Sewage operatives at five sewage treatment plants (n = 59) and controls not exposed to sewage (n = 55) were examined to determine work-related symptoms and inflammatory responses. Symptoms were elicited using a questionnaire, and spirometry was performed. Inflammatory markers were determined in blood and nasal lavage. Workplace endotoxin and hydrogen sulfide were measured and adeno- and enterovirus antibodies were evaluated in blood. Gastrointestinal and airway symptoms, joint pains, unusual tiredness, and toxic pneumonitis were more common among operatives, and the proportion of blood neutrophils was higher among operatives as compared with controls. A relationship was found between several reported symptoms and the inflammatory markers. Hydrogen sulfide levels were very low. Endotoxin levels were generally low, but high at some work sites. Key words: inflammation; endotoxin; sewage operatives; enterovirus; work-related symptoms.

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Health effects among operatives in sewage treatment plants have been studied, and various work related symptoms have been described.1,2 The most commonly reported have been irritation of the eyes, nose, and airways, fever, fatigue, headache, and gastrointestinal tract symptoms.3–12 A recent nationwide survey in Sweden has confirmed increased risks for these symptoms, as well as joint pains, among sewage operatives.13 The symptom profiles in this and previous studies are quite consistent.

Some studies have investigated the risks for infectious diseases, often using determinations of specific antibodies in the blood. There are studies reporting an increased prevalence of antibodies against hepatitis A among operatives,3,14–18 but there are also studies not showing such increases.19,20 Flu-like symptoms with fever, shivering, and headache have been related to exposure to Legionella pneumophila21 and Leptospira.22,23

Endotoxins are powerful inflammatory agents.29 It is thus of interest to investigate whether the work-related symptoms among sewage plant operatives could be related to inflammation. For this purpose a study was undertaken among a sample of sewage plant operatives previously investigated13 and controls not exposed to sewage water. A questionnaire was used to determine the presence of work-related symptoms, blood and nasal lavage (NAL) samples were drawn to determine inflammatory markers, and lung function tests were performed. The Ethics Committee of the Faculty of Medicine in Gothenburg approved the study.

MATERIAL AND METHODS

Exposure

The sewage treatment plants in five municipalities (Borås, Falkenberg, Kungsbacka, Skövde, and Mariestad/Skara) in western Sweden were included. The procedures for sewage treatment were similar in the different municipalities (aluminum chloride, polyacrylamide and bacterial cultures were used in the purification process).

Based on previous experiences,1 exposure determinations focused on endotoxin and hydrogen sulfide. Personal and stationary samplers were used. Air samples were taken on two different occasions separated by one week at each work site. For endotoxin determinations, air was drawn through Isopore filters (ATTP 0.8 μm, Millipore, Cambridge, MA, USA) using open filter cassettes. For personal samplers, the filters were placed in the breathing zone and connected to a portable pump (Gil-Air 3 SC, Gilian® personal air sampler, Gilian Instrument Corp., USA) with a flow rate of 2 L/min for at least four hours. The pumps were switched off during the coffee/lunch breaks. Air sam-
samples by stationary samplers were taken at a flow rate of 5 L/min for 60 minutes. Double samples using parallel filters were taken at each stationary measurement. The amounts of airborne endotoxin on the filters were determined using the Limulus assay.30

The amounts of hydrogen sulfide at certain work sites judged to have high risks for exposure (e.g., after transportation of sewage water in long pipes and when handling sludge) were determined with stationary equipment (GMI Personal Monitor, Scotland). Air samples were taken for 180 minutes with registrations each minute of the values in parts per million (ppm).

Population

All operatives at the included sewage plants who were in daily contact with sewage were invited to participate and all volunteered (n = 59). Controls were recruited among other municipal workers not exposed to sewage, such as workers in drinking water plants and gardeners. All subjects were examined on a regular workday and the investigations were performed from September 2001 to May 2002. The population characteristics are presented in Table 1.

