ORIGINAL ARTICLE

Cross-shift study of exposure–response relationships between bioaerosol exposure and respiratory effects in the Norwegian grain and animal feed production industry

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ABSTRACT

Objective We have studied cross-shift respiratory responses of several individual bioaerosol components of the dust in the grain and feed industry in Norway.

Methods Cross-shift changes in lung function and nasal congestion, as well as in respiratory and systemic symptoms of 56 exposed workers and 36 referents, were recorded on the same day as full-shift exposure to the inhalable aerosol fraction was assessed. Exposure–response associations were investigated by regression analysis.

Results The workers were exposed on average to 1.0 mg/m3 of grain dust, 440 EU/m3 of endotoxin, 6 μg/m3 of β-1,3-glucans, 17×104/m3 of bacteria and 4×104/m3 of fungal spores during work. The exposure was associated with higher prevalence of self-reported eye and airway symptoms, which were related to the individual microbial components in a complex manner. Fatigue and nose symptoms were strongest associated with fungal spores, cough with or without phlegm was associated with grain dust and fungal spores equally strong and wheeze/tight chest/dyspnoea was strongest associated with grain dust. Bioaerosol exposure did not lead to cross-shift lung function decline, but several microbial components had influence on nose congestion.

Conclusions Exposure to fungal spores and dust showed stronger associations with respiratory symptoms and fatigue than endotoxin exposure. The associations with dust suggest that there are other components in dust than the ones studied that induce these effects.

INTRODUCTION

Grain dust exposure may induce respiratory symptoms in grain handlers, and measures for reducing the dust exposure have constantly attention in the grain industry. Employees at grain elevators and compound feed mills may be exposed to large amounts of grain dust,1 frequently leading to symptoms from the airways and pulmonary functional changes such as asthma.2 Acute and short-term effects, such as cross-shift lung function decline and toxic pneumonitis, have been observed during a workday,3–6 and long-term exposure may lead to permanently decreased lung function,7–9 although results are not always consistent.10–11

The pathological response to grain dust exposure suggests inflammatory and allergic pathways, although exact mechanisms are under debate. The cell wall component of Gram-negative bacteria, endotoxin, has potential to exert several of the observed health effects caused by grain dust exposure,12,13 and many studies from the grain industry include endotoxin measurements. On the basis of in vitro studies, animal experiments and epidemiological studies, endotoxin has been proposed as a target for measurements of occupational health hazards related to grain dust exposure.14 However, grain dust also contains several other components, such as mycotoxins, tannins, lectins, endotoxins, fungal spores and β-1,3-glucans, that may induce immunological, allergic or toxic responses, and interact with the effects of endotoxin.12

What this paper adds

▸ Occupational exposure to grain dust may induce respiratory symptoms in grain handlers, but the dose–response patterns between grain dust exposure and inflammatory response or changes in respiratory function are not consistent between studies. This may be due to differential effects of individual grain dust components, such as mycotoxins, tannins, lectins, endotoxins, fungal spores and β-1,3-glucans, and differences in grain dust composition.

▸ This is the first study in the grain and animal feed industry that has investigated the cross-shift respiratory response to exposure for several individual bioaerosol components of the grain dust.

▸ Bioaerosol exposure did not lead to cross-shift lung function decline, but several microbial components were associated with nose congestion and self-reporting of symptoms in a complex manner.

▸ Exposure to fungal spores and grain dust showed stronger associations with respiratory effects and fatigue than endotoxin exposure.

▸ The strong associations with grain dust suggest that there are other components in grain and feed dust than the ones studied that induce respiratory effects and that remain to identify.
Exposure assessment

The dose–response patterns between grain dust exposure and inflammatory response or changes in respiratory function are contradictory between studies, possibly due to a differential effect of the individual grain dust components. Furthermore, the microbial composition of the dust will vary between crop types, years/weather conditions, geographic regions, companies and job groups.14 This exposure variability may dilute and even bias exposure–response patterns, and should therefore be considered. Few exposure studies in the grain and animal feed industry have included microbial components, and studies of lung function have been conducted without exposure measurements. Only few exposure–response studies have assessed exposure simultaneously with health examinations. To the best of our knowledge, no study has reported exposure–response associations of other exposure components than grain dust and endotoxins. Thus, information on exposure–response associations with other bioaerosol components is needed to understand health effects after grain dust exposure.

Effects of grain dust exposure on nose congestion by the use of acoustic rhinometry (AR) have, to the best of our knowledge, not been studied before. AR provides objective measurements of the cross-sectional area and volume of the nasal cavity, which may reveal inflammatory responses reflected as swelling of the nose mucosa.15

In the present study, we therefore aimed to investigate the cross-shift respiratory response of grain dust exposure, and their associations with individual bioaerosol components of the dust. Cross-shift lung function, nasal congestion and symptoms were recorded on the same day as full-shift exposure to inhalable grain dust, endotoxin, bacteria, fungal spores and β-1,3-glucans were assessed.

MATERIALS AND METHODS

Company and job description

Twenty companies of grain elevators and compound feed mills in the Norwegian grain industry geographically distributed throughout 20 municipalities in 9 counties in central and southeastern Norway were included in this study. Recruitment and description of companies and work tasks is previously published.14

Study population

The study population comprised all employees working day shifts; altogether, 68 grain dust exposed workers and 38 assumed unexposed administrative workers as referents. Two workers assigned to the reference group were excluded because of health reasons they had been replaced from exposed jobs to office work, leaving 36 referents. The exposed workers included 28 workers in the grain elevator department, 17 workers in the compound feed mill department, 6 workers in grain elevator and compound feed mill departments and in transport workers. Characteristics of the study population are given in table 1. All participants gave their written informed consent on participation in the study. All, but one, of the workers who received the written information agreed to participate. The one who denied participated in the exposure measurements only. The Regional Ethical Committee of South-East Norway and the Norwegian Data Inspectorate approved the study.

