FACULTY OF HEALTH SCIENCES
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Dust and endotoxin exposure in animal farming populations

Formulating the basis for a model-based exposure assessment approach

PhD dissertation

Ioannis Basinas

Faculty of Health Sciences
Aarhus University
2011

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To my wife, Emmanouela, and my son, Christos. You have been my inspiration through all these years of hardship…….

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List of publications

The present PhD dissertation is based on data from the SUS 12 studies, which are analyzed and summarized in the three manuscripts included as appendixes at the end of the dissertation.

I. Basinas I, Sigsgaard T, Heederik D, Takai H, Omland Ø, Andersen NT, Wouters IM, Bønløkke J, Kromhout H, Schlünssen V. Exposure to inhalable dust and endotoxin among Danish pig, dairy, poultry and mink farmers; results from the SUS cohort study. Manuscript submitted for publication.

II. Basinas I, Schlünssen V, Heederik D, Sigsgaard T, Smit LAM, Samadi S, Omland Ø, Hjort C, Madsen Am, Skov S, Wouters IM. Sensitization to common allergens and respiratory symptoms in endotoxin exposed workers: a pooled analysis. Accepted for publication in the Journal of Occupational and Environmental Medicine.

III. Basinas I, Schlünssen V, Takai H, Heederik D, Omland Ø, Sigsgaard T, Kromhout H. Work tasks and stable characteristics associated with the levels of exposure to inhalable dust and endotoxin among Danish pig Farmers. Draft manuscript.

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Definitions

Organic Dust: A complex mixture of particulate matter and aerosolized biological material comprising from human and animal dander, plant and skin fragments, high molecular allergens, pollen, viruses, fungal spores and hyphae, bacteria (viable and non-viable) and their constituents.[1]

Endotoxins: Also referred to as Lipopolysaccharides (LPS); strong pro-inflammatory molecules[1] comprising of a long polysaccharide complex chain bound to a lipid A component (Figure 1).[2] Allocated in the external cell-wall membrane of gram-negative bacteria are released to the environment primarily through cell replication, death or lysis.[3] Endotoxins are considered as one of the main and biologically most active constituents of organic dusts.[4]

Exposure assessment: An independent study or an integrated part of an epidemiological study aiming to describe the basic characteristics (route, pathway, source) and the dimensions (concentration, frequency, duration) of human exposure to a specific chemical, biological or physical agent.[5]

Exposure variability: The amount of variation in exposure intensity of a particular substance through time or between persons.[5-7]

Exposure determinant: A factor either a workplace (e.g. ventilation, temperature) or a worker (e.g. task) characteristic that influences the level of exposure.[8]

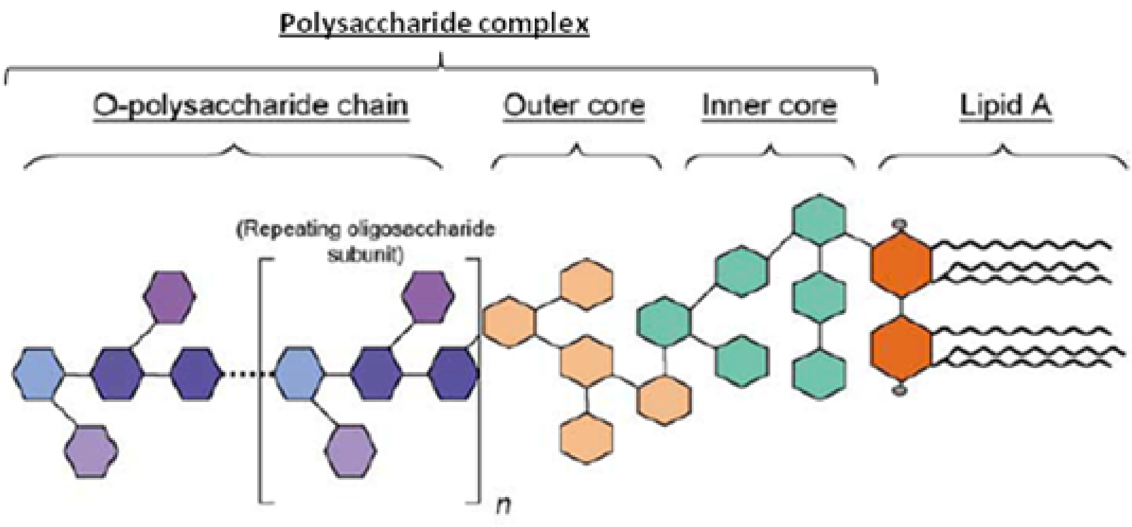

Figure 1. Structure of an LPS derived from Gram-negative bacteria. Slightly modified picture adapted from Erridge et al.[9]

Summary in English

Good exposure assessment practices are fundamental both for the risk identification and management as well as for the assessment of causality in epidemiological studies. The recent focus on gene-environment interactions in asthma causality has increased the demand for more reliable and precise exposure assessment methodologies due to the need to detect smaller effects. However, in farming populations both exposure and risk estimations are hampered by a large inter-and intra-individual exposure variation, and as a consequence traditional quantitative exposure assessment based solely on measurements is costly and a methodological challenge.

The present thesis deals with the foundation and the basic exposure assessment principles applied in the SUS cohort. The study was initiated with the aim to provide the cohort with valid exposure estimates through modelling approaches, and thereby to increase its potential to explore gene-environment interactions and identify exposures driving the harmful and beneficial effects of farming. The assessment for dust and endotoxin exposure in the cohort is described, and the results are further deployed in exposure-response analysis.

A screening questionnaire was used to identify the remaining active farming population of the initial SUS cohort comprising of 2000 farming apprentices. Based on the results 54 pig, 26 dairy and 3 mink farms were selected. Furthermore, contacts with 3 poultry farms were established through the Danish agricultural advisory service. 507 personal inhalable dust samples were collected from 327 farmers employed in the selected farms. Measurements in pig and dairy farmers were full-shift and performed during summer and winter, whereas poultry and mink farmers were monitored during 4 well-defined production stages. Tasks performed by the farmers were recorded in self-administrative dairies, and information on exposure determinants was collected through walk-through surveys. The sampled dust was analysed gravimetrically and its endotoxin content was determined by the Limulus amebocyte lysate assay.

The measurements showed an overall geometric mean exposure of 2.5 mg/m^3 (range <LOD-47.8) for dust and 992.3 EU/m^3 (range <LOD-374,579) for endotoxin. Pig and poultry farmers were highest exposed; though, levels above the currently available exposure limit for dust (3 mg/m^3) and the suggested threshold limit for endotoxin (90 EU/m^3) were common also among cattle and mink farmers. Simple random-effect analysis on pig and cattle farmers showed a substantial day-to-day variability in exposure that increased from indoors-to-outdoors work.

Determinants of personal exposure to dust and endotoxin for pig farmers were explored using linear mixed effect models. Again, indoor versus outdoor work was seen to play a dominant role on the exposure variability. Indoor working tasks related to intense animal activity or handling of feed material in storage areas increased exposure, which in contrast decreased during field work. High pressure washing was a factor increasing endotoxin exposure. Stable characteristics determining dust exposure were related to feeding practices and the ventilation

type. For endotoxin the most important determinants were the use of dry feed and the slatted floor coverage. Feeding practices could solely explain all the between-farms variability in dust and endotoxin exposure.

The measured levels of endotoxin exposure were used to estimate exposure at baseline for the SUS cohort population. A pooled cross-sectional analysis was performed using data from four studies of employees occupationally exposed to microbial exposures, including the SUS cohort. Exposure-response relationships between endotoxin exposure, allergic sensitization, asthma and other respiratory disorders were assessed using exposure estimates derived from simple study-specific job-exposure matrices based on more than 1200 quantitative exposure measurements. The analysis confirmed the currently available literature by showing a dual effect of endotoxin exposure and further suggested possible stronger protective effects on farming populations.

The study show that Danish livestock farmers remain exposed to high levels of dust and endotoxin exposure potentially hazardous for their respiratory health. Preventive initiatives are needed to create a safer working environment for the farmers. The results from the analysis for exposure determinants among pig farmers can be used for an initial prevention strategy established on the basis of personal protective equipment for specific work tasks. Furthermore, the results demonstrate the potential of the collected data to allow for a breakthrough on the identification of determinants of personal exposure in farming, and to provide the further analyses of the cohort with the needed valid exposure estimates. The later is confirmed by the results of the included epidemiological study.

However, more have to be done. The developed models for pig farmers will have to be expanded and a similar analysis performed for cattle farmers. The identification of determinants of personal exposure will hopefully reveal the driving sources of exposure variability within farmers, and will provide the research team with essential information for the development of the most efficient exposure assignment to be followed within the cohort. The described estimation process for dust and endotoxin serve as a model for the analytical approach that will be followed for the rest of the exposures of interest in the cohort like common allergens, glucans and *archae* bacteria.

Dansk Resumé

Valide eksponeringsvurderinger er afgørende for både risiko identifikation og risikohåndtering samt for kausalitetsvurderinger i epidemiologiske studier. Den aktuelle fokus på betydningen af gen-miljø interaktioner i relation til astma ætiologi har øget behovet for mere akkurate og præcise metoder til eksponeringsvurderinger for at kunne detektere mindre effekter. Blandt landmænd er både eksponerings og risiko estimeringen dog besværliggjort af en stor inter- og intra-individuel variation i eksponering, og som en konsekvens er traditionelle kvantitative eksponeringsvurderinger, der udelukkende er baseret på målinger, særdeles omkostningstunge og en metodologisk udfordring.

Denne afhandling omhandler de grundlæggende eksponeringsvurderingsprincipper, der anvendes i SUS-kohorten. Undersøgelsen blev igangsat med det formål at generere valide eksponeringsvurderinger på individniveau for SUS-kohortens deltagere via en modelleringstilgang, og derved øge mulighederne for at identificere gen-miljø interaktioner samt både skadelige og gavnlige virkninger af landbrugseksponeringer. I afhandlingen beskrives, hvordan estimeringen af støv og endotoksin niveauer i kohorten blev foretaget, og det beskrives også hvordan disse estimater er brugt i eksponerings-respons analyser.

Vi brugte et screenings spørgeskema til at identificere den del af den oprindelige SUS kohorte (2000 landbrugsskoleelever), der stadig var aktive landmænd. På baggrund af resultaterne blev 54 svinebrug, 26 gårde med malkekvæg og 3 minkfarme udvalgt. Desuden blev der etableret kontakt til 3 fjerkræbesætninger gennem Videncentret for Landbrug. Der blev indsamlet 507 personbårne målinger af inhalerbart støv fra 327 landmænd ansat på de udvalgte gårde. Målingerne af svine- og mælkeproducenter foregik over hele arbejdsdagen. Der blev foretaget gentagne målinger sommer og vinter. Fjerkræ- og minkavlere blev målt i 4 veldefinerede produktions stadier.

Landmændenes arbejdsopgaver blev registreret i selvadministrerede dagbøger, og oplysninger om faktorer af betydning for eksponeringen blev indsamlet ved en arbejdshygiejnisk gennemgang af gårdenes forskellige afdelinger. Støvprøverne blev analyseret gravimetrisk, og deres indhold af endotoksin blev bestemt med et Limulus amebocyte lysat assay.

Målingerne viste en gennemsnitlig geometrisk middelværdi på 2,5 mg/m^3(<LOD - 47,8) for støv og 992,3 EU/m^3 (interval <LOD – 374.579) for endotoksin. Svine- og fjerkræavlere var de højest eksponerede, men selv blandt kvæg- og minkavlere var niveauerne ofte over den gældende grænseværdi for støv (3 mg/m^3), og den anbefalede grænseværdi for endotoksin (90 EU/m^3). Simple random-effekt analyser for svine- og kvægavlere viste en betydelig dag-til-dag variation i eksponering, som øgedes fra indendørs til udendørs arbejde.

Determinanter for svineproducenternes eksponering for støv og endotoksin blev analyseret i lineære mixed effekt modeller. Indendørs vs. udendørs arbejde spillede igen en dominerende rolle for variabiliteten i eksponeringen. Indendørs arbejdsopgaver relateret til høj aktivitet blandt dyrene samt til håndtering af foderstoffer i lagerområder øgede eksponeringen, mens

markarbejde sænkede eksponeringen. Højtryksspuling øgede endotoxin eksponeringen betydeligt.

Staldkarakteristika af betydning for eksponeringsniveauerne for støv var fodringsmåde og type af ventilation, mens det for endotoxin var forekomsten af tør fodring og spaltegulv. Fodringspraksis kunne alene forklare hele variationen i støv og endotoxin eksponering mellem gårde.

De målte niveauer af endotoxin blev brugt til at estimere eksponeringen ved baseline for SUS-kohortens medlemmer. Der blev gennemført en pooled tværsnits analyse på data fra fire studier af personer erhvervsmæssigt eksponeret for organisk støv, herunder SUS-kohorten. Vi foretog eksponerings-respons analyser af endotoksin eksponering og IgE medieret sensibilisering, astma og andre luftvejslidelser med eksponeringsvurderinger, der stammede fra simple studie-specifikke job-eksponerings-matricer, baseret på mere end 1200 kvantitative eksponerings- målinger. Analyserne bekræftede den foreliggende litteratur, idet de viste en tvetydig effekt af endotoxin eksponering, og yderligere antydede de en stærkere beskyttende effekt for landbrugsbefolkninger.

Undersøgelsen viser, at danske husdyravlere fortsat udsættes for høje koncentrationer af støv og endotoxin, som potentielt kan forårsage luftvejssygdom. Forebyggende initiativer er nødvendige for at skabe et mere sikkert arbejdsmiljø for landmænd. Resultaterne fra analysen af determinanter for eksponering blandt svineproducenter kan bruges i en indledende forebyggelsesstrategi baseret på bl.a. anvendelsen af personlige værnemidler til specifikke arbejdsopgaver. Desuden demonstrerer resultaterne de indsamlede datas potentiale til at skabe et gennembrud i identifikationen af determinanter, der er af betydning for den enkeltes eksponering i landbruget. Analyserne tyder på, at data er velegnede til at udvikle valide individuelle eksponeringsestimater for SUS-kohortens deltagere, hvilket også demonstreres i det inkluderede epidemiologiske studie.

Men der skal gøres mere. Modellerne for svineproducenter skal videreudvikles, og tilsvarende analyser skal udføres for kvægavlere. Den videre identifikationen af determinanter for den enkelte persons eksponering kan forhåbentligt afdække de afgørende kilder til eksponeringsvariation for landmænd, og vil betyde at forskergruppen i fremtiden har de redskaber, der skal til, for at undersøge og udvælge de mest valide mål for eksponering for den enkelte. Den beskrevne proces for estimering af støv og endotoksin vil tjene som model for den analytiske tilgang, som vil blive brugt til at estimere andre relevante eksponeringer i kohorten som allergener, glucaner og Archae bakterier.

1 Introduction

Good exposure assessment practices are fundamental both for the risk identification and management as well as for the assessment of causality in epidemiological studies. The recent focus on gene-environment interactions in asthma causality has further increased the need for improvement of the currently available exposure assessment methodologies due to the necessity to detect smaller effects.[10]

When it comes to asthma causality, farming populations have emerged as highly important due to their unique exposure environment and the low prevalence of atopic asthma and sensitization when compared to the general population.[11] However, exposure assessment in farming populations is cumbersome and costly because farm entities tend to be small-sized and disperse over large distances.[12] Furthermore, the personal exposure levels of farmers tend to vary considerably both between- and within individuals. This variation, if not addressed, can result in large measurement errors,[13] which can hamper risk estimations and consequently the ability to assess gene-environment interactions.

The present PhD dissertation deals with the improvement of the applied methodologies for exposure assessment in farming populations. The dissertation represents the initial steps of a nested study within the 15th year follow-up of the SUS cohort (SUS12) aiming to develop a model-based exposure assessment method for dust and Microbial Associated Molecular Pattern (MAMP) exposures. In this framework, the thesis opens with a brief overview of the health effects of farming in a historical and current prospective, and the general aim and scopes of the SUS12 study are presented. An overview of reported levels of dust and endotoxin exposure within animal farming environments is then given, followed by a discussion on the implications of variability in exposure and risk assessment and an overview of determinants of exposure to dust and endotoxin among animal farmers. Next, the status and characteristics of the Danish primary animal production is presented. The aims of the present study are then introduced and a brief description of the applied methodologies follows. The main results of the three manuscripts (from now on referred to using their Roman numerical; i.e. I, II, III) composing the core of the present work are summarized in the following section, which is followed by a discussion both in respect to the findings and applied methodologies as well as to the future perspectives of this work. The thesis concludes with an Appendix that includes examples of the field surveys and the three manuscripts with their online supplements.

1.1 Respiratory effects of farming: historical overview and current findings

The hazardous nature of the farming environment has been acknowledged even before Ramazzini's milestone work *De Morbis Artificum Diatriba* [Diseases of Workers]. Olaus Magnus, a Swedish archbishop, is reported as the first to notate the health effects of farming among grain threshers in 1555.[12,14] There was sparse focus on the farmers' health until the late 1970's when Donham et al.[15] published results from a study among Iowa swine confinement workers that reported increased respiratory health symptoms in relation to high levels of measured gaseous exposures. Donham and colleagues results triggered the researchers' interest on the health effects of farming. Since then, numerous studies have assessed the health status of farmers either in relation to specified exposures or, most frequently, in simple comparisons with non-farming populations.

The farming environment is rich in terms of exposure agents.[16,17] Farmers are routinely exposed to chemical, mineral, microbial, plant, and animal originated agents through all 3 routes of exposure (i.e. inhalation, dermal absorption, and ingestion). Though, the inhalation route is considered to be far the most important concerning respiratory health. The respiratory effects of farming have been reviewed in details by Schencker et al.[12] and Omland.[18] Several updates of these reviews have been published, either focusing on specific exposures and settings or on specific symptoms.[19-25] In general, epidemiological studies show farmers to have higher respiratory morbidity in comparison to both other occupational groups and the general population. Particular disorders more prevalent among farmers include asthma and asthma like symptoms, chronic bronchitis, organic dust toxic syndrome (ODTS), allergic alveolitis, bronchial hyperresponsiveness, and acute or chronic decline in lung function. Most of the literature is based on cross-sectional studies, but longitudinal studies have confirmed an increased incidence of chronic bronchitis and accelerated decline in lung function in relation to long-term exposure to farming.

Specific agents associated with respiratory disorders among farming populations include ammonia (NH_3),[12,18,26] hydrogen sulfide (H_2S),[26] and organic dusts from microbial and animal origin like allergens, endotoxins, and glucans.[12,18,27-29] Of those, organic dust constituents are considered the most important, with endotoxins being the most investigated agents.[4,30,31] Endotoxins, complex lipopolysaccharide molecules with strong pro-inflammatory capabilities,[1] are associated to several acute and systemic respiratory disorders, including organic dust toxic syndrome (ODTS), bronchial hyper-responsiveness, airway inflammation, chest tightness, cough, shortness of breath, wheezing, and chronic bronchitis.[20,22,26-28,32] Beside organic dusts, other inorganic agents (e.g. silica and quartz)[12,26] or chemical substances (e.g. disinfectants)[33,34] have also been suggested to pose a challenge to the farmers' respiratory tract. Though, according to Heederik et al.[10] and Sigsgaard et al.,[13] studies with integrated exposure assessments have been very focused, and especially in relation to asthma few in numbers, despite the increased awareness and recognition of the possible influential presence of other causal agents (e.g. antibiotics) on top of the previously mentioned.

In the late 90's, results emerging from epidemiological studies in Central and Northern Europe suggested a lower risk for atopic sensitization and asthma in children with than without a farm childhood.[35-37] This protective effect was attributed to high exposure to microbial agents of primarily bacterial and fungal origin,[38] a speculation reaffirmed in studies using quantitatively measured exposures, mainly house-dust endotoxin.[39-42] Results from studies among adolescent[43] and adult populations[44-46] suggested a persistent protective effect of farm childhood into adulthood. In 2002, Portengen et al.,[47] in an analysis of the baseline SUS cohort health data, reported a lower prevalence of IgE-mediated sensitization among current farmers compared to rural non farmers. The decreased risk appeared to be independent of farm childhood, thus implying a potential for a protective effect even of current farming against sensitization. Consistent with the findings of Portengen et al., later studies among farming[45,48,49] and rural-dwelling[46] populations showed lower risks for allergy or asthma symptoms in persons combining exposure to farming during both childhood and adulthood.

In 2004, Eduard and colleagues published results from a cross-sectional study among Norwegian farmers describing relationships between exposure to microbial agents and ammonia, asthma *per se*, and serum-defined sensitization.[50] Using personal exposure monitoring, the authors reported the prevalence of asthma among sensitized farmers to decrease with increased exposure to endotoxin, fungal spores, or ammonia. On the contrary, elevated levels of exposure among atopic workers appeared to increase the likelihood of asthma. In addition, cross-sectional investigations among farmers[51] and agricultural industry workers[27,28] from the Netherlands showed endotoxin exposure to decrease the likelihood of sensitization, but at the same time to increase the risk for respiratory symptoms and bronchial hyper-responsiveness. These findings, although in support of the suggested protective effect of farming on sensitisation, highlight the complexity of the interactions between environment, exposure, and disease.

Currently, asthma and allergic diseases are recognized as the outcomes of complex interactions between environmental exposures, heredity, and epigenetic changes; a fact that explains the existence of different phenotypes and levels of disease severity.[10,52,53] In this context, as previously comprehensively reviewed,[10,52,54,55] several studies has been initiated to enlighten the mechanisms underlying the complex interplay between environmental exposures and the individuals' genetic makeup that determine immune responses leading to asthma and allergies. However, the focus on occupational populations has been relatively small despite the potential of serving as a model for understanding asthma.[10] Considering adult farming populations, very few studies have been published;[56-58] regardless that the first analysis describing farming as an effect modifier on the association between α-1-Antitrypsin (A1A) and Bronchial hyperresponsiveness was published by the SUS cohort team as early as in the mid-90s.[59] The studies suggest farming exposure to modify the risk for asthma and allergy only to a limited extend, highlighting that the assessment of gene-environment interactions is a rather difficult process that requires *a priory* powered design to detect small effects and emphasis on precise and reliable measurements for genotypes, phenotypes, and exposures.[10] Given the complexity of exposure assessment in farming populations,[13] the establishment of state of the art gene-environment analyses in studies focused on the long-term effects of the farm environment on allergy and lung function as well as the different phenotypes of asthma, poses a challenge.

1.2 The SUS study

The SUS study was initiated in 1992 with the aims to a) describe the prevalence and incidence of respiratory symptoms in a farming environment and b) investigate the effect of farming on the development of allergy, asthma and respiratory disease.[60] The study population included all 2458 second year students at the farming schools of Denmark and a control group of 967 conscripts in the Danish army. Recruitment occurred between February 1992 and February 1994, and overall 2004 farming school students (81%) and 592 conscripts (61%) gave consent to participate in the study. Of the farming school students that gave consent, 40 (2%) failed to participate in the baseline clinical investigations. The final population sample consisted of 1964 students and 407 randomly selected conscripts. Through the years the study produced 14 peer-reviewed publications the most interesting findings of which are summarized in Table 1.

Table 1 Main results of the SUS study published in peer-reviewed articles. (Slightly modified table adapted from Elholm et al.[60])

Reference	Main findings
61	The resistance of the mini-Wright flow meter causes less variation in recordings but reduce peak expiratory flow.
62	Skin prick tests to house dust and storage mites were more prevalent among controls compared with male and female farming students; size of house dust mite weal and number of positive skin prick reactions were associated with BHR.
63	No relation between asthma and farming exposure was seen, but lung function was slightly reduced in the male farming students compared with male controls. Prevalence of asthma was related to smoking, female sex, and family history of asthma and allergy.
64	The shape of the maximum expiratory flow-volume curve reflects exposure in farming.
65	S and Z α_1-antitrypsin alleles are risk factors for BHR in young farmers indicating a gene/environment interaction.
66	LDS using FEV_1 is the best spirometric index to measure short time repeatability of histamine bronchial testing.
47	Farming students born and raised on a farm had lower prevalence of allergic symptoms, BHR, positive SPT, and specific IgE than students raised on a farm.
67	Selenium measured both in serum and urine is associated with mild asthma and atopy.
68	For asthmatics the time domain index αl_{75} was found to discriminate better among the flow indices applied whereas for BHR the LDS using FEV_1 was superior to other measures of BHR.
69	CD3 levels in young farmers correlate with respirable dust exposure levels during work in swine-confinement housing.
70	Exposure to work-related levels of swine dust upregulates CD106 in human alveolar macrophages.
71	A single exposure to organic dust in non-naïve non-exposed volunteers induces long lasting symptoms of endotoxin tolerance.
57	CD14/-260 and CD14/-651 promoter polymorphisms associated with atopy prevalence in young adults with farm exposure.

Abbreviations: BHR=Bronchial hyperresponsiveness; LDS=Log dose slope; SPT=Skin prick test.

In 2006, the 15th year follow-up of the study was initiated aiming to assess the role of exposure to microbe-associated molecular patterns (MAMPs) on the development of allergy and respiratory disease, and to describe interactions between genes, diseases and environmental exposure both independently and within the GABRIEL post genome FP6 project (www.gabriel-fp6.org) to which it is a part of. The Gabriel consortium is a pan European multidisciplinary study that aims to disentangle the genetic and environmental causes of asthma, and to lead the development of new preventative and therapeutic strategies to combat the asthma epidemic in Europe and worldwide. The SUS cohort together with 3 other organic dust exposed cohorts comprises the industrial cohort component of the consortium, which is established in close collaboration between the School of Public Health, Aarhus University and Institute of Risk assessment Sciences (IRAS), University of Utrecht, the Netherlands.

