

# Exposure to Cotton Dust Endotoxin and Respiratory Health: The Shanghai Textile Workers Study

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## Part I

Basic concepts of cotton  
dust-related disease

## Process of cotton textile production

- Opening
- Cleaning
- Carding
- Drawing
- Combing
- Roving
- Spinning
- Weaving





## SYMPTOM / SYNDROME CHARACTERISTICS

### Acute responses

<b>Mill fever</b>	Febrile response upon first exposure(s)
<b>Byssinosis</b>	Chest tightness towards end of first day of work-week after several years of exposure
<b>Acute broncho-constriction</b>	Drop in FEV <sub>1</sub> over a work-shift

### Chronic responses

<b>Chronic bronchitis</b>	Cough and sputum
<b>Chronic dry cough</b>	Cough without sputum
<b>Airway hyperreactivity</b>	Usually after long-term exposure
<b>Chronic airflow obstruction</b>	Decreased pre-shift FEV <sub>1</sub> after chronic exposure

## BYSSINOSIS: ETIOLOGY- ENDOTOXIN

1. "Mill fever": almost certainly caused by endotoxin with syndrome reproduced in volunteers (1960's); and also found in sewer workers and others working with decaying plant material.
2. Not clear to date whether the mechanism involves stimulation of the same inflammatory receptors by endotoxin and by cotton dust.

3. Model cardroom studies (North Carolina) have shown that acute drops in FEV<sub>1</sub> have correlated better with endotoxin than dust in volunteers specifically selected for their reactivity to cotton dust. Swedish studies found similar results in the experimental setting.

## BYSSINOSIS: MECHANISMS OF DISEASE

Pathogenesis still unclear: Three proposed mechanisms:

1. Direct release of histamine triggered by cotton dust components
2. Immunological responses (principally antibody mediated) to cotton dust antigens
3. Inflammatory responses triggered by endotoxin

## Part II

### Respiratory effects of early exposure to cotton dust

*An 18-month follow-up in newly hired cotton textile workers*

## BACKGROUND

Limited knowledge about pulmonary response after initiation of exposure

The features of previous studies:

- cross-sectional design
- experiment in model cardrooms
- non-exposed volunteers
- both smokers and non-smokers

## OBJECTIVES

1. To observe natural history of cotton dust-related health problems in previously unexposed newly-hired workers, especially in the magnitude of changes in lung function and non-specific airway responsiveness.
2. To identify potential roles of pre-existing atopy and early symptoms in the changes of lung function.
3. To assess the relationships between pulmonary responses and exposure levels.

## SUBJECTS

Original cohort: 225 female newly-hired workers in 3 cotton mills in Shanghai, China

- assigned to the yarn preparation areas
- without cardio-respiratory disease & symptoms
- not previously exposed to industrial dusts
- lifelong non-smokers
- average age of 18 years (16 - 29 years)

At baseline	240	
		– 9 males, 5 with respiratory diseases, and 1 lacking data
Enrolled cohort	225 (100%)	
		– 31 drop out
At 3-months	194 (86%)	
		– 58 drop out
At 12-months	136 (60%)	
		– 35 drop out
At 18-months	101 (45%)	

Description of participating subjects

## METHODS

- Skin-prick testing at baseline using six common local antigens.
- Standardized respiratory symptoms questionnaire.
- Pulmonary function tests: Before and after shift ventilatory tests using standardized techniques.
- Methacholine challenge testing to measure nonspecific airway responsiveness using a dose strategy of 0.5, 1, 2, 5, 10 and 25 mg/ml.

## Statistical analysis

### Lung function data analysis:

- acute changes: ? FEV<sub>1</sub> over the follow-up time
- longitudinal changes: changes in FEV<sub>1</sub> and FVC (using pre-shift values) over the follow-up period

**Changes in airway responsiveness:** differences in decrease of FEV<sub>1</sub> in response to each dose of methacholine between follow-up and baseline surveys

## Environmental Measurement Data by Mill

	—Mill 1—		—Mill 2—		—Mill 3—	
	N	Means (SD)	N	Means (SD)	N	Means (SD)
<b>Cotton dust (mg/m<sup>3</sup>)</b>						
3-months	42	0.42 (0.34)	48	0.39 (0.32)	48	0.46 (0.52)
12-months	56	0.32 (0.33)	—	—	56	1.05 (1.19)
18-months	56	0.47 (0.53)	—	—	—	—
<b>Endotoxin (mg/m<sup>3</sup>)</b>						
3-months	42	0.22 (0.51)*	48	1.36 (2.07)	48	1.07 (1.84)

Geometric means is expressed; N = sample number;

\*Significantly lower (p<0.05) in comparison with Mill 2 or Mill 3.

