



# Work Tasks as Determinants of Grain Dust and Microbial Exposure in the Norwegian Grain and Compound Feed Industry

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## ABSTRACT

**Objectives:** The grain and compound feed industry entails inevitable risks of exposure to grain dust and its microbial content. The objective of this study was therefore to investigate task-dependent exposure differences in order to create knowledge basis for awareness and exposure reducing measures in the Norwegian grain and compound feed industry.

**Methods:** A total of 166 samples of airborne dust were collected by full-shift personal sampling during work in 20 grain elevators and compound feed mills during one autumn season and two winter seasons. The personal exposure to grain dust, endotoxins,  $\beta$ -1 $\rightarrow$ 3-glucans, bacteria, and fungal spores was quantified and used as individual outcomes in mixed models with worker nested in company as random effect and different departments and tasks as fixed effects.

**Results:** The exposure levels were highest in grain elevator departments. Exposure to endotoxins was particularly high. Tasks that represented the highest and lowest exposures varied depending on the bioaerosol component. The most important determinants for elevated dust exposure were cleaning and process controlling. Cleaning increased the dust exposure level by a factor of 2.44 of the reference, from 0.65 to 1.58 mg m<sup>-3</sup>, whereas process controlling increased the dust exposure level by a factor of 2.97, from 0.65 to 1.93 mg m<sup>-3</sup>. Process controlling was associated with significantly less grain dust exposure in compound feed mills and the combined grain elevators and compound feed mills, than in grain elevators. The exposure was reduced by a factor of 0.18 and 0.22, from 1.93 to 0.34 mg m<sup>-3</sup> and to 0.42 mg m<sup>-3</sup>, respectively, compared with the grain elevators. Inspection/maintenance, cleaning, and grain rotation and emptying were determinants of higher exposure to both endotoxin and  $\beta$ -1 $\rightarrow$ 3-glucans. Seed winnowing was in addition a strong determinant for endotoxin, whereas mixing of animal feed implied higher  $\beta$ -1 $\rightarrow$ 3-glucan exposure. Cleaning was the only task that contributed significantly to higher exposure to bacteria and fungal spores.

**Conclusion:** Cleaning in all companies and process controlling in grain elevators were the strongest determinants for overall exposure, whereas seed winnowing was a particular strong determinant of endotoxin exposure. Exposure reduction by technical intervention or personal protective equipment should therefore be considered at work places with identified high exposure tasks.

**KEYWORDS:** bacteria;  $\beta$ -1 $\rightarrow$ 3-glucans; endotoxin; exposure assessment; fungal spores; grain industry

## INTRODUCTION

Grain dust is generated during threshing and other handling of grain. Employees at grain elevators and compound feed mills may be exposed to large amounts of grain dust during work, frequently leading to symptoms from the airways and functional changes such as asthma (Broder *et al.*, 1984). Acute and short term effects such as cross-shift reduction in lung function and toxic pneumonitis (organic dust toxic syndrome/grain fever) have been observed during a working day (Corey *et al.*, 1982; doPico *et al.*, 1983; Manfreda *et al.*, 1986; Huy *et al.*, 1991), and chronic exposure may lead to permanently decreased lung function (Melbostad *et al.*, 1997; Swan and Crook, 1998; Spurzem *et al.*, 2002).

Grain dust is a heterogeneous mixture of inorganic soil particles, plant fragments, insect-and mite body parts and faeces, viable and non-viable microorganisms, and their bioactive components such as endotoxins,  $\beta$ -1,3-glucans and mycotoxins (Smith, 1989). Each microbial component may exert health effects. Health effects resulting from grain dust inhalation may, therefore, be stronger associated to specific microbial components than to the dust level (Creasia *et al.*, 1990; Douwes *et al.*, 2003).

Highly variable dust and microbial exposure levels have previously been shown in the grain and animal feed industry; inhalable dust exposure ranging from GM (GSD) 0.8 (2.7) to 9.8 (4.6) mg m<sup>-3</sup>, endotoxin exposure from 5.9 (8.6) to 628 (5.9) EU m<sup>-3</sup>, fungal exposure from  $2.3 \times 10^3$  (2.6) cfu m<sup>-3</sup> to  $4 \times 10^6$  (5.9) spores m<sup>-3</sup>,  $21 \times 10^4$  m<sup>-3</sup> bacteria and 7.4 (5.6)  $\mu$ g m<sup>-3</sup> of  $\beta$ -1,3-glucans (Smid *et al.*, 1992; Spaan *et al.*, 2006; Halstensen *et al.*, 2013). As working in this industry clearly entails inevitable risks of exposure to grain dust and its microbial contents, it is valuable to identify possible sites, tasks, and technical solutions that determine particular high exposure. An increased awareness of the exposure risks will enable the companies and workers to take necessary measures or precautions in order to keep a safe work environment. Determinants of high exposure may form the basis for the priority of exposure preventive measures. Identification of determinants of low exposure may be equally important in this respect, by indicating safe conditions.