A comprehensive quantitative exposure assessment approach based on personal measurements of dust and MAMP exposure was included in the follow-up study to allow assessment of gene-environment interactions, and to disentangle the limitations of the qualitative exposure assessment approach adapted at baseline. The quantitative exposure assessment form the core of all analysis presented in the current dissertation, and it is used to estimate exposure at both baseline and follow-up. As an example, the measurements are used to model exposure at baseline and to perform a pooled analysis within the framework of the GABRIEL consortium. The results of this study are summarised in Manuscript II.

1.3 Dust and endotoxin exposure in animal farming populations

Within the last decades the exposure conditions in animal farming have been the subject of numerous exposure assessment studies. A large part of this literature is published in engineering periodicals, conferences and conference proceedings, thus complicating the performance of any comprehensive review on the reported levels of dust and endotoxin exposure within animal production facilities. Nevertheless, I have made a review of the applied sampling methodology and the reported levels of total or inhalable personal dust and endotoxin exposure for studies on active sampling published in PUBMED indexed periodicals during the last 30 years. The literature search was performed using the following keywords: *personal, exposure, dust, endotoxin, swine, pig, hog, poultry, broiler, layer, cattle, cow, dairy, farm, farmers, agricultural* or *agriculture*. Searches were performed in blocks of minimum 3 words with one of the terms *exposure, dust* or *endotoxin* always included. Additional references were obtained through the reference list in the identified publications.

Due to the topic of the present PhD thesis only studies related to cattle, pig, and poultry farming were reviewed. Experimental studies or studies involving monitoring of non-farmers as well as those measuring exposure solely among slaughters, processing workers, or poultry catchers were excluded. In case of several publications reporting exposure estimates from the same measurement series, the one presenting original values with the most adequate and detailed methodology description was used; though, supplementary information was extracted from the other publications. When findings were reported in one or more publications using both time-weighted and original estimates, the later findings were used. In addition, summary statistics from log-transformed concentrations were preferred. Overall, the literature search resulted in 28 publications reporting dust and endotoxin levels from 27 different measurement series among farmers (Table 2). Of those, 12 were on pig farmers, 5 on poultry farmers, and 6 on cattle farmers; whereas the remaining 4 studies were comparative studies reporting exposure among several agricultural production sectors.

Approximately half of the included publications reported the well defined "inhalable" dust and endotoxin exposure fraction (defined as the mass fraction of total airborne particles inhaled through the nose and mouth, typically these particles have a mean aerodynamic diameter of <100 μm), while the remaining reported the less well defined "total" fraction (all dust particles irrespectively of their size, in Denmark defined as dust sampled by sampler inlet velocity of 1.25 m/s) of dust and/or endotoxin making therefore direct comparisons difficult. On top of that, many of the identified studies do not have adequate descriptions of the applied sampling methodology. Crucial information like the type of sampler and the sampling flow, the applied sampling strategy (e.g. full-shift or task based monitoring), the monitoring time, or even the range of measured concentrations are absent, which further complicate comparisons of the studies. Furthermore, the studies vary considerably in methods of extraction and analysis used for endotoxin determination, i.e. all three methods (endpoint, chromogen-kinetic and turbometric-kinetic) of the *Limulus amoebocyte lysate* (LAL) assay are used, and even the newer recombinant factor C (rFC) bioassay for endotoxin determination is applied in a few studies.

Table 2 Dust and endotoxin concentrations alongside sampling characteristics from personal measurements reported in the literature.

Reference	Farm characteristics		Sampling characteristics					Measure	Dust (mg/m^3)			Endotoxin (EU/m^3)				Strategy
	Type	n	Fraction	Sampler*	Filter	Flow rate (l/min)	Sampling Time (hr)**		N	Average	Range	N	Average	Range	CTRY	
Pig farmers																
Haglind and Rylander, 1987[72]	n.s	19 farms	Total	n.s	n.s	n.s	n.s	AM	≤29	4.9	2.2-15.2	≤29	n.s	200-19,000^	SE	FS
Holness et al., 1987[73]	Finishing	36 farms	Total	n.s	PVC	2.0	9	GM	53	2.06#	0.27-12.81	n/a	n/a	n/a	CA	FS
Donham et l., 1989[74]	n.s	30 buildings	Total	n.s	n.s	n.s	n.s	AM	n.s	6.8	1.8-21.7	n.s	2,400^	200-11,000	SE	n.s
Christensen et al., 1992[75]	Breeding	11 farms	Total	CFC	CN	1.9	5.9	AM	22	4.13	1.12-6.76	22	640^	90-1,200	DK	OS
Vinzents and Nielsen, 1992[76]	Breeding	11farms	Total	CFC	CN	1.9	n.s	GM	23	4.00	n.s	23	702^	n.s	DK	OS
Vinzents and Nielsen, 1992[76]	Breeding	2 farms	Total	CFC	CN	1.9	~3.3	GM	16	3.11#	n.s	16	789#^	n.s	DK	TB
Choudat et al., 1994[77]	n.s	28 buildings	Inhalable	n.s	PVC	n.s	n.s	AM	4	3.63	1.63-7.51	n/a	n/a	n/a	FR	n.s
Donham et al., 1995[78]	n.s	107 farms	Total	n.s	CA	2.0	n.s	GM	201	4.53	n.s	201	202	n.s	US	n.s
Preller et al., 1995[79]	All	198 farms	Inhalable	PAS6	TF	2.0	8.3 (5.2-10.4)	GM	360	2.4#	0.3-26.6	350	920#	56-15,030	NL	FS
Simpson et al., 1999[80]	Breeding	11 sites	Inhalable	IOM	GF	2.0	4.7	GM	27	5.78	0.76-19.09	27	6,600^	600-149,923	GB	FS
Radon et al., 2002[17]	All	n.s	Inhalable	GSP	GF	3.5	2.3 (1.3-4.3)	MDN	40	3.95	71.1-13.8	40	580^	13-11,017	DK	OS
Radon et al., 2002[17]	All	n.s	Inhalable	GSP	GF	3.5	0.9 (0.2-2.8)	MDN	100	5.00	<LOD-76.7	100	763^	0.1-20,901	DE	OS
Spaan, et al., 2006[81]	n.s	n.s	Inhalable	GSP	GF	3.5	>1.8††	GM	6	2.6	1.6-5.4	6	1,510	992-6,970	NL	FS
Melbostad and Eduard, 2001[82]	n.s	n.s	Total	CFC	PC	1.0	<1h	GM	29-32	3.1	n.s	29-32	23,000	n.s	NO	TB
Mc Donnell et al., 2008[83]	Weaners	5 buildings	Inhalable	IOM	GF	n.s	6-8	MDN	12	4.69	0.25-7.6	n.s	n.s	n.s	IE	FS$
Mc Donnell et al., 2008[83]	Finishing	5 buildings	Inhalable	IOM	GF	n.s	6-8	MDN	6	2.31	1.9-5.0	n.s	n.s	n.s	IE	FS$
Mc Donnell et al., 2008[83]	Farrowing	5 buildings	Inhalable	IOM	GF	n.s	6-8	MDN	10	1.49	0.29-4.4	n.s	n.s	n.s	IE	FS$
Mc Donnell et al., 2008[83]	Dry sow	5 buildings	Inhalable	IOM	GF	n.s	6-8	MDN	11	1.1	0.25-3.5	n.s	n.s	n.s	IE	FS$
Mc Donnell et al., 2008[83]	General	5 buildings	Inhalable	IOM	GF	n.s	6-8	MDN	8	2.99	1.1-5.6	n.s	n.s	n.s	IE	FS$

Table 2 Continued.

Reference	Farm characteristics		Sampling characteristics					Measure	Dust (mg/m^3)			Endotoxin (EU/m^3)				Strategy
	Type	n	Fraction	Sampler*	Filter	Flow rate (l/min)	Sampling Time (hr)**		N	Average	Range	N	Average	Range	CTRY	
Kim et al., 2008[84]	Finishing	150 buildings	Total	CFC	GF	2.0	2-3	AM	n.s	3.02	0.64–6.67	n/a	n/a	n/a	KR	TB
Bonlokke et al., 2010[85]	Finishing	n.s	Total	CFC	PVC	2.0	~4 (0.7-7.3)	MDN†	41	2.39-3.8	0.61-10.24	n/a	n/a	n/a	CA	OS
Bonlokke et al., 2010[85]	Finishing	n.s	Total	CFC	GF	2.0	~4 (0.7-7.3)	MDN†	n/a	n/a	n/a	41	6,553-25,690	1,800-69,096	CA	OS
O'Shaughnessy et al., 2010[86]	Gestation/ Farrowing	2 facilities	Inhalable	IOM	PVC	2.0	~ 7	GM†	34	0.83-3.76#	n.s	34	400-2,500#	n.s	US	FS
Cattle farmers																
Virtanen et al., 1988[87]	Dairy	18 farms	Total	n.s	CA/CN	2-20	n.s	AM	31	2.4	0.2-7.4	n/a	n/a	n/a	FI	n.s
Kullman et al., 1998[88]	Dairy	85 farms	Inhalable	IOM similar	PVC	2.0	4-6	GM	159	1.78#	0.007-53.6	194‡	647#	25.4-34,800	US	OS
Nieuwenhuijsen et al., 1999[89]	Dairy	2 farms	Inhalable	IOM	PVC	2.0	n.s††	GM†	17	0.3-0.62	n.s	17	10.9-120.4	n.s	US	TB
Melbostad and Eduard, 2001[82]	n.s	n.s	Total	CFC	PC	1.0	<1h	GM	33-36	1.2	n.s	33-36	2,200	n.s	NO	TB
Berger et al., 2005[90]	n.s	23 farms	Inhalable	n.s	GF	3.5	n.s	MDN	23	1.78	0.25-58,22	n/a	n/a	n/a	DE	n.s
Firth et al., 2006[91]	Dairy	18 farms	Inhalable	IOM	n.s	2.0	4	MDN	18	0.6	n.s	n/a	n/a	n/a	NZ	TL
Spaan, et al., 2006[81]	Dairy	n.s	Inhalable	GSP	GF	3.5	>1.8††	GM	8	1.3	0.4-2.3	8	560	62-2,230	NL	FS
Spaan, et al., 2006[81]	Dairy/ breeding	n.s	Inhalable	GSP	GF	3.5	>1.8††	GM	4	1.5	0.7-2.7	4	1,570	444-3,860	NL	FS
Saito et l., 2009[92]	Diary	n.s	Inhalable	IOM	PVC	2.0	6-8	GM	n/a	n/a	n/a	17	752	n.s	US	FS
Saito et l., 2009[92]	Feedlot	n.s	Inhalable	IOM	PVC	2.0	6-8	GM	n/a	n/a	n/a	48	1,097	n.s	US	FS
Burch et al., 2010[93]	Feedlot	n.s	Inhalable	IOM	PVC	2.0	n.s	GM	55	2.4#	n.s	55	943#	n.s	US	FS
Burch et al., 2010[93]	Diary	n.s	Inhalable	IOM	PVC	2.0	n.s	GM	15	2.4#	n.s	n.s	n.s	n.s	US	FS
Poultry farmers																
Lenhart et al., 1990[94]	Broilers	22 farms	Inhalable	CFC	PVC	1.5	0.25-1.5	GM	>26	24.2	12.9-78.2	>26	2,100^	530-9,200	US	TL
Nieuwenhuijsen et al., 1999[89]	n.s	1 farms	Inhalable	IOM	PVC	2.0	n.s††	GM†	11	1.77-6.67	n.s	11	222.3-1,861.2	n.s	US	TB
Golbabaei and Islami., 2000[95]	Parental stock	4 barns	Total	n.s	TF	1.5	n.s	AM†	n.s	7.1-21.3	n.s	n.s	206^	n.s	IR	n.s

Table 2 Continued.

Reference	Farm characteristics		Sampling characteristics					Measure	Dust (mg/m^3)			Endotoxin (EU/m^3)				Strategy
	Type	n	Fraction	Sampler*	Filter	Flow rate (l/min)	Sampling Time (hr)**		N	Average	Range	N	Average	Range	CTRY	
Golbabaei and Islami., 2000[95]	Layers	3 barns	Total	n.s	TF	1.5	n.s	AM†	n.s	10.5-15.8	n.s	n.s	142^	n.s	IR	n.s
Golbabaei and Islami., 2000[95]	Broilers	6 barns	Total	n.s	TF	1.5	n.s	AM†	n.s	3.7-4.2	n.s	n.s	187^	n.s	IR	n.s
Golbabaei and Islami., 2000[95]	Control rooms	n.s	Total	n.s	TF	1.5	n.s	AM†	n.s	1.1-3.1	n.s	n.s	68-138^	n.s	IR	n.s
Melbostad and Eduard, 2001[82]	n.s	n.s	Total	CFC	PVC	1.0	<1h	GM	24-32	5.0	n.s	24-32	4200	n.s	NO	TB
Donham et al., 2002[96]	Layers, broilers, turkey & shacklers	n.s	Total	CFC	PVC	1.0-2.0	n.s	AM	238	6.5	0.02-81.33	236	1,589	0.24-39,267	US	FS
Radon et al., 2002[17]	Layers & broilers	n.s	Inhalable	GSP	GF	3.5	0.5 (0.2-2.2)	MDN	40	7.01	0.42-21.75	40	2,576^	190-16,348	CH	OS
Whyte, R.T. 2002[97]	Layers, barn houses	n.s	Inhalable	IOM	GF	2.0	n.s	AM	12	10.8	n.s	n.a	n.a	n.a	UK	FS
Whyte, R.T. 2002[97]	Layers, battery	n.s	Inhalable	IOM	GF	2.0	n.s	AM	9	4.8	n.s	n.a	n.a	n.a	UK	FS
Whyte, R.T. 2002[97]	Layers, barn houses	n.s	Inhalable	IOM	GF	2.0	n.s	AM †	55	5-71	n.s	n.a	n.a	n.a	UK	TB
Kirychuk et al., 2006[98]	Broiler & Turkey	ns	Total	CFC	GF	2.0	1.6	AM	80	9.56	n.s	80	7,484	n.s	CA	OS
Kirychuk et al., 2006[98]	Layers, cages	ns	Total	CFC	GF	2.0	2.7	AM	31	7.57	n.s	31	9,544	n.s	CA	OS
Spaan, et al., 2006[81]	Layers	n.s	Inhalable	GSP	GF	3.5	>1.8††	GM	2	9.5	6.6-14	2	2090	1,716-2,550	NL	FS
Spaan, et al., 2006[81]	Broilers	n.s	Inhalable	GSP	GF	3.5	>1.8††	GM	2	4.2	4.0-4.4	2	880	520-1,500	NL	FS
Spaan, et al., 2006[81]	Layers, free-range	n.s	Inhalable	GSP	GF	3.5	>1.8††	GM	6	3.6	1.6-11	6	2140	360-8,120	NL	FS

Abbreviations and explanations: n=Number; n.s=Non specified; n.a=Not available; AM=Arithmetic mean; GM=Geometric mean; RNG=Range; CTRY=Country ISO abbreviation; CFCM=Close faced Millipore; CFC=Close faced; CN=Cellular nitrate; CA= Cellulose acetate; GF=Glass-fibre; PC=Polycarbonate; PVC=Polyvinylchloride; TF=Teflon; OS=Only stable work; TB=Task-based; FS=Full-shift; TL=Time limited

TWA values; *Total samplers defined only by the inlet function (open/closed); **Given based on the info provided; ^Transformed value using a 1ng eq to10 EU standard; $Excluding brakes; ‡Includes area measurements; †Range of averages; ††Values given for a larger sample of measurements

Nevertheless, full-shift average levels of inhalable dust in the included studies were between 0.8 and 10.8 mg/m^3 and for inhalable endotoxin between 400 and 6,600 EU/m^3. Pig and poultry farmers appear to be somewhat higher exposed than cattle farmers, and this pattern seems to be consistent when looking also at the "total" dust and endotoxin exposure estimates. The highest average for endotoxin exposure is reported among pig farmers, but it is derived through task based measurements and thereby it is heavily influenced by the short sampling duration. The results from studies measuring exposure solely during stable work suggest poultry farmers to be highest exposed both in respect to dust and endotoxin exposure. These findings are in accordance with the results from studies measuring dust and endotoxin exposure with the use of stationary sampling (Table 3). However, stationary sampling tends to somewhat underestimate the level of exposure, as supported by studies assessing exposure by both stationary and personal monitoring.[74,77,84,87,88,90]

The evident large variation in the reported concentrations can partly be attributed to the different sampling and analytical methods,[99-101] but most importantly to the alternating daily working tasks of the farmers and the several environmental and engineering factors that influence the dust and endotoxin exposure within animal buildings.[79,102,103] Specifically, in a re-analysis of data from a study among Californian livestock and arable farmers, the average exposure concentrations within farmers were estimated to lie within a 63-folds difference for dust, and within 523-folds for endotoxin.[13] Similarly, Preller and colleagues,[104] in a study with repeated exposure measurements among Dutch pig farmers, reported daily dust and endotoxin exposure concentrations to average within a range of 9- and 21-folds, respectively. In the same population, variations within average personal concentrations were within a 4-fold range. This specific study represents a landmark in the field of exposure assessment among farming populations as it included a detailed discussion of the implications and the handling of exposure variability in an epidemiological context, and was followed by a comprehensive evaluation of factors affecting exposure both in the personal and temporal (day-to-day) level. In the following chapters these findings are discussed along with those from other studies.

Table 3 Dust and endotoxin concentrations from stationary measurements reported in a sample of selected studies.

Reference	Farm characteristics		Fraction	Sampling Time (hr)*	Measure	Dust (mg/m^3)			Endotoxin (EU/m^3)			
	Type	n				n	Average	Range	n	Average	Range	CTRY
Pig farmers												
Clark et al., 1983[105]	n.s	8 farms	Total	1	AM	18	3.08	1-8	18	1200^	n.s	SE
Donham et al., 1986[106]	Farrowing/ Nursery/ Finishing	21 buildings	Total	n.s	AM	126	6.25	n.s	n/a	n/a	n/a	US
Attwood et al., 1986[107]	Finishing	4 buildings	Total	6	GM	26	1.32	0.69 – 2.29	14	627^	80-2520	NL
Attwood et al., 1987[102]	Finishing	~70 buildings	Total	6	GM	70	2.82	0.47- 9.55	70	1200^	350-3130	NL
Attwood et al., 1987[102]	Farrowing & nursing	~100 buildings	Total	6	GM	100	4.9	1.29 – 23.48	96	1280^	230-4450	NL
Donham et l., 1989[74]	n.s	30 buildings	Total	3-6	AM	60	4.3	1.4-8.3	60	1800^	400-3.300	SE
Cormier et al., 1990 [108]	Farrowing/ finishing	4	Total	1	AM	20	3. 85	n.s	n/a	n/a	n/a	CA
Crook et al., 1991[109]	Finishing	20 houses	Total	n.s	AM†	120	1.66-21.04	n.s	n.s	n.s	n.s	UK
Dutkiewicz et al., 1994[110]	Farrowing/ finishing	5 houses	Total	n.s	AM†	10	3.03-14.05	n.s	10	18800-750000^	n.s	PL
Zelda et al., 1994[111]	All	50 buildings	Total	n.s	MDN	n.s	2.77	1.71-5.02	n.s	5,427	438-41,307	CA
Mackiewicz B., 1998[112]	Breeding	3 farms	Total	n.s	AM	n.s	8.76	2.02-14.05	n.s	228,000^	18,800-312,500	PL
Takai et al., 1998[113]	All	134 buildings	Inhalable	12	AM	256‡	2.19	n.s	n.s	n.s	n.s	EU
Seedorf et al. 1998[114]	All	110 buildings	Inhalable	12	AM†	n.s	n.s	n.s	n.s	523-1865^	n.s	EU
Choudat et al., 1994[77]	??	28 buildings	Total	n.s	AM	21	2.41	0.29-8.55	n/a	n/a	n/a	FR
Choudat et al., 1994[77]	??	28 buildings	Inhalable	n.s	AM	28	1.82	0.23-6.71	n/a	n/a	n/a	FR
Chang et al., 2001[115]	All, open style	30 buildings	Ambient	6	AM	90	0.24	0.03-1.11	60	140	14.4-818	TW
Duchaine et al., 2001[116]	n.s	7 buildings	Total	~4	GM†	21	0.58-1.89		21	3927	729-18425	CA
Schierl et al., 2007[117]	Finishing houses	4 buildings	Inhalable	1 or 6	MDN	n.s	n.s	n.s	18	668.7	43.2-7,469	DE
Kim et al., 2008[84]	All	150 buildings	Total	8	AM	300	1.88	0.53–4.37	n/a	n/a	n/a	KR
Thorne et al., 2009[118]	Finishing	4 buildings	Inhalable	4	GM	40	1.91	0.16-7,05	40	3100	59-57800	US

Table 3 Continued.

Reference	Farm characteristics		Fraction	Sampling Time (hr)*	Measure	Dust (mg/m^3)			Endotoxin (EU/m^3)			
	Type	n				n	Average	Range	n	Average	Range	CTRY
Thorne et al., 2009[118]	Hoop finishing	3 buildings	Inhalable	4	GM	30	1.4	0.01-5.47	30	3250	48-37700	US
Létourneau et al., 2010[119]	Finishing	18 buildings	Total	4	AM†	54	1.02-1.77	0.49-2.83	54	3170-51900	n.s	CA
Cattle farmers												
Virtanen et al., 1988[87]	Dairy	18 farms	Total	n.s	AM†	36	1.4-1.5	0.3-6.3	n/a	n/a	n/a	FI
Kullman et al., 1998[88]	Dairy	85 farms	Total	4-6	GM	211	0.74#	0.007-6.5	n.s	n.s	n.s	US
Takai et al., 1998[113]	Beef/ dairy/ calves	118 buildings	Inhalable	12	AM	231‡	0.38	n.s	n.s	n.s	n.s	EU
Seedorf et al. 1998[114]	Beef/ dairy/ calves	67 buildings	Inhalable	12	AM	n.s	n.s	n.s	n.s	74-639^	n.s	EU
Berger et al., 2005[90]	n.s	23 farms	Inhalable	n.s	MDN	31	0.24	0.01-2.43	32	36	4-561	DE
Schierl et al., 2007[117]	Dairy	4 houses	Inhalable	1 or 6	MDN	n.s	n.s	n.s	22	16.9	2.8-16.9	DE
Schierl et al., 2007[117]	Beef	1 house	Inhalable	1 or 6	MDN	n.s	n.s	n.s	6	557.9	124-1,025	DE
Dutkiewicz et al., 1994[110]	Dairy/ calves	4 stables	Total	n.s	AM†	8	0.25-0.8	n.s	8	12.5-125^	n.s	PL
Poultry farmers§												
Clark et al., 1983[105]	Layers, cage	5 units	Total	1 h	AM	7	2.34	0.95-3.68	7	3100^	1200-5000	SE
Jones et al., 1984[120]	Broiler	3	Total	4-6	AM	9	4.4	0.02-11	9	396.7	240-590	US
Reynolds et al., 1994[121]	Turkeys	36 facilities	Total	n.s	GM†	23	1.2-7.6#	0.4-10.7	23	208-10,960#	16-30,544	US
Takai et al., 1998[113]	Broilers and Layer	81 buildings	Inhalable	12	AM	162‡	3.6	n.s	n.s	n.s	n.s	EU
Seedorf et al. 1998[114]	Broilers and Layer	64 buildings	Inhalable	12	AM†	n.s	n.s	n.s	n.s	3389-8604^	n.s	EU
Schierl et al., 2007[117]	Layers	3 houses	Inhalable	1 or 6	MDN	n.s	n.s	n.s	18	463.2	21.8-21,933	DE
Schierl et al., 2007[117]	Turkeys	1 houses	Inhalable	1 or 6	MDN	n.s	n.s	n.s	6	1902	467.1-5292	DE
Rylander and Cavalheiro., 2006[122]	n.s	n.s	n.s	0.5-1	AM	n.s	n.s	n.s	n.s	4100^	100-10,030	SE
Nimmermark et al., 2009[123]	Layers	9 facilities	Total	n.s	GM	14	2.7	0.71-17.65	n.a	n.a	n.a	NO
Kirychuk et al., 2010[124]	Layers, cages	15 buildings	Total	4 hr	GM	30	1.69	n.s	30	1513.1	n.s	CA

Table 3 Continued.

Reference	Farm characteristics		Fraction	Sampling Time (hr)*	Measure	Dust (mg/m³)			Endotoxin (EU/m³)			
	Type	n				n	Average	Range	n	Average	Range	CTRY
Kirychuk et al., 2010[124]	Floor-housed	15 buildings	Total	4 hr	GM	30	4.62	n.s	30	2504.1	n.s	CA
Rimac et al., 2010[125]	Layers, cage	2 farms	Total	n.s	MDN†	10	0.35-1.1	0.2-1.5	3	n.s	230-238.3	HR

Abbreviations and explanations: n=Number; n.s=Non specified; n.a=Not available; AM=Arithmetic mean; GM=Geometric mean; RNG=Range; CTRY=Country ISO abbreviation; §Chicken production unless otherwise stated; # =TWA values; *Given based on the info provided; ^Transformed value using a 1ng eq to10 EU standard; †Range of averages; ‡Given as number of field measurements, actual number of collected samples probably larger.

