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DISSERTATION

ACUTE RESPIRATORY EFFECTS AND ENDOTOXIN EXPOSURE DURING WHEAT HARVEST IN NORTHEASTERN COLORADO

Submitted by

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In partial fulfillment of the requirements

for the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

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March 28, 2000

WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY SUSAN MARIE VIET ENTITLED "ACUTE RESPIRATORY EFFECTS AND ENDOTOXIN EXPOSURE DURING WHEAT HARVEST IN NORTHEASTERN COLORADO" BE ACCEPTED AS FULFILLING IN PART THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

Committee on Graduate Work

Co-Advisor

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ABSTRACT OF DISSERTATION

ACUTE RESPIRATORY EFFECTS AND ENDOTOXIN EXPOSURE DURING WHEAT HARVEST IN NORTHEASTERN COLORADO

Acute cross-shift respiratory changes were evaluated at 26 farms in Northeastern Colorado during the summer of 1994 wheat harvest. Trained field staff administered a questionnaire to harvest workers to gather information on respiratory health, past occupational exposures, and smoking status. Each worker was also asked to rank 10 acute symptoms on a scale of 1 to 5 before they began harvest work for the day. Spirometry was performed immediately after the questionnaire was completed using NIOSH's HF5 spirometric system. Each participant wore a high flow personal air sampling pump with a 35-mm, closed-face cassette and fiber glass filter. Participants were observed throughout the workshift to determine tasks performed and personal protective equipment worn. At the end of the workshift, spirometry and ranking of the 10 acute symptoms were conducted again. Total dust exposure was determined gravimetrically. Total endotoxin was measured by the Limulus amoebocyte lysate (LAL) assay.

The 98 harvest workers included in the study ranged in age from 18 to 80 years. The gender distribution included 90 males and 8 females. Ten percent of the workers had airway obstruction, as indicated by the pre-shift spirometry test results. Fifty percent of the workers were smokers or ex-smokers. Tasks included

combine operators, auger operators, grain truck drivers, grain cart drivers, and supervisors. Total dust exposures ranged from 0.09 to 15.33 mg/m^3 (geometric mean 0.83 mg/m^3), with 8 percent of workers exposed above the threshold limit value of 4 mg/m^3. Total endotoxin exposures ranged from 4.4 to 744.4 EU/m^3 (geometric mean 54.2 EU/m^3), with 33 percent of workers exposed above levels associated with symptoms and lung function effects. At these exposure levels, 60 percent of workers experienced a cross-shift change in at least one respiratory symptom. The respiratory index (sum of cross-shift change in eight acute respiratory symptoms) was significantly correlated with both total dust and endotoxin exposure. Peak expiratory flow rate was found to decrease over the workshift in a manner similar to that experienced by cotton workers. Cross-shift changes in the spirometric variables were associated with smoking status, age, presence of airway obstruction, and history of chronic respiratory symptoms, but not with dust or endotoxin exposure. This study is the only known study that has examined cross-shift symptom and lung function change and dust and endotoxin exposure during harvest activities, which may be applicable to workers in other agricultural settings.

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I must extend my most heartfelt thanks to the farmers and workers who agreed to participate in the study. I wanted to be adopted several times during the study.

DEDICATION

This work is dedicated to Michael, who never doubted that I could complete this program and has supported me throughout the years of study and time spent apart. I'm finally coming home to stay.

I must also dedicate this to Evan, who did not receive as much of my attention as he should have during the time spent on this endeavor, but who, hopefully, will better appreciate the work involved in reaching his dreams of becoming an engineer. He has sustained my sense of humor during all these years.

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Chapter 1

Introduction

Ramazzini (1713) was the first to report that grain dust exposure caused health effects, including skin, eye, throat, and lung irritation, among grain workers. Since that time, both acute and chronic pulmonary and non-pulmonary effects of grain dust exposure have been studied. Acute pulmonary effects associated with present-day grain dust exposures include occupational asthma, dyspnea, chest tightness, dry cough, wheezing, expectoration, and short-term reduction in lung function (Manfreda et al.,1986). Acute nonpulmonary effects include conjunctivitis, rhinitis, dermatitis, headache, fever and chills, flushed face, and pain in joints and muscles (Brown,1988). If fever accompanies acute pulmonary or non-pulmonary symptoms, the diagnosis has historically been called "grain fever," or more recently "organic dust toxic syndrome (ODTS)" (Rask-Anderson,1989a). Chronic health effects associated with grain dust exposure include asthma, chronic bronchitis, farmer's lung or hypersensitivity pneumonitis (HP), non-specific chronic obstructive pulmonary disease (COPD), and long-term reduction in lung function (Schenker et al.,1991; doPico et al.,1977). Chronic adverse effects are likely to result from repetitive acute inflammatory responses to grain dust exposure.

The most important evidence that grain dust can cause permanent, irreversible lung damage comes from the large number of prevalence and longitudinal studies of U.S. and Canadian grain worker cohorts (Chan-Yeung et al.,1992a

and 1981; Becklake et al.,1980b; Broder et al.,1979; doPico et al.,1977). These studies have shown that grain workers generally have a significantly higher prevalence of respiratory symptoms and disease and reduced lung function as compared to unexposed workers from the same region of similar age, height, and smoking habits. These extensive investigations have also clarified the contributions of smoking, ethnicity, layoff, duration of employment, and allergic status to the prevalence of respiratory symptoms and disease among grain workers. It has been shown that workers who leave the grain industry have higher prevalence of symptoms as compared to those who remain employed (Broder et al.,1985). This "healthy worker" effect has undoubtedly led to an underestimate of the adverse effects of grain dust exposure.

The epidemiological studies that address grain dust exposures have mostly been performed on grain elevator workers who have regular exposure to grain dust. Other exposed worker groups, in particular farmers and dock workers, have not been as well studied. The majority of farms across the U.S. are small family operations or employ less than 10 workers, and are exempt from OSHA regulations and general duty inspections (Popendorf,1985). Thus, exposure levels have not been measured for farm workers on a regular or systematic basis. Farmers may be exposed to grain dust during harvesting and storage, feeding of livestock, or during cleaning and maintenance of silos or bins. While their exposure has been estimated at only 40 to 60 days per year, farm worker exposure has been estimated to range from half that of grain elevator workers to as

high or higher than grain elevator workers (Beard,1994; May,1993; Warren,1989; Donham,1986a). It has been suggested that modern agricultural practices, such as confined feeding operations, may result in further increased exposures (Popendorf,1985). In addition, farmers have multiple hazardous exposures, including toxic gases from animal confinement buildings and manure pits, fertilizers, pesticides, animal dander and feces, infectious agents, and moldy hay and grain dusts, such as barley, corn, cotton, wheat, rice, sorghum, and soybean (Zejda et al.,1993b; Chan-Yeung et al.,1992b; Popendorf et al.,1985; Pratt et al.,1984). Likewise, dock workers have varied intensity of grain dust exposure and other hazardous exposures, such as asbestos and mineral dusts, depending on what materials are being received and exported. The cumulative effect of multiple exposures and varied intensity on these other exposed groups is unknown. Thus, studies of farmers and dock workers provide less convincing evidence of the effects of grain dust exposure.

Most of the epidemiological studies have assigned worker exposure by years of employment, exposure category (e.g. high or low), or very limited available air monitoring data. Acute and chronic respiratory effects and reduced pulmonary function have generally correlated with these proxy exposure measures. Few studies have developed dose-response relationships between an individual's exposure and health effects. In those that have, only acute effects have been examined and only grain dust has been monitored. For example, doPico et al. (1983) showed that cross-shift complaints of respiratory symptoms and change in

FVC, $FEF_{50\%}$, and $FEF_{25\%}$ correlated with total dust exposure, and Corey et al. (1982) found that cross-shift change in $FEF_{50\%}$ was related to respirable dust exposure. Even fewer studies have looked at dose-response by specific component of grain dust.

The specific etiologic agent in grain dust (and other organic dusts) is unknown. In fact, it is still unclear if the grain itself or a contaminant(s) initiates the toxic reaction. Grain dust is a heterogeneous mixture of organic and inorganic constituents (Brown,1988). The biologic activity of the dust varies with particle size distribution, grain variety, and contaminant levels. Numerous common contaminants include plant matter, insects, mites, hair, feathers, excreta, bacterial endotoxins, fungal mycotoxins, pesticides, fertilizers, soil (silica), lubricating oil, metal fragments, and paint chips (Beard,1994; Schenker et al.,1991). The specific composition of grain dust varies with location, altitude, season, humidity, amount of rain, age of the grain, and methods of storage, handling, and processing of the grain (doPico,1994; Donham,1986a). More than one of the possible constituents is likely to act as an etiologic agent, although microorganisms have been implicated in many of the acute and chronic health effects.

Proposed mechanisms by which grain dust exerts its toxic action include simple physical irritation, allergic response to fungi, grain mites and weevils; activation of the alternative complement pathway; and inflammatory reaction to mycotoxins or endotoxins (Menkes et al.,1989). Several authors have proposed that a

hypersensitivity reaction to foreign proteins may be involved (Warren et al.,1974; Dickie et al.,1967). Mycotoxins or fungal spores in the dusts have long been considered the responsible agent for acute response to grain dust and ODTS, but several studies dispute this theory (Emanuel et al.,1989 and 1975). While HP is thought to be an immune response to thermophilic actinomycetes, it is proposed that bacterial endotoxins in organic dust may also play a role (Von Essen et al.,1990b; Dutkiewicz et al.,1985).

Endotoxin is now believed to be the responsible agent for the majority of acute grain dust-related respiratory effects. Recent animal and human exposure studies have shown that endotoxin increases proinflammatory cytokines when interacted with alveolar macrophages (Deetz et al.,1997; Jagielo et al.,1996; Schwartz et al.,1995; Clapp et al.,1994). Cosma and Martinez (1999) have shown that endotoxin is responsible for 70% of the inflammatory response by rat alveolar macrophages exposed to wheat and corn dust *in vitro*. Human inhalation studies have shown that endotoxin in cotton dust is associated with dose-dependent airflow obstruction (Castellan et al., 1987; Rylander et al.,1985). Several epidemiological studies have demonstrated associations between endotoxin exposure among swine building, animal feed industry, and cotton workers and respiratory symptoms or pulmonary function declines (Donham et al.,1989; Smid et al.,1992b; Rylander et al.,1993).

Farmers in Northeastern Colorado have elevated dust exposures during wheat harvest (Beard,1994). Endotoxin levels in the dust are in the range at which mucous membrane irritation and acute pulmonary function changes have been documented (Olenchock,1994; Haglind et al.,1984). Because of these exposures, and the fact that farmers have been seldom studied during harvest, the correlation of cross-shift symptoms and lung function change with dust and endotoxin exposure during harvest is critical to further assessment of the acute effects of grain dust and the role played by endotoxin.

Chapter 2

Literature Review

A great deal of research has been conducted in the area of grain dust exposure and associated respiratory diseases. This chapter summarizes the research regarding grain dust components, respiratory symptoms and conditions, risk factors for respiratory effects, and possible mechanisms of disease in grain-exposed populations.

2.1 Extent of Grain Dust Exposure

Fixed agricultural operations are the third largest source of particulate pollution in the United States (Donham,1986a). U.S. agricultural operations produce nearly 1.8 million tons of dust each year, mostly from grain elevators (Donham,1986a). The estimated number of people at risk of exposure to grain dust varies depending on both the specific dust and the occupations included. Grain dust is only one of a group of agents referred to as "organic dusts" which include corn, barley, raw cotton, silage, hay, feed, and flour that are thought to have similar mechanisms of toxicity (doPico,1992; Rask-Anderson,1989a; Rylander,1986). Grain handling has evolved into a complex system involving primary and secondary production, storage, inspection, transportation, marketing, and consumption. Thus, exposed occupations include farmers, truckers, grain elevator workers, dock workers, and feed, flour, and seed mill workers (Chan-Yeung et al.,1992b).

The exact number of exposed workers is difficult to estimate since so many occupations are involved. NIOSH estimated that over 500,000 workers were exposed to grain dust on a continuous basis in grain elevator and related industries and 2 to 3 million farm workers on a periodic or seasonal basis in U.S. grain elevators, flour, feed and seed mills, and grain transportation activities (doPico,1986; Popendorf,1985). These estimates grow to 10 to 12 million agricultural workers exposed to all organic dusts in all phases of production and use (e.g. dairy, beef, pork and cash grain farmers), thus comprising the largest population at risk of occupational lung disease in the United States (Chan-Yeung et al.,1992b; doPico,1992; Hurst et al.,1990; Popendorf,1985).

In addition, large populations in other countries have grain dust exposures similar to the U.S. For example, the annual grain harvest in the United Kingdom had increased from 13.2 million tons in 1977 to 24.5 million tons in 1987 with an estimated 100,000 people exposed occupationally to grain dust (Blainey,1990). An estimated 100,000 workers are occupationally exposed to grain dust in Canada (Chan-Yeung et al.,1979).

In Colorado, there are over 200 grain handling facilities with an estimated 800 workers (Colorado Agricultural Statistics,1992). In addition, there are approximately 15,600 farms with wheat acreage and an estimated 62,400 full-time and seasonal grain workers (Colorado Agricultural Statistics,1992).

2.2 Components of Grain Dust

Grain dust is a complex and variable mixture of approximately 60 to 75% organic materials and 25 to 40% inorganic materials (Brown,1988; Yoshida et al.,1980). Grain dust is produced by the abrasion and attrition of grain kernels each time the grain mass is handled. Approximately 3 to 4 pounds of dust are produced per ton of grain processed through a grain elevator system (Farant,1989). Grain dust composition, and thus biologic activity, varies with particle size distribution, grain variety, and decomposition products. The specific composition of grain dust also varies with location, altitude, season, humidity, amount of rain, age of the grain, pest management practices, and methods of storage, handling, and processing of the grain (Donham,1986a). Table 2.1 lists the common constituents and contaminants found in naturally-occurring, heterogeneous grain dust mixtures.

2.2.1 Airborne Grain Dust Levels

Airborne grain dust concentrations have been shown to vary with elevator, task performed, throughput volume, housekeeping efforts, ventilation, degree of enclosure, humidity, age of the grain, and extent to which the grain has been cleaned and manipulated (Warren,1989; Farant,1989; Brown,1988). Table 2-2 summarizes total and respirable dust exposures reported for various grain dust exposure situations.

Table 2.1 Common constituents found in grain dust

Grain dust constituents	Reference
Grain components: Cereal grain fragments (wheat, barley, oats, rye, corn, rice, etc.) Oil seeds (rapeseed, linseed, sunflower) Pulses (edible seeds of legumes) Weed seeds	Becklake,1980a
Decomposition products of cereal grains, oil seeds, and pulses	Chan-Yeung et al.,1985
Microorganisms: Bacteria and endotoxins Fungi and mycotoxins	Smalley et al.,1986; Beard,1994; Lacey,1980
Insects, insect parts and feces	Beard,1994; Schenker et al.,1991
Mites	Beard,1994; Schenker et al.,1991
Hairs, feathers, and excreta of rodents and birds	Becklake,1980a
Soil and trace elements (silica)	Yoshida et al.,1980
Chemicals (fertilizers, pesticides, herbicides)	Schenker et al.,1991
Other contaminants (lubricating oil, metal fragments, paint chips)	Schenker et al.,1991

Airborne grain dust concentrations are lognormally distributed with geometric means varying from less than 2 to 109 mg/m^3 (ACGIH, 1995). In the United States, environmental surveys of grain handling facilities have shown a total dust level range of less than 1 to 253 mg/m^3 and a respirable dust level range of less than 1 to 31 mg/m^3 (Beard,1994; Brown,1988; Jannsen,1980; doPico et al.,1977). In Canada, surveys of grain handling facilities have shown higher levels - a total

Table 2.2 Airborne grain dust concentrations

Occupational exposure	Grain dust concentrations	Reference
Grain elevators (Wisconsin and Minnesota)	10 to 253 mg/m^3 (8-hr TWAs)	doPico et al.,1977
Feed mills, grain elevators (northeastern Colorado)	0.5 to 32 mg/m^3	Jannsen,1980
6 exporting grain elevators (USA)	0.12 to 132 mg/m^3 total) 2.2 to 7.4 mg/m^3 (means, total) 0.005 to 31 mg/m^3 (respirable) 0.18 to 0.91 mg/m^3 (means, respirable)	Brown,1988
Grain elevators (Northeastern Colorado)	8.5 to 41.0 mg/m^3	Beard,1994
Feed mill, grain elevator, seed cleaning plant (Canada)	2.8 to 26 mg/m^3 (means, total)	Schrag et al.,1972
8 terminal, 9 transfer, and 14 country elevators (Canada)	0.2 to 781 mg/m^3 (total) 1.4 to 109 mg/m^3 (means, total) ND to 76 mg/m^3 (respirable) 0.3 to 14 mg/m^3 (means, respirable)	Farant et al.,1980
Country grain elevators (Canada)	0.3 to 890 mg/m^3 (total)	Yoshida et al.,1980
Terminal grain elevators (Canada)	3.7 to 17 mg/m^3 (personal means, total)	Chan-Yeung et al.,1981
5 grain pits (Sweden)	4 to 53 mg/m^3 (total)	Brown,1988
Grain mill (South Africa)	1.6 to 19.9 mg/m^3 (total) 0.6 to 3.5 mg/m^3 (respirable)	Fonn et al.,1993a
Flour mills (Sudan)	20 to 190 mg/m^3 (respirable)	Fakhri,1922
Silo unloading (Pennsylvania)	0.6 to 129 mg/m^3 (total) 0.2 to 25 mg/m^3 (respirable)	Morey,1990
Farms at harvest (Northeastern Colorado)	4.1 to 15.2 mg/m^3	Beard,1994
Swine confinement buildings (Sweden)	6.3 mg/m^3 (means, total) 0.5 mg/m^3 (means, respirable)	Donham et al.,1986
Pig houses (Great Britain)	1.7 to 21.0 mg/m^3 (means, total)	Crook et al.,1991
Piggeries and poultry coops (Finland)	12.7 mg/m^3 (means, total)	Louhelainen et al.,1987b
Dairy barns (Wisconsin)	0.7 mg/m^3 (mean, total) 1.8 mg/m^3 (means, inhalable) <0.1 mg/m^3 (means, respirable)	Kullman et al.,1998

dust level range of less than 1 to 890 mg/m^3 and a respirable dust level range of less than 1 to 76 mg/m^3 (Farant et al.,1980). Similar grain dust exposure ranges have been reported in other countries (Fonn et al.,1993a; Brown,1988). The highest exposures have been found in the elevator receiving tunnel and the transfer gallery, and areas with inadequate or nonexistent ventilation. The job tasks with the highest exposures in elevators were sweeping, weighing and bagging operations, and work near free fall of grain into hoppers and unenclosed seed cleaning machines.

Although grain dust exposure on farms is generally intermittent, some farm activities result in higher exposures than grain elevators, e.g. during silo unloading, especially when the silage is very dry, and during short-term "trucking loading activities" (Beard,1994; Morey,1989). Burg et al. (1984) reported total dust levels as high as 230 mg/m^3 around corn combine harvesters. Others have reported high dust levels around livestock operations and in piggeries, where the dust is composed primarily of grain particles and fecal matter (Crook et al.,1991; Louhelainen et al.,1987a and 1987b; Donham et al.,1986b). Vinzents et al. (1992) showed that total dust levels decreased when fat was added to livestock food.

Dust levels have been dramatically reduced in grain handling facilities over the last two decades (Chan-Yeung et al.,1992b). Advances in dust control technology have resulted in lower dust levels, generally below 10 mg/m^3, in North

American grain elevators (doPico,1994). By 1985, 94% of Canadian elevators had installed ventilation equipment (doPico,1994). When Farant (1989) repeated a survey at transfer elevators in Canada 10 years later, the dust levels were 5 to 20-fold lower due to improved controls and work practices.

Particle Size

The particle size distribution of the dust is strongly related to the type of grain. The respirable dust mass is characteristically 17% for barley, 18% for wheat, and 7% for corn (Farant,1989). However, respirable values as high as 40% and above have been reported (Broder,1979). This grain type distinction seems to support workers' complaints being greater for barley and wheat dust than for corn dust exposure.

Yoshida et al. (1980) reported a mass mean diameter (MMD) of 3.0 to 8.5 um for grain dust inside country elevators in Saskatchewan. Williams et al. (1964) reported geometric mean diameters (GMD) in country grain elevators of 3.1 um (GSD = 1.7 um) for oat dust and 2.1 um (GSD = 2.0 um) for wheat dust. Parnell et al. (1986) reported the MMD for settled rice, corn, wheat, soybean, and sorghum dusts to range from 10.7 to 14.0 um, with rice having the smallest diameter. Average count mean diameters (CMD) for total dust distributions range from 0.47 to 0.65 um in Northeastern Colorado grain elevators, while the CMD of dust collected at a control site was 0.10 um (Kramer,1978). Beard (1994) reported

mean mass median aerodynamic diameters (MMAD) of 4.2 to 7.3 um for Colorado farms and 3.1 to 8.2 um for Colorado grain elevators. Airborne dusts in swine confinement buildings had a GMD of 2.9 um (Donham et al.,1986b).

Exposure Standards

The Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL) for grain dust (barley, oat, and wheat) is 10 mg/m^3 as total dust (OSHA,1989). However, there are many studies showing that grain dust exposure results in excess health effects at levels below 10 mg/m^3 total dust. Grain dust-induced chronic bronchitis has been observed at exposures between 4 and 10 mg/m^3 (doPico et al.,1981). Eye and nasal irritation, rhinitis, wheezing, cough, breathlessness, sputum production, and dyspnea have been reported at total grain dust concentrations as low as 0.1 mg/m^3 and respirable dust concentrations as low as 0.2 mg/m^3 (Chan-Yeung et al.,1981; DoPico et al.,1982b; Broder et al.,1985). Airway obstruction has been observed at exposures between 5 and 10 mg/m^3 total grain dust (Kennedy et al.,1994; Fonn et al.,1993a; Huy et al.,1991; doPico et al.,1983; Corey et al.,1982). Enarson et al. (1985a) calculated that at airborne dust concentrations above 5 mg/m^3, grain workers were at serious risk of lung function decline over time. Based on these and other studies, the American Conference of Governmental Industrial Hygienists (ACGIH) established a threshold limit value (TLV) for grain (barley, oat, and wheat) dust of 4 mg/m^3 as

total dust (ACGIH, 1994-1995). NIOSH has adopted this same level as a recommended exposure limit (REL).

2.2.2 Grain and Decomposition Products

The major component of grain dust arises from the various grains and their decomposition products. It has been found that certain symptoms are precipitated by, or are more intense upon, exposure to a particular grain. Durum wheat, barley, rye and spring wheat, and oats, in decreasing order, are the most bothersome grains in terms of inducing symptoms (Chan-Yeung et al.,1985; doPico et al.,1977; Williams et al.,1964). Durum wheat has been experimentally shown to induce airway constriction regardless of atopic status (doPico et al.,1981; Jacobs et al.,1994). Fewer complaints have been reported for corn and rice dust exposure (doPico et al.,1977).

The chief characteristic of grain dust, when viewed under a scanning electron microscope, is a mixture of the hairlike trichome particles with agglomerated globular particles (Yoshida et al.,1980). Trichomes are found on the surface of wheat and barley. Duke (1935) believed four cases of asthma among wheat flour mill workers were caused by sensitization to the trichomes. He showed that the reaction to scratch tests with bran and germ fractions gave less reaction than tests with the hairs. However, the health significance of these particles has not been further studied and is currently unknown.

Grain dusts have a 20 to 25% protein content (Rylander,1986; Donham et al.,1986b). Kramer (1978) determined the mean percentage of protein-bearing particles in airborne grain elevator dust in Colorado to range from 36 to 45% of the total dust particulates, while dust under ambient conditions contained 6% protein-bearing particulate. The mean percentage of respirable total dust was 98.5% and-protein-bearing particulates, 97.2%. Several authors have proposed that symptoms resulting from exposure to grain dust may be due to a foreign protein reaction.

Starch particles, lectins, and lymphocyte mitogens are also present in grain dust (Goynes et al.,1986). Starch particles tend to agglomerate into larger particles, many of which are still less than 10 um in diameter and available for penetration into lung airways. Plant lectins, which can cause erythrocyte agglutination and hemolysis, are found in barley, corn, and rye, but not in durum wheat, oat or spring wheat (Olenchock et al.,1986). Lymphocyte mitogens could contribute to lymphocyte-mediated reactivity after inhalation (Olenchock et al.,1986).

2.2.3 Microorganisms

While a wide variety of microorganisms are found in grain dust, the microflora changes through the process of harvesting, storage, and handling (Lacey,1980). The predominant organisms depend on the time the dust is produced and other factors such as season of the year, geographical location, grain type, water content of the grain, temperature, pH, aeration, and storage practices (Lacey,1994b;

Smalley,1986). Many of the microorganisms are well-known allergens (Lacey,1980).

Fungi/molds and Mycotoxins

The predominance of one fungal type or another in grain dust is most strongly dependent on the temperature, moisture content, and type of grain. *Cladosporium, Alternaria*, and other species of field fungi are more common in recently harvested grain, while *Aspergillus, Penicillium,* and *Actinomycetes* predominate under storage conditions (Beard,1994; Smalley,1986; Palmgren et al.,1983; Lacey,1980). *Verticullium* and *Actinomycetes* were dominant in settled swine confinement building dusts (Donham et al.,1986b). The fungal species also vary with geography, e.g. the most prevalent species present in New Orleans grain elevators differ from those present in Minnesota elevators (Palmgren et al.,1983). Differences between year and crops are usually quantitative rather than qualitative (Lacey,1980).

Up to $200x10^6/m^3$ airborne fungal spores (predominately *Cladosporium*) have been found around grain harvesters, $75x10^6$ spores/m^3 around workers unloading grain trucks, and $958x10^6$ spores/m^3 in elevators (Darke et al.,1976; Farant et al.,1980). Considerably more spores are present in the air of unventilated elevators (Farant et al.,1980).

Spores are released in large numbers during farming operations (Lacey,1994b). Fungal concentrations have been reported as follows: $8.3x10^4$ cfu/m^3 (mean) during winter wheat shipment in northeastern Colorado (Beard,1994); 130 to 15,300 spores/m^3 at animal feed production facilities (Smid et al.,1992a); 7,000 to $160x10^6$ spores/m^3 during dry silage unloading (Morey,1989); $0.02x10^6$ to $13.7x10^6$ spores/m^3 on farms in Finland (Kotimaa et al.,1987); as high as 10^9 spores/m^3 when handling a moldy hay crop (Popendorf et al.,1985); and an average of 300 and 500 cfu/m^3 in swine and poultry confinement buildings, respectively (Clark et al.,1983).

During harvest, fungal concentrations are surprisingly high. Lacey (1987) found $3.5x10^6$ to $212x10^6$ spores/m^3 around the combine cutter bar, front pick-up reel, and rear discharge, and $0.5x10^6$ to $34x10^6$ spores/m^3 in the driver's breathing zone. Concentrations were greatest in warm, still weather and when harvesting downwind. Beard (1994) found airborne fungi concentrations between $0.47x10^4$ and $17.2x10^4$ cfu/m^3 during harvest at northeastern Colorado farms. Depending on filter types and maintenance, cabs supplied with filtered air can reduce fungal levels to 5% of outside air levels (Lacey,1987).

Mycotoxins are products of secondary fungal metabolism, and may be found in the hyphae and spores as well as secreted directly into the grain (Lacey et al.,1994a; Palmgren et al.,1983). Aflatoxin, a known carcinogen and hepatotoxin, is a mycotoxin specific to the fungal strain *Aspergillus* (Dvorackova,1976). Other

mycotoxins found in grains include T-2 toxins, deoxynivalenol, fumonisin, zearalenone, and ochratoxin (Lacey et al.,1994a).

Aflatoxins have not typically been found in dust samples from grain elevators (Sorensen et al.,1986; Palmgren et al.,1983). However, aflatoxin has been detected in numerous corn and rice operations, ranging from 0.04 to 92 ng/m^3 during corn harvest and unloading (Selim et al.,1998); 3,850 ng/g in front of the combine, 1,360 ng/g in the combine cab, and 52,000 ng/g while transferring corn to the truck; and 130 ppb in airborne dust at a Georgia corn dumping station (Sorensen et al.,1986); 124 to 4,849 ng/m^3 during corn bin cleaning (Burg et al.,1984); and 26 pg/m^3 and 10 pg/m^3 in respirable dust samples in India rice mill work and storage areas, respectively. Levels as high as 50,421 ng/m^3 aflatoxin have been measured in enclosed animal feeding buildings (Ghosh et al.,1997). Aflatoxin concentrations are generally lower in the total dust samples than respirable dust samples (Ghosh et al.,1997).

Bacteria and Endotoxins

Both gram-positive and gram-negative bacteria are found in agricultural dusts, but gram-negative bacteria pose the greater health concern. Gram-negative bacteria are commonly found in agricultural environments where large quantities of grain and other organic agricultural dusts are generated, such as stored grains, hay, silage, composted wood chips, bulk cottons, mushrooms, and animal confinement

(Olenchock,1994; Donham et al.,1989). Bacteria are typically more numerous than fungi in granaries (Lacey,1994b). Gram-negative bacteria and their toxins have been identified in other occupational environments, including paper and pulp mills, wastewater treatment plants, and buildings where humidification systems are in operation (Liesivuori et al.,1994; Olenchock,1994; Mamolen et al.,1993; Olenchock et al.,1990b).

The gram negative bacterial rods, especially *Erchichia herbicola* and members of *Streptomyces*, *Staphylococci*, and *Actinomycetes* predominate under both field and storage conditions (Dutkiewicz,1978a). *Erwinia herbicola, Psuedomonas, Bacillus, Enterobacter agglomerans, Serratia,* and *Actinobacter* have also been isolated from silo and elevator dust deposits (Brown,1988; Cuthbert et al.,1980; Dutkiewicz,1978a). Beard (1994) found *E. agglomerans* and *Pseudomonas* to predominate under both harvest and winter storage conditions in Colorado. There appears to be as seasonal variation in bacterial populations in airborne dust (deLucca et al.,1987).

Concentrations of viable bacteria as high as $1x10^5$ spores/m^3 have been found at grain stores, cleaning rooms, and feed producing plants (Morey et al.,1989). Counts for Minnesota grain elevator workers ranged up to $3.6x10^6$ spores/m^3 as compared to municipal workers' counts of up to $1.4x10^5$ spores/m^3 (Smalley,1986). During silo unloading, concentrations of airborne bacteria ranged from $1.1x10^6$ to $3.5x10^9$ microorganisms, with the highest concentrations present when the silage

was very dry (Morey,1989). Beard (1994) found that airborne bacterial concentrations ranged from $7.6x10^4$ to $116x10^4$ cfu/m^3 at northeastern Colorado farms and elevators during harvest and winter shipment, with approximately one-third identified as gram-negative species. Colorado farmers received exposures during harvest comparable to terminal elevator workers, and Colorado country elevator workers received exposures greater than terminal workers (Beard,1994).

Large concentrations of bacteria are also found in animal facilities. Dutkiewicz et al. (1978a) found as many as $595x10^3$ viable bacteria/m^3 and $7.7x10^6$ viable bacteria/m^3 in swine and poultry confinement buildings, respectively. *Staphylococci, Corynebacteria, and Streptococci* were most frequently isolated. Clark et al. (1983) found an average of 300 $x10^3$ bacterial cfu/m^3 and 420 $x10^3$ cfu/m^3 in swine and poultry confinement buildings, respectively. Crook et al. (1991) found that total airborne microorganisms ranged from 10^5 to 10^7 cfu/m^3 in pig houses in Great Britain, with most of the organisms identified as gram-positive bacteria.

Bacterial antigens include primarily endotoxins, which are released from the cell wall into the environment after lysis of the bacteria or may be shed during periods of active cell growth (Olenchock,1994). Endotoxins are heat-stable lipopolysaccharides that profoundly affect humoral and cellular host mediation, including complement and coagulation, systems and cause leukocytosis and other local inflammatory responses (Morrison,1985).

Endotoxins have been found in both airborne and settled grain and other agricultural dusts (Wirtz et al.,1984a; Olenchock et al.,1990b and 1986). They are found in high concentrations in hay, cotton, and flax dusts (Buick et al.,1994; Siegal et al.,1991; Rylander et al.,1985; Pernis et al.,1961). Olenchock et al. (1983) showed that endotoxin concentration in bulk cotton varied with geographic source. While endotoxin was present in all bulk size fractions, there was a trend for higher endotoxin concentration in larger particle sizes. Washing bulk cotton before the carding process lowered both bulk and airborne endotoxin concentrations.

Blaski et al. (1996b) found mean endotoxin exposures of 2,372 EU/m^3 (10 EU/m^3 equals approximately 1 ng/m^3) for 172 grain workers in Iowa. Monthly average endotoxin levels in New Orleans terminal elevators ranged from 3 to 53 EU/m^3 over a one-year period (deLucca et al.,1987). A seasonal variation was seen in that both respirable and settled dust endotoxin levels were highest in the fall months and lowest in January (deLucca et al.,1987).

Total airborne endotoxin levels as high as $160x10^3$ EU/m^3 have been reported when emptying grain driers; $88x10^3$ EU/m^3 when moving birds in a poultry house; $88x10^3$ EU/m^3 for silo unloading; $27x10^3$ EU/m^3 for chopping dairy bedding; $16x10^3$ EU/m^3 for the opening area in a Shanghai cotton mill; and $9x10^3$ EU/m^3 for a poultry shackling line (Olenchock,1994,1990b, and 1982; Leistikow et al.,1989). Elevated levels have also been found in animal facilities: inhalable endotoxin levels from 25 to 34,800 EU/m^3 in dairy barns (geometric mean 647 EU/m^3)

(Kullman et al.,1998); inspirable endotoxin concentrations from 2 to 18,700 EU/m^3 at eight animal feed production facilities (Smid et al.,1992a); from 250 to 300 EU/m^3 in pig houses in Great Britain (Crook et al.,1991); and an average of 1,200 EU/m^3 and 3,100 EU/m^3 in swine and poultry confinement buildings, respectively (Clark et al.,1983).

Lundholm et al. (1986) collected 59 air samples in a general farm environment. They found 29 samples had endotoxin levels above 2,000 EU/m^3. During harvest, they found levels from 3 x10^4 to 1x x10^6 EU/m^3 outside the tractor cabin, but below 2,000 EU/m^3 inside the ventilated cabin. Beard (1994) found lower endotoxin levels (27 to 237 EU/m^3) during harvest in Colorado. Todd (1999) found endotoxin levels as high as 1.7 x10^6 EU/m^3 in Colorado corn bins.

While there are no exposure standards for endotoxins, the threshold for acute pulmonary function changes has been estimated to be as little as 330 EU/m^3 for smoking cotton workers and 90 EU/m^3 for a mixed population (Haglind et al.,1984). The International Committee on Occupational Health concluded that endotoxin exerts different effects at different concentrations as listed in Table 2.3 (Olenchock,1994).

Table 2.3 Effects of endotoxin at different exposure concentration levels

Endotoxin Concentration	Effect
200 to 500 EU/m^3	Mucous membrane irritation
1,000 to 2,000 EU/m^3	Acute bronchoconstriction
10,000 to 20,000 EU/m^3	Organic dust toxic syndrome (ODTS)

2.2.4 Arthropods

The primary arthropods implicated in allergic and respiratory reaction to grain dust are various species of storage mites and, to a lesser extent, grain weevils. However, other insects and insect fragments, such as grasshoppers and beetles, may be found in grain dust.

Mites are known to cause allergy (Terho,1990; Ingram et al.,1979; Cuthbert et al.,1979). The mite species most often reported in granaries, livestock-feeding facilities, barns, grain storage/transfer facilities, hay, and straw are *Tyrophagus, Glycyphagus, Acarus, Euroglyphus, Lepidoglyphus, Chortoglyphus,* and *Gohieria* (Lacy,1980; Cuthbert et al.,1980; Arlian et al.,1984). Growth and development of mites depend on moisture, temperature, light, mechanical disturbance, predation, nutrition, and species competition (Krantz,1961). Temperature and moisture are considered the most important factors. In fact, fresh hay and dry bales of hay contained no recognizable numbers of storage mites, while hay stored for a year in Iceland typically had 50,000 live mites/kg (Hallas et al.,1987; Leskinen et al.,1987).

There were more mites in Finnish cow house hay than in hay stores (Leskinen et al.,1987). In cow houses, the predominant species was *A. siro,* while in hay stores *Tydeus*, which is resistant to cold, was more common. Scottish farmers were found to be exposed to large numbers of mites when feeding cattle (Ingram et a.,1979). In general, dust from Canadian grain elevators contains fewer mites than dust from British elevators (Blainey et al.,1989). The frequency of occurrence of mites was not different for wheat, barley, and oats (Griffiths et al.,1976). The house dust mites *D. pteronyssinus* and *D. farinae*, responsible for house dust allergy, have not been found in grain dust.

The primary weevil found in grain, *Situphilus granarius*, has been shown to induce hypersensitivity (Lacy,1980; Warren et al.,1974). As many as 200 grain weevils per gram of grain dust have been reported (Jacobs,1994). The most common insects in stored Colorado wheat were found to be beetles and grasshoppers (Beard,1994).

2.2.5 Animal Debris

Animal debris, including particles and excreta from rodents and birds, are frequently found in grain dust. These have not been quantitated in grain dust nor have potential health effects on grain workers been studied.

