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ENDOTOXIN EXPOSURE AND CHANGES IN SHORT-TERM PULMONARY FUNCTION AMONG SEWAGE WORKERS

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Abstract
Objectives: The inhaled endotoxin is considered as a causative factor in the process of acute bronchial obstruction, which can be measured by a decrease in forced expiratory volume in 1 s (FEV_1). The aim of this study was to assess endotoxin exposure among sewage treatment plant workers (STPW) and its effect on across-shift changes in respiratory airflow. **Material and Methods:** A group of 78 STPW from a large sewage treatment plant was studied. Inhalable dust for endotoxin assessment was collected using personal aerosol samplers. Endotoxin was assayed with the kinetic, chromogenic *Limulus* amebocyte lysate test. Across-shift spirometric measurements were performed on Mondays, after 2-days absence from work, with the use of portable spirometer. The forced vital capacity (FVC), and FEV_1 parameters were analyzed. Multifactor regression modeling was performed to determine parameters significantly associated with endotoxin exposure. **Results:** The concentration of inhalable dust and endotoxin ranged from 0.01–1.38 mg/m^3 and 0.68–214 endotoxin units per cubic meter of air (EU/m^3), respectively. Endotoxins were characterized with the skewed distribution (arithmetic mean (AM) = 38.8 EU/m^3, geometric mean (GM) = 15.4 EU/m^3, geometric standard deviation (GSD) = 4.21). Through the use of multifactor analysis, which excluded the main confounders (inhalable dust and smoking habit) it was found that, despite low levels of endotoxin, it had significant impact on the observed across-shift decline in FEV_1 ($p = 0.044$). For this parameter, the regression slope was additionally calculated ($r = –0.017$, $p = 0.071$). **Conclusions:** Relatively low levels of endotoxin among sewage treatment plant workers may cause small, but significant across-shift declines in FEV_1. The observed relationship was independent of organic dust concentrations and smoking habit. The respiratory protection should be provided for STPW.

Key words:
Sewage workers, Endotoxin exposure, Inhalable dust, Smoking, Lung function, Regression analysis

INTRODUCTION

Bioaerosols are complex mixtures containing a number of agents that can cause inflammation or lung function changes, like endotoxin, allergens, mycotoxins and (1→3)-β-D-glucans. According to current knowledge, endotoxin is characterized as the main inflammatory factor in occupational environments [1]. The inhaled endotoxin has a causative role in the acute bronchial obstruction, which can be measured by a decrease in forced expiratory volume in 1 s (FEV_1). The change in FEV_1 is usually measured before and after workshift and is presented as the "across-shift ΔFEV_1" [2].

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Endotoxin-related pulmonary function changes have been shown in experimental studies [3] as well as in a number of field studies. They covered various occupational groups such as: cotton workers [4], poultry workers [5], animal feed and grain workers [6] and potato processing workers [7]. Unfortunately, similar studies in sewage treatment plants are still insufficient. Exposure to endotoxin has been also assessed in this branch, however, the analysis was focused on evaluation of the relationship with workers' health symptoms, mainly from the respiratory tract [8,9]. In the newest study, Heldal et al. [10] tried to assess across-shift changes of lung function according to endotoxin exposure among sewage workers; however, no relationship was found.

All these facts show that there is still a need to carry out in-depth study of this phenomenon among sewage workers. The aim of this work was to assess endotoxin exposure among sewage treatment plant workers and its effect on short-term changes in respiratory airflow.

MATERIAL AND METHODS

Population under study

A group of 78 sewage treatment plant workers (STPW) was studied in the Combined Sewage Treatment Plant in Central Poland. Workers were divided into 5 subgroups according to the phase of the treatment process where they worked:

- mechanical treatment workers (MTW),
- biological treatment workers (BTW),
- sewage sludge treatment workers (SSTW),
- operation control workers (OCW),
- administrative workers (ADW).

