Exposure to inhalable dust and endotoxins in agricultural industries†

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Endotoxin is a well-known bacterial toxin that causes several health effects. Animal faeces and plant materials contaminated with bacteria have been identified as important determinants of organic dust related endotoxin exposure. Although high exposure to organic dust and endotoxins has been described regularly in agricultural industries, a detailed overview of levels of airborne exposure to endotoxins in the agricultural industry, as well as a systematic comparison between several specific branches using the same exposure assessment protocols are lacking. In this study, personal endotoxin exposure in a broad spectrum of agricultural industries was investigated and possible determinants of exposure were explored. 601 personal inhalable dust samples were taken in 46 companies of three agricultural industrial sectors: grains, seeds and legumes sector (GSL), horticulture sector (HC) and animal production sector (AP), with 350 participating employees. Dust and endotoxin levels were determined gravimetrically and by using the Limulus Amoebocyte Lysate (LAL) assay, respectively. Basic descriptive analysis and elaborate analysis of variance were performed. Mean exposure levels were high, with large differences between sectors and between companies within the sectors. Highest dust and endotoxin exposures were found in companies of the GSL sector. In all three sectors exposure was higher in the primary production part compared to the (industrial) products processing part of the sector. The Dutch proposed health based occupational exposure limit (50 EU m^{-3}) and temporary legal limit (200 EU m^{-3}) for endotoxin were often exceeded. Differences in exposure between workers were larger than the day-to-day variability. Identified determinants increasing exposure levels were company, dustiness of the product and contact with animals/faeces. 'Wet' processes resulted in less dusty working environments and thus lowered endotoxin exposure. Overall, exposure to endotoxins over the whole range of agricultural industries is high. A 10–1000 fold reduction in exposure is needed to reduce endotoxin related health risks.

Background

Many workers in the agricultural industry are exposed to organic dusts, which are known to be harmful to the respiratory tract. Endotoxins are ubiquitous contaminants of organic dusts and are probably a major causative agent in health problems associated with organic dust exposure.[1–4] Endotoxins are chemically complex constituents of the outer membrane of Gram-negative bacteria and airborne endotoxins are directly related to the occurrence of these bacteria. During cell growth and after cell death lysis occurs, resulting in the release of endotoxins into the environment. Lipopolysaccharides (LPS) are responsible for most of the biological properties characteristic of bacterial endotoxins.[5,6] Animal faeces and plant materials contaminated with bacteria are known to be important determinants of organic dust related endotoxin exposure.[7] Microbiological growth can occur during culturing, processing, storage, and transport of agricultural products, under specific conditions in which bacteria thrive well. High occupational endotoxin exposure is therefore prevalent in agricultural and related industries.[3,4,7–9]

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Inhalation is thought to be the major route of endotoxin exposure in the working environment. Inhaled endotoxin causes respiratory and systemic inflammatory responses. Acute symptoms after inhalation of high levels of endotoxin are dry cough and shortness of breath, accompanied by a decrease in lung function, fever reactions, shivering and malaise. Dyspnoea, headache and joint aches may also occur a few hours after exposure. Furthermore, epidemiological studies suggest that chronic exposure, to on average much lower levels, may lead to accelerated lung function decline and Chronic Obstructive Pulmonary Disease (COPD).[10–13] On the other hand, recent literature has suggested a possible protective effect of environmental and occupational endotoxin exposure on the risk of atopic sensitization.[14–16]

In The Netherlands, the Dutch Expert Committee on Occupational Standards has recommended a health based exposure limit of 50 EU m^{-3} for exposure to airborne endotoxin in the working environment, averaged over an 8-hour working day. Several studies, experimental as well as epidemiological, have shown that endotoxins can cause respiratory effects at concentrations around this standard (50–100 EU m^{-3}).[17–19]

During the 80's and the start of the 90's, exposure to endotoxin was investigated in several agricultural industries. However, comparison of exposure levels in the agricultural industry at large is difficult, as only certain branches have been investigated. Additionally, most studies were performed by different laboratories using different measurement and analytical techniques. More importantly, most studies comprised small measurement series and important information about sampling and analytical methods was either lacking or differed between studies.[20–29] For example, measured dust fractions differed between studies or were unknown, and personal exposure measurements were not always performed. Some large scale studies are available for specific agricultural industries like pig farming, dairy barns, animal feed industry and potato industry, but studies were limited by investigating only one industry at a time.[30–35] Comparison of endotoxin exposure in a limited number of different industries has only twice been reported.[36,37]

Therefore, this study investigated exposure to endotoxins in a broad spectrum of agricultural industries, using personal exposure measurements and similar sampling and analytical methods. Results are compared with proposed exposure limits, and possible determinants of exposure are explored.

Materials and methods

Study population and design

This study was conducted in a total of 46 companies in The Netherlands, with collaboration of three national employers' organizations: 'Grains, Seeds and Legumes sector' (GSL, 14 companies), 'Horticulture sector' (HC, 21 companies) and 'Animal Production sector' (AP, 11 companies) (Table 1). The GSL sector consists of the culturing, harvesting, (industrial) processing and trade of grains, seeds, legumes, derivatives and related products. The HC sector contains indoor nurseries and outdoor culturing of flowers, vegetables and plants, preparation and trade of mushroom compost, and industrial processing and trade of horticulture products. The AP sector consists of production of dairy products, meat and eggs on farms and the (industrial) processing of these products, with emphasis on abattoirs.

