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PAPER

Exposure to inhalable dust and endotoxin among Danish livestock farmers: results from the SUS cohort study†

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Studies on personal dust and endotoxin concentrations among animal farmers have been either small or limited to a few sectors in their investigations. The present study aimed to provide comparable information on the levels and variability of exposure to personal dust and endotoxin in different types of animal farmers. 507 personal inhalable dust samples were collected from 327 farmers employed in 54 pig, 26 dairy, 3 poultry, and 3 mink farms in Denmark. Measurements in pig and dairy farmers were full-shift and performed during summer and winter, while poultry and mink farmers were monitored during 4 well-defined production stages. The collected samples were measured for dust gravimetrically and analyzed for endotoxin by the Limulus amebocyte lysate assay. Simple statistics and random-effect analysis were used to describe the levels and the variability in measured dust and endotoxin exposure concentrations. Measured inhalable dust levels had an overall geometric mean of 2.5 mg m^{-3} (range <LOD to 47.8) and endotoxin of 988 EU m^{-3} (range <LOD to 374 000). The highest dust and endotoxin concentrations were measured among pig and poultry farmers, and were the lowest among dairy and mink farmers, respectively. Exposure among pig and cattle farmers was characterised by a substantial day-to-day variability that increased from the indoor to outdoor working environment. Only mink farmers complied with the Danish occupational exposure limit for total dust (3 mg m^{-3}). More than 93% of our measurements exceeded the recently proposed Dutch exposure-limit for endotoxin (90 EU m^{-3}). These findings suggest animal farmers to be exposed to high levels of dust and endotoxin consistent with an increased risk of developing respiratory symptoms and diseases. The development of preventive strategies to reduce exposure will require in-depth identification of factors that affect day-to-day variability in exposure.

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Introduction

Denmark is a major producer and exporter of agricultural products. With an annual pig production exceeding 25 million and a 30% share of the global mink production, Denmark is the world's largest pig-meat-exporting and mink-pelt-producing nation and has a substantial dairy and poultry production with an annual export value of two billion Euros.[1,2] The Danish primary farm sector consisted in 2008 of approximately 43 000 professional holdings with an average size of 63 hectares. Of

Environmental impact

The present study describes the levels and the variability in personal dust and endotoxin exposure in four different types of animal farmers: pig, dairy, poultry and mink. It shows that animal farmers remain exposed to high levels of dust and endotoxin and that the distribution and magnitude of variability in their exposure depend strongly on the type of production and the working environment. Given the limited number of comparative large-sized studies on different types of animal farmers and the absence of insight into the variability structure in their personal exposure to dust and endotoxin, the present study should be of interest for many readers including epidemiologists and occupational hygienist as well as Danish policy makers.

those, approximately 35% were specialized in livestock production (mainly pig and cattle farming), 39% were solely crop producers, and 26% were primarily crop farms carrying out sideline livestock production activities.[1]

Agricultural workers have an increased risk for acute and chronic respiratory disorders; their respiration is routinely challenged by intense exposure to several chemical and biological substances such as pesticides and odorous gases, and organic and inorganic dusts.[3–6] Exposure to organic dusts (also referred to as bio-aerosols) including inflammatory and allergenic microbial agents (moulds, bacteria, virus and allergens) and pathogen associated molecular patterns (*e.g.* endotoxin, glucans and peptidoglycans) is suggested to have a distinctive role in the development of allergic and non-allergic respiratory diseases and lung function impairment.[7] Among the organic dust constituents, one of the most intensively studied and potentially influential agents related to respiratory health is endotoxin.[6] In particular, exposure to endotoxin, lipopolysaccharide-containing fragments of the cell-wall of Gram-negative bacteria,[7] induces non-atopic asthma, bronchial hyper-responsiveness, and lung function decline, but at the same time it appears to decrease the risk of atopic disease.[8–10] Most agricultural environments are highly contaminated with endotoxins,[11] but, most frequently, peak levels in personal exposure are reported among workers in livestock confinement buildings, particularly pig and poultry farmers.[3,11–13] However, exposure intensities within farming environments are known to vary considerably temporally, spatially as well as personally, depending on the type of production, performed task, and different environmental and farm characteristics that are present.[14] In addition, sampling in exposure assessment studies of farming populations is hampered by the small size of the operations and the large distances between farm entities.[4]

Numerous studies have assessed dust and endotoxin concentrations present in primary animal production environments. However, simultaneous investigations of multiple types of production with comparable measurement strategies and devices are sparse.[11,13,15–18] Most of these studies determined exposure using area measurements,[15–17] while the rest either included only a limited number of farming types[13,18] or few personal measurements among animal farmers.[11] In addition, the magnitude of the variability in dust and endotoxin exposure concentrations in animal farmers has also rarely been reported, and always without the ability to compare between different types of animal farmers.[19,20] Furthermore, despite the size of the Danish primary agriculture, reports of personal exposure levels of Danish farmers are limited,[13,21] and to our knowledge, no study has described exposure levels among mink farmers.

Therefore, the present paper aims: (a) to provide comparable information on personal dust and endotoxin exposure levels of farmers in different types of primary animal production, (b) to elucidate the nature and magnitude of exposure variability within and between livestock farmers, and (c) to gain insight into the temporal variability in personal exposure concentrations throughout different stages of poultry and mink production.

