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ENDOTOXIN EXPOSURE IN SEWAGE TREATMENT WORKERS: INVESTIGATION OF EXPOSURE VARIABILITY AND COMPARISON OF ANALYTICAL TECHNIQUES

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Abstract: Introduction: Objectives were to give an overview of endotoxin exposure and its determinants in sewage treatment workers, and to study exposure to culturable and non-culturable microorganisms and the applicability of the LAL assay in this work environment. Material and methods: In 43 Dutch sewage treatment plants 470 full-shift, 123 task-based personal and 54 stationary inhalable dust samples were collected. Endotoxin concentration was determined with the LAL-assay. Mixed effects models were used to investigate possible determinants of exposure. Simultaneous parallel filter samples, impinger samples and viable total bacteria and Gram-negative bacterial samples were taken to compare analytical techniques. Filter and impinger samples were analyzed with the LAL-assay, gas chromatography-mass spectrometry (GC-MS) and fluorescence microscopy. Results: Endotoxin exposure levels were moderate to low (geometric mean personal exposure 27 EU/m³, stationary 33 EU/m³, task-based 64 EU/m³), yet differences between jobs and sources and some determinants of exposure were identified. Exposure varied more from day to day than between workers. Concentrations in filter samples were higher and more consistent than in impinger samples. Fungi and Gram-positive bacteria were found in higher levels than Gram-negative bacteria. The LAL assay and GC-MS showed comparable endotoxin levels. Discussion and conclusion: Endotoxin exposure in Dutch sewage treatment workers was relatively low. Comparison of sampling and analytical techniques suggests that the LAL-assay did not result in much exposure misclassification. It thus seems justified to perform filter measurements in combination with the LAL-assay to measure endotoxin exposure in sewage treatment plants.

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Key words: endotoxin, exposure variability, analyzing techniques, culturable and non-culturable microorganisms, sewage.

INTRODUCTION

Sewage treatment is one of the components of waste control to decrease the environmental burden and to control disease in the human population. The sewage treatment process, as schematically shown in Figure 1, removes human pathogens and other physical, chemical and biological contaminants from wastewater by physical, chemical and biological processes, ultimately resulting in a waste stream, sludge and effluent [17]. Much of the biological matter is converted by microorganisms, which thus are present in the sewage treatment plant environment. Sewage and sludge produce a number of gases such as hydrogen sulfide, ammonia, and carbon monoxide. Furthermore, chemicals are

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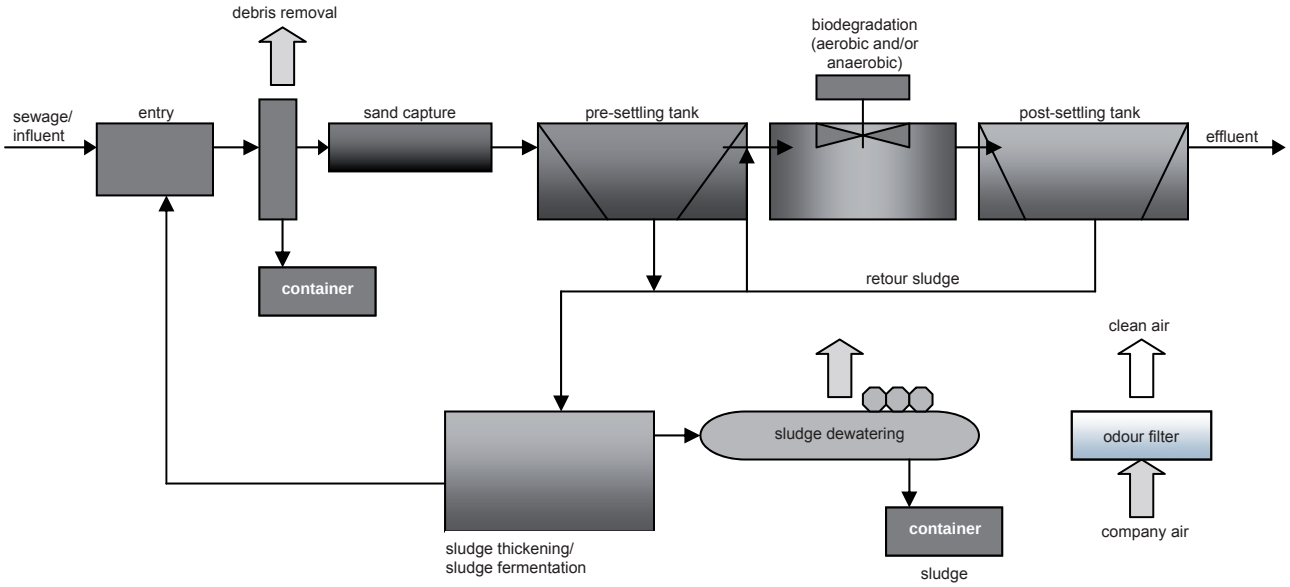

Figure 1. Schematic overview of sewage treatment process in The Netherlands.

used for the treatment of liquid waste and in the cleaning and maintenance of the plants. Consequently, workers in sewage treatment plants are exposed to a large variety of chemicals and microorganisms and their products, among which are endotoxins [17].

Endotoxins are lipopolysaccharides (LPS) present in the outer membrane of Gram-negative bacteria, and inhaled endotoxin is a well-known toxin with high pro-inflammatory potency. Exposure to endotoxin has been associated with several health effects in various agricultural and industrial environments [14, 26]. An increased prevalence of (work-related) airway, flu-like, gastrointestinal and neurological symptoms and joint pain has been observed in sewage workers [2, 5, 8, 13, 15, 16, 24, 30, 35], and in several studies endotoxin exposure has been suggested as the most probable cause of these symptoms [2, 24, 30, 35]. Inhalation is thought to be the most important route of exposure, e.g. after aerosol formation, although contact with raw sewage or sludge (dermal and ingestion) might also play a role [20].