1.4 Exposure variability and implications

The issue of exposure variability and its implications has been discussed in details in papers and book chapters on exposure assessment related subjects. A thorough discussion on the implications of exposure variability in occupational epidemiology can be found in a review paper by Loomis and Kromhout,[7] whereas Burdorf and Tongeren[126] provide a brief overview of the historical discussions and developments on the issue. For epidemiological studies in farming populations the issue is comprehensively addressed in a relatively recent discussion paper by Kromhout and Heederik.[13]

Variations in exposure can apply both between persons and through time, and in the case of groups of populations also between groups.[7] In the context of a single group of workers variability can be distinguished into two levels: (a) between-workers (also referred to as personal or inter-individual) and (b) within-workers (also referred to as temporal or intra-individual), and it is analytically characterized by a systematic and a random component based on the source of variation.[127] Sources of systematic variation in exposure include differences in workplace settings, machinery and equipment, and environmental parameters (e.g. temperature) as well as personal working characteristics (e.g. working tasks). Random variation is any variations that cannot be explained by factors of the systematic component (e.g. individual workers habits and peculiarities, or measurement errors caused by investigators).[127] Understanding of variability in exposure and recognition of its sources within a workplace is essential for the design of both measurement and exposure control strategies.[6]

In general, the presence of a substantial variability in exposure bears a potential for measurement error and misclassification.[7] In the presence of repeated measurements of exposure variability can be quantitatively estimated using random-effect ANOVA (analysis of the variance) models. These estimates can be used along with developed equations[128] that allow calculations on the sample-size and attenuation in exposure-response relationships to optimize sampling strategies, provide guidelines on the exposure assignment in epidemiological studies (e.g. grouped vs. individual approach), and in combination with information on exposure determinants and empirical modeling techniques, to improve the precision of the exposure estimates.[7]

One of the best examples of the implications of exposure variability in an epidemiological setting is found on the study among Dutch pig farmers by Preller and colleagues.[104] Exposure to inhalable dust and endotoxin was measured twice (summer and winter) by personal monitoring of 198 farmers. The authors used a one-way random effect ANOVA model and estimated the ratio between the within- and between-variance for measured endotoxin exposure to be equal to 4.7. Using this ratio (λ), the number of repeated measurements included (n) and the following equation as given by Liu et al.,[128]

$$b = \beta\,(1+\lambda/n)^{-1}$$

where b and β are the observed and true values of the regression coefficient, respectively

the authors estimated that a direct use of their measured endotoxin exposure levels would have underestimated the value of the true regression coefficient by 70%, hampering the chance for observing clear exposure-response relationships. To increase precision in exposure the authors developed an empirical model based on measured endotoxin levels and information on farm characteristics and work tasks. Predictions were based on information on the farmers' working tasks for 7 consecutive days both summer and winter collected by the same self-administered activity dairy that was distributed to the farmers during the measurements. The developed model explained 37% of the within-workers variability in endotoxin exposure, and it was used to predict long-term average exposure for every farmer included in the study. The predicted exposure estimates decreased the potential attenuation in the exposure-response relationships to 50% when considering only the 2 measurement days and to 8% when all 14 days with information were used. The measured levels showed no association with lung function, whereas the modeled exposure showed a considerable decrease in lung function with increasing exposure, a trend significant for farmers without chronic respiratory symptoms.

The study of Preller et al. clearly shows both the effects of a substantial variability in exposure as well as the benefit of the use of empirical models in order to minimize these effects. Such approaches are essential in epidemiological studies among populations with predominant variation in exposure within-workers, which in principal require an increased number of repeated measurements per worker, a practice that can increase substantially both the effort and cost of research.[13] Farmers, as nicely shown by Kromhout and Heederik,[13] are clearly a population that shares the above characteristics.

1.5 Determinants of exposure to dust and endotoxin among animal farmers

Determinants of dust and, to a lesser extent, endotoxin exposure have been a subject of investigation in observational and experimental studies primarily in relation to pig farming.[16,83,84,109,111,113-115,117,129-131] In general, the results suggest use of wet feed, ad-libitum feeding practices, smaller animal density, use of colza oil, spraying of water, vacuum cleaning, and use of electrostatic filters to reduce dust exposure. Season is consistently shown as an important determinant for stable exposures with higher levels reported during winter than summer seasons. However, this pattern is stronger in pig and poultry stables than in cattle stables. Additionally, increased animal activity and use of litter seem to increase exposure levels. Full concrete and slatted floors are associated with increased exposure concentrations, but findings seem somewhat inconsistent. Ventilation, air temperature, and humidity seem also to be of importance. In poultry, as recently reviewed by Just et al.,[132] additional important determinants include the age of the chicks and the type of the housing system (floor vs. cages).

In observational settings most investigations were descriptive using stationary measurements and focusing on the type of stable, the season and/or type of ventilation, and the bedding and flooring. In one of the earliest studies assessing factors related to both dust and endotoxin exposure in piggeries, Atwood et al.[102] reported airborne dust to be inversely related to the age of the animals (r =-0.36) and time interval (weeks) since last cleaning (r=-0.22), and positively with the indoor temperature (r=0.35) and the animals/m^3 (r=0.44). Differences in the measured dust concentrations were found also between different feeding practices with stables using wet feed having the lowest levels. Endotoxin was correlated only with the level of carbon dioxide (CO_2), which was used as an indicator for the stable air quality. In Canada, Duchaine et al.[133] examined relationships between dust, endotoxin, ammonia, CO_2 and bacterial counts and dirtiness (scale 1-10), ventilation, indoor air temperature and humidity, outdoor temperature, number of pigs, building and room size, frequency of emptying the manure, type of feed, and the number of ventilators in 8 buildings. The authors found endotoxin to associate only with the number of pigs (r=0.7), and the number of bacteria (r=0.6). Dust levels were reported to be significantly higher during the winter. In a more recent study, Thorne et al.[118] assessed the effect of season, temperature, wind, number of pigs, type of stable, and humidity on ambient bioaerosol concentrations inside hoop and conventional finishing stables. The authors were able to explain more than 70% of the variability in dust and endotoxin concentrations with season, barn type, number of animals, humidity, and temperature being the most important factors affecting exposure.

In one of the very few studies trying to grab determinants of personal dust and endotoxin exposure among (but not limited to) cattle farmers, Nieuwenhuijsen et al.[89] performed task-based personal measurements during milking, feeding, animal moving, animal handling and scraping of cow stalls in combination with other frequently performed tasks like building or equipment reparations, high pressure washing, and field work. In general, animal handling, milking, and feeding were associated with the highest dust levels compared to all cattle-specific working tasks included; for endotoxin the task with the highest level was feeding. Other non-cattle working tasks with high dust and endotoxin levels included the reparation of equipment, the maintenance of buildings and the harvesting of crops. It has to be mentioned, however, that the specific study composed a small measurement series with only 17 measurements in cattle-related tasks.

In a study with personal measurements among Korean pig farmers, Kim et al.[84] reported lower dust levels in natural and mechanical ventilated buildings with a deep-pit manure system compared to scraped manure designs or littered buildings. In addition, Holness et al.[73] found higher dust levels in Canadian farmers using floor feeding practices compared to those using automatic feeding, while Vinzents and Nielsen[76] were unable to observe clear differences in dust and endotoxin levels of pig farmers working close to and far from animals. On the contrary, O'Shaughnessy et al.,[86] in a study among American pig breeders aiming to promote use of respirators in certain working processes, found greater dust concentrations in tasks related to animal movement during the weaning process. The authors based their results on task-based analysis in linear regression using photometer readings and time-weighted estimates derived from full-shift personal sampling.

Finally, in the most comprehensive observational evaluation so far, Preller et al.[79] used empirical modelling approaches to gain id-depth knowledge on the determinants of personal dust and endotoxin exposure of pig farmers. Exposure levels to inhalable dust and endotoxin were obtained by seasonal (summer/winter), personal monitoring of 198 farmers located in south-eastern Holland. Information on 95 distinct farm characteristics were collected though walk-through surveys, and all farmers filled in their working tasks on detailed activity diaries covering one week per measurement season. Using classical step-wise regression techniques, the authors fitted linear models that finally resulted in more than 30% of the variability in exposure being explained by 10 tasks and 10 farm characteristics for dust, and 12 tasks and 8 farm characteristics for endotoxin. The predictors for dust exposure included low outside temperature and tasks like feeding, castrating, ear tagging, floor sweeping, removal of dry manure, and teeth cutting. Important farm characteristics were the presence of dry manure, a dusty overall environment or a dusty feeding path, the use of pig starter, and wet feeding practices. For endotoxin, exposure decreased by the presence of a convex floor, the use of automated dry feeding, and the air sucking through the pit. Exposure to endotoxin increased as a consequence of a full slatted floor, use of floor heating and a working environment deteriorated by dust. Among others, the most highly associated tasks with endotoxin exposure included ear tagging, teeth cutting, floor sweeping, and iron injections.

Despite animal farming being well-established as a major occupational hazard, from the above it becomes evident that comprehensive observational evaluations of determinants of personal exposures to dust and endotoxin among animal farmers are limited for pig farmers, and almost totally absent for cow farmers. Such studies can be integrated into epidemiological designs for exposure predictions, and they can be highly important for the establishment of preventive strategies as they benefit from allowing the assessment of multiple exposure determinants in real working conditions with a great degree of generalizability.[134]

1.6 The primary animal production in Denmark

The Danish primary agriculture sector has been heavily industrialized during the last decades. Now it is characterised by increased specialization, high productivity and one of the world's most strict legislations concerning animal-welfare and production quality. Denmark is in fact the largest producer and exporter of mink-pelts and pork-meat, respectively. Furthermore, the country holds significant dairy and poultry productions that by exports contribute to the Danish economy with more than 2 billion Euros on an annual basis.[135,136] Structurally, the number of professional farm holdings rapidly decreased in the last decades (Figure 2). Several large multi-corporate farms have emerged through this change, whereas the tendency for even greater land and production accumulation persists. Currently, the average size of a Danish farm stands at approximately 63 hectares; one of the largest averages in Europe. As of 2008, the Danish primary agriculture sector consisted of 43,415 holdings with a size larger than 5 hectares. Of those, approximately 35% specialized in livestock production (mainly pig and cattle farming), 39% solely produced crops, and 26% were mixing crop with livestock production activities.[136]

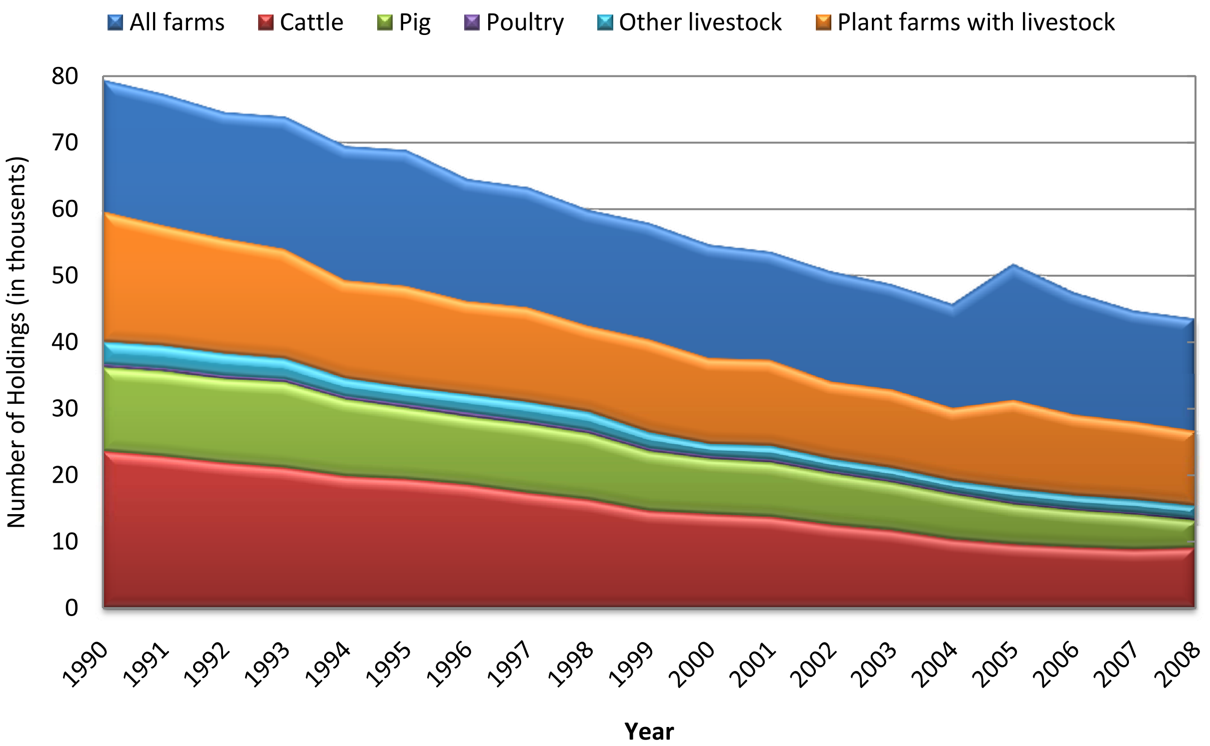

Figure 2 Structural changes in the Danish primary animal production sector over the last 18 years. Results are given for the overall and per farm-type change in farm numbers. Figures do not include farms with a size smaller than 5 hectares. (Source; Denmark statistics).

In terms of human labour, during the same period (i.e. 2007) the agriculture sector provided direct or indirect employment to more than 100,000 individuals, a number corresponding to approximately 4% of the total labour force of Denmark.(Denmark statistic, 2007)

2 Objectives

The overall objective of the exposure assessment part of the SUS study is to develop a model-based assessment method for dust and MAMP exposure of farmers in order to assess current and retrospective exposure in the framework of the SUS cohort. The underlining hypothesis is that the use of modelled exposure estimates can improve precision of the risk estimates and thereby the ability to detect smaller effects, which is a pre-requirement of any gen-environment interaction analysis.

The present thesis formulates the basis for this model-based assessment method focusing on the initial steps for the development of the models for dust and endotoxin exposure. This involves the design and result of a field study measuring dust and endotoxin exposure in the farmers' workplace, and the use of these measurements in a model for baseline exposure which results are used to estimated health effects in the framework of the GABRIEL consortium. Furthermore, it covers the initial steps of the data preparation and analysis necessary to the development of an empirical model to be used in exposure predictions within the cohorts' subpopulation of pig farmers. The specific aims for the three manuscripts comprising the present thesis are:

Manuscript I: To describe the levels of dust and endotoxin exposure in different types of Danish farmers. To provide information on the magnitude and distribution of the variability in exposure, and hence to deliver the basic structure for the predictive models.

Manuscript II: To use the measured endotoxin levels in the SUS follow-up (Manuscript 1) in order to estimate exposure in the baseline SUS population, and then to investigate whether and how endotoxin exposure predicts the likelihood of allergic sensitization and airway disease in a pooled analysis of the four GABRIEL industrial populations.

Manuscript III: To initiate the modeling process by exploring factors and working tasks determining the level of personal exposure among pig farmers.

3 Materials and Methods

The SUS exposure study is multidisciplinary using different methods to assess exposure of farmers. Despite that all methods were included in the field work and were part of the current authors' working tasks during his PhD studies, only the relevant to the presented scientific work in the three manuscripts are summarised in the sections that follow. Overall, all work presented in the present dissertation is based on the dust and endotoxin exposure measurements performed. Therefore, most of the present chapter is dedicated to the design and performance of the exposure field measurements and to the methodology used to determine the farmers' personal levels of dust and endotoxin exposure. The statistical and epidemiological concepts applied in each of the three manuscripts are discussed briefly in separate paragraphs.

3.1 Study population and farm selection

The present work is an integrated component of the 15-year follow-up of the SUS cohort. Consequently, its study population in principal comprises all 1,964 participants of the initial cohort.[60] In the intermediate period between the baseline and the 15-year follow-up investigation several changes in the occupational status of the participants of the initial cohort were to be expected. An *a priori* identification of the remaining active farming population was essential for the development and the design of the exposure assessment. Therefore, 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 distributed to all the participants. In total, 1,156 participants completed the questionnaire resulting in participation rate of 59%. This was increased to 63% by integrating comparable information on current and previous employment, and farm characteristics for another 83 participants, available from the exposure scheme used in the clinical part of the study. Overall, 34% of the participants were full-time employed in farming, most (77%) in farms located in the area of Jutland (Table 4). Of all identified active farmers, 78% were pig and cattle farmers, 3.8% mink farmers, 12.3% crop farmers, 0.4% poultry farmers, and 5.4% combined different types of animal production. Due to increased logistics and cost, as well as due to the similar distribution of farm types per region in our population and the overall Danish farming population, we decided to restrict out investigation to the area of Jutland.

Table 4 Distribution of full-time farmers per type of specialization and geographical area in the SUS12 cohort population (n=1239).

Farm type	Geographical area, n (%)			
	Denmark	**Jutland**	**Zealand**	**Funen**
Cattle, dairy	106 (25.1)	91 (28.0)	11 (16.9)	4 (12.1)
Cattle, beef	18 (4.3)	16 (4.9)	1 (1.5)	1 (3.0)
Pigs	206 (48.7)	159 (48.9)	28 (43.1)	19 (57.6)
Mink	16 (3.8)	15 (4.6)	0 (0)	1 (3.0)
Crop	52 (12.3)	25 (7.7)	22 (33.8)	5 (15.2)
Poultry	2 (0.4)	2 (0.7)	0 (0)	0 (0)
Other, mixed	23 (5.4)	17 (5.2)	3 (4.6)	3 (9.1)
Total	423	325	65	33

The selection and enrollment processes within the study applied different principles for the different types of farms to be included, and it is schematically described in Figure 3. For pig farmers selection was randomly performed after stratification by the farm size, which was calculated on the basis of animal units (AU; the needed number of type-specific animals to produce an equivalent of 100 kg of Nitrogen containing manure).[137] In particular, stratification was made using the first and last quartiles of the size distribution as cut-off levels, and 25 pig farmers were randomly selected from each size category (75 in total). On the contrary, dairy cattle (n=33) and mink farmers (n=3) were selected at random from the corresponding sub-groups of the study population. After selection, 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 to participate and an interview date was agreed upon. For employed selected farmers consent for participation in the study was also requested by the farm owners. Of the selected 111 farmers, 16 (11 pig and 5 cattle) were excluded and 12 (11 pig and 1 cattle) did not want to participate in the study, see Figure 3. The final population consisted of 54 pig farmers, 26 dairy cattle farmers, and 3 mink farmers. Through the Danish Agricultural Advisory Service contact was established with 3 poultry farmers (1 with broiler and 2 with layer production), which were all included.

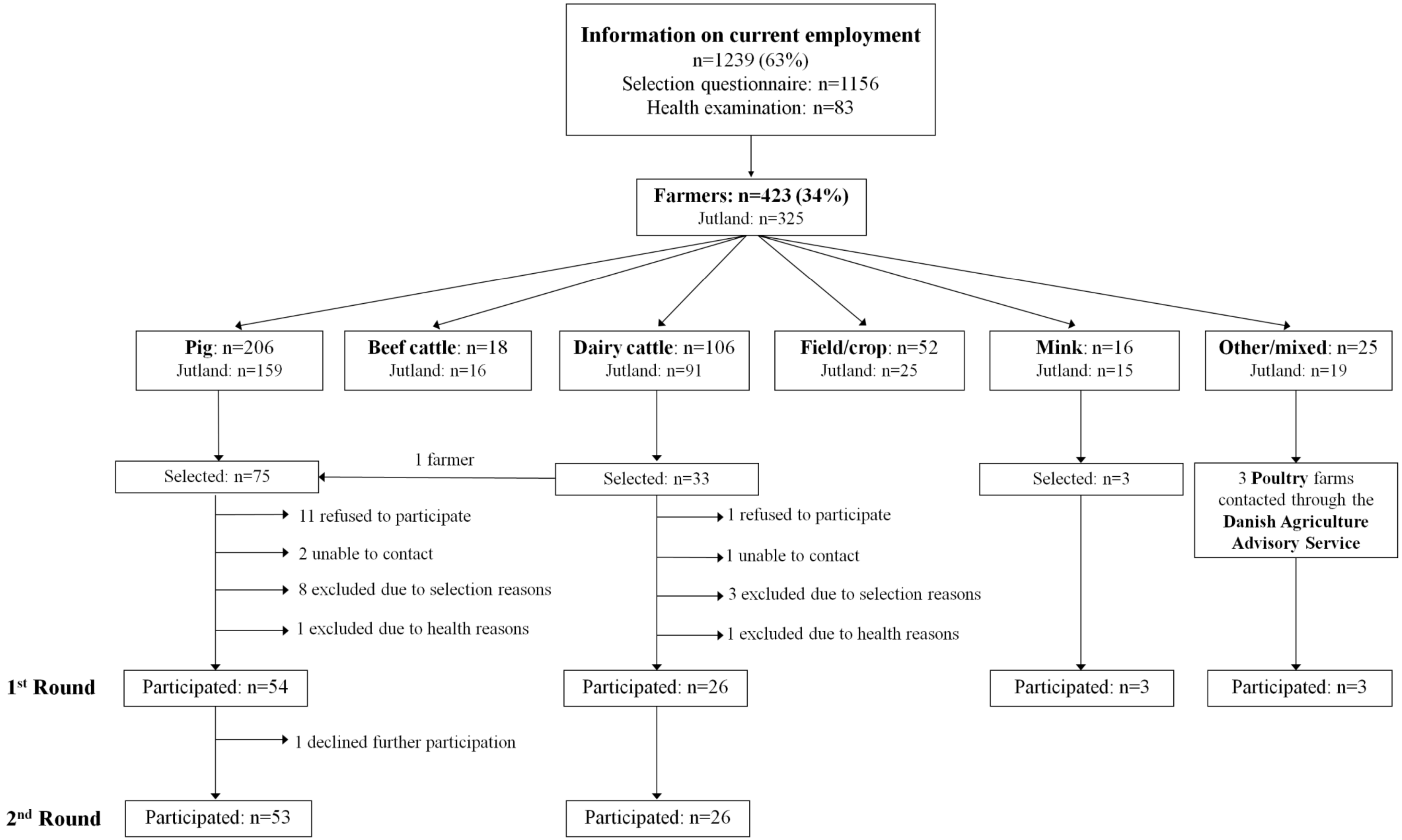

Figure 3 Schematic description of the selection and recruitment of farmers within the SUS exposure assessment study.

3.2 Farm visits, measurement strategies and data collection

Interviews were performed in person, either with the SUS participant or with the farm owner (when the two were different) and, in most cases, in presence of all workers of the farm. During each interview information related to the general production characteristics (i.e. number of employees, collaborations, number and type of animals, size, unit structure and locations, building infrastructure) and farm practices (i.e. agriculture form, cleaning and disinfection schedules and frequency, manure handling) applied by the company were obtained using structured schemes. Explanation concerning the project was provided, including demonstration of the equipment and instructions regarding the surveys to be used, and the days of the measurement visits were established.

For all selected pig and cattle farms two seasonal measurement visits were scheduled between 1st of May and 1st of October for summer and between 17th of November and 3rd of April for winter. In all cases, the visit day was randomly chosen among available working days. All workers on the farm were asked to be monitored, and more than 90% agreed to participate. Sampling was performed throughout the whole working shift including both stable and field work. All farmers were requested to document their working tasks in structured activity diaries with a 30 minute interval checklists. Due to the presence of non-Danish speakers the diaries were made available also in English. The diaries covered one week per visit starting from the day of the measurement. Detailed instructions on the use of the diary were provided to all participants. In addition, farm characteristics, engineering parameters, and the hygienic conditions present in each department of the visited farm were registered through walk-through surveys performed during the visiting days. The inspections were usually performed during the morning and notations were kept in farm-type specific, pre-fixed inspection sheets designed to allow assessment for more than 120 well-defined characteristics. An outline of the information collected through the different schemes is given in Table 5. The interview form along with the walkthrough survey and the activity diary used for pig farmers can be found in the Appendices I, II and III. In all visits the outdoor temperature was measured, using a portable weather station (OBH Nordica A/S, Taastrup, Denmark) with a measurement accuracy of ±1 ^{0}C.

The sampling strategy aiming at full-shift monitoring was also applied to workers in the 2 poultry layer farms included, but each farm was only measured one time. In contrast, measurements in the mink and poultry broiler farms were task-based aiming to determine the level of exposure in different stages of the production. Specifically, mink farms were visited during the breeding, whelping, furring, and pelting stages, and the broiler farm during the preparation of the stables and when the chicks aged 1-2 days (1^{st} week), 21-22 days (3^{rd} week), and 1-7 days before being harvested (5^{th} week). All mink and poultry farmers were requested to fill in detailed activity diaries for a number of consecutive days, and their farms were subject to walk-through surveys performed under the same principles as for the pig and cattle farmers.

Table 5 Outline of information acquired for every company, department and worker.

Company level (Interview)	**Department level** (Walk-though survey)	**Worker level** (Personal sheets and activity diaries)
Ownership and collaboration characteristics (year of acquisition, food supply etc.) Production methods (e.g. conventional, organic, all-in-all-out etc,) Total number and type of animals (overall and per unit) Total size in hectares Land usage Farm plan (number, location and type of compartment) Cleaning Disinfection Use of biofuels Slurry tank handling	Type Housed animals (number and type) Animal accommodation (number and type) Ventilation type Flooring type Use of showering Feeding characteristics (method, type and preparation) Manure handling (removal method and frequency) Hygienic conditions (feed path, dung accumulation, level of floor dampness, overall) Cleaning (overall and areal practices including method and frequency) Disinfection (method, agent, frequency)	Personal characteristics (e.g. age, educational level, working experience) Working tasks

3.3 Dust sampling

Dust sampling was carried out according to the basic principles for personal sampling of aerosols.[138] Glass-fibre (GFA) filters with a 37mm diameter (Whatman international Ltd, Maidstone, UK) were mounted on plastic adaptations of the German GSP samplers (CIS; JS Holdings, Stevenage, UK).[139] The samplers were attached to a silicone tube and safety clipped at the upper part of the chest of the farmers covering their breathing zone (Figure 4). Duplicate sampling was performed to allow assessment of endotoxin, glucan, allergens and microbial contamination using different analytical techniques. Each sampler was attached to a pre-calibrated AirChek XR5000 portable pump (SKC Inc., Eighty Four, PA, USA) at an operational flow of 3.5 l/min. Airflow calibration was preformed with a rotameter and the flow consistency was re-checked at the end of each measurement. Blank filters subjected to the same conditions as the sampled ones, but not subjected to the sampling process, were included for every farm unit visited and for any worker solely working outdoors.