Study design

The field team rigged the equipment at the work site and informed the participating workers on a Monday, and performed exposure measurements and health examinations on a Tuesday. For two companies, exposure measurements and health examinations were performed on a Wednesday. The employees were examined before work (baseline) and after work in a cross-shift design. The examinations included spirometry, gas diffusion tests and AR. The employees were asked to abstain from smoking at least 1 hour before the health examinations. Blood samples were collected after shift for atopy testing and haemoglobin measurements. After work, the employees filled in a questionnaire reporting possible work-related health complaints that day or last week. Thirteen referents and 25 exposed workers were examined over, respectively, 3 and 5 months in the winter, whereas 23 referents and 43 exposed workers were examined over, respectively, 3 and 5 months in the autumn of 2008.

Aerosol exposure measurements and analyses

Fifty-six full-shift personal inhalable samples were collected with PAS-6 personal inhalable samplers16 and portable pumps (PS101; National Institute of Occupational Health, Oslo, Norway) for 6–8 hours with a flow rate of 2 L/min. Samples were analysed for grain dust, endotoxins, bacteria, fungal spores and β-1,3-glucans as previously described.1 In brief, dust was lysed by Limulus amoebocyte lysate assay and endotoxins were counted by scanning electron microscopy, endotoxins were analysed by enzyme immunoassay. The job groups and analyses with other bioaerosol components is needed to understand health effects after grain dust exposure.
Questionnaire
After work shift, the participants filled out a questionnaire based on the ATS recommended respiratory disease questionnaire for epidemiological research that included questions on respiratory protective equipment (RPE) use, smoking habits, work history, symptoms of the airways, eyes, skin and gastrointestinal and systemic symptoms experienced during or after work on the day of the examinations. A member of the research team was available for questions from the participants.

Spirometry
After demonstration of the procedure, the spirometry tests were performed with a Jaeger Master screen PFT spirometer (Erich Jaeger GmbH & Co. KG, Würzburg, Germany) according to the ATS/ERS 2005 criteria with the person sitting and breathing through the mouth piece using a nose clip. The spirometer was calibrated daily with a 3 L syringe. The best of any three efforts, regardless of curve, of the forced vital capacity (FVC) and the forced expired volume in the first second (FEV1) were used in the analyses. Results of five and six workers before and after shift, respectively, were excluded due to technical reasons. Ninety-five of the 104 workers had acceptable tests before and after shift (91%). FEV1/FVC and the percentage of the predicted values for FEV1 (FEV1, predicted) and FVC (FVC, predicted) were calculated using reference values for a normal population from central Norway. Lung function at baseline is expressed as arithmetic mean (AM) (minimum–maximum) of actual values in L, and mean per cent of predicted values according to age, height and weight. Cross-shift changes in lung function are expressed in mL.

Gas diffusion tests
The transfer factor for pulmonary carbon monoxide (CO) uptake was measured by single-breath CO diffusion (Jaeger Master Screen PFT, Erich Jaeger GmbH & Co. KG) according to the ATS/ERS 2005 criteria. Tests of seven workers before and after shift were excluded due to technical reasons. Ninety-five of the 104 workers had acceptable tests before and after shift (91%). FEV1/FVC and the percentage of the predicted values for FEV1 (FEV1, predicted) and FVC (FVC, predicted) were calculated using reference values for a normal population from central Norway. Lung function at baseline is expressed as arithmetic mean (AM) (minimum–maximum) of actual values in L, and mean per cent of predicted values according to age, height and weight. Cross-shift changes in lung function are expressed in mL.

Acoustic rhinometry
The nasal cavity dimensions were measured by AR (Rhin2100, Rhino Metrics AS, Lyng, Denmark) with the worker in a seated position, using a hand-held sound wave tube and an anatomical nasal adapter. The mean of three efforts, regardless of curve, of the forced vital capacity (FVC) and the forced expired volume in the first second (FEV1) were used in the analyses. Results of five and six workers before and after shift, respectively, were excluded due to technical reasons. Ninety-five of the 104 workers had acceptable tests before and after shift (91%). FEV1/FVC and the percentage of the predicted values for FEV1 (FEV1, predicted) and FVC (FVC, predicted) were calculated using reference values for a normal population from central Norway. Lung function at baseline is expressed as arithmetic mean (AM) (minimum–maximum) of actual values in L, and mean per cent of predicted values according to age, height and weight. Cross-shift changes in lung function are expressed in mL.

Statistical analysis
Observations of workers not using RPE on the day of investigation were selected (n=92). Differences in symptom prevalence between exposed workers and referents were tested by Pearson’s χ2 test, and a p value of ≤0.05 was considered statistically significant. Exposure differences between job groups were tested by comparing geometric means (GM) in independent sample t tests. Associations between exposure and cross-shift respiratory changes or symptoms were investigated by using individual bioaerosol exposure values. The exposure values were used categorised at three levels and as continuous variables after log10-transformation. Associations with cross-shift changes in lung function or nose congestion were investigated using univariate general linear models with the health parameter as independent, linear exposure variables as dependent covariates and categorical exposure variables as fixed factors. Associations with self-reported symptoms were investigated by logistic regression models. Before logistic regression analyses, all nose symptoms, all eye symptoms, cough+cough with phlegm and all pulmonary symptoms, respectively, were grouped. Confounding effects of age, gender smoking, atopy, farm childhood and contact with farm and pet animals were tested and included in the models when the effect was significant, that is, at a p value of below 0.05 and when changing the β coefficient >20%.