In view of the planned introduction of a health based occupational exposure limit for endotoxin, the aim of this study was to give an overview of exposure to endotoxin in sewage treatment plants. Since determinants of exposure had not been studied in this industry before, this was also incorporated in the study. Although exposure was exceptionally low, the occurrence of health effects was directly related to the endotoxin exposure measured [30]. This might be an indication that either the LAL assay underestimates exposure to endotoxin, at least in this occupational environment, or that other microorganisms with comparable health effects occur in conjunction with determined endotoxin exposure. Therefore, an additional experiment was performed to explore exposure to (viable) microorganisms and endotoxin at sewage treatment plants, and to investigate the relative amount of Gram-negative bacteria to the total microbial load. Furthermore, the performance of the LAL assay relative to the chemical analysis of endotoxin in this work environment was investigated in filter and impinger samples in order to compare sampling and analytical techniques.

MATERIAL AND METHODS

Exposure study. From the 27 Dutch Water Boards, which are responsible for the treatment of sewage in The Netherlands, 21 agreed to participate in the study. Personal inhalable dust and endotoxin exposure was measured in 225 workers from 43 sewage treatment plants. The measurements were performed in 3 periods (June-July, August-October and November 2003), and 8 individual measurements were taken in July 2004. One to 6 measurements per worker were obtained, depending on availability of the workers in the 3 sampling periods, leading to a total of 470 measurements. Furthermore, 54 stationary and 123 task-based measurements were performed to identify possible sources of endotoxin exposure or activities leading to a high endotoxin exposure.

Full-shift and task-based personal inhalable dust samples were collected using GSP sampling heads (JS Holdings) with 37 mm glass fibre filters (Whatman GF/A) in combination with Gilian GilAir5 portable pumps at a flow of 3.5 liter/min. The sampling head was placed on the shoulder of the worker, near the breathing zone, with the inlet facing forward. For the stationary measurements the same equipment was used, with the sampling head placed on a tripod (height 1.5 m) during a full shift. On each sampling day a control filter (field blank) was included, which was handled in the same way as the other samples, except for the actual sampling. The loaded filters were stored at -20°C prior to extraction.

The filters were pre- and post-weighed on an analytical balance in a conditioned room with stable temperature and humidity meeting US EPA criteria, to determine the amount of dust on the filters gravimetrically. Inhalable dust concentrations below the limit of detection (LOD) were assigned a value of 2/3 of the LOD of the balance, which was 0.05 mg. For extraction, each filter was immersed in 5 ml of pyrogen-free water with 0.05% Tween-20 in a glass tube and rocked vigorously for 1 hr at room temperature on a horizontal shaker. After 15 min of centrifugation at 1,000 xG, 1 ml supernatant per sample was collected, vortexed, and 4 aliquots of 0.1 ml, and the remaining 0.6 ml were stored in pyrogen-free glass tubes at -20°C until analysis. Endotoxin concentration in extracts was assayed with the kinetic chromogenic Limulus Amoebocyte Lysate (LAL) method (Cambrex, Verviers, Belgium; lot no. lysate 1L676S, lot no. standard 2L20090 (RSE/CSE ratio 11.5 EU/ng)), in which pyrogen-free water +0.05% Tween-20 was used as assay solution. Samples were assayed at an initial dilution of 1:5, and retested at higher dilutions (up to 1:100) when the measured concentration was too close to the upper detection limit of the assay. 95% of all samples were analyzed in duplicate. Samples with endotoxin levels below the limit of detection (LOD) were assigned a value of 2/3 of the mean LOD of the sampling runs in that period, which was 0.05 EU/ml for all 3 assay periods.

Information about job title, workplace, work activities during the measurements, work environment, etc., was obtained from the workers included in the study. Furthermore, additional information about the job, work activities in general, and use of personal protective equipment was available from a questionnaire, which is described elsewhere [30]. In each plant information about process characteristics and other possible determinants was gathered with a company checklist. Information about weather characteristics for each sampling day was obtained from the website of the Royal Netherlands Meteorological Institute (www.knmi.nl).

Data were analyzed with SAS statistical software (version 9e; SAS Institute, Cary, NC, USA). Endotoxin concentrations were log-normally distributed. Therefore, all calculations were performed with natural log-transformed concentrations. Crude descriptive endotoxin and inhalable dust exposure levels were calculated as arithmetic mean (AM), geometric mean (GM) and geometric standard deviation (GSD) for the full-shift and task-based personal and stationary measurements. Spearman correlations were calculated between inhalable dust and endotoxin concentrations.

Between-worker and day-to-day (within-worker) variance in exposure were determined by applying mixed effects models, with worker identity as a random factor in order to correct for possible correlation between repeated measurements in the same worker. Any 2 repeated measurements of the same worker were assumed to have equal correlation (a compound symmetric covariance structure). Between- and within-variance components were estimated by using a restricted maximum likelihood method (REML). Process characteristics, and information about job, work activities, workplace, weather conditions, etc., were introduced as fixed effects to investigate possible determinants of exposure [19, 22]. For this analysis, a part of the dataset was used (417 out of 470 measurements), because the measurements with missing data for one or more of the determinants of interest were removed from the dataset for stability of the analysis.

Comparison of methods. An additional experiment was carried out to compare several sampling and analytical methods. Five measurement series of 4.5–6 h (for the filter and impinger measurements) were performed on 3 days in August 2004, 2 at the sludge dewatering department and 3 at the debris removal department of a sewage treatment plant. Each measurement series was placed close to each other and consisted of:

• Two simultaneous measurements with the N6-modification of the Andersen sampler and Becker VT3 pumps at a flow of 28.3 l/min. One sampler was equipped with a Tryptone Soya Agar (TSA, for total bacteria, measurement duration 8 min) and one with a Tryptone Soya Agar + 0.001% Kristal violet (TSA+KV, for Gram-negative bacteria, measurement duration 10 min). The measurements started at the same time, were positioned on a tripod at 1.5 m, and were performed shortly after the start of the other measurements at the same location. The plates were incubated at 37°C for 48 h, and the colonies counted at 24 and 48 h. The number of colonies was corrected according to the positive hole conversion method, and then converted to colony forming units per m^3 (CFU/m^3) on the basis of the sampling volume.