Figure 4 A farmer with the two GSP samplers clipped near his breathing zone while hand feeding weaners.

3.4 Gravimetric analysis

The amount of the collected dust was estimated by weighing the filters before and after sampling. In both cases an equilibration period of minimum 24 hours (temperature of 22 ^{0}C, relative humidity of 45%) preceded the filter weighing, which was performed with an analytical scale (Mettler-Toledo Ltd, Greifensee, Switzerland) with a 0.1 μg precision. The lower limit of detection (LOD) per filter was 0.074 mg, and the results were expressed as mg/m^3 based on the volume of the air sampled during monitoring. The later was derived automatically by the sampling pump, and could be verified through time measurement observations made by the investigators.

3.5 Endotoxin extraction and analysis

Sample extraction and endotoxin determination was performed in one of the duplicate samples, which was randomly chosen, as described previously.[100] Briefly, the samples were extracted in pyrogen-free water (PFW) with 0.05% (v/v) Tween-20. Five ml of extraction solution per sample was used. The samples were thoroughly rocked for 60 minutes on a Multi Reax digital shaker (Heidolph Instruments GmbH, Schwabach, Germany) and then centrifuged for 15 min at 1000 g. Then, the supernatant was harvested and stored (-20^{o}C) in aliquots of 0.1 ml. One of the aliquots was used for endotoxin analysis, which was performed in duplicate using a quantitative kinetic chromogenic Limulus Amboecyte Lysate (LAL) test (Kinetic-QCL 50-650U kit, Lonza, Walkersville, Maryland, USA). The assay was prepared in PFW at a dilution of 1:200, and the endotoxin concentration was determined using a 12 point standard curve (0.01 to 25 EU/ml) obtained from an Escherichia coli (O55:B5) reference. The assays' LOD was 0.0137 EU/ml, and as for dust the results were expressed in EU/m^3 using the volume of air sampled during monitoring.

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3.6 Handling of Non-detectable exposure concentrations

Overall, all measurement series involved the use of 210 field blanks. The SD of the weight change in all (n=210) blind filters was equal to 0.0246 mg. Thus, the *"analytical limit of detection"* for dust was equal to 0.0738 mg. The average sampling time for all the 1038 (Table 6) collected filters was 337 min (SD 103 min). The method limit of detection (MLOD) for dust in our samples was therefore equal to: $0.0738mg/(3.5\ l/min \times 337\ min \times 0.001\ l/min)$ = 0.0626 mg/m^3 of dust. In respect to endotoxin only half (n=105) of the available field blanks were extracted and analyzed and most had levels below the detection limit of the endotoxin assay. Thus, the average LOD of the assay (Mean=13.69 EU per filter; range 12-26 EU/filter) was used. The LOD of the assay expressed as EU/ml was equal to 0.0137. The average sampling time of the analyzed filters (n=519) was 339 min (SD 102.3 min). The MLOD for endotoxin expressed in EU/m^{-3} was equal to *13.69231 EU/filter/(3.5 l/min × 339 min × 0.001 l/min) = 11.52 EU/m^{-3}*. Three samples had non-detectable dust and endotoxin levels. These filters were assigned a dust and endotoxin level equal to the 2/3 of the LOD of the corresponding assay (0.042 mg/m^3 and 7.68 EU/m^3 for the dust and endotoxin assay, respectively).

Table 6 Performed farm visits and number of collected personal samples (in duplicate) per type of farm during the SUS12 exposure assessment study.

Type of measurements	Farm visits (n)	Collected samples (n)
Dairy, morning shift	52	238
Dairy, afternoon shift*	--	16
Pig	105	730‡
Mixed beef and pig	2	4
Minks^	4	16
Broilers†	5	28
Layers	2	6
Total	170	1038

*Afternoon measurements were performed in 5 regular visits in cattle farms where we prolonged our stay and used new filters during the afternoon shift. These measurements were excluded by the analysis presented in Manuscript I in order to maintain homogeneity in working practices.
^Measurements in minks were performed in 4 different stages (mating, growth, grading and pelting). One measurement in pelting did not involve work with minks and thus was excluded.
†Measurements in Broilers were divided into 4 production stages (stable preparation, 1st week, 3nd week, and 5th week of chicks age). All measurements were task-based but when possible the intermediate field work was included using separate filters. 3 field measurements (6 samples), were collected in this fashion, these measurements are also excluded.
‡Includes 4 measurements from 2 workers involved in mixed dairy and pig production activities.

3.7 Manuscript I

Manuscript I is a descriptive paper on the design and the sampling and analytical methodology applied for dust and endotoxin determination within the study. In addition, it reports descriptive information about performed visits and number of measurements as well as number of workers enrolled, and it summarises the measured levels of personal exposure to dust and endotoxin in all four types of livestock farmers included. Finally, it touches upon the structure and distribution of the variability in exposure, under simple descriptive concepts for mink and poultry farmers, and through variance components analysis for pig and cattle farmers.

3.8 Manuscript II

Manuscript II deals with the pooled analysis (GABRIEL industrial) on the health effects of adult endotoxin exposure taking farm childhood into consideration, and using data from four studies of endotoxin exposed workers including veterinary students, biofuel and agricultural industry workers, and farm apprentices (the SUS study population). The establishment of the common database was based on a thorough assessment of the comparability of the questionnaires used by the four studies, while data on IgE-mediated sensitization were available from either skin prick tests (2 studies) or serological test (2 studies). Exposure to endotoxin was estimated by study-specific job-exposure matrices based on exposure measurements performed within the studies; for SUS participants this was achieved by combining the baseline questionnaire information on the farmers' previous occupations and the results of the measurement series described in Manuscript I.

Overall, data from 3,883 individuals (Table 7) were used in the statistical analysis with endotoxin exposure treated consecutively as categorical and continuous, in order to explore relationships between endotoxin, IgE-mediated sensitization, wheeze, asthma, chronic bronchitis, organic dust toxic syndrome, hay fever, and self-reported allergic symptoms. Apart from the combined analysis, stratifications by study population, atopic status, and farm childhood were performed.

Table 7 Study populations: Eligible and included subjects in the GABRIEL industrial populations pooled analysis.

Study population	Eligible subjects	Included subjects*	Country	Reference
Farm apprentices (SUS)	1964	1933	DK	60
Biofuel workers	232	176	DK	140
Agricultural industry workers	901	877	NL	27
Veterinary students	901†	897	NL	141

* Subjects were excluded due to missing information on exposure and potential confounders and due to limitation in age (>65 years). †Only subjects enrolled between June 16 and October 4, 2006 were included.

3.9 Manuscript III

The third paper, a working draft, deals with the initial analysis and data exploration of the collected survey field data along with the measurement results for pig farmers, in order to explore the basic determinants of dust and endotoxin exposure levels. For this purpose the activity diary data were used to explore tasks explaining variability in pig farmers, whereas the influence of stable characteristics was examined only in indoor workers at the selected pig farms. For the later only a small portion of the collected data on farm characteristics mainly related to the applied ventilation, flooring, hygienic conditions and type of feed were used. Overall, the effect of 22 distinct working tasks and 22 farm characteristics was explored using mixed effect linear models (Table 8).

Table 8 Outline of the developed database and basic information for working tasks performed by 231 pig farmers employed in 54 Danish pig farms, and farm characteristics for a sub-group of 181 workers including direct animal exposure.

Working tasks and environment[a]	**n**	**Department characteristics (cut-off time level)** [b]	**n**	**Coding (Median) ‡**
Indoor Environment	353	Outdoor temperature	268	Continuous (12 ^{0}C)
		Housing [a]		
Tasks inside animal areas		Animals in a loose housing system	58	Continuous (15 %)
Controlling	243	Animals housed in batch pens	205	Continuous (39 %)
Weighing	40	Animal housed in crates (including farrowing)	211	Continuous (64 %)
Moving breeding animals	111	Ventilation		
Moving weaners and finishers	139	Mechanical with neutral pressure (>60%)	15	Present (1) or absent (0)
Handling and nursing piglets (ear tagging, castrating, cutting tails)	116	Mixed type(including natural)	19	Present (1) or absent (0)
		Mechanical with negative pressure (>60%)	234	Ref
Inseminating	112	Mechanical with pit exhaust [a]	48	Continuous (10.3%)
Scanning	13	Heating		
Injection or handling sick animals	171	Floor heating (>50%)	168	Present (1) or absent (0)
Handling dead animals	93	Radiator heating (>50%)	63	Present (1) or absent (0)
Feed preparation and manual feeding	181	Floor type		
Automatic feeding (adjusting/inspecting)	138	Full slatted floor (>50%)	22	Present (1) or absent (0)
Bedding preparation and disposition		Mostly slatted (>50%)	101	Present (1) or absent (0)
Removing manure (in pens and stalls)	86	Mostly concrete	145	Ref
Sweeping or scraping corridors	54	Deep litter	38	Continuous (6.8 %)
Washing with high pressure	72	Showering (water) applied [a]	83	Continuous (42.8 %)
Disinfecting pens/stalls/stables	17	Feeding characteristics		
Repair and maintenance of animal buildings/feed room and installations	77	Dry feed (>80%)	121	Present (1) or absent (0)
		Dry and wet feed	50	Present (1) or absent (0)
Tasks outside animal areas		Wet feed (>80%)	97	Ref
Office work	35	Ad-libitum feeding method	157	Continuous (33.7 %)
Handling feed and seeds in barns and work relating to silos or drying plants	37	Hygienic conditions		
		Floor conditions		
Repairing/maintaining machinery & equipment (e.g. tractor, track, harvester)	48	Wet floor (>80%)	85	Present (1) or absent (0)
		Wet floor	83	Present (1) or absent (0)
Handling manure tanks and dunghills	4	Dry floor (>80%)	100	Ref
Work in the fields (working the soil, sowing, harvesting, applying fertilizers etc.)	15	Very dusty feeding path	73	Continuous (11.1 %)
		Very high dung accumulation	104	Continuous (11.3 %)
		Disinfected with bacterial agents (only endotoxin)	202	Continuous (63.6 %)

Abbreviations and explanations: n=Number of observations; [a] For all pig farmers included; [b] Only for workers with a full-indoor working shift and time spend dealing with the characteristics, cut-off level indicates the level of stable working time used to consider the characteristic present; ‡Median value of portion of time spend with the presence of a characteristic for continuous values estimated for positive values.

3.10 Methods of statistical analysis

Both dust and endotoxin exposure concentrations were log-transformed prior to all analysis due to a log-normal distribution (evaluated graphically and with Kolmogorov–Smirnov tests). Descriptive statistics concerning levels of exposure were given either as geometric means with geometric standard deviations or as medians and range. In Manuscript II non-parametric statistical methods were applied for comparisons between categorical or continuous covariates, whereas in Manuscripts I and III parametric methods were used. Variance component analysis and exploration of determinants of dust and endotoxin exposure were performed using linear mixed effect models. These analyses were performed with the worker and the farm id as random effects, and, in Manuscript III, with the exposure determinants as fixed effects. Finally, exposure-response relationships in Manuscript II were explored using logistic regression and generalized additive modelling (smoothing). All analysis was performed using SAS software version 9.2.

4 Summary of Results

The main results of the present work are summarised below in separate paragraphs for each of the three included manuscripts. Further details on these findings can be found in the manuscripts attached as appendices.

4.1 Manuscript I

Overall, 170 farm measurement visits were performed during the SUS exposure field study. The number of measurements performed and the number of workers participating along with the general measurement characteristics are shown in Table 9.

Table 9 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.

					Sampling duration, hour			
Farming type	**n**	**f**	**k**	**n/k**	**AM (SD)**	**Range**	**<LOD for dust, N**	**<LOD for endotoxin, N**
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 & 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

Abbreviations: 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.

The measured levels of dust and endotoxin exposure per type of production are summarized in Figure 5. In general, exposure was high in all four types of livestock farmers included; but measured exposure concentrations showed a large variation with a range between <LOD and 47.8 mg/m^3 for inhalable dust, and between <LOD and 374.600 EU/m^3 for endotoxin. Inhalable dust concentrations were highest in poultry farmers, followed by pig, mixed, mink and dairy farmers. The exposure pattern for endotoxin was relatively similar. The highest GM exposure was seen among pig farmers (1495 EU/m^3) and the lowest among mink farmers (214 EU/m^3). The Danish occupational exposure limit (OEL) for total dust (3 mg/m^3) was complied with only in mink farmers, whereas for endotoxin more than 93% of all our

measurements exceeded the recently proposed health-based exposure-limit for endotoxin (90 EU/m^3).

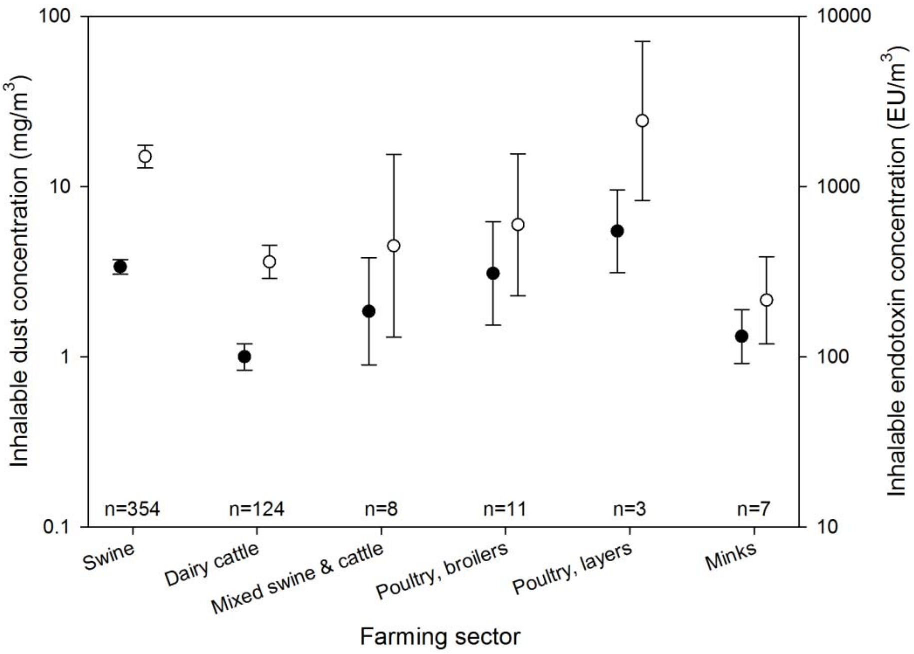

Figure 5 Inhalable dust (●) and endotoxin (○) exposure levels (geometric mean ± 95% confidence intervals) obtained by personal sampling in different types of Danish animal farms.

Seasonal patterns with higher measured dust and endotoxin levels during the summer season were observed among pig farmers. Among poultry broiler farmers the levels of exposure increased up to 10-folds between the 1st and the 5th week of the chicks' growth circle. On the contrary, the mink production circle was not characterized by any patterns in the measured personal levels of dust and endotoxin exposure (Figure 6).

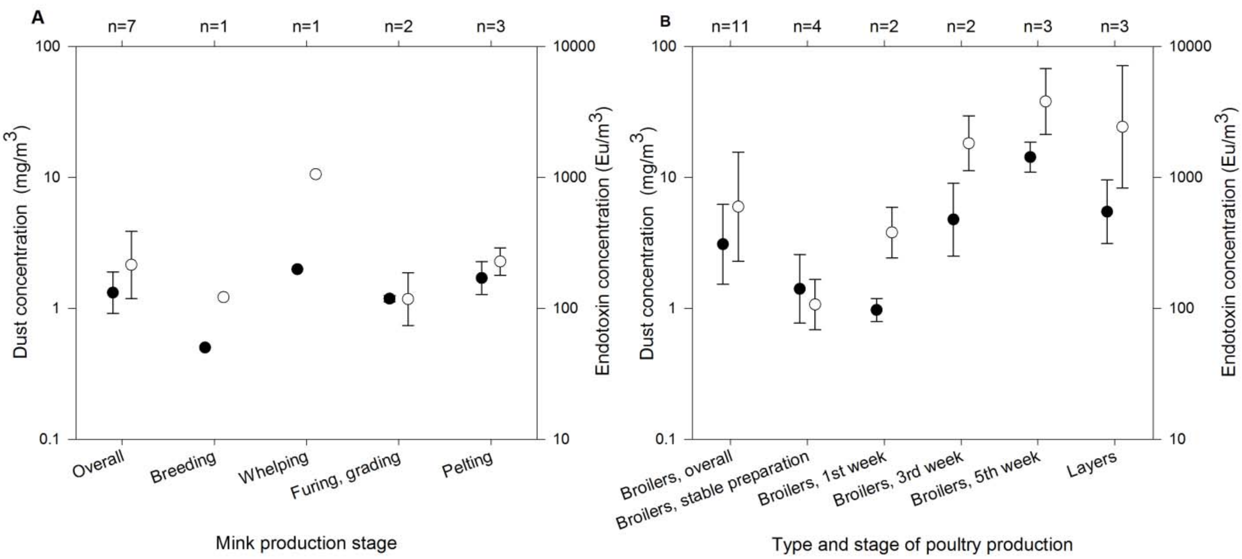

Figure 6 Inhalable dust (●) and endotoxin (○) concentrations (geometric mean ± 95% confidence intervals), measured in different stages of mink (A) and poultry (B) production farms.

Analysis of the variance components for dust and endotoxin among pig and cattle farmers showed a large within-worker variability compared to between-worker variability. Among cattle farmers' considerable differences in variation between workers were present for both dust and endotoxin. Differences in exposure between farms were small among pig farmers but substantial for cattle farmers. Grouping of farmers by their working environment (indoors, combined in- and outdoors, outdoors) showed a considerable increase in within-workers variability when moving from indoors to outdoors.

4.2 Manuscript II

Overall, the results of the pooled analysis in the different types of regression models used were consistent for most of the investigated health-endpoints. The results suggested current endotoxin exposure to be inversely associated with allergic sensitization and hay fever, and positively associated with organic dust toxic syndrome (ODTS) and chronic bronchitis (for levels above 100 EU/m^3). The effect of potential confounders (gender, age, farm childhood, family history, and smoking habits) was mostly small and results remain consistent even after adjustment for study. These findings were significant and are summarised, along with those for asthma and wheeze, in the smoothed curves shown in Figure 7.

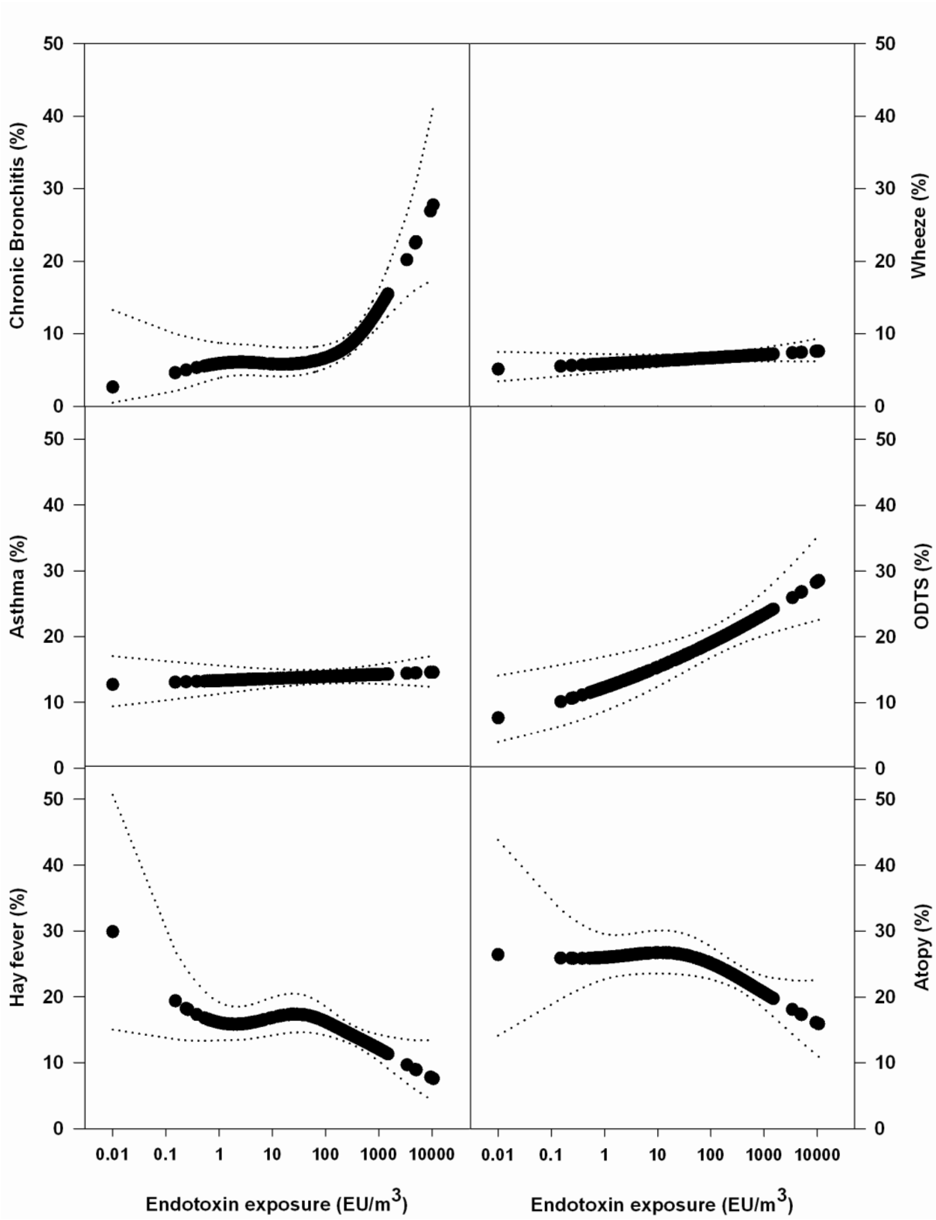

Figure 7 Smoothed relationships between endotoxin exposure, chronic bronchitis, wheeze, asthma, ODTS, hay fever and atopy for the pooled study population. ········ : ± 95% confidence intervals. Results are adjusted for gender, age, farm childhood, atopic predisposition, smoking and study.

Although that descriptive analysis showed considerable heterogeneity for both population characteristics and exposure across the four study populations included, no major differences in the estimated relationships for the sub-populations of agricultural industry workers, veterinary and farm apprentices were seen for most of the health outcomes under investigation. These three populations accounted for approximately 95% of the overall population.

Atopy was not a significant effect modifier for asthma and wheeze, and in stratified analysis there were no clear patterns for the relationships between endotoxin and those symptoms. However, when assessing the relationships between adult endotoxin exposure, farm childhood and health symptoms, the effect of endotoxin exposure on atopy depended on the presence of a farm childhood. In particular, among workers with a farm childhood there was no association between endotoxin exposure and atopy, but in workers without a farm childhood a negative dose dependent trend between endotoxin exposure and atopy was observed.

The results from analysis with farm childhood as the main exposure are shown in Table 10. As it can be seen, farm childhood was protective for chronic bronchitis, asthma and asthma symptoms, hay fever, self-reported allergy and atopy, not influenced by potential confounders, including endotoxin exposure.

Table 10 Univariate and multiple logistic regression analysis describing associations between farm childhood, and the health symptoms of interest.

Symptom	Univariate analysis		Model 1[a]		Model 1[b]	
	OR	95% CI	OR	95% CI	OR	95% CI
Chronic bronchitis	0.88	0.65 to 1.19	0.97	0.69 to 1.36	1.00	0.71 to 1.40
Wheezing	0.62	0.50 to 0.78	0.69	0.54 to 0.88	0.70	0.54 to 0.89
Asthma	0.61	0.51 to 0.73	0.71	0.58 to 0.88	0.72	0.59 to 0.89
Hay fever	0.50	0.40 to 0.62	0.63	0.50 to 0.79	0.63	0.50 to 0.79
Self-reported allergy	0.49	0.40 to 0.59	0.65	0.52 to 0.81	0.66	0.53 to 0.83
Atopy	0.61	0.50 to 0.74	0.63	0.51 to 0.77	0.62	0.51 to 0.77

[a] results are adjusted for study, gender, age (continuous), atopic predisposition and smoking habits.
[b] results are adjusted for study, gender, age (continuous), atopic predisposition, smoking habits and for current exposure to endotoxin.

4.3 Manuscript III

The results of the linear mixed effect analysis with tasks as fixed effects are presented in Table 11. Following stepwise regression, the working environment along with 11 distinct tasks explained 38% of the overall variability in dust exposure, and for endotoxin 28% was explained by the environment and 6 tasks. Strong exposure predictors for dust exposure included the “handling of feeding materials in barns and silos”, the “feed preparation and manual feeding”, and tasks related to the movement and handling of pigs. For endotoxin, apart from working tasks related to the feed barn, the high-pressure washing of stables was the strongest stable task related to an increased level of exposure. The performance of field work was the task explaining most of the variability in exposure.