RESULTS
Bioaerosol exposure
The GM and SD (geometric standard deviation (GSD)) of dust exposure of all workers was 1.0 mg/m3 (2.9). The highest exposure was observed in compound feed mills (GM 1.6 mg/m3, GSD 4.0, table 2).

The GM (GSD) concentration of endotoxin was 444 (4.8) EU/m3, β-1,3-glucans 6 (4.6) μg/m3, bacteria 17×107/m3 (5.9) and fungal spores 4×107/m3 (3.3). The highest concentrations of all components were in the grain elevator department, except bacterial exposure, which was highest among transport
workers (30×10⁴/m³ (2.5), table 2). Endotoxin and β-1,3-glucan exposure levels were strongly correlated (Pearson correlation coefficient r_p=0.93), whereas moderate correlations were observed between β-1,3-glucan and dust, between β-1,3-glucan and fungal spores and between endotoxin and grain dust exposure levels (r_p=0.59–0.66). Endotoxin, bacteria and β-1,3-glucan levels were 3.5–4 times higher for workers using RPE compared with those who did not (p=0.02 for all), whereas the exposure level for grain dust and fungal spores was similar (not shown).

**Table 2** Aerosol exposure of grain and animal feed industry workers in Norway not using respiratory protective equipment

| Aerosol exposure | All job groups | Grain elevator | Compound feed mill | Grain elevator and feed mill | Grain transport |
|------------------|----------------|----------------|--------------------|------------------------------|----------------|
| N                | 56             | 28             | 17                 | 6                            | 5              |
| Grain dust (mg/m³) |               |                |                   |                              |                |
| GM (GSD)         | 1.0 (2.9)      | 0.9 (2.5)      | 1.6 (4.0)          | 0.5 (1.8)                    | 0.5 (2.0)      |
| 25th centile     | 0.5            | 0.6            | 0.6                | 0.3                          | 0.3            |
| 75th centile     | 2.0            | 1.4            | 4.4                | 0.9                          | 0.9            |
| Minimum–maximum  | 0.09–14        | 0.1–7.0        | 0.09–14            | 0.2–1.0                      | 0.3–1.5        |
| Endotoxin (EU/m³) |               |                |                   |                              |                |
| GM (GSD)         | 444 (4.8)      | 852 (3.9)      | 348 (3.4)          | 107 (5.0)                    | 146 (7.6)      |
| 25th centile     | 194            | 395            | 229                | 18                           | 48             |
| 75th centile     | 1306           | 1700           | 766                | 428                          | 670            |
| Minimum–maximum  | 15–10 940      | 15–10 940      | 26–2700            | 17–530                       | 41–5320        |
| Bacteria (×10⁴/m³) |               |                |                   |                              |                |
| GM (GSD)         | 17 (5.9)       | 25 (6.8)       | 18 (3.3)           | 2 (5.5)                      | 30 (2.5)       |
| 25th centile     | 6.5            | 13             | 7                  | 0.3                          | 16             |
| 75th centile     | 55             | 96             | 33                 | 10                           | 58             |
| Minimum–maximum  | 0.3–240        | 0.3–230        | 1–240              | 0.3–15                       | 11–130         |
| Fungal spores (×10⁴/m³) |       |                |                   |                              |                |
| GM (GSD)         | 4 (3.3)        | 4 (3.7)        | 3 (3.1)            | 3 (2.9)                      | 3 (2.3)        |
| 25th centile     | 1.4            | 1.3            | 1.5                | 1.3                          | 1.4            |
| 75th centile     | 2              | 21             | 4                  | 5.4                          | 6.5            |
| Minimum–maximum  | 1.0–50         | 1.1–48         | 1.1–50             | 1.3–20                       | 1.0–7.8        |
| β-1,3-Glucans (µg/m³) |             |                |                   |                              |                |
| GM (GSD)         | 6 (4.6)        | 11 (3.9)       | 5 (4.1)            | 1 (2.5)                      | 2 (6.0)        |
| 25th centile     | 2.3            | 4              | 2                  | 0.6                          | 0.6            |
| 75th centile     | 19             | 23             | 15                 | 3.2                          | 12             |
| Minimum–maximum  | 0.3–151        | 0.3–151        | 0.4–42             | 0.4–3                        | 0.6–40         |

GMs with identical letters are significantly different (p≤0.05). GM, geometric means.

workers exposed to endotoxin levels above 360 EU/m³ (OR 6.4, p≤0.05), β-1,3-glucan exposure above 3.7 µg/m³ (OR 6.4, p≤0.05) and fungal spor exposure between 1.6×10⁴/m³ and 5.4×10⁴/m³ (OR 6.1), but not significantly in the highest fungal exposure category (OR 3.9).