• Three measurements with liquid impingers, 2 filled with 15 ml pyrogen-free water (PFW) and one filled with 15 ml 9% saline solution, in combination with Gilian GilAir5 portable pumps set at a flow of 2 l/min, positioned at 1.5 m on tripods. After the measurement on the same day, the amount of liquid was checked, filled up to 20 ml, vortexed, and divided in the following way:

– 2 × 1 ml was stored in tubes to which 0.05% Tween was added, vortexed, and stored at -20°C until analysis with the LAL-assay

– 5 ml was stored in two 5-ml tubes, and stored at -20°C until transport on dry-ice to Lund, Sweden, for analysis with gas chromatography-mass spectrometry (GC-MS)

– 5 ml was filtered through a 25 mm polycarbonate membrane filter (Whatman Nuclepore®, 0.2 μm pore size), the filter dried and stored in a cassette at room temperature until transport to Oslo, Norway, for analysis with fluorescence microscopy (FM)

– 4 × 1 ml was plated on 2 TSA agars and 2 TSA+KV agars, after which the agars were treated as described above.

On 2 of the 3 measurement days, an impinger field blank was also collected, of which the liquid was divided over the analytical methods in the same way as the other samples.

Table 1. Characteristics of personal, stationary and task-based measurements in sewage treatment plants.

	Personal	Stationary	Task-based
# measurements	470	54	123
# sewage treatment plants	43	22	28
# workers	225	–	82
# measurements per worker	1–6	–	1–5
Mean sampling time (hr)	6.4 (range 1.3–8.2)	5.9 (range 0.5–8.7)	0.9 (range 0.1–5.0)
Missing endotoxin concentration	2	–	-
<LOD for endotoxin	2	3	16
Mean CV% of duplicate endotoxin analyses	19.0 (range 0–90)	19.2 (range 0–90)	18.9 (range 0–150)
Missing dust concentration	75	4	6
<LOD for dust	26	31	85
Correlation between dust and endotoxin exposure	0.37 ($p < 0.0001$)	0.27 ($p = 0.056$)	0.14 ($p = 0.127$)

From the liquid impingers with the saline solution, 2 × 2 ml was stored in tubes to which 0.05% Tween was added, vortexed, and stored at -20°C until analysis with the LAL-assay.

• A sampling run with a previously described parallel sampler [4, 31], which enabled the simultaneous collection of 10 close to identical airborne samples using PAS6 sampling heads, of which 8 were equipped with 25 mm glass fiber filters (Whatman, GF/A) and 2 with 25 mm polycarbonate membrane filters (Whatman Nuclepore®). The filters were then divided over the various treatments:

– 2 glass fiber filters were stored in petri dishes at -20°C until further extraction and analysis with the LAL assay

– 2 glass fiber filters were stored in cassettes at -20°C until transport on dry-ice to Lund, Sweden, for analysis with GC-MS

– 2 polycarbonate filters were stored in cassettes at room temperature until transport to Oslo, Norway, for analysis with FM

The other 4 glass fiber filters were used in another experiment, which will not be discussed in this paper. Furthermore, per analytical method 2 field blanks were collected over the measurement days.

The filters assigned to the LAL-assay were extracted, and analyzed together with the impinger samples in our laboratory in the same way as described earlier in this paper.

With GC-MS, the samples were analyzed for the chemical markers 3-hydroxy fatty acids of various length (3-OHFAs C_{10}-C_{18}, marker for endotoxin) and muramic acid (MuAc, marker for peptidoglycan), as described previously. The LPS concentration was computed as the sum of nanomoles of individual 3-OHFA with chain lengths 10–16 divided by 4 to account for the 4 molecules of 3-OHFAs assumed per molecule of LPS [27, 29, 33].

With FM, the fluorescence staining causes recognition of microorganisms between other particles and microorganisms present in complex aggregates [4]. Both viable and non-viable bacteria and fungi were counted. The particles on the filter were resuspended and analyzed using the modified CAMNEA method by staining with acridine orange and counting with an epifluorescence microscope [6].

RESULTS

In total, 647 measurements in 43 sewage treatment plants were performed, of which 470 were full-shift personal, 123 task-based personal and 54 stationary measurements. More characteristics of the measurements are given in Table 1. For part of the samples no dust weight could be estimated due to errors during the weighing procedure, and for 2 samples the endotoxin concentration could not be estimated. The endotoxin concentration of 115 out of 124 field blanks was below the LOD (range detectable field blanks 2.0–5.3 EU per sample). Thus, contamination during handling of the filters and assembling of the sampling heads was unlikely. Of the loaded samples, 21 were below the LOD for endotoxin and 142 below the LOD for inhalable dust, most of them being task-based measurements. The average coefficient of variation (CV%) for duplicate analyses was 18%.

Table 2 shows the endotoxin and inhalable dust exposure levels for the different types of measurements, overall and divided over, respectively, functions, locations and tasks. The geometric mean personal, stationary and task-based endotoxin concentrations were moderate, with levels of 27 EU/m³, 33 EU/m³ and 64 EU/m³, respectively. The highest personal exposure levels were found in operators and sludge workers, which were statistically higher than the reference group management (Tab. 3). The highest dust concentrations were found in mechanics and sludge workers. The results of the stationary measurements indicate that the highest endotoxin levels are found in the front end of the process has, whereas the highest dust concentrations were found during sludge dewatering. Sludge dewatering and manufacturing of polymers showed the highest task-based endotoxin and dust levels, respectively. Overall, the correlation between measured dust and endotoxin exposure levels was low (Tab. 1).

Table 2. Endotoxin (EU/m³) and inhalable dust (mg/m³) exposure levels for personal, stationary and task-based measurements in sewage treatment plants.