The study is part of the exposure assessment for the fifteenth year follow-up of the SUS project, a Danish prospective cohort study that aims to investigate the effect of farming exposures on respiratory diseases and allergy in a population of 1964 young Danish farmers.[22]

Materials and methods

Selection of farms

Details on the design and methodology of the SUS study can be found elsewhere.[22,23] A screening exposure questionnaire addressing current and past employment in farming, type of farm and basic farm characteristics (location area, size, number and type of animals) was used to identify the remaining active farming population of the initial SUS cohort. In total, 1156 participants (participation rate 59%) completed the questionnaire. In addition, information on current and previous employment, and farm characteristics for another 83 participants was available from an exposure scheme, comparable to the screening questionnaire, used in the clinical investigation part of the study. Overall, 423 (34%) participants reported still to be full-time employed in farming, most of them (77%) in farms located in the area of Jutland. Of those, 78% were pig and cattle farmers, while the remaining were mink (3.8%), crop (12.3%), poultry (0.5%), and combined animal production (5.4%) farmers (see ESI, Table A1† for details). For efficiency reasons and due to no systematic differences when compared to the distribution of different farms in Denmark,[24] we decided to restrict our investigation to Jutland.

The size of the pig farms was estimated from animal units (AU; the needed number of type-specific animals to produce an equivalent of 100 kg of nitrogen containing manure),[25] and the population was divided into three groups using the first and the last quartiles of the size distribution as cut-off levels. Twenty five pig farmers were randomly selected from each size group (75 in total). In addition, 33 dairy cattle and 3 mink farmers were randomly selected from the corresponding groups of farmers in the study population. The selected farmers were approached by phone and if they were still full-time employed in Jutland, in a primarily pig, mink or dairy farm, they were asked for an interview date. When the farm owner and the SUS participant were not the same individual, then the farm owner was also asked to give consent. Of the selected 111 farmers, 12 (11 pig and 1 cattle) were reluctant to participate in the study, and 16 (11 pig and 5 cattle) were excluded due to poor health ($n = 2$), inability to establish contact ($n = 3$), part-time employment ($n = 6$), or due to migration or change of occupation ($n = 5$). The resulting population consisted of 54 pig farms, 26 dairy cattle farms and 3 mink farms. In addition, contacts with 2 layer (one with enriched cages and one with a single tier system) and 1 broiler poultry farms were obtained from the Danish Agricultural Advisory Service. A graphical representation of the selection process can be seen in the ESI (Fig. A1†).

Farm visits

During the interview general information on the company (*e.g.* number of employees and units, type of production, locations, number and type of animals, *etc.*) was obtained. Two (summer and winter) measurement visits were scheduled for all selected pig and dairy cattle farms. All measurements were performed on randomly chosen working days during 2008–2009. Summer visits were carried out between 1st of May and 1st of October and winter visits between 17th of November and 3rd of April. Almost all farms combined animals with crop production and four (2 pig

Table 1 General production characteristics of participating pig, dairy cattle, poultry and mink farms. Characteristics are presented as median (range) values by type of farming and system of production

	Pig farms						Poultry farms			
Characteristic	Breeding improvement or multiplying ($n = 4$)	Farrowing/sow units ($n = 15$)	Integrated (farrow-to-finish) ($n = 22$)	Finishing ($n = 12$)[b]	Free-range ($n = 1$)	Cattle farms ($n = 26$)[c]	Broilers ($n = 1$)	Layers, cages ($n = 1$)	Layers, floor ($n = 1$)	Mink farms ($n = 3$)
Full-time employees[a]	5.5 (4–11)	3 (1–13)	4.5 (1–9)	3 (1–4)	4	3 (1–9)	6	5	4	1 (1–2)
Production units[a]	1.5 (1–2)	1 (1–3)	2 (1–4)	2 (1–3)	2	1 (1–3)	3	1	1	1
Animal compartments[ad]	7.5 (5–8)	8 (5–14)	10 (3–28)	5 (2–9)	5	4 (3–9)	12	1	1	14 (12–14)
Animal stock[a]										
Boars	4 (2–5)	6 (3–16)	5 (2–16)		3					
Sows	477 (250–1150)	670 (310–2200)	465 (148–1200)		680					
Young sows	260 (100–2000)	120 (40–450)	75 (0–300)		200					
Weaners	1300 (900–4000)	2100 (900–10 300)	1825 (450–6000)	800 (0–2500)	1500					
Slaughter pigs	1278 (500–2400)	50 (0–480)	1950 (600–7000)	2030 (600–5700)	1000					
Dairy cows						155 (42–470)				
Heifers						75 (0–400)				
Calves						63 (0–240)				
Bulls						0 (0–1)				
Layering hens								45 000	16 400	
Broilers							425 000			
Mink breeding stock										1900 (1800–3100)
Mink pelts										10 700 (10 000–17 000)
Land use, hectares[e]										
Overall size	409 (175–1200)	168 (0–550)	264 (96–850)	240 (90–800)	280	178 (42–530)	370	865	117	93 (2–120)
Meadow	27 (23–40)	10 (0–70)	2.5 (0–100)	11 (0–45)		10 (0–60)				0 (0–15)
Cereal cultivation	354 (142–800)	115 (552–400)	218 (92–620)	156 (72–580)	170	46 (0–250)	370	800	100	86 (0–93)
Roughage cultivation		0 (0–20)	0 (0–20)	0 (0–18)		95 (30–500)		35		
Beet cultivation						0 (0–18)				
Seed cultivation	29 (0–40)	16 (0–80)	0 (0–60)	8 (0–210)		0 (0–50)				0 (0–15)
Forestry	35 (0–91)	0 (0–10)	0 (0–50)	0 (0–30)		0 (0–160)		30	7	0 (0–4)
Potato cultivation			0 (0–50)	0 (0–32)	70					
Rapeseed cultivation		0 (0–30)	0 (0–100)	0 (0–37)		0 (0–11)				
Free land for sows					30					

[a] Number of observations. [b] Includes 2 farms with sideline beef production activities. [c] Includes 2 farms with sideline slaughter pig production activities. [d] A compartment within a farm unit is considered as distinct when housing animals from different production stages and including different stable characteristics (*e.g.* ventilation, feeding method, flooring). For poultry and mink farms, compartments refer to distinct animal buildings and mink sheds, respectively. [e] Only main production activities are summarized. Remaining land is commonly uncultivated, covered by installations and roads or used for cultivation of other non-common crops.

and 2 cattle) combined pig with cattle farming (Table 1). All workers on the selected farms were included in the personal measurements, and more than 90% participated. Sampling was performed during the whole morning working-shift of the farmers including both field and stable work. Daily tasks were documented by all farmers in detailed activity diaries covering one week per season, starting from the measurement day.

