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Size-selective Assessment of Respirator Protection against Airborne Fungi and (1→3)-*β*-D-glucan in Farms

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ABSTRACT

(1→3)-*β*-D-glucan is a major component of the fungal cell wall. It is commonly used to evaluate human exposure to fungi. A personal sampling system was developed to size-selectively evaluate the workplace protection factors (WPFs) of N95 filtering facepiece respirators (FFRs) and surgical masks (SMs) against fungi. This field study was performed with human subjects wearing an N95 FFR or a SM during farming activities. The geometric means of the WPFs of N95 FFRs and SMs were 156.2 and 12.2 for the total culturable fungi, 55.4 and 9.0 for the total fungi, and 10.5 and 11.1 for (1→3)-*β*-D-glucan. The WPFs for N95 FFRs against fungal contaminants were mostly greater than those for SMs; however, about 4.8%–35.0% of WPFs in the spore size range > 1.8 μm were still below 10 (the assigned protection factor designated for N95 FFRs by the US Occupational Safety and Health Administration). The WPFs of N95 FFRs and SMs against culturable fungi and (1→3)-*β*-D-glucan increased with the particle size. The total (1→3)-*β*-D-glucan significantly correlated with the total fungi ($r = 0.588$, $p < 0.001$) and total culturable fungi ($r = 0.463$, $p = 0.002$), which suggests that (1→3)-*β*-D-glucan can be used as an indicator to assess respiratory protection against airborne fungi on agricultural farms.

Keywords: Culturable fungi; Total fungi; (1→3)-*β*-D-glucan; Protection; Respirator.

INTRODUCTION

Fungi are classified as a type of bioaerosols. It has been found that exposure to fungi can be associated with respiratory diseases such as decreased lung functions, allergies and asthma (Bornehag *et al.*, 2005; Edwards *et al.*, 2012; Roy *et al.*, 2017; Tarigan *et al.*, 2017). Epidemiological studies have discovered that moisture and fungi in the air are correlated with respiratory diseases in children and adults (Institute of Medicine, 2004; Thacher *et al.*, 2017). Apart from causing respiratory and allergy-related problems in humans, fungi also cause infections (e.g., Aspergillosis) and other toxic responses. The mechanism for toxic responses is induced by secondary metabolites such as mycotoxins. However, the components of the fungal cell wall (such as (1→3)-*β*-D-glucan) are also known to cause toxic reactions (Levetin, 1995; Husman, 1996) and to exacerbate allergic asthma (Zhang *et al.*, 2017). Additionally, exposure to volatile organic compounds produced by fungi may cause atypical or non-specific symptoms such as headache, eye, nose, and throat irritation, fatigue, and others (Miller, 1992). Hence, some countries have proposed occupational exposure limits for total microorganisms. They propose that the range be kept between 5000–100,000 CFU m^{-3} (National Labour Inspection of Denmark, 1989; Swan, *et al.*, 2003). Beijer *et al.* (2002) divided the concentration of (1→3)-*β*-D-glucan into "high" and "low". A concentration greater than 4 ng m^{-3} is considered as a "high" level, while a concentration lower than 2 ng m^{-3} is counted as a "low" level.

Taiwan is located in a sub-tropical climatic region and is surrounded by the sea. The weather is warm and humid, suitable for agricultural development. According to Taiwan's 2013 Directorate–General of Budget, Accounting and Statistics, Executive Yuan, R.O.C (Taiwan), 3 million people out of Taiwan's population of 23 million live and work in agricultural environments (Taiwan Directorate-General of Budget, 2013). Farming environments are known for their high concentrations of fungi. Chief among these are swine farms (Sowiak *et al.*, 2012), poultry farms (Lee *et al.*, 2006), and grain farms (Roy and Thorne, 2003). Apart from farms, peripheral housing close to farming areas is also known to have greater concentrations of fungi compared to average housing (Lis *et al.*, 2008). The concentrations of airborne fungi in farms from this study were published in 2014 (Lee *et al.*, 2014). The study results indicated that concentrations of culturable fungi in corn farms and mushroom farms were both higher than the proposed occupational exposure limits (5000–100,000 CFU m^{-3}). Swine farms and poultry farms

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also had concentrations of airborne culturable fungi that were close to the exposure limits. In addition, the concentrations of (1→3)-*β*-D-glucan at the agricultural farms (Lee *et al.*, 2014) we tested all exceeded 4 ng m^{-3}, which Beijer *et al.* (2002) classified as a "high" exposure level. This indicates that the participating farmers at these particular farms in this study have a high risk of exposing themselves to high concentrations of fungal contaminants.

As a farm covers a massive area, there is a high variation in sources of fungal contamination. For example, animal feed, grains, and soil were found to contain high levels of allergenic fungi (Weikl *et al.*, 2015). Fungi can spread via farming work, crop cultivation, and harvesting. Therefore, in order to lower the risk of farmers exposing themselves to high concentrations of fungi, the use of respirators is more feasible than engineering control. There are two types of respirators: air-supplying respirators (ASR) and air-purifying respirators (APR). As APRs are easier to maintain, less of a hindrance for the user (Han *et al.*, 1997), lighter in weight and more convenient, they are more commonly used by farm workers (Popendorf *et al.*, 1995). Two types of APRs are often used by healthcare workers to combat the spread of airborne infectious diseases: N95 filtering facepiece respirators (FFR) and surgical masks (SM). In addition to providing protection, N95 FFRs and SMs have demonstrated its ability in bioaerosol shielding for human breath (Xu *et al*., 2017), N95 FFRs have shown less filter penetration, fewer face seal leaks, and less total inward leakage than surgical masks under laboratory conditions (Lee *et al.*, 2008; Grinshpun *et al.*, 2009; Smith *et al.*, 2016). However, there is still insufficient data to definitively show that N95 FFRs provide better protection against fungal contaminants in agricultural farms. Hazardous particles enter the respirator through leaks in the filter material and the face seal. Normally, the efficiency of the filter is determined by filtration efficiency, and face seal leaks are evaluated by fit factors (FF). When evaluating the protection efficiency of respirators during work, a workplace protection factor (WPF) examination should be conducted as well. The WPF is a measure of the protection provided in the workplace by a properly selected, fit-tested, and functioning respirator that is correctly worn and used in the specific conditions of that workplace (NIOSH, 1995; AIHA, 2002). In order to determine the actual protection level that the respirators tested can provide, this study conducted a WPF measurement on workers wearing respirators while performing farming activities.

