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# RESPIRATORY PROTECTION AGAINST BIOAEROSOLS IN AGRICULTURE

**P.I.:** Tiina Reponen, Ph.D., Professor

Department of Environmental health

University of Cincinnati, P.O. Box 670056

Cincinnati, OH 45267-0056

Tel: 513-558-0571, Fax: 513-558-2263

Email: Tiina.Reponen@uc.edu

**Co-investigators:**

Sergey Grinshpun, Ph.D., Roy McKay, Ph.D., Rakesh Shukla, Ph.D.

University of Cincinnati

Susan Jones, Ph.D., Gordon Jones,

Western Kentucky University

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**FINAL PERFORMANCE REPORT 10/24/11**

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## LIST OF TERMS AND ABBREVIATIONS

APF = Assigned Protection Factor

ER = Elastomeric Half-facepiece Respirator

EU = Endotoxin Unit

FFR = Filtering Facepiece Respirator

GM = Geometric Mean

GSD = Geometric Standard Deviation

MIF = Mean Inspiratory Flow Rate

OC = $C_{out}$ = Outside concentration (concentration measured outside the respirator)

OPC = Optical Particle Counter

P = Penetration

PF = Protection Factor

PS particle = Polystyrene particle

RL = Reporting Limit

WPF = Workplace Protection Factor

**ABSTRACT**
**Title:** Respiratory Protection against Bioaerosols in Agriculture
**Investigator:** Tiina Reponen, Ph.D., Professor, Department of Environmental Health, University of Cincinnati, P.O. Box 670056, Cincinnati, OH 45267-0056, Tel: 513-558-0571, Fax: 513-558-2263, Email: Tiina.Reponen@uc.edu
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**Final Report Abstract:**
Background: Current guidelines for respirator use and selection are based primarily on data collected for particle mass without size-selective data on particle concentrations. Furthermore, very little information is available on the protection provided by respirators against biological particles.
Methods: Personal sampling setup developed in a previous NIOSH grant permitted simultaneous determination of workplace protection factors (WPFs) for biological and non-biological particles. In the current study, WPF was measured on 8 farms for 25 subjects wearing an N95 filtering facepiece respirator (FFR) and an N95 elastomeric respirator (ER). Aerosol concentrations were measured simultaneously inside and outside the respirator using an optical particle counter (OPC) for the size range of 0.7–10 μm to obtain size-selective WPFs. Two filter samplers collected particles for subsequent analysis of WPF for particle mass, endotoxin, (1-3)-β-D-glucan, and fungal spores. Laboratory experiments were conducted with various types of non-biological and biological particles using a respirator partially sealed on manikin face simulating realistic faceseal leakage. Total penetration was measured as in the field study. Additionally, filter penetrations were measured after the FFR was fully sealed on the manikin face using mean inspiratory flow rates of 15, 30, and 85 L/min. Filter penetrations were deducted from total penetrations to determine faceseal penetrations.
Results: For the ER, geometric mean (GM) WPFs were 172, 321, 1013, 2097 and 2784 for particle diameters of 0.7–1.0, 1.0–2.0, 2.0–3.0, 3.0–5.0, and 5.0–10.0 μm, respectively. Corresponding values for the FFR were 67, 124, 312, 909, and 2089. WPFs for the ER were significantly higher than the FFR for all particle size ranges when calculated from the OPC data. However, when assessing WPFs against particle mass, endotoxin, (1-3)-β-D-glucan, and fungi, no significant differences between the two respirator types were found. GM WPFs for the two types of respirators combined were 154, 29, 18, 19 and 176 for endotoxin, fungal spore count, (1→3)-β-D-glucan, total particle mass, and total particle number, respectively. The differences in the WPFs between different types of contaminants were statistically significant. Careful statistical data analysis indicated that the differences between contaminants were due to differences in the sensitivity of analytical methods. Laboratory experiments with non-biological particles representing the size range of bacteria and fungal spores (0.7 - 4 μm) confirmed that WPF decreases with decreasing particle size. Laboratory experiments with model bacteria (*Pseudomonas fluorescence*) and fungi (*Penicillium citrinum*) did not show differences between WPFs measured by OPC and by microbiological methods. Laboratory experiments also showed that spherical particles had 2.0-2.8 times higher penetration through faceseal leaks and 1.1-1.5 times higher penetration through filter media than fibers of similar aerodynamic diameter.
Conclusions: The results show WPFs for the ER were higher than the FFR for all particle size ranges and WPFs for both respirator types decreased with decreasing particle size. Results also indicate that differences in WPFs observed between different contaminants may be attributed to

differences in the sensitivity of analytical methods to detect low inside concentrations, rather than the nature of particles (biological or non-biological).

**SECTION 1**

**Significant Key Findings:**

- WPFs for the N95 elastomeric respirator were higher than the N95 filtering facepiece respirator for all particle size ranges.
- WPFs decreased with decrease in particle size
- Differences were observed in the WPFs against different contaminants (particle mass, endotoxin, (1-3)-β-D-glucan, and fungi), but these were found to be due to differences in the sensitivity of analytical methods rather than the nature of particles.
- Laboratory experiments showed that spherical particles had 2.0-2.8 times higher penetration through faceseal leaks and 1.1-1.5 higher penetration through filter media than fibers of similar aerodynamic diameter.

**Translation of Findings:**

- Both types of half mask air purifying respirators used in this study (N95 elastomeric and N95 filtering facepiece) achieved WPFs above the OSHA Assigned Protection Factor (APF) of 10.
- This study supports the OSHA APF of 10 for N95 half mask respirators worn by agricultural workers for protection against. commonly encountered non-biological and biological particles.
- Better protection was offered by N95 elastomeric respirator compared to N95 filtering facepiece respirator.
- For the assessment of WPFs, direct reading particle counter appears to provide data that can be used for estimating the protection provided against spherical or near spherical particles of different nature (biological vs. non-biological particles). The benefit for these devices is their low detection limit that allows the measurement of very low concentration inside the respirator.
- Since fibers have less penetration through filter media and faceseal leaks than spherical particles, assessment of penetration for spherical particles provides a more conservative estimate for protection factor studies.

**Outcomes/Impact:**

- Results are helpful for the selection of respirators to be used for protection against various types of biological particles.
- The finding of particle size differences and sensitivity of analytical methods used to calculate WPFs provides valuable information for future WPF studies.

## SECTION 2 – SCIENTIFIC REPORT

### A. BACKGROUND

There are about 3 million farm workers in the United States (USDA-NASS, 2005). Of these, 2 million are farm owners or family members working on the farm. Although large family and commercial farms account for over half of the total value of agricultural production, small family farms constitute 91% of all farms (U.S. Bureau of Labor Statistics, 2005). Disorders of the upper and lower respiratory track have been reported after exposure to a variety of work environments on farms. The types of agricultural environments most commonly associated with respiratory complaints include grain farming and handling, working in animal confinement units, and dairy farming (Von Essen and Donham, 1997). Among the agents that can cause respiratory diseases, organic dusts, including bacteria and fungal spores, are the most ubiquitous agents in agriculture (Jacobs, 1994; Von Essen and Donham, 1997). The pulmonary and systemic response to endotoxin from gram-negative bacteria in farm dust is particularly important in the causation of illness in exposed workers (Reynolds et al., 1996; Viet et al., 2001; Von Essen and Romberger, 2005). There is some evidence that peptidoglycan from gram-positive bacteria also plays a role in causing illness (Zhiping et al., 1996). Exposure to fungi is also important as it can cause inflammation and has been associated with lung disease (Milanowski, 1998; Shahan et al., 1994; Radon et al., 2002).

The concentrations of airborne bacteria and fungal spores typically range from $10^4$ up to $10^9$ cfu/m$^3$ in agricultural environments (Donham et al., 1989; Dutkiewicz et al., 1989; Crook et al., 1991; Dutkiewicz et al., 1994; Thorne et al., 1994; Krahmer et al., 1998). In contrast, indoor air environments typically have 2-5 decades lower concentrations, from $10^2$ to $10^3$ cfu/m$^3$ (Reponen et al., 1992; DeKoster and Thorne, 1995; Rao et al., 1996: Niemeier et al., 2005). The mass concentrations for total dust in agricultural environments (0.7 to 95.4 mg/m$^3$) (Molocznik, 2002; Roy and Thorne, 2003) have also been found to be much higher than those measured in non-agricultural indoor environments (0.02 to 0.44 mg/m$^3$) (Schneider et al., 2003), and often exceed the exposure limit for total dust (15 mg/m$^3$, OSHA, 1993) and organic dust (5 mg/m$^3$, Swedish National Board of Occupational Safety and Health, 1994). Agricultural workers exposed to high concentrations of dust and bioaerosols frequently experience acute respiratory symptoms, such as cough, chest tightness, dyspnea, wheezing, rhinitis, which often develop into more severe respiratory diseases, such as occupational asthma, bronchitis, organic dust toxic syndrome, and farmer's lung (Terho et al. 1987; Lacey and Crook, 1988; Malmberg and Rask-Andersen, 1990; de Pico, 1994; Melbostad et al., 1997; Von Essen and Romberger, 2005; Dosman et al., 2005). An emerging health issue is the occurrence of multi-drug resistant bacterial pathogens in animal confinements. Recently, Chapin et al. (2005) reported that 98% of bacteria isolated from air samples collected in swine confinements expressed high level of resistance to at least two commonly prescribed antibiotics.

Because the application of engineering controls is limited due to the diverse nature of bioaerosol sources in agricultural environments, the use of respirators is in many cases the best option available for reducing the exposure of workers. The OSHA Respiratory Protection Standar*d (29 CFR Part 1910.134)* does not apply specifically to agricultural workplaces. Furthermore, there is limited guidance for respiratory protection against bioaerosols other than those regarding tuberculosis, Severe Acute Respiratory Syndrome (SARS) and Avian flu (CDC, 1994; 1999; 2005a; 2006). The CDC guidelines (CDC, 1994) include performance criteria for respirators to be used by health-care workers against *M. tuberculosis* bacteria. All respirators that

are certified by NIOSH (42 CFR Part 84; CDC, 1995) satisfy the current CDC guidelines and are, therefore, authorized for use in health care facilities. Among the nine categories of air purifying respirator filters available for use with particulate exposures, the N95 filtering facepiece respirators are the least expensive and most frequently used in general work environments and in agriculture (BLS/NIOSH, 2003; Doney et al., 2005). N95 respirators were also recommended by CDC to be used during renovation of moldy buildings after the flooding caused by hurricane Katrina in New Orleans area (CDC, 2005b). The number "95" in this designation means that the filtration efficiency of the respirator is at least 95% at the most penetrating particle size (ca. 0.1 to 0.3 μm). The "N" series designation refers to a challenge aerosol of sodium chloride. Respirators with less efficient filtration characteristics are not certified. One of our earlier respirator studies has shown that the filtration efficiency of N95 filters is 99.5% or higher for particles of the bacterial size range (Qian et al., 1998). For fungal spores that are mostly about 2-3 μm in aerodynamic size (Reponen et al., 1996), the filtration efficiency is expected to be even higher. Therefore, it is reasonable to assume that the filtration efficiency of N95 filtering facepiece respirators is sufficient for bioaerosols in agricultural environments. Face-seal leakage, however, may result in unacceptable levels of microorganism penetration inside the respirator (Lee et al., 2004c). Our pilot-scale field study (Lee et al., 2005b) showed inadequate protection by N95 filtering facepiece respirators against microorganisms in actual agricultural work environments, where the fit of the respirator to the wearer's face may vary, resulting in leakage of particles from the ambient environment inside the respitaor.

Agricultural workers are more likely to wear personal protective equipment against noise and pesticides than against dust (Niewenhuijsen et al., 1996). An earlier study showed that 30% of swine producers usually wore dust masks when working inside a barn. However, no apparent protective effect of dust masks was observed because of inappropriate use of the respirators and impaired respiratory health underlying an individual's decision to begin respiratory protection (Zejda et al., 1993). A later study by the same group showed that wearing fit-tested respirators decreased acute health effects of swine confinement workers (Senthilsevan et al., 1999). After that, several other studies have shown the positive health effects of wearing a respirator in agricultural environments (Obase et al., 1999; Larsson et al., 2002, Palmgren et al., 2004; Dosman et al., 2000). However, these studies did not measure the degree of protection provided by respirators, which is usually expressed as Workplace Protection Factor (WPF).

WPF is a measure of the actual protection achieved in the workplace while the respirator is properly worn and used during normal work activities. WPF is expressed as the ratio of the ambient to the in-facepiece concentration:

$$\mathrm{WPF} = \frac{\text{Concentration outside the respirator}}{\text{Concentration inside the respirator}} \quad (1)$$

It is a measure of the protection provided in the workplace, under the conditions of the workplace, by a properly selected, fit-tested, and functioning respirator that is correctly worn and used (CDC, 1995; AIHA, 2002). Only a small WPF database is currently available, which limits OSHA's ability to regulate the Assigned Protection Factors (APF) for different types of respirators. There is considerable controversy around the OSHA APFs for filtering facepiece respirators. Based upon a limited number of published WPF studies, OSHA has assigned an APF of 10 for filtering facepieces as well as elastomeric half-facepiece respirators (OSHA, 2006). However, many experts in the respirator community feel these two facepieces offer different levels of respiratory protection. Arguments against the APF of 10 are based in part upon concerns that the workplace atmospheres used in these studies had relatively large particle sizes

which may not reflect performance in other work environments where smaller particle sizes are encountered. Thus, there is a need to broaden the WPF database for a variety of occupational environments, personal protection practices, and the particle size ranges of airborne contaminants.

Previous studies of the WPF against dust have been performed by collecting the mass of the total dust or specific metals inside and outside the respirators without investigating the respiratory protection for individual particle size ranges (Myers et al., 1996 and 1998; Zhuang et al., 1996). Furthermore, beyond our pilot-scale field study (Lee et al., 2005b) there appears to be no other information on the WPF against bioaerosols. There is only one earlier report on WPFs in agricultural environments (Popendorf et al., 1995).

Table I summarizes the study design of previous peer-reviewed studies that investigated WPF for half-facepiece respirators (both disposable and elastomeric). These studies included 7-25 subjects and altogether 22-70 WPF data points. None of these studies measured WPF against particles of different sizes or against biological particles. The two studies that included both disposable and elastomeric respirators (Myers et al., 1996 and Myers and Zhuang, 1998) did not find any difference in the WPF between these two respirator types. This may be due to their study designs. Within each study, different study procedures were used for different subjects: some subjects were tested only with one respirator while others were tested multiple times or with multiple respirators. Furthermore, multiple sites were used for testing but the different types of respirators were not consistently tested at all sites. Therefore, there is a need for more controlled WPF studies that would overcome the limitations of previous ones. Furthermore, there is need to obtain information on WPF against biological particles and against particles of different sizes.

In our original NIOSH grant, we found a significant effect of the particle size and particle type (biological vs. non-biological) on the WPF. Furthermore, the particle size was found to depend on the farming type (Lee et al., 2005b, 2006). Therefore, the protection provided by respirators against particles should ideally be determined dynamically using size-selective method during the worker's normal work routines. Previously there was no field-compatible method available to measure the WPF in real-time or to measure WPF against biological particles. In our previous NIOSH grant (1 RO1 OH 04085), we developed a new method to measure WPF, which allows addressing the existing knowledge gaps as explained below. Our pilot-scale study conducted in New Orleans affected by hurricane Katrina indicates that elastomeric half-facepiece respirators may offer better protection against fungal spores than disposable filtering facepiece respirators (although both respirator types have been assigned an APF=10 [OSHA, 2006]).

In this study, the new technique was used to measure real-time the protection provided by respirators in agricultural environments. Two types of respirators were included: disposable N95 filtering facepiece respirator and an elastomeric half-facepiece respirator. Number concentration and size distribution of particles was measured in real time simultaneously inside and outside of the respirator. In parallel with the dynamic measurements, samples were collected and analyzed for total particle mass, total fungal and bacterial counts, as well as for β-glucan and endotoxin. The results of this study document the range of respirator WPFs against dust and bioaerosols for farmers when they perform their usual farming activities. Laboratory-based experiments were performed to confirm the field-based results.