A full-shift measurement approach was also applied for workers in the 2 layer farms. In contrast, measurements in the broiler and mink farms were task based. Mink farms were visited during the breeding, whelping, furring, and pelting production stages and the broiler farm during the preparation of the stables and when the chicks aged 1–2 days (1st week), 21–22 days (3rd week), and 1–7 days before being harvested (5th week).

The monitoring time for included pig, mink and poultry layer farmers represents the whole working period within the day, whereas for cattle farmers the morning working shift, depending on the production management practices followed, represents either the whole working period or the working-shift with the longest duration in a day. For broiler poultry farmers monitoring was performed only during stable work.

Sampling and analytical methods

Dust sampling was carried out using a conductive plastic inhalable conical sampler (CIS; JS Holdings, Stevenage, UK)[26] mounted with a 37 mm glass-fibre (GFA) filter (Whatman International Ltd, Maidstone, UK). The samplers were strapped on duplicate (one at each side) at the upper part of the chest of the farmers, and a silicone rubber tube connected each sampler to a pre-calibrated at an operational flow of 3.5 l min^{-1} AirChek XR5000 portable pump (SKC Inc., Eighty Four, PA, USA). Field blanks were included at a rate of at least one per farm unit visited. The collected dust was estimated gravimetrically. An equilibration period of a minimum of 24 hours (22 °C, 45% relative humidity) preceded filter weighing, which was performed using a Mettler UMT2 analytical scale (Mettler-Toledo Ltd, Greifensee, Switzerland) with a 0.1 µg precision. The lower limit of detection (LOD) was 0.074 mg per filter. Results were expressed as mg m^{-3}.

Sample extraction and endotoxin analysis were performed as described by Spaan *et al.*[27] in one of the duplicate dust samples that was randomly chosen. Briefly, the extraction of the samples was performed in 5 ml of pyrogen-free water (PFW) with 0.05% (v/v) Tween-20. The samples were initially shaken for 60 minutes on a Multi Reax digital shaker (Heidolph Instruments GmbH, Schwabach, Germany) and then centrifuged for 15 min at 1000*g*. Subsequently, 1 ml of the supernatant was removed, aliquoted in four 0.1 ml portions, and stored at −20 °C. The extracts were analysed for endotoxin in PFW (1 : 200 dilution) using a quantitative kinetic chromogenic Limulus Amebocyte Lysate (LAL) test (Kinetic-QCL 50-650U kit, Lonza, Walkersville, Maryland, USA). Analysis was performed in duplicate, and the endotoxin concentration was estimated by an *Escherichia coli* (O55:B5) derived standard curve with 12 potency points (0.01 to 25 EU ml^{-1}). The assays' LOD was 13.69 EU per filter and results were expressed as EU m^{-3}. Dust results only from samples analysed for endotoxin were used for the present analysis.

All measured inhalable dust and endotoxin concentrations below the limits of detection were assigned a 2/3 value of the corresponding LOD.

Statistical analysis

All statistical analyses were performed using log-transformed values because exposure distributions appeared to be lognormal. As a result, measures of spread and location of exposure are presented as geometric means (GMs) with a geometric standard deviation (GSD). The corresponding arithmetic mean (AM) is also given. Analysis of the variance (ANOVA) and paired Student's *t*-tests were used to compare groups and seasons, respectively. Relationships between dust and endotoxin concentrations were investigated using Pearson correlation coefficients.

Mixed effect linear models (PROC MIXED) were used to estimate variance components of dust and endotoxin exposure for pig and dairy cattle farmers.[28,29] A multilevel approach was applied as a two-step procedure. At first the models were fitted with only the worker id as a random effect, while in the second step also farm was introduced to allow assessment of exposure variability at three levels: between-farms (${}_{bf}\sigma^2$), between-workers (${}_{bw}\sigma^2$), and within-workers (${}_{ww}\sigma^2$). The models were further stratified by the farmers usual working environment (indoors, outdoors, mixed in- and outdoor) using the information from the activity diaries. With only two repeated measurements available, a compound symmetric covariance structure was assumed, and estimations were based on the restricted maximum likelihood (REML) approach. The fold-range variations in dust and endotoxin exposure between farms, between workers, and within workers were estimated as the ratio between the 97.5 and 2.5 percentiles of the distribution of the log-transformed corresponding variance component.[30]

All data were analysed in SAS version 9.2 (SAS Institute Inc, Cary, NC, USA) using two-sided hypothesis testing at a 5% level.

Results

The general production characteristics of the selected farms are summarized in Table 1. More than 55% of the visited pig farms included production in multiple units from several different departments built between 1850 and 2007. Consequently, stable characteristics (*e.g.* heating, flooring, ventilation and feeding system) changed across departments based on the building recommendations for maximum productivity existing during each specific period. Of the selected 26 dairy farms, 5 milked their cows with robots, 2 used a pipe milking system and 19 had a separate milking area with a conventional or rotary milking parlour installed. Dairy cows were commonly housed in loose housing systems with the exception of the 2 farms applying pipe milking where a tie-up system was applied. Another 6 farms included at least one stable housing heifer or dry cows in tie-ups whereas huts were used to house calves in 8 of the selected farms.