	Endotoxin (EU/m³)					Inhalable dust (mg/m³)		
	N	AM	GM	GSD	range	N	AM	range
Personal measurements								
Overall	*468*	*71.0*	*26.9*	*3.7*	*0.2–2093*	*394*	*0.4*	*0.0–23.5*
Office workers	21	14.9	8.5	2.7	1.6–97.9	18	0.1	0.0–0.2
– Management / office	18	15.1	8.0	2.9	1.6–97.9	15	0.1	0.0–0.2
– Administration / house keeping	1	20.6				1	0.2	
– Analist	2	10.3	9.9	1.5	7.4–13.3	2	0.1	0.1–0.1
Technician	82	46.6	24.9	2.8	2.9–702	69	0.6	0.0–15.7
– Electrical engineer	15	27.6	19.5	2.4	6.2–86.1	12	0.2	0.0–0.9
– Mechanic	67	50.8	26.3	2.8	2.9–702	57	0.7	0.0–15.7
Operator	258	77.5	28.0	4.0	0.2–2093	211	0.3	0.0–3.8
Sludge worker	107	84.8	32.4	3.7	1.4–1506	96	0.5	0.0–23.5
Stationary measurements								
Overall	*54*	*110.3*	*32.9*	*6.4*	*0.2–1397*	*50*	*0.3*	*0.0–12.6*
Supply sewage and debris removal	12	180.5	51.3	5.3	1.8–1397	10	0.1	0.0–0.2
Sewage treatment process	12	36.3	12.6	4.8	2.1–204	11	0.0	0.0–0.1
Sludge dewatering	30	111.9	40.4	7.1	0.2–458	29	0.5	0.0–12.6
Task-based measurements								
Overall	*123*	*178.6*	*64.4*	*4.3*	*2.2–2135*	*117*	*0.5*	*0.1–4.3*
Supply sewage & debris removal	29	151.7	86.6	2.6	15.6–1100	27	0.4	0.1–1.7
Sewage treatment process	41	140.0	40.9	4.6	2.2–1317	40	0.5	0.1–2.8
Manufacture polymers	3	29.3	19.3	3.4	5.4–60.8	3	1.8	0.2–4.3
Sludge dewatering	40	272.6	112.9	4.1	2.2–2135	37	0.4	0.1–0.8
Sludge drying	2	271.0	199.5	3.2	87.6–454	2	0.5	0.1–0.8
Sludge transfer/reloading	8	37.1	15.9	4.1	3.0–123	8	0.6	0.2–1.4

Day-to-day variability in endotoxin exposure was larger than the differences between workers in average exposures, with the between- and within- worker variance components being 0.39 and 1.37, respectively (Tab. 3). No clear determinants of day-to-day variability in exposure could be identified; some climate characteristics (precipitation, wind direction, and relative humidity), month in which the measurements took place and measured dust concentration explained only 1–7% of the variability over time (Tab. 3). The combination of all climate variables available from the national survey points of the KNMI explained 11% of the variability in endotoxin exposure over time. Between-worker variability was mainly reduced by introducing function category, plant and cleaning during the measurement day as fixed effects (Tab. 3). Also, process characteristics such as kind of debris removal, type of aeration tank and system explained some of the between-worker variance. However, many of the variables available from the company checklist, time registration and questionnaire had no effect on the variance components and are therefore not mentioned in Table 3. The combination of function category and plant explained 56% of the between-worker variability. Furthermore, the combination of climate variables, hygiene facilities of a plant (industrial clothing, washing clothes at the plant, changing clothes before entering canteen and the procedure for use of personal protective devices), information on tasks and task duration registered during the measurement, variables concerning process characteristics and a combination of variables from the questionnaire (cleaning activities, cleaning with effluent, eating/drinking during work, showering at end of the day, frequency washing work clothes, place washing work clothes, place changing clothes) explained 13%, 18%, 10%, 13% and 28% of the between-worker variability, respectively (data not shown).

Figure 2 shows the results of the various viable and non-viable sampling and analytical methods. Because of the limited number of observations, the data are only presented graphically. The concentration measured in the filter samples were generally higher than those measured in the impinger samples. Furthermore, for filter samples the highest concentrations were generally found in the sludge dewatering department, whereas the difference between sludge dewatering and debris removal was less distinct in the impinger samples. As shown in Figure 2A, some sampling runs resulted in fairly comparable results when analyzed with the LAL assay, others differed considerably.