The microbial exposure in the grain industry has been shown to vary considerably between workplaces and seasons, although the dust exposure levels were

more similar (Halstensen *et al.*, 2013). It may therefore be insufficient to assess the grain dust exposure by aerosol mass only, and exposure assessment of possible microbial components may be necessary. As the microbial exposure also may differ between tasks, a task-based exposure assessment will be optimal. To be able to do task-based assessments, it is necessary to have detailed information on the work in addition to personal measurements. A thorough characterization of exposure and determinants of exposure will not only provide a strong basis for future evaluations of exposure risk and preventive measures, but may also improve studies of health effects associations in epidemiological studies.

Our objective was to characterize task-dependent exposure differences at Norwegian grain elevators and compound feed mills in order to create knowledge and awareness of the exposure risks and variations in the industry. The study is part of a cross-sectional study on inflammatory and respiratory effects related to exposure to grain dust and its constituents.

## METHODS

### Departments, work tasks, and sampling strategy

A representative selection of grain elevators and compound feed mills, altogether 52 companies or departments, were invited to participate in the study. Twenty companies agreed to participate and were included in this study. The companies were geographically distributed throughout 20 municipalities in nine counties in central and south-eastern Norway. Eleven of the companies had both grain elevator department and compound feed mill department, six of the companies had grain elevator department only, and three of the companies had compound feed mill department only. The companies differed in technical solutions, age and size. At five of the companies were transport workers also included in the study.

In the grain elevators, the grain was loaded into the elevator, sorted, winnowed, dried, rotated, moved, stored, and unloaded. In compound feed mills, the grain was milled and mixed with other nutrients and pressed into feed pellets. Transport work included loading, driving and unloading of grain and animal feed. Sampling was performed in these three main work places. In 11 of the companies, the operators worked in both the grain elevator department and in

the compound feed mill department each day. Samples from workers at these 11 companies contained a mixture of airborne dust from both workplaces and were, therefore, grouped separately in a combined grain elevator and compound feed mill group.

The workers registered work place, work task, breaks, and use of respiratory protection device throughout the day on a card small enough to keep in the pocket. Based on these notes we distinguished 10 tasks that described the work either in grain elevators, compound feed mills or both (Table 1). Since the workers had one or more of these tasks during a shift, each exposure measurement mostly covered several tasks. Samples from workers that did not specify their task at all were grouped as unspecified work. The number of samples including the specific tasks for each workplace/department is given in Table 2.

Full shift personal sampling of airborne dust was conducted on two consecutive days on the same persons (73 workers in total). Sampling was done during winter at 7 companies and in the harvesting season at 13 companies. One-day sampling was repeated the year after for ten of the companies. Five of the companies that were visited the first winter (2008) were also visited the next winter (2009). Five of the companies that were visited in autumn (2008) were also visited the following winter (2009). The resulting collection of samples thus included day-to-day variability, season-to-season variability, and year-to-year variability. Various types of grain was handled in the grain elevators and compound feed facilities at all times, including winter and summer wheat, barley, rye, maize, and soya. The dust collected thus derived from various sources, and the exposure measurements could not be related to a specific grain type. The grain types handled during sampling were therefore not registered. Natural variability due to grain type and quality is thus included in the sample variability, but not specifically evaluated.

#### Personal sampling of airborne dust

Personal airborne grain dust samples were collected from the workers' breathing zone. The samples were collected in parallel on pre-weighed 25 mm diameter polycarbonate (PC) filters with 0.8  $\mu\text{m}$  pore size (Millipore Corporation, Billerica, MA, USA) and on 25 mm diameter glass fiber filters (Whatman GF/A, Whatman, Maidstone, MA, USA) mounted in PAS-6 personal inhalable aerosol sampler cassettes (van der Wal, 1983)

using in-house manufactured portable pumps (PS101; National Institute of Occupational Health, Oslo, Norway) for 6–8 h with a flow rate of 2 l  $\text{min}^{-1}$ .

#### Sample preparation and analysis of microbial content

PC filters were weighed in an air conditioned room at 19–21°C and 38–42% relative humidity using a Sartorius AG MC 210p analytical balance (Sartorius AG, Göttingen, Germany) before being transferred to small petri dishes for further preparation. The glass fiber filters were stored at –20°C in 47 mm micro petrislides (Millipore). Airborne dust collected on the PC filters was suspended in 5 ml of 0.1 % Tween-80 by ultrasound treatment for 3 min in a Sonorex RK 510H ultrasonic bath (Bandelin Electronic GmbH & Co. KG, Berlin, Germany). One aliquot (0.5–1 ml) of the dust suspension was stained with acridine orange for quantification of bacteria by epifluorescence microscopy as previously described (Heldal *et al.*, 1996). Another aliquot (0.5–1 ml) of the dust suspension was filtered on a PC filter with 0.4  $\mu\text{m}$  pore size (Millipore) for quantification of fungal spores by scanning electron microscopy (SEM) previously described (Halstensen *et al.*, 2013). Endotoxins and  $\beta$ -1 $\rightarrow$ 3-glucans were extracted from glass fiber filters and analyzed as previously described (Douwes *et al.*, 1995, 1996). The exposure levels of grain dust and microbial components have been published previously (Halstensen *et al.*, 2013).

