Ann. Occup. Hyg., Vol. 50, No. 7, pp. 731–736, 2006

doi:10.1093/annhyg/mel026

Influence of Different Cleaning Practices on Endotoxin Exposure at Sewage Treatment Plants

M. J. VISSER[1], S. SPAAN[1]*, H. J. J. M. ARTS[2], L. A. M. SMIT[1] and D. J. J. HEEDERIK[1]

[1]*Environmental and Occupational Health Division, Institute for Risk Assessment Sciences, Utrecht University, PO Box 80176, 3508 TD Utrecht, The Netherlands;*
[2]*Arboprofit, Voorwillenseweg 18, 2806 ZG Gouda, The Netherlands*

Received 26 October 2005; in final form 19 April 2006; published online 16 June 2006

Exposure to endotoxin at sewage treatment plants is associated with an increased prevalence of work-related symptoms in sewage workers. Since cleaning activities are regarded as an important determinant of endotoxin exposure, workers' endotoxin exposure levels during different cleaning activities were compared in an experimental setting. Variables considered were water used (tap water, surface water or effluent), water pressure (low or high pressure, and a fire hose with the mouth open or obstructed), presence of mechanical ventilation and the distance between the worker and the object to be cleaned (concentration gradient). Experimental cleaning scenarios were defined, during which endotoxin exposure was measured with personal and stationary air sampling. Data were statistically analyzed with mixed effects models. The water used for cleaning appeared to have a large influence on endotoxin exposure, especially the use of effluent, which caused a factor 2.4 increase in exposure. Use of high pressure did not significantly add to the exposure. Use of a fire hose with fully opened mouth (spout opening) led to a 3-fold decrease in exposure when compared with a partially obstructed mouth. The presence of mechanical ventilation decreased endotoxin concentration in a room, provided that the capacity of the ventilation system was sufficient. The worker's distance to the object that was cleaned did not significantly influence exposure.

Keywords: cleaning; effluent; endotoxin; pressure; sewage treatment plant; ventilation; wastewater

INTRODUCTION

Sewage treatment workers can be professionally exposed to a wide variety of chemical and biological agents. There is an increased prevalence of airway, flu-like, gastrointestinal and neurological symptoms, and joint pains in sewage workers (Khuder *et al*., 1998; Friss *et al*., 1999; Rylander, 1999; Douwes *et al*., 2001; Thorn *et al*., 2002a). In several recent studies, exposure to endotoxin has been suggested to be the most probable cause of these symptoms (Rylander, 1999; Douwes *et al*., 2001; Thorn and Kerekes, 2001; Smit *et al*., 2005). Endotoxin is a cell wall component of Gram-negative bacteria that is released when bacteria die and lysis occurs. Since high amounts of Gram-negative bacteria are present in sewage, it always contains endotoxins. Acute effects of inhaled endotoxin observed in healthy volunteers, who inhaled pure endotoxin, are dry cough, shortness of breath, decreased lung function, fever and malaise. Several hours after exposure chest tightness, joint pains and headache can occur. Chronic exposure to endotoxin may lead to chronic bronchitis and a decreased lung function (Heederik and Douwes, 1997; Michel, 2000). In the Netherlands, a temporary legal limit for endotoxin was set at 200 EU (Endotoxin Units) m^{-3} as the mean exposure over an 8 h work shift. However, this limit was withdrawn because of feasibility issues, mainly in agricultural industries. The health-based recommended occupational exposure limit (HBROEL) for endotoxin is 50 EU m^{-3} (Heederik and Douwes, 1997).

