Scientific
Research
Publishing

Occupational Exposure to Endotoxin in PM$_{2.5}$ and Pre- and Post-Shift Lung Function in California Dairy Workers

Veronica E. Arteaga[1], Diane C. Mitchell[2], Georg E. Matt[3], Penelope J. E. Quintana[4], Joshua Schaeffer[5], Stephen J. Reynolds[5], Marc B. Schenker[2], Frank M. Mitloehner[1]

[1]Department of Animal Science, University of California, Davis, USA
[2]Department of Public Health Sciences and Center for Health and the Environment, University of California, Davis, USA
[3]Department of Psychology, San Diego State University, San Diego, USA
[4]Graduate School of Public Health, San Diego State University, San Diego, USA
[5]Department of Environmental and Radiological Health Sciences, Colorado State University, Fort Collins, USA
Email: fmmitloehner@ucdavis.edu

Abstract

Individual exposures to particulate matter and endotoxin in dairies have increased as operations have transitioned to larger herd sizes. A cross-sectional study at 13 California dairies and one non-dairy control facility was conducted to determine associations between endotoxin concentrations measured in fine particulate matter (PM$_{2.5}$) and respiratory function in these workers. Previous research found that dairy work was associated with acute airway obstruction in comparison to controls. While endotoxin levels in PM$_{2.5}$ have been previously reported, their effects on lung function have not been studied among California dairy workers. This paper also examined correlations with PM$_{2.5}$ concentration, inhalable particulate matter (IPM), and endotoxin in IPM. Personal samples collected from a total of 185 dairy workers and 45 control workers were included in the analyses. Findings showed that dairy facilities had higher (p < 0.001) endotoxin in PM$_{2.5}$ when compared with the control facility (3.3 EU/m^3, 0.6 EU/m^3, respectively). Endotoxin in PM$_{2.5}$ was weakly correlated with PM$_{2.5}$ concentration (r^2 = 0.16, p < 0.05) and IPM endotoxin (r^2 = 0.35, p < 0.01), but not with IPM concentration. When controlling for age, ethnicity, smoking, height and personal protective equipment use, PM$_{2.5}$ endotoxin exposure was associated with lower cross-shift in forced expiratory volume in 1 second (FEV$_1$) and forced vital capacity (FVC) only among taller workers (p = 0.006). This is the first study to present personal occupational exposures to endotoxin in PM$_{2.5}$ measured on Californian dairies. Despite lower levels than in other industries, such as poultry farms, this study suggests that some workers may be affected, and efforts should

be aimed at mitigating pollutants in order to maintain endotoxin concentrations below recommended levels.

Keywords

Agriculture, Particulate Matter, Concentrated Animal Feeding Operations, Respiratory Health

1. Introduction

As food sources have changed with industrialization and population growth, small farms are being replaced by large-scale operations that offer lower cost and higher productivity [1]. Herd sizes have increased in the past two decades, with the largest growth seen in California's San Joaquin Valley (SJV) [1]. According to the USDA, dairy cattle and milk production farms spent $2.8 billion on hired labor in 2007 [2]. The majority of farm workers are low income, immigrants from Mexico and Latin/South America [3]. Occupational exposures of concern in dairy operations include heat and cold stress, acute injury, ergonomic issues, silage gases, and exposure to particulate matter (PM) [4]-[7].

Chronic exposure to organic PM can lead to decreased lung function, chronic bronchitis, byssinosis, asthma, and chronic obstructive pulmonary disease (COPD) [8]. Components of PM in large dairies include soil particles, bedding materials, litter, fecal matter, and feed. The biogenic components include bacteria, fungi, and viruses [1]. Aerosolization of PM depends on many factors such as temperature, wind velocity, air ventilation rate, manure water content, storage time, spreading technique of manure, feed, worker effect, and animal activity [9]. Higher rates of occupational asthma, chronic bronchitis, hypersensitivity pneumonitis, and cancer have been reported in dairy workers [7]. Organic dust toxic syndrome (ODTS) is a disease commonly seen in dairy farmers. ODTS is a flu-like disease that can cause fever, chills, muscle and joint pains, and fatigue [10]. Prevalence rates of obstructive pulmonary disorders were reportedly higher in poultry workers with longer exposure to PM less than 10 μm in diameter (PM_{10}) and PM less than 2.5 μm in diameter ($PM_{2.5}$) regardless of smoking status [11]-[13].

This study focused on the potential occupational risks of exposure to $PM_{2.5}$ endotoxin on California dairies. Endotoxin, also known as lipopolysaccharide (LPS) complex, is a heat stable, toxic molecule originating from the cell membrane of gram-negative bacteria. LPS is made up of a lipid A portion, core, and O-antigen. The lipid A portion of the LPS complex is responsible for its toxicity [14]. Major sources of endotoxin are animal feces and bacterial-contaminated plants [15]. When the cell undergoes lysis, endotoxin is released, and is aerosolized with other PM components [16]. Certain tasks, such as cleaning and animal handling, are also associated with higher exposures [17] [18]. Health risks associated with agricultural endotoxin exposure include acute fever and inflammatory reactions in the respiratory tract, accompanied by cough, chest tightness, shortness of breath, wheezing, and mucous membrane irritation [19]-[21]. Changes in lung function appear more closely associated with changes in endotoxin than other contaminants in broiler and layer operations [12]. Exposure has also been associated with chronic phlegm and cross-shift changes in FEV_1 [11] [12] [22].

There are no current endotoxin exposure limits regulated in the US by Occupation Safety and Health Administration (OSHA) [16], although there is a recommended exposure limit of 90 EU/m^3 by the Dutch Committee on Occupational Standards and a suggested threshold limit value of 4 mg/m^3 by the American Conference of Governmental Industrial Hygienists (ACGIH) [23] [24]. Regulation of endotoxin exposure is hampered due to variation among measurement methods, which leads to discrepancies between studies. Method standardization could minimize inter-study variability and facilitate establishing occupational exposure limits or guidelines in occupational settings [16] [25] [26]. Furthermore, particle size must be considered when measuring endotoxin exposure. Most studies in agricultural settings have evaluated total suspended particulates and/or inhalable particulates in relation to health effects. However, endotoxin levels in respirable particles may not be well predicted by PM concentration [11]. Thus, examining worker health in relation to endotoxin on different PM size fragments is important.

The present study is part of the California Dairy Environmental Health Research Initiative (Cal-DEHRI). Previous studies on these workers have reported a significant increase in respiratory symptoms in dairy workers compared with controls [27] and a significant decrease in cross-shift FEV_1 and FVC measured in dairy workers

as compared with controls [28]. Dairy farm job task also influenced endotoxin exposures in IPM pollutants [29]. In the paper by Mitchell *et al.* [30] exposure to endotoxin in the TSP size fraction and shift-length was associated with decrease in FVC. However, this analysis used a larger dairy worker sample size of 205. In the present study, endotoxin in $PM_{2.5}$ size fraction collected on personal samplers and effects on pulmonary function were investigated. It is hypothesized that exposure to $PM_{2.5}$ endotoxin would be higher among dairy workers and would have a greater respiratory health effect on the dairy workers in comparison to the controls workers.

