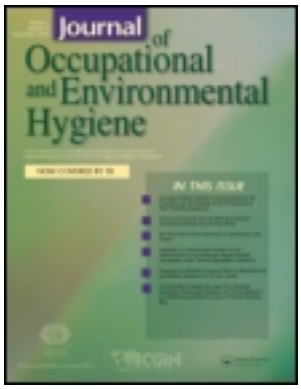

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Characterization of Endotoxin Collected on California Dairies Using Personal and Area-Based Sampling Methods

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Endotoxin, found in the cell wall of gram negative bacteria, is an important contributor to the biological activity of agriculture particulate matter (PM). We analyzed endotoxin in PM collected on 13 California dairies and from the breathing zone of 226 workers during the summer months of 2008. Two particle size fractions were measured: $PM_{2.5}$ and inhalable PM. Recombinant factor C assays were used to analyze biologically active endotoxin, while gas chromatography coupled with mass spectrometry in tandem was used to quantify total lipopolysaccharide. Biologically active endotoxin concentrations in the inhalable PM size fraction from area-based samples ranged from 11–2095 EU/m^3 and from 45–2061 EU/m^3 for personal samples. Total endotoxin in the inhalable PM size fraction ranged from 75–10,166 pmol/m^3 for area-based samples and 34–11,689 pmol/m^3 for personal samples. Area-based geometric mean concentrations for biologically active endotoxin and total endotoxin in $PM_{2.5}$ and inhalable PM size fractions were 3 EU/m^3, 149 EU/m^3, 60 pmol/m^3, and 515 pmol/m^3, respectively. Personal geometric mean concentrations in the inhalable PM size fraction were 334 EU/m^3, and 1178 pmol/m^3. Biologically active and total endotoxin concentration variation was best explained by meteorological data, wind speed, relative humidity, and dairy waste management practices. Differences in endotoxin concentration and composition were found across locations on the dairy.

Keywords 3-OHFA, agriculture, dairy, endotoxin, particulate matter

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INTRODUCTION

Endotoxin is a substantial contributor to the biological activity of particulate matter (PM) in agricultural settings.[1] Endotoxin is a lipopolysaccharide (LPS), which is a component of the cell wall of gram-negative bacteria and is essential in transport and recognition for the cell.[2] Studies have linked exposure to endotoxin with inflammatory responses in the respiratory system[3–5] as well as decreased pulmonary function,[4,6–8] persistent wheeze, chronic cough, allergic and non-allergic rhinitis, chronic bronchitis,[1,8,9] and organic dust toxic syndrome (ODTS).[10,11] Dairy farm workers are at an increased risk for lung disorders, such as chronic bronchitis,[12,13] and studies conducted in Lithuania and Wisconsin dairy farms identified elevated concentrations of endotoxin.[14] A yearlong study conducted at Idaho dairies demonstrated elevated endotoxin concentrations by using upwind and downwind sampling.[15] Studies of Colorado dairy workers performing a variety of tasks also found elevated concentrations of endotoxin and total lipopolysaccharide (3-OHFA) associated with increases in inflammatory markers and decreases in pulmonary function.[5,13,16] The National Health Council of the Netherlands has established a recommended threshold value for endotoxin as 90 EU/m^3.[17]

Previous studies have been conducted mostly on dairies in cold climates, where small numbers of animals are housed in enclosed facilities. In California, typically, dairies house at least 1000 cows, causing the dairies to be classified as concentrated animal feeding operations (CAFOs). In addition, California dairies house animals mostly in open freestall barns or dry lot corrals. Many of the larger dairies in California are modern, with efficient flushing systems for waste removal. The different configuration of California dairies and the increased number of cows housed compared with cold climate dairies may cause endotoxin concentrations to differ compared to those previously reported for cold climate dairies. Evaluation of endotoxin concentrations and subsequent worker exposure in CAFOs[18,19] and other agricultural industries[20–22] is

needed because of the concern raised about the impact of endotoxin exposure on worker health.[23,24]

Endotoxin LPS includes 3-hydroxy fatty acids (3-OHFAs) of varying chain lengths (C_8-C_{18}). Differences in bacterial species present lead to different chain length proportions of 3-OHFAs.[16,25] Chain length concentrations of 3-OHFAs vary between indoor micro-environments.[26,27] Different areas on a dairy farm may have distinct endotoxin chain length concentration profiles based on unique activities or sources.[16] For example, the grain storage area, the area where feed is stored and mixed, and the milking parlor where cows are milked, may differ in endotoxin composition based on bacterial ecology of these distinct environments. It is of interest to quantify mass of various chain lengths as specific chain lengths may be representative of bacteria that may or may not be more harmful than others.[25,28] Some investigators[29] have stated that longer chain lengths may represent Actinobacteria, although this has not been established.

To find out if large California dairies produce high levels of endotoxin and if areas within the dairies show distinct endotoxin chain lengths and can be used to differentiate endotoxin between locations, we will report on the following: (1) endotoxin concentrations associated with two particle size fractions, $PM_{2.5}$ and inhalable PM, using both biological and chemical analysis for various locations within dairies; (2) the impact that individual dairy and daily differences have on endotoxin concentration; and (3) whether areas within the dairy have distinct endotoxin chain length identifiers potentially related to differences in bacterial sources within the dairy.

METHODS

Study Design

Personal and area-based samples were collected from 13 dairies and from the breathing zone of 226 dairy workers between May and September of 2008 as part of a study to investigate respiratory health outcomes due to endotoxin exposure. Recruitment information has been published by Eastman et al.[30] Area-based samples ($PM_{2.5}$ and inhalable PM) were collected on multiple days at multiple locations on each dairy. Integrated personal exposure samples, which measured inhalable PM, were collected from dairy workers over the time of their entire shift. All 13 dairies had more than 1000 lactating cows. The areas sampled included the milking parlors, freestall barns, dry lot corrals, hospital/maternity locations, calf hutches, and grain storage areas. Not all locations were sampled at each dairy on each day; rather, locations were selected on a daily basis according to where more workers were located, the availability of equipment, and to minimize interference with dairy operations. Upwind, downwind, and central locations on each dairy were also measured for endotoxin concentrations, with upwind concentrations representing background endotoxin concentrations for the region. The central location, the most central area that we could logistically sample, was used as a reference location.

The amount of biologically active endotoxin in 225 of the 226 inhalable PM personal samples, 73 of the $PM_{2.5}$ area-based samples and 113 of the inhalable PM area-based samples, was quantified by recombinant factor C (rFC) analysis. Results from this type of analysis are often simply referred to as "endotoxin values," but we refer to this measurement as "biologically active endotoxin." Chemical analysis was used to determine 3-OHFAs in 225 of the inhalable PM personal samples, 49 of the $PM_{2.5}$ area-based samples, and 73 of the inhalable PM area-based samples. Personal samples were classified among various job types, with some jobs not fitting into one of these categories; thus, they were included in a miscellaneous category that was not included in all analyses. Area-based samples were similarly classified among various areas on each dairy, with some samples placed in a location that was classified as miscellaneous. This article refers to 3-OHFAs as "total endotoxin." Chemical analysis through the use of GC/MS measures total endotoxin present; however, it does not quantify levels of biologically active endotoxin.