Only one of the initially selected 80 pig and dairy farms was not visited twice due to the owners loss of interest in the study. Overall, 327 workers employed in 86 farm corporations (in the further treated as 89 due to the presence of the mixed production farmers) were monitored resulting in the collection of 507 personal inhalable dust samples within the 170 measurement

Table 2 Overall and type-specific sampling characteristics of personal measurements on Danish pig, cattle, poultry, and mink farmers. Measurements were collected between March 2008 and May 2010[a]

Farming type	*n*	*f*	*k*	*n*/*k*	Sampling duration, hour		<LOD for dust, *N*	<LOD for endotoxin, N
					AM (SD)	Range		
Dairy cattle	124	26	77	1–2	4.8 (1.8)	0.9–12	2	1
Pigs	354	53	231	1–2	6.1 (1.4)	1.1–9.2	1	1
Mixed, cattle and pigs	8	4	4	2	5.4 (1.3)	3.4–6.9	0	1
Poultry, broilers	11	1	5	1–5	2.5 (0.7)	1.6–3.7	0	0
Poultry, layers	3	2	3	1	6.2 (1.9)	4.2–7.9	0	0
Minks	7	3	7	1	6.1 (0.5)	5.6–6.8	0	0
Overall	507	89	327	1–5	5.7 (1.7)	0.9–12	3	3

[a] *n*, total number of personal measurements taken; *f*, number of involved farms; *k*, number of farmers sampled; *n*/*k*, number of measurements per farmer; AM, arithmetic mean; SD, standard deviation.

visits performed. Details with respect to the number of farms, workers and measurement characteristics along with the number of repeated measurements per worker are given in Table 2. The measurement duration varied considerably between farmers. The longest measurements were performed in farmers involved in field work and the shortest among cattle farmers nursing calves or heifers. Only 3 samples were below the LODs for dust and endotoxin respectively, mainly in relation to short-duration sampling in office or outdoor performed tasks.

A summary of the measured inhalable dust and endotoxin levels per type of farming is shown in Table 3 and Fig. 1. The results of the seasonal personal measurements in pig and cattle farmers are also shown. The GM exposure for all monitored farmers was 2.5 mg m^{-3} (GSD 3.0) for personal inhalable dust and 988 EU m^{-3} (GSD 4.8) for endotoxin. Average inhalable dust and endotoxin concentrations differed significantly between farm categories ($p < 0.0001$). The highest average dust and endotoxin exposure concentrations were seen among poultry and pig farmers, with the latter group having the highest observed individual concentrations. Pig farmers were on average 3-fold higher exposed than cattle farmers, who had the lowest GM inhalable dust exposure. The average endotoxin concentrations were lowest for mink farmers.

The observed exposure concentrations for both pig and cattle farmers were higher in winter than in summer, statistically significant only among pig farmers ($p < 0.0001$). Pearson correlations between seasons were modest for both dust ($r = 0.48$, $p < 0.0001$) and endotoxin exposure ($r = 0.33$, $p < 0.0001$) with a relatively similar pattern for pig and cattle farmers (see ESI, Fig. A2† for details). The overall Pearson correlation coefficient between dust and endotoxin was 0.69, whereas the farm type-specific correlations ranged from moderate to strong (Table 3).

The dust and endotoxin exposure concentrations in the different stages of the mink and poultry broiler production are

Table 3 Personal inhalable dust and endotoxin exposure levels in different types of Danish animal farmers. Results are presented overall, per type and (if applicable) per season[a]

Farming type and season	*n*	Inhalable dust/mg m^{-3}			Endotoxin/EU m^{-3}			*r*
		AM	GM (GSD)	Min–Max	AM	GM (GSD)	Min–Max	
Pigs								
Overall	354	4.9	3.4 (2.6)	<LOD to 47.8	6240	1490 (4.4)	<LOD to 374 000	0.62***
Summer	181	4.3	2.8 (2.6)†	0.1–47.8	5950	1090 (4.2)†	14.4–374 000	0.66***
Winter	173	5.5	4.1 (2.5)	<LOD to 20.0	6550	2080 (4.2)	<LOD to 285 000	0.54***
Cattle								
Overall	124	1.6	1.0 (2.7)	<LOD to 9.8	759	358 (3.6)	<LOD to 5890	0.63***
Summer	62	1.5	0.9 (2.5)	0.2–9.8	512	286 (3.2)	18–3400	0.64***
Winter	62	1.8	1.1 (2.9)	<LOD to 9.4	1010	448 (4.0)	<LOD to 5890	0.61***
Mixed, cattle and pigs								
Overall	8	2.9	1.9 (2.8)	0.4–8.9	900	448 (6.0)	<LOD to 2910	0.72*
Summer	4	2.9	2.2 (2.5)	0.7–6.0	1230	868 (2.7)	251–2910	0.46
Winter	4	3.0	1.6 (3.6)	0.4–8.9	569	231 (9.9)	<LOD to 1090	0.82
Poultry								
Overall	14	5.7	3.5 (2.9)	0.7–18.3	1960	805 (4.9)	61–7090	0.83**
Layers	3	5.9	5.5 (1.6)	3.1–8.3	3330	2430 (2.6)	1162–7090	0.86
Broilers	11	5.7	3.1 (3.3)	0.7–18.3	1580	596 (5.1)	61–6420	0.82**
Minks	7	1.4	1.3 (1.6)	0.5–2.3	301	214 (2.2)	93–1050	0.61
Overall	507	4.0	2.5 (3.0)	<LOD to 47.8	4620	988 (4.8)	<LOD to 374 000	0.69***

[a] *n*, number of measurements; AM, arithmetic mean; GM, geometrical mean; GSD, geometrical standard deviation; *r*, Pearson correlations between measured dust and endotoxin concentrations; † significantly different ($p < 0.0001$) than winter, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$.