2. Methods

2.1. Facilities

Workers were recruited from 13 free-stall dairies with over 1000 cows in California's San Joaquin Valley. Each farm was visited for 2 to 7 consecutive days. The control facility was a vegetable processing plant in Gilroy, CA. The control was chosen because it had similar socio-demographic characteristics as the dairy workers and lacked exposure to animals. Single cross-shift measurements of each worker were collected from June to October 2008. Further details of participant recruitment and health measurements are described in [28].

2.2. Subjects

Following UC Davis Institutional Review Board approval, subjects were recruited with the following eligibility: age 18+ years, male, able to wear the samplers for their entire work-shift (more than 6 hrs), speak English or Spanish, and able to perform Pulmonary Function Tests (PFTs). Detailed health measurements, demographics, study design, and analysis can be found in [27] and [28]. This study had a high recruitment rate at 93% of the workers consenting to take part in the study. A total of 226 dairy workers and 52 control workers were enrolled. In the present paper 185 dairy workers and 45 control workers were used in the analysis. Subjects were excluded for the following reasons: 29 dairy workers and 4 control workers did not have usable spirograms, 12 dairy and 3 controls workers with $PM_{2.5}$ concentrations falling more than 1.5 inter-quartile ranges above the 75th or below the 25th quartile in boxplots, as in [29]. As mentioned in [28], participants excluded from analysis because of spirometer values that were not acceptable were defined as those greater than 8% variability in pre- or post-shift between maneuvers. Pre- and post-shift questionnaires were administered by trained interviewers. For dairy workers, the pre-shift questionnaire included demographics, health history, current health, work history, and the post-shift questionnaire asked about tasks performed on the dairy the day they wore the personal monitor and symptoms, as well as other behaviors, such as Personal Protective Equipment (PPE) use. PPE included use of mask, N95 respirator, or cartridge respirator. Use of a bandana was not included in the definition of using PPE. For control workers, a simplified version of the dairy questionnaire was given. This included demographics, health history, current health, and work history, but did not include tasks.

2.3. Respiratory Health Assessments

Pulmonary function was measured using an Easy One Portable Spirometer (NDD Medical Technologies, Andover, MA). The National Institute for Occupational Safety and Health (NIOSH) protocol was followed and technicians were NIOSH certified. Statistical modeling of pulmonary function changes examined a mixed effects model that jointly examines pre- and post-FEV_1 and FVC by including a shift factor (before the start of the shift and at the end of the shift). FEV_1 is the volume of air forcefully expelled from the point of maximum inhalation in the first second and is an indicator of obstructive conditions reducing exhalation speed. FVC is the maximum volume of air exhaled forcefully from the point of maximum inhalation and indicates restrictive conditions and very severe obstructive diseases.

2.4. Exposure Assessments

A single cross-shift measurement was performed on each enrolled worker. Workers wore personal monitors for the duration of their work-shift, including lunch and breaks. Sampling methods and analysis can be found in further detail in [29]. Briefly, workers were fitted with a personal monitor backpack. The backpack included one Leland Legacy personal sampling pump (SKC, Inc., Eighty Four, PA) connected to a GK2.05SH (KTL) cyclone sampler and a SKC button sampler (SKC, Inc. 225 - 360, Eighty Four, PA) via Tygon tubing. The GK2.05SH

(KTL) cyclone sampler was fitted with a Teflon 37 mm, Millipore filter with a pore size of 0.45 μm (Fisher, FHLP03700). The SKC button sampler was fitted with a 3.0 μm pore size, Teflon 25 mm, Millipore PTFE filter (Fisher, FSLW02500). Airflow to both samplers was adjusted through a Swagelok needle valve (Swagelok, Solon, OH). Airflow for both personal and area samplers were calibrated using a Defender-series (Bios International, Butler, NJ) electron piston volumetric gas flow meter at 3.5 L/min for the cyclone sampler and 4.0 L/min for the button sampler.

2.5. Endotoxin Analysis

Filter samples were analyzed for biologically active endotoxin using recombinant Factor C (rFC) assay (Lonza, Inc., Walkersville, MD). Samples were shipped over night on dry ice to Colorado State University for rFC analysis. The method was described in detail in [29]. Any result below the limit of detection (LOD) was assigned a value of LOD divided by the square root of 2.

2.6. Statistical Analysis

Stata 12 software was used for all statistical analyses. Exposure measures (*i.e.*, IPM, $PM_{2.5}$, endotoxin in IPM fraction and $PM_{2.5}$ fraction) were log-transformed to correct for positive skew and mean-centered to reduce multicollinearity when examining interactions. Multiple linear regression models were estimated for exposure measures to examine differences between dairy and control workers. Mixed linear regression models were estimated to examine the association between worker exposure to pollutants (explanatory variables) and cross-shift lung function as measured by FEV_1 and FVC (response variables). Lung function changes and interactions by pollutant exposures during the shift were examined as time main and interaction effects, where the pre-shift lung function measure was coded as time 1 and the post-shift measure as time 2 (pre-shift minus post-shift lung function measures). Separate analyses were conducted for FEV_1 and FVC lung function measures. In each case, the lung function measure at pre- and post-shift was the response variable, and the four exposure measures, time (pre-shift and post-shift) and other covariates (age, race, height, years in the US, country or origin, education level, family income, smoking status, exposure to second-hand smoke, number of days since last day off, PPE use, years worked on a dairy, length of work-shift, hours spent in an enclosed cab, dairy facility, herd size, tasks, RH, and temperature) were entered as explanatory variables (**Table 1**). In the first stage of the analysis, all explanatory variables were entered as main effects. Interaction effects of exposure measures with shift and the other covariates were entered in stage two. Statistically nonsignificant interaction effects and nonsignificant main effects not involved in interaction effects were then dropped one at a time, and the model was re-estimated. This parsimonious final model was then further examined using Stata's margin command to better understand the main and interaction effects supported by the model.

3. Results

3.1. Demographics and Smoking History

The male dairy workers averaged 32.9 years (SD = 10.9), mostly (94.2%) Latino immigrants from Mexico, and had spent on average 12 years in the US (**Table 1**). Participants in the control site were close in age (Mean = 34.8 years, SD = 11.6) and were also mostly Latino immigrants from Mexico (p < 0.001). Dairy workers reported a median yearly income between $20,000 and $30,000, and control workers' median income was between $10,000 and $20,000. The majority of the dairy workers described themselves as being Indigenous (Central and South) American Indian (59%) and White (32%). About half of the dairy workers had six or fewer years of formal education, while half or fewer of the control workers had fewer than 9 years of education (**Table 1**). About 25.9% of dairy workers and 13.3% of controls were current smokers (**Table 2**). Among current and former dairy worker smokers, the average pack-years was 5.5. About 23% dairy workers reported being exposed to second-hand smoke at work, and about 23% reported being exposed to second-hand smoke at home (**Table 2**). Control workers had lower pack-years (3.72, p < 0.001) and 11% reported being exposed to second-hand smoke at home (**Table 2**).

3.2. Work History and Shift Observed

Table 3 shows the work history and personal protective equipment (PPE) use for both dairy and control workers.

Table 1. Demographics of participants with usable pulmonary function test.