Apart from fungal spores, fungal fragments and hyphae can also be suspended in the air. Fungal fragments smaller than 1 μm exist as well (Seo *et al.*, 2009; Lee *et al.*, 2014). Fungal fragments and hyphae are potential allergens (Green *et al.*, 2005). Experimental studies have indicated that the concentrations of fungal spores in buildings contaminated by fungi might not be greater than those in non-contaminated buildings (Chew *et al.*, 2003). The study results revealed that taking concentrations of fungal spores and fragments into account increased the association with allergy severity (Delfino *et al.*, 1997). This implies that the particle size of fungal spores and fragments should be considered when we evaluate respiratory protection against fungi. Since (1→3)-*β*-D-glucan is a major constituent of the fungal cell wall, it is frequently used as an index to evaluate fungi and fungal fragments (Seo *et al.*, 2009; Lee *et al.*, 2014). Therefore, this study utilized (1→3)-*β*-D-glucan as an index for fungal fragments. This was accomplished by using a two-stage bioaerosol cyclone sampler to size-selectively collect fungal spores and fragments from the air.

Currently, fungal spore analysis is conducted using either a culturable method or a non-culturable method. The culturable method involves placing the sample on an agar plate, placing the plate into an incubator for 1 week, and then calculating the colonies on the plate. The non-culturable method is conducted by collecting fungal spores from the filter, dying them with colored dye, then counting the numbers under the microscope. It was discovered that two non-culturable methods (light microscopy with phenosafranin and epi-fluorescence microscopy with acridine orange) yielded the same outcome, with no significant statistical difference and a correlation coefficient close to 1 (Lee *et al.*, 2014). Even though the non-culturable method and the culturable method showed a direct relation in terms of fungal spore concentration, the non-culturable method produced higher values than the culturable method by 10–14 times. Hence, this study also investigated the effect of different fungal spore counting methods on the protection level provided by respirators.

The aim of this study is to use our newly developed personal sampling system to size-selectively determine WPF values provided by N95 FFRs and SMs against airborne fungi and (1→3)-*β*-D-glucan in agricultural farms. The effects of fungal contaminant particle size on respirator protection was also explored while comparing WPF results. We investigated WPF results for both fungi and (1→3)-*β*-D-glucan in this study and this also gave us an opportunity to further explore the association between WPF values for (1→3)-*β*-D-glucan and WPF values for fungi.

METHODS

Development of a Personal Sampling System

A sampling system was developed to size-selectively assess respirator protection against airborne fungal contaminants in agricultural farms. The design concept was based on our previously developed personal sampling system, which had been used to evaluate respirator protection against airborne dust and microorganisms in the laboratory and in agricultural farms (Lee *et al.*, 2004, 2005a, b). This previous personal sampling system was described in detail by Lee *et al.* (2004). In short, there were two sampling lines (an in-facepiece sampling line and an ambient sampling line) to collect air samples inside and outside the respirator. Each sampling line consisted of a sampling probe, two adaptors, a 0.5-inch diamter of Tygon tubing, a metal sampling chamber, an optical particle counter, a 25-mm filter cassette, and a pump. A silica gel/Nafion dryer was installed to remove moisture from human-exhaled air in the in-facepiece sampling line.

In order to size-selectively collect airborne fungi and

fungal fragments in the present study, we used a two-stage bioaerosol cyclone sampler (model BC221) instead of the 25-mm filter cassette. This sampler is composed of two screw-top 1.5 mL microcentrifuge tubes (Model 506-624, Fisher Scientific, USA) and a 37-mm filter holder with a 0.8 μm polycarbonate filter (Millipore Inc., Ireland). The 50% cut-off diameters of the first and second tubes are 1.8 μm and 1.0 μm at an air flow rate of 3.5 L min^{-1}. The filter directly after the second tube is used to sample particles smaller than 1.0 μm. The size fractions of < 1.0 μm, 1–1.8 μm, and > 1.8 μm represent fungal fragments, a mixture of fungal fragments and spores, and fungal spores respectively. The sampler's detailed description and aerosol collection performance was stated in Lindsley *et al.* (2006).

The previously developed silica gel/Nafion dryer would quickly become saturated with moisture and had to be replaced frequently, especially in heavy workloads or in hot and humid weather. We modified the silica gel/Nafion dryer by providing additional dry air instead of installing silica gels to create humidity gradients like those found in commercially available Nafion dryers. The modified dryer was composed of a bundle of 12 lengths of Nafion tubing (2.8 mm outer diameter and 127 mm length) placed inside an acrylic cylinder (38 mm outer diameter and 152 mm length). At a sampling flow of 3.5 L min^{-1} and a dry air flow (RH = 20–30%) of 20 L min^{-1}, the modified dryer was capable of reducing the RH of human exhaled air from 91–93% to < 68.7%. This prevents hygroscopicity and the agglomeration of collected particles. In order to make the modified dryer portable and compatible for field study, we used ambient air as dry air. The RH of ambient air varied, and should be preconditioned before introduction to the modified dryer. Ambient air was preconditioned and filtered by a HEPA filter (HEPA capsule, Pall Corp., NY, USA) and an Al_2O_3 dryer (330 g Al_2O_3 pellets in an acrylic cylinder with an outer diameter of 58 mm and a length of 267 mm). When the Al_2O_3 dryer was saturated with moisture, its indicator (chloride cobalt) changed color from blue to pink to indicate that it had to be replaced. At a flow rate of 20 L min^{-1}, the Al_2O_3 dryer demonstrated its ability to make dry air RH < 37% in 120 minutes when ambient air RH = 55–66%. The RH of dry air was controlled at < 42% in 60 minutes and < 56% in 120 minutes when ambient air RH = 90%. These results could be used as a basis for Al_2O_3 dryer replacement in field trials. The personal sampling system along with the modified dryer used in this study is presented in Fig. 1.

WPF Measurements Conducted in Agricultural Farms

Our field measurements were conducted in four farms: two types of animal confinements (swine and poultry), one corn farm, and one mushroom cultivation farm. Detailed information on farming activities, farm characteristics, and methods for analyzing airborne fungi and (1→3)-*β*-D-glucan are presented in Lee *et al.* (2014). Field samples were collected using a personal sampling system, which was also described in detail by Lee *et al.* (2014). This was worn by a human subject wearing a respirator. N95 filtering facepiece respirators (Model 8210; 3M, St. Paul, MN, USA) and surgical masks (Model 1827; 3M, St. Paul, MN, USA) were tested for each subject in each field experiment.

