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Endotoxin Exposure and Symptoms in Wastewater Treatment Workers

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Background *Wastewater treatment workers can be exposed to biological and chemical agents resulting in work-related health effects. The aim of this study was to investigate work-related symptoms in these workers.*
Methods *Questionnaire data of 468 employees from 67 sewage treatment plants is evaluated. Personal endotoxin exposure (8 hr measurements; n = 460) was measured in a sample of workers in three different periods over 1 year.*
Results *Endotoxin exposure ranged from 0.6 to 2093 endotoxin units (EU)/m^3, the geometric mean exposure was low (27 EU/m^3). Factor analysis yielded three clusters of correlated symptoms: "lower respiratory and skin symptoms," "flu-like and systemic symptoms," and "upper respiratory symptoms." Symptoms appeared to be more prevalent in workers exposed to endotoxin levels higher than 50 EU/m^3. A significant dose-response relationship was found for "lower respiratory and skin symptoms" and "flu-like and systemic symptoms" ($P < 0.05$).*
Conclusions *Wastewater treatment workers reported a wide range of symptoms that may be work-related. Microbial exposures such as endotoxin seem to play a causal role.* Am. J. Ind. Med. 48:30–39, 2005. *© 2005 Wiley-Liss, Inc.*

KEY WORDS: wastewater treatment workers; sewage; endotoxin; bioaerosol exposure; symptoms; epidemiology; factor analysis

INTRODUCTION

Wastewater treatment workers are potentially exposed to a variety of biological and chemical agents that may result in work-related health effects. Wastewater processed at treatment plants could be a source of human infection with pathogens including *Giardia lamblia*, hepatitis A virus, *Legionella pneumophila*, and *Ascaris lumbricoides* [Palmer et al., 1995; Baggi et al., 2001; Nelson and Darby, 2001; Caccio et al., 2003]. Despite a few cross-sectional studies reporting an increased hepatitis A virus seropositivity among wastewater treatment workers [Cadilhac and Roudot-Thoraval, 1996; Brugha et al., 1998], a historical prospective study and cross-sectional studies of clinical hepatitis A did not demonstrate a higher incidence in workers exposed to wastewater, suggesting that wastewater workers are not at higher risk of contracting clinical hepatitis A [Lerman et al., 1999; Glas et al., 2001; Venczel et al., 2003]. The risk of other infectious or parasitic diseases like leptospirosis, roundworm infection, and giardiasis was investigated by determining antibody levels or parasitologic examinations of stool samples [Clark et al., 1976, 1984]. These studies did not conclusively indicate an elevated risk of infections from exposure to wastewater or sludge, although infectious disease sporadically occurred in isolated clusters.

While the risk of contracting an infection appears to be minimal, a number of studies show that respiratory symptoms, flu-like symptoms, and gastrointestinal symptoms are more common in wastewater treatment workers [Khuder et al., 1998; Rylander, 1999; Douwes et al., 2001]. Bacterial endotoxin exposure has been related to fever and other flu-like symptoms among workers in plants where

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sludge was heat-dried into powder [Rylander et al., 1976; Mattsby and Rylander, 1978]. Respiratory symptoms, fatigue, joint pains, and diarrhea among workers in conventional wastewater treatment plants were also attributed to endotoxin exposure [Rylander, 1999]. Associations between levels of total bacteria, rod-shaped bacteria and symptoms such as headache and fatigue were found in wastewater workers, but in this study no associations were found for endotoxin exposure [Melbostad et al., 1994]. In general, measured endotoxin concentrations in wastewater treatment plants are low, although increased levels were found at indoor worksites like the sludge dewatering area [Douwes et al., 2001; Thorn et al., 2002; Prażmo et al., 2003]. Moreover, peak exposures during specific tasks like cleaning or sludge treatment may cause adverse health effects in susceptible workers.

Most previous studies examining symptoms and microbial exposure in wastewater treatment workers were relatively small or did not relate personal exposure measurements to health outcomes. Therefore, a large-scale study was conducted to measure endotoxin exposure in a representative sample of wastewater treatment workers and to assess work-related health effects using a questionnaire. In this study we aimed to investigate associations between a wide range of symptoms and potential determinants of microbial exposure. Furthermore, we attempted to identify clusters of highly correlating symptoms using factor analysis to investigate associations between clustered symptoms and exposure variables.

