Endotoxin and hydrogen sulphide exposure and effects on the airways among waste water workers in sewage treatment plants and sewer net system

KK Heldal[1], AD Austigard[3], KH Svendsen[2], E Einarsdottir[1], LO Goffeng[1], LI Sikkeland[4,5], KC Nordby[1]

[1]National Institute of Occupational Health, Oslo

[2] Department of Industrial Economics and Technology Management, NTNU, Trondheim

[3]Working Environment Office, Trondheim Municipality, Trondheim

[4]Institute of clinical medicine, Faculty of Medicine, University of Oslo

[5]Department of Respiratory Medicine, Rikshospitalet, Oslo University Hospital

Corresponding author:

Kari Kulvik Heldal

Kari.heldal@stami.no

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Abstract

Background: The purpose of this study is to investigate whether airborne exposure to endotoxins, hydrogen sulphide (H_2S) and inhalable particles negatively impacts the respiratory system and inflammatory blood proteins in sewage plant and sewer net system workers, and further to determine dose-response associations between exposure and health outcomes.

Methods: In total, 148 waste water workers (WWWs) from urban and rural sewage plants and the sewer net system participated. One hundred and twenty-one workers were exposed to sewage, 46 from sewage plants and 75 from the sewer net system. Twenty-seven workers were characterized as little or not exposed and served as an internal reference group. Personal inhalable samples were analysed for endotoxins (Limulus assay), particle dust (gravimetrically) and *Salmonella* and *Yersinia* spp. (polymerase chain reaction method, PCR). Levels of H_2S were measured using personal electro chemical sensors. Intercellular adhesion molecule 1(ICAM-1), interleukin 8 (IL-8), surfactant protein D (SP-D), club cell protein 16 (CC16) and macrophage inflammatory protein (MIP) were determined by enzyme-linked immunosorbent assay and C-reactive protein (CRP) by an HS-MicroCRP assay in blood samples.

Results: Workers in sewage plants were exposed to significantly higher levels of endotoxins compared to workers in the sewer net system (median 55 EU/m^3 (4–262 EU/m^3) and median 27 EU/m^3 (1–304 EU/m^3), respectively). The estimated H_2S index showed higher values when working in the sewer net system (median 3.1 (0.5–78.1) compared to workers at the sewage plants (median 1.3 (0.5–9.3), and the most excessive exposure was collecting sewage from cesspools (273 ppm). No viable airborne *Salmonella* and *Yersinia spp*. were detected. The exposed workers had significantly higher CRP compared to the referents (1.2 µg/ml (0.1–19.0 µg/ml) and 0.8 µg/ml (0.1–5.0 µg/ml), respectively) and lower forced expiratory volume in 1 s (FEV1 %) (92.6 %, SD 14.6) and 102.0 %, SD10.1, respectively), with numbers given as mean and standard deviation. The serum concentration of CRP was significantly and negatively associated with FEV_1% (β = - 7.7, R^2 = 0.05) and FVC% (β = - 8.5, R^2 = 0.08), and the serum concentration of ICAM-1 with the estimated exposure to H_2S (β = - 19.9, R^2 = 0.07).

Conclusion: Despite moderate levels of endotoxin and H_2S exposure, the results indicate an impact of these agents on lung function and the adhesion molecule ICAM-1, and a low-grade systemic inflammation was indicated in increased levels of CRP.

Introduction

Waste water workers (WWWs) at sewage treatments plants are exposed to a complex mixture of non-infectious and infectious microorganisms, microbial components and toxic gases. Several studies have shown that the range of symptoms are also broad, extending from central nervous system (CNS), respiratory, and gastrointestinal symptoms to infection and flu-like symptoms (Lundholm and Rylander, 1983, Melbostad et al., 1994, Douwes et al, 2001, Thorn et al., 2002, Heldal et al., 2010). Of all the airborne exposure associated with handling waste water at sewage treatment plants, hydrogen sulphide (H_2S) from degradation of organic material in the sewage and endotoxins from Gram-negative bacteria are of particular interest (Adelson and Sunchine, 1966, Beauchamp et al., 1984, Latinen et al., 1994, Farahat and Kishk, 2010). High-dose acute exposure to H_2S can be extremely hazardous, being able to cause pulmonary oedema (> 300 ppm) and sudden unconsciousness (> 500 ppm) or death by even a single exposure at levels above 1,000 ppm (NEG 2001). However, repeated exposure to low levels of H_2S (1–10 ppm) may possibly result in chronic symptoms from the CNS, such as fatigue, headache, poor memory and concentration difficulties (Tvedt et al., 1991, Richardson, 1995, Watt et al., 1997, Lee et al., 2007, Farahat and Kishk, 2010). Whether effects result from short-duration high exposure peaks or from slightly elevated average H_2S exposure is of interest in the analysis of associations with health outcomes, and levels and variation of exposure in the work environment may be important to study in relation to CNS-dysfunction. Results across industry-based studies warrant caution, are partly conflicting and suffer from methodological shortcomings regarding confounding, selection bias and other bias-related problems (Lewis & Copley 2015, Lim et al., 2016).