2.2.6 Pesticides

The primary chemical residues in grain dust are pesticides -- herbicides to control weeds and insecticides to control various insects and their larvae. Fumigation with pesticides may occur during transportation by truck or freight to large elevators, during transfer into the elevators, periodically during long-term storage, and during discharge into barges, ships, or rail cars (Brown,1988). Pesticides currently used on grain in the U.S. include methyl bromide, aluminum and magnesium phosphide, and malathion. Grain farmers in northeastern Colorado have reported use of chlorpyrifos methyl (Reldan™), malathion, and aluminum phosphide (Beard,1994). Carbon disulfide, carbon tetrachloride, ethylene dibromide, and ethylene dichloride have been banned for use in the U.S. (Brown,1988), but may be used in other countries.

Because pesticides are applied to the grain surface, it is likely that dust generated by grain handling operations which abrade the surface, may have higher concentrations of these chemicals than the bulk grain (Palmgren et al.,1984). This supposition was confirmed in Australian studies showing 18 to 30 times more malathion in settled dust than whole grain. Malathion levels ranged from 0.17 to 32 ug/g in grain dust collected at terminal elevators in the New Orleans area, while no diazinon concentrations were above the detectable limit of 0.01 ug/g (Palmgren et al.,1984).

In addition to pesticide residue in the grain dust, farmers and grain workers may also be exposed to pesticides and other chemicals directly. Flaherty (1976) reported that out of 310 grain handlers surveyed, 57% encountered health problems form pesticide exposure at work and 11% had been taken to a physician as a result of pesticide exposure. Neuropsychiatric effects have been observed in grain elevator workers exposed to carbon disulfide (Peters et al.,1982). Bronchial asthma has been attributed to organophosphate insecticide exposure (Weiner,1961). Several studies have suggested possible increased lung cancer due to pesticide exposure, with farmers with lung cancer reporting more extensive exposure to herbicides, grains, and diesel exhaust than siblings who did not have lung cancer (McDuffie et al.,1991).

2.2.7 Inorganic Components

The inorganic components of grain dust include soil, silica, silicates, and compounds containing trace elements (Farant et al.,1980). The free silica content of grain dust has been considered as a possible factor in the development of pulmonary fibrosis. Farant and Moore (1980) suggested three possible sources of free silica in grain dust: 1) soil contamination of harvested grain; 2) inclusion of quartz-containing soil particles on the growing kernels of grain; and 3) quartz cells formed in *Graminae* genera of grasses such as wheat, barley, oats, and other cereal grains. Asbestos fibers may be shed into the air when soil is worked agriculturally in areas where asbestos deposits are present (Abraham,1982).

Application of pesticides formulated in talc presents a potential asbestos exposure as well, since talc is often contaminated with asbestos fibers.

The mean quartz content of total dust was 6.5%, 4.8%, and 2.1% in settled grain dust at country elevators, terminal elevators and transfer elevators, respectively (Farant et al.,1980). Williams et al. (1964) reported the free silica content of total airborne grain dust to be 8.9% and 7.4% in oat and wheat dust, respectively. Beard (1994) reported the respirable silica concentration ranged from <0.02 to 0.07 mg/m^3 at farms and elevators during harvest. The content of quartz and other inorganic matter appears to be related to the amount of cleaning the grain has experienced (Farant et al.,1980).

2.3 Respiratory Symptoms and Conditions Associated with Grain Dust

Occupational exposure to grain dust results in both pulmonary and non-pulmonary symptoms and conditions (Brown,1988). Pulmonary symptoms include wheezing, cough, shortness of breath, and dyspnea. Non-pulmonary symptoms include eye, nose, and skin irritation, fever, malaise, and muscle fatigue. Pulmonary diseases and conditions include mucous membrane irritation (MMI), asthma, acute and chronic bronchitis, acute and chronic hypersensitivity pneumonitis (HP), organic dust toxic syndrome (ODTS), and restrictive disease (Schenker et al.,1991; Popendorf,1985). The seriousness of respiratory effects of grain and organic dust exposure are reflected in an

increased proportional mortality ratio (PMR) and increased fatal respiratory disability among more heavily-exposed agricultural workers both in the U.S. and Europe (Notkola et al.,1992; Thelin,1991; Cohen et al.,1953). Smith et al. (1941) attributed 28% of all deaths among 241 grain handlers to respiratory disease. An increased PMR has been observed for emphysema among grain, flour and feed mill workers, and for asthma among California agricultural workers (Merchant,1987). In addition, respiratory symptoms and diseases can cause missed work days, reduced efficiency, and thus reduced income (DoPico et al.,1992).

2.3.1 Historical Reports

The association of adverse health effects due to inhalation of grain dust was first documented by the Catholic Archbishop of Sweden in 1555, who noted that threshing indoors could result in illness and death (Hurst et al.,1990). Several centuries later, Ramazzini (1713) observed that workers engaged in sifting and measuring grain all developed "shortness of breath and obstinate cough and...asthma...and rarely reached old age," and that after a harvest, "the hospitals of Rome fill up with reapers who have fallen sick." He also noted that bakers and millers were liable to "coughs, short breath, hoarseness, and finally, asthma." In 1832, Thackrah described corn flour millers as "annoyed with morning cough and expectoration, and some are asthmatic at an early age" (Mieklejohn,1957).

In the 20th century, physicians began reporting case studies of acute and chronic illness related to grain dust exposure. Campbell (1932) reported acute symptoms, including initial fever, transient cough and wheeze, and extreme dyspnea, lasting three or more weeks in five patients exposed to moldy hay in Westmoreland, England. Several other case reports were compiled from this area in England and termed "broncho-mycoses feniseciorum (of haymakers)" (Fawcitt,1936a and 1936b). Duke (1935) reported on four cases of bronchial asthma among wheat flourmill workers, concluding that the responsible structures were the wheat hairs or surface scales of wheat, or both. In 1944, a case of severe dyspnea and dry cough, diagnosed as silicosis, was attributed to unloading grain from rail cars (Heatley,1944). In 1946, Tornell (1946) proposed that fungi was the probable cause of "thresher's disease," characterized by emaciation, nightly fever, expectoration and sometimes rales following threshing of moldy grain, previously thought to be colds contracted during the drafty work.

Cohen and Osgood (1953) found that among 11 grain workers with respiratory disability, all had worked in the environment of crude grain dust for 10 to 27 years and attributed their symptoms to inhaled dust. The workers noted that the onset of symptoms was gradual and had progressed until the symptoms became disabling, especially dyspnea. Examination showed evidence of chronic bronchitis and recurrent bronchial obstruction. In eight of the 11 workers, clinically apparent emphysema eventually developed. X-ray examination of the grain handlers showed several cases of diffuse mottling of the lungs. Autopsies of three of the

grain handlers showed marked fibrosis and emphysema with cor pulmonare present in two of the workers.

2.3.2 Symptoms of Respiratory Dysfunction

The symptoms most often present among workers exposed to grain dust are cough, phlegm and expectoration, wheeze, chest tightness or dyspnea, shortness of breath, and flu-like symptoms or fever (Chan-Yeung et al.,1992b). Reduced pulmonary function and acute (cross-shift, cross-week, or cross-season) pulmonary function changes, as measured by spirometry, are also symptoms of grain dust exposure. These symptoms are generally dose-related (doPico,1992; Huy,1991). One or more of these symptoms have been reported in from 47% to 83% of grain workers (Williams et al,1964; Tse et al.,1973). Table 2.4 summarizes reported prevalence rates of individual respiratory symptoms associated with grain dust exposures in grain worker and farming populations.

Grain handlers

The first extensive prevalence study of respiratory symptoms and illnesses among grain handlers was conducted by Smith et al. (1941) in New York state. Several other large cross-sectional surveys without control groups were conducted in the subsequent decades, including populations of 1066 Canadian country elevator terminal workers (Sheridan et al.,1980), 300 grain elevator workers in Wisconsin and Minnesota (doPico et al.,1977); and 502 grain workers in Saskatchewan

Table 2.4 Prevalence of respiratory symptoms associated with wheat dust exposure

Respiratory Symptom	Grain Handlers		Farmers	
	Prevalence	References	Prevalence	References
Cough	16-83% 1-11%(bakers)	1-2,4,6,8-9, 15,17-18,20	7-73% 60-67%(swine)	5,10
Expectoration	18-79%	1-2,4,6-7,11, 17-18,20,17	15-18% 17-56%(swine)	5,10,14
Shortness of breath	29-32% 20%(bakers)	4,7-8,22	8-42% 30-32%(swine)	5,19
Wheezing	5-65% 7-20%(bakers)	1-2,4,5-6,8,12, 20-22	8-56% 27-29% (swine)	7,10,14,19
Chest tightness	6-71%	1-2,6,8-9,15, 18,20	8-41% 36%(swine)	5,22
Flu-like episodes (with dusty work)	0-33% 0%(bakers)	1-2,4,6,8-9, 17-18,20	5-35% 10-30% (swine)	3,5,10,13,16
Abnormal pulmonary function	4-42% 5.5% (bakers)	1-2,4,5,8-9, 11,18,21	Not reported	7,22

References (Only the first author's name is provided for conciseness):
1-Becklake,1980b 2-Broder,1979 3-Calvahiero,1995 4-Chan-Yeung,1992a 5-Donham,1989 6-doPico,1977 7-Dosman,1988 8-Herbert,1981 9-Kleinfeld,1968 10-Manfreda,1986 11-McDuffie,1991 12-Prichard,1984 13-Rask-Anderson,1989a 14-Schwartz,1995 15-Sheridan,1980 16-Siegel,1991 17-Smith,1941 18-Tse,1973 19-Warren,1987 20-Williams,1964 21-Yach,1985 22-Zejda,1993a

(Williams et al.,1964). In addition, a number of smaller cross-sectional studies looked at specific exposed populations: 68 grain elevator agents in Manitoba (McCarthy et al.,1985), 24 Australian workers loading ships with grain (Gandevia et al.,1980), 80 English dock workers handling barley (Tse et al.,1973), and 55 New York grain scoopers (Kleinfeld et al.,1968).

To confirm that the prevalence of various symptoms and diseases found among grain workers were elevated above the prevalence among nonexposed workers, a

number of cross-sectional studies have included unexposed control groups. These study populations have compared 41 Australian grain terminal workers to 10 public works maintenance employees (James et al.,1990), 582 South African grain mill workers to 153 paper packaging workers (Yach et al.,1985), 310 Wisconsin and Minnesota grain elevator workers to 239 outdoor city service workers (doPico et al.,1983), 248 Wisconsin and Minnesota grain elevator workers to 192 outdoor city service workers (doPico et al.,1984), 63 Alberta grain terminal workers to 63 office workers (Herbert et al.,1981), 103 St. Lawrence grain elevator workers and longshoremen to 39 unexposed port workers (Becklake et al.,1980b), 573 Port of Vancouver grain handlers to civic workers (Chan-Yeung et al.,1980), and 441 Thunder Bay grain handlers to 180 outside civic workers (Broder et al.,1979).

The prevalence of the various symptoms among grain handlers varies widely depending on the population, with cough reported by 16-83% of workers, expectoration and phlegm reported by 18-79% of workers, shortness of breath reported by 29-32% of workers, wheezing reported by 5-65% of workers, chest tightness reported by 6-71% of workers, and flu-like episodes during dusty work reported by 0-33% of workers. Despite the wide range, in almost all cases where a control group was included, the prevalence of symptoms was greater among the grain-exposed workers. In one study, the only significant difference was that grain handlers who smoked reported more shortness of breath than office workers who smoked (Herbert et al.,1981).

A study of 118 dock workers showed distinctly different respiratory health patterns than a comparison group of 128 civic workers and a comparison group of 555 grain elevator workers. The prevalence of chronic cough and phlegm was eight-fold higher among dock workers than the civic workers and no different than grain elevator workers (Dimich-Ward et al.,1995). Dock workers experienced dyspnea less often than grain elevator workers, but experienced eye and skin irritation more often than either comparison group. They reported more grain fever episodes, probably due to higher levels of exposure.

Respiratory symptoms have also been examined among flour mill workers who work with both bulk and ground grains. Among 100 Sudan flour mill workers, 29% had chronic productive cough, 22% experienced chest tightness on exposure, and 18% exhibited bronchial asthma (El Karim et al.,1986). In a similar study at a flour mill in France, a higher prevalence of cough and phlegm were found among mill workers as compared to a referent group (Giminez et al.,1995).

Among bakers, who work with ground grains only, the prevalence of all respiratory symptoms is lower than among flour mill workers and grain handlers (Prichard,1984; Armentia et al.,1992; Kleinfeld et al.,1968). There are no reports of a grain fever type reaction among bakers. The frequency of positive skin prick tests to common allergens declined with employment duration, suggesting that the more sensitized bakers were leaving the industry (Prichard et al.,1984).

Jannsen (1980) performed one of the first cross-shift exposure/response studies in feed mills and grain elevators. He administered a respiratory symptoms questionnaire to 37 workers before and after a workshift. Total dust concentrations ranged from 0.5 to 32 mg/m^3, but most were less than 5 mg/m^3. He reported a positive correlation between dust concentration and cough and irritation of the eyes and nose.

Farmers

In general, fewer farmers report symptoms related to grain dust exposure than elevator workers. The prevalence of cough among farmers has been reported from 7 to 73%, expectoration and phlegm 15-18%, shortness of breath 8-42%, wheezing 8-56%, and chest tightness 8-41% (doPico,1992; Warren et al.,1987; Manfreda et al.,1986). Dosman et al. (1987) showed that the prevalence of phlegm, wheeze, shortness of breath, and chronic bronchitis was increased for 1,824 Saskatchewan farmers as compared to 556 control subjects.

Few significant differences in symptoms have been reported among cattle, grain, or mixed farming groups. One study did show that northeastern Colorado farmers producing cash grains had lower prevalence of symptoms than livestock producers (Champney,1994). However, swine farmers have been shown to exhibit higher incidences of symptoms as compared to other farmer groups (Donham et al.,1989; Dosman et al.,1988). The prevalence for listed

symptoms, as well as frequent chest colds, chest illness, and history of pneumonia were all reported about twice as frequently by swine workers as other farmers. Symptoms were related to years spent working with swine. A study of 181 farmers showed that shortness of breath, dry cough, or wheezing occurred during work for 39% of pig farmers, but only 5% of dairy farmers (Iversen et al.,1990b). Poultry farmers have been shown to have a prevalence of the various respiratory symptoms intermediate between farmers and swine confinement workers (Leistikow et al.,1989).

In 1976, Darke et al. (1976) reported on respiratory symptoms among 65 English farmers harvesting wheat, barley, and oats. They found 23% had at least one respiratory symptom, with cough, wheezing, and breathlessness being severe enough to prevent work. No fever was reported. Respiratory distress increased through the harvest period and disappeared after harvest. One-fourth of the subjects reported respiratory distress when working on combine harvesters or near grain driers and elevators. The symptoms usually developed after several years working on a farm, but occurred in some during the first harvest year. Atopic and nonatopic, and smoking and nonsmoking farmers were equally affected. Peak flow rates were generally below normal before harvest began, but no significant cross-shift changes were noted. Chest radiographs were normal. Similarly, James et al. (1986) found that 18% of seasonal harvest workers developed wheeze, breathlessness, and chest tightness, but no fever, and had declines in FEV_1. Chan-Yeung et al. (1992b)

considered this to be an acute respiratory illness quite different from those experienced by grain elevator workers. The presence of respiratory symptoms, atopy, and NSBH before employment were important predictors for acute symptoms changes over harvest (Cookson et al.,1986).

Reduced Pulmonary Function

Continued or repeated exposure to grain dust is thought to cause a gradual loss of pulmonary function. Thus, many of the cross-sectional studies have conducted spirometry on the subjects (James et al.,1990; Yach et al.,1985; doPico et al.,1984,1983, and 1977; Herbert et al.,1981; Becklake et al.,1980b; Chan-Yeung et al.,1978 and 1980; Broder et al.,1979; Tse et al.,1973). Spirometry is defined as the measurement of volume change during a clearly defined forced vital capacity maneuver, a complete maximum exhalation made as rapidly as possible following a maximum inhalation. A reduction (<80%) in forced vital capacity (FVC) with constant ratio of forced expiratory volume in one second to FVC (FEV_1/FVC) can indicate a restrictive disease, while decreases in FEV_1 and FEV_1/FVC (<70%) generally indicates obstructive disease (McDuffie et al.,1991). When FVC < 80% and FEV_1/FVC is between 70 and 80%, the effect is generally considered a mixed restrictive and obstructive effect. Abnormal pulmonary function values have been reported in between 4 and 42% of grain handlers, but only 7% of control worker subjects (doPico,1994). Lung function tests, including FEV_1, FVC, and mid-expiratory

flowrate ($FEF_{25-75\%}$), have generally been lower for grain workers compared to controls. Airway obstruction, as measured by FEV_1/FVC, $FEF_{25-75\%}$, and $FEF_{50\%}$, was more prevalent for grain workers with chronic bronchitis than those without (doPico et al.,1984). One study found that Alberta grain workers had better FVC and FEV_1 than the general population at all ages and smoking history (Siemens et al.,1980). However, only 216 of the 1,545 grain workers were nonsmokers, and the general population is not considered the best comparison group. Pollock et al. (1980) did not find differences in lung function between granary workers and controls, except that the 32% of Scottish granary workers with acute symptoms had reduced lung function as compared to asymptomatic granary workers and controls.

The available evidence suggests that smoking grain workers develop respiratory abnormalities independent of, and in excess of those expected by smoking alone (doPico,1994; Hurst et al.,1990; Becklake et al.,1980b; Dosman,1977). Dosman et al. (1980) found lower mean $FEF_{25-75\%}$ for nonsmoking grain workers as compared to nonsmoking control subjects. Herbert et al. (1981) found a significantly lower FEV_1/FVC for grain handlers (mean FEV_1/FVC = 60%) who smoked as compared to age-matched office worker controls who smoked, but there was no difference in spirometric values between nonsmoking groups. Broder et al. (1979) found significantly lower FEV_1 for nonsmoking grain workers as compared to outdoor civic workers at one elevator, but not at another elevator.

Pulmonary function among dock workers was similar to civic workers and significantly higher than grain elevator workers (Dimich-Ward et al.,1995). The paradox between increased symptoms and better lung function was explained by the difference in exposure patterns. Dock workers are exposed intermittently to grain dust, but work more overtime and are more likely to wear a dust mask than elevator workers (Dimich-Ward et al.,1995). The intermittent exposure may allow for some recovery of lung function and enhance worker sensitivity to the effects of grain dust. Among the dock workers, subjective estimates of exposure duration were associated with lower FEV_1.

A respiratory health study of 118 flour mill workers and 164 unexposed manufacturing workers in eastern France showed no difference in baseline pulmonary function parameters or prevalence of asthma (Massin et al.,1995). The prevalence of chronic cough and chronic bronchitis was significantly higher for the mill workers. Personal inhalable dust concentrations ranged from 0.6 to 58 mg/m^3. A dose-response relationship was observed between dust exposure and chronic respiratory symptoms and airways hyperresponsiveness. In the comparative study of grain handlers and bakers, 27% of grain handlers, but only 5.5% of bakers, had an FEV_1 below the lower limit of the normal range (Kleinfeld et al.,1968).

A secular decline in pulmonary function was first reported by Chan-Yeung et al. (1981). They examined 373 grain workers during the winter of 1975-76 and the

summer of 1978. The average annual decline in FEV_1 was 87 ml for grain workers and 28 ml for control civic workers. Since that time, several investigators have presented similar findings (Pahwa et al.,1994; Kennedy et al.,1994; Zejda et al.,1992; Tabona et al.,1984; Becklake et al.,1980b). Positive skin reaction to common allergens (atopy), presence of respiratory symptoms, and initial lung function in grain workers were not predictors of subsequent decline in lung function over time (Tabona et al.,1984). Age, cumulative exposure, and metacholine responsiveness were positively associated with decline in lung function (Tabona et al.,1984; Chan-Yeung et al.,1981). Chronic grain dust exposure seems to accelerate the decline in lung function among grain handlers, especially in older age groups (doPico,1994).

Cross-shift and cross-week changes in lung function were also correlated with subsequent decline in lung function over time (Tabona et al.,1984; Chan-Yeung et al.,1981). This finding led the authors to conclude that the acute bronchoconstrictive effect of grain dust exposure may lead to irreversible airway obstruction. Other studies have confirmed that, in the presence of bronchial hyperresponsiveness, change in FEV_1 over a workshift more precisely predicts the trend in FEV_1 decline over time in both grain handlers and cotton workers (Glindmeyer et al.,1994; Enarson et al., 1988; Tabona et al.,1984). However, bronchial responsiveness did not predict long term decline in FEV_1 in one group of Canadian grain handlers (doPico et al.,1977).

In a longitudinal survey of 1,211 grain elevator workers, subjects who reported persistent wheeze had the largest mean annual change in FEV_1 and FVC (Senthiselvan et al.,1996). New onset of wheeze, but not cough or phlegm, was significantly related to the annual rate of change of pulmonary function. A concern is that, even though dust concentrations in elevators have declined progressively over the course of longitudinal studies, grain workers continue to have more respiratory symptoms and lower lung function that the civic workers (Chan-Yeung et al.,1992a). This could mean that grain dust levels have not been reduced sufficiently, or that changes in lung function are affected by past exposures.

Seventy-seven workers with low seniority were tracked monthly over the winter of 1978 to determine how periods of layoff affected respiratory health (Broder et al.,1980). A transient decrease in respiratory symptoms was observed among 41 men who were laid off for an average of 82 days. The 36 men who continued to work showed an increase in respiratory symptoms even though it was a period of reduced grain movement – probably due to the cold temperatures. Upon rehire, the laid-off workers reported an increase in rhinitis, cough, and sputum production, and a decrease in shortness of breath and respiratory illness. Pulmonary function parameters improved in workers after several months of being laid off, showing that longitudinal changes in respiratory variables are at least partially reversible. For these same workers, FEV_1 and $FEF_{50\%}$ declined after rehire. This same reversible effect was

demonstrated among young Australian seasonal workers (James et al.,1990). These findings suggested that continuously employed workers are at greater risk for chronic inflammatory disease.

Among three groups of grain elevator workers with more than 20 years of exposure – those with respiratory symptoms, those without symptoms, and retired workers, the retired workers had the lowest mean FEV_1, while the workers without the symptoms had the highest FEV_1 (Kennedy et al.,1994). The retired grain and civic workers from this cohort were evaluated an average of six years after their retirement (Kennedy et al.,1994). The retired grain workers had significantly lower lung function and a more rapid decline in lung function as compared to the civic workers. These findings suggest that chronic lung functions changes due to grain dust exposure are irreversible at the later stages of life.

The most consistent difference in the pulmonary function of gain handlers as compared to controls has been a small decrease in FEV_1 (Broder,1989). Enarson et al. (1985a) showed an average decline in FEV_1 of >100 ml/year, and calculated that the FEV_1 decline should not occur at airborne dust concentrations below 5 mg/m^3. A pooled analysis of four studies among Dutch feed mill and grain elevators and Canadian grain elevators and docks confirmed a negative dose-response relationship between FEV_1 and both current and cumulative exposure (Peelen et al.,1996). Slopes for the dose-

response relationship differed by a factor of three to five between industries. This is especially significant in light of different methods of exposure assessment and estimation used in the studies.

Among farmers, the prevalence of reduced pulmonary function would be expected to be approximately that of the non-farmers because many of the acute respiratory conditions associated with grain exposure do not result in long-term reduced pulmonary function (May,1990). However, while prevalence was not reported, a group of dairy farmers in France had lower mean FVC, FEV_1, $FEF_{25-75\%}$, and peak expiratory flowrate (PEFR) than a control group matched on gender, age, height, and smoking status (Dalphin et al.,1989). Likewise, farmers in England and Wales, especially those working in dairy/silage, have significantly lower lung function than industry controls as measured by FEV_1 and $FEF_{25-75\%}$, despite the fact that chronic diseases such as bronchitis were not elevated (possibly due to more stringent diagnostic criteria) and the prevalence of smoking among the farmers was lower (Heller et al.,1986). Dosman et al. (1987) had also found a decrease in FVC and FEV_1, as well as chronic bronchitis, in 1800 Canadian farmers as compared to a non-farming control population. Symptomatic farmers had significantly lower FEV_1 than asymptomatic farmers.

Reduced lung function has also been reported among swine workers. Dosman et al. (1988) found slight decreases in FVC and FEV_1, with retained FEV_1/FVC,

for 504 swine-producing farmers versus 448 other farmers. In another study, a lower mean FEV_1/FVC and $FEF_{25-75\%}$ was found for swine farmers as compared to nonfarming controls (Cormier et al.,1991). Donham et al. (1989) found reduced $FEF_{50\%}$, $FEF_{75\%}$, and FEV_1/FVC, with normal FEV_1 and FVC, among Swedish swine workers. Likewise, Zejda et al. (1993b) reported significantly lower FVC, FEV_1, FEV_1/FVC, and $FEF_{25-75\%}$ for 249 swine producers as compared to 251 grain farmers. This defect was related to exposure hours per day and age. Tielemans et al. (1994) reported that mean total dust exposure was inversely related to FVC and FEV_1 among 390 male animal feed workers. Several studies have found increased cough, sputum production, and chronic bronchitis, without corresponding differences in lung function, but these had small subject numbers and average exposure periods (Larrson et al.,1992; Holness et al.,1987; Donham et al.,1984). A longitudinal decline in FEV_1 among Danish farmers was associated with pig farming and smoking status (Iversen et al.,1990b).

Acute Pulmonary Function Changes

In addition to surveys of symptoms and pulmonary function associated with exposure to grain dust, a number of studies have reported acute changes in pulmonary function following short-term exposure - after bronchial provocation, and over the course of a workshift, workweek, or harvest season.

Clinical trials have shown immediate significant falls in FEV_1, $FEF_{50\%}$, and $FEF_{75\%}$ among symptomatic grain handlers and controls after 2 hour exposure to grain dust, independent of atopic status, previous exposure, or bronchial hyperresponsiveness (doPico,1982a). Dock workers demonstrated a mean decline in FEV_1 of 141 ml after 30 minutes, and 291 ml after eight hours of wheat dust provocation (Gandevia et al.,1980). FEV_1 declines were significantly smaller for control subjects exposed to phosphate rock dust - only 43 ml and 130 ml for 30 minutes and eight hours, respectively. Those workers with productive cough showed the largest decrease. There was no evidence of alveolitis or complement activation, or change in static compliance or diffusing capacity (doPico,1982a). The response was associated with fever and leukocytosis in almost all cases, but may or may not be associated with a change in other respiratory symptoms (doPico et al.,1982a; Davies et al.,1976). The acute bronchoconstriction following a single exposure can last for up to 72 hours or recur daily (Chan-Yeung et al.,1979; Davies et al.,1976).

Cross-shift, cross-week, and cross-season studies of grain workers have demonstrated slight but significant drops in several lung function parameters (see Table 2.5). The mean FEV_1 and FVC dropped after one workshift and workweek in 485 Vancouver grain workers (Chan-Yeung et al.,1980). In contrast, there was slight but significant increase in FEV_1 and FVC for a comparison group of 65 noncedar sawmill workers. Some of the grain workers

Table 2.5 Acute lung function changes in grain-exposed study populations

Study[1]	Subjects/ Controls	Period	ΔFEV_1	ΔFVC	$\Delta FEV_1/FVC$	Other change
Grain: Chan-Yeung, 1980	610/136	Shift Week	-/+* -/+*	-/+* -/+*	—	$FEF_{25-75\%}$: 98/94%p
Corey,1982	35/15	Shift Week	+4%/NR -6/-3%*	0/NR -4/0%*	+0.2%/NR —	— $\Delta V_{max50\%}$: -8/-9%
DoPico,1983	248/192	Shift	-0.25%/ +1.41%	-0.95%/ 0.25%*	—	$\Delta V_{max50\%}$: -1/+3%*
James,1986	119/0 (mean age 23)	Season	-74ml	—	—	—
Yach,1986	582/153	Week	-4.8%/ -3.3%*	-1.9%/ +2.7%*	—	$\Delta FEF_{25-75\%}$: -14.8/ -0.8%*
Revsbech,1989	132/0	Shift	—	—	—	$\Delta PEFR$: +5.9%
James,1990	41/10	Week	-321ml/ NR*	-350ml/ NR*	80%p/ NR*	—
Flour: El Karim,1986	100/30	Shift	-290ml/ -70ml*	-280ml/ +50ml*	-3.2%/ +0.1%*	—
Swine: Donham,1989	37/0	Shift	-1%/NR	-1%/NR	—	$\Delta FEF_{50\%}$: -6%/NR $FEF_{75\%}$: -5%/NR

[1] References provide only the first author's name for conciseness.
* Significant difference in change between exposed and unexposed groups.
%p = percent of predicted value.
NR = Not reported.

experienced a greater than 10% drop in FEV_1 over the workshift. This report demonstrated cross-shift acute airways response as well as further lung function decrement across the work week.

Corey et al. (1982) showed that cross-shift changes in FEV_1, FVC, TLC, and $FEF_{50\%}$ were compatible with obstructive changes for 34 grain workers. In contrast, they found that changes over the workweek were more that of a restrictive defect. Daily lung function tests of 34 grain workers showed that FVC, FEV_1 and FEV_1/FVC decreased from Monday to Wednesday, and then were stable until Friday. All spirometric variables increased over each work shift. For the 15 comparison civic workers, only $FEF_{50\%}$ and $FEF_{25\%}$ decreased from pre- to post-shift, indicating airway obstruction without restriction. Correlation of these pulmonary decrements with dust exposures showed that 50% of the grain workers underwent a daily decrease of at least 923 ml/sec in $FEF_{50\%}$ and 310 ml/sec $FEF_{25\%}$ for each 1 mg/m^3 increase in respirable dust concentration. The multiple regression coefficients ranged from 0.52 to 0.77, but partial coefficients for dust concentration were not provided.

DoPico et al. (1983) looked at diurnal variation in pulmonary function and symptoms and workshift dust exposure among 283 grain workers and 192 service workers. There was a significant pre-shift to post-shift percentage change for FVC, $FEF_{50\%}$, and $FEF_{75\%}$ among grain workers, even after correction for age, height and smoking. The effect on FVC was so substantial in grain workers that the validity of the flow rate measurements was altered due to shifting the volume at which measurements were made. A significant correlation was also found between total dust concentration and report of

cough, expectoration, dyspnea, and eye irritation; and pre- to post-shift change in leukocyte count among grain workers.

In a study of grain elevator workers, Revsbech et al. (1989) showed that the mean diurnal change in PEFR among 132 grain elevator workers was 5.9%. The diurnal change was higher among smokers, and symptomatic and older workers, and marginally and independently related to exposure category among grain elevator workers. Respiratory symptoms, total IgE, and duration of occupation were not related to the observed variation, while dust exposure level, age and smoking were related to the variation.

Two surveys of 309 and 582 South African grain mill workers showed that those exposed to high levels of grain had higher lung function values at the beginning of the work week (Fonn et al.,1993b; Yach et al.,1985). Over the week, FEV_1 and FVC remained unchanged or reduced for workers in the high exposure level, but increased for workers in the low and medium exposure levels. The researchers attributed the difference to a healthy worker effect. Total dust, bacteria, and fungi were all significant predictors of lung function change (Fonn et al.,1993b).

McCarthy et al. (1985) found cross-shift decreases in FVC and V_{max50} among six men working with barley at an English dock, while five workers loading a non-dusty cargo did not experience changes. Among 100 Sudan flour mill workers,

58% experienced a drop in cross-shift FEV_1 and FVC (El Karim et al.,1986). In a similar study of 142 flour mill workers in France, the workers had lower pre-shift PEFR and $FEF_{75\%}$, but similar FVC and FEV_1 as compared to a referent group (Giminez et al.,1995). While both groups showed similar cross-shift reductions, the mean change for nonasthmatics was greater for the control group.

Zuskin et al. (1991) showed that swine workers with positive skin reactions had larger cross-shift reductions in $FEF_{25\%}$ and $FEF_{50\%}$ than swine workers with negative skin reactions. The baseline FEV_1, $FEF_{25\%}$ and $FEF_{50\%}$ were all lower in the swine workers with positive skin reactions. They concluded that skin testing might be useful in identifying workers at risk for chronic lung disease. Donham et al. (1989) reported that cross-shift changes in pulmonary function among Swedish swine workers was significantly correlated with time spent working in swine buildings, personal total and respirable dust levels, and area endotoxin levels. Among nonsmokers, a significant dose-response relation was seen between change in FEV_1 and total endotoxin. A less significant dose-response was seen between cross-shift change in $FEF_{25-75\%}$ and endotoxin. The FEV_1 decrement began at about 0.18 ug/m^3 endotoxin, in agreement with studies on endotoxin and cotton dust. This relationship was not observed for nonsmokers, or respirable endotoxin levels. The other significant predictors for change in FEV_1 were smoking and baseline FEV_1.

In summary, studies have shown that cross-shift changes in lung function are related to level of dust exposure, but not to age, duration of employment, smoking status, or atopy (Corey et al.,1982; doPico et al.,1983). About 4 to 11% of grain workers have a fall in FEV_1 of >10% across the workshift with the degree of bronchoconstriction related to grain dust exposure (doPico et al.,1983; Corey et al.,1982; Chan-Yeung et al.,1980). In addition, previously unexposed workers develop acute decreases in lung function when exposed to high concentrations of grain dust (doPico et al.,1983). There is limited information on the prevalence of cross-shift changes among farmers during harvest or dock workers loading and unloading grain (Chan-Yeung et al.,1992b).

2.3.3 Respiratory Conditions

There are six categories of grain-related respiratory conditions, or diseases: 1) allergic and non-allergic mucous membrane irritation (MMI), 2) acute febrile syndromes, such as organic dust toxic syndrome (ODTS), 3) asthma, 4) acute and chronic bronchitis, 5) acute and chronic hypersensitivity pneumonitis (HP), and 6) restrictive pulmonary disease (doPico,1992; Chan-Yeung et al.,1992b; Schenker et al.,1991; Richerson,1990; Dosman et al.,1987; Rylander,1986; Hutcheon et al.,1984). Each of these conditions is discussed below. A summary of the prevalence of each condition among grain handlers and farmers is presented in Table 2.6. Mechanisms of grain dust-induced disease are mentioned briefly here

as they relate to suspect etiological agents for each condition, and are discussed in further detail in Section 2.5.

Table 2.6 Prevalence of respiratory conditions associated with wheat dust exposure

Respiratory Condition	Grain Handlers		Farmers	
	Prevalence	References	Prevalence	References
Allergies/MMI	77-80%	6	20-57% 39%(swine)	5,15
Flu-like episodes (ODTS)	0-33% 0%(bakers)	1,6,20	5-35% 10-30% (swine)	5,15
Asthma	2.4-3.3% 5-7%(bakers)	16,19,22	3-15% 10-30%(swine)	3,7,11,13,17,21
Chronic bronchitis	10-57% 30%(bakers)	1,6,8,18,20	6-33% 12-70%(swine)	2,4-5,7,9-12, 22
Hypersensitivity pneumonitis (HP)	Not reported	---	0.02-15% 10-15%(swine)	5,14,19,21-22

References (Only the first author's name is provided for conciseness):
1-Chan-Yeung,1992a 2-Cormier,1991 3-Cuthbert,1979 4-Dalphin,1989 5-Donham,1989 6-doPico,1977 7-Dosman,1988 8-El Karim,1986 9-Holness,1987 10-Husman,1987 11-Iversen,1988 12-Leistikow,1989 13-Lefcoe,1974 14-Malmberg,1988a 15-Manfreda,1986 16-Prichard,1984 17-Senthiselvan,1992 18-Sheridan,1980 19-Terho,1990 20-Tse,1973 21-Warren,1987 22-Zejda,1993a

Mucous Membrane Irritation (MMI)

Mucous membrane irritation (MMI) consists of multiple symptoms, including cough, expectoration, sore throat, nasal stuffiness and irritation, rhinnorhea and catarrahl rhinitis, skin irritation, and eye irritation including acute and subacute conjunctivitis and keratitis (doPico,1994; Rylander,1994b; Hurst et al.,1990; Broder et al.,1979).

The mechanism of grain dust-related MMI is unknown and may involve either simple physical irritation and the inflammatory process, or the immunological or allergic process (doPico,1994; Richerson,1989). Components of grain dust with physically irritating properties include the husk, bran, endosperm, germ, trichomes, mites (grain itch), and silica (Hurst et. al.,1990; Hogan et al.,1986). The allergen responsible for allergic MMI has been difficult to identify and is likely to be more than one grain dust component. Different allergens are probably important to different individuals. For example, Marx et al. (1993) showed that Wisconsin dairy farmers were more likely than controls to have immunologic sensitivities to house dust, storage mites, grain smuts, *Cladosporium*, *Aspergillus*, and cattle. Several grain dust components have been identified as important allergens, such as durum wheat and the mite *Glycophagus destructor* (Jacobs et al.,1994; Chan-Yeung et al.,1992b). Storage mites appear to pose the greatest allergic threat to farmers who work in damp environments or humid crop storage areas (Iversen et al.,1990a; Terho et al.,1990; van Hage-Hamsten et al.,1987). Blainey et al. (1988) demonstrated a 60% prevalence of storage mite-specific IgE among European grain storage workers who complained of cough, wheezing or breathlessness. This high prevalence of storage mite reactivity was not observed among Canadian workers, suggesting that difference in storage practices may lead to greater antigenic exposure by European workers (Manfreda et al.,1989). Asthma, allergic rhinitis, and atopic eczema are closely related and possibly the result of the same disease mechanism as MMI (Evans,1993).

