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Comparison of Workplace Protection Factors for Different Biological Contaminants

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This study compared workplace protection factors (WPFs) for five different contaminants (endotoxin, fungal spores, (1→3)-β-D-glucan, total particle mass, and total particle number) provided by an N95 elastomeric respirator (ER) and an N95 filtering facepiece respirator (FFR). We previously reported size-selective WPFs for total particle numbers for the ER and FFR, whereas the current article is focused on WPFs for bioaerosols and total particle mass. Farm workers (n = 25) wore the ER and FFR while performing activities at eight locations representing horse farms, pig barns, and grain handling facilities. For the determination of WPFs, particles were collected on filters simultaneously inside and outside the respirator during the first and last 15 min of a 60-min experiment. One field blank per subject was collected without actual sampling. A reporting limit (RL) was established for each contaminant based on geometric means (GMs) of the field blanks as the lowest possible measurable values. Depending on the contaminant type, 38–48% of data points were below the RL. Therefore, a censored regression model was used to estimate WPFs ($WPF_{censored}$). The $WPF_{censored}$ provided by the two types of respirators were not significantly different. In contrast, significant differences were found in the $WPF_{censored}$ for different types of contaminants. GMs $WPFs_{censored}$ 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 $WPF_{censored}$ was more strongly associated with concentrations measured outside the respirator for endotoxin, fungal spores, and total particle mass except for total particle number. However, when only data points with outside concentrations higher than 176×RL were included, the WPFs increased, and the association between the outside concentrations and the WPFs became weaker. Results indicate that difference 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).

Keywords agriculture, bioaerosol, respirator, workplace protection factor

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INTRODUCTION

Aerosols in agricultural environments contain inorganic and organic dust, including bioaerosols, that may contribute to the higher prevalence of respiratory symptoms and diseases among farmers than among a general population.[1] Endotoxin, a cell-wall component for Gram-negative bacteria, is commonly found in agricultural settings, for example, in grain handling sites and poultry and swine confinements.[2,3] Endotoxin induces significant airway inflammation and dysfunction.[4] Fungal spores and their constituent, (1→3)-β-D-glucan, are also often elevated in agricultural environments.[5] Exposure to fungi and its components is known to be associated with asthma[6]and allergic alveolitis.[7] Respiratory protection is often the only feasible way to reduce bioaerosol and dust exposures to agricultural workers on farms.

The federal Occupational Safety and Health Administration (OSHA) Respiratory Protection Standard (29 CFR Part 1910.134) is not applicable to many agricultural environments due to exclusion of such workplaces.[8] When respiratory protection is required for compliance with OSHA standards, selected respirators must be certified by National Institute for Occupational Safety and Health (NIOSH). However, NIOSH certification (42 CFR Part 84) does not include testing with biological particles.[9] The performance of respirator filters against biological particles has been shown to depend on aerodynamic size;[10] however, the penetration of rod-shaped bacteria has been reported to be lower than spherical bacteria of the same aerodynamic size.[11] Very little is known regarding how particle characteristics (i.e., size, shape, and density)

affect faceseal leakage, which may account for most of the total penetration into the respirator.[12]

The efficiency of respirators used in the workplace can be expressed as a workplace protection factor (WPF), defined as a ratio of the concentration of airborne contaminant outside the respirator to that inside the respirator, measured under the conditions of the workplace using a properly selected, fit-tested, and functioning respirator while it is correctly worn.[13] WPF studies have investigated the performance of elastomeric respirators (ERs) and filtering facepiece respirators (FFRs) for airborne particles.[14–18] However, most of these studies did not involve bioaerosols. To our knowledge, only one previous study (conducted by our research group) investigated WPF for biological particles.[16] WPFs provided by one model of N95 filtering facepiece respirator were studied in agricultural environments for culturable bacteria and fungi, total fungi, and total particle numbers in five different size ranges (0.7–1, 1–2, 2–3, 3–5, 5–10 μm). It was found that WPFs for fungi were lower than those for total particles in the same size range.[16]

To further investigate this phenomenon, a follow-up study included two different types of respirators and five different contaminants for the measurement of WPFs in agricultural settings. One model of ER with N95 filters and one model of N95 FFR were included. Total particle numbers in the five size ranges specified above and the total particle mass concentration were measured outside and inside the respirator. In addition, endotoxin, fungal spores, and (1→3)-β-glucan were collected concurrently.