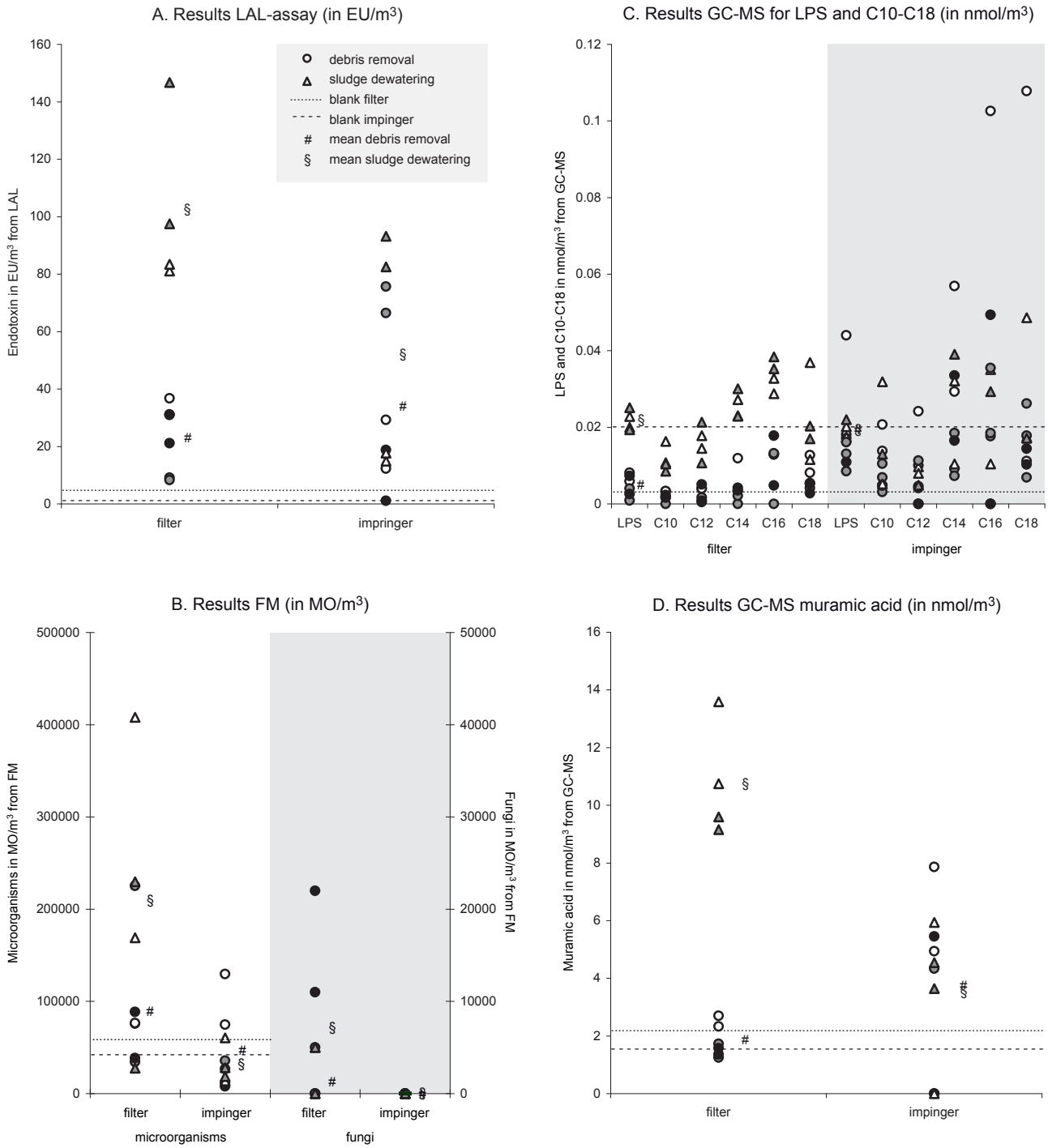

Figure 2. Comparison of results between 2 sampling methods (filters and impingers) per analytical method; the results are grouped by sampling location (● for debris removal and ▲ for sludge dewatering) and per sampling run (fill color).

The saline solution of impingers was analyzed solely with the LAL assay; they resulted all in non-detectable concentrations and were not considered any further (data not shown).

The mean amount of microorganisms found per m³ with fluorescence microscopy was 4.1×10^4 for impinger measurements and 1.4×10^5 for filter measurements, which is slightly elevated but not very high. A small part of these microorganisms was identified as fungi in the filter samples; no fungi were found in the impinger samples (Fig. 2B).

Figure 2C and 2D show the results of the GC-MS analyses for 3-OH FAs (endotoxin) and MuAc (peptidoglycan), respectively. Overall, the amounts of 3-OH FAs and MuAc

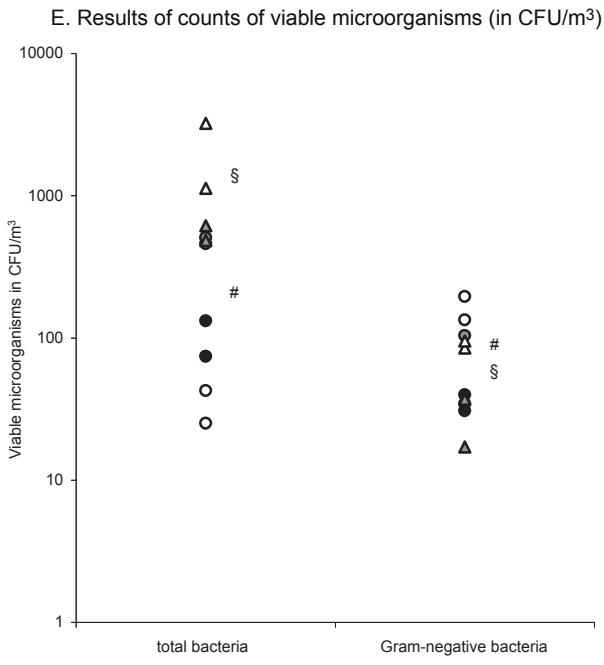

Figure 2 – continuation.

were low. A reasonably high part of the impinger measurements analyzed for 3-OH FAs were below the highest value measured in the field blanks, as were most of the filter samples from the debris removal. In both filter and impinger samples, concentrations of longer chain 3-OH FAs were higher. The variation in MuAc concentrations in filter samples was larger than in impinger samples, although many of the samples taken during debris removal were below the highest value measured in the filter field blanks.

The results of the viable measurements are shown in Figure 2E. During the counting of the formed colonies on the plates it appeared that also many fungi colonies had formed on the plates. The number of fungal colonies in samples from debris removal often exceeded the upper detection limit (>399 colonies). In samples from sludge dewatering almost no fungi were found (data not shown). In most sampling runs the amount of total bacteria was higher than the amount of Gram-negative bacteria, although the levels themselves were low to moderate. The highest amounts of total bacteria were found in the sludge dewatering department, and the highest amounts of Gram-negative bacteria were found in the debris removal department. Plating part of the impinger liquid on agars resulted in virtually no growth of colonies (data not shown). The total amount of both viable and non-viable microorganisms was in the order of 10^4–10^5, and thus the proportion of viable microorganisms (10^3–10^4) was 1–10%.

The ratio of the endotoxin bioactivity (EU) and the LPS concentration is referred to as the potency (EU/nmol LPS) [23]. This potency ranged from 3,600–5,500 EU/nmol LPS for filters per sampling run, and from 60–7,900 EU/nmol LPS for impinger samples. The mean potency was higher in filter samples than in impinger samples (AM 4,600 vs. 2,800 EU/nmol LPS) However, the mean potencies per sampling location (debris removal and sludge dewatering) were approximately the same for both collection methods (data not shown).

DISCUSSION AND CONCLUSIONS

Dust and endotoxin exposure levels in Dutch sewage treatment plants were relatively low, although differences between functions and tasks were observed. Apart from debris removal and sludge dewatering, sewage treatment was mostly situated outdoors, with some covered sections, which explains the relatively low exposure levels found. The generally higher endotoxin levels of stationary and task-based measurements compared to personal full-shift measurements suggest that working in certain parts of the installation and/or performing certain tasks is associated with higher endotoxin exposure. Yet workers normally spend only a relatively short part of the working day in these parts of the installation and/or performing these tasks. This results in relatively low exposures over the whole working day, as possible peak exposures are diluted over the rest of the day, when they work mainly in control rooms with low exposure.

The low endotoxin levels found are in accordance with those found in other studies on endotoxin exposure in sewage treatment plant workers [2, 18, 21, 34]. These studies also showed higher exposure with ambient measurements in sludge dewatering areas [2], at specific worksites, with highest values found for worksites located indoors, during agitation of wastewater [34], and during tasks with expected high peak exposure [18]. Scandinavian studies [10, 16, 24], an American [12], and a Polish study [9] in sewage treatment workers, and in the wastewater treatment part of wood processing plants [28], have shown much higher endotoxin concentrations. The fact that, for instance, in Scandinavia many phases of the wastewater treatment process are typically located indoors due to the low ambient temperature may be an explanation for the higher endotoxin levels found there.

