

The Chemical Composition of Standard Cotton Dust

by Linda N. Domelsmith* and Ralph J. Berni*

Cotton dust samples from Cotton Incorporated were investigated by X-ray fluorescence and proximate analysis methods. These dust samples are known as "standard cotton dust" and have been used by many researchers investigating the causative agent(s) and physiological mechanisms of byssinosis. Silicon, calcium, potassium, and aluminum were present in relatively high concentrations (1-4%) in the dust fractions. The ash content of the dust fractions increased as the fraction particle size decreased. Proximate analyses demonstrate that "noncellulosic organics" are the major class of constituents (35-45%) in cotton dust. Cellulose comprises only 10-15% of the dust, while water-extractable materials comprise approximately 20% of the dust. Capillary gas chromatography performed on silylated, freeze-dried, aqueous extracts of the $\leq 38 \mu\text{m}$ dust fraction revealed the presence of phosphate, malate, arabitol, citrate, fructose, glucose, and mannitol.

Introduction

Searches for the causative agent(s) of byssinosis, proposed models for acute and chronic physiological response to cotton dust inhalation, and even the definition of the term, "byssinosis," have been objects of considerable research, debate, and controversy over the past decade (1-7). Direct comparison of experimental findings has been hampered by use of cotton dusts or leaf and bract materials from various sources. To alleviate this problem, Cotton Incorporated proposed production and use of a single source cotton dust that would be made available to cotton dust researchers worldwide (8). Fractions of $\leq 38 \mu\text{m}$, 38-75 μm , and $\geq 75 \mu\text{m}$ cotton dust were sieved from crude dust collected in a West Texas cotton mill by Cotton Incorporated in 1981-1982 and 1982-1983. Samples of "standard cotton dust" furnished by Cotton Incorporated have been used by researchers in the United States, Sweden, and Wales to study the chemical, physical, biological, and physiological properties of cotton dust. Use of this dust allowed scientists to compare their data and hypotheses with those produced by other scientists. In order to provide fundamental data on the chemical composition of "standard cotton dust," we have chemically characterized "standard dust" fractions by X-ray fluorescence and proximate analysis methods. In addition, silylated, freeze-dried, aqueous extracts of the $\leq 38 \mu\text{m}$ fraction were analyzed by capillary gas chromatography.

Experimental

The proximate analysis method consists of a serial solvent extraction procedure followed by gravimetric analysis to determine moisture content as the loss on oven-drying overnight at 110°C, water-extractables as the loss on extraction with boiling 0.1% Triton X-100 in deionized water, ethanol-extractables as the loss on extraction with boiling 100% ethanol, ethanolamine-extractables as the loss on extraction with boiling ethanolamine, cellulose as the loss on extraction with 1.00 M cupriethylenediamine (cuene), and insoluble residue. The method of Brown and co-workers was modified through use of Triton X-100 in the initial extraction to ensure thorough wetting of the dust sample (9).

Ash content is determined by slowly heating sample replicates to 725°C. The replicates are then ashed at 725°C for 2 hr.

Elemental analyses were performed by energy-dispersive X-ray fluorescence on an EG&G Ortec Spectrometer.

Samples were prepared for gas chromatographic analysis by extracting 40 mg of dust with 1.0 mL of pyrogen-free water for 1 hr at room temperature, passing the solution through a syringe filter (0.45 μm , Gelman Acrodisc), and pipetting 200 μL portions of the filtrate into derivatization vials; 10 μL of glutaric acid standard (equivalent to 2.0×10^{-5} g) was added, and the solutions were freeze-dried at 40 mTorr; 200 μL of Tri-Sil was added to each vial and was allowed to react at 50°C in a heating module. Gas chromatograms were recorded after 0.5 hr and 1.5 hr reaction times. Gas chromatography was performed on an HP 5880 Chromatograph

*Southern Regional Research Center, P. O. Box 19687, New Orleans, LA 70179.

Table 1. Proximate analyses of "standard cotton dust" fractions for 1981-1982 sample series.

	Dust fraction			Types of compounds involved
	$\geq 75 \mu\text{m}$	38-75 μm	$\leq 38 \mu\text{m}$	
Moisture	8.2 ± 0.5^a	8.5 ± 0.5	6.9 ± 0.3	Water and volatile organic compounds
Extraction solvent ($T^\circ\text{C}$)				
Water (100°C)	18.5 ± 0.3	20.3 ± 0.3	18.6 ± 0.3	Polar organic molecules and some inorganic salts
Ethanol (78°C)	2.4 ± 0.6	1.6 ± 0.4	3.1 ± 1.1	Waxes, organic compounds
Ethanolamine (171°C)	45.0 ± 0.7	42.9 ± 0.8	34.6 ± 1.4	Noncellulosic organics
Cuene (25°C)	10.5 ± 1.6	13.8 ± 0.8	11.6 ± 0.3	Cellulose
Residue	15.5 ± 1.5	13.0 ± 0.4	25.2 ± 2.5	Insoluble materials of plant and soil origin

^a Mean \pm SEM, $n = 4$.