Sample Collection

A GK2.05SH (KTL) cyclone sampler (BGI Inc., Waltham, Mass.) with a cut point of 2.5 μm an airflow of 3.5 L/min, which has been shown by other studies to have good agreement with the PQ200 (BGI Inc.) Federal Reference Method $PM_{2.5}$ sampler,[31] was used to collect $PM_{2.5}$. Teflon 37-mm Millipore filters with a pore size of 0.45 μm (FHLP03700; Fisher, Pittsburgh, Pa.) were used to collect the particles from the cyclone sampler. An SKC button sampler (225-360; SKC, Inc., Eighty Four, Pa.) with a curved multi-orificed inlet for collecting all suspended PM smaller than 100 μ in aerodynamic diameter with an airflow of 4.0 L/min was used to collect the inhalable PM size fraction. Inhalable PM concentrations (aerodynamic size distribution 0–100 μm) represent aerosols deposited anywhere in the respiratory tract. Each button sampler was fitted with a Teflon 25-mm Millipore PTFE filter with a pore size of 3.0 μm (FSLW02500; Fisher). Tygon tubing connected the samplers to a Swagelok needle valve (Swagelok, Solon, Ohio) for flow adjustment. The samplers were attached to a high-flow Leland legacy personal sampling pump (SKC, Inc.). Personal samplers were attached to a backpack at breathing height, while area-based measurement samplers were attached to a custom sampling board at 1.2–2.7 m, based on logistical constraints. Pumps were warmed up for 15–20 min prior to use. Airflow was measured pre- and post-sampling using a Defender-series electronic piston volumetric gas flow meter (Bios International, Butler, N.J.). Samples were stored at –20°C between collection and equilibration for post sample weighing.

Gravimetric Analysis

Filters were pre- and post-weighed using a Cabh = 35 Micro Balance (Thermo Fisher Scientific, Inc., Waltham, Mass.). Filters were equilibrated for 24–48 hr in temperature- and humidity-controlled cleanroom prior to being pre and post weighed at least two times. The microbalance was calibrated

at the beginning of each weighing session for accuracy, and a quality check was performed after every tenth filter to account for fluctuation. Any shift of ± 0.002 mg was cause for reweighing of the 10 previous filters.

Biological Analysis

Following the method previously outlined by Saito et al.[16] and Thorne et al.,[32] $PM_{2.5}$ and inhalable PM samples were extracted from the filters by vortexing filters in a TWEEN (10 mL at 0.05%) solution with pyrogen-free water for 1 hr at 20–22°C. The solution containing the samples was then split into two aliquots. One aliquot was analyzed for biologically active endotoxin using the rFC assay (Lonza PyroGene), which detects activation of factor C by utilizing a fluorogenic substrate. The samples, 100 μL of blank, and endotoxin standard (*Escherichia coli* 055:B5) were added to a 96 well plate. The plates were then pre-incubated for 10 min at 37°C. A mixture of 100 μL of rFC enzyme solution, buffer, and fluorogenic substrate at a 1:4:5 ratios were then added. The plates were incubated for 1 hr at 37°C. Fluorescence (excitation 380 nm, emission 440 nm) was read at 0 and 1 hr in a fluorescence microtiter plate reader (Biotek Instruments, Winooski, Vt.). The "blank plate" reading was subtracted from the 1-hr reading. Values were corrected using the average of three blank sample wells. The log endotoxin concentration (EU/mL) was plotted against the log difference in fluorescence (RFU) in a linear regression curve to obtain endotoxin concentration. Linear axes and a second order polynomial regression curve were used for the standards. Blanks taken in the field and plate well blanks along with spiking assays were used for quality control in order to account for field contaminates and lab factors affecting fluorescence, such as pyrogen-free water, reagent water, centrifuge tubes, pipette tips, and microplates. Dilution of some samples was necessary; in these cases a fifty-fold dilution was performed.

Chemical Analysis (GC-MS/MS)

The chemical analysis method used has been detailed previously.[25] Samples were extracted as in the rFC method, and aliquots were stored in the freezer at –80°C prior to chemical analysis. The samples were lyophilized at –50°C in preparation of chemical analysis. Standards (Matreya LLC, Pleasant Gap, Pa.) of 3-OHFAs with carbon chain lengths of C_8–C_{10} and C_{12}–C_{18} were analyzed at the following concentrations: 0, 1, 2, 5, 20, 100, and 500 ng. The samples and standard solutions were spiked with a surrogate of C_{11} at 25 ng. This particular chain length was employed because previous studies have shown an absence of C_{11} in agricultural PM.[16] The spiked samples and standards were digested in methanolic HCl overnight at 85°C. This process allows for breakdown of the ester and amide bonded 3-OHFAs and formation of 3-hydroxy fatty acid methyl esters (3-OH FAMEs) through an acid catalyzed esterification reaction. Samples were subsequently diluted with 1 mL of pyrogen-free water and spiked with 10 μL of 100 μg/mL pentadecanol as a keeper solvent. Solid phase extraction (SPE) of the 3-OH FAMEs was conducted using Strata-X 60 mg/3 mL polymeric reversed phase columns (Phenomenex, Torrance, Calif.). The columns were conditioned with 1 mL of diethyl ether and 1 mL of pyrogen-free water. After the samples were loaded, they were pulled drop-wise under vacuum for 20 min. The 3-OH FAMEs were eluted from the column using diethyl ether and dried under nitrogen. The sample was then derivatized using 50 μL BSTFA/1% TMCS and 5 μL pyridine at 85°C for 30 min to form trimethylsilyl esters at the 3-OH position. Cooled samples were diluted using heptane to a volume of 100 μL.

Derivatized 3-OH FAMEs were separated using gas chromatography (Quattro Micro GC; Waters Corp., Milford, Mass.). A DB-5 ms capillary column (30 mm × 0.25 mm I.D., 0.25 μm film thickness; J&W Scientific, Folsom, Calif.) was used. An autosampler (7683B; Agilent, Santa Clara, Calif.) set in the splitless mode was used for injecting samples with an inlet temperature of 280°C. Oven temperature was ramped up from 90 to 250°C at 5°C/min and from 250 to 290°C at 20°C/min, finally holding at 290°C for 5 min. The interface temperature with the detector was set to 300°C. The carrier gas utilized during analysis was ultra-high purity helium at constant flow of 1 mL/min.

The GC was coupled with a mass spectrometer with tandem detection (Quattro Micro GC-MS/MS; Waters Corp.). The detector mode was multiple reactions monitoring (MRM) with fragment ions formed using 10eV of collision energy. The two MRM transitions, quantitation and confirmation, retention time frames were: C_8 m/z 231>189, 131; C_9 m/z 245>203, 131; C_{10} m/z 259.02>217, 131; C_{11} m/z 273>241, 131; C_{12} m/z 287>255, 131; C_{13} m/z 301>269, 131; C_{14} m/z 315>283, 131; C_{15} m/z 329>297, 131; C_{16} m/z 343>311, 131; C_{17} m/z 357>325, 131; and C_{18} m/z 371>339, 131. The limit of detection (LOD) and limit of quantification were 0.5 ng and 1 ng, respectively, as determined using a signal to noise ratio with a three- and six-fold increase.

Data Analysis

The unit of analysis for our multilevel data was the day- and dairy location-specific record of measurements collected from sampled dairy farms for area-based measurements and daily exposure value per worker. Summary statistics and graphical methods were used to describe the distribution of endotoxin concentrations by location on the dairy. Potential outliers were identified in box plots as those falling more than 1.5 interquartile ranges below the 25th (above the 75th) quartile. Concentrations were log transformed for regression analysis. Pearson correlation coefficients were calculated between various concentration measures of endotoxin. The mean proportion of each chain length (C_8-C_{18}) concentration for 3-OHFAs was used to evaluate chain length differences between locations.

