filter membranes with the same approximate amount and type of cotton dust were sent for analysis to laboratories that 'routinely' perform endotoxin analyses. Each of these laboratories performed the analysis using the methodology common to their laboratory. The results showed that intralaboratory variations were very small; but large and significant interlaboratory variations were observed. In the second part of the study, filter membranes with cotton dust were again sent to the same laboratories where the analyses were performed as before but with a common extraction protocol. The partial results from the second part of the study showed that again intralaboratory variations were very small and again significant variation existed between laboratories. However, where a common extraction protocol was used, the differences in results between the laboratories was reduced considerably which suggested strongly that further standardization might reduce the differences even more and possibly to the point that interlaboratory results might become directly comparable.

These findings came about partially because uniform vertically elutriated cotton dust samples were made available for study (Perkins et al., 1996). When the dust samples were originally made, testing beyond what has been described was not foretold. However, since these results have become public, the need for additional samples developed and we have again taken the initiative to collect the dust at the USDA, Cotton Quality Research Station, Clemson, SC. It is the purpose of this report to describe the methodology used to generate additional cotton dust samples and to describe the samples generated.

General Methodology

Cotton dust with three different endotoxin concentrations on two different support filters was collected. For this study, PVC (polyvinyl chloride) and glass filters were used. PVC was used because it is the standard filter prescribed by the Cotton Dust Standard (historical information is greatest on and PVC is the industry standard). Glass was used because many laboratories have shown a preference to using glass filters -- possibly for its lower cost and the elimination of the need for support pads as required with PVC filters. The initial thought for generating cotton dust with different endotoxin concentrations was to use cottons from growing regions known to usually produce dusts with low endotoxin concentrations and growing regions known to usually produce high endotoxin concentrations (Fischer et al., 1989; Olenchock et al., 1984; Simpson & Marsh, 1985). For example, California and Mississippi cottons, respectively, would be such a choice. However, the bacterial profiles of cotton from different regions differ significantly (Chun and Perkins, 1997b). Dust endotoxin concentrations were obtained from cottons grown in the same region. Better grade cottons tend to have lower dust and the dust is of lower endotoxin concentrations than that of lower grade cottons (Fischer et al., 1982, 1986; Godby et al., 1995). As a starting point for a possible test and based on having as many as 13 laboratories, working with 2 filter types, examining 3 endotoxin concentrations, and using only 3 sample replicates per laboratory, a minimum of 234 filter membranes was needed. It was decided early on that since dust generation was such a time consuming, labor intensive and costly endeavor, that as many dust laden sample filters would be collected at this time as possible to anticipate further endotoxin assay testing.

The facilities for dust generation at CQRS and general protocol for dust collection have been described by Chun and Perkins (1997a), and Perkins et al. (1996) and will not be described in detail here. The general approach by Perkins et al. (1996) was followed except where noted. The earlier dust collection study showed no difference in endotoxin level due to location and position of the vertical elutriators (VE) so dust collection duration, air flows etc,

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were adjusted to optimize collection of 0.3 – 0.8 mg of dust/filter as needed. The cotton with the expected lowest level of cotton dust and endotoxin was processed first followed by the cottons expected to produce higher levels of cotton dust and endotoxin.

Cotton

Cotton was purchased from the Eastern Trading Company, Inc. (620 Rutherford St, Greenville, SC 29609) and arrived at our location July 1, 1998. The cotton was grown in the Mississippi Delta region from the 1997-harvest year and consisted of 12 bales of strict low middling (grade 41) and 12 bales of low middling light spotted (grade 53) cottons. High Volume Instrument (HVI) data on the cottons delivered is given in Table 1. Only 9 bales of the strict low middling and 9 bales of the low middling light spotted were used.