When handling sewage, endotoxins can be present in levels exceeding those that may give rise to symptoms and illness (Rylander 1999). Symptoms from the airways and impaired lung function among sewage plant workers compared to controls have been related to endotoxin exposure (Douwes et al., 2001, Thorn and Beijer 2004, Heldal et al., 2010). Recent studies have reported associations between exposure when handling sewage-related dust and both specific inflammatory pneumoproteins and systemic inflammatory biomarkers (Heldal et al., 2013, 2016). Both the inflammatory effects of endotoxins and irritant effects of H_2S may have the respiratory system as a target organ. The potential effect from exposure to a mixture of these contaminants in low levels on the airways is less studied (Lee et al., 2007).

Most of the published studies regarding exposure and health effects when handling sewage were carried out in waste water treatment facilities; thus, published reports regarding work in the sewer net including sewer underground pipes, chambers and pumps are scarce (Neumann et al, 2002, Duquenne et al., 2014).

The aim of this study is to investigate whether airborne exposure to endotoxin, H_2S and inhalable particles negatively impact the respiratory system and central nervous symptoms in WWWs, both at sewage plants and in the sewer net system. Further, we aimed to detect exposure to selected variables of pathogenic microorganisms *Salmonella* and *Yersinia spp*. by personal sampling. A further aim was to investigate dose-response associations between exposure to endotoxin and health outcomes and to quantitate possible synergic effects of exposure on the respiratory system.

METHODS

Description of work tasks

The sewer net system is a complex network collecting domestic waste water, rain water and industrial effluent for treatment at sewage plants. It comprises underground pipe galleries, channels and pump stations leading wastewater to the plants. Raw waste water carries a large amount of suspended matter that may deposit in the pipes and disturb the waste water flow and even obstruct the pipes. The sewage treatment process removes physical, chemical and biological contaminants from the waste water by coarse filtering of small particles flocculated after addition of chemicals ($FeCl_3$, $Al_2(SO_4)_3$) in sedimentation tanks. The sediment sludge is then dewatered by pressing or centrifugation. The main tasks for workers at the urban sewage plants were daily inspection and cleaning activities. The work at the rural plants was more complex and additional tasks were inspection and cleaning of the network system such as pipes, pits and pump stations connected to the plant.

Study population

Workers from four sewage treatment plants, being representative of municipal plants operated to treat wastewater from large urban cities (Oslo, Trondheim), and from four sewage treatment plants in small rural communities (Steinkjer, Støren, Klæbu, Selbu) were invited to participate in the study. Workers handling waste water in the sewer net system consisting of sewage pipes and pump stations were recruited from the same areas. In total, 148 workers (99%) of the invited workers accepted to participate (Table 1). One hundred and twenty-one workers were characterized as exposed to sewage, 46 from sewage treatment plants and 75 from the sewer net system including underground pipe galleries, channels and pump stations. Based on a questionnaire of job operations and our own inspections, 27 workers from the same working sites were characterized as low or not exposed to toxicants from the sewage. These data include workers from the TV-inspection group which have minimal contact with waste water, workers dealing with the fresh water net system in ditches and workers with administration tasks with lower than 10% exposed working time. Workers relocated to administrative work because of health problems were not included in the reference group. This combined low-exposed group had a similar socioeconomic status as the exposed group, and they served as an internal reference group. At the beginning of the study, all participants were invited to the National Institute of Occupational Health in Oslo or St. Olav's Hospital in Trondheim for a health examination consisting of spirometry, and a blood sample was collected for determination of biomarkers. Participants completed a self-administrated questionnaire of work-related symptoms, including questions regarding airway symptoms (irritations and cough), gastro-intestinal symptoms (nausea and diaré) and neuropsychological symptoms from the central nervous system (tiredness, concentration difficulties and forgetfulness). Smoking and respiratory health status was recorded by a general questionnaire (Melbostad et al., 2001). The data were collected from April to June 2015. The study was approved by the Regional Medical Ethics Board. All participants gave their written informed consent.

Spirometry

Pulmonary function was measured by spirometry which included measures of forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC) (Spirare SPS 330, Diagnostica, Oslo, Norway) according to the joint ERS/ATS guidelines (Miller et al., 2005). The same technician performed all tests in each of the test locations. Predicted values were based on European values for a reference population

(Quanjer et al., 1993). Spirometric airflow limitation was defined by a FEV_1/FVC ratio < 70 % and FEV_1 < 80% of predicted value based (ERS, 1993).

Blood sampling and protein analysis in blood

Blood samples were collected from workers and referents at approximately the same hour of the day, between 10 and 12 AM. Blood samples were collected in vacuum tubes containing ethylenediaminetetraacetic acid (EDTA) for plasma or without additives for serum (BD Diagnostic, Plymouth, UK). Plasma samples was used for measuring protein levels of Surfactant protein D (SpD), Club Cell protein16 (CC16), Interleukin 8(IL-89, Intercellular Adhesion Molecule (ICAM-1) and Macrophage inflammatory protein (MIP)-1alpha (or CCL3) using a DuoSet enzyme-linked immunosorbent assay kit obtained from R&D systems (Stillwater, MN, USA).