## Respiratory Symptoms<sup>†</sup> [% (N)] Over 18-month Observation

	All workers	Atopics	Nonatopics
3- months	7.9 (8)	6.5 (2)	8.6 (6)
12- months	2.0 (2)	0.0 (0)	2.9 (2)
18- months	1.0 (1)	0.0 (0)	1.4 (1)

<sup>†</sup>Including either dry cough or cough with phlegm.

The calculation was based on 101 workers who participated all of the three follow-up surveys.

## Changes in spirometry after work by respiratory symptoms reported at three-months

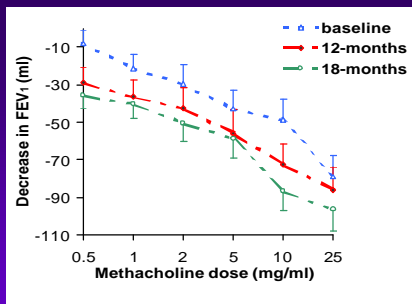
	All workers	Symptomatic	Asymptomatic
<b>FVC (ml)<sup>†</sup></b>			
3-months	39 (201)	3 (156)	42 (204)
12-months	-124 (181)	-177 (150)*	-119 (184)
<b>FEV<sub>1</sub> (ml)<sup>†</sup></b>			
3-months	43 (163)	7 (84)	46 (168)
12-months	-70 (154)	-66 (60)	-70 (154)
<b>?FEV<sub>1</sub> (ml)</b>			
3-months	-18 (90)	-26 (71)	-18 (92)
12-months	-24 (81)	-61 (64)**	-21 (82)
<b>No. ?FEV<sub>1</sub> ≥-5%</b>			
3-months	21/194 (10.8%)	4/20 (20.0%)	17/174 (9.8%)
12-months	15/136 (11.0%)	7/15 (46.7%)*	8/121 (6.6%)

<sup>†</sup> Relative to baseline data (mean and SD). \* p < 0.05 \*\* p < 0.01 when compared with asymptomatic subgroup (t-test).

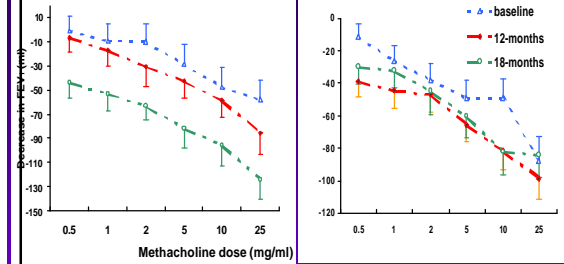
## Changes<sup>†</sup> in spirometric parameters over 18-months

	All workers	Atopics	Nonatopics
<b>FVC, ml</b>			
3-months	17.6 (19.1)	67.5 (31.5)*	-5.7 (23.5)
12-months	-157.3 (19.5)	-158.9 (34.8)**	-156.6 (23.7)**
18-months	-165.6 (24.7)**	-144.3 (48.1)**	-175.4 (28.6)**
<b>FEV<sub>1</sub>, ml</b>			
3-months	49.1 (17.8)**	78.4 (17.6)**	35.5 (24.6)
12-months	-77.5 (16.1)**	-66.4 (16.4)**	-82.0 (21.7)**
18-months	-40.8 (19.5)*	-12.2 (23.6)	-52.6 (25.7)*
<b>?FEV<sub>1</sub>, ml</b>			
3-months	-31.1 (9.3)	-38.1 (15.4)	-27.7 (11.7)
12-months	-27.7 (7.0)	-33.3 (15.5)	-25.3 (7.5)
18-months	-24.7 (8.4)	-39.5 (17.5)	-18.2 (9.3)