MATERIALS AND METHODS

Study Population

All 27 Dutch Water Boards that are responsible for treatment of wastewater were contacted and asked if their employees would participate in the study. Twenty-one out of the 27 Water Boards agreed to participate and a total of 67 wastewater treatment plants were visited. Questionnaires were administered to all 470 employees who were present during visits to the plants between June and September 2003. All subjects gave written consent.

Questionnaire

A shortened and adapted version of a questionnaire developed specifically for bioaerosol related health effects in the waste recycling and composting industry was used [Wouters, 2003]. The questionnaire consisted of three parts: personal and work characteristics; health symptoms; and smoking habits. The questionnaire included questions about general respiratory symptoms, diarrhea, allergies, and flu-like symptoms resembling organic dust toxic syndrome (ODTS) during the past 12 months. Furthermore, participants reported for 27 symptoms how often these occurred during the past year (daily, weekly, monthly, or almost never). A subject was considered to have a positive response for one of the 27 symptoms if the symptom occurred at least monthly.

Questions regarding general respiratory symptoms could be compared to a general Dutch population sample of the European Community Respiratory Health Survey (ECRHS) [Rijcken et al., 1996]. All questionnaires were checked the same day by a trained researcher who asked participants to complete missing answers.

Endotoxin Exposure Assessment

Personal endotoxin exposure (8 hr measurements; $n = 460$) was measured in 216 workers from 40 wastewater treatment plants. One to six samples per worker were collected during three different periods (June–July, August–October, and November 2003). Inhalable dust was sampled on 37 mm glass fiber filters (Whatman GF/A) using GSP sampling heads at a flow rate of 3.5 L/min and portable pumps (Gilian GilAir5). Endotoxin extraction and analysis by the Limulus Amebocyte Lysate (LAL) assay were performed as described previously [Douwes et al., 1995]. Concentrations below the limit of detection were assigned 2/3 of the concentration corresponding to the average limit of detection. Results were expressed as endotoxin units (EU)/m^3 (1 EU $\sim$ 0.1 ng).

Statistical Analysis

Data were analyzed using SAS statistical software (SAS System for Windows, version 8.02; SAS Institute, Cary, NC). Explorative factor analysis was used for the 27 symptoms to identify clusters of symptoms that were reported regularly in combination with each other [Kleinbaum et al., 1988]. Instead of evaluating individual symptoms we studied the occurrence of these relatively independent, meaningful clusters (or "factors") to gain better insight in the associations between health symptoms and exposure categories. Factors were retained if eigenvalues were greater than 1.0. After orthogonal varimax rotation factors were identified by the factor pattern matrix, symptoms with factor loadings greater than 0.3 were included in a factor. Reliability coefficients (Cronbach's alpha) were calculated to describe the internal consistency of the extracted factors. A subject's positive response for a factor was defined as a minimum number of individual positive symptoms within that particular factor. This cut-off point was arbitrarily chosen, such that approximately 15% of the subjects would have a positive response for each factor, which is sufficient for a meaningful quantitative analysis.

In cross-sectional studies, the prevalence ratio (PR) is often a more interpretable and meaningful statistic than the odds ratio [Skov et al., 1998]. We, therefore, calculated prevalence ratios and 95% confidence intervals (95% CI) by log-binomial regression analysis to describe associations between (clustered) symptoms and measures of exposure. A starting value of −4 for the intercept was used to prevent convergence problems [Deddens et al., 2003]. Prevalence ratios were adjusted for possible confounders age, gender, and smoking habits.

RESULTS

Personal Characteristics and Symptom Prevalence

The questionnaire response rate was 99.6%; 468 employees returned a completed questionnaire. Personal characteristics are presented in Table I. According to data on job title, tasks and job area, participants were categorized as ''office staff'' or ''operators and maintenance workers.'' Office staff were those workers who reported working in an office the majority of time, including laboratory and domestic personnel. Some office employees reported occasional tasks involving exposure to sewage or sludge. Operators and maintenance workers included technicians, sludge workers, and general operators.