#### Statistical analysis

Exposure variables were skewed to the right and were ln-transformed before statistical analysis. In 7 and 38 % of the samples, respectively, there were no bacteria or fungal spores detected. In order to be able to ln-transform these data before parametric statistical analyses, zero values were substituted by one bacterium or spore divided by the square root of two followed by filter area observed in the microscope and volume adjustment (Oehlert *et al.*, 1995). The exposures were summarized by geometric means (GMs) with geometric standard deviation (GSD) of the total mean, of the mean between companies (BC), between workers (BW), and within workers (WW).

Exposure was modelled by linear mixed effect regression in order to account for the correlation between repeated measurements. Determinants of exposure (departments and tasks) were treated as

**Table 1. Description of work tasks.**

Work task	N <sup>a</sup>	Description of work
Inspection/maintenance	50	Inspection and maintenance in the plant (both grain elevators and compound feed mills) including filter change, change of matrix in the compound feed press, valve adjustments, error search, and repairs
Cleaning	32	Sweeping with broom, vacuum cleaning with a central vacuum cleaner. This work task could also include cleaning of filters, e.g. in the winnowing machines.
Grain reception	29	Reception of grain, soy, and maize. Work in control room and unloading area, fill-up of raw materials using truck in the unloading shaft.
Process controlling	19	Controlling the grain drier, winnowing, and/or the compound feed production. This was mainly done from control rooms with automatic systems (remote control). This work task was closely connected with inspection/maintenance.
Filling sacks	48	Automatic or manual filling of grain or compound feed in small or large sacks inside or outside the plant.
Mixing compound feed	16	Fill-up and mixing of ingredients for compound feed, including grain, maize, calcium carbonate, fat, and a ready-to-use mix of vitamins and amino acids (premix) necessary for the particular compound feed. This work was connected to the compound feed mills only).
Grain rotation and emptying	22	Grain rotation at the grain dryer, emptying of grain for transport. Grain moving or rotation was either air-driven (suck-and-blow), elevator-driven, or done by passive emptying (gravitation).
Seed winnowing	4	Winnowing of seed grain were done with specialized machines with several different sieves intended for seed grain
Transport	13	Transport work included, loading, driving, and unloading of grain and compound feed in lorry or tank lorry with or without a suction device. The operators were often standing in the loading/unloading area. The work could include collection of grain at the producers, waiting, and washing of the lorry in the end of the day.
Unspecified work	12	Workers have not described their work at all
Low exposure work	37	Work that <i>a priori</i> were defined as low exposure work included testing of falling number, laboratory work, office work, meeting, storage work, packing, truck driving, and grain sampling

<sup>a</sup>Number of measurements that included each particular work task.

fixed effects and worker nested in company as random effects. Tasks were modelled as dichotomous variables (task performed yes/no). The models were built by

stepwise including all tasks, departments, and interactions between one task at a time and departments. Determinants that significantly improved the models

**Table 2. Overview of the number of samples including each work tasks by department.**

Work task	Department			
	Grain elevator	Compound feed mill	Grain elevator and compound feed mill	Transport
1 Inspection/maintenance	26	13	11	0
2 Cleaning	22	7	3	0
3 Grain reception	21	4	4	0
4 Filling sacks	7	5	7	0
5 Process controlling	12	25	11	0
6 Mixing compound feed	0	13	2	0
7 Grain rotation and emptying	13	2	7	0
8 Seed winnowing	3	0	1	0
9 Transport	0	0	0	13
10 Low exposure work	17	7	12	0
11 Unspecified work	8	2	2	0

as judged by a  $P$ -value  $<0.05$  in likelihood ratio (LR) tests were kept in the models. Only 13 samples from transport were collected, and further grouping of these samples into tasks was inapplicable. Therefore, grain transport was not included in the models. The fit of the models was assessed by inspecting the distributions of the residuals and the plots of standardized residuals versus predicted values. Separate models were constructed for inhalable dust, endotoxins,  $\beta$ -1 $\rightarrow$ 3-glucans, bacteria, and fungal spores.

The restricted maximum likelihood algorithm was used to estimate variance components. To quantify the contribution of the fixed effects to the BC, BW, and WW variance components, values of the various components obtained under the mixed effect model were compared with those from a mixed effect model without fixed effects. The software package STATA 13 for Windows (Stata Corp LP, TX, USA) was used for the statistical analyses.

The effect of determinants is shown as factors by back-transformation of the ln transformed regression coefficients. Exposure levels of different combinations of tasks can be computed from the regression models as follows:

$$E = e^{c+b1+b2\dots} = e^c \times \text{effect}_{\text{determinant1}} \times \text{effect}_{\text{determinant2}}$$

where  $E$  = exposure,  $c$  = constant of the regression model, and  $b1$  and  $b2$  = regression coefficients of the determinants 1 and 2.

