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

Shu-An Lee[1*], Chien-Hua Liao[1], Tsai-Yu Lin [2]

[1] Department of Environmental Engineering and Science, Feng Chia University, No. 100, Wenhwa Rd., Seatwen, Taichung 40724, Taiwan, Republic of China.

[2] Department of Applied Mathematics, Feng Chia University, No. 100, Wenhwa Rd., Seatwen, Taichung 40724, Taiwan, Republic of China.

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 workplace protection factors (WPF) provided by N95 filtering facepiece respirators (FFR) and surgical masks (SM) against fungi. This field study was performed with human subjects wearing an N95 FFR or a SM in farming activities. The geometric means (GM) of the WPFs of N95 FFRs and SMs were 156.2 and 12.2 for total culturable fungi, 55.4 and 9.0 for total fungi, and 10.5 and 11.1 for (1→3)-*β*-D-glucan. WPFs for N95 FFRs against fungal contaminants were mostly greater than those for SMs; however, still about 4.8%-35.0% of WPFs in the spore size range (> 1.8 μm) were 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 increasing particle size. Total (1→3)-*β*-D-glucan significantly correlated with total fungi ($r = 0.588$, $p < 0.001$) and total culturable fungi ($r = 0.463$, $p = 0.002$). This indicates that (1→3)-*β*-D-glucan could be used as an indicator to assess respirator protection against airborne fungi in agricultural farms.

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

INTRODUCTION

[*] Corresponding author. Tel: +886-4-24517250 ext. 5234; Fax: +886-4-24517686

E-mail address: salee@fcu.edu.tw

Fungi are classified as a type of bioaerosols. It has been found that exposure to fungi can be associated with respiratory diseases such as allergies and asthma (Bornehag et al., 2005; Edwards et al., 2012; Roy 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 (Husman, 1996; Levetin, 1995) 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 5000-100000 CFU m^{-3} (Health and Safety Executive, 2003; National Labour Inspection of Denmark, 1989). 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 (5000-100000 CFU m^{-3}). Swine farms and poultry farms 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 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). N95 FFRs have shown less filter penetration, fewer face seal leaks, and less total inward leakage than surgical masks under laboratory conditions (Grinshpun et al., 2009; Lee et al., 2008; 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 (AIHA, 2002; Federal Register 60:110, 1995). 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 1μm exist as well (Lee et al., 2014; Seo et al., 2009). 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

118 further explore the association between WPF values for (1→3)-β-D-glucan and WPF values for
119 fungi.
120
121 ## METHODS
122 ***Development of a personal sampling system***
123 A sampling system was developed to size-selectively assess respirator protection against
124 airborne fungal contaminants in agricultural farms. The design concept was based on our
125 previously developed personal sampling system, which had been used to evaluate respirator
126 protection against airborne dust and microorganisms in the laboratory and in agricultural farms
127 (Lee et al, 2004, 2005a and 2005b). This previous personal sampling system was described in
128 detail by Lee et al. (2004). In short, there were two sampling lines (an in-facepiece sampling line
129 and an ambient sampling line) to collect air samples inside and outside the respirator. Each
130 sampling line consisted of a sampling probe, two adaptors, a 0.5-inch diamter of Tygon tubing, a
131 metal sampling chamber, an optical particle counter, a 25-mm filter cassette, and a pump. A silica
132 gel/Nafion dryer was installed to remove moisture from human-exhaled air in the in-facepiece
133 sampling line.
134 In order to size-selectively collect airborne fungi and fungal fragments in the present study, we
135 used a two-stage bioaerosol cyclone sampler (model BC221) instead of the 25-mm filter cassette.
136 This sampler is composed of two screw-top 1.5 mL microcentrifuge tubes (Model 506-624,
137 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 measurement 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 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. $WPF_{\text{total culturable}}$

211 fungi, $WPF_{total\ (1\rightarrow3)\text{-}\beta\text{-D-glucan}}$ vs. $WPF_{total\ fungal\ spores}$, FFs vs. $WPF_{total\ culturable\ fungi}$, FFs vs. $WPF_{total\ fungal}$

212 spores, and FFs vs. $WPF_{total\ (1\rightarrow3)\text{-}\beta\text{-D-glucan}}$. WPF values for fungal contaminants whose

213 concentrations were not detected inside the respirator were calculated using half of the detection

214 limit for inside concentrations (Cho et al., 2011; Lee et al., 2005).