For each outcome, separate mixed-effects (multilevel) regression models were used to assess whether mean log-transformed PM concentrations varied between locations or with variation in other measured within-dairy or between-dairy characteristics. All specific locations (i.e., milking parlor,

freestall barn) were compared with the central location as the reference location using binary indicator variables. In addition to the location factor, regression models included between-dairy and within-dairy covariates, with the selection of candidate covariates based on expert judgment. Candidate covariates with insufficient variation in our sample were removed from further consideration. The final selection of covariates to include in models relied on empirically comparing candidate models based on Akaike and Bayesian information criteria statistics to protect model selection against over-fitting bias. A restricted maximum likelihood setting was used in the final model. Candidate within-dairy covariates included daily meteorological measurements (relative humidity, ambient temperature, soil temperature, and wind speed) and time of day variables. Dairy facility level covariates were facility age; number of freestall cows (lactating cows); number of dry lot animals (heifers and non-lactating cows); if heifers or calves were kept on adjoining lands; number of milking cows in the milking parlor at any one time; frequency of milkings; acreage of dairy; type of waste handling (i.e., lagoon storage, compost, off-site disposal); frequency of flushing freestall barn lanes; frequency of manure removal; and use of dust suppression. While calves are housed in separate individual pens, their numbers were accounted for under the freestall covariate because calf hutch housing is more closely related to the concrete floor housing in the freestall barns than the dirt floored dry lot corrals. Any chain length measurement below the limit of detection was assigned a value of LOD divided by the square root of 2. Statistical analysis was performed using SAS version 9.2 (SAS institute, Cary, N.C.).

RESULTS

Area-based inhalable PM samples collected in the dry lot corrals had the greatest sample mean concentration of biologically active endotoxin relative to other locations (Figure 1). Personal exposure samples collected from individuals engaged in rebedding had the greatest sample mean concentrations for biologically active endotoxin (Figure 1). Only those workers who spent greater than 75% of their shift performing one task were included in the boxplots. Personal samples collected from workers engaged in feeding had the lowest sample mean concentration (Figure 1). Arithmetic mean personal endotoxin exposure concentrations in the inhalable PM size fraction (biologically active = 453 EU/m^3, N = 225; total endotoxin = 1682 pmol/m^3, N = 191) were approximately 1.7 times higher than the arithmetic mean of area-based concentrations (biologically active = 261 EU/m^3, N = 113; total endotoxin = 862 pmol/m^3, N = 73). For biologically active endotoxin (EU/m^3) and total endotoxin (pmol/m^3) that was associated with $PM_{2.5}$, the two areas that had the highest sample mean concentrations were the grain storage areas and dry lot corrals (Table I). Areas with the highest biologically active and total endotoxin sample mean concentrations for the inhalable PM size fraction were the dry lot corrals and central location, demonstrating different trends by PM size.

Correlations

We calculated Spearman correlation coefficients between a variety of mass and endotoxin measures, specifically:

1. Area-based $PM_{2.5}$ (inhalable PM) mass concentrations and biologically active endotoxin (Table II).
2. Area-based $PM_{2.5}$ (inhalable PM) mass concentrations and total endotoxin (Table II).
3. Biologically active endotoxin and total endotoxin (area-based $PM_{2.5}$ and inhalable PM, personal inhalable PM size fractions) (Table III).
4. Endotoxin associated with $PM_{2.5}$ and inhalable PM (biologically active and total endotoxin) (Table III).

The correlation between $PM_{2.5}$ and both biologically active and total endotoxin was weak (defined by correlation coefficients <0.40) (Table II). In contrast, there was a moderate (defined by correlation coefficients >0.60) correlation between area-based and personal exposure inhalable PM concentrations, and both biologically active and total endotoxin. This indicates that $PM_{2.5}$ mass concentration cannot be used to forecast endotoxin concentrations but that inhalable PM mass concentrations are somewhat predictive. Correlation between biologically active endotoxin in $PM_{2.5}$ and biologically active endotoxin in inhalable PM (EU/m^3) was strong (Table III), demonstrating that knowing the biologically active endotoxin concentration in one size-fraction can be somewhat predictive of the concentration in another size-fraction. In contrast, no correlation was found between total endotoxin in the two size-fractions. This may be partially explained by examining the correlation between biologically active and total endotoxin in the two size-fractions. In the $PM_{2.5}$ size fraction, the correlation was not significant between the two methods, while in the area-based inhalable PM size fraction there was a strong association ($r = 0.76$, $P < 0.05$, $N = 73$) between the two methods. The correlation for personal inhalable PM exposure ($r = 0.67$, $P<0.05$, $N = 191$) was also strong between the two methods.

The endotoxin concentrations per particle mass were also calculated for area-based samples ($PM_{2.5}$/inhalable PM (EU/mg), Table III). The arithmetic mean concentration for biologically active endotoxin was 170 EU/mg (N = 51) in $PM_{2.5}$ and 579 EU/mg (N = 113) in the inhalable PM size fraction. For total endotoxin, the concentration was 2655 pmol/mg (N = 51) for $PM_{2.5}$ and 1800 pmol/mg (N = 73) for inhalable PM. Based on these mean values, the concentration of biologically active endotoxin in the $PM_{2.5}$ size fraction was 29% of that found in inhalable PM; however, it was 148% for total endotoxin, indicating that larger particles are more enriched for biologically active endotoxin than for total endotoxin by mass.

3-OHFA Chain Lengths

To identify any differences in 3-OHFA chain length profile of endotoxin LPS, the mean proportion of each 3-OHFA chain length in the inhalable PM size fraction was determined for

TABLE I. Total Endotoxin (3-OHFAs) and Biologically Active Endotoxin (rFC) by Location Within Dairies

	$PM_{2.5}$										Inhalable PM									
	3-OHFA (pmol/m^3)					rFC (EU/m^3)					3-OHFA (pmol/m^3)					rFC (EU/m^3)				
	N	Mean	Median	STD	90th	N	Mean	Median	STD	90th	N	Mean	Median	STD	90th	N	Mean	Median	STD	90th
Freestall barns	12	56	57	16	75	12	3.5	1.7	3.5	6.3	16	602	610	409	1421	17	246	189	188	567
Dry lot corrals	4	70	74	14	82	4	8.6	8.5	2.5	12	5	1343	705	1326	3517	5	866	763	729	2100
Milking parlors	19	64	61	20	103	19	6.1	3.9	8.5	16	24	1035	644	941	2725	29	229	180	191	465
Upwind	0					0					1		586			5	15	16	3	19
Downwind	0					0					0					7	251	93	402	1150
Central	2	67				2	13				13	1267	542	2686	882	29	321	308	283	640
Calf hutches	4	59	60	8	68	4	2.3	2.4	0.75	3.1	4	148	146	43	202	4	61	61	30	96
Grain storage	5	80	67	43	148	5	3.8	2.2	4.8	12	5	445	558	310	791	5	220	114	229	515
Maternity	4	57	44	26	96	4	0.72	0.63	0.63	1.6	4	341	248	312	788	5	67	64	37	108
Hospitals	1		66			1		3.9			1		497			5	237	273	118	379

Note: Samples from other locations (N = 2) where excluded from the table due to not fitting in any of the established locations.

