

ORGANIC DUSTS

Second Collection of Card-Generated, Vertically Elutriated Dust for Comparison Endotoxin Assays

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INTERPRETIVE SUMMARY

In an earlier work, dust samples on polyvinyl chloride filters were produced by an elaborate cotton blending and dust collection protocol. The dust samples produced were uniform, vertically elutriated dust samples. These samples were used in a two-part endotoxin assay study of various laboratories. The results from the study prompted the desire to continue the study.

The supply of dust samples from the original dust collection was exhausted, which meant that additional dust samples would have to be generated. To get the needed additional samples, cotton from three sources was carded and the dust collected on two types of filters using vertical elutriators in the model cardroom at the Cotton Quality Research Station in Clemson, SC.

More than 3000 filter samples, each with 0.3 to 0.8 mg cotton dust, were collected, which should satisfy the supply needs of the anticipated endotoxin assay study. This paper describes the cotton blending procedure and the dust collection process, as well as the dust sample population.

ABSTRACT

Previously, an elaborate cotton blending and dust collection protocol was developed and implemented to produce uniform, vertically elutriated dust samples that were used in a two-part interlaboratory endotoxin assay study. The results from that study generated interest in extending the interlaboratory endotoxin assay study. To satisfy this need, a second

series of dust samples on glass filters, as well as on the polyvinyl chloride filters used previously, were collected. Three sources of cotton were used. The cottons were blended to produce a uniform and homogenized cotton, and then carded. The dust collection was made on vertical elutriators using the model cardroom at the Cotton Quality Research Station in Clemson, SC. Dust samples containing a low, medium and high endotoxin concentration were obtained. A full 120 separate collection runs were made and they resulted in more than 1500 dust samples on polyvinyl chloride filters and 1500 dust samples on glass filters, each with 0.3 to 0.8 mg cotton dust per filter. The dust samples collected should satisfy the need for dust samples for present and future endotoxin assay studies.

At the 1998 Beltwide Cotton Conferences, the results of the first phase of an endotoxin assay round-robin study were presented (Chun et al., 1998). This was followed by a report on the preliminary results of the second phase of the same study at a workshop sponsored by the American Conference of Governmental Industrial Hygienists held at Chapel Hill, NC (Chun et al., 1999a).

A summary of the highlights of the first phase and the complete results of the second phase were presented at the 1999 Beltwide Cotton Conferences (Chun et al., 1999b). 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 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 again showed that intralaboratory variations were

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small and significant variation existed between laboratories. However, when a common extraction protocol was used, the differences in results between the laboratories were reduced considerably, which suggested strongly that further standardization might reduce the differences even more, 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). Sufficient number of dust samples were originally collected only for the tests described. However, since the above results have become public, the need for additional tests developed, and additional dust samples were collected at the USDA, Cotton Quality Research Station in 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 was collected with three different endotoxin concentrations on two different support filters; polyvinyl chloride (GLA-5000 Membrane, 5 μm 37 mm, PVC membrane filter)¹, and glass (Type A/E Glass Fiber Filter 1 μm 37 mm). Both were from Pall Gelman Sciences, Ann Arbor, MI.

The polyvinyl chloride filters were used because they are the standard filter prescribed by the Cotton Dust Standard, a great deal of historical information on its usage exists, and polyvinyl chloride is the textile industry standard. Glass was used because many laboratories have shown a preference to using glass filters—possibly because they cost less, do not need support pads as required with polyvinyl chloride filters, and because their endotoxin recovery is often greater than that of polyvinyl chloride (Thorne et al., 1997). For aqueous samples, though, polyvinyl chloride filters maybe a better choice for recovery (Woods and Jacobs, 1997).

The initial thought for generating cotton dust with different endotoxin concentrations was to use

cottons from two growing regions; (i) one known to usually produce dusts with low endotoxin concentrations and (ii) one known to usually produce high endotoxin concentrations (Fischer et al., 1989; Olenchock et al., 1984; Simpson and Marsh, 1985). For example, dusts from California cottons are known to produce low concentrations, while dust from Mississippi cottons is often high. But 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 (whiter and less trash) cottons tend to have lower dust content and the dust is of lower endotoxin concentrations than those 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 two filter types, examining three endotoxin concentrations, and using only three sample replicates per laboratory, a minimum of 234 filter membranes would be needed. It was decided early on that because dust generation was such a time consuming, labor intensive, and costly endeavor, numerous dust-laden sample filters would be collected in anticipation of further endotoxin assay testing.

The facilities for dust generation at the Cotton Quality Research Station, Clemson, SC, 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, so dust collection duration, air flows, etc., were adjusted to optimize collection of 0.3 to 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., in Greenville, SC, and arrived at Cotton Quality Research Station on 1 July 1998. The

¹Mention of a trademark, warranty, proprietary product or vendor does not constitute a guarantee by the USDA and does not imply approval or recommendations of the product to the exclusion of others that also may be suitable.