Table 2. Ash content of "standard cotton dust" fractions for 1981-1982 series.

	Dust fraction ^a		
	$\geq 75 \mu\text{m}$	38-75 μm	$\leq 38 \mu\text{m}$
Ash, %	14.6 ± 0.2^a	18.8 ± 0.2	34.2 ± 0.1

^a Mean \pm SEM, $n = 4$.

equipped with a crosslinked dimethyl silicone high-performance capillary column (12.5 m, 0.20 mm I.D.) and a flame ionization detector. The following temperature and flow parameters were used: injector, 200°C; detector, 250°C; oven temperature program, 100°C for 1.00

Table 3. Elemental content of "standard cotton dust" fractions for 1981-1982 sample series.

	$\geq 75 \mu\text{m}$ $n = 1^b$	38-75 μm $n = 3$	$\leq 38 \mu\text{m}$ $n = 5$
Silicon, %	0.3 ^a	0.3-1.6	2.9-4.3
Calcium, %	1.8	1.6-1.8	1.8-1.9
Potassium, %	1.6	1.6	1.3-1.4
Aluminum, %	1.2	1.1	1.5-1.9
Magnesium, %	0.62	0.58-0.83	0.66-0.96
Chlorine, %	0.57	0.52-0.72	0.48-0.74
Sulfur, %	0.40	0.39-0.53	0.38-0.55
Iron, %	0.08	0.06-0.09	0.28-0.36
Zinc, ppm	19	17-27	27-41
Manganese, ppm	36	54-73	82-141

^a Range for $\geq 75 \mu\text{m}$ fraction would be expected to vary similarly to that of 38-75 μm fraction.

^b Number of replicates (n) is determined by quantity of sample available for analyses.

Table 4. Sample-to-sample and year-to-year variations in elemental content of "standard cotton dust" samples.

	$\geq 38 \mu\text{m}$ fractions ^a		
	1981-1982 ^b	1981-1982 ^b	1982-1983 ^b
Silicon, %	4.17 ± 0.10	3.37 ± 0.31	3.73 ± 0.14
Calcium, %	1.90 ± 0.02	1.79 ± 0.01	2.63 ± 0.07
Aluminum, %	1.85 ± 0.02	1.55 ± 0.04	1.89 ± 0.12
Potassium, %	1.37 ± 0.03	1.29 ± 0.01	1.17 ± 0.14
Magnesium, %	0.93 ± 0.03	0.87 ± 0.01	1.09 ± 0.03
Chlorine, %	0.68 ± 0.03	0.72 ± 0.02	1.27 ± 0.02
Sulfur, %	0.55 ± 0.01	0.52 ± 0.03	0.86 ± 0.02
Zinc, %	0.0035 ± 0.0004	0.0028 ± 0.0001	0.081 ± 0.010

^a Mean \pm SEM, $n = 2$.

^b Samples received as separate shipments from Cotton Inc.

Table 5. Identities and retention times of major peaks in capillary gas chromatograms of silylated extracts of "standard cotton dusts".

Peak identity	t_r , min
Phosphate	4.49
Glycerol	4.79 ^a
Glutarate	6.84 ^b
Malate	9.01
Arabitol	14.85
Citrate	16.31
Fructose	16.56, 16.70, 16.78
Glucose	18.28, 21.33
Mannitol	19.81

^a Partly contaminant.

^b Internal standard.

min, 5.00°C/min to 180°C, 180°C for 10 min, 10°C/min to 210°C, and 210°C for 11 min; 3.7 mL/min He carrier; and 100:1 split ratio.

Results and Discussion

Proximate analyses data shown in Table 1 demonstrate that cotton dust is composed primarily of ethanolamine-extractable material (35-45%) and water-extractable material (ca. 20%). The boiling ethanolamine extraction procedure is used to remove a class of compounds known as "noncellulosic organics." Water extraction removes polar organic molecules and some of the inorganic salts, particularly the potassium, calcium, aluminum, and magnesium salts. Cellulose, the major constituent of raw cotton lint, accounts for only 10 to 15% of the dust. This is comparable to the amount of cellulose (ca. 10%) found in dried cotton leaf tissue (unpublished observation). Cellulose contents ranging from 38 to 59% have been reported for "standard dust" samples (8). However, this report did not indicate the method of cellulose determination. Our method removes water-soluble, ethanol-soluble, and ethanolamine-soluble substances prior to dissolution of the cellulose. Our method takes advantage of the structure of plant materials in that the outer layers of extracellular and cellular material are removed before the cellulose framework is dissolved.