Additionally, workers grouped in the highest exposure category of endotoxin, fungal spores and β-1,3-glucans reported more fatigue than referents (OR 3.0–3.5, table 4), although only the association with β-1,3-glucans was significantly different from the referents. Linear exposure–response associations between reported symptoms and some log₁₀-transformed bioaerosol concentrations among exposed workers were observed (table 4). In particular, fungal spor exposure was related to increased reporting of fatigue, cough with and without phlegm, wheezing/tight chest/dyspnoea and nose symptoms in a dose-dependent manner. Cough with and without phlegm was linearly related with log₁₀ grain dust and log₁₀ fungal spores, with similar strength (OR 3.6 and OR 3.2, respectively). Wheezing/tight chest/dyspnoea was linearly related to grain dust exposure, fungal spores, β-1,3-glucans and endotoxin, although grain dust was most important (OR 26). Fatigue was linearly related to fungal spores and β-1,3-glucans. To disen- tangle which component was most important, we adjusted the effect of one significant component for another pairwise in the regression models. This showed that grain dust and fungal spores were associated with cough and cough with phlegm with similar strength (OR 2.5 and 2.3, respectively, p<0.02), fungal spores and glucan exposure were associated with fatigue with similar strength (OR 2.3 and 1.7, respectively, p<0.03), but dust exposure was the single most important component for wheeze/ chest tightness/dyspnoea.
such as self-reported symptoms, lung function, CO diffusion capacity and nose congestion was studied in a cross-shift design. Bioaerosol exposure during a work shift with handling of grain and compound feed was associated with increased self-reporting of eye and airway symptoms after work shift. Bioaerosol exposure was not associated with cross-shift lung function decline, but several microbial components affected nose congestion. Self-reported airway symptoms were related to microbial components of the grain dust in a complex manner, showing differential associations between individual symptoms and the individual bioaerosol components. This suggests that each component of the grain dust may give differential effect on inhalation, which is in agreement with the a priori hypothesis.

The exposed workers reported more respiratory symptoms than the referents, which could indicate that inhalation of grain dust still might cause health problems in this industry. Symptoms such as fever attacks, fatigue, muscle pain and cough are typical symptoms of toxic pneumonitis, and these symptoms were in our study more often reported by exposed workers than by referents. The reporting of cough among exposed workers was more pronounced in this study compared with a cross-sectional study of animal feed workers.

Several symptoms were associated with bioaerosol exposure levels, although the mean dust exposure level was relatively low. The lowest observed effect level (LOEL) cross-shift for cough and cough with phlegm, wheezing/tight chest/dyspnoea and eye symptoms was 1.3 mg/m³ inhalable grain dust, which is somewhat lower than the 2.1 mg/m³ no observed adverse effect level of total dust for respiratory symptoms previously reported by grain handlers. The LOEL of 1.6 x 10⁴/m³ fungal spores for eye symptoms was in the same range as previously reported among Norwegian farmers, whereas the LOEL of 5.5 x 10⁴/m³ fungal spores for cough and cough with phlegm in the present study was lower. The highest exposure category of endotoxin (1200–11 000 EU/m³) and β-1,3-glucan (19–150 μg/m³) was associated with cough and cough with phlegm, but the linear dose–response relationship among exposed workers was not statistically significant, indicating that the exposure to endotoxin and glucan may not be as important for the reporting of these symptoms as grain dust and fungal spores. The exposure to GM 0.83 (0.09–15.2) mg/m³ of total dust and GM 54.2 (4.4–744) EU/m³ of endotoxin has been associated with respiratory symptoms reported by wheat harvest workers. In the present study, fatigue, wheezing/tight chest/dyspnoea and nose symptoms reported by exposed workers were associated with fungal spore exposure in a dose-dependent manner. However, the symptom prevalence ratios were not significantly different from the referents, so no LOEL could be identified from the categorical groups of fungal exposure levels. In spite of the high prevalence, eye symptoms were not linearly associated with any of the measured aerosol components. The fact that reported eye symptoms in exposure categories of all aerosol components nevertheless were significantly different from the referents indicates that eye symptoms may occur at low exposure levels. The present study shows LOELs at normal conditions in the grain industry. The bioaerosol exposure can be higher and dominated by different microorganisms when handling problematic materials, as shown in a case study where workers handling problematic grass seeds developed toxic pneumonitis.

A lung function <100% of predicted at baseline may indicate a detrimental effect of long-term grain dust exposure, although the cross-shift change in lung function was small and inconsistent within the study population. Cross-shift lung function reductions seemed to be associated with the highest exposure

### Table 3 Prevalence of self-reported acute symptoms in grain and animal feed industry workers in Norway

| Symptoms                  | Exposed (n=56) | Referents (n=36) | p Value, χ²† |
|---------------------------|----------------|-----------------|-------------|
| Nausea and/or vomit       | 1 (2)          | 2 (6)           | 0.32        |
| Diarrhoea                 | 2 (4)          | 0               | 0.25        |
| Headache                  | 17 (30)        | 8 (22)          | 0.39        |
| Fatigue                   | 17 (30)        | 9 (25)          | 0.58        |
| Fever attack              | 6 (11)         | 0               | 0.04        |
| Muscular pain             | 15 (27)        | 9 (25)          | 0.85        |
| Influenza/cold            | 12 (21)        | 9 (25)          | 0.69        |
| Sore throat               | 6 (11)         | 3 (8)           | 0.71        |
| Cough                     | 19 (34)        | 5 (14)          | 0.03        |
| Cough with phlegm         | 11 (20)        | 2 (6)           | 0.06        |
| Cough with phlegm         | 20 (22)        | 6 (17)          | 0.05        |
| Tight chest               | 3 (5)          | 4 (11)          | 0.32        |
| Wheezing                  | 6 (11)         | 0               | 0.04        |
| Dyspnoea                  | 7 (13)         | 3 (8)           | 0.51        |
| Wheezing/tight chest/dyspnoea | 10 (18)     | 6 (17)          | 0.59        |
| Nose itch/sting           | 6 (11)         | 2 (6)           | 0.38        |
| Runny nose                | 16 (29)        | 8 (22)          | 0.50        |
| Σ Nose symptoms           | 19 (34)        | 9 (10)          | 0.43        |
| Eye itch/sting            | 12 (21)        | 2 (6)           | 0.04        |
| Runny eyes                | 6 (11)         | 1 (3)           | 0.16        |
| Σ Eye symptoms            | 13 (23)        | 2 (6)           | 0.03        |
| Hand itch/sting           | 2 (4)          | 0               | 0.25        |
| Skin rash                 | 4 (7)          | 1 (3)           | 0.37        |
| Σ Skins symptoms          | 5 (9)          | 1 (3)           | 0.24        |

Bold values indicate p≤0.05. *n (%) of the study population without respiratory protective equipment. †p Value calculated between exposed workers and referents.