Serum was obtained after coagulation for 60 min at room temperature and centrifugation for 15 min at 1,550g. The serum samples were frozen in NUNC® cryotubes at -25°C no more than 2 h later and kept frozen until analysis. CRP was measured by a HS-MicroCRP assay with an immunoturbidmetric assay with latex-bounded anti-CRP antibody (Tina QuantRoche, Roche Diagnostic Corporation, Germany). Atopy for respiratory allergens were tested in serum using a Phadiatop test (FEIA, UniCAP system, Fürst Laboratory, Norway).

Sampling strategy

The measurements were performed in all eight sewage treatment plants with connected sewer net system. Personal samples were collected of workers equipped with a rucksack with two pumps connected to cassettes fixed to the straps of the rucksack in the breathing zone. One of the cassettes was for endotoxin analysis, the other for gravimetric determination of particulates and polymerase chain reaction (PCR) for determination of *Salmonella* and *Yersinia* spp. A hydrogen sulphide sensor was fixed on straps in the breathing zone of the worker.

All exposure measurements were carried out from the beginning of the day and until lunchtime (sampling time 4–5 hours) while most of the practical work was performed. Throughout the sampling period, the workers recorded their work operations, the use of personal protective device and breaks. The sampling was performed on the available workers during the sampling period (January to December 2015), both in the summer and winter period at each plant, in order to include seasonal variations. The number of samples performed at each plant is listed in Table 2.

Personal sampling of airborne endotoxins, dust and pathogens

Exposure to endotoxins, dust particles and bacteria (Salmonella and Yersinia spp.) was assessed in samples collected with inhalable PAS6 cassettes (Personal Air Samplers with a 6 mm inlet manufactured at the workshop of the NIOH, Oslo) (Van der Wal 1983). The cassettes were connected to two pumps (PS103) operated at a flow rate of 2.0 l/min. The sampling time was approximately 4–5 hours. Aerosols for the determination of dust and viable bacteria (Salmonella and Yersinia spp.) were collected on polycarbonate filters with pore size 0.8 µm (Poretics, Osmonics, Livermore, USA), while endotoxins were collected on glass fibre filters (Whatman GF/A, Maidstone, USA). The samples were stored at +4^{O}C and transported to the laboratory by car the same or the following day for analyses. The dust mass was determined gravimetrically in a climate-controlled weighing room. Endotoxins were extracted with 0.05% (vol/vol) Tween 20 (Merck Schuchard) in pyrogen-free water, rocked vigorously at room temperature and centrifuged at 1,000 g for 10 min (Douwes et al.1995). The suspension was analyzed by a quantitative kinetic chromogenic Limulus amoebocyte lysate assay according to the manufacturer's instructions (Cambrex, New Jersey, USA).

As a part of standard procedure airborne bacteria were pre-cultivated in order to reach a higher concentration of cells of the target pathogens (*Salmonella spp*. and *Yersinia spp.)* suitable for detection by the *PCR* method (BAX System PCR Assay for *Salmonella* spp. and ISO/TS 18867:2015 for *Yersinia* spp.). Filters were put in 100 ml of buffered peptone water (BPW, at pH 7.2), shaken gently for 15–30 minutes and then the suspension was divided in two aliquots for further analysis. The buffer with material from the filters was incubated for growth as follows: *Salmonella* at 37^{o}C for 16–20 hours, *Yersinia* at 25^{o}C for 20–24 hours. The two cultures were used for DNA extraction with the paramagnetic bead method and then analyzed based on Real Time PCR assay with Taqman. This analysis was performed by a commercial laboratory (Eurofins Steins Laboratorium A/S, Veje, Denmark).

Calculation of hydrogen sulphide index

Hydrogen sulphide (H_2S) was measured using direct reading instruments with logging OdaLog L2/LL (ThermoFisher Scientific Inc. Australia) with a quantification range of 0.1–200 ppm, Dräger GasVisi X-am 500 and Dräger Pac 700 (Dräger Safety, Norway) and 0–100 ppm, respectively. The concentrations were measured every 15 sec and the average of each interval of 15 sec was recorded using data loggers. H_2S exposure was registered by the number of peaks recorded above 0.1 ppm (H_2S01), 1 ppm (H_2S1), 5.0 ppm (H_2S5) and 10.0 ppm (H_2S10), as well as the duration of the peaks. To combine these measurement results into a single value, a hydrogen sulphide index was computed, as earlier described (Austigard et al., 2018):

Hydrogen sulphide index:

$$= H_2S_{01} * 0.1 + H_2S_{duration01} * 0.1 + H_2S_1 + H_2S_5 * 5 + H_2S_{duration5} * 5 + H_2S_{10} * 10 + H_2S_{max}$$

A background sulphide level was estimated as $0.4/\sqrt{2}=0.28$, where 0.4 is the lowest calculated sulphide index, and all results with a 0 sulphide index were replaced with this value.

Statistical analyses

The variables were summarized with median, minimum and maximum values. Thereafter, parametric statistical methods were used. Continuous variables were log-transformed to achieve normal distribution when the skewness exceeded 2.0, and accordingly, all exposure and biomarker variables, except for ICAM-1, were log-transformed. One-way analysis of variance (ANOVA) was used when more than two groups were compared with a post-hoc LSD test (least significant difference test). Multiple linear regression (backward strategy) was used to assess associations between dependent and exposure variables simultaneously and to study interaction effects between exposures on lung function. General linear models of relevant parameters were used to calculate group estimates, adjusted for smoke, age, body-mass index (BMI) and atopy. Exposure was modelled by linear mixed effect regression to account for the correlation between repeated measurements. Determinants of exposure were treated as fixed effects. The models were built stepwise including work operations, work place, season and the amount of high-pressure flushing. Determinants that significantly improved the models as judged by a p-value <0.05 in likelihood ratio tests were kept in the models.