MMI is common among both grain elevator workers and farmers (Schenker et al.,1991; doPico et al.,1977). Up to 80% of grain elevator operators report skin irritation and other MMI symptoms (Hogan et al.,1986; doPico et al.,1984). MMI symptoms have been reported in up to 57% of farmers (Manfreda et al.,1986). Swine workers have a higher reported prevalence of MMI symptoms, especially burning or watering eyes, than vegetable and grain farmers, most likely due to the variety of irritant gases in their work environment (Carvalheiro et al.,1995; Donham et al.,1984).

Organic Dust Toxic Syndrome (Grain Fever)

Organic dust toxic syndrome (ODTS) is an acute respiratory condition characterized by fever, dry cough, headache, chest tightness, fatigue, aches, wheezing, chills, and other flu-like symptoms. Onset is usually within 4 to 12 hours of exposure to large amounts of respirable organic dust, a renewal of exposure after absence from a week or more of work, or an unusually heavy exposure at any time (NIOSH,1994; Williams et al.,1964). Symptoms persist from several hours to several days. The severity can necessitate at least one day of bedrest (Rask-Anderson,1989a). Typically, no radiographic abnormalities are noted, and pulmonary function remains approximately normal. Patients have normal breath sounds and arterial oxygen saturation (May et al.,1986). Often the only significant findings are fever and an elevated white blood cell count (Rask-Anderson,1989a). If obtained, bronchoalveolar

lavage (BAL) and lung biopsy reveal polymorphonuclear leukocytic alveolitis, bronchitis with no granulomata, and increased neutrophils (Von Essen et al.,1990a; Rask-Anderson,1989a). Lavage fluids produce large numbers of fungal spores and bacterial species (doPico,1992). The syndrome is short-lived and not associated with an long-term sequelae or chronic disability (Rylander,1986; Cotton,1982). Some workers experience reduced symptoms after prolonged periods of nonexposure, but not on recurrent exposures, suggesting development of tolerance (doPico,1986; Rylander,1986). ODTS is distinct from hypersensitivity pneumonitis (HP) in the lack of reactivity to farmer's lung antigen, BAL findings, and that most individuals who experience a high exposure become symptomatic (Marx et al.,1990; doPico,1986; May et al.,1986). Emanuel et al. (1989) found that the median age for farmers with ODTS was the fourth decade, while farmers with HP were in the sixth decade. ODTS is also distinct from silo-filler's disease, which is an acute reaction to oxides of nitrogen created in enclosed silo structures (Zwemer et al.,1992).

ODTS has been referred to by a multitude of names over the years. Since it was originally diagnosed among patients who had handled moldy hay or grain or opened silos, it was thought that the fungal components of grain caused the disease. It was therefore referred to as pulmonary mycotoxicosis or "silo unloaders' syndrome" (May et al.,1986; Emanuel et al.,1975). Because the febrile reaction was similar to the acute form of allergic alveolitis, others referred to the syndrome as grain fever, atypical farmers lung or precipitin-

negative farmers lung (Edwards et al,1974). In 1985, it was proposed that the syndrome be consistently referred to as ODTS to eliminate the confusion (doPico,1986).

While the mechanism of injury in ODTS is not known, proposed mechanisms include complement activation to produce C3, C4, and C5a, alveolar macrophage stimulation to produce IL-1, and macrophage release of cytokines. The results of biopsy and BAL suggest that the disease is secondary to an acute inflammatory response triggered by inhaled toxic dusts (Marx et al.,1990). One theory is that ODTS involves a Type III hypersensitivity reaction to fungal organisms (Morey,1989; Olenchock et al.,1990b; Cotton,1982). However, mycotoxins have not been consistently present in inhaled moldy material (Emanuel et al.,1989). The lack of correlation between history of ODTS and positive skin reactions, presence of precipitins to fungi, grain or grain dusts, and total IgE levels all argue against an immunologic mechanism, as does the fact that unexposed controls experience fever upon exposure (DoPico et al.,1982a and 1977; Warren et al.; 1987 and 1974). Another theory suggests that ODTS is a syndrome like metal fume fever and a foreign protein reaction might be involved (Von Essen et al.,1990a). Still others have suggested that ODTS is a nonspecific immune response to exposure to excess grain dust that results in an amplification of the number of immune cells and inflammatory mediators compared to a typical allergic response (Lewis et al.,1989).

While ODTS was initially attributed to mycotoxin, recent work suggests that mycotoxins do not cause a fever reaction (Emanuel et al.,1989; May et al.,1989). Instead, experimental data now suggests that bacterial endotoxins, mold glucans, tannin and tannin-like substances, and histamine present in organic dusts are all possible causative agents (Rylander,1994a; Olenchock et al.,1986; Skea et al.,1986; Warren et al.,1986). Human macrophages are sensitive to gram negative bacteria and endotoxin *in vitro* (Olenchock,1994; Reitschel,1992).

A link between endotoxin and byssinosis (mill fever) has been established, and recent data has strengthened the relationship between endotoxin and ODTS (Cosma et al.,1999; Von Essen et al.,1990a). Bacterial endotoxins are now believed to be responsible for the pathogenesis of ODTS (Popendorf et al.,1985).

ODTS occurs frequently among grain handlers. The incidence of flu-like episodes ranges from 0 to 33% among grain workers surveyed (Kleinfeld,1968). Because ODTS occurs only when the organic dust exposure is high, it is rarely reported in Vancouver grain terminals, where grain is partially cleaned. The prevalence of ODTS has been reported as from 5% to 35% in general farmers, with some estimates as high as 41% (NIOSH,1994; Warren et al.,1987). Farmers who work with swine and other animals have a similar incidence of ODTS as compared to farmers who work with grain and vegetable crops (Carvalheiro et al.,1995; Zejda et al.,1993a). In a survey of

6,267 farms in Sweden and Finland, respiratory symptoms consistent with ODTS were reported at a lifetime prevalence of 6% and two-year incidence of 1% (Rask-Anderson,1989a; Malmberg et al.,1988a; Terho et al.,1987a). Based on a study of 198 subjects with similar exposure to moldy grain, there do not appear to be any unique host factors which predispose an individual to this acute illness (Emanuel et al.,1989).

Asthma

It is well known that grain dust exposure may cause asthma and asthma-like syndromes (Zejda et al.,1993a; Rylander,1986). Definitions of asthma vary, but the hallmarks of occupational asthma include work-related bronchoconstriction, eosinophilia, abnormal spirometry, and specific and/or nonspecific airway hyperresponsiveness (Karol et al.,1994). Chest x-ray changes are typically not present (Rylander,1986). Some subjects demonstrate an allergic response, others a nonallergic response, and in some individuals, both forms of asthma may be concurrent (Fabbri et al.,1993; Bates et al.,1992; Enarson et al.,1988). Due to the high prevalence of atopy in the population, the severity of asthmatic reactions vary from simple increased airway inflammation to bronchial hyperreactivity to fatal hypoxia (Barnes,1993; Greenberger,1993; Shearer et al.,1993). If exposure continues after diagnosis, asthma persists and may even deteriorate as shown by increased airways hyperresponsiveness and chronic

airflow limitation (Fabbri et al.,1993). However, cessation of exposure is not always associated with improvement in symptomology (Fabbri et al.,1993).

Historically, occupational asthma was thought to arise from an immunological mechanism, the release of inflammatory chemicals from mast cells triggered by IgE and antigen reactions. However, allergy is present in only about 25% of adult asthmatics (Bigby et al.,1992). Several other proposed mechanisms for grain dust-induced asthmatic bronchoconstriction are particle irritation (perhaps involving complement mediation), induction by pharmacological agents, and the presence of inflammation (Broder, 1989; Rylander,1986). At present, the acute or immediate, Type I allergic asthmatic phase airway smooth muscle contraction and mucosal edema are thought to be caused by mast cell activation (Fabbri et al.,1993; Cockcroft,1989; Richerson,1989; Rylander,1986). The late asthmatic phase (Type III reaction) with increased airways hyperresponsiveness is thought to be caused by accumulation of inflammatory macrophage/lymphocyte cell activation and exudate in the airway walls and lumen (Fabbri et al.,1993; Busse et al.,1993a and 1993b). The role of mediators, such a prostaglandins, leukotrienes, and neutrophils have been investigated, but no clear evidence has been presented (Busse et al.,1993b; Rylander,1986).

The components of grain dust responsible for asthmatic reactions are unknown, but a variety have been implicated over the years: animal danders and proteins, general fungal spores and contaminants, storage mites,

pesticides, grain weevils, wheat hairs, and wheat rust (*Puccinia graminis*) (Lacey,1994b; Senthilsevan et al.,1992; Schenker et al.,1991; Merchant,1987; Warren et al.,1983; Weiner,1961; Jiminez et al.,1947; Duke,1935; Cadham,1924). However, because of the variability of grain dust composition, attempts to identify the specific allergens responsible for individual cases of grain dust-induced asthma have been difficult and only reported in a few cases.

The prevalence of asthma in the general adult population has been difficult to establish due to inconsistent definitions of the disease, lack of uniform diagnostic criteria, and physician diagnostic bias (Evans,1993; Dodge et al.,1980). The prevalence of occupational asthma, and grain dust asthma in particular, is even more difficult to estimate. Studies have typically reported prevalence rates by specific commodity, e.g. coffee bean, wood dust, tea, and mushroom production (Schenker et al.,1991). In addition, it is difficult to separate chronic bronchitis with reduced FEV_1 and wheezing from occupational asthma. Occupational asthma is thought to represent only about 2% of all asthma cases in the general U.S. population (May,1990). Smokers and nonsmokers seem to be affected to an equal degree (Rylander,1986).

Where bronchial hyperresponsiveness has been measured, asthma can be found in only a minority of symptomatic grain workers (Blainey et al.,1989). Chan-Yeung et al. (1993) found no difference between the prevalence of asthma among grain workers and a control group of civic workers (2.4 and

2.7%, respectively), but they attributed this to pre-employment screening, workers leaving the grain industry, and the difficulty of measuring asthma in cross-sectional surveys. The prevalence among grain handlers of symptoms associated with asthma, namely chest tightness, wheezing, dyspnea and airway obstruction ($FEV_1/FVC > 70\%$), ranges from 16 to 56% (doPico,1992; Tse et al.,1973; Kleinfeld et al.,1968). In many surveys of grain workers, especially those in North America, cough, sputum and chronic bronchitis are more common than wheeze or self-reported asthma (Dosman et al.,1980; Broder et al.,1979; doPico et al.,1977). Asthma has been reported to affect up to 7% of bakers (Bates et al.,1992).

Unlike grain workers, farmers may not have the same opportunity to select careers or switch jobs, and the prevalence of asthma is believed to be generally higher among farmers (Zejda et al., 1993a). Asthma has been reported in 15% of farmers in the Orkney Islands, with symptoms aggravated by exposure to hay, straw or grain dust in barns (Cuthbert et al.;1979); 12% in retired farmers and 30% in swine farmers, as compared to only 4% in non-farmers (Zejda et al.,1993a); 7.7% among 1,222 Danish farmers (Iversen et al.,1988); 8.8% among 1,072 Canadian workers, higher than firemen, chemical workers, or doctors (Lefcoe et al.,1974); 3.3% among 1500 farmers, based on data in the Finnish Register of Occupational Disease (Terho,1990); and 3% among Manitoba farmers (Warren et al.,1980). Dosman et al. (1987) reported that 27% of a mixed group of farmers had respiratory wheeze compared to 10%

of nonfarmers. In a cross-sectional questionnaire study of 1,634 adult men and women in Canada, Senthiselvan et al. (1993) determined the significant predictors for both asthma and wheezing to be grain farming and smoking. Age, level of education, and physical activity were not significant predictors for either asthma or wheezing.

Chronic Bronchitis

Probably the most common respiratory disease resulting from exposure to grain dust is chronic bronchitis and airways obstruction (Merchant,1987). The present generally accepted definition of chronic bronchitis is daily cough and sputum for at least three months of the year for two consecutive years (Morgan,1995). Chronic bronchitis is usually, but not always associated with dyspnea and decreased ventilatory capacity, as reflected by a lower FEV_1 with normal or slightly reduced FVC (Morgan,1995; Killburn,1986). Chronic bronchitis is further reflected by hypertrophy and hyperplasia of the bronchial mucous glands and goblet cells of the airways (Bates et al.,1992). Individuals with chronic bronchitis may develop substantial and disabling airway obstruction later in life (Chan-Yeung et al.,1985).

Chronic bronchitis is associated with multiple exposures related to vegetable dusts, as well as steel workers, coal miners, foundry men, textile workers, gold miners, and cement and bauxite workers (Morgan,1995; Merchant,1987).

Chronic bronchitis is a result of persistent inflammation of the airway epithelial, bronchial, and alveolar tissue (Cooper et al.,1986). Continued exposure results in increased adhesions, temporary airways collapse, loss of airway structure, reduced pulmonary function, and finally chronic obstructive pulmonary disease (Jennings et al.,1992). The excess in cough, expectoration, wheezing, and shortness of breath and reduced FEV_1 among grain handlers is consistent with the interpretation that long-term exposure to grain dust, rather than a specific agent, causes chronic bronchitis (Chan-Yeung et al.,1992b; Broder,1989; Dosman et al.,1980).

Chronic bronchitis has been reported in up to 35% of non-smoking grain handlers and 57% of smoking grain handlers (doPico,1992). Dosman et al. (1980) found a 23% prevalence of chronic bronchitis among nonsmoking grain workers as compared to 3% for nonsmoking control subjects. Numerous epidemiological studies have reported that from 16 to 83% of grain handlers have chronic productive cough. In an investigation of lifetime nonsmoking grain workers, Dosman et al. (1977) showed that the increased prevalence of chronic bronchitis and airflow obstruction was dose-related to grain dust exposure.

The prevalence of chronic bronchitis among farmers has been difficult to determine. Morgan et al. (1975) reported the prevalence of chronic bronchitis to be as high as 33% in mostly-smoking Welsh farmer populations. Iversen et al. (1988) found that 24% of 1,222 Danish farmers reported chronic bronchitis. In contrast,

Manfreda et al. (1989) found no association between mixed farming or grain farming and cough, phlegm, wheeze, or chronic bronchitis among 1,900 Manitoba farmers. Likewise, Husman et al. (1987) found no difference in the 3.5% prevalence of chronic bronchitis between Finnish farmers and non-farmers, but did report a three-fold higher incidence among farmers over a six-year study period. OSHA reports that 5.6 to 21% of non-smoking farmers have chronic bronchitis - three times the risk of non-smokers in other industries (OSHA,1994). Another study showed that chronic bronchitis occurred twice as often among nonsmoking, atopic farmers as among nonsmoking, nonatopic farmers (Terho,1987b). Among grain farmers, those who used a harvester with a sack loader were more likely to have chronic bronchitis (Vohlonen et al.,1987).

The association with chronic bronchitis appears to be greater for livestock farmers than grain farmers (Zejda et al.,1993b; doPico,1992; Cormier et al.,1991; Donham et al.,1984). For example, Heller et al. (1986) only observed an increased rate of chronic bronchitis among Welsh farmers engaged in dairy or silage work. The prevalence of chronic bronchitis among dairy farmers in France was 12% compared to 6% for a matched control group (Dalphin et al.,1993). In the dairy group, bronchitis was more common among nonsmokers and subjects over 40 years old, and associated with previous acute respiratory syndromes and farmer's lung, but not exposure.

Swine confinement workers have the highest prevalence of chronic bronchitis of any farm group (Notkola et al.,1992, Vohlonen et al.,1987). Dosman et al. (1988) reported that 13% of swine farmers had chronic bronchitis as compared to 8% of control farmers. Another study of 488 Canadian swine building workers showed that 18% had chronic bronchitis as compared to 12% for nonfarming controls subjects (Cormier et al.,1991). Among 1,222 Danish farmers, the prevalence of bronchitis was twice as high among pig farmers (Iversen et al.,1988). In addition, swine workers who spent more than three hours per day in swine buildings had a higher prevalence of chronic bronchitis (21% versus 13%) than workers who spent less time working with swine (Donham et al.,1989). This leads to the conclusion that the complex mixture of gases and dusts found in confinement farming leads to occupational asthma and chronic bronchitis (Donham et al.,1989 and 1986).

Hypersensitivity Pneumonitis (Farmer's Lung)

Hypersensitivity pneumonitis (HP), or extrinsic allergic alveolitis, is a term applied to a group of allergic lung diseases that arise from sensitization and recurrent exposure in a variety of industrial and agricultural settings (Bates et al.,1992). Three clinical manifestations of HP have been described: acute, subacute, and chronic (Fuller,1953). The signs and symptoms of acute HP are similar to acute ODTS episodes, including fever, malaise, rigors, myalgia, dyspnea, and rales that occur four to eight hours after exposure and subside in 3 to 5 days (Schenker et al.,1991; Malmberg et al.,1988a; Davies et al.,1976).

In the subacute disease, symptoms are less severe and protracted over a 10 to 14 day period (Gurney,1992). Unlike ODTS, diffuse, nodular and patchy infiltrates may be seen on x-ray (Gurney et al.,1991; Molina et al.,1989). Pulmonary function reveals a restrictive pattern for the acute disease, with decreases in tidal volume, lung capacity, diffusion capacity, and arterial oxygenation. In chronic disease, fatigue, dyspnea, anorexia, and weight loss are present (Gurney,1992). The symptoms persist and lead to interstitial pulmonary fibrosis and scarring with parenchymal contraction or honeycombing, scarring, and chronic obstructive respiratory insufficiency (Zejda,1993a; Fink,1993: Gurney,1992).

HP is a recurrent disease that may occur with minor exposures to the inciting agent. Darke et al. (1976) noted that after exposure was discontinued, even a subsequent light exposure to grain dust provoked symptoms that usually recurred during subsequent harvests. A case report of a dairy farmer developing a severe attack following workplace challenge led to the recommendation that provocation tests not be used for diagnosis of HP (Kokkarinen et al.,1992). However, recurrent episodes do not seem to be required for progression to pulmonary fibrosis. While the occurrence of an acute episode of ODTS presents the opportunity for sensitization, there is no evidence that ODTS leads to HP (Emanuel et al.,1989).

The acute HP response can often be controlled with bronchodilators, but the late response is resistant (Fink,1993). Other treatments, such as corticosteroids, are effective for the acute disease, but slow pulmonary function recovery (Kokkarinen et al.,1993; Schenker et al.,1991). While workers diagnosed with HP are advised to avoid further exposure, exposure usually continues after diagnosis with recurrence of symptoms (Schenker et al.,1991; Braun et al.,1979). Unfortunately, HP patients who continue to work are also the most likely agricultural workers to have abnormal pulmonary function, chest radiographs, and chronic bronchitis (Braun et al.,1979).

HP was first reported by Campbell (1932) as a bronchomycosis among farmers exposed to extremely moldy hay. Several other physicians published similar case reports over the next few years, attributing the disease to *Aspergillus, Monilia*, or possibly grass particles (Fuller,1953; Tornell,1946; Fawcitt,1936a). Dickie and Rankin (1958) described the disease as an acute granulomatous interstitial pneumonitis observed in farmers who handled moldy grains or tobacco. Thus, the disease has often been referred to as "farmer's lung." It was postulated that the cause was sensitization on repeated exposure to molds or products of molds. The antigens of *Micropolyspora faeni* and *Thermoactinomyces vulgaris*, present in moldy hay and silage, have been shown via inhalation challenge to induce the disease (Fink,1993; Darke,1976; Williams et al.,1964). Kotimaa et al. (1987) found that the total mean fungal spore population was significantly higher on the farms of HP patients than on reference

farms of their sibling. More recent investigators have attributed HP to agricultural exposure to fungi and bacteria, animal danders and proteins, and insects (Merchant,1987). Glucans in mold cell walls are known to cause granulomas and have also been implicated in HP (Rylander,1994a).

While moldy hay and silage are often implicated as the relevant HP exposure, malt, sugar cane, wood, cheese, mushroom, and aviary workers have also experienced the disease (Fink,1993; doPico,1992; Schenker et al.,1991). In fact, the original classification of these diseases was based upon the source of dust exposure, e.g. farmer's lung, maple bark disease, malt worker's lung, bagassosis, suberosis, etc. (Towey,1932). The discovery of common cellular pathophysiologic mechanisms has led investigators to rename these disorders as "organic dust diseases" (Schenker et al.,1991; Flaherty et al.,1976). In some instances, there has been no clear association between HP and contact with organic dusts, e.g. in the case where four of 27 office workers developed HP due to contamination of an air conditioner with a thermophilic actinomycete (Banaszak et al.,1970). All these diseases, including farmer's lung, are now referred to as hypersensitivity pneumonitis (HP). Other industrial compounds, such as nickel, welding fumes, trimetallic anhydride and epoxy resins, have also been associated with HP (Fink,1993; Patterson et al.,1991).

HP is a particular type of acute and chronic inflammation in the peripheral airways and alveoli (Rylander,1986). Early on, the mechanism of HP was considered to be

a Type III antibody-antigen type reaction, and blood precipitins to the antigen were sought (Rylander,1986). However, only the IgA and IgM (not IgE) antibodies *to M. faeni* discriminated between farmer's lung patients and control subject (Patterson et al.,1976). Likewise, Ojanen (1992) showed that the presence of IgG antibodies to *T. vulgaris* and IgA antibodies to *A. fumigatus* correctly grouped 94% of Finnish farmer's lung cases, noting that selection of microbes for serological diagnosis should be determined locally.

Von Essen et al. (1990b) showed that nonsmoking, nonasthmatic farmers had an elevated bronchitis index (elevated bronchial neutrophils and alveolar lymphocytes) during harvest that fell significantly after harvest. Alveolar lymphocytes and macrophages were elevated in the farmers' post-harvest BAL fluids, reflecting alveolar and airways inflammation during harvest that may contribute to chronic alveolitis. Vogelmeier et al. (1993) showed that neutrophils in patients with farmer's lung are primed for an enhanced respiratory burst as compared to unexposed volunteers. While HP is strongly associated with an immunological response to molds, direct stimulation of cytokines may play a role as well (Michel et al.,1994).

To date, radiologic findings, pulmonary function, BAL findings, lung biopsy, and immune function (skin tests and precipitins) have been considered useful to document exposure, but not very useful in the prediction of HP (LaLancette et al.,1993; Fink,1993; doPico,1992; Marx et al.,1990 and 1978; Flaherty,1982;

Patterson et al.,1976; Warren et al.,1974). In particular, dairy and other agricultural workers may have BAL lymphocytosis without clinical symptoms, and HP symptoms without lung involvement have been reported (Cormier et al,1993 and 1984). It is generally agreed that antibodies are gross indicators of exposure, not the causative mechanism. The pathogenesis of HP in farmers is now thought to involve a Type III antigen-antibody reaction for the acute symptoms, and a Type IV cell-mediated delayed hypersensitivity response by effector T-lymphocytes, as well as nonimmunologic reactions (Pepys,1993; Gurney,1992; Richerson,1989). Fibrosis, a key component in late stage HP, is related to activation of macrophages, which in turn activate fibroblasts to secrete collagen (Fink,1993; Rylander,1986). Several immune reactions involving IgG_4, which is found only in the lung and binds to mast cells similar to IgE, have also been proposed (Flaherty,1982).

It has been suggested that the thermophilic organisms which may cause farmer's lung upon exposure to damp hay and grain, may also be initiators of HP in grain handlers (Chan-Yeung,1985). However, HP has not been reported among grain worker populations, apparently due to the generally lower moisture and microbial numbers in elevator dust (Kryda et al.,1993; Chan-Yeung et al.,1992b; Warren,1989). These organisms are rarely found in airborne grain dust (Dutkiewicz et al., 1978a). Furthermore, precipitins to *M. faeni* have not been commonly found among grain handlers or swine building workers (Cormier et al.,1991; Warren et al.,1974; Darke et al.,1976; Dutkiewicz et al.,1978b).

Blackadder (1980) did identify extrinsic allergic alveolitis among 5.2% of a Scottish malt worker population. Because precipitating antibodies against *A. clavatus,* but not *A. fumigatus,* were positively correlated with the clinical findings, he concluded that a Type III allergic reaction to *A. clavatus* was responsible for the alveolitis in malt workers.

The prevalence of HP among various farming populations is difficult to estimate. It has been estimated at between 0.1 and 15% of farmers at risk, with climatic conditions, agricultural practices, and type of farming thought to have significant effects (Zejda,1993a; Marx et al.,1990). In addition, seasonal variation in cases is observed, with an autumn and winter following a wet summer having the highest case incidence (Chan-Yeung et al.,1992b, Molina et al.,1989). A survey of farmers in Orkney, Scotland indicated that 2.3% to 8.6% had farmer's lung (Grant et al.,1972). In Finland, the prevalence of allergic alveolitis among farmers was found to be 0.2 to 9% (Malmberg et al.,1988a and 1988b). The prevalence among dairy farmers has been estimated between 0.04 and 0.3% (Bates et al.,1992). Among poultry farmers, the prevalence ranges from 3 to 15% (Bates et al.,1992). A cross-sectional study of 471 farmers and dairy producers in Wisconsin showed that 3.9% had farmer's lung based on questionnaire response (Madsen et al.,1976). In Scandinavia, the incidence rate of HP was reported as 2 to 4.4 per 10,000 farmers, correlated with measures of daily rainfall, number of days with rain fall, and amount of time spent in cow houses (doPico,1992; Terho et al.,1987a). Among grain farmers, those who used grain

driers with unheated air were more likely to have farmer's lung (Vohlonen et al.,1987). In Northern Ireland, the prevalence of HP was estimated at 0.5% based on antibody presence, delayed symptoms, and restricted pulmonary physiology, but 4.9% reported previous diagnosis of farmer's lung (Stanford et al.,1990). The disease is more common among nonsmokers (Warren et al.,1977). Because of all the factors involved, HP is more prevalent in England and Scandinavia than the United States and Canada, and among livestock farmers than grain farmers (Gurney,1992; Vohlonen et al.,1987).

The disparity in reported prevalence of HP likely arises from the inability of questionnaires to distinguish between HP and ODTS. In general, HP is considered to be a rare disease (Von Essen et al.,1990a). The prevalence seems to be dropping due to education and improved work practices (Merchant,1987).

The concentration of various microorganisms and duration of exposure required for development of HP have not been determined. Colorado wheat farmers are not expected to have increased HP due to dry wheat conditions during harvest, storage, and handling, and limited duration of exposure (Beard,1994).

Restrictive (Interstitial) Pulmonary Disease

The term restrictive disease usually means reduced lung capacity, or decreased FVC with little change in FEV_1/FVC. Restrictive diseases associated with organic dust exposure include the chronic effects of HP discussed above, fibrosis due to silica exposure, and interstitial lung disease due to pesticide exposure, primarily paraqaut (Schenker et al.,1991). The latter two have not been well studied among grain and other agricultural workers. This is thought to be due to the low incidence of reported disease, the difficulty in diagnosis, the lack of a recognized link between exposure and disease, and the migrant nature of some exposed populations.

The reports of restrictive lung disease based on radiological evidence provide mixed results. However, a survey of 3000 grain workers revealed no radiographic evidence of silicosis (Cotton et al.,1983). Only one case of silicosis from exposure to grain dust has been reported (Heatley et al.,1944). An early report of 26 cases of pneumoconiosis among dock workers dealing with grains and seeds could possibly have been caused by exposure to minerals and metals to which they were also exposed, rather than grain dust (Dunner et al.,1946). Pulmonary fibrosis and heavy deposits of silica have been found by radiography or autopsy in several career farmers (Schenker et al.,1991; Abraham,1982).

Smith et al. (1941) observed pulmonary fibrosis on x-ray in 3% of 216 New York grain handlers. Upon examination of chest x-rays, Kavoussi (1974) found rounded or irregular opacities in one or both lungs of 13% of 465 grain handlers in Teheran, the severity of which was related to duration of employment. Kleinfeld et al. (1968) found that the incidence of pulmonary fibrosis in grain handlers was 2.3% as compared to 0.9% and 1.3% found in large groups of carpet mill and wood workers, respectively. In addition, they noted that roentgenographic changes seen in the grain handlers were not characteristic of silicosis. Grain dust had previously been shown induce interstitial granulomata in the absence of free silica in rabbits within five days after exposure (Williams et al.,1964).

Radiologic studies of agricultural populations in Bulgaria, Russia, and Holland indicate restrictive disease. However, the large epidemiological studies in Canada and the U.S. have found no radiographic evidence of diffuse interstitial or alveolar lung disease among grain and agricultural workers (Chan-Yeung et al.,1992a; Kennedy et al.,1991; doPico et al.,1984 and 1977; Becklake et al.,1980; Broder et al.,1979).

Lung function tests in recent studies indicate that organic dusts may pose a risk for restrictive disease among both grain and agricultural workers. Cross-sectional epidemiologic studies have shown restrictive or mixed restrictive/obstructive pulmonary function among Canadian farmers, swine producers, and grain

handlers, and Californian grape producers compared to controls (Chan-Yeung et al.,1992a; Gamsky et al.,1992: Dosman et al.,1987 and 1988; Hutcheon et al.,1984). Cross-shift and other short-term longitudinal studies have found reduced FVC and restrictive lung disease in grain handlers (Hutcheon et al.,1984; doPico et al.,1983; Chan-Yeung et al.,1980). McDuffie et al. (1991) reported 12% obstructive, 5% restrictive, and 2.4% mixed restrictive/obstructive defects among 3,098 male grain handlers in Canada based on spirometry. Dosman et al. (1987) found slightly reduced FVC and FEV_1 among 1800 Canadian farmers as compared to non-farmers. However, a survey of chronic respiratory disorders related to farming suggested impairment of lung function was not due to grain dust exposure, once smoking and age had been taken into account (Manfreda et al.,1989). Dosman et al. (1988) found a mixed restrictive/obstructive defect in swine farmers, with slight reductions in FVC and FEV_1 observed among former farmers compared to controls were attributed to hog confinement work, rather than grain dust exposure.

2.4 Risk Factors for Grain Dust-Induced Disease

There appear to be several predisposing host risk factors that may effect the frequency, type, and progression of grain dust-induced disease among exposed workers. The relative importance of these factors depends on the clinical syndrome. For acute responses, such as grain fever and cross-shift lung function changes, the degree of exposure seem to be more important than

host factors. For chronic disease, such as bronchitis and asthma, both environmental and host factors are important.

2.4.1 Smoking

Smoking is the strongest host risk factor contributing to the occurrence of respiratory symptoms among grain-exposed populations. This is because most of the symptoms associated with grain exposure are also positively associated with smoking (Huy et al.,1991). Most studies show that 1) nonsmoking grain and agricultural workers have a higher rate of respiratory symptoms, chronic bronchitis, and reduced pulmonary function compared to unexposed, nonsmoking controls (doPico et al.,1992; Chan-Yeung et al.,1992b; Dosman et al.,1980), and 2) smoking grain and agricultural workers have a higher rate of respiratory symptoms and reduced pulmonary function compared to nonsmoking grain workers (Zejda et al.,1993a; Huy et al.,1991; Manfreda et al.,1986; Herbert et al.,1981; doPico et al.,1977; Warren et al.,1974 and 1987; Tse et al.,1973; Kleinfeld et al.,1968; Williams et al.,1964).

A few studies have presented somewhat different findings. Becklake (1980b) found that while nonsmoking grain workers had fewer respiratory symptoms, their lung function was no better than a comparable group of smoking grain workers. Corey et al. (1982) did not find a difference in acute symptoms between smoking and nonsmoking grain elevator workers. Herbert et al. (1981)

found no difference in prevalence of symptoms or spirometric results between nonsmoking grain handlers and office workers matched by age, but they did find higher levels of reported dyspnea and lower FEV_1/FVC for smoking grain handlers as opposed to smoking office workers. Smoking grain workers and controls reported the same elevated frequency of chronic cough (64%) (Dosman et al.,1980). Manfreda et al. (1986) showed that grain fever was less common among smoking Manitoba farmers, although respiratory symptoms were increased among the smoking farmers.

Most studies have found the effects of grain dust and smoking among grain handlers to be additive (Menkes,1989; doPico et al.,1984; Chan-Yeung et al.,1980; Broder et al.,1979). Cotton et al. (1983) found that the combined effects of smoking and grain dust exposure on peripheral airways tests were additive except in the least exposed group of young cereal grain workers (five years or less) where a synergistic effect was observed. Interestingly, doPico et al. (1977) reported that the odds of developing respiratory symptoms from grain dust were independent of, and usually greater than, the odds of developing them from smoking.

A synergistic effect between grain exposure and smoking on lung dysfunction has also been reported among grain workers (Herbert et al.,1981; Dosman,1977). Sheridan et al. (1980) showed that smoking workers had a lower $FEF_{25-75\%}$ than nonsmoking workers and smoking controls, suggesting a

greater effect on airways obstruction among smoking workers. Regardless of smoking habits, airway obstruction, as measured by lowered FEV_1/FVC, was more prevalent among grain workers (doPico et al.,1984). Regardless of smoking habits, an annual decrease in FEV_1 was observed among 587 grain workers over age 50 (Chan-Yeung et al,1981). The annual decline in $FEF_{25-75\%}$ observed among grain workers over age 50 was significantly greater for the nonsmoking grain workers as compared to the nonsmoking controls. Chen et al. (1991) showed that the combined effect of smoking and grain exposure on lung function and the prevalence of chronic bronchitis was additive in women, but not men.

While many studies suggest that the effects of grain dust and cigarette smoking are synergistic, this has not been confirmed (Gandevia et al.,1980; Chan-Yeung et al.,1980). Such a synergism could be the result of grain induced enhancement of airway obstruction, or cigarettes could make the airways more susceptible to airway obstruction by grain dust (Manfreda et al.,1989). It is known that cigarette smoking retards clearance of particles from the lung, effectively increasing exposure duration in smoking workers. Confusing the issue is the fact that particle penetration into the airways may be less in smokers than in nonsmokers (Becklake et al.,1980b).

The effect of smoking on respiratory disease among farmers is more difficult to assess. Warren (1977) showed that HP was less common among smoking

grain workers. DoPico et al. (1984) found that the odds of chronic bronchitis and wheezing at work were increased 4.4-fold and 4.8-fold, respectively, by grain handling and 2.9-fold and 1.9-fold by smoking. In contrast, higher levels of chronic bronchitis are seen in nonsmoking farmers as compared to smoking farmers (Dalphin et al.,1989). This may be due to overestimation of smoking in farmers, who smoke in the open air and often do not inhale the smoke (Dalphin et al.,1989). It may also be due to confounding by farmer's lung disease, which is closely associated with chronic bronchitis and more common in nonsmokers (Dalphin et al.,1989; Warren,1977). Both dairy and swine farmers with respiratory symptoms had a greater number of smokers as compared to respective farming groups without disease (Zejda et al.,1993a).

Case control studies have shown that both smoking and grain dust exposure, taken separately appear to be associated with a similar decline in lung function. The large number of studies of different design and populations that support this conclusion provides strong evidence of an additive effect. Since airway obstruction appears to be detectable at the small airways, the target site for inhaled grain dust may be similar to cigarette smoke. Thus, advanced obstructive disease in grain handlers may be indistinguishable from chronic obstructive disease found in smokers (Dosman,1977).

2.4.2 Atopy

Another host factor that may be of importance to grain dust-induced disease, especially asthma, is atopy (Zejda et al.,1992). Atopy is generally defined as the hereditary tendency for an individual to mount an IgE response of mucous membranes to low doses of common allergens (doPico,1986). The most common method of assessing atopic status is by one or more skin-prick test reactions to common environmental allergens. About 30% of the general population is atopic (Evans,1993).

Many studies show a correlation between grain exposure and positive skin tests. Immediate cutaneous sensitivity to grain extracts and fungal contaminants has been associated with symptoms (doPico et al.,1977; Warren et al.,1974). In Australian seasonal grain handlers, atopic subjects are more likely to develop symptoms (Gandevia,1980). Most grain farmers with asthma caused by storage mites are atopic (Blainey et al.,1989; Cuthbert et al.,1979). Similarly, hypersensitivity reactions to skin tests are more common among symptomatic workers (doPico et al.,1977; Tse et al.,1980; Warren et al.,1974).

Conversely, other studies have not found a relationship between skin tests and symptoms (Becklake et al.,1980b; Gerrard et al.;1979). Airways response to durum wheat challenge appeared to be independent of atopic status (doPico et al.,1981). Herbert et al. (1981) showed that 18% of workers were atopic with no

lung dysfunction, while only 3% of workers had positive reactions to barley, oat or wheat dust extract. DoPico et al. (1979) showed a correlation between skin reactivity and wheezing, but not other respiratory symptoms, among grain workers. Atopy has not been shown to correlate with longitudinal decline in lung function (Tabona et al.,1984; Pollock et al.,1980).

Most researchers have not found a relationship between respiratory symptoms or past history of grain fever and other immunological tests, including gamma-globulins, precipitins to grain dust, immunological blood tests for specific antibodies, e.g. RAST and ELISA, and family history of allergy and asthma (Manfreda et al.,1986; Evans,1993; Broder et al.,1983; Herbert et al.,1981; Chan-Yeung et al.,1979; doPico et al.,1977; Warren et al.,1974; Tse et al.,1973). The serum precipitins provide evidence of exposure to inhaled organic dusts, but precipitin-negative individuals may exhibit clinical symptoms (doPico et al.,1976).