### Lung function

At baseline, the lung function as per cent of predicted showed no significant differences between exposed workers and referents (table 1). The AM FVC% of predicted and the FEV₁% of predicted were above 90% for all groups, except the FEV₁% for smokers (87%). The AM FEV₁/FVC% was between 76% and 78% for all workers. The CO diffusion capacity was above 80% for all workers (78%) except for smokers (87%). The AM FEV₁/FVC% was between 76% and 80% for all worker groups, except for smokers (77%), atopics (79%) and glucan may not be as important for the reporting of these symptoms as grain dust and fungal spores. The reporting of cough among exposed workers was more pronounced in this study compared with a cross-sectional study of animal feed workers.

Several symptoms were associated with bioaerosol exposure levels, although the mean dust exposure level was relatively low. The lowest observed effect level (LOEL) cross-shift for cough and cough with phlegm, wheezing/tight chest/dyspnoea and eye symptoms was 1.3 mg/m³ inhalable grain dust, which is somewhat lower than the 2.1 mg/m³ no observed adverse effect level of total dust for respiratory symptoms previously reported by grain handlers. The LOEL of 1.6 x 10⁴/m³ fungal spores for eye symptoms was in the same range as previously reported among Norwegian farmers, whereas the LOEL of 5.5 x 10⁴/m³ fungal spores for cough and cough with phlegm in the present study was lower. The highest exposure category of endotoxin (1200–11 000 EU/m³) and β-1,3-glucan (19–150 μg/m³) was associated with cough and cough with phlegm, but the linear dose–response relationship among exposed workers was not statistically significant, indicating that the exposure to endotoxin and glucan may not be as important for the reporting of these symptoms as grain dust and fungal spores. The exposure to GM 0.83 (0.09–15.2) mg/m³ of total dust and GM 54.2 (4.4–744) EU/m³ of endotoxin has been associated with respiratory symptoms reported by wheat harvest workers.

In the present study, fatigue, wheezing/tight chest/dyspnoea and nose symptoms reported by exposed workers were associated with fungal spore exposure in a dose-dependent manner. However, the symptom prevalence ratios were not significantly different from the referents, so no LOEL could be identified from the categorical groups of fungal exposure levels. In spite of the high prevalence, eye symptoms were not linearly associated with any of the measured aerosol components. The fact that reported eye symptoms in exposure categories of all aerosol components nevertheless were significantly different from the referents indicates that eye symptoms may occur at low exposure levels. The present study shows LOELs at normal conditions in the grain industry. The bioaerosol exposure can be higher and dominated by different microorganisms when handling problematic materials, as shown in a case study where workers handling problematic grass seeds developed toxic pneumonitis.

A lung function <100% of predicted at baseline may indicate a detrimental effect of long-term grain dust exposure, although the cross-shift change in lung function was small and inconsistent within the study population. Cross-shift lung function reductions seemed to be associated with the highest exposure

### Nose congestion

All workers had moderate nose congestion, as shown by a rhino- metric D-factor of 0.5 for referents and 0.6 for exposed workers (table 1).

Significant reductions of the nasal dimensions in the anterior 2 cm of the nasal mucosa were associated with exposure for several aerosol components (table 5). However, the dose–response patterns were inconsistent, although a weak trend could be seen for grain dust and fungal spore exposure. No significant association with continuous log-transformed exposure variables was observed (not shown).

### DISCUSSION

Exposure for several individually measured bioaerosol components in the Norwegian grain industry and health outcomes...
categories of all bioaerosol components, although no linear trends were seen. Our results seem to be in line with the long-
term decline in lung function that is commonly observed in
studies of grain workers, whereas cross-shift effects are less con-
sistent. Corey et al found a baseline decline of FVC, FEV1,
maximal expiratory flow at 50% of FVC (MEF50) and maximal
respiratory function at 75% of FVC (MEF75) from Monday to
Friday, but increases in FVC and FEV1 cross-shifts and cross-
week among grain elevator workers. Smid et al demonstrated a
cross-week FEV1 decline, but no cross-shift FEV1 decline
among animal feed workers. Cross-shift lung function changes
have been observed among grain handlers and wheat harvest
workers.26 Whereas cross-shift change in wheat harvest workers
was not consistently related to the dust or endotoxin expos-
ure,27 where decreases in FVC were found among grain hand-
lers significantly correlated with increasing grain dust exposure,
and workers exposed to AM 3.3 (SD 7) mg/m³ of total dust had a
cross-shift FVC decline of 46 mL.4 In five cross-sectional
surveys over a period of 12 years, Chan-Yeung et al14
consistently found that grain workers had more respiratory
symptoms and lower lung function compared with civic
workers, despite a progressive reduction in dust levels in the
grain elevators from GM (SD) 10.1 mg/m³ (3.3) in 1974 to
1.9 mg/m³ (3.4) in 1988. The latter is similar to the dust level
in the present study.