## RESULTS

### Exposure levels of grain dust and microbial components

The overall exposure was GM (GSD)  $1.0 \text{ mg m}^{-3}$  (3.7) of grain dust,  $662 \text{ EU m}^{-3}$  (5.9) of endotoxins,  $7.4 \mu\text{g m}^{-3}$  (5.6) of  $\beta$ -1 $\rightarrow$ 3-glucans,  $22 \times 10^4 \text{ m}^{-3}$  (7.9) of bacteria, and  $4 \times 10^4 \text{ m}^{-3}$  (3.4) of fungal spores (Table 3). The  $\text{GSD}_{\text{WW}}$  was larger than the  $\text{GSD}_{\text{BW}}$  or  $\text{GSD}_{\text{BC}}$  for grain dust, bacteria, and fungal spores. For endotoxins and of  $\beta$ -1 $\rightarrow$ 3-glucans was the  $\text{GSD}_{\text{BW}}$  larger than the  $\text{GSD}_{\text{WW}}$  and  $\text{GSD}_{\text{BC}}$  (Table 3). Transport workers were exposed to GM (GSD)  $0.5 \text{ mg m}^{-3}$  (2.2) of grain dust,  $112 \text{ EU m}^{-3}$  (4.0) of endotoxins,  $1.4 \mu\text{g m}^{-3}$  (3.4) of  $\beta$ -1 $\rightarrow$ 3-glucans,  $8 \times 10^4 \text{ m}^{-3}$  (5.5) of bacteria, and  $2 \times 10^4 \text{ m}^{-3}$  (2.0) of fungal spores.

### Season was not an exposure determinant

Inclusion of season as a random effect nested in worker and company did not improve the models ( $P > 0.1$ ). The possibility for different dependence between repeated samples collected two consecutive days, and

**Table 3. GM exposure and variances in the Norwegian grain and compound feed industry.**

Exposure parameter	<i>n</i>	GM	GSD <sub>TOT</sub>	GSD <sub>BC</sub>	GSD <sub>BW</sub>	GSD <sub>WW</sub>
Grain dust (mg m <sup>-3</sup> )	166	1.0	3.67	2.00	1.96	2.46
Endotoxin (EU m <sup>-3</sup> )	166	662	5.93	1.89	4.05	2.52
β-1→3-glucan (μg m <sup>-3</sup> )	166	7.4	5.58	1.71	3.87	2.55
Bacteria (counts m <sup>-3</sup> )	166	22 × 10 <sup>4</sup>	7.92	2.42	2.46	5.13
Fungal spores (counts m <sup>-3</sup> )	165	4 × 10 <sup>4</sup>	3.42	2.00	1.85	2.30

GSD<sub>TOT</sub>, geometric standard deviation of the mean; GSD<sub>BC</sub>, geometric standard deviation of the mean between companies; GSD<sub>BW</sub>, geometric standard deviation of the mean between workers; GSD<sub>WW</sub>, geometric standard deviation of the mean within workers.

each of these and the single sample collected the year after, was thus ruled out. As this also showed that there was no significant difference between seasons, the season determinant was excluded in the following analyses.

#### Exposure determinants of grain dust

Working in the compound feed mills was associated with higher dust exposure than working in grain elevators, whereas work in combined grain elevators and compound feed mills gave similar dust exposure level as grain elevators for most tasks except process controlling (Table 4). All registered work tasks contributed to the exposure level of grain dust, but cleaning and process controlling implied particularly high exposure for grain dust. Cleaning increased the exposure level by a factor of 2.44 of the reference (from 0.65 to 1.58 mg m<sup>-3</sup>), whereas process controlling increased the dust exposure level by a factor of 2.97 (from 0.65 to 1.93 mg m<sup>-3</sup>). However, the latter seemed to apply for grain elevators only, because process controlling was associated with significantly less grain dust exposure in compound feed mills and the combined grain elevators and compound feed mills, than in grain elevators. The exposure was reduced with a factor of 0.18 and 0.22, from 1.93 to 0.34 mg m<sup>-3</sup> and to 0.42 mg m<sup>-3</sup>, respectively, compared with the grain elevators. The greatest effect of all work tasks was associated to seed winnowing (3.0), but the contribution was not statistically significant. The fixed-effects determinants in the mixed model reduced the BC, BW and WW variances of grain dust exposure by 16, 55, and 6%, respectively. The fixed effects explained totally 20% of the variance in the grain dust model.

#### Exposure determinants of endotoxins and β-1→3-glucans

The exposure to endotoxins and β-1→3-glucans was highest in grain elevator departments, about five times higher than in the compound feed mill departments or the combined grain elevator and compound feed mills (Table 5). Furthermore, the exposure to endotoxins was particularly high when working with seed winnowing, inspection/maintenance, cleaning, and grain rotation and emptying. Unspecified work was also associated with increased exposure to endotoxins. The fixed-effects determinants in the mixed model increased the BC variance of endotoxin exposure by 44%, but reduced the BW and WW variances by 43 and 8%, respectively. The fixed effects explained totally 24% of the variance in the endotoxin model.

Mixing of compound feed was the strongest determinant for β-1→3-glucan exposure (Table 4), but inspection/maintenance, cleaning, and grain rotation and emptying also contributed significantly to a higher glucan exposure. The fixed-effects determinants in the mixed model increased the BC and WW variances of β-1→3-glucan exposure by 64 and 4%, respectively, but reduced the BW variance by 57%. The fixed effects explained totally 24% of the variance in the β-1→3-glucan model.

