

Airway inflammation in waste handlers exposed to bioaerosols assessed by induced sputum

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Airway inflammation in waste handlers exposed to bioaerosols assessed by induced sputum. K.K. Heldal, A.S. Halstensen, J. Thorn, W. Eduard, T.S. Halstensen. ©ERS Journals Ltd 2003.

ABSTRACT: Work-associated lower airway inflammation in waste collectors was examined by induced sputum and correlated with the bioaerosol exposure.

Organic waste collectors (n=25) underwent induced sputum collection and spirometry before work on Monday and the following Thursday. Total cells, cell differentials, interleukin (IL)-8 and eosinophilic cationic protein were determined. Personal full-shift exposure measurements were performed Monday, Tuesday and Wednesday and analysed for total bacteria, fungal spores, endotoxins and $\beta_{(1-3)}$ -glucans.

The percentage of neutrophils (46–58%) and the IL-8 concentration (1.1–1.4 ng·mL⁻¹) increased from Monday to Thursday. Forced expiratory volume in one second (FEV₁) was significantly reduced on Thursday, and the decrease in FEV₁/forced vital capacity correlated with the increase in the percentage of neutrophils. The median exposure to endotoxin (range 7–180 EU·m⁻³) and $\beta_{(1-3)}$ -glucan (range 5–220 ng·m⁻³) was correlated with the increase in IL-8.

Bioaerosol exposure during waste collection induced an inflammatory response in the lower airways, characterised by neutrophils and interleukin-8 secretion, that influenced the lung function. The inflammatory response was related to microbial components in the bioaerosol and was more pronounced for endotoxin than $\beta_{(1-3)}$ -glucan exposure. No associations were found for mould spores or bacteria.

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Collection of source-separated organic waste has introduced new health hazards, predominantly respiratory inflammation, for an increasing number of workers. Bioaerosols generated from decaying organic waste contain bacteria, bacterial and fungal spores, and microbial components like endotoxins and $\beta_{(1-3)}$ -glucans that may induce inflammation [1, 2]. Inhalation of endotoxins induces the influx of neutrophils and the production of pro-inflammatory cytokines and biomarkers, which is reflected in airway fluids. Endotoxins are regarded as the most potent inflammatory component in bioaerosols [3]. However, exposure to endotoxins during waste collection is usually low compared with the "no-effect level" for airway inflammation [2–6]. Yet, increased levels of neutrophils and their product, myeloperoxidase (MPO), in the nasal lavage from waste collectors and compost workers were related to the endotoxin exposure during work [1, 5]. Animal and human inhalation studies have suggested that $\beta_{(1-3)}$ -glucans have inflammatory properties. $\beta_{(1-3)}$ -glucans are regarded as a marker of fungal spores, but can also be present in some bacteria and plant material. The response in the airways is different from that induced by inhaled endotoxins. This probably depends on the structure and physical properties of $\beta_{(1-3)}$ -glucans [7]. Animal studies have shown that $\beta_{(1-3)}$ -glucan exposure causes an eosinophil dominated inflammatory response, which decreases after simultaneous endotoxin exposure [8]. Conversely, in a similar animal model, a single exposure to $\beta_{(1-3)}$ -glucans is reported to reduce the inflammatory response to endotoxins, while repeated exposures have induced a synergistic inflammatory effect between $\beta_{(1-3)}$ -glucans and endotoxins [7]. These studies suggest that bioaerosol exposure induced inflammatory responses

associated with different components in the aerosol with a possible complex interaction between the microorganisms or microbial cell wall components.

In this study, induced sputum was used to assess airway inflammation in waste collectors. Sputum examination has become an important noninvasive method for direct investigation of the degree of lower airway inflammation. At present, this method has proven its feasibility mainly in human challenge studies to reveal mechanisms of asthma and other lung diseases. A few small studies of working populations have also been reported [2, 9]. The purpose of this study was to assess the various cell types and mediators of inflammation in the induced sputum of waste collectors and to correlate these findings with the exposure to different components in the bioaerosol generated when collecting household waste.