	Dairy workers (n = 185)				Control workers (n = 45)			
	Mean (SD)	Median	n	%	Mean (SD)	Median	n	%
Age (years)	32.9 (10.9)	31			34.8 (11.6)	32.5		
Years spent in the US	12.08 (8.1)	10			8.9 (8.9)	5		
Height (cm)	169.4 (5.9)	169			165.0 (7.2)	166		
BMI	27.0 (4.1)	27.2			27.7 (3.1)	27.4		
Age (years)								
19 - 25			57	30.8			12	26.7
26 - 35			66	35.7			14	31.1
36 - 45			37	20.0			10	22.2
46 - 55			18	9.7			8	17.8
56 - 70			7	3.8			1	2.2
Yearly family income								
$0 - 10,000			9	4.9			10	22.2
$10,001 - 20,000			35	18.9			18	40.0
$20,001 - 30,000			103	55.7			7	15.6
$30,001 - 50,000			24	13.0			6	13.3
$50,001+			1	0.5			1	2.2
No response			13	7.0			3	6.7
Education								
None			5	2.7			0	0.0
Primary (grades 1-6)			90	48.6			14	31.1
Junior high (grades 7-9)			57	30.8			21	46.7
High school (grades 10-12)			30	16.2			8	17.8
Some college (grade > 12)			3	1.6			2	4.4
Ethnicity								
Latino			171	92.4			40	88.9
Non-Latino			9	4.9			1	2.2
No response			5	2.7			4	8.9
Race								
White			61	32				
Indigenous Indian/S American Indian			109	59				
Other			8	5				
Declined			7	4				
Country of origin								
United States			4	2.2			3	6.7
Mexico			171	92.4			42	93.3
Other Central/South America			5	2.7			0	0.0
Portugal			5	2.7			0	0.0

Table 2. Smoking exposures for dairy and control workers.

	Dairy workers (n = 185)				Control workers (n = 45)			
	Mean (SD)	Median	n	%	Mean (SD)	Median	n	%
Never smokers			101	54.6			31	68.9
Former smokers			36	19.5			8	17.8
Current smokers			48	25.9			6	13.3
Pack years[a]	5.5 (7.1)	3.1			3.72 (4.7)	1.7		
Cigarettes per day[b]	8.2 (1)	5			4.5 (1.5)	2.5		
Shift use			25	13.5			0	0.0
Cigarettes per shift[c]	4 (3.3)	3			0	0		
Exposure to second-hand smoke								
None			97	52.4			27	60.0
At home only			42	22.7			13	28.9
At work only			43	23.2			5	11.1
At home and work			3	1.6			0	0.0
Time exposed at work during observed shift (mins)	26.9 (7.5)	10			0.2 (0.2)	0.5		

[a]Pack-years calculated among ever smokers, n = 79; 74 dairy workers and 15 control workers, 2 missing. [b]Cigarettes per day among ever smokers. [c]Cigarettes per shift among workers who smoked during shift, n = 25 dairy workers.

Table 3. Work history, history of PPE use, and observed shift characteristics and PPE use.

	Dairy workers (n = 185)				Control workers (n = 45)			
	Mean (SD)	Median	n	%	Mean (SD)	Median	n	%
Work history								
Years in agriculture	19.2 (12.6)	17.0			8.3 (6.5)	6.0		
Years working on any dairy	8.7 (8.8)	5.0			0	0.0		
History of PPE use[a]								
More than half the time			16	8.7			14	31.1
Up to half the time			26	14.0			13	28.9
Never or rarely			142	76.8			18	40.0
No response			1	0.5			0	0.0
Observed shift								
Length of work-shift (hrs)	9.1 (0.9)	9.0			8.6 (0.2)	8.0		
Time spent working in dusty environment (mins)	145 (200)	1.0			2.4 (3.8)	0.0		
# of days worked since last day	3.1 (2.1)	3.0			3.9 (1.6)	4.0		
Mins spent in an enclosed cab[b]	245 (62)	0			0	0		
PPE use[a]								
Yes			1	0.5			2	4.5
No			26	14.1			43	95.5
No response			158	85.4			0	0.0

[a]Includes use of mask, N95 respirator, or cartridge respirator. [b]Only 15 dairy workers who spent time in enclosed cab included.

Dairy workers had spent more time working in an agricultural setting than the controls with a mean of 19.2 years (SD = 12.6) and 8.3 years (SD = 6.5), respectively. None of the controls had previously worked on a dairy. PPE use was higher (p < 0.001) among the control workers at 60%. Only 22.7% of the dairy workers reported using PPE either up to half of the time or more than half of the time during their shift. During the observed shift none of the dairy workers had reported wearing PPE more than half of the time. On average dairy workers' shifts were half an hour longer than the control workers, at 9.1 hrs (SD = 0.9) as compared to 8.6 hrs (SD = 0.2), respectively.

3.3. Personal Exposures to Endotoxin and Dairy and Control Workers

Personal exposures to IPM, $PM_{2.5}$ and endotoxin in both fractions are reported in Table 4. Comparison using t-test of the log transformed exposure means reveals statistically higher exposures for dairy workers versus control workers with respect to exposure to endotoxin in IPM (p < 0.001, $r^2 = 0.487$) and endotoxin in $PM_{2.5}$ (p < 0.001, $r^2 = 0.240$). Geometric mean personal exposure to endotoxin in IPM and $PM_{2.5}$ in dairy workers was 331.5 EU/m^3 and 12.5 EU/m^3, respectively. Mean exposure to endotoxin in IPM and $PM_{2.5}$ was over 26 times and 5 times, respectively, higher in dairy workers than in control workers. T-tests were also used to compare log-transformed exposures to particulate matter, which also were higher in dairy workers versus controls, IPM (p < 0.001, $R^2 = 0.092$) and $PM_{2.5}$ (p < 0.001, $R^2 = 0.092$) when controlling for tobacco use and second-hand smoke exposure. Personal exposure to IPM and $PM_{2.5}$ geometric mean was about 2 times (800 $\mu g/m^3$) and 1.5 times (33 $\mu g/m^3$) higher in dairy workers than in the control workers. None of the dairy worker $PM_{2.5}$ endotoxin samples were below LOD, but 15.5% of the control samples were below LOD. The correlations between IPM and $PM_{2.5}$, $PM_{2.5}$ concentration and $PM_{2.5}$ endotoxin, IPM concentration and IPM endotoxin for both dairy and control workers are demonstrated in Figure 1. Personal exposure to $PM_{2.5}$ endotoxin was correlated with $PM_{2.5}$ and IPM endotoxin ($r^2 = 0.157$, p < 0.001, and $r^2 = 0.348$, p < 0.001, respectively). An interaction with different linear relationship between $PM_{2.5}$ endotoxin and IPM endotoxin was observed (Figure 1(d)). Data were collected during the summer, with average daily temperature of 28°C and average relative humidity (RH) of 38%. Bivariate temperature and RH were not correlated with $PM_{2.5}$ endotoxin (p = 0.307, $r^2 = 0.025$, and p = 0.749, $r^2 = 0.032$, respectively).

Table 4. Occupational personal exposure to IPM[a] ($\mu g/m^3$), $PM_{2.5}$[b] ($\mu g/m^3$), IPM endotoxin (EU/m^3), and $PM_{2.5}$ endotoxin (EU/m^3) for dairy workers and control workers.