215

216 **RESULTS AND DISCUSSION**

217

218 ***Fit factors of surgical masks and N95 filtering facepiece respirators***

219 Fig. 2 shows the FFs acquired for N95 FFRs and SMs under 6 different types of exercises. Fig.

220 2(a) shows the FFs acquired for N95 FFRs under different types of exercises. It was discovered

221 that the FF from the first normal breathing (geometric mean (GM) = 553.3) was higher than other

222 types of exercises. The lowest FF value (GM = 319.7) was seen when bending. After conducting

223 the Kruskal-Wallis test, it was found that the 6 different types of exercises did not cause

224 statistical significance ($p = 0.35$) in FF values. Fig. 2(b) is the FFs acquired for SMs under

225 different types of exercises. The results show that the FF value acquired from talking (GM = 5.4)

226 was the highest. The FF value from the last normal breathing was the lowest (GM = 2.8). After

227 conducting the Kruskal-Wallis test, it was found that the 6 different types of exercises did not

228 cause significant statistical difference ($p = 0.31$) in FF values. The FFs measured by Crutchfield

229 et al. (1999) for full- and half-facepiece elastomeric respirators indicated that lower FFs would be

230 acquired during the talking and bending exercises. The lower FFs obtained for the talking
231 exercise were likely attributed to sampling artifacts than to actual exercise dynamics. The
232 bending over exercise was more predictive of poor respirator fit. N95 FFRs, full- and half-
233 facepiece elastomeric respirators are all in the 'tight-fitting respirator with sturdier mask material'
234 category. Hence, a lower FF was obtained for N95 FFRs in this study during the bending over
235 exercise. SMs belong to the 'loose-fitting respirator' category. As they do not provide a good
236 fitting to the facial surface, particle penetration through face seal leaks is far greater than through
237 the normal filter (Grinshpun et al., 2009). This causes lower FFs during head turning.
238 Furthermore, SM material is relatively soft in comparison to that of N95 FFRs, causing an
239 unstable fixing of the sampling probe. This results in variable FF outcomes due to sampling
240 artifacts.

241 According to the OSHA 29 CFR 1910.134 regulation for FFs, the FF values of N95 FFRs must
242 be equal or greater than 100. The GM of FF values for N95 FFRs in this study was 331.1, while
243 that for SMs was 3.5. The FF of N95 FFRs was greater than that of SMs by 94.6 times. The
244 percentage of N95 FFRs passing the fit test was 95.2%, while Lee et al. (2011) found it to be
245 between 8% and 100%. The greater FF values for N95 FFRs compared to SMs was perhaps due
246 to the differences in filter efficiency and face seal leaks. Grinshpun et al. (2009) found that the
247 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, 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

285 WPFs were smaller than APF = 10 against microorganisms. Laboratory experiments also found
286 that N95 FFRs and EN-certified FFP series respirators might not achieve the expected protection
287 level against particles in the viral and bacterial size range (Lee et al, 2008; 2016).

288 Fig 3. shows the protection results of N95 FFRs and SMs against fungal contaminants by
289 particle size. The dotted line indicates OSHA's regulated APF value (APF = 10) for tight-fitting
290 half-masks. Fig. 3(a) shows that the GMs of WPFs of N95 FFRs against culturable fungi were
291 209.9, 48.1, and 6.4 respectively for particle sizes of > 1.8 μm, 1-1.8 μm and < 1 μm. This
292 indicates that WPFs decreased with decreasing particle size. After conducting the Kruskal-Wallis
293 test, the effect of particle size on WPF values was found to be statistically significant ($p = 0.008$).
294 The percentages of WPFs against culturable fungi exceeding APF = 10 for N95 FFRs at particle
295 sizes of > 1.8 μm, 1-1.8 μm and < 1 μm were 95.2%, 66.7% and 30.0% respectively. The
296 percentage of WPFs greater than APF = 10 decreased as the particle size decreased. On the other
297 hand, the GMs of WPFs against culturable fungi for SMs at particle sizes of > 1.8 μm, 1-1.8 μm
298 and < 1 μm were 12.2, 15.2 and 4.4 respectively. This also shows a decrease in WPF values with
299 decreasing particle size. After performing the Kruskal-Wallis test, the results showed that particle
300 size had a statistically significant effect ($p = 0.048$) on the WPFs. The percentages of WPFs
301 against culturable fungi exceeding APF = 10 for SMs at particle sizes of > 1.8 μm, 1-1.8 μm and
302 < 1 μm were 52.4%, 57.9% and 10.0% respectively, similar to those for N95 FFRs. The