The ash contents of the standard dust fractions increase with decreasing particle size (Table 2). This trend of increasing ash content with decreasing particle size is also observed for cotton dust samples generated by

a variety of techniques (10). The $\leq 38 \mu\text{m}$ fraction has a high ash content: 34%. This is not unexpected for Texas cotton dust samples. For example, cardroom dust samples from three Texas-grown cottons were shown to have ash contents of 31 to 36% (11). The high ash contents were attributed to soil contamination.

Consistent with the trends in ash contents, elemental analyses show higher levels of silicon, iron, zinc, and manganese in the $\leq 38 \mu\text{m}$ fraction than in the ≥ 75 and 38–75 μm fractions (Table 3). Silicon, iron, and manganese are commonly found in soils. Variations of silicon, iron, manganese, and ash content with particle size indicate that soil particles in the dust are smaller on the average than many of the particles of plant origin. Thus, for these fractions, the inorganic content of the dust increases with decreasing particle size.

In order for a dust to serve as a standard, it is necessary that samples taken from the standard be chemically invariant. Elemental analyses of two separate 1981–1982 samples and one 1982–1983 sample were conducted to test for variations. The results are shown in Table 4. Variations in the levels of several important elements are minor. Calcium, chlorine, sulfur, and zinc levels were slightly higher in the 1982–1983 sample than

in the 1981–1982 samples. The $\leq 38 \mu\text{m}$ standard cotton dust samples have silicon contents of 3–4%, calcium contents of 2%, aluminum contents of 2%, potassium contents of 1%, and magnesium contents of 1%. By comparison, Wirtz and Olenchok (12) investigated several grain dusts, including flax dust, and found silicon contents of 0.35 to $> 1\%$, calcium contents of 0.1 to $> 1\%$, aluminum contents of 0.05 to $> 1\%$, potassium contents of 0.17 to $> 1\%$, and magnesium contents of 0.039 to $> 1\%$.

Gas chromatograms of derivatized, freeze-dried, aqueous extracts of $\leq 38 \mu\text{m}$ standard cotton dust show approximately 10 to 15 major volatile components other than those present in controls (Fig. 1). Retention time matching and peak enhancement studies are consistent with assignment of ten of these peaks to phosphate, malate, arabitol, citrate, fructose (three major peaks), glucose (two major peaks), and mannitol. Retention times and peak identities are shown in Table 5. These compounds are characteristic of plant extracts and have been found in aqueous extracts of cotton lint (13,14), cardroom dust (15), and cotton bract (unpublished observation). Glycerol, an expected plant product, is also present in the controls. It is leached from the plastic syringe filters. These chro-