Material and methods

Population

The survey was performed at a waste company in a community near Oslo, in which separation of household waste was implemented in 1997. The organic waste fraction, mainly consisting of food and garden waste, was stored in closed containers and collected every fortnight. The survey took place in the summers of 1999 and 2000. In total, 25 waste collectors participated voluntarily in the survey (84% participation rate), of which 22 collected the organic waste and three collected the paper waste fraction of the household waste. The main work tasks for the collectors were emptying containers

and driving trucks. The workers had been employed for 1.0 yr (median) at the company (range 1–8 yrs). The median age was 32 yrs, range of 17–53 yrs. Current smokers included 88% (n=22) of workers, with an arithmetic median tobacco consumption of 11 (range 0–34) pack-yrs. Sixteen waste collectors tested voluntarily for respiratory allergens by the Phadiatop test (fluorescent enzyme immunoassay (FEIA), UniCap system; Pharmacia Diagnostic AB, Uppsala, Sweden). Five of these collectors (31%) revealed atopy.

Study design

Medical examination of the waste collectors was performed at the local hospital. The examination consisted of spirometry and sputum collection before working on Monday morning and before working on the following Thursday morning. The workers were asked not to smoke 1 h before the medical examination. Personal full-shift exposure was monitored on each day between the medical examinations. All subjects gave their informed consent and the study was approved by the Regional Medical Ethics Board.

Sampling and analysis of exposure

Inhalable aerosols were collected by personal sampling with two personal aerosol sampler cassettes (inlet 6 mm; National Institute of Occupational Health, Oslo, Norway) simultaneously. One sample was collected on a glass fibre filter (Whatman GF/A; Whatman, Maidstone, MA, USA) and analysed for endotoxins and $\beta_{(1-3)}$ -glucans by specific, quantitative, kinetic and chromogenic *Limulus amoebocyte-lysate* assays [10, 11]. The other sample was collected on a polycarbonate filter with pore size 0.8 μm (Poretics; Osmonics, Livermore, MN, USA). In these samples, rod-shaped and spherical bacteria were determined by fluorescence microscopy [12], and spores from fungi and actinomycetes were counted in a scanning electron microscope [13]. The exposure to bacteria was expressed both as total bacteria counted (the sum of spherical, rod-shaped bacteria and actinomycetes spores), as well as the number of actinomycetes spores, spherical bacteria and rod-shaped bacteria separately.

Induced sputum

The induction procedure of PIN *et al.* [14] was used with some modifications. After bronchodilatation (β_2 -agonist, 200 μg salbutamol inhalation), induction was performed by 7-min inhalation periods of 3 and 5% hypertonic saline using an ultrasonic nebuliser (DeVilbiss; Sunrise medical, Somerset, PA, USA) following a baseline spirometry. The inhalation was stopped if a sputum sample of good quality was obtained. Spirometry was also performed after the induction periods for safety reasons. If forced expiratory volume in one second (FEV1) decreased >20% from the baseline value, the sputum induction was discontinued.

Sputum processing and cell counting

Samples were processed according to the "plug method" described by PIZZICHINI *et al.* [15] with some modification. The supernatants after dithiothreitol incubation and centrifugation were added to a protease inhibitor cocktail (soya bean trypsin inhibitor, aprotinin, β_1 -antitrypsin, pepstatin A, 1,10-phenanthroline, ethylene diamine tetra-acetic acid and

benzamidine; Sigma, St Louis, MO, USA) and frozen to -20°C until analysis. The total cell count and the cell viability of the cell samples were determined using Trypan blue exclusion (0.4%) and a Bürker chamber. Visually identified squamous epithelial cells were excluded from the counts to estimate the total cell concentration. The cell samples were cyto-centrifuged and stained with May-Grünwald-Giemsa. Samples were considered adequate if the differential counts contained <25% squamous cells. At least 400 nonsquamous cells were counted. Stratified counting was performed on eosinophils and lymphocytes to improve the precision. Thus, percentages of lymphocytes and eosinophils were based on 2,000–3,000 cells. The results were expressed as 10^6 cells $\cdot\text{mL}^{-1}$ sputum or as a percentage of the total nonsquamous epithelial cell count. In the study population, 21 waste collectors delivered adequate sputum samples, 20 of which collected organic waste and one collected paper waste.