Representative companies within the relevant sectors were contacted to participate in the study. During the selection procedure companies with technology that reflected future trends were preferred, which led to a bias in favor of more modern companies. Furthermore, measurements were partly performed during selected activities when exposure was expected to be high, for example during cyclic activities like harvesting, based on information from previous studies and literature.

The study was conducted over a 10-month period (December 2001–September 2002).

In principle all workers of a company were included in the study. In large companies (> 10 employees), 10 subjects were selected to be included in the study. Selection was based on relevant work areas and jobs in the companies to obtain a representative overview of exposure to organic dust and endotoxin during a typical work shift for each industry. Sampling was performed on two days, in most companies on two consecutive days, with as many repeated measurements as possible, depending on the availability and willingness of workers. In total 350 workers participated and 601 measurements were collected, of which 251 were repeated measurements on one subject. Mean sampling time was 7.3 hours (range 1.8–10.1 hours).

Exposure measurements

Full-shift personal inhalable dust samples were collected using Gilian Gilair5 portable constant-flow pumps at a flow rate of 3.5 L min^{-1}, in combination with conductive plastic conical inhalable samplers (CIS), manufactured after the example of the German GSP (JS Holdings, UK). Samplers were equipped with 37 mm glass fiber filters (Whatman GF/A, UK). These sampling heads are less sensitive to changes in wind speed, an important factor when measuring in open air. Moreover, they maintain adequate performance, and sample in agreement with the inhalable dust convention.[38] The sampling head was placed on the shoulder of the worker, near the breathing zone, with the inlet facing forward. Each sampling day a control filter was included. Dust samples were stored at −20 °C after collection until further processing. Duration of storage ranged from a week until a few months because extractions and analyses were performed after collection of all samples. A previous study showed for house dust that storage at −20 °C before extraction does not affect endotoxin concentration.[39] Although storage of extracts for several months at −20 °C before analysis does not affect endotoxin concentrations,[39,40] repeated freeze and thaw cycles of extracts lower endotoxin concentrations.[40,41] In this study, extracts were stored in several aliquots and each aliquot is used only once to avoid repeated freezing of extracts.

The amount of dust on filters was determined gravimetrically by pre- and post-weighing of filters on an analytical balance in an EPA (US) criteria conditioned room. Extraction

Table 1 Personal geometric mean (GM) and geometric standard deviation (GSD) of endotoxin, inhalable dust and relative amount of endotoxin per mg dust in agricultural industries. Results are expressed overall and per sector