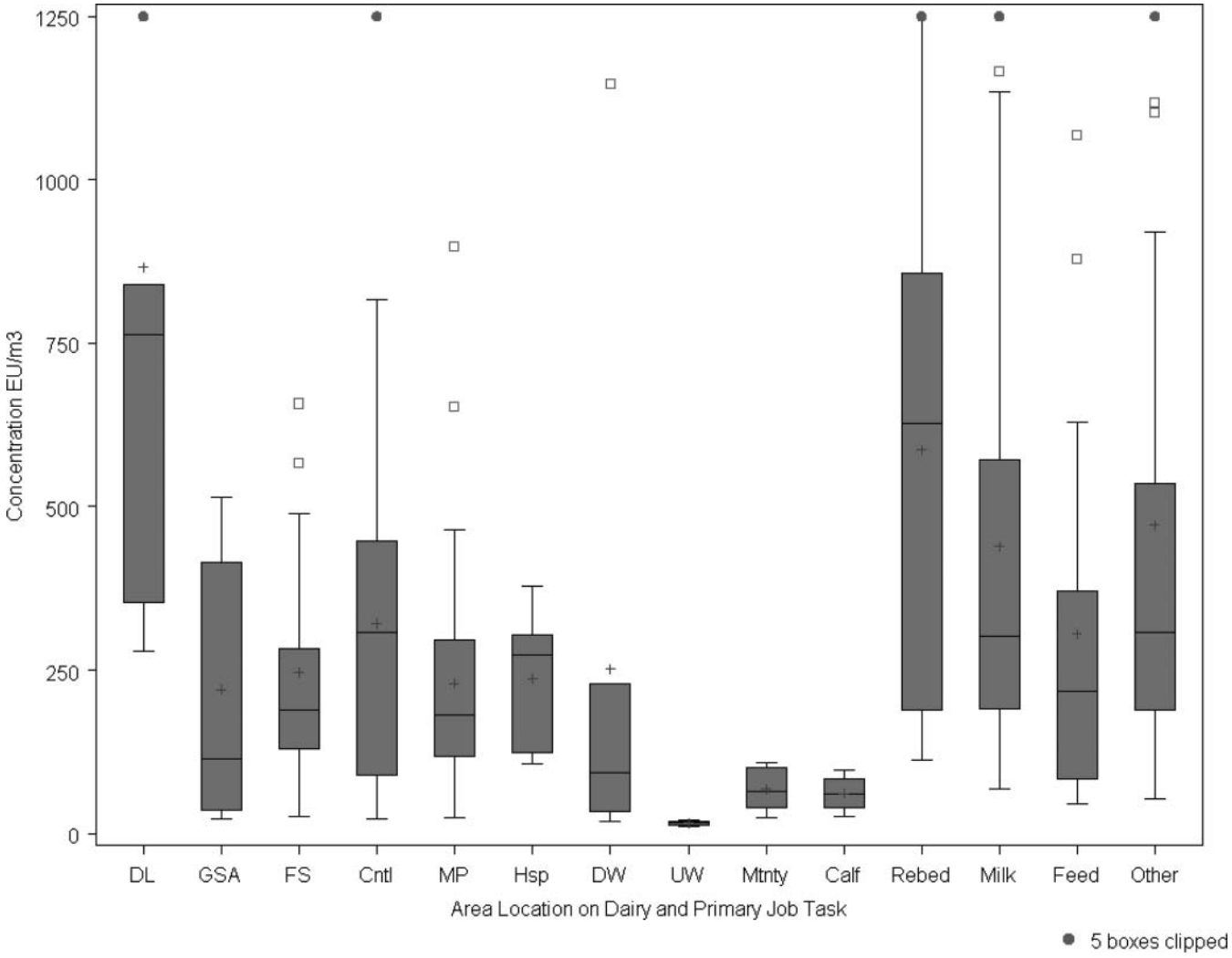

FIGURE 1. Distributions for biologically active endotoxin (rFC) in inhalable PM size particulate matter across locations within the dairy and primary job tasks within the dairy. Only those workers who performed one job task for 75% or more of their work shifts were included. Boxplots represent the maximum, minimum, 75th percentile, 25th percentile, and median values. The mean value is represented by (+) symbol, while outliers (represented by small square) are defined by values that are 1.5× interquartile ranges above the 75th and below the 25th percentile. Boxes clipped are those boxplots that have values excluded for scale purposes. Locations and tasks abbreviated as follows: dry lot corral (DL N = 5), grain storage area (GSA N = 5), freestall barns (FS N = 17), central (Cntl N = 29), milking parlor (MP N = 29), hospital (Hsp N = 5), downwind (DW N = 7), upwind (UW N = 5), maternity (Mtnty N = 5), calf hutches (Calf N = 4), rebedding (Rebed N = 9), milking (Milk N = 111), feed (Feed N = 18), and Other (N = 46).

each location and job task (Figure 2). Not all chain lengths were measurable in all samples. The grain storage and calf hutch locations, along with the job task of feeding, had different chain length profiles compared with other locations and tasks; there was a trend toward a greater contribution from C_8–C_{12} and a lesser contribution from longer chain lengths C_{13}–C_{18}. In the calf hutch location, C_8 was 7% of the total 3-OHFA chain length profile and in the grain storage area C_8 was 11%. In other locations, C_8 was between 2 and 4% of the total chain length profile. Similar to C_8, C_{12} was also highest in the calf hutch (25%) and grain storage (17%) areas. In other locations, C_{12} was approximately 7–9% of the total chain length profile.

Previous studies[16] have shown that correlations exist between even chain lengths and biologically active endotoxin. We calculated Pearson correlation coefficients between the different 3-OHFA chain lengths, mass, and biologically active endotoxin for samples collected from personal and area-based samplers in the inhalable PM size fraction. Strong correlation was found among all of the longer chain lengths, C_{13}–C_{18}, for both personal and area-based samples, as can be seen in Table IV. In addition, we found correlation between biologically active endotoxin and chain lengths C_{10}–C_{18} for both personal and area-based measures (Table IV), with the strongest correlation for C_{13} ($r = 0.71$, $P<0.0001$, $N = 191$) for personal samples, and C_{15} ($r = 0.75$, $P<0.0001$, $N = 72$), C_{16} ($r = 0.73$,

TABLE II. Spearman Correlation Coefficients Between Both $PM_{2.5}$ and Inhalable PM Mass and Both Biologically Active and Total Endotoxin

	N	Area Based	N	Personal Exposure
$PM_{2.5}$ ($\mu g/m^3$)/rFC (EU/filter)	51	0.24	—	—
$PM_{2.5}$ ($\mu g/m^3$)/3-OHFAs (pmol/filter)	51	–0.14	—	—
IPM ($\mu g/m^3$)/rFC (EU/filter)	113	**0.79**	225	**0.66**
IPM ($\mu g/m^3$)/3-OHFAs (pmol/filter)	73	**0.73**	191	**0.70**

Notes: $p < 0.05$ in bold. IPM, inhalable PM.

TABLE III. Spearman Correlation Coefficients Between Size Fraction and Between Biologically Active and Total Endotoxin (Inhalable PM)

	N	$PM_{2.5}$/IPM (EU/m^3)	$PM_{2.5}$/IPM (EU/mg)
Biologically active (rFC)	50	0.64	0.54
	N	$PM_{2.5}$/IPM (pmol/m3)	$PM_{2.5}$/IPM (pmol/mg)
Total endotoxin (3-OHFA)	49	−0.17	−0.007
	N	rFC (EU/m^3)/3-OHFA (pmol/m^3)	rFC (EU/mg)/3-OHFA (pmol/mg)
Area based (PM2.5)	51	0.23	0.33
Area based (IPM)	73	0.76	0.49
Personal exposure (IPM)	191	0.67	0.40

Notes: $p < 0.05$ in bold. IPM, inhalable PM.

P<0.0001, N = 191) and C_{17} (r = 0.73, P<0.0001, N = 191) for area-based samples. Also observed was a good correlation between particle mass and numerous chain lengths, as can be observed in Table IV.