#### Exposure determinants of bacteria and fungal spores

The exposure to bacteria and fungal spores was highest in grain elevator departments, ~2–4 times higher than in compound feed mill departments or combined grain elevators and compound feed mills (Table 6). Cleaning was the only work task that had a significantly higher contribution to the exposure level, and was, with an effect of 3.6 times the reference level, a particularly

**Table 4. Linear mixed effect models of exposure determinants for grain dust.**

	In grain dust model (n=153) <sup>b</sup>		
	Random effects model B (SE)	Mixed effects model B (SE)	Effects e <sup>B</sup>
<b>Determinant</b>			
Constant	-0.01 (0.18)	-0.43 (0.29)	
<b>Work task</b>			
Inspection/maintenance		0.33 (0.21)	1.39
Cleaning		0.89 (0.24) <sup>***</sup>	2.44
Grain reception		-0.24 (0.27)	0.79
Process control		1.09 (0.37) <sup>**</sup>	2.97
Filling sacks		-0.08 (0.34)	0.92
Mixing compound feed		0.22 (0.43)	1.24
Grain rotation and emptying		0.40 (0.28)	1.49
Seed winnowing		1.11 (0.61)	3.03
Unspecified work		0.73 (0.47)	2.08
Low exposure work		-0.40 (0.24)	0.67
<b>Department<sup>a</sup></b>			
Compound feed mill		0.46 (0.42)	1.58
Combined grain elevator and compound feed mill		-0.03 (0.36)	0.97
Grain elevator (ref)		ref	1
<b>Interaction</b>			
Process control × department			
1. Compound feed mill		-1.73 (0.56) <sup>**</sup>	0.18
2. Combined grain elevator and compound feed mill		-1.53 (0.61) <sup>*</sup>	0.22
	<b>Variance (SE)</b>	<b>Variance (SE)</b>	
<b>Between-company (BC)</b>	0.38 (0.22)	0.32 (0.20)	
<b>Between-worker (BW)</b>	0.42 (0.22)	0.19 (0.18)	
<b>Within-worker (WW)</b>	0.99 (0.16)	0.93 (0.16)	
<b>% Variance explained by fixed effects</b>		20	

SE, standard error. Example calculation: Grain dust exposure of worker doing process control and cleaning in compound feed mill department: background level × effect of process control × effect of cleaning × effect of compound feed mill × effect of process control × compound feed mill department =  $e^{-0.43} \times 2.97 \times 2.44 \times 1.58 \times 0.18 = 1.34 \text{ mg m}^{-3}$ .

<sup>a</sup>Grain elevator is reference.

<sup>b</sup>Samples from transport (n = 13) are excluded.

<sup>\*\*\*</sup>P ≤ 0.001; <sup>\*\*</sup>P ≤ 0.01; <sup>\*</sup>P ≤ 0.05.

**Table 5. Linear mixed effect models of exposure determinants for endotoxins and  $\beta$ -1,3-glucans.**

Determinant	In endotoxin model ( $n = 153$ ) <sup>b</sup>			In $\beta$ -1,3-glucan model ( $n = 153$ ) <sup>b</sup>		
	Random effects model B (SE)	Mixed effects model B (SE)	Effects e <sup>B</sup>	Random effects model B (SE)	Mixed effects model B (SE)	Effects e <sup>B</sup>
<b>Constant</b>	6.56 (0.24)	6.57 (0.35)***		2.15 (0.22)	2.12 (0.35)***	
<b>Work task</b>						
Inspection/ maintenance		0.64 (0.22)***	1.90		0.65 (0.24)**	1.92
Cleaning		0.54 (0.26)*	1.72		0.68 (0.28)*	1.97
Grain reception		0.04 (0.30)	1.04		0.06 (0.32)	1.06
Process controlling		0.25 (0.31)	1.28		0.17 (0.32)	1.19
Filling sacks		0.55 (0.41)	1.73		0.45 (0.42)	1.57
Mixing compound feed		0.57 (0.43)	1.77		1.04 (0.46)*	2.83
Grain rotation and emptying		0.63 (0.31)*	1.88		0.73 (0.34)*	2.08
Seed winnowing		1.83 (0.64)**	6.23		1.19 (0.70)	3.28
Unspecified work		1.42 (0.54)**	4.14		0.80 (0.56)	2.23
Low exposure work		-0.06 (0.54)	0.94		-0.25 (0.29)	0.78
<b>Department<sup>a</sup></b>						
Compound feed mill		-1.53 (0.41)***	0.22		-1.49 (0.41)***	0.23
Combined grain elevator and compound feed mill		-1.61 (0.42)***	0.20		-1.30 (0.42)**	0.27
Grain elevator		ref	1		ref	1
	<b>Variance (SE)</b>	<b>Variance (SE)</b>		<b>Variance (SE)</b>	<b>Variance (SE)</b>	
<b>Between-company (BC)</b>	0.34 (0.34)	0.49 (0.34)		0.28 (0.29)	0.46 (0.30)	
<b>Between-worker (BW)</b>	2.05 (0.53)	1.16 (0.44)		1.67 (0.47)	0.71 (0.35)	
<b>Within-worker (WW)</b>	0.92 (0.14)	0.85 (0.16)		1.08 (0.17)	1.12 (0.21)	
<b>% Variance explained by fixed effects</b>		24			24	

SE, standard error.