**Table I. Peer-reviewed studies on WPF of half-facepiece respirators (elastomeric and disposable)**

| Author | Respirator type(s) | Contaminant | Sampling time | Number of subjects | Number of WPFs |
|---|---|---|---|---|---|
| Cohen, 1984 | 1 model of disposable mercury vapor respirator | Mercury | 10-30 min | 7 | 26 |
| Dixon and Nelson, 1984 | 1 model of elastomeric respirator with organic vapor and HEPA filter | Particulate lead | 30-120 min | 11 | 37 |
| Lenhart and Campbell, 1984 | 1 model of elastomeric respirators with HEPA filters | Particulate lead | ~ 8 hours | 25 | 25 |
| Reed et al., 1987 | 1 model of disposable dust/mist respirator | Total dust mass | 6 hours | 7 | 22 |
| Galvin et al., 1990 | 1 model of elastomeric respirator with organic cartridge | Styrene | 60 min | 13 | 63 |
| Wallis and Menke, 1993 | 1 model of disposable dust/mist respirator | Particulate Mn | 30-40 min | unknown | 70 |
| Myers et al., 1996 | 1 model of disposable and 3 models of elastomeric dust/mist respirators | Particulate Zn and Pb | 1-4 hours | 25 | 66 |
| Zhuang and Myers 1996 | 3 models of elastomeric respirators with HEPA filters | Particulate Ti and Cr | 1-4 hours | 22 | 36 |
| Myers and Zhuang, 1998 | 2 models of disposable and 3 models of elastomeric dust/mist respirators | Particulate Fe | 1-4 hours | 16 | 54 |
| Weber and Mullins, 2000 | 1 model of elastomeric respirator with dust/mist filter and organic vapor cartridge | Styrene | 23-88 min | 19 | 46 |

This study gives unique new information on the following aspects: 1) size-selective data on the WPF against particles, 2) real-time data on the WPF against particles, 3) data on WPF against biological particles, 4) data on the effect of particle characteristics on the WPF, and 5) data on the WPFs obtained with two common respirator types: N95 filtering facepiece and elastomeric half-facepiece respirators. This study belongs to NORA2 sector "Agriculture, Forestry and Fishing".

## HYPOTHESIS

In agricultural environments, WPF against biological particles is significantly lower than that against non-biological particles due to the differences in aerodynamic particle size and shape. The WPFs measured for elastomeric half-facepiece respirators are higher and have smaller variation than those measured for N-95 filtering facepiece respirators.

## SPECIFIC AIMS

1. Assess the range of WPFs for N-95 filtering facepiece and half-facepiece elastomeric respirators against particles and bioaerosols in agricultural environments.
2. Assess the contribution of factors (aerodynamic size and shape of particles) that could cause the difference in WPF between biological and non-biological particles**,** under controlled laboratory conditions, under field conditions, and through theoretical modeling.

## SPECIFIC AIM 1

***Assess the range of WPFs for N-95 filtering facepiece and half-facepiece elastomeric respirators against particles and bioaerosols in agricultural environments***

### B.1 Procedures

***Test Subjects, Sites, and Respirators***

Twenty-five healthy farm workers ranging in age from 20 to 30 years old voluntarily participated in this study. Among 25 subjects, one Hispanic male and six females were included to reflect the gender and racial make-up of farmers in Ohio and Kentucky (which are very close to the US average). Altogether eight farms were included representing three different types: two horse/livestock pavilions, three pig barns, and three grain handling sites. The activities on farms of these types were expected to generate high aerosol concentrations with a wide particle size range. The selected farms were typical of those in the south central region of the US**.**

The respirators tested in the study were represented by an elastomeric half-facepiece respirator (ER) equipped with N95 filters and an N95 filtering facepiece respirator (FFR). The ER was available in three sizes, whereas FFR was available in two sizes. The respirators used for this study were selected due to their high success rates in passing routine quantitative fit testing, as determined from our clinical experience (i.e., both respirators had good fitting characteristics).

**Table II. Summary of the field testing sites on agricultural farms.**

| Farm Types | Number of subjects tested | | Sampling time | Activity |
|---|---|---|---|---|
| | Male | Female | | |
| Grain Handling 1 (Grain Bin) | 3 | | August 2008 | Shoveling, sweeping |
| Grain Handling 2 (Commodities/grain/feed dealer) | 2 | | December 2008 | Walking; unloading grain |
| Grain Handling 3 (Grain Bin) | 3 | | October 2009 | Shoveling, sweeping |
| Horse Farm 1 (Horse/livestock pavilion) | 1 | 3 | January 2008 | Sweeping, spreading hay |
| Horse Farm 2 (Horse/livestock pavilion) | 4[A] | | March 2009 | Sweeping |
| Pig Barn 1 (Confinement swine farrowing/nursery barn) | | 3[B] | March 2008 | Sweeping, feeding |
| Pig Barn 2 (Confinement swine finishing barn) | 3 | | June 2008 | Sweeping, scraping |
| Pig Barn 3 (Confinement swine barn) | 3[A] | | June 2009 | Cleaning with air blowers |

[A] One subject on this farm failed fit test to the filtering facepiece respirator

[B] Missing data for one subject with a filtering facepiece respirator due to an instrument malfunction

All subjects signed a consent form approved by the University of Cincinnati's Institutional Review Board and obtained medical clearance using an on-line questionnaire prior to field testing. (Janssen and McCullough 2010) All subjects were asked not to smoke for at least one hour prior to field testing and male subjects were clean shaven. Study subjects were trained to wear both respirators according to manufacturer's instructions. All subjects passed

user seal checks prior to fit testing. Fit testing was performed using a TSI PortaCount Plus with an N95 companion (TSI, Inc., St. Paul, MN). In order to minimize systematic errors in results, the type of respirator (ER or FFR) to be worn first was randomly assigned to the first subject each testing day. Respirator type was then alternated for all subsequent subjects.

Voluntary respirator use among this farming population was generally intermittent and varied considerably. Some farmers only wore respirators during activities that they perceived to be dusty operations. During preliminary studies, we observed that some study subjects did not tolerate wearing of respirators more than 1 hour at moderate to strenuous work loads. Preliminary studies also confirmed that sufficient particle counts could be obtained for sample times of 30 minutes. Consequently, our subjects wore the ER and FFR while performing their daily activities, such as spreading hay, feeding livestock, and shoveling. Table II summarizes the activities at each site. Among 25 subjects, two subjects failed fit testing to the FFR (one on Horse Farm 2 and the other in Pig Barn 3). In addition, data were missing on one subject wearing the FFR in Pig Barn 1 due to an instrument malfunction. Thus, data for 3 subjects were excluded from the FFR dataset.

## B.2 Methodology

### *Particle Measurement*

Aerosol particle concentrations inside and outside the respirator were measured with a personal sampling system described in an earlier WPF-study conducted in agricultural environments (Lee, Grinshpun et al. 2005). Briefly, as shown in Figure 1, the personal sampling system consists of two identical sampling lines each with a sampling probe, a sampling chamber, an optical particle counter (HHPC-6, Hach Company, Loveland, CO ), and a pump (Leland Legacy, SKC Inc., Eighty Four, PA). The optical particle counter measures particle number concentration in five size channels: 0.7–1.0, 1.0–2.0, 2.0–3.0, 3.0–5.0, and 5.0–10.0 μm. The corresponding mean sizes of these channels are 0.85, 1.5, 2.5, 4, and 7.5 μm. Using a DryCal DC-Lite calibrator (Bios International Corporation, Butler, NJ), the pump was adjusted to maintain a total sampling flow of 10 L/min. Particle concentrations were measured simultaneously inside and outside the respirator during the first and last 15 minutes of a 60-minute experiment. The sampling time was intentionally less than respirator wear time to avoid moisture condensation inside sample tubing. For every subject, size-selective WPFs were calculated in one-minute intervals and then averaged over the 30-minute sampling time. WPFs were also calculated for "all" particle sizes after combining particle concentrations from each of the five particle size channels.

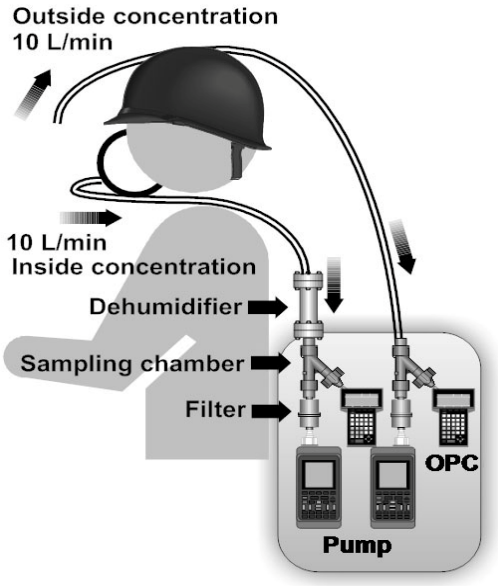



Figure 1. Schematic presentation of the personal sampling setup.

### *Collection of Bioaerosol and Particle Mass Samples*

Particles were collected on a polycarbonate filter (a pore size of 3.0 μm and a diameter of 25 mm, Millipore, Billerica, MA) loaded in a cassette (225-1107, SKC Inc., Eighty Four, PA) for bioaerosol analysis (endotoxin, fungal spores and (1→3)-β-D-glucan). One cassette was connected with the inside sampling line and another cassette was connected with the outside sampling line. The filters and cassettes

were cleaned and sterilized before collecting samples in the field. Each filter was placed in a 10-ml pyrogen free tube containing 5 ml of Tween 80 solution (0.05% in pyrogen free water) for cleaning. The tube was vortexed for 1 minute and agitated in an ultrasonic bath for 15 minutes. The filter was then rinsed twice with pyrogen free reagent water (Associates of Cape Cod Inc., East Falmouth, MA) and air-dried in a biosafety hood (SterilchemGARD Class II, Type B2, The Baker Company Inc., Stanford, ME). The compartments of the filter holder except O-rings were soaked in a beaker of soap water for 10 minutes then agitated in an ultrasonic bath for an additional 10 minutes. The compartments were rinsed with tap water for 10 minutes and agitated again with autoclaved water for 10 minutes. Subsequently, the compartments were autoclaved for 15 minutes after being air-dried in the biosafety hood. O-rings were soaked in 70% ethanol for 30 minutes and air-dried in the biosafety hood because these are not autoclavable.

A portion (2.8 L/min) of the total sampling flow (10 L/min) was passed into the optical particle counter. The remaining air flow (7.2 L/min) was diverted to the filter to collect bioaerosols. Flow rates were calibrated using a DryCal DC-Lite calibrator (Bios International Corporation, Butler, NJ). Bioaerosols were collected during the first and last 15 minutes of the 60-minute experiment onto one pair of filter samplers collecting inside and outside the respirator. Separate bioaerosol samples were not collected for the first and last 15-min in order to obtain sufficient amount of analyte, especially inside the respirator. After sampling, the filter cassette was covered with aluminum foil, and kept in a disinfected icebox during the transportation from the field to the laboratory. Total particle mass, endotoxin, fungal spore count, and (1→3)-β-D-glucan concentration were analyzed as described below.

***Extraction for Bioaerosol Analysis***

Bioaerosols collected on filters were extracted immediately after the filters were analyzed gravimetrically. Each filter was placed into a 10-ml sterile pyrogen free tube containing 9 ml of extraction solution (0.05% Tween 80 in pyrogen free water). Tubes were vortexed for 2-minutes followed by 15-minutes agitation in an ultrasonic bath. The extracted solution was divided into aliquots for further analysis. Preparation for microscopic counting of fungal spores was conducted immediately after filter extraction. Aliquots for endotoxin and β-glucan assays were stored at in -20°C for up to two weeks before analysis.

***Endotoxin Analysis***

Endotoxin was determined using an endotoxin-specific *Limulus* Amebocyte Lysate (LAL) kinetic chromogenic assay (Pyrochrome Associates of Cape Cod Inc., East Falmouth, MA) with an absorbance microplate reader (ELx808, BioTek Instrument Inc., Winooski, VT) as described earlier (Adhikari, Jung et al. 2009) The absorbance was measured every 60 sec for 180 min, and converted into Endotoxin Units (EU/$m^3$).

***Fungal Spore Count***

A 1 ml aliquot of the extracted solution was filtered through a 13-mm mixed cellulose ester (MCE) filter with pore size of 1.2 μm (Millipore Corporation, Billerica, MA) using an analytical stainless-steel vacuum filter holder (Fisher Scientific, Pittsburgh, PA). After filtration, the filter was placed on a microscopic glass slide, made transparent and stained as described previously (Adhikari, Martuzevicius et al. 2003). Fungal spores were counted under a bright light microscope and converted into concentration units (spores/$m^3$).

***(1→3)-β-D-glucan Analysis***

Concentration of (1→3)-β-D-glucan was assessed by the β-D-glucan-specific kinetic choromogenic LAL assay (Glucatell Kit, Associates of Cape Cod Inc., East Falmouth, MA) with the above-mentioned absorbance microplate reader, as described before (Adhikari, Jung et al. 2009). The results were converted into concentration units (ng/$m^3$).

***Total Particle Mass***

Particle mass was determined by weighing the filter with a microbalance (M5, Mettler-Toledo Inc., Columbus, OH). Weighing was typically performed one day before and after sampling. Before weighing, filters were placed in a desiccator overnight and weighed in triplicate to calculate averages for unloaded and loaded filters. Immediately before weighing, all filters were exposed to a static neutralizer (Staticmaster 2U500, NRD LLC, Grand Island, NY) to neutralize static charge on filters to avoid interference.

***Field Blanks***

One field blank per subject (total of 25 field blanks) was collected. Blank filters were loaded into a filter cassette and treated just like field samples, except there was no sample flow. All field blanks were analyzed by weighing and subjected to analysis of biological contaminants as described above. All values were converted to airborne concentration units using an average sampling volume of 0.218 $m^3$, for the 30 minute sampling time. Geometric means (GMs) of field blanks for endotoxin, fungal spore count, (1→3)-β-D-glucan, and total particle mass were, 4 EU/$m^3$, 2,436 spores/$m^3$, 5.3 ng/$m^3$, and 25 μg/$m^3$ respectively. These concentrations are referred to as "reporting limits" (RL) for each contaminant throughout this paper. With the same average sampling volume, analytical detection limits for endotoxin, fungal spore count, (1→3)-β-D-glucan, and total particle mass were 2.2 EU/$m^3$, 277 spores/$m^3$, 0.1 ng/$m^3$, and 1 μg/$m^3$, respectively. Theoretical detection limit for total particle number is 5 particles/l (one particle for each channel), and a RL for total particle number could not be determined because particle concentrations could not be measured from the field blanks.

**B.3 Statistical Analysis**

Among the contaminants quantified in this study, concentrations measured outside the respirator below the respective RL were discarded from entire data sets to avoid significant underestimation of WPFs: four data sets for fungal spore count and three data sets for total particle mass. Concentrations measured inside the respirator below their respective RL varied from 0 to 48 % (endotoxin: 48%, (1→3)-β-D-glucan: 38%, fungal spore: 41%, total particle mass: 42%, and total particle number: 0%). Geometric means (GMs) and geometric standard deviations (GSDs) of WPFs were evaluated using three statistical approaches for the treatment of inside concentration below the RL. These three approaches are: (1) "excluded" refers the exclusion of a WPF value when inside concentration was below the RL for each contaminant ($WPF_{excluded}$); (2) "replaced" refers to the traditional approach of using 50% of the RL for inside concentration below the RL ($WPF_{replaced}$); (3) "censored" refers to treatment of inside values less than the RL using a censoring regression method described below ($WPF_{censored}$). Censoring regression is a method based on maximum likelihood estimates and allows both left censoring (above certain cut-off values) and right censoring (below certain cut-off values). In censoring regression, censoring values can be varied between observations in a dependent variable. Censoring regression has been shown to be accurate for both non-detected and detected

data.(Liu, Lu et al. 1997; Helsel 2005) In this study, results were right-censored because the minimum value for WPFs is theoretically 1. These three approaches for handling inside concentration below the RL for each contaminant were compared using one-way analysis of variance. Log-transformation was done for each of the continuous variables to induce normality.