The study covered all workers in the plant who had worked morning shift from 6 a.m. to 2 p.m. during measurement period. All included participants were men. Characteristics of the study population are shown in Table 1. On average, subjects were 43 years old and worked for 8.5 years in this sewage plant.

Endotoxin sampling and analysis

Total airborne dust for endotoxin assessment was collected with the use of personal Apex pumps (Casella, Ltd., UK) and 7-hole inhalable aerosol samplers (Casella, Ltd., UK) with 25 mm glass fiber (GF/F) filters (Whatman, Poland). The sampling time was 6 h with a calibrated air flow of 2 l/min. Measurements were conducted every Monday from the beginning of June through July. In each subject 1 measurement was carried out. After each sampling day, the filters were placed into sterilized cassette containers and stored frozen

Table 1. Study population by age, smoking habit and years of employment

Parameter	Sewage treatment plant workers (N = 78)					
	MTW	BTW	SSTW	OCW	ADW	total (M)
Workers [n]	9	13	14	28	14	
Age [years]	39	46	41	46	40	43.0
Smokers [n]						
current or previous	6	5	7	20	5	9.0
never	3	8	7	8	9	7.0
Employment in this plant [years]	12	9	8	9	6	8.5

MTW – mechanical treatment workers; BTW – biological treatment workers; SSTW – sewage sludge treatment workers; OCW – operation control workers; ADW – administrative workers; M – mean.

at –20°C. Endotoxins were analyzed after all the samples were collected.
Endotoxin was assayed with the LAL (*Limulus* amebocyte lysate) test in a kinetic, chromogenic version (Lonza, Ltd., USA). The samples of inhalable dust collected on glass fiber filters were extracted with 10 ml of LAL water with an addition of 0.05% Tween 20. All samples were shaken for 60 min and then centrifuged at 1000×G for 15 min. Endotoxin concentrations were measured with a temperature-controlled microplate reader SpectraMax Plus384 (Molecular Devices, USA) at a wavelength of 405 nm and 37°C. The results were obtained by means of comparing the samples to the standard curve, which was generated from 2-fold serial dilutions of 15 EU/ng *Escherichia coli* 055:B5 control standard endotoxin (CSE). Endotoxin values below the detection limit (3% of samples) were substituted by the lowest determined value divided by the square root of 2. Results were expressed in endotoxin units per cubic meter of air (EU/m^3).

Spirometry

The spirometry was performed using a portable spirometer Spirolab II (MIR, Italy), which has digital turbine flow sensor that required no calibration and complied with the stringent American Thoracic Society [11] 24/26 waveforms. Across-shift spirometric measurements were performed on Mondays, after 2-day absence from work, just before beginning of a working shift and after minimum 6 h of continuous work. At least 3 acceptable forced expiratory surveys were obtained from each subject on each test occasion and measurement with the highest values in forced vital capacity (FVC) and FEV_1 were used for further analysis. The FVC, and FEV_1 parameters were analyzed. Across-shift changes were calculated for each of them. Each worker was asked not to smoke for at least 1 h before spirometry. Additionally the subjects were inquired about current and previous smoking habits as well as employment period in this plant. Only 1 out of 78 spirometry results was unreliable and was excluded from the analysis. The study was conducted under an approved study protocol from the Regional Bioethical Committee.

Statistical methods

For endotoxin and inhalable dust concentrations, the arithmetic (AM) and geometric mean (GM) were calculated as the measure of central tendency, while geometric standard deviation (GSD) was used as indicator of variation. Normality of the distributions of exposure variables were tested by Shapiro-Wilk statistic. Concentration values of endotoxin and inhalable dust were log-transformed for regression analysis.
The evaluation of the changes in the spirometry parameters during a working shift was performed with the use of a linear regression model, in which the morning measure results were included as a predictor variable. In this way, the influence of the parameters that were constant during the work shift and affected the spirometry results was eliminated. We adjusted for strong predictors even if they were not confounders to gain some precision in standard errors of estimates [12].
The parameters describing the exposure during a work shift (endotoxins, inhalable dust and smoking status) were examined individually in a univariate linear regression model. A multifactor model was created to account for parameters significantly correlated with the spirometric variables. All statistics were calculated by R software package (Free Software Foundation, USA). For statistical inference, a $p < 0.05$ was considered significant.