	Dairy workers							
	Geomean	95% CI	% samples < LOD	Min	25th	50th	75th	Max
IPM ($\mu g/m^3$)	800.0	735.7, 870.0	0.0	191.0	564.3	757.8	1129.1	4949.5
$PM_{2.5}$ ($\mu g/m^3$)	33.0	29.8, 36.4	0.0	7.1	20.5	30.4	50.2	252.7
IPM endotoxin (EU/m^3)	331.5	294.8, 372.8	0.0	45.2	188.7	312.4	583.4	2061.3
$PM_{2.5}$ endotoxin (EU/m^3)	3.3	2.9, 3.8	0.0	0.3	1.7	3.1	6.9	29.5
	Control workers							
	Geomean	95% CI	% samples < LOD	Min	25th	50th	75th	Max
IPM ($\mu g/m^3$)	408.4	338.8, 594.7	0.0	112.5	273.9	328.1	690.5	1477.5
$PM_{2.5}$ ($\mu g/m^3$)	19.6	14.8, 25.9	0.0	2.1	10.5	18.9	33.8	168.4
IPM endotoxin (EU/m^3)	12.5	6.2, 25.2	0.0	0.0	4.5	10.9	60.6	1279.4
$PM_{2.5}$ endotoxin (EU/m^3)	0.6	0.4, 0.9	15.5	0.0	0.2	0.4	0.8	12.1

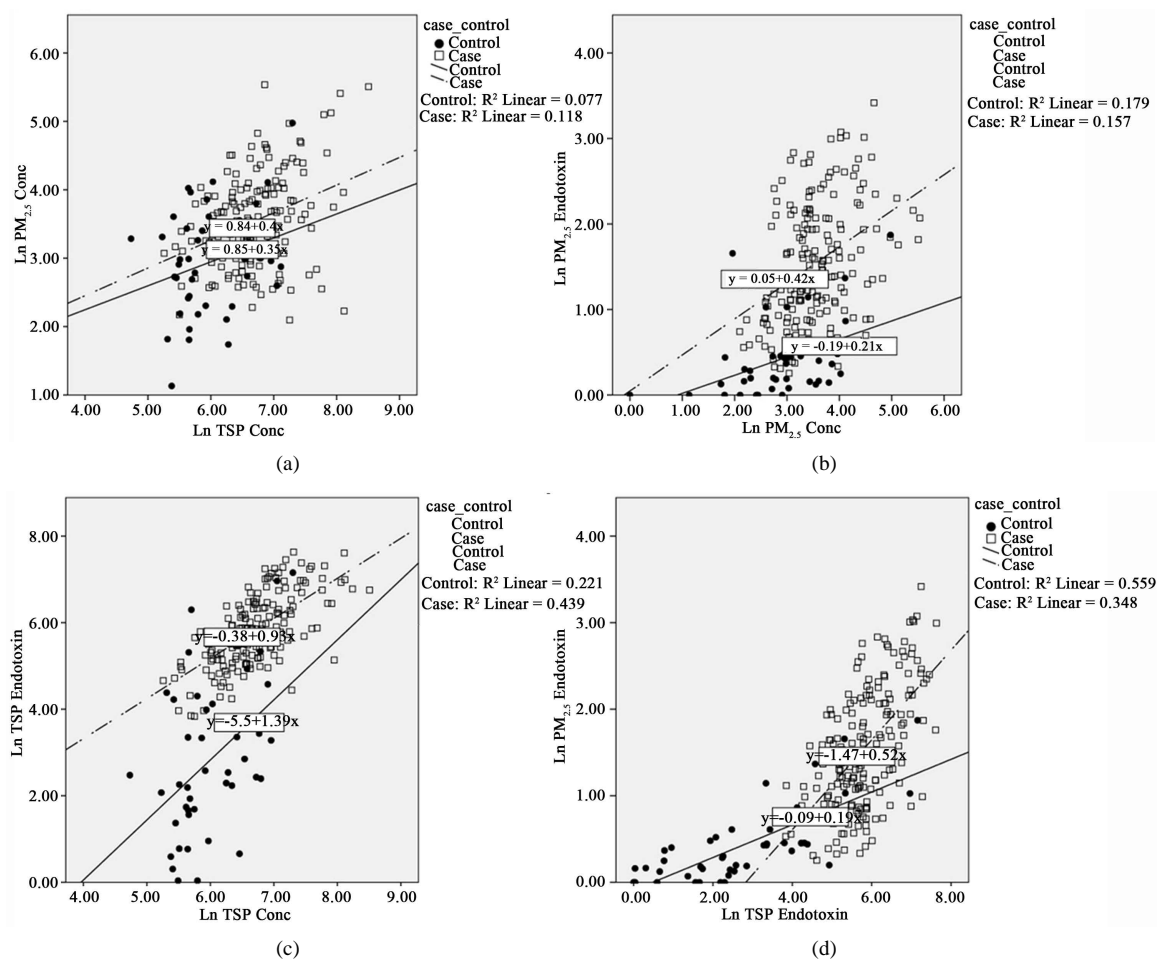[a]Inhalable particulate matter (IPM). [b]Particulate matter less than 2.5 μm in diameter ($PM_{2.5}$).

Figure 1. Scatter plots by dairy workers and controls of natural log of (a) IPM concentration ($\mu g/m^3$) and $PM_{2.5}$ concentration ($\mu g/m^3$); (b) $PM_{2.5}$ concentration ($\mu g/m^3$) and $PM_{2.5}$ endotoxin (EU/m^3); (c) IPM concentration ($\mu g/m^3$) and IPM endotoxin (EU/m^3); and (d) IPM endotoxin (EU/m^3) and $PM_{2.5}$ endotoxin (EU/m^3).

3.4. Cross-Shift Changes in Pulmonary Function

No significant difference was observed in baseline FEV_1 and FVC between dairy workers and control workers (p = 0.180, R^2 = 0.524 and p = 0.218, R^2 = 0.543, respectively) after controlling for age and height; smoking and second-hand exposure were not significant. The subgroup of dairy workers observed in the current paper experienced a crude-change cross-shift decline in FEV_1 and FVC of −44.3 mL and −35.6 mL, respectively, in comparison to controls, who had cross-shift levels in FEV_1 and FVC of +18.6 mL and +60.8 mL, respectively. There was a significant difference between dairy and control workers in cross-shift FEV_1 and FVC when controlling for age and height (p = 0.029, R^2 = 0.021 and p = 0.006, R^2 = 0.037, respectively). Cross-shift FEV_1 and FVC in dairy workers declined significantly (t = −3.53 (184) p = 0.001, and, t = −2.75 (184), p = 0.006, respectively). In controls, cross-shift FEV_1 (t = 0.79 (44), p = 0.436) did not change and FVC increased (t = 2.12 (44), p = 0.04). Findings in the current paper are consistent with results reporting lower pulmonary function in dairy workers than in controls in a larger set of subjects from the same facilities [28].

3.5. Association between Personal Exposures to Endotoxin and Pulmonary Function

A mixed linear regression was performed for FEV_1 among dairy and control workers. Statistically significant covariates included: age, race, number of days since last day off, PPE use, height, and temperature (**Table 5**). Neither current or former smoker status nor exposure to second-hand smoke were significant in the models in either dairy and control facilities. Age-related decrease was much higher in dairy workers versus control workers

Table 5. Coefficients, p-values, and 95% CI for multivariate mixed regression modeling of dairy workers cross-shift pulmonary lung function measures.