Some cross-sectional surveys have even shown a lower prevalence of atopy, as well as a lower atopic family incidence for asthma, in grain workers than controls (doPico et al.,1977; Broder et al.,1979; Gerrard et al.,1979). This could be an effect of pre-employment screening or a personal self-selection ("healthy worker" effect). Grain workers have a lower prevalence of positive skin tests three months after hire than controls, and this difference increases with length of employment (Dosman et al.,1991). Individuals who had left employment between the 1977 and 1980 health surveys were younger, had a shorter

employment duration, and reported a greater prevalence of cough, eye irritation, and shortness of breath than the grain workers who remained employed, after adjustment for age, pack-years of smoking, and duration of employment. No differences were found between the civic workers who left employment and those who remained (Broder et al.,1985). While the population seemed stable, it could not be determined whether pre-employment selection occurred, i.e. whether individuals at risk avoided the industry (Herbert et al.,1981). Since grain dust exposure is estimated at a total of less than 60 days per year for the average farmer, it had been suggested the "healthy worker" effect may not be a confounding factor in farming studies (Chan-Yeung,1992b).

Blaski et al. (1996a) compared spirometric change and BAL measures of lower respiratory tract inflammation (TNF-alpha, IL-1, IL-6, IL-8) between nonsmoking atopic and nonatopic subjects. Both groups developed significant airflow obstruction and inflammation following inhalation of corn dust extract. Importantly, the magnitude of the measures did not differ significantly between atopic and nonatopic groups. They concluded that atopic status plays, at most, a minor role in development of grain dust-induced disease. The general disparity between positive skin tests and symptoms, including asthma, has led most researchers to conclude that atopy is not a meaningful factor in diagnosis of disease.

2.4.3 Nonspecific Bronchial Hyperresponsiveness

Nonspecific bronchial hyperresponsiveness (NSBH) may be a host factor in grain dust-induced disease. In a carefully designed study, Chan-Yeung et al. (1979 and 1980) showed that both NSBH (metacholine challenge) and specific bronchial hyperresponsiveness (grain dust challenge) were increased among grain workers who reported respiratory symptoms. Gerrard et al. (1979) found that a group of lifetime nonsmoking grain handlers matched for age with nonsmoking control subjects of similar lung function had a greater bronchial response to histamine. Cotton (1982) has suggested that the greater incidence of cough and wheezing in smoking workers compared with unexposed workers may also be due to greater increased airway reactivity. Swine workers were not found to be more hyperresponsive on metacholine challenge than a reference group of office workers (LaLancette et al.,1992).

Some studies have shown that grain workers with NSBH have a greater decline in lung function during follow-up studies. During a six-year follow-up, Tabona et al. (1984) showed that NSBH was a predictor of longitudinal decline in FEV_1. However, they did not test for NSBH until the follow-up examination and thus did not have baseline information. Enarson et al. (1985b) found that FEV_1, atopy, presence of symptoms, and cumulative grain dust exposure were all important predictors of NSBH. In a subset of these workers studied over six years, those with NSBH at the onset had a greater decline in lung function as

compared to those without NSBH (Enarson et al.,1988). However, the number of subjects was relatively small by the end of the follow-up period to draw any meaningful conclusions.

Some researchers argue that repeated exposure to grain dust increases airway responsiveness, eventually leading to chronic obstruction (Dales et al.,1988). However, several longitudinal studies did not observe a seasonal change in airway responsiveness among grain handlers (Dales et al.,1988; James et al.,1986). Bronchial responsiveness did not predict long term decline in FEV_1 in Canadian grain handlers (doPico et al.,1977).

It is not clear whether bronchial hyperreactivity is a pre-disposing factor or acquired after chronic exposure to grain dust (Flaherty,1982). It appears to be a secondary phenomenon which demonstrates, rather than predicts, whether a given worker will experience pulmonary effects from grain dust (Broder,1989).

2.4.4 Alpha-1-Antitrypsin

Intermediate deficiency of alpha-1-antitrypsin, or MZ phenotype, occurs in about three percent of the population. Deficiency of this serum protease inhibitor predisposes a person to chronic obstructive lung disease, but there is considerable debate about whether the intermediate deficiency has the same effect (Chan-Yeung et al.,1992b). Horne et al. (1980) first suggested that grain

handlers with the MZ phenotype might have a greater risk of lung function abnormalities when they found that 2 MZ phenotypes among 17 grain workers had been compensated for chronic obstructive pulmonary disability. This represented a five-fold risk over what would be expected based on the frequency of MZ phenotypes in the population. In an earlier larger study, doPico et al. (1977) did not observe this relationship. Likewise, Chan-Yeung et al. (1978) did not see a relationship between the MZ heterozygotes and pulmonary disease among 1,138 men working in sawmills and grain elevators. At this time, it is unknown whether the MZ phenotype is an important risk factor for grain dust-related disease.

2.4.5 Ethnicity

Horne et al. (1989) suggested that ethnicity may be a significant factor in the development of airflow obstruction in grain handlers. They found a significantly greater prevalence of airflow obstruction among Canadian grain workers of British ancestry as compared to workers of German or eastern European ancestry. Age and smoking pack-years were also significantly associated. The authors had no information about diet or other factors on these results. Based on one report, it is unclear whether ethnicity is an important risk factor for grain workers.

2.5 Mechanisms Associated with Adverse Pulmonary Effects

The mechanism by which grain dust, or one or more of its constituents, exerts acute and chronic respiratory effects is unknown. The respiratory tract can respond to grain dusts via several mechanisms, including acute and chronic immunological and inflammatory responses, or a combination of these (Rylander,1986). In addition, these may be accompanied by increased airways hypersensitivity (Rylander,1986). At the alveolar level, the macrophage is the primary target cell (Rylander,1986). The macrophages phagocytize particles deposited in the lung, and digest them, transport them out of the lung, or present them to immune competent cells, which later form antibodies against the particle (Rylander,1986). The macrophages also secrete chemotactic substances, which recruits neutrophils and platelets (Rylander,1986). These various cells secrete mediators that initiate different parts of the inflammatory response. The classical mediator is histamine, which is released from mast cells and platelets, but there a host of other mediators, including leukotrienes, cyclooxygenases, and lipooxygenases (Rylander,1986). The mediators serve as chemoattractants and activators of other cells (Rylander,1994a). The different mediators and cell types can act in series or groups and, thus, it is unlikely any effect is induced by one mechanism or one cytokine. In addition, there is evidence that different components of grain dust are responsible for asthma, febrile reactions, and bronchitis (Donham, 1986a). As discussed above, other risk factors such as smoking, atopy, and duration of exposure may play a role.

Cell and animal modeling, as well as human studies of specific mechanisms have helped to isolate and study the effects of the possible causative and modulating factors (Karol et al.,1994). The major mechanisms that have been studied are discussed below.

2.5.1 Simple Irritation

Because only isolated instances of grain dust-related pulmonary effects were explained by allergy, Broder (1989) proposed that an irritant effect was plausible. Direct, nonspecific mechanical irritation by dust particles results in hyperemia and watery discharge from cells (Hurst et al.,1990). When the particles cannot be easily eliminated by the normal inflammatory response, damage may be done to the lung tissue, eroding epithelial cells and exposing sensory nerve endings, and finally increasing airways responsiveness and lowering the threshold for adverse effect (Busse et al.,1993b; Bigby et al.,1992). This lowered threshold may play a role in exacerbating the physical irritation and resultant inflammatory process involved in the allergic reaction to grain dust (doPico,1992; Hurst et al.,1990).

Tse et al. (1980) suggested that grain dust could induce airway obstruction by stimulating the irritant receptors in the bronchial airways, i.e. in addition to an allergenic response. They suggested that chronic stimulation could lead to the pathologic changes that are the basis for chronic grain dust-induced diseases. Other have agreed with this mechanism, noting that nasal and eye irritation are dose-dependent and appear to be non-specific response to dusts

(Flaherty,1982). However, some dusts are more irritating than others. Durum wheat, barley and rye have been reported to be the most irritating (doPico et al.,1980). In addition, it has been suggested that irritation of the irritant receptors by grain dust can elicit a reflex bronchoconstriction (Flaherty,1982). The initial bronchoconstriction then lowers the threshold for stimulation, thus augmenting and perpetuating the response. It is unkown how grain dust gains access to the irritant receptors.

2.5.2 Allergenic/immunologic Response

The immunologic or allergic response to foreign particles in the lung involves interaction between cell-bound IgE and an allergen, which culminates in the release of mediators from mast cells and induction of smooth muscle contraction (Flaherty,1982). Immediate skin tests are often used to evoke IgE-mediated allergic reactions, while serum precipitating antibodies are used to test for previous exposure and sensitization to a specific allergen (Flaherty,1982). Variability in study results can arise from lack of standardization of extraction and testing techniques, as well as antigen source and preparation.

Grain, either as itself or as flour, and its constituents are well known inducers of IgE-mediated sensitivity reactions. DoPico et al. (1981) found immediate or isolated late responses to durum wheat extract challenge in 5 of 11 grain

workers, while none reacted to insect or mite extract, concluding that the wheat itself caused the asthmatic reaction. Chan-Yeung et al. (1979) found either immediate or dual asthmatic responses for 6 of 22 grain workers, but no control subjects, challenged with grain dust extract. However, airflow obstruction may result from this mechanism in some, but not all workers, since inhalation challenge-induced allergic reactions are observed in individuals with negative skin reaction (doPico et al.,1981; Chan-Yeung et al.,1979). This could be due to the fact that the allergic or asthmatic response is manifested by the local immune system in the lung (Flaherty,1982).

Precipitating antibodies against whole grains are rare among grain workers (Chan-Yeung et al.,1980; Dutkiewicz et al.,1978b; Tse et al.,1980). Lewis et al. (1986) found that the prevalence of serum IgE antibodies to grain dust extract antigens was higher than IgE antibodies to whole grain extract antigens for 175 grain terminal workers, and that IgE antibodies to grain dust antigens by RAST correlated well with skin test results. No association was found between allergic symptoms and precipitins to wheat or grain dust or to positive skin tests for molds among flour mill workers in Sudan (Fakhri,1992). These results imply that either some non-grain component of the dust is responsible for allergic reaction, or a non-allergic mechanism is responsible for the effect.

Baker's asthma appears to be an allergy to inhalation of cereal flours. Barber et al. (1989) isolated a barley salt-soluble protein as a major IgE-binding component of

sera from baker's asthma patients. Similarly, wheat salt-soluble proteins are IgE-binding components in these same sera (Gomez et al.,1990). A substantial fraction of the salt-soluble proteins in wheat and barley endosperms are represented by the single family of alpha-amylase/trypsin inhibitors (Barber et al.,1989). Of 12 bakers with due to sensitization, 10 were sensitized to fungal amylase (Smith et al.,1996). However, sensitization to flour is low (about 1 in 1,000) among bakers (Smith et al.,1996).

It has been proposed that an allergic reaction to fungi may be responsible for many of the respiratory responses to grain dust exposure. However, studies of allergic reaction to fungi in grain workers have reported inconsistent findings. As with whole grain extracts, precipitating antibodies against fungal components are rare among grain workers (Chan-Yeung et al.,1980; Dutkiewicz et al.,1978b; Tse et al.,1980). Tse et al. (1980) found that some but not all of 40 grain workers had a positive skin reaction to fungal extracts. These same workers had a higher prevalence of symptoms when exposed to grain dust than when exposed to molds. These results showed that fungal antigens do not represent the major allergenic component of grain dust.

When tested by crossed-immunoelectrophoretic techniques, grain dust from five Vancouver grain elevators demonstrated a significant number of shared antigenic determinants with cotton dust extract and five fungal extracts (O'Neil et al.,1988). The researchers concluded that the antigenic properties of grain and cotton dust

are due to fungal organisms. They suggested that previous attempts to detect immune reactivity in grain workers by skin testing may not have been effective due to the use of commercial extracts of fungal contaminants.

While the mechanism of grain dust asthma is unknown, there is evidence that allergy is important (Salvaggio et al.,1986a). Immediate and/or delayed asthmatic reactions have been demonstrated following provocative inhalation challenge with grain dust extracts (Cotton et al.,1983). The immediate asthmatic reaction following grain dust challenge has the same onset timing, maximum reaction and recovery timing as allergen-induced bronchoconstriction (Chan-Yeung et al.,1979). The inhibition of the immediate reaction by disodium eromglycate and the late reaction by beclomethasone also point in favor of this hypothesis. Serum precipitins have been demonstrated in asthmatic grain workers, but these do not correlate with severity of disease (Tse et al.,1973).

Asthma and allergic rhinitis among grain handlers have been associated with allergy to storage mites present in grain dusts (Blainey et al.,1989; doPico et al.,1982b; Cuthbert et al.,1979; Ingram et al.,1979; Warren, et al.,1974). Davies et al. (1976) attributed recurrent nocturnal attacks of asthma in a grain handler to allergy to the storage mite, *Glycyphagus destructor*. Warren et al. (1983) demonstrated sensitization to the storage mite, *Lepidoglyphus destructor* in 12% of asthmatic grain workers in Canada. In a study of 133 Essex grain store or terminal workers (95% of available work force), the

association between work-related respiratory symptoms and positive skin test or specific IgEs to storage mites was confirmed (Blainey et al.,1989). They concluded that allergy to storage mites is an important determinant for respiratory disease in British grain workers. This might be partially explained by higher water content (typically greater than 15%) of grain grown in the U.K. Chan-Yueng et al. (1979) found only nonspecific serum precipitin bands in 22 grain elevator workers and 11 controls. They also found that exposure to crude grain dust elicited a stronger bronchial reaction than grain dust extract, concluding that the allergenic component was not well extracted from the dust. It is possible that different allergens are responsible for asthmatic reaction in different subjects. Arlian et al. (1984) showed that individuals with storage mite allergy to *Tyrophagus putrescentiae* could produce allergic antibodies against different specific allergens from mite bodies or their feces or both. Blainey et al. (1989) did not find associations between symptoms and skin tests or specific IgEs to the housedust mite, *Dermatophagoides. pteronyssinus,* in symptomatic grain store workers.

Some studies have concluded that storage mites are the most important allergens causing bronchial asthma and rhinitis in the farming environment (Iversen et al.,1990a; Blainey et al.,1988; Cuthbert et al.,1979). In Sweden, IgE-mediated allergy to storage mites is a greater problem in rural populations than farmer's lung (van Hage-Hamsten et al.,1987). Bronchial provocation studies have confirmed that storage mite extract elicits an asthma-like response in farmers (van Hage-

Hamstem et al.,1987; Ingram et al.,1979). The highest prevalence of positive skin tests in Swedish farmers is to *Lepidoglyphus destructor*, also the most common mite in barn dusts (van Hage-Hamsten et al.,1994). Katila and Mantyjarvi (1987) followed 292 Finnish farm workers over a six-year period. They showed that, among men with precipitins against immunogenic molds, the risk of occupationally disabling respiratory disease was three times higher than for precipitin-negative farmers of the same age.

Iversen et al. (1994) showed that swine confinement workers had raised IgE levels to mites and grain species, but not to swine proteins. In a study of 20 people employed in swine barns in Finland, sensitization to a number of antigens was found (Katila et al.,1981). IgG precipitins against swine antigen were found in only two workers, and precipitins against feed antigens were found in 12 workers. No IgE antibodies were found. Sensitization was correlated with exposure but not symptoms. In a similar study in Sweden, precipitating antibodies were found to pig dander in 14, pig feed in 5, and swine dust in 3 of 20 swine confinement workers (Larrson et al.,1992). Skin tests with swine extracts were all negative. Others have reported specific allergies to storage mites, not related to those of *Dermatophagoides pteronyssinus*, among subjects who were not farmers, but had contact with small animals or livestock food (Hardel et al.,1987).

Revsbech and Dueholm (1990) found that among bakers with nasal or pulmonary symptoms, there was no difference in prevalence or strength of response to skin

prick tests or RAST to three storage mite species. However, all the bakers sensitized to flour were also sensitized to storage mites. Thus, they suggested that if a baker becomes sensitized to flour, he will also be more prone to develop specific IgE antibodies toward storage mites. Likewise, Tee et al. (1992) found that 251 U.K. bakers were more likely sensitized to house dust mites rather than storage mites, and none were sensitized to storage mites and not house dust mites. The prevalence of co-sensitization to the mite *Lepidoglyphus destructor* among 43 patients with IgE-mediated hypersensitivity was 30% (Armentia et al.,1992). Patients with either daily or indirect contact with wheat flour exhibited a low degree of cosensitization to *Dermatophagoides pteronyssinus* (Armentia et al.,1992). Among farmers and individuals with occasional contact with wheat flour, the prevalence of co-sensitization was 42%. The prevalence among patients with daily contact with wheat flour (bakers and pastry factory worker) was 63% (Armentia et al.,1992).

Frankland and Lunn (1965) reported on two laboratory workers in a grain mill who developed allergy to the grain weevil protein. Lunn (1966) then found a positive skin response to weevil extract in 57% of 75 mill workers. He conducted inhalation provocation tests on 25 of the workers with a positive skin response. Two workers showed significant lung function changes, while no controls showed such changes. He concluded that grain weevil sensitivity was a factor in mill worker's asthma. Lunn et al. (1967) also demonstrated a

pulmonary hypersensitivity in a young woman who worked in a laboratory with grain weevils.

Some grain handlers have been diagnosed as having a Type I allergic reaction to grain dust, variably associated with rhinitis and conjunctivitis (doPico et al.,1981; Chan-Yeung et al.,1979; Warren et al.,1974). It seems unlikely that this a significant mechanism in gain dust-induced disease, since the prevalence grain asthma is similar to control populations, grain handlers have lower atopy, and positive skin reactions correlate poorly with respiratory symptoms (Broder,1989).

Other studies have suggested a Type III allergic reaction, since this mechanism is at least partially the basis of HP and grain weevil asthma (Broder,1989). However, late skin responses are not usually observed among grain handlers (Chan-Yeung et al.,1979; Warren et al.,1974). Chest x-rays suggest that HP is rare among grain handlers (doPico et al.,1984; Becklake et al.,1980b; Broder et al.,1979). Thus, it is unlikely that there is common involvement of Type III allergic reaction upon grain dust exposure (Broder,1989).

In summary, chronic exposure to grain dust stimulates the immune system, as evidenced by an increase in IgE, IgA and IgG that is related to length of employment and blunted by smoking (doPico,1994). Serum-precipitating antibodies to grain dust, fungi, and other grain components are found only in a

small proportion of grain handlers, and poor correlation reduces their diagnostic value for disease (doPico,1994). The role of IgE-mediated allergic sensitization remains controversial.

2.5.3 Inflammatory Response

Inflammation is a response to a foreign agent that involves a series of cellular and molecular interactions that are intended to stop tissue destruction, remove the agent, and return body tissue to normal function (Doherty et al.,1993). Inflammatory responses observed in grain exposed workers and animal models include airway smooth muscle constriction, increased mucous production, accumulation of inflammatory cells in the airway, fever, and elevated circulating white blood cells (Broder,1989; Cooper et al.,1986). The continual presence of inflammatory cells, such as neutrophils, eosinophils, lymphocytes, and monocytes, in the lung may result in release of enzymes, pharmacologic compounds, and peptide hormones or cytokines, and eventually lead to fibrosis and other chronic lung diseases (Doherty et al.,1993).

Two stages of inflammation of the bronchial epithelium are distinguished: acute inflammation and chronic bronchitis (Rylander,1986). Acute inflammation presents as rhinitis, upper airway irritation, and increased mucous secretion (Rylander,1986). The cell mechanism is most likely the polymorphonuclear neutrophil, which is mobilized into the exposed site (Rylander,1986). Long-term

exposure to grain dust has been associated with a neutrophilic bronchitis (Rylander,1986). Neutrophils release mediators of inflammation that serve to amplify the inflammatory response. In vitro studies have suggested direct and indirect mechanisms for recruitment of neutrophils. In rats, intratracheal instillation of grain dust initiated an acute inflammatory reaction with influx of neutrophils into the air spaces and lung interstitium (Brown,1988). Neutrophil chemotaxis has been observed when both guinea pig and human alveolar macrophages are incubated with grain dust extract (Von Essen et al.,1988). *In vitro* challenge of bovine bronchial epithelial cells with grain extract elicited an indirect neutrophil chemotactic activity (Von Essen et al.,1994). Continuous insult to the respiratory epithelium results in an increase in goblet cells, enlargement of subepithelial glands, and thickening of the mucous, thus modifying the ability of the smooth muscle to contract (Rylander,1986; Flaherty,1982). Both the acute and chronic neutrophilic inflammation may be accompanied by bronchial hyperreactivity (Rylander,1986).

Microorganism-induced or enhanced mediator release has been suggested as a possible mechanism in organic dust related disease (Norn,1994; doPico et al.,1981). Acute airways constriction may occur as a result of release of a number of pulmonary mediators, e.g. histamine (Doherty et al.,1993). DoPico et al. (1981) showed that human response to durum wheat was inhibited by cromolyn sulfate, an inhibitor of mast cell degranulation, suggesting a histamine- or mediator-induced bronchial reaction. Histamine increases

epithelial permeability, thereby facilitating the entrance of microorganisms, mediators and other insulting particles, leading to bronchoconstriction and airway inflammation. Histamine release is obtained from a wide spectrum of gram-positive and gram-negative bacteria (Norn,1994). *Staphylococcus aureus* has been shown to trigger release of histamine in BAL cells of nonatopic individuals (Norn,1994). Further, histamine release caused by allergens and nonimmunologic reactions was enhanced by bacteria, endotoxins and fungal spores (Norn,1994). Chan-Yeung et al. (1987) showed that human lung fragments respond to grain dust extract by releasing histamine and leukotrienes (B4, D4, and E4), independent of the complement pathway or cell cytotoxicity. Because mediators were not released in the presence of house dust extract, they suggested that grain mites were not the responsible agent.

The emergence of the bronchoalveolar lavage (BAL) technique has allowed investigators to look at the inflammatory response to grain dust *in vivo*. These studies have led to the conclusion that grain dust activates alveolar macrophages and the subsequent nonimmunologic release of IL-1, IL-6, IL-8, tumor necrosis factor (TNF-alpha), and other pyrogens (Lewis et al.,1989; Richerson,1989). IL-1, which mediates many of the symptoms of acute inflammation (fever, chills, malaise), may be 500-fold higher then normal following heavy exposure to organic dusts (Rylander,1994a). Rat alveolar macrophages have been stimulated to produce interleukin-1 and neutrophils in response to inhalation of agricultural dust extracts (Lewis et al.,1989). Further

studies showed that IL-1 levels may be artificially elevated in human serum and plasma, and thus the absolute value may not be a valuable biomarker of pulmonary inflammation (Lewis et al.,1992). Mice exposed to airborne grain dust at 1200-5400 mg/m^3 for 2 to 28 days developed increased pulmonary macrophage response (Menkes et al.,1989). Rabbits exposed to 20 mg/m^3 total grain dust for 24 weeks, 5 days per week, developed inflammation of the alveoli and an interstitial pneumonitis within five days and still present at six months, consistent with a Type IV nonimmunologic hypersensitivity reaction (Stepner et al.,1986). *M. faeni* stimulated strong release of proinflammatory cytokines IL-1 and TNF-a from nonfarmer volunteer and mice alveolar macrophages (Michel et al.,1991). The BAL fluid of 20 swine confinement workers was found to have increased total cells and granulocytes with normal levels of lymphocytes and macrophages (LaLancette et al.,1992).

Because of its potency as an inflammatory mediator and bronchoconstricting agent, ability to activate and recruit eosinophils, and capacity to prime leukocytes to respond to otherwise subthreshold levels of cytokines, platelet-activating factor (PAF) is thought to be involved in the pathogenesis of grain-induced asthma (Page,1992). Eosinophils are the predominant cells found in lavage fluid during late asthmatic reaction (Busse et al.,1993a). PAF release has been detected in the BAL fluid of asthmatic subjects (Page,1992). While the mechanisms whereby PAF can induce airways hyperresponsiveness have been investigated, clinical studies are underway to determine whether PAF

antagonists will reduce asthmatic response (Page,1992). Thrombocytopenic purpura, a disease associated with platelet activation, does not result in airway obstruction (Cooper et al.,1986). Chest tightness is possibly caused by temporary accumulation of platelets in the pulmonary capillaries after organic dust exposure (Rylander,1994b). No studies have demonstrated antibodies related to the severity of chest tightness (Haglind et al.,1984).

Nasal lavage has been used to study acute inflammatory reaction of the upper respiratory tract. Blaski et al. (1996b) found that nasal lavage from grain workers had more total cells, more squamous epithelial cells, and more neutrophils than postal workers' nasal lavage samples. However, these results did not correlate with ambient grain dust or endotoxin exposures, lung function, or airflow obstruction.

In a review of inflammatory mechanisms and cotton and grain dust exposure, Cooper et al. (1986) concluded that insufficient evidence had been presented for any proposed mechanism. He concurred that the pulmonary alveolar macrophage is plausibly the initiating cell, but noted that the function of polymorphonuclear leukocytes is unclear. Likewise, the roles of eosinophils, neutrophils, mast cells, PAF, and lymphocytes in bronchoconstriction is unknown.

2.5.4 Hypersensitivity Reaction

Hypersensitivity reactions to any of the potential grain dust antigens may be important in the development of pulmonary symptoms in exposed workers. The respiratory symptoms which normally appear after exposure to grain dust are either immediate wheezing or a slower onset of tightness of the chest. Also, when challenged with a grain dust extract in the laboratory, immediate and late asthmatic reactions are observed (Warren et al.,1974; Chan-Yeung et al.,1985).

Two types of hypersensitivity appear to be involved. The immediate symptoms of cough, wheezing, tightness across the chest, and dyspnea have been characterized as a Type III reaction with immune complex formation, complement fixation, interaction of mediators, and prominent inflammation (Kryda et al.,1993). The reaction occurs when IgE molecules on the surface of the mast cells bind with antigen (Gurney,1992). The binding causes the release of pharmacologically-active substance such as histamine, 5-hydroxytryptamine, bradykinin, and slow-reacting substances of anaphylaxis from the mast cells. The tissue reactions occur within minutes of antigen introduction and are manifested by development of a wheal if introduced into the skin, or by bronchoconstriction if inhaled. The timing of the acute response supports this made of immune reaction (Kryda et al.,1993).

The mechanism involved in the late respiratory reaction is less well understood but it has been theorized that this is a Type IV hypersensitivity reaction. Histopathology demonstrates a mononuclear infiltrate with granuloma formation (Kryda et al.,1993). The major supporting evidence is the increased BAL lymphocyte count (Kryda et al.,1993). The Type IV delayed cell-mediated hypersensitivity reaction seems to be most important in development of disease (Gurney,1992). Serum precipitins are usually present in HP patients consistent with an immune complex and complement-mediated (Arthrus type) response (Merchant,1987).

Warren et al. (1974) demonstrated a good correlation between hypersensitivity reactions on skin testing and bronchial provocation with crude grain dust extract. They stated that it is unclear which constituent might be the main antigen causing hypersensitivity. However, they concluded that molds and fungi, grasses, and weed pollens are unlikely sources because skin hypersensitivity to these does not correlate with hypersensitivity to grain dust. Further, precipitins against arthropods common in grain dust, e.g. the grain weevil *Sitophilus granarius,* were not detected in the subjects examined.

While serum precipitins suggest the Arthrus-type reaction is important, others have not found good correlation between serum precipitins and HP. Approximately 10% of patients with HP do not have precipitating antibodies

(doPico et al.,1976). Many dairy farmers and pigeon breeders have serum precipitins without clinical disease (Gurney,1992).

2.5.5 Complement Activation

The complement system is the primary humoral mediator of antigen-antibody reactions and is composed of at least 15 serum proteins capable of reacting with one another, with antibodies, or with cell membranes (Olenchock et al.,1980). These interactions lead to various events, including lysis of cells, bacteria and viruses, mediation of inflammatory processes, induction of other cellular systems, histamine release, migration of leukocytes, and phagocytosis. Complement fragment C5a is a potent chemotactic factor for human neutrophils. The complement system works through two pathways. The classical pathway may be activated by antigen-antibody complexes, aggregated immunoglobulins, or by substances such as DNA, proteins, or enzymes. The alternative pathway may be activated by aggregated immunoglobulins, or by complex polysaccharides, lipopolysaccharides, and trypsin-like enzymes.

Components of both grain and cotton dust have been shown to activate complement *in vitro* and *in vivo* (Cooper et al.,1986). Serum from patients with HP has been shown to activate complement, as have respirable fractions of moldy hay and grain dusts and fungi (Marx et al.,1975; Edwards et al.,1974).

Olenchock et al. (1980) showed that various grain dusts activate the alternate complement pathway *in vitro* by converting the third complement component (C3PA) to the activator form (C3A) in a dose-dependent manner. Rye, spring wheat, and barley were more potent activators *in vitro* than oats, durum wheat, corn, and sunflower seeds. This was interesting in that workers have reported that rye, barley, and spring wheat are the most bothersome (doPico et al.,1977). However, the specific dust component that activates the system was not identified. While endotoxin was present in every dust tested, it was not believed to be solely responsible for the observed decreases in serum complement levels, because dusts with similar endotoxin levels did not convert C3PA to the same extent.

Wirtz et al. (1984b) showed that spring wheat dust extract activated both the classical and alternate complement pathways. Follow-up experiments indicated that the activators were not proteins or nucleic acids, but were carbohydrates and macromolecular. While endotoxin was present, one researcher attributed a significant portion of the classical pathway activator to a grain-derived tannin (Skea et al.,1986). Another researcher reported acute *in vitro* pulmonary effects with neutrophil influx from inhalation of tannin (Rohrbach,1994). However, others have concluded that while tannins may contribute to cytotoxic inflammation following chronic exposure, it is unlikely that tannins are the cause of any acute lung effects (Lacey et al.,1994a).

This alternate pathway of activation is produced by many substances, including moldy hay and silage (Edwards et al.,1974; Olenchock,1989b). Three mold species present in grain dust were shown to activate complement, as well as result in the release of leukotriene B_4 and increase in superoxide anion production *in vitro* (Lacey et al.,1994a).

The activation of complement *in vivo* has not been demonstrated in humans responding to grain dust inhalation exposure (doPico,1994). DoPico et al. (1981) did not find complement activation by either pathway during bronchial challenge of 11 subjects with durum wheat dust extract. Dusts that do not cause bronchoconstriction, such as asbestos, activate complement *in vitro*, suggesting that this may be a nonspecific effect not related to bronchoconstriction (Cooper et al.,1986). While complement activation can account for part of the inflammatory reaction to grain dust, this mechanism is not sufficient to explain all the signs and symptoms associated with such exposure (Lewis et al.,1989).

2.5.6 Endotoxin as a Causative Agent

Exposure to gram-negative bacterial antigens has been shown to increase the risk of sensitization in grain-exposed persons (Dutkiewicz et al.,1978b; deLucca et al.,1984). Grain workers frequently have precipitating antibodies to the gram-negative bacteria *E. herbicola* (Dutkiewicz et al.,1978b and 1985). Likewise,

skin reactions to *E. herbicola* are significantly correlated with reactions to grain dust and show a close relationship to degree of exposure (Dutkiewicz et al.,1978b). Endotoxin in gram negative bacteria has been suggested as a cause of acute symptoms such as chest tightness, cough, shortness of breath, fever, and wheezing, as well as a variety of agricultural disease conditions, including ODTS, HP, weaver's cough, mill fever, and others (Thelin et al.,1991; Merchant,1987; Donham et al.,1986a).

Endotoxins are the heat stable, lipopolysaccharide-protein complexes of the cell wall of gram-negative bacteria (Lacey, 1980). The phospholipid, Lipid A, is responsible for the majority of toxic and pyrogenic effects of endotoxins (Rietschel et al.,1992). The polysaccharide chains are connected by a core oligosaccharide to the lipid part of the molecule (Rylander,1994c). The poysaccharide carries the principal immunogenic determinants specific for the organism from which it was derived. The determinants are made up of small numbers of hexose or pentose units, and constitute the somatic antigens of the organism. After inhalation, intact bacterial cells and endotoxin in grain dust are taken up by phagocytosis, and the macrophages respond by producing free oxygen radicals, recruitment of neutrophils, and production of chemotactic factors, enzymes, and cytokines, such as IL-1, TNF-alpha, and PAF (Olenchock,1994; Rylander,1994c and 1989).

Human and animal cells have been shown to be extremely sensitive to the effects of endotoxin *in vitro* (Morrison et al.,1985). Exposure to mycotoxin and

grain dust extract with low endotoxin content produced noncytotoxic histamine release in rat peritoneal cells, while grain dust extract with high endotoxin content produced a cytotoxic histamine release (Warren et al.,1986; Sorenson et al.,1986). Extracts of grain dust also induced release of histamine and leukotrienes from human lung fragments in a dose-dependent manner (Chan-Yeung et al,1987). The release of these mediators was independent of complement pathway, cell cytotoxicity, or immunologic mechanisms. Brown et al. (1991) showed that neither wool nor grain dust nor purified endotoxin produced direct injury to A549 alveolar epithelial cells as measured by direct lysis and cell detachment. They concluded that recruitment of inflammatory cells into the lung was therefore a plausible cause of airways obstruction seen in the workforce. Cosma and Martinez (1999) showed that endotoxin was responsible for 70% of the acute inflammatory response (IL-1, IL-6, and TNF-alpha release) by rat alveolar macrophages exposed to wheat and corn dust *in vitro*, while 20 metals and silica did not significantly increase the inflammatory response.

Effects of exposure of a variety of laboratory animals to inhaled endotoxin correlates well with endotoxin-exposed workers' signs and symptoms (Burrell,1984). Rabbits developed fever, circulating leukopenia, histopathologic changes in the lung, and marked changed in arterial O_2 tensions following challenge with purified endotoxin aerosol (Olenchock et al.,1982). There were increased leukocytes in rat airways after exposure to aerosolized *E. Coli*

endotoxin (Olenchock et al.,1982). Guinea pigs showed granulocyte recruitment following challenge with aerosolized *S. typhosa* endotoxin (Olenchock et al.,1982). These findings correlate with cellular studies and support the hypothesis that bacterial endotoxin is important in the pulmonary reaction to organic dusts.

Several studies have examined the relationship between endotoxin exposure and acute respiratory symptoms. In a clinical trial of asthmatic subjects, inhalation of purified lipopolysaccharide (LPS) induced local bronchial inflammation, systemic inflammatory response, and increased blood ACTH level suggesting that clinical manifestations of asthma are influenced by substances such as endotoxin (Michel,1994). Rylander et al. (1989) found that one-half of 77 naïve subjects exposed to endotoxin developed fever, about one-third experienced chest tightness, and a small, but dose-related decrease in FEV_1 and transfer factor were observed. Pernis et al. (1961) exposed human subjects to endotoxin intravenously and obtained slight fever within 15 minutes (peaking at approximately 1-1/2 hours and 3-5 hours), dry cough, and shortness of breath, but no leukocytosis. There was a drop in FEV_1 for two of eight normal subjects. When sufficient endotoxin dosage was given, an initial leukopenia preceded the pyrexia, followed by a leukocytosis involving mainly an increase in the number of immature granulocytes (Rylander,1994c). In healthy nonsmoking volunteers exposed to LPS by inhalation, BAL revealed a 100-fold increase in neutrophils, a three-fold increase in lymphocytes, elevated

fibronectin concentration, and a decrease in alveolar macrophage phagocytosis (Sandstrom et al.,1994).

Recent studies have focused on endotoxin as the etiologic agent in cotton dusts (Olenchock,1990a). In studies of cotton workers, mean cross-shift decline in FEV_1, presence of byssinosis symptoms, increase in blood neutrophils, and the extent of decrease in FEV_1 on metacholine challenge was related to endotoxin concentration in the workplace, but not to total dust exposure (Rylander et al.,1993 and 1985; Olenchock et al.,1994 and 1990b; Castellan et al.,1987; Haglind et al.,1984). These studies report a threshold of 90-330 EU/m^3. Boehlacke et al. (1984) showed FEV_1 decrements among all 32 volunteers exposed to endotoxin for six hours in a cotton card room. Washing the cotton markedly reduced the airborne endotoxin concentration and FEV_1 decrement. Boehlacke et al. (1984) found an association between the endotoxin content of bulk cotton and pulmonary function decrements for asthmatic cardroom workers only. These findings support the theory that endotoxin has a causative role in the acute response to cotton dust.

Associations between endotoxin concentration and chronic lung disease have also been reported. In a study of 443 cotton textile mill workers from two mills in Shanghai, a dose-response trend was observed between current endotoxin levels (10 to 200 EU/m^3) and chronic bronchitis (Kennedy et al.,1987). This relationship did not exist between dust concentration and bronchitis.

Several studies of the effects of endotoxin have included grain handlers. Respiratory symptoms among 410 grain handlers were strongly associated with endotoxin exposures and only marginally associated with total dust (Schwartz et al.,1995). Both protein and messenger RNA cytokines were increased, and eosinophils and lymphocytes remained the same, in the BAL of 15 nonsmoking, nonasthmatic, nonatopic grain handlers six hours after challenge with lipopolysaccharide from corn dust extract (Clapp et al.,1994). These authors concluded that airway response to grain dust is an acute inflammatory response to an inhaled toxin, such as endotoxin. Borm et al. (1996) investigated cytokine (TNF, Il-6, Il-8) release and several proinflammatory proteins in plasma of 12 workers exposed to an average of 20 mg/m^3 grain dust in a fodder plant. No cross-week lung function changes were observed. However, TNF release increased seven-fold across the week. In addition to cytokine release, investigators have shown several peripheral pathways to amplify the response to endotoxins (Borm et al.,1996).