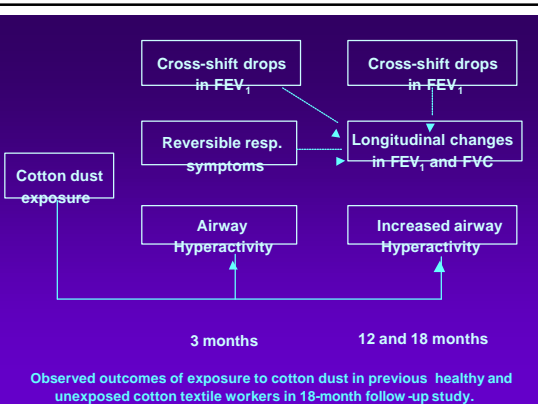
<sup>†</sup> Relative to baseline data (mean and SE). \* p < 0.05 \*\* p < 0.01 in comparison with baseline data (paired t-test). No statistical significance between the atopics and nonatopics.



Methacholine dose-response characteristics of total workers (n=101) over 18-month follow-up observations.

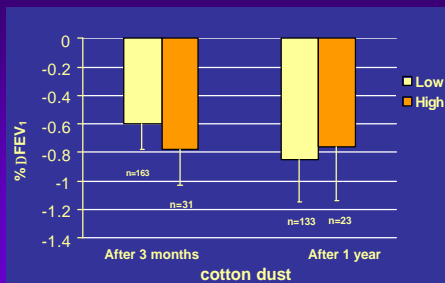


Methacholine dose-response characteristics of atopic workers (left) and nonatopic workers (right) over 18-month follow-up observation

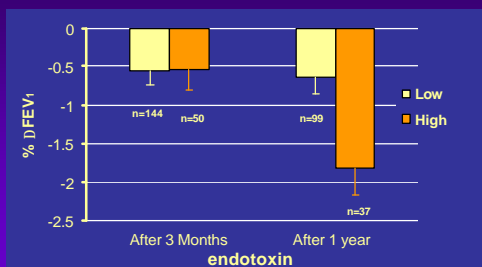


Observed outcomes of exposure to cotton dust in previous healthy and unexposed cotton textile workers in 18-month follow-up study.

### Cross-shift changes in FEV<sub>1</sub> at three months and one year



### Cross-shift changes in FEV<sub>1</sub> at three months and one year



### GEE Analysis Results for Cross-shift Changes in FEV<sub>1</sub> Based on 12-month Data

	Estimate (SE)	p value
Intercept	540.44 (326.15)	0.09
Symptoms <sup>†</sup>	- 67.35 (25.01)	<0.01
High endotoxin <sup>§</sup>	- 26.53 (13.30)	0.04
High cotton dust	- 7.93 (23.96)	0.74
Mill	- 44.54 (11.15)	<0.0001
Age (year)	11.11 (4.15)	0.007
Height (cm)	- 4.41 (2.02)	0.03

<sup>†</sup> reported at 3-months; <sup>§</sup> measured at three-months only

### GEE Analysis Results for Cross-shift Changes in FEV<sub>1</sub> Based on 18-month Data

	Estimate (SE)	p value
Intercept	13.37 (15.09)	0.38
Atopy	- 23.29 (12.93)	0.07
Symptoms <sup>†</sup>	- 37.02 (16.47)	0.02
Mill 2 vs 1	- 53.95 (14.84)	<0.001
Mill 3 vs 1	- 33.98 (17.02)	0.05
High cotton dust	- 27.77 (19.30)	0.51
High endotoxin <sup>‡</sup>	8.88 (11.26)	0.45

<sup>†</sup> reported at 3-months; <sup>‡</sup> measured at three-months only

## CONCLUSIONS (1)

1. Exposure to cotton dust in naïve workers is associated with substantial acute and longitudinal decrements in lung function and increasing non-specific airway responsiveness.
2. Early, reversible, nonspecific respiratory symptoms may be related to subsequent loss of lung function in cotton textile workers.

## CONCLUSIONS (2)

3. Atopy may be an important risk factors for cotton dust - related acute drops in lung function and increasing airway responsiveness.
4. The results imply that endotoxin is more closely related to the acute drop in FEV<sub>1</sub>, but the current data were insufficient to assess either exposure-response relationship or the etiologic importance of endotoxin and cotton dust.