	FEV$_1$, L/sec			
	Coef.		95% CI	p-value
Age	−0.029	−0.034	−0.024	**<0.001**
Race				
White				
Indigenous Indian/S American Indian	0.081	−0.042	0.199	0.199
Other	−0.430	−0.713	0.003	**0.003**
Days worked since last day off	−0.024	−0.050	0.002	0.075
LnPM$_{2.5}$ endotoxin (EU/m^3)[a]	−0.046	−0.129	0.564	0.564
PPE history use	0.224	0.076	0.003	**0.003**
Height[a]	−0.016	−0.054	0.023	0.424
Temperature[a]	−0.005	−0.010	0.000	**0.038**
	FVC, L/sec			
	Coef.		95% CI	p-value
Age	−0.029	−0.035	−0.023	**<0.001**
LnPM$_{2.5}$ endotoxin (EU/m^3)[a]	−0.022	−0.121	0.076	0.652
PPE history use	0.194	−0.023	0.370	**0.031**
Height (10 cm)[a]	0.065	0.052	0.078	**<0.001**
Temperature[a]	−0.006	−0.012	−0.001	**0.018**

[a]Data centered.

(p < 0.001), decreasing 1 ml instead of 0.2 ml per 10 years. Older control workers also had lower pre- and post-shift FEV$_1$ (p < 0.001), however, for every 10 year increase in age, FEV$_1$ declined by 0.2 ml. With respect to race, dairy workers who identified as being "Other" had lower FEV$_1$ than those who identified as being White (p = 0.023). However, workers who identified as being Indigenous Indian/S American Indian and those who declined did not differ from those who identified as being White (p = 0.596). There was no significant difference in FEV$_1$ among control workers who identified as being Indigenous Indian/S American Indian, those who declined and "Other" (p = 0.824, p = 0.293, and 0.273, respectively). Workers wearing a PPE showed better cross-shift FEV$_1$ by 0.3 ml (p < 0.001) than those who did not. There was no significant change observed in FEV$_1$ among control workers and use of PPE (p = 0.090). Finally, the more days dairy workers had worked in a row, was marginally significant in decrease of cross-shift FEV$_1$ (p = 0.075). The more days workers had worked in a row was not significant in cross-shift FEV$_1$ among control workers (p = 0.309).

Analyses showed significant interactions between PM$_{2.5}$ endotoxin exposure and dairy worker height and time of FEV$_1$ measure (pre-, post-shift) and temperature of sampling day. There was no significance observed between PM$_{2.5}$ endotoxin exposure and control worker height and time of FEV$_1$ measure and temperature of sampling day (p = 0.112 and p = 0.409, respectively). The observed interactions for dairy workers indicate that the effect of exposure on FEV$_1$ differed by worker height and that cross-shift changes depend on temperature. To further study these interactions, we examined the conditional relationships for workers at different heights (mean ± 1SD: 163 cm, 169 cm, 175 cm) and for sampling work-shifts with different temperatures (mean ± 1SD: 16˚C, 22˚C, 28˚C). For the endotoxin exposure-by-height interaction, findings show a statistically significant negative association among taller workers (p = 0.006) and no association among workers of average or shorter height (p

= 0.268 and p = 0.274, respectively). Further investigations of cross-shift by temperature interaction showed a significant cross-shift decline in FEV_1 when temperature was $\geq 28°C$ (p = 0.019 and p = 0.002, respectively). When workers worked during shifts when temperature was relatively low (16°C) there was no significant cross-shift change in FEV_1 (p = 0.820).

A mixed linear regression was also performed for dairy and control workers FVC results (**Table 5**). Statistically significant covariates included: age, PPE use, height, temperature, and $PM_{2.5}$ endotoxin. Older dairy and control workers had lower pre- and post-shift FVC (p < 0.001 and p = 0.003, respectively). As with FEV_1, FVC decreased by about 1 ml for every 10 years increase in age among dairy workers and 0.2 ml among control workers. Dairy and control workers who reported a history of PPE use showed better FVC than workers who did not (p = 0.031 and 0.041, respectively). Taller dairy and control workers had higher cross-shift FVC than short workers (p < 0.001 and p < 0.001, respectively). A 10 cm increase in height of dairy and control workers was associated with a 0.65 and 0.56 L/sec larger cross-shift FVC, respectively.

Similar to our findings for FEV_1, an interaction between dairy worker height and exposure to $PM_{2.5}$ endotoxin was observed. To further investigate this interaction, the conditional relationships between FVC and $PM_{2.5}$ endotoxin for workers at different heights (mean ± 1SD: 163 cm, 169 cm, 175 cm) were examined. These analyses showed a significant negative association among the taller dairy workers (p = 0.003) between $PM_{2.5}$ endotoxin exposure and FVC, a significant positive association between shorter workers (p = 0.028), and no association among average height workers (p = 0.102 and p = 0.652). There was no significant association between height and exposure to $PM_{2.5}$ endotoxin in control workers at shorter, average, and taller heights (p = 0.783, p = 0.510, and p = 0.512, respectively). The interaction between temperature and time was also further investigated to determine temperature effects on cross-shift FVC. These analyses showed a decline in cross-shift FVC (p = 0.021) at higher temperature and no association when temperatures were average or below average among dairy workers (p = 0.078 and p = 0.806, respectively). There was no significant interaction between temperature and time (pre- minus post-shift) with respect to FVC for control workers (p = 0.594).

4. Discussion

Personal exposure to endotoxin in $PM_{2.5}$ among Californian dairy workers has not been previously reported. Here we show that dairy workers had about five times higher personal exposure to $PM_{2.5}$ endotoxin compared to controls, which is similar to Mitchell *et al.* [30] who found total inhalable endotoxin was an order of magnitude higher among the same worker. This is likely the case because dairy facilities have more biogenic sources originating from the animals and daily practices when compared to other non-livestock agricultural settings [21]. Poole *et al.* [31] demonstrated higher endotoxin concentrations present in samples collected at animal farms, along with other biogenic material than samples collected at residential homes. Personal $PM_{2.5}$ endotoxin geometric mean in the dairy facilities in this study was 3.3 EU/m^3, over 200 times higher than ambient geometric mean $PM_{2.5}$ endotoxin measured in a metropolitan area (0.015 EU/m^3) [32].

Job tasks, variation in dairy manure management, meteorological data, wind speed, and RH have been reported to influence personal and area IPM endotoxin concentration on these dairies [29]. The influence of these variables on endotoxin in $PM_{2.5}$ was of interest in this study. The dairies studied here were open-lot and open-freestall, which are thought to be beneficial over enclosed operations because of the higher ventilation rate. However, higher IPM has still been measured downwind of an open-lot and open-freestall dairy facility compared to upwind measurements [33]. Endotoxin exposure in dairies is still high in comparison to other work environments [21] [34].