Sewage workers can be exposed to endotoxins through contact with raw sewage or sludge, for example, after development of aerosols (small, airborne water droplets) by workers' activities. Since inhalation of aerosols is the main exposure route for endotoxin and cleaning activities generally produce high levels of aerosols, cleaning activities are

*Author to whom correspondence should be addressed.
Tel: +31-30-253-9474; fax: +31-30-253-9499;
e-mail: S.Spaan@iras.uu.nl

regarded as an important determinant of exposure on sewage treatment plants (STPs) (Rylander, 1999; Smit *et al.*, 2005). Therefore, this study aims to quantify sewage workers' endotoxin exposure during different cleaning activities. Experimental exposure scenarios were created, comparing different ways of cleaning with respect to kind of water used, water pressure used, presence of mechanical ventilation, and distance between the worker and the object to be cleaned (concentration gradient).

METHODS

Study design

This study is part of a larger study on endotoxin exposure and health effects of wastewater treatment workers (Smit *et al.*, 2005). Exposure data will be presented in a different paper. The study described here focuses on the influence of different cleaning practices on endotoxin exposure. At three different STPs cleaning activities were carried out in several experimental settings. The STP's were selected from those participating in the larger study based on the possibility to use different kinds of water and water pressures and the presence of mechanical ventilation. A number of cleaning activities were selected where there was an expected risk of contact with sewage water or sewage sludge, because it is known that these contain high concentrations of endotoxin, especially the sludge (Thorn *et al.*, 2002b). These objects were cleaned with varying use of water (stored tap water, effluent or surface water from a nearby river), pressure (high pressure, or low pressure using a fire hose) and mechanical ventilation (on or off). The objects were two influent screens where large pieces of waste are removed, a grit chamber where particles can settle before the sewage is treated further, a sludge buffer tank, a heat exchanger (Fig. 1), and three types of sludge dewatering installations, namely a centrifuge, a belt press and a filter press. Besides these objects, measurements were taken during the cleaning of spilled sludge on the ground outside on the plant area. Table 1 shows which variations in cleaning methods were considered at each measured object. In two experiments, a fire hose was used with an obstructed mouth (to increase pressure), as well as with a fully opened mouth to see if there was a significant difference in pressure. The presence of a concentration gradient was also studied, by taking three stationary measurements at different distances (<1, 1–2 and >2 m) from the object to be cleaned. Personal exposure measurements were taken on the worker who performed the cleaning activities.

Fig. 1. Cleaning of heat exchanger using low pressure.

Measurements

Both personal and stationary measurements were conducted with portable Gilian Gilair5 pumps, in combination with GSP sampling heads (JS Holdings) and 3.7 cm glass fiber filters (Whatman GF/A, UK). The pumps were set to a flow of 3.5 l min^{-1}. After sampling, filters were stored at −20°C. Endotoxin extractions were performed as described earlier (Douwes *et al.*, 1995). In summary, filters were extracted in 5 ml pyrogen-free water with 0.05% Tween-20 and shaken for 1 h. Then the samples were centrifuged at 1000 *g* (2094 r.p.m.) for 15 min. After centrifuging, the supernatant was transferred to pyrogen-free glass tubes and stored at −20°C. Extracts were analyzed with the kinetic chromogenic *Limulus* amoebocyte lysate (LAL) assay (BioWhittaker; lysate lot no. 1L676S, standard lot no. 2L0090, RSE/CSE ratio 11.5 EU ng^{-1}). Also, two samples of the effluent used for cleaning were taken on two different days at Plant B, and one sample was taken of the surface water used for cleaning at Plant A. These samples were centrifuged and then treated in the same way as the filter extracts.

Statistical analysis

Statistical analyses were performed using SAS statistical software (The SAS System for Windows, version 8.02, SAS Institute Inc.). Endotoxin exposure values were log-normally distributed and therefore log-transformed values were used. Separate analysis were carried out for personal and stationary measurements. The influence of water, pressure, ventilation and distance on endotoxin exposure was analyzed by multilevel regression analysis using the MIXED MODELS procedure in which 'experiment' was introduced as a random effect, to correct for possible correlation between duplicate samples. Different models were tested to identify determinants with a significant contribution, and models were judged on their fit and significance of individual variables. The possible presence of a concentration gradient was studied in separate ANOVA models for each different cleaning activity.