	Endotoxins/EU m^{-3}			Inhalable dust/mg m^{-3}			Endotoxins per mg dust	
Industry	*N*	GM (GSD)	Range	*N*	GM (GSD)	Range	*N*	GM (GSD)
Overall	587	230 (8.6)	1.6–191 430	591	0.8 (4.5)	<0.1[a]–99	587	270 (4.4)
(a) Grains, seeds and legumes sector								
Overall	188	580 (8.5)	2.3–149 060	190	1.5 (5.3)	<0.1–99	188	375 (4.9)
Primary production	**15**	**2700 (4.5)**	**96–41 200**	**15**	**2.5 (4.3)**	**0.3–56**	**15**	**1090 (2.9)**
Potato cultivation	2	310 (5.4)	96–1030	2	2.0 (3.9)	0.8–5.2	2	160 (1.4)
Flax culture and processing	10	4470 (3.7)	685–41 200	10	4.1 (4.0)	0.6–57	10	1090 (1.7)
Arable farming, grain harvest	3	2100 (2.5)	1032–5790	3	0.5 (2.1)	0.3–1.2	3	3980 (1.6)
(Industrial) processing	**173**	**500 (8.4)**	**2.3–149 060**	**175**	**1.4 (5.4)**	**<0.1–99**	**173**	**340 (4.9)**
Meal/flour tillage and processing	16	280 (7.7)	19–28 240	17	1.5 (3.0)	0.2–7.3	16	200 (3.4)
Animal feed industry	20	470 (4.4)	24–4930	20	1.1 (3.7)	<0.1–7.5	20	520 (2.3)
Grinding industry	17	2810 (4.1)	257–35 940	18	2.4 (5.5)	<0.1–17	17	800 (5.9)
Rice hulling plant	16	1110 (7.6)	95–149 060	16	3.1 (6.0)	0.3–80	16	360 (1.9)
Industrial bakery	12	49 (7.4)	2–3030	12	1.2 (3.0)	0.3–11	12	40 (5.5)
Corn processing	14	710 (7.3)	36–30 720	14	7.4 (3.6)	0.7–42	14	90 (4.8)
Grain transshipment and derivatives	19	2150 (9.0)	113–131 480	19	6.7 (5.1)	0.8–99	19	320 (3.4)
Malting plant	8	3720 (4.3)	291–20 030	8	0.7 (1.5)	0.4–1.3	8	5125 (3.0)
Grass drying plant	5	2900 (6.2)	179–20 180	5	3.7 (4.0)	0.5–18	5	780 (1.6)
Coffee-roasting plant and tea trading	19	140 (3.4)	12–2030	19	0.7 (2.5)	0.2–2.7	19	200 (3.1)
Sugar production (sugar beets)	27	130 (4.0)	9–2520	27	0.2 (2.7)	<0.1–1.3	27	575 (3.9)
(b) Horticulture								
Overall	291	170 (6.9)	1.6–191 430	293	0.6 (3.7)	<0.1–35	291	265 (3.8)
Culturing vegetables, flowers and plants (glasshouse)	**120**	**110 (4.3)**	**1.6–4130**	**122**	**0.5 (3.2)**	**<0.1–11**	**120**	**205 (3.1)**
Mushroom nursery/growing	17	81 (4.0)	3–1350	17	0.2 (4.2)	<0.1–0.9	17	375 (5.6)
Chicory nursery/growing	19	140 (2.6)	35–770	19	0.8 (1.6)	0.4–2.0	19	165 (2.0)
Cut flowers nursery/growing (tulips)	13	66 (1.9)	30–330	13	0.3 (1.4)	0.2–0.6	13	195 (1.8)
Cut flowers nursery/growing (roses)	18	27 (2.8)	5–180	18	0.3 (1.5)	0.1–0.7	18	90 (2.0)
Pot-plants nursery (ficus)	8	48 (6.7)	2–1490	8	0.3 (2.5)	0.1–2.4	8	155 (3.6)
Tomatoes nursery	10	69 (2.5)	14–340	10	0.8 (1.7)	0.4–1.9	10	83 (1.8)
Cucumber and paprika nursery	14	160 (2.2)	36–650	16	0.3 (6.3)	<0.1–2.4	14	275 (2.0)
Flower bulbs nursery	15	430 (3.5)	10–1930	15	1.0 (2.1)	0.3–4.1	15	410 (3.0)
Flower bulbs nursery	6	1120 (4.5)	108–4130	6	2.6 (2.3)	1.1–11.4	6	435 (3.3)
Culturing vegetables, flowers and plants (outdoor)	**50**	**110 (2.5)**	**8.6–450**	**50**	**0.9 (2.4)**	**0.1–9.2**	**50**	**120 (2.5)**
Hardy nursery stock and trading	19	130 (1.9)	25–310	19	1.4 (1.8)	0.3–3.2	19	90 (1.5)
Hardy nursery stock	10	110 (2.4)	19–350	10	1.2 (2.8)	0.5–9.2	10	95 (3.2)
Gardening company	5	150 (2.3)	55–450	5	0.9 (1.9)	0.4–1.7	5	175 (1.3)
Gardening company	16	75 (3.2)	9–450	16	0.4 (2.0)	0.1–1.2	16	170 (3.5)
Compost preparation/trade and trade	**77**	**860 (9.8)**	**14–191 430**	**77**	**1.2 (5.1)**	**0.1–35**	**77**	**680 (2.6)**
Mushroom compost preparation	20	240 (3.1)	18–2430	20	0.6 (2.7)	0.1–2.6	20	380 (2.4)
Flower bulb trade	16	390 (1.8)	107–1220	16	1.7 (1.9)	0.2–2.7	16	655 (1.7)
Onion trade	20	25 930 (2.7)	4025–191 430	20	14.4 (1.5)	6.7–35	20	1795 (2.0)
Mushroom compost preparation	21	210 (3.3)	14–1780	21	0.4 (1.7)	0.1–1.2	21	535 (2.3)
Industrial processing	**44**	**61 (4.9)**	**4.9–1200**	**44**	**0.3 (1.9)**	**<0.1–1.5**	**44**	**240 (5.9)**
Vegetable slicing plant	9	39 (3.9)	9–590	9	0.1 (4.2)	<0.1–0.5	9	270 (6.8)
Dried subtropical fruit	15	19 (2.3)	5–150	15	0.4 (1.8)	0.2–1.5	15	50 (2.0)
Vegetable and fruit canning industry	19	140 (3.4)	12–2030	12	0.3 (1.2)	0.2–0.3	8	255 (3.9)
Vegetable and fruit freezing industry	8	49 (3.1)	11–280	8	0.2 (1.5)	0.1–0.3	12	1575 (2.2)
(c) Animal production sector								
Overall	108	110 (9.3)	2.0–8120	108	0.7 (4.0)	<0.1–21	108	170 (4.9)
Primary production	**27**	**1190 (3.1)**	**62–8120**	**27**	**2.4 (2.2)**	**0.4–14**	**27**	**505 (2.4)**
Dairy farming	8	560 (3.9)	62–2230	8	1.3 (1.8)	0.4–2.3	8	440 (2.9)
Dairy farming and cattle breeding	4	1570 (2.5)	444–3860	4	1.5 (6.1)	0.7–2.7	4	1030 (1.7)
Poultry farm (eggs)	2	2090 (1.3)	1716–2550	2	9.5 (1.7)	6.6–14	2	220 (2.2)
Poultry farm (chickens for meat)	2	880 (2.1)	520–1500	2	4.2 (1.1)	4.0–4.4	2	210 (2.0)
Poultry farm (free-range hens)	5	2140 (3.6)	360–8120	5	3.6 (2.1)	1.6–11	5	600 (2.4)
Pig farm (with own pulp feed installation)	6	1510 (2.1)	992–6970	6	2.6 (1.6)	1.6–5.4	6	575 (1.7)
(Industrial) processing	**81**	**51 (6.8)**	**2.0–6230**	**81**	**0.4 (3.7)**	**<0.1–21**	**81**	**115 (5.0)**
Poultry abattoir	14	310 (7.0)	27–6230	14	1.5 (5.3)	0.2–21	14	210 (1.6)
Calf abattoir	12	120 (11.8)	3–3480	12	0.2 (4.9)	<0.1–2.1	12	510 (5.0)
Cow/cattle abattoir	19	31 (5.2)	2–820	19	0.3 (1.9)	0.1–1.9	19	110 (5.4)
Pig/swine abattoir	16	28 (3.4)	2–220	16	0.3 (1.6)	0.1–0.6	16	90 (3.1)
Meat processing	20	23 (3.6)	3–1420	20	0.6 (3.3)	0.1–11	20	400 (4.9)

[a] Non-detectable dust concentration, <0.1 mg m^{-3}.

was done as described previously, under pyrogen-free conditions.[40] Briefly, filters were immersed in 5 ml 0.05% Tween20 in pyrogen-free water and rocked vigorously for one hour at room temperature. After 15 minutes of centrifugation at 1000 *G* (=2094 rpm), supernatant was harvested and stored in 0.1 ml aliquots at −20 °C until analysis.