Table 11 Effect of working activities (per 1 min) on the log-transformed personal level of exposure to dust (mg/m^3) and endotoxin (EU/m^3) among Danish pig farmers. Results estimated on the basis of 354 measurements performed in 231 farmers employed in 54 farms.

	n	**PPE (n)**	**MDN (min)**	**Dust**			**Endotoxin**		
				β	***e***	***p***	***β***	***e***	***p***
Naïve Model									
Intercept				1.2114	0.0616	<.0001	7.3074	0.0806	<.0001
${}_{bf}\sigma^2$				0.041	0.035	0.1168	0.002	0.049	0.484
${}_{bw}\sigma^2$				0.193	0.097	0.0195	0.184	0.218	0.2004
${}_{ww}\sigma^2$				0.663	0.088	<.0001	1.972	0.250	<.0001
Model with tasks and environment									
Intercept				0.4213	0.1726	0.0181	5.7989	0.2821	<.0001
Indoor working environment				0.0051	0.0019	0.0105	0.0160	0.0030	<.0001
Moving breeding animals	111	3	55	0.0019	0.0009	0.048			
Moving weaners and finishing pigs	139	4	30	0.0023	0.0007	0.0007	0.0024	0.0010	0.0198
Handling and nursing piglets (ear tagging, castrating, cutting tails etc.)	116	5	90	0.0019	0.0006	0.0028			
Injection or handling sick animals	171	6	45	0.0022	0.0009	0.0209			
Feed preparation and manual feeding	181	10	40	0.0033	0.0012	0.0094			
Washing with high pressure	72	9	90				0.0045	0.0011	<.0001
Disinfection	17	4	30				-0.0178	0.0046	0.0002
Repair and maintenance of animal buildings/feed room and stable installations	48	0	30	0.0020	0.0007	0.0031			
Handling feed and seeds in barns and work relating to silos or drying plants	37	5	40	0.0070	0.0012	<.0001	0.0053	0.0019	0.0062
Work in the fields (working the soil, sowing, harvesting, applying fertilizers)	15	0	210	-0.0049	0.0009	<.0001	-0.006	0.0014	<.0001
Office work	35	0	60	-0.0043	0.001	<.0001	-0.0048	0.0016	0.004
${}_{bf}\sigma^2$				0.029	0.0224	0.0952	0.113	0.0638	0.0382
${}_{bw}\sigma^2$				0.058	0.0628	0.1763	0		
${}_{ww}\sigma^2$				0.490	0.0657	<.0001	1.438	0.1164	<.0001
Explained within worker variability				26%			28%		
Explained total variability				36%			28%		

Abbreviations and explanations: n=number of observations; PPE=number of cases reported for use of personal protection equipment; MDN=median time spent on an activity estimated only for positive responses on the day of the measurements; *β*=regression coefficient; *e*=standard error; *p*=p-value; ${}_{bf}\sigma^2$=between-farm variance; ${}_{bw}\sigma^2$=between-worker (within-farms) variance; ${}_{ww}\sigma^2$=within-worker (day-to-day) variance.

An analysis in the sub-population of indoor workers on the effect of farm characteristics resulted in 2 relatively small models that however were able to explain almost all of the variability in exposure between farms (Table 12). The use of dry feed was the most influential stable characteristic for both dust and endotoxin exposure. Other important factors associated with an increase in the levels of exposure included the use of an ad-libitum feeding system and the type of ventilation for dust, and for endotoxin the slatted floor coverage. The type of animal housing used seemed also to be of importance.

Table 12 Mixed effect models results on determinants of log-transformed personal dust (mg/m^3) and endotoxin (EU/m^3) exposure among indoor pig farmers. All characteristics are estimated on the worker level.

	Dust			**Endotoxin**		
	β	***e***	***p***	***β***	***e***	***p***
Naïve Model						
Intercept	1.3923	0.0584	<.0001	7.5224	0.0801	<.0001
$_{bf}\sigma^2$	0.053	0.0315	0.0469	0.056	0.062	0.1836
$_{bw}\sigma^2$	0.094	0.0673	0.0806	0		
$_{ww}\sigma^2$	0.406	0.0654	<.0001	1.325	0.124	<.0001
Model with determinants						
Intercept	1.3077	0.1109	<.0001	7.4371	0.2014	<.0001
Outdoor temperature	-0.025	0.0048	<.0001	-0.0408	0.0085	<.0001
Ventilation (1/0)						
Mostly neutral pressure	0.3582	0.1849	0.0563			
Mixed type (incl. natural)	0.2640	0.1641	0.1116			
Mostly negative pressure	Ref					
Feed type (1/0)						
Dry	0.4296	0.113	0.0003	0.5568	0.1693	0.0015
Dry and wet	0.3577	0.1308	0.0077	0.6996	0.2056	0.001
Wet	Ref			Ref		
Ad-libitum feeding system[a]	0.0046	0.0016	0.0054			
Floor type (1/0)						
Full slatted				0.6146	0.2592	0.0201
Mostly slatted				0.2086	0.1507	0.17
Mostly concrete				Ref		
Floor condition (1/0)						
Wet floor	-0.249	0.1107	0.0275			
Mixed floor condition	-0.057	0.093	0.5448			
Dry floor	Ref					
$_{bf}\sigma^2$	0.010	0.0242	0.337	0		
$_{bw}\sigma^2$	0.117	0.0558	0.0179	0.082	0.1566	0.3013
$_{ww}\sigma^2$	0.304	0.0495	<.0001	1.118	0.1779	<.0001
Explained bf variability	81%			100%		
Explained total variability	23%			9%		

Abbreviations and explanations: [a] per portion (1%) of overall time spend on the presence of a characteristic; *β*=regression coefficient; *e*=standard error; *p*=p-value; $_{bf}\sigma^2$=between-farm variance; $_{bw}\sigma^2$=between-worker (within-farms) variance; $_{ww}\sigma^2$=within-worker (day-to-day) variance.

5 Discussion

The present work covers the foundation and the basic exposure assessment principals applied within the exposure part of the SUS cohort; a work that was initiated in recognition of the potential of the study to allow a longitudinal evaluation of the effects of the farm environment on allergy and lung function as well as to the different phenotypes of asthma.

In the first manuscript using well established methodologies we explored the levels of dust and endotoxin that Danish animal farmers are exposed to. We reported dust and endotoxin levels among pig, poultry, and cattle farmers in ranges similar to previous studies that used comparable sampling[17,81] and analytical methodologies.[81] For mink farmers, though, there are no previous studies describing the levels of dust or endotoxin exposure, neither on a personal nor on a stationary level. The large reported number of samples above the currently available occupational exposure limits for dust and endotoxin suggest that, despite the long-running discussion on the health effects of farming,[12,18,31] animal farmers remain exposed to potentially hazardous dust and endotoxin concentrations. This implies that the development of effective workplace exposure control and prevention strategies, including a wider adaptation of proven methods of exposure reduction (e.g. ionization, spraying of colza oil etc), optimization of the management practices and educational training of farmers (e.g. on the use of respiratory protection), is of outmost importance. Besides the high levels of dust and endotoxin exposure, the study results quantitatively demonstrated the presence of high variability in personal exposure among pig, cattle and poultry farmers. For poultry broiler farmers, in consistency with previous publications,[95,142,143] this study clearly showed that the level of dust and endotoxin exposure is rising with the age of the chicks; a finding that can most likely be attributed to the increasing deterioration of the hygienic conditions inside the broiler houses within the growth circle, as previously reported.[132] In contrast, for mink farmers, the task-based assessment approach that we applied showed absence of considerable variations in exposure between the examined production stages. This could partly be a result of the widely open and natural ventilated mink sheds; though, the similarity in measured levels within pure production stages (breeding, furring, grading) and pelting, which was performed in a completely enclosed environment, argues against this explanation.

The observed large within-workers variability for pig and cattle farmers, apart from demonstrating a large potential for measurement error, supports our decision on using a modelling based approach for assessment of exposure within our study. As thoroughly discussed in Manuscript I, if we solely based our future analysis on the measured exposure levels we should expect, for example, an attenuation of at least 50% in the estimated health associations for dust and 80% for endotoxin given our variability estimates among pig farmers.

Our focus on farms located in Jutland, together with the applied selection of pig farmers that was stratified by size, and the relatively low number of collected measurements for mink and poultry farmers can be considered as potential limitations in our study. However, our decision to concentrate the investigation in Jutland was taken after considering the distribution of farms in our study, and the similarities to the overall farm distribution in Denmark; less than 10% of the Danish farms involved in production and rearing of pigs and dairy cows are located in Zealand.[144]

Our decision to sample pig farms based on their size distribution was taken at the start of the study with the aim to have a final sample of 75 farms with an equal size representation. For every drop-out a replacement was supposed to be drawn. Nevertheless, this was not fullfilled due to logistics and due to more power (more included subjects pr. farm) than expected. To clarify if selection bias took place a sensitivity analysis was performed, which compared the final size distributions to the initial sample as well as the derived personal dust and endotoxin levels across the different farm size strata. Both comparisons showed no statistically significant differences in the given distributions, thereby suggesting that such bias was unlikely to have occurred.

The relatively small numbers of measurements and the sampling strategy that we applied for mink and poultry farmers were decided on the basis of the small number of mink and poultry farmers in our study population, and aiming on a final exposure assessment using a job-exposure matrix. Stages included in the monitoring of mink farmers were selected taking into account the differences in the number of housed animals between the beginning and the end of the production cycle, as well as the intense animal handling required during the whelping and weaning stages. For broiler poultry farmers a similar approach was taken based on the literature that reported differences in exposure during the chicks' growth cycle.[95,120]

In the second Manuscript, the exposure level results are used to estimate dust and endotoxin exposure in the baseline SUS population. A pooled analysis is then established to explore the health effects of endotoxin exposure in adulthood taking exposures that occurred during childhood into consideration. Strengths of the study include an increased power to explore exposure-response relationships and the chance to assess heterogeneity across different populations using a standardised setting.

The study results suggest a dual-effect of endotoxin exposure in adulthood by showing a strong protective effect of occupational endotoxin exposure on allergic sensitization and hay fever and at the same time an increase in the risk for organic dust toxic syndrome and chronic bronchitis. The inverse associations for atopy and hay fever observed among agricultural workers and veterinary and farming school students compared to the biofuel workers suggest that the protective effects of endotoxin might be stronger when the exposure is agriculturally related. Nevertheless, that specific conclusion is limited to an indication due to the small size of the included population of biofuel workers and the much lower exposure levels that they experience. An earlier study among Dutch pig farmers showed inverse dose-response relationships between endotoxin exposure and allergic sensitization consistent with an increased risk for bronchial hyperresponsiveness.[51] Similar findings were reported among agricultural industry workers,[27,28] and Eduard et al., [50] in a study among Norwegian farmers, found endotoxin exposure to decrease the risk of atopic asthma but to increase the risk of non-atopic asthma. These results show the presence of diverse responses to endotoxin exposure especially in relation to atopic status.

We tried to confirm the role of atopy by applying analysis for endotoxin and asthma symptoms stratified by atopic status. In contrast to the Norwegian study, we did not observe an exposure-response relationship between endotoxin and atopic wheeze despite the increased power that we applied. Potential explanations for this discrepancy between the two studies could be population exposure differences (i.e. type of endotoxin, type of organic dust, and level of exposure) as well as methodological and analytical differences between the two studies (i.e. differences in the exposure assessment strategies, cut-off levels, and in asthma and atopy definitions used). However, the interpretations are further complicated by the lack of clarification of the relevance of current exposure among subjects with a farm childhood.

In our study farm childhood is suggested as being protective against allergic sensitization and allergic disease during both childhood and adulthood. However, information on farm childhood was not available in the studies among Norwegian[50] and Dutch[51] farmers. In another study, Dutch agricultural industry workers with a farm childhood were reported to have a lower prevalence of hay fever compared to those without a farm childhood.[27] In addition, in the same population a strong decrease in allergic sensitization with endotoxin exposure between 35 and 1000 EU/m^3 was observed, but only among individuals without a farm childhood.[28] For individuals with a farm childhood the prevalence of allergic sensitization remain constantly low irrespectively of the level of endotoxin exposure. In our pooled analysis, farm childhood showed strong protective associations against both allergic and asthma symptoms, and when stratified by farm childhood the results confirmed those reported in the Dutch agricultural industry workers study.

These findings support the relevance of current endotoxin exposure with respect to the protective effects against sensitization. One should not, in general, expect large decreases in symptom occurrence among persons that already experienced the beneficial effects of exposure during a farm childhood. However, it could simply be the result of a healthy worker selection. The young age and the short time since commencement of exposure to endotoxin for the majority of our study population contradicts the presence of a large effect of such bias, but still the possibility of occurrence cannot be excluded. Given the limitations of the self-reported exposure to farming during childhood, the importance of adult exposure in the presence of early life exposures to farming can be properly addressed only in studies with longitudinal designs. These should include studies in workers with measured exposure during childhood and a follow-up of both health and exposure since the beginning of their farming carriers.

Besides the possibility of selection bias, the pooled analysis could be subject to further limitations considering the demonstrated heterogeneity in the demographic and exposure characteristics among the four included populations, the use of a combined skin-prick and specific IgE atopy definition, and the possibility of exposure misclassification within the SUS and the biofuled worker studies. Thorough discussions on these issues are given in Manuscript II.

The study presented in Manuscript III is an integrated part of the SUS exposure assessment process; it is the first steps of modelling development, and an initial response to the results of Manuscript I, which suggested farmers to be exposed to high levels of dust and endotoxin consistent with an increased risk for respiratory disease. These findings were in spite of the current industrialization and relatively large degree of automation applied on many working processes inside and even outside concentrated animal feeding operations (e.g. use of milking robots in dairy production, computer controlled or robot delivered feeding systems etc.).

The mixed effect models exploring relationships between personal exposure and different working tasks performed by the farmers suggest the environment to play a dominant role on the demonstrated exposure variability. Indoor work in relation to task with active animals or to feed handling in storage areas is postulated as the main source of exposure, whereas field working tasks are shown to be associated to lower exposure levels for both dust and endotoxin. These findings are not unexpected given the previous results from studies among Polish[145] and American[89] farmers. It is very likely that a similar analysis on working tasks for cattle farmers in our study population will provide, at least to a certain extent, similar results.

For stable characteristic our analysis demonstrated the applied feeding practices (feeding type and method) and the type of ventilation to be determinants of dust exposure and the feeding type and the slatted floor coverage for endotoxin exposure. The most important exposure determinant though was the type of feed, which explained all the given between-farms variability for both dust and endotoxin exposure. These findings, although that they do not give clear clues for the development of exposure control strategies, demonstrate the potential of our data to allow for a breakthrough on the identification of determinants of personal exposure. This exercise is very important given that most of the currently available information is derived from studies performed in the 1980s and 1990s. Since then, the pig production and, in general, the production of all livestock animals has evolved with new stable designs and new technologies implemented.

From an epidemiological prospect, these observations have a considerable impact on our study design and exposure assignment as they contain valuable information for the future planning and performance of the modelling for pig farmers. Given the possibility of a group-based approach, these findings will imply that the maximal contrast among pig farmers can possibly be obtained by a grouping strategy based on scale of involvement in outdoor activities and when indoor workers based on the type of feed used in the farm and their involvement in certain animal related tasks with high exposure including feed storage activities. Given the small number of included determinants and the exploratory nature of our study the findings on stable characteristics can be considered only as indicative considering their prevention and exposure control prospective. They suggest feeding practices, ventilation, flooring, and hygienic conditions to be potential areas for further investigation. Contrary to stable characteristics, the derived information from the tasks model provide vital information with regard to the development of prevention strategies based on the use of Personal Protective Equipment (PPE). Such strategies have been recently advertised as a cost-effective way for reducing the health effects on workers inside animal buildings.[25,146]

The external and internal validity of the specific study are subject to the same issues concerning the population representativeness and the possibility of selection bias as the whole SUS exposure assessment study. Moreover, our results, for both working tasks and stable characteristics, showed good agreement with the literature; especially in relation to the study of Preller et al.,[79] which is the most comprehensive existing observational evaluation of determinants of personal exposure to dust and endotoxin in pig farmers.

The modelling process was limited to two separate models for (a) working tasks and (b) stable characteristics. Such an approach ignores the fact that processes occur under certain working and environmental conditions. The variability in exposure is determined by several factors including human behaviour, working processes and spatial workplace characteristics. However, all our estimations, including dust measurements and calculations for stable characteristics, were performed on the personal level. Most previous studies on determinants in farming populations have been either experimental or source-oriented using stationary sampling. In general, stationary sampling tends to underestimate the personal level of exposure, and when a worker is not included the effect of the human factor cannot be determined.[134] For pig farmers the identification of exposure determinants is further complicated by their very alternating working tasks and their tendency to work within several workplaces that usually bear different characteristics. As a result, task-based sampling approaches become inefficient because of the involved small time intervals per task and department.[79] Nevertheless, a combined model could provide us with essential information regarding the development of effective exposure control strategies, and its development is included in our future scopes.

6 Concluding remarks and perspectives

The SUS exposure assessment is one of the largest studies on personal bioaerosol exposure of livestock farmers, and for Denmark the first comprehensive attempt within almost two decades to document the exposure status of livestock farmers. The measured levels of dust and endotoxin exposure show that, in spite of 30 years long debates and scientific interest, livestock farmers in Denmark remain highly exposed to hazardous dust and endotoxin exposure concentrations. Also from an international perspective these findings stress the need for a re-evaluation of the exposure conditions in livestock environments. Most of the information available on dust and endotoxin exposure and its determinants in livestock environments is derived from studies performed during the 1980s and 1990s. During the last two decades production has intensified, farming practices as well as used technologies have been altered, and new legislations have been implemented (mainly to improve animal welfare).

Our preliminary results on working tasks can be a tool for Danish authorities and agricultural advisors for an initial action plan based on the promotion of use of protective equipment; an action that seems necessary given our measured levels of exposure. Overall, our analysis on determinants has the potential to provide us with valuable information for the development of preventive initiatives including exposure control strategies. This will eventually lead to a safer working environment for the farmers with a reduced risk for respiratory symptoms. For this purpose the developed models for pig farmers will have to be extended by including working tasks and information on further determinants including the use of disinfectants, manure handling, animal intensity, and even animal housing. Different model building strategies and data mining approaches will have to be considered in order to optimize the ability to recognise factors of importance. An extension of the analysis for determinants on the sub-population of cattle farmers has also to be performed. The specific research activity is of highly importance given the currently limited information available.

The SUS exposure assessment was initiated with the aim to provide the cohort with valid exposure estimates, and thereby to increase the study's potential to identify exposures driving the harmful and beneficial effects of farming. The pooled GABRIEL analysis comprises the first use of the derived exposure estimates. The specific study serves as a good example of the potential strengths of performing well powered analysis by combing good quality exposure and health data. Its results confirmed the currently available literature, whereas the pooling exercise and standardization of the study-specific methodologies enabled the finalization and validation of the initial GABRIEL industrial cohorts' database. With the addition of collected lung function and genetic data the analysis can proceed to its next analytical phase, which given the current reported results appears to be very promising. The potential limitations of the study concerning differences in the applied health and exposure sampling methodologies indicate the need for a greater degree of standardization in future studies both in respect to the collected health and exposure data.

The identification of determinants of personal exposure will not directly lead to an accurate and valid exposure assessment to be used in the context of the SUS cohort. However, it is a step necessary to reveal the driving sources of exposure variability within farming populations. Such information is essential in order to develop the most efficient exposure assignment to be followed within the cohort. This implies an investigation on the variability distribution in different levels using different grouping. Furthermore, the determinant models will guide the development of a short and efficient questionnaire to be used to collect information that will eventually allow estimation of exposure for the whole population of the cohort. Finally, the estimation process for dust and endotoxin are a model for the SUS study for the analytical approach that will be followed for the rest of the exposures of interest including common allergens, glucans and *archae* bacteria.

7 References

1. Douwes J, Thorne P, Pearce N, Heederik D. Bioaerosol health effects and exposure assessment: progress and prospects. Ann Occup Hyg 2003;47:187-200.

2. Williams KL. Endotoxin Structure, Function, and Activity. In: Endotoxins:67-90.

3. Williams KL. Endotoxin Relevance and Control Overview. In: Endotoxins:27-45.

4. Sigsgaard T, Omland Ø, Thorne PS. Asthma-like diseases in agriculture. In: Sigsgaard T, Heederik D, eds. Occupational Asthma. Basel, Switzerland: Birkhauser Basel; 2010:163-83.

5. Nieuwenhuijsen MJ. Exposure assessment in occupational & environmental epidemiology. Oxford: Oxford University Press; 2003.

6. Burdorf A. Identification of determinants of exposure: consequences for measurement and control strategies. Occup Environ Med 2005;62:344-50.

7. Loomis D, Kromhout H. Exposure variability: concepts and applications in occupational epidemiology. Am J Ind Med 2004;45:113-22.

8. Kromhout H, van Tongeren M, Burstyn I. Design of Exposure Measurement Surveys and their Statistical Analyses. In: Occupational Hygiene: Blackwell Publishing Ltd; 2008:124-44.

9. Erridge C, Bennett-Guerrero E, Poxton IR. Structure and function of lipopolysaccharides. Microbes and Infection 2002;4:837-51.

10. Kauffmann F, Castro-Giner F, Smit LAM, Nadif R, Kogevinas M. Gene-environment interactions in occupational asthma. In: Sigsgaard T, Heederik D, eds. Occupational Asthma. Basel, Switzerland: Birkhauser Basel; 2010:205-28.

11. Braun-Fahrlander C. The role of the farm environment and animal contact for the development of asthma and allergies. Clin Exp Allergy 2001;31:1799-803.

12. Schenker MB, Christiani D, Cormier Y, et al. Respiratory health hazards in agriculture. Am J Resp Crit Care 1998;158:S1-S76.

13. Kromhout H, Heederik D. Effects of errors in the measurement of agricultural exposures. Scand J Work Environ Health 2005;31 Suppl 1:33-8; discussion 5-7.

14. Rask-Andersen A, Omland Ø. Bio-Aerosols and Organic Dusts. In: Vuyst P, Gevenois P, eds. Imaging of Occupational and Environmental Disorders of the Chest: Springer Berlin Heidelberg; 2006:31-66.

15. Donham KJ, Rubino M, Thedell TD, Kammermeyer J. Potential health hazards to agricultural workers in swine confinement buildings. J Occup Med 1977;19:383-7.

16. Donham KJ, Popendorf W, Palmgren U, Larsson L. Characterization of dusts collected from swine confinement buildings. Am J Ind Med 1986;10:294-7.

17. Radon K, Danuser B, Iversen M, et al. Air contaminants in different European farming environments. Ann Agric Environ Med 2002;9:41-8.

18. Omland O. Exposure and respiratory health in farming in temperate zones--a review of the literature. Ann Agric Environ Med 2002;9:119-36.

19. Heederik D, Sigsgaard T. Respiratory allergy in agricultural workers: recent developments. Curr Opin Allergy Clin Immunol 2005;5:129-34.

20. Radon K. The two sides of the "endotoxin coin". Occup Environ Med 2006;63:73-8.

21. Liebers V, Raulf-Heimsoth M, Bruning T. Health effects due to endotoxin inhalation (review). Arch Toxicol 2008;82:203-10.

22. Liebers V, Bruning T, Raulf-Heimsoth M. Occupational endotoxin-exposure and possible health effects on humans. Am J Ind Med 2006;49:474-91.

23. Sigsgaard T, Bonefeld-Jorgensen EC, Hoffmann HJ, Bonlokke J, Kruger T. Microbial cell wall agents as an occupational hazard. Toxicol Appl Pharmacol 2005;207:310-9.

24. Heederik D, Sigsgaard T, Thorne PS, et al. Health effects of airborne exposures from concentrated animal feeding operations. Environ Health Perspect 2007;115:298-302.

25. Donham KJ. Community and occupational health concerns in pork production: a review. J Anim Sci 2010;88:E102-11.

26. Eduard W, Pearce N, Douwes J. Chronic bronchitis, COPD, and lung function in farmers: the role of biological agents. Chest 2009;136:716-25.

27. Smit LA, Heederik D, Doekes G, Blom C, van Zweden I, Wouters IM. Exposure-response analysis of allergy and respiratory symptoms in endotoxin-exposed adults. Eur Respir J 2008;31:1241-8.

28. Smit LA, Heederik D, Doekes G, Lammers JW, Wouters IM. Occupational endotoxin exposure reduces the risk of atopic sensitization but increases the risk of bronchial hyperresponsiveness. Int Arch Allergy Immunol 2010;152:151-8.

29. Douwes J. (1-->3)-Beta-D-glucans and respiratory health: a review of the scientific evidence. Indoor Air 2005;15:160-9.

30. Zejda JE, Dosman JA. Respiratory disorders in agriculture. Tuber Lung Dis 1993;74:74-86.

31. Kirkhorn SR, Garry VF. Agricultural lung diseases. Environ Health Perspect 2000;108 Suppl 4:705-12.

32. Rylander R. Endotoxin and occupational airway disease. Curr Opin Allergy Clin Immunol 2006;6:62-6.

33. Preller L, Heederik D, Boleij JS, Vogelzang PF, Tielen MJ. Lung function and chronic respiratory symptoms of pig farmers: focus on exposure to endotoxins and ammonia and use of disinfectants. Occup Environ Med 1995;52:654-60.

34. Preller L, Doekes G, Heederik D, Vermeulen R, Vogelzang PF, Boleij JS. Disinfectant use as a risk factor for atopic sensitization and symptoms consistent with asthma: an epidemiological study. Eur Respir J 1996;9:1407-13.

35. Braun-Fahrlander C, Gassner M, Grize L, et al. Prevalence of hay fever and allergic sensitization in farmer's children and their peers living in the same rural community. SCARPOL team. Swiss Study on Childhood Allergy and Respiratory Symptoms with Respect to Air Pollution. Clin Exp Allergy 1999;29:28-34.

36. Von Ehrenstein OS, Von Mutius E, Illi S, Baumann L, Bohm O, von Kries R. Reduced risk of hay fever and asthma among children of farmers. Clin Exp Allergy 2000;30:187-93.

37. Riedler J, Eder W, Oberfeld G, Schreuer M. Austrian children living on a farm have less hay fever, asthma and allergic sensitization. Clin Exp Allergy 2000;30:194-200.