The arithmetic mean concentration for the sum of even chain lengths was at least two times that for the sum of the odd chain lengths. The Spearman correlation coefficient between inhalable PM concentration and even chain lengths (r = 0.71, P < 0.001, N = 69) was slightly higher than that between odd chain lengths (r = 0.63, P < 0.001, N = 71). The Spearman correlation coefficient between $PM_{2.5}$ concentration and even chain lengths was much weaker (r = 0.25, P = 0.04, N = 68), and there was a negative correlation coefficient with odd chain lengths (r = −0.31, P = 0.03, N = 51), most likely due to many chain lengths in the $PM_{2.5}$ size fraction being below the detection limit. The Spearman correlation coefficient between biologically active endotoxin and even (odd) chain lengths was similar. C_{12} was the dominant chain

TABLE IV. Pearson Correlation Coefficients Between Inhalable PM Mass, Biologically Active Endotoxin and 3-OHFA Chain Lengths for Both Personal (top half) and Area-Based (bottom half) Samples

		rFC	IPM	C_8	C_9	C_{10}	C_{12}	C_{13}	C_{14}	C_{15}	C_{16}	C_{17}	C_{18}	
A	rFC		**0.66**	0.37	0.28	0.58	0.47	**0.71**	0.49	0.55	0.52	0.59	0.51	P
R	IPM	0.77		0.47	0.38	**0.65**	0.48	**0.65**	0.44	0.56	0.46	0.53	0.52	E
E	C_8		0.29		**0.83**	**0.70**	0.25	0.46	0.19	0.33	0.30	0.29	0.31	R
A	C_9		0.23	0.87		**0.74**	0.40	0.40	0.27	0.26	0.23	0.20	0.20	S
	C_{10}	0.55	0.65	0.66	0.73		**0.60**	**0.60**	0.42	0.47	0.43	0.45	0.45	O
	C_{12}	0.59	0.63	0.38	0.52	**0.75**		0.55	**0.65**	0.51	0.53	0.54	0.52	N
	C_{13}	0.64	0.65	0.44	0.45	0.69	0.64		**0.70**	**0.80**	**0.77**	**0.81**	**0.74**	A
	C_{14}	0.69	0.60	0.36	0.41	0.57	0.69	0.72		**0.68**	**0.72**	**0.74**	**0.63**	L
	C_{15}	**0.75**	0.67	0.32	0.41	0.61	0.72	**0.85**	**0.82**		**0.81**	**0.87**	**0.77**	
	C_{16}	0.73	0.63	0.25	0.30	0.51	0.58	**0.80**	**0.76**	**0.87**		**0.92**	**0.93**	
	C_{17}	0.73	0.64	0.27	0.30	0.51	0.57	**0.80**	0.73	**0.91**	**0.89**		**0.92**	
	C_{18}	0.65	0.64	0.35	0.27	0.46	0.45	0.72	0.59	**0.78**	**0.85**	**0.87**		

Notes: Bold values represent the top 25% of coefficients. Personal coefficients are on the top half of the table, and area-based coefficients are on the bottom half. Only those pairs with correlation coefficients with a $P < 0.05$ are shown.

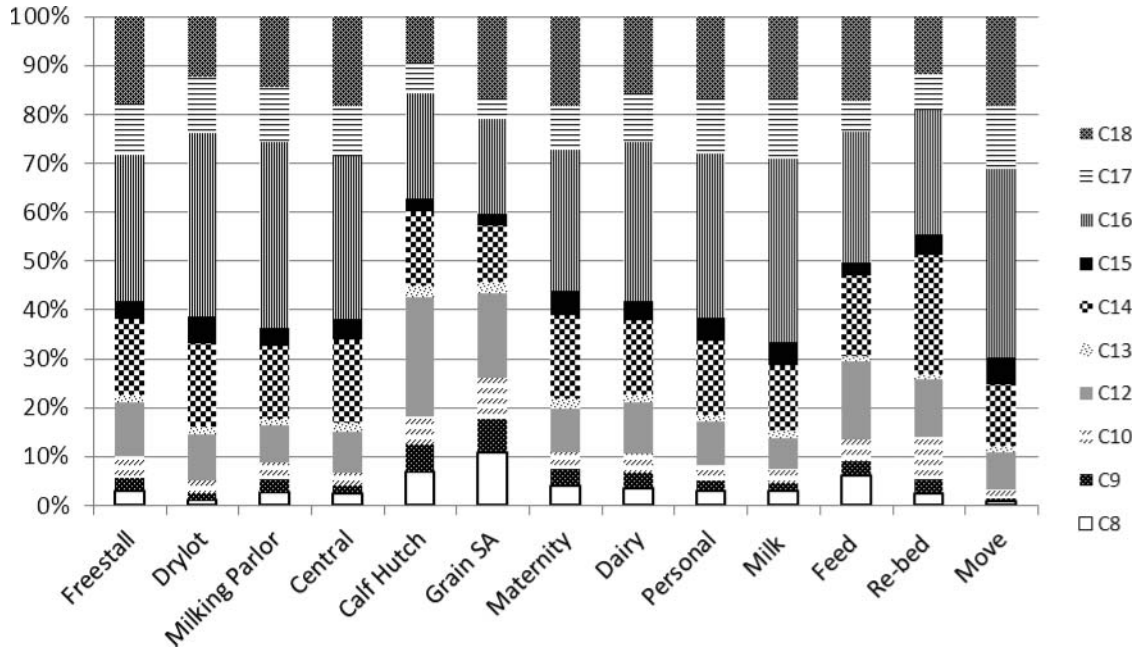

FIGURE 2. 3-OHFA chain length, C_8-C_{18}, profiles for area locations and personal tasks within 13 dairies. Locations are freestall barns (Freestall N = 15-16), drylot corral (Drylot N = 5), milking parlor (N = 22-24), central (N = 12-13), calf hutch (N = 4), grain storage area (Grain SA N = 5), maternity (N = 4), and all locations (Dairy, N = 69-73, inclusive of all previous listed areas as well as other area samples that did not fall into one of these categories). Personal exposure categories included milkers (Milk N = 69-78), feeding (Feed N = 10-15), rebedding (Re-bed N = 5), those who moved animals (Move N = 7-9), and all personal samples (Personal N = 165–191 includes those in categories listed, plus all others that did not fall into one of these categories). Values calculated from picomoles in inhalable PM size fraction. N is variable due to missing values for some chain lengths used to calculate the percentage, with C8 accounting for most missing values.

length for $PM_{2.5}$, while the dominant chain length was C_{16} for inhalable PM. This difference is likely due to regional background sources of $PM_{2.5}$ influencing the chain length profile in this size fraction[33] (Figure 3).

Regression Models

The mixed-effects models for biologically active and total endotoxin in the inhalable PM size fraction from area-based samplers had similar dairy and day level covariates (Table V), with the biologically active endotoxin model used to determine the covariates. For biologically active endotoxin, wind speed, relative humidity, flushing frequency, and whether composting was performed on the dairy were the variables that best explained variation in the outcome of log-transformed biologically active endotoxin concentrations (EU/m^3). The regression model with biologically active endotoxin concentration as an outcome confirmed that the dry lot corrals had significantly (GM ratio 2.5, P = 0.02) higher endotoxin concentrations, whereas the calf hutch and maternity locations had lower endotoxin concentrations relative to the central location. Using regression model estimate values to compare upwind and downwind concentrations, we found that downwind had significantly higher (P < 0.001) biologically active endotoxin concentrations relative to the upwind dairy location (Table V). For total endotoxin, two covariates had a significant impact on concentrations; there were higher concentrations when composting (P < 0.01) was conducted and lower concentrations with increased flushing (P = 0.04) (Table V). The outcome demonstrated that there were significantly lower (GM ratio 0.3, P = 0.01) total endotoxin concentrations in the calf hutches compared with the central location. We note that there was a larger sample size (N = 113 vs. 73) for biologically active endotoxin, which might explain why more significant differences were found for biologically active endotoxin compared to total endotoxin.