Analysis of interleukin-8

The concentration of interleukin (IL)-8 was measured by enzyme-linked immunosorbent assay (R&D Systems, Oxon, UK). Spiking experiments in 21 samples from seven workers with recombinant IL-8 concentrations of $\geq 20\%$ of the IL-8 content in the samples, revealed an average of 36% recovery (range 0–72%), suggesting underestimation of IL-8 concentration in sputum. Running the standard curve in several sputum samples revealed individual curves slightly skewed to the right. The average of two standard curves run in sputum samples with low IL-8 concentration was then used to recalculate the IL-8 concentration in all samples. The recovery rose to 65% (range 0–203%). Re-running all statistics revealed slight changes in significance for some of the relationships. However, since it appeared that all workers had an individual sputum-based standard curve, the use of a sputum-based curve on the average of the two workers sputum to calculate the IL-8 concentrations in all workers, was not more or less correct than using the buffer-based standard curve. In order to compare these results with other published reports, the authors decided to use the data based on the buffer-based standard, but have provided the statistical influence of the tested relationships in the results. The concentrations were expressed as $\text{ng}\cdot\text{mL}^{-1}$. The detection limit was 31 $\text{pg}\cdot\text{mL}^{-1}$.

Analysis of eosinophil cationic protein

Eosinophil cationic protein (ECP) was measured by an FEIA technique (UniCAP ECP FEIA, Pharmacia Diagnostics AB). The concentrations were expressed as $\text{ng}\cdot\text{mL}^{-1}$. The detection limit was 2.0 $\text{ng}\cdot\text{mL}^{-1}$.

Spirometry

A spirometer (Vitalograph 2170; Spirotrac IV, Ennis, Ireland) was used and forced vital capacity (FVC) and the FEV1 were measured according to the American Thoracic Society guidelines [16]. The same trained nurse performed all the tests. The largest FEV1 value was selected and compared with predicted values [17]. Subjects with spirometric airflow limitation were defined as those with a ratio of FEV1/FVC of <70% and a FEV1 <80% of predicted value [18].

Questionnaires

The participants completed a self-administered questionnaire including questions on respiratory symptoms that they

had experienced on the day of exposure measurements. This questionnaire had been used previously in investigations of organic dust-related effects [19].

Statistical analysis

Exposure was estimated for each worker by the median of the exposures measured on Monday, Tuesday and Wednesday. Nonparametric tests were used as many values were below the detection limit and the distributions were skewed to the right. The associations between continuous exposure variables were analysed with Spearman correlation coefficients. The levels of sputum parameters in workers with self-reported symptoms on at least one day of exposure measurements, were compared with those without symptoms by the Mann-Whitney U-test. Paired comparisons of sputum parameters and lung function data on Monday and Thursday were performed by Wilcoxon signed-rank tests. Differences were considered statistically significant at $p < 0.05$.

Results

Exposure levels

The exposure levels to microbial agents and inhalable dust are listed in table 1. Exposure levels to endotoxins were relatively low, and only 12% of the samples were $>100 \text{ EU}\cdot\text{m}^{-3}$ ($\sim 10 \text{ ng}\cdot\text{m}^{-3}$), suggested to be a no-effect level for airway inflammation [3]. Dust was intercorrelated to total bacteria (Spearman rank correlation coefficient (r_s)=0.7) and endotoxins (r_s =0.6). Other intercorrelations were low ($r_s < 0.3$).

Inflammatory cells and biomarkers in induced sputum

Total and differential cell counts and inflammatory biomarkers in induced sputum on Monday and Thursday are shown in table 2. The percentage and concentrations of neutrophils, as well as the concentration of eosinophils and IL-8, increased significantly from Monday to Thursday. Both the increase in neutrophils and IL-8 correlated with the increase in ECP (r_s =0.68, $p < 0.01$, and r_s =0.62, $p < 0.05$, respectively). The increase in IL-8 from Monday to Thursday ($p < 0.05$) and the correlation with neutrophil increase also

Table 1. – Exposure of 25 waste collectors to bacteria, fungal spores, endotoxins, $\beta_{(1-3)}$ -glucans and inhalable dust

Agents	Exposure level		
	Arithmetic mean	Median	Range
Total bacteria [#] $10^6\cdot\text{m}^{-3}$	1.20	0.80	0.06–3.80
Rod-shaped $10^6\cdot\text{m}^{-3}$	0.010	0.004	0–0.10
Spherical $10^6\cdot\text{m}^{-3}$	1.10	0.80	0.06–3.70
Actinomycetes spores $10^6\cdot\text{m}^{-3}$	0.03	0	0–0.40
Fungal spores $10^6\cdot\text{m}^{-3}$	0.4	0.2	0–2.0
Inhalable dust $\text{mg}\cdot\text{m}^{-3}$	0.55	0.37	0.10–2.10
Endotoxins $\text{EU}\cdot\text{m}^{-3}$	31	13	7–180
$\beta_{(1-3)}$ -glucans $\text{ng}\cdot\text{m}^{-3}$	59	52	5–220

Data are presented as measures of the individual median exposures from Monday to Thursday. [#]: total bacteria, the sum of spherical and rod-shaped bacteria estimated by fluorescence microscopy, and actinomycetes spores estimated by scanning electron microscopy.