Example of calculation: GM (geometric mean) exposure to endotoxin in a worker from rural plant/net in the autumn and at Selbu with more than 1 to 3 episodes of flushing during the work shift:
$0.745 \times 2.64 \times 3.35 \times 22.64 \times 0.79 = 117.8$ EU/m^3
AM (Arithmetic mean: exp[log-transformed exposure + $0.5(\sigma^2_B + \sigma^2_W)$] (Friesen et al., 2006):
endotoxin exposure = GM x $e^{(0.5xvarians)}$= 117.8 x $e^{0.595}$=117.8 x 1.81=213.6 EU/m^3
Variance within workers (W)= 1.15, variance between workers (B)= 0.04

All analyses were performed using IBM SPSS Statistics 20 (IBM, Armonk NY).

RESULTS

Exposure levels of airborne endotoxins and dust particles

In total, 160 airborne samples were collected during a period of one year (2015) and analyzed for endotoxins and dust exposure. Because a limited number of electrochemical sensors were available, only 93 personal measurements of H_2S were performed. The work place, typical work operations, seasons and number of flushing operations during a day contributed to the explanation of the exposure concentration levels. A descriptive overview of the levels of exposure to endotoxins and dust particles, and H_2S-index, considering these determinants are shown in Table 2.

The endotoxin exposure level in the 160 observations obtained during handling of sewage at sewage plants or in the sewer net system were moderate with a highest concentration of 342 EU/m^3 measured during 1–3 flushing operations in a working day. Inspection of the sewer net system (TV-inspection), (median 7 EU/m^3 (1–150 EU/m^3)) and grease handling (median 13 EU/m^3 (5–70 EU/m^3)) showed the lowest exposure levels during different working operations ($p<0.05$). The exposure levels also varied by season, with the highest concentration during the springtime (median 56 EU/m^3 (6–152 EU/m^3), $p<0.05$). The inhalable dust exposure was low (0.1–1.9 mg/m^3), with no significant differences between working operations. No viable *Salmonella* and *Yersinia spp.* were detected in any of the air samples (not shown). The correlations between the exposure variables endotoxin and dust were low (r_s = -0.07 – 0.13).

Estimated exposure levels based on a linear mixed effect model of the 160 observations (Table 5) of the determinants were used in the assessment of associations between exposure and health effects among 121 sewage plants and sewer net workers. Workers in the sewage plants were significantly more exposed to endotoxins compared to those working in the sewer net system (median 55 EU/m^3 (4–262 EU/m^3) and median 27 EU/m^3 (1–304 EU/m^3), respectively), but showed significantly lower values of H_2S index than workers in the sewer net system (median 1.3 (0.5–9.3) and median 3.1 (3.1–78.1), respectively).

Measurements of H_2S

Twenty-nine percent of the 93 measurements of H_2S were below the detection limit (0 and 0.1 ppm). Nine percent of all hydrogen sulphide recordings showed peaks above 10 ppm; in addition, 15% have peaks of 5–10 ppm, 35% have peaks of 1–5 ppm and 65% have peaks of 0.1–1 ppm. Pump stations and small sewage plants, work in sewer net system and collecting sewage from cesspools all had shifts with H_2S peaks above 10ppm. The job with most excessive exposure to hydrogen sulphide was collecting sewage from cesspools (273 ppm), with 15% of all shifts having peaks above 10 ppm.

Health effects

Work-related symptoms

In total, 148 WWWs, 121 exposed and 27 referents participated in the health examination of the study. The prevalence of work-related symptoms was generally higher among sewage plant and sewer workers compared to referents, but only airway symptoms were significantly higher (respectively 33 % and 11 %). In particular, cough (57 %), nausea (43 %) and fever attack (35 %) were often reported among exposed workers. There was no difference in reported symptoms between workers at sewage plants and the sewer net system. The same tendency was observed with symptoms from the central nervous system (CNS). Concentration difficulties (32 %), forgetfulness (47 %) and tiredness (46 %) were the symptoms most often reported among the sewage plant and sewer workers.

Spirometry

Table 3 summarizes the lung function measures of referents, sewage plant and sewer net workers. The predicted FEV_1 % and FVC % were lower for all exposed workers (92.6 % and 97.5%) compared to the referents (102.0 % and 106.4 %), respectively, in analyses adjusted for smoking, atopy, age and BMI. FEV_1 % predicted was also lower among the sewage plant working group (93.0%) and sewer net working group (92.4%) compared to the referents. However, there were no significant differences in the lung function measures between the sewage plant and the sewer net working group. Nine of non-smoking workers (8%) showed moderate spirometric airflow limitation according to the European Respiratory Society (ERS) criteria (FEV_1<80% of predicted value and FEV_1/FVC ratio <70%).