Referents and exposed workers had moderate nose congestion
as shown by a D-factor of 0.5 and 0.6, respectively. Exposed
workers had cross-shift reduction in area and volume of the
anterior part of the nose. This suggests a local in
flammation in the anterior part of the nose. In the anterior part of the nose, the mucosa extends
highly exposed workers had more nose symptoms and lower lung function compared with civic
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1.9 mg/m³ (3.4) in 1988. The latter is similar to the dust level
in the present study.

Table 4 Odds ratios of self-reported acute symptoms by categorical and linear bioaerosol exposure among grain and animal feed industry
workers in Norway

| Bioaerosol exposure | Fatigue OR 95% CI | Coughs/phlegm OR 95% CI | Wheeze/tight chest/dyspnoea OR 95% CI | Nose symptoms OR 95% CI | Eye symptoms OR 95% CI |
|---------------------|-------------------|--------------------------|-------------------------------------|-------------------------|------------------------|
| Grain dust (mg/m³)  |
| 0 (referents)       | 1.0               | 1.0                      | 1.0                                 | 1.0                     | 1.0                    |
| 0.09-0.6            | 1.3 (0.3 to 4.5)  | 1.1* (0.2 to 5.3)        | –                                   | 0.4                     | 0.08 to 2.1            |
| 0.61–1.3            | 1.3 (0.3 to 3.8)  | 3.0* (0.8 to 11)         | 0.6† (0.3 to 2.6)                   | 0.08 to 2.1             | 5.0                     |
| 1.31–14             | 1.6 (0.3 to 5.3)  | 5.5* (1.5 to 20)         | 4.0† (1.1 to 15)                    | 0.6                     | 0.08 to 2.1            |
| per 10-fold increase| 1.7§ (0.5 to 5.8) | 3.6                      | 1.0 (1.3)                           | 26 (3.2 to 213)         | 2.5                     |
| Endotoxin (EU/m³)  |
| 0 (referents)       | 1.0               | 1.0                      | 1.0                                 | 1.0                     | 1.0                    |
| 15–35               | 1.1 (0.3 to 3.8)  | 1.8                      | 0.5 (1.4 to 7)                      | 1.0                     | 0.4                     |
| 360–1000            | 0.7               | 2.5                      | 0.7 (1.4 to 7)                      | 1.0                     | 0.4                     |
| 1200–11000          | 3.4               | 1.0 (12)                 | 5.7 (1.5 to 21)                     | 10 (1.6 to 20)          | 2.6                     |
| per 10-fold increase| 1.8§ (0.7 to 4.4) | 0.7                      | 0.3 (1.4)                           | 3.8                     | 1.0 (14)               |
| Bacteria (/m³)      |
| 0 (referents)       | 1.0               | 1.0                      | 1.0                                 | 1.0                     | 1.0                    |
| 0.28×10⁶–14.99×10⁶ | 1.6               | 0.5 (1.5 to 3.8)         | 5.0 (1.4 to 17)                     | 1.7                     | 0.4                     |
| 15×10⁴–47×10⁴       | 1.4               | 0.4 (1.4 to 5.5)         | 0.5 (1.4 to 5.5)                    | 0.5 (0.9 to 2.7)        | 0.1                     |
| 58×10⁴–240×10⁴      | 0.8               | 0.2 (1.4 to 7)           | 0.7 (1.4 to 5.5)                    | 0.1 (0.2 to 5.0)        | 0.3                     |
| per 10-fold increase| 0.8§ (0.4 to 1.6) | 0.7                      | 0.3 (1.4)                           | 0.3                     | 0.1                     |
| Fungal spores (/m³) |
| 0 (referents)       | 1.0               | 1.0                      | 1.0                                 | 1.0                     | 1.0                    |
| 1×10⁴–1.5×10⁴       | 0.7               | 0.2 (1.7)                | 0.6 (1.4 to 7)                      | 1.0                     | 0.4                     |
| 1.6×10⁴–5.4×10⁴     | 1.1               | 0.3 (1.8)                | 0.8 (1.5 to 7)                      | 1.8                     | 0.5                     |
| 5.5×10⁴–50×10⁴      | 3.0               | 0.9 (10)                 | 5.0 (1.3 to 19)                     | 2.3                     | 0.6 (9.0)              |
| per 10-fold increase| 3.4§ (1.1 to 10)  | 3.2                      | 1.1 (10 to 9.4)                     | 3.7                     | 1.0 (13)               |
| β-1,3-Glucans (µg/m³) |
| 0 (referents)       | 1.0               | 1.0                      | 1.0                                 | 1.0                     | 1.0                    |
| 0.30–3.60           | 0.8               | 0.2 (1.7)                | 1.7 (1.4 to 6.3)                    | 0.6                     | 0.1 (3.1)              |
| 3.70–18.9           | 0.9               | 0.3 (1.9)                | 0.5 (1.5 to 6.8)                    | 0.8                     | 0.2 (3.5)              |
| 19.0–150            | 2.6               | 1.0 (6.9)                | 9.0 (2.2 to 37)                     | 2.8                     | 0.7 (11)               |
| per 10-fold increase| 1.5§ (1.0 to 2.3) | 2.1                      | 0.9 (5.0)                           | 3.5                     | 1.0 (12)               |