Because each subject wore two types of respirators (ER and FFR), observations could not be considered independent. Under this situation, regression models may underestimate standard errors. To adjust regression model estimates for clustering, an alternative, more robust approach for calculating standard errors was applied.(Aerts, Molenberghs et al. 2002) WPFs for different contaminants were compared using censored regression after accounting for clustering. To identify factors associated with each WPF, univariate censored regressions were used (STATA; StataCorp LP, College Station, TX, SAS 9.2; SAS Institute Inc., Cary, NC).(Hardin and Hilbe 2003) Respirator type, gender and farm types were considered as cofactors for each WPF. Variables significant at the 5% level with univariate analysis were considered for multivariate censored regression. Standard deviations for regression coefficients were adjusted for clustering. Possible interaction effects were also assessed before finalizing the regression model. Censored regression was also used for the analysis of the association between WPFs and concentrations measured outside the respirator. P-value of 0.05 was considered significant for all analysis.

## B.4 Results

### *Results obtained by the optical particle counter*

Normalized size-selective number concentrations of particles measured outside the respirator for three different farm types are presented in Figure 2. Total particle number concentration varied from $1.2\times10^6$ to $1.7\times10^8$ particles/$m^3$. The multivariate analysis assessed the effect of farm type and particle size on the outside concentrations. Interaction was found between farm type and particle size and therefore, the model was adjusted for this interaction. On average, horse farms had an 11-fold higher geometric mean outside concentration than grain handling sites ($p\leq0.0001$). There was, however, no significant difference in the concentrations between the grain handling and the pig barns ($p=0.101$).

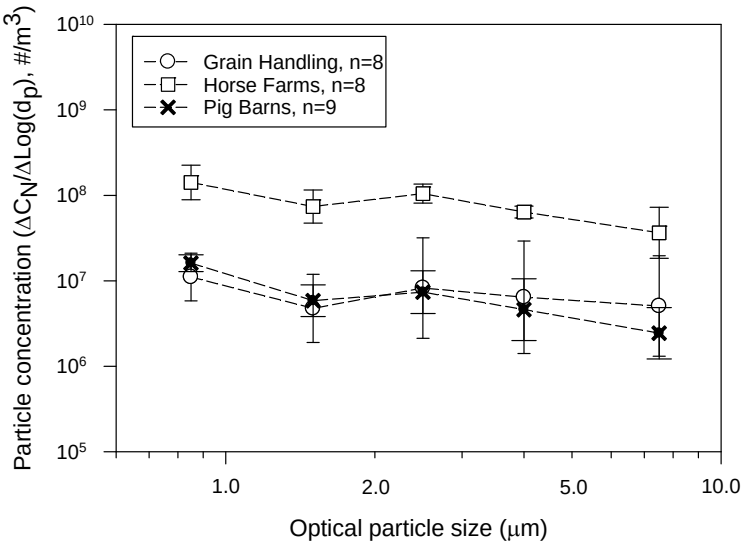



Figure 2. Normalized outside particle number concentrations at three different farm types. The symbols present geometric means, and error bars present geometric standard deviations.

The average of WPFs during the first15 minutes was compared with those from the last 15 minutes. Result showed no statistically significant difference between WPFs for the two periods (ER: p=0.76, FFR: p=0.89). Therefore, an average over the 30-minute sampling time was used for further data analyses.

Figure 3 presents the WPFs provided by the two types of respirators as a function of particle size. For the ER, geometric means (GMs) were 172, 321, 1013, 2097, and 2784 for particle sizes of 0.7–1.0, 1.0–2.0, 2.0–3.0, 3.0–5.0, and 5.0–10.0 μm, respectively. Corresponding values for the FFR were 67, 124, 312, 909, and 2089. Another observation from Figure 3 is that the WPFs were higher for the ER than the FFR in all size ranges.

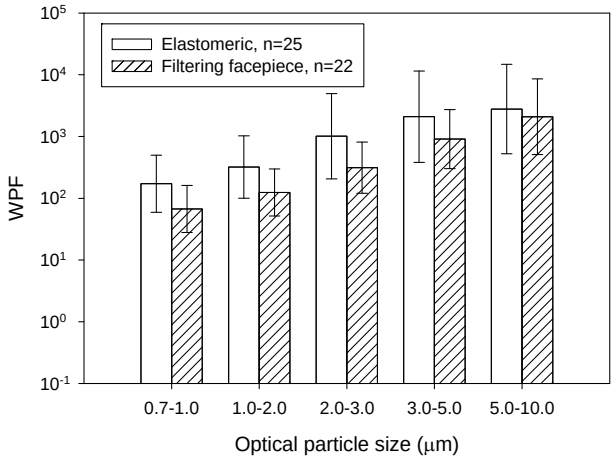



Figure 3. Workplace protection factor (WPF) provided by elastomeric respirator and filtering facepiece respirator for particles of different sizes (n=the number of subjects). The histograms present geometric means, and the error bars present geometric standard deviations.

Table III compares the 5$^{th}$ percentiles of WPFs for the ER and FFR. For both respirator types, all particle size selective WPFs were higher than the assigned protection factor (APF) of 10 for half facepiece respirators (OSHA 2006) . The 5$^{th}$ percentiles for the ER were higher than those for the FFR for all five particle size ranges. Similar trend was seen when WPFs were calculated from the total number concentration of particles. The 5$^{th}$ percentiles of the WPFs for the ER and FFR indicate a similar trend: WPFs increased as particle size increased.

In the univariate analysis, the WPF was found to be significantly associated with respirator type, farm type, particle size, and outside concentration, whereas no association was found with gender of the respirator wearer. WPFs measured on horse farms were higher than those measured on the other farm types. A high co-linearity between outside concentration and farm type was observed. This indicates that the difference in WPFs between farm types was mainly due to differences in outside concentration. The possible interaction effects between particle size and respirator type, farm type and particle size, and respirator type and farm type were also explored. The results on the multivariate analysis assessing factors that affect the WPF are summarized in Table IV. In the final multivariate model, only respirator type and particle size remained significant. The WPFs were 2.4 times higher for the ER than for the FFR ($p \leq 0.0001$). Furthermore, the size-selective WPFs increased significantly with the increase in particle size.

**Table III. Comparison of the 5th percentiles of the workplace protection factor (WPF) for the elastomeric respirator and the filtering facepiece respirator.**

| | 5th percentile | |
|---|---|---|
| | N95 elastomeric | N95 filtering facepiece |
| | n=25 | n=22 |
| 0.7 – 1.0 μm | 27.8 | 16.2 |
| 1.0 – 2.0 μm | 43.0 | 32.2 |
| 2.0 – 3.0 μm | 61.5 | 48.0 |
| 3.0 – 5.0 μm | 131.5 | 86.0 |
| 5.0 – 10.0 μm | 250.0 | 223.4 |
| Total: all particle sizes combined[A] | 63.8 | 44.0 |

[A] WPF values were calculated from the total number concentrations (by adding up all the number concentrations for each size range).

The association between WPFs and total outside/inside concentrations was further investigated by a correlation analysis. The correlation coefficient was -0.41 ($p = 0.005$) for the inside concentration and 0.31 ($p=0.03$) for the outside concentration (data not shown).

**Table IV. Multivariate analysis results for log-transformed workplace protection factors assessed by the generalized estimating equation.**

| Variables | Regression Estimates (95%Confidence Interval) | p-value |
|---|---|---|
| **Group** | Regression coefficient[A] | |
| Filtering facepiece | Reference | |
| Elastomeric | 0.88 ( 0.55, 1.22 ) | ≤ 0.0001 |
| **Size** | | |
| 0.7 – 1.0 μm | Reference | |
| 1.0 – 2.0 μm | 0.63 ( 0.53, 0.73 ) | ≤ 0.0001 |
| 2.0 – 3.0 μm | 1.71 ( 1.41, 2.01 ) | ≤ 0.0001 |
| 3.0 – 5.0 μm | 2.62 ( 2.21, 3.03 ) | ≤ 0.0001 |
| 5.0 – 10.0 μm | 3.42 ( 2.79, 4.05 ) | ≤ 0.0001 |

[A]The regression estimates are log-transformed. For example, the elastomeric respirator had $e^{0.88} = 2.4$ times higher geometric mean than the filtering facepiece respirator.

### *Results obtained from the filter samples*

Airborne concentrations measured outside the respirator for four different contaminants (endotoxin, fungal spore count, (1→3)-β-D-glucan, and total particle mass) are summarized in Table V. Airborne concentrations of endotoxin varied from 7 to $8.4\times10^5$ EU/m$^3$ (1 to 84,000 ng/m$^3$, based on the conversion formula 10 EU = 1 ng (Malyala and Singh 2008). Corresponding values for fungal spores ranged from 3,226 to $9.9\times10^6$ spores/m$^3$, whereas (1→3)-β-D-glucan varied from 34 to $6.0\times10^4$ ng/m$^3$. Total particle mass concentration varied from 0.17 to 13.7 mg/m$^3$. As reported above, (Cho, Jones et al. 2010).

**Table V. Airborne concentrations of different contaminants measured outside the respirator at eight agricultural settings**

| | Endotoxin | Fungal spores | β-glucan | Total particle mass |
|---|---|---|---|---|
| | EU/m$^3$ | spores/m$^3$ | ng/m$^3$ | mg/m$^3$ |
| Reporting limit (RL) | 4 | 2,436 | 5.3 | 0.025 |
| N | 48 | 44 | 48 | 45 |
| n (outside concentration greater than 10×RL) | 46 | 36 | 46 | 41 |
| AVE | 51,603 | 1,174,102 | 4,672 | 2.7 |
| GM | 3,267 | 172,299 | 476 | 1.6 |
| MIN | 7 | 3,226 | 34 | 0.17 |
| MAX | 840,311 | 9,938,877 | 60,329 | 13.7 |

Table VI presents GMs and GSDs of WPFs for each contaminant and number of data points used for the treatment of data below the RL: $WPF_{censored}$, $WPF_{replaced}$, and $WPF_{excluded}$. $WPF_{censored}$ and $WPF_{replaced}$ included all data points even if inside concentration was below the RL. $WPF_{excluded}$ had less data points due to the exclusion of the data below the RL. Although the respective GM and GSD estimates for the WPFs made by the three data adjustment methods were not significantly different from each other, $WPF_{replaced}$ demonstrated slightly higher WPFs for all contaminants.

**Table VI. WPFs based on three methods for the treatment of values below the reporting limit[A,B,C].**

| Contaminant | $WPF_{censored}$[A] | | | $WPF_{replaced}$[B] | | | $WPF_{excluded}$[C] | | | ANOVA |
|---|---|---|---|---|---|---|---|---|---|---|
| | n[D] | GM | GSD | n | GM | GSD | n | GM | GSD | p |
| Endotoxin | 48 | 154.1 | 28.7 | 48 | 282.8 | 10.5 | 25 | 135.8 | 14.7 | 0.47 |
| Fungal spore count | 44 | 29.0 | 8.1 | 44 | 39.2 | 5.9 | 26 | 27.3 | 5.7 | 0.67 |
| β-glucan | 48 | 18.1 | 12.6 | 48 | 34.5 | 8.9 | 30 | 14.6 | 9.9 | 0.23 |
| Total particle mass | 45 | 18.5 | 4.3 | 45 | 33.1 | 3.6 | 26 | 18.3 | 3.2 | 0.08 |
| Total particle number | 47 | 176.2 | 3.2 | 47 | 176.2 | 3.2 | 47 | 176.2 | 3.2 | 1.00 |

[A] Values below reporting limit were treated by the censoring regression model.
[B] Values below reporting limit were replaced by ½ of the reporting limit.
[C] Values below reporting limit were excluded.
[D] 48 = 23 of FFR + 25 of ER and 47 = 22 of FFR + 25 of ER due to an instrument malfunction with FFR for total particle number. Four data sets for fungal spores and three data sets for total particle mass were discarded because outside concentrations were below RL.

Figure 4 compares $WPF_{censored}$ for both respirators by contaminant type (endotoxin, fungal spore count, (1→3)-β-D-glucan, total particle mass, and total particle number). For the ER, GMs were 151, 29, 24, 20, and 269 for endotoxin, fungal spore count, (1→3)-β-D-glucan, total particle mass, and total particle number, respectively. Corresponding values for the FFR were 158, 29, 14, 17, and 109, respectively. The censored regression showed no significant difference between WPFs provided by the two types of respirators but revealed significant differences for different contaminants. $WPF_{censored}$ for fungal spore count, (1→3)-β-D-glucan, and total particle mass were significantly lower than those for total particle number. $WPF_{censored}$ for fungal spore count, (1→3)-β-D-glucan and total particle mass were similar to each other. No

significant difference was found between $WPF_{censored}$ for endotoxin and total particle number. Since the two respirator types produced statistically similar WPFs, the data were combined for further data analysis. For consistency, WPFs for total particles were also combined for the current analysis even though they were found to be different between respirator types.

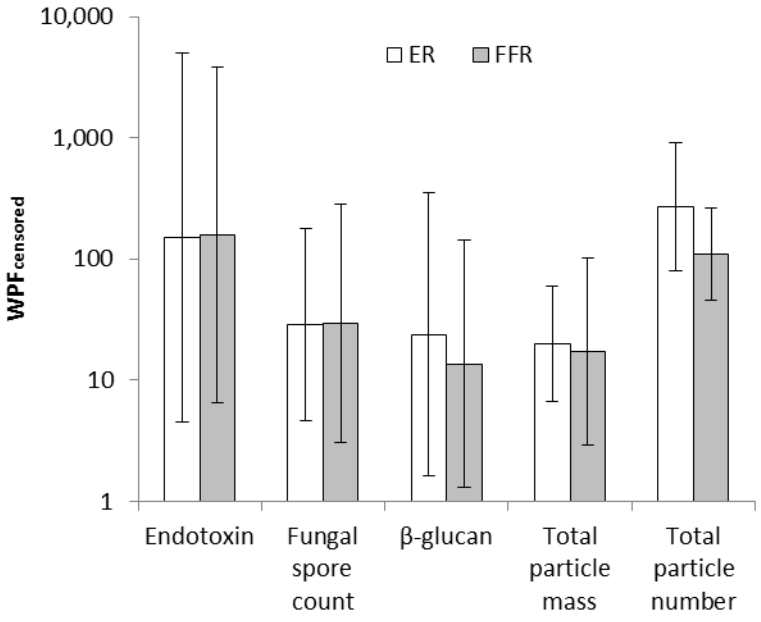



Figure 4. Comparison of workplace protection factors ($WPF_{censored}$) provided by elastomeric (ER) and filtering facepiece respirator (FFR) for different types of contaminants (endotoxin, fungal spores, β-glucan, total particle mass and total particle number). Censoring regression method showed no significant difference between the $WPF_{censored}$ provided by the two types of respirators but showed significant differences between $WPF_{censored}$ for different types of contaminants. The histograms present geometric means and the error bars present geometric standard deviations (upper value: GM×GSD, lower value: GM/GSD). For ER, n=25, 22, 25, 23, and 25 for endotoxin, fungal spores, β-glucan, total particle mass and total particle number, respectively. Particle size-selective GMs were 110, 204, 580, 1380, and 2364 for size channels 0.7-1, 1-2, 2-3, 3-5, and 5-10 µm, respectively. $WPF_{censored}$ for all contaminants shown in Figure 2A were significantly lower than the $WPF_{censored}$ measured size selectively by the optical particle counter (Figure 5B), except for endotoxin. The endotoxin $WPF_{censored}$ was statistically similar to particle size ranges of 0.7-1 and 1-2 µm (p=0.77 and 0.56, respectively).

Figure 5A compares the $WPF_{censored}$ for the three bioaerosols (endotoxin, fungal spore count, and (1→3)-β-D-glucan) and total particle mass. Figure 5B compares particle number for the five particle size ranges. All WPFs in Figure 5 represent the combined performance of both half mask respirators (ER & FFR) using censored regression treatment. Combined GMs of $WPF_{censored}$ were 154, 29, 18, 19, and 176 for endotoxin, fungal spore count, (1→3)-β-D-glucan, total particle mass, and total particle number, respectively.