Comprehensive analysis of the WPF results based on the total particle number has been reported in a previous paper.[19] Briefly, the 5th percentiles WPF for total particles for the ER and FFR were higher than the OSHA-assigned protection factor (APF) of 10 for a half-mask respirator. Geometric means (GMs) of WPFs for the ER were 172, 321, 1013, 2097, and 2784 for particles of 0.7–1.0, 1.0–2.0, 2.0–3.0, 3.0–5.0, and 5.0–10.0 μm, respectively, and corresponding values for the FFR were 67, 124, 312, 909, and 2089.[19] Thus, the ER provided higher WPFs for total particles than the FFR in all size ranges, and the WPFs for both respirators increased with an increase in particle size. The current article reports WPFs for bioaerosols and total particulate mass. WPFs for different contaminants were compared with each other and to the previously reported data on size-selective WPFs based on total particle numbers.

METHODS

Field Study Design

Field study design has been described in detail by Cho et al.[19] In brief, 25 farm workers wore the ER and FFR while performing activities at eight locations representing pig barns, horse farms, and grain handling sites. Six females were included to reflect the gender make-up of farmers. Among 25 subjects, two subjects failed the fit-test with the FFR. Thus, those two data sets were excluded for further analysis. Two to four subjects participated at each study location.

Particle Number Measurement

Particle concentrations inside and outside the respirator were simultaneously measured using a specially developed personal sampling system as described previously.[19] The sampling system consists of two identical sampling lines, each one including a sampling probe, a sampling chamber, an optical particle counter (HHPC-6; Hach Company, Loveland, Colo.), a filter sampler for collection of bioaerosols, and a pump (Leland Legacy; SKC Inc., Eighty Four, Pa.). The total sampling flow rate was 10 L/min. All instruments were placed in a sampling back bag and connected with sampling tubing and filter cassette, and then fixed with cable ties onto the sampling back bag to avoid interference by movement while subjects were doing activities.

The optical particle counter measured particle number concentration in five size channels: 0.7–1, 1–2, 2–3, 3–5 and 5–10 μm. Particle concentrations were determined concurrently inside and outside the respirator during the first and last 15 min of the 60-min experiment to avoid moisture condensation inside sample tubing. For every subject, size-selective WPFs were calculated in 1-min intervals and averaged over the entire 30-min sampling time. There was no significant difference between the average WPFs obtained during the first and last 15 min of sampling.[19]

Collection of Bioaerosol and Particle Mass Samples

Particles were collected on a polycarbonate filter (Millipore, Billerica, Mass.) with a pore size of 3.0 μm and a diameter of 25 mm, and loaded in a cassette (225–1107, SKC Inc.) 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. 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 min and agitated in an ultrasonic bath for 15 min. The filter was then rinsed twice with pyrogen-free reagent water (Pyrochrome Associates of Cape Cod Inc., East Falmouth, Mass.) and air dried in a biosafety hood (Sterilchem-GARD Class II, Type B2; The Baker Company Inc., Stanford, Maine).

The compartments of the filter holder, except O-rings, were soaked in a beaker of soap water for 10 min then agitated in an ultrasonic bath for an additional 10 min. The compartments were rinsed with tap water for 10 min and agitated again with autoclaved water for 10 min. Subsequently, the compartments were autoclaved for 15 min after being air dried in the biosafety hood. O-rings (non-autoclavable) were soaked in 70% ethanol for 30 min and air dried in the biosafety hood.

A portion (2.8 L/min) of the total sampling flow (10 L/min) was passed into the optical particle counter. The remaining airflow (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, N.J.). Bioaerosols were collected during the first and last 15 min of the 60-min

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 so as to obtain a 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 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.