4.1. Correlation between PM and Endotoxin in Different Size Fractions

Personal exposures to $PM_{2.5}$ endotoxin was weakly correlated with $PM_{2.5}$ concentration ($r^2 = 0.16$, p < 0.05) and inhalable PM endotoxin ($r^2 = 0.35$, p < 0.01), but not with inhalable PM concentration. Endotoxin in IPM in a study by Burch *et al.* [15] found moderate correlation with IPM measured in air (r = 0.4 to 0.6). A study that looked at total and respirable PM and endotoxin poultry farms found significant correlations between both size fractions and endotoxin in both size fractions [35]. Endotoxin concentration might vary from PM concentrations due to its dependence on particle source, composition, and season [36] [37]. The sampling environment (indoor vs. outdoor) also influences correlation between endotoxin in different size fragments and PM concentration. In two separate studies in indoor poultry facilities, endotoxin in IPM ranging from 230 to 284 EU/m^3 and IPM cor-

related better than endotoxin in respirable PM ranging from 236.1 to 490.6 EU/m^3 and respirable PM concentration [11] [38].

4.2. Lung Function and Endotoxin Exposure

In this study, we found that higher endotoxin exposure in the PM$_{2.5}$ size fraction appeared to be associated with reduced lung function, but only in taller workers. Specifically, we found significant negative associations between endotoxin exposure and FEV$_1$ as well as FVC among taller workers. There was a marginally significant exposure to PM$_{2.5}$ endotoxin among taller workers (p = 0.075). This novel finding, however, should be replicated with a larger sample size to further investigate the relationship between PM$_{2.5}$ endotoxin exposure and lung function. Smoking background and primary task was further explored to observe if there was any differences in regards to workers' height (*i.e.* taller workers had a greater history of smoking). No significant differences were observed among workers height and smoking background. There was also no significant differences among height and tasks on the dairy, however, maintenance workers were a bit taller with an average height of 174.2 cm, but there were only 5 workers in this group. In this study, higher daily average temperatures were associated with the cross-shift declines in FEV$_1$ and FVC. Donaldson *et al.* [39] observed decreased FEV$_1$ (at 2.20 mL/˚C) and FVC (at 3.64 mL/˚C) during the warmest and coolest weeks in a one year study in a population of COPD patients living in London. Decrease in FEV$_1$ and FVC was also observed among children with asthma at 20˚C in Australia [40]. The warmer temperature causes strain in the airways, causing decrease in lung function [41]. Although there was no interaction observed between warmer temperature work environment and exposure to PM$_{2.5}$ endotoxin in the main effect model, the combination of both could further exacerbate lung function.

The number of days worked since the last day off was marginally significant with respect to cross-shift FEV$_1$. Workers who worked more days in a row had lower cross-shift FEV$_1$. Bonlokke *et al.* [42] found cross-shift FVC decrease of 3.1% among swine building workers returning to work after four days of either being off of work or using respiratory protection. They found a loss of adaptation from the time they were not exposed noted by decrease in both baseline FVC and immune reactions when returning to work. Endotoxin was thought to be the main culprit. In this study, reported PPE use, which included masks, N95 respirators, or cartridge respirators, was positively correlated with cross-shift FEV$_1$ and FVC. Wearing PPE during the shift may have protected workers from exposure to PM$_{2.5}$ endotoxin. The majority of studies observing respiratory health and particulate and endotoxin exposure either had not reported PPE use, omitted workers who used a PPE because of low numbers, or found low use [9] [15] [43] [44]. PPE has also been found to be worn incorrectly [43]. Mirabelli *et al.* [44] found only 11% of the participants reported wearing a mask all the time. In this study 9.7% of the dairy workers and 33.3% of the control workers reported wearing PPE more than half of their work-shift, although none of them reported wearing a mask more than half of the time during the observed shift. Among California agricultural workers, higher education was positively correlated to mask use. Smoking was negatively correlated with mask use in the same group. Ex-smokers were more likely to wear a mask compared to smokers and non-smokers [45]. PPE use is important for secondary prevention of PM. However, trying to maintain low levels of pollutants through better mitigation practices should be the primary concern.

4.3. Limitations

Limitations included the need for a larger sample size so that smaller associations between lung function and PM$_{2.5}$ endotoxin exposure may be detected with better statistical power. In observational studies such as this, confounding is a concern. In this case, control workers had lower smoking prevalence and secondhand smoke exposure than dairy workers. Our analyses, however, showed that neither was a significant covariate in FEV$_1$ and FVC mixed linear regression models. As discussed in [28], selection bias might be present because of the healthy worker effect. This bias was reduced by selecting similar age and ethnicities in dairy and control worker populations. Finally, the study design examined cross-shift changes in lung function and endotoxin exposure on a specific day. The health effects of exposure, however, are likely to be cumulative over months or years of exposure. It would have also been beneficial to test other possible bioaerosols, such as gram positive bacteria or molds/fungi, which may have given more insight as to workers exposure and effects on lung function. Current findings could be Type I errors and any significant association between respiratory health and exposure could have possibly arisen by chance, however, not very likely given the observed level of significance in the mixed

linear regression model. Future studies, therefore should apply longitudinal designs, in which the cumulative effects of endotoxin exposure can be observed over longer periods of time.

5. Conclusion

This study found a negative association between personal exposure to $PM_{2.5}$ endotoxin exposure and decreases in dairy worker lung function (cross-shift) among taller workers. Higher temperatures during work-shifts were found to be associated with cross-shift decline in FEV_1 and FVC, and self-reported use of PPE appeared protective. Replications in other dairies, locations, and other measures, such as gram positive bacteria or molds/fungi, are necessary. Further investigation on how tasks on the dairy influence exposure to $PM_{2.5}$ endotoxin can help in elucidating the source of $PM_{2.5}$ endotoxin, and potential health effects. Longitudinal studies in lung function could potential help in better understanding the cumulative effect of endotoxin exposure. In future studies, assessing task-specific exposure is important to mediate efforts to reduce decline in worker lung function and pollutant exposure.

Acknowledgements

References

[1] US Environmental Protection Agency (2004) Risk Assessment Evaluation for Concentrated Animal Feeding Operations: 6.0 Air Transport and Deposition. US Environmental Protection Agency. EPA/600/R-04/042. http://nepis.epa.gov

[2] US Department of Agriculture (2007) 2007 Census of Agriculture: Farm Labor. http://www.agcensus.usda.gov/Publications/2007/Online_Highlights/Fact_Sheets/Economics/farm_labor.pdf

[3] US Department of Labor (2010) The National Agricultural Workers Survey, 2010. Washington DC. http://www.doleta.gov/agworker/report/major.cfm

[4] Douphrate, D.I., Fethke, N.B., Nonnenman, M.W., Rosecrance, J.C. and Reynolds, S.J. (2012) Full Shift Arm Inclinometry among Dairy Parlor Workers: A Feasibility Study in a Challenging Work Environment. *Applied Ergonomics*, **43**, 604-613. http://dx.doi.org/10.1016/j.apergo.2011.09.007

[5] Hagevoort, G.R., Douphrate, D.I. and Reynolds, S.J. (2013) A Review of Health and Safety Leadership and Managerial Practices on Modern Dairy Farms. *Journal of Agromedicine*, **18**, 265-273. http://dx.doi.org/10.1080/1059924X.2013.796905