<sup>a</sup>Grain elevator is reference.<sup>b</sup>Samples from transport ( $n = 13$ ) are excluded.\*\*\* $P \leq 0.001$ ; \*\* $P \leq 0.01$ ; \* $P \leq 0.05$ .

strong determinant for bacterial exposure. Grain rotation and emptying, seed winnowing, and unspecified work also contributed to a higher exposure. This was, however, not statistically significant. The fixed-effects determinants of the bacteria model did not reduce the

BC, or WW variances, but explained 100% of the BW variance, and only 10% of the total variances. The fixed-effects determinants of the fungal model reduced the BC, BW, and WW variances by 14, 35, and 6%, respectively, and explained totally 13% of the variance.

**Table 6. Linear mixed effect models of exposure determinants for bacteria and fungal spores.**

Determinant	In bacteria model ( <i>n</i> = 153) <sup>b</sup>			In fungal spore model ( <i>n</i> = 153) <sup>b</sup>		
	Random effects model, B (SE)	Mixed effects model, B (SE)	Effects e <sup>B</sup>	Random effects model, B (SE)	Mixed effects model, B (SE)	Effects e <sup>B</sup>
<b>Constant</b>	12.4 (0.28)	12.24 (0.48)***		10.61 (0.20)	10.75 (0.30)***	
<b>Work task</b>						
Inspection/ maintenance		0.37 (0.32)	1.45		0.18 (0.19)	1.20
Cleaning		1.29 (0.39)***	3.63		0.53 (0.23)*	1.70
Grain reception		0.02 (0.44)	1.02		0.13 (0.26)	1.14
Process controlling		0.43 (0.41)	1.54		0.17 (0.25)	1.19
Filling sacks		0.13 (0.52)	1.14		-0.11 (0.32)	0.90
Mixing compound feed		0.52 (0.63)	1.68		0.24 (0.37)	1.27
Grain rotation and emptying		0.81 (0.45)	2.25		0.27 (0.27)	1.31
Seed winnowing		1.38 (0.95)	3.97		0.62 (0.56)	1.86
Unspecified work		1.25 (0.72)	3.49		-0.20 (0.44)	0.82
Low exposure work		-0.41 (0.38)	0.66		-0.06 (0.23)	0.94
<b>Department<sup>a</sup></b>						
Compound feed mill		-1.43 (0.52)**	0.24		-0.97 (0.32)**	0.38
Combined grain elevator and compound feed mill		-0.92 (0.52)	0.4		-0.81 (0.33)**	0.44
Grain elevator		ref	1		ref	1
	<b>Variance (SE)</b>	<b>Variance (SE)</b>		<b>Variance (SE)</b>	<b>Variance (SE)</b>	
<b>Between-company (BC)</b>	0.88 (0.49)	1.15 (0.55)		0.58 (0.28)	0.50 (0.24)	
<b>Between-worker (BW)</b>	0.69 (0.44)	0		0.26 (0.16)	0.17 (0.15)	
<b>Within-worker (WW)</b>	2.80 (0.42)	2.79 (0.36)		0.87 (0.14)	0.82 (0.13)	
<b>% Variance explained by fixed effects</b>		10			13	

SE, standard error.

<sup>a</sup>Grain elevator is reference.<sup>b</sup>Samples from transport (*n* = 13) are excluded.\*\*\**P* ≤ 0.001; \*\**P* ≤ 0.01; \**P* ≤ 0.05.

## DISCUSSION

This study describes the exposure levels and variability for grain dust, endotoxin, glucans, bacteria, and fungal spores in the Norwegian grain and compound feed

industry. As previously reported, the dust and microbial exposure in this industry is highly variable, and can reach health impairing levels (Smid *et al.*, 1992; Spaan *et al.*, 2006; Halstensen *et al.*, 2013). Our hypothesis

was that the variability could be explained by different workplaces/departments and tasks, and that these determinants may be of different importance for each exposure component.

The exposure to endotoxin was high. Otherwise, the exposure levels were moderate, but the GSDs reflected high variability. The variability in exposure to grain dust and microorganisms was overall mainly related to the day-to-day differences within the same worker, as indicated by higher  $GSD_{WW}$  than  $GSD_{BC}$  and  $GSD_{BW}$ . This corroborates the need to look more closely at the exposure related to different workplaces and tasks. The variability in endotoxin and glucan exposure was higher between different workers, as shown by higher  $GSD_{BW}$  than  $GSD_{BC}$  and  $GSD_{WW}$ . This furthermore demonstrates why it is important to do separate exposure assessments for each exposure component.

Whereas the exposure to grain dust was slightly higher in the compound feed mills than the grain elevators or combined grain elevators and compound feed mills, the exposure to endotoxins,  $\beta$ -1 $\rightarrow$ 3-glucans, bacteria, and fungal spores was highest in grain elevators. This indicated that the microbial content of the dust differs between the work places, and was an expected result considering the different raw materials used in the different departments. Departments are quite crude exposure determinants that are often used in exposure assessments, because it is easy to group workers in departments in many industries and companies. Differences in exposure levels between departments have also previously been shown particularly for grain dust and microbial components in the grain and compound feed industry (Halstensen *et al.*, 2013). Departments were therefore important fixed effects in our mixed models. However, when many different tasks are carried out in one department, such as in the Norwegian grain and compound feed industry, a more detailed assessment is necessary in order to evaluate the exposure differences between workers, sites, and tasks.