## Part III Respiratory effects of long term exposure to cotton dust

*A fifteen-year (1981 – 1996) observation*

## Questions to be answered

1. Is long-term or extended exposure to cotton dust associated with excess chronic declines in lung function?
2. What is the significance of respiratory symptom persistence for chronic changes in lung function among cotton dust exposed population?
3. Does the mechanism of cotton dust induced disease involve exposure to endotoxin, to cotton dust or both?

## SUBJECTS

- | 447 workers from yarn preparation areas of two cotton textile mills in Shanghai, China
- | 472 silk workers as controls from a silk textile mill in the same city
- | Exclusion criteria: any history of respiratory disease before entering work at the mills

## METHODS

- Standardized respiratory symptoms questionnaire.
- Symptom definition: Byssinosis, chest tightness at work, chronic cough, chronic bronchitis, dyspnea.
- Environmental assessment: Inhalable dust sampling using vertical elutriator ( $\leq 15$  micron AED); Gravimetric and endotoxin analysis on air samples.
- Pulmonary function tests: Before and after shift ventilatory tests using standardized techniques.

## Study Population at Baseline and follow-up Surveys

	Cotton Workers		Silk Workers	
	N	(%)	N	(%)
<b>Questionnaire</b>				
1981	447	(100.0)	472	(100.0)
1986	384	(85.9)	403	(85.4)
1992	394	(88.1)	393	(83.3)
1996	346	(77.4)	338	(71.6)
<b>Lung function testing</b>				
1981	447	(100.0)	472	(100.0)
1986	356	(79.6)	374	(79.2)
1992	366	(81.9)	331	(70.1)
1996	339	(75.8)	336	(71.2)

## Respiratory symptoms (%) in cotton and silk textile workers

	Prevalence at baseline	Cumulative incidence
<b>Byssinosis</b>		
Cotton	7.5 (5.3-10.5)	21.5 (17.1-25.7)
Silk	0.0	0.0
P value	< 0.0001	< 0.0001
<b>Chest tightness</b>		
Cotton	7.7 (5.5-10.7)	24.9 (20.7-29.7)
Silk	0.2 (0.1-0.3)	10.1 (7.1-13.6)
P value	< 0.0001	< 0.0001
<b>Chronic bronchitis</b>		
Cotton	21.4 (17.8-25.6)	9.1 (6.5-12.2)
Silk	7.1 (5.4-10.4)	7.1 (4.6-10.4)
P value	< 0.0001	> 0.05
<b>Chronic cough</b>		
Cotton	19.1 (15.9-23.4)	9.0 (6.5-12.2)
Silk	6.5 (4.9-9.7)	6.6 (4.3-9.8)
P value	< 0.0001	> 0.05
<b>Dyspnea (+2)</b>		
Cotton	14.9 (11.8-18.6)	22.6 (20.1-29.6)
Silk	3.8 (2.3-6.0)	21.3 (18.3-27.8)
P value	< 0.0001	> 0.05

## Persistence of Respiratory Symptoms (%; 95%CI)

	1 time	2 times	3 or 4 times
<b>Byssinosis</b>			
Cotton	23.1 (18.4-27.9)	9.9 (6.5-13.3)	1.3 (0.3-2.6)
Silk	0.0	0.0	0.0
P value			< 0.0001
<b>Chest tightness</b>			
Cotton	20.8 (16.2-25.4)	7.3 (4.3-10.2)	1.3 (0.3-2.6)
Silk	8.9 (5.7-12.2)	1.7 (2.3-3.2)	0.0
P value			< 0.0001
<b>Chronic bronchitis</b>			
Cotton	14.2 (10.3-18.1)	5.9 (3.3-8.6)	5.9 (3.8-8.6)
Silk	10.7 (7.1-14.2)	3.1 (1.1-5.1)	1.7 (0.2-3.2)
P value			< 0.01
<b>Chronic cough</b>			
Cotton	14.5 (10.6-18.5)	5.0 (2.5-7.4)	5.3 (2.8-7.8)
Silk	9.3 (5.9-12.6)	4.5 (2.1-6.8)	1.7 (0.2-3.2)
P value			< 0.01
<b>Dyspnea (+2)</b>			
Cotton	24.1 (19.3-28.9)	9.2 (6.0-12.5)	7.3 (4.3-10.2)
Silk	23.4 (18.5-28.2)	5.8 (3.2-8.5)	1.4 (0.4-2.7)
P value			< 0.001