[6] Reynolds, S.J., Clark, M.L., Koehncke, N., von Essen, S., Prinz, L., Keefe, T.J., Mehaffy, J., Bradford, M., Crammer, B., Davidson M.E., Yang, I.V. and Burch, J.N. (2012) Pulmonary Function Reductions among Potentially Susceptible Subgroups of Agricultural Workers in Colorado and Nebraska. *Journal of Occupational and Environmental Medicine*, **54**, 632-641. http://dx.doi.org/10.1097/JOM.0b013e31824d2e1c

[7] Reynolds, S.J., Nonnenmann, M., Basinas, I., Davidson, M., Elfman, L., Gordon, J., Kirychuch, S., Reed, S., Schaeffer, J.W., Schenker, M.B., Schlunssen, V. and Sigsgaard, T. (2013) Systematic Review of Respiratory Health among Dairy Workers. *Journal of Agromedicine*, **18**, 219-243. http://dx.doi.org/10.1080/1059924X.2013.797374

[8] Guillam, M.T., Pedrono, G., Le Bouquin, S., Huneau, A., Gaudon, J., Leborge, R., Dewitte, J. and Segala, C. (2013) Chronic Respiratory Symptoms of Poultry Farmers and Model-Based Estimates of Long-Term Dust Exposure. *Annals of Agricultural and Environmental Medicine*, **20**, 307-311.

[9] Donham, K.J., Cumro, D. and Reynolds, S.J. (2002) Synergistic Effects of Dust and Ammonia on the Occupational Health Effects of Poultry Production Workers. *Journal of Agromedicine*, **8**, 57-76. http://dx.doi.org/10.1300/J096v08n02_09

[10] Rylander, R. (2002) Endotoxin in the Environment—Exposure and Effects. *Journal of Endotoxin Research*, **8**, 241-252. http://dx.doi.org/10.1179/096805102125000452

[11] Kirychuk, S., Dosman, J., Reynolds, S.J., Wilson, P., Senthilselvan, A., Feddes, J.J.R. Classen, H.L. and Guenter, W. (2006) Total Dust and Endotoxin in poultry Operations: A Comparison between Cage and Floor Housing and Respiratory Effects in Workers. *Journal of Occupational and Environmental Medicine*, **48**, 741-748. http://dx.doi.org/10.1097/01.jom.0000216215.39521.3c

[12] Senthilselvan, A., Beach, J., Feddes, J., Cherry, N. and Wenger, I. (2011) A Prospective Evaluation of Air Quality and Workers' Health in Broiler and Layer Operations. *Occupational and Environmental Medicine*, **68**, 102-107. http://dx.doi.org/10.1136/oem.2008.045021

[13] Viegas, S., Faisca, V.M., Dias, H., Clerigo, A., Carolino, E. and Viegas, C. (2013) Occupational Exposure to Poultry Dust and Effects on the Respiratory System in Workers. *Journal of Toxicology*, **76**, 230-239. http://dx.doi.org/10.1080/15287394.2013.757199

[14] Rietschel, E.T., Kirikae, T., Schade, U., Ulmer, A.J., Holst, O., Brade, H., Schmidt, G., Mamat, U., Grimmecke, H.D., Kusumoto, S. and Zahringer, U. (1993) The Chemical Structure of Bacterial Endotoxin in Relation to Bioactivity. *Immunobiology*, **187**, 169-190. http://dx.doi.org/10.1016/S0171-2985(11)80338-4

[15] Burch, J.B., Svendsen, E., Siegel, P.D., Wagner, S.E., von Essen, S., Keefe, T., Mehaffy, J., Martinez, A.S., Brdford, M., Baker, L., Cranmer, B., Saito, R., Tessari, J., Linda, P., Andersen, C., Christensen, P., Koehncke, N. and Reynolds, S.J. (2010) Endotoxin Exposure and Inflammation Markers among Agricultural Workers in Colorado and Nebraska. *Journal of Toxicology and Environmental Health*, **73**, 5-22. http://dx.doi.org/10.1080/15287390903248604

[16] Duquenne, P., Marchand, G. and Duchaine, C. (2012) Measurement of Endotoxins in Bioaerosols at Workplace: A Critical Review of Literature and a Standardization Issue. *The Annals of Occupational Hygiene*, **57**, 137-172. http://dx.doi.org/10.1093/annhyg/mes051

[17] Simpson, J.C., Niven, R.M., Pickering, C.A., Oldham, L.A., Fletcher, A.M. and Francis, H.C. (1999) Comparative Personal Exposures to Organic Dusts and Endotoxin. *The Annals of Occupational Hygiene*, **43**, 107-115. http://dx.doi.org/10.1093/annhyg/43.2.107

[18] Whyte, R.T. (2002) Occupational Exposure of Poultry Stockmen in Current Barn Systems for Egg Production in the United Kingdom. *British Poultry Science*, **43**, 364-373. http://dx.doi.org/10.1080/00071660120103639

[19] Riva, D.R., Magalhaes, C.B., Lopes, A.A., Lancas, T., Malm, O., Valenca, S.S., Saldiva, P.H., Faffe, D.S. and Zin, W.A. (2011) Low Dose of Fine Particulate Matter ($PM_{2.5}$) Can Induce Acute Oxidative Stress, Inflammation and Pulmonary Function Impairment in Healthy Mice. *Inhalation Toxicology*, **23**, 257-267. http://dx.doi.org/10.3109/08958378.2011.566290

[20] Rylander, R. and Carvalheiro, M.F. (2006) Airway Inflammation among Workers in Poultry Houses. *International Archives of Occupational and Environmental Health*, **79**, 487-490. http://dx.doi.org/10.1007/s00420-005-0072-5

[21] Spaan, S., Wouters, I., Oosting, I., Doekes, G. and Heederik D. (2006) Exposure to Inhalable Dust and Endotoxins in Agricultural Industries. *Journal of Environmental Monitoring*, **8**, 63-72. http://dx.doi.org/10.1039/B509838F

[22] Radon, K., Schulze, A., van Strien, R., Ehrenstein, V., Praml, G. and Nowak, D. (2005) Respiratory Health and Allergy among Young Adults from Rural Areas of Lower Saxony, Germany. *Proceedings of* 50*th Annual Meeting of the German Society for Medical Informatics*, *Biometrics and Epidemiology*, Freiburg, 15 September 2005.

[23] American Conference of Governmental Industrial Hygienists (ACGIH) (2001) Documentation of the TLVs and BEIs, 7th Edition.

[24] Dutch Expert Committee on Occupational Standards (DECOS) (2010) Endotoxins: Health-Based Recommended Occupational Limit. Publication No. 2010/04OSH. The Health Council of the Netherlands, The Hague.

[25] Reynolds, S.J., Thorne, P.S., Donham, K.J., Croteau, E.A., Kelly, K.M., Lewis, D., Whitmer, D.J.J., Heederik, J.D., Connaughton, I., Koch, S., Mamberg, P., Larsson, B.M. and Milton, D.K. (2002) Interlaboratory Comparison of Endotoxin Assays Using Agricultural Dusts. *American Industrial Hygiene Association Journal*, **63**, 430-438. http://dx.doi.org/10.1080/15428110208984731

[26] Thorne, P.S., Perry, S.S., Saito, R., O'Shaughnessy, P.T., Mehaffy, J., Metwali, N., Keefe, T., Donham, K.J. and Reynolds, S.J. (2010) Evaluation of the *Limulus* Amebocyte Lysate and Recombinant Factor C Assays for Assessment of Airborne Endotoxin. *Applied and Environmental Microbiology*, **76**, 4988-4995. http://dx.doi.org/10.1128/AEM.00527-10

[27] Eastman, C., Mitchell, D.C., Bennett, D.H., Tancredi, D.J., Mitloehner, F.M. and Schenker, M.B. (2010) Respiratory Symptoms of California's Dairy Workers. *Field Actions of Science Reports*, Special Issue 2, 1-6.