Among the sewage operatives a higher proportion had been vaccinated against hepatitis. None of the sewage operatives had physician-diagnosed asthma, as compared with five of the 55 controls.

Questionnaire

The participants were interviewed using a questionnaire similar to the one used in a previous study.13 It explored the presence of symptoms affecting the nose, eyes, airways, and central nervous system, joint pains, and gastrointestinal symptoms during the most recent two weeks. The prevalence of toxic pneumonitis during the most recent 12 months was determined by the question: “Have you had episodes of flu-like symptoms such as fever, chills, malaise, muscle or joint pain, and perhaps also cough, breathlessness and weakness, and felt completely well the following day?” Chronic bronchitis was defined as cough with sputum for at least three months a year for a period of at least two years. Special questions explored whether the subject had a physician-diagnosed allergy, eczema, or asthma. Finally, the questionnaire explored lifetime smoking habits and current alcohol use. Questions were also posed on vaccination programs against hepatitis among the operatives.

Determination of Atopy

The presence in serum of specific IgE antibodies against common airborne allergens (birch, timothy, mugwort, house dust mite d1 and d2, cat, dog, horse, and cladosporium) was determined using the Phadiatop assay based on ImmunoCAP technology (CAP Phadiatop FEIA, Pharmacia Diagnostics AB, Uppsala, Sweden).

Inflammatory Markers in Blood

Blood samples were collected with vacutainer technique in tubes with EDTA for leukocyte counts and tubes with sodium citrate to collect plasma for fibrinogen analyses. Blood in non-prepared tubes was used to collect serum after 60 minutes of coagulation. Serum and plasma were stored at –70°C until analyses.

Total leukocyte counts were performed using a Bürker chamber and microscope. Differential cell counts were made on smears prepared with May–Grünwald/Giemsa stain. The results were expressed as percentages of total leukocytes.

Eosinophilic cationic protein (ECP) was assayed in serum by a fluorescent enzyme immunoassay technique (CAP ECP FEIA, Pharmacia Diagnostics AB, Uppsala, Sweden) and expressed as µg/L (detection limit 2.0 µg/L).

The amount of C-reactive protein (CRP) in serum was quantified using a sensitive double-sandwich ELISA. Rabbit anti-human CRP, peroxidase-conjugated rabbit anti-human CRP, and human serum CRP calibrator were used (DAKO A/S, Glostrup, Denmark). Anti-CRP, 15 mg protein/L in a 0.10-M phosphate-buffered saline, pH 7.2, was incubated overnight in a maxisorp micro plate (NUNC, Roskilde, Denmark), 100 µL per well. The plate was then washed five times with washing and dilution buffer (0.1 M phosphate, 0.50 M NaCl, 0.05 % Tween 20, pH 7.2). The calibrator and samples, diluted in the same buffer, were added and incubated for one hour. The washing was repeated and conjugated anti-CRP, 163 µg immunoglobulin/L was added and incubated for one hour. After washing, the substrate 3,3’,5,5’-tetramethylbenzidin (TMB, R&D Systems, Abingdon, UK) was added, and the reaction...
was stopped after approximately 20 minutes with 1 M phosphoric acid. Light absorbance was measured at 405 nm. All incubations were done at room temperature on a microplate shaker, 700 rpm (detection limit 0.7 µg/L).

Measurements of cytokines focused on monocyte/macrophage-derived cytokines as markers of inflammation. Interleukin 6 (IL-6), IL-8, and tumor necrosis factor α (TNFα) were analyzed in serum using ELISA kits (IL-8 and IL-6; PeliKine-compact ELISA, CLB, Holland; detection limit 0.8 pg/mL). For TNFα we used Quantikine high-sensitive (R&D Systems, Abingdon, UK; detection limit 0.5 pg/mL). L-selectin was measured in serum as a marker of neutrophil recruitment/activation (Parameter Human sL-selectin, R&D Systems, Abingdon, UK; detection limit 1.0 ng/mL).