Table 1 †. High-volume instrument data of the strict low middling and the low middling light spotted cotton from the 1997 harvest year grown in the Mississippi Delta region.

Bale ID	Gr-1	Str	Mic	GPT	Lng	Unf	Col-g	Colrd	Colb	Trmt
<u>Strict low middling cotton, cotton A</u>										
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
<u>Low middling light spotted cotton, cotton B</u>										
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

† Bale ID = Bale identification number; Gr-1 = Classifier'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.

cotton had been 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 data on the cottons delivered are given in Table 1. Only nine bales of each grade 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).

During the time since the work described in Perkins et al. (1996), some of the machinery at Cotton Quality Research Station has fallen into disrepair, so an alternate but more efficient blending method was used. Instead of blending the cotton and re-baling the cotton before forming cotton laps (a thin batt—0.044077 to 0.047468 g cm⁻² (13 to 14

oz/sq yd)— of cotton rolled up as a cylinder and weighing about 18 to 19 kg (40 to 42 lb)), the laps from Cotton A and Cotton B, were formed directly. To do this, three bales from Cotton A were randomly chosen at a time.

Each of the three bales was placed behind and then fed into a separate blending hopper (Syncromatic Blending System, Fibers Control Corporation, 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 in large wheel boxes and 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, which consists of a spiked beater and a lap-forming section.

As cotton passed through the beater, small tufts were produced that were subsequently formed into the lap. 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 was made into laps, one lap each was randomly selected from lap groups A1, A2, and A3 and processed through the finishing picker until all the laps were processed to obtain approximately 0.047478 g cm⁻² (14 oz/sq yd) laps. The same was done for Cotton B.

Next, two-thirds of the laps from Cotton A were randomly chosen, four laps at a time, to be processed through the finishing picker. These laps were then wrapped in brown paper and stored in plastic bags 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 two laps randomly chosen from the remaining Cotton A and two laps from the remaining Cotton B. These laps were then wrapped in brown paper and stored in plastic bags until carded for dust production. This is a highly efficient 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 (Model GMW-4000; General Metal Works, Cleaves, OH) were used for dust collection. Only the cone portion of the elutriator was used. To augment the supply of elutriators at Cotton Quality Research Station, additional units were borrowed from the National Institute for Occupational Safety and Health in Morgantown, WV.

The elutriators were hung in three rows 16, 8, and 6 per row. Normally, the aerosol analysis monitor filter cassettes (M000-037-A0, Millipore Corp., Bedford, MA) are used in the open-faced configuration, but it was found just before dust collection was to begin that some of the elutriators from the Cotton Quality Research Station and all of them from the National Institute of Occupational Safety and Health (NIOSH) would accommodate only the filter cassettes in the closed-faced configuration. Therefore, because all the elutriators can operate in the closed-face configuration, dust collection was made in that configuration.

This procedure results in a more concentrated location of the dust on the filters compared with the more diffuse collection in the open-faced method. The elutriators were numbered sequentially, the odd numbered ones were fitted with polyvinyl chloride filters, and the even numbered ones were fitted with glass filters.

Flow rates of the critical orifices were calibrated with a Gilan Gilibrator-2 (Sensidyne, Inc., Clearwater, FL) 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., Lincoln Park, NJ) and stored in the dark at room temperature ($\sim 22 \pm 1$ °C) until used.

Statistical Analysis

Data were analyzed on a personal computer using the mainframe release 6.12 of SAS (SAS, Statistical Analysis System for Windows, version 4.0950; SAS Institute Inc., Cary, NC) for making mean comparisons. Otherwise additional testing and data manipulation were done with Microsoft EXCEL

97 SR-1 for Windows 95 and plotted using SigmaPlot for Windows Version 4.01 (SPSS, Inc., USA).

RESULTS AND DISCUSSION

In total, 120 collection runs or lots were made, each supplying approximately 30 usable dust samples per run, 15 on polyvinyl chloride filters and 15 on glass filters. These provided 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. Even with the longer times used, the filters had an average lower dust/filter weight (Table 2 and Figs. 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. These runs tended to contain, on an average, more dust than from Cotton A (Table 2, Fig. 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 custom filter samples could be made if needed (Chun and Perkins, 1996). If needed, we would use Microdust and Trash Monitor (MTM, Zellweger Uster, Inc., Technologies, Knoxville, TN).

On an average, more dust was collected on the glass than on the polyvinyl chloride filters (Table 2,

Table 2. Average dust weight on Glass and PVC filters on Cottons A, AB and B.