[28] Eastman, C., Schenker, M.B., Mitchell, D.C., Tancredi, D.J., Bennett, D.H. and Mitloehner, F.M. (2013) Acute Pulmonary Function Change Associated with Work on Large Dairies in California. *Journal of Occupational and Environmental Medicine*, **55**, 74-79. http://dx.doi.org/10.1097/JOM.0b013e318270d6e4

[29] Garcia, J., Bennett, D., Tancredi, D., Schenker, M.B., Mitchell, D.C., Reynolds, S.J., Silva, R., Dooley, G.P., Mehaffy, J. and Mitloehner, F.M. (2012) Characterization of Endotoxin Collected on California Dairies Using Personal and Area-Based Sampling Methods. *Journal of Occupational and Environmental Hygiene*, **9**, 580-591. http://dx.doi.org/10.1080/15459624.2012.713301

[30] Mitchell, D., Armitage, T.L., Schenker, M.B., Bennett, D.H., Tancredi, D.J., Langer, C.E., Reynolds, S.J., Dooley, G., Mehaffy, J. and Mitloehner, F.M. (2015) Particulate Matter, Endotoxin, and Worker Respiratory Health on Large Cal-

ifornian Dairies. *Journal of Occupational and Environmental Medicine*, **57**, 79-87.
http://dx.doi.org/10.1097/JOM.0000000000000304

[31] Poole, J., Dooley, G., Saito, R., Burrell, A.M., Bailey, K.L., Romberger, D.J., Mehaffy, J. and Reynolds, S.J. (2010) Muramic Acid, Endotoxin, 3-Hydroxy Fatty Acids, and Ergosterol Content Explain Monocyte and Epithelial Cell Inflammatory Responses to Agricultural Dusts. *Journal of Toxicology*, **73**, 684-700.
http://dx.doi.org/10.1080/15287390903578539

[32] Carty, C., Gehring, U., Cyrys, J. Bischod, W. and Heinrich, J. (2003) Seasonal Variability of Endotoxin in Ambient Fine Particulate Matter. *Journal of Environmental Monitoring*, **5**, 953-958. http://dx.doi.org/10.1039/b308488d

[33] Dungan, R.S. and Leytem, A.B. (2011) Ambient Endotoxin Concentrations and Assessment of Offsite Transport at Open-Lot and Open-Freestall Dairies. *Journal of Environmental Quality*, **40**, 462-467.
http://dx.doi.org/10.2134/jeq2010.0363

[34] Nieuwenhuijsen, M.J., Noderer, K.S., Schenker, M.B., Vallyathan, V. and Olenchock, S. (1999) Personal Exposure to Dust, Endotoxin, and Crystalline Silica in California Agriculture. *The Annals of Occupational Hygiene*, **43**, 35-42.
http://dx.doi.org/10.1093/annhyg/43.1.35

[35] Huneau-Salaün, A, Le Bouquin, S., Bex-Capelle, V., Huonnic, D., Balaine, L., Guilam, M.T., Squizani, F., Segala, C. and Michel, V. (2011) Endotoxin Concentration in Poultry Houses for Laying Hens Kept in Cages or in Alternative Housing Systems. *British Poultry Science*, **52**, 523-530. http://dx.doi.org/10.1080/00071668.2011.617728

[36] Heinrich, J., Pitz, M., Bischof, W., Krug, N. and Born, P.J.A. (2003) Endotoxin in Fine ($PM_{2.5}$) and Coarse ($PM_{2.5-10}$) Particle mass of Ambient Aerosols. A Temporo-Spatial Analysis. *Atmospheric Environment*, **37**, 3659-3667.
http://dx.doi.org/10.1016/S1352-2310(03)00467-9

[37] Reynolds, S.J., Nakatsu, J., Tillery, M., Keefe, T., Mehaffy, J., Thorne, P.S. Donham, K., Nonnenmann, M., Golla, V. and O'shaughnessy, P. (2009) Field and Wind Tunnel Comparison of Four Aerosol Samplers Using Agricultural Dusts. *The Annals of Occupational Hygiene*, **53**, 585-594. http://dx.doi.org/10.1093/annhyg/mep021

[38] Rimac, D., Macan, J., Varnai, V.M., Vucemilo, M., Matkovic, K., Prester, L., Orcht, T., Trosic, I. and Pavicic, I. (2010) Exposure to Poultry Dust and Health Effects in Poultry Workers: Impact of Mould and Mite Allergens. *International Archives of Occupational and Environmental Health*, **83**, 9-19. http://dx.doi.org/10.1007/s00420-009-0487-5

[39] Donaldson, G.C., Seemungal, T., Jeffries, D.J. and Wedzicha, J.A. (1999) Effect of Temperature on Lung Function and Symptoms in Chronic Obstructive Pulmonary Disease. *European Respiratory Journal*, **13**, 844-849.
http://dx.doi.org/10.1034/j.1399-3003.1999.13d25.x

[40] Li, S., Baker, P.J., Jalaludin, B.B., Marks, G.B., Denison, L.S. and Williams, G.M. (2014) Ambient Temperature and Lung Function in Children with Asthma in Australia. *European Respiratory Journal*, **43**, 1059-1066.
http://dx.doi.org/10.1183/09031936.00079313

[41] Spicer, W.S., Reinke, W.A. and Kerr, H.D. (1966) Effects of Environment upon Respiratory Function. II. Daily Studies in Patients with Chronic Obstructive Lung Disease. *Archives of Environmental Health*, **13**, 753-762.
http://dx.doi.org/10.1080/00039896.1966.10664660

[42] Bonlokke, J.H., Veillette, M., Meriaux, A., Duchaine, C. and Cormier, Y. (2012) Work-Related Health Effects in Swine Building Workers after Respiratory Protection Use. *Journal of Occupational and Environmental Medicine*, **54**, 1126-1132. http://dx.doi.org/10.1097/JOM.0b013e31825461f4

[43] Health and Safety Executive (HSE) (2010) RR655 Exposure to Dust and Bioaerosols in Poultry Farming.

[44] Mirabelli, M., Chatterjee, A., Arcury, T., Mora, D.C., Blocker, J.N., Grzywacz, J.G., Chen, H., Marín, A.J., Schulz, M.R. and Quandt, S.A. (2012) Poultry Processing Work and Respiratory Health of Latino Men and Women in North Carolina. *Journal of Occupational and Environmental Medicine*, **54**, 177-183.
http://dx.doi.org/10.1097/JOM.0b013e31823d86f2

[45] Mitchell, D. and Schenker, M.B. (2008) Protection against Breathing Dust: Behavior over Time in Californian Farmers. *Journal of Agricultural Safety and Health*, **14**, 189-203. http://dx.doi.org/10.13031/2013.24350