303 percentage of WPFs greater than APF = 10 decreased as the particle size decreased. However,
304 under the same particle size range, the percentage for N95 FFRs was greater than that of SMs.
305 Fig. 3(b) shows that the GMs of WPFs for N95 FFRs against (1→3)-*β*-D-glucan at particle
306 sizes of > 1.8 μm, 1-1.8 μm and < 1 μm were 42.7, 7.7 and 4.2 respectively. The WPFs decreased
307 as particle size lowered. After performing the Kruskal-Wallis test, it was discovered that the
308 effect of particle size on WPFs was statistically significant (p = 0.01). The percentages of WPFs
309 exceeding APF = 10 for N95 FFRs against (1→3)-*β*-D-glucan at particle sizes of > 1.8 μm, 1-1.8
310 μm and < 1 μm were 65.0%, 26.7%, and 9.1% respectively. The percentage of WPFs greater than
311 APF = 10 decreased as particle size reduced. The GMs of WPFs for SMs against (1→3)-*β*-D-
312 glucan at particle sizes of > 1.8 μm, 1-1.8 μm and < 1 μm were 19.5, 5.7, and 3.2 respectively,
313 indicating a decrease in WPF values with decreasing particle size. After performing the Kruskal-
314 Wallis test, it was observed that the effect of particle size on WPFs was statistically significant (p
315 = 0.03). The percentages of WPFs exceeding APF = 10 for SMs against (1→3)-*β*-D-glucan at
316 particle sizes of > 1.8 μm, 1-1.8 μm and < 1 μm were 60.0%, 35.3% and 9.1% respectively. The
317 above results indicate that particle size had an effect on the WPFs for both N95 FFRs and SMs
318 against culturable fungi and (1→3)-*β*-D-glucan. It also shows that WPFs decreased with
319 decreasing particle size.

320 Previous studies had not taken into account nor discussed the effect of particle size on the
321 protection efficiency of respirators against fungal contaminants. The results of this study revealed
322 that the WPFs of both N95 FFRs and SMs against culturable fungi and (1→3)-*β*-D-glucan, as
323 well as the percentage of WPFs exceeding APF = 10, increased as particle size increased. Lee et
324 al. (2005) found that the WPFs of N95 FFRs were different for different fungal genera/groups,
325 and increased with increases in fungi size. In our results published in 2014, we have already
326 proven that variable sizes and groups of fungi existed within present farm environments (Lee et
327 al., 2014). Furthermore, (1→3)-*β*-D-glucan that existed within variable particle sizes also
328 affected the protection efficiency of respirators. A point worth noting is that, when particle size is
329 smaller than 1 μm, the WPFs of both N95 FFRs and SMs against fungal contaminants are smaller
330 than APF = 10. Therefore, fungi that release small fragments containing (1→3)-*β*-D-glucan, such
331 as *Aspergillus versicolor* and *Stachybotrys hartarum* (Seo et al., 2009), should be paid special
332 attention to when respirator protection is required.
333 Another interesting note is that the concentration of fungi decreased as the particle size
334 decreased, which further caused no detection of fungi inside the respirator. The percentage of
335 samples where culturable fungi concentrations were not detected inside the N95 FFRs at particle
336 sizes of > 1.8 μm, 1-1.8 μm and < 1 μm were 28.6%, 61.9%, and 60.0% respectively. For SMs,
337 they were 9.5%, 31.6%, and 70.0% respectively. The percentages for most of the N95 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

357 coefficients were 0.463 ($p = 0.002$) for the WPF of (1→3)-*β*-D-glucan and culturable fungi, and
358 0.588 ($p < 0.001$) for the WPF of (1→3)-*β*-D-glucan and total fungi. These values were in the
359 middle of weak ($r = 0.3$) and moderate ($r = 0.6$) positive linear relationships and were statistically
360 significant ($p < 0.05$). The equations provided in Fig. 4 can be used to estimate the WPF of these
361 fungi when (1→3)-*β*-D-glucan is performed. This means the WPF of fungi can be assessed by
362 importing the WPF of (1→3)-*β*-D-glucan into the equation. In these cases, the time for fungi
363 cultivation can be minimized, and the time and manpower needed for microscopic fungal spore
364 counting can be reduced. As the concentration of culturable fungi is influenced by viability and
365 culturability, the culturable method is not necessarily more accurate for obtaining the
366 concentration of total fungi than spore counting by light microscopy. In this context, the WPF for
367 (1→3)-*β*-D-glucan had a stronger association with the WPF for total fungi than with the WPF for
368 cultural fungi. On the other hand, due to the variation of (1→3)-*β*-D-glucan content within each
369 fungus as well as the variation in sensitivity of different methods for analyzing fungal
370 contaminants, only moderate correlations were found among the WPFs for fungal contaminants
371 in this study.