TABLE I. Personal Characteristics of 468 Dutch Wastewater Treatment Workers

	Office staff[b] (n = 97)	Operators and maintenance workers (n = 371)
Gender (% male)**	77.3	97.8
Age in years[a,†]	47.3 (9.9)	43.3 (10.1)
Length of employment in years[a,***]	14.9 (10.2)	12.6 (9.0)
Smoking habits (%)		
Current	25.8	28.6
Ever	38.1	30.8
Never	36.1	40.7
Tasks (%)		
Cleaning**	21.9	85.2
Cleaning with effluent**	13.5	68.7
Raw sewage treatment**	39.2	72.0
Sludge treatment**	45.4	82.5
Personal hygiene (%)		
Eating/drinking during work*	24.7	36.9
Shower after work**		
At the plant	6.2	29.9
At home	48.5	57.9

*$P < 0.05$.
**$P < 0.001$; Chi-square test.
***$P < 0.05$.
†$P < 0.001$; *t*-test.
[a]Arithmetic mean (standard deviation).
[b]Includes laboratory and domestic personnel.

The two categories of workers did not differ with respect to smoking habits. Office staff included a higher proportion of female workers, were older than operators and maintenance workers and had worked longer in the wastewater treatment industry.

Prevalence of individual self-reported symptoms among the two categories of wastewater workers is given in Table II. No significantly increased prevalence ratios were found when symptoms for operators and maintenance workers were compared to symptom prevalence among office staff. Correction for potential confounders of age, gender, and smoking did not affect associations between job category and symptoms.

Respiratory Symptoms Compared to a General Population Sample

The prevalence of respiratory symptoms was compared for wastewater treatment workers and a sample of 2,698 Dutch adults [Rijcken et al., 1996]. Mean age was higher in the general population sample (45.5 years vs. 44.2 years; *t*-test; $P < 0.05$) and among the general population sample were more females (50.3% vs. 6.4%; Chi-square; $P < 0.001$) and more current smokers (36.7% vs. 28.0%; Chi-square; $P < 0.001$). Table III shows the crude prevalence and prevalence ratio after adjustment for age, gender, and smoking habits. Asthma, daily cough, daily cough up phlegm and shortness of breath in rest were significantly elevated in wastewater workers (prevalence ratios ranging from 1.43 to 2.61). Wastewater workers had a significantly lower prevalence for waking up due to cough or due to chest tightness (PR 0.63 and 0.66, respectively). Results did not change meaningfully after correction for age, gender, and smoking or after exclusion of females or current smokers.

Factor Analysis

Three clusters of correlated symptoms explained 85.6% of the variance (Table IV). Exploratory factor analysis yielded: ''lower respiratory and skin symptoms,'' ''flu-like and systemic symptoms,'' and ''upper respiratory symptoms.'' The symptom ''heart palpitations'' did not load significantly on any factor and was therefore excluded from analysis. ''Shivering'' was included in ''flu-like and systemic symptoms,'' although its factor loading was less than 0.3 (0.28). None of the individual symptoms loaded greater than 0.35 on more than one factor. Cronbach's alpha was 0.80 for ''lower respiratory and skin symptoms,'' 0.76 for ''flu-like and systemic symptoms,'' and 0.76 for ''upper respiratory symptoms'' which is sufficient considering that 0.7 is an acceptable reliability coefficient [Nunnally, 1978].

In addition, factor analysis was applied separately for operators and maintenance workers (n = 371) and office staff

TABLE II. Individual Symptoms Present Once a Month or More Often During the Past 12 Months Among 468 Dutch Wastewater Workers by Job Category