RESULTS

Inhalable dust and endotoxin exposure

Detailed results of inhalable dust and endotoxin exposure among STPW are presented in Table 2. The concentration of airborne inhalable dust was in the range 0.01–1.38 mg/m^3 with an AM = 0.12 mg/m^3

and GM = 0.10 mg/m^3, GSD = 2.04. Endotoxin concentration levels ranged 0.68–214 EU/m^3. Based on AM, the highest levels of endotoxin exposure were found in sewage sludge treatment workers – SSTW (89.5 EU/m^3) while the workers the biological treatment (BTW and the mechanical treatment departments (MTW) were less exposed (63.7 EU/m^3 and 49.7 EU/m^3, respectively). As shown in Table 2, relatively large differences between AM and GM were due to the skewed distribution of endotoxin values in each analyzed group. A weak positive correlation between the level of inhalable dust and endotoxin concentrations was found ($r = 0.33$, $p = 0.003$).

Assessment of lung function

The results of the assessment of lung function in STPW show that the mean values of the 2 basic spirometric parameters, FVC and FEV_1, declined during the workshift (Table 3). The study showed that the occupational exposure described above can influence this small reduction of spirometry results (Table 4).

In particular, through the use of multifactor analysis, which excluded the main confounders (inhalable dust and smoking habit) it was found that, despite low levels of endotoxin, it had significant impact on the observed across-shift decline in FEV_1 ($p = 0.044$). For this parameter, the regression slope was additionally calculated, however, its value was not significant ($r = -0.017$, $p = 0.071$). The influence of smoking status on FEV_1 ($p = 0.05$) was shown to be on the border of significance. However, the analysis showed that smoking was a significant factor strongly influencing the decline in FVC ($p = 0.003$). In this study, there was no evidence that the across-shift changes of the analyzed spirometric parameters would have been affected by the concentration of inhalable dust.

Table 3. Spirometric parameters in sewage treatment plant workers (STPW) by department

Occupational group	FVC [l]		FEV_1 [l]	
	before workshift	after workshift	before workshift	after workshift
Total	4.64	4.60	4.00	3.98
MTW	4.99	4.87	4.32	4.24
BTW	5.04	4.96	4.21	4.13
SSTW	4.80	4.74	4.08	4.10
OCW	4.49	4.50	3.90	3.91
ADW	4.21	4.15	3.70	3.71

FVC – forced vital capacity; FEV_1 – forced expiratory volume in 1 s.
Other abbreviations as in Table 1.

Table 2. Inhalable dust and endotoxin concentrations in sewage treatment plant workers (STPW) by department

Occupational group	Dust samples [n]	Inhalable dust				Endotoxin			
		AM [mg/m^3]	GM [mg/m^3]	GSD	range (min.–max)	AM [EU/m^3]	GM [EU/m^3]	GSD	range (min.–max)
Total	78	0.13	0.10	2.04	0.01–1.38	38.8	15.4	4.21	0.68–214
MTW	9	0.14	0.10	2.87	0.01–0.46	49.7	34.2	2.82	4.04–116
BTW	13	0.10	0.07	2.65	0.01–0.24	63.7	45.7	2.23	18.5–207
SSTW	14	0.24	0.16	2.20	0.05–1.38	89.5	27.3	9.79	0.68–214
OCW	28	0.13	0.11	1.62	0.04–0.47	16.7	10.8	2.55	1.85–85.0
ADW	14	0.09	0.07	1.89	0.02–0.20	6.09	4.41	2.22	1.26–23.3

AM – arithmetic mean; GM – geometric mean; GSD – geometric standard deviation; min. – minimal value; max – maximal value.
Other abbreviations as in Table 1.