Endotoxin concentration in extracts was assayed using a quantitative kinetic chromogenic Limulus Amoebocyte Lysate (LAL) method (BioWhittaker; lot no. lysate 1L6765, lot no. standard 2L0090 (RSE/CSE ratio 11.5 EU ng^{-1})).[40] Samples were assayed at an initial dilution of 1 : 20, and when the measured concentration was too close to the upper detection limit of the assay, retested at higher dilutions up to a maximum of 1 : 1000. Potential enhancement or inhibition was evaluated by testing samples in serial dilutions, but no significant deviation from parallelity to the calibration line was observed.

Worker and company information

A self-administered checklist was used to obtain information from the workers included in the study on job, job title, workplace, work activities, work environment and use of protective equipment. In each company information about process characteristics and other possible determinants of organic dust and/or endotoxin exposure was gathered by interviewing someone from the executive staff with use of a purpose-developed checklist.

Statistical analysis

Data were analyzed with SAS statistical software (version 8e; SAS Institute, Cary, NC, USA). Inhalable dust and endotoxin concentrations below the limits of detection (LOD) were assigned a value of two thirds of the detection limit, which was 0.01 mg in the case of dust. For endotoxin the detection limit varied from 1.3 to 3.0 EU per filter, depending on the day of analysis and the plate the analyses were performed on.

Levels of exposure were natural log transformed before statistical analysis. Distributions of dust and endotoxin exposure were examined to ascertain lognormal distributions. Crude descriptive exposure levels were calculated as geometric mean (GM) with geometric standard deviation (GSD) for each sector and company. GM and GSD of sectors and subsectors were used to calculate the chance of exceeding the Dutch occupational exposure limit of nuisance dust (10 mg m^{-3}), the proposed health based occupational exposure limit for endotoxin (50 EU m^{-3}), and the as of January 1st 2003 implemented temporary legal limit for endotoxin (200 EU m^{-3}),[8] as described in Boleij *et al.*[42] Spearman correlations were calculated between inhalable dust and endotoxin concentrations.

Determinants of exposure were explored by mixed effect analysis of variance in order to correct for possible correlation between repeated measurements.[43] Sector, company and process characteristics or activities were introduced as fixed effects, while worker identity was introduced as a random effect. The mixed-effect models are specified by the following expression:

$$Y_{ij} = \mu_y + \beta_1 + \ldots + \beta_p + \chi_i + \varepsilon_{ij}$$

for $i = 1, \ldots, k$ (workers) and $j = 1, \ldots, n_i$ (repetitions of the *i*th worker), where Y_{ij} is the log-transformed exposure level. In this model, μ_y represents an overall intercept for the group that corresponds to mean background exposure (log-transformed); $\beta_1, \ldots, \beta_p$ are fixed effects; χ_i is the random effects of the *i*th worker; and ε_{ij} is the random effect of the *j*th measurement effect of the *i*th worker. It is assumed that $\chi_{i(k)}$ and $\varepsilon_{j(ik)}$ are each normally distributed and mutually independent, with zero means and between-worker (${}_{bw}\sigma^2$) and within-worker (${}_{ww}\sigma^2$) variances. Separate models were constructed for inhalable dust and endotoxin exposure. Variances are estimated as between-worker and within-worker variance components.

Results

Exposure to inhalable dust and endotoxin

Of the 601 collected samples, 10 dust and 14 endotoxin samples were lost during extraction and analysis. Thus, dust and endotoxin data were available for 591 and 587 samples, respectively. Of these samples, 7 were below the LOD of dust and 49 below the LOD of endotoxin. All control filters resulted in endotoxin concentrations below LOD, thus contamination during mounting of the samplers did not occur. Inhalable dust and endotoxin exposure is summarized in Fig. 1 and 2 and described in more detail in Table 1. The overall geometric mean concentration was 0.8 mg m^{-3} for inhalable dust and 230 EU m^{-3} for endotoxins, with distinctly more spreading in endotoxin exposure (GSD 4.5 for dust *vs.* 8.6 for endotoxin). These large variances were also observed within the sectors, indicating considerable variation in exposure between workers or between days for a worker in all sectors, especially for endotoxin. Overall, highest mean exposure levels were found in the GSL sector. Dust and endotoxin exposure levels were slightly higher in the HC sector than in the AP industry. However, large differences in exposure were found between companies within each sector.

In the GSL sector (Table 1a, Fig. 1 and 2) a difference between exposure during primary production (culture and harvest of the products) and further industrial processing and trade could be observed. In primary GSL production, endotoxin exposure was high in almost every company and function (GM = 2700 EU m^{-3}). This was due to both a fairly high dust exposure (GM = 2.5 mg m^{-3}), and relatively high amounts of endotoxin per mg dust (Fig. 3). It should be noted that the measurements in the primary production part of this sector were worst-case scenarios, namely the measurements during the harvesting of grain ($n = 3$) and flax ($n = 10$) and the cultivation of potatoes ($n = 2$). Mean endotoxin and dust exposure levels during processing in the GSL sector were lower, being 500 EU m^{-3} and 1.4 mg m^{-3}, respectively.