Farm workers were less willing to participate in field testing. In order to investigate the protection of the respirators against airborne fungi and (1→3)-*β*-D-glucan, 8 healthy subjects enrolled in the study and followed the farm workers to perform the same farming activities as they did at work. They were all students (7 males and 1 female, aged 20–28) from Feng Chia University. Measured results of facial characteristics of student subjects were listed as follows: values for the distance from the menton to the top of the head ranged from 19.4 to 24.9 cm; for the bitragion breadth, they were 12.9 to 15.0 cm; and for the lip width, they were 4.4 to 5.6 cm. A total of 42 samples (21 samples for N95 FFRs and 21 samples for SMs) were collected in

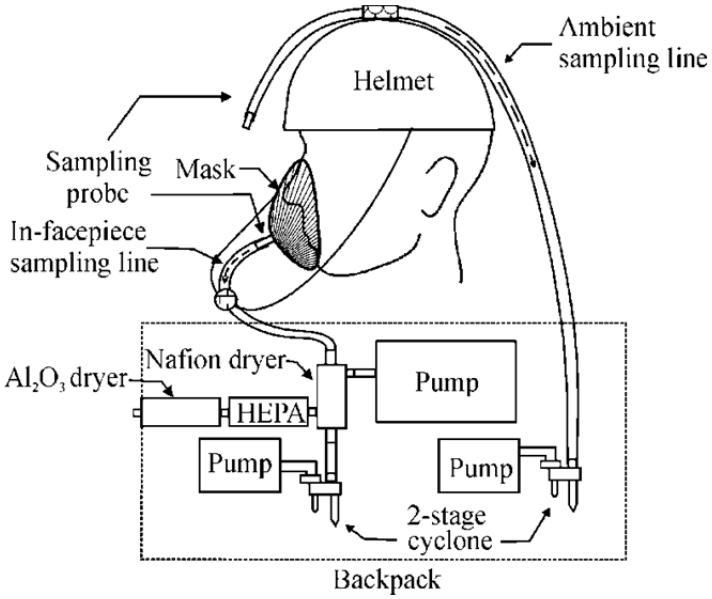

Fig. 1. Personal sampling system.

four farms. All subjects recruited in the study had to pass a medical clearance evaluation and perform a fit test before participating in field testing. The medical clearance evaluation was conducted using the questionnaire specified in OSHA standard 1910.134, Appendix A (OSHA, 1998). Human testing in this study had been approved by the Institutional Review Board of China Medical University Hospital, Taichung, Taiwan (approval number DMR96-IRB-210). Each test subject provided written informed consent where the possible risks of the field test were addressed. All subjects were required not to have beards or stubble on their face, and not to smoke for one hour before the test.

The respirator fit test was performed for each subject prior to his or her involvement in the field test. Before fit testing, each subject was trained and instructed on how to wear the respirator properly. The instructions followed the manufacturer's guidance for the use of the respirator. Fit testing was conducted with a TSI Portacount Plus in connection with an N95 companion (TSI, Inc., St. Paul, MN, USA) in compliance with the 6-exercise protocol (OSHA, 1998). A fit factor of 100 or above in the quantitative fit test constituted a pass for N95 FFRs. The subject then donned the respirator equipped with the personal sampling system. In each farming environment, each subject performed farming activities that lasted for about 60 minutes.

Data Analysis

Data obtained in the field study was organized in Microsoft Excel 2016. Plots were made by SigmaPlot 10.0. Statistical tests were performed with SPSS 12.0 for Windows (SPSS Inc., USA). P-values of < 0.05 were considered significant. WPFs and FFs were not normally distributed. The Mann-Whitney U test was used to examine the difference in three types of WPF values between N95 FFRs and SMs: the WPF for total culturable fungi ($WPF_{\text{total culturable fungi}}$), the WPF for total fungal spores ($WPF_{\text{total fungal spores}}$), and the WPF for total (1→3)-β-D-glucan ($WPF_{\text{total (1→3)-}\beta\text{-D-glucan}}$). The differences in fit factors among fit testing exercises were examined by the Kruskal-Wallis test. The differences in $WPF_{\text{total culturable fungi}}$ and $WPF_{\text{total (1→3)-}\beta\text{-D-glucan}}$ among particle sizes were also examined through the Kruskal-Wallis test. To approach normality for the regression analysis, WPFs and FFs were transformed using base-10 logarithms. Pearson correlation coefficients were obtained to examine the associations: $WPF_{\text{total (1→3)-}\beta\text{-D-glucan}}$ vs. $WPF_{\text{total culturable fungi}}$, $WPF_{\text{total (1→3)-}\beta\text{-D-glucan}}$ vs. $WPF_{\text{total fungal spores}}$, FFs vs. $WPF_{\text{total culturable fungi}}$, FFs vs. $WPF_{\text{total fungal spores}}$, and FFs vs. $WPF_{\text{total (1→3)-}\beta\text{-D-glucan}}$. WPF values for fungal contaminants whose concentrations were not detected inside the respirator were calculated using half of the detection limit for inside concentrations (Cho *et al.*, 2011; Lee *et al.*, 2005).

RESULTS AND DISCUSSION

Fit factors of Surgical Masks and N95 Filtering Facepiece Respirators

Fig. 2 shows the FFs acquired for N95 FFRs and SMs under 6 different types of exercises. Fig. 2(a) shows the FFs acquired for N95 FFRs under different types of exercises. It was discovered that the FF from the first normal breathing (geometric mean (GM) = 553.3) was higher than other types of exercises. The lowest FF value (GM = 319.7) was seen when bending. After conducting the Kruskal-Wallis test, it was found that the 6 different types of exercises did not cause statistical significance ($p = 0.35$) in FF values. Fig. 2(b) is the FFs acquired for SMs under different types of exercises. The results show that the FF value acquired from talking (GM = 5.4) was the highest. The FF value from the last normal breathing was the lowest (GM = 2.8). After conducting the Kruskal-Wallis test, it was found that the 6 different types of exercises did not cause significant statistical difference ($p = 0.31$) in FF values. The FFs measured by Crutchfield *et al.* (1999) for full- and half-facepiece elastomeric respirators indicated that lower FFs would be acquired during the talking and bending exercises. The lower FFs obtained for the talking exercise were likely attributed to sampling artifacts than to actual exercise dynamics. The bending over exercise was more predictive of poor respirator fit. N95 FFRs, full- and half-facepiece elastomeric respirators are all in the "tight-fitting respirator with sturdier mask material" category. Hence, a lower FF was obtained for N95 FFRs in this study during the bending over exercise. SMs belong to the "loose-fitting respirator" category. As they do not provide a good fitting to the facial surface, particle penetration through face seal leaks is far greater than through the normal filter (Grinshpun *et al.*, 2009). This causes lower FFs during head turning. Furthermore, SM material is relatively soft in comparison to that of N95 FFRs, causing an unstable fixing of the sampling probe. This results in variable FF outcomes due to sampling artifacts.