Dust was generated from blended strict low middling (Cotton A), low middling light spotted (Cotton B), and a 1:1 mixture of low middling light spotted and strict low middling cottons (Cotton AB). Since the work by Perkins et al. (1996), some of the machinery at CQRS has fallen into disrepair so an alternate blending method was used which in actuality results in more uniformly blended cotton. Instead of blending the cotton and re-baling the cotton before forming cotton laps, laps from Cotton A and Cotton B, were formed directly. To do this, three bales from Cotton A were randomly chosen at a time. One of the 3 bales were placed behind and then fed into a separate blending hopper (Syncromatic Blending System, Fibers Control Corporation, P.O. Box 1358, Gastonia, NC). The delivery from each hopper fell onto an endless belt to form a sandwich blend of the cotton from the three bales. The sandwich blend, theoretically contained equal portions of cotton from each of the three bales. The cotton was picked up at this point in large wheel boxes and was transported manually to the blending finisher picker (Aldrich Machine Works, Greenwood, SC) where the laps were made. The cotton was placed on a large apron behind the picker. The picker consists of a spiked beater and a lap forming section. As cotton passes through the beater, small tufts are produced and these subsequently are formed into a "lap" that is a thin batt (13-14 oz/sq. yd.) of cotton rolled up as a cylinder weighing about 40-42 pounds. All of the blended cotton was processed into laps. These laps were labeled as laps A1. This was repeated for the remaining Cotton A bales to create a group of laps A2 and A3. After all the cotton were made into laps, one lap was randomly selected from lap groups A1, A2 and A3 and processed through the finishing picker until all the laps were processed to obtained approximately 14 oz/sq. Yd laps. The same was done for Cotton B. Next, 2/3 of the laps from Cotton A were processed through the finishing picker, 4 laps at a time, randomly chosen. These laps were then wrapped in brown paper and stored in plastic bag until carded for dust production. The same was done for the laps from Cotton B. Finally to get the intermediate cotton, the remaining laps were processed through the finishing picker, using 2 laps from the remaining Cotton A and 2 laps from the remaining Cotton B, randomly chosen. These laps were then wrapped in brown paper and stored in plastic bags until carded for dust production. This is an ultimate blending scheme that ensures that any one pound of cotton fed to the card is essentially identical to any other pound of cotton fed.

Vertical Elutriators

Thirty vertical elutriators (VE's, Model GMW-4000; General Metal Works, 145 S. Miami Ave, Cleaves OH 45002) were used for dust collection. Only the 'cone portion' of the VE was used. To augment the supply of VE's at CQRS, additional VE's were borrowed from the National Institute of Occupational Safety and Health (NIOSH) in Morgantown, WV. The VE's were hung in three rows with 16, 8 and 6 VE in the rows, respectively. Normally the aerosol analysis monitor filter cassettes (M000-037-A0, Millipore Corporation, 80 Ashby Road, Bedford, MA 01730) are used in the 'open-faced' situation, but it was found just before dust collection was to begin that some of the CQRS and all of the NIOSH VE's would only accommodate the filter cassettes in the 'closed-faced' situation. For this reason, the dust collection was made in the 'closed-faced' situation that results in a more concentrated location of the dust on the filters compared to the more diffuse collection in the 'open-faced' method. The VE's were numbered sequentially and the odd numbered VE's were fitted with PVC filters (GLA-5000; Pall Gelman Sciences, 600 South Wagner Road, Ann Arbor, MI 48103-9019) and the even-numbered VE's were fitted with glass filters (Type A/E; Pall Gelman Sciences, 600 South Wagner Road, Ann Arbor, MI 48103-9019). Flow rates of the critical orifices were calibrated with a Gilan Gilibrator-2 (Sensidyne, Inc., 16333 Bay Vista Dr., Clearwater, FL 33760) to fall within standards. Dust collection duration time was altered as required to collect approximately 0.3 to 0.8 mg of cotton dust per filter. Each weighed dust laden membrane was transferred to a 50-ml screw-top polypropylene conical tube (Falcon® 2998; Becton Dickinson and Co., 2 Bridgewater Lane, Lincoln Park, New Jersey 07035) and stored in the dark at room temperature (~22°±1°C) until used.

Statistical Analysis

Data were analyzed using release 6.12 of SAS (SAS, Statistical Analysis System; SAS system for Windows version 4.0950; SAS Institute Inc., Cary, NC, USA) for making mean comparisons. Otherwise additional testing and data manipulation were done with Microsoft EXCEL 97 SR-1 for Windows 95 (Microsoft Corporation, USA) and plotted using SigmaPlot for Windows Version 4.01 (SPSS, Inc., USA).

Results and Discussion on Dust Samples Collected

In all, 120 collection runs or lots were made, each supplying approximately 30 usable dust samples per run – 15 on PVC filters and 15 on glass filters to provide ample samples for several round robin endotoxin assay tests. Cotton A dust generation was low as expected due to the lower anticipated dust potential which necessitated longer collection times; and even with the longer times used, the filters had an average lower dust/filter weight, Table 2 and Figures 1 & 2. Twenty-eight runs or lots of Cotton A were made, using all of the Cotton A laps. Because of the higher dust potential, collecting dust from Cotton AB and B required less time for collection and more runs were possible which resulted in 44 and 48 lots of Cotton AB and Cotton B, respectively; and these runs tended to contain on an average, more dust than from Cotton A, Table 2 and Figure 1. Not all of the Cottons B and AB were used -- two or more laps of both Cotton AB and Cotton B are being held in reserve so that additional MTM generated custom filter samples could be made if needed.