Table VII presents the associations between log-transformed $WPF_{censored}$ and log-transformed concentrations measured outside the respirator for each contaminant. A relatively strong association between $WPF_{censored}$ and outside concentration was found for endotoxin, fungal spore count, (1→3)-β-D-glucan, and total particle mass. In contrast, no association was found for total particle number between $WPF_{censored}$ and outside concentration.

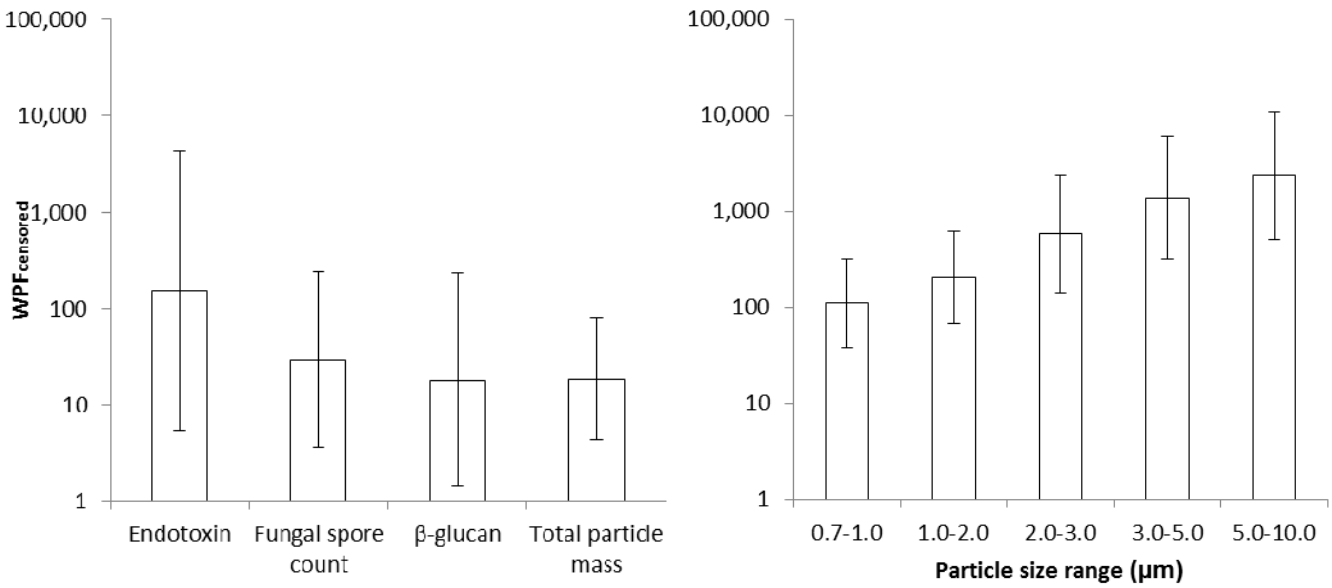



Figure 5. Comparison of workplace protection factors ($WPF_{censored}$) for three types of bioaerosols (endotoxin, fungal spores and β-glucan) and particle mass with those for number concentration in five particle size ranges. $WPF_{censored}$ for endotoxin in Figure 5A was statistically similar to WPF for the two smallest particles sizes (0.7-2.0 µm) in Figure 5B. The histograms present geometric means and the error bars present geometric standard deviations (upper value: GM×GSD, lower value: GM/GSD, n=48, 44, 48, and 45 for endotoxin, fungal spore, β-glucan, and total particle mass in Figure A, and n=47 in Figure B).

**Table VII. Association between $WPF_{censored}$ and outside concentrations for five contaminants.**

| Contaminant | Regression Estimates (95% Confidence Interval) | | |
|---|---|---|---|
| | n | Regression Coefficient[A] | p-value |
| Endotoxin | 48 | 0.68 (0.50, 0.86) | < 0.001 |
| Fungal spore count | 44 | 0.71 (0.54, 0.87) | < 0.001 |
| β-glucan | 48 | 0.96 (0.72, 1.20) | < 0.001 |
| Total particle mass | 45 | 0.95 (0.74, 1.15) | < 0.001 |
| Total particle number | 47 | 0.14 (-0.10, 0.38) | 0.24 |

[A]For example, 1% increase of the average of outside concentration for endotoxin yields 0.68% increase in the average of WPF.

The association between $WPF_{censored}$ and the outside concentrations for total particle numbers was weaker than those for the rest of the contaminants. At the same time, the highest non-size-selective $WPF_{censored}$ (176) was observed for total particle number (Table VI). Therefore, we further analyzed the data by examining the effect of low outside concentration on the association between $WPF_{censored}$ and outside concentration (Table VIII). Using the data on the total particle number as the reference point, we divided the data in two groups: (1) outside concentrations above or equal to 176×RL and (2) outside concentrations below 176×RL. For the group 1, the re-calculated GMs of $WPF_{censored}$ for endotoxin, fungal spores, (1→3)-β-D-glucan, and total particle mass were 502,113, 267, and 75, respectively. Corresponding values for the group 2 were 2, 9, 6, and 14, respectively. Compared to $WPF_{censored}$ estimated using all data points, $WPF_{censored}$ for the group 1 increased, whereas $WPF_{censored}$ for the group 2 decreased for all contaminants . The regression coefficient was recalculated for endotoxin. The other contaminants did not have sufficient number of data points when the outside concentrations

below 176×RL were excluded. The re-calculated regression coefficient for endotoxin decreased from 0.68 to 0.20, which was similar to the value obtained for total particle number (0.14).

**Table VIII. $WPF_{censored}$ including only data points which had outside concentration larger than 176 × reporting limit or smaller than 176 × reporting limit.[A]**

| Contaminant | WPF (OC≥176×RL) Group 1 | | | WPF (OC<176×RL) Group 2 | | |
|---|---|---|---|---|---|---|
| | n | GM | GSD | n | GM | GSD |
| Endotoxin | 37 | 502.2 | 6.0 | 11 | 2.3 | 1.8 |
| Fungal spore count | 13 | 112.8 | 5.3 | 31 | 8.5 | 5.0 |
| β-glucan | 10 | 266.8 | 14.4 | 38 | 5.5 | 3.5 |
| Total particle mass | 4 | 75.1 | 2.2 | 41 | 14.3 | 4.1 |

[A]WPFs including all data points are presented in Table VI.

Factors potentially affecting $WPF_{censored}$ (respirator type, gender, and farm type) were explored by the univariate and multivariate censored regression. In the univariate analysis, gender was not significantly associated with $WPF_{censored}$ for total particle mass. In all the other univariate models, gender and farm type were significantly associated with $WPF_{censored}$. Most of these associations disappeared in the multivariate censored regression. Only farm type remained a significant factor for $WPF_{censored}$ for (1→3)-β-D-glucan. GM $WPF_{censored}$ was highest at the grain handling sites. The outside concentration of (1→3)-β-D-glucan was significantly higher at the grain handling sites compared to other types of farms (p=0.02).

## DISCUSSION

All particle size distributions measured in this study appear to be similar to those measured during grain harvesting and unloading in our pilot study (Lee, Adhikari et al. 2006). In contrast to the current study, in the pilot study we found that the contribution of large particles (>2 μm) in these workplaces was greater than that measured in animal confinements. The difference may be attributed to the differences in human and animal activities taking place in these two studies. A study in Iowa (O'Shaughnessy, Donham et al. 2010) measuring dust exposures in swine confinements using personal photometers, showed that work tasks performed near moving animals resulted in the highest exposure. The total number concentrations of particles (non-normalized) over the entire size range of 0.7–10.0 μm varied from $1.2 \times 10^6$ to $3.3 \times 10^7$ particles/$m^3$ at grain handling sites and in pig barns and from $1 \times 10^7$ to $1.7 \times 10^8$ particles/$m^3$ on horse farms. In our pilot study, corresponding concentrations ranged from $4.4 \times 10^6$ to $5.8 \times 10^7$ particles/$m^3$ at grain harvesting and from $1.7 \times 10^6$ to $2.9 \times 10^7$ particles/$m^3$ in animal confinements (Lee, Adhikari et al. 2006) reported that. Thus, the outside concentrations obtained in the current our study at grain handling sites and in pig barns were similar to those in our pilot study; however, higher concentrations were measured on horse farms in the current study.

Airborne bioaerosols concentrations reported in earlier studies in agricultural farms have varied widely ranging from 2 to $3.8 \times 10^5$ EU/$m^3$ for endotoxin (Roy and Thorne 2003) 1000 to $10^9$ spores/$m^3$ for fungal spores (Lacey and Dutkiewicz 1994) and 87 to $2.8 \times 10^5$ ng/$m^3$ for (1→3)-β-D-glucan (Roy and Thorne 2003) . The corresponding values in the current study are similar to those previously reported. Total particle mass concentration reported previously for agricultural settings varied from 0.7 to 95.4 mg/$m^3$(Molocznik 2002; Roy and Thorne 2003). Corresponding values in the present study were also within the range of previously reported

values. Thus, airborne concentrations for the five contaminants in the current study are representative for agricultural environments.

The size-selective WPFs for both respirators were higher than those reported for another model of FFR in our pilot study (Lee, Adhikari et al. 2005) (21, 28, 51, 115, and 270, respectively). While the reasons for differences in the in WPFs are not known with certainty, we believe differences in fitting characteristics between respirators are a plausible explanation. Differences in filter efficiency may be another factor, although likely of smaller magnitude. WPFs for both respirators in the current study increased with increasing particle size, which is consistent with the results of our pilot study (Lee, Adhikari et al. 2005) However, it is discrepant with a previous hypothesis (Janssen and McCullough 2010) based on measurement of the WPF of an ER with P100 filters suggesting that WPFs are not particle size-dependent. The investigators found relatively large particles on the in-facepiece samples and hypothesized that WPFs should not depend on the particle size because both large and small particles enter the respirators during temporary leakage. As indicated in Table III, the 5$^{th}$ percentile of the ER calculated over all particle sizes in our study was 63.8; for the study conducted by Janssen and McCullough the corresponding value was 51.5. Following this finding we concluded that these two types of respirators have similar performance when assessed non-size selectively. However, the most distinguishable difference between the quoted and the present study is the basis for determining the WPF. While Janssen and McCullough (2010) calculated WPFs based on mass over all size ranges, WPFs in this study were based on simultaneous measurement of the number of particles within specific size ranges.

Between the two respirator models tested in this study, the ER provided a higher level of performance than the FFR. This finding was not surprising since the ER selected for this study was based upon our fit testing experience with local companies. The selected ER comes in three sizes (versus two for the FFR), consistently achieves high fit factors, and is reported by users to maintain acceptable fit during use. A previous WPF study (Myers, Zhuang et al. 1996) reported no difference in the performance of ER or FFR at different workplaces. However, the filter materials used in their study may not be directly comparable with N95 filters used in our study as their study was conducted before the issuance of new certification regulations (OSHA 1995). Performance characteristics and the selection of respirators (within the same category) may also be a consideration whenever a small number of models are compared. WPF performance ranges are expected and the actual performance of any two models is not known until they are evaluated. Consequently two models could be selected from the two tails of WPF studies while another study could select models near the mean.

The observed correlations were consistent with several WPF studies demonstrating that log-transformed WPFs were significantly, negatively correlated with log-transformed inside concentrations rather than outside concentrations (Myers, Zhuang et al. 1996; Myers and Zhuang 1998; Janssen, Nelson et al. 2007) No clear explanation, however, was previously offered for this correlation. The outside concentration could theoretically affect the WPF under high loading conditions as respirator efficiency may change due to excessive particle load on the respirator filter. The latter increases pressure drop through the filter, which changes the balance of air flowing through filter and faceseal leaks. Mathematically, WPFs have correlations with both outside and inside concentrations because WPF is the ratio of the concentration of particles outside the respirator to the concentration of particles inside the respirator. Negative correlation between the WPF and inside concentration could occur when outside concentration does not vary much, but the WPF varies due to different fitting of the respirator on the wearers' faces. Thus,

the presence or lack of correlation appears to be a reflection of the variation in the outside concentration and in the respirator's ability to form a good seal on the wearer's face.

Previously, most WPF studies have not taken field blanks into account when WPFs were calculated. However, it should be noted that field blank values conceptually indicate the minimum detectable values in workplaces. In contrast, detection limits indicate the minimum analytical value in laboratory conditions. This distinction is particularly important for low concentration measured inside well-fitting respirators, which is common for bioaerosols. Therefore, we decided to use the GM of field blanks as the RL rather than the analytical detection limit to determine the lowest possible measurable value for each contaminant. In this study we also considered the treatment of values that fell below the RL. Several WPF studies (Weber and Mullins 2000; Bidwell and Janssen 2004; Janssen, Nelson et al. 2007) have replaced concentrations less than the detection limit by a 50 or 70% of the detection limit. However, this replacement method is known to lead to inaccurate statistics and poor and misleading regression models (Helsel 1990). We compared three different statistical approaches: excluded observations ($WPF_{excluded}$), replacement ($WPF_{replaced}$), and censored regression ($WPF_{censored}$). While no statistical difference was found between the three methods, the commonly used replacement method ($WPF_{replaced}$) generally produced higher WPFs. This replacement method may overestimate true WPFs. Moreover, $WPF_{replaced}$ and $WPF_{excluded}$ are not recommended when more than 15% of data set are nondetected because arbitrarily replaced concentrations potentially introduce a false trend or cancels out a real trend in the samples (Helsel 2005). The censoring regression used for $WPF_{censored}$ is considered to provide a more accurate method for computing statistics on all data points including both nondetected and detected data (Liu, Lu et al. 1997; Helsel 2005). This is particularly true for this study where more than 15% of the data was below the RL. Consequently, the current study employed the censoring regression for the estimation of WPFs based on the RL.

$WPF_{censored}$ for fungal spore count, (1→3)-β-D-glucan, and total particle mass were significantly lower than those for total particle number. This might be attributed to the difference in the sensitivity of the analytical methods to detect high WPFs, which relates to the RL and the concentration of the respective contaminant outside and inside the respirator. The highest GM of $WPF_{censored}$ (176) was observed for total particle number. In order to obtain this high WPF (i.e., to obtain measurable level inside the respirator), the minimum outside concentration for the contaminant needed to be 176 times the respective RL. However, only 8.9, 29.5, 20.8, and 77.1% of the outside concentrations for total particle mass, fungal spore count, (1→3)-β-D-glucan, and endotoxin, were above this value, respectively. When we included only data points for outside concentrations above or equal to 176×RL, all GM WPFs increased. This suggests that the outside concentration for many samples were not high enough to obtain a WPF of 176. In contrast, when counting only data points for outside concentrations below 176×RL, all GM WPFs decreased. This indicates that higher values of WPFs are closely related to higher outside concentrations. Alternatively, the respective RL should be at least 176 times smaller than the outside concentrations to obtain a WPF of 176. RLs for total particle mass, fungal spore count, and (1→3)-β-D-glucan, were 64, 71, and 90 times smaller than the GM of the outside concentrations, respectively. In contrast, the ratio for endotoxin was 817. The similarity in $WPF_{censored}$ for total particle mass, fungal spore count, and (1→3)-β-D-glucan appears to be attributed to proportionally lower outside concentrations and higher RL compared to those of endotoxin.

The effect of outside concentrations on censored WPF is further supported by the association between the $WPF_{censored}$ and outside concentrations. All $WPF_{censored}$ results were significantly associated with the outside concentrations of respective contaminants except for total particle number. As shown with endotoxin data, the effect of the outside concentration on the $WPF_{censored}$ became weaker when outside concentrations below 176×RL were excluded. This explains why WPFs for total particle number were not associated as strongly with the outside concentrations as those of bioaerosols. Consequently, the differences in the sensitivity of the analytical methods to detect low inside concentrations may be the reason for the differences found in the WPFs for different contaminants.