According to the OSHA 29 CFR 1910.134 regulation for FFs, the FF values of N95 FFRs must be equal or greater than 100. The GM of FF values for N95 FFRs in this study was 331.1, while that for SMs was 3.5. The FF of N95 FFRs was greater than that of SMs by 94.6 times. The percentage of N95 FFRs passing the fit test was 95.2%, while Lee *et al.* (2011) found it to be between 8% and 100%. The greater FF values for N95 FFRs compared to SMs was perhaps due to the differences in filter efficiency and face seal leaks. Grinshpun *et al.* (2009) found that the number of particles penetrating through face seal leaks in N95 FFRs and SMs far exceeded the number of particles penetrating through the filter medium. SMs had greater particle penetration through filters and face seal leaks compared to N95 FFRs.

Workplace Protection Factors of Surgical Masks and N95 Filtering Facepiece Respirators

Table 1 presents the protection results of N95 FFRs and SMs against fungal contaminants for study participants in the farm. The results show that the GMs of WPF values respectively for N95 FFRs and SMs. They were 156.2 and 12.2 for culturable fungi, 55.4 and 9.0 for total fungi, and 10.5 and 11.1 for (1→3)-β-D-glucan. After conducting the Mann-Whitney U test, it was discovered that there was a significant statistical difference in WPFs between N95 FFRs and SMs for culturable fungi ($p = 0.02$) and total fungi ($p = 0.03$), but no statistical significant difference for (1→3)-β-D-glucan ($p = 0.37$). Despite (1→3)-β-D-glucan,

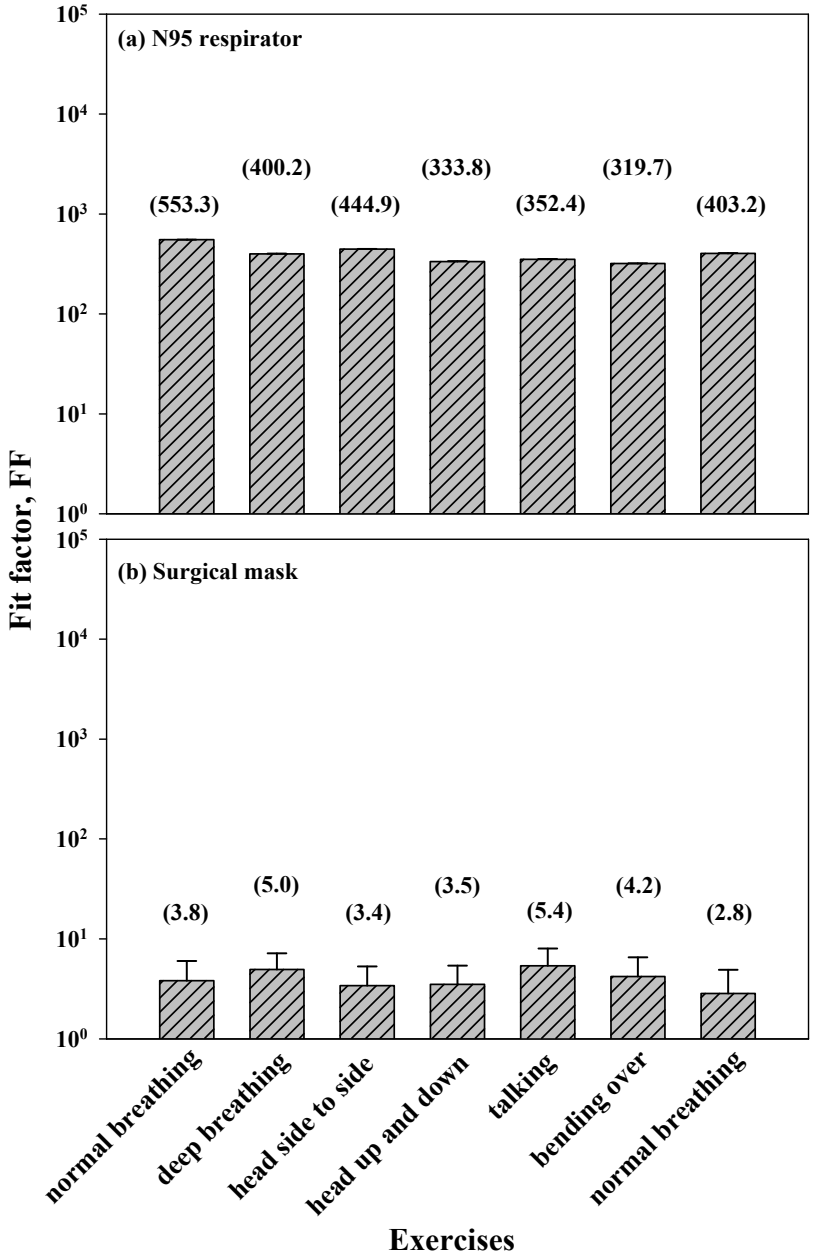

Fig. 2. The effect of fit testing exercises on fit factors: (a) N95 respirator; (b) surgical mask. The number in parentheses represents the geometric mean of the observations. (n = 21)

Table 1. Protection provided by N95 respirators and surgical masks against fungal pollutants. (n = 21)

	Culturable fungi		Total fungi		(1→3)-*β*-D-glucan	
	N95	Surgical Mask	N95	Surgical Mask	N95	Surgical Mask
Fifth percentile	4.2	3.4	2.5	1.0	1.0	1.1
Geometric mean	156.2	12.2	55.4	9.0	10.5	11.1
(Geometric standard deviation)	(16.4)	(2.3)	(8.6)	(3.1)	(7.8)	(3.3)