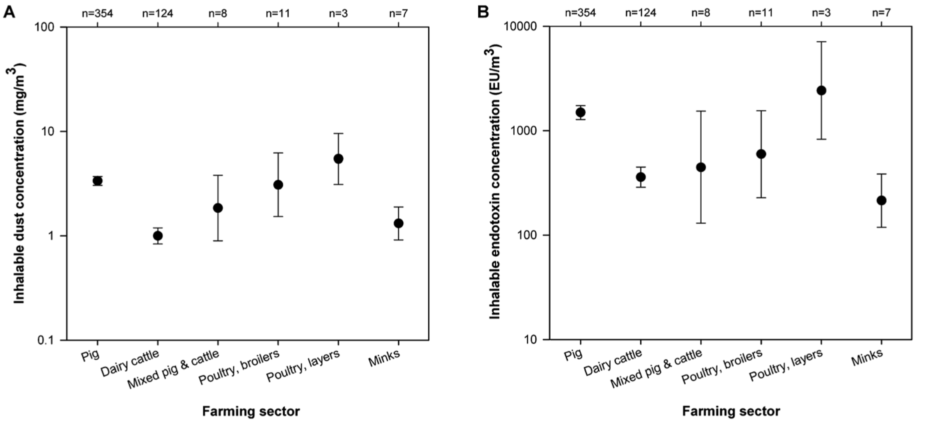

Fig. 1 Inhalable dust (A) and endotoxin (B) exposure levels (geometric mean ± 95% confidence intervals) obtained by personal sampling in different types of Danish animal farms.

given in Table 4. Overall, exposure levels through almost all the different stages of the mink production were moderate and relatively comparable to the levels measured among cattle farmers. The highest concentrations for dust were measured during the pelting stage and for endotoxin during whelping (Fig. 2). In contrast, exposure measurements in broiler farmers showed a wide range in exposure levels primarily in relation to the presence and growth of chicks and the conditions inside the stable (*i.e.* accumulation of manure and feed residues). In particular, the personal exposure concentrations for both dust and endotoxin exposure showed a greater than 10-fold increase between the 1st and the 5th week of the chicks age. In comparison to the layer production, lower dust and endotoxin exposure levels were found when the chicks were young, but this pattern reversed when the chicks reached their final growth stage (Fig. 2).

The results of the random effect models with and without the farm level are summarized in Table 5. Overall, considerable variability in exposure concentrations was seen both between- and within-workers. In all cases, the within-workers variability (day-to-day variability) was larger than the between-workers variability in exposure concentrations irrespective of the type of exposure. The between-worker variance was similar for dust and endotoxin, but day-to-day variability for endotoxin was considerably higher than for dust. Cattle farmers had higher between-workers variability than pig farmers, whereas pig farmers showed larger day-to-day variability especially for endotoxin concentrations in which daily concentrations varied within a 250-fold range. Introduction of the farm level (Model 2) into the models had limited effect on the estimated variance components for dust and endotoxin among pig farmers. For cattle farmers, however, farm explained 28% and 52% of the between-worker variance for endotoxin and dust exposure concentrations, respectively. When grouped by farm, pig and cattle farmers appeared to have similar between- and within-variance for inhalable dust concentrations, implying that variance components can be pooled across groups of farmers for inhalable dust.

When farmers were grouped by animal type and working environment, the day-to-day variability in dust and endotoxin concentrations increased substantially from an enclosed to an open (outdoor) working environment among both pig and cattle farmers (Table 6). For pig farmers, the day-to-day variability clearly dominated variability in all working environments, whereas for cattle farmers' within- and between-workers variability was mostly similar. Division of the total variability into 3 components (between-farm, between-worker and within-workers) was possible only among workers working indoors due to the small

Table 4 Personal dust and endotoxin exposure levels measured in different stages of the Danish mink and poultry production[a]

		Inhalable dust/mg m^{-3}			Endotoxin/EU m^{-3}		
Farming type and stage of production	*n*	AM	GM (GSD)	Min–Max	AM	GM (GSD)	Min–Max
Minks							
Overall	7	1.4	1.3 (1.6)	0.5–2.3	301	214 (2.2)	93–1050
Breeding	1	0.5	—	—	121	—	—
Whelping	1	2.0	—	—	1050	—	—
Furring/grading	2	1.2	1.2 (1.0)	1.1–1.2	121	118 (1.4)	93–149
Pelting	3	1.8	1.7 (1.3)	1.5–2.3	231	227 (1.2)	178–264
Poultry broilers							
Overall	11	5.6	3.1 (3.3)	0.7–18.3	1580	596 (5.1)	61–6420
Stable preparation	4	1.6	1.4 (1.9)	0.7–3.0	115	107 (1.6)	61–179
1st week	2	1.0	1.0 (1.1)	0.9–1.1	389	379 (1.4)	302–476
3rd week	2	5.0	4.8 (1.6)	3.4–6.6	1870	1820 (1.4)	1421–2330
5th week	3	14.5	14.3 (1.3)	11.5–18.4	4130	3790 (1.7)	2314–6420

[a] *n*, number of measurements; AM, arithmetic mean; GM, geometrical mean; GSD, geometrical standard deviation.