## Adjusted odds ratios for byssinosis in relation to cumulative exposure levels in cotton workers

	Exposure to endotoxin	Exposure to dust
<b>Whole group (n = 429)</b>		
Low	0.6 (0.29 - 1.32)	1.6 (0.70 - 3.46)
Middle	1.4 (0.54 - 3.35)	1.0 (0.44 - 2.15)
High	1.9 (1.02 - 3.51)	1.8 (1.03 - 3.32)
<b>Male (n = 203)</b>		
Low	0.5 (0.17 - 1.62)	2.5 (0.86 - 7.32)
Middle	2.5 (0.50 - 4.05)	0.8 (0.26 - 2.59)
High	1.9 (0.86 - 3.96)	2.4 (1.13 - 5.10)
<b>Female (n = 226)</b>		
Low	0.6 (0.18 - 1.95)	0.6 (0.17 - 1.84)
Middle	1.0 (0.25 - 3.71)	0.6 (0.20 - 1.97)
High	1.7 (0.48 - 5.64)	1.0 (0.37 - 2.66)

The odds ratios were adjusted by age, years worked, smoking status and gender (for whole group) in GEE models. The lowest levels of cumulative exposure were as reference. Statistically significant odds ratios are underlined.

## Annual Changes<sup>‡</sup> in Lung Function in Cotton and Silk Textile Workers

	FEV <sub>1</sub> , ml/yr		FVC, ml/yr	
	Cotton	Silk	Cotton	Silk
<b>Total</b>	-32.3(1.0)	-29.4(1.0)*	-20.1(1.2)	-15.3(1.3)**
<b>Male</b>	-42.2 (1.8)	-38.0 (1.9)	-27.8 (2.1)	-23.6 (2.3)
<b>Female</b>	-24.8 (1.6)	-22.4 (1.5)	-14.7(1.6)	-9.0 (1.6)
<b>Smokers<sup>‡</sup></b>	-43.7 (2.0)	-39.3 (2.1)	-27.5 (2.5)	-24.3(3.1)
<b>Nonsmokers</b>	-40.4 (3.2)	-40.1 (2.5)	-27.5 (4.0)	-23.6 (3.7)

<sup>‡</sup> The values were adjusted by age at mid-point during follow-up period and height at final survey; sex and smoking status were also adjusted in calculations.

### GEE Analysis for Longitudinal Changes in Lung Function (Cotton and silk workers)

	FEV <sub>1</sub> , ml		FVC, ml	
	Estimate (SE)	p value	Estimate(SE)	p value
Sex, male	548 (162)	< 0.001	695 (244)	< 0.001
Height, cm	46.3 (6.8)	< 0.001	60.2 (7.2)	< 0.001
Age, yr	-45.7 (7.6)	< 0.001	-16.5 (3.8)	< 0.001
Endotoxin (high)	-281 (130)	< 0.05	—	—
? FEV <sub>1</sub> , L	-732 (135)	< 0.001	-380 (157)	< 0.05
Chronic bronchitis	-189 (81.4)	< 0.05	—	—
Chronic cough	-212 (72.6)	< 0.01	—	—
Dyspnea (2+)	-161 (81.3)	< 0.05	—	—
Chest tightness	-235 (116)	< 0.05	—	—
Bronchitis * year	-198 (66.2)	< 0.01	—	—