#### Work tasks as determinants of exposure

The workers in the present study had several tasks each day, and the samples included exposure from all these tasks. The time used for each task was not known, although personal sampling was performed for 6–8 h during an ordinary shift. In contrast to conditions

where workers have separate tasks the whole work day, multivariate statistical modeling with all tasks included was necessary to find the tasks representing highest and lowest exposure risk. Although this approach overcomes the limitation of the unknown proportional contribution of dust to a sample from the various tasks, it may influence the precision of the models. However, the significance of the estimated regression coefficients is likely to be conservative.

Although most work tasks contributed to exposure, the mixed models revealed that some work tasks contributed in particular to increased exposure, and that the exposure to each component was determined by a somewhat different set of work tasks. Cleaning and seed winnowing were work tasks associated with increased exposure to all investigated bioaerosol components. The large contribution of these tasks was shown in all models, and demonstrates their general importance as exposure determinants. These tasks were strongest associated with exposure to bacteria and endotoxins, as shown by the slightly weaker effect on the exposure to  $\beta$ -1 $\rightarrow$ 3-glucans and fungal spores. According to the endotoxin model, the estimated exposure level will be 1350 EU m<sup>-3</sup> when cleaning is the only task performed. Simpson *et al.* (1999) also demonstrated high endotoxin exposure during cleaning in grain silos (median 650 EU m<sup>-3</sup>). Seed winnowing was performed in the grain elevator department only, and because this task was carried out only four times, the contribution was not statistically significant in the most models. The estimated exposure level when performing this task only would be over 4000 EU m<sup>-3</sup> when calculated from the endotoxin model. Cleaning of grass seed, maize seeds, cereal seed, and vegetable seeds has previously been associated with high endotoxin exposure in the agricultural seed processing industry (Smit *et al.*, 2006). As a consequence of their findings of exposure levels up to 274 000 EU m<sup>-3</sup> during cleaning of grass seeds, sieving rooms were closed and thoroughly cleaned, and dust reducing measures were implemented before resuming this task. Subsequent measurements showed reduced endotoxin levels, and several other work tasks were demonstrated to contribute stronger to endotoxin and dust exposure than seed cleaning. The highest exposed task was mixing and dumping of seeds, which increased the exposure relative to lab work, the lowest exposed task, with a factor of 74. Tasks explained 81%

of the variability in endotoxin levels between workers at cereal seed plants, whereas the WW variability was not influenced by task. SEM of the seeds revealed microbial contamination, more prevalent on the cereal seed than on the grass or vegetable seeds (Smit *et al.*, 2006). However, Madsen *et al.* (2012) found a very high level of microorganisms when working with grass seeds, and that the exposure levels varied between seed batches. Working with problematic batches with high degree of microbial contamination also generated more dust (Madsen *et al.*, 2012).

In addition to cleaning, process controlling was a strong determinant for increased grain dust exposure. This finding is somewhat unexpected as this task is mainly done from control rooms with remote control, separated from the grain or feed. However, the interaction between process controlling and departments showed that the exposure increase was mainly associated with grain elevators. This suggests that the task was different in grain elevators and compound feed mills departments. Due to the nature of the processes involved in compound feed production, the humidity in compound feed departments is higher than in grain elevators. This may at least partly be connected with the observed exposure difference between the departments associated with process controlling. Possible bias due to dependence between process controlling and other dusty tasks, such as cleaning or inspection/maintenance, were ruled out by checking which other tasks the process controllers did the same day. Inspection/maintenance was the strongest determinant for increased endotoxin and  $\beta$ -1 $\rightarrow$ 3-glucan exposure, reflecting high-exposure situations such as filter change, valve adjustments, and repairs. There was also an elevated effect for the other bioaerosol components, although not significant.

Grain rotation and emptying imply moving of grain and feed, which is likely to generate dust. Indeed, this was a determinant of increased exposure to endotoxin, glucan, and bacteria. Various methods of grain rotation or emptying, such as air-driven, elevator driven, or passive (gravitation), were included in the measurements. There were, however, too few samples to statistically compare the level of dust generated by each method.

Mixing compound feed was a relatively strong determinant for endotoxin and  $\beta$ -1 $\rightarrow$ 3-glucan exposure, although the task was only performed in

compound feed mill departments. This task includes handling of ingredients for compound feed that are not only grains, but also fat and premix, that are not believed to be a microbial source. When we nevertheless found a particular contribution to endotoxin and  $\beta$ -1 $\rightarrow$ 3-glucan exposure from this task, we speculate that the reason may be that the grains used for compound feed production had a higher degree of microbial contamination than grain used for other purposes. Indeed, legislations allow a higher level of microbial contamination in grain used to animal feed than grain for human consumption (European Commission 2006a,b). It is important to include this knowledge in the increased awareness of different exposure patterns associated with different tasks. Working with grain intended for different purposes might represent different microbial exposure risks. Spankie and Cherrie identified increased awareness of respiratory diseases caused by exposure to grain dust among the people employed in the British grain industry, and a greater focus on minimizing contamination of grain by microorganisms. Testing of grain for mites, insects, mycotoxin, and endotoxins before delivery acceptance and demand of keeping record of such tests is implemented on British mills (Spankie and Cherrie, 2012).