Day-to-day variability is the major source of exposure variability, most probably caused by a combination of variation in work activities, changing weather conditions and differences in the supply and composition of influent. A combination of weather conditions also explained part of the day-to-day variability in endotoxin exposure. The plant a worker worked on, in combination with function, explained most of the variability between workers. However, hygiene associated variables also explained 28% of the difference between workers.

Although clear determinants of exposure were not observed, some characteristics were associated with a higher endotoxin concentration, for instance, workers who change their working clothes at home instead of at the plant. Furthermore, workers involved in cleaning activities had

Table 3. Between- and within-worker variability and percentage of explained variance, and univariate relative effect of variables on endotoxin levels (EU/m³), in 417 out of 470 personal measurements without missing data.

Variable	BW	WW	e^{β}	95% CI e^{β}
Worker only	0.39	1.37		
Function	0.33 (15%)	1.37 (0%)		
– Administration			2.67	0.19–37.88
– Analist			1.28	0.19–8.88
– Electrical engineer			2.59**	0.96–6.93
– Mechanic			3.46*	1.61–7.45
– Operator			3.80*	1.89–7.67
– Sludge worker			4.55*	2.17–9.55
– Management			ref	
Function category	0.32 (18%)	1.37 (0%)		
– Operator			3.50*	1.84–6.64
– Sludge worker			4.18*	2.11–8.30
– Technician			2.99*	1.50–5.98
– Office workers			ref	
Plant (n = 38)	0.24 (38%)	1.38 (-1%)		
Debris removal (uncovered *vs.* covered)	0.34 (13%)	1.37 (0%)	1.64*	1.22–2.20
Type of aeration tank	0.35 (10%)	1.37 (0%)		
– carrousel			ref	
– oxidation tank			4.85*	1.58–14.83
– aeration tank			1.33	0.93–1.92
– other			1.09	0.69–1.73
Type of aeration system	0.32 (18%)	1.38 (-1%)		
– fine bubbles aeration			1.32	0.83–2.11
– point aeration (covered)			1.34	0.81–2.22
– point aeration (uncovered)			3.81*	1.90–7.63
– other			ref	
Load of installation	0.38 (3%)	1.36 (1%)		
– high			1.87*	1.12–3.13
– low			1.24	0.91–1.70
– ultra low			ref	
Maintenance	0.36 (8%)	1.37 (0%)		
– external			4.39*	1.03–18.73
– external and internal			1.35**	0.99–1.85
– internal (all workers)			ref	
Industrial clothing	0.35 (10%)	1.37 (0%)		
– changing clothes at home			2.22*	1.36–3.61
– changing clothes at work			1.20	0.86–1.67
– changing both at home and at work			ref	
% supply of domestic waste water[#]	0.40 (-3%)	1.38 (-1%)	0.99*	0.978–0.998
Use of personal protective devices[$]	0.33 (15%)	1.38 (-1%)		
– yes, for specific work activities			1.23	0.56–2.70
– yes, during majority of the work day			2.45*	1.05–5.72
– yes, both			1.18	0.49–2.83
– no use of PPD			ref	
Cleaning as part of work activities (yes *vs.* no)[$]	0.35 (10%)	1.37 (0%)	2.16*	1.31–3.55
Cleaning during work day (yes *vs.* no)[&]	0.31 (21%)	1.38 (-1%)	1.63*	1.26–2.10

Variable	BW	WW	e^{β}	95% CI e^{β}
Number of cleaning activities during day[&]	0.29 (26%)	1.40 (-2%)		
Working at sludge dewatering during day (yes vs. no)[&]	0.38 (3%)	1.37 (0%)	1.29**	0.98–1.69
Precipitation (mm)[#%]	0.39 (0%)	1.34 (2%)	1.04*	1.01–1.07
Length precipitation (hours)[#%]	0.39 (0%)	1.34 (2%)	1.09*	1.03–1.16
Wind direction (16 categories measured)[%]	0.41 (-5%)	1.29 (6%)		
Relative humidity[#%]	0.39 (0%)	1.36 (1%)	1.02*	1.002–1.04
Month in which measured	0.39 (0%)	1.31 (4%)		
– June			5.82*	1.40–24.15
– July			7.20*	1.70–30.57
– August			3.93*	1.91–16.98
– September			7.66*	1.85–31.77
– October			4.77*	1.10–20.65
– November			9.41*	2.18–40.59
– December			ref	
Dust concentration (lognormally transformed)[^]	0.40 (7%)	1.30 (7%)	1.36*	1.21–1.54

BW between-worker variability, WW within-worker variability, * $p<0.05$, ** $0.05<p<0.10$, ref reference category, [#] continuous variable, [$] variables from questionnaire, [&] variable from time observation during measurement, [%] climate variables, [^] in smaller part of data, without missing data for dust (n = 342, worker only BW = 0.43, WW = 1.40).

higher endotoxin exposure. An experimental study showed that cleaning with tap water or surface water instead of effluent, and lowering the water pressure during cleaning, as well as mechanical ventilation, significantly lowered endotoxin exposure [36]. Good hygiene practices and adequate cleaning protocols could thus reduce endotoxin exposure. Furthermore, some process characteristics were associated with a higher endotoxin exposure. The extent to which process water or sludge is being moved and/or agitated seems to have an effect on endotoxin exposure, which has been found previously [10, 34]. Covering sources of exposure may be a way to further reduce endotoxin exposure, although working indoors results in higher exposure levels [10, 18] and maintenance thus should be performed with caution.

Beforehand, variation in weather conditions over seasons was thought to be a determinant of exposure, but no clear differences between measurement series or seasons were found. A Swiss study also found no differences in endotoxin exposure between seasons (summer and winter) [18].