A modified Clauss method was used to quantify fibrinogen in plasma. The time to coagulation was recorded after the plasma was supplemented with an excess of thrombin. The analyses were performed at the laboratory for clinical chemistry at Borås Hospital, Borås, Sweden.

**Inflammatory Markers in NAL**

Nasal lavage was performed according to the method of Wihl et al.,\(^{31}\) with some modifications. The subject blew his nose at a maximum of 20 and a minimum of 10 minutes before the lavage. During the lavage, the subject was sitting with the head bent forward, in a "writing position." A 6-mL volume of saline, 20–22° C, was instilled into the nostril using a 10-mL syringe attached to a "nose olive." The lavage fluid was flushed back and forth five times and the process was repeated in the other nostril using the same lavage fluid. The retrieved amount of lavage fluid after washing both nostrils was usually about 4 mL. The fluid was transferred to a plastic centrifuge tube and kept on ice. Within 20 minutes the sample was centrifuged at 2,000 × g for 5 minutes. The supernatant was collected and immediately frozen at −20° C. The samples were then stored at −70° C until analyses.

Myeloperoxidase (MPO) was assayed by an enzyme-linked immunoassay ( MPO-EIA R&D Systems, Abingdon, UK; detection limit 1.6 µg/L). ECP, IL-6, IL-8, and TNFα were measured in NAL using the methods described above.

**Entero- and Adenovirus in Serum**

The antibody titers towards entero- and adenovirus in serum were determined by a complement-binding reaction, using a hemolytic system with sheep red blood cells coated with autoantibodies. The analyses were performed at the laboratory for clinical virology, Sahlgrenska University Hospital, Göteborg, Sweden. The result was considered positive if antibodies could be detected. The results were presented as percentages of subjects with a positive test.

**Liver Enzymes**

The amounts of alanine aminotransferas (S-ALAT), aspartate aminotransferase (S-ASAT), and alkaline phosphate (S-ALP) in serum were quantified by measuring the enzymatic activity by photometry. The analyses were performed at the laboratory for clinical chemistry at Borås Hospital, Borås, Sweden.

**Spirometry**

Spirometry was performed using standard techniques. A Vitalograph model S with a PFT printer was used and calibrated every morning with a 1-liter syringe. The subjects performed at least three technically acceptable trials according to ATS criteria, and the largest values for the forced expiratory volume in one second (FEV₁) and the forced vital capacity (FVC) were registered and compared with predicted values.\(^ {32}\)

**Statistical Analyses**

The evaluation focused on a comparison of inflammatory markers between operatives and controls and the relationships between inflammatory markers and symptoms. The symptoms studied can be caused by factors other than those present at the sewage plants, and thus an evaluation was also made for the whole material (operatives and controls amalgamated). Three symptom groups were defined: 1) *airway* symptoms = breathlessness, wheezing, and cough with phlegm or dry cough; 2) *gastrointestinal* symptoms = soft stools or nausea; and 3) *general* symptoms = joint pains, unusual tiredness, headache, heaviness in the head, or concentration difficulties. A positive response was defined as reporting of one or more of the symptoms within each group.

As smoking and asthma may cause symptoms similar to the symptoms caused by sewage and may have effects on the inflammatory markers investigated, data from non-smokers and non-asthmatics were analyzed separately. No influence of either could be found on the extent of symptoms or inflammatory markers.

Analyses of different variables were made using parametric tests in the case of normal distributions and nonparametric tests for non-normal distributions (χ² or Fischer’s exact test and the Mann–Whitney test). Differences were considered significant at \( p < 0.05 \).
a work site in Borås (116 and 185 ng/m³) using personal and/or stationary sampler equipment. Measurements performed after the previously published data30 confirmed this pattern. The highest recorded endotoxin values were found in the flocculoreaction room (4.2 and 17.5 ng/m³) and the sludge thickener (9.9 and 10.8 ng/m³) in Kungsbacka.