On an average, more dust is collected on the glass than on the PVC filters, Table 3 and Figures 2 & 3. The differences between the PVC and glass filters were significant for all cotton sources. Strangely, the difference was not significant with Cotton AB, a 1:1 mix of Cotton A and B even though the differences for both cottons were highly significant, Table 3. The higher dust retention by the glass filters is not unexpected since its average pore size is one micron compared to 5 microns for the PVC filter. Other filter characteristics to consider is that the glass filter has a slower flow rate, 45 Lpm/cm² vs. 53 Lpm/cm² at 0.7 bars (10 psi) and is much thicker, 457 μm, compared to the PVC filter, 152.4μm (Pall Gelman Sciences, 1998). Also, severe extraction procedures on the glass filter may cause the glass filter to disintegrate (personnel communiqué). Further research will have to determine whether these differences play a meaningful role for endotoxin determination assay.

The goal of this project, to generate cotton dust samples, was accomplished. Over 3,000 dust samples on filter media have been collected which should provide sufficient test material for the current proposed round robin test, filter medium and endotoxin concentrations, and tests to be determined later.

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Disclaimer

Mention of a trademark, warranty, proprietary product or vendor does not constitute a guarantee by the U.S. Department of Agriculture and does not imply approval or recommendations of the product to the exclusion of others which may also be suitable.

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Table 1[1]. HVI data of the strict low middling and the low middling light spotted cotton from the 1997 harvest year grown in the Mississippi Delta region.

Strict Low Middling Cotton, Cotton A										
Bale ID	Gr-1	Str	Mic	GPT	Lng	Unf	Col-g	Colrd	Colb	Trmt
10538	41-4	34	5.3	27.6	1.07	83	41-1	75	78	9
10540	41-4	36	5.0	27.7	1.11	82	51-1	71	76	9
10558	41-4	35	5.1	29.4	1.10	83	41-1	74	79	4
10572	41-4	35	5.2	28.7	1.08	82	41-2	74	74	8
10937	41-4	34	5.0	29.3	1.07	82	41-2	74	75	8
48553	41-4	36	3.4	30.3	1.13	91	51-1	71	76	5
907021	41-3	34	4.0	30.4	1.05	83	31-2	77	78	4
907339	41-4	36	5.0	26.5	1.11	82	41-1	76	77	6
949412	41-4	36	4.8	25.3	1.13	82	41-2	75	68	8
960439	41-4	36	4.8	27.3	1.12	83	41-3	73	85	5
961423	41-4	34	4.8	26.4	1.07	82	41-3	73	84	7
999181	31-3	37	5.3	29.0	1.14	82	21-3	78	94	3
Low Middling Light Spotted Cotton, Cotton B										
Bale ID	Gr-1	Str	Mic	GPT	Lng	Unf	Col-g	Colrd	Colb	Trmt
24101	43-6	35	4.1	24.7	1.10	81	53-3	62	107	13
24102	53-5	36	4.2	25.9	1.11	80	53-3	63	108	10
24103	53-7	36	4.3	27.1	1.12	83	53-2	62	104	19
24104	53-6	36	3.9	27.3	1.13	81	53-4	61	105	17
24105	53-6	37	3.9	26.1	1.14	82	50-2	61	103	18
24106	53-6	36	3.8	27.5	1.13	81	53-2	62	104	17
24107	53-6	36	3.8	27.2	1.12	82	53-2	62	101	15
24152	53-6	35	4.0	27.6	1.10	83	53-1	65	101	15
24153	53-7	35	3.9	27.2	1.09	82	53-1	64	101	13
24155	53-6	35	4.0	26.2	1.10	82	43-2	65	106	14
24188	53-6	36	3.9	27.1	1.11	83	53-1	64	106	12
24202	53-7	35	4.1	26.0	1.10	82	43-2	66	104	9

[1] Bale ID = Bale identification number; Gr-1 = Classer's grade; Str = staple length, 1/32 of an inch; Mic = micronaire reading, microgram/inch; GPT = grams/tex; Lng = upper half mean length; UNF = uniformity index, mean/upper half mean; Col-g=Color grade; Colrd = color reflectance; Colb = color + b; Trmt = percent trash.

Table 2. Overall average dust weight from Cotton A, AB and B.