372 Prior to the participants taking part in this study, they needed to go through the procedure of fit
373 testing in order to inspect the respirator fit. To further elaborate the relationship between FFs and
374 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 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; (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 increase with increasing 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 culturable fungi is affected by fungal viability and culturability. This in turn affects the sensitivity of fungal analysis, and subsequently leads to lower WPFs for culturable fungi as compared to 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. This subsequently causes the WPFs for (1→3)-*β*-D-glucan to be lower than those for fungi. About 4.8%-35.0% of WPFs against fungal contaminants for N95 FFRs were below 10 in the spore size range (> 1.8μm). This indicates the possibility that the American OSHA regulation standard for N95 FFRs (APF = 10) may overestimate the protection they actually provide against fungal contaminants. Both the FF and the WPF for (1→3)-*β*-D-glucan show medium statistically significant relationships with the WPF for fungi. This means that both values 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 in agricultural farms.

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556 **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 (Geometric standard deviation)	156.2 (16.4)	12.2 (2.3)	55.4 (8.6)	9.0 (3.1)	10.5 (7.8)	11.1 (3.3)

Note : Total fungi were measured using light microscopy with phenosafranin

557

Figure Captions

Fig. 1. Personal sampling system.

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)

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}; $LOD_{(1\rightarrow3)\text{-}\beta\text{-D-glucan}}$ = 68.4 pg m^{-3}); APF = assigned protection factor.

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)

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)