	Office staff[c] (n = 97)		Operators and maintenance workers (n = 371)	
Symptoms	**%**	**PR**	**%**	**PR (95% CI)**
Respiratory symptoms				
Cough	17.5	1.0	16.7	0.81 (0.48–1.35)
Cough up phlegm	8.3	1.0	17.0	1.60 (0.78–3.28)
Shortness of breath (SOB)	12.4	1.0	11.1	1.00 (0.51–1.96)
Wheezing	7.2	1.0	7.6	1.01 (0.43–2.39)
SOB and wheezing	5.2	1.0	7.0	1.31 (0.48–3.63)
Chest tightness	9.3	1.0	8.6	0.74 (0.34–1.61)
Stuffed nose	27.8	1.0	26.7	0.79 (0.54–1.15)
Runny nose	17.5	1.0	17.5	0.78 (0.46–1.32)
Sneezing	22.7	1.0	20.0	0.71 (0.46–1.12)
Dry or sore throat	8.3	1.0	14.0	1.55 (0.72–3.34)
Skin and eye symptoms				
Itchy, red eyes	18.6	1.0	14.8	0.69 (0.41–1.15)
Itchy, red skin (>2 days)	6.2	1.0	6.7	1.24 (0.47–3.25)
Rash	10.3	1.0	10.8	1.46 (0.68–3.14)
Flu-like and systemic symptoms				
Headache	17.5	1.0	28.3	1.51 (0.92–2.48)
Heavy head	11.3	1.0	14.6	1.27 (0.65–2.47)
Dizziness	11.3	1.0	8.6	0.69 (0.34–1.40)
Unusual tiredness	20.6	1.0	20.0	0.79 (0.49–1.28)
Troubles concentrating	9.3	1.0	10.2	1.14 (0.53–2.48)
Fever	2.1	1.0	1.9	0.65 (0.13–3.29)
Shivering	3.1	1.0	2.4	0.71 (0.17–2.93)
Joint pain	15.5	1.0	20.2	1.40 (0.84–2.33)
Muscle pains (not from sport activities)	10.3	1.0	11.1	1.06 (0.54–2.09)
ODTS[a]	20.6	1.0	30.7	1.45 (0.92–2.29)
Gastrointestinal symptoms				
Nausea	4.1	1.0	6.7	1.17 (0.37–3.68)
Heartburn	12.4	1.0	13.5	0.77 (0.42–1.40)
Loss of appetite	2.1	1.0	6.5	2.15 (0.44–10.46)
Vomiting	1.0	1.0	2.4	2.47 (0.18–33.80)
Diarrhea[b]	26.8	1.0	40.4	1.47 (1.00–2.15)
Other				
Heart palpitations	4.1	1.0	3.8	0.88 (0.30–2.63)

Unadjusted prevalence and prevalence ratio (95% confidence interval) adjusted for age, gender and smoking habits.
[a]ODTS, flu-like symptoms resembling organic dust toxic syndrome, at least once during past 12 months.
[b]At least once during past 12 months.
[c]Includes laboratory and domestic personnel.

(n = 97). Analysis of symptoms among operators and maintenance workers yielded comparable factors to those found in the entire study population. On the other hand, a different factor structure was obtained for office staff. No meaningful symptom patterns were observed within the six factors that were retained in these workers.

Table V shows the distribution of symptom clusters among the study population by number of prevalent individual symptoms within the cluster. More than half of the participants had no positive response for any individual symptom in each symptom cluster. Almost 70% of the participants did not report any of the ''lower respiratory and

TABLE III. Prevalence and Prevalence Ratio (95% CI; Adjusted for Age, Smoking Habits and Gender) of Respiratory Symptoms Present at Least Once During Past 12 Months Among Wastewater Workers (n = 468) and a General Population Sample (n = 2698) in The Netherlands

	General population		Wastewater workers	
	%	PR	%	PR (95% CI)
Cough symptoms				
Daily cough	15.8	1.0	21.4	1.48 (1.20–1.82)***
Daily cough up phlegm	10.0	1.0	15.4	1.54 (1.19–1.98)***
Awakened due to cough	31.8	1.0	15.2	0.63 (0.50–0.79)***
Shortness of breath, dyspnea, wheezing				
SOB at rest	7.6	1.0	9.2	1.48 (1.04–2.09)*
Exercise induced SOB	21.0	1.0	19.0	1.07 (0.86–1.32)
Awakened due to SOB	6.4	1.0	6.8	1.28 (0.86–1.89)
Wheezing	23.8	1.0	18.2	0.87 (0.71–1.08)
Wheezing with SOB	15.7	1.0	10.2	0.75 (0.56–1.01)
Wheezing without a cold	13.0	1.0	12.3	1.06 (0.80–1.40)
Awakened due to chest tightness	12.1	1.0	7.1	0.66 (0.46–0.95)*
Dyspnea going one flight of stairs	19.3	1.0	15.4	1.03 (0.81–1.30)
Dyspnea versus contemporaries	3.1	1.0	3.3	1.39 (0.77–2.49)
Asthma				
Asthma attack (ever)	4.9	1.0	9.0	1.79 (1.24–2.59)**
Asthma attack doctor diagnosed	4.7	1.0	6.9	1.49 (0.99–2.26)
Asthma attack last year	1.6	1.0	2.8	2.59 (1.25–5.35)*
Asthma medication use	2.5	1.0	4.3	1.84 (1.07–3.15)*

*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$; Log-binomial regression.