Inhalable dust exposure was low in most HC companies (GM = 0.6 mg m^{-3}), except for the onion trade (GM = 14.4 mg m^{-3}). In contrast, exposure to endotoxins varied greatly, with highest exposure in the onion trade company (GM > 25 000 EU m^{-3}) and fairly low exposures in other companies, including the industrial processing of vegetables (GM = 61 EU m^{-3}) (Table 1b). The ratio of endotoxin per mg dust varied as well, with most endotoxin per mg dust in onion trading

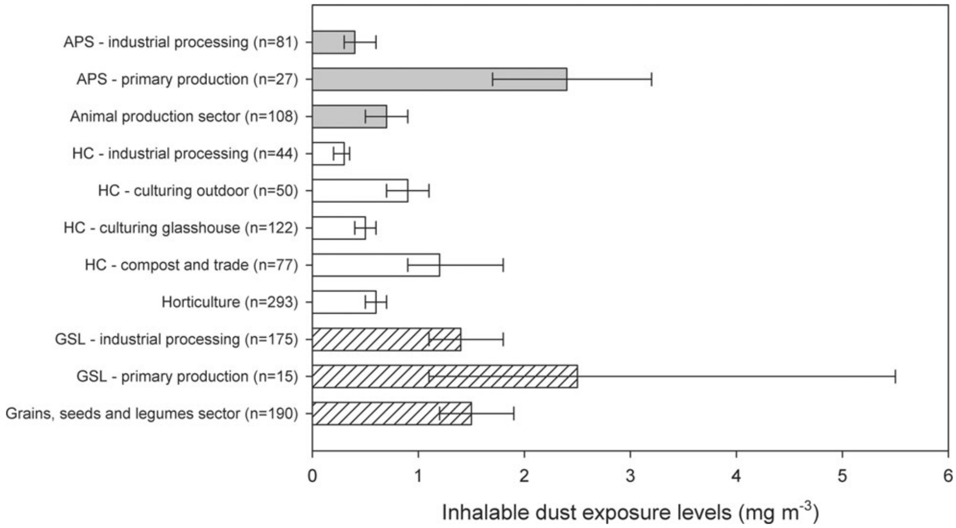

Fig. 1 Inhalable dust exposure (GM and 95% CI) levels in three sectors and subsectors of the agricultural industry.

(Fig. 3). In general, endotoxin exposure levels were similar for different jobs within a company. However, some companies had a few highly exposed jobs, *e.g.* in the mushroom nursery, during mushroom compost preparation, in the flower bulb nursery and the cucumber and paprika nursery (data not shown). Roughly, endotoxin exposure seemed to depend on the type of process, the handled products and thus most likely the occurrence of microbiological growth in the products, and the level of dust exposure.

In the AP sector, exposure to dust was overall moderate (GM = 0.7 mg m^{-3}), except for farming (GM = 3.6–9.5 mg m^{-3}) (Table 1c, Fig. 1). There was a wide range in endotoxin exposure (GM = 110, range 2.0–8120 EU m^{-3}), with highest exposure levels found in poultry and dairy farming (Table 1c, Fig. 2). During primary production, almost all farm workers were highly exposed to dust (GM = 2.4 mg m^{-3}) as well as endotoxin (GM = 1190 EU m^{-3}), whereas during further (industrial) processing high exposures were only found in small specific parts of the companies where workers had direct contact with animals (front end of the process) or animal waste (data not shown). The amount of endotoxin per mg dust was also higher for primary production compared to exposure during industrial processing of products of the AP sector (Fig. 3).

Correlation

The correlation coefficient (r) between inhalable dust and endotoxin was 0.69 for all measurements, and 0.67, 0.59 and 0.66 for the 'Grains, Seeds and Legumes', 'Horticulture' and the 'Animal Production', respectively. This coefficient squared gives the explained variance (R^2), which for dust levels explained at maximum 48% of the variance in endotoxin exposure levels. Indeed, there were large differences in exposure levels between companies of a sector and in the ratio of endotoxin per mg dust.

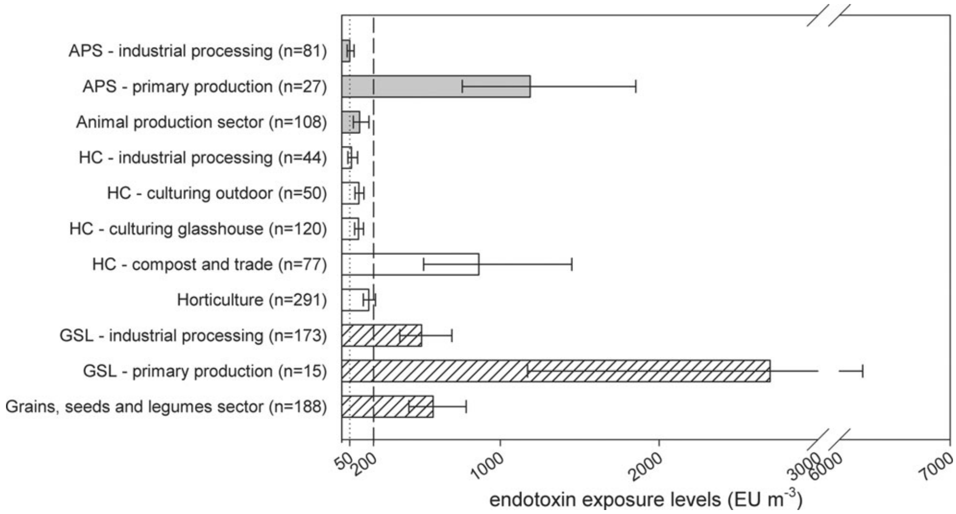

Fig. 2 Endotoxin exposure (GM and 95% CI) levels in three sectors and subsectors of the agricultural industry.