Note: Total fungi were measured using light microscopy with phenosafranin

the WPF values for N95 FFRs were larger than those for SMs by approximately 1.0–12.8 times. Our results were similar to those found by Lee *et al.* (2008), who found that protection factors (PF) of N95 FFRs against NaCl particles of sizes between 0.04–1.3 μm were higher than those of SMs by 8–12 times. The highest WPF value of both N95 FFRs and SMs was found for culturable fungi, while the lowest was found for (1→3)-*β*-D-glucan. These results were similar to those obtained by Cho *et al.* (2011). Cho *et al.* (2011) discovered that the WPF GMs of N95 elastomeric

respirators for total fungi and (1→3)-*β*-D-glucan respectively were 29 and 24, compared to 29 and 14 for N95 FFRs. The difference in WPF results was perhaps due to a difference in sensitivity of the methods of analyzing fungal contaminants. The concentration of culturable fungi would be affected by viability and culturability while the concentration of total fungi was determined by direct spore counting under light microscopy, which is less affected by fungal viability and culturability. Therefore, using the WPF values obtained by light microscopy may yield a more genuine result for the actual protection of respirators against fungi. (1→3)-*β*-D-glucan is part of the fungal cell wall, and therefore fungal spores are not the only place where (1→3)-*β*-D-glucan is found. They also exist in fungal fragments or attach to small particles (Seo *et al.*, 2009). Particle size is known to affect the protection factors of respirators. Respirators were found to be less protective when facing smaller particles (Lee *et al.*, 2005a, 2008, 2016, 2017), and hence we had the lowest WPF value for (1→3)-*β*-D-glucan in this study. The fifth percentile of WPFs against culturable fungi, total fungi and (1→3)-*β*-D-glucan WPFs were 4.2, 2.5, and 1.0 for N95 FFRs, and 3.4, 1.0, and 1.1 for SMs. Despite the fact that the assigned protection factors (APF) for N95 FFRs against fungal contaminants were higher than those for SMs, they had yet to reach OSHA's half-mask standard requirement of APF = 10. The percentages of WPFs exceeding APF = 10 for N95 FFRs and SMs against fungal contaminants were as follows: 90.5% and 52.4% for culturable fungi, 81.0% and 42.9% for total fungi, and 38.1 and 52.4% for (1→3)-*β*-D-glucan. Due to differences in particle properties and particle loss in face seal leaks, the WPFs of N95 FFRs for different fungal contaminants may be varied. Lee *et al.* (2005a) found more than 50% of the N95 FFR WPFs were smaller than APF = 10 against microorganisms. Laboratory experiments also found that N95 FFRs and EN-certified FFP series respirators might not achieve the expected protection level against particles in the viral and bacterial size range (Lee *et al.*, 2008, 2016).

Fig. 3 shows the protection results of N95 FFRs and SMs against fungal contaminants by particle size. The dotted line indicates OSHA's regulated APF value (APF = 10) for tight-fitting half-masks. Fig. 3(a) shows that the GMs of WPFs of N95 FFRs against culturable fungi were 209.9, 48.1, and 6.4 respectively for particle sizes of > 1.8 μm, 1–1.8 μm and < 1 μm. This indicates that WPFs decreased with decreasing particle size. After conducting the Kruskal-Wallis test, the effect of particle size on WPF values was found to be statistically significant ($p = 0.008$). The percentages of WPFs against culturable fungi exceeding APF = 10 for N95 FFRs at particle sizes of > 1.8 μm, 1–1.8 μm and < 1 μm were 95.2%, 66.7% and 30.0% respectively. The percentage of WPFs greater than APF = 10 decreased as the particle size decreased. On the other hand, the GMs of WPFs against culturable fungi for SMs at particle sizes of > 1.8 μm, 1–1.8 μm and < 1 μm were 12.2, 15.2 and 4.4 respectively. This also shows a decrease in WPF values with decreasing particle size. After performing the Kruskal-Wallis test, the results showed that particle size had a statistically significant effect ($p = 0.048$) on the WPFs. The percentages of WPFs against culturable fungi exceeding APF = 10 for SMs at particle sizes of > 1.8 μm, 1–1.8 μm and < 1 μm were 52.4%, 57.9% and 10.0% respectively, similar to those for N95 FFRs. The percentage of WPFs greater than APF = 10 decreased as the particle size decreased. However, under the same particle size range, the percentage for N95 FFRs was greater than that of SMs.

Fig. 3(b) shows that the GMs of WPFs for N95 FFRs against (1→3)-*β*-D-glucan at particle sizes of > 1.8 μm, 1–1.8 μm and < 1 μm were 42.7, 7.7 and 4.2 respectively. The WPFs decreased as particle size lowered. After performing the Kruskal-Wallis test, it was discovered that the effect of particle size on WPFs was statistically significant ($p = 0.01$). The percentages of WPFs exceeding APF = 10 for N95 FFRs against (1→3)-*β*-D-glucan at particle sizes of > 1.8 μm, 1–1.8 μm and < 1 μm were 65.0%, 26.7%, and 9.1% respectively. The percentage of WPFs greater than APF = 10 decreased as particle size reduced. The GMs of WPFs for SMs against (1→3)-*β*-D-glucan at particle sizes of > 1.8 μm, 1–1.8 μm and < 1 μm were 19.5, 5.7, and 3.2 respectively, indicating a decrease in WPF values with decreasing particle size. After performing the Kruskal-Wallis test, it was observed that the effect of particle size on WPFs was statistically significant ($p = 0.03$). The percentages of WPFs exceeding APF = 10 for SMs against (1→3)-*β*-D-glucan at particle sizes of > 1.8 μm, 1–1.8 μm and < 1 μm were 60.0%, 35.3% and 9.1% respectively. The above results indicate that particle size had an effect on the WPFs for both N95 FFRs and SMs against culturable fungi and (1→3)-*β*-D-glucan. It also shows that WPFs decreased with decreasing particle size.

Previous studies had not taken into account nor discussed the effect of particle size on the protection efficiency of respirators against fungal contaminants. The results of this study revealed that the WPFs of both N95 FFRs and SMs against culturable fungi and (1→3)-*β*-D-glucan, as well as the percentage of WPFs exceeding APF = 10, increased as particle size increased. Lee *et al.* (2005) found that the WPFs of N95 FFRs were different for different fungal genera/groups, and increased with increases in fungi size. In our results published in 2014, we have already proven that variable sizes and groups of fungi existed within present farm environments (Lee *et al.*, 2014). Furthermore, (1→3)-*β*-D-glucan that existed within variable particle sizes also affected the protection efficiency of respirators. A point worth noting is that when particle size is smaller than 1 μm, the WPFs of both N95 FFRs and SMs against fungal contaminants are smaller than APF = 10. Therefore, fungi that release small fragments containing (1→3)-*β*-D-glucan, such as *Aspergillus versicolor* and *Stachybotrys hartarum* (Seo *et al.*, 2009), should be paid special attention to when respirator protection is required.