TABLE IV. Clusters of Correlating Symptoms and Percentage of Explained Variance as Determined by Factor Analysis (Factor Loadings After Orthogonal Varimax Rotation Between Brackets)

Lower respiratory and skin symptoms	Flu-like and systemic symptoms	Upper respiratory symptoms
30.3	**Explained variance (%) 29.8**	**25.4**
Shortness of breath and wheezing (0.77)	Trouble concentrating (0.56)	Sneezing (0.65)
Wheezing (0.72)	Unusual tiredness (0.53)	Runny nose (0.58)
Chest tightness (0.68)	Heavy head (0.49)	Stuffed nose (0.57)
Shortness of breath (0.60)	Dizziness (0.47)	Itchy, red eyes (0.45)
Itchy, red skin (0.45)	Loss of appetite (0.45)	Cough (0.43)
Cough up phlegm (0.40)	Nausea (0.43)	Dry or sore throat (0.42)
Rash (0.35)	Joint pain (0.42)	
	Headache (0.42)	
	Vomiting (0.42)	
	Fever (0.38)	
	Muscle pain (0.37)	
	Heartburn (0.35)	
	Shivering (0.28)	

TABLE V. Symptom Cluster Prevalence (%) Among Dutch Wastewater Workers (n = 468) by Number of Prevalent Individual Symptoms Within the Cluster

	Number of symptoms						
Symptom cluster	**0**	**≥ 1**	**≥ 2**	**≥ 3**	**≥ 4**	**≥ 5**	**≥ 6**
Lower respiratory and skin symptoms	69.7	30.3	14.5[a]	9.0	5.3	4.1	1.9
Flu-like and systemic symptoms	51.1	48.9	34.2	22.4	15.8[a]	8.6	4.7
Upper respiratory symptoms	55.6	44.4	29.9	19.2[a]	9.8	4.9	1.9

[a]Cut-off point for a positive response for a symptom cluster.

skin symptoms.'' The cut-off point for a positive response for ''lower respiratory and skin symptoms'' was a minimum of two symptoms (14.5% of the subjects), a minimum of four symptoms for ''flu-like and systemic symptoms'' (15.8% of the subjects), and a minimum of three symptoms for ''upper respiratory symptoms'' (19.2% of the subjects).

Endotoxin Exposure

Personal 8 hr endotoxin exposure was low with a geometric mean of 27 EU/m^3 (geometric standard deviation = 3.7; n = 460). Endotoxin levels ranged from 0.6 to 2093 EU/m^3. Subjects with more than one personal sample were assigned the arithmetic mean of their measurements, which resulted in 216 personal endotoxin exposure levels ranging from 1.5 to 1081 EU/m^3. Endotoxin exposure was categorized into three groups: 0–50 EU/m^3, >50 to 200 EU/m^3, and >200 EU/m^3, based on the proposed health-based occupational exposure limit of 50 EU/m^3 for endotoxin as recommended by The Health Council of The Netherlands [1998] and the tentative feasibility limit of 200 EU/m^3.

Association Between Symptom Clusters and Exposure

Associations between dichotomized symptom clusters and exposure variables (adjusted for age, gender, and smoking habits) are shown in Table VI. Associations between endotoxin exposure and symptoms were investigated in the 216 subjects with available endotoxin exposure data. Symptoms appeared to be more prevalent in workers exposed to endotoxin levels higher than 50 EU/m^3. A significant dose-response relationship was found for ''lower respiratory and skin symptoms'' and ''flu-like and systemic symptoms'' (test for trend; $P < 0.05$).