Schwartz et al. (1995 and 1994) showed that endotoxin responsiveness is critical to the development of grain dust-induced inflammatory response measured by BAL and FEV_1 in grain and swine workers. Healthy and asthmatic subjects have shown airway inflammation and fibronectin after challenge with LPS and workplace exposure to swine dust (Michel et al.,1994; Sandstrom et al.,1994; Larsson et al.,1992). Exposure to endotoxin in the animal feed industry led to a greater decrease in lung function as compared to other inspirable dusts

measured (Donham et al.,1989). In a study of 315 animal feed workers in the Netherlands, Smid et al. (1992b) had similar findings in that chronic lung function changes and symptoms were related more to present and historic endotoxin concentrations than to inspirable dust concentrations. These findings were confirmed during a five-year follow-up study of 50 of the animal feed workers (Heederik et al.,1994). Zejda et al. (1994) reported an increased prevalence of cough and chronic bronchitis symptoms and reduced FVC in 54 swine producers that correlated with endotoxin levels, but not respirable dust levels or exposure groups. Cough depended significantly on the interaction with ammonia and endotoxin. A threshold level was reported as 10 EU/m^3.

Rask-Anderson et al. (1989b) and Malmberg et al. (1988b) found that the endotoxin concentrations were high, but similar for farms with farmers who had experienced febrile reactions or allergic alveolitis and farms with symptomless farmers. They concluded that endotoxin was not responsible for the symptoms. However, they noted that 75% of the endotoxin content resided in the non-respirable fraction. They suggested that the LAL assay used for endotoxin analysis might be sensitive to some other component of grain dust. Other researchers have reported that peptidoglycans, mannans, and dextrans activate the limulus assay, but with less sensitivity (Pratt et al.,1994; Rask-Anderson et al.,1989b).

While endotoxins have been implicated as a possible cause of acute febrile reaction and other symptoms for several decades, there are several reasons that it has not been possible to prove it is the causative agent, including the complex composition of the grain dust, the variety of bacteria present in different dust samples, variable toxicity of different endotoxins, use of purified LPS in many experiments, and possible interactions between endotoxin and other dust components. The current paradigm is that endotoxin produces its bronchial effects through release of proinflammatory mediators (Schwartz et al.,1995). It needs to be shown that endotoxin levels in grain dust will release IL-1 by macrophages *in vivo*. The requirement for high dust levels is consistent with the proposal that bacterial endotoxin causes ODTS, since while endotoxin is a known pyrogen, it is present in low concentrations in grain dust (Broder,1989).

2.6 Summary and Research Needs

The many grain dust studies have shown that grain dust is not a biologically inert substance. Grain dust is a complex mixture of many materials, several of which may cause disease. Robust epidemiological investigations including unexposed controls groups have shown that a number of acute and chronic respiratory symptoms may result from and are related to the level of grain dust exposure. The studies all show that cough, phlegm, wheeze, and dyspnea occur more frequently, and mean FEV_1 and FVC are lower, among grain elevator workers and farmers than control groups (when available), irrespective

of smoking habits. Diseases that have been associated with grain dust exposure include allergies and allergic rhinitis, asthma, bronchitis, periodic febrile episodes, and hypersensitivity pneumonitis. It is likely that the chronic health effects result from repetitive acute inflammatory responses to grain dust exposure. There is evidence that the cross-shift change in lung function with dust exposure is predictive of longitudinal decline in lung function. Chronic obstructive disease is the most likely outcome of prolonged exposure, but interstitial lung disease occurs uncommonly in a few workers. Exposure-response relationships have been established by measuring exposures and contrasting the prevalence of symptoms, disease, and lung function change between exposed and unexposed groups. The consistency of findings in different studies using different designs in different cohorts and different countries constitute strong epidemiological evidence of the harmful effects of grain dust exposure.

Numerous studies to elucidate the mechanisms of grain dust-induced disease have also been conducted. These suggest that chronic bronchitis may be the result of irritant effects and related to total dust inhaled, asthma may result from sensitivity to grain mites and other specific allergens, HP seems to be the result of exposure to high levels of mold spores, and high dust concentrations and endotoxin may be important factors in acute febrile episodes. However, many questions remain concerning both the constituent and mechanisms of effect of grain dust on the lung. There are a number of difficulties in determining the

etiology of grain dust diseases, including:

- the presence of multiple diseases and etiologies,
- the variable composition of grain dust,
- subtle clinical differences between diseases (e.g. HP and ODTS),
- the use of different names for the same disease (e.g. HP has been called allergic alveolitis, farmer's lung, thresher's lung, etc.),
- the same disease may arise from non-agricultural exposures (e.g. HP can also result from exposure to cheese, cork, tea, contaminated air conditioning systems, and metal fumes),
- several diseases may co-exist in the same patient (Bates et al.,1992),
- reliance on medical questionnaires, rather than lung function measurements and physician diagnosis, and
- various predisposing host factors of unknown importance.

A final, but significant problem in understanding the etiology of grain related diseases is the fact that many of the studies have not related pulmonary symptoms with quantitative dust and dust component exposures. Even when quantitative total or respirable dust measures have been made, they have not characterized other components in the dust. The various studies utilize different sampling and analytical methods. Thus, dose-response relationships have not been adequately determined for the individual components. There is a need to determine a safe level of exposure to grain dust and perhaps for its individual constituents, perhaps endotoxin, to protect worker's health (doPico,1994).

Chapter 3

Purpose and Scope

3.1 Purpose of the Study

The primary purpose of this study was to determine the relationship between total dust and endotoxin exposures and cross-shift respiratory symptom and pulmonary function changes among wheat harvest workers over one harvest workday. NIOSH has recommended that research be conducted to establish the relationship between the recognized health effects of exposure to grain dust and the various constituents of the dust (Brown,1988). This study supports NIOSH's recommendation as related to acute respiratory health effects and exposure to total dust and endotoxin in wheat dust among the poorly studied harvest worker population.

Wheat was selected as the study grain for several reasons. First, many studies indicate that wheat has greater adverse health effects than other grains, e.g. corn. In fact, most of the studies conducted on elevator workers have involved primarily wheat dust exposure. Also, a wheat dust exposure characterization has been recently conducted on northeastern Colorado elevators and farms (Beard,1994). In addition, wheat is the number one grain crop in Colorado. Thus, a study of wheat dust exposure supplements previous studies and provides health and exposure information relevant to Colorado farmers.

Cross-shift studies are commonly used to evaluate acute health effects of exposure. A number of studies have demonstrated cross-shift changes in both respiratory symptoms and pulmonary function related to total dust exposure in grain elevator workers. However, because harvest is a very busy time and involves a mobile population, few studies have included workers in the fields harvesting the grain. Recent measurements in northeastern Colorado found concentrations of total dust and endotoxin during harvest comparable to those found in grain elevators, but the study did not evaluate respiratory effects among the exposed workers. Since grain composition changes as it is processed, correlation of respiratory symptom and pulmonary function changes with dust exposures during harvest will add valuable information to the existing literature.

A number of wheat dust components have been implicated in causing or exacerbating respiratory disease in exposed workers. It has been suggested that the acute airways obstruction and febrile response associated with grain dust exposure may be caused by endotoxin. In fact, in the recent dust characterization study in northeastern Colorado, endotoxin levels were above reported threshold levels for acute health effects. Thus, comparison of endotoxin exposure with acute respiratory changes during harvest will help to elucidate a possible dose-response relationship.

Finally, an improved understanding of the etiology and acute health effects of wheat dust will supplement studies of worker groups exposed to the other organic dusts, as well as support agricultural exposure reduction and personal protection efforts.

3.2 Study Design and Scope

Based on the identified research needs, a cross-shift study was designed in which harvest workers on northeastern Colorado farms were identified as a high-risk group of individuals exposed to variable levels of dust during the course of a wheat harvest workshift. Respiratory symptoms and work history were obtained via a questionnaire. Total dust and endotoxin exposures were measured for each subject over a single workshift. Cross-shift changes in respiratory symptoms and pulmonary function were assessed via a symptom questionnaire and spirometry, respectively. This study was designed to answer the following specific research questions:

1. What were the characteristics of wheat harvest workers on northeastern Colorado farms in 1994?

2. What were the wheat harvest workers' exposures to total dust and endotoxin during a harvest workshift? How was total dust exposure related to total endotoxin exposure?

3. What was the prevalence of chronic respiratory symptoms/illness among the wheat harvest worker population?
   a. By standard questions (for chronic respiratory symptoms)?
   b. By baseline spirometric determination (for chronic respiratory illness)?

4. What variables were related to chronic respiratory symptoms/illness among the wheat harvest workers?

   a. By standard questions (for chronic respiratory symptoms)?
   b. By baseline spirometric determination (for chronic respiratory illness)?

5. How well did detection of chronic respiratory symptoms by spirometry compare to detection by standard chronic respiratory symptom questions among wheat harvest workers?

6. What were the acute respiratory changes in lung function over a harvest workday?
   a. By post-pre shift spirometric values?
   b. By post-pre shift self-report in symptoms?

7. How were the acute respiratory changes over a harvest workday related to dust exposures?

8. How were the acute respiratory changes over a harvest workday related to endotoxin exposures?

9. What other variables were related to changes in acute respiratory symptoms?

The study was limited to 26 farms in northeastern Colorado during the 1994 harvest season. It is assumed that total dust and endotoxin exposures vary by region and areas of the world due to different farming methods, varieties of wheat, etc. In addition, it is assumed that varying humidity and rain create different regional grain dust composition at harvest and, thus, different exposure patterns.

The study subjects were harvest workers present on farms whose owners had agreed to participate in the study. Thus, the sample population was a convenience cluster sample, i.e. farms were not randomly selected, but subjects included all eligible workers on each farm. Personal information, work history and medical history, as well as data on potential confounding factors, were gathered via a questionnaire administered to each subject. There were no surrogate respondents.

The research focused on total dust and endotoxin exposures only. Endotoxin exposure has been highly correlated with respiratory function among other agricultural worker groups, e.g. cotton workers and swine workers (Olenchock,1994a; Zejda et al.,1994). Every subject was monitored for both total dust and endotoxin for one entire workshift (if less than 6 hours) or at least the first 6 hours of harvest work on the day the study team arrived at the farm. Some subjects continued to work after the study team left the farm.

Cross-shift effects of grain dust exposure were determined from pre- and post-shift spirometric values and pre- and post-shift self-report of respiratory symptoms. Specific acute conditions, such as ODTS, acute bronchitis or HP, or asthma-like syndrome, were not identified.

Chapter 4

Methods and Materials

4.1 Summary

A cross-shift study was designed to determine if changes in self-reported symptom severity and pulmonary function among harvest workers were related to total dust and endotoxin exposure over a workshift. The disease outcomes investigated included cross-shift changes in six spirometric variables and eight self-reported symptoms. Individual dust and endotoxin exposures were measured for harvest workers in northeastern Colorado during one 1994 wheat harvest workshift. A respiratory health and occupational history questionnaire was also administered to each participant. The field results were statistically analyzed to evaluate the relationships between dust and endotoxin concentrations and cross-shift changes in acute symptoms and pulmonary function. Prevalence rates for history of respiratory symptoms and abnormal spirometric results among study subjects were compared to other agricultural and non-agricultural populations to determine if there were any significant differences. Also, the sensitivity, specificity, and positive and negative predictive values of self-reported history of respiratory symptoms via questionnaire were determined with respect to the baseline spirometric test results. The CSU Human Research Committee approved the study design, procedures, questionnaires, and consent forms prior to implementation.

4.2 Farm Selection and Recruitment

Subjects were selected via a purposive two-step process. First, farm owners were screened and recruited into the study. Then all harvest workers at the recruited farms were recruited for participation. The study was limited to farms in northeastern Colorado on which winter wheat was grown. This was primarily for logistical reasons, but also because grain dust exposures had been previously characterized for this population (Beard,1994). Fifteen counties were included in the recruitment effort: Adams, Arapahoe, Boulder, Douglas, Elbert, Kit Carson, Larimer, Lincoln, Logan, Morgan, Phillips, Sedgwick, Washington, Weld, and Yuma Counties. Farms with over 1,000 acres of wheat on which three or more workers were involved in harvest-related work each day were targeted for the study. An estimated 975 farms with over 1,000 acres in wheat were present in the study area (Colorado Agricultural Statistics,1987). Smaller farms with fewer workers were targeted as study back-up locations.

Potential farm owner participants in the study area were recruited from various sources, including:

1. References from Colorado State University agricultural extension agents and other rural contacts, including the High Plains Intermountain Center for Agricultural Health and Safety (HI-CAHS) Advisory Board, Colorado Wheat Administrative Committee, Colorado Agricultural Leadership Council, Kit Carson Rural Nursing Association, and several grain cooperative associations.
2. Farmers who had participated in a previous grain dust characterization study (Beard,1994).

From March through June 1994, the owners of farms identified in the study area were called to determine the acreage of winter wheat production and the number of workers expected during each harvest workday, and to solicit participation. Notices (Appendix A) were included in Colorado grain association newsletters and distributed at HI-CAHS functions to inform potential participants about the study. In addition, a follow-up letter was sent out to farm owners who agreed to participate or who requested further information about the study (Appendix B). A second phone call was made approximately one week after sending out the information letter to again request participation by the farm owner.

Using correlation coefficients reported for lung function change (FEV_1) versus total endotoxin exposure among swine workers of 0.35-0.42 (Zejda,1994) and versus total dust exposure among grain elevator workers of 0.52-0.77 (doPico,1977), a confidence interval of 0.27-0.67 for the correlation coefficient was expected if this study included 100 subjects (Zumbrunnen,1994). Thus, every attempt was made to ensure that enough farms were available from which to recruit 100 subjects.

4.3 Subject Selection and Recruitment

Beginning in June 1994, participating farm owners were called to obtain anticipated harvest dates and to schedule the study team visit. Upon arrival at the farm, all harvest workers present were asked to participate in the study and to sign

the informed consent form. When consent was received, the eligibility screener was administered.

If the subject met the screening criteria, the full questionnaire was administered and pre-shift spirometry and dust sampling were performed immediately thereafter.

Harvest workers involved in this study realized the following benefits from participating in the study:

1. A no-cost spirometric screen and dust exposure monitoring.
2. The results of the spirometric screen and total dust sampling and a brochure on the use and selection of respiratory protection that may be advised. Appendix C presents the basic format of the letter of results that was submitted to each subject.
3. Direct access to health and safety professionals from the HI-CAHS program. The farm operators were provided the HI-CAHS address and phone number. In addition, some of the field team staff were HI-CAHS professionals.
4. It was intended that field staff use the opportunity for formal and informal instruction in any hazards observed during harvest. Further, any farm health and safety questions asked by the operator or workers during the study were addressed by the field staff.
5. Study participants were offered a CSU ball cap or $10 cash in appreciation of their time and to offset any lost wages. This compensation was given to each participant at the completion of the post-shift spirometric test.
6. Study subjects with abnormal spirometric values were offered a free follow-up diagnostic examination at one of three clinics participating in the HI-CAHS grant work (see Appendix C).

While the subject sampling method was not random, it provided a population with a wide range of exposures, which allowed for quantitative assessment.

4.4 Instrument Design

A number of data collection instruments were developed for the study. These included farm information, phone log, farm/subject link, and informed consent forms; screener, smoking history, pre-shift and post-shift questionnaires; HF5 spirometry log sheet; dust and endotoxin data collection forms; field observations form; and request for health and safety information form. These are presented in Appendix D.

A pilot test of the screener, smoking history, and pre- and post-shift questionnaires was conducted. The draft questionnaires were administered in-person to four interviewers for the Colorado Farm Family Health and Hazard Surveillance (CFFHHS) Study (Xiang et al.,1999). These interviewers were asked to consider whether the questions were understandable, flowed well, and answered the questions of interest. Because of their experience in interviewing farming subjects and their technical knowledge of farming activities, the CFFHHS interviewers provided valuable feedback in terms of both worker reaction and interview technique. Questionnaires were modified based on the comments and findings of the pilot interviews. The study field staff administered the questionnaires to ensure

efficient and complete data recording, as well as to ensure the subjects understood the questions.

Farm Information Form: The Farm Information Form (F1) included farm-related information that was gathered by telephone at the first and all subsequent contacts with each farm owner. This information documented the owner's agreement to participate and provided information necessary for the field teams to prioritize scheduling.

Phone Log: The Phone Log was used to summarize useful information for future scheduling of visits to each farm, e.g., expected or actual harvest start dates, or days the team visit could not be made.

Farm/Subject Link: The Farm/Subject Link Form (F2) was used to list all harvest workers present at each farm, whether or not they became subjects. If a worker refused to participate in the study, the reason was recorded on this form. If the subject did not refuse to participate, but was excluded for another reason, this was recorded on the form. The subject ID number assigned to each worker was recorded next to the subject's name on this form. This was the only form on which the subject's name was recorded.

Informed Consent Form: The Informed Consent Form (CONF) listed the primary benefits and potential risks associated with study participation. This form was

required and approved by the Colorado State University Human Research Committee. Each potential participant was provided a copy of the form, and asked to read and sign the form prior to any interviewing or sampling activity. One copy of this form was given to the subject; a second copy was retained for the study file.

Subject Screener Questionnaire: The Subject Screener Questionnaire (INC) listed criteria for which a subject would not be eligible to participate in the study. If a subject answered "yes" to any of the screener questions, he or she was not included in the study. All willing workers on the farms visited were included in the study, unless they 1) were under 18 years of age, 2) were on medication for recent surgery, cold, or heart or lung disease, 3) had a collapsed lung in the previous six months, or 4) would be using a bronchodilator or corticosteroid medication during that work day. Both male and female harvest workers, including farm owners and operators, were included in the study. Workers performing all harvest tasks were included to ensure that a wide range of exposures was represented.

Pre-Shift Questionnaire: The Pre-Shift Questionnaire (PRE) was administered immediately after the screener and before workday wheat harvest exposure began. This questionnaire included questions focusing on acute and chronic respiratory symptoms and work history questions:

- The acute symptoms questions (H1 - H12) were based on Lawther et al. (1970) amended for oral presentation and including 10 symptoms previously reported at a prevalence of 11-77% among grain workers (doPico,1977). Acute

symptoms were those associated with eye, nose, and throat irritation, cough, productive cough, phlegm, wheezing, dyspnea, chest discomfort, fevers or chills. In addition, tingling fingers and blurred vision were included to serve as control symptoms, i.e. symptoms not expected to be associated with dust exposure. Each subject was asked to rate the severity of each acute symptom based on how they felt at that moment. The rating was on a scale of 1 to 5, with 1 indicating no problem, 2 indicating a mild problem, 3 indicating a moderate problem, 4 indicating a severe problem, and 5 indicating a very severe problem. A laminated symptom rating card that listed these ratings was handed to each subject.

- Chronic respiratory symptoms questions H13 - H27 were based on the Respiratory Conditions and Allergies module of the CFFHHS Principal Operator's Questionnaire. The CFFHHS questions were based on 1) the Third National Health and Nutrition Examination Surveys (NHANES III) Adult Respiratory and Allergy Section (NCHS, 1990) and 2) proposed questions for identifying ODTS (Rylander,1990). It should be noted that the participants were not asked directly whether they had a diagnosed chronic respiratory disease. The presence of asthma, chronic bronchitis and emphysema are inferred from each participant's responses about symptoms in the last 12 months. Question H28 asked about family history of allergies, asthma, chronic bronchitis, and emphysema as recommended in the Epidemiology Standardization Project (Ferris,1978).

- The work history question (W1) addressed previous exposures to visible dusts or metal fumes for more than one hour per day, gases or vapors, or herbicides and insecticides. Questions were also included to estimate how many years the subject had lived and worked on a farm (W2 - W3) and harvested wheat (W4 - W5). Recent exposure to wheat dust was assessed (W7 - W8). The employment category of harvest worker (family member, local hire, or custom cutter) was also determined.

Smoking History Questionnaire: The Smoking History Questionnaire (S) included questions to determine the smoking status and pack-years of smoking for each subject. In addition, information needed to properly interpret the spirometric results was collected. Smoking questions were modified from the CFFHHS Principal Operator's Questionnaire (Xiang et al.,1999).

Post-Shift Questionnaire: The Post-Shift Questionnaire (POST) was administered after at least six hours of harvest work or, if the subject worked less than 6 hours, at the end of the harvest workday. This questionnaire was designed to address cross-shift changes in acute symptoms. Thus, questions were identical to the pre-shift acute symptom questions except that they had been re-ordered to reduce the possibility of subjects attempting to match their pre-shift responses. To assess possible response biases, each subject was also asked to estimate the amount of dust exposure experienced during the day, whether they had used medications, or smoked during the day. In addition, questions were asked to supplement and verify

team field notes regarding harvest tasks performed and any respiratory protection worn.

HF5 Spirometry Log Sheet: The HF5 Spirometry Log Sheet was used to record information needed to properly interpret the spirometric results and validate the computer output. Information included date, time, spirometer identification, team leader, ambient temperature and barometric pressure, the age, weight, gender, and race of the subject, whether the subject had smoked or eaten a heavy meal in the past hour or had an upper respiratory infection in the past three weeks, and the total number of maneuvers and quality code. Because height is a critical variable in assessing normal spirometric values, height was measured using a solid tape measure while the subject stood on a plywood platform. The height of heels and subject with heels was recorded on the first page of the Pre-Shift Questionnaire.

Dust and Endotoxin Data Collection Forms: Several forms were developed for recording sampling data. The Filter Weight Record was used to record pre- and post-sampling filter weights. The Pump Calibration Log Form was used to record pump flow rate calibration data before and after each day's sampling, and the barometric pressure and temperature at each calibration location. The Field Data Collection Form was used to record the subject identification number, sampling pump used, the time, temperature, pressure and humidity when the pump was turned on and off, and chain of custody information. The barometric pressure was measured in millimeters of mercury with an altimeter. The temperature was

measured in degrees Celsius using a glass mercury thermometer. Humidity was measured using a Cole-Palmer humidity meter.

Field Observations Form: At several hour intervals throughout the day, the tasks performed by each subject, e.g. cleaning grain bins, loading and unloading grain, combine or truck driving, or auger operation, were recorded on the Field Observations Form. Other important exposure variables, such as use of respiratory protection, observation of visible dust, wetting of dusty operations, enclosed tractors, or other dust sources, were recorded. The duration of each task was recorded to the best of each team member's ability.

Request for Health and Safety Information Form: The Request for Health and Safety Information Form was used to record any question or request for literature or assistance in agricultural health or safety. Four subjects made such requests, which were forwarded to HI-CAHS staff for responses.

4.5 Measurement of Respiratory Function

Spirometry has been adopted from the clinical setting for use in epidemiological studies because it is simple, convenient, safe, inexpensive, noninvasive, sensitive to early detection of disease, highly reproducible for a particular individual, and useful in the detection of changes from baseline over time (Eisen,1987; NIOSH,1981). While a number of other diagnostic tests have

been utilized to determine the responsible agent or mechanism of grain-induced disease, including bronchial reactivity to methacholine, allergy skin prick and conjunctival provocation tests, serum precipitins, total IgE, chest radiographs, serum complement levels and leukocyte counts following exposure, and bronchioalveolar lavage (BAL), these are too expensive, time-consuming, or invasive to use in a workplace setting (Chan-Yeung et al.,1979; Warren et al.,1974).

Spirometry is the measurement of lung volume changes during clearly defined respiratory maneuvers. The forced vital capacity (FVC) measurement involves a maximum exhalation made as rapidly as possible following a maximum inhalation. The spirogram is a graphic recording of volume displayed against time, while the flow-volume curve is a graph of expiratory flow rate displayed against volume. Three parts of the FVC maneuver have been described (Miller,1986c). Phase I is effort-dependent. Phase II, or critical flow, shows effort-independent constant deceleration. Phase III is terminal leakage. The spirometric parameters measured during this study include:

- Forced vital capacity (FVC) – the total volume expired during the forced maneuver.
- Forced expiratory volume in one second (FEV_1) – the volume of air exhaled from a full inhalation in one second.
- Forced expiratory volume in six seconds (FEV_6) – the volume of air exhaled from a full inhalation in six seconds.
- Ratio of FEV_1 to FVC (FEV_1/FVC) – the portion of the FVC expired in one second (the FEV_1/FVC is between 70 and 80% in normal subjects.)

- Forced expiratory flow rate, 25% to 75% ($FEF_{25\%-75\%}$) - the flow rate for the middle half of the maximal expiration, determined by the effort-independent part of the maneuver (calculated as the slope of the line connecting the points on the spirogram at which 25% and 75% of volume have been expired.)
- Peak expiratory flow rate (PEFR) - the maximum forced expiratory flow obtained within the first 15% of the volume expired and sustained for 10 milliseconds, determined by the effort-dependent part of the maneuver.

Pre-shift spirometry tests were conducted on each subject before beginning harvest work for the day. Post-shift spirometry tests were conducted at the end of the work day immediately upon conclusion of exposure monitoring. Spirometry was conducted by trained staff using NIOSH's HF5 system, which features a rolling seal spirometer, an IBM-compatible notebook computer, automated calibration and operation, comparison of the subject's performance, and a real-time display of flow-volume curves. The system exceeds the minimum recommendations of the American Thoracic Society and NIOSH for spirometry performance (Hankinson,1986b; NIOSH,1981; Ferris,1978).

All spirometry tests were conducted in accordance with NIOSH's Spirometry Procedure Manual (NIOSH,1992). The spirometer was calibrated and leak tested with a 3-liter syringe before testing subjects each morning and evening. The acceptable average of the difference between three calibration checks was +/-3 percent. These criteria have been shown to provide data robust enough for statistical analyses for cross-shift studies (Townsend,1984). Each team leader also

performed a lung function test on themselves before each morning and evening session.

The pre- and post-shift testing temperature was maintained within 5°F of each other to allow for accurate cross-shift comparisons (Hankinson et al.,1986a). To achieve this, the spirometer was kept out of the direct sunlight. On cool mornings, the van was heated to about 80-85°F. For post-shift testing, the van was air conditioned to the pre-shift temperature.

The spirometer was cleaned with rubbing alcohol and the cover was sealed with stopcock grease after each morning session. A new mouthpiece and clean sensor was used for each subject tested. Hoses and noseclips were cleaned by soaking for one minute in a 1 ounce bleach:1 gallon water solution followed by air drying.

Three acceptable FVC maneuvers were obtained from each subject. Subjects were in a sitting position which is acceptable to the American Thoracic Society (Ferris,1978). Each subject wore a noseclip during the maneuver. An acceptable maneuver included adequate understanding, an unhesitating start, apparent maximal effort with smooth continuous exhalation, and absence of cough, glottis closure, second inspiration, leak or blockage. The end of the expiration maneuver was defined as that point when, after at least six seconds, the volume change was less than 25 ml or flow was less than 0.05 L/sec. The HF5 spirometer system was set up to graphically display the flow-volume curve during the maneuver as this

curve permits easier visual comparison of expiration efforts (Boushey et al.,1982). The HF5 system presented FVC, FEV_1, and PEFR results immediately following each test. These features enabled the team member to determine when three acceptable tests had been completed. Up to eight maneuvers were obtained to achieve the desired reproducibility in three maneuvers, i.e. the three best FVCs were within +/-5% of each other, the three best FEV_1s were within +/-5% of each other, and the two best PEFRs were within +/-10% of each other. These reproducibility specifications are in accordance with NIOSH's recommendations for occupational spirometry (Hankinson,1986b; NIOSH,1981).

The computer software converted lung volumes and flow rates from ambient temperature and barometric pressure to values for body temperature, pressure saturated (BTPS) values (NIOSH,1992), and then calculated FVC, FEV_1, FEV_6, $FEF_{25-75\%}$, PEFR, and FVC/FEV_1 from the spirograms for each subject. The starting points for FEV_1 and FEV_6 were obtained by backward extrapolation, i.e. the zero time point was determined by extending the steepest portion of the tracing backward to compensate for a hesitant start. If the extrapolated volume exceeded 10% of the FVC, the test was not included. The largest of the three FVCs was recorded as the FVC for the test. The largest of the three FEV_1s was recorded as the FEV_1, even if the two values did not occur on the same maneuver. The FEV_6 was determined in the same manner. In working populations, less than 3% of the subjects are unable to perform reproducible and acceptable spirometry tests (Miller,1986c). Individuals who are unable to perform reproducible tests are

generally those with the most disability, and thus irreproducible results were not excluded from this study (Eisen,1987).

The computer files were returned to NIOSH for data abstraction and review by a pulmonary physiologist. Percent of predicted spirometric values were calculated from Knudson (1983) for the age, gender, and height of each subject. Reports were returned to Colorado State University for use in the study data analysis and for inclusion in reports to each study participant.

4.6 Exposure Monitoring

Each subject was monitored for total dust and endotoxin for the entire workshift, or at least 6 hours, depending on work schedules. The field staff also described the tasks performed by each subject during the workday, e.g. cleaning grain bins, loading and unloading grain, or combine or truck driving. Other important exposure variables, such as use of respiratory protection, wetting of dusty operations, or enclosed tractors, were recorded.

Total Dust and Endotoxin Sampling

NIOSH Method 0500 for personal sampling of total dust was used for sample collection (NIOSH,1993). Sampling was performed with pre-weighed, 37 mm glass

fiber filters (Gelman A/E Lot 234712) housed in 2-piece, closed-face cassettes (Millipore Lot H3HM99538).

Study results have been mixed as to the proper filter to use for endotoxin collection. Olenchock (1990a) reported that filter type had little consequence on the results. Gordon et al. (1992) reported higher extractable endotoxins from cotton dust sampled with cellulose ester and PVC filters as compared to glass fiber filters. Cosma and Ufferfilge (1994) found a significantly higher extractable endotoxin when sampling grain dust at elevators in Colorado with glass fiber filters as compared to PVC or cellulose acetate filters. Glass fiber filters have also been shown to yield significantly higher endotoxin activity than PVC filters when the chromogenic *Limulus* amoebocyte lysate (LAL) assay was used to analyze poultry and swine confinement aerosols (Thorne et al.,1997). Based on these last two reports, glass fiber filters were selected for the study. Both total dust and endotoxin were collected on the same filter for each subject.

The filters were desiccated overnight and weighed in the laboratory (Mettler H5AR analytical balance, SN 67117) prior to field use. The balance had weighing capabilities to 0.01 mg and reproducibility of ± 0.02 mg. Dust weights were determined to the nearest 0.01 mg. Ten percent of all filters were desiccated another 24 hours and re-weighed to ensure a stable weight had been obtained. At this initial weighing, each cassette was assigned a unique, sequential three-digit identification number beginning with the letter W (e.g. W001).

Sampling was conducted with cassettes in a "closed-face" configuration, i.e. with only the inlet plug removed from the cassette. There have been mixed reports regarding the efficiency of sampling with open-face versus closed-face cassettes. While Bien and Corn (1971) suggested that sampling through small diameter inlets could reduce collection efficiency of large particles, Breslin and Stein (1975) infer a 95 to 100% collection efficiency for particles up to 20 um using a 4 mm diameter inlet (i.e. closed-face cassette) at 3 lpm. Vincent (1995) points out that closed-face sampling tends to undersample the inhalable fraction of dust under workplace conditions. Fidino (1979) showed that significantly more paint was collected with open-face cassettes as compared to closed-face cassettes when sampling industrial paint spraying operations (average ratio of 1.3). The one study which examined the effect of inlet diameter on grain sampling showed a mean of 12 total dust samples of 103 mg/m^3 for open-face and 51 mg/m^3 for closed-face sampling (Arlington,1979). As a result of these findings, Janssen (1979) used open-face cassettes, but concluded that closed-face cassettes would have prevented loss of dust from the filters. In a study of total dust and microorganisms, Morey (1989) found comparable results between closed-face cassettes and glass impinger collection. The closed-face cassette configuration was selected for this study for several reasons. While a possible large particle or gravimetric bias could be significant for agents which exert a systemic influence, grain dust primarily affects the respiratory system. Also, for this study, there was concern about loss of dust from the filter face during the long sampling period.

The cassette was attached to the sampling pump via a 30- to 34-inch section of flexible tubing. The pump was attached to each subject's belt and the cassette was positioned on the lapel in the "breathing zone" 6 to 9 inches from the subjects mouth (Figure 4-1). SKC Airchek (Model 50, 224-43XR or 224-PCXR7) or Gilian Hi-Flow (Model HFS113A) personal sampling pumps were operated between 1.5 and 2.0 liters per minute for the observation period. Pumps were fully charged before each field use and calibrated via a primary standard (electronic or manual bubble meter) before and after each sample collection. Temperature and pressure were recorded at each calibration and at the beginning and end of each sample period. The final air volumes were corrected as needed for differences between calibration and sampling conditions. Humidity was recorded at the beginning and end of the sampling period.

Cassettes were immediately sealed and placed in a styrofoam cooler with ice after sample collection. Cassettes were transferred to a standard freezer upon arrival back at Colorado State University, approximately 2 to 12 hours after sample collection. Each field team submitted one field blank filter cassette with each day's samples as a quality assurance sample.

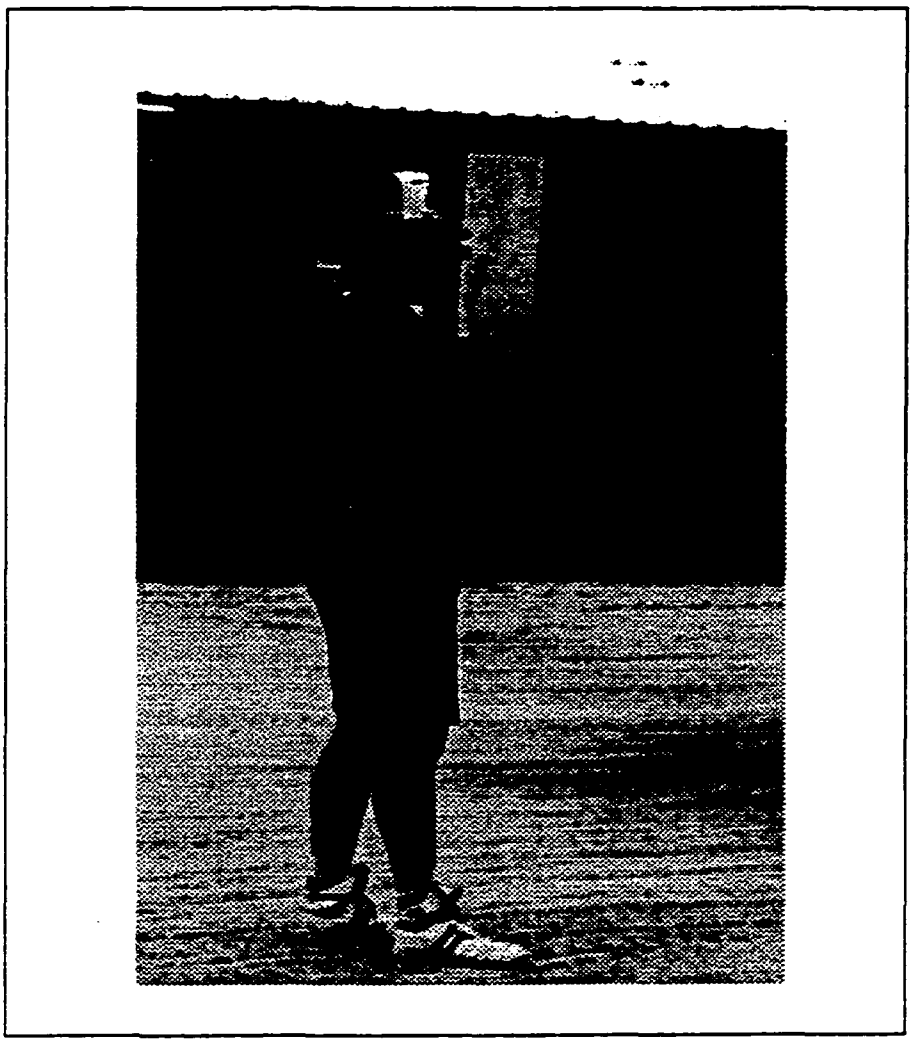

Figure 4.1 Subject Wearing Sampling Equipment

Laboratory Analysis for Total Dust and Endotoxin

The filters were desiccated after sample collection as described above. Total dust analysis was then performed gravimetrically in accordance with NIOSH Method 0500 for nuisance dust (NIOSH,1993). The pre-collection filter weight was subtracted from the post-collection weight to determine the weight of dust sampled.

After weighing, filters were placed in 50-ml sterile polypropylene conical centrifuge tubes with screw-on caps (Cole-Parmer #H-06325-50). Tubes were placed in a standard freezer until shipment on dry ice to NIOSH's laboratory in Morgantown, WV for endotoxin analysis. At the laboratory, 10 ml of sterile, pyrogen-free water was added to each sample tube [Mull,1994; Olenchock et al.,1989a]. The tubes were rocked gently at room temperature for 60 minutes. The supernatant was transferred into sterile 15 ml centrifuge tubes and centrifuged at 2200 rpm, 4°C, for 10 minutes. Four ml of supernatant were then transferred to sterile 4 ml NUNC vials for storage at -85°C. Endotoxin was determined on 5-fold dilutions of these latter supernatants using the quantitative chromogenic modification of the *Limulus* Amoebocyte Lysate (LAL) assay by BioWhittaker Kinetic-QCL test kit (BioWhittaker,1994). Multiple dilutions allow for an assessment of inhibition or enhancement of endotoxin level by interfering chemicals or proteins in the sample (Hollander et al.,1993).