Smid *et al.* (1992) grouped animal feed workers into categories by job titles, and each category contained one or more job titles with similar tasks and working environments. Job categories were more important than facilities in which the workers were employed in explaining dust and endotoxin levels in the animal feed industry. In their study, the unloaders were exposed to the highest dust and endotoxin levels; GM (GSD) 9.8 mg m<sup>-3</sup> (4.6) and 28.5 ng m<sup>-3</sup> (5.4) (corresponding to ~285 EU m<sup>-3</sup>), respectively. An even higher exposure could be estimated for 8 h of work with grain reception, the corresponding task in our study (742 EU m<sup>-3</sup> calculated from the model). Although the exposure was high, grain reception did not contribute significantly compared with other tasks. Unloaders in Smids study worked mainly in barges, whereas in our study grain was unloaded from lorries down through shafts at the grain elevators. The exposure differences between job categories in their study were the same for endotoxin and dust, whereas in our study this varied depending on the bioaerosol component. The tasks were performed indoors or in an outdoor area mostly shielded from weather and wind. Although we cannot completely exclude the

possibility that the weather in some instances influenced the exposure level, the weather was not believed to have significant impact on the exposure levels or the variances in the models.

Twelve of the samples included unspecified tasks, which in our models turned out as determinants of increased exposure to grain dust,  $\beta$ -1 $\rightarrow$ 3-glucans, bacteria, and particularly endotoxins. We do not know what kind of tasks these were, or whether or not they were different for each sample. Although they most likely were some of the listed tasks, but not registered as such, a large degree of uncertainty was associated to the unspecified tasks.

Only 13 samples were collected from grain transport. This obstructed the possibility to do meaningful statistical analysis that might have differentiated between various types of transport vehicles (passive loading and unloading versus vacuum suction for example), and tasks related to grain and compound feed transport. Therefore, we cannot exclude that these and other determinants have effect on the exposure in this work category. However, the exposure levels were lower in this group compared with other grain workers.

#### Variances explained by fixed effects

The high GSDs for several job categories observed by Smid *et al.* (crane drivers had GSD = 8.9 for endotoxin and 4.9 for dust) suggested that exposure variances were not only explained by job category, and possibly that tasks within the same category should be characterized in more detail. GSD<sub>TOTAL</sub> were also high in the present study (3.42–7.93), but most of the exposure variances were related to the differences between workers. Modeling exposures by departments and tasks showed that the determinants explained 10–24% of the total exposure variances, but were distributed differently between BC, BW, and WW variances for each exposure component. The determinants explained mainly the BW variances of all exposure parameters, possibly reflecting that the workers have different tasks within the companies. We could, however, not differentiate this by their job title. The reason that only 4–8% of the WW variances could be explained by the fixed effect, may be that there were only small changes in the working conditions during the repeated measurements. Although some tasks of particular importance for exposure were identified and could explain some of the BW variance, most of the variance could not be explained by the investigated determinants. This may be due to natural variation in microbial

contamination level of raw materials which will not be explained by departments or tasks. Furthermore, as the average of task frequency and time is modeled by the use of dichotomous variables, variable number of tasks performed between workers will result in further unexplained variance. The organization of work at the facilities prevented a more detailed registration of the work that had been carried out. Changes in the grain industry over the last 20 years have resulted in workers typically now undertaking several tasks where in the past, there would have been a number of people undertaking individual tasks (Spankie and Cherrie, 2012). Tasks or departments could not explain the differences in endotoxin, glucan and bacteria exposure between companies, as suggested by the increased BC variance in the models when including fixed effects. However, a considerable difference between companies of 20–30% of the total variances in the models, suggests that options for exposure prevention by technical and organizational means exist.

Determinants that in general might be expected to have impact on the exposure, although not shown in this study, are season, year, and region. Season or weather may particularly influence the microbial content of grain and grain dust. Region may similarly influence the microbial content indirectly by representing various microbial flora and climatic conditions. As knowledge, technology, legislations, and organizations develop, may time (year) on the long term become a determinant reflecting organizational changes in work and society.

#### Potential for exposure abatement

The presented models explaining the exposure with tasks and departments as determinant represents an improvement of the exposure assessment in the Norwegian grain and compound feed industry. Characterization of the exposure at work as detailed as possible is valuable in both epidemiological studies and later health risk evaluations. The findings in the present study may furthermore be used to reduce exposure. The identified high exposure tasks, such as seed winnowing and cleaning, should be object for higher awareness, either by dust-reducing interventions at sites where the high exposure tasks are performed or by use of personal protective equipment particularly connected to these tasks. A particular focus on endotoxin exposure seems to be needed to improve the exposure conditions in the grain industry.

## CONCLUSION

In line with our hypothesis, some tasks represented higher exposure than others, and these tasks varied depending on the bioaerosol component. Exposure levels were highest in grain elevator departments, even when adjusting for tasks. Cleaning and process controlling were the strongest determinant for overall exposure, whereas seed winnowing was a particular strong determinant of endotoxin exposure. Particular awareness of exposure reduction by technical intervention or personal protective equipment should therefore be taken at work places with identified high exposure tasks.

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