Table 4. Linear regression analysis of parameters describing the exposure during the work shift and spirometric parameter changes

Parameter	Single-factor model			Multifactor model		
	β	SE	p	β	SE	p
Endotoxin[a]						
FVC	–0.045	0.028	0.116	–0.052	0.027	0.058
FEV_1	–0.037	0.020	0.071	–0.042	0.021	0.044*
Inhalable dust[b]						
FVC	0.051	0.058	0.376	0.109	0.056	0.055
FEV_1	–0.018	0.045	0.683	0.019	0.047	0.689
Smoking status[c]						
FVC	–0.126	0.047	0.009*	–0.144	0.047	0.003*
FEV_1	–0.079	0.044	0.078	–0.087	0.044	0.050

In a multifactor model adjusted for: [a] smoking status and inhalable dust exposure; [b] smoking status and endotoxin exposure; [c] endotoxin and inhalable dust exposure.
β – β coefficient; SE – standard error of β.
* Statistically significant.
Other abbreviations as in Table 3.

DISCUSSION

The aim of this study was to assess only short-term changes in pulmonary function of STPW exposed to endotoxin. Inhalable dust concentrations among sewage workers were small and well below the Polish occupational exposure limit of 4 mg/m^3 for organic dust [13]. Perhaps the type of primarily water-based aerosols emitted at workplaces could be responsible for the low levels of inhalable dust.

The mean endotoxin concentrations were nearly 2 orders of magnitude lower than the Polish reference value for endotoxin of 2000 EU/m^3 [14]. This value has been proposed for concentrations determined with the use of volumetric methods, for rooms contaminated with organic dust. It is true that, in the present study, the demonstrated concentrations of organic dust were low, but these workplaces could not be classified as public facilities, hence the use of this value for this type of occupational environment was fully justified. Comparing the obtained data with recommendations prepared by the Dutch Expert Committee on Occupational Safety in collaboration with the Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals [2], about 14.2% of individual results were above the health-based occupational exposure limit of 90 EU/m^3.

Taking the latter into consideration, none of the mean concentrations in analyzed occupational subgroups exceeded that value. The highest endotoxin exposures (89.5 EU/m^3) were found in the group of sewage sludge treatment workers, and confirmed that the treatment processes in this job category were significant sources of airborne endotoxins. The concentrations determined in that subgroup corresponded to data reported by Spaan et al. [15]; however, the variability in endotoxin exposure was twice lower than in the present study (GM = 32.4 EU/m^3, GSD = 3.7). In other studies, the trend is similar. Thorn et al. [16] as well as Douwes et al. [10] measured the highest endotoxin concentrations among workers employed in the processes of sludge dewatering (1850 EU/m^3 and 85.6 EU/m^3, respectively). Also the work of Heldal et al. [10] showed that the exposure to endotoxin was higher in sewage plants with sludge treatment (GM = 72 EU/m^3, GSD = 5.9) compared to plants without that process (GM = 12 EU/m^3, GSD = 8.9). Similar to other studies on endotoxin exposure in different working environments [10,15,17], it was also found that variability in endotoxin exposure was generally larger than the variability in dust exposure.

The 1st study on sewage workers health reported widespread inflammaging symptoms, increased airway responsiveness, but no effect on FEV_1 [18]. Contradictory data were reported in a study from Croatia [19], however, there were no explanations for the reasons of this phenomenon. Our paper describes the 1st research carried out in sewage treatment plant showing that relatively low exposure to endotoxin can induce airflow obstruction illustrated by small but significant across-shift declines in FEV_1. Importantly, the reported relationships were independent of inhalable dust concentrations as well as smoking status.