The above discussion is further corroborated by the lack of association between the $WPF_{censored}$ for specific bioaerosol types and the $WPF_{censored}$ for particles in the five particle size ranges. The bioaerosols measured in this study are known to have different size ranges. The aerodynamic size of the common airborne fungal spores is above 1.8 μm, whereas bacteria can be as small as 0.6 μm (Reponen, Willeke et al. 2001). During agricultural operations, mechanical disturbance is expected to aerosolize larger aggregates (Nieuwenhuijsen, Kruize et al. 1998). Endotoxin and (1→3)-β-D-glucan can occur as either attached to intact spores, cells, or in the submicrometer size range after the rupture of the cell wall. In a concurrent study, we investigated the size range of airborne endotoxin and (1→3)-β-D-glucan side-by-side with the WPF testing and found that 96.5% of airborne endotoxin and 96.7% of airborne (1→3)-β-D-glucan were in the size range >1.0 μm (Singh, Reponen et al. 2010). The $WPF_{censored}$ for endotoxin was statistically the same as the $WPF_{censored}$ for particles in size ranges of 0.7-1 and 1-2 μm, which is consistent with the particle size observed for endotoxin. In contrast to what one might expect based on the particle size of fungal spores and (1→3)-β-D-glucan, $WPF_{censored}$ for these contaminants were consistently lower than all the size-selective $WPF_{censored}$ for particles in the size range of 0.7-10 μm. The findings reported in this paper agree with our earlier WPF-study(Lee, Adhikari et al. 2005) in which we found that WPFs for culturable fungi and total fungi were lower than those for total particles in the same size range. Possible explanations were presented but no conclusive reason for this discrepancy could be deducted from those results. As discussed above, we now have data suggesting that this discrepancy may be attributed to the sensitivity of the biological assay in detecting low inside concentrations. It appears that the effect of particle size is masked by the effect of the assay sensitivity for bioaerosols. Furthermore, this may partially explain why we did not detect a difference in WPFs between respirator types for bioaerosols, but did detect differences in WPFs for particle number using an optical particle counter.

Our results indicate the bioaerosol assays for bacteria count, endotoxin, fungal spore count and (1→3)-β-D-glucan may not be sensitive enough to detect small difference compared to the optical particle counter. This explains why the bioaerosol assay could not detect the difference in WPFs between respirator types, which was detected by the optical particle counter.

Relatively high sampling flows in this study were used. Possible positive as well as negative effects of using high sampling flows were described in earlier investigations.(Lee, Adhikari et al. 2005; Cho, Jones et al. 2010; Reponen, Lee et al. 2010) Briefly, high sampling flow increases the likelihood of detecting contaminant inside the respirator, which is especially important for bioaerosols as shown in this study. Furthermore, as the direction of sampling flow inside the respirator is opposite to the direction of inhalation, smaller sampling rates compared to breathing rates would induce sampling bias especially for larger particles. On the other hand,

higher sampling flow rates may decrease the penetration of particles through filter media as well as faceseal leakage due to impaction losses.

In this study, concentrations measured inside the respirator were not corrected for deposition losses within the respiratory tract. These losses are expected to be similar for biological and no-biological particles. We have earlier reported that after correcting for respiratory deposition, protection factors decreased for all tested particle sizes (0.04 – 10 μm) (Reponen, Lee et al. 2010; Lee, Adhikari et al. 2005). Based on the correction factors presented before (Lee, Adhikari et al. 2005), our WPFs may be overestimated by a factor of 1.2-1.8. However, the trends in particle-size selective protection factors remain the same. Moreover, in the current study, GMs of $WPF_{censored}$ for endotoxin was 5.3, 8.5, and 8.3 times higher than those for fungal spore count, (1→3)-β-D-glucan, and total particle mass, respectively. Corresponding ratios for total particle numbers were 6.1, 9.7, and 9.5, respectively. Thus, it is unlikely that the difference in $WPF_{censored}$ is caused by respiratory deposition.

## B. SPECIFIC AIM 2

***Assess the contribution of factors (aerodynamic size and shape of particles) that could cause the difference in WPF between biological and non-biological particles, under controlled laboratory conditions, under field conditions, and through theoretical modeling***

### C.1 Procedure

***Experimental set-up for manikin study***

The manikin experiments were conducted using an experimental set-up shown in Figure 7. A breathing manikin wearing the same type of N95 FFR as in the field study was placed in a walk-in test chamber (volume = 24.3 $m^3$). The manikin used for the study is commercially available (Allen Display$^{SM}$) and is made of hard plastic with smooth facial surfaces. The manikin breathed at three different MIF cyclic breathing rates of 15, 30 and 85 L/min, which simulate the human breathing rate during rest, medium work load, and strenuous work load, respectively. MIF is defined as a ratio of the tidal inspiratory volume to the inspiratory duration. Cyclic flow was produced by an electromechanical breathing simulator described in detail by Haruta et al. (2009) (Koken Ltd, Japan). Briefly, an electromechanical drive-cylinder connected to two air cylinders is the primary mechanical component of the breathing simulator. As the electromechanical cylinder moves back and forth, a sinusoidal air flow is generated. A HEPA-filter was placed between the manikin and the breathing simulator to prevent re-entry of particles into the respirator cavity by the exhalation air.

Particle concentrations inside and outside the respirator were measured by the same personal sampling system used in the field study. In each experiment, particle concentrations were determined over a period of 15 min and the measurement was repeated three times. The particle penetration (P, %) was calculated by dividing the particle concentration inside the respirator ($C_{in}$) by that outside the respirator ($C_{out}$) and expressed in percent:

$$P\ (\%) = 100 \cdot C_{in} / C_{out} \qquad (2)$$

Penetration through respirator filter ($P_{filter}$) was determined by a similar testing conducted with a fully sealed respirator (glued to the manikin face with silicon). The seal was verified using a bubbling solution that was applied to the interface between a manikin and the respirator. The total penetration ($P_{total}$) was determined with the respirator only partially sealed on the manikin face as described below.

For this study, one objective was to simulate faceseal leakage that results in similar PF as measured in the field. Particle size distributions and concentrations that occur in agricultural environments were collected from data obtained from the first 13 human subjects among 25 human subjects. This also provided information regarding the level of protection (WPF) offered by the specific respirator to be used in the subsequent manikin study. Several different sealing configurations on the manikin were tested in order to select the configuration that showed protection factors closest to the WPF collected from 13 subjects. Table IX presents the sealing configuration on a manikin that resulted in similar total penetration to the one measured under the field conditions.

The length of sealing from the cheekbone towards the chin was 11 cm on both left and right sides of the respirator. This configuration was selected for further manikin experiments to simulate faceseal leakage. $P_{total}$ was determined for the partially sealed respirator. For

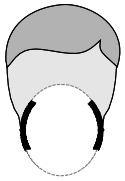
comparison with the WPF data obtained in the field, $P_{total}$ was converted to Protection Factor (PF):

$$PF = 100/P_{total} \qquad (3)$$

$P_{filter}$ and $P_{total}$ were determined particle size selectively and separately for the three respiration flow rates. The experiments under the three flow rates were conducted in random order. Penetration through the faceseal leakage ($P_{faceseal}$) was calculated as follows (Grinshpun et al., 2009):

$$P_{faceseal} = P_{total} - P_{filter} \quad (4)$$

**Table IX. Sealing configuration selected to simulate faceseal leakage on a manikin.**

| Partially sealed condition | |
|---|---|
| Sealed length | 11 cm x 2 |
| Unsealed length | 16 cm |
| Sealed: solid line<br>Unsealed: dash line |  |

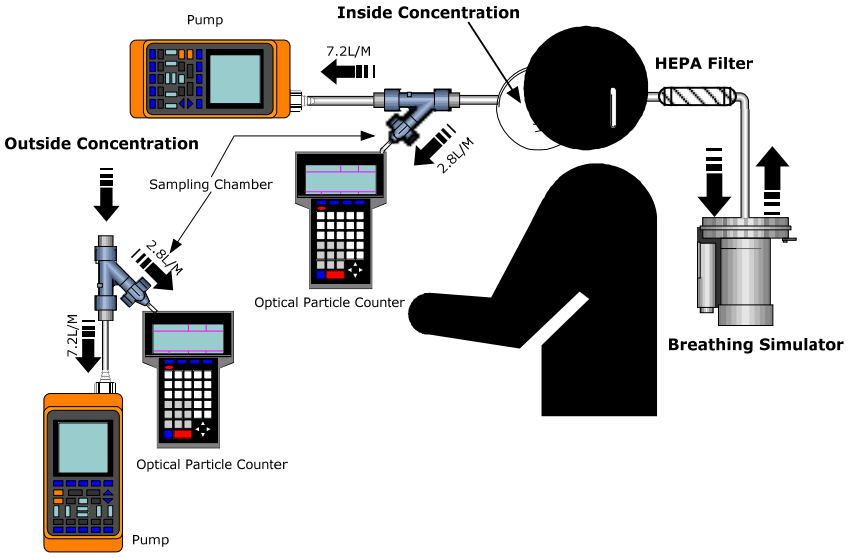



Figure 7. Experimental set-up for manikin-based testing of particle penetration through filter and face seal leakage.

## C.2 Methodology

### *Generation of non-biological test dust*

A Collison nebulizer with NaCl solution has been widely used to generate challenge aerosols in previous studies related to filter performance (Eninger, Honda et al. 2008; Lee,

Grinshpun et al. 2008). Most of the particles aerosolized by this method are in the size range of 0.01 to 1 μm (Balazy, Toivola et al. 2006), which is low relative to the bacterial and fungal size ranges. Thus, we needed larger test particles and consequently a different aerosolization methodology. Since particles in the field experiment were well distributed from 0.7 to 10 μm, we chose a Koken-manufactured nebulizer to generate test dust (ISO 12103-1 A1, Powder Technology Inc., USA) ranging from 1 to 20 μm. This nebulizer was originally used to generate 2-μm silica particles for the filter testing program at Koken Ltd. Due to the high water-solubility of the test dust, it was mixed with 2-propanol instead of water. The challenge aerosol was mixed with filtered-dry air of 100 L/min and passed through a $^{85}$Kr charge neutralizer (3054, TSI Inc, USA) to attain the Boltzmann charge distribution. An air blower with a capacity of approximately 25.5 $m^3$/min was utilized for air mixing in the chamber. The challenge particles were continuously produced for about 15 min in the beginning of the experiment to attain airborne particle concentration of 70,000 particles/L and then, intermittently atomized to maintain the desired concentration. The coefficient of variation for the concentration generated during entire experiment was 0.04. A concentration of 10 particles/L per size channel was set as the minimum acceptable level inside the respirator and is referred from this point on, as the detection limit. The maximum concentration that can be measured with the HHPC-6 optical particle counter is 70,000 particles/L. Thus, the minimum theoretical penetration that could be measured with this set-up was 0.01 %.

***Bioaerosol generation***

Two strains were selected to represent bacteria and fungi as the biological test particles: *Pseudomonas fluorescens* (ATCC 13525, ATCC, Manassas, Virginia) and *Penicillium citrinum* (ATCC 28752, ATCC, Manassas, Virginia), respectively. *P. fluorescens* are Gram-negative bacteria (Neidhart, Ingraham et al. 1990) and are often used as representatives of environmentally sensitive bacteria in laboratory studies (Wang, Reponen et al. 2001; Mainelis, oacute et al. 2002). *P. citrinum* is a well known airborne hyphomycete fungus, which is commonly found in rice seeds and the phylloplane of wheat (Pravindra Chary and Reddy 1988; Singh and Rai 1990). Test particle concentrations inside and outside the respirator were determined during 15 minutes and the measurement was repeated three times. Bacteria count and endotoxin were determined in the experiment conducted with *P. fluorescens* whereas fungal spore count and (1→3)-β-D-glucan were determined from experiments conducted with *P. citrinum*. Simultaneously with the bioaerosol collection, the optical particle counter determined particle number concentrations in the size range of 0.7-10.0 μm.

One field blank was prepared for each of three-repeated experiments and the limit of quantification was determined similarly as in the field study.

***Challenge Aerosols for fiber testing***

Fibers were prepared by crushing Pall glass fiber filter pads. To avoid aggregation of fibers during dispersion, fibers were dispersed at 10 wt. % in a 0.2 wt. % cationic surfactant solution (cetyltrimethylammonium bromide). To obtain size distribution of aerosolized fibers, fibers were aerosolized as described above and collected on mixed cellulose ester (MCE) filters (Millipore Corporation, Billerica, MA, USA). Collected fibers (Figure 2) were counted under a microscope, and fiber lengths were measured by Motic software. As shown in Figure 13, aerosolized fibers had a median length of 5 μm (a mean diameter of 1 μm and calculated aerodynamic diameter, $d_{ae}$=1.39 μm).

Monodisperse polystyrene particles with a mean physical diameters of 1.01 μm (PS I) and 1.54 μm (PS II) (Bangs Laboratories, Inc., Fishers, IN, USA) were used to represent spherical particles. Calculated aerodynamic diameters for PS I and PS II were 1.05 and 1.58 μm, respectively, bracketing the mean aerodynamic size of fibers. PS particles were prepared at 1 wt. % in distilled water.

*Effect of isopropanol*

It has been reported that electret respirator filters loose their electrostatic charge if treated by isopropanol. A treatment consisting of dipping a FFR in 2-propanol for 15 sec and air-drying overnight resulted in 30% higher penetration compared to untreated respirators (Martin and Moyer 2000). To assure that atomized 2-propanol did not affect particle penetration in our experiments, an additional manikin experiment was conducted to examine filter penetration with and without aerosolizing isopropanol. Monodisperse polystyrene spheres (PS) of 2.03 μm were used to challenge an N95 FFR that was completely sealed on the manikin face for the measurement of filter penetration. Completely sealed condition was expected to provide the worst-case scenario on the possible effect of 2-propanol in reducing the electrostatic forces in the filter material. First, PS particles were atomized by a Collison nebulizer (BGI Inc., USA), mixed with filtered-dry air of 100 L/min and passed through the $^{85}Kr$ charge neutralizer. PS particles were generated continuously for 2 hrs to attain sufficient particle concentration in the chamber. Then, filter penetration was measured for 15 min and repeated with three different manikins. After assuring that the concentration of PS particles continued to be sufficiently high for further testing, the Collison nebulizer was replaced by the Koken nebulizer containing 2-propanol. Continuously atomized 2-propanol was mixed with filtered-dry air of 100 L/min and passed through the charge neutralizer. After 30 min, while continuing the generation of 2-propanol, filter penetration was measured for 15 min and repeated using the three manikins. The filter penetrations of PS alone and of PS with 2-propanol were 0.025% and 0.029%, respectively. The difference between these two values was not significant (t-test: p=0.82). Therefore, it was concluded that aerosolized 2-propanol was unlikely to affect the filter penetrations measured in this study.

*Statistical analysis*

All statistical analyses were performed using SAS 9.1.3 software (SAS Institute Inc., Cary, NC). Analyses of variance were performed with penetration as the dependent variable separately for $P_{filter}$ and $P_{faceseal}$ and for the fraction of particles penetrating though the faceseal vs through the filter ($P_{faceseal}/P_{filter}$). The $P_{filter}$ and $P_{faceseal}$ values were square-root transformed and $P_{faceseal}/P_{filter}$-fractions were log-transformed to approximate normality. General linear model (PROC GLM) was used to construct two-factor models with interaction to relate penetrations and $P_{faceseal}/P_{filter}$ –fractions with breathing rate and particle size. Adjusted mean penetrations of all levels of breathing rate and particle size were obtained through a Least Squares MEANS statement in PROC GLM. These predicted (adjusted) penetration values are listed for one factor (breathing flow rate) adjusted for the other factor (particle size) and vice versa in Tables. Paired t-test was conducted to study the difference in particle concentration measured in the field vs. in the laboratory.

**C.3 Results**

Figure 8 shows the laboratory and field aerosol concentrations and size distributions measured outside the respirator. In the largest particle size range (5 - 10 μm, mean diameter = 7.5 μm), the ambient concentration generated in the laboratory was approximately 250 particles/L (normalized value, $\Delta C_N/\Delta Log(D_p) = 5.2 \times 10^6$ #/m$^3$ as shown in Fig. 8). This resulted in concentrations below the detection limit for all in-facepiece measurements. Therefore, results obtained for particles larger than 5 μm were excluded from this study. Results for filter penetration from the previous particle size range (mean diameter = 4 μm) were also excluded from the analysis because, the inside concentration was below the detection limit when the respirator was sealed to the manikin, despite an outside concentration of approximately 1000 particles/L. Because filter penetration was negligible, it was assumed that the faceseal penetration was equal to the total penetration for 4-μm particles. The size distribution of the challenge aerosol generated in the laboratory was close to that in the field in the size range from 0.7 to 5 μm (paired t-test: p=0.977).