Table 1. Mean endotoxin exposure values (EU m^{-3}) measured during different cleaning tasks

STP	Water used	Pressure used	Ventilation	Personal samples		Stationary samples	
				N	GM (range) (EU m^{-3})	*N*	GM (range) (EU m^{-3})
Cleaning influent screens							
A	Tap water	Low	On	2	61 (61–61)	3	70 (48–87)
A	Tap water	Low	Off	2	89 (89–89)	3	86 (72–103)
A	Effluent	Low	On	2	163 (153–175)	3	156 (129–213)
A	Effluent	Low	Off	2	222 (183–269)	1	282 (—)
B	Surface water	Low	On	4	236 (152–648)	12	131 (71–196)
B	Surface water	Low	Off	2	405 (320–514)	6	310 (250–399)
Cleaning centrifuge							
B	Surface water	Low		2	216 (189–248)	6	198 (138–318)
Cleaning belt press							
A	Tap water	High	On	4	37 (26–45)	3	29 (26–34)
A	Tap water	High	Off	3	209 (135–290)	3	144 (102–248)
A	Effluent	Low	On	8	165 (43–601)	6	187 (45–868)
A	Effluent	High	On	8	59 (6–379)	6	78 (10–363)
A	Effluent	High	Off	4	363 (337–416)	3	380 (327–508)
Cleaning filter press							
C	Tap water	Low		2	140 (132–149)	2	72 (60–88)
C	Tap water	High		2	804 (729–887)	2	583 (513–662)
Cleaning sludge buffer tank							
A	Tap water	Low	On	4	52 (30–120)	2	17 (12–24)
A	Tap water	Low	Off	6	28 (4–71)	2	38 (38–38)
A	Tap water	High	Off	2	107 (105–108)	2	91 (78–107)
A	Effluent	Low	On	4	107 (70–361)	2	59 (51–68)
A	Effluent	Low	Off	4	123 (71–205)	2	153 (54–434)
Cleaning of spilled sludge, outside							
B	Effluent	Low		2	8 (7–9)	3	7 (7–7)
B	Surface water	Low		4	29 (12–176)	12	9 (6–21)
Cleaning grit chamber							
B	Surface water	Low	On	0	—	3	6 (4–11)
B	Surface water	Low	Off	2	26 (10–71)	3	7 (7–8)
Cleaning heat exchanger							
A	Tap water	Low		4	71 (67–75)	1	53 (—)
A	Effluent	Low		4	249 (175–305)	1	267 (—)

RESULTS

A total of 81 personal samples and 92 stationary samples were used in the regression analysis. Sampling time varied from 23 min to ~3.5 h for personal samples, and from 16 min to ~7 h for stationary samples. The mean duration of the cleaning tasks was 34 min. Table 1 shows the mean endotoxin concentrations found during the different cleaning activities. Among the personal measurements endotoxin concentrations ranged from 4 to 887 EU m^{-3}. The arithmetic mean was 175 EU m^{-3} (SD 176) and the geometric mean was 103 EU m^{-3} [geometric standard deviation (GSD) 3.1]. A majority (77% of the samples) exceeded the Dutch HBROEL of 50 EU m^{-3}, and the temporal legal limit of 200 EU m^{-3} was exceeded in 30% of the samples. Among the stationary samples, the concentration range was 4–868 EU m^{-3}, with an arithmetic mean of 146 EU m^{-3} (SD 165) and a geometric mean of 67 EU m^{-3} (GSD 4.3).