Cotton Source †, ‡	Average dust per filter, mg/filter		
	Glass & PVC filters§	Glass filters	PVC filters
A***	0.45c	0.49	0.41
AB	0.61b	0.62	0.60
B***	0.65a	0.68	0.62
A, B and AB***		0.62	0.56

† Cotton A = strict low middling; Cotton B = low middling light spotted; Cotton AB = 1:1 blend of the two just described.

‡ t-test, average dust weight difference between PVC and glass filters is equal to zero: *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$.

§ Mean separation within columns by Duncan's multiple range test, 5% level. Means with the same letter are not significantly different.

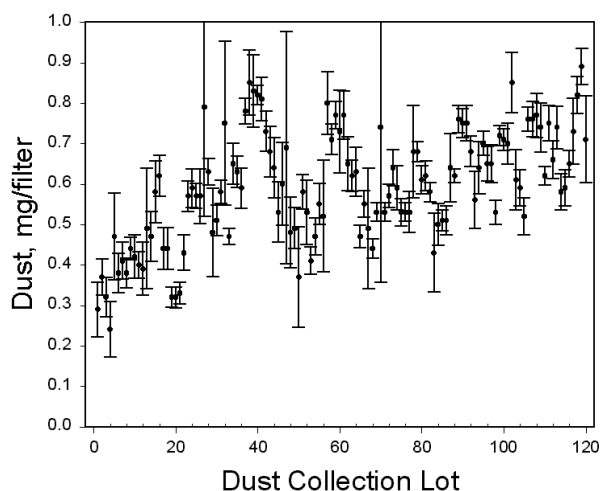


Fig. 1. Average dust collected on glass and PVC filter for each collection run/lot— Lots 1–28 from Cotton A; lots 29–72 from Cotton AB; and lots 73–120 from Cotton B. Each half-error bar represents 2 s.e.

Fig. 2). The overall differences between the polyvinyl chloride 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 2).

The higher dust retention by the glass filters was expected because its average pore size is 1μ , compared with 5μ for the polyvinyl chloride filter. Other filter characteristics to consider were 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 μ m, compared with the polyvinyl chloride filter, 152.4 μ m (Pall Gelman Sciences, 1998. The Filter Book. Ann Arbor, MI, p. 192.).

Also, severe extraction procedures on the glass filter may cause the glass filter to disintegrate (Chen, Teh-hsun B., personal communication) so an additional step is needed before analysis—that of centrifugation to clear suspended glass fibers. Further research will have to determine whether these differences play a meaningful role in endotoxin determination assays.

The goal of this project, to generate cotton dust samples, was accomplished. More than 3000 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.

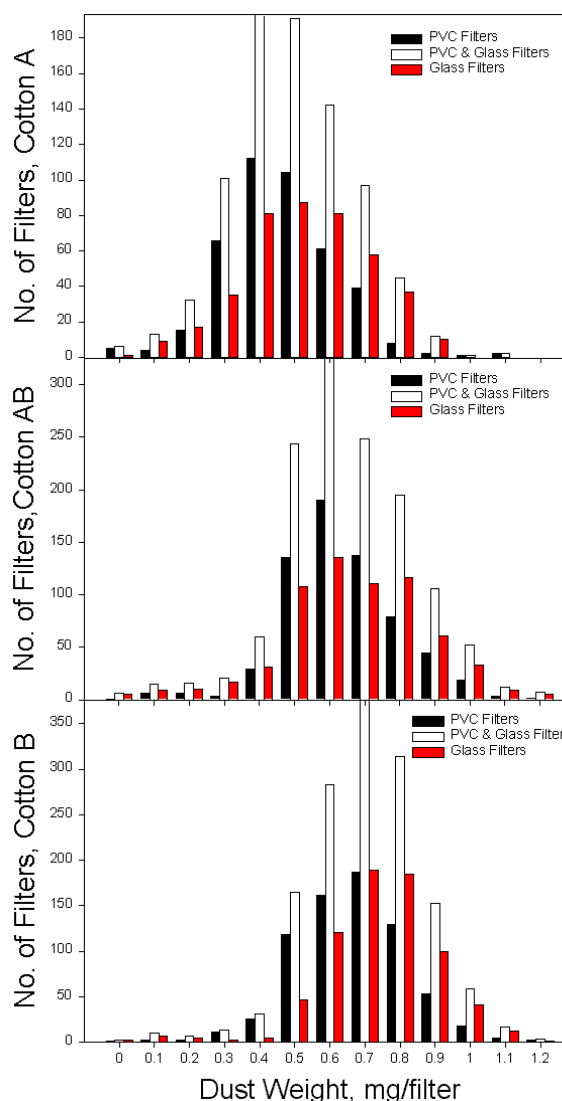


Fig. 2. Frequency of filters to the dust weight on all filters and on the glass or on the PVC filters. Cotton A = strict low middling; Cotton B = low middling light spotted; Cotton AB = 1:1 blend of the two just described.

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