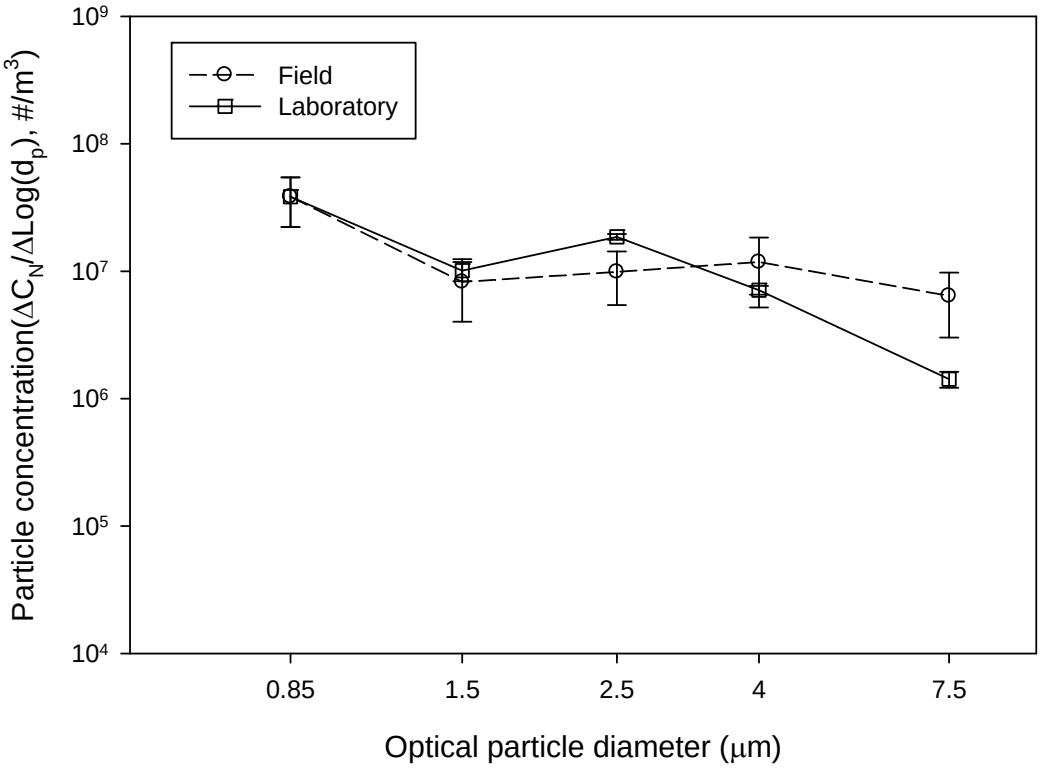



Figure 8. Mean of outside concentration in four field experiments and in all laboratory experiments. The concentration in the laboratory was averaged over all laboratory experiments (completely sealed and partially sealed respirator tested under three respiration flow rates), and the concentration in the field was averaged from four agricultural farms where WPF was measured for 13 human subjects. The symbols present means, and error bars present 95% confidence intervals.

Figure 9 compares PF-values measured under the partially sealed condition in the laboratory at different MIF cyclic breathing rates with WPF-values obtained in the field study. Generally, WPF in the field and PF in the laboratory showed particle size dependence, increasing with the increase in the particle size. PF also consistently increased within each size range with

the increase in the cyclic breathing flow. These trends are expected given that the test particles are relatively large so that their motion and collection is governed primarily by impaction and interception mechanisms. The unadjusted WPF-values (not adjusted for size and breathing flow) ranged from 12 to 9,531 and had a mean value of 515 when averaged over all particle sizes for all subjects. The unadjusted PF-values measured in the laboratory varied from 71 to 1,161 and most were within 95% confidence interval of WPF-values measured in the field.

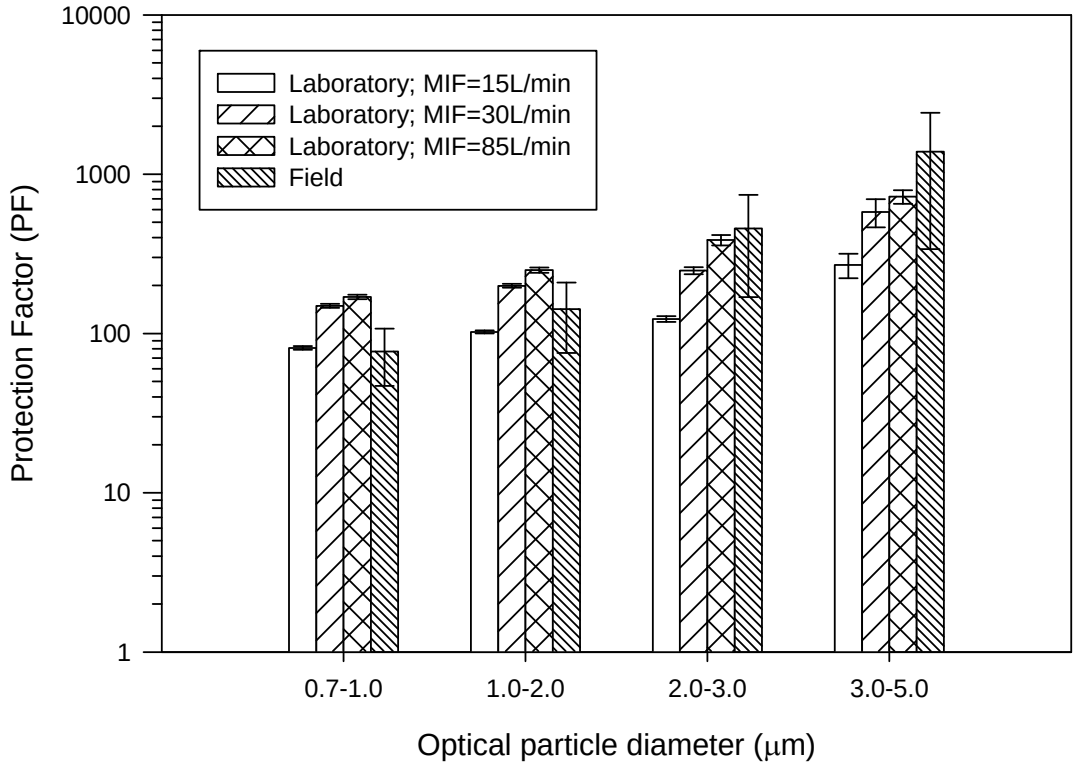



Figure 9. WPF measured in the field for 13 agricultural workers and in the laboratory for three different Mean Inspiration Flow (MIF) cyclic breathing rates under partially sealed condition. The histograms present means, and error bars present 95% confidence intervals.

Total penetration, filter penetration and faceseal penetration measured in the manikin experiments at three different MIF cyclic breathing rates at different particle sizes are shown in Figure 10, and the results obtained by the general linear model are summarized in Tables X and XI. The unadjusted values for the faceseal penetration varied from 0.11 to 1.07 % and those for the filter penetration were between 0.04 and 0.19%. Within each breathing flow rate, the faceseal penetration and the filter penetration decreased with the increase in the particle size. This decrease was statistically significant ($p<0.001$; Table X). Also, the faceseal penetration significantly decreased with the increase in the MIF cyclic breathing rate ($p<0.001$; Table XI). In contrast, the filter penetration slightly increased with the increase in the breathing rate ($p=0.02$; Table XI). Maximum faceseal and filter penetration were observed at the particle size of 0.85 μm, which was the smallest particle size included in this study. The aerosol fraction penetrating through the faceseal leak relative to the fraction penetrating through the filter material increased significantly with the increase in particle size ($p<0.001$) and with the decrease

in the breathing rate ($p<0.001$), varying from 6.2 to 16.1 at MIF = 15 L/min, from 2.9 to 6.2 at MIF = 30 L/min, and from 1.9 to 4.1 at MIF = 85 L/min.

**Table X. Penetration at different particle sizes adjusted for breathing rate.**

| Particle size (μm) | Faceseal penetration (%) | Filter penetration (%) |
|---|---|---|
| 0.85 | 0.62 | 0.17 |
| 1.50 | 0.48 | 0.11 |
| 2.50 | 0.40 | 0.05 |
| 4.00 | 0.21 | Below detection limit |
| p-value | < 0.001 | <0.001 |

**Table XI. Penetration at different MIF cyclic breathing rates adjusted for particle size.**

| MIF (L/min) | Faceseal penetration (%) | Filter penetration (%) |
|---|---|---|
| 15 | 0.74 | 0.10 |
| 30 | 0.34 | 0.10 |
| 85 | 0.25 | 0.11 |
| p-value | < 0.001 | 0.02 |

Figure 11 demonstrates how the faceseal penetration correlated with the total penetration and the filter penetration. Significant correlation was demonstrated between the faceseal penetration and the total penetration ($R^2 = 0.97$). However, no correlation was found between the faceseal penetration and the filter penetration when all data were included in the analysis ($R^2 = 0.07$). When data were analyzed separately for each respiration flow rate, significant correlations were found between faceseal and filter penetration: $R^2=0.96$ at MIF=15 L/min, $R^2=0.90$ at MIF=30 L/min, and $R^2=0.91$ at MIF=85 L/min.

Figure 12 presents the comparison of protection factors (PFs) against different types of bioaerosols generated in the laboratory. In the experiment with *P. fluorescens*, PFs against total particles was calculated based on the particle number concentration in the size range of 0.7 - 10 μm to facilitate comparison with the field data presented in Figure 1. However, 98.7% of total particles were measured in the size range of 0.7-3.0 μm because pure bacterial strain was used and the degree of particle aggregation was small. GM PFs against bacteria count, endotoxin and particle number were 8.1, 116.1 and 38.8, respectively. Significant difference was found in PFs between bacteria count and endotoxin ($p=0.03$). Like *P. fluorescens*, PFs against total particles for *P. citrinum* were based on the particle concentration in the size range of 0.7 – 10 μm. In this case, 94.1% of total particles were in the size range of 0.7-3.0 μm. GM PFs against fungal spore count, (1→3)-β-D-glucan and particle number were 97.1, 59.0 and 46.0, respectively. No significant difference was found in PFs against fungal spore count, (1→3)-β-D-glucan, and total particle number. However, it should be noted that the PFs against (1→3)-β-D-glucan is an underestimate because two of three inside concentrations were below RL and were replaced by half of the RL.

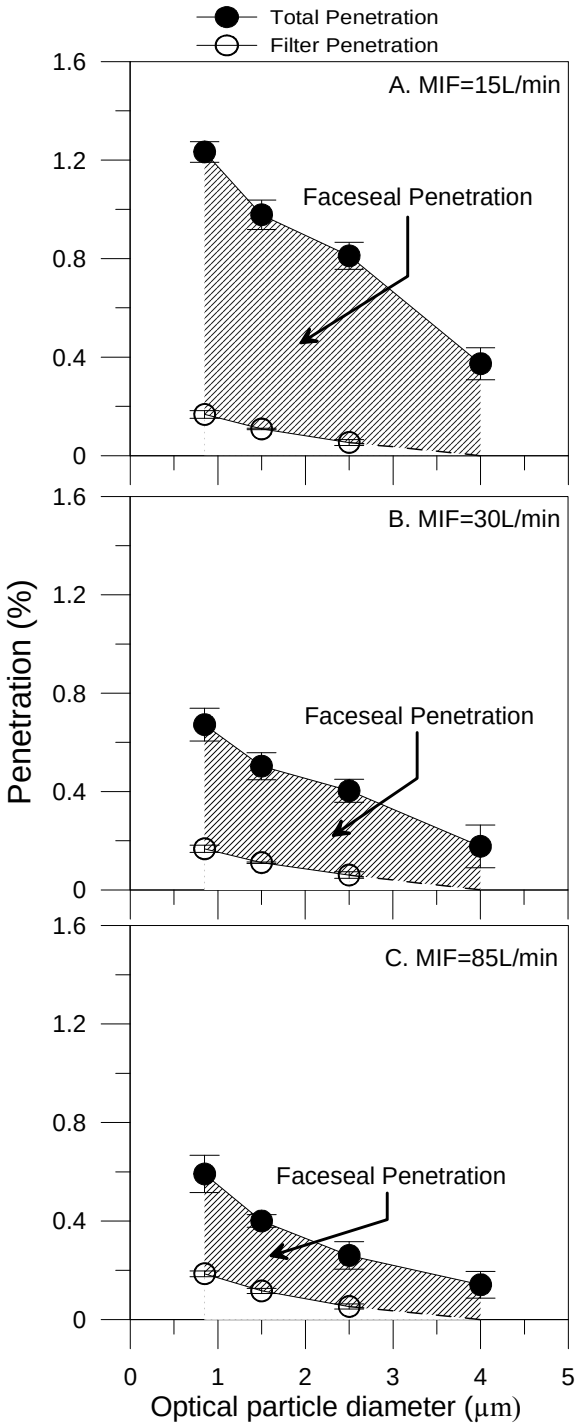



Figure 10. Comparison of total penetration, filter penetration and face seal penetration at three different Mean Inspiratory Flow (MIF) breathing rates. The symbols present means, and error bars present 95% confidence intervals.

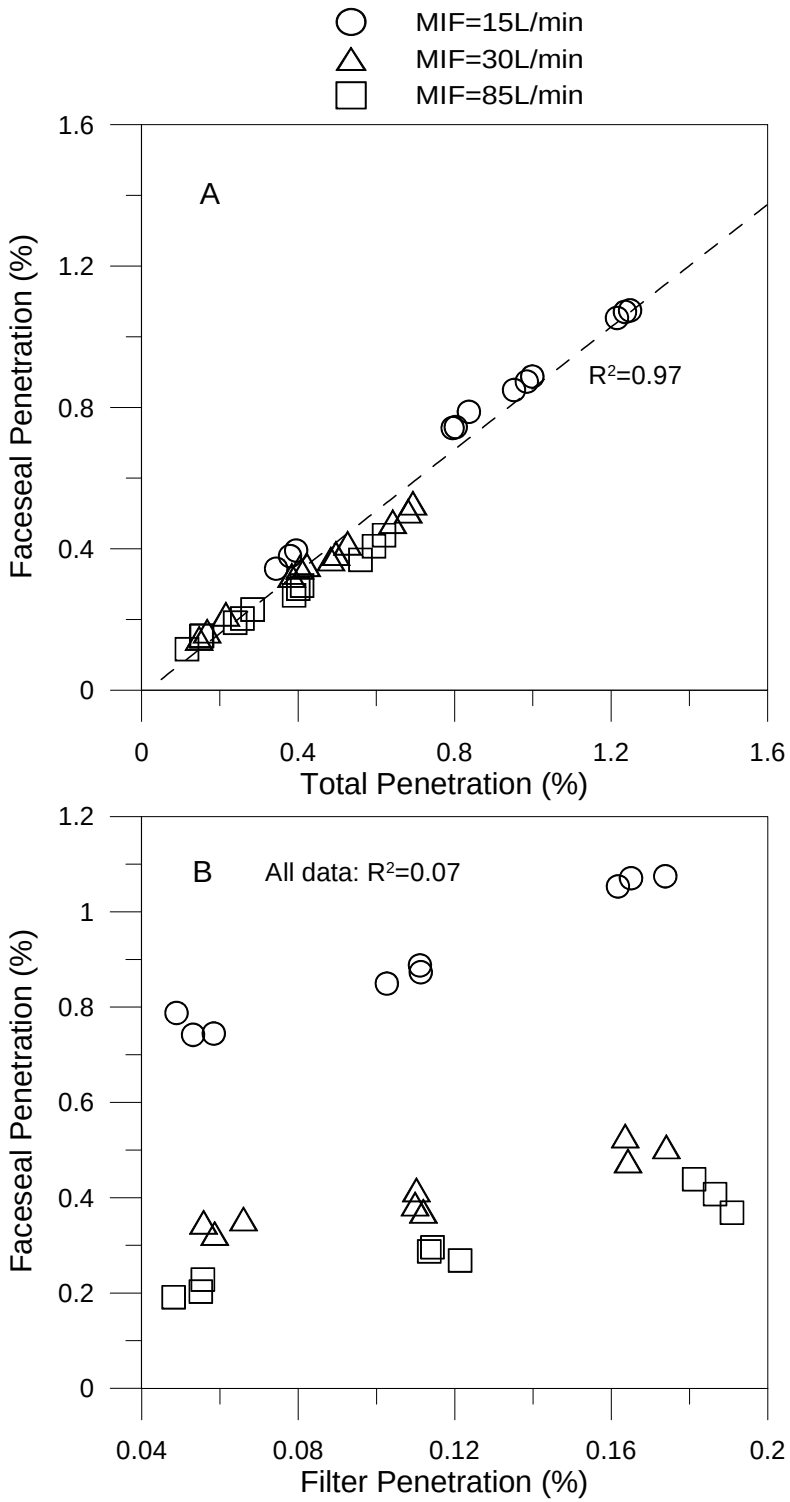



Figure 11. Correlation between faceseal penetration and total penetration (A) and between faceseal penetration and filter penetration (B).