Another interesting note is that the concentration of fungi decreased as the particle size decreased, which further caused no detection of fungi inside the respirator. The percentage of samples where culturable fungi concentrations were not detected inside the N95 FFRs at particle sizes of > 1.8 μm, 1–1.8 μm and < 1 μm were 28.6%, 61.9%, and 60.0% respectively. For SMs, they were 9.5%, 31.6%, and 70.0% respectively. The percentages for most of the N95

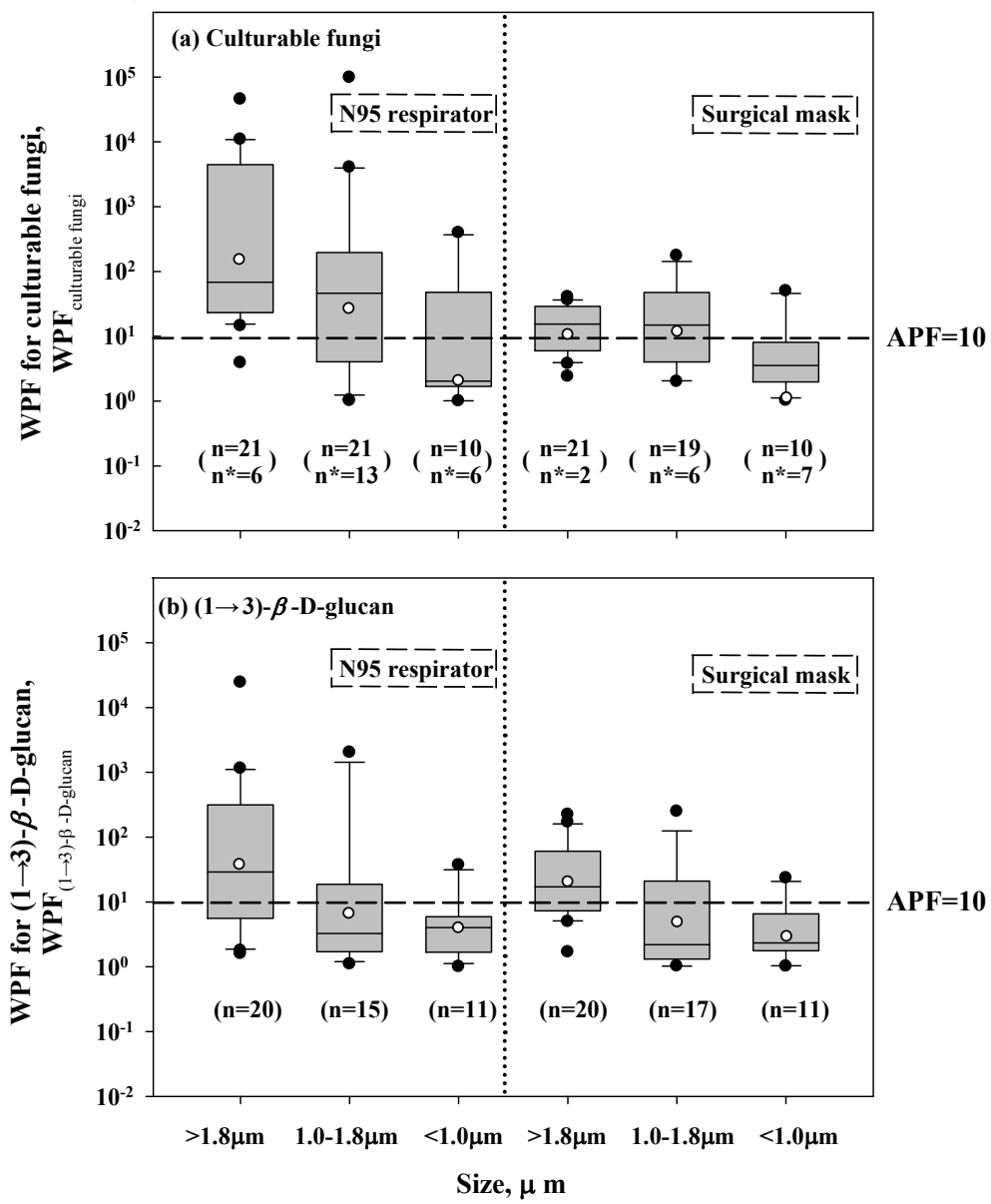

Fig. 3. Protection provided by N95 respirators and surgical masks against fungal contaminants: (a) culturable fungi; (b) (1→3)-β-D-glucan. The boxplot shows the following: hollow dots represent geometric mean; solid dots means outliers; horizontal lines from bottom represent 5%, 25%, 50%, 75%, 95%; n represents the number of observations; n* represents the number of those observations in which the in-facepiece concentrations were below the LOD and the WPFs were calculated using half the LOD ($LOD_{culturable\ fungi}$ = 156 CFU m^{-3}; $LOD_{(1\rightarrow3)\text{-}\beta\text{-}D\text{-}glucan}$ = 68.4 pg m^{-3}); APF = assigned protection factor.

FFRs were higher than those for SMs. However, (1→3)-β-D-glucan was always detected inside the respirator when it was present outside the respirator. Cho *et al.* (2011) discovered that when calculating WPF values, higher results were obtained by using half the detection limit as a replacement value when no concentration was detected inside the respirator. This caused an overestimate of the WPFs. This might be attributed to the difference in sensitivity of the analytical methods used to detect the concentrations of contaminants inside and outside the respirators. If the concentration of contamination outside the respirator is insufficient, or is non-detectable, then we are unable to demonstrate the actual WPF value. This in turn will cause limitations in the application of study results.

When OSHA set the APF value of half-masks, they did not consider the characteristics (biological or non-biological) and size of particles, the types of masks (filtering facepiece or elastomeric facepiece), the filtration efficiency of filter material, work environments or other factors which may affect the protection of respirators. Our study results recommend that when constructing a respiratory protection program for farmers against fungal contamination in agricultural farms, the above-mentioned factors should be taken into consideration.