The time-weighted average (TWA) exposures for airborne dust and endotoxin were determined for each subject by dividing the total weight of dust or units of endotoxin on each filter by the volume of air sampled. The 8-hour TWA (8-hr TWA) was then determined by multiplying the TWA exposure by the number of hours sampled and dividing by 8 hours.

4.7 Field Team Preparation and Training

Each farm was visited by a two-person team including a team leader and assistant. Four team leaders and seven assistants were trained and equipped for the study. The team leaders were responsible for maintaining approximately 20 farm files, calling each farm contact to determine probable harvest dates and scheduling farm visits for the team. At the farm, the team leaders were responsible for introducing the team and explaining the study to the workers. The team leaders were also responsible for handling any sensitive topic, such as a worker's concern about regulatory enforcement. The team leaders conducted spirometry on each subject. Team leaders were all experienced industrial hygiene professionals, and three were certified industrial hygienists. The team assistants were responsible for collecting air samples on the subjects, including pump calibration and all sample handling. Team assistants were students or faculty members involved in the agricultural health and safety field. The team members shared in explaining the consent form, administering the questionnaires, and recording field observations. The same team member conducted both the pre-shift and post-shift interview on the same subject.

The following training was provided to the team members:

1. In June 1994, NIOSH staff provided 3 days of spirometric training to all team leaders.
2. Team assistants without prior field experience received a full day of training in air sampling.

3. All team members received a full day of training in study-specific procedures, field forms and questionnaire administration.
4. A Field Procedures Manual was developed and provided to each team for reference in the field, as needed.

Three Colorado State University vans were available and equipped for the duration of the study period (Figure 4.2). A list of equipment provided in each van is presented in Table 4-1. Window covers were constructed from cardboard covered with aluminum foil to keep direct sunlight from inside the van and to reflect heat during the day. The spirometer was placed in the travel case after the pre and post-shift measurements.

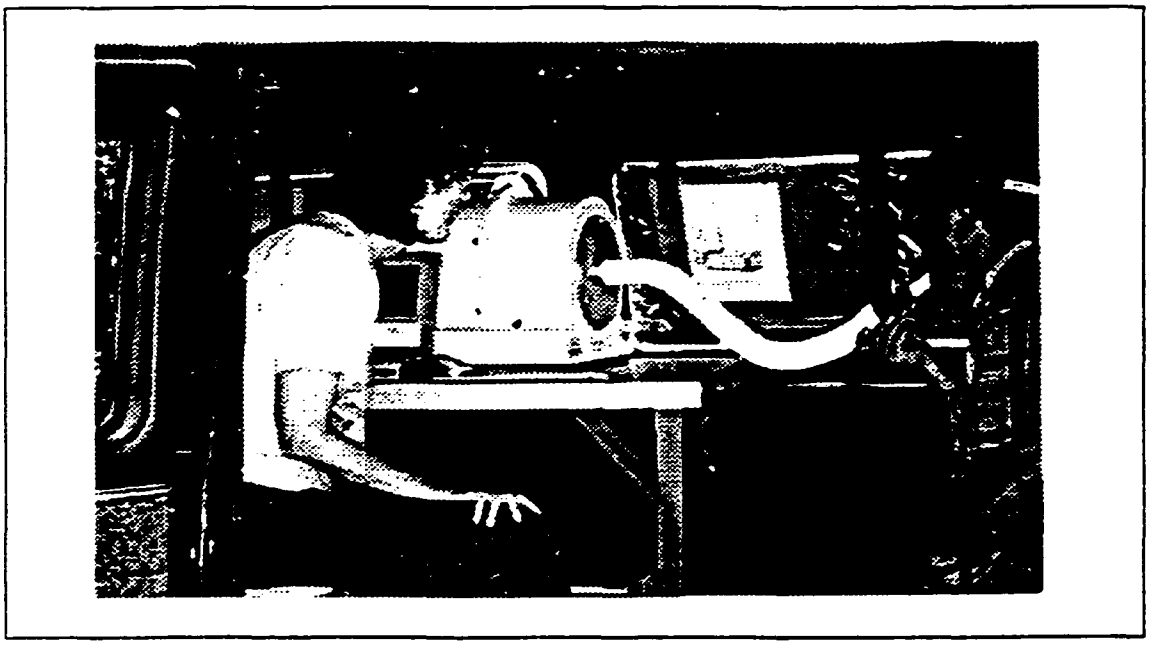

Figure 4.2 Van Setup – Conducting Spirometry

Table 4.1 Field equipment and supplies provided in each van

Miscellaneous:	Spirometry Equipment:
Map and directions to farm(s)	Large blue trunk/spirometer
Gas credit cards	Calibration syringe
Water container	1 box mouthpieces (14/visit)
1 chair	Hose labels/rubberbands
1 stool	Gauze pads
Table	Altimeter
Trash can	Humidity/temp gauge
Whisk broom	AA batteries
Power inverter	Large garbage bags
Extension cord	Stopcock grease
3-to-2 prong adapter	Rubbing alcohol
Aluminized window covers	Paper cups/paper towels
Band-Aids	Tape measure
CSU Ball Caps	Plywood Platform
	Hoses (7/visit)
Field Forms:	Laptop computer and floppies
	Noseclips (14/visit)
Field Procedure Manual	
Spirometry manual	Sampling Equipment:
Farm Folder(s)	
Disbursement Envelope	Clipboard
Phone logs	Cooler with blue packs
Farm/subject links	7 High-flow personal air sampling pumps
Confidentiality forms	
Questionnaires	Pump recharging unit - overnight
HF5 log sheets	Bubble meter - overnight
Pump calibration forms	Filter cassettes - 18/visit
Data collection forms	7 3-foot lengths of tygon tubing
Field observation forms	7 luer adapters
H&S request forms	7 lapel clips/safety pins
HI-CAHS packets	7 webbed belts
	1/2 inch waterproof tape
Personal items:	1 roll duct tape
	Watch
Watch	Sharpie markers/pens
Sunscreen	Ziploc baggies - quart size
Hat	Symptoms card
Sunglasses	
Overnight bag	

4.8 Data Control and Storage

Each county in the study area was assigned a sequential two-digit number starting with the letter C. Each farm was assigned an identification number when the owner agreed to participate in the study. Farm identification numbers were omprised of the county number followed by a sequential farm number. For example, C01F01 was farm number one in county number one. A Farm File was also established when each farm owner was enrolled into the study. The Farm File was a manila folder into which all information from the farm was inserted. Initially the file contained only the Farm Information Form (F-1), Phone Log, and Farm/Subject Link Form (F-2). After signing the confidentiality form, each subject was assigned a unique subject identification number linked to each farm identification number. For example, C01F01S03 was the third subject enrolled at farm number one in county number one. The air samples were linked to each subject by simply adding the subject identification number to the cassette number, e.g. W001-C02F03S09. The information linking the data was kept in a file cabinet in a locked room with limited access. Only the principal investigator and co-investigator had access to the linked data forms. Data were entered into computer files with only the subject identification number. No information linking specific individuals with their responses or exposures was available or presented in reports. The subject's response to each question on the questionnaires was assigned a code as indicated on the instruments.

All data from the study, including questionnaire response codes and quantitative values for exposure and spirometry, were entered into dBase V by subject identification number. A series of data logic checks were run to ensure the accuracy of data entry (e.g. the number of years that a subject lived on a farm was equal to or less than their age, and only allowable responses were present). The data were then transferred as an ASCII file to SAS for data analysis.

4.9 Data Analysis

The collection of discrete and continuous data (see Table 4.2), the large number of potential inter-relationships, and the non-random cluster design required that several statistical methods be employed in the data analysis. This made it possible to identify the important variables and eliminated those of little or no significance from further consideration. All calculations were performed using SAS™ software (SAS,1985).

Simple descriptive statistics were used to characterize basic study variables. Frequencies were calculated for discrete variables. Mean, median, range and standard deviation were calculated for continuous variables. Simple linear regression was used to show the relationship between some variables (e.g. total dust exposure versus endotoxin exposure, total dust exposure versus humidity). Fisher's exact chi-square analysis (2-tailed) was then used to determine whether a significant association between the variable and response existed. T-

Table 4.2 Study variables

Discrete variables	Quantitative variables
Dependent:	Dependent:
8 acute symptoms cross-shift change: I/D/NC	Percent change in spirometric variables:
Respiratory Index*: -3 to 7	FVC, FEV_1, FEV_6, FEV_1/FVC, $FEF_{25-75\%}$, PEFR
Obstruction: Y/N	
Independent:	Independent:
Demographic: Gender: M/F Race: White, non-white Ethnicity: Hispanic, non-Hispanic Education: 8 classes Employer: Farm family, local worker, custom cutter	Demographic: age, height, weight, BMI
Smoking status: Y/Ex/N/passive Smoking during shift: Y/N	Smoking: packs/day, years smoked, pack-yrs
Use of respirator during shift: Y/N Use of medications during shift: Y/N Pre-shift dust exposure: Y/N Task: 5 categories	Occupational: Years in agriculture Years in harvest Days/year harvest Days harvest in 1994
Past use of herbicides: Y/N Past use of insecticides: Y/N Past work with visible dusts: Y/N Past work with visible metal fumes: Y/N Past work with gases/vapors: Y/N	Total dust exposure (TWA, Log) Endotoxin exposure (TWA, Log) Endotoxin/dust ratio
History of chronic illness: Y/N Family history of chronic illness: Y/N	

* Respiratory Index equals the sum of the eight acute symptom changes.

tests (2-tailed) were used to determine whether there was a difference between discrete groups for a continuous variable. The prevalence of existing respiratory conditions was compared to other reported prevalences using the z-test for difference in proportions (Fleiss,1981).

A number of general linear models were used to provide an assessment of the association between continuous and discrete variables. Tukey's GLM procedure was used to test the association between the five job categories and total dust (or endotoxin) exposure. The simple GLM procedure was used to test the association between exposure and cross-shift change in acute symptoms. The NPAR1WAY (nonparametric) procedure was used to compare the respiratory index with discrete variables. The association of total dust and endotoxin exposure with change in lung function over the workday was accomplished via backward logistic regression analysis, including the following nine variables as co-variates: smoking status, age, dust exposure in morning before study visit, respirator use during shift, medication use during shift, history of herbicide exposure, presence of obstruction, number of days harvesting in 1994, and history of any chronic respiratory symptom (SAS,1985; Kleinbaum et al.,1978). This analysis allowed for the determination of the independent variable(s) possessing the greatest degree of association with the respiratory change outcome of interest.

Cross-tabulation was used to form contingency tables between discrete variables. Correlation coefficients were used to assess the relationship between some

discrete and continuous variables. For example, Pearson's correlation coefficient was used to compare the worker's estimation of dust exposure and changes in cross-shift acute symptoms. Spearman correlation (nonparametric) was used to compare the respiratory index to quantitative variables.

To compare the results of the spirometry exams with the self-reported respiratory conditions, contingency tables were constructed from which the specificity, sensitivity, and predictive values of both positive and negative tests were calculated. The spirometry result was used as the "gold standard" in determining how well a subject's self-report compared to a measurable parameter both with and without controlling for smoking.

4.9 Control of Confounders/Bias

In any occupational study, the "healthy worker effect" is a potential confounder in that respiratory-sensitive individuals may have migrated away from farm work to other jobs in the community. This should tend to reduce observed differences among subjects. The effect is not expected to be as great among family farmers as among grain elevator workers (Manfreda et al.,1986; Chan-Yeung et al.,1992b; doPico et al.,1992). However, custom cutters were also included in this study. Custom cutters represent a population in which severely affected individuals may have been forced to change jobs. Thus, the study population may be "healthier" than the general population. Evaluation of past history of respiratory disease and

occupation from the questionnaire should help clarify whether this effect is present in the study population.

Whenever questionnaires are used, recall error is a potential source of bias. The study questionnaire limited recall about chronic respiratory conditions to the last 12 months. Thus, recall bias about symptom history is not likely to be a significant source of bias (Horne,1989).

Subjects were asked to rate the severity of acute symptoms before and after the same workshift. There existed the possibility that subjects would alter responses to match (or not) the pre- and post-shift responses. While this problem could not be totally eliminated, the post-acute symptom questions were scrambled so the respondent was less likely to repeat the same answers by memorization of the pre-responses. Two false symptoms were also included (blurred vision and tingling fingers). In addition, each subject was asked to categorize his/her dust exposure during the day, with this response analyzed in relation to reported symptoms to determine whether reported changes were based on perceived dust exposure.

There are a number of confounding factors that may affect an individual's response to grain dust. Two such variables are smoking status and past grain dust exposure. In order to evaluate the significance of smoking status in this study, both a qualitative smoking category and a quantitative value of pack-years was assigned to each subject. The qualitative smoking categories were: current smokers, past

smokers, passive smokers, and those who had never smoked (smoked less than 100 cigarettes total in their lifetimes). Years of grain dust exposure was evaluated for each subject based on response to the questions "W2. How many years have you lived on any farm?", "W3. How long have you worked on any farm?" and "W4. How many years have you worked harvesting wheat?"

In a cross-shift study, bias can occur during subject selection or loss of individuals during the study. An evaluation of characteristics of individuals who refused to participate was performed. No subject was "lost to follow-up" and all subjects completed the post-spirometric examination.

There are a number of technical and subject factors that can influence spirometric values. Up to three percent of the variation in between-individual measurements can be attributed to technical variation (Becklake,1986). Technical variation was controlled by using instrumentation and methods that met the American Thoracic Society criteria, trained and accredited spirometry technicians, computer-driven algorithms for test acceptability, detailed procedures, and consistent calculations (Becklake et al.,1993; Enright,1992; Dawson et al.,1982; NIOSH,1981; Ferris,1978). Subject factors were controlled as follows:

- *Gender, age, height:* Gender, age, and height are significant factors in normal lung function values. Prediction equations for 'normal limits' of FVC, FEV_1, and $FEF_{25-75\%}$ based on gender, age, and height were utilized for this study (Dockery et al.,1985; Knudson,1983).

- *Race:* Differences in pulmonary function values related to race and ethnic backgrounds have been generally overlooked in epidemiological surveys. Black persons residing both in Africa and the New World have smaller height coefficients for lung volumes and flows than Caucasians (Miller,1986b). Useful data for Mexican-Americans is limited (Clausen,1982). Horne et al. (1989) found that British grain workers had a significantly greater prevalence of airflow obstruction than Swedish grain workers. Race and ethnicity questions were included in the study questionnaire. However, the study population was primarily white and non-Hispanic.

- *Environmental Exposures/Smoking:* Smoking is probably the most important environmental exposure affecting spirometric tests. Duration of smoking and pack years, including passive smoking, account for up to 15% of the residual variation between individuals after accounting for age, height, and gender, and explains almost all the lung function decrease among smokers (Becklake et al.,1993; Miller,1986c). Smoking immediately before spirometry may reduce flow rates, but are unlikely to diminish FVC or FEV_1 (Miller,1986c). Smoking history and smoking before each spirometric test were recorded for each subject in this study.

- *Body position:* Spirometric values decrease as the body moves from a standing to sitting to semi-recumbent to supine position (Becklake et al.,1993). Subjects were seated in the field van in this study as part of the effort to assure consistent pre- and post-spirometer temperatures. However, both tests were conducted in the seated position and percent change in spirometric value should not be affected.

- *Circadian rhythm:* FVC, PEFR, and FEV_1 display diurnal rhythm, rising in the morning to a maximum in the afternoon, then falling to a minimum during the night. Cross-shift changes in spirometric variables were compared to

cross-shift changes in other populations to determine the significance of the change.

- *Weight:* Spirometric results are positively correlated with weight to the extent that increased weight means increased growth, height, or muscle mass. Beyond this (e.g. with obesity), spirometric values decrease with greater weight. However, only two percent of the variation between individuals can be explained by a difference in weight, it is often excluded from predictive equations (Becklake, 1993; Clausen,1982). Weight was recorded for this study and body mass index included an independent variable.

- *Upper respiratory infection:* Simple upper respiratory infection has not generally been found to decrease spirometric results in adults. However, subjects were asked if they had had an upper respiratory in the previous two weeks before the study.

- *Muscular development:* There is evidence that athletes, divers, miners, and those whose work activities develop the pectoral muscles have a relatively greater FVC compared to FEV_1. Short term strength training resulted in only a 4% increase in FVC (Miller,1986c). Muscular development was not addressed in this study.

- *Tracheal diameter:* Tracheal cross-sectional diameter correlates with PEFR but not with flows at lower lung volume (Miller,1986c). A chest radiograph is required to determine tracheal diameter, so it was not addressed in this study.

- *Socioeconomic factors:* Spirometric values are lower in socioeconomically disadvantaged children and adults (Becklake et al.,1993). Aspects which may be responsible include nutrition, crowding with increased frequency of

respiratory infections, access to health care, and increased atmospheric pollution in the home, community, and workplace (Becklake et al.,1993; Miller,1986c). Socioeconomic information was not obtained from the study subjects.

- *Alcohol:* Epidemiological studies have either shown an inverse relationship between alcohol consumption and pulmonary function or no relationship when smoking is controlled. Questions about alcohol consumption were not asked in this study.

- *Time of year:* The time of year influences spirometric values, perhaps due to difference in levels of pollutants or incidence of respiratory infections. The study occurred during a six-week period in the summer, so time of year was not considered a significant factor in the study.

- *Altitude:* While altitude is thought to significantly influence spirometric results due to decreased air density, the relationship between normal spirometric values and the altitude history of an individual is not well defined (Miller,1986c; Clausen,1982). Altitude history was not obtained for this study. However, three-fourths of the subjects were local residents.

Factors such as different farming methods, humidity, and rain create different regional grain dust composition at harvest and, thus, different exposure patterns. This confounder was controlled by performing the study in a limited geographical area during a single harvest season.

Finally, some small error in measurement of filter weights and sampling data, such as time of sampling, are present. Because there was a detectable quantity of dust and endotoxin on all samples, the problem of censored data did not exist.

Chapter 5

Results and Discussion

Grain dust has been shown to cause acute respiratory effects in exposed workers. The causative agent for the acute effects is unknown, but endotoxin has been implicated as a possible cause. Harvest workers represent an exposed worker group that has not been extensively studied. An earlier study in northeastern Colorado indicated that harvest workers have grain dust exposures that are as high or higher than elevator workers (Beard,1994). The primary purpose of this study was to evaluate the existing respiratory health of harvest workers, determine cross-shift respiratory effects, and to correlate the effects of total dust and endotoxin exposure with cross-shift respiratory changes.

5.1 Study Participation Rates

The 1994 growing season was extremely hot and dry so that the wheat crops ripened and were harvested sooner and in a shorter time period than typical. Thus, the field teams were not able to visit all the recruited farms during the shortened harvest season. Figure 5.1 shows the 15 counties included in the recruitment area and the 11 counties in which the final study farms were located.

The participation rates for both farm owners and harvest workers are shown in Table 5.1. Eighty-six farm owners were contacted before the 1994 winter wheat

harvest. Of these, 12 farms were ineligible due to lack of a wheat crop that season or too few harvest workers. Seven farm owners refused to participate due to harvest being too busy or concern for regulations. Thus, 67 farm owners had agreed to participate when harvest season began.

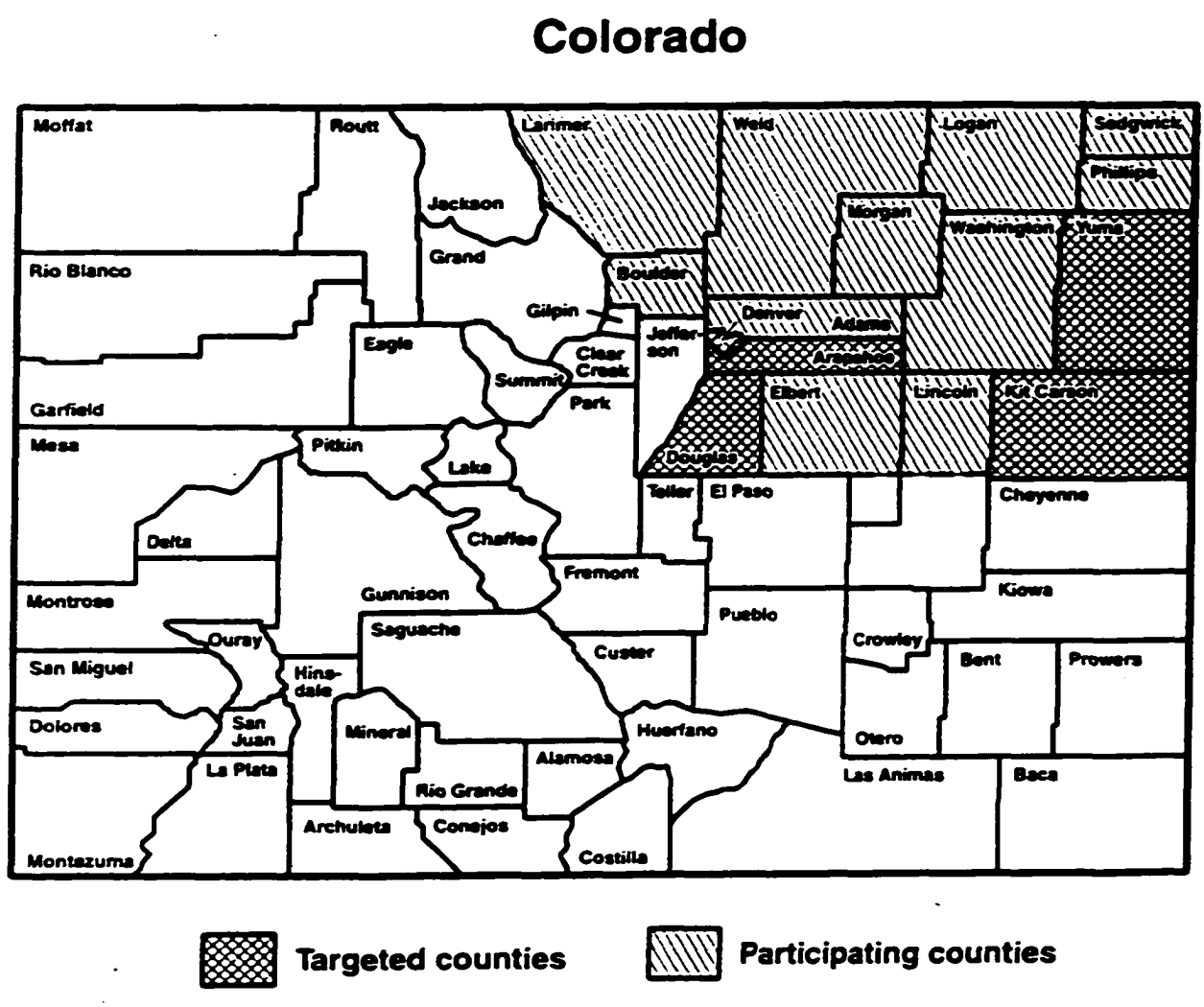

Figure 5.1 Counties Targeted and Participating in the Study

Of a total of 129 workers present at the 26 farms visited, 98 were recruited into the study. Fourteen workers did not meet the eligibility criteria for age and health condition. Eight workers had already started working in the field when the field team arrived at the farm. Three workers could not be included because the field team did not have enough air sampling pumps for all the eligible workers. Only five percent (6 of the 129 available workers) refused to participate. Four refusals came from farm owners who claimed to be too busy. Two workers were just not interested in participating.

Table 5.1 Study participation and refusal rates

Participant/refusal group	Number	Reason(s) for ineligibility/refusal
Farm owners:		
Contacted	86	
Willing to participate	67 (78%)	
Ineligible	12 (14%)	6 - Too few workers 6 - No wheat in 1994
Refusals	7 (8%)	4 - Too busy 3 - Too many regulations
Total farms in study	26	
Harvest workers:		
Present during field visit	129	
Ineligible	25 (11%)	14 - Ineligible 8 - In fields too early 3 - Not enough pumps
Refusals	6 (5%)	4 - Too busy 2 - Not interested
Total workers in study	98	

5.2 Population Description

Table 5.2 presents basic qualitative demographic characteristics of the subjects enrolled in the study by gender. There were 90 male and 8 female subjects. One male subject was black. Two of the male subjects considered themselves Hispanic. All other subjects were non-Hispanic and Caucasian. It was decided that there were not enough subjects in the non-white race and Hispanic ethnicity classes to analyze these variables further.

The largest number of subjects was in the 30-39 year-old age group (n=27). The second largest number of subjects was in the 19-24 year-old age group (n=23). The most frequent education level was high school graduate (n=29). However, a large number of subjects had at least some college (n=50) and others had attended technical school beyond high school (n=8). There was no statistically significant difference between the male and female subjects in the frequency of these demographic characteristics.

Table 5.3 presents the quantitative physical characteristics of the study subjects. The mean ages of the men and women were 38 and 34 years, respectively. For the men, this age was comparable to the 38 to 40 year mean age of grain elevator worker populations previously studied (Chan-Yeung et al.,1992a; Broder et al.,1979; doPico et al.,1977). The study population was somewhat younger than

previously-studied farmer populations whose mean ages were 42 and 45 for men and 41 for women (Dosman et al.,1987; Manfreda et al.,1986).

Table 5.2 Demographic characteristics of the study population by gender

Characteristic	Number of individuals (percent)[1]		p[2]
	Male - 90	Female - 8	
Age groups:			
19-24 yrs	21(23%)	2(25%)	0.869
25-29 yrs	10(11%)	0(0%)	
30-39 yrs	23(26%)	4(50%)	
40-49 yrs	17(19%)	2(25%)	
50-59 yrs	7(8%)	0(0%)	
60-69 yrs	8(9%)	0(0%)	
≥70 yrs	4(4%)	0(0%)	
Race:			
White	89 (99%)	8 (100%)	1.00
Black	1 (1%)	0 (0%)	
Hispanic:			
Yes	2 (2%)	0 (0%)	1.00
No	88 (98%)	8 (100%)	
Education:			
≤ 8th grade	4 (4%)	0 (0%)	0.90
Some high school	6 (7%)	1 (12.5%)	
High school graduate	26 (29%)	3 (37.5%)	
Some technical school	2 (2%)	0 (0%)	
Technical school graduate	6 (7%)	0 (0%)	
Some college	22 (24%)	3 (37.5%)	
College graduate	22 (24%)	1 (12.5%)	
Post-graduate degree	2 (2%)	0 (0%)	

[1] Column percents [2] Fisher's exact X^2 (2-tailed) between males and females

Not unexpectedly, the study men were taller and heavier, and had higher body mass indices (BMI = weight/height^2) than the women. The men were slightly taller and heavier than other male grain worker populations studied, whose mean height ranged from 172 to 177 cm, and mean weight ranged from 82 to 83 kg (Chan-Yeung et al.,1992a; Broder et al.,1979; doPico et al.,1977).

Twenty-seven (30%) of the men in the study were obese based on a BMI greater than 27.8 kg/m^2, while six women (75%) were obese based on a BMI of greater than 27.3 kg/m^2 (Taber,1995).

Table 5.3 Physical characteristics of the study population by gender

Characteristic	Mean	Range	Standard deviation	p[1]
Age (years):				
All subjects	38	18-76	15	
Male	38	18-76	15	
Female	34	19-48	9	0.423
Height (cm):				
All subjects	176	157-192	8	
Male	178	160-192	7	
Female	166	157-170	5	<0.0001
Weight (lb):				
All subjects	187	115-350	38	
Male	191	130-350	36	
Female	141	115-200	26	0.0003
Body mass index (kg/m^2):				
All subjects	26.7	19.5-51.4	4.9	
Male	27.0	19.5-51.4	5.0	
Female	23.0	19.5-31.9	4.0	0.0283

[1] T-test (2-tailed) between males and females

Table 5.4 presents the smoking characteristics of the study subjects. At the time of the study, 19 (19%) of the subjects were current smokers, 30 (31%) were ex-smokers, and 49 (50%) had never smoked. Thirteen of the nonsmokers were considered passive smokers, i.e. they reported that other people smoked in their homes. There were no significant differences between men and women in smoking status. The percentage of smokers in the study

population was much lower than in previously-studied grain elevator populations, whose smoking rates have ranged from 35 to 60% (Chan-Yeung et al.,1992a; Broder et al.,1979; doPico et al.,1977), and farming populations, whose smoking rates have ranged from 30 to 34% (Dosman et al.,1987; Manfreda et al.,1986).

Table 5.4 Smoking characteristics of the study population

Smoking Status	Number of individuals (percent)[1]		p[2]
	Male	Female	
Smoking status I:			
Current smoker (n=19)	17 (19%)	2 (25%)	0.18
Ex-smoker (n=30)	30 (33%)	0 (0%)	
Passive smoker (n=13)	11 (12%)	2 (25%)	
Never smoked (n=36)	32 (36%)	4 (50%)	
Smoking status II:			
Smoker (n=49) *(current + ex)*	47 (52%)	2 (25%)	0.27
Non-smoker (n=49) *(passive + never)*	43 (48%)	6 (75%)	
Smoking history for smokers (current plus ex-smokers)	**Mean**	**Range**	**Standard deviation**
Packs of cigarettes smoked/day (n=49)	1.08	0.05-3.50	0.91
Number years smoked (n=49)	19.01	0.50-59.5	16.31
Smoking pack-years:			
All smokers	15.65	2-42	10.22
Males	15.13	2-42	10.04
Females	28.00	23-33	7.07

[1] Column percents [2] Fisher's exact X^2 (2-tailed) between males and females

The 49 smokers (current plus ex-smokers) reported smoking between 1 cigarette per day and 3.5 packs of cigarettes per day (mean=1.08 packs) for between 6 months and 59.5 years (mean=19.0 years). The range of smoking pack-years was from 2 to 42 (mean=15.65 pack-years). Cigar and pipe smoking has been included in the calculation, but only three subjects reported smoking cigars who did not also smoke cigarettes. The smoking pack-years was comparable to the mean of 14 pack-years for farmers, but lower than the mean of 22 pack-years for grain elevator workers previously reported (Dosman et al.,1987; Broder et al.,1979).

Table 5.5 presents the agricultural history characteristics of the study subjects. Eighty-seven of the subjects had lived on a farm during their lifetimes, ranging from 1 to 76 years (mean=16 yrs). Thirty-seven currently lived on the study farm. The subjects had worked on farms for 1 to 65 years (mean=14 yrs) and had harvested wheat for 1 to 55 years (mean=14 yrs). These exposure years are slightly higher than the range of means of 8 to 14 years reported for grain elevator populations, and lower than the mean of 22 years for a farmer population (Chan-Yeung et al.,1992a; Manfreda et al.,1986; Broder et al.,1979; doPico et al.,1977).

Forty-eight percent of the subjects were study farm family members, 26% were local hires, and 24% were custom cutters. The average number of days the subjects spent harvesting wheat ranged from 4 to 180 days (mean=35 days). In 1994, the subjects had spent between 0 and 50 days (mean=14 days) harvesting

Table 5.5 Agricultural work history characteristics of the study population

Work history characteristic	Frequency	Mean	Range	Standard deviation
Ever lived on a farm	87 (89%)			
Years lived on a farm, n=87		28 yrs	1-76 yrs	16 yrs
Live on study farm, n=87	37 (38%)			
Years worked on any farm		23 yrs	1-65 yrs	14 yrs
Years harvesting wheat		16 yrs	1-55 yrs	14 yrs
Days per year harvesting wheat		34 days	4-180 days	42 days
Days harvesting wheat in 1994		14 days	0-50 days	13 days
Work on study farm as:				
- Family member	48 (49%)			
- Local hire	26 (26%)			
- Custom cutter	24 (24%)			
Ever worked in job breathing herbicides/insecticides: n=59	59 (60%):			
-2,4-D(aryloxyalkanoic acid)	26 (44%)			
-Roundup(aminoacidglyphosate)	8 (14%)			
-Orthophosphates	5 (8%)			
-Bladex (cyanizine)	5 (8%)			
-Banvel (benzoic acid)	4 (7%)			
-All herbicides/insecticides	1 (2%)			
-Other (Phosgene, Treflan, Pyrethrin, Eradicane)	5 (5%)			
-Don't know	5 (8%)			
Breathed dust on morning before study: n=28	28 (29%)	22 min	2-74 min	18 min
- Unload truck	7 (25%)			
- Blow filter or radiator	13 (46%)			
- Drive on dirt road	5 (18%)			
- Clean livestock pen	1 (4%)			
- Feed livestock	1 (4%)			
- Bale hay	1 (4%)			
Wore dust mask during day	4 (4%)			

n=98 unless otherwise specified

wheat prior to the study date. Twenty-eight of the subjects reported breathing dust in the morning before the study team arrived at the farm, most commonly to unload

a truck that had been filled the evening before or blowing dust off equipment during maintenance work.

Fifty nine of the subjects reported previous work using herbicides or insecticides, primarily 2,4-D. However, only five subjects reported work with organophosphate pesticides, which has been associated with bronchial asthma (Weiner,1961).

Four subjects wore a dust mask during the day of the study, but only for short periods during very dusty operations, such as when wheat was being dumped from the truck into the auger vessel. Of these, two wore a paper dust mask and two wore a half-face air-purifying respirator with HEPA filters. The four subjects were all male: a 19-year-old custom cutter, a 32 year old local hire, a 38-year-old family worker, and a 57-year-old local hire (the only smoker who wore a mask). In studies at grain elevators, many more workers (between 45 and 69%) reported wearing masks during dusty work (Broder et al.,1979).

Table 5.6 presents the non-farming work history characteristics of the study subjects. Non-farming jobs held by the subjects included welding, painting, ammonia work, coal work, firefighting, gas station work, and other miscellaneous jobs. The subjects had worked at these jobs for 1 to 10 years.

Table 5.6 Non-farming work history characteristics of the study population

Work history characteristic	Frequency	Mean (years)	Range (years)	Standard deviation (years)
Ever worked in job breathing visible dust for >1 hr/day:				
- Coal, n=1	1 (1%): 1 (100%)	3	NA	NA
Ever worked in job breathing visible metal fumes for >1hr/day:				
- Welding, n=14	14 (14%): 14 (100%)	3.2	1-10	2.7
Ever worked in job breathing gases/vapors for >1hr/day:	19 (19%):			
- Welding, n=19	1 (5%)	5	NA	NA
- Fire fighting	2 (11%)	6.0	5-7	1.4
- Painting	7 (37%)	2.3	1-5	1.9
- Ammonia work	3 (16%)	2	2-2	NA
- Rubber industry	1 (5%)	1	NA	NA
- Gas station work	2 (11%)	1.5	1-2	0.7
- Construction	1 (5%)	1	NA	NA
- Vinyl chloride manufacture	1 (5%)	10	NA	NA
- Printing	1 (5%)	1	NA	NA

n=98 unless otherwise specified NA = not applicable

5.3 Subject Exposures

The geometric mean, range and geometric standard deviation (GSD) for the 8-hr TWA subject exposures to total airborne dust and endotoxin, and for the ratio of endotoxin to dust concentration, are presented in Table 5.7.

Figure 5.2 shows that the frequency of airborne dust concentrations follows an approximately lognormal distribution. The geometric mean total dust was 0.83 mg/m^3 and the arithmetic mean was 1.32 mg/m^3. Only one subject was exposed

Table 5.7 8-hour TWA dust and endotoxin exposures for the study subjects

8-hr TWA Exposure	Geometric Mean	GSD	Arithmetic Mean	Range
Total dust (mg/m^3)	0.83	2.44	1.32	0.09-15.33
Endotoxin (EU/m^3)	54.24	3.27	104.6	4.4-744.4
Endotoxin/dust ratio (EU/mg)	56.99	2.38	82.8	6.1-525.7

above OSHA's PEL of 10 mg/m^3 total dust, while eight subjects were exposed at or above the ACGIH TLV of 4 mg/m^3 total dust. The dust exposures and number of exceedances above the occupational exposure limits found during the study were comparable to exposures found during an earlier study of grain elevator workers in northeastern Colorado. Janssen (1980) found a mean personal dust exposure of 0.7 mg/m^3 with 6 of 204 exposure samples greater than 10 mg/m^3. During the 1992 harvest, the mean personal total dust exposure of 8.4 mg/m^3 was significantly higher than that observed in the present study (Beard,1994). This is likely due to the fact that the Beard study targeted worse-case exposures. Nonetheless, the upper exposure limits were comparable between the two studies (1992 upper limit: 15.2 mg/m^3).

Figure 5.3 shows that the frequency of airborne endotoxin exposures also follows an approximately lognormal distribution[1]. The geometric mean endotoxin was 54.24 EU/m^3 and the arithmetic mean was 104.6 mg/m^3. Thirty-two subjects were exposed to endotoxin levels greater than 90 EU/m^3, the level suggested as a

[1] Because of this distribution, the logarithms of the endotoxin and total dust exposure measures were used in all statistical calculations.

threshold for acute mucous membrane irritation and pulmonary change (Haglind et al.,1984). The mean endotoxin exposure was comparable to the mean of 106 EU/m^3 (range 61.5 to 237 EU/m^3) found during the 1993 wheat harvest (Beard,1994).