Results of the regression analysis are shown in Tables 2 and 3. Because for some measurements information on ventilation was missing, a separate model for ventilation was built using only data without missing data. The intercept represents a reference exposure, which is constructed from variables that give the lowest exposure. In this model the use of tap water, use of low pressure and cleaning of spilled sludge outside on the ground were set as references. The regression coefficients show the increase in exposure caused by a certain cleaning activity. For example, the use of effluent caused a more than 2-fold

Table 2. Result of mixed effects models investigating determinants of endotoxin exposure in sewage treatment plants during cleaning tasks

Variable	Personal ($N = 81$)		Stationary ($N = 92$)	
	e*	95% CI	e*	95% CI
Intercept[a]	9.3†	1.1–76.3	3.3†	1.4–7.5
Use of surface water	1.8	0.3–12.5	2.2	0.9–5.7
Use of effluent	2.4†	1.1–5.1	2.6†	1.4–4.8
Use of high pressure	1.3	0.5–3.6	1.4	0.7–2.9
Fire hose, mouth obstructed[b]	3.0	0.5–18.0	1.3	0.5–3.1
Cleaning grit chamber	1.6	0.1–20.1	0.9	0.3–2.9
Cleaning sludge buffer tank	4.7	0.6–40.0	10.8†	3.7–31.2
Cleaning belt press	5.3	0.6–50.3	14.5†	5.4–39.1
Cleaning influent screens	8.2†	1.4–50.0	21.3†	10.4–43.7
Cleaning heat exchanger	9.3	1.0–89.6	22.4†	5.2–96.7
Cleaning centrifuge	13.1†	1.0– 166.8	26.9†	8.1–88.8
Cleaning filter press	31.4†	2.5–391.8	53.1†	15.2–185.5

N: number of samples; $^{\dagger}P < 0.05$; *regression coefficient.
[a]The intercept represents a combination of reference variables that gave the lowest exposures, namely cleaning of spilled sludge outside, with stored tap water and low pressure.
[b]This variable was only measured at the influent screens and the cleaning of spilled sludge outside, and only with surface water.

Table 3. Result of mixed effects models investigating the effect of mechanical ventilation on endotoxin exposure during different cleaning activities

Variable	Personal			Stationary		
	N	e*	95% CI	N	e*	95% CI
Overall						
Intercept (ventilation off)[a]	63	108.7†	59.6–198.3	65	118.9†	66.0–214.2
Ventilation on[a]		0.8	0.4–1.9		0.7	0.3–1.4
Influent screens						
Intercept (ventilation off)[b]	14	199.9†	68.7–581.7	28	180.0†	119.5–271.3
Ventilation on[b]		0.8	0.2–2.8		0.7	0.4–1.1
Belt press						
Intercept (ventilation off)[b]	27	263.1†	77.1–897.8	21	234.3†	81.2–676.3
Ventilation on[b]		0.3	0.1–1.3		0.4	n.e.
Buffer tank						
Intercept (ventilation off)[b]	20	56.9†	21.8–148.5	10	80.9†	19.5–336.6
Ventilation on[b]		1.3	0.3–5.7		0.4	0.1–2.4

N: number of samples, n.e.: not estimated, $^{\dagger}P < 0.05$; *regression coefficient.
[a]Adjusted for water, pressure and object.
[b]Adjusted for water and pressure.

increase in personal exposure compared with the use of tap water, and cleaning of the filter press gave 31 times higher personal exposure than cleaning of spilled sludge outside on the ground. The regression coefficients for the stationary data are higher than those of the personal data for most variables, but generally point in the same direction.

In the mixed effects models the variance in endotoxin level, adjusted for differences between duplicate samples, decreased from 1.08 (GSD 2.8) to 0.93 (GSD 2.6) after introduction of the variable ‘object’ (8 levels). Addition of the variable ‘water’ (3 levels) further reduced the variance to 0.80 (GSD 2.4). Addition of the variable ‘pressure’ (3 levels) did not further reduce the variance, and inclusion of the variables ‘water’ or ‘pressure’ in the model without ‘object’ had only a marginal effect.