Measurements of airborne hydrogen sulfide generally showed low values, in the region of 0 ppm. The highest recorded value was 6.6 ppm at the sewage plant in Falkenberg.

As most of the operatives (64%) had been vaccinated against hepatitis, no evaluation could be performed on the risk of contracting hepatitis infection when working with sewage water.

The different symptoms reported among operatives and controls are shown in Table 2. The prevalences of certain airway symptoms, nonspecific gastrointestinal symptoms, joint pains and central nervous system symptoms were higher among operatives as compared with controls.

There was no difference in lung function values between the sewage operatives and controls (data not shown).

Data on blood cells, inflammatory markers, virus antibodies, and liver enzymes among operatives and controls are shown in Table 3. A higher proportion of neutrophils and a lower proportion of lymphocytes were found among the sewage operatives as compared with controls.

In the analyses of operatives in each individual sewage treatment plant compared with controls, some differences were found in certain parameters. As the number of operatives was rather small in each plant and no consistent pattern could be found with respect to exposures, these differences are not reported. Regarding exposure data, higher proportions of the operatives in the Skövde and Mariestad/Skara plants had enterovirus antibodies in blood as compared with controls (40% vs 9%, \( p < 0.05 \) and 33% vs 9%, \( p = 0.08 \)). When comparing all subjects with and without enterovirus antibodies in blood (yes/no), no significant difference in inflammatory markers was found (data not shown).

Inflammatory markers in NAL among operatives and controls are shown in Table 4. There was no difference in inflammatory markers between sewage operatives and controls. Data on TNFα/H9251 are not reported, since only a few of the subjects had values above the detection limit.

When the whole material (sewage operatives and controls) was analyzed, subjects who reported general

<table>
<thead>
<tr>
<th>TABLE 2. Extent of Different Symptoms Reported by Operatives and Controls</th>
<th>Operatives</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breathlessness</td>
<td>8.5%</td>
<td>5.5%</td>
</tr>
<tr>
<td>Chronic bronchitis</td>
<td>8.5%</td>
<td>7.3%</td>
</tr>
<tr>
<td>Congested nose</td>
<td>27.1%</td>
<td>25.5%</td>
</tr>
<tr>
<td>Wheezing chest</td>
<td>3.4%</td>
<td>12.7%</td>
</tr>
<tr>
<td>Cough with phlegm</td>
<td>18.6%</td>
<td>7.3%</td>
</tr>
<tr>
<td>Dry cough</td>
<td>13.6%</td>
<td>10.9%</td>
</tr>
<tr>
<td>Toxic pneumonitis</td>
<td>25.4%*</td>
<td>5.5%</td>
</tr>
<tr>
<td>Eye irritation</td>
<td>20.3%</td>
<td>18.2%</td>
</tr>
<tr>
<td>Unusual tiredness</td>
<td>30.5%*</td>
<td>5.5%</td>
</tr>
<tr>
<td>Headache</td>
<td>23.7%</td>
<td>20.0%</td>
</tr>
<tr>
<td>Headaches in the head</td>
<td>22.0%</td>
<td>14.5%</td>
</tr>
<tr>
<td>Concentration difficulties</td>
<td>10.2%</td>
<td>5.5%</td>
</tr>
<tr>
<td>Soft stools</td>
<td>32.2%†</td>
<td>12.7%</td>
</tr>
<tr>
<td>Nausea</td>
<td>6.8%</td>
<td>0%</td>
</tr>
<tr>
<td>Joint pains</td>
<td>35.6%†</td>
<td>15.4%</td>
</tr>
</tbody>
</table>

* \( p < 0.01 \), † \( p < 0.05 \), compared with controls.