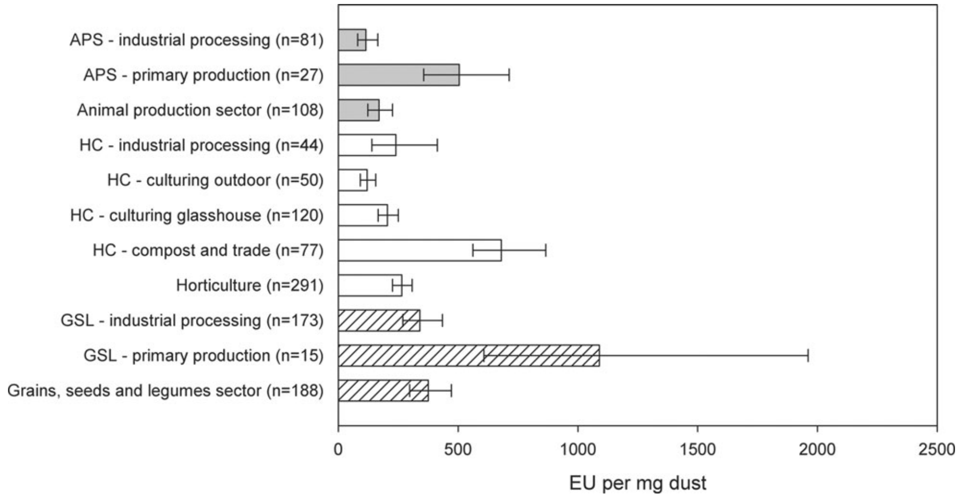

Fig. 3 Relative amount of endotoxin (EU per mg dust) (GM and 95% CI) levels in three sectors and subsectors of the agricultural industry.

Comparison with exposure limits

In Table 2 the chances of exceeding occupational exposure limits for endotoxin and dust are presented. While only 5% of all inhalable dust measurements were above the occupational exposure limit for nuisance dust of 10 mg m^{-3}, 53% of the endotoxin measurements exceeded the temporary legal limit of 200 EU m^{-3} and 76% were above the proposed health based exposure limit of 50 EU m^{-3}. Both limits for endotoxin were exceeded in all sectors, with the highest chance in primary production companies.

Determinants of exposure

Day-to-day and between-worker variances of exposure were 0.9 and 3.7 for endotoxin, and 0.6 and 1.8 for inhalable dust exposure, respectively. Thus, differences in exposure between workers were considerably larger than variation in exposure from day-to-day. Dustiness of the product processed and 'short *versus* long work cycles' explained some but only a little of the day-to-day variability for a worker (data not shown). Presence of waste water, dustiness of the product, and contact with animals explained differences between workers in dust and endotoxin exposures. Effect estimates on exposure levels of the above described and other possible determinants are presented in Table 3. Presence of waste water, process water, exhaust ventilation, a cyclic process, an industrial scale process and continuous exposure patterns were associated with lower exposure levels of both dust and endotoxin. Presence of faeces was associated with lower dust and higher endotoxin exposure. Type of company, presence of animals and a prolonged cycle (with seasonal variation) were associated with higher exposure levels of both dust and endotoxin. Stratified analysis for the three sectors and subsectors generally showed a similar pattern for the effect of determinants, although some determinants disappeared due to a lack of diversity within the sector. The strongest determinants explained some of the observed dissimilarities between sectors. For example, exposure in the GSL sector was increased by the presence of remnant products (products that remain during the process and in some cases can be used in other industries like animal feed) and bulk product and decreased by presence of recirculating process water. In the HC sector remnant products, dustiness of the product and process water were important explanatory variables, and in the AP sector contact with living animals explained most of the differences.

Table 2 Calculated percentage of exceeding exposure limits for endotoxin and inhalable dust, overall and per sector

	Endotoxins		Inhalable dust
	>50 EU m^{-3}	>200 EU m^{-3}	>10 mg m^{-3}
Overall	76%	53%	5%
Grains, seeds and legumes sector	**87%**	**69%**	**13%**
Primary production	100%	96%	17%
Industrial processing	86%	67%	13%
Horticulture	**74%**	**47%**	**2%**
Industrial processing	55%	23%	0%
Compost and trade	89%	74%	10%
Culturing glasshouse	69%	33%	0%
Culturing outdoor	80%	25%	0%
Animal production sector	**64%**	**40%**	**3%**
Primary production	100%	95%	3%
Industrial processing	50%	24%	1%

Discussion

In this study, exposure to inhalable dust and endotoxin in a broad spectrum of agricultural industries has been investigated. Mean inhalable dust and endotoxin exposure levels were highest in the grains, seeds and legumes (GSL) sector. Exposure in the horticulture (HC) sector is slightly higher than in the animal production (AP) sector. Within the different sectors large differences between companies and between jobs were noted. Additional subdivision within the sectors revealed that highest exposures occur in the primary production phases of grains, seeds, legumes and animal products, mushroom compost preparation and trade of horticulture products: both dust as well as endotoxin exposure levels were high. The lowest concentrations were found in the industrial processing of

Table 3 Relative effect (compared to the reference) of exposure determinants on endotoxin and inhalable dust levels with all variables in the mixed regression model