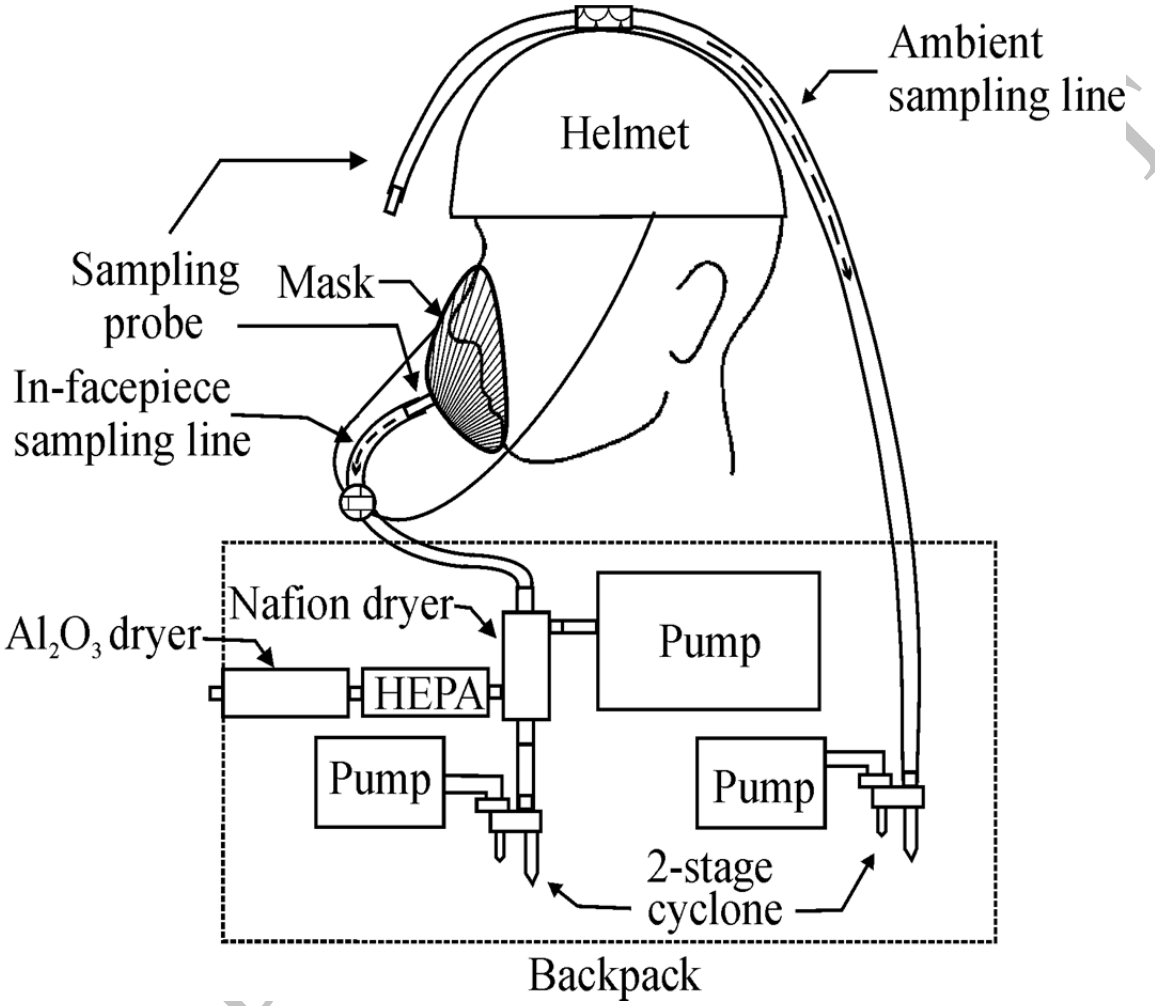
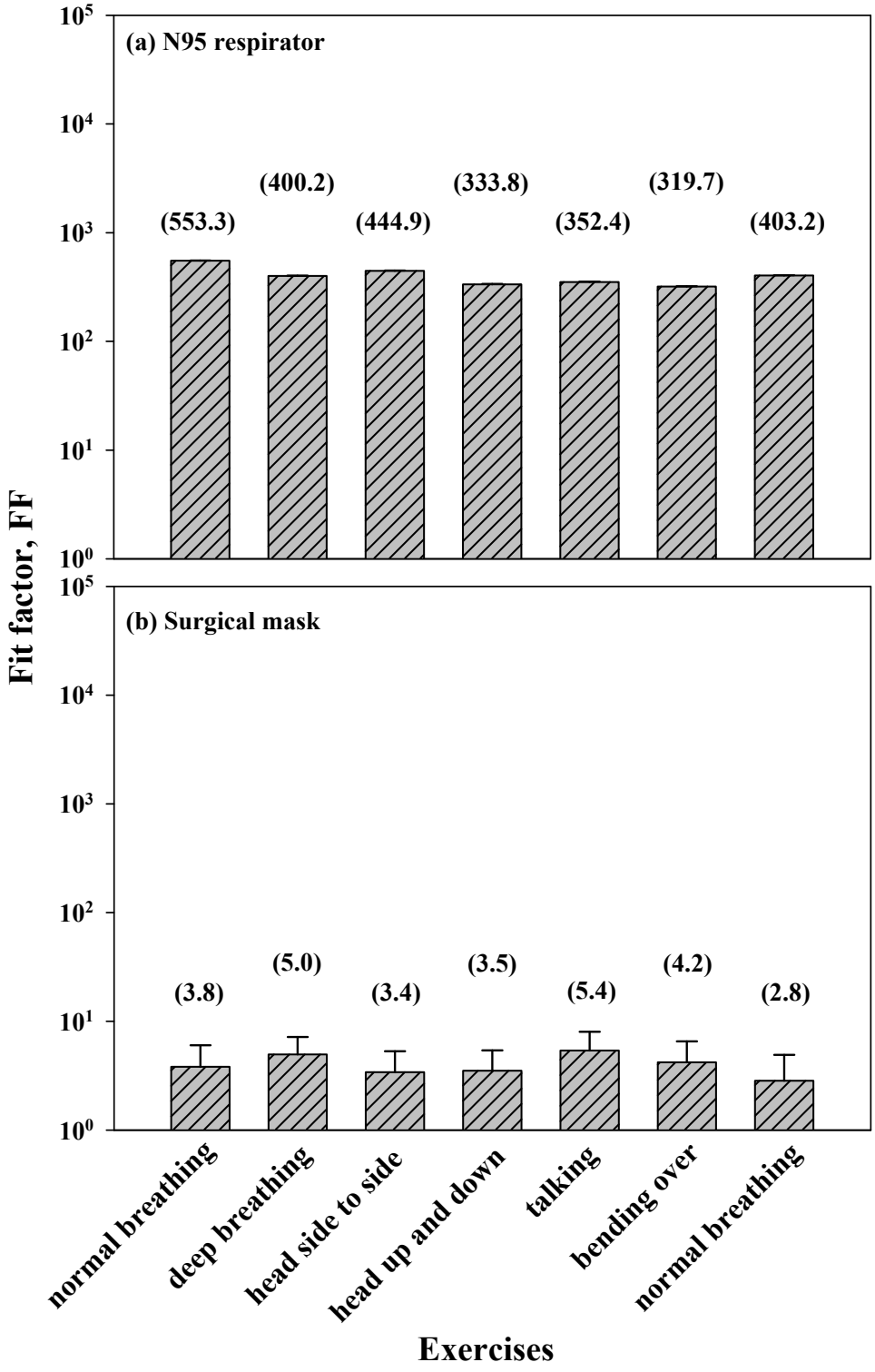