Table 2. – Percentage and concentration of nonsquamous cells and mediators in induced sputum of 21 waste collectors

Induced sputum parameters	Monday	Thursday
Total cells $\times 10^6\cdot\text{mL}^{-1}$	1.39 (0.43–7.10)	2.35 (0.26–11.64)
Macrophages $\times 10^6\cdot\text{mL}^{-1}$	0.65 (0.22–3.41)	0.95 (0.13–3.62)
Neutrophils $\times 10^6\cdot\text{mL}^{-1}$	0.50 (0.08–4.90)	1.23* (0.12–7.95)
Eosinophils $\times 10^6\cdot\text{mL}^{-1}$	0.002 (0–0.05)	0.007* (0–0.05)
Lymphocytes $\times 10^6\cdot\text{mL}^{-1}$	0.004 (0–0.28)	0.005 (0–0.08)
Macrophages %	53 (21–96)	40 (10–79)
Neutrophils %	46 (4–75)	58* (22–90)
Eosinophils %	0.2 (0–1.2)	0.3 (0–4.3)
Lymphocytes %	0.2 (0–1.5)	0.3 (0–1.5)
IL-8 $\text{ng}\cdot\text{mL}^{-1}$	1.14 (0.05–32.12)	1.40* (0.06–51.36)
ECP $\text{ng}\cdot\text{mL}^{-1}$	60.9 (4.8–523)	71.7 (8.0–1800)

Data are presented as median (range). IL: interleukin; ECP: eosinophil cationic protein. *: $p < 0.05$ significance level between Thursday and Monday (Wilcoxon signed-rank test).

maintained significance for sputum-based IL-8 standard curves (r_s =0.67, $p < 0.001$).

Spirometry

Table 3 summarises the lung function variables of the waste collectors measured on Monday morning before work shift and the changes in lung function variables from Monday to Thursday. One worker was omitted because the expiration was terminated too early. Two workers showed spirometric airflow limitation.

A significant decline in FEV1 and FEV1 % of predicted values was observed from Monday to Thursday. Although the decrease in FEV1/FVC from Monday to Thursday did not reach significance ($p=0.08$), the decrease correlated with the increase in the percentage of neutrophils (r_s =–0.51, $p < 0.05$; fig. 1a). For the sputum-based standard curve, the increase in IL-8 from Monday to Thursday correlated with the decrease in FEV1/FVC (r_s =–0.45, $p < 0.05$). Tobacco consumption in pack-years and age were also risk factors for the decline in FEV1/FVC (r_s =–0.48, $p < 0.05$, and r_s =–0.46, $p < 0.05$, respectively).

Work-related symptoms

The prevalence of any respiratory work-related symptom was 29%, chest tightness 14% and cough with or without phlegm 10%. No associations between symptoms and exposure or symptoms and induced sputum variables were found.

Table 3. – Lung function variables in waste collectors

Lung function variables	Arithmetic mean	SD
FEV1 % pred [#]	90.1	10.5
FVC % pred [#]	99.6	13.4
FEV1/FVC %	80.3	12.7
Δ FEV1 [¶] L	–0.12*	0.2
Δ FEV1/FVC% [¶]	–1.34**	3.9
Δ FEV1 % pred [¶]	–3.0*	4.4

FEV1: forced expiratory volume in one second; % pred: % predicted; FVC: forced vital capacity. [#]: lung function on Monday morning before the work shift compared with the European Respiratory Society standard population (never-smokers, without symptoms, age 18–70 yrs); [¶]: difference between Thursday and Monday morning. *: $p < 0.05$; **: $p < 0.01$ (Wilcoxon signed-rank test). n=24.