	Endotoxin/EU m^{-3}				Inhalable dust/mg m^{-3}			
	Overall	Grains, seeds and legumes sector	Horticulture	Animal production sector	Overall	Grains, seeds and legumes sector	Horticulture	Animal production sector
Working mainly inside (I), outside (O) or both (IO) on worker level	IO = ref.	IO = ref.	IO = ref.	IO = ref.	IO = ref.	IO = ref.	IO = ref.	IO = ref.
	I: 0.9	I: 0.5 [a]	I: 1.2	I: 3.3 [b]	I: 0.9	I: 0.6	I: 1.2	I: 1.8
	O: 0.7	O: 1.7	O: 0.3 [b]	O: 0.9	O: 0.8	O: 0.9	O: 0.6	O: 3.0
Working mainly inside (I), outside (O) or both (IO) on company level	IO = ref.	IO = ref.	IO = ref.	IO = ref.	IO = ref.	IO = ref.	IO = ref.	IO = ref.
	I: 0.9	I: 0.4	I: 0.8	I: 1.3	I: 0.9	I: 5.0	I: 0.5 [a]	I: 2.3
	O: 1.3	O: —	O: 2.0	O: —	O: 2.5 [b]	O: —	O: 2.5 [a]	O: —
Contact with living animals[c]	6.8 [b]	—	—	15.8 [b]	3.9 [b]	—	—	4.5 [b]
Remnant products[c]	2.5[b]	116[b]	6.0[b]	1.1	0.9	2.0	1.5	1.1
Waste water[c]	0.3[b]	0.9	0.1[b]	—	0.3[b]	0.5[a]	0.1[b]	—
Process water[c]	0.7	0.4	0.6	—	1.7	1.1	1.1	—
Recirculating process water[c]	0.9	0.03[b]	—	—	0.4[b]	1.4	—	—
Ventilation[c]	0.3[b]	0.4	0.5	—	0.5[b]	0.1[b]	0.4[b]	—
Faeces[c]	1.9[a]	—	1.2	1.1	0.9	—	0.5[a]	0.6
Cyclic process (company)[c]	0.7	5.3[a]	1.5	1.2	0.5[b]	0.5	0.6	0.5[b]
Bulk product[c]	3.3[b]	8.1[b]	3.5[b]	—	0.7[a]	0.8	0.4[b]	—
Dustiness of product[c]	5.2[b]	—	10.4[b]	1.6	2.9[b]	—	7.6[b]	1.6
Industrial process[c]	0.3[b]	—	1.7	0.3	0.6[b]	—	1.3	0.2
Exposure variable *vs.* continued	0.2[b]	—	0.3[b]	—	0.4[b]	—	0.8	—
Cycle short *vs.* prolonged	4.3[b]	—	4.8[b]	—	1.8[b]	—	1.6	—

ref. = reference variable.[a] $0.05 < p < 0.1$. [b] $p < 0.05$. [c] (0/1) dummy variable: present *versus* absent (absent is reference).

animal as well as horticulture products, with the exception of the front end of the abattoirs, when workers have contact with living animals or animal waste. The industrial processing of grains and related products results in fairly high exposure levels, where in most cases the endotoxin exposure is high when dust exposure is high.

The sample of companies included in the study was not random, as the width of the study and time available limited the number of companies included in the study, which might have resulted in selection bias. This was not likely to have happened as a qualitative walk through in comparable companies of a number of industries showed no large differences between those companies included for sampling in comparison with the others. Also, not all workers of a company were included in the study, but workers were selected taking into account as many relevant functions and activities as possible. Therefore, no distortion of the results from this perspective is expected. On the other hand, most data in primary production of the GSL sector came from worst-case measurements. In these industries exposure occurs during specific activities, for example during harvesting, which is conducted during a limited period, and the exposure pattern might be quite different during the rest of the year because of other activities and/or crops. Determined exposure levels thus only represent specific periods. In contrast, the processing of these products continues throughout the year, as well as the work on animal farms.

Both inhalable dust and endotoxin showed a reasonably large variability, but the variation in endotoxin was much larger (GSD 8.6 *versus* 4.5 for dust levels). This difference may be partly due to a larger analytical error, since the assessment of endotoxin requires a much more complicated procedure involving extraction of filters, storage of extracts, and dilution and testing in the LAL assay, compared to 'only' weighing of filters for dust analyses. A larger analytical error however does not explain all of the difference in variance of exposure between endotoxins and dust. The larger variance in endotoxin exposure is most likely due to large variation in microbiological activity in the products and processes of the different branches. This is confirmed by the varying endotoxin content of the dust, which showed considerable differences between and within the various sectors.

It is known that assessment of endotoxin exposure may differ considerably between groups when different sampling, extraction, analysis, and storage procedures are employed. Differences between laboratories are usually within an order of magnitude and vary according to the type of dust,[40,41,44] which compromises comparisons between results obtained by different groups. Nonetheless, in The Netherlands comparable techniques have been used in the past, which simplifies comparison with previous Dutch studies in animal production and GSL sectors, although for other industries data is lacking. The exposure levels found in the animal production companies are comparable with measurements conducted on pig farmers in The Netherlands with mean exposure levels of about 1820 EU m^{-3} (56–8250 EU m^{-3}; $n = 182$) in Summer and 1680 EU m^{-3} (11–15 030 EU m^{-3}; $n = 168$) in Winter.[31,45] In other studies of pig farmers outside The Netherlands comparably high endotoxin exposures were found.[36,37] Previous studies in poultry farmers also showed high exposure levels ranging from 0.24–39 167 EU m^{-3} for total endotoxin and 0.35–694 EU m^{-3} for respirable endotoxin.[22,46] Total endotoxin exposure levels found in poultry slaughter houses for workers handling living poultry ranged from 200 to 15 000 EU m^{-3}, which is in the same range as we found,[23] although even higher exposure levels have been found.[37] The results from the animal feed industry are also within the range of earlier investigations in and outside The Netherlands, although the range of exposure in the Dutch studies was larger (2 to 18 700 EU m^{-3}).[33,34,37] The endotoxin concentrations in the sugar beet processing company were even higher than those in an earlier study in the same industry (range 9–2521 *versus* 25–350 EU m^{-3}).[47] Comparable results are also found in studies that investigated a few different agricultural industries at the same time.[36,37]