DISCUSSION AND CONCLUSION

In the present study the personal geometric sample mean concentrations in the inhalable PM size fraction for biologically active endotoxin (334 EU/m^3 N = 225 for rFC) was half that for personal exposure concentrations for dairy workers in Colorado and Nebraska, whereas the concentrations were similar for total endotoxin (1178 pmol/m^3 STD = 2.5 N = 191 for 3-OHFAs).[16] Noteworthy is that while the sample means were similar for total endotoxin, the Colorado/Nebraska study excluded C16, which we found to be the most prevalent chain length in the inhalable PM size fraction. When we compared our area-based concentrations with the personal concentrations in the Colorado/Nebraska study, we found that the geometric sample mean for our area-based total endotoxin concentrations (60 pmol/m^3 STD = 1.4 N = 51 for $PM_{2.5}$ and 515 pmol/m^3 STD = 2.6 N = 73 for inhalable PM) were approximately 2.5 times lower. Concentrations for the area-based biologically

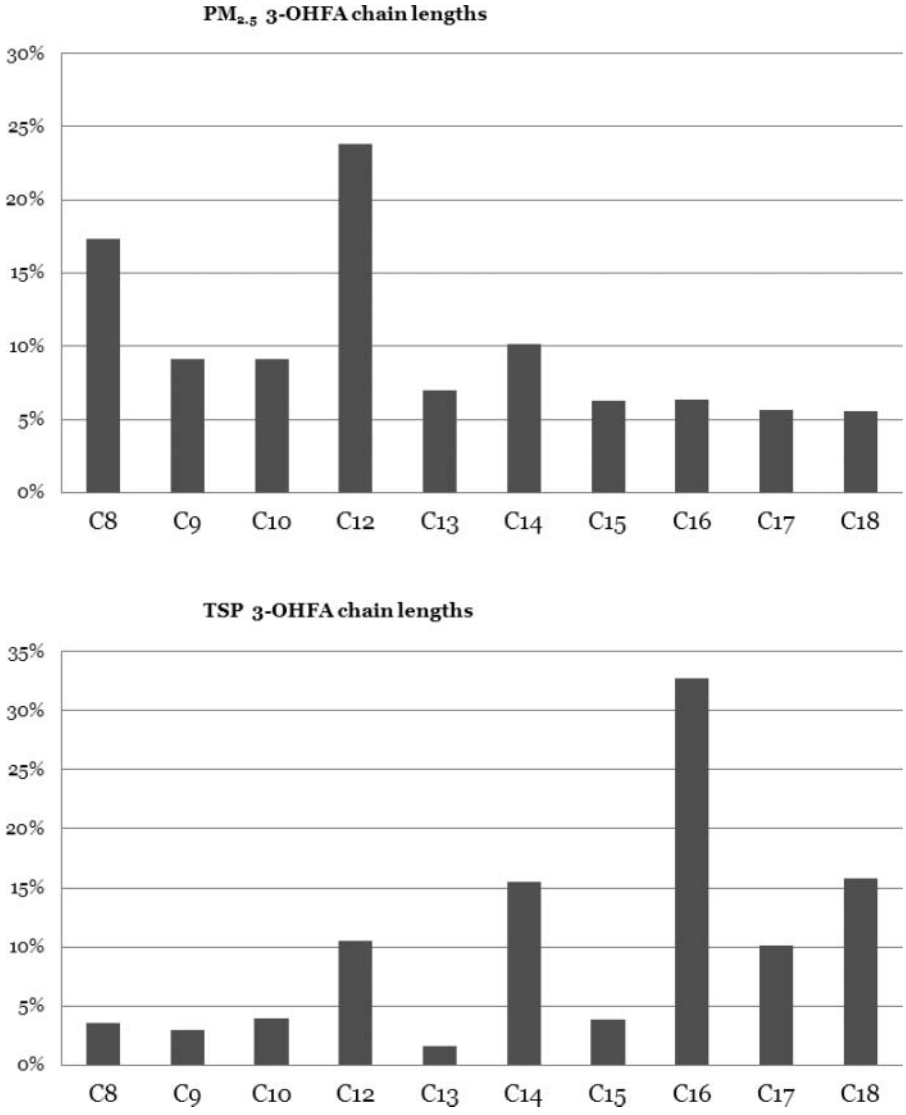

FIGURE 3. Percentage of 3-OHFAs by chain length in $PM_{2.5}$ (4.3a) and inhalable PM (4.3b) size fractions (area based measurements)

active endotoxin in the inhalable PM size fraction (2.9 EU/m^3 N = 51 for $PM_{2.5}$ and 149 EU/m^3 N = 113 for inhalable PM) in our study were five times lower.[16] The measurements in Colorado and Nebraska encompassed all four seasons, with more closed facilities and higher concentrations in colder months.

A study conducted in Wisconsin of both area-based and personal measured inhalable and respirable biologically active endotoxin reported (inhalable) geometric mean endotoxin concentrations (EU/m^3) that were twice the geometric mean concentrations that we found for personal measures and were 4.3 times higher than the concentrations that we found for area-based measures in the inhalable PM size fraction.[3] Comparing the respirable geometric mean in the Wisconsin study with the geometric mean concentration for endotoxin in the $PM_{2.5}$ size fraction (area-based), we found that our geometric mean value was five times lower. Similar concentrations to those that we found were reported from a study in Idaho looking at total biologically active endotoxin.[34] A different study also conducted in Colorado and Nebraska that investigated total endotoxin exposure by dairy workers reported a geometric mean concentration that was similar to what we found for personal exposure.[5]

While our observed concentrations appear lower in most cases than those reported in previous studies, different samplers were utilized across studies, which may have impacted measured concentrations. In addition, some studies we cite, specifically, Dungan and Leytom[34] and Kullman et al.,[3] have utilized the Limulus amoebocyte lysate (LAL), which is known to have variation,[35] whereas we have used the recombinant factor C assay.

Concentrations of endotoxin collected in another study[25] as settled PM from dairy farms were similar to the mass normalized endotoxin concentrations (EU/mg) from our personal samplers of total PM (481 EU/mg N = 225) and lower than what we found for area-based concentrations (579 EU/mg

TABLE V. Mixed-Effects Model Outcome for Total Endotoxin (3-OHFAs, N = 73) and Biologically Active Endotoxin (rFC, N = 113) Using Dairy, Day, and Location Covariates

	Biologically Active Endotoxin (rFC)				Total Endotoxin (3-OHFA)			
	GM Ratio	**p-value**	**Lower**	**Upper**	**GM Ratio**	**p-value**	**Lower**	**Upper**
Dairy covariates								
Wind speed	0.85	**0.03**	0.7	1.0	0.97	0.64	0.8	1.1
Relative humidity	0.97	< **0.01**	0.9	1.0	1.00	0.81	0.98	1.03
Flushing frequency	0.5	**0.01**	0.3	0.8	0.6	**0.04**	0.4	0.98
Compost	3.2	< **0.001**	2.0	4.9	2.0	< **0.01**	1.3	3.1
Locations								
Dry lot corral	2.5	**0.02**	1.1	5.4	1.5	0.34	0.6	3.6
Grain storage area	0.6	0.17	0.3	1.3	0.6	0.24	0.3	1.4
Freestall	1.0	0.99	0.6	1.6	0.8	0.43	0.4	1.5
Milking parlor	0.9	0.71	0.6	1.4	1.2	0.59	0.7	2.1
Calf hutches	0.3	**0.01**	0.1	0.8	0.3	**0.01**	0.1	0.8
Hospital	0.9	0.88	0.4	2.1	0.5	0.45	0.1	3.0
Maternity	0.4	**0.04**	0.2	1.0	0.5	0.11	0.2	1.2
Upwind	0.05	<**.0001**	0.02	0.1	0.5	0.49	0.1	3.1
Downwind	0.44	**0.01**	0.2	1.0	.	.	.	.
Estimate								
Freestall vs. dry lot	0.4	**0.03**			0.5	0.13		
GSA vs. freestall barns	1.7	0.19			1.3	0.53		
GSA vs. dry lot	4.3	<**.01**			2.5	0.08		
Upwind vs. downwind	0.1	<**.0001**			.	.	.	.