Apart from which object was cleaned, kind of water used for cleaning appeared to have the largest influence on endotoxin exposure, with especially effluent causing a large increase in exposure. Use of high pressure did not have an overall significant effect, but when using a fire hose, there appeared to be a 3-fold difference in exposure between using it with its mouth opened or obstructed. Presence of mechanical ventilation did not have a significant effect on the measured endotoxin concentrations, but did decrease endotoxin levels in the room in most cases.

The relation between the distance of the worker to the object and his endotoxin exposure (concentration gradient) was also examined, by comparing stationary samples taken at three different distances during the experiments. However, a significant concentration gradient could not be found in any of the experiments (ANOVA, $P > 0.05$), although the expected negative trend (decreasing concentration with increasing distance) was seen at the filter press and the grit chamber.

Furthermore, two effluent samples and one surface water sample were taken. Samples of effluent and surface water used on the participating STPs showed that the endotoxin concentration was high (though highly variable) in the two effluent samples (900 EU ml^{-1} and 40 000 EU ml^{-1}), and low in the surface water sample (400 EU ml^{-1}).

DISCUSSION AND CONCLUSIONS

The geometric mean of endotoxin concentrations found in this study (82 EU m^{-3}) is in the same range as the geometric mean of 72 EU m^{-3} found for task-based personal sampling in the exposure part of the larger study (Smit *et al*., 2005). In that exposure study, the same methods for sampling and analysis were used and sampling duration was comparable, although measured tasks were not executed in an experimental context. This indicates that the experiments of this study were conducted under realistic circumstances. Thorn *et al*. (2002b) found a geometric mean of 13 EU m^{-3} for personal measurements that were partly task-based, using the same sampling method but a different filter type. Both studies found a range of concentrations (2–2135 and 10–272 EU m^{-3}, respectively) comparable to this study (3–887 EU m^{-3}). Although 70% of the measured mean exposures were below the temporal legal limit of 200 EU m^{-3}, the proposed health-based exposure limit of 50 EU m^{-3} was exceeded in a majority of the samples (77%), which indicates a possible health risk for the workers. However, the occurrence of health effects not only depends on endotoxin concentration in a room, but also on duration of different tasks, the use of personal protection equipment, personal hygiene and individual susceptibility for endotoxins (Michel, 2000; Smit *et al*., 2005).

The models in Tables 2 and 3 can be used to compare a worker's endotoxin exposure during different cleaning activities by multiplying the exponent of the variable's regression coefficient (as shown in the tables) with the intercept. The predicted exposure levels are based on the geometric means. For example, the exposure of a worker who has been cleaning spilled sludge outside, using stored tap water and low pressure, can be estimated at 9.3 EU m^{-3} (the intercept). If this worker had been cleaning the influent screens, his exposure can be estimated at $(9.3 * 8.2) = 76.3$ EU m^{-3}, and if he had been using effluent instead of stored tap water to clean the influent screens, his estimated exposure would be $(9.3 * 8.2 * 2.4) = 183$ EU m^{-3}. However, the models are based on a small number of samples and confidence intervals are large. Therefore, the results should not be interpreted as quantitatively precise estimates, but as indications of the influence of different cleaning methods on exposure to endotoxin.

In the multivariate mixed effects models, endotoxin exposure during cleaning activities appeared to be highly influenced by the kind of water used. Use of effluent increased the endotoxin exposure by more than a factor 2 compared with use of tap water. Cleaning with surface water, however, did not cause a significant increase in personal exposure compared to cleaning with tap water. The influence on exposure of a certain kind of water is determined by the endotoxin concentration in that water, which was indeed higher in the effluent than in the surface water. However, the endotoxin concentration of effluent can vary considerably between different STPs, and also within the same STP in time. This is illustrated by the large difference in endotoxin concentration between our two effluent samples, both taken at Plant A (900 and 40 000 EU ml^{-1}). Consistently, Jorgensen *et al*. found endotoxin concentrations ranging from <3.1 to 12 500 EU ml^{-1} in effluent samples from several advanced STPs in the US, while multiple samples of one STP produced a concentration range from 60 to 6000 EU ml^{-1} (Jorgensen *et al*., 1976, 1979). Because of the high variation in endotoxin content of effluent, the factor found with regression analyses may in fact be underestimated in some situations.