38. von Mutius E, Braun-Fahrlander C, Schierl R, et al. Exposure to endotoxin or other bacterial components might protect against the development of atopy. Clin Exp Allergy 2000;30:1230-4.

39. Gereda JE, Leung DY, Thatayatikom A, et al. Relation between house-dust endotoxin exposure, type 1 T-cell development, and allergen sensitisation in infants at high risk of asthma. Lancet 2000;355:1680-3.

40. Braun-Fahrlander C, Riedler J, Herz U, et al. Environmental exposure to endotoxin and its relation to asthma in school-age children. N Engl J Med 2002;347:869-77.

41. Douwes J, van Strien R, Doekes G, et al. Does early indoor microbial exposure reduce the risk of asthma? The Prevention and Incidence of Asthma and Mite Allergy birth cohort study. J Allergy Clin Immunol 2006;117:1067-73.

42. Sordillo JE, Hoffman EB, Celedon JC, Litonjua AA, Milton DK, Gold DR. Multiple microbial exposures in the home may protect against asthma or allergy in childhood. Clin Exp Allergy 2010;40:902-10.

43. Ernst P, Cormier Y. Relative scarcity of asthma and atopy among rural adolescents raised on a farm. Am J Respir Crit Care Med 2000;161:1563-6.

44. Kauffmann F, Oryszczyn MP, Maccario J. The protective role of country living on skin prick tests, immunoglobulin E and asthma in adults from the Epidemiological study on the Genetics and Environment of Asthma, bronchial hyper-responsiveness and atopy. Clin Exp Allergy 2002;32:379-86.

45. Koskela HO, Happonen KK, Remes ST, Pekkanen J. Effect of farming environment on sensitisation to allergens continues after childhood. Occup Environ Med 2005;62:607-11.

46. Radon K, Schulze A, Nowak D. Inverse association between farm animal contact and respiratory allergies in adulthood: protection, underreporting or selection? Allergy 2006;61:443-6.

47. Portengen L, Sigsgaard T, Omland O, Hjort C, Heederik D, Doekes G. Low prevalence of atopy in young Danish farmers and farming students born and raised on a farm. Clin Exp Allergy 2002;32:247-53.

48. Smit LA, Zuurbier M, Doekes G, Wouters IM, Heederik D, Douwes J. Hay fever and asthma symptoms in conventional and organic farmers in The Netherlands. Occup Environ Med 2007;64:101-7.

49. Douwes J, Travier N, Huang K, et al. Lifelong farm exposure may strongly reduce the risk of asthma in adults. Allergy 2007;62:1158-65.

50. Eduard W, Douwes J, Omenaas E, Heederik D. Do farming exposures cause or prevent asthma? Results from a study of adult Norwegian farmers. Thorax 2004;59:381-6.

51. Portengen L, Preller L, Tielen M, Doekes G, Heederik D. Endotoxin exposure and atopic sensitization in adult pig farmers. J Allergy Clin Immunol 2005;115:797-802.

52. Yang IA, Savarimuthu S, Kim ST, Holloway JW, Bell SC, Fong KM. Gene-environmental interaction in asthma. Curr Opin Allergy Clin Immunol 2007;7:75-82.

53. Holloway JW, Yang IA, Holgate ST. Genetics of allergic disease. J Allergy Clin Immunol 2010;125:S81-94.

54. Castro-Giner F, Kauffmann F, de Cid R, Kogevinas M. Gene–environment interactions in asthma. Occupational and Environmental Medicine 2006;63:776-86.

55. von Mutius E. Gene-environment interactions in asthma. J Allergy Clin Immun 2009;123:3-11.

56. Smit LA, Heederik D, Doekes G, et al. Endotoxin exposure, CD14 and wheeze among farmers: a gene-environment interaction. Occup Environ Med 2011.

57. Smit LA, Bongers SI, Ruven HJ, et al. Atopy and new-onset asthma in young Danish farmers and CD14, TLR2, and TLR4 genetic polymorphisms: a nested case-control study. Clin Exp Allergy 2007;37:1602-8.

58. LeVan TD, Von Essen S, Romberger DJ, et al. Polymorphisms in the CD14 Gene Associated with Pulmonary Function in Farmers. Am J Respir Crit Care Med 2005;171:773-9.

59. Sigsgaard T, Brandslund I, Rasmussen JB, Lund ED, Varming H. Low normal alpha-1-antitrypsin serum concentrations and MZ-phenotype are associated with byssinosis and familial allergy in cotton mill workers. Pharmacogenetics 1994;4:135-41.

60. Elholm G, Omland O, Schlunssen V, Hjort C, Basinas I, Sigsgaard T. The cohort of young Danish farmers - A longitudinal study of the health effects of farming exposure. Clin Epidemiol 2010;2:45-50.

61. Pedersen OF, Rasmussen TR, Omland O, Sigsgaard T, Quanjer PH, Miller MR. Peak expiratory flow and the resistance of the mini-wright peak flow meter. Eur Respir J 1996;9:828-33.

62. Sigsgaard T, Hjort C, Omland O, Miller MR, Pedersen OF. Respiratory health and allergy among young farmers and non-farming rural males in Denmark: the SUS study. J Agromedicine 2004;9:223-38.

63. Omland O, Sigsgaard T, Hjort C, Pedersen OF, Miller MR. Lung status in young Danish rurals: the effect of farming exposure on asthma-like symptoms and lung function. Eur Respir J 1999;13:31-7.

64. Omland O, Sigsgaard T, Pedersen OF, Miller MR. The shape of the maximum expiratory flow-volume curve reflects exposure in farming. Ann Agric Environ Med 2000;7:71-8.

65. Sigsgaard T, Brandslund I, Omland O, et al. S and Z alpha1-antitrypsin alleles are risk factors for bronchial hyperresponsiveness in young farmers: an example of gene/environment interaction. Eur Respir J 2000;16:50-5.

66. Omland O, Miller MR, Sigsgaard T, Pedersen OF. The short-term repeatability of histamine bronchial testing in young males. The SUS study. Respir Med 2001;95:287-91.

67. Omland O, Deguchi Y, Sigsgaard T, Hansen JC. Selenium serum and urine is associated to mild asthma and atopy. The SUS study. J Trace Elem Med Biol 2002;16:123-7.

68. Miller MR, Sigsgaard T, Omland O, Pedersen OF. Time domain and flow indices of bronchial hyperresponsiveness: association with asthma symptoms, atopy and smoking. Eur Respir J 2002;20:86-91.

69. Hoffmann HJ, Iversen M, Brandslund I, et al. Plasma C3d levels of young farmers correlate with respirable dust exposure levels during normal work in swine confinement buildings. Ann Agric Environ Med 2003;10:53-60.

70. Hoffmann HJ, Iversen M, Takai H, Sigsgaard T, Omland O, Dahl R. Exposure to work-related levels of swine dust up-regulates CD106 on human alveolar macrophages. Am J Ind Med 2004;46:378-80.

71. Hoffmann HJ, Iversen M, Sigsgaard T, et al. A single exposure to organic dust of non-naive non-exposed volunteers induces long-lasting symptoms of endotoxin tolerance. Int Arch Allergy Immunol 2005;138:121-6.

72. Haglind P, Rylander R. Occupational exposure and lung function measurements among workers in swine confinement buildings. J Occup Med 1987;29:904-7.

73. Holness DL, O'Blenis EL, Sass-Kortsak A, Pilger C, Nethercott JR. Respiratory effects and dust exposures in hog confinement farming. Am J Ind Med 1987;11:571-80.

74. Donham K, Haglind P, Peterson Y, Rylander R, Belin L. Environmental and health studies of farm workers in Swedish swine confinement buildings. Br J Ind Med 1989;46:31-7.

75. Christensen H, Vinzents P, Nielsen BH, Finsen L, Pedersen MB, Sjøgaard G. Occupational exposures and health among Danish farmers working in swine confinement buildings. Int J Industr Ergo 1992;48:6.

76. Vinzents P, Nielsen BH. Variations in exposures to dust and endotoxin in Danish piggeries. Am Ind Hyg Assoc J 1992;53:237-41.

77. Choudat D, Goehen M, Korobaeff M, Boulet A, Dewitte JD, Martin MH. Respiratory symptoms and bronchial reactivity among pig and dairy farmers. Scand J Work Environ Health 1994;20:48-54.

78. Donham KJ, Reynolds SJ, Whitten P, Merchant JA, Burmeister L, Popendorf WJ. Respiratory dysfunction in swine production facility workers: dose-response relationships of environmental exposures and pulmonary function. Am J Ind Med 1995;27:405-18.

79. Preller L, Heederik D, Kromhout H, Boleij JS, Tielen MJ. Determinants of dust and endotoxin exposure of pig farmers: development of a control strategy using empirical modelling. Ann Occup Hyg 1995;39:545-57.

80. Simpson JC, Niven RM, Pickering CA, Oldham LA, Fletcher AM, Francis HC. Comparative personal exposures to organic dusts and endotoxin. Ann Occup Hyg 1999;43:107-15.

81. Spaan S, Wouters IM, Oosting I, Doekes G, Heederik D. Exposure to inhalable dust and endotoxins in agricultural industries. J Environ Monit 2006;8:63-72.

82. Melbostad E, Eduard W. Organic dust-related respiratory and eye irritation in Norwegian farmers. Am J Ind Med 2001;39:209-17.

83. Mc Donnell PE, Coggins MA, Hogan VJ, Fleming GT. Exposure assessment of airborne contaminants in the indoor environment of Irish swine farms. Ann Agric Environ Med 2008;15:323-6.

84. Kim KY, Ko HJ, Kim YS, Kim CN. Assessment of Korean farmer's exposure level to dust in pig buildings. Ann Agric Environ Med 2008;15:51-8.

85. Bonlokke JH, Meriaux A, Duchaine C, Godbout S, Cormier Y. Seasonal variations in work-related health effects in swine farm workers. Ann Agric Environ Med 2009;16:43-52.

86. O'Shaughnessy PT, Donham KJ, Peters TM, Taylor C, Altmaier R, Kelly KM. A task-specific assessment of Swine worker exposure to airborne dust. J Occup Environ Hyg 2010;7:7-13.

87. Virtanen T, Vilhunen P, Husman K, Happonen P, Mantyjarvi R. Level of airborne bovine epithelial antigen in Finnish cowsheds. Int Arch Occup Environ Health 1988;60:355-60.

88. Kullman GJ, Thorne PS, Waldron PF, et al. Organic dust exposures from work in dairy barns. Am Ind Hyg Assoc J 1998;59:403-13.

89. Nieuwenhuijsen MJ, Noderer KS, Schenker MB, Vallyathan V, Olenchock S. Personal exposure to dust, endotoxin and crystalline silica in California agriculture. Ann Occup Hyg 1999;43:35-42.

90. Berger I, Schierl R, Ochmann U, Egger U, Scharrer E, Nowak D. Concentrations of dust, allergens and endotoxin in stables, living rooms and mattresses from cattle farmers in southern Bavaria. Ann Agric Environ Med 2005;12:101-7.

91. Firth H, Herbison P, Mc Bride D. Dust and noise exposures among farmers in Southland,New Zealand. Int J Environ Health Res 2006;16:155-61.

92. Saito R, Cranmer BK, Tessari JD, et al. Recombinant factor C (rFC) assay and gas chromatography/mass spectrometry (GC/MS) analysis of endotoxin variability in four agricultural dusts. Ann Occup Hyg 2009;53:713-22.

93. Burch JB, Svendsen E, Siegel PD, et al. Endotoxin exposure and inflammation markers among agricultural workers in Colorado and Nebraska. J Toxicol Environ Health A 2010;73:5-22.

94. Lenhart SW, Morris PD, Akin RE, Olenchock SA, Service WS, Boone WP. Organic Dust, Endotoxin, and Ammonia Exposures in the North Carolina Poultry Processing Industry. Appl Occup Environ Hyg 1990;5:611-18.

95. Golbabaei F, Islami F. Evaluation of workers' exposure to dust, ammonia and endotoxin in poultry industries at the province of Isfahan, Iran. Ind Health 2000;38:41-6.

96. Donham KJ, Cumro D, Reynolds S. Synergistic effects of dust and ammonia on the occupational health effects of poultry production workers. J Agromedicine 2002;8:57-76.

97. Whyte RT. Occupational exposure of poultry stockmen in current barn systems for egg production in the United Kingdom. Br Poult Sci 2002;43:364-73.

98. Kirychuk SP, Dosman JA, Reynolds SJ, et al. Total dust and endotoxin in poultry operations: comparison between cage and floor housing and respiratory effects in workers. J Occup Environ Med 2006;48:741-8.

99. Douwes J, Versloot P, Hollander A, Heederik D, Doekes G. Influence of various dust sampling and extraction methods on the measurement of airborne endotoxin. Appl Environ Microbiol 1995;61:1763-9.

100. Spaan S, Doekes G, Heederik D, Thorne PS, Wouters IM. Effect of extraction and assay media on analysis of airborne endotoxin. Appl Environ Microbiol 2008;74:3804-11.

101. Spaan S, Heederik DJ, Thorne PS, Wouters IM. Optimization of airborne endotoxin exposure assessment: effects of filter type, transport conditions, extraction solutions, and storage of samples and extracts. Appl Environ Microbiol 2007;73:6134-43.

102. Attwood P, Brouwer R, Ruigewaard P, et al. A study of the relationship between airborne contaminants and environmental factors in Dutch swine confinement buildings. Am Ind Hyg Assoc J 1987;48:745-51.

103. Heber AJ, Stroik M, Nelssen JL, Nichols DA. Influence of Environmental-Factors on Concentrations and Inorganic Content of Aerial Dust in Swine Finishing Buildings. T Asae 1988;31:875-81.

104. Preller L, Kromhout H, Heederik D, Tielen MJ. Modeling long-term average exposure in occupational exposure-response analysis. Scand J Work Environ Health 1995;21:504-12.

105. Clark S, Rylander R, Larsson L. Airborne bacteria, endotoxin and fungi in dust in poultry and swine confinement buildings. Am Ind Hyg Assoc J 1983;44:537-41.

106. Donham KJ, Scallon LJ, Popendorf W, Treuhaft MW, Roberts RC. Characterization of dusts collected from swine confinement buildings. Am Ind Hyg Assoc J 1986;47:404-10.

107. Attwood P, Versloot P, Heederik D, de Wit R, Boleij JS. Assessment of dust and endotoxin levels in the working environment of Dutch pig farmers: a preliminary study. Ann Occup Hyg 1986;30:201-8.

108. Cormier Y, Tremblay G, Meriaux A, Brochu G, Lavoie J. Airborne microbial contents in two types of swine confinement buildings in Quebec. Am Ind Hyg Assoc J 1990;51:304-9.

109. Crook B, Robertson JF, Glass SA, Botheroyd EM, Lacey J, Topping MD. Airborne dust, ammonia, microorganisms, and antigens in pig confinement houses and the respiratory health of exposed farm workers. Am Ind Hyg Assoc J 1991;52:271-9.

110. Dutkiewicz J, Pomorski ZJH, Sitkowska J, et al. Airborne Microorganisms and Endotoxin in Animal Houses. Grana 1994;33:85-90.

111. Zejda JE, Barber E, Dosman JA, et al. Respiratory health status in swine producers relates to endotoxin exposure in the presence of low dust levels. J Occup Med 1994;36:49-56.

112. Mackiewicz B. Study on exposure of pig farm workers to bioaerosols, immunologic reactivity and health effects. Ann Agric Environ Med 1998;5:169-75.

113. Takai H, Pedersen S, Johnsen JO, et al. Concentrations and emissions of airborne dust in livestock buildings in Northern Europe. Journal of Agricultural Engineering Research 1998;70:59-77.

114. Seedorf J, Hartung J, Schroder M, et al. Concentrations and emissions of airborne endotoxins and microorganisms in livestock buildings in Northern Europe. Journal of Agricultural Engineering Research 1998;70:97-109.

115. Chang CW, Chung H, Huang CF, Su HJ. Exposure assessment to airborne endotoxin, dust, ammonia, hydrogen sulfide and carbon dioxide in open style swine houses. Ann Occup Hyg 2001;45:457-65.

116. Duchaine C, Thorne PS, Meriaux A, Grimard Y, Whitten P, Cormier Y. Comparison of endotoxin exposure assessment by bioaerosol impinger and filter-sampling methods. Appl Environ Microbiol 2001;67:2775-80.

117. Schierl R, Heise A, Egger U, et al. Endotoxin concentration in modern animal houses in southern Bavaria. Ann Agric Environ Med 2007;14:129-36.

118. Thorne PS, Ansley AC, Perry SS. Concentrations of bioaerosols, odors, and hydrogen sulfide inside and downwind from two types of swine livestock operations. J Occup Environ Hyg 2009;6:211-20.

119. Letourneau V, Nehme B, Meriaux A, Masse D, Duchaine C. Impact of production systems on swine confinement buildings bioaerosols. J Occup Environ Hyg 2010;7:94-102.

120. Jones W, Morring K, Olenchock SA, Williams T, Hickey J. Environmental study of poultry confinement buildings. Am Ind Hyg Assoc J 1984;45:760-6.

121. Reynolds SJ, Parker D, Vesley D, Janni K, McJilton C. Occupational Exposure to Organic Dusts and Gases in the Turkey Growing Industry. Applied Occupational and Environmental Hygiene 1994;9:493 - 502.

122. Rylander R, Carvalheiro MF. Airways inflammation among workers in poultry houses. Int Arch Occup Environ Health 2006;79:487-90.

123. Nimmermark S, Lund V, Gustafsson G, Eduard W. Ammonia, dust and bacteria in welfare-oriented systems for laying hens. Ann Agric Environ Med 2009;16:103-13.

124. Kirychuk SP, Reynolds SJ, Koehncke NK, et al. Endotoxin and dust at respirable and nonrespirable particle sizes are not consistent between cage- and floor-housed poultry operations. Ann Occup Hyg 2010;54:824-32.

125. Rimac D, Macan J, Varnai VM, et al. Exposure to poultry dust and health effects in poultry workers: impact of mould and mite allergens. Int Arch Occup Environ Health 2010;83:9-19.

126. Burdorf A, Van Tongeren M. Commentary: variability in workplace exposures and the design of efficient measurement and control strategies. Ann Occup Hyg 2003;47:95-9.

127. Peretz C, Goren A, Smid T, Kromhout H. Application of mixed-effects models for exposure assessment. Ann Occup Hyg 2002;46:69-77.

128. Liu K, Stamler J, Dyer A, McKeever J, McKeever P. Statistical methods to assess and minimize the role of intra-individual variability in obscuring the relationship between dietary lipids and serum cholesterol. J Chronic Dis 1978;31:399-418.

129. Seedorf J, Hartung J, Schroder M, et al. A survey of ventilation rates in livestock buildings in Northern Europe. Journal of Agricultural Engineering Research 1998;70:39-47.

130. Gustafsson G. Factors affecting the release and concentration of dust in pig houses. Journal of Agricultural Engineering Research 1999;74:379-90.

131. Gustafsson C. Investigations of factors affecting air pollutants in animal houses. Ann Agric Environ Med 1997;4:203-15.

132. Just N, Duchaine C, Singh B. An aerobiological perspective of dust in cage-housed and floor-housed poultry operations. J Occup Med Toxicol 2009;4:13.

133. Duchaine C, Grimard Y, Cormier Y. Influence of building maintenance, environmental factors, and seasons on airborne contaminants of swine confinement buildings. AIHAJ 2000;61:56-63.

134. Burstyn I, Teschke K. Studying the determinants of exposure: a review of methods. Am Ind Hyg Assoc J 1999;60:57-72.

135. EFBA. The European Fur Breeders' Association - Annual report 2009. Available from http://www.efba.eu/download/annual_report/2009/index.html (accessed 08 February 2011); 2009.

136. Landbrug og Fødevarer. Dansk landbrug i tal 2009. [The Danish agriculture in figures 2009]. An anual report by the Danish Agriculture and Food Council. Axelborg, Copenhagen: The Danish Agriculture and Food Council; 2009.

137. Dansk landbrugsrådgivning. Håndbog til drifts-planlægning. [Knowledge Centre for Agriculture. Manual for farm operation]. 46 ed. Århus: Landbrugsforlaget. p. 20-29.; 2007.

138. Mark D. The Sampling of Aerosols: Principles and Methods. In: Occupational Hygiene: Blackwell Publishing Ltd; 2008:185-207.

139. Kenny LC, Aitken R, Chalmers C, et al. A collaborative European study of personal inhalable aerosol sampler performance. Ann Occup Hyg 1997;41:135-53.

140. Schlunssen V, Madsen AM, Skov S, Sigsgaard T. Does the use of biofuels affect respiratory health among male Danish energy plant workers? Occup Environ Med 2010.

141. Samadi S, Spithoven J, Jamshidifard AR, et al. Allergy among veterinary medicine students in The Netherlands. Occup Environ Med 2011.

142. Oppliger A, Charriere N, Droz PO, Rinsoz T. Exposure to bioaerosols in poultry houses at different stages of fattening; use of real-time PCR for airborne bacterial quantification. Ann Occup Hyg 2008;52:405-12.

143. Senthilselvan A, Beach J, Feddes J, Cherry N, Wenger I. A prospective evaluation of air quality and workers' health in broiler and layer operations. Occup Environ Med 2011;68:102-7.

144. Statistics Denmark. BDF07: Farms by region, type, farms and area (2006-2010): HDYR07: Livestock by county, unit and type (2006-2010): PELS2: Furred animals by region, unit and type of farm (2007-2009). Available at http://www.statbank.dk/statbank5a/default.asp?w=1440 (accessed 08 April 2011). (Accessed at

145. Molocznik A. Qualitative and quantitative analysis of agricultural dust in working environment. Ann Agric Environ Med 2002;9:71-8.

146. Von Essen S, Moore G, Gibbs S, Larson KL. Respiratory Issues in Beef and Pork Production: Recommendations From an Expert Panel. Journal of Agromedicine 2010;15:216-25.

Appendix I

Interview scheme

INTERVIEW SKEMA

A) GENERELLE OPLYSNINGER

Interviewer: ____

Interview dato: _____________ _____________ _____________

Gårdens adresse 1:

1 Gade og husnummer: ____

2 Postnummer: _____________ 3 By: ____

Gårdens adresse 2:

4 Gade og husnummer: ____

5 Postnummer: _____________ 6 By: ____

Interviewedes ID:

7 Fornavn(e): ____

8 Efternavn: ____

9 Gade og husnummer: ____

10 Postnummer: _____________ 11 By: ____

12 Telefon: ___________________________ 13 Telefon: ___________________________

14 E-mail:______________________________________

15 Er interviewede ejer eller en af ejerne af gården?

1 ❑ *Ja*

2 ❑ *Nej ⇒ Hvilken status har vedkommende da?:* ______________________________________

16 Er interviewede en SUS12 deltager?

1 ❑ *Ja ⇒ SUS deltager ID:* ___________________

2 ❑ *Nej*

1

B) OPLYSNINGER OM GÅRDENS PERSONALE

17 Antal og type beskæftigede på gården:

Type af beskæftigede	Antal
1 ☐ Fuldtids ansat	…………………………………
2 ☐ Deltids ansat	…………………………………
3 ☐ Voksent familiemedlem (≥ 15 år)	…………………………………
4 ☐ Barn i familien (<15 år)	…………………………………

18 Anvendes der husbondafløser eller landbrugsvikar i forbindelse med ferie, sygdom etc?

1 ☐ *Ja*

2 ☐ *Nej*

C) EJERSKAB TIL GÅRDEN OG SAMARBEJDSFORHOLD

19 Er gården købt eller lejet?

1 ☐ *Købt*

2 ☐ *Lejet*

20 Hvilket årstal blev gården købt eller lejet? __________ (årstal)

21 Drives gården sammen med andre gårde eller indgår den i et samarbejde med andre ?

1 ☐ *Ja ⇒ Hvordan?:* ____

2 ☐ *Nej*

22 Benyttes i driften ydelser fra maskinstation eller andre leverandører/aftagere?

1 ☐ Ja *⇒ gå til spørgsmål 23*

2 ☐ Nej *⇒ gå til spørgsmål 24*

23 Udføres en eller flere af de følgende opgaver af maskinstation eller anden leverandør?

1 ☐ Transport af handlede dyr (købt eller solgt)

2 ☐ Spredning af gylle eller gødning

3 ☐ Markarbejde

4 ☐ Bedækning eller insemination

5 ☐ Andet: ________________

24 Leveres en eller flere af de følgende produkter udefra?

1 ❑ *En væsentlig* del af foder forbruget

2 ❑ Strøelse

3 ❑ Andet

D) GENERELLE GÅRD OG PRODUKTIONS KARAKTERISTIKA

25 Hvad er gårdens vigtigste produktionstype? Hvilken form for landbrug anvendes der på gården? *(Sæt gerne flere krydser)*

Produktionstype/Landbrugsform	Konventionelt	Under ændring	Økologisk
1 ❑ Kvæg, malkebesætning	❑	❑	❑
2 ❑ Kvæg, kødkvæg	❑	❑	❑
3 ❑ Svin, avlsbesætning	❑	❑	❑
4 ❑ Svin, opformeringsbesætning	❑	❑	❑
5 ❑ Svin, slagtesvinebesætning	❑	❑	❑
6 ❑ Svin, blandet produktion	❑	❑	❑
7 ❑ Fjerkræ	❑	❑	❑
8 ❑ Geder og/eller får	❑	❑	❑
9 ❑ Mink	❑	❑	❑
10 ❑ Planteavl	❑	❑	❑
11 ❑ Andet: _______________	❑	❑	❑

26 Hvilket antal af de følgende dyr er der på gården?

Typer af dyr	Aktuelle antal	Produktion/år
Malke køer	……………………………	……………………………
Kødkvæg	……………………………	……………………………
Kalve	……………………………	……………………………
Kvier	……………………………	……………………………
Stude/ungtyre	……………………………	……………………………
Tyre	……………………………	……………………………
Orner	……………………………	……………………………
Søer	……………………………	……………………………
Polte	……………………………	……………………………
Smågrise	……………………………	……………………………
Slagtesvin	……………………………	……………………………
Æglæggere	……………………………	……………………………
Slagtekyllinger	……………………………	……………………………
Geder	……………………………	……………………………
Får	……………………………	……………………………
Mink	……………………………	……………………………
Heste	……………………………	……………………………
Andet: ………………………	……………………………	……………………………
Andet: ………………………	……………………………	……………………………

27 Hvilke fodertyper (dvs. grovfoder, kraftfoder, våd, tør eller blandet foder) anvendes hovedsagelig til de forskellige typer af dyr?