Self-reported exposure data were investigated for the entire study population. No associations were found between symptom clusters and job title, whereas cleaning tasks were positively and significantly associated with the prevalence of ''lower respiratory and skin symptoms'' (PR 1.88; $P < 0.05$). Positively elevated PRs were also found for cleaning tasks using effluent (treated wastewater) and ''lower respiratory and skin symptoms'' (PR 1.78; $P < 0.05$) and ''flu-like and systemic symptoms'' (PR 1.63; $P < 0.05$). ''Flu-like and systemic symptoms'' seemed more common in workers who handled raw sewage and in workers who used to eat or drink during their work (PR 1.59 and 1.48; $P < 0.10$). ''Upper respiratory symptoms'' were not associated with self-reported exposure variables. A very strong association existed between ''lower respiratory and skin symptoms'' and length of employment (>20 years vs. = 0–4 years or <4 years, PR 6.58; $P < 0.001$). This association increased strongly after adjustment for age; the uncorrected PR was 2.06 ($P < 0.05$). Length of employment was not associated with the other two symptom clusters. Except for years of employment, correction for potential confounders did not affect estimated prevalence ratios.

Since ODTS and diarrhea were not included in factor analysis, we separately investigated associations between these two symptoms and (self-reported) exposure variables. ODTS was more prevalent in workers exposed to endotoxin levels higher than 200 EU/m^3 (PR 2.03; 95% CI 1.20–3.44). A significantly increased PR was found for diarrhea in workers exposed to endotoxin levels between 50 and 200 EU/m^3 (PR 1.45; 95% CI 1.03–2.05). Significantly increased PRs were found for ODTS and diarrhea in operators compared to office staff (ODTS: PR 1.66; 95% CI 1.04–2.64, diarrhea: PR 1.52; 95% CI 1.02–2.27). The prevalence of diarrhea was also higher in technicians (PR 1.55; 95% CI 1.01–2.37), whereas sludge workers did not differ from office staff with respect to ODTS and diarrhea prevalence. Cleaning tasks and eating or drinking during work were positively associated with ODTS (PR 1.55; 95% CI 1.05–2.29 and PR 1.39; 95% CI 1.03–1.87, respectively).

DISCUSSION

Wastewater treatment workers exhibit symptoms that are in accordance with symptoms reported by other populations that are occupationally exposed to biological agents including endotoxin. A relevant study related to this topic was recently conducted among organic domestic waste

TABLE VI. Associations Between Symptom Clusters and Measures of Exposure in Wastewater Workers in The Netherlands Expressed as Prevalence Ratio (PR) With 95% Confidence Intervals (95% CI), Adjusted for Age, Gender, and Smoking Habits

		Lower respiratory and skin symptoms	Flu-like and systemic symptoms	Upper respiratory symptoms
	n	PR (95% CI)	PR (95% CI)	PR (95% CI)
Endotoxin				
$<$50 EU/m^3	141	1.0	1.0	1.0
$>$50–200 EU/m^3	63	1.13 (0.54–2.38)	1.12 (0.55–2.26)	1.63 (0.88–3.02)
$>$200 EU/m^3	12	1.79 (0.62–5.16)	2.02 (0.83–4.88)**	1.79 (0.84–3.84)**
Job title				
Office staff	97	1.0	1.0	1.0
Technician	109	1.09 (0.52–2.29)	0.67 (0.33–1.38)	0.88 (0.48–1.61)
Operator	202	1.29 (0.67–2.45)	0.96 (0.54–1.72)	0.94 (0.55–1.60)
Sludge worker	60	0.80 (0.31–2.07)	0.92 (0.43–1.98)	0.78 (0.38–1.62)
Tasks				
Cleaning	332	1.88 (1.03–3.42)**	1.40 (0.83–2.38)	0.92 (0.61–1.40)
Cleaning with effluent	263	1.78 (1.08–2.95)**	1.63 (1.02–2.61)**	1.01 (0.68–1.48)
Raw sewage treatment	305	1.49 (0.88–2.50)	1.59 (0.96–2.62)*	1.32 (0.85–2.04)
Sludge treatment	350	1.56 (0.85–2.87)	1.17 (0.68–2.01)	1.06 (0.66–1.72)
Personal hygiene				
Eating/drinking during work	158	1.22 (0.77–1.92)	1.48 (0.97–2.27)*	0.92 (0.62–1.37)
No shower after work	88	0.94 (0.52–1.69)	0.85 (0.48–1.52)	0.96 (0.59–1.57)
Length of employment				
0–4 years or $<$ 4 years	116	1.0	1.0	1.0
$>$4–13 years	118	1.96 (0.93–4.10)*	1.58 (0.89–2.80)	1.23 (0.73–2.06)
13–20 years	110	2.22 (0.95–5.18)*	0.72 (0.33–1.54)	0.81 (0.40–1.60)
20 years	122	6.58 (2.49–17.35)***	1.05 (0.46–2.40)	1.79 (0.83–3.86)