It is suggested that the wet environment and frequent generation of aerosols could cause clogging of filters [1]. Use of liquid impingers could be an alternative in these situations. Comparison of filter and impinger samplers resulted in variable outcomes with respect to the analysis technique used, i.e. LAL-assay, GC-MS and FM. Except for 3-OH FAs in impingers, the highest concentrations were found in filter samples, which suggest no major role in clogging. Filter samples could better distinguish the difference in composition of microorganisms in the air of the departments compared to impinger samples. Although it has been suggested that the activity of liquid inside the impingers might result in lysis of microorganisms, and thus more endotoxin available in the LAL assay, this did not seem to be the case here. However, part of the liquid had 'evaporated' from the impingers due to the fierce bubbling of the liquid during the measurements. This loss was replenished in order to divide the sample for the different applications in the experiment, which might have diluted the ultimately measured concentrations. This does not pose a problem when only liquid evaporates during sampling, but does alter the outcomes when droplets leave the impinger. Furthermore, bacteria prefer staying on the border of liquid and air, and therefore could differentially disappear in larger quantities when droplets are formed. Our data, however, showed both lower bacterial and fungal levels in impinger samples, which suggests that differential loss of bacteria had not occurred. The kind of liquid may also have influenced the measured concentration. The endotoxin concentrations in all 9% saline impinger samples were below LOD. Perhaps also solely pyrogen-free water is not the optimal sampling solution for impinger measurements due to osmosis or related mechanisms, thus affecting the cells in the solution. Other studies that compared filter and impinger sampling methods concluded that the performance of both methods depends on the airborne endotoxin levels. However, in these studies impinger measurements generally resulted in higher and less variable endotoxin levels [3, 32].

In a simultaneously performed questionnaire study a dose-response relation between endotoxin exposure and some health symptoms was found [30]. The relatively low exposure levels suggest that, apart from some exceptions, endotoxin itself may play only a minor part in causing possible health effects in sewage treatment workers. The results of the additional experiment carried out also point in that direction, with more viable Gram-positive bacteria and fungi than Gram-negative bacteria, and the presence

of muramic acid in the filter and impinger samples. Other studies also found low endotoxin concentrations and/or reasonably high concentrations of fungi and bacteria, of which Gram-positive bacteria were dominant [21]. The total numbers of bacteria in extracts of endotoxin samples ranged from 10^7–10^9 bacterial cells per m^3, somewhat higher than the amounts found in our study, although these concentrations may have been overestimated through disturbance of counting by glass fibers from the filters and other non-bacterial particles that show fluorescence [11]. Oppliger *et al.* (2005) showed more cultivable bacteria indoors than outdoors. Climatic parameters seemed to have a significant effect on the mean airborne concentration of fungi (in summer higher than in winter), but not on total bacteria, Gram-negative bacteria and endotoxin [18]. Since the experimental measurements were performed in summer on a relatively hot day, this could explain the rather high amounts of fungi found.

The results of the LAL-assay did not differ substantially from the GC-MS analysis. Both the analysis with FM and GC-MS showed a relatively low exposure to bacteria. Only in sludge dewatering 3-OH FAs and MuAc were found. The viable measurements accordingly showed that bacteria occurred mainly in sludge dewatering, the majority being Gram-positive bacteria, which is confirmed by the concentrations of MuAc found in this department. In the debris removal department exposure to viable microorganisms was also reasonably high, but these were mainly fungi.

It is known that the LAL assay particularly measures free (unbound) endotoxin [7]. In cell-bound endotoxin most of the lipid A is a covalent part of the membrane and thus does not activate Limulus enzymes. However, experimental data suggest that cell-bound LPS may still be highly biologically active when inhaled [25]. Results from the GC-MS analysis indicated a very low endotoxin exposure, whereas the LAL assay suggested also low but slightly elevated exposure levels. In combination with the differences between filter and impinger samples, and the fact that the viable as well as the non-viable measurement techniques showed the presence of microorganisms at sewage treatment plants, these results do not rule out that some fungi and peptidoglycans may have interfered with the LAL-assay. This interference is of little consequence in the case of high exposure levels, but may cause some exposure misclassification in case of moderate exposure levels in combination with the presence of relatively high amounts of viable bacteria and fungi, as found in this study. It should be noted that the additional experiment consisted only of a limited set of measurements, and thus no very firm conclusions could be drawn.

In conclusion, endotoxin exposure in Dutch sewage treatment plants is moderate to low, although the results indicate differences in exposure levels between jobs, locations, and tasks performed. Exposure varied more from day-to-day than between workers, and some determinants of exposure could be identified. Comparison of sampling and analytical techniques suggests that it seems justified to perform filter measurements in combination with the LAL-assay to measure endotoxin exposure in sewage treatment plants, although some interference of other microorganisms or their products cannot be ruled out.