Typer af dyr	Fodertyper
……………………………………	……………………………………
……………………………………	……………………………………
……………………………………	……………………………………
……………………………………	……………………………………
……………………………………	……………………………………

28 Hvor stort et areal dyrkes der totalt under gården: ____________ hektar

29 Hvordan blev landbrugsarealet dyrket sidste år? Angiv afgrøden og antal hektar brugt til den.

(*Sæt gerne flere krydser)*

Arealanvendelse	Antal hektar
1❑ Eng	……………………..
2❑ Korndyrkning	……………………..
3❑ Grovfoder	……………………..
4❑ Roer	……………………..
5❑ Frøavl	……………………..
6❑ Skovbrug	……………………..
7❑ Frugtplantage	……………………..
8❑ Grøntsager	……………………..
9❑ Andet: ……………………………	……………………..
10❑ Andet: ……………………………	……………………..

Spørgsmål 26 og 27-28 besvares kun,
hvis gårdens hovedproduktion er enten svine- eller kvægavl.

30 Hvis der produceres enten slagtesvin eller kødkvæg, hvordan er produktionssystemet da indrettet?

1❑ Kontinuerlig udskiftning *(dyr indtræder og udtræder af produktionen under særlige individuelle omstændigheder, fx vægt)*

2❑ Alt ind/alt ud *(dyr af same alder eller vægt købes, opfodres, sælges eller slagtes sammen)*

31 Er svineproduktionen registreret under det danske SPF system?

1❑ Ja ⇒ *Fortsæt til spørgsmål 28.*

2❑ Nej ⇒ *Fortsæt til spørgsmål 29.*

32 Hvilen aktuel SPF status har besætningen?

1❑ Rød

2❑ Blå

3❑ Grøn

33 Bruges der biobrændsel til opvarmning på gården? (Halm, træflis, træspåner, savsmuld, træpiller, kul samt korn og afrens.)

1 ❑ Ja

2 ❑ Nej

E) GENERELLE OPLYSNINGER OM BYGNINGERNES INDRETNING

34 Angiv hvilke bygningstyper og antallet af disse gården består af?

Bygningstyper		Antal
1 ❑	Stald med blandet brug	…………..
2 ❑	Løbestald	…………..
3 ❑	Drægtighedsstald	…………..
4 ❑	Farestald	…………..
5 ❑	Poltestald	…………..
6 ❑	Smågrisestald	…………..
7 ❑	Slagtesvinestald	…………..
8 ❑	Syge- og aflastningsstald	…………..
9 ❑	Karantænestald	…………..
10 ❑	Udleveringsstald	…………..
11 ❑	Traditionel kostald med båse	…………..
12 ❑	Kostald med løsdrift	…………..
13 ❑	Malkestald	…………..
14 ❑	Kalvestald	…………..
15 ❑	Foder rum	…………..
16 ❑	Lade	…………..
17 ❑	Plan silo	…………..
18 ❑	Tårn silo	…………..
19 ❑	Maskinhus	…………..
20 ❑	Gylletank	…………..
21 ❑	Stuehus	…………..
22 ❑	Andet: …………………………………..	…………..

35 Appendiks 1 er en kortskitse af gården. Nummerer de forskellige bygninger med tal fra 1-100, og skriv de tildelte tal på bygningerne på kortet.

36 De nummererede bygninger i spørgsmål 35 beskrives med hensyn til **bygningstype** via betegnelse eller nummerering fra spørgsmål 34 (2/Løbestaldstald; 3/Drægtighedsstald osv.), deres omtrentlige **størrelse** i m^2, jeres **egene benævnelse**, **opførelses år** og **renoverings år**.

Bygnings nr.	Bygningstype	M^2	Egen benævnelse	Opførelses år	Renoverings år
1	………………	……	…………………	…………….	…………….
2	………………	……	…………………	…………….	…………….
3	………………	……	…………………	…………….	…………….
4	………………	……	…………………	…………….	…………….
5	………………	……	…………………	…………….	…………….
6	………………	……	…………………	…………….	…………….
7	………………	……	…………………	…………….	…………….
8	………………	……	…………………	…………….	…………….
9	………………	……	…………………	…………….	…………….
10	………………	……	…………………	…………….	…………….
11	………………	……	…………………	…………….	…………….
12	………………	……	…………………	…………….	…………….
13	………………	……	…………………	…………….	…………….
14	………………	……	…………………	…………….	…………….
15	………………	……	…………………	…………….	…………….
16	………………	……	…………………	…………….	…………….
17	………………	……	…………………	…………….	…………….
18	………………	……	…………………	…………….	…………….
19	………………	……	…………………	…………….	…………….
20	………………	……	…………………	…………….	…………….
21	………………	……	…………………	…………….	…………….
22	………………	……	…………………	…………….	…………….

F) RENGØRING OG DESINFEKTION

37 Er der en regelmæssig (stram) rengøringsplan for bygningerne?

[1] ❑ Ja ⇒ *Fortsæt til spørgsmål 39.*

[2] ❑ Nej ⇒ *Fortsæt til spørgsmål 38.*

38 **Hvis nej i spørgsmål 37**, hvor tit sker der da rengøring I bygningerne?

1❑ Der sker slet ingen rengøring ⇒ *Fortsæt til spørgsmål 42*

2❑ Der rengøres sporadisk uden nogen plan

3❑ Der rengøres efter en løs plan

39 Hvilke rengøringsformer anvendes der?

1❑ Fejning.

2❑ Støvsugning

3❑ Højtryks rensning

4❑ Vandslange

5❑ Andet: ………………………………

40 Er der ansat et særligt rengørings personel?

1❑ Ja ⇒ *Fortsæt til spørgsmål 42.*

2❑ Nej ⇒ *Fortsæt til spørgsmål 41.*

41 Er der hyret et særligt rengøringsfirma til opgaverne?

1❑ Ja

2❑ Nej

42 Bruges der desinficerende stoffer i rengøringen?

1❑ Ja ⇒ *Fortsæt til spørgsmål 43.*

2❑ Nej ⇒ *Fortsæt til* ***Afsnit G.***

43 Angiv hvilke desinficerende stoffer der anvendes:

1 ……………………………………………….

2 ……………………………………………….

3 ……………………………………………….

4 ……………………………………………….

44 Hvor bruges de desinficerende stoffer?

1❑ I den generelle rengøring af alle bygningerne

2❑ I den generelle rengøring af nogle af bygningerne ⇒ *Fortsæt til spørgsmål 44.a*

3❑ Til rengøring af specielle områder (stier, rør anlæg etc.) ⇒ *Fortsæt til spørgsmål 44.a*

44.a Angiv de bygninger og/eller områder og/eller opgaver, hvor der bruges desinficerende stoffer:

1 …………………………………………… 6 ……………………………………………
2 …………………………………………… 7 ……………………………………………
3 …………………………………………… 8 ……………………………………………
4 …………………………………………… 9 ……………………………………………
5 …………………………………………… 10 …………………………………………

45 Hvor tit bruges de desinficerende stoffer?

[1]❑ Dagligt
[2]❑ Hver uge
[3]❑ Hver måned
[4]❑ Hver 2-3 måned
[5]❑ Hver 6 måned
[6]❑ Hver 6-12 måned

G) OPLYSNINGER OM BRUGEN AF GYLLETANK

*Hvis der bruges gylletank besvares spørgsmål 46, ellers fortsættes til **Afsnit H**.*

46 Hvor mange dage om året røres der i gylletank?

[1]❑ Forår ⇒ Antal dage: ______
[2]❑ Efterår ⇒ Antal dage: ______

H) KOMMENTARER

Har du ting du synes vi bør vide om dit arbejde eller bemærkninger til dette interview, vil vi meget gerne høre dem:

……
……
……
……
……
……

SLUT PÅ INTERVIEWET

Appendix II

Walkthrough survey for pig stables

D. Characteristics per Animal Department - pigs

Farm no.: ____ **1** ***Department no.:*** ____ **2** ***Farm Unit:*** ___________ **3** ***Season:*** ___________

4. Type of building/sector:

1 ❑	Mixed use
2 ❑	Serve-/ insemination stall
3 ❑	Gestation stall
4 ❑	Farrowing house
5 ❑	Gilt house
6 ❑	Pig weaning house
7 ❑	Finishing house
8 ❑	Delivery/Quarantine room
9 ❑	Sick animal accommodation
10 ❑	Other:________________

5. No of Sectors in the building (If applicable): ____

6. Average size of the sectors (If applicable) : ____m^2

7. Year of construction: _______

8. Year till last renovation (if applicable): _____

9. Dimensions:

a. Length: ______m

b. Width: ______m

c. Height: ______m

10. Type and number of housed animals:

	Type	Num.	Num/sect
1 ❑	Sows	____	____
2 ❑	Piglets/ weaners	____	____
3 ❑	Fattening pigs	____	____
4 ❑	Gilts	____	____
5 ❑	Boars	____	____

11. Housing arrangement:

	Type	Num.	N/sect
1 ❑	Free stall housing	___	___
2 ❑	Batch pens	___	___
3 ❑	Serving stall	___	___
4 ❑	Farrowing Crates	___	___
5 ❑	Crates	___	___
6 ❑	2 climate pen	___	___
7 ❑	Free access cubicles	___	___
8 ❑	Pen with ESF equipment	___	___
9 ❑	Sick animal accommodation	___	___

12. Is the feeding room combined with the department?

1 ❑ Yes 2 ❑ No

13.a Type of Ventilation used:

1 ❑ Mechanical with positive pressure

2 ❑ Mechanical with negative pressure

3 ❑ Mechanical with neutral pressure

4 ❑ Natural with controlling device

5 ❑ Natural without controlling device

13.b Ventilation rate: ________ m^3/min

14. Type of heating:

1 ❑ None

2 ❑ Floor heating

3 ❑ Air heating

4 ❑ Beam heating

5 ❑ Radiator heating

15. Type of floor coverage:

	Type of floor	% of coverage
1 ❑	Deep litter	________
2 ❑	Concrete	________
3 ❑	Slatted flooring	________
4 ❑	Bedding	________

16. Type of bedding, amount and % of coverage:

	Type	% of total	Amount used/time
1 ❑	None	________	
2 ❑	Straw	________	____kg/_____
3 ❑	Wood chips	________	____kg/_____
4 ❑	Sawdust	________	____kg/_____
5 ❑	Mat	________	

17.a When does showering taking place?

1 ❑ Never

2 ❑ Summer

3 ❑ Winter

4 ❑ All year

17.b Is colza oil used by spaying for dust binding?

1 ❑ Yes 2 ❑ No

19.a Type of feed used :

- 1 ☐ Liquid
- 2 ☐ Dry
- 3 ☐ Mixed

19.b Method of feed preparation:

- 1 ☐ Manual
- 2 ☐ Partly mechanical
- 3 ☐ Automatic

19.c Method of feeding:

- 1 ☐ Manual
- 2 ☐ Partly mechanical
- 3 ☐ Automatic

19.d Food accessibility to animals

- 1 ☐ Ad libitum
- 2 ☐ With time intervals

19.e Accumulation of dirt in the feeding corridor:

- 1 ☐ Low
- 2 ☐ Medium
- 3 ☐ High

20.a Method of dung removal:

- 1 ☐ Manual
- 2 ☐ Mechanical with scraper
- 3 ☐ Vacuuming through slurry pits
- 4 ☐ Mechanical with tractor

20.b Frequency of dung removal:

- 1 ☐ Daily
- 2 ☐ Weekly
- 3 ☐ Every 2 weeks
- 4 ☐ Every 2-8 weeks
- 5 ☐ More than 8 weeks

20.c Accumulation of dung in the building:

- 1 ☐ Low
- 2 ☐ Medium
- 3 ☐ High

21. General Hygiene level in building:

- 1 ☐ Low
- 2 ☐ Medium
- 3 ☐ High

22 Condition of the floor?

- 1 ☐ Dry
- 2 ☐ Wet
- 3 ☐ Very wet

23.a Schedule for general cleaning:

- 1 ☐ None
- 2 ☐ Sporadically cleaned with no schedule
- 3 ☐ Loosely cleaned based on a scheduled
- 4 ☐ Tight schedule

23.b Methods used during the general cleaning?

- 1 ☐ Sweeping
- 2 ☐ High pressure
- 3 ☐ Other: _________
- 4 ☐ Other: _________

Frequency of general cleaning:___(times/_____)

24. Regular cleaning in individual animal accommodations

	Method	Frequency
1 ☐	Sweeping	___times/_____
2 ☐	High pressure	___times/_____
3 ☐	Manual scraper/shovel	___times/_____
4 ☐	Mechanical scraper/robot	___times/_____
5 ☐	Other: __________	___times/_____

25. Methods & frequency of regular cleaning in corridors:

	Method	Frequency
1 ☐	Sweeping	___times/_____
2 ☐	High pressure	___times/_____
3 ☐	Manual Scraper/shovel	___times/_____
4 ☐	Other: __________	___times/_____

26.a Use of detergents/disinfectants during:

- 1 ☐ General cleaning
- 2 ☐ Cleaning in individual animal accommodations
- 3 ☐ Cleaning of the feeding corridor

26.b Type of disinfectants used during cleaning:

a) Namely:_______________

b) Namely:_______________

26.c Frequency of disinfection: ___times/_______

Appendix III

Activity diary for pig farmers

Pigs **Measuring day**

Date of filling in: I__I__I__I__I__I__I (dd/mm/yy) **Filled in by:** ______________________________ **SUS ID:** _______ **Farm ID:** _______

	Working task	Time (in 30 minutes intervals)																													
		05:00		06:00		07:00		08:00		09:00		10:00		11:00		12:00		13:00		14:00		15:00		16:00		17:00		18:00		19:00	
		00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30
	Inside the animal building																														
1	Controlling	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
2	Weighing	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
3	Moving breeding animals	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
4	Moving and loading weaners	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
5	Moving and loading slaughter swine	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
6	Handling and nursing piglets (ear tagging, castrating, cutting tails)	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
7	Inseminating	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
8	Injection or handling sick animals	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
9	Handling dead animals	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
10	Preparing feed	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
11	Spreading feed manually	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
12	Automatic feeding (adjusting/inspecting)	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
13	Preparing bedding	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
14	Disposing bedding	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
15	Removing manure (in pens and stalls)	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
16	Sweeping or scraping the corridors	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
17	Washing with high pressure	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
18	Disinfecting pens/stalls/stables	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
19	Repair and maintenance of animal buildings/feed room and installations	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
20	Other (indicate): ____________	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
	Outside the animal building																														
21	Repairing/maintaining machinery & equipment (e.g. tractor, track, harvester)	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
22	Handling manure tanks and dunghills	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
23	Handling of feeding materials, seeds, grains in the barn	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
24	Work relating to silos or drying plants	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
25	Work in the fields (working the soil, sowing, harvesting, applying fertilizers)	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
26	Other (indicate): ____________	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
	Use of protection equipment																														
27	Wearing breath protection equipment	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
	Breaks in work																														
28	Break	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑

Pigs **2nd day**

Date of filling in: |__|__|__|__|__|__| (dd/mm/yy) **Filled in by:** ______________________ **SUS ID:** _______ **Farm ID:** _______

	Working task	**Time (in 30 minutes intervals)**																													
		05:00		**06:00**		**07:00**		**08:00**		**09:00**		**10:00**		**11:00**		**12:00**		**13:00**		**14:00**		**15:00**		**16:00**		**17:00**		**18:00**		**19:00**	
		00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30
	Inside the animal building																														
1	Controlling	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
2	Weighing	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
3	Moving breeding animals	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
4	Moving and loading weaners	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
5	Moving and loading slaughter swine	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
6	Handling and nursing piglets (ear tagging, castrating, cutting tails)	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
7	Inseminating	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
8	Injection or handling sick animals	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
9	Handling dead animals	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
10	Preparing feed	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
11	Spreading feed manually	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
12	Automatic feeding (adjusting/inspecting)	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
13	Preparing bedding	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
14	Disposing bedding	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
15	Removing manure (in pens and stalls)	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
16	Sweeping or scraping the corridors	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
17	Washing with high pressure	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
18	Disinfecting pens/stalls/stables	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
19	Repair and maintenance of animal buildings/feed room and installations	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
20	Other (indicate): ____________	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
	Outside the animal building																														
21	Repairing/maintaining machinery & equipment (e.g. tractor, track, harvester)	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
22	Handling manure tanks and dunghills	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
23	Handling of feeding materials, seeds, grains in the barn	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
24	Work relating to silos or drying plants	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
25	Work in the fields (working the soil, sowing, harvesting, applying fertilizers)	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
26	Other (indicate): ____________	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
	Use of protection equipment																														
27	Wearing breath protection equipment	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
	Breaks in work																														
28	Break	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑

Pigs 3rd day

Date of filling in: |__|__|__|__|__|__| (dd/mm/yy) **Filled in by:** ________________ **SUS ID:** ________ **Farm ID:** ________

	Working task	Time (in 30 minutes intervals)																													
		05:00		06:00		07:00		08:00		09:00		10:00		11:00		12:00		13:00		14:00		15:00		16:00		17:00		18:00		19:00	
		00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30
	Inside the animal building																														
1	Controlling	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
2	Weighing	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
3	Moving breeding animals	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
4	Moving and loading weaners	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
5	Moving and loading slaughter swine	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
6	Handling and nursing piglets (ear tagging, castrating, cutting tails)	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
7	Inseminating	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
8	Injection or handling sick animals	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
9	Handling dead animals	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
10	Preparing feed	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
11	Spreading feed manually	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
12	Automatic feeding (adjusting/inspecting)	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
13	Preparing bedding	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
14	Disposing bedding	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
15	Removing manure (in pens and stalls)	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
16	Sweeping or scraping the corridors	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
17	Washing with high pressure	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
18	Disinfecting pens/stalls/stables	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
19	Repair and maintenance of animal buildings/feed room and installations	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
20	Other (indicate): ______________	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
	Outside the animal building																														
21	Repairing/maintaining machinery & equipment (e.g. tractor, track, harvester)	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
22	Handling manure tanks and dunghills	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
23	Handling of feeding materials, seeds, grains in the barn	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
24	Work relating to silos or drying plants	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
25	Work in the fields (working the soil, sowing, harvesting, applying fertilizers)	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
26	Other (indicate): ______________	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
	Use of protection equipment																														
27	Wearing breath protection equipment	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
	Breaks in work																														
28	Break	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑

Pigs **4th day**

Date of filling in: I__I__I__I__I__I__I (dd/mm/yy) **Filled in by:** ________________________ **SUS ID:** _______ **Farm ID:** _______

	Working Task	**Time (in 30 minutes intervals)**																													
		05:00		**06:00**		**07:00**		**08:00**		**09:00**		**10:00**		**11:00**		**12:00**		**13:00**		**14:00**		**15:00**		**16:00**		**17:00**		**18:00**		**19:00**	
		00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30
	Inside the animal building																														
1	Controlling	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
2	Weighing	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
3	Moving breeding animals	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
4	Moving and loading weaners	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
5	Moving and loading slaughter swine	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
6	Handling and nursing piglets (ear tagging, castrating, cutting tails)	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
7	Inseminating	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
8	Injection or handling sick animals	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
9	Handling dead animals	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
10	Preparing feed	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
11	Spreading feed manually	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
12	Automatic feeding (adjusting/inspecting)	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
13	Preparing bedding	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
14	Disposing bedding	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
15	Removing manure (in pens and stalls)	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
16	Sweeping or scraping the corridors	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
17	Washing with high pressure	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
18	Disinfecting pens/stalls/stables	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
19	Repair and maintenance of animal buildings/feed room and installations	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
20	Other (indicate): ____________	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
	Outside the animal building																														
21	Repairing/maintaining machinery & equipment (e.g. tractor, track, harvester)	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
22	Handling manure tanks and dunghills	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
23	Handling of feeding materials, seeds, grains in the barn	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
24	Work relating to silos or drying plants	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
25	Work in the fields (working the soil, sowing, harvesting, applying fertilizers)	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
26	Other (indicate): ____________	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
	Use of protection equipment																														
27	Wearing breath protection equipment	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
	Breaks in work																														
28	Break	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑

Pigs **5th day**

Date of filling in: I__I__I__I__I__I__I (dd/mm/yy) **Filled in by: ____________________________________** **SUS ID: _______** **Farm ID: _______**

	Working Task	Time (in 30 minutes intervals)																													
		05:00		06:00		07:00		08:00		09:00		10:00		11:00		12:00		13:00		14:00		15:00		16:00		17:00		18:00		19:00	
		00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30
	Inside the animal building																														
1	Controlling	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
2	Weighing	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
3	Moving breeding animals	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
4	Moving and loading weaners	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
5	Moving and loading slaughter swine	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
6	Handling and nursing piglets (ear tagging, castrating, cutting tails)	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
7	Inseminating	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
8	Injection or handling sick animals	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
9	Handling dead animals	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
10	Preparing feed	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
11	Spreading feed manually	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
12	Automatic feeding (adjusting/inspecting)	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
13	Preparing bedding	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
14	Disposing bedding	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
15	Removing manure (in pens and stalls)	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
16	Sweeping or scraping the corridors	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
17	Washing with high pressure	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
18	Disinfecting pens/stalls/stables	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
19	Repair and maintenance of animal buildings/feed room and installations	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
20	Other (indicate): ______________	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
	Outside the animal building																														
21	Repairing/maintaining machinery & equipment (e.g. tractor, track, harvester)	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
22	Handling manure tanks and dunghills	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
23	Handling of feeding materials, seeds, grains in the barn	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
24	Work relating to silos or drying plants	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
25	Work in the fields (working the soil, sowing, harvesting, applying fertilizers)	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
26	Other (indicate): ______________	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
	Use of protection equipment																														
27	Wearing breath protection equipment	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
	Breaks in work																														
28	Break	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑

Pigs **6th day**

Date of filling in: I__I__I__I__I__I__I (dd/mm/yy) **Filled in by:** ______________________________ **SUS ID:** _______ **Farm ID:** _______

	Working task	Time (in 30 minutes intervals)																													
		05:00		06:00		07:00		08:00		09:00		10:00		11:00		12:00		13:00		14:00		15:00		16:00		17:00		18:00		19:00	
		00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30
	Inside the animal building																														
1	Controlling	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
2	Weighing	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
3	Moving breeding animals	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
4	Moving and loading weaners	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
5	Moving and loading slaughter swine	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
6	Handling and nursing piglets (ear tagging, castrating, cutting tails)	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
7	Inseminating	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
8	Injection or handling sick animals	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
9	Handling dead animals	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
10	Preparing feed	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
11	Spreading feed manually	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
12	Automatic feeding (adjusting/inspecting)	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
13	Preparing bedding	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
14	Disposing bedding	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
15	Removing manure (in pens and stalls)	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
16	Sweeping or scraping the corridors	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
17	Washing with high pressure	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
18	Disinfecting pens/stalls/stables	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
19	Repair and maintenance of animal buildings/feed room and installations	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
20	Other (indicate): ______________	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
	Outside the animal building																														
21	Repairing/maintaining machinery & equipment (e.g. tractor, track, harvester)	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
22	Handling manure tanks and dunghills	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
23	Handling of feeding materials, seeds, grains in the barn	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
24	Work relating to silos or drying plants	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
25	Work in the fields (working the soil, sowing, harvesting, applying fertilizers)	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
26	Other (indicate): ______________	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
	Use of protection equipment																														
27	Wearing breath protection equipment	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
	Breaks in work																														
28	Break	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑

Pigs **7th day**

Date of filling in: I__I__I__I__I__I__I (dd/mm/yy) **Filled in by:** ____________________ **SUS ID:** _______ **Farm ID:** _______

	Working task	Time (in 30 minutes intervals)																													
		05:00		06:00		07:00		08:00		09:00		10:00		11:00		12:00		13:00		14:00		15:00		16:00		17:00		18:00		19:00	
		00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30	00	30
	Inside the animal building																														
1	Controlling	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
2	Weighing	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
3	Moving breeding animals	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
4	Moving and loading weaners	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
5	Moving and loading slaughter swine	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
6	Handling and nursing piglets (ear tagging, castrating, cutting tails)	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
7	Inseminating	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
8	Injection or handling sick animals	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
9	Handling dead animals	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
10	Preparing feed	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
11	Spreading feed manually	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
12	Automatic feeding (adjusting/inspecting)	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
13	Preparing bedding	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
14	Disposing bedding	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
15	Removing manure (in pens and stalls)	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
16	Sweeping or scraping the corridors	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
17	Washing with high pressure	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
18	Disinfecting pens/stalls/stables	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
19	Repair and maintenance of animal buildings/feed room and installations	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
20	Other (indicate): ____________	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
	Outside the animal building																														
21	Repairing/maintaining machinery & equipment (e.g. tractor, track, harvester)	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
22	Handling manure tanks and dunghills	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
23	Handling of feeding materials, seeds, grains in the barn	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
24	Work relating to silos or drying plants	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
25	Work in the fields (working the soil, sowing, harvesting, applying fertilizers)	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
26	Other (indicate): ____________	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
	Use of protection equipment																														
27	Wearing breath protection equipment	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
	Breaks in work																														
28	Break	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑

Manuscripts

Manuscript I

Basinas I, Sigsgaard T, Heederik D, Takai H, Omland Ø, Andersen N.T, Wouters I.M, Bønløkke J.H, Kromhout H, Schlünssen V. *Exposure to inhalable dust and endotoxin among Danish livestock farmers: results from the SUS cohort study.* Submitted

Title:

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

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Abstract

Studies on personal dust and endotoxin concentrations among animal farmers have been either small or limited to 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 2.4 mg/m^3 (range <LOD-47.8) and endotoxin of 991 EU/m^3 (range <LOD-374,579). The highest dust and endotoxin concentrations were measured among pig and poultry farmers, and 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 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.