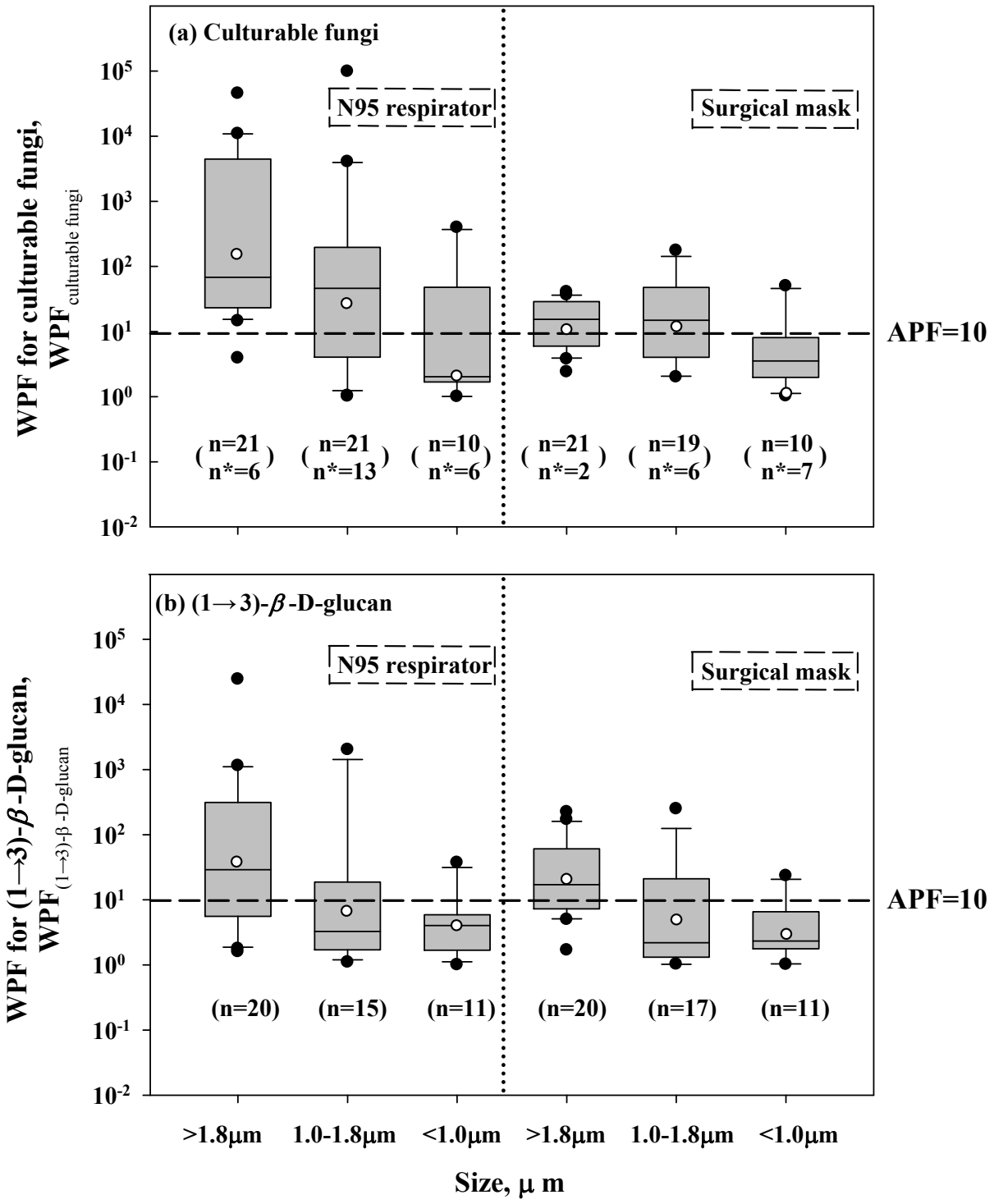
Fig. 1.