Bold values indicate p<0.05.
—too few samples in this category.
Associations are expressed as OR with 95% CIs when compared to referents (=OR 1). Workers without respiratory protective equipment (RPE), n=92.
*Adjusted for gender.
†Adjusted for gender and age.
‡Exposed workers only, n=56.
§OR for a 10-fold increase in exposure level
¶Adjusted for age.
Table 5  Cross-shift changes in lung function and nose congestion by exposure category among grain and animal feed industry workers in Norway*

| Exposure          | ΔFVC (mL) | ΔFEV1 (mL) | ΔD_{LCO} (mmol/min/Pa) | AR ΔM1 (cm²) | AR ΔV1 (cm³) | AR ΔM2 (cm³) | AR ΔV2 (cm³) |
|-------------------|-----------|------------|------------------------|--------------|--------------|--------------|--------------|
| Grain dust (mg/m³) |           |            |                        |              |              |              |              |
| 0 (refere)ns      | 0         | 0          | 0                      | 0            | 0            | 0            | 0            |
| 0.09–0.6          | 67 (–60 to 194) | 57 (–46 to 161) | 153 (–238 to 544) | –0.08 (–0.19 to 0.04) | –0.08 (–0.31 to 0.14) | 0.03 (–0.08 to 0.14) | 0.06 (–0.77 to 0.89) |
| 0.61–1.3          | 65 (–50 to 181) | –27 (–121 to 67) | –87 (–440 to 265) | –0.15 (–0.26 to –0.05) | –0.23 (–0.43 to –0.02) | 0.005 (–0.01 to 0.10) | 0.30 (–0.46 to 1.1) |
| 1.31–14           | 29 (–84 to 142) | 17 (–76 to 109) | –64 (–410 to 281) | –0.12 (–0.22 to –0.02) | –0.16 (–0.36 to 0.05) | 0.03 (–0.13 to 0.07) | 0.006 (–0.74 to 0.76) |
| Endotoxin (EU/m³) | 0 (refere)ns | 0          | 0                      | 0            | 0            | 0            | 0            |
| 0.15–350          | 46 (–72 to 165) | 11 (–86 to 108) | 235 (–129 to 598) | –0.18 (–0.29 to –0.08) | –0.28 (–0.49 to –0.07) | 0.03 (–0.07 to 0.014) | 0.40 (–0.39 to 1.19) |
| 360–1000          | 103 (–5 to 211) | 61 (–27 to 149) | –147 (–475 to 181) | –0.10 (–0.20 to 0.004) | –0.15 (–0.34 to 0.05) | 0.002 (–0.10 to 0.09) | 0.06 (–0.78 to 0.67) |
| 1200–11 000       | –19 (–144 to 106) | –62 (–164 to 40) | –95 (–480 to 289) | –0.08 (–0.19 to 0.04) | –0.05 (–0.28 to 0.17) | –0.05 (–0.15 to 0.06) | 0.09 (–0.74 to 0.91) |
| Bacteria (counts/m³) | 0 (refere)ns | 0          | 0                      | 0            | 0            | 0            | 0            |
| 0.28×10⁶–1.499×10⁴ | 65 (–47 to 177) | –14 (–101 to 73) | 191 (–156 to 537) | –0.16 (–0.26 to –0.05) | –0.24 (–0.45 to –0.04) | 0.02 (–0.08 to 0.12) | 0.17 (–0.60 to 0.93) |
| 15×10⁴–47×10⁴    | 90 (–24 to 204) | 109 (20 to 198) | –37 (–384 to 310) | –0.09 (–0.19 to 0.02) | –0.14 (–0.34 to 0.06) | –0.008 (–0.11 to 0.09) | 0.11 (–0.64 to 0.86) |
| 58×10⁴–240×10⁴   | –15 (–141 to 110) | –78 (–176 to 20) | –240 (–613 to 133) | –0.11 (0.23 to 0.006) | –0.09 (–0.032 to 0.13) | –0.03 (–0.14 to 0.08) | 0.09 (–0.74 to 0.92) |
| Fungal spores (counts/m³) | 0 (refere)ns | 0          | 0                      | 0            | 0            | 0            | 0            |
| 1×10³–1.5×10⁴    | 18 (–94 to 130) | 45 (–49 to 138) | 159 (–200 to 518) | –0.10 (–0.20 to 0.01) | –0.15 (–0.36 to 0.06) | 0.01 (–0.09 to 0.11) | 0.08 (–0.67 to 0.83) |
| 1.6×10³–5.4×10⁴  | 143 (29 to 258) | 38 (–57 to 133) | –138 (–497 to 221) | –0.13 (–0.24 to –0.02) | –0.13 (–0.35 to –0.08) | –0.05 (–0.15 to 0.05) | –0.22 (–0.99 to 0.54) |
| 5.5×10³–50×10⁴   | –6 (–123 to 111) | –52 (–150 to 45) | –65 (–424 to 293) | –0.13 (–0.24 to –0.02) | –0.21 (–0.43 to 0.006) | 0.03 (–0.08 to 0.13) | 0.54 (–0.24 to 1.33) |
| β-1,3-Glucans (µg/m³) | 0 (refere)ns | 0          | 0                      | 0            | 0            | 0            | 0            |
| 0.30–3.60         | 70 (–46 to 186) | 30 (–66 to 126) | 44 (–318 to 407) | –0.13 (–0.24 to –0.02) | –0.17 (–0.39 to 0.04) | 0.08 (–0.02 to 0.18) | 0.51 (–0.25 to 1.28) |
| 3.70–18.8         | 86 (–21 to 194) | 31 (–59 to 120) | 19 (–322 to 360) | –0.11 (–0.22 to –0.01) | –0.20 (–0.40 to –0.01) | 0.08 (–0.17 to 0.009) | 0.26 (–0.97 to 0.45) |
| 19.0–150          | –29 (–158 to 100) | –139 (–531 to 254) | –104 (–500 to 290) | –0.12 (–0.24 to 0.004) | –0.09 (–0.32 to 0.14) | 0.01 (–0.10 to 0.12) | 0.24 (–0.60 to 1.08) |

Values are presented as coefficients (95% CI). Bold values indicate p<0.05.