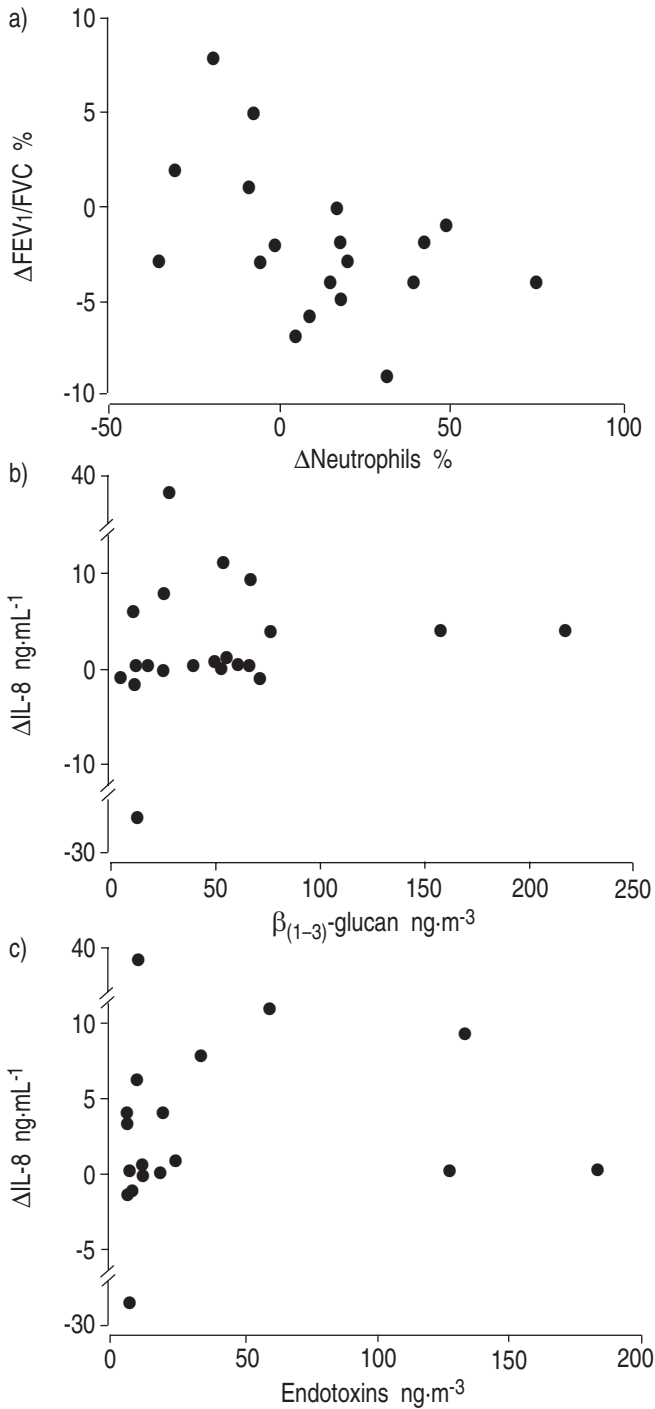


Fig. 1.—Spearman correlations (r_s) between lung function, inflammatory cells and mediators in sputum and exposure. a) Δ Forced expiratory volume in one second (FEV₁)/forced vital capacity (FVC) % and Δ neutrophils % from Monday to Thursday ($n=20$; $r_s=0.51$, $p<0.05$), b) Δ interleukin (IL)-8 and median exposure levels to $\beta_{(1-3)}$ -glucans from Monday to Thursday ($n=21$; $r_s=0.38$, $p=0.05$), and c) Δ IL-8 and median exposure levels to endotoxins from Monday to Thursday ($n=21$; $r_s=0.55$, $p<0.05$).

Associations between exposure and inflammatory mediators in sputum

The increased sputum level of IL-8 from Monday to Thursday correlated significantly with the median exposure to endotoxins ($r_s=0.55$, $p<0.05$) and close to significantly with

the median exposure to $\beta_{(1-3)}$ -glucans ($r_s=0.49$, $p=0.09$; figs 1b and 1c). For the sputum-based standard curve, the endotoxin correlation maintained significance ($r_s=0.51$, $p<0.05$), while the $\beta_{(1-3)}$ -glucan correlation lost significance.

Discussion

This study suggests that even moderate exposure to bioaerosols during collection of organic household waste induces a lower airway inflammation dominated by neutrophils. The increased percentage of neutrophil granulocytes in sputum from the start of work on Monday to the start of work on Thursday correlated not only with increases in the inflammatory mediators IL-8 and ECP, but also with a decrease in FEV₁/FVC. The exposure during waste handling apparently induced the inflammation, since an increase in IL-8 correlated with the exposure to endotoxins and possibly $\beta_{(1-3)}$ -glucans. No associations between respiratory symptoms and inflammatory markers were found, possible due to the relatively small changes in the inflammatory response.