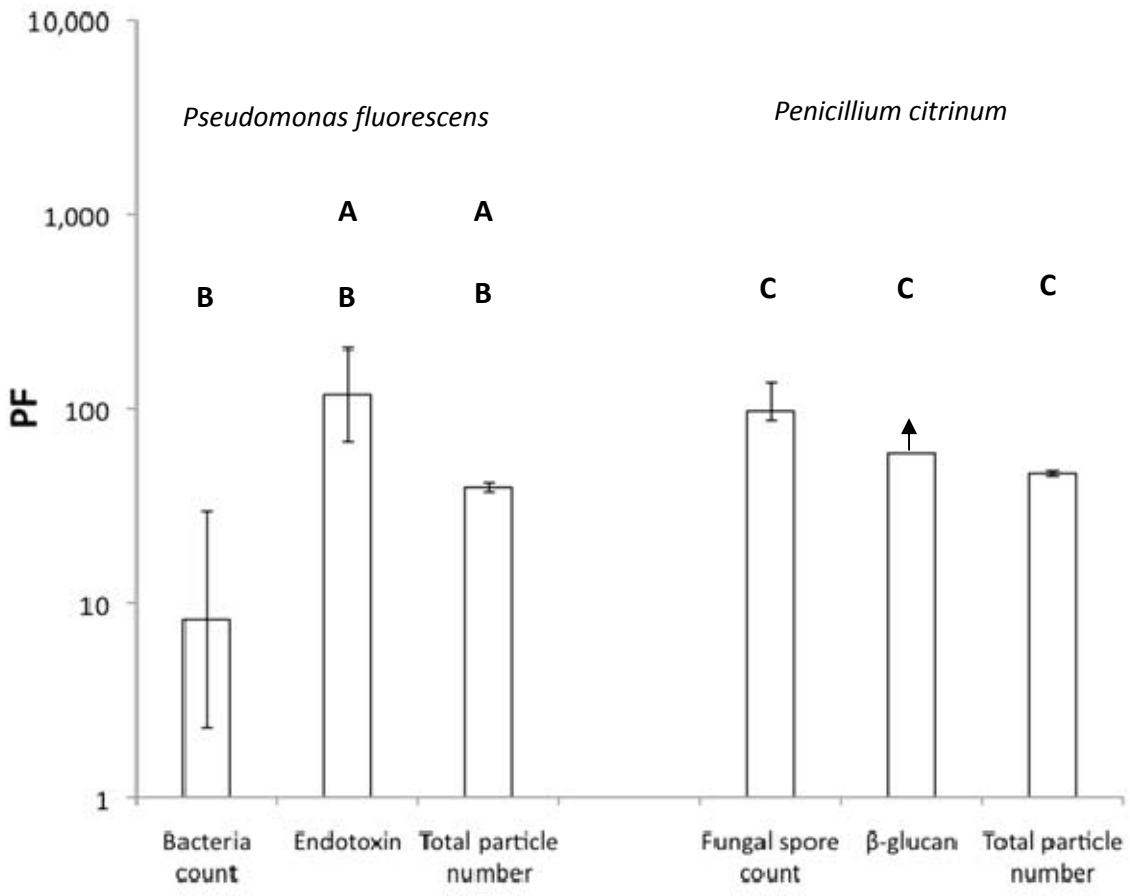



Figure 12. Comparison of protection factors (PFs) against different types of contaminants generated in the laboratory. For β-glucan, two of three inside concentrations were below detection limit and were replaced by ½ of the detection limit. Histograms and error bars present geometric means and geometric standard deviations of 3 repeats, respectively. WPFs against contaminants marked with the same capital letter were not significantly different from each other.

Filter penetration of fibers are compared with that of PS I ($d_{ae}$=1.05 μm) in Figure 14. Geometric means (GMs) of filter penetration of fibers were 0.06, 0.09, and 0.08 % at MIF of 15, 30, and 85 L/min, respectively. Corresponding values for PS I were 0.07, 0.12, and 0.12 %, respectively. All filter penetration of PS I were significantly higher than that of the fibers ($p \leq 0.001$) at each breathing flow.

Figure 15 shows the comparison of faceseal penetration between fibers and PS I at different breathing rates. GMs of faceseal penetration of fibers were 0.40, 0.14, and 0.09 % at MIF of 15, 30, and 85 L/min, respectively. Corresponding values for PS I were 0.96, 0.41, and 0.17 %, respectively. Faceseal penetrations for both types of particles were greater than the respective filter penetrations ($p \leq 0.001$). PS I showed 2.0 – 2.8 times higher faceseal penetration compared to fibers at the three breathing rates. This could be attributed to the increased interception of fiber vs. spherical particles, but the smaller $d_{ae}$ of PS I particles could also contribute to this difference. Faceseal penetrations of both particles decreased as breathing rate increased ($p \leq 0.001$). This can be explained by greater effect of impaction and interception occurring at higher air velocities.

In order to elucidate the effect of aerodynamic size vs. shape on the observed differences, an additional experiment was conducted with the next largest available PS particle, PS II ($d_{ae}$=1.58 µm). In this experiment, an N95 FFR was tested at a MIF of 30 L/min. Figure 16 illustrates the comparison of penetration of particles with different aerodynamic diameters through filter medium and faceseal leakage. GMs of filter penetration and faceseal penetration of PS II were 0.14 % and 0.36 %, respectively. These values were close to the corresponding values (0.12 and 0.41%) for PS I ($d_{ae}$=1.05 µm), but significantly (p=0.003) higher than corresponding values for the fiber particles with $d_{ae}$ of 1.39 µm (0.09 and 0.14 %). In addition, dynamic shape factors were considered to cover different shapes of fibers. Dynamic shape factors found in literature[1] are varied from 1 (sphere) to 1.88 (talc), and calculated aerodynamic diameter of fibers based on two extreme cases is in the range of 1.15 – 1.58 µm. This range is in between calculated aerodynamic diameter of PS I and PS II. Filter and faceseal penetration of fibers should be between those obtained for PS I and PS II if the aerodynamic diameter is the dominant factor governing the particle deposition mechanisms. Filter and faceseal penetration of fibers, however, were significantly lower than those of PS I and PS II. Moreover, the ratios of faceseal penetration to filter penetration for fibers were 6.4, 1.6, and 1.1 at MIF of 15, 30, and 85 L/min. Corresponding values for PS I were 13.6, 3.3, and 1.5, respectively.

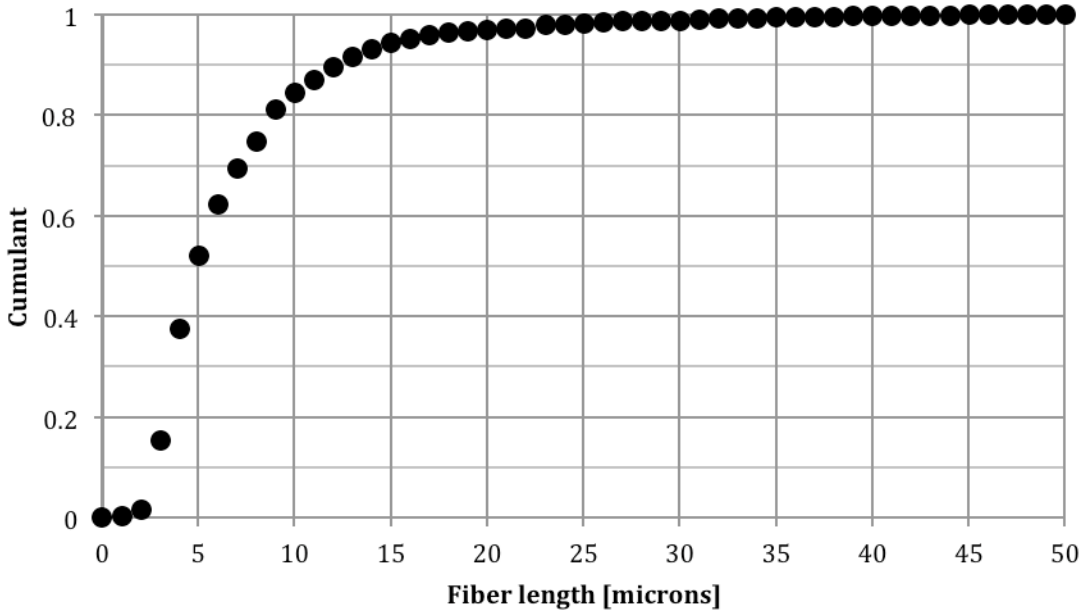



Figure 13. Cumulative length of fibers collected on MCE filter.

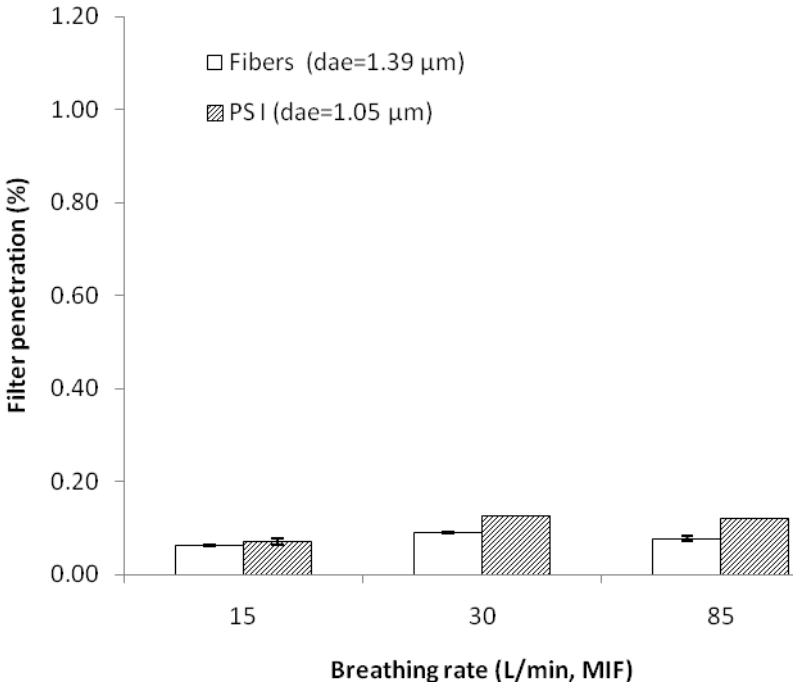



Figure 14. Comparison of filter penetration between fibers and PS particles at different breathing rates. The histograms present geometric means, and error bars present geometric standard deviations.

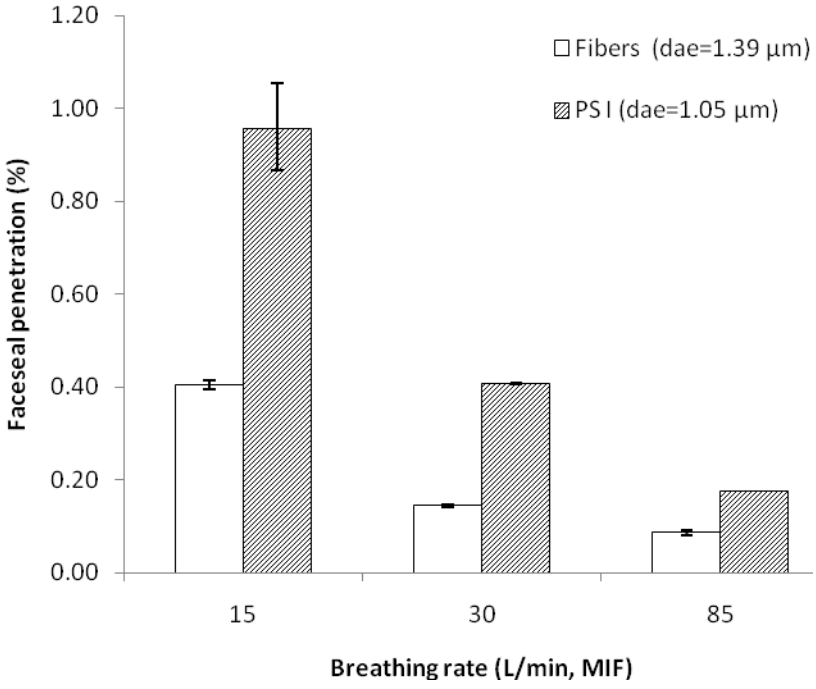



Figure 15. Comparison of faceseal penetration between fibers and PS particles at different breathing rates. The histograms present geometric means, and error bars present geometric standard deviations.

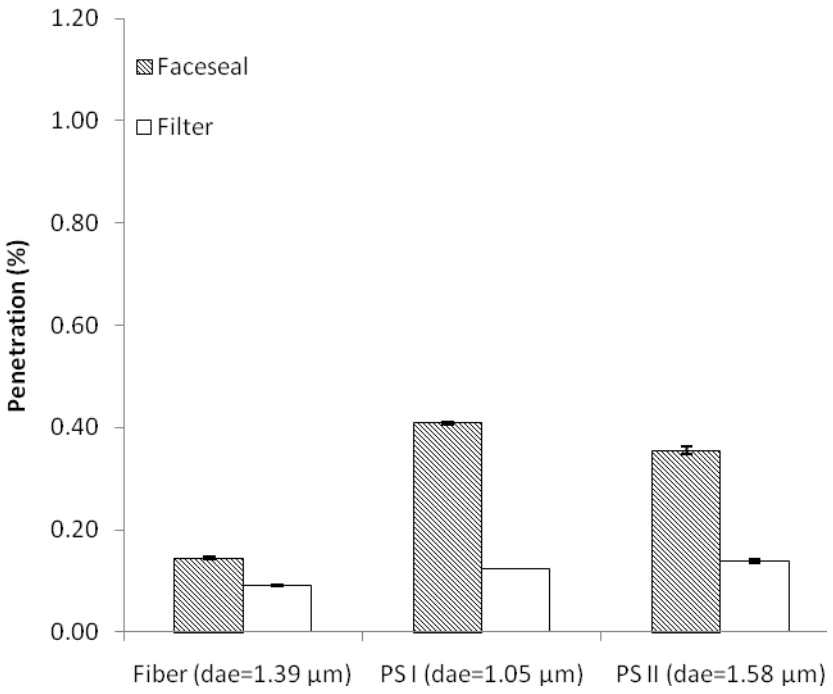



Figure 16. Comparison of penetrations of particles with different aerodynamic diameters at MIF of 30 L/min. The histograms present geometric means, and error bars present geometric standard deviations.