Biomarkers of inflammation

The results are shown in Table 4. The acute phase protein CRP was elevated in all exposed workers compared to the reference group, median (range) 1.2 µg/ml, (0.1–19.0 µg/ml) and 0.8 µg/ml (0.1–5.0 µg/ml, p<0.05), respectively. Workers at the sewer net system (0.3–19.0 µg/ml) demonstrated higher CRP levels compared to workers at the sewage treatment plants (0.1–14.0 µg/ml, p<0.05). The concentration of CC16 was significantly lower among the sewer net workers compared to the referents (median 27 ng/ml (7–300 ng/ml) and 34 ng/ml (5–152 ng/ml), respectively, p<0.05). The level of adhesion molecule ICAM-1 in all exposed workers was close to significantly higher than the referents (median 98 ng/ml (25–237 ng/ml) and 75 ng/ml (26–174 ng/ml, respectively, p=0.06). No other of the proteins measured showed significant differences between workers and referents or between working groups.

The concentration of CRP was negatively associated with the lung function variables FEV_1% predicted (β = -7.7, R^2 = 0.05, p<0.01) (Figure 1) and FVC% predicted (β = -8.5, R^2 = 0.08, p<0.01), adjusted for BMI, age and smoking.

Associations between exposure and health effects

The associations between the concentration of biomarkers, lung function variables and exposure were studied using linear regression analyses. The concentration of ICAM-1 was negatively associated with the estimated exposure to H_2S (H_2S index) (β = - 19.9, R^2 = 0.09, p<0.05). This association was also significant for sewer net workers only (β = - 52.6, R^2 = 0.07, p<0.05). The absolute value of FEV_1 (3.39 l, ±0.7) was inversely associated with endotoxin exposure (β = - 0.22, R^2 = 0.18, p<0.05), and this association was close to significant regarding FEV_1 % of predicted value (p=0.07). No interaction between the exposure to endotoxin and H_2S index was observed on the association with any of the analysed lung function variables.

Discussion

Exposure to endotoxins during the handling of sewage was higher when working at the sewage treatment plants compared to the sewer net system. The exposure to H_2S was higher when working in the sewer net system compared to the sewage plants, and alarmingly high when collecting sewage from cesspools. A work-related weak inflammatory effect on the airways was observed as CRP, particularly among the sewer net workers, increased with impaired lung function. Exposure to endotoxin was associated with lower FEV_1. Furthermore, exposure to H_2S was associated with ICAM-1. Because exposure levels to H_2S and endotoxin exposure were not correlated, the observed exposure-response associations found may be regarded as independent.

Overall, the exposure level of endotoxins was moderate, both when working at the sewage plants or in the sewer net system. Season and flushing as work operations were the most important determinants to exposure to both endotoxins and H_2S. The endotoxin exposure level measured at the sewage plants is comparable to other studies of WWWs (1–4000 EU m^3) (Smit et al., 2005, Spaan et al., 2008, Heldal et al., 2010, Cyprowski et al., 2015). However, the occurrence of high levels of endotoxin exposure as measured earlier in Norwegian sewage plants (3200 EU/m^3) was not observed in this study (Heldal et al., 2010). The exposure during work in the sewer net system has been less studied. In a French exploratory study of sewer net workers, the endotoxin measurements ranged from 8 – 420 EU/ m^3 and were thus comparable to the present study (1–304 EU/ m^3) (Duquenne et al., 2014). However, the number of measurements in that study were few (n=39) and the sampling devices were not worn by sewer workers but by an independent person close to the worker, possibly resulting in a lower exposure. The exposure to H_2S was in general low when working in the sewage plants and sewer net system, but peaked to high concentrations when handling sewage and transporting sludge (5–270 ppm). This result is comparable with the study of Lee et al. (2007), where 140 workers at sewage plants and sewer net system in USA were exposed to peak levels from 42 to 122 ppm during collection and dewatering of sludge.

Respiratory symptoms among WWWs may often be associated with exposure to endotoxins, while CNS symptoms are known to be associated with exposure to H_2S. High prevalence of symptoms, both from the airways and central nervous system, were observed in this study. There was no significant difference in the prevalence of self-reported symptoms between sewage plant and sewer net system workers, although the sewage plant workers tended to report symptoms more often than the sewer net workers. A high percentage of workers reported a feeling of fever and influenza-like symptoms after work among both groups of workers (35%), and the prevalence was higher than earlier reported among 44 sewage plant workers in Norway (0%) and 2% among 371 Dutch sewage workers (Smit et al., 2005). CNS-related symptoms such as tiredness (46%) and concentration difficulties (32%) were also more often reported compared to 141 sewage plant workers in a Dutch study (6% and 1%, respectively) (Douwes et al., 2001), but at similar prevalences among 59 Swedish sewage plant workers (31% and 10%, respectively) (Thorn et al., 2004). However, exposure to H_2S was not measured in these studies. In a case report, several workers in the sewer net system developed airway symptoms such as cough and sore throat, in addition to influenza-like symptoms, which persisted several weeks after incidences of probable high H_2S exposure (Watt et al., 1997). However, personal exposure to H_2S was not measured in that study, and exposure was recorded subjectively based on the smell of gas. In the same case report, an impact on the lung function (FEV_1) was observed among several workers up to one year after exposure to highly odorant gas when removing a broken pipe (Watt et al., 1997), but no associations between the self-reported symptoms and exposure to endotoxins or H_2S were observed.