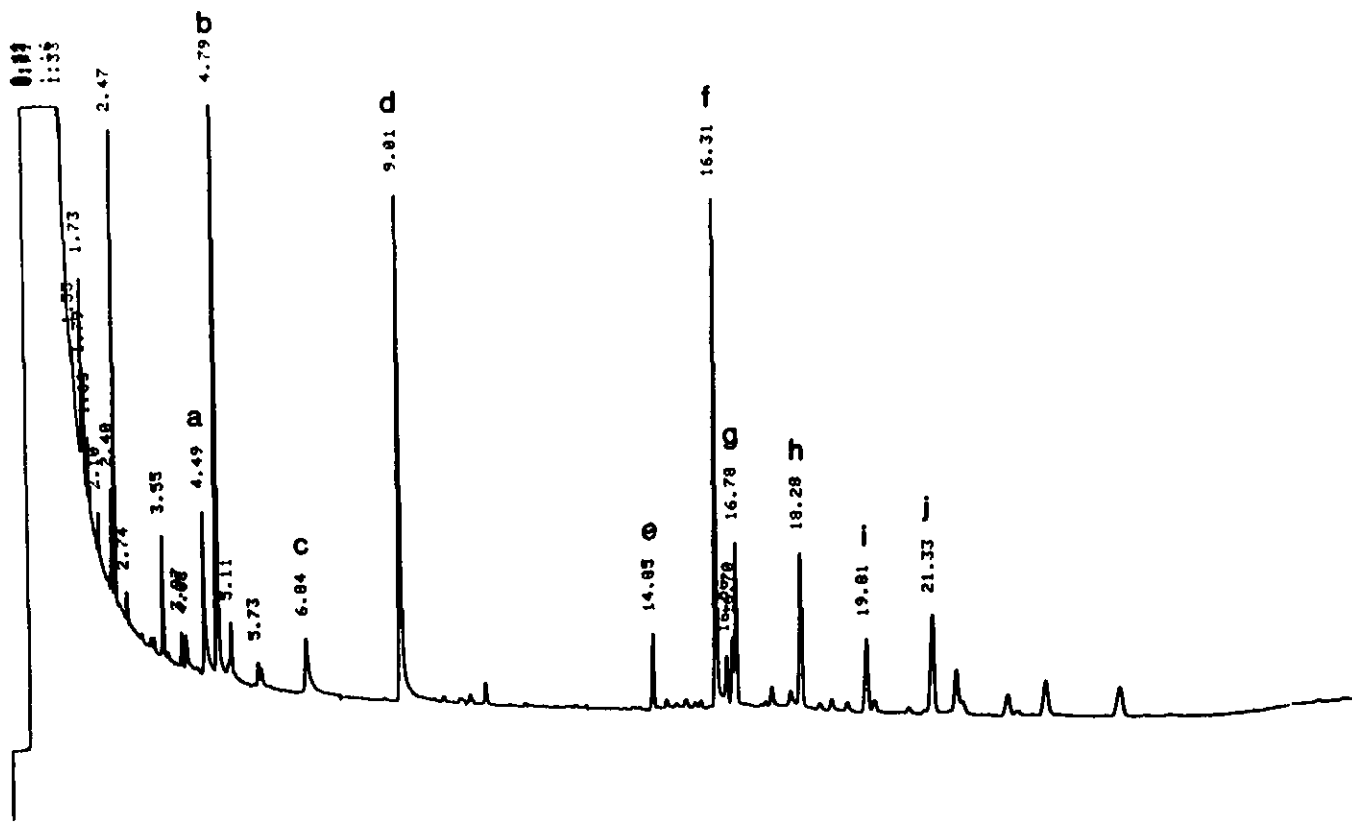


FIGURE 1. Capillary gas chromatogram of silylated, freeze-dried, aqueous extract of $\leq 38 \mu\text{m}$ fraction of "standard cotton dust"; (a) phosphate, (b) glycerol (partly contaminant), (c) glutarate (internal standard), (d) malate, (e) arabitol, (f) citrate, (g) fructose (three peaks), (h) α -D-glucose, (i) mannitol; (j) β -D-glucose.

matographic studies demonstrate that vegetative matter is an important component of the standard cotton dust. The high levels of malate and citrate and the relatively low levels of arabitol and mannitol indicate that the vegetative matter in the dust was recently photosynthetically active and was only in the early stages of degradation at the time of harvest (15).

The level of senescence or degradation of the vegetative matter may be an important determinant of pulmonary response to cotton dust. Studies in this area are continuing in our laboratories.

Conclusions

Standard cotton dust contains approximately 20% water-extractable material, approximately 40% ethanolic extractable material, and 10 to 15% cellulose. Ash contents and levels of silicon, iron, and manganese of standard cotton dust fractions increase with decreasing particle size. Silicon, calcium, potassium, aluminum, and magnesium compounds are present in the 1 to 4% range in the dust fractions. The chemical composition of standard cotton dust fractions from 1981-1982 and 1982-1983 are similar. Malate, citrate, fructose, glucose, arabitol, and mannitol are major components of derivatized, freeze-dried, aqueous extracts of standard cotton dust. These elemental, proximate, and chromatographic analyses support the contention that standard cotton dust can be an important resource for all scientists trying to develop meaningful bioassays for byssinosis, in order to eventually identify and eliminate the causes of the disease.

We thank R. R. Jacobs of Cotton Incorporated, Raleigh, NC, for samples of "standard cotton dust." We thank Chauncey Williams for proximate and ash analyses, Biagio Piccolo and Charles McCombs for X-ray fluorescence analyses, and Sharon McDonald and Virginia Fernandez for gas chromatographic analyses.

Use of a company and/or product name does not imply approval or recommendation of the product or company to the exclusion of others which may also be suitable.