*Workers without RPE were selected (n=92).

AR, acoustic rhinometry; ΔFEV1, difference in forced expired volume in the first second; ΔFVC, difference in forced vital capacity; ΔD_{LCO}, difference in CO diffusion capacity of the lung; RPE, respiratory protective equipment.
over a smaller surface area than the interior part. Measurements of mucosal swelling in the anterior part are therefore likely to be more accurate and may better reflect an inflammatory response of dust exposure.

There were no significant difference in lung function between exposed workers and referents. This could be due to reduced acute effects of dust exposure, a kind of tolerance previously shown among pig farmers and grain handlers with long-term exposure of organic dust.\textsuperscript{3}\textsuperscript{30} Lung function below 100\% of predicted may indicate that a long-term effect of exposure has already reduced the lung function, thus supporting this view. It is also plausible that individuals sensitive to exposure change jobs to avoid hazardous exposure, leaving the ones who cope behind, thus resulting in a selection of healthy worker into exposed jobs. It may furthermore be possible that referents were sporadically exposed, which reduces the exposure contrasts between the groups. Several referents and exposed workers also had a farm or animals at home. There were no dose–response association between exposure and cross-shift lung function changes in spite of relatively high exposure levels. This may be due to a large proportion of large particles in the inhalable fraction, probably being most active in the upper airways, whereas the pulmonary effects are expected to occur from smaller particle sizes in the thoracic and respirable aerosol fraction. Although spores and bacteria are likely to protrude in the thoracic and alveolar region of the lungs, it is possible that they stick to larger fragments or are present as aggregates that deposit higher up in the airways. The associations between aerosol components, and AR nose effects and reported nose symptoms indicate that this was the case. On the other hand, spirometry and gas diffusion tests do not always reflect all processes in the development of airway diseases. Self-reporting of symptoms seemed to be more sensitive, but this may be due to the participants’ greater focus on occupational exposure, resulting in over-reporting of respiratory symptoms.\textsuperscript{31} Validity and reliability are general problems with questionnaires. However, we believe that specificity problems were limited in this study because the selection of questions was adapted to the study by relating to a priori defined possible effects of the relevant exposure components, and related in time to the workday. The guidance of participants during filling in the questionnaire also reduced possible misunderstandings of the questions. Given that the low lung function among workers is caused by long-term occupational exposure, the increased reporting of symptoms from the airways is in agreement with another study from occupational settings.\textsuperscript{32} In clinical settings, however, self-reported symptoms seldom correlate with objective measurements of lung function such as FEV\textsubscript{1}.\textsuperscript{33}\textsuperscript{34} All of the above will lead to an underestimation of exposure–response relationships in the grain elevator and compound feed mill industry, and should be considered when interpreting the results of this study. On the other hand, the selection of workers without RPE will reduce dilution problems in the exposure–response associations. Multiple testing might have led to spurious significant findings, but the number of positive associations found are greater than expected taking multiple testing into account. Significant associations between symptoms and exposure measurements are more plausible when a linear trend along the exposure categories is shown, such as between several symptoms and spore and dust exposure. The significant associations between exposure and cross-shift lung function increases and AR associations without dose–response trends are more likely to be spurious.

Previous publications from this study showed that although grain dust exposure was low, microbial exposure levels exceeded health-based recommended values\textsuperscript{3} and exposure levels varied between job groups and tasks.\textsuperscript{40} The present study shows how bioaerosol components individually and combined affected the respiratory health of workers in grain elevators, compound feed mills and transport in a complex manner. Differential linear associations between individual symptoms and the exposure level of individual bioaerosol components demonstrated this. This suggests that each component of the grain dust may give differential effect on inhalation. Fatigue and nose symptoms were strongest associated with fungal spores; cough and cough with phlegm were associated with grain dust and fungal spores with equal strength. Wheeze/tight chest/dyspnoea was strongest associated with grain dust, indicating that components of the dust not measured are involved. Some candidate effect inducers may be a large number of microbial metabolites previously detected in grain dust.\textsuperscript{83} It is also likely that some additives of animal feed could have effects.

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REFERENCES

1. Halteisen AS, Heldal KK, Wouters IM, et al. Exposure to grain dust and microbial components in the Norwegian grain and compound feed industry. Ann Occup Hyg 2013;57:1105–14.
2. Broder I, Hutcheon MA, Mintz S, et al. Changes in respiratory variables of grain handlers and civic workers during their initial months of employment. Br J Ind Med 1984;41:94–9.
3. Corey P, Hutcheon M, Broder I, et al. Grain elevator workers show work-related pulmonary-function changes and dose-effect relationships with dust exposure. Br J Ind Med 1982;39:330–7.
4. doPico GA, Reddan W, Anderson S, et al. Acute effects of grain dust exposure during a work shift. Am Rev Respir Dis 1983;128:399–404.
5. Manfreda J, Holford-strevens V, Cheang M, et al. Acute symptoms following exposure to grain dust in farming. Environ Health Perspect 1986;66:73–80.
6. Huy T, De Schipper K, Chan-yeung M, et al. Grain dust and lung function—dose-response relationships. Am Rev Respir Dis 1991;144:1314–21.
7. Palha P, Dosman JA, McDuffie HH. Effects of grain dust on lungs prior to and following dust remediation. J Occup Environ Med 2008;50:1394–400.
8. Post W, Heederik D, Houba R. Decline in lung function related to exposure and selection processes among workers in the grain processing and animal feed industry. Occup Environ Med 1998;55:349–55.
9. Spurzem JR, Romberger DJ, Van Essen SG. Agricultural lung disease. Clin Chest Med 2002;23:795–810.