Relationships between Workplace Protection Factors and Fit Factors

Fig. 4 presents the regression plots for associations between the WPF for (1→3)-*β*-D-glucan, the WPF for culturable fungi, and the WPF for total fungi. We found that the correlation coefficients were 0.463 ($p = 0.002$) for the WPF of (1→3)-*β*-D-glucan and culturable fungi, and 0.588 ($p < 0.001$) for the WPF of (1→3)-*β*-D-glucan and total fungi. These values were in the middle of weak ($r = 0.3$) and moderate ($r = 0.6$) positive linear relationships and were statistically significant ($p < 0.05$). The equations provided in Fig. 4 can be used to estimate the WPF of these fungi when (1→3)-*β*-D-glucan is measured. This means the WPF of fungi can be assessed by importing the WPF of (1→3)-*β*-D-glucan into the equation. In these cases, the time for fungi cultivation can be minimized, and the time and manpower needed for microscopic fungal spore counting can be reduced. As the concentration of culturable fungi is influenced by viability and culturability, the culturable method is not necessarily more accurate for obtaining the concentration of total fungi than spore counting by light microscopy. In this context, the WPF for (1→3)-*β*-D-glucan had a stronger association with the WPF for total fungi than with the WPF for cultural fungi. On the other hand, due to the variation of (1→3)-*β*-D-glucan content within each fungus as well as the variation in sensitivity of different methods for analyzing fungal contaminants, only moderate correlations were found among the WPFs for fungal contaminants in this study.

Prior to the participants taking part in this study, they needed to go through the procedure of fit testing in order to inspect the respirator fit. To further elaborate the relationship

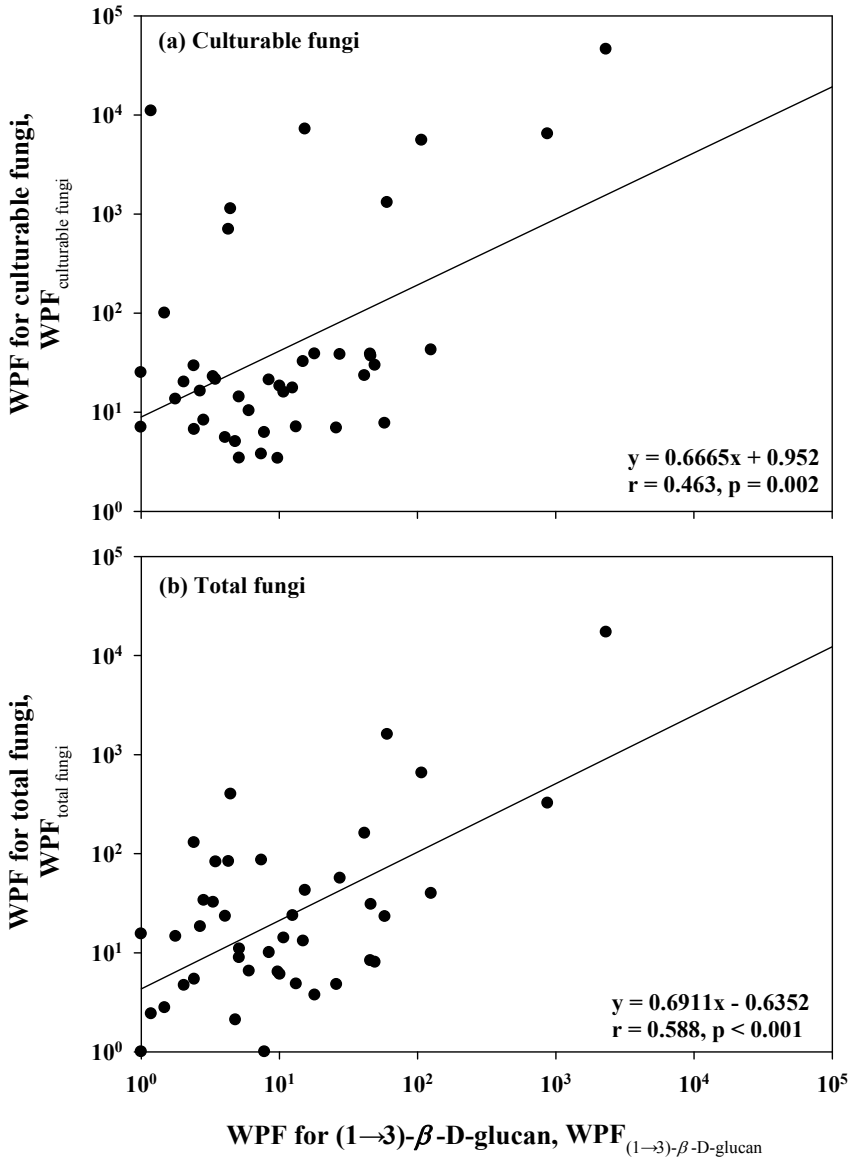

Fig. 4. Correlation between WPFs for fungi and WPFs for (1→3)-*β*-D-glucan: (a) culturable fungi; (b) total fungi. Total fungi were measured using light microscopy with phenosafranin; y = log (the WPF for fungi); x = log (the WPF for (1→3)-*β*-D-glucan). (n = 42)

between FFs and WPFs, regression analysis was performed on the FFs towards the effect on the WPFs. This is shown in Fig. 5. We found that the correlation coefficients were 0.559 ($p < 0.001$) for culturable fungi, 0.437 ($p = 0.004$) for total fungi, and –0.002 ($p = 0.992$) for (1→3)-β-D-glucan. The associations between FFs and WPFs for culturable fungi and total fungi were statistically significant in moderate positive linear relationships, but no relationship was found for (1→3)-β-D-glucan. Consequently, the FF values of the respirator can be predictive of the WPFs for culturable