The effect of using high pressure was measured at three different objects: the filter press, belt press and sludge buffer tank. At the filter press (Fig. 2), the use of high pressure caused a high and significant increase in exposure. However, the filter press was cleaned using only tap water. After correction for the kind of water used the effect of high pressure disappeared, which indicates that the kind of water used for cleaning was a more important determinant of endotoxin exposure than the pressure used. Nevertheless, there appeared to be a 3-fold difference between using a fire hose with its mouth either fully opened or obstructed. Because this experiment was done at only two objects while using only surface water, in the total model the use of a fire hose with its mouth fully opened was collated with the use of low pressure. In all other experiments, use of low pressure meant using a fire hose, but the degree of opening of the mouth was not registered. So, in case the mouth of the fire hose had been (partially) obstructed

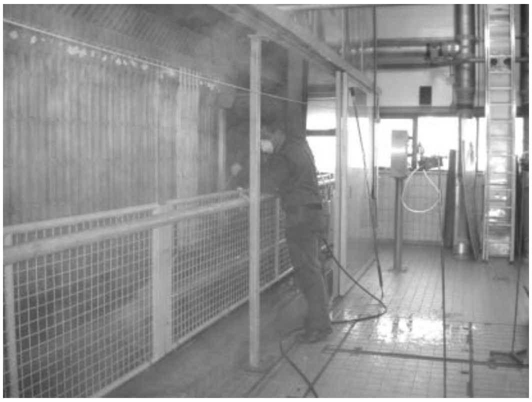

Fig. 2. Cleaning of filter press with high pressure.

during cleaning with low pressure, the effect of obstructing the mouth could have been slightly underestimated. However, separate analysis of the effect of obstructing the mouth of the fire hose (within the experiment) resulted in a very similar estimate, indicating that the assumption was legitimate.

Although not significant, the presence of mechanical ventilation decreased the endotoxin concentration in most rooms. However, ventilation needs to be well tuned for a room to reach a sufficient effect. At the buffer tank, for example, an improvised form of ventilation was constructed by hanging a ventilation hose through a hole in the covering of the closed tank. The data obtained from the personal samples showed no protective effect of ventilation, while the data from stationary samples did show an effect. This can be explained by the fact that the stationary samples were taken near the roof of the tank, near the ventilation hose, while the workers were walking at the bottom of the tank. Apparently, this ventilation hose did not have enough power to ventilate the whole tank.

The presence of a concentration gradient could not be demonstrated in this study. Because there was limited space around some objects, stationary samples were not always placed at similar distances from the object. If the pumps could have been placed at constant distances from the object, with a better dispersal, a concentration gradient may have been visible. However, from a practical point of view, concentration gradients over distances of more than 1–1.5 m are not directly relevant for workers' exposure.

In conclusion, endotoxin exposure during cleaning activities on STP's is mainly influenced by the kind of water used for cleaning and which object is cleaned, and to a lesser extent by the pressure used. Among the different cleaning activities performed in this study, cleaning of a filter press contributed most to the exposure. The use of effluent increased exposure more than 2-fold, while the use of surface water did not significantly increase the exposure compared to using tap water. When using a fire hose, fully opening the mouth may decrease exposure with about a factor 3 compared to using it with the mouth obstructed. Therefore, it is recommended to avoid the use of effluent for cleaning activities, and maintain as low pressure as possible when cleaning. The worker's distance to the object that is cleaned does not significantly influence exposure. Furthermore, the presence of mechanical ventilation can decrease endotoxin concentration in a room, provided that the capacity of the ventilation system is well tuned.

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