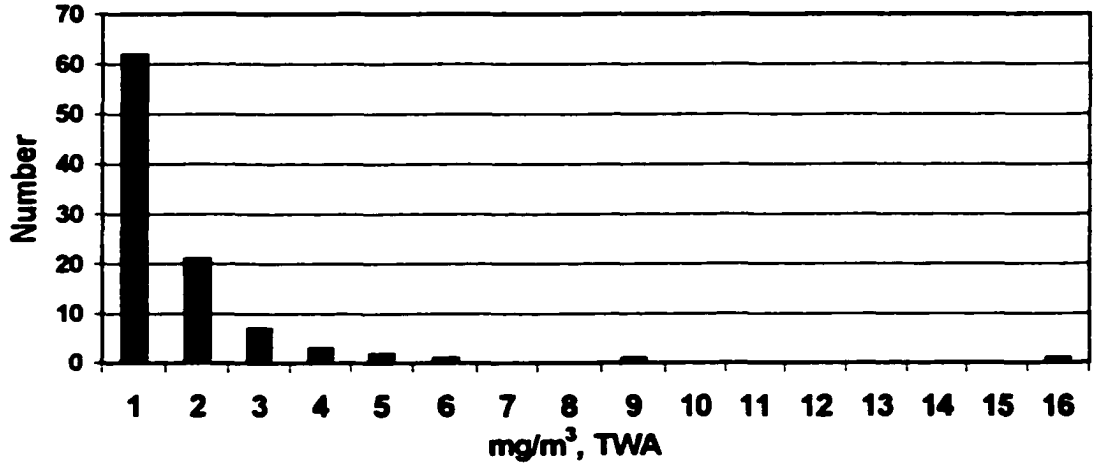

Figure 5.2 Frequency Distribution of Total Dust Exposures

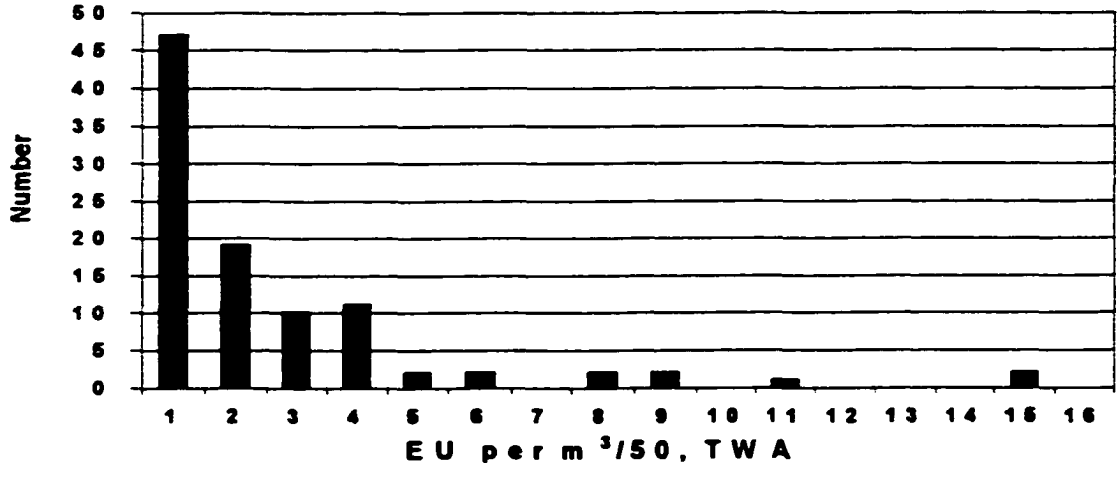

Figure 5.3 Frequency Distribution of Endotoxin Exposures

Since there is currently no occupational standard for endotoxin, it was of interest to compare the relationship between total dust and endotoxin exposures to determine how well total dust measurements predict endotoxin levels. In Figure 5.4, the logarithms of total dust exposure are plotted against the logarithms of endotoxin exposures. The correlation coefficient, r, between these two exposures was 0.69, indicating a moderately good relationship.

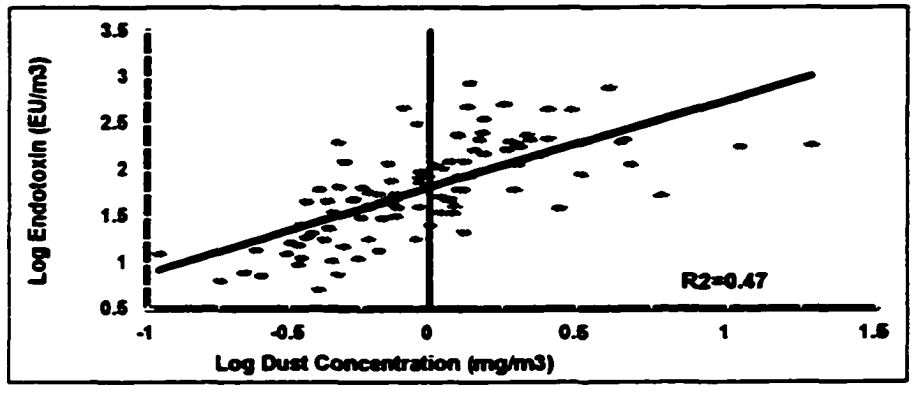

Figure 5.4 Total Dust Versus Endotoxin Exposure

The study included all the harvest workers at the study farms. Thus, a number of job categories were represented with different potential exposures. Table 5.8 presents the mean, range and standard deviation for exposure by each of six job categories. Figure 5.5 depicts the exposures graphically. While the "maintenance" and "other" category had the highest total dust and endotoxin exposure, there were only one and two persons in these categories, respectively. The high exposures for these jobs are likely due to tasks such as blowing the grain dust out of the engine and radiator. The similarity of the mid-range exposures for auger operators and

truck drivers is likely due to exposure to grain dust in a similar process state. In fact, many truck drivers actually operate the auger for part of the workday. While the combine operators had the lowest total dust and endotoxin exposure (as would be expected since almost all the cabs were enclosed and ventilated), their endotoxin to dust ratio was the highest of all job categories. This is probably due to the fact that the dust was not a mixture of grain dust and road dust, to which the truck drivers and other workers were likely exposed. It might also be due to the fact that only small particles enter the cab, and these may likely be the particles containing higher concentrations of endotoxin.

Table 5.8 8-hour TWA dust and endotoxin exposures by job category

Job category	Statistical parameter	Total dust (mg/m^3)	Endotoxin (EU/m^3)	Endotoxin/dust ratio (EU/mg)
Combine operator (n=47)	Geo.Mean Range GSD	0.51 0.09-3.93 2.01	36.3 5.3-725.9 3.22	63.0 13.7-525.7 2.24
Truck driver (n=31)	Geo.Mean Range GSD	1.19 0.34-15.33 2.08	78.5 4.3-434.1 2.57	57.9 7.6-285.0 2.27
Cart driver (n=4)	Geo.Mean Range GSD	1.13 0.25-8.62 4.59	45.7 7.1-404.8 6.90	31.8 12.5-224.2 3.73
Auger operator (n=13)	Geo.Mean Range GSD	1.58 0.33-5.12 2.15	83.6 10.3-744.5 3.63	47.3 6.1-214.8 2.94
Maintenance worker (n=1)	Geo.Mean Range GSD	2.20 NA NA	132.1 NA NA	55.2 NA NA
Other (n=2)	Geo.Mean Range GSD	1.59 0.82-3.07 2.53	85.9 83.5-88.4 1.04	46.3 25.4-85.0 2.34
	p[1]	0.005	0.062	0.341

[1] GLM-Tukey's test between job category and exposure
SD = standard deviation NA = not applicable

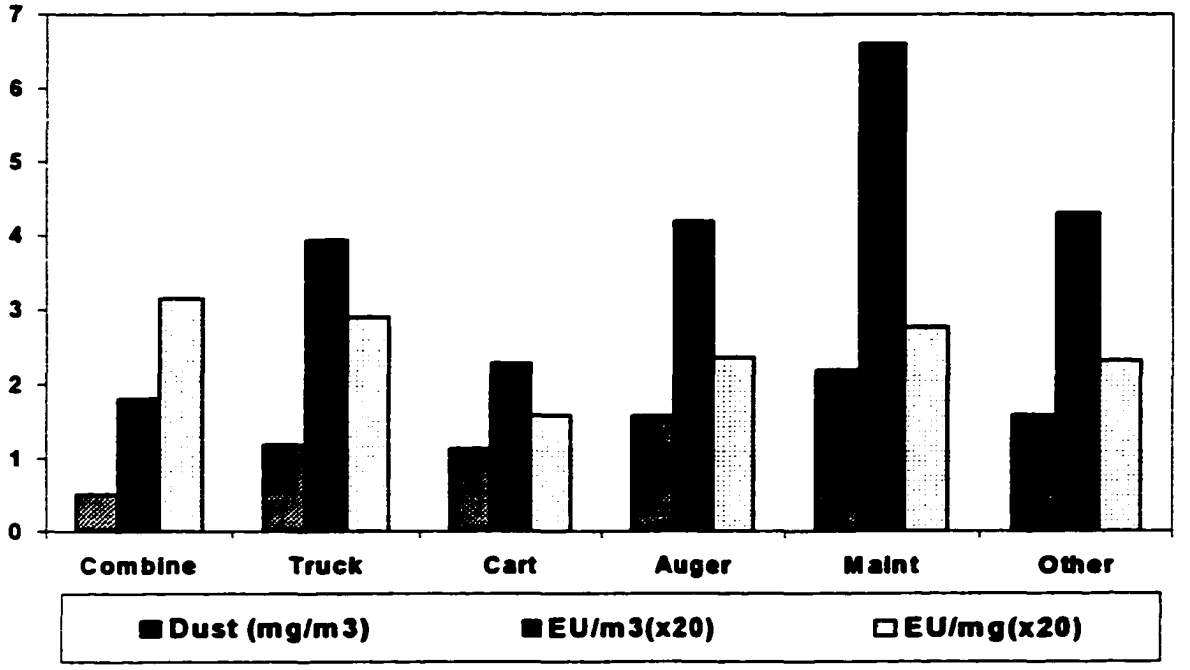

Figure 5.5 Mean Exposures by Job Category

Finally, it was of interest to determine whether ambient humidity was related to the airborne dust and endotoxin exposures. The mean humidity during the study was 32.4% (range 20-47%). The correlation coefficient, r, was 0.089 for the linear regression of dust concentration and humidity; and 0.077 for endotoxin concentration and humidity. Thus, humidity did not have a significant effect on airborne dust or endotoxin exposures.

5.4 History of Respiratory Symptoms

Table 5.9 presents the prevalence of self-reported chronic respiratory symptoms by smoking status category among the study population. Prevalence

rates were not examined by farm or county due the limited number of subjects in each group. However, this was not expected to be a significant factor based on previous findings of no significant difference in smoking patterns or reported respiratory symptoms in northeastern Colorado counties (Champney,1994).

Referring to Table 2.4, the prevalence of cough, phlegm, shortness of breath, wheezing, chest tightness with dusty work, and flu-like symptoms among the study population was comparable to the large farmer populations studied previously. The 26 to 29% prevalence of family history of asthma was higher than the 12% reported in other farmer studies. The 2 to 6% family history of chronic bronchitis was lower than the 7% found among grain workers (Broder et al.,1979).

There was no statistically significant difference between study smokers and non-smokers in their self-report of any respiratory symptom. However, smokers had significantly more episodes of wheezing in the last 12 months ($p=0.057$) and somewhat more flu-like episodes in their lifetimes ($p=0.123$) than non-smokers. While studies with larger populations have found significant differences between smokers and non-smokers with regard to respiratory symptoms, this study supports the conclusion that smokers have somewhat more respiratory symptoms.

Table 5.10 lists the tasks that were associated with past episodes of chest tightness and flu-like symptoms among the study subjects. The most frequently reported tasks associated with both chest tightness and flu-like symptoms

Table 5.9 Prevalence rates and extent of self-reported chronic respiratory symptoms by smoking status

Self-reported chronic respiratory symptom	Number of subjects (per 100) or extent of symptom[1]		p
	Current/ex smokers (n=49)	Never smoked (n=49)	
Cough (3 consecutive mos) (n=9)	6 (12%)	3 (6%)	0.487[2]
- Mean number years with cough	5.8 Range: 2-15 SD: 5.0	7.0 Range: 4-10 SD: 3.0	0.728[3]
Phlegm (3 consecutive mos) (n=14)	9 (18%)	5 (10%)	0.387[2]
- Mean number of years with phlegm	7.8 Range: 1-30 SD: 10.2	8.4 Range: 3-15 SD: 4.9	0.919[3]
Shortness of breath on slight hill (n=11)	6 (12%)	5 (10%)	1.00[2]
Wheezing (past 12 mos) (n=24)	12 (24%)	12 (24%)	1.00[2]
- Mean number of wheezing episodes	5.6 Range: 1-14 SD: 5.6	2.2 Range: 1-5 SD: 1.6	0.057[3]
- Mean number of doctor's visits	0.5 Range: 0-3 SD: 0.9	0.8 Range: 0.6 SD: 1.7	0.618[3]
- Mean number of hospitalizations	0	0	NA
Pneumonia (past 12 mos) (n=1)	0 (0%)	1 (2%)	1.00[2]
Sinus problem (past 12 mos) (n=31)	15 (31%)	16 (33%)	1.00[2]
Cold or flu (past 12 mos) (n=70)	34 (69%)	36 (74%)	0.823[2]
- Mean typical number of colds per year	1.9 Range: 1-5 SD: 1.0	1.8 Range: 1-4 SD: 0.7	0.509[3]
Chest tightness (with dusty work) (n=30)	15 (31%)	15 (31%)	1.00[2]
Flu-like symptoms (past 12 mos) (n=8)	4 (8%)	4 (8%)	1.00[2]
Flu-like symptoms (ever) (n=23)	9 (18%)	14 (29%)	1.00[2]
- Mean number of flu-like episodes in life	7.8 Range: 1-30 SD: 9.6	3.0 Range: 1-12 SD: 3	0.123[3]
- Mean time period of flu-like episode	24.1 Range: 1-48 SD: 18.8	23 Range: 12-36 SD: 6	0.848[3]
Family history: Allergies (n=46)	22 (48%)	24 (49%)	1.00[2]
Asthma (n=27)	14 (29%)	13 (26%)	1.00
Chronic bronchitis (n=4)	3 (6%)	1 (2%)	0.617
Emphysema (n=8)	5 (10%)	3 (6%)	0.715

[1] Column percents [2] Fisher's exact X^2 test (2-tailed) between smoking classes
[3] T-test (2-tailed) between smoking classes SD=standard deviation NA=not applicable

involved working with wheat dusts. The other reported tasks involved working with corn, beans, seeds, livestock, hay and pear dusts.

Table 5.10 Tasks associated with chest tightness and flu-like symptoms among the study subjects

Task groups	Frequency	
	Chest tightness with dusty work (ever), n=30	Flu-like symptoms with dusty work (ever), n=23
Cleaning wheat bins or elevator, shoveling wheat	17 (57%)	15 (65%)
Repair or maintenance of combine	5 (17%)	0 (0%)
Combine operation	3 (10%)	2 (9%)
Any wheat dust exposure	3 (10%)	0 (0%)
Moving wheat, unloading wheat truck, auger operation	2 (7%)	1 (4%)
Clean corn bins	1 (3%)	1 (4%)
Harvest corn	1 (3%)	0 (0%)
Clean bean bins	0 (0%)	1 (4%)
Cleaning seeds	0 (0%)	1 (4%)
Clean livestock pens	0 (0%)	1 (4%)
Baling hay	0 (0%)	1 (4%)
Harvest pears	0 (0%)	1 (4%)

Fisher's exact chi-square tests were used to determine the significance of the association between self-reported symptoms and past exposure to herbicides, and non-farming exposure to visible dust, metal fumes, and gases or vapors.

The only significant associations were that self report of sinus in the past 12 months was associated with past exposure to visible metal fumes (p=0.010) and the report of any respiratory symptom was slightly associated with past exposure to herbicides (p=0.10).

5.5 Baseline (pre-shift) lung function

While the pre-shift spirometry results were primarily obtained for comparison to the post-shift spirometry results, the pre-shift results were also used to determine if any subject had evidence of existing disease at the start of the study. The results of spirometry indicate obstructive disease if 1) the FEV_1/FVC is less than 70%, or 2) the FVC is less than 70% of predicted. A restrictive disease is indicated when the observed FVC/predicted FVC is less than 80%. Thus, spirometry can detect respiratory disease only in advanced stages or when a subject is currently experiencing an acute respiratory reaction.

No subject exhibited restrictive disease via spirometric testing. Ten of the 98 (10%) subjects had a pre-shift FEV_1/FVC less than 70% indicating obstructive disease. Characteristics of these 10 subjects are compared to the 88 subjects without obstruction in Table 5.11. All subjects with airways obstruction were male. Eight of the 10 were or had been smokers, i.e. they had smoked at least 100 cigarettes in their lifetimes. The mean age (p=<0.001) and mean years of farm work (p=0.003) were both significantly higher for the subjects with obstruction. Smoking status, but

Table 5.11 Characteristics of study subjects with and without decreased lung function (obstruction)

Characteristic	Subjects with obstruction (n=10)	Subjects without obstruction (n=88)	p
Gender:			
Male	10 (100%)	80 (91%)	1.00[1]
Female	0 (0%)	8 (9%)	
Mean age	58 years	35 years	<0.001[2]
Smoking status:			
Non-smoker	2 (20%)	47 (53%)	0.091[1]
Smoker (ever smoked)	8 (80%)	41 (47%)	
Mean smoking pack-years	19.5	19.0	0.965[2]
Mean years farm work	35 years	21 years	0.003[2]
Work on farm as:			
Family member	5 (50%)	44 (50%)	1.00[1]
Local hire	3 (30%)	23 (26%)	1.00
Custom cutter	2 (20%)	21 (24%)	1.00

% are column percents.
[1] Fisher's exact chi square test (2-tailed) between decreased lung function classes
[2] T-test (2-tailed) between decreased lung function classes

not pack-years, was marginally associated with obstruction (p=0.091). There was no difference in obstruction based on gender or employee status (family member, local worker, or custom cutter). Interestingly, of the four subjects who wore a mask during the study, the only smoker who wore a mask also had obstruction.

The prevalence rate of 10% obstruction found during this study falls in the range of 4 to 42% abnormal pulmonary function reported for grain elevator workers (see Table 2.4). It was also comparable to the prevalence of 13% found in a general

population survey of males ages 20-69, all smoking categories, in Glenwood Springs, CO (Miller,1986a and 1986d). The population size was not provided in the Glenwood Springs study.

5.6 Comparison of self-reported history of chronic respiratory symptoms with reduced lung function (obstruction)

The ability of self-reported respiratory symptoms to predict actual reduced lung function is important to epidemiologists, who often use questionnaires rather than spirometry to determine prevalence of disease in a population. Table 5.12 presents the prevalence rates for self-reported respiratory symptoms for subjects with and without actual reduced lung function. Subjects with obstruction would be expected to have higher prevalence of the various symptoms. However, there are no significant differences between the two groups, except that family history of allergies is more common among subjects without obstruction (p=0.079).

Table 5.13 presents the sensitivity, specificity, predictive value of a positive test, and predictive value of a negative test for smoking and non-smoking study subjects. Self-reported response to the chronic respiratory symptom question is the test and the spirometric finding is the standard. While it has been reported that farmers exposed to cigarette smoke more readily identify decreased lung function (Champney,1994), this is seen by improved sensitivity, specificity, and

Table 5.12 Self-report of respiratory symptoms by subjects with and without decreased pulmonary function (obstruction)

Respiratory symptom	Subjects with decreased pulmonary function (n=10)	Subjects with no decreased pulmonary function (n=88)	p[1]
Cough (3 consecutive mos)	1 (10%)	8 (9%)	1.00
Phlegm (3 consecutive mos)	3 (30%)	11 (12%)	0.152
Shortness of breath	2 (20%)	9 (10%)	0.312
Wheezing (past 12 mos)	3 (30%)	21 (24%)	0.703
Sinus problem (past 12 mos)	2 (20%)	29 (33%)	0.497
Pneumonia (past 12 mos)	0 (0%)	1 (1%)	1.00
Cold or flu (>3 in past 12 mos)	1 (10%)	12 (14%)	1.00
Chest tightness (dusty work)	2 (20%)	30 (34%)	0.491
Flu-like symptoms (dusty work)	2 (20%)	20 (23%)	1.00
Any respiratory symptom above	7 (70%)	65 (74%)	0.722
Family history:			
Allergies	2 (20%)	44 (50%)	0.079
Asthma	2 (20%)	25 (28%)	0.722
Chronic bronchitis	0 (0%)	4 (4%)	1.00
Emphysema	2 (0%)	6 (7%)	0.188

% are column percents.
[1] Fisher's exact chi square test between those with and without decreased pulmonary function

predictive value of a positive test only for self report of "sinus problem in the last 12 months" and "any respiratory symptom" for smoking subjects in the present study. Self-reported cough, phlegm, and shortness of breath showed improved sensitivity, specificity, and predictive value of a positive test for nonsmoking subjects.

Table 5.13 Ability of self-reported chronic respiratory symptoms to predict obstruction

Respiratory symptom	Obstruction (spirometry)					
	Smokers (n=49)			Non-smokers (n=49)		
	Y	N	Test parameter	Y	N	Test parameter
Cough (3 consecutive mos)			Sens = 0%			Sens = 50%
Yes	0	6	Spec = 85%	1	2	Spec = 96%
No	8	35	PV(+) = 0%	1	45	PV(+) = 33%
			PV(-) = 81%			PV(-) = 98%
Phlegm (3 consecutive mos)			Sens = 25%			Sens = 50%
Yes	2	7	Spec = 83%	1	4	Spec = 91%
No	6	34	PV(+) = 22%	1	43	PV(+) = 20%
			PV(-) = 85%			PV(-) = 98%
Shortness of breath			Sens = 12%			Sens = 50%
Yes	1	5	Spec = 88%	1	4	Spec = 91%
No	7	36	PV(+) = 17%	1	43	PV(+) = 20%
			PV(-) = 83%			PV(-) = 98%
Wheezing (past 12 mos)			Sens = 25%			Sens = 50%
Yes	2	10	Spec = 76%	1	11	Spec = 76%
No	6	31	PV(+) = 17%	1	36	PV(+) = 8%
			PV(-) = 84%			PV(-) = 84%
Sinus problem (past 12 mos)			Sens = 25%			Sens = 0%
Yes	2	13	Spec = 68%	0	16	Spec = 66%
No	6	28	PV(+) = 13%	2	31	PV(+) = 0%
			PV(-) = 82%			PV(-) = 94%
Pneumonia (past 12 mos)			Sens = 0%			Sens = 0%
Yes	0	0	Spec = 100%	0	1	Spec = 98%
No	8	41	PV(+) = 0%	2	46	PV(+) = 0%
			PV(-) = 84%			PV(-) = 96%
Chest tightness (dusty work)			Sens = 12%			Sens = 50%
Yes	1	14	Spec = 66%	1	16	Spec = 66%
No	7	27	PV(+) = 7%	1	31	PV(+) = 6%
			PV(-) = 79%			PV(-) = 97%
Flu-like symptoms (dusty work - ever)			Sens = 12%			Sens = 50%
			Spec = 80%			Spec = 74%
Yes	1	8	PV(+) = 11%	1	12	PV(+) = 8%
No	7	33	PV(-) = 82%	1	34	PV(-) = 97%
Cold or flu (>3 in past 12 mos)			Sens = 12%			Sens = 0%
Yes			Spec = 83%			Spec = 89%
No	1	7	PV(+) = 12%	0	5	PV(+) = 0%
	7	34	PV(-) = 83%	2	42	PV(-) = 95%
Any respiratory symptom above			Sens = 75%			Sens = 50%
Yes	6	29	Spec = 83%			Spec = 23%
No	2	12	PV(+) = 12%	1	36	PV(+) = 3%
			PV(-) = 83%	1	11	PV(-) = 92%

For all tests, sensitivity was low while specificity was generally high. This means that the ability of the self-reported respiratory symptom question to correctly identify those with obstruction was poor. Conversely, the ability of spirometry to correctly identify those without respiratory symptoms was good.

5.7 Cross-Shift Lung Function Changes

Figure 5.6 shows the mean pre- and post-shift lung function values. While the changes do not appear large, the mean cross-shift decrease in FVC, FEV_1 and FEV_6 were all statistically significant. Cross-shift decreases in FVC and FEV_1 have been most often reported among grain workers, while controls experience smaller decreases or increases in these parameters (see Table 2.5). In a study of farmers, the cross-shift decrease in FVC and FEV_1 was -1% (Donham et al.,1989). Slightly larger cross-shift decreases were found during this study. While the changes are statistically significant, it is important to note that biologically significant changes would have required cross-shift changes of 10 to 20%, depending on the lung function parameter.

The numerical values for the cross-shift lung function changes are summarized in Table 5.14 for all subjects and for subjects with and without obstruction. The cross-shift changes in FEV_6 and $FEF_{25-75\%}$ were significantly different for subjects with obstruction as compared to those without obstruction. For subjects with

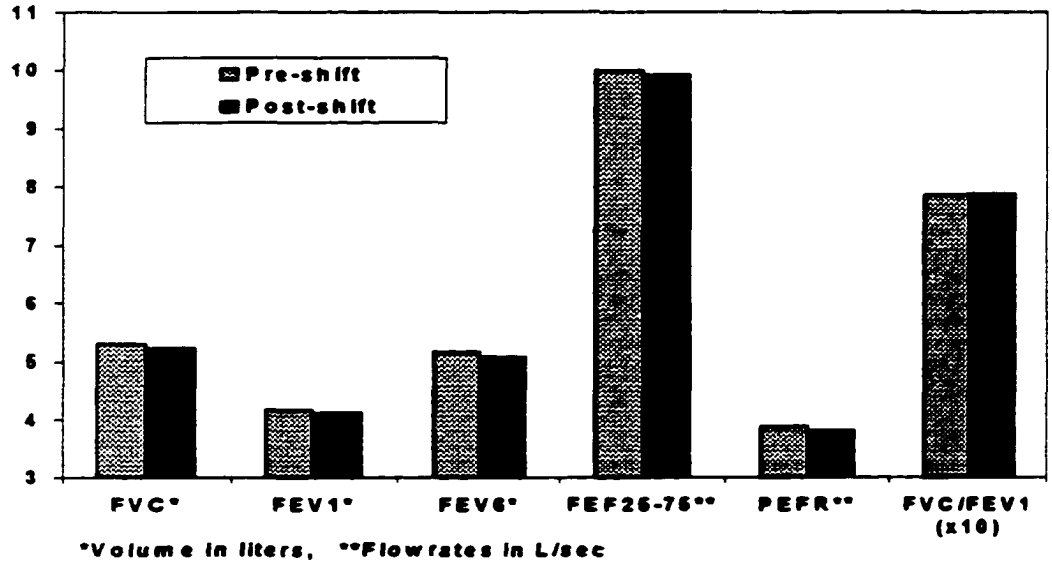

Figure 5.6 Pre- and Post-Shift Lung Function Values

obstruction, FEV_6 increased slightly (+1.2%) over the workshift, while FEV_6 decreased slightly (-1.8%) for subjects without obstruction. For subjects with obstruction, $FEF_{25-75\%}$ increased significantly (+11.4%) over the workshift, while $FEF_{25-75\%}$ decreased slightly (-1.9%) for subjects without obstruction. The cross-shift changes in FEV_1 and PEFR were marginally different for subjects with obstruction as compared to those without obstruction. For subjects with obstruction, FEV1 and PEFR increased slightly (+1.3% and +2.9%, respectively) over the workshift, while FEV_1 and PEFR decreased slightly (-1.6% and –0.7%, respectively) for subjects without obstruction. These findings were not unexpected as the subjects with obstruction had already compromised lungs. Other studies have reported similar findings, e.g. asthmatic flour mill workers have a greater

cross-shift change in lung function than the nonasthmatic workers (Giminez et al.,1995).

Table 5.14 Cross-shift change in lung function values

Cross-shift change in lung function value	All Subjects (n=98)	P[1]	Subjects with obstruction (n=10)	Subjects without obstruction (n=88)	P[2]
FVC, % Δ	-1.479	0.0001	0.042	- 1.652	0.159
FEV_1, % Δ	-1.288	0.0089	1.340	- 1.587	0.066
FEV_6, % Δ	-1.525	0.0001	1.225	-1.838	0.014
FEF_{25-75}, % Δ	-0.608	0.6724	11.377	-1.970	0.004
FEV_1/FVC, % Δ	0.159	0.5818	1.205	0.040	0.221
PEFR, % Δ	-0.376	0.5242	2.952	-0.754	0.056

[1] Matched pairs t-test between pre- and post-lung function value
[2] T-test (2-tailed) between those with and without obstruction

Because there is a normal diurnal variation in lung function parameters, it is important to assess whether the cross-shift changes experienced by the study subjects differ from unexposed populations. While general population diurnal changes in lung function are well-reported, only cross-shift changes in FEV_1 and PEFR are reported for unexposed worker populations comparable to the study subjects.

In a population of 944 unexposed blue-collar workers, the cross-shift change in FEV_1 was +0.8% (Ghio et al.,1991; Petersen et al.,1985). While the study subjects experienced a cross-shift decrease in FEV_1, the difference between

the two groups was not statistically significant. Also, the reduction in FEV_1 experienced by study subjects was no different than the reduction experienced by young, healthy subjects exposed to inert dust in an environmental chamber (Anderson et al.,1979).

Diurnal variation in flow rates is inherently more variable than lung volumes. For random samples of the general population, the mean percent diurnal (morning to evening) change in PEFR ranges from +4.9% to +8.9%, significantly different than the decrease seen in the study population (Quackenboss et al.,1991; Higgins et al.,1989; Hetzel et al.,1980). A study of asthmatics in the general population showed a mean diurnal increase in PEFR of as high as +51% (Quackenboss et al.,1991). Blue-collar workers have been shown to experience a cross-shift increase in PEFR of +2.1 to +11.6%, which is statistically different than the current study subjects' decrease of -0.4% ($p<0.05$ by z-test) (Neukirch et al.,1992; Ghio et al.,1991). In a group of cotton mill workers, linear declines in FEV_1, FVC, and PEFR were seen over the course of the workday and workweek (Field et al.,1979). Declines in FEV_1 and FVC were not apparent by the end of the week, supporting the suggestion that PEFR may be the more sensitive indicator of exposure effects (Eisen et al.,1993). The declines observed among cotton workers are larger, but in the same direction as the subjects in the current study.

5.8 Cross-Shift Change in Respiratory Symptoms

Table 5.15 presents the number of subjects by smoking status who reported an increase or decrease in each acute respiratory symptom over the course of the workshift. All subjects not listed reported no cross-shift change in the symptom. No significant difference was found between the two smoking classes for self-report of change in cross-shift symptoms, except for "mucous". Smokers generally reported decreased mucous accumulation, while non-smokers reported increased mucous accumulation at the end of the day as compared to the start of the day.

Table 5.15 Subjects who reported a cross-shift change in respiratory symptoms by smoking status

Respiratory symptom	Number of subjects reporting a change in respiratory symptom						p[1]
	Current/ex smokers (n=49)			Never smoked (n=49)			
	Increase	N/C	Decrease	Increase	N/C	Decrease	
Cough	2	39	8	6	31	12	0.669
Mucous	2	37	10	9	6	4	**0.015**
Eye irritation	12	33	4	14	30	5	1.00[2]
Nose irritation	5	35	9	10	33	6	0.272
Throat irritation	6	8	5	9	38	2	0.361
Chest discomfort	3	5	1	6	41	2	1.00
Chest wheezing	0	46	3	0	47	2	NA
Shortness of breath	3	43	3	6	43	0	0.182
Fever or chills	0	49	0	0	49	0	NA
Tingling fingers	0	49	0	2	45	2	NA
Blurred vision	0	49	0	2	46	1	NA

[1] Fisher's exact X^2 test (2-tailed) between smoking classes
N/C = No change in reported symptom over the workshift.

In order to determine whether any reported cross-shift change in respiratory symptom was associated with exposure, a linear regression was run for each acute respiratory symptom and the logarithm of total dust, endotoxin, and endotoxin to dust ratio with smoking status included in the model. There were no significant relationships found between exposure and change in any individual respiratory symptom.

By examining Table 5.15, it is clear that the majority of subjects reported no change in most of the respiratory symptoms. Thus, an additional measure, called the "respiratory index", was created. The index was the sum of the responses for changes in all the listed symptoms, thus providing a measure of whether any symptom changed as well as the extent of change, i.e. how large a change or the number of symptoms that changed. Table 5.16 shows that the reported indices ranged from –3 to +7.

Table 5.17 presents the association between the respiratory index and independent variables thought to affect cross-shift change in respiratory symptoms. A positive and significant association was found between the respiratory index and both total dust and endotoxin exposure. This means that the number and/or extent of respiratory symptoms increased over the work shift as exposure to both total dust and endotoxin increased.

Table 5.16 Frequency of respiratory index values among the study subjects

Respiratory Symptom Change Index	Frequency (n=98)
-3	3 (3%)
-2	9 (9%)
-1	19 (19%)
0	38 (39%)
1	13 (13%)
2	8 (8%)
3	2 (2%)
4	2 (2%)
5	2 (2%)
6	1 (1%)
7	1 (1%)

Other variables suspected to affect cross-shift respiratory symptoms, such as number of days harvested before the study, years of farm and harvesting work, report of chronic respiratory symptoms, presence of lung obstruction, job category, and whether the subject took over-the-counter medication during the day, were not related to the respiratory index.

It was thought that cross-shift changes in lung function parameters might be related to the respiratory index. Some studies of grain workers have shown larger falls in FEV_1 and $FEF_{50\%}$ among symptomatic workers (doPico,1982a). However, a relationship between cross-shift symptoms (respiratory index) and cross-shift lung function was not demonstrated among the current study subjects.

Table 5.17 Association of independent subject variables with the respiratory index

Independent subject variable (n=98)	Spearman Correlation Coefficient	p
Log TWA total dust exposure	0.217	0.036[1]
Log TWA endotoxin exposure	0.212	0.036
Log TWA endotoxin/dust	0.103	0.31
Number of days harvested before study	0.095	0.35
Number of years harvesting wheat	-0.004	0.96
Years of farm work	-0.087	0.39
Age	-0.091	0.37
Δ FVC, % L	-0.157	0.12
Δ FEV_1, % L	-0.151	0.12
Δ FEV_6, % L	-0.088	0.39
Δ FEF_{25-75}, % L/min	-0.030	0.77
Δ FVC/FEV_1	-0.079	0.44
Δ PEFR, % L/min	-0.087	0.39
Any chronic respiratory symptom		0.67[2]
Obstruction		0.19
Job category		0.73
Took medication for respiratory symptoms during the day		0.92
Subject rating of amount of dust breathed during day		0.74

[1] Quantitative variables – Spearman correlation coefficient with respiratory index;
[2] Qualitative variables – NPAR1WAY one way analysis of variance with respiratory index

Importantly, the subject's self-rating of his/her exposure level during the day was not related to the respiratory index. This implies that the perception of exposure level did not affect the self-reporting of respiratory symptoms. Related to this issue is the subject's self-rating of exposure level as compared

to their actual exposure. The Pearson correlation coefficients for subject's self-rating of exposure versus actual exposure were –0.065 (p=0.52) for total dust, 0.070 (p=0.49) for endotoxin, and 0.193 (p=0.056) for endotoxin to dust ratio. This means that the subjects were not able to accurately estimate their dust or endotoxin exposure levels. However, as the endotoxin concentration in the dust increased, they reported higher dust levels. This may mean that higher endotoxin concentration, not exposure, causes the acute reaction, which is interpreted as higher dust exposure by the subjects.

5.9 Models of Cross-shift Lung Function Change

A backward regression model was employed to determine the significance of independent variables thought to be associated with cross-shift change in each lung function parameter and the respiratory index. The results are listed in Table 5.17. The independent variables included in the models are listed at the bottom of the table.

Four variables met a 0.05 level of significance for the cross-shift change in FVC: age, smoking status, whether the subject smoked before the pre-test, and whether the subject smoked before the post-test. Age and smoking before the pre-test were negatively associated with change in FVC.

Four variables met a 0.05 level of significance for the cross-shift change in FEV_1 and FEV_6: age, obstruction, whether the subject smoked before the pre-test, and whether the subject smoked before the post-test. Age and smoking before the pre-test were negatively associated with change in both FEV_1 and FEV_6.

Only two variables met a 0.05 level of significance for the cross-shift change in the ratio FEV_1/FVC: chronic respiratory symptoms and whether the subject smoked before the post-test.

Three variables significantly explained the cross-shift change in $FEF_{25-75\%}$: obstruction, smoking status, and whether the subject smoked before the post-test. Only one variable, obstruction, significantly explained the cross-shift change in PEFR.

Finally, three variables met a 0.05 level of significance for the respiratory index: total dust exposure, age, and whether the subject smoked before the post-test. The model for respiratory index was the only one where exposure was a significant explanatory variable. There was also a small measure of protection for respiratory index associated with age. This may be related to the healthy worker effect. Those individuals most severely affected by the respiratory hazards are likely to have changed jobs or avoid harvest activities.

It is well known that smoking causes many different respiratory symptoms and diseases, so it was not surprising to find that smoking was significantly associated with the respiratory index and change in lung function parameters.