A lower level of the lung-specific pneumoprotein CC16 and a close to significant higher level of the adhesion molecule ICAM-1 were observed among the sub group of sewer net workers compared to the referents. This result may indicate early signs of both lung specific and systemic inflammation, which has been previously reported among sewage plant workers exposed to sewage dust (Heldal et al., 2013, 2016). A lower concentration of CC16 compared to the reference suggested that chronic exposure may compromise the synthesis or secretion of the protein. In addition, a dose-response relationship between exposure to bacterial cells and CC16 measured in blood taken the same day as the exposure was measured for that individual, indicated a more acute response in a previous study (Heldal et al., 2013). Among the same sewage plant workers, an association between sewage dust exposure and elevated serum concentration of the adhesion molecule ICAM were observed, suggesting that the increase in ICAM-1 may be initiated by a local inflammatory response in the pulmonary tissue caused by exposure to bacteria and dust (Heldal et al., 2016). In the present study, we found associations between estimated exposure to H_2S and serum concentration of ICAM-1, indicating that H_2S may also contribute to the observed inflammatory response. ICAM-1 could play a pivotal role in acute and possibly in chronic inflammation (Beck-Schimmer et al., 2002, Courmier and Israël-Assayag, 2004). Animal studies have shown that ICAM-1 contributes in the development of acute lung inflammation by adhesion of neutrophils to alveolar epithelial cells after instillation of bacterial LPS in rats (Beck-Shimmer et al., 1997, 2002).

The most plausible causative agent of the observed nonspecific inflammatory reaction is endotoxins (Rylander and Jacobs, 1999, Thorn 2001). The elevated level of CRP observed in this study, particularly among sewer net workers, suggests a systemic inflammatory response, which may originate from the airways. In agreement with an earlier study among sewage plant workers (Heldal et al., 2016), we found that the elevated level of CRP was associated with impaired lung function among the WWWs. However, no association between exposure to endotoxins and levels of CRP was observed among the WWWs in previous studies. A study among 33 greenhouse workers showed that CRP levels positively associated with endotoxin exposure (Madsen et al., 2016). These workers were higher exposed to endotoxins (med 66 EU/m^3, range 1–3100 EU/m^3) compared to our study. On the other hand, neither elevated levels of CRP (GM 1.7 mg/l) compared to the control group (1.3 mg/l) nor association to the exposure were found among grain workers (n=67) exposed to even higher levels of endotoxins (GM 777 EU/m^3, GSD 5,53) (Straumfors et al., 2018).

We observed an association between the relatively low exposure level of endotoxins and the absolute value of FEV_1, indicating that the exposure during handling sewage had an impact on lung function. An impairment of lung function variables (FEV_1 %

predicted and FVC % predicted) compared to the referents were observed earlier among workers exposed to sewage dust (Heldal et al., 2010), however, with no association to the exposure. A significant impact on the lung function (FEV_1) related to the relatively moderate exposure level to endotoxin (1–214 EU/m^3) was also reported among 78 sewer workers in a Polish study (Cyprowski et al., 2015), at an exposure level of endotoxin comparable to the present study (1–340 EU/m^3).

In this study, we have investigated a presumed interaction between combined exposure to endotoxins and H_2S on the lung function among WWWs. The inflammatory airway effects of endotoxins are well known (Thorn 2001, Heldal et al., 2013) as is the impact on lung function being related to H_2S exposure (Richardson, 1995). However, no such interactions were observed in our study.

Conclusion

In summary, the results from this study demonstrate that sewer net workers are more exposed to incidental H_2S peaks than workers at sewage plants, and less exposed to endotoxins. However, despite moderate levels of endotoxin and H_2S exposure, the results indicate an impact of these agents on lung function and the adhesion molecule ICAM-1, respectively. The results also signify a low-grade systemic inflammation shown by an increased level of CRP, particularly among workers at the sewer net system, a relation which was associated with an observed lower lung function among the workers. Exposure to dust and gases among sewer and sewage treatment plant workers should be reduced in order to prevent lowered respiratory function and negative health effects from inflammation.

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Table 1. Characteristics of the study population

Characteristics	Referents	Workers		
		All	Sewage plant	Sewage net system
	n=27	n=121	n=46	n=75
Male, %	96	94	89	97
Age, year[A]	40.5 (7.7)	44.6 (9.5)	42.9 (11.0)	45.7 (8.3)
Atopy positive, %	29,6	22	27,3	18,9
Smoking. %	11,1	20,7	17,4	22,7
Body mass index, BMI [A]	28.4 (5.2)	28.6 (5.1)	27.7 (4.8)	29.2 (5.2)

A: Average (standard deviation)

Table 2. Descriptive statistics for the concentration of endotoxin, dust particles and calculated H2S index for the determinants work place, work operations, seasons and degree of flushing