<table>
<thead>
<tr>
<th>TABLE 3. Inflammatory Markers and Cells in Blood, Viral Antibodies, and Liver Enzymes among Operatives and Controls, Medians and Ranges</th>
<th>Operatives</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECP</td>
<td>7.1 µ/L (2.0–29.2)</td>
<td>7.6 µ/L (2.1–53.1)</td>
</tr>
<tr>
<td>IL-8</td>
<td>6.7 pg/mL (0.9–74.3)</td>
<td>5.2 pg/mL (1.0–22.0)</td>
</tr>
<tr>
<td>IL-6</td>
<td>1.4 pg/mL (0.1–17.8)</td>
<td>1.7 pg/mL (0.8–6.2)</td>
</tr>
<tr>
<td>TNFα</td>
<td>1.8 pg/mL (0.7–32.3)</td>
<td>1.9 pg/mL (0.7–13.9)</td>
</tr>
<tr>
<td>L-selectin</td>
<td>0.9 mg/L (0.5–2.0)</td>
<td>1.0 mg/L (0.5–1.3)</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>2.5 g/L (1.1–4.4)</td>
<td>2.4 g/L (1.3–4.7)</td>
</tr>
<tr>
<td>CRP mg/L</td>
<td>1.1 mg/L (0.1–21.6)</td>
<td>0.8 mg/L (0.1–10.6)</td>
</tr>
<tr>
<td>ASAT</td>
<td>0.4 µkat/L (0.3–0.9)</td>
<td>0.4 µkat/L (0.2–0.8)</td>
</tr>
<tr>
<td>ALAT</td>
<td>0.4 µkat/L (0.1–1.5)</td>
<td>0.4 µkat/L (0.2–0.9)</td>
</tr>
<tr>
<td>ALP</td>
<td>2.6 µkat/L (1.4–5.3)</td>
<td>2.7 µkat/L (1.5–4.9)</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>2% (0–8)</td>
<td>2% (0–8)</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>40% (18–59)*</td>
<td>44% (26–88)</td>
</tr>
<tr>
<td>Monocytes</td>
<td>3% (0–7)</td>
<td>4% (0–14)</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>56% (34–76)†</td>
<td>50% (11–70)</td>
</tr>
<tr>
<td>Adenovirus antibodies</td>
<td>21%</td>
<td>17%</td>
</tr>
<tr>
<td>Enterovirus antibodies</td>
<td>16%</td>
<td>9%</td>
</tr>
</tbody>
</table>

* \( p < 0.01 \), † \( p < 0.05 \), compared with controls.
symptoms (defined in the method section) had significantly higher CRP values in blood compared with those who did not report such symptoms (median = 1.1 mg/L, range = 0.14–21.6, n = 55 vs median=0.79 mg/L, range = 0.11–5.2, n = 57, p < 0.05). Subjects reporting joint pains had a tendency to higher CRP values in blood (median = 1.1 mg/L, range = 0.21–21.6, n = 30 vs median=0.83 mg/L, range = 0.11–5.2, n = 82, p = 0.06). Subjects who reported congested nose had significantly higher IL-8 levels in NAL compared to those who did not report congested nose (median = 317 pg/mL, range = 48–2,800, n = 29 vs median = 215 pg/mL, range = 30–1,866, n = 83, p < 0.05).

Subjects who reported airway symptoms or gastrointestinal symptoms had significantly lower FEV1 values as percentage of predicted as compared with those who did not report such symptoms (median = 95%, range = 71–128, n = 30 vs median = 102%, range = 71–137, n = 84, p = 0.05 and median = 93%, range = 71–121, n = 34 vs median = 103%, range = 71–137, n = 80, p < 0.05, respectively).

### DISCUSSION

The sewage treatment plants in five municipalities in western Sweden were included in the study. They were selected based on practical aspects and similarities of sizes, numbers of operatives employed, and procedures for cleaning sewage. The controls were recruited among municipal personnel not exposed to sewage who were similar in socioeconomic status to the sewage operatives. The operatives and controls were also similar with respect to age, years of employment, gender, smoking habits, alcohol use, and prevalence of atopy. There were some subjects with physician-diagnosed asthma among the controls, while there was no sewage operative with that diagnosis, which may indicate a healthy-worker effect.