Although the variables included in the models were significant at reasonable p-values, the R-square values for models were small, suggesting that the significant variables could not explain the majority of the variation in the dependent variable. Therefore, the models do not provide reliable models from which to make inferences. The highest R-square obtained was 0.310. Ultimately this means that lung function changes by spirometry and respiratory index are not well explained by total dust or endotoxin exposure, or the other significant variables. However, given the poor harvest and lower than normal dust levels (most below the TLV), a strong relationship between dust and respiratory response would not be expected.

Table 5.18 Regression analysis: Independent variables significantly associated with change in lung function values and respiratory index (n=98)

Cross-shift lung function change	Significant independent variable(s)	Parameter estimate	p-value	Intercept	R^2
FVC, % Δ	Smoking status	1.549	0.034	0.313	0.266
	Age	-0.062	0.006		
	Pre-smoke	-10.039	<0.001		
	Post-smoke	9.154	<0.001		
FEV_1 % Δ	Obstruction	3.329	0.0362	1.366	0.294
	Age	-0.085	0.0081		
	Pre-smoke	-10.890	<0.001		
	Post-smoke	13.170	<0.001		
FEV_6, % Δ	Obstruction	3.612	0.002	1.267	0.310
	Age	-0.083	0.001		
	Pre-smoke	-8.926	<0.001		
	Post-smoke	9.099	<0.001		
FEV_1/FVC, % Δ	Chronic respiratory symptoms	-1.309	0.0381	0.828	0.130
	Post-smoke	2.204	0.008		
FEF_{25-75}, % Δ	Obstruction	14.953	0.001	0.091	0.144
	Smoking status	-6.792	0.025		
	Post-smoke	8.831	0.044		
Δ Peak Flow, % Δ	Obstruction	3.706	0.056	-0.754	0.038
Index of respiratory symptom, Δ	Total dust exposure (log TWA)	1.081	0.017	1.192	0.120
	Age	-0.020	0.085		
	Post-smoke	-1.11	0.030		

Independent variables included in models: Total dust exposure (log TWA), endotoxin exposure (log TWA), age, obstruction, smoking status, smoke before pre-test, smoke before post-test, mask, pre-test exposure, number days in 94 harvest, chronic symptom, past herbicide exposure

Chapter 6

Summary and Recommendations

The primary purpose of this research was to examine the relationship between cross-shift changes in 1) lung function and 2) respiratory symptoms and total dust and endotoxin exposure. A study was designed in which the disease outcomes measured were cross-shift changes in six spirometric variables and eight self-reported acute symptoms. The cohort included 98 harvest workers in northeastern Colorado during one 1994 wheat harvest workshift. Individual exposure to dust and endotoxin were measured for each worker in the study. Respiratory health and occupational history questionnaires were also administered to each participant. The field results were statistically analyzed to evaluate the relationships between the exposures and disease outcomes. Additional information obtained included prevalence rates for history of chronic respiratory symptoms and abnormal spirometric results among study subjects and the ability of self-reported respiratory symptoms to predict actual lung dysfunction (as determined by spirometry) for the study population.

This research adds new data about worker exposure to dust and endotoxin during harvest in northeastern Colorado, and the relationship between those exposures and cross-shift respiratory changes. It is the only known study that compares cross-shift lung function change and dust and endotoxin exposure during harvest activities, which may be applicable to workers in other

agricultural situations. This research confirms that dust exposure can be high at farms during harvest. Further, it was shown that endotoxin exposure could also be high during harvest – even for combine operators in ventilated cabs. This research supports findings by Darke et al. (1976) and James et al. (1986) that showed no fever development, but demonstrated a slight decline in FEV_1 over a harvest workshift. Additionally, it was shown that the respiratory symptoms questionnaire might not adequately predict existing lung disease within this cohort.

6.1 Exposures during Harvest

Although grain handling has become highly mechanized, exposure to grain dust is still widespread. Even for the relatively modest harvest yield in this study, workers were exposed to a geometric mean dust concentration of 0.83 mg/m^3, with 8 (8%) of the workers exposed to levels over the TLV of 4 mg/m^3. Thirty-two (33%) workers were exposed to endotoxin levels above 90 EU/m^3, the level associated with symptoms and lung function effects. Endotoxin level was positively correlated with dust level.

The highest dust exposures were for those working at maintenance, auger operation, and truck driving tasks. Surprisingly, the endotoxin to dust ratio exposure was highest for the combine operators. This was unexpected, as almost all study combines had ventilated cabs. This is significant because

combine operators are often ignored in sampling schemes on the assumption that the ventilated cabs reduce or eliminate exposures. While the study exposures were not high enough to elicit ODTS, the long term effect or possible additive effects of grain dust exposure and other farm exposures on lung function is unknown.

6.2 Cross-shift Change in Respiratory Symptoms

The exposure data indicated that acute cross-shift respiratory effects would be mild, with slight mucous membrane and mild bronchoconstriction expected. In fact, 60 subjects reported a change in at least one acute respiratory symptom. Individual correlation analysis showed that the respiratory index (the sum of reported changes in all eight acute respiratory symptoms) was significantly related to total dust and endotoxin exposure. Multiple regression analysis showed that the respiratory index was related to total dust exposure, age, and smoking before the final spirometry test.

6.3 Cross-Shift Change in Lung Function

Mild, but measurable cross-shift decreases in lung volumes (FVC, FEV_1, and FEV_6) were observed. Moreover, PEFR was found to decrease over the day, similar to that experienced by cotton workers, as compared to the general unexposed working population whose PEFRs typically increase over the day.

Multiple regression analysis showed that smoking, age, presence of obstruction, and history of chronic respiratory symptoms, but not dust or endotoxin exposure, were associated with cross-shift change in the spirometric variables. More specific studies would have to be undertaken to validate any causal links suggested.

6.4 Prevalence of Lung Dysfunction among Harvest Workers

The prevalence rates for self-reported chronic respiratory symptoms ranged from 6 to 24%. Compared to the general male population, chronic respiratory symptoms were lower among the harvest workers (Miller, 1986). Thirty-one percent of the subjects reported having experienced chest tightness and 18 to 29% had experienced flu-like symptoms during dusty work.

An additional objective was to determine if a measurable reduction in lung function by spirometry was associated with self-reported respiratory symptoms. In general, the association was weak. The sensitivity, specificity, and positive predictive value (PV+) were all low. They were improved among non-smokers as compared to smokers. However, report of any symptom was more reliable for smokers than nonsmokers.

6.5 Sources of Error

The small number of respondents for a given variable, e.g. only four subjects wore masks during the day, may have produced outliers that affected the statistical significance of the variable.

Recall bias, i.e. the ability of the subjects to accurately recall respiratory symptoms, is potential source of error. It should not have been too great in this study because the chronic respiratory symptom questions were asked for the last year, or ever, and the acute symptom questions covered respiratory symptom changes during only one day.

It is also possible that several hours are required after exposure is discontinued before lung function changes are maximal. In this study, we tested after the 6 to 9 hours of work, or at the end of the workshift, i.e. no time period passed between work exposure and the measurement.

6.6 Recommendations

A number of recommendations have been made in previous studies conducted in agricultural environments. Some technologies, such as cyclones, filters, or exhaust fans reduce dust levels, but are often too expensive for small country elevators (Frye,1982). Drying equipment may reduce organisms, but may

increase the immediate release of endotoxin and dust. While ventilation has been added to harvest equipment over the years (generally for comfort, not dust control), this study shows that endotoxin levels may still be elevated in ventilated cabs. The use of water or oil misting of certain processes has been shown to reduce dust levels and health effects, but is often technically difficult or unfeasible (Senthilselvan et al.,1997). Further, the growth of spores resulting from misting must be balanced with decreased dust exposure. Use of lactic-acid producing bacterium to prevent hay deterioration was not effective at reducing airborne microbial or endotoxin levels in the barn microenvironment (Duchaine et al.,1999).

While substitution or technology change is preferred over personal protective devices, it is recommended that NIOSH-approved air-purifying respirators be worn by workers during dusty operations, such as when unloading wheat from trucks or operating augers (NIOSH,1994). These respirators have been shown to reduce pulmonary and systemic effects associated with farmer's lung under experimental challenge and during farm activities (Kusaka et al.,1993; Mueller-Wening et al.,1989, Cuthbert et al.,1983). It is important to note that while a reduction was observed, pulmonary and systemic symptoms were not completely prevented. Lacey (1994b) noted that respirators must effectively filter spores down to 1 um in diameter.

The few workers in this study who wore respirators reported reduced symptoms when wearing them. Because 2 of the 4 subjects wore paper dust masks, some education on respirator selection would be useful. Further, education has been shown to be a more important factor in respirator acquisition than symptoms of bronchitis or farmer's lung (Virolainen et al.,1987).

Another recommendation is that an exposure level based on endotoxin (rather than total dust) should be considered. Endotoxin was associated with respiratory symptoms in this and many other epidemiology studies, and has been shown to be responsible for at least 70% of the inflammatory response to grain dust in cells.

Lastly, there is a need for an improved respiratory questionnaire for assessment of pulmonary function. The current questionnaire does not predict changes in pulmonary function very well, at least among the current population.

6.7 Further Research

Based on the scope of this research, future research areas might include:

- A study investigating lung function changes and endotoxin exposure during higher dust activities, such as working in the grain bins. Beard (1994) showed that dust and endotoxin levels are highest in enclosed work areas and storage bins in northeastern Colorado.

- Focused (case-study) endotoxin testing should be done during more productive crop years to ascertain the full range of possible exposures. In particular, these investigations should examine the differences in cab design and maintenance and exposure. It would be useful to include full dust characterization during such focused studies.
- Study in which spirometry is conducted over a period of hours following exposure rather than immediately at the conclusion of exposure. The cellular effects from endotoxin exposure have been shown to peak at three hours following exposure. In addition, ODTS symptoms begin several hours after exposure.
- Study of lung lavage, blood, or sputum inflammatory mediators (T-cell subsets or monocyte reactivity) in exposed workers and correlated with lung function changes. Blood tests would be easier to obtain from agricultural workers than lung lavage samples.

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APPENDIX A

STUDY NOTICE

Please Post

ATTENTION!

WHEAT FARMERS
in
Adams, Arapahoe, Douglas, Elbert, Kit Carson, Larimer, Lincoln, Logan, Morgan, Phillips, Sedgwick, Washington, Weld, and Yuma Counties

During the Summer 1994 winter wheat harvest, Colorado State University High Plains Inter-Mountain Center for Agricultural Health and Safety (HI-CAHS) will be conducting

A STUDY OF LUNG FUNCTION IN HARVEST WORKERS

What is involved?

A CSU team will arrive at your farm on one morning during harvest - before activities start. They will ask workers to:
1) answer a few questions about work history and health status.
2) blow into the tube of a lung function test machine.
3) wear an air sampling pump on their belt (approximately 2 pounds) during the workday.
The lung function test will be repeated in the late afternoon during dinner break or at the end of the day.

Who will we study?

All workers 18 years of age and older will be asked to participate.

What's in it for you and the workers?

Direct access to health and safety information: Any questions you have about health and safety issues on your farm can be addressed by the experienced field team members or the CSU HI-CAHS staff (EPA Pesticide Worker Standard, OSHA rollover protection).
Free report: Each participant will receive a written report of their breathing test and airborne dust level results.
CSU Ball Cap or $10 cash in appreciation of each participant's time.

ALL NAMES AND INFORMATION WILL BE CONFIDENTIAL
NO REGULATORY AGENCIES ARE INVOLVED IN THIS STUDY.

Interested? Need more details?

Call 1-800-622-8673 (please mention the Wheat Harvest Study)

APPENDIX B

STUDY FARM FOLLOW-UP LETTER

, 1994

RE: CSU STUDY OF LUNG FUNCTION IN HARVEST WORKERS

Dear :

Thank you for your interest in work we are planning at the Center for Agricultural Health and Safety. This summer we will perform a study looking at small changes in lung function which occur during winter wheat harvest activities. The study hopes to determine whether farm workers experience symptoms similar to those seen in grain elevator workers. Further, components in grain dust which may responsible for health effects will be determined.

I know harvest is a busy time for you. Less than 45 minutes of each worker's time will be required - on one day only. They will be asked a few questions about work and health history, blow into the tube of a breathing test machine, and wear a lightweight air sampling pump on their belt. Our work will be mostly done in the early morning (before harvest activities begin) and at the evening dinner break. Each participant will receive a report of their breathing test and dust levels and a small gift of our appreciation for their time.

The study team will include health and safety staff from CSU. You or your operator should feel free to discuss any health and safety issues of concern while we are at the farm (such as the EPA Pesticide Worker Protection Standard or OSHA's new rollover protection requirements).

I will call you in a week or two to see if you have any further questions. In the meantime, don't hesitate to call 1-800-622-8673.

Sincerely,

Susan Marie Viet

APPENDIX C

STUDY SUBJECT RESULTS LETTER

Example Follow-up Report to Study Participants

[CSU Letterhead]

Date

Name and Address

Dear Mr. (or Ms.)

Thank you again for participating in the Wheat Harvest Study.

Enclosed are the results of your lung function test. This exam was not intended for clinical diagnosis and is presented only to inform you of the results. We suggest you have the report included in your medical records. The spirometry test reports on various measures of lung function. The forced vital capacity (FVC) indicates how much air you blow out after taking a deep breath. The forced expiratory volume in one second (FEV1) indicates how much of that air you blew out in the first second. Your test results are compared to an expected or predicted value for a non-smoking person of the same age, height, sex, and race.

Interpretation of your spirometry test indicates the following:

Insert Spirometry Interpretations

SPIROMETRY INTERPRETATIONS: POSSIBLE RESPONSES

(NOTE: Based on pre-shift FVC and FEV1 values)

IF: > 70% FEV1/FVC or < LLN for the best FVC, then insert:
Your breathing tests were within normal limits of the predicted values.

IF: 61 % to 69 % FEV1/FVC, then insert:
Your breathing tests were slightly below normal limits of the predicted values. We suggest during your next annual physical to have your doctor consider all relevant information and provide you with a clinical interpretation.

IF: < 60% FEV1/FVC, then insert:
Your breathing tests were not within normal limits of the predicted values. We suggest you contact your doctor and provide him/her with the results. Your doctor will consider all relevant information and provide you with a clinical interpretation.

IF B OR C (<60% OR 61% to 69%) then insert:
If you wish the services of a physician specializing in occupational health, HI-CAHS has arranged for study participants to have additional diagnostic services with an occupational health clinic. These diagnostic services would be provided to you **free of charge** and will be billed to HI-CAHS. If you are interested in further evaluation by an occupational physician, please contact the following clinic, **and identify yourself as a HI-CAHS Wheat Harvest Study participant.** (Any additional treatment costs must be paid for by the patient.)

Greeley Foundation Medical Clinic
CHAMPS (Corporate Health and Medical Programs, Inc.)
1900 16th St.
Greeley, CO 80631
(303) 350- 2471

We measured the total airborne dust levels on the day we were at your worksite. The airborne total dust level while you were working that day was:

Insert Dust Value and Interpretations

DUST VALUE AND INTERPRETATION: POSSIBLE RESPONSES

If less than 10 mg/m^3, then insert:

The total grain dust levels to which you were exposed were below the Occupational Safety and Health Administration (OSHA) acceptable maximum permissible exposure level of 10 mg/m^3.

If more than 10 mg/m^3, then insert:

The total grain dust levels to which you were exposed were above the Occupational Safety and Health Administration (OSHA) acceptable maximum permissible exposure level of 10 mg/m^3. If you are regularly exposed to similar grain dust levels in future work, you should consider using a dust mask or respirator to reduce the amount dust you breath. Enclosed is a brochure explaining respiratory protective equipment.

HI-CAHS consultants are willing to provide additional services, such as assistance in the selection and/or fit testing of respiratory protection. If you require such assistance or have specific questions concerning this report, please feel free to contact our office at (800) 622-8673.

Sincerely,

Susan Marie Viet
Study Investigator

APPENDIX D

DATA COLLECTION INSTRUMENTS

F-1: FARM INFORMATION

To be completed at the time of agreement to participate:

Date _____ Initials_____ Farm Code # _____

Farmer Name _____ Phone(H)_____
Farm Address _____ (W)_____
_____
_____ County _____

Expected start date(s) of winter wheat harvest_____

Acres of farm_____ Acres in winter wheat_____

Number of workers (during typical harvest day) ___ custom cutters ___ family/local
Number of days to complete harvest _____

Directions to home:

_____
_____
_____
_____

Field Contact Name/phone:_____

To be completed during confirmation of study team visit:

Where to meet before harvest activities begin?_____

Directions to meeting location (if different from above):

_____
_____
_____
_____

What time do you expect to start cutting wheat on date of visit?_____

Other Comments:

_____
_____
_____
_____

WHEAT HARVEST STUDY: PHONE LOG FARM #_____ Page __of__

Date	Init.	Phone number	Contact name	Results/Summary

F-2: FARM/SUBJECT LINK

Date _____ Farm Code # _____ Initials_____

Farmer Name _____

Worker Name (List as UNK if no name):	Subject identification #s/ Reason for refusal

Total Number of workers: Present _____
Refused _____
Study Subjects _____

COLORADO STATE UNIVERSITY
INFORMED CONSENT TO PARTICIPATE IN A RESEARCH PROJECT

PROJECT TITLE: Acute Respiratory Response to Grain Dust During Winter Wheat Harvest in Northeastern Colorado

PRINCIPAL INVESTIGATOR: Roy Buchan

CO-INVESTIGATORS: Susan Marie Viet, Philip Bigelow, Lorann Stallones, Alan Tucker, Bruce Belleville

CONTACT NAME AND PHONE NUMBER FOR QUESTIONS/PROBLEMS: Susan Viet (303)491-6151

SPONSOR OF THE PROJECT: National Institute for Occupational Safety and Health

PURPOSE OF THE RESEARCH: The purposes of the study are to determine the change in lung function at the beginning and end of the work day and to measure dust exposure during the work day.

PROCEDURES/METHODS TO BE USED: The study includes information to be obtained from a personal interview; spirometric (breathing) test at the beginning and end of the workday; and personal air sampling during the day.

RISKS INHERENT IN THE PROCEDURES: The known risks associated with this study include light-headedness during the breathing test. Thus, the test is conducted in a seated position. People who are taking medication for lung or heart disease should not participate in this study. I understand that it is not possible to identify all potential risks in an experimental procedure, but I believe that reasonable safeguards have been taken to minimize both the known and potential, but unknown, risks.

BENEFITS: The benefits of the study include a no cost spirometric screen, the results of the spirometric screen and dust levels, direct access to health and safety information from the field team members and a small gift of appreciation for your time (choice of CSU ball cap or $10 cash).

I choose _____ CSU ballcap
_____ $10 Cash

You will receive your gift when you complete the test this evening.

 Subject Initials _____ Date _____

CONFIDENTIALITY: All data collected in this study will be kept separate from individual identifying information, such as name, address, and phone number. Any information published will not include individual names, addresses or phone numbers. Data will be published in the form of summary information only.

LIMITATION OF LIABILITY: Because Colorado State University is a publicly-funded, state institution, it may have only limited legal responsibility for injuries incurred as a result of participation in this study under a Colorado law known as the Colorado Governmental Immunity Act (Colorado Revised Statutes, section 24-10-101, et seq.). In addition, under Colorado law, you must file any claim against the University within 180 days after the date of the injury.

In light of these laws, you are encouraged to evaluate your own health and disability insurance to determine whether you are covered for any injuries you might sustain by participating in this research, since it may be necessary for you to rely on your individual coverage for any such injuries. If you sustain injuries which you believe were caused by Colorado State University or its employees, we advise you to consult an attorney.

Questions concerning treatment of subjects' rights may be directed to LaVina Matzdorff at (303)491-6355.

PARTICIPATION: I understand that my participation in this research is voluntary. If I decide to participate in the study, I may withdraw my consent and stop participating at any time without penalty or loss of benefits to which I am otherwise entitled.

I have read and understand the information stated and willingly sign this consent form. My signature also acknowledges that I have received, on the date signed, a copy of this document containing 2 pages.

_____ _____

Subject name (printed) Subject Signature

_____

_____

_____

(Subject mailing Address)

_____ _____

Field team member signature Date

INC-1: Subject # C____F____S____ Date_____ Time_____am pm Initials_____

Height(heels)_____in Height(heels plus subject)_____in Height(subject)_____in

First I need to verify your eligibility in the study. Please feel free to ask me for more details if you don't understand a question.

I1. Are you under 18 years of age? YES [1] NO [2]

I2. Are you on medication today for any of the following [READ LIST]?

	YES	NO	
I2a.	[1]	[2]	Recent surgery
I2b.	[1]	[2]	Coronary heart disease
I2c.	[1]	[2]	Stroke
I2d.	[1]	[2]	High blood pressure
I2e.	[1]	[2]	Chronic bronchitis
I2f.	[1]	[2]	Emphysema
I2g.	[1]	[2]	Asthma
I2h.	[1]	[2]	Cold [OTHER THAN ASPIRIN,TYLENOL, ETC FOR PAIN]
I2i.	[1]	[2]	Other lung disease [IF YES, Specify lung disease_____]

		YES	NO
I3.	Will you use a bronchodilator or corticosteroid medication today?	[1]	[2]
I4.	Have you had a collapsed lung (or pneumothorax) in the last 6 months?	[1]	[2]

[IF "YES" TO ANY OF THE ABOVE QUESTIONS, STOP IMMEDIATELY. YOU NEED TO THANK THE SUBJECT AND INFORM THEM THEY ARE NOT ELIGIBLE TO PARTICIPATE IN THE STUDY.]

INTERVIEWER COMMENTS:

_____

_____

_____

PRE-1: HEALTH QUESTIONS

Now I have some questions to ask related to your health. Please rate the severity of the following symptoms based on how you feel RIGHT NOW on a scale of 1 to 5. Here is a card showing the choices. 1 indicates no problem, 2 indicates a mild problem, 3 indicates a moderate problem, 4 indicates a severe problem, and 5 indicates a very severe problem.

		(no problem) 1	(mild) 2	(moderate) 3	(severe) 4	(very severe) 5	DK	NR
H1.	Eye irritation	[91]	[92]	[93]	[94]	[95]	[77]	[99]
H2.	Blurred vision	[91]	[92]	[93]	[94]	[95]	[77]	[99]
H3.	Nose irritation	[91]	[92]	[93]	[94]	[95]	[77]	[99]
H4.	Amount of mucous	[91]	[92]	[93]	[94]	[95]	[77]	[99]
H5.	Chest wheezing or whistling	[91]	[92]	[93]	[94]	[95]	[77]	[99]
H6.	Shortness of breath	[91]	[92]	[93]	[94]	[95]	[77]	[99]
H7.	Chest discomfort	[91]	[92]	[93]	[94]	[95]	[77]	[99]
H8.	Tingling fingers	[91]	[92]	[93]	[94]	[95]	[77]	[99]
H9.	Throat irritation	[91]	[92]	[93]	[94]	[95]	[77]	[99]
H10.	Cough	[91]	[92]	[93]	[94]	[95]	[77]	[99]

H11. Do you usually bring up mucous when you cough?

[1] YES
[2] NO
[77] DK
[99] NR

H12. Do you feel feverish or have chills right now?

[1] YES
[2] NO
[77] DK
[99] NR

H13. Do you usually cough on most days for 3 consecutive months or more during the year?

[1] YES
[2] NO - [IF NO, GO TO H15]
[77] DK
[99] NR

H14. For how many years have you had this cough? _____ years
[77] DK
[99] NR

H15. Do you bring up phlegm (or mucous) on most days for 3 consecutive months or more during the year?

[1] YES
[2] NO - [IF NO, GO TO H17]
[77] DK
[99] NR

H16. For how many years have you had trouble with phlegm (or mucous)? _____ years
[77] DK
[99] NR

H17. Are you troubled with shortness of breath when hurrying on level ground or walking up a slight hill?

[1] YES
[2] NO
[77] DK
[99] NR

H18. Have you had wheezing or chest whistling at any time in the past 12 months?

[1] YES
[2] NO - [IF NO, GO TO H22]
[77] DK
[99] NR

H19. How many episodes of wheezing or chest whistling have you had in the past 12 months?

_____ episodes
[77] DK
[99] NR

H20. How many times did you go to a doctor's office or a hospital emergency room for one of these episodes of wheezing or chest whistling in the past 12 months?

_____ times
[77] DK
[99] NR

H21. How many times were you hospitalized for these episodes of wheezing or chest whistling in the past 12 months?

_____ times
[77] DK
[99] NR

H22. During the past 12 months, have you had any of the following conditions [READ LIST]?

	YES	NO	DK	NR
a. Sinus problem (not a cold)	[1]	[2]	[77]	[99]
b. Pneumonia	[1]	[2]	[77]	[99]
c. Cold or flu	[1]	[2]	[77]	[99]

[IF YES, How many times per year do you typically have a cold?]

_____ times
[77] DK
[99] NR

H23. Does your chest ever feel tight in relation to dusty work?

[1] YES [Specify dust/task_____]
[2] NO
[77] DK
[99] NR

H24. **During the past 12 months, have you had any episodes of flu-like symptoms 3 to 8 hours following dusty farm work (fever, shivering, cough, tiredness, weakness, muscle and joint pains)?**

[1] YES - Specify task/dust_____ - **[GO TO H26]**
[2] NO
[77] DK
[99] NR

H25. **Have you EVER had episodes of flu-like symptoms 3 to 8 hours following dusty farm work (fever, shivering, cough, tiredness, weakness, muscle and joint pains)?**

[1] YES - Specify task/dust_____
[2] NO - **[GO TO H28]**
[77] DK
[99] NR

H26. How many of these flu-like episodes have you had in your lifetime? _____ episodes
[77] DK
[99] NR

H27. How long do these flu-like episodes usually last?

_____ hours _____ days [77] DK [99] NR

H28. Do any relatives (on your side of the family) have **[READ LIST]**

	YES	NO	DK	NR	
a. Allergies?	[1]	[2]	[77]	[99]	
b. Asthma?	[1]	[2]	[77]	[99]	
c. Chronic Bronchitis?	[1]	[2]	[77]	[99]	**[COUGH WITH PHLEGM MOST OF THE TIME]**
d. Emphysema?	[1]	[2]	[77]	[99]	**[BREATHLESSNESS ON EXERTION]**

PRE-5: WORK HISTORY

Now I have some questions to ask related to your work.

W1. Have you ever worked in a job(s) in which you breathed [READ LIST]

	YES	NO	DK	NR
a. visible dust for more than 1 hour per day? (e.g. farming,mining)	[1]	[2]	[77]	[99]

[IF YES, Specify Dust(s)_____Years at job_____]
_____Years at job_____]

b. visible metal fumes for more than 1 hour per day? [1] [2] [77] [99]
(e.g. welding,foundry,smelter)

[IF YES, Specify Fume(s)_____Years at job_____]
_____Years at job_____]

c. gases/vapors? [1] [2] [77] [99]
(e.g. gas station,painting,ammonia,dry cleaner)

[IF YES, Specify Gas/vapor_____Years at job_____]
_____Years at job_____]

d. herbicides/insecticides? [1] [2] [77] [99]

[IF YES, Specify chemical(s)_____Years at job_____]
_____Years at job_____]

W2. Have you ever lived on ANY farm? YES [1] NO [2] DK [77] NR [99] - [IF NO, GO TO W3]

2a. How long have you lived on ANY farm? ___yrs ___months [77] DK [99] NR

2b. Do you live on THIS farm? [1] YES [2] NO [77] DK [99] NR

W3. How long have you worked on ANY farm? ___yrs ___months [77] DK [99] NR

W4. How many years have you worked harvesting wheat? _____yrs [77] DK [99] NR

PRE-6: WORK HISTORY (continued)

W5. How many days per year do you work harvesting wheat? ___days [77] DK [99]NR

W6. How many days have you harvested wheat this year? _____days [77] DK [99] NR

W7. What time did you finish harvest work yesterday? _____ am/pm [77]DK [99]NR

W8. Have you breathed any dust this morning?

[1] YES [2] NO [77] DK [99] NR

[IF NO, PROMPT FOR CLEANING COMBINES, FEEDING LIVESTOCK]

[IF YES, Specify dust/task/duration_____]

		YES	NO	DK	NR	
W9.	Do you work on this farm as a family member?	[1]	[2]	[77]	[99]	- [IF YES, GO TO S1]
W10.	Do you work on this farm as a local hire?	[1]	[2]	[77]	[99]	- [IF YES, GO TO S1]
W11.	Do you work on this farm as a custom cutter?	[1]	[2]	[77]	[99]	

[IF NO, Specify employment on farm _____]

S-1: Smoking

I now have some questions to ask about your smoking history.

S1. Have you ever smoked tobacco?

[1] YES
[2] NO - [IF NO, GO TO S8]
[77] DK
[99] NR

S2. In your lifetime, have you smoked [READ LIST]

	YES	NO	DK	NR
a. more than 100 cigarettes (approximately 5 packs)	[11]	[12]	[77]	[99]
b. at least 20 cigars	[11]	[12]	[77]	[99]
c. at least 20 pipefulls of tobacco	[11]	[12]	[77]	[99]

[IF 9A,B, AND C ARE ALL NO, GO TO S8]

S3. At what age did you start smoking tobacco fairly regularly? _____ years
[77] DK
[99] NR

S4. Do you smoke now? [INCLUDES OCCASSIONAL SMOKING]

[1] YES - [IF YES, GO TO S6]
[2] NO
[77] DK
[99] NR

S5. About how long has it been since you last smoked regularly?
___ months
___ years
[77] DK
[99] NR

[AFTER THIS QUESTION, GO TO S8]

S6. On average,
a. how many cigarettes do you smoke now? _____ per_____ [77] DK [99] NR

b. how many cigars do you smoke? _____ per_____ [77] DK [99] NR

c. how many pipefulls of tobacco do you smoke? _____ per_____ [77] DK [99] NR

S-2: Smoking (continued)

S7. Have there been periods (longer than a year) when you quit smoking?

[1] YES
[2] NO
[77] DK
[99] NR

S8. When you are at your home, are you exposed to smoke from other people's cigarettes, pipes or cigars?

[1] YES
[2] NO
[77] DK
[99] NR

S9. What is the highest grade or year of school you completed, including technical schools?

[41] Eighth grade or less
[42] Some high school
[43] High school graduate or GED certificate
[44] Some technical school
[45] Technical school graduate
[46] Some college [PROMPT FOR GRADUATE]
[47] College graduate [PROMPT FOR POST GRADUATE]
[48] Post graduate or professional degree
[77] DK
[99] NR

INTERVIEWER COMMENTS:

POST-1: Subject # C___F___S___ Date_____ Time _____ am pm Initials_____

I have some final questions to ask related to your health. Please rate the severity of the following symptoms based on how you feel RIGHT NOW on a scale of 1 to 5. Here is a card showing the choices. 1 indicates no problem, 2 indicates a mild problem, 3 indicates a moderate problem, 4 indicates a severe problem, and 5 indicates a very severe problem.

		(no problem) 1	(mild) 2	(moderate) 3	(severe) 4	(very severe) 5	DK	NR
P1.	Chest discomfort	[91]	[92]	[93]	[94]	[95]	[77]	[99]
P2.	Tingling fingers	[91]	[92]	[93]	[94]	[95]	[77]	[99]
P3.	Throat irritation	[91]	[92]	[93]	[94]	[95]	[77]	[99]
P4.	Eye irritation	[91]	[92]	[93]	[94]	[95]	[77]	[99]
P5.	Blurred vision	[91]	[92]	[93]	[94]	[95]	[77]	[99]
P6.	Nose irritation	[91]	[92]	[93]	[94]	[95]	[77]	[99]
P7.	Amount of mucous	[91]	[92]	[93]	[94]	[95]	[77]	[99]
P8.	Chest whistling or wheezing	[91]	[92]	[93]	[94]	[95]	[77]	[99]
P9.	Shortness of breath	[91]	[92]	[93]	[94]	[95]	[77]	[99]
P10.	Cough	[91]	[92]	[93]	[94]	[95]	[77]	[99]

P11. Do you feel feverish or have chills right now?

[01] YES
[02] NO
[77] DK
[99] NR

P12. Did you take any medications today for any of the symptoms I have just asked you about?

[1] YES
[2] NO
[77] DK
[99] NR

POST-2:(continued)

P13. Compared to a typical harvest workday, the amount of wheat dust you were exposed to today was [READ LIST]

[51] Less than average
[52] Average
[53] More than average
[77] DK
[99] NR

P14. At any time today, did you breath an above average amount of dust?

[1] YES - [IF YES, Specify task/number of hours_____]
[2] NO
[77] DK
[99] NR

P15. How many hours have you worked harvesting wheat today? _____ hours
[77] DK
[99] NR

P16. During harvest work today, have you

	YES	NO	DK	NR	
a. Drive a combine?	[1]	[2]	[77]	[99]	[IF YES: How long? _____hours]
b. Drive a wheat truck?	[1]	[2]	[77]	[99]	[IF YES: How long? _____hours]
c. Run a wheat auger?	[1]	[2]	[77]	[99]	[IF YES: How long? _____hours]
d. Perform other tasks?	[1]	[2]	[77]	[99]	[IF YES: Specify task_____]

P17. Did you wear a face mask or a respirator today?

[1] YES [IF YES, Specify type_____]
[2] NO [IF BANDANA WORN, CHECK HERE_____]
[77] DK
[99] NR

P18. Did you smoke cigarettes, pipe tobacco or cigars today?

[1] YES [IF YES, Specify number/item_____]
[2] NO
[77] DK
[99] NR

INTERVIEWER COMMENTS:

_____

_____

HF5 SPIROMETRY LOG SHEET

Study No: _____ Computer #: _____

Location/FARM #: _____ Sensor #: _____

Date: __/__/__ PAGE #: ____

STD	SUBJECT'S REF ID	TID	DATE	T°C	BP	AGE	HT	SEX	RACE #	SM	URI	#	TLM WT
								M F	0 1 2 3 4 H	Y N	Y N		
								M F	0 1 2 3 4 H	Y N	Y N		
								M F	0 1 2 3 4 H	Y N	Y N		
								M F	0 1 2 3 4 H	Y N	Y N		
								M F	0 1 2 3 4 H	Y N	Y N		
								M F	0 1 2 3 4 H	Y N	Y N		
								M F	0 1 2 3 4 H	Y N	Y N		
								M F	0 1 2 3 4 H	Y N	Y N		
								M F	0 1 2 3 4 H	Y N	Y N		
								M F	0 1 2 3 4 H	Y N	Y N		
								M F	0 1 2 3 4 H	Y N	Y N		
								M F	0 1 2 3 4 H	Y N	Y N		
								M F	0 1 2 3 4 H	Y N	Y N		
								M F	0 1 2 3 4 H	Y N	Y N		
								M F	0 1 2 3 4 H	Y N	Y N		
								M F	0 1 2 3 4 H	Y N	Y N		
								M F	0 1 2 3 4 H	Y N	Y N		
								M F	0 1 2 3 4 H	Y N	Y N		
								M F	0 1 2 3 4 H	Y N	Y N		
								M F	0 1 2 3 4 H	Y N	Y N		
								M F	0 1 2 3 4 H	Y N	Y N		
								M F	0 1 2 3 4 H	Y N	Y N		
								M F	0 1 2 3 4 H	Y N	Y N		

SID=Subject ID; TID=Technician ID; SM=Have you smoked tobacco in the past hour?
URI=Have you had a respiratory infection in the past 3 weeks?; #=Number of trials (blows)
RACE: 0=White; 1=Black; 2=Asian/Pacific; 3=Am. Indian/Eskimo; 4=Other; H=Hispanic Origin
TLM = time last meal; WT = weight

CSU Wheat Harvest Study: Filter Weight Record

Type Filter:_____

Filter ID	Pan Wt (mg)	Pan plus filter wt (mg)	Pre-weight (mg)	Date/ Time/ Init.	Pan Wt (mg)	Pan plus filter wt (mg)	Post-weight (mg)	Date/ Time/ Init.	Total Dust (mg)
w251									
w252									
w253									
w254									
w255									
w256									
w257									
w258									
w259									
w260									
QA									

Winter Wheat Harvest Study: Pump Calibration Log

Farm #C___F___ Page ___ of ___

Date/ Location	Time am/pm	Press/ Temp	Pump #	Calibration device/id#	Time 1 (sec)	Time 2 (sec)	Time 3 (sec)	Average (sec)	Flow (lpm)	Initials

CSU Wheat Harvest Study: Field Data Collection Form FARM #C___F___ Page ___of___

SAMPLE INFORMATION

Subject#	C F S	C F S	C F S	C F S	C F S
Sample #					
Pump #					
Time on/off					
Flow on/off (lpm)					
Total time (min)					
Temp on/off					
Pressure on/off					
Humidity on/off					
Volume (l)					
Corr. Volume (l)					

CHAIN OF CUSTODY

SIGNATURE	DATE	TIME	ACTION/STORAGE/COMMENTS
			Completed day's sample collection

WHEAT HARVEST STUDY:FIELD OBSERVATIONS Farm #C___F___ Date_____ Initials_____ Page ___ of ___

Subject #	Worker's activities observed during the sampling period: Note task(s) performed and approx. duration, any respiratory protection worn, other engineering controls, etc.
C__F__S__	
C__F__S__	
C__F__S__	
C__F__S__	
C__F__S__	

Other comments about activities on the farm:

_____

_____

WHEAT HARVEST STUDY: REQUEST FOR HEALTH AND SAFETY INFORMATION

Farm # C____F____ Date_____ Initials_____

Name/Address/Phone	Question/need/request	Follow-up status