## C.4 Discussion

Most of the laboratory protection factors (PF) were within the 95% confidence interval of the WPFs measured in the field evaluation. This demonstrates that the positioning of the respirator on the manikin closely simulated the size of faceseal leakage in the field study. In both cases, the WPF and laboratory PF increased with increasing particle size. This is consistent with previous laboratory and field studies (Chen, Ruuskanen et al. 1990; Lee, Grinshpun et al. 2005). The laboratory PF also increased with flow rate. This also agrees with previous studies and can be explained by greater effect of impaction and interception that occurs at higher air velocities (Chen, Ruuskanen et al. 1990; Huang, Chen et al. 2007). It should be noted that we studied a relatively well-fitting respirator having a mean PF of 660 and a minimum PF of 71 (measured at particle size of 0.85 µm and flow rate of 15 L/min). As pointed out by Chen et al. (1990), the effect of particle size on the faceseal penetration may be enhanced for well-fitting respirators; the smaller the leak and the larger the particle size, the greater the effect of impaction in removing particles during their passage through the leak.

Similar to total penetration, faceseal penetration decreased with an increase in particle size and breathing rate. Although dynamic change of the fitting of the respirator to the dummy head could potentially contribute to our observation, no visual deformation of the respirator was observed even at MIF of 85L/min. Furthermore, our finding agrees with the results reported by Chen et al. (1990), who used fixed leaks (circular tubes varying in diameter) and reported that faceseal penetration decreased with an increase in particle size and breathing rate. This trend should be expected as impaction and interception mechanisms dominate with increasing particle

size and air velocity, particularly in the supermicrometer size range (Huang, Chen et al. 2007). We conclude that change of fitting of the respirator to the dummy head due to flow rate appears to be negligible.

Filter penetration also decreased with increasing particle size, consistent with classic filtration theory. However, the slight increase in filter penetration with increased breathing rate was not expected (Table XI) and appears to be opposite to the findings reported by Chen and Willeke (1990), especially for particles larger than 2 μm. This discrepancy may be partially explained by the fact that Chen and Willeke compared constant inhalation flows whereas we used sinusoidal breathing pattern, which more closely simulates the human breathing. Even though the unadjusted values of filter penetration did not considerably differ and are relatively low (varied between 0.05 and 0.2 %), filter penetration was significantly affected by particle size and breathing rate. Low filter penetration was expected as the challenge particles were large, 0.7 - 10 μm. Esbaugh et al. (2009) reported that when the penetration approaches zero, the influence of flow rate has less of an effect on penetration.

The ratio of particles penetrating through the faceseal leak relative to those penetrating through the filter varied from 1.9 to 16.1. This suggests that faceseal penetration accounted for most of the total penetration and consequently, affects the level of protection more than the filter penetration. The results are in accordance with the findings of Coffey et al. (1998) who reported that faceseal leakage was the largest component of total penetration for a given respirator (Coffey, Zhuang et al. 1998).

The fraction of faceseal penetration relative to total penetration decreased with decreasing particle size and an increase in cyclic MIF. This appears to be similar to what was reported by Chen and Willeke (1990). Furthermore, Grinshpun et al. (2009) found a similar trend for smaller particles (0.04 – 1 μm). The faceseal penetration correlated highly with the total penetration. This was anticipated as the faceseal penetration accounted for most of the total penetration. It was also accordance with the findings by Coffey et al. (1998) who reported that total penetration was significantly correlated with faceseal leakage. However, they did not measure breathing flow rates of human subjects when total penetration was determined and measured filter penetration at one flow rate of 31.4 L/min. As a consequence, no correlation between filter penetration and faceseal leakage was observed because, as shown in our study, this correlation is dependent upon flow rate. In our study, the faceseal penetration correlated with the filter penetration only when analyzed separately for each breathing flow. For example, the faceseal penetration at 30 L/min correlated with the filter penetration at 30 L/min but not with the filter penetration at 15 L/min. This was because the faceseal penetration was affected by the respiration flow more strongly than the filter penetration.

To further elucidate the effect of assay sensitivity, WPFs determined in the agricultural settings were compared with PFs measured in the laboratory against bacteria count, endotoxin, fungal spore count and (1→3)-β-D-glucan. No significant difference was found in PFs against fungal spore count, (1→3)-β-D-glucan, and total particle number. In the laboratory study, we were able to generate sufficiently high outside concentration of fungal spores, so that the sensitivity of the fungal counting and (1→3)-β-D-glucan assay was high enough to reach the same PF as for total particle number.

However, significant difference was found in PFs between bacteria count and endotoxin. Moreover, like WPFs, lowest PFs were measured in bacteria count. It should be noted that bacteria collected from the agricultural settings included both Gram-negative and Gram-positive bacteria but bacteria count in the laboratory was determined by only one Gram-negative

bacterium. Nevertheless, when the outside bacteria concentration generated in the laboratory was compared with the respective LQ, it was found that maximum PF possible to detect for bacteria with the current configuration of the experimental conditions was 18.

The finding that filter penetrations were of spherical particles were higher than those of fibers is consistent with the findings by Ortiz et al.[5] Furthermore, all values were below 5 %, which is required for the tested respirator according to NIOSH respirator certification.[9] The ratio of filter penetration for PS I to that of fibers varied from 1.1 to 1.5. Values for filter penetration of the fibers in this study were in similar ranges reported previously.[4-6]

Similar to what was found with the polydisperse non-bilogical test dust (see above), faceseal penetrations for fibers were greater than the respective filter penetrations confirming that most penetration through respirators occurs through faceseal leakage. In this particular case, the above statement holds true even when the respirator is considered to have an acceptable seal to verified by fit testing (i.e., fit factor was > 100). The lower faceseal penetration of fibers compared to spherical particles can be explained by increased interception. The experiments with fibers indicate that spherical particles penetrate more through faceseal leakage compared to fibers, and the length of fibers rather than the calculated mean aerodynamic diameter is a prevailing factor on deposition mechanisms through the tested respirator type.

The limitation of the laboratory experiment is that the largest particle size range (mean diameter = 7.5 μm) was excluded from the analysis due to low particle concentration outside the respirator. Consequently, it was not possible to compare experimental data with field data for this particle size. Another limitation is that the experiments were conducted using a breathing manikin with fixed faceseal leakage. Even though no visual deformation of the respirator was observed during the experiment, shape and size for faceseal leakage are unknown. Faceseal leakage likely fluctuates when a worker is wearing a respirator. The manikin-based set-up used in this study has the advantage of investigating factors affecting faceseal and filter penetrations of hazardous substances that cannot be studied in human subjects. In the future, the set-up and testing protocol utilized in this study can be used to investigate the faceseal penetration of hazardous substances, such as allergens or toxic fungal spores.

## D. CONCLUSIONS

The results show WPFs for the ER were higher than the FFR for all particle size ranges, and WPFs for both respirator types decreased with decreasing particle size. However, compared to the APF=10, both types of respirators provided expected level of respiratory protection for workers against particle numbers in agricultural farms. The 5$^{th}$ percentiles for the ER and FFR were higher than the APF of 10 and varied from 28 to 250 for ER and from 16 to 223 for FFR.

The performance of two types of half mask respirators was determined for five different types of contaminants. $WPFs_{censored}$ in this study were not significantly different between the two types of respirators, but were significantly different for the type of contaminant. GMs of $WPF_{censored}$ were 154, 29, 18, 19 and 176 for endotoxin, fungal spore count, (1→3)-β-D-glucan, total particle mass and total particle number, respectively. The outside concentrations of endotoxin, fungal spore count, and total particle mass affected the respective WPFs more than those of total particle number. However, the WPFs increased and the effect of the outside concentrations on the WPFs became less significant when the outside concentrations were above or equal to 176×RL. Results indicate that particle size, not the nature of particles (biological or non-biological) determines the WPFs. The observed differences may be attributed to the difference in the sensitivity of the analytical methods to detect high WPFs at the concentration levels prevailing at our field sites.

The manikin-based set-up used in this study has the advantage of investigating factors affecting faceseal and filter penetrations of hazardous substances that cannot be studied in human subjects. Results with a well-fitted N95 FFR indicate that most of the particles penetrate into the respirator through the faceseal leakage, which decreases with an increase in the respiration flow rate and with an increase in the particle size.

Filter penetration of the three different test particles were well below 5 %, which is required for the filter material of the tested respirator. Spherical particles had 1.1-1.5 higher filter penetration and 2.0 -2.8 times higher faceseal penetration compared to fibers at the three breathing rates, and faceseal penetrations of both spherical and fiber particles decreased as breathing rate increased .

The results indicate that spherical particles penetrate more through respirator filters and faceseal leaks compared to fibers of similar diameter. This difference is more pronounced for faceseal leaks than for filter penetration, even for respirators considered to have an acceptable fit. The length of the fibers rather than the aerodynamic diameter is a prevailing factor on deposition mechanisms through the tested respirator type.

For the assessment of WPFs, direct reading particle counter appears to provide data that can be used for estimating the protection provided against spherical or near spherical particles of different nature (biological vs. non-biological particles). The benefit for these devices is their low detection limit that allows the measurement of very low concentration inside the respirator. Results can be used for the selection of respirators against various types of biological particles. The results will also guide future WPF studies.

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## F. PUBLICATIONS

### *Journal Articles*

1. Kyungmin Jacob Cho, Tiina Reponen, Roy MaKay, Rakesh Shukla, Hiroki Haruta, Padmini Sekar, Sergey A. Grinshpun: [2010] Large Particle Penetration through N95 Respirator Filters and Facepiece Leaks with Cyclic Flow. The Annals of Occupational Hygiene 54 (1): 68-77.
2. Kyungmin Jacob Cho, Susan Jones, Gordon Jones, Roy MaKay, Sergey A. Grinshpun, Alok Dwivedi, Rakesh Shukla, Umesh Singh, Tiina Reponen: [2010] Effect of Particle Size on Respiratory Protection Provided by Two Types of N95 Respirators on Agricultural Settings. Journal of Occupational and Environmental Hygiene 7: 622-627.
3. Kyungmin Jacob Cho, Tiina Reponen, Roy Mckay, Atin Adhikari, Umesh Singh, Alok Dwivedi, Rekesh ShuklaR, Susan Jones, Gordon Jones, Sergey Grinshpun: [2011] Comparison of Workplace Protection Factors for Different Biological Contaminants. Journal of Occupational & Environmental Hygiene. 8:417-425.
4. Tiina Reponen, Shu-An Lee, Sergey A. Grinshpun, Erik Johnson, Roy McKay: [2011] Effect of fit-testing on the protection offered by N95 filtering facepiece respirators against fine particles in a laboratory setting. The Annals of Occupational Hygiene 55:264-271.
5. Umesh Singh, Kyungmin Jacob Cho, Sergey A. Grinshpun, Atin Adhikari, Linda Levin, Brett Green, Tiina Reponen: [2011]. Airborne endotoxin and β-glucan in fine particles in agricultural and home environments. Aerosol & Air Quality Research 11:376-386.
6. Kyungmin Jacob Cho, Leonid Turkevich, Matthew Miller, Roy McKay, Sergey Grinshpun, KwonChul Ha, and Tiina Reponen: [2011] Penetration of fibers through filter media and faceseal leakage of N95 filtering facepiece respirators with cyclic flow (manuscript in preparation)

### *Presentations*

1. Tiina Reponen [2008] Respiratory protection against fungal particles. In: Roundtable 251: Remediation Microbial Exposure Assessment. AIHCE 2008, Minneapolis, MN.
2. Kyungmin Jacob Cho, Tiina Reponen, Susan Jones, Gordon Jones, Roy MaKay, Umesh Singh, Atin Adhikari, Rakesh Shukla, , Sergey A. Grinshpun: [2009] Workplace Protection Factors of N95 Respirators against Non-Biological Particles on Agricultural Farms. AIHCE 2009 Toronto, Canada.
3. Tiina Reponen, Shu-An Lee, Sergey A. Grinshpun, Eric Johnson, Roy McKay: [2010] Effect of Fit-Testing And Particle Size on The Protection Offered by N95 Filtering Facepiece Respirators Against Fine Particles in a Laboratory Setting. AIHCE 2010, Denver, CO.
4. Kyungmin Jacob Cho, Tiina Reponen, Roy McKay, Atin Adhikari, Umesh Singh, Alok Dwivedi, Rakesh Shukla, Susan Jones, Gordon Jones, Sergey A. Grinshpun: [2011] Workplace Protection Factors for Two Types of N95Respirators Used on Farms for Respiratory Protection against Bioaerosols. AIHCE 2011, Portland, Oregon, May 14-19.

### *Posters*

1. Tiina Reponen, Kyungmin Jacob Cho, Umesh Singh, Roy McKay, Rakesh Shukla, Sergey Grinshpun: [2009] Respiratory Protection against Bioaerosols. NIOSH PPT Stakeholders Meeting, Pittsburgh, PA, 2009.
2. Tiina Reponen, Kyungmin Jacob Cho, Roy McKay, Umesh Singh, Alok Dwivedi, Rakesh Shukla, Susan Jones, Gordon Jones, Sergey Grinshpun: [2010] Effect of Particle Size on

Respiratory Protection Provided by Two Types of N95 Respirators on Farms. NIOSH PPT Stakeholders Meeting, Pittsburgh, PA, 2010.

3. Umesh Singh, Tiina Reponen, Kyungmin Jacob Cho, Atin Adhikari, Sergey A. Grinshpun: [2010] Comparison of Exposures to Airborne Endotoxin and (1→3)-β-D-glucan in fine particle size fraction collected in farm and home environments. Abstract in the students' poster session, AIHCE'2010.
4. Kyungmin Jacob Cho, Roy McKay, Atin Adhikari, Umesh Singh, Alok Dwivedi, Rakesh Shukla, Susan Jones, Gordon Jones, Sergey Grinshpun, Tiina Reponen: [2011] Comparison of Workplace Protection Factors for Different Biological Contaminations on Agricultural Farms. NIOSH PPT Stakeholders Meeting, Pittsburgh, PA, 2011.
5. Kyungmin Jacob Cho, Leonid Turkevich, Mark Miller, Roy McKay, Sergey A. Grinshpun, K.C. Ha, Tiina Reponen: [2011] Fiber and spherical particle penetration through N95 filtering facepiece respirators with cyclic flow. NIOSH NORA Manufacturing sector conference, Cincinnati, Ohio, 2011.
6. Tiina Reponen, Umesh Singh, Kyungmin Jacob Cho, Atin Adhikari, Linda Levin, Brett Green, Sergey A. Grinshpun: [2011] Airborne endotoxin and β-glucan in fine particles in agricultural and home environments. Trømso Organic Dust Symposium, Trømso, Norway, 2011.

Program Director/Principal Investigator (Last, First, Middle):

# Inclusion Enrollment Report

**This report format should NOT be used for data collection from study participants.**

**Study Title:** Respiratory Protection against Bioaerosols in Agriculture

**Total Enrollment:** 25 **Protocol Number:** 99-05-11-01-EE

**Grant Number:** RO1 OH 04085

**PART A. TOTAL ENROLLMENT REPORT: Number of Subjects Enrolled to Date (Cumulative) by Ethnicity and Race**

| Ethnic Category | Females | Males | Sex/Gender Unknown or Not Reported | Total |
|---|---|---|---|---|
| Hispanic or Latino | | 1 | | 1 ** |
| Not Hispanic or Latino | 6 | 18 | | 24 |
| Unknown (individuals not reporting ethnicity) | | | | |
| **Ethnic Category: Total of All Subjects*** | 6 | 19 | | 25 * |
| **Racial Categories** | | | | |
| American Indian/Alaska Native | | | | |
| Asian | | | | |
| Native Hawaiian or Other Pacific Islander | | | | |
| Black or African American | | | | |
| White | 6 | 19 | | 25 |
| More Than One Race | | | | |
| Unknown or Not Reported | | | | |
| **Racial Categories: Total of All Subjects*** | 6 | 19 | | 25 * |

**PART B. HISPANIC ENROLLMENT REPORT: Number of Hispanics or Latinos Enrolled to Date (Cumulative)**

| Racial Categories | Females | Males | Sex/Gender Unknown or Not Reported | Total |
|---|---|---|---|---|
| American Indian or Alaska Native | | | | |
| Asian | | | | |
| Native Hawaiian or Other Pacific Islander | | | | |
| Black or African American | | | | |
| White | | 1 | | 1 |
| More Than One Race | | | | |
| Unknown or Not Reported | | | | |
| **Racial Categories: Total of Hispanics or Latinos**** | | | | 1 ** |

* These totals must agree.
** These totals must agree.