Changes of that parameter were much lower than those observed in experiment conducted by Castellan et al. [3] with simulation tests where healthy volunteers inhaled a given amount of endotoxins in cotton dust. A strong correlation was found to occur between endotoxin concentrations and percentage of changes in FEV_1 (coefficient of correlation $r = -0.74$, $p < 0.0001$). In our study we found a weak negative linear correlation ($r = -0.017$, $p = 0.071$) between these variables. This difference is likely a consequence of the narrow range of low endotoxin concentrations among STPW. In Castellan's study, the ranges were much wider: 60–7790 EU/m^3, while in our study the range among STPW was 0.68–214 EU/m^3, and most of the results did not exceed the level of 90 EU/m^3. Additionally, Castellan's study was affected by a remarkable selection bias because of the preference for including reactive volunteers without respiratory symptoms who demonstrated reproducible responses to endotoxin during screening. In our on-site research, this kind of selection was impossible; however, the measurements were conducted on Mondays, after 2-days absence from work, when the endotoxin-induced symptoms were expected to be most severe [20].

Due to negligible levels of main irritating and toxic gas – hydrogen sulfide (much below the Polish minimum admissible concentration (MAC) value of 2 mg/m^3) [14], we could exclude this factor from the analysis.

The results described above are encumbered with some weaknesses. The observed across-shift changes in FEV_1 can also arise due to the circadian rhythm. In healthy subjects, they can be as high as 1–2% of the decline in FEV_1 [21]. Previous studies have shown widespread symptoms among sewage treatment workers [22] which, together with observed changes in spirometry before and after work-shift, could be caused by other immunoreactive agents of microbial origin which were not analyzed, like (1→3)-β-D-glucans or peptidoglycans. Especially (1→3)-β-D-glucans (components of fungal cell wall) may be responsible for declines in FEV_1 [23], as well as they can show additive or synergistic effects with bacterial endotoxins [24]. It was found that concentrations of (1→3)-β-D-glucans in sewage treatment plant can range between 1.38–11 ng/m^3 [25]. It also does not seem reasonable to exclude that our results are free from the healthy worker effect [26].

The presented research revealed that airway inflammation may occur at endotoxin levels below 90 EU/m^3 – the proposed health-based occupational limit [2]. Similar relationships were also observed by Zock et al. [7] among potato processing workers and Milton et al. [27] in a fiberglass manufacturing facility. In the 1st case, significant across-shift falls in lung function were found above 53 EU/m^3 and in the other one – above 40 EU/m^3. In the present study, the positive significant reaction of the respiratory tract occurred when the geometric mean endotoxin concentration reached 15.4 EU/m^3.

All these 3 cases have no implication on the previously established occupational health limit; however, they show that, in some occupational environments, special circumstances may occur in which the respiratory tract response may appear at lower endotoxin concentrations. It should be noted that exposure to endotoxin in our study was determined using a 6-h sampling value. This fact could increase the risk that relevant peak exposures were diluted. It has previously been shown that exposure to endotoxin in sewage treatment plants varies much between work sites [16]. It has been also found that the peak values of 15–20 min duration are sufficient to cause an inflammatory response [28].

Additionally, the inflammatory response may be due to qualitative variability of Gram-negative bacteria in each environment, which can cause differences in real endotoxin activity inhaled into the lungs. The study of Hansen et al. [29] has revealed that endotoxin activity depends on the species of bacteria from which it is derived. Moreover, it has been noted that this potential may differ depending on the method of measurement (LAL assay vs. A549 bioassay). In LAL assay, endotoxin derived from *Escherichia coli* and *Salmonella enteritidis* showed the greatest potential, but when the A549 bioassay was used (it can measure the amount of IL-8 released from the pulmonaty epithelial cells) the greatest activity was shown by the endotoxin from *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. It should be noted that the previous study carried out in this sewage treatment plant showed Gram-negative rods of *Pseudomonas* and *Burkholderia* genera as predominant in the air at workplaces [30].

CONCLUSIONS

This study demonstrated small, but significant across-shift declines in FEV_1, associated with endotoxin exposure among sewage treatment plant workers. These changes were found at relatively low levels of endotoxin. The results revealed that this association was independent of organic dust concentrations and smoking habit. Due to the airway inflammation observed in sewage treatment plant workers, it is advisable to provide exhaust ventilation and respiratory protection, particularly for operators of sewage sludge treatment processes.

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