Sample Analysis

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 min followed by 15 min 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 –20°C for up to 2 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., Woods Hole, Mass.) with an absorbance microplate reader (ELx808; BioTek Instrument Inc., Winooski, Vt.) as described by Adhikari et al.[20] 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) 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.[21] Fungal spores were counted under a bright light microscope as described by Adhikari et al.[21] 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; Pyrochrome; Associates of Cape Cod, Inc., Woods Hole, Mass.) with the above-mentioned absorbance microplate reader, as described by Adhikari et al.[20] 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, Ohio). 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, N.Y.) 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-min sampling time. Geometric means of field blanks for endotoxin, fungal spore count, (1→3)-β-D-glucan, and total particle mass were 4 EU/m^3, 2436 spores/m^3, 5.3 ng/m^3, and 0.025 mg/m^3, respectively. These concentrations are referred to as "reporting limits" (RL) for each contaminant throughout this article. 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 was 5 particles/L, which translates to one particle for each channel. An RL value for total particle number could not be determined because particle concentrations could not be measured from the field blanks.

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 38 to 48% (endotoxin 48%; (1→3)-β-D-glucan 38%; fungal spore 41%; and total particle mass 42%). Geometric means 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 to 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 cutoff values) and right censoring (below certain cutoff 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.[22,23] 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.[24] 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, Texas; SAS 9.2; SAS Institute Inc., Cary, N.C.).[25] Respirator type, gender, and farm types were considered as co-factors 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.

RESULTS

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 I. Airborne concentrations of endotoxin varied from 7 to 8.4 $\times$ 10^5 EU/m^3 (1 to 84,000 ng/m^3 based on the conversion formula:[26] 10 EU = 1 ng). Corresponding values for fungal spores ranged from 3226 to 9.9 $\times 10^6$ spores/m^3. (1→3)-β-D-glucan varied from 34 to 6.0 $\times$ 10^4 ng/m^3. Total particle mass concentration varied from 0.17 to 13.7 mg/m^3. As reported previously, total particle number concentration varied from 1.2 $\times 10^6$ to 1.7 $\times 10^8$ particles/m^3.[19]

Table II 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 the inside concentration was below the RL. $WPF_{excluded}$ had fewer 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.

Figure 1 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. 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 with our previous study, WPFs for total particles were also combined for the current analysis even though they were previously found to be different between respirator types.[19]

Figure 2A compares the $WPF_{censored}$ for the three bioaerosols (endotoxin, fungal spore count, and (1→3)-β-D-glucan) and total particle mass. Figure 2B compares particle number for the five particle size ranges. All WPFs in Figure 2 represent the combined performance of both half-mask respirators (ER and 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. 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

TABLE I. Airborne Concentrations of Different Contaminants Measured Outside the Respirator at Eight Agricultural Settings

	Endotoxin (EU/m^3)	Fungal Spores (spores/m^3)	β-glucan (ng/m^3)	Total Particle Mass (mg/m^3)
Reporting limit (RL)	4	2436	5.3	0.025
n	48	44	48	45
n (outside concentration greater than 10 $\times$ RL)	46	36	46	41
AVE	51,603	1,174,102	4672	2.7
GM	3267	172,299	476	1.6
MIN	7	3226	34	0.17
MAX	840,311	9,938,877	60,329	13.7

TABLE II. WPFs Based on Three Methods for the Treatment of Values Below the RL

	$WPF_{censored}$[A]			$WPF_{replaced}$[B]			$WPF_{excluded}$[C]			ANOVA
Contaminant	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 RL were treated by the censoring regression model.
[B]Values below RL were replaced by 1/2 of the reporting limit.
[C]Values below RL 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.

lower than the $WPF_{censored}$ measured size selectively by the optical particle counter (Figure 2B) 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). Table III 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.