Work elements			Exposure					
			Endotoxin, Eu/m^3		Dust particles, mg/m^3		H_2S, index	
			n	Median (Range)	n	Median (Range)	n	Median (Range)
Work place	Urban sewage plants	VEAS	21	24 (3-303)	22	0.2 (0.1-1.9)	11	0.9 (0.3-36.7)
		Bekkelaget	14	50 (5-342)	13	0.3 (0.1-1.4)	11	1.0 (0.3-7.4)
	Rural plants and net system	Støren	2	121 (57-185)	2	0.4 (0.3-0.5)	2	2.8 (0.3-5.5)
		Klæbu	8	77 (5-271)	9	0.3 (0.1-1.6)	8	8.4 (0.3-177.5)
		Selbu	8	119 (3-193)	8	0.2 (0.1-0.7)	7	13.1 (3.7-281.0)
		Steinkjer	8	7 (2-61)	26	0.2 (0.1-0.8)	15	4.4 (0.3-338.6)
	Urban network sytem	Oslo VAV	39	9 (2-304)	40	0.2 (0.1-1.8)	14	0.4 (0.3-75.1)
		TrheimVAV	60	18 (1-152)	14	0.4 (0.1-0.8)	25	0.7 (0.3-25.8)
Work operations	Inspection	urban plants	37	36 (3-342)	45	0.3 (0.1-1.9)	31	0.7 (0.3-36.7)
		rural plants/network	23	30 (2-271)	22	0.2 (0.1-1.6)	25	5.5 (0.3-281.0)
		urban network	34	7 (1-150)	19	0.3 (0.1-1.8)	12	0.5 (0.3-55.5)
	Septic/sludge		44	37 (2-304)	35	0.3 (0.1-1.5)	25	3.0 (0.3-338.6)
	Greace		10	13 (5-70)		-		-
	TV-inspection/ditches		12	9 (3-30)	13	0.2 (0.1-1.2)	4	5.1 (1.0-8.0)
Season		Winter	53	8 (2-193)	34	0.3 (0.1-1.8)	26	3.4 (0.3-281.0)
		Spring	18	56 (6-152)	14	0.4 (0.1-0.8)	14	0.7 (0.3-5.3)
		Summer	37	17 (1-304)	46	0.2 (0.1-1.6)	25	6.3 (0.3-338.6)
		Authum	52	26 (2-342)	40	0.3 (0.1-1.9)	28	0.3 (0.3-55.5)
Flushing		No	51	12 (2-271)	51	0.3 (0.1-1.8)	34	0.6 (0.03-55.5)
		1 to 3 times	50	38 (3-342)	47	0.3 (0.1-1.9)	31	1.2 (0.3-25.8)
		more than 3 times	56	17 (1-304)	36	0.2 (0.1-1.1)	28	7.6 (0.3-338,6)

Descriptive statistics expressed with number of measurements and the corresponding median, minimum and maximum value (range) of exposure to three deifferent agents influencing the WWW's

Table 3. Lung function in workers and referents

Parameter	Referents (n=27)	Workers: All (n=119)	Workers: Sewage plant (n=45)	Workers: Sewer net (n=74)
	AM (SD)[A]	AM (SD)	AM (SD)	AM (SD)
FEV_1 % of predicted[B]	102.0 (10.1)[abc]	92.6 (14.6)[a]	93.0 (16.4)[b]	92.4 (13.4)[c]
FVC % of predicted[B]	106.4 (11.5)[a]	97.9 (12.9)[a]	99.7 (13.1)	96.8 (12.7)
FEV_1/FVC	0.79 (0.1)	0.77 (0.1)	0.76 (0.1)	0.78 (0.1)

A: AM=Arithmetic mean, SD=standard deviation
B: Predicted using European normal values for sex, age and height
*p<0.05 (GLM test) adjusted for age, smoking and BMI (body mass index
a: between referents and all workers
b: between referents and plant workers
c: between referents and net workers

Table 4. The concentrations of biomarkers in sewage plant and sewer system workers and referents

	Referents				Workers											
					All				Sewage plants				Sewe system			
Biomarkers	n[A]	AM	Med	(range)	n	AM	Med	(range)	n	AM	Med	(range)	n	AM	Med	(range)
CRP (ug/ml)[abc]	27	1.4[ab]	0.8	(0.1-5.0)	118	2.6	1.2[a]	(0.1-19.0)	44	1.9[c]	1.2	(0.1-14.0)	74	3[bc]	1.5	(0.3-19)
ICAM-1 ng/ml	27	84	75	(26-174)	118	97	90	(25-237)	44	88	83	(36-188)	74	102	98	(25-237)
IL-8 pg/ml	26	13	84	(6-56)	118	26	12	(4-389)	44	37	13	(4-389)	74	18	10	(4-278)
Sp-D pg/ml	27	14	8	(0.4-159)	118	14	7	(0.3-146)	44	12	7	(0.3-74)	74	14	7	(0.3-146)
CC16 ng/ml[b]	26	44	34	(5-152)	118	35	30	(7-131)	44	39	36	(8-98)	74	32[b]	27	(7-130)
MIP1a pg/ml	27	99	9	(0.1-748)	111	65	8	(0.3-735)	44	53	6	(0.1-735)	74	65	6	(0.1-650)
MIP1b pg/ml	26	25	22	(4-63)	110	22	71	(2-71)	43	22	18	(2-71)	68	21	19	(0.1-69)

A: number of measurements

AM=aritmetic mean, Med (range) =median and range (lowest - highest value)