The sputum induction procedure itself is an inflammatory stimulus and may cause increase in inflammatory cells and mediators [20]. Therefore, a time lag from the first to the second sputum induction of 72 h was used, as proposed by NIGHTINGALE *et al.* [21] to reduce possible effects caused by the induction. Tobacco consumption may have modified the correlation between change in lung function and the percentage of neutrophils, indicating that heavy smokers may have a chronic high level of neutrophils in the lower airways. This is in agreement with earlier studies [15]. Mass significance may explain some of the associations between exposure and effects, since 40 associations were tested and two associations are likely to be found with a statistical probability of 0.05. However, all the associations found are biologically plausible.

A comparison of the results in the present study with a study of healthy, nonsmoking, nonatopic subjects ($n=114$) [22], suggested that sputum samples contained approximately the same number of cells, except for a higher percentage of neutrophils (48% *versus* 27%) in the waste collector workers. The low eosinophil concentration suggested a nonspecific inflammation mediated by neutrophils. A neutrophil response was further supported by an increased level of the neutrophil chemoattractant IL-8 from Monday to Thursday. Similar results have been reported recently in nasal lavage among waste collectors [5].

Although the eosinophil concentration in sputum was low, it increased significantly from Monday to Thursday in the present study. An increase in sputum eosinophils is regarded as a characteristic of asthma, but only one worker showed an eosinophil percentage $>2\%$, suggested as a limit for eosinophilic asthma [14].

There was no significant increase in ECP from Monday to Thursday, although the change in ECP correlated with the increase in neutrophils and IL-8. Activated macrophages and neutrophils are producers of IL-8. Also, in patients with chronic obstructive pulmonary disease, an increase in neutrophils in induced sputum was related to the increase in ECP and IL-8 after inhalation of the $\beta_{(1-3)}$ -glucan [23]. The results in the present study therefore suggest a neutrophil-dominated inflammatory response among the waste collectors.

In addition to a significant decrease in FEV₁ from Monday to Thursday, a correlation between the decrease in FEV₁/FVC and the neutrophil influx was found, indicating an acute airway obstruction caused by neutrophils.

Endotoxin exposure was associated to the increase in IL-8 from Monday to Thursday in induced sputum. The acute effects seen after human challenge with endotoxin are

characterised by an influx of neutrophils and an increase in pro-inflammatory cytokines (IL-1, IL-6, tumour necrosis factor- α , and IL-8) reflected in airway fluids [24]. The reason for such short-term effects on long-term changes is not clear. Chronic inflammation in the airways, as occurs in chronically exposed workers, may subsequently result in chronic respiratory symptoms and chronic loss of lung function. Several epidemiological studies of cotton workers, animal feed workers and grain handlers have shown associations between chronic decline in lung function (particularly FEV₁) and exposure to endotoxin-contaminated dust [25–27].

The increase in IL-8 also tended to correlate to the $\beta_{(1-3)}$ -glucan exposure. In an animal inhalation study, $\beta_{(1-3)}$ -glucans did not cause neutrophilia 24 h after a single exposure [7]. However, in repeated exposures, endotoxin and $\beta_{(1-3)}$ -glucans induced an increase in inflammatory cells in a synergistic way. Therefore, it cannot be excluded that the low levels of endotoxin in combination with $\beta_{(1-3)}$ -glucan exposure during waste collection may induce an inflammatory response among the workers.

Associations between endotoxin exposure and IL-8 and MPO levels have previously been shown in nasal lavage of waste collectors and compost workers in the Netherlands [1, 5]. Thus, the inflammatory response in the nose showed similarities with the inflammatory response in the lower airways. This may be secondary to the size of causal agents as it may deposit both in the upper and lower airway. The majority of particles generated from waste have an aerodynamic diameter >10 μm [28], which are predominantly deposited in the nose and pharynx region. However, when inhaling through the mouth, e.g. during the heavy work that waste collectors carry out, larger particles also deposit in the trachea and further down.

In conclusion, exposure to bioaerosols during waste collection may induce a lower airway inflammation that can be observed in induced sputum. The inflammatory response was characterised by a neutrophil infiltration with an increased secretion of interleukin-8 from Monday to Thursday, concurrent with a decrease in forced expiratory volume in one second. These inflammatory responses showed relationships to the endotoxins and possible $\beta_{(1-3)}$ -glucans exposure, but not to fungal spores and total bacteria.

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