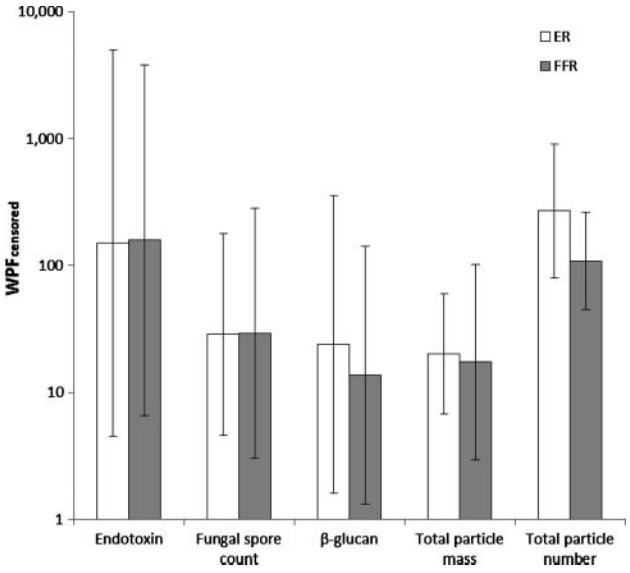

FIGURE 1. Comparison of workplace protection factors ($WPF_{censored}$) provided by elastomeric (ER) and filtering facepiece respirator (FFR) for different types of contaminants (endotoxin, fungal spores, β-glucan, total particle mass, and total particle number). Censoring regression method showed no 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. Corresponding n for FFR are 23, 22, 23, 22, and 22, respectively.

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 IV). Therefore, we further analyzed the data by examining the effect of low outside concentration on the association between $WPF_{censored}$ and outside concentration. Using the data on the total particle number as the reference point, we divided the data into two groups: (1) outside concentrations above or equal to 176 × RL, and (2) outside concentrations below 176 × RL. For Group 1, the recalculated 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 Group 2 were 2, 9, 6, and 14, respectively. Compared with $WPF_{censored}$ estimated using all data points, $WPF_{censored}$ for Group 1 increased, whereas $WPF_{censored}$ for 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 recalculated regression coefficient for endotoxin decreased from 0.68 to 0.20, which was similar to the value obtained for total particle number (0.14).