REFERENCES

- Honeybourne, D., Wales, D. S., Watson, A., Lee, W. R., and Sagar, B. F. Byssinosis—Causative Agent and Clinical Aspects (a Critical Literature Research Review). Shirley Institute, Publication S.43, England, 1982.
- Statement of the Industry/Government/Union Task Force, Washed Cotton Evaluation/Cotton Dust Research to OSHA's Proposed Rulemaking, Occupational Exposure to Cotton Dust, Docket No. H-052E, 1983.
- Rylander, R., and Holt, P. G. Macrophages-neutrophil-platelet interaction as a mechanism for byssinosis? In: Proceedings of the Seventh Cotton Dust Research Conference (P. J. Wakelyn and R. R. Jacobs, Eds.), National Cotton Council and Cotton Incorporated, Memphis, 1983, pp. 32-33.
- Karol, M. H., Sinagoga, L. A., Burke, S., Ellakkani, M., Keleti, G., Sykora, J., Alarie, Y., and Weyel, D. Characterization of the bacterial and endotoxin content of cotton dust causing respiratory reactions in guinea pigs. In: Proceedings of the Eighth Cotton Dust Research Conference (P. J. Wakelyn and R. R. Jacobs, Eds.), National Cotton Council and Cotton Incorporated, Memphis, 1984, pp. 80-81.
- Ellakkani, M., Alarie, Y., Weyel, D., and Karol, M. Concentration-response relationship for the acute respiratory response to inhaled cotton dust in guinea pigs. In: Proceedings of the Eighth Cotton Dust Research Conference (P. J. Wakelyn and R. R. Jacobs, Eds.), National Cotton Council and Cotton Incorporated, Memphis, 1984, pp. 82-83.
- Alarie, Y., Ellakkani, M., Weyel, D., Mazumdar, S., and Karol, M. Monday post-shift respiratory response in guinea pigs following inhalation of cotton dust. In: Proceedings of the Eighth Cotton Dust Research Conference (P. J. Wakelyn and R. R. Jacobs, Eds.), National Cotton Council and Cotton Incorporated, Memphis, 1984, pp. 84-86.
- Rooke, G. B. Byssinosis in the United Kingdom. In: Proceedings of the Eighth Cotton Dust Research Conference (P. J. Wakelyn and R. R. Jacobs, Eds.), National Cotton Council and Cotton Incorporated, Memphis, 1984, pp. 5-6.
- Jacobs, R. R. *In vivo* and *in vitro* evaluations of the "standard cotton dust." In: Proceedings of the Eighth Cotton Dust Research Conference (P. J. Wakelyn and R. R. Jacobs, Eds.), National Cotton Council and Cotton Incorporated, Memphis, 1984, pp. 30-34.
- Brown, D. F., and Berni, R. J. Collection of large quantities of respirable cotton dust for byssinosis research. *Textile Res. J.* 47: 152-154 (1977).
- Berni, R. J., Domelsmith, L. N., Goynes, W. R., and Karol, M. Proximate, elemental, and microscopical analyses of "standard cotton dusts." In: Proceedings of the Ninth Cotton Dust Research Conference (P. J. Wakelyn and R. R. Jacobs, Eds.), National Cotton Council and Cotton Incorporated, Memphis, 1985, pp. 32-36.
- Domelsmith, L. N., Berni, R. J., Cocke, J. B., and Perkins, H. H., Jr. Chemical differentiation of card dusts from the 1983 Area of Growth/Varieties Study: I. Proximate and elemental analyses. In: Proceedings of the Ninth Cotton Dust Research Conference (P. J. Wakelyn and R. R. Jacobs, Eds.), National Cotton Council, Memphis, 1985, in press.
- Wirtz, G. H., and Olenchock, S. A. Elemental analysis of airborne grain dusts. *Environ. Sci. Health.* B19: 379-391 (1984).
- Roberts, C. W., Koenig, H. S., Merrill, R. G., Cheung, P. S. R., and Perkins, H. H., Jr. Implications of monosaccharides in sticky cotton processing. *Textile Res. J.* 46: 374-380 (1976).
- Roberts, C. W., Cheung, P. S. R., and Perkins, H. H., Jr. Implications of monosaccharides in sticky-cotton processing. Part II: Effects of growing conditions on fiber contaminants. *Textile Res. J.* 48: 91-96 (1978).
- Domelsmith, L. N., Berni, R. J., Perkins, H. H., Jr., and Cocke, J. B. Chemical differentiation of card dusts from the 1983 Area of Growth/Varieties Study: II. Capillary gas chromatographic analyses of derivatized extracts. In: Proceedings of the Ninth Cotton Dust Research Conference (P. J. Wakelyn and R. R. Jacobs, Eds.), National Cotton Council and Cotton Incorporated, Memphis, 1985, pp. 42-46.