584

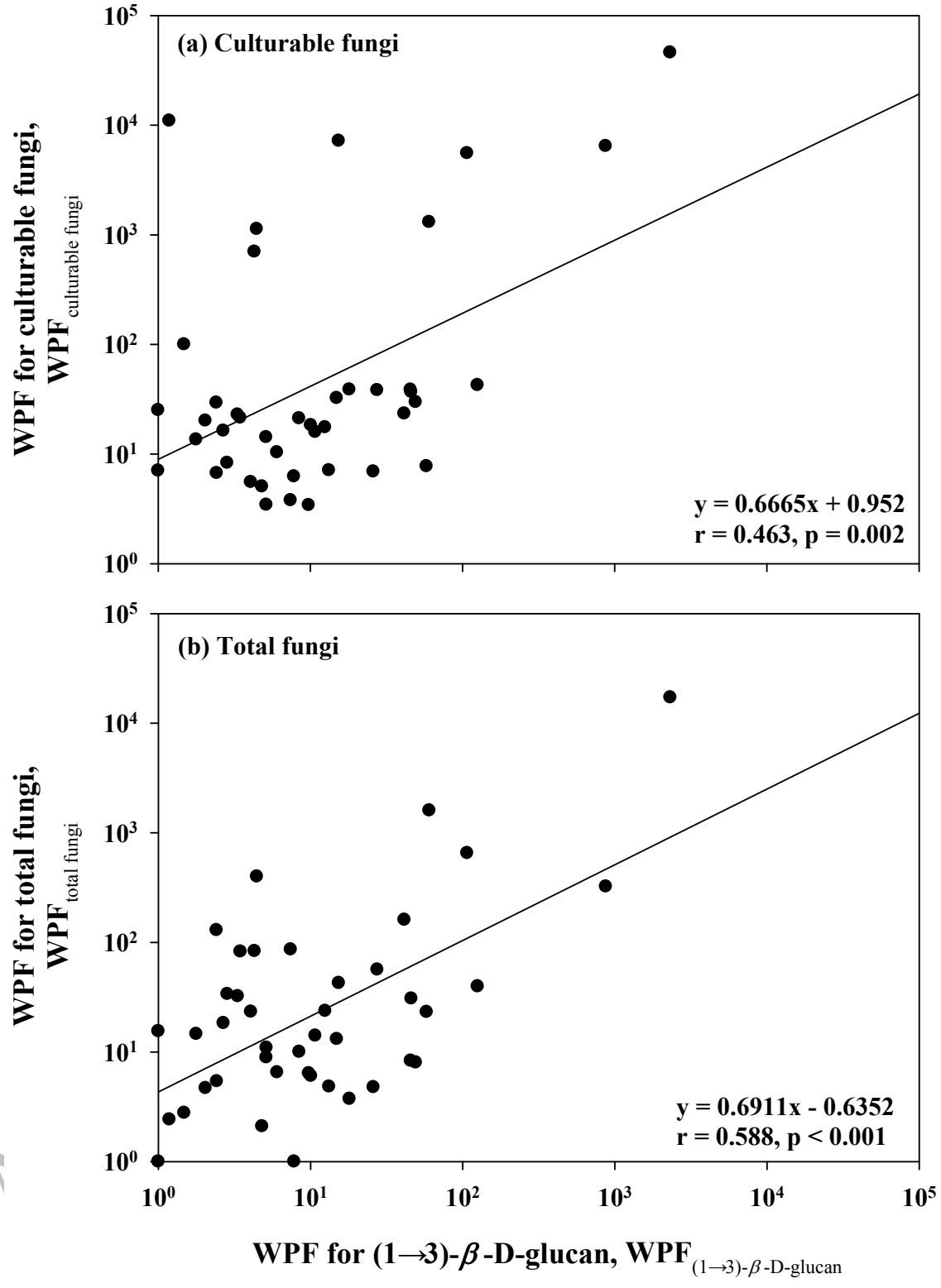
585 **Fig. 2.**

586


(a) Culturable fungi
WPF for culturable fungi, WPF culturable fungi
N95 respirator
Surgical mask
APF=10
(n=21 n*=6)
(n=21 n*=13)
(n=10 n*=6)
(n=21 n*=2)
(n=19 n*=6)
(n=10 n*=7)
(b) (1→3)-β-D-glucan
WPF for (1→3)-β-D-glucan, WPF (1→3)-β-D-glucan
N95 respirator
Surgical mask
APF=10
(n=20)
(n=15)
(n=11)
(n=20)
(n=17)
(n=11)
>1.8μm
1.0-1.8μm
<1.0μm
>1.8μm
1.0-1.8μm
<1.0μm
Size, μ m