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REFERENCES

1. Contal P, Simao J, Thomas D, Frising T, Callé S, Appert-Collin JC, Bémer D: Clogging of fibre filters by submicron droplets. Phenomena and influence of operating conditions. *J Aerosol Sci* 2004, **35**, 263-278.
2. Douwes J, Mannetje A, Heederik D: Work-related symptoms in sewage treatment workers. *Ann Agric Environ Med* 2001, **8**, 39-45.
3. Duchaine C, Thorne PS, Meriaux A, Grimard Y, Whitten P, Cormier Y: Comparison of endotoxin exposure assessment by bioaerosol impinger and filter-sampling methods. *Appl Environ Microbiol* 2001, **67**, 2775-2780.
4. Eduard W, Blomquist G, Herbert Nielsen B, Kulvik Heldal K: Recognition errors in the quantification of micro-organisms by fluorescence microscopy. *Ann Occup Hyg* 2001, **45**, 493-498.
5. Friss L, Norback D, Edling C: Self-reported asthma and respiratory symptoms in sewage workers. *J Occup Health* 1999, **41**, 87-90.
6. Heldal K, Skogstad A, Eduard W: Improvements in the quantification of airborne micro-organisms in the farm environment by epifluorescence microscopy. *Ann Occup Hyg* 1996, **41**, 437-447.
7. Jorgensen JH, Smith RF: Measurement of bound and free endotoxin by the Limulus assay. *Proc Soc Exp Biol Med* 1974, **146**, 1024-1031.
8. Khuder SA, Arthur T, Bisesi MS, Schaub EA: Prevalence of infectious diseases and associated symptoms in wastewater treatment workers. *Am J Ind Med* 1998, **33**, 571-577.
9. Krajewski JA, Cyprowski M, Szymczak W, Gruchała J: Health complaints from workplace exposure to bioaerosols: a questionnaire study in sewage workers. *Ann Agric Environ Med* 2004, **11**, 199-204.
10. Laitinen S, Kangas J, Kotimaa M, Liesivuori J, Martikainen PJ, Nevalainen A, Sarantila R, Husman K: Workers' exposure to airborne bacteria and endotoxins at industrial wastewater treatment plants. *Am Ind Hyg Assoc J* 1994, **55**, 1055-1060.
11. Laitinen S, Nevalainen A, Kotimaa M, Liesivuori J, Martikainen PJ: Relationship between bacterial counts and endotoxin concentrations in the air of wastewater treatment plants. *Appl Environ Microbiol* 1992, **58**, 3774-3776.
12. Lee JA, Johnson JC, Reynolds SJ, Thorne PS, O'Shaughnessy PT: Indoor and outdoor air quality assessment of four wastewater treatment plants. *J Occup Environ Hyg* 2006, **3**, 36-43.
13. Lee JA, Thorne PS, Reynolds SJ, O'Shaughnessy PT: Monitoring risks in association with exposure levels among wastewater treatment plant workers. *J Occup Environ Med* 2007, **49**, 1235-1248.
14. Liebers V, Bruning T, Raulf-Heimsoth M: Occupational endotoxin - exposure and possible health effects on humans. *Am J Ind Med* 2006, **49**, 474-491.
15. Lundholm M, Rylander R: Work related symptoms among sewage workers. *Br J Ind Med* 1983, **40**, 325-329.

16. Melbostad E, Eduard W, Skogstad A, Sandven P, Lassen J, Sostrand P, Heldal K: Exposure to bacterial aerosols and work-related symptoms in sewage workers. *Am J Ind Med* 1994, **25**, 59-63.

17. Mulloy KB: Sewage workers: toxic hazards and health effects. *Occup Med* 2001, **16**, 23-38.

18. Oppliger A, Hilfiker S, Vu Duc T: Influence of seasons and sampling strategy on assessment of bioaerosols in sewage treatment plants in Switzerland. *Ann Occup Hyg* 2005, **49**, 393-400.

19. Peretz C, Goren A, Smid T, Kromhout H: Application of mixed-effects models for exposure assessment. *Ann Occup Hyg* 2002, **46**, 69-77.

20. Pillai SD, and Ricke SC: Bioaerosols from municipal and animal wastes: background and contemporary issues. *Can J Microbiol* 2002, **48**, 681-696.

21. Prażmo Z, Krysińska-Traczyk E, Skórska C, Sitkowska J, Cholewa G, Dutkiewicz J: Exposure to bioaerosols in a municipal sewage treatment plant. *Ann Agric Environ Med* 2003, **10**, 241-248.

22. Rappaport SM, Weaver M, Taylor D, Kupper L, Susi P: Application of mixed models to assess exposures monitored by construction workers during hot processes. *Ann Occup Hyg* 1999, **43**, 457-469.

23. Reynolds SJ, Milton DK, Heederik D, Thorne PS, Donham KJ, Croteau EA, Kelly KM, Douwes J, Lewis D, Whitmer M, Connaughton I, Koch S, Malmberg P, Larsson BM, Deddens J, Saraf A, Larsson L: Interlaboratory evaluation of endotoxin analyses in agricultural dusts - comparison of LAL assay and mass spectrometry. *J Environ Monit* 2005, **7**, 1371-1377.

24. Rylander R: Health effects among workers in sewage treatment plants. *Occup Environ Med* 1999, **56**, 354-357.

25. Rylander R, Bake B, Fischer JJ, Helander IM: Pulmonary function and symptoms after inhalation of endotoxin. *Am Rev Respir Dis* 1989, **140**, 981-986.

26. Rylander R, Jacobs RR: Endotoxins in the environment - a criteria document. *Int J Occup Environ Health* 1997, **3**, S1-S48.

27. Saraf A, Larsson L, Burge H, Milton D: Quantification of ergosterol and 3-hydroxy fatty acids in settled house dust by gas chromatography-mass spectrometry: comparison with fungal culture and determination of endotoxin by a Limulus amebocyte lysate assay. *Appl Environ Microbiol* 1997, **63**, 2554-2559.

28. Sarantila R, Reiman M, Kangas J, Husman K, Savolainen H: Exposure to endotoxins and microbes in the treatment of waste water and in the industrial debarking of wood. *Bull Environ Contam Toxicol* 2001, **67**, 171-178.

29. Sebastian A, Larsson L: Characterization of the microbial community in indoor environments: a chemical-analytical approach. *Appl Environ Microbiol* 2003, **69**, 3103-3109.

30. Smit LA, Spaan S, Heederik D: Endotoxin exposure and symptoms in wastewater treatment workers. *Am J Ind Med* 2005, **48**, 30-39.

31. Spaan S, Heederik DJ, Thorne PS, Wouters IM: Optimization of airborne endotoxin exposure assessment: effects of filter type, transport conditions, extraction solutions, and storage of samples and extracts. *Appl Environ Microbiol* 2007, **73**, 6134-6143.

32. Stephenson DJ, Lillquist DR, DeRosso FD, Greene DD, White G: Side-by-side comparison of three sampling methods for aerosolized endotoxin in a wastewater treatment facility. *J Environ Health* 2004, **67**, 16-19.

33. Szponar B, Larsson L: Use of mass spectrometry for characterising microbial communities in bioaerosols. *Ann Agric Environ Med* 2001, **8**, 111-117.

34. Thorn J, Beijer L, Jonsson T, Rylander R: Measurement strategies for the determination of airborne bacterial endotoxin in sewage treatment plants. *Ann Occup Hyg* 2002, **46**, 549-554.

35. Thorn J, Kerekes E: Health effects among employees in sewage treatment plants: A literature survey. *Am J Ind Med* 2001, **40**, 170-179.

36. Visser MJ, Spaan S, Arts HJ, Smit LA, Heederik DJ: Influence of different cleaning practices on endotoxin exposure at sewage treatment plants. *Ann Occup Hyg* 2006, **50**, 731-736.