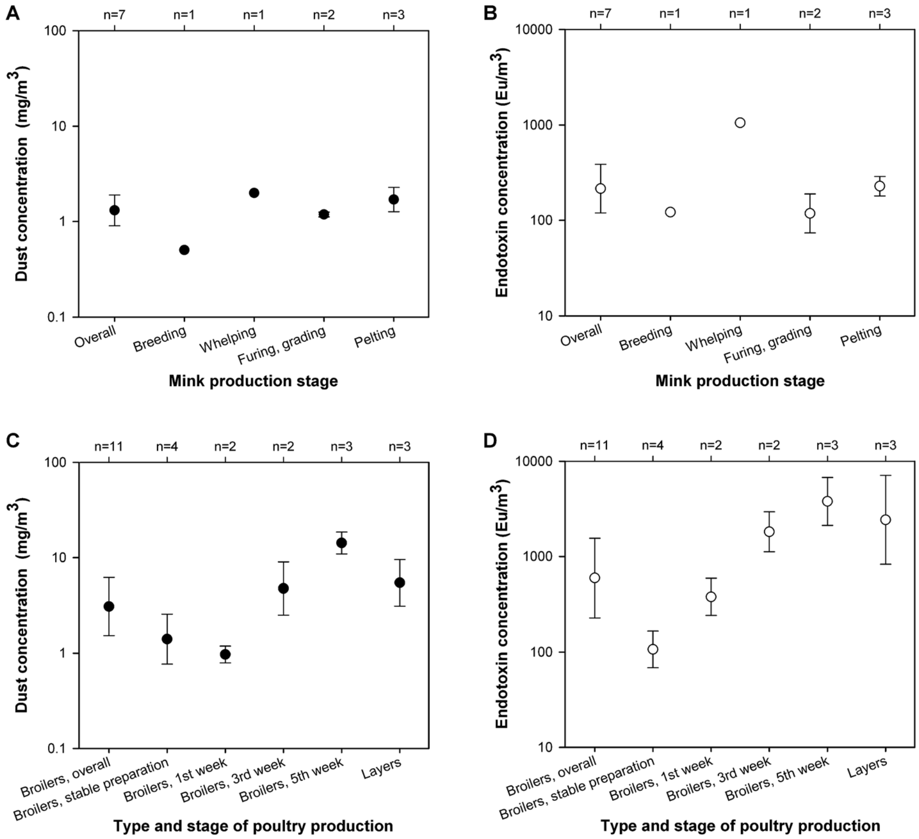

Fig. 2 Inhalable dust (●) and endotoxin (○) concentrations (geometric mean ± 95% confidence intervals), measured in different stages of mink (A and B) and poultry (C and D) production farms.

number of repeated measurements in other working environments (Table 6). The between-farm component did not considerably affect the within- and between-worker variability structure for dust and endotoxin exposure among cattle indoor workers. Among pig indoor workers the between-farms variation for both dust and endotoxin exposure was also small, indicating the presence of minimal differences in average exposure concentrations between individual pig farmers employed in different farms.

Discussion

The present study describes the inhalable dust and endotoxin exposure levels in different types of livestock farmers in Denmark. This is one of the largest exposure assessment studies using personal measurements in primary animal farming and one of the very few that report the magnitude of the variability in dust and endotoxin exposure concentrations among different types of animal farmers. To our knowledge this is also the first study to report personal dust and endotoxin concentrations among mink farmers.

We have included a large number of pig and cattle farms in our study; although, the representativeness of the Danish farms in our sample might have been altered by our choice to randomly select pig farms based on their size distribution. However, participating pig farms did not differ significantly in size from farms in the initial sample population, and they even had an

Table 5 Variance components for dust and endotoxin exposure in Danish pig and dairy cattle farmers

		Inhalable dust						Endotoxin					
	n	${}_{\mathrm{bf}}\sigma^2$	${}_{\mathrm{bw}}\sigma^2$	${}_{\mathrm{ww}}\sigma^2$	${}_{\mathrm{bw}}R_{0.95}$	${}_{\mathrm{ww}}R_{0.95}$	λ	${}_{\mathrm{bf}}\sigma^2$	${}_{\mathrm{bw}}\sigma^2$	${}_{\mathrm{ww}}\sigma^2$	${}_{\mathrm{bw}}R_{0.95}$	${}_{\mathrm{ww}}R_{0.95}$	λ
Model 1[a]													
Pigs	354	—	0.25	0.64	6.95	23.00	2.6	—	0.19	1.99	5.49	250.53	10.5
Cattle	124	—	0.44	0.57	13.57	19.21	1.3	—	0.47	1.18	14.82	70.14	2.5
Model 2[b]													
Pigs	354	0.04	0.19	0.65	5.55	23.51	3.4	0.00	0.19	1.99	5.44	250.46	10.6
Cattle	124	0.20	0.21	0.60	5.94	20.73	2.9	0.12	0.34	1.19	9.78	72.06	3.5

[a] Model with worker as random effect. [b] Model with farm and worker (within farm) as random effects; n, total number of personal measurements taken; ${}_{\mathrm{bf}}\sigma^2$, between-farm variance; ${}_{\mathrm{bw}}\sigma^2$, between-worker (within-farm) variance; ${}_{\mathrm{ww}}\sigma^2$, within-worker (day-to-day) variance; ${}_{\mathrm{bw}}R_{0.95}$, ratio of the 2.5$^{\mathrm{th}}$ and 97.5$^{\mathrm{th}}$ percentile of the between-worker variance of the log-normally distributed exposure; ${}_{\mathrm{ww}}R_{0.95}$, ratio of the 2.5$^{\mathrm{th}}$ and 97.5$^{\mathrm{th}}$ percentile of the within-worker variance of the log-normally distributed exposure; λ, ratio of within- and between-worker variance.

Table 6 Variance components by usual working environment for dust and endotoxin exposure in Danish pig and dairy cattle farmers

				Inhalable dust						Endotoxin					
	n	f	k	$_{bf}\sigma^2$	$_{bw}\sigma^2$	$_{ww}\sigma^2$	$_{bf}R_{0.95}$	$_{bw}R_{0.95}$	$_{ww}R_{0.95}$	$_{bf}\sigma^2$	$_{bw}\sigma^2$	$_{ww}\sigma^2$	$_{bf}R_{0.95}$	$_{bw}R_{0.95}$	$_{ww}R_{0.95}$
Pigs															
Indoor[a]	266	45	177	0.04	0.12	0.47	2.24	3.87	14.75	0.09	0.08	1.55	3.17	3.06	130.73
Mixed, in- and outdoor[b]	62	26	35	—	0.21	0.75	—	5.92	29.73	—	0.00	1.90	—	0.00	221.23
Outdoor[b]	26	15	19	—	0.00	1.96	—	0.00	241.60	—	0.00	4.45	—	0.00	3911.45
Cattle															
Indoor[a]	71	20	47	0.07	0.59	0.48	2.79	20.08	14.99	0.12	0.52	1.04	3.93	16.97	54.61
Mixed, in- and outdoor[b]	43	20	24	—	0.10	0.67	—	3.38	24.72	—	0.58	0.61	—	19.68	21.17
Outdoor[b]	10	5	6	—	0.43	0.83	—	12.99	35.60	—	0.00	2.80	—	0.00	702.23