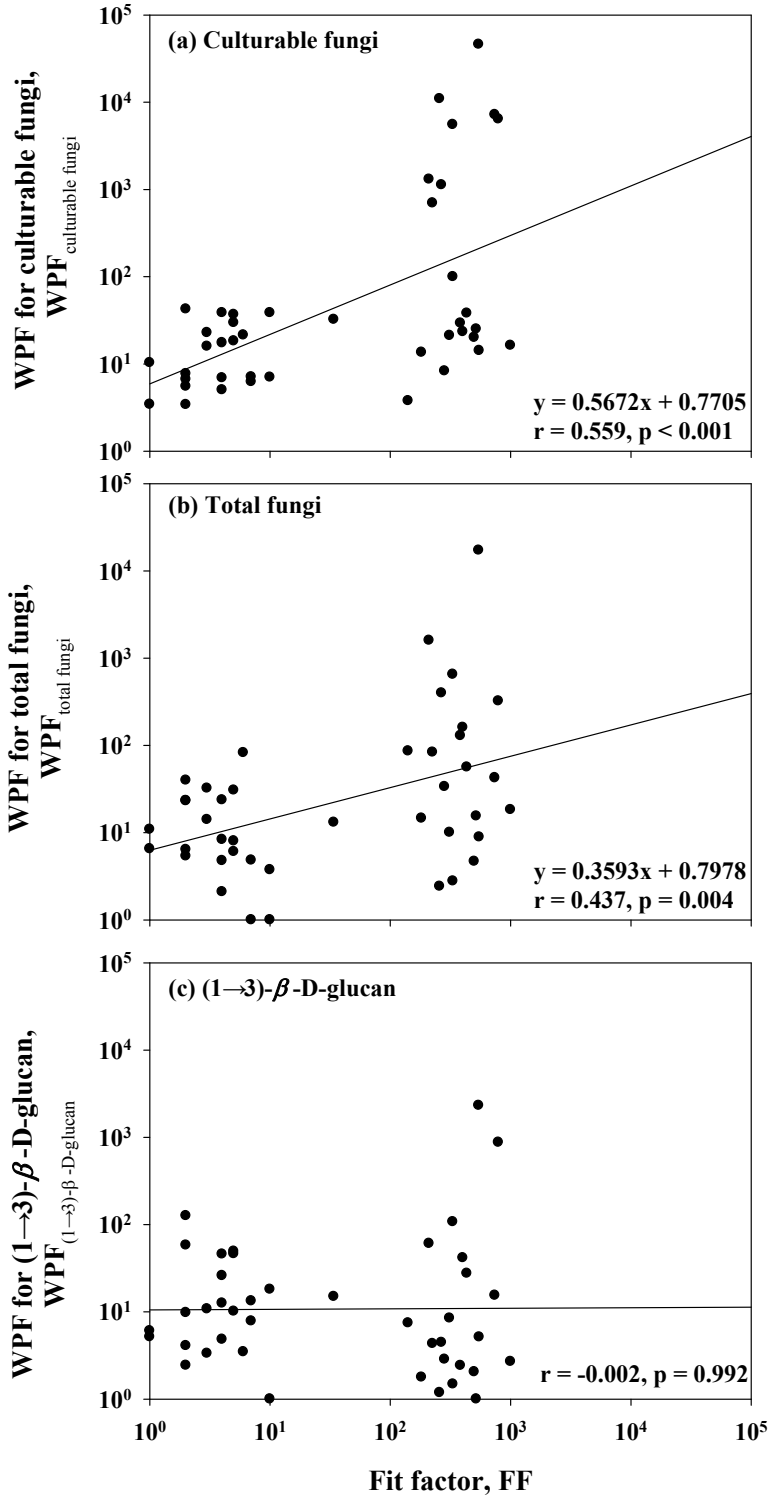

Fig. 5. Association between FFs and WPFs for fungal contaminants: (a) culturable fungi; (b) total fungi; (c) (1→3)-β-D-glucan. Total fungi were measured using light microscopy with phenosafranin; y = log (the WPF for fungal contaminants); x = log (the FF). (n = 42)

fungi and total fungi using the equation given in the study. However, this equation was not suitable for estimating the WPF for (1→3)-*β*-D-glucan.

A moderate correlation between FFs and PFs was also found for the following: N95 FFRs against airborne irons in the welding workplace (Han, 2002); EN-specified FFP respirators and surgical masks against NaCl aerosols (Lee *et al.*, 2016); full facepiece respirators with HEPA filters (Lee *et al.*, 2017). A strong association was found for half elastomeric respirators with P-100 filters against airborne irons at a steel foundry. This indicates that FF was a meaningful indicator of respirator performance in the workplace (Zhuang *et al.*, 2003). However, no correlation between FFs and WPFs was found for half-mask facepiece respirators (Zhuang and Myers, 1996).

Fit test means, under the regulation of OSHA, that the respirator fit was measured under different types of facial movements carried out by the participants. It does not take into account other means of contamination leakage, such as damage to the respirator or penetration through filter material. WPF measurement considers not only other means of contamination leakage, but also other working environments and conditions which may cause physical and physiological burdens that further affect respiratory protection efficiency. In comparison to the TSI Portacount Plus with N95 companion, which focuses on the measurement of particles, WPF measurement focuses on real contaminants that exist in the workplace. Therefore, the type and size of contaminants, level of work burden, type of work, time and moisture level while wearing the respirator will affect the relationship between FFs and WPFs. Dixon and Nelson (1984) explained the reason behind the relationship between FFs and WPFs as follows: (1) Standard fit test exercises are unable to reflect the actual workers' movements in working conditions; (2) breathing rates during fit tests are not the same as those in actual working conditions; (3) the reagent used in fit tests may be different from those that exist in actual working environments, causing different leaks through the face seal; and (4) water content in exhaled air may lower FF values. Zhuang *et al.* (2003) discovered that the variation of WPFs and FFs was greater when FFs were greater than 10, which in return lowered the correlation between WPFs and FFs. Moreover, a low correlation might also be produced by variable sensitivity in different analytical methods (Cho *et al.*, 2011).

CONCLUSIONS

The protection provided by N95 FFRs and SMs against airborne fungi and (1→3)-*β*-D-glucan increases with the particle size. The WPFs of N95 FFRs against airborne fungi are greater than those of SMs by 1.0–12.8 times. The concentration of the culturable fungi is affected by fungal viability and culturability, which in turn affects the sensitivity of the fungal analysis and leads to higher WPFs for the culturable fungi than the total fungi. (1→3)-*β*-D-glucan is part of the fungal cell wall, which exists in fungal spores and fungal fragments or attaches to the surface of particles, thus causing the WPFs for (1→3)-*β*-D-glucan to be lower than those for fungi. About 4.8%–35.0% of the WPFs against fungal contaminants for the N95 FFRs were below 10 for the spore size range > 1.8 μm, indicating the possibility that the American OSHA regulation standard for N95 FFRs (APF = 10) may overestimate the protection they actually provide. Both the FF and the WPF for (1→3)-*β*-D-glucan show medium statistically significant relationships with the WPF for fungi, which means that either value can be used as an indicator for evaluating the protection provided by respirators against airborne fungi. The results of this study can be referenced for setting future standards for respiratory protection from fungi on agricultural farms.

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