587

588 **Fig. 3.**



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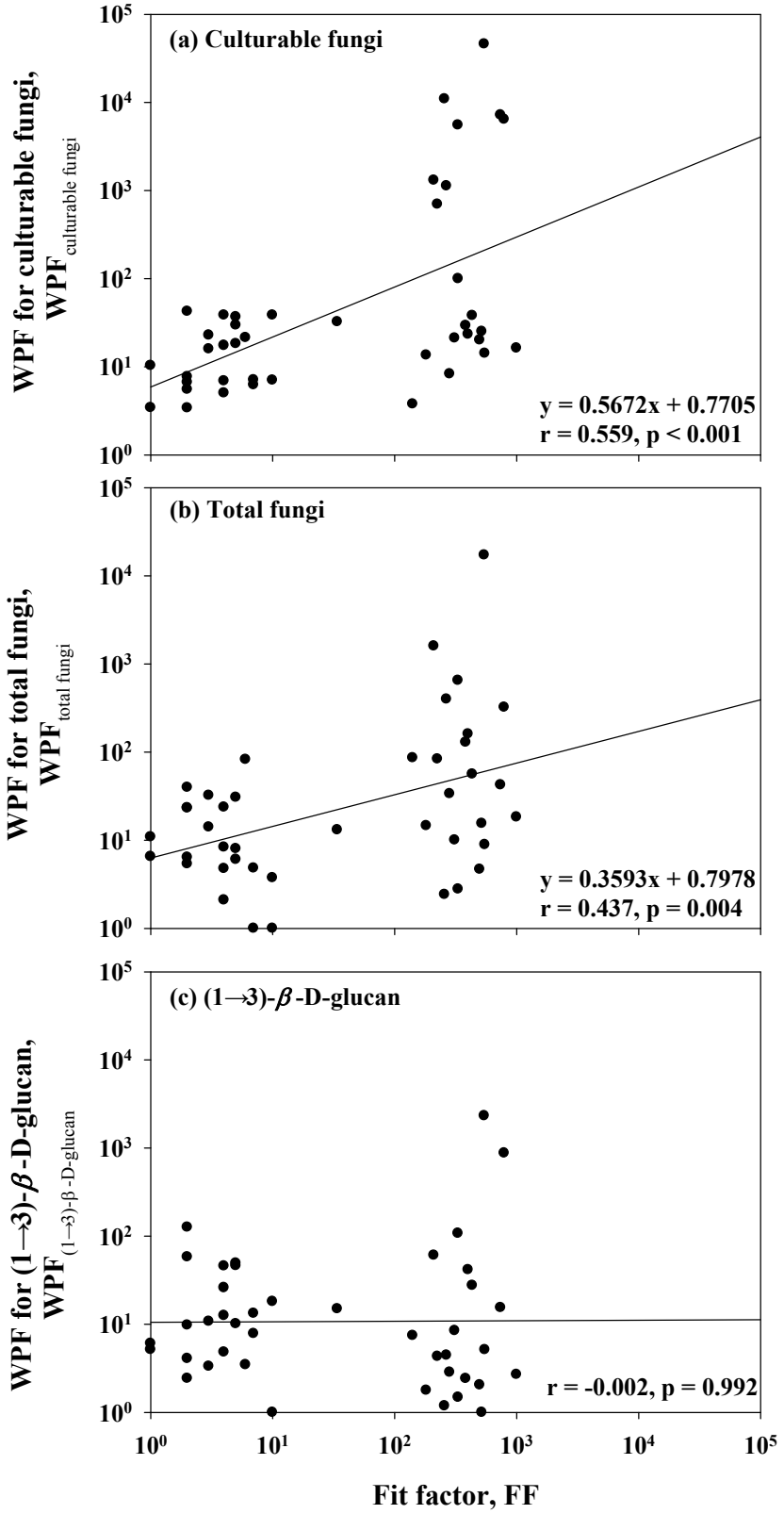
591 **Fig. 4**.


(a) Culturable fungi
y = 0.5672x + 0.7705
r = 0.559, p < 0.001
(b) Total fungi
y = 0.3593x + 0.7978
r = 0.437, p = 0.004
(c) (1→3)-β-D-glucan
r = -0.002, p = 0.992
WPF for culturable fungi, WPF culturable fungi
WPF for total fungi, WPF total fungi
WPF for (1→3)-β-D-glucan, WPF (1→3)-β-D-glucan
Fit factor, FF

592

593 **Fig. 5.**