In Horticulture only a few comparisons can be made. The concentrations found in mushroom growing are comparable with an earlier study.[37] However, endotoxin exposure in glasshouses was higher than has been found in Spanish measurements (GM 110 EU m^{-3} *vs.* 0.36 ng m^{-3}), but here difference in technique used to assess endotoxin levels may also account for much of the apparent difference.[36]

It can be concluded that in general exposure levels derived from this current study are in agreement with earlier investigations in similar agricultural settings. Albeit a more thorough and detailed comparison of exposure levels in the future would require standardization of measurement and analytical methods for endotoxin exposure.

Several determinants were associated with exposure, *e.g.*, the presence of waste water, process water, ventilation, cyclic process and an industrial scale process are associated with lower dust and endotoxin exposure. Contact with faeces was associated with higher endotoxin but lower dust levels. As information about most determinants of exposure was only available at the company level, interpretation of differences between workers was not possible.

Presence of water in the process of industries was expected to increase endotoxin exposure, as previously reported for potato processing [19,48] and the paper industry.[49,50] Surprisingly, the presence of water in the industries in the current study seemed more important for the reduction of dust and endotoxin exposure levels. Since production of consumption goods and the use of water in the process are bound to strict hygienic rules, water recycling was not common. This time water itself appeared not to be a source of microbes, but aided in reducing exposure levels.

The type of company, presence of animals, dustiness of the product, bulk production and prolonged exposure are associated with a higher exposure level, as might have been expected. Endotoxins that originate from faeces, microbial growth in contaminated plant material on the land or during storage have been associated previously with high organic dust exposure.[7] Type of company explained most of the variability between workers, suggesting that together with the specific determinants mentioned, other unidentified determinants of exposure play a role. This was further supported by the fact that inclusion of company decreased the effect estimates of all other variables (data not shown). However, there is still little knowledge about the origin of endotoxin exposure in the studied sectors. There are large differences in the amount of microbiological growth and different sources of exposure might play a role.[7] Improvement of the explanatory models may be obtained by including more personal information and detailed descriptive information on microbial growth and determinants of microbial growth, which were unfortunately not available.

There was only little day-to-day variability in exposure within workers, and we were not able to find factors that explained variation in exposure from day-to-day. This might be due to the fact that repeated measures were derived from two successive days with almost no change of working conditions. In future studies, more repeated measurements over a larger time period have to be performed to be able to distinguish possible determinants of within-worker variance.

From several studies, experimental as well as epidemiological, endotoxins appear to be related to (respiratory) health effects at relatively low concentrations (50–100 EU m^{-3}).[4,18,19] Since many measurements were above the temporary Dutch legal limit of 200 EU m^{-3} as well as the proposed health based exposure limit of 50 EU m^{-3}, a potential health risk exists. In every company workers were exposed to concentrations above 200 EU m^{-3}, and in the companies of the GSL sector almost all jobs had an exposure above 200 EU m^{-3}. Even considering overestimation of exposure in primary production of the GSL sector, considerable exceedance of limits occurs. It is clear that efforts should be made to lower the exposure to endotoxins drastically.

During the study, a qualitative assessment of currently applied exposure control measures was executed. Control measures like forced ventilation and local exhaust ventilation were mostly not available and if present, were frequently insufficient. Personal protective devices were often present. However, based on theoretical protection factors and determined exposure levels in these companies, they are not able to protect workers sufficiently. In addition, many workers do not use them properly, which would lower theoretical protection factors further. Thus, to create a healthy working environment for workers in these industries considering endotoxin exposure, exposure levels need to be reduced by a factor of 10–100 and sometimes by a factor 1000 or more. The current control measures do not and cannot result in such reduction factors. Changes of processes, procedures and control measures will be necessary and this will require large (technical) interventions and investments from the companies.

Investigation of health effects was not the scope of the current investigation. Previous studies of pig farmers, the animal feed industry and potato industry showed the adverse respiratory health effects of endotoxin exposure.[19,34,41] More recently, a possible protective effect of endotoxins in the development of atopy and asthma in children has been found.[14,15] A recent study showed that this might apply in an adult population as well.[16] Thus, to fully understand the impact of occupational exposure to endotoxins in these sectors, future investigations should focus on both protective and adverse effects and should also take into account individual sensitivity of people after endotoxin exposure.[51,52]

Conclusion

This study gives insight into endotoxin exposure in a broad spectrum of agricultural industries. Overall, it can be concluded that exposure to endotoxin in the many different parts of the agricultural industry is high. Inhalable dust and endotoxin exposure is the highest in the primary production cycle of a sector, and lower in the following cycle: (industrial) processing and trade. However, the exposure to dust and endotoxin varies greatly and seems to be dependent on the process and the products processed and produced in the company. More detailed information about possible exposure determinants is needed to fully understand differences in exposure between industries and between workers within an industry. Moreover, exposure levels exceed health-based exposure limits, indicating a possible health risk for workers in these industries. In the current situation 'good housekeeping' and the present control measures are not enough to realize a desirable reduction of the exposure; this can only be realized through structural exposure control measures.

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