[a] Model with farm and worker (within farm) as random effects. [b] Model with worker as a random effect; n, number of personal measurements; f, number of farms visited; k, number of farmers sampled; $_{bf}\sigma^2$, between-farm variance; $_{bw}\sigma^2$, between-worker (within-farm) variance; $_{ww}\sigma^2$, within-worker (day-to-day) variance; $R_{0.95}$, ratio of the 2.5th and 97.5th percentile of the corresponding (between-farm, between-worker, within-worker) variance of the log-normally distributed exposure.

average size that was comparable to the one reported (232 *vs.* 239 AU) for all pig farms in Denmark.[1] Participating pig farms covered production systems from both the breeding improvement and production branches of the Danish pig industry, including breeding, multiplying, sow, integrated (farrow-to-finish), finishing and multi-site herds. Farm characteristics like animal housing, ventilation, feeding equipment, flooring type, manure storage, litter usage as well as farming practices varied considerably both between and within farms, primarily depending on the applied production system and the year of construction of the individual farm compartments. In addition, analysis of the variance by farm size showed no statistically significant differences in the personal dust and endotoxin exposure levels between small, medium, and large sized farms (not shown). Therefore, it is unlikely that the applied selection process has biased the representativeness of our pig farm sample.

Overall, Danish animal farmers in our sample were exposed to substantial dust and endotoxin concentrations, irrespective of the applied type of production; though, as expected, the highest personal dust and endotoxin exposure concentrations were measured among pig farmers (47.8 mg m^{-3} and 374 000 EU m^{-3}, respectively) and poultry farmers (18.3 mg m^{-3} and 7090 EU m^{-3}, respectively). A notion for the health impact of these levels can be obtained by looking at the number of exceedances in relation to currently available occupational exposure limits (OELs). In general, although not directly comparable, 47% of our measurements exceeded the 3 mg m^{-3} Danish OEL for total organic dust.[31] A number of methods for extrapolation of the "total dust" to inhalable dust have been suggested.[32–34] Liden and colleagues,[35] in a comparative study of the Swedish open-face sampler with the IOM sampler, which included measurements of organic dust, proposed a conversion factor of 2 for the recalculation of the OELs from total to inhalable dust. Madsen *et al.*[36] in an exposure assessment study among Danish greenhouse workers reported a mean ratio of 1.6 between the personal dust levels measured with the GSP inhalable sampler and the closed-face Millipore cassette (the standard aerosol sampler used for total dust measurements in Denmark). Using this conservative conversion factor of 1.6 the Danish OEL for total dust can be recalculated to an exposure level of approximately 4.8 mg m^{-3} of inhalable dust. Twenty-eight % of our measurements were above this level. For endotoxin, the Health Council of The Netherlands recently recommended a health-based exposure limit of 90 EU m^{-3}.[37] This newly proposed limit was exceeded by more than 93% of our measurements. Several recent studies have indicated exposure related respiratory symptoms and bronchial hyperresponsiveness starting at levels between 100 and 200 EU m^{-1}.[9,38,39] Thus, it is evident that Danish farmers are exposed to dust and endotoxin concentrations consistent with an increased risk of developing respiratory symptoms and diseases.

When compared with earlier studies, our personal exposure dust levels for pig, cattle and poultry farmers (GMs of 3.4, 1.0, and 3.5 mg m^{-3}; respectively) are similar to those previously reported among Dutch farmers (GMs of 2.6, 1.4 and 4.6 mg m^{-3} for pig, cattle and poultry farmers, respectively),[11,20] but slightly lower compared to the levels found within the "European farmer's" study (median of 4.0, 5.0 and 7.0 mg m^{-3} for Danish pig, German pig and Swiss poultry farmers, respectively).[13] Both studies preformed their sampling using similar techniques with the ones used in the present study. The observed slightly higher dust levels for German pig and Swiss poultry farmers in the European farmer's study can, at least partly, be explained by differences in farm characteristics and practices between countries,[17] and most importantly by the cyclic measurement strategy that we followed for poultry farmers.

Our inhalable endotoxin exposure levels for pig, poultry and cattle farmers are comparable to those reported in other studies that used personal measurements.[11,20,40,41] However, the interpretation of such comparisons is complicated by the lack of standardization in sampling and analytical methods across studies.[27,42] Our endotoxin results can best be compared to the results from the Dutch study of Spaan *et al.*[11] as similar measurement and analytical protocols were used. In the Dutch study the endotoxin exposure concentrations for cattle and poultry farmers ranged from 62 to 3860 EU m^{-3} and from 360 to 8120 EU m^{-3} respectively, which are similar to the ranges we found (range <LOD to 5890 and 61 to 7090 EU m^{-3} for cattle and poultry farmers, respectively). The higher endotoxin levels among pig farmers in our study (range: <LOD to 374 000 EU m^{-3}) compared to the levels (range 992 to 6970 EU m^{-3}) of Spaan and

colleagues probably reflect the larger number of measurements and consequently the wider variety of working tasks that we included. Inter-laboratory variations[43,44] are of minor importance in the context of interpreting the percentage of measurements above exposure limits; even in the case of an overestimation of the exposure concentrations of up to 10-fold several measurements will still be above the proposed exposure limit for endotoxin.