The prevalences of nonspecific gastrointestinal symptoms, joint pains, toxic pneumonitis, and central nervous system symptoms were higher among operatives as compared with controls. The results are similar to those reported in a previous nationwide survey and in several previous smaller studies. This indicates that the sample recruited for this study was representative of operatives in sewage treatment plants in general.

Determinations of inflammatory markers in blood and NAL were performed. A significant higher proportion of neutrophils in blood was found among the sewage operatives as compared with the controls, but no difference could be detected in the other markers of inflammatory determined. It is known from previous studies that inhalation of endotoxin causes a neutrophil-dominated inflammation and that blood neutrophil activation can be recorded after very low concentrations of inhaled endotoxin. According to the measurements performed in these sewage treatment plants, the levels of endotoxin exposure were generally low, but occasional high amounts of airborne endotoxin could be generated under certain conditions. Toxic pneumonitis has been related to endotoxin exposure. In this study, 25% of the sewage operatives reported that they had experienced toxic pneumonitis, indicating that airborne endotoxin in amounts inducing inflammation could be regarded as a workplace exposure risk in sewage treatment plants.

Regarding the symptoms as such in the total population, relationships were found between several of the symptoms and inflammatory markers. This suggests that airway, gastrointestinal, and general symptoms such as fatigue and joint pains can be related to an inflammatory response. However, whether such an inflammatory response was related to sewage exposure could not be evaluated, as the unexposed controls were included in the analyses. Additional analyses comparing the relationships between symptoms and inflammatory markers among sewage operatives and controls, separately, would have been optimal, but such analyses were not possible due to the small sample size.

In conclusion, the results confirm that sewage operatives experience certain symptoms as described in previous studies. They also had a higher proportion of neutrophils in blood, suggesting an influence by workplace exposure. Workplace exposures to inflammatory agents such as endotoxins and viruses may occur.

The authors thank Dag Söderberg, Gunilla Arvidsson, Erika Kerekes, and Rose-Marie Olofsson for technical assistance during exposure measurements and analyses of endotoxin as well as clinical examinations and analyses.

### References


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### Table 4. Inflammatory Markers in NAL among Operatives and Controls, Medians and Ranges

<table>
<thead>
<tr>
<th>Marker</th>
<th>Operatives (n = 59)</th>
<th>Controls (n = 55)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPO</td>
<td>255 µg/L (40–1180)</td>
<td>273 µg/L (25–9146)</td>
</tr>
<tr>
<td>ECP</td>
<td>2.1 µg/L (2.0–42.9)</td>
<td>2.4 µg/L (2.0–104.0)</td>
</tr>
<tr>
<td>IL-8</td>
<td>188 pg/mL (44–2800)</td>
<td>277 pg/mL (30–1866)</td>
</tr>
<tr>
<td>IL-6</td>
<td>2.4 pg/mL (0.8–212.3)</td>
<td>1.9 pg/mL (0.8–100.0)</td>
</tr>
</tbody>
</table>

COVER NOTE

The scene on the cover is from a Library of Congress collection of photographs that demonstrate exposures to asbestos in the back shops in the Western United States. In this picture, workers smoke on their lunch break surrounded by asbestos dust on the floor, the engine jacket, and the engine itself. This photograph is by Jack Delano, a remarkable man who photographed the railroads in the 40s for the Office of War Preparedness. A true workers' advocate, he was an immigrant from Ukraine who changed his name to Delano because of his admiration for Franklin Delano Roosevelt. (Photo courtesy of the Library of Congress Prints & Photographs Collection; descriptive information courtesy of Daniel T. Teitelbaum, MD.)