Note: P < 0.05 in bold.

N = 113 for inhalable PM and 170 EU/mg N = 51 for $PM_{2.5}$).

When comparing the dairy endotoxin concentrations with urban and rural background levels for Fresno and Southern California,[36,37] we found that the dairy concentrations were considerable higher, signifying that the dairies are likely sources for endotoxin.

Within the dairy, rebedding was the job task that was associated with the highest concentrations of endotoxin,[33] while for area-based measurements the dry lot corral was the location on the dairy that had the greatest mean concentration. In addition, though levels of endotoxin observed in our study were lower than some values observed in other studies mentioned previously, the levels observed where higher than those recommended. The National Health Council of the Netherlands has recommended a threshold value of 90 EU/m^3.[17] Based on these recommendations it may be necessary to perform future studies looking at how to reduce exposure, potentially by wearing respiratory protection or other intervention, while performing job tasks that lead to higher levels of endotoxin.[33] Based on the correlation between PM and endotoxin and the separate analysis of PM,[33] we see that tasks and locations that have elevated PM also tend to have elevated levels of biologically active endotoxin for both personal and area-based measurements.

We found that correlations between both 3-OHFA chain lengths (C_8-C_{18}) and biologically active endotoxin for personal and area-based measurements were similar to those seen in previous studies.[16] While correlations were observed in both even and odd numbered 3-OHFA chain lengths, the concentrations for the even chain lengths were greater. The present study demonstrated that a third of the total endotoxin comprised the C_{16} 3-OHFA chain length in the inhalable PM size fraction. Previous studies have excluded the C_{16} chain length.[16] The present work also revealed a different chain length profile by size fraction, with the most predominant chain length for endotoxin in the $PM_{2.5}$ size fraction being C_{12}, whereas for the inhalable PM size fraction it was C_{16}.

We found that different locations on dairies have distinct profiles due to chain length proportional differences likely because of the different bacterial sources. Other studies have shown that distinct 3-OHFA chain lengths predominate in specific bacterial species.[38] Two areas that differed, the calf hutches and grain storage areas, would be expected to have different sources of bacteria. The calf hutches house young animals, and their waste differs from older animals based on different animal digestive physiology.[39] Feed is stored in the grain storage, and thus, the source of 3-OHFAs is primarily non-waste-derived bacteria.

Mixed-effect regression models illustrated that dairy and day level variation for biologically active endotoxin was explained by wind speed and relative humidity. This finding is consistent with previous studies that have shown association with these variables.[34,40] In addition, the more frequently

the freestall within the dairy is flushed, the lower the endotoxin concentration, as waste products are removed more often, resulting in a decrease in accumulated waste that could contribute to endotoxin. Composting on the dairies increased the endotoxin concentration by as much as 200%. Composting involves an aerobic microbial processes that might lead to this increase in endotoxin, as studies have shown that *E. coli* 0157: H-7[41] and coliform,[42] both of which are gram-negative bacteria, can multiply in dairy waste during this process. However, a cause and effect relationship between this process and airborne endotoxin concentrations has not been verified. The model using total endotoxin as an outcome in comparison with the abovementioned model looking at biologically active endotoxin followed the same trends outlined above.

By analyzing both biologically active and total endotoxin using two distinct analysis methods in two-size fractions, we were able to determine associations between PM size and analysis method. Smaller size fractions contained less biologically active endotoxin than larger size fractions, yet we observed the inverse relationship for total endotoxin concentrations by mass. The chain length profiles differed by location, which may be relevant as distinct chain lengths may have different impacts on human health. We see that the relationship between total and biologically active endotoxin varies between the two PM size fractions. This is potentially important to consider when designing health studies as it is not yet clear whether biologically active, total, or specific 3-OHFA chain lengths drive health impacts. The present work did not explain the relative impact of endotoxin associated with PM size fraction; the smaller size is more likely to penetrate deeply into the respiratory system, but the larger particles seem to have longer chain lengths (C_{16}) associated with them. The differences observed between size fractions and different analysis methods demonstrate a need for further in-depth study of bacterial endotoxin by size fraction and chemical composition. Follow-up physiological experiments would prove beneficial for further understanding chain length-related health effects.

In conclusion, we found that endotoxin is elevated in specific locations (dry lot corral) and while performing specific tasks (rebedding), 3-OHFA profiles differ across locations within the dairy, and a correlation exists between biologically active endotoxin in $PM_{2.5}$ and inhalable PM, but not for total endotoxin.

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REFERENCES

1. **Ad Hoc Committee of the Assembly on Environmental and Occupational Health, American Thoracic Society:** Respiratory Health Hazards in Agriculture. Conference report. *Am. J. Respir. Crit. Care Med. 158*(*5*), *Supplement. Part 2*. 1998.
2. **Rietschel, E.T., T. Kirikae, F.U. Schade, et al.:** Bacterial, endotoxin-molecular relationships of structure to activity and function. *FASEB J. 8*:217–225 (1994).
3. **Kullman, G.J., P.S. Thorne, P.F. Waldron, et al.:** Organic dust exposures from work in dairy barns. *Am. Ind. Hyg. Assoc. J. 59*:403–413 (1998).
4. **Liebers, V., T. Bruning, M. Raulf-Heimsoth:** Occupational endotoxin-exposure and possible health effects on humans. *Am. J. Ind. Med. 49*:474–491 (2006).
5. **Burch, J.B., E. Svendsen, P.D. Siegel, et al.:** Endotoxin exposure and inflammation markers among agricultural workers in Colorado and Nebraska. *J. Toxicol. Environ. Health A. 73*:5–22 (2010).
6. **Reynolds, S.J., K.J. Donham, P. Whitten, J.A. Merchant, L.F. Burmeister, and W.J. Popendorf:** Longitudinal evaluation of dose-response relationships for environmental exposures and pulmonary function in swine production workers. *Am. J. Ind. Med. 29*:33–40 (1996).
7. **Donham, K.J., D. Cumro, S.J. Reynolds, and J.A. Merchant:** Dose-response relationships between occupational aerosol exposures and cross-shift declines of lung function in poultry workers: Recommendations for exposure limits. *J. Occup. Environ. Med. 42*:260–269 (2000).
8. **Kirychuk, S.P., J.A. Dosman, S.J. Reynolds, et al.:** Total dust and endotoxin in poultry operations: Comparison between cage and floor housing and respiratory effects in workers. *J. Occup. Environ. Med. 48*:741–748 (2006).
9. **Schenker, M.B., J.A. Farrar, D.C. Mitchell, et al.:** Agricultural dust exposure and respiratory symptoms among California farm operators. *J. Occup. Environ. Med. 47*:1157–1166 (2005).
10. **Vogelzang, P.F.J., J.W.J. Van Der Gulden, H. Folgering, and C.P. Van Schayck:** Organic dust toxic syndrome in swine confinement farming. *Am. J. Ind. Med. 35*:332–334 (1999).
11. **Smit, L.A.M., I.M. Wouters, M.M. Hobo, W. Eduard, G. Doekes, and D. Heederik:** Agricultural seed dust as a potential cause of organic dust toxic syndrome. *Occup. Environ. Med. 63*:59–67 (2006).
12. **Gaint, M., I. Thaon, V. Westeel, et al.:** Twelve-year longitudinal study of respiratory status in dairy farmers. *Eur. Respir. J. 30*:97–103 (2007).
13. **Reynolds, S.J., J. Nakatsu, M. Tillery, et al.:** Field and wind tunnel comparison of four aerosol samplers using agricultural dusts. *Ann. Occup. Hyg. 53*(*6*):585–594 (2009).
14. **Bakutis, B., E. Monstviliene, and G. Januskeviciene:** Analyses of airborne contamination with bacteria, endotoxins and dust in livestock barns and poultry houses. *Acta Veterinaria Brno. 73*:283–289 (2004).
15. **Dungan, R.S., A.B. Leytem, and D.B. Bjorneberg:** Year-long assessment of airborne endotoxin at a concentrated dairy operation. *Aerobiologia. 26*:141–148 (2010).
16. **Saito, R., B. Cranmer, J. Tessari, et al.:** Recombinant factor C (rFC) assay and gas chromatography/mass spectrometry (GC/MS) analysis of endotoxin variability in four agricultural dusts. *Ann. Occup. Hyg. 53*(*7*):713–722 (2009).
17. **Health Council of the Netherlands:** *Endotoxins. Health-Based Recommended Occupational Exposure Limit*. No. 2010/04OSH. The Hague: Health Council of The Netherlands, 2010.
18. **Heederik, D., T. Sigsgaard, P.S. Thorne, et al.:** Health effects of airborne exposures from concentrated animal feeding operations. *Environ. Health Perspect. 115*:298–302 (2007).