In our study, the measured dust and endotoxin exposure levels among pig farmers were significantly higher during the winter than the summer season. These findings are in agreement with results of earlier studies that measured dust or endotoxin concentrations in different seasons using either personal[41,45,46] or stationary[15,17] measurements. The higher dust and endotoxin exposure concentrations found in the winter can largely be attributed to higher ventilation rates that are normally used inside pig stables during the summer season.[17,47,48] However, potential differences in time spent working outdoors and applied farming practices (*e.g.* increased use of cooling by spraying water during summer) between the two seasons may also play a role.

We examined dust and endotoxin exposure patterns at a personal level within different stages of mink and poultry broiler production. Our poultry broiler results are supported by those from a recent Canadian study[49] that included personal winter and summer measurements in 2 stages (0–2nd week and 4–6th week) of the broiler fattening period, and reported dust and endotoxin levels to significantly increase with flocks' age during the summer season. Similarly, Oppliger *et al.*[50] in a Swiss study that described levels of microbial exposure in 12 poultry broiler operations, found stationary measured dust and endotoxin exposure levels to increase by up to 4- and 10-fold, respectively, between the beginning (chicks aged 1 to 2 days) and end (1 day before harvest) of the chicks' growth cycle. The routine activity patterns followed by the workers during the broiler fattening stage suggest this trend to be primarily associated with increased animal activity and size, which are aggravated by deterioration of stables' hygienic conditions during the chicks' growth. However, other factors, such as air temperature, relative humidity and ventilation rate, may also have played a role.[51] Measurements on stable preparation were performed one day prior to arrival of the chicks. The moderately high dust concentrations observed during this stage probably reflect the performed litter (wood chips) disposal. We did not include measurements during the bird catching and stable cleaning stages as both are being performed by external contractors in Denmark. Personal dust and endotoxin exposure concentrations across mink production, in contrast with the poultry broiler production, were not characterised by any clear patterns. The variability in exposure concentrations between breeding, furring, and pelting stages was small despite differences in working tasks or in numbers of housed animals between the three stages. Differences in environmental settings seem also to be of minimal influence as pelting in contrast to animal tending is performed in a completely enclosed environment. However, as pointed out by the higher levels measured during the whelping stage, the small variability could be a result of the few measurements that we included.

A prime objective of the present study was to provide information on size and variability in personal dust and endotoxin exposure concentrations for pig and cattle farmers. In general, day-to-day variability in dust and endotoxin exposure concentrations exceeded between-worker variability in both farming groups, but the pattern was strongest among pig farmers. A recent analysis of a large database with more than 2000 measurements in endotoxin exposed workers also reported higher within- than between-worker variability among primary animal production workers (mainly pig farmers).[20] Moreover, in an earlier study that included repeated seasonal (summer/winter) measurements on 198 Dutch pig farmers,[19] the average endotoxin concentrations between and within farmers were estimated to lie within a 4- and 20-fold difference, respectively. Our results for pig farmers showed a similar range in the average endotoxin concentrations between farmers (${}_{\mathrm{bw}}R_{0.95} = 5.5$), but our fold-range in average daily concentrations for both all and only indoor workers was somewhat higher (${}_{\mathrm{ww}}R_{0.95} = 250$ and ${}_{\mathrm{ww}}R_{0.95} = 130$, respectively) suggesting the presence of even larger day-to-day variability than the one reported by Preller *et al.* In addition, the observed increasing day-to-day variability when moving from an indoor to an outdoor environment is in accordance with the findings from another large database on inhalatory chemical exposures.[52] The larger between-farm and between-worker variability observed among cattle farmers can probably be explained by more distinct differences in farm characteristics, larger degree of task specialization, and more continuous working tasks seen among this group compared to pig farmers. As an example, the milking system (robots, parlours or pipes) used in a farm determines the performance and time that a farmer will spend on milking activities; farmers using parlour or pipe milking systems will spend large portions or even their whole working-shifts just milking.

These findings have implications for both exposure assessment and epidemiological risk assessment studies. In general, prospective exposure assessment studies in farming populations should design their sampling strategies always in view of the size of the exposure variability depending on the type of production and working environment. The large between-farm and within-worker variability among cattle farmers stresses the need for inclusion of sufficient repeated measurements on a large number of farms in order to increase the precision of the personal exposure estimates. In contrast, precision of exposure estimates for pig farmers seems to greatly depend on the day-to-day variability, and hence to the number of repeated personal measurements included. Mathematical equations that allow sample-size and bias estimations based on the presence and magnitude of exposure variability within-workers have been available in the literature.[53] For example, given our sample and dust results (Table 5, Model 1) for pig farmers a minimal bias on exposure–response estimations of 50% should be expected in a hypothetical direct use of our individual measured exposure estimates. A bias reduction to a maximum value of 10% will require acquisition of at least 10-times more measurements per worker. However, collection of such amounts of samples per individual is not an option as they are much too expensive and time-consuming given the distances between farms and large number of farms involved. The consequences of this substantial variability for epidemiological studies in agricultural populations have been addressed in detail in a previous discussion paper by Kromhout and Heederik.[14] The direct or indirect use of individual exposure estimates without proper handling of the issue of variability will usually result in a misclassification error that will, in most cases, tend to attenuate or even totally obscure exposure–response associations. This problem can largely be handled by the use of predicted exposure estimates based on empirical modelling approaches.[19]

Conclusions

The present study shows that animal farmers in Denmark are exposed to high and variable dust and endotoxin exposure levels. Pig and poultry farmers are highest exposed, but levels above the currently available exposure limits are common also among cattle and mink farmers. The chicks' growth cycle is an important determinant for dust and endotoxin exposure of broiler farmers. Exposure levels among pig and cattle farmers are characterised by a predominant, large, and increasing from indoor-to-outdoor working environment day-to-day variability. In order to optimize exposure assessment for epidemiological studies of these farmer populations collection of information on tasks for several days alongside repeated measurements will be of crucial importance. Gaining in-depth knowledge of determinants affecting exposure will also be essential in order to develop effective control and prevention strategies to reduce dust and endotoxin exposure among animal farmers.

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