### GEE Analysis for Longitudinal Changes in Lung Function (Cotton workers only)

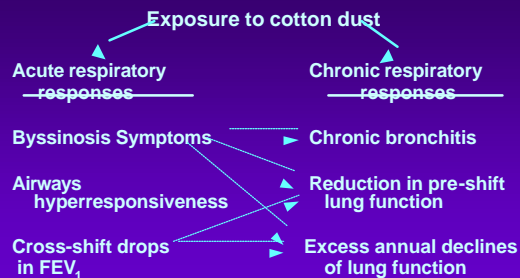
	FEV <sub>1</sub> , ml		FVC, ml	
	Estimate (SE)	p value	Estimate(SE)	p value
Sex, male	530 (188)	< 0.001	693 (250)	< 0.001
Height, cm	45.3 (9.8)	< 0.001	59.8 (9.1)	< 0.001
Age, yr	-44.1 (14.0)	< 0.001	-14.2 (11.8)	< 0.05
Years worked, yr	-35.0 (4.9)	< 0.05	-26.8 (11.0)	< 0.05
Endotoxin (high)	-334 (125)	< 0.01	-308 (142)	< 0.05
? FEV <sub>1</sub> , L	-916 (150)	< 0.001	-678 (23.3)	< 0.001
Byssinosis	-336 (118)	< 0.01	—	—
Chest tightness	-181 (93.2)	0.05	—	—
Dyspnea (2+)	-194 (104)	0.06	—	—
Bronchitis * year	-140 (57.4)	< 0.05	—	—

### Effects of Respiratory Symptom Persistence on Longitudinal Changes in Lung Function in Cotton Workers<sup>‡</sup>

	FEV <sub>1</sub> , ml		FVC, ml	
	Estimate (SE)	p-value	Estimate(SE)	p-value
Byssinosis <sup>†</sup>				
1	39 (90)	NS*	109 (95)	NS
2	-118 (154)	NS	-224 (132)	NS
≥ 3	-414 (64)	< 0.001	-142 (173)	NS
Chest tightness				
1	-68 (92)	NS	-15 (89)	NS
2	-69 (143)	NS	-16 (134)	NS
≥ 3	-924 (292)	< 0.01	-549 (97)	< 0.001
Chronic bronchitis				
1	1 (89)	NS	1 (94)	NS
2	-66 (111)	NS	-16 (121)	NS
≥ 3	-281 (149)	< 0.05	-27 (134)	NS

<sup>‡</sup> Sex, age, height, years worked, cumulative exposure to endotoxin and cross-shift changes in FEV<sub>1</sub> were simultaneously adjusted. <sup>†</sup> Categories 1, 2 and ≥3 versus category 0 (all negative), respectively. \* p < 0.05

### Relationship of acute airway change to chronic obstruction



## Part IV.

### Preliminary data of gene-environment interactions in cotton textile workers

### New progress:

#### -Gene-environment interactions in lung function decline of cotton textile workers

- Hypothesis
  - Genetic polymorphisms of genes involved in innate immunity, endotoxin metabolism, and inflammation may modify the association between endotoxin exposure and lung function decline
- Genotyping method
  - Taqman
  - PCR-RFLP
  - PCR-pyrosequencing

## TLR4 polymorphisms in Chinese Population

- TLR4 Asp299Gly and Thr339Ile polymorphisms were genotyping in 491 cotton and silk textile workers
- No homozygous or heterozygous variants were found in either polymorphisms
- These two polymorphisms are very rare in Chinese populations

## Joint effects of mEH polymorphism and endotoxin exposure

	Endotoxin exposure	
	Silk group	Cotton group
<b>mEH Tyr113His polymorphism</b>		
Tyr/Tyr	1.00	1.68 (0.71-3.96)
Tyr/His	0.60 (0.26-1.43)	1.37 (0.63-3.01)
His/His	0.77 (0.25-2.32)	2.71 (1.69-6.70)

\*Logistic regression model adjusted for age, sex, height, and smoking status. The odds ratios are for rapid decliners (decrease in FEV<sub>1</sub>>40 ml/yr) vs. slow decliners (decrease in FEV<sub>1</sub><40 ml/yr).

## Conclusions (1)

1. Long-term exposure to cotton dust is associated with higher incidence of respiratory symptoms and accelerated chronic loss of pulmonary function.
2. Years worked in cotton mills, acute drops in FEV<sub>1</sub> and respiratory symptoms such as byssinosis, chest tightness at work and chronic bronchitis were contributing factors for chronic loss of pulmonary function.

## Conclusions (2)

3. The persistence of respiratory symptoms may predict the magnitude of chronic obstructive impairment among cotton textile workers.
4. Byssinosis and chronic loss of pulmonary function were more closely related to cumulative exposure to endotoxin than to cotton dust itself.
5. mEH polymorphism may modify the effect of endotoxin exposure on lung function decline.

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