*$P < 0.10$.
**$P < 0.05$.
***$P < 0.001$; Log-binomial regression.

collectors and compost workers [Wouters, 2003]. In this study, a clearly elevated prevalence of symptoms was associated with increased exposure to endotoxin and inhalable dust. The prevalence of a number of respiratory symptoms among wastewater treatment workers appeared to be moderately increased compared to a sample of Dutch adults. This finding is in agreement with a Swedish study, which also showed an increased self-reported asthma prevalence among wastewater workers compared to a reference population [Friis et al., 1999]. The same symptoms—asthma, daily cough, daily cough up phlegm, and shortness of breath—were also elevated in employees of composting facilities. Wouters [2003] suggested that bioaerosol exposures may contribute to development of these respiratory symptoms in compost workers.

The prevalence of symptoms among office staff in the wastewater treatment industry did not differ from operators and maintenance workers in the same industry, which agrees with findings by Khuder et al. [1998]. An explanation could be that a part of the office personnel in this industry has occasional, specialized tasks that may involve incidental high exposure to sludge or sewage, whereas some of the operators and maintenance workers are working in the control room or machine shop the majority of time, where bioaerosol exposure is low or absent. In addition, Clark et al. [1985] found that inexperienced sewage workers reported a higher incidence of gastrointestinal illness than experienced workers, suggesting that employees who are exposed to sewage or sludge on a regular basis may develop some tolerance for microbial agents. Infrequent exposures in some of the office staff could therefore lead to symptoms that are not elicited in more regularly exposed workers.

The wide range of symptoms reported in this study is in agreement with previous studies in the wastewater treatment industry [Lundholm and Rylander, 1983; Scarlett-Kranz et al., 1987; Zuskin et al., 1993; Melbostad et al., 1994; Khuder et al., 1998; Rylander, 1999; Douwes et al., 2001]. Instead of evaluating these individual symptoms, we studied the prevalence of three clusters of highly correlating symptoms. These three clusters ‘‘lower respiratory and skin symptoms,'' ''flu-like and systemic symptoms,'' and ''upper respiratory symptoms'' resembled the symptom clusters found in a previous study among sewage workers [Douwes et al., 2001]. In the present study systemic symptoms correlated with flu-like symptoms and unexpectedly, lower respiratory symptoms appeared to be correlated with skin symptoms, which also occurred when a different number of factors was extracted. It is not very clear why lower respiratory symptoms are clustered with skin symptoms, although both lower respiratory symptoms and skin symptoms may be induced by tasks such as cleaning that could lead to inhalation as well as dermal exposures. Clusters of symptoms that were identified in compost workers were considerably different, which was thought to be due to more specific variation in exposure in sewage workers [Wouters, 2003]. This supposition is supported by the observation that operators and maintenance workers had a relatively clear and meaningful pattern of symptoms, while office staff did not show interpretable symptom clusters. More heterogeneous exposure and different tasks among the latter category of workers could be the cause of this difference.

Chemical exposures could be among the causative agents in the wastewater treatment environment. Sulfur compounds such as hydrogen sulfide are a cause of anxiety among some wastewater workers, and serious incidents have been reported in waste management and sewer workers [Watt et al., 1997; Hendrickson et al., 2004]. Hydrogen sulfide levels are monitored continuously in most facilities, and portable monitors are available during performance of specific tasks. However, hydrogen sulfide levels are generally low and are unlikely to cause health effects [Melbostad et al., 1994]. A few studies mention health effects in sewage workers due to accidental or long-term exposure to other chemicals such as organic solvents and hexachlorocyclopentadiene (HCCPD), an intermediate compound in the manufacture of pesticides [Morse et al., 1979; Kraut et al., 1988]. Although health effects due to chemical exposures cannot be excluded, it is implausible that they are a main cause of symptoms in this large sample of wastewater treatment plants that receive mainly domestic wastewater.