19. **Thorne, P.S.:** Environmental health impacts of concentrated animal feeding operations: Anticipating hazards—Searching for solutions. *Environ. Health Perspect. 115*:296–297 (2007).
20. **Clark, C.S., R. Rylander, and L. Larsson:** Levels of gram-negative bacteria, apsergillus-fumigatus, dust, and endotoxin at compost plants. *Appl. Environ. Microbiol. 45*: 1501–1505 (1983).
21. **Rylander, R., P. Haglind, and M. Lundholm:** Endotoxin levels in cotton dust and FEV1 decrement in cotton mill workers. *Am. Rev. Respir. Dis. 127i*:156 (1983).
22. **Heederik, D., R. Brouwer, K. Biersteker, J.S.M. Boleij:** Relationship of airborne endotoxin and bacteria levels in pig farms with the lung-function and respiratory sympotms of farmers. *Int. Arch. Occup. Environ. Health. 62*:595–601 (1991).
23. **Rylander, R., and M.F. Carvalheiro:** Airways inflammation among workers in poultry houses. *Int. Arch. Occup. Environ. Health. 79*:487–490 (2006).
24. **Heederik, D., and I.M. Wouters:** Endotoxin exposure and health effects: Reevaluating the evidence and new research initiatives. *Gefahrstoffe Reinhaltung Der Luft. 67*:357–360 (2007).
25. **Poole, J.A., G.P. Dooley, R. Saito, et al.:** Muramic acid, endotoxin, 3-hydroxy fatty acids, and ergosterol content explain monocyte and epithelial cell inflammatory responses to agricultural dusts. *J. Toxicol. Environ. Health A. 73*:684–700 (2010).
26. **Hines, C.J., D.K. Milton, L. Larsson, et al.:** Characterization and variability of endotoxin and 3-hydroxy fatty acids in an office building during a particle intervention study. *Indoor Air. 10*:2–12 (2000).
27. **Hines, C.J., M.A. Waters, L. Larsson, M.R. Petersen, A. Saraf, and D.K. Milton:** Characterization of endotoxin and 3-hydroxy fatty acid levels in air and settled dust from commercial aircraft cabins. *Indoor Air. 13*:166–173 (2003).
28. **Dehus, O., T. Hartung, and C. Hermann:** Endotoxin evaluation of eleven lipopolysaccharides by whole blood assay does not always correlate with Limulus amebocyte lysate assay. *J. Endotox. Res. 12*(*3*):171–180 (2006).
29. **Sebastian, A., B. Szponar, and L. Larsson:** Characterization of the microbial community in indoor environments by chemical marker analysis: An update and critical evaluation. *Indoor Air. 15*(*Suppl 9*):20–26 (2005).
30. **Eastman, C., D.C. Mitchell, D.H. Bennett, D.J. Tancredi, F.M. Mitloehner, and M.B. Schenker:** "Respiratory Symptoms of California's Dairy Workers. Field Actions Science Reports." [Online] Available at http://factsreports.revues.org/492 (accessed June, 15, 2011).
31. **Yanosky, J.D., and D.L. Macintosh:** A comparison of four gravimetric fine particle sampling methods. *J. Air Waste Manage. Assoc. 51*:878–884 (2001).
32. **Thorne, P.S., S.S. Perry, R. Saito, et al.:** Evaluation of the Limulus amebocyte lysate and recombinant factor C assays for assessment of airborne endotoxin. *Appl. Environ. Microbiol. 76*:4988–4995 (2010).
33. **Garcia, J., D.H. Bennett, D.J. Tancredi, et al.:** Occupational exposure to particulate matter and endotoxin for California dairy workers. *Int. J. Hyg. Environ. Health.* [In Press] Epub ahead of print May 12, 2012.
34. **Dungan, R.S., and A.B. Leytem:** Airborne endotoxin concentrations at a large open-lot dairy in southern Idaho. *J. Environ. Qual. 38*:1919–1923 (2009).
35. **Alwis, K.U., and D.K. Milton:** Recombinant factor c assay for measuring endotoxin in house dust: Comparison with LAL, and (1 -> 3)-beta-D-glucans. *Am. J. Ind. Med. 49*:296–300 (2006).
36. **Mueller-Anneling, L., E. Avol, J.M. Peters, and P.S. Thorne:** Ambient endotoxin concentrations in PM10 from Southern California. *Environ. Health Perspect. 112*:583–588 (2004).
37. **Tager, I.B., F.W. Lurmann, T. Haight, S. Alcorn, B. Penfold, and S.K. Hammond:** Temporal and spatial patterns of ambient endotoxin concentrations in Fresno, California. *Environ. Health Perspect. 118*:1490–1496 (2010).
38. **Saraf, A., L. Larsson, H. Burge, and D. Milton:** Quantification of ergosterol and 3-hydroxy fatty acids in settled house dust by gas chromatography mass spectrometry: Comparison with fungal culture and determination of endotoxin by a Limulus amebocyte lysate assay. *Appl. Environ. Microbiol. 63*:2554–2559 (1997).
39. **Drackley, J.K.:** Calf nutrition from birth to breeding. *Vet. Clin. North Am. Food Anim. Pract. 24*:55–86.(2008).
40. **Allen, J., K. Bartlett, M. Graham, and P. Jackson:** Ambient concentrations of airborne endotoxin in two cities in the interior of British Columbia, Canada. *J. Environ. Monit. 13*: 631–640 (2011).
41. **Shepherd, M.W., P.F. Liang, X.P. Jiang, M.P. Doyle, and M.C. Erickson:** Fate of *Escherichia coli* O157:H7 during on-farm dairy manure-based composting. *J. Food Prot. 70*:2708–2716 (2007).
42. **Mote, C.R., B.L. Emerton, J.S. Allison, H.H. Dowlen, and S.P. Oliver:** Survival of coliform bacteria in static compost piles of dairy waste solids intended for freestall bedding. *J. Dairy Sci. 71*:1676–1681 (1988).