$p<0.05$ a: between refernts and all workers, b: between referents and net workers, c: between sewage plant and net workers

Table 5. Linear mixed effect model (MEM) of exposure determinants for endotoxin, dust particles (ln values) and H_2S index

	ln endotoxin			ln dust particles			H_2S index		
Determinants	MEM B	(SE)	e^B	MEM B	(SE)	e^B	MEM B	(SE)	e^B
Intercept	-0.295	0.742	0.745	-1.67*	0.28	0.19	5.02*	1.35	151.4
Work operation									
Rural plant/net	0.97	0.60	2.64	0.16	0.34	1.17	-1.73	1.51	0.18
Septic	1.26*	0.46	3.53	0.15	0.30	1.16	-0.42	1.46	0.66
Sewage plants	1.23	0.77	3.42	-0.21	0.28	0.81	-2.55	1.52	0.08
Grease	1.01	0.64	2.75	-					
Sewer net	0.32	0.44	1.38	0.02	0.30	1.02	0	0	1
TV inspec/ditches	0	0	1	0	0	1			
Seasons									
Autumn	1.21*	0.27	3.35				-1.36*	0.65	0.26
Summer	0.61*	0.29	1.84				-1.10	0.82	0.33
Winter	1.56*	0.36	4.76				-2.49*	0.88	0.08
Spring	0	0	1				0	0	1.00
Work place									
Veas	2.02*	0.54	7.54				-1.14	1.10	0.32
Bekkelaget	2.26*	0.59	9.58				0.07	1.22	1.07
Oslo	2.15*	0.67	8.58				-2.80*	1.09	0.06
Støren	4.19*	1.04	66.02				-1.90	1.33	0.15
Klæbu	3.19*	0.75	24.29				-0.38	0.69	0.68
Selbu	3.12	0.74	22.64				0.57	0.90	1.77
Trondheim	1.65*	0.61	5.21				-2.07*	1.00	0.13
Steinkjer	0	0	1				0	0	1
Flushing									
Not	-0.78*	0.26	0.46	0.56*	0.21	1.75	-1.10*	0.47	0.33
1 to 3 times	-0.23	0.26	0.79	0.61*	0.21	1.84	-1.16*	0.45	0.31
More than three tomes	0	0	1	0	0	1	0	0	1.00

SE, Standard error. *p<0.05

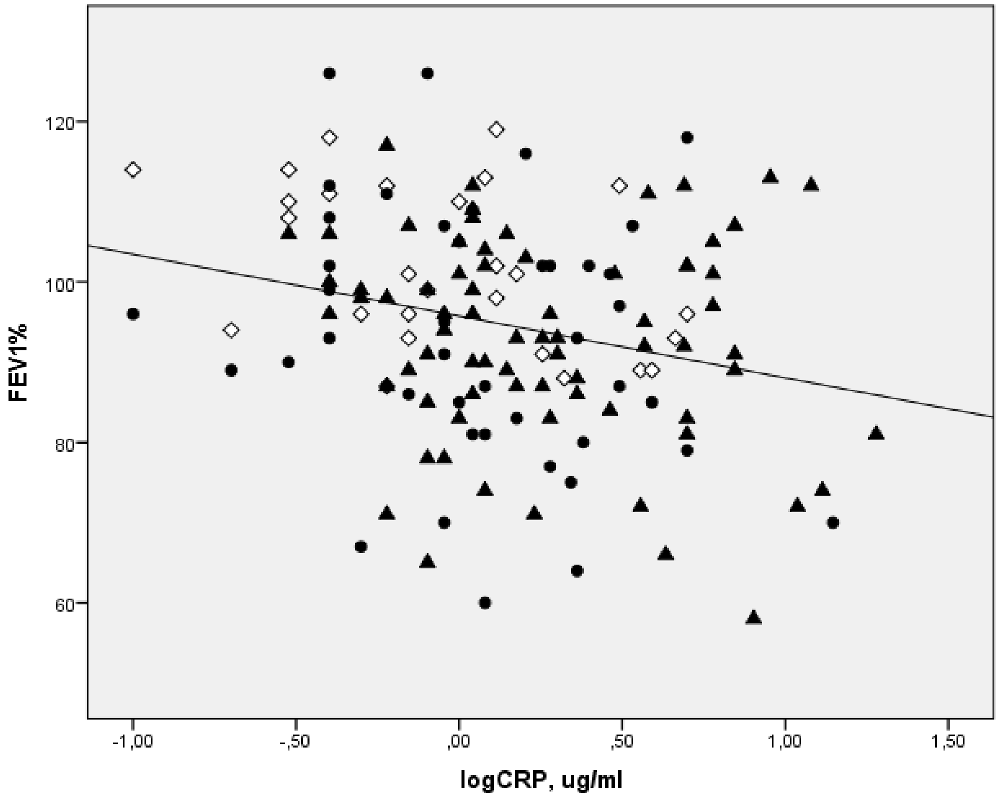

Figure 1. Relationship between serum concentration of CRP in 27 referents (open diamond symbol), 46 sewage plant workers (filled round symbol) and 75 sewer net workers (filled triangle symbol) and FEV1 in % of predicted (β = -7.7, R^2=0.05, p<0.01)