Altogether, it seems more reasonable to focus on microbial exposures. In this study moderately increased prevalence ratios were found for upper respiratory, flu-like and other symptoms in subjects exposed to endotoxin levels higher than 50 EU/m^3. Although these associations were not significant, a dose-response relationship existed for ''lower respiratory and skin symptoms'', ''flu-like and systemic symptoms'', and ODTS.

Somewhat higher endotoxin concentrations were measured in a study in the waste composting industry, and symptom prevalence among compost workers appeared to be approximately twice as high as in wastewater workers [Wouters, 2003]. In a recent study, levels of airborne microorganisms and endotoxin at different sites of a sewage treatment plant in Poland were determined and the microbial species composition was studied [Prażmo et al., 2003]. Microbiological examination of air samples showed the presence of 20 potentially pathogenic species or genera of bacteria and fungi having allergenic and/or immunotoxic properties such as *Acinetobacter calcoaceticus*, *Penicillium* sp., and *Alternaria alternata*. In the Polish study, comparably low endotoxin concentrations were found (0–52 EU/m^3), and levels of viable microorganisms were relatively low as well ($<2.4–70.7 \times 10^2$ CFU/m^3), although a more detailed interpretation cannot be given as the methodology used differed from more current sampling methods. It appears that endotoxin in itself plays only a minor role given the generally low levels, and that airborne endotoxin should be considered as a relatively good marker for general microbial exposure. However, with the LAL-assay only free, cell wall dissociated, endotoxins are measured, whereas cell bound endotoxins are not detectable [Sonesson et al., 1990]. Total amounts of endotoxin in wastewater treatment plants could therefore be underestimated.

An increased prevalence of diarrhea was found in operators and technicians but, unexpectedly, not in sludge workers. An explanation could be that sludge workers usually treat digested sludge, in which many potential pathogens like fecal bacteria and enteroviruses have been destroyed [Carrington et al., 1991]. Lundholm and Rylander [1983] postulated that the etiological agents for gastrointestinal symptoms in sewage workers were enterotoxins from gram-negative bacteria; in a later study endotoxin was suggested as the causal agent [Rylander, 1999]. However, no conclusive evidence was provided to support both hypotheses.

Associations between health effects and measures of self-reported exposure to wastewater seemed to be stronger in a previous Dutch study that was conducted in 1993 [Douwes et al., 2001]. Many wastewater treatment plants in The Netherlands applied odor-reducing and occupational safety measures over the last 10 years, reducing exposure to dust, aerosols, sludge and sewage. It is likely that these measures have had a positive effect on wastewater workers' health.

In this study we used self-reported data on health effects and exposure variables such as job title, tasks and personal hygiene. It may have been difficult for the workers to answer the health questions accurately as we inquired about symptoms during the past 12 months. Although we cannot exclude that recall bias influenced results, it is unlikely that recall differed systematically among exposed and nonexposed workers. It is possible that participants exaggerated symptom occurrence as a result of the attention that was paid to endotoxin and its adverse health effects, in particular those workers who are often exposed to sludge or sewage or those who believe that their symptoms are work-related. However, sludge workers who were expected to report a high symptom prevalence did not show increased prevalence ratios.

Non-differential information bias due to inaccurate classification of workers into broadly defined exposure categories may be a more important limitation of this study. Exposure variables such as raw sewage treatment, sludge treatment and cleaning tasks are not very specific. Because most workers found it difficult to indicate how much time they spent on tasks in different areas of the plant it was necessary to categorize these variables into crude ‘‘yes/no’’ categories. This qualitative definition may have weakened associations between self-reported exposure and symptoms.

Finally, it is possible that the ‘‘healthy worker effect,’’ which means that a working population is relatively healthier than the general population, may have biased our results. If healthier employees stay longer with the same job, associations between exposure and health will be underestimated. However, participants who were employed more than 20 years reported more lower respiratory and skin symptoms than less experienced workers, suggesting that a healthy worker effect did not seriously influence this study. The increased prevalence of lower respiratory and skin symptoms among more experienced workers could not be explained by job title or tasks, hence it seems to be an indication that long-term exposure to agents in sewage or sludge elicited these symptoms. More senior workers may as well have experienced higher concentrations and exposures of a different character before exposure reducing measures took place.

In conclusion, wastewater treatment workers reported a wide range of symptoms that may be work-related. Three clusters of highly correlating symptoms showed positive associations with endotoxin exposure and cleaning tasks (with and without using effluent).

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