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

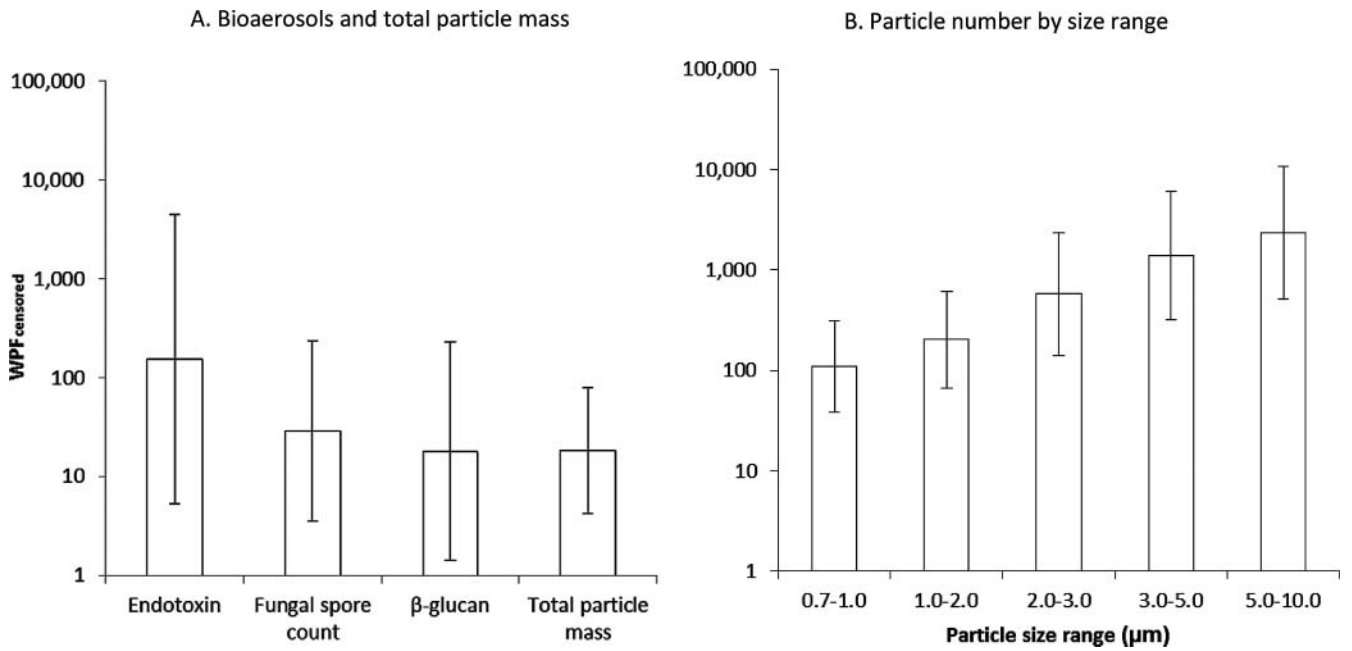

FIGURE 2. Comparison of workplace protection factors ($WPF_{censored}$) for three types of bioaerosols (endotoxin, fungal spores and β-glucan) and particle mass with those for number concentration in five particle size ranges. $WPF_{censored}$ for endotoxin in Figure 2A was statistically similar to WPF for the two smallest particles sizes (0.7–2.0 μm) in Figure 2B. The histograms present geometric means, and the error bars present geometric standard deviations (upper value: GM $\times$ 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).

concentration of (1→3)-β-D-glucan was significantly higher at the grain handling sites compared with other types of farms ($p = 0.02$).

DISCUSSION

Airborne concentrations reported in earlier studies in agricultural farms have varied widely, ranging from 2 to 3.8×10^5 EU/m^3 for endotoxin,[5] 1000 to 10^9 spores/m^3 for fungal spores,[27] and 87 to 2.8×10^5 ng/m^3 for (1→3)-β-D-glucan.[5] Corresponding values in the present 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.[5,28] Corresponding values in the present study were also within the range of previously reported values. The total particle number concentration was also similar to values previously reported in agricultural settings.[19] Thus, airborne concentrations for the five contaminants in the present study are representative for agricultural environments.

Previously, most WPF studies have not taken field blanks into account when WPFs were calculated. However, 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[15,29,30] have replaced concentrations less than the detection limit by 50 or 70% of the detection limit. However, this replacement method is known to lead to inaccurate statistics and poor and misleading regression models.[31] 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 the data set are non-detected because arbitrarily replaced concentrations potentially introduce a false trend or cancels out a real trend in the samples.[22] The censoring regression used for $WPF_{censored}$ is considered to provide a more accurate method for computing statistics on all data points, including both non-detected and detected data.[22,23] This is particularly true for this study where more than 15% of the data were 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

TABLE III. 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.

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. 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 with 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.[32] During agricultural operations, mechanical disturbance is expected to aerosolize larger aggregates.[33] Endotoxin and (1→3)-β-D-glucan can occur as either attached to intact spores or 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

TABLE IV. $WPF_{censored}$ Including Only Data Points that Had Outside Concentration Larger than 176 × RL or Smaller than 176 × RL

Contaminant	WPF (OC ≥ 176 × RL)			WPF (OC < 176 × RL)		
	Group 1			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

Note: WPFs including all data points are presented in Table II.

96.7% of airborne (1→3)-β-D-glucan were in the size range >1.0 μm.[34] 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 article agree with our earlier WPF study[16] 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.

In the previous investigation,[19] the effect respirator type, farm type, gender, and particle size on WPFs was explored by univariate and multivariate analysis. Results showed that only respirator type and particle size remained significant in the multivariate analysis. However, in this investigation, only farm type remained significant only for (1→3)-β-D-glucan in the multivariate censored regression. This can be explained by the higher (1→3)-β-D-glucan concentration in the grain handling sites compared with two other farm types.

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.[16,19,35] 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 with 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 non-biological particles. Reponen et al.[35] and Lee et al.[16] reported that after correcting for respiratory deposition, protection factors decreased for all tested particle sizes (0.04–10 μm). Based on the correction factors presented by Lee et al.,[16] 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 were 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.

CONCLUSIONS

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. 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 $176 \times$ 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.

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