Cotton Source[1]	Average dust per filter, mg/filter[2]
A	0.45[c]
AB	0.61[b]
B	0.65[a]

[1] Cotton A = strict low middling; Cotton B = low middling light spotted; Cotton AB = 1:1 blend of strict low middling to low middling light spotted.
[2] Mean separation within columns by Duncan's multiple range test, 5% level. Means with the same letter are not significantly different.

Table 3. Average dust weight on Glass and PVC filters on Cottons A, AB and B. .

	Average dust per filter, mg/filter	
Cotton Source[1, 2]	Glass Filters	PVC Filters
A, B and AB***	0.56	0.62
A***	0.49	0.41
AB	0.62	0.60
B***	0.68	0.62

[1] Cotton A = strict low middling; Cotton B = low middling light spotted; Cotton AB = 1:1 blend of strict low middling to low middling light spotted.
[2] t-test, average dust weight difference between PVC and Glass filters is equal to zero: *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$.

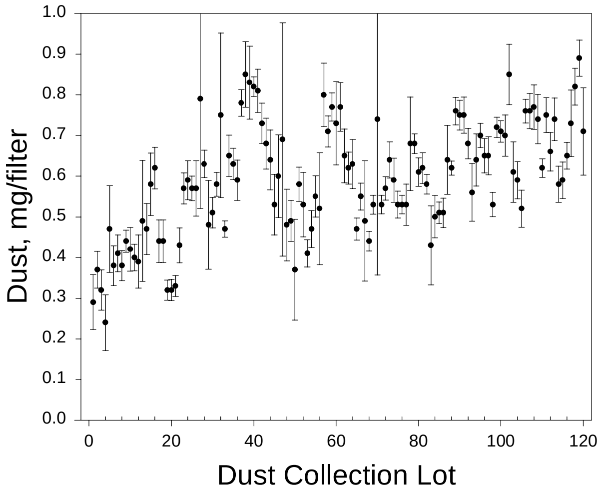

Figure 1. Average dust collected on glass and PVC filter for each collection run/lot -- Lots 1-28 from Cotton A; lots 29-72 from Cotton AB; and lots 73-120 from Cotton B. Each half-error bar represents 2 s.e.

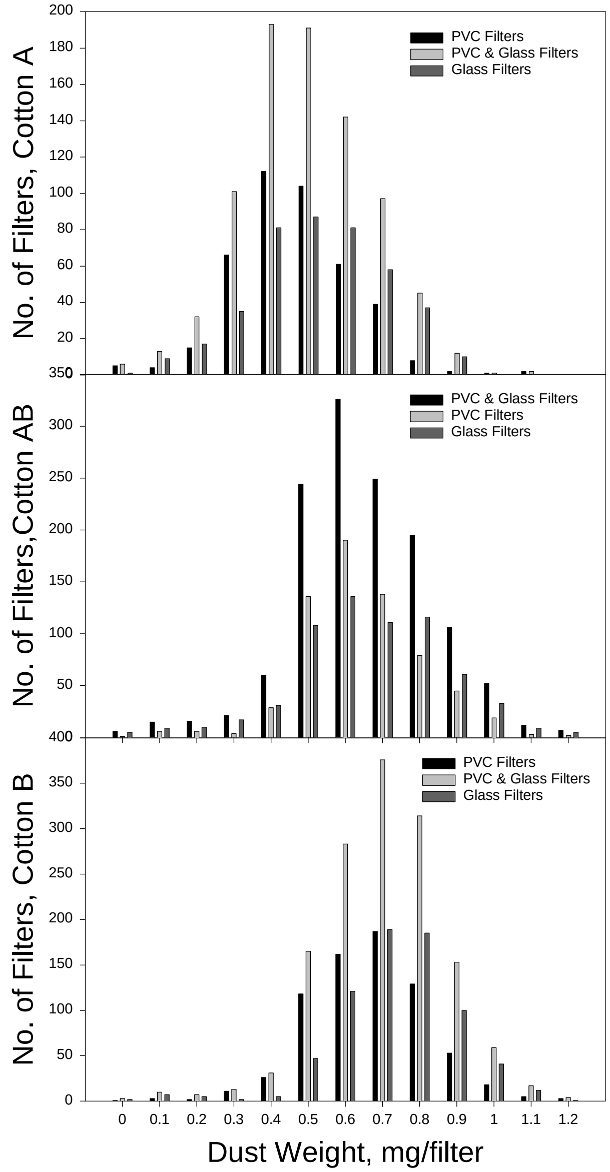

Figure 2. Frequency of filters to the dust weight on all filters and on the glass or on the PVC filters. Cotton A = strict low middling; Cotton B = low middling light spotted; Cotton AB = 1:1 blend of strict low middling to low middling light spotted.