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. Furthermore, the country has a substantial dairy and poultry production that annually supplies the Danish economy with approximately two billion Euros through its exports.[1, 2] The Danish primary farm sector consisted in 2008 of approximately 43.000 professional holdings with an average size of 63 hectares. Of those, approximately 35% was specialized in livestock production (mainly pig and cattle farming), 39% were solely crops 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 dust (also referred to as bio-aerosols) is suggested to have a distinctive role in the development of allergic and non-allergic respiratory disease and lung function impairment.[7] In particular, exposure to endotoxins, lipopolysaccharide 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 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 1,964 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, 1,156 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 appendix, 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),[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 farmers, 26 dairy cattle farmers and 3 mink farmers. 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 appendix (Figure 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) were 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 animal with crop production and four (2 pig and 2 cattle) combined pig with cattle farming. All workers on the selected farms were included in the personal measurements, and more than 90% participated. Sampling was performed during the whole 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).

Sampling and analytical methods

Dust sampling was carried out using a plastic inhalable GSP sampler (CIS; *JS Holdings,* Stevenage, UK)[26] mounted with a 37mm 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 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 minimum 24 hours (22 ^{0}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 was 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^{o}C. The extracts were analysed for endotoxin in PFW (1:200 dilution) using a quantitative kinetic chromogenic Limulus Amboecyte 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). The assays' LOD was 0.0137 EU/ml 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 (GM) 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-steps procedure. At first the models were fitted with only the worker id as a random effect, while in a 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

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 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 1. 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.

Table 1 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.

Farming type	n	f	k	n/k	Sampling duration, hour AM (SD)	Range	<LOD for dust, N	<LOD for endotoxin, N
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 & 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

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.

A summary of the measured inhalable dust and endotoxin levels per type of farming is shown in Table 2 and Figure 1. The results of the seasonal personal measurements in pig and cattle farmers are also shown. The GM exposure for all monitored farmers was 2.4 mg/m^3 (GSD 3.0) for personal inhalable dust and 991 (GSD 4.7), EU/m^3 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 later group having the highest observed individual concentrations. Pig farmers were on average 3-folds 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 ($r=0.32$, $p<0.0001$) with a relatively similar pattern for pig and cattle farmers (see appendix, Figure 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 2).

Table 2 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.

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 - 47.8	6241	1495 (4.3)	<LOD - 374579	0.62***
Summer	181	4.3	2.8 (2.6)	0.1 - 47.8	5949	1088 (4.2)	14.4 - 374579	0.66***
Winter	173	5.5	4.0 (2.5)	<LOD - 20.0	6546	2085 (4.2)	<LOD - 285264	0.54***
Cattle								
Overall	124	1.6	1.0 (2.7)	<LOD - 9.8	759	361 (3.6)	<LOD - 5886	0.61***
Summer	62	1.5	0.9 (2.6)	0.2 - 9.8	512	286 (3.2)	18 - 3401	0.64***
Winter	62	1.8	1.1 (2.9)	<LOD - 9.4	1006	455 (3.9)	<LOD - 5886	0.59***
Mixed, cattle & pigs								
Overall	8	2.9	1.8 (2.8)	0.4 - 8.9	900	448 (6.0)	<LOD - 2910	0.71*
Summer	4	2.9	2.2 (2.5)	0.7 - 6.0	1232	868 (2.7)	251 - 2910	0.46
Winter	4	3.0	1.6 (3.6)	0.4 - 8.9	569	231 (9.9)	<LOD - 1090	0.80
Poultry								
Overall	14	5.7	3.5 (3.0)	0.7 - 18.3	1955	805 (4.9)	61 - 7092	0.83**
Layers	3	5.9	5.5 (1.6)	3.1 - 8.3	3329	2426 (2.6)	1162 - 7092	0.85
Broilers	11	5.6	3.1 (3.3)	0.7 - 18.3	1580	596 (5.1)	61 - 6424	0.82**
Minks	7	1.4	1.3 (1.6)	0.5 - 2.3	301	214 (2.2)	93 - 1054	0.62
Overall	507	4.0	2.4 (3.0)	<LOD - 47.8	4615	991 (4.7)	<LOD - 374579	0.69***

n, number of measurements; AM, arithmetic mean; GM, geometrical mean; GSD, geometrical standard deviation; r, Pearson correlations between measured dust and endotoxin concentrations; *p<0.05, **p<0.01, ***p<0.0001.

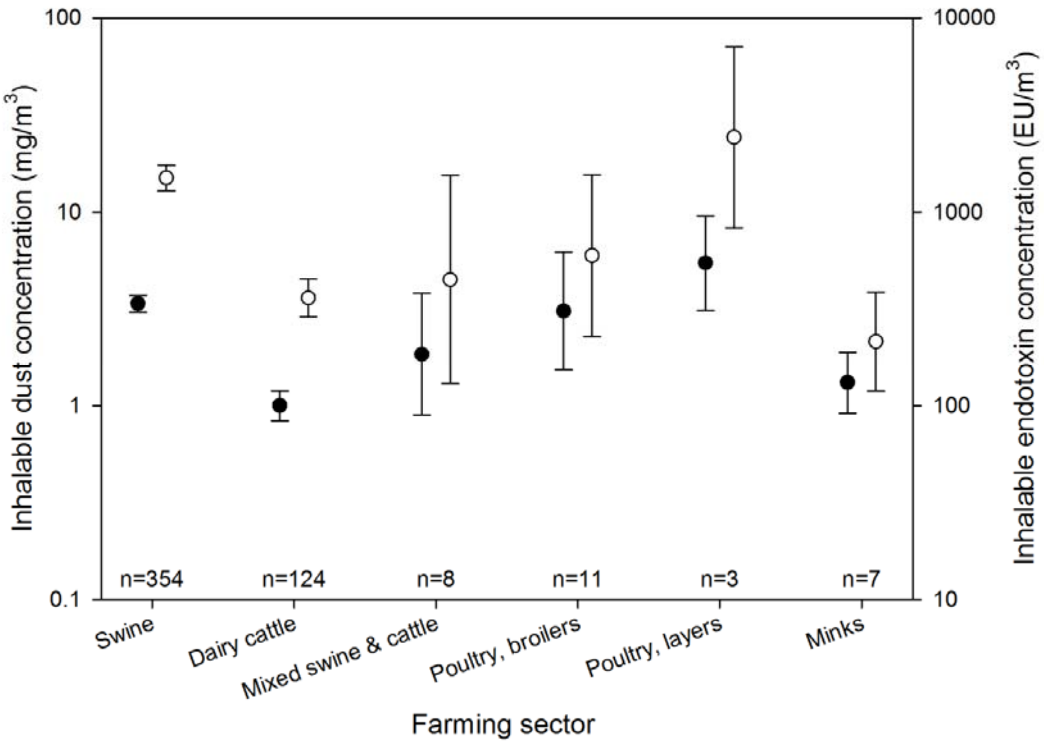

Figure 1 Inhalable dust (●) and endotoxin (○) exposure levels (geometric mean ± 95% confidence intervals) obtained by personal sampling in different types of Danish animal farms.

The dust and endotoxin exposure concentrations in the different stages of the mink and poultry broiler production are given in Table 3. Overall, exposure was moderate through almost all the different stages of the mink production. The highest concentrations for dust were measured during the pelting stage and for endotoxin during whelping (Figure 2A). On the contrary, 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 (Figure 2B).

Table 3 Personal dust and endotoxin exposure levels measured in different stages of the Danish mink and poultry production.

Farming type and stage of production	n	Inhalable dust (mg/m^3)			Endotoxin (EU/m^3)		
		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 - 1054
Breeding	1	0.5	--	--	121	--	--
Whelping	1	2.0	--	--	1054	--	--
Furring/grading	2	1.2	1.2 (1.0)	1.1 - 1.2	121	118 (1.4)	93 - 149
Pelting	3	1.7	1.7 (1.3)	1.4 - 2.3	231	228 (1.2)	178 - 264
Poultry Broilers							
Overall	11	5.6	3.1 (3.3)	0.7 - 18.3	1580	596 (5.1)	61 - 6424
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 (1.2)	0.9 - 1.1	389	379 (1.4)	302 - 476
3rd week	2	5.0	4.8 (1.6)	3.4 - 6.6	1874	1818 (1.4)	1421 - 2326
5th week	3	14.5	14.3 (1.3)	11.5 - 18.3	4132	3788 (1.7)	2314 - 6424

n, number of measurements; AM, arithmetic mean; GM, geometrical mean; GSD, geometrical standard deviation.

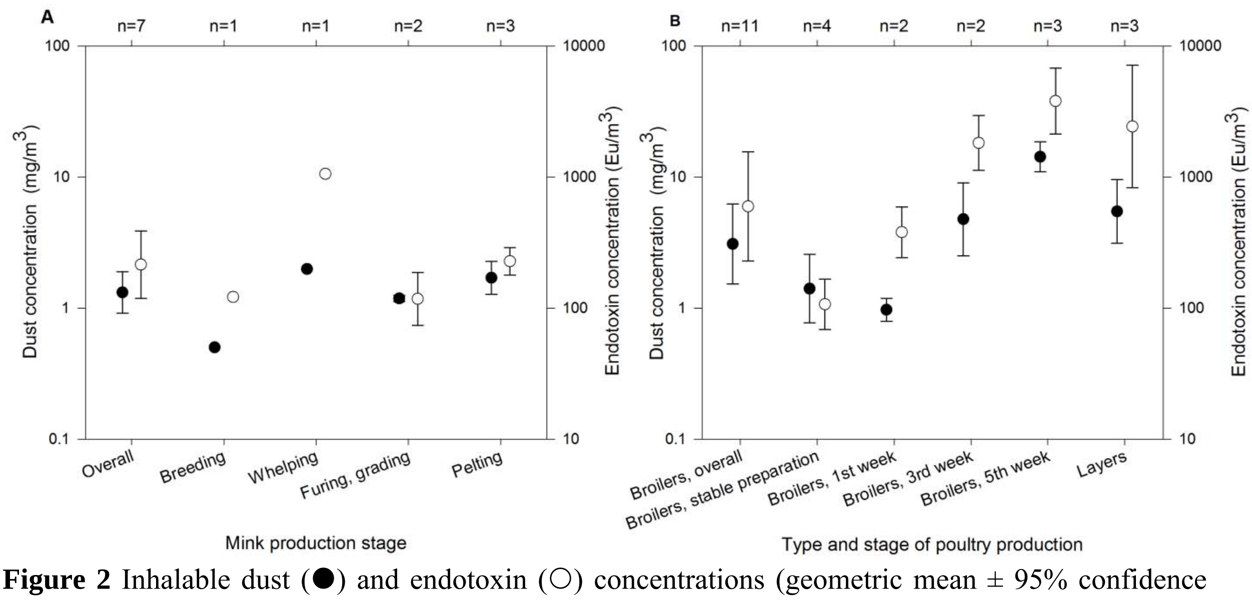

Figure 2 Inhalable dust (●) and endotoxin (○) concentrations (geometric mean ± 95% confidence intervals), measured in different stages of mink (A) and poultry (B) production farms.

The results of the random effects models with and without the farm level are summarized in Table 4. 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 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 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 55% of the between-worker variance for dust and endotoxin 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.

Table 4 Variance components for dust and endotoxin exposure in Danish pig and dairy cattle farmers.

	n	Inhalable dust						Endotoxin					
		$_{bf}\sigma^2$	$_{bw}\sigma^2$	$_{ww}\sigma^2$	$_{bw}R_{0.95}$	$_{ww}R_{0.95}$	λ	$_{bf}\sigma^2$	$_{bw}\sigma^2$	$_{ww}\sigma^2$	$_{bw}R_{0.95}$	$_{ww}R_{0.95}$	λ
Model 1[a]													
Pigs	354	--	0.25	0.65	6.98	23.79	2.7	--	0.19	1.97	5.40	245.97	10.6
Cattle	124	--	0.46	0.58	14.10	19.74	1.3	--	0.47	1.16	14.62	67.71	2.5
Model 2[b]													
Pigs	354	0.04	0.19	0.66	5.60	24.30	3.4	0.00	0.18	1.97	5.36	245.90	10.7
Cattle	124	0.20	0.21	0.61	6.11	21.30	2.9	0.12	0.34	1.17	9.67	69.57	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; $_{bf}\sigma^2$, between-farm variance; $_{bw}\sigma^2$, between-worker (within-farms) variance; $_{ww}\sigma^2$, within-worker (day-to-day) variance; $_{bw}R_{0.95}$, ratio of the 2.5th and 97.5th percentile of the between-worker variance of the log-normally distributed exposure; $_{ww}R_{0.95}$, ratio of the 2.5th and 97.5th percentile of the within-worker variance of the log-normally distributed exposure; λ, ratio of within- and between-worker variance.

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 5). 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 number of repeated measurements in other working environments (Table 5). 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.

Table 5 Variance components by usual working environment for dust end endotoxin exposure in Danish pig and dairy cattle farmers.

	n	f	k	Inhalable dust						Endotoxin					
				$_{bf}\sigma^2$	$_{bw}\sigma^2$	$_{ww}\sigma^2$	$_{bf}R_{0.95}$	$_{bw}R_{0.95}$	$_{ww}R_{0.95}$	$_{bf}\sigma^2$	$_{bw}\sigma^2$	$_{ww}\sigma^2$	$_{bf}R_{0.95}$	$_{bw}R_{0.95}$	$_{ww}R_{0.95}$
Pigs															
Indoor[a]	266	45	177	0.04	0.12	0.47	2.24	3.94	14.82	0.09	0.08	1.53	3.16	2.98	126.98
Mixed, in- & outdoor[b]	62	26	35	--	0.21	0.76	--	5.94	30.19	--	0.00	1.90	--	0.00	221.23
Outdoor[b]	26	15	19	--	0.00	2.05	--	0.00	274.30	--	0.00	4.45	--	0.00	3911.45
Cattle															
Indoor[a]	71	20	47	0.06	0.60	0.49	2.54	20.81	15.36	0.13	0.50	1.00	4.15	16.09	50.84
Mixed, in- & outdoor[b]	43	20	24	--	0.13	0.67	--	4.08	24.59	--	0.58	0.61	--	19.68	21.17
Outdoor[b]	10	5	6	--	0.45	0.89	--	13.93	40.43	--	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.

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; though, 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 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, irrespectively of the applied type of production; though, as expected, the highest dust and endotoxin exposure concentrations were measured among pig farmers (47.8 mg/m3 and 374,600 EU/m3, respectively) and poultry farmers (18.3 mg/m3 and 7,092 EU/m3, 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.[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 3.6, 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 3,860 EU/m^3 and from 360 to 8,120 EU/m^3 respectively, which are similar to the ranges we found (range <LOD-5996 and 61-7,092 EU/m^3 for cattle and poultry farmers, respectively). The higher endotoxin levels among pig farmers in our

study (range: <LOD-374,600 EU/m^3) compared to the levels (range 992-6,970 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 case of an overestimation of the exposure concentrations of up to 10-folds 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 winter than 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] Though, potential differences in the applied farming practices 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-folds, respectively, between the beginning (chicks aged 1 to 2 days) and end (1 day before harvest) of the chicks' growth circle. The routine activity patterns followed by the workers during the broiler fattening stage suggest this trend to be primarily associated to 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 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 (${}_{bw}R_{0.95}$=5.4), but our fold-range in average daily concentrations was somewhat higher (${}_{ww}R_{0.95}$=245) 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 spent 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 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. On the contrary, 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 literature.[53] For example, given our sample and dust results (Table 4, Model 1) for pig farmers a minimal bias on exposure estimation of 50% should be expected. A bias reduction to a maximum value of 10% will require acquisition of at least 10-times more measurements per worker. Though, collection of such amounts of samples per individual are 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 details 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 circle 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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Reference List

1 Landbrug og Fødevarer, *Dansk landbrug i tal 2009. [The Danish agriculture in figures 2009]. An anual report by the Danish Agriculture and Food Council.*, The Danish Agriculture and Food Council, Axelborg, Copenhagen, 2009.

2 EFBA, *The European Fur Breeders' Association - Annual report 2009. Available from http://www.efba.eu/download/annual_report/2009/index.html (accessed 08 February 2011)*, 2009.

3 O. Omland, *Ann Agric Environ Med*, 2002, **9**, 119-136.

4 M. B. Schenker, D. Christiani, Y. Cormier, H. Dimich-Ward, G. Doekes, J. Dosman, J. Douwes, K. Dowling, D. Enarson, F. Green, D. Heederik, K. Husman, S. Kennedy, G. Kullman, Y. Lacasse, B. Lawson, P. Malmberg, J. May, S. McCurdy, J. Merchant, J. Myers, M. Nieuwenhuijsen, S. Olenchock, C. Saiki, D. Schwartz, J. Seiber, P. Thorne, G. Wagner, N. White, X. P. Xu, M. Chan-Yeung and A. E. O. Hlth, *Am J Resp Crit Care*, 1998, **158**, S1-S76.

5 S. R. Kirkhorn and V. F. Garry, *Environ Health Perspect*, 2000, **108 Suppl 4**, 705-712.

6 T. Sigsgaard, Ø. Omland and P. S. Thorne, in *Occupational Asthma*, eds. T. Sigsgaard and D. Heederik, Birkhauser Basel, Basel, Switzerland, Editon edn., 2010, pp. 163-183.

7 J. Douwes, P. Thorne, N. Pearce and D. Heederik, *Ann Occup Hyg*, 2003, **47**, 187-200.

8 W. Eduard, J. Douwes, E. Omenaas and D. Heederik, *Thorax*, 2004, **59**, 381-386.

9 L. A. Smit, D. Heederik, G. Doekes, J. W. Lammers and I. M. Wouters, *Int Arch Allergy Immunol*, 2010, **152**, 151-158.

10 P. F. Vogelzang, J. W. van der Gulden, H. Folgering, J. J. Kolk, D. Heederik, L. Preller, M. J. Tielen and C. P. van Schayck, *Am J Respir Crit Care Med*, 1998, **157**, 15-18.

11 S. Spaan, I. M. Wouters, I. Oosting, G. Doekes and D. Heederik, *J Environ Monit*, 2006, **8**, 63-72.

12 M. J. Nieuwenhuijsen, K. S. Noderer, M. B. Schenker, V. Vallyathan and S. Olenchock, *Ann Occup Hyg*, 1999, **43**, 35-42.

13 K. Radon, B. Danuser, M. Iversen, E. Monso, C. Weber, J. Hartung, K. Donham, U. Palmgren and D. Nowak, *Ann Agric Environ Med*, 2002, **9**, 41-48.

14 H. Kromhout and D. Heederik, *Scand J Work Environ Health*, 2005, **31 Suppl 1**, 33-38; discussion 35-37.

15 R. Schierl, A. Heise, U. Egger, F. Schneider, R. Eichelser, S. Neser and D. Nowak, *Ann Agric Environ Med*, 2007, **14**, 129-136.

16 J. Seedorf, J. Hartung, M. Schroder, K. H. Linkert, V. R. Phillips, M. R. Holden, R. W. Sneath, J. L. Short, R. P. White, S. Pedersen, H. Takai, J. O. Johnsen, J. H. M. Metz, P. W. G. G. Koerkamp, G. H. Uenk and C. M. Wathes, *Journal of Agricultural Engineering Research*, 1998, **70**, 97-109.

17 H. Takai, S. Pedersen, J. O. Johnsen, J. H. M. Metz, P. W. G. G. Koerkamp, G. H. Uenk, V. R. Phillips, M. R. Holden, R. W. Sneath, J. L. Short, R. P. White, J. Hartung, J. Seedorf, M. Schroder, K. H. Linkert and C. M. Wathes, *Journal of Agricultural Engineering Research*, 1998, **70**, 59-77.

18 J. C. Simpson, R. M. Niven, C. A. Pickering, L. A. Oldham, A. M. Fletcher and H. C. Francis, *Ann Occup Hyg*, 1999, **43**, 107-115.

19 L. Preller, H. Kromhout, D. Heederik and M. J. Tielen, *Scand J Work Environ Health*, 1995, **21**, 504-512.

20 S. Spaan, J. Schinkel, I. M. Wouters, L. Preller, E. Tielemans, E. T. Nij and D. Heederik, *Ann Occup Hyg*, 2008, **52**, 303-316.

21 P. Vinzents and B. H. Nielsen, *Am Ind Hyg Assoc J*, 1992, **53**, 237-241.

22 G. Elholm, O. Omland, V. Schlunssen, C. Hjort, I. Basinas and T. Sigsgaard, *Clin Epidemiol*, 2010, **2**, 45-50.

23 T. Sigsgaard, C. Hjort, O. Omland, M. R. Miller and O. F. Pedersen, *J Agromedicine*, 1997, **4**, 63-78.

24 StatBank Denmark, *Statistics Denmark. BDF07: Farms by region, type, farms and area (2006-2010): HDYR07: Livestock by county, unit and type (2006-2010): PELS2: Furred animals by region, unit and type of farm (2007-2009). Available at http://www.statbank.dk/statbank5a/default.asp?w=1440 (accessed 08 April 2011).*

25 Dansk landbrugsrådgivning, *Håndbog til drifts-planlægning. [Knowledge Centre for Agriculture. Manual for farm operation].* 46 edn., Landbrugsforlaget. p. 20-29., Århus, 2007.

26 L. C. Kenny, R. Aitken, C. Chalmers, J. F. Fabries, E. Gonzalez-Fernandez, H. Kromhout, G. Liden, D. Mark, G. Riediger and V. Prodi, *Ann Occup Hyg*, 1997, **41**, 135-153.

27 S. Spaan, G. Doekes, D. Heederik, P. S. Thorne and I. M. Wouters, *Appl Environ Microbiol*, 2008, **74**, 3804-3811.

28 C. Peretz, A. Goren, T. Smid and H. Kromhout, *Ann Occup Hyg*, 2002, **46**, 69-77.

29 S. M. Rappaport, M. Weaver, D. Taylor, L. Kupper and P. Susi, *Ann Occup Hyg*, 1999, **43**, 457-469.

30 S. M. Rappaport, *Ann Occup Hyg*, 1991, **35**, 61-121.

31 Arbejdstilsynet, *At-vejledning. Grænseværdier for stoffer og materialer. [Danish Working Environment Authority. Limit values for substances and materials]. Publication no. C.0.1*, The Danish Working Environment Authority, Copenhagen, 2007.

32 P. S. Vinzents, Y. Thomassen and S. Hetland, *Ann Occup Hyg*, 1995, **39**, 795-800.

33 M. A. Werner, T. M. Spear and J. H. Vincent, *Analyst*, 1996, **121**, 1207-1214.

34 S. C. Soderholm, *Appl Occup Environ Hyg*, 1993, **8**, 453-457.

35 G. Liden, B. Melin, A. Lidblom, K. Lindberg and J. O. Noren, *Appl Occup Environ Hyg*, 2000, **15**, 263-276.

36 A. M. Madsen, V. M. Hansen, S. H. Nielsen and T. T. Olsen, *Ann Occup Hyg*, 2009, **53**, 129-138.

37 DECOS, *Endotoxins: health based recommended exposure limit. A report of the Health Council of the Netherlands, publication no. 2010/04OSH*, Health Council of the Netherlands, The Hague, 2010.

38 L. A. Smit, D. Heederik, G. Doekes, C. Blom, I. van Zweden and I. M. Wouters, *Eur Respir J*, 2008, **31**, 1241-1248.

39 U. Latza, M. Oldenburg and X. Baur, *Arch Environ Health*, 2004, **59**, 519-525.

40 G. J. Kullman, P. S. Thorne, P. F. Waldron, J. J. Marx, B. Ault, D. M. Lewis, P. D. Siegel, S. A. Olenchock and J. A. Merchant, *Am Ind Hyg Assoc J*, 1998, **59**, 403-413.

41 L. Preller, D. Heederik, H. Kromhout, J. S. Boleij and M. J. Tielen, *Ann Occup Hyg*, 1995, **39**, 545-557.

42 S. Spaan, D. J. Heederik, P. S. Thorne and I. M. Wouters, *Appl Environ Microbiol*, 2007, **73**, 6134-6143.

43 D. T. Chun, K. Bartlett, T. Gordon, R. R. Jacobs, B. M. Larsson, L. Larsson, D. M. Lewis, J. Liesivuori, O. Michel, D. K. Milton, R. Rylander, P. S. Thorne, E. M. White, M. E. Brown, V. S. Gunn and H. Wurtz, *Am J Ind Med*, 2006, **49**, 301-306.

44 S. J. Reynolds, P. S. Thorne, K. J. Donham, E. A. Croteau, K. M. Kelly, D. Lewis, M. Whitmer, D. J. Heederik, J. Douwes, I. Connaughton, S. Koch, P. Malmberg, B. M. Larsson and D. K. Milton, *AIHA J (Fairfax, Va)*, 2002, **63**, 430-438.

45 J. H. Bonlokke, A. Meriaux, C. Duchaine, S. Godbout and Y. Cormier, *Ann Agric Environ Med*, 2009, **16**, 43-52.

46 P. T. O'Shaughnessy, K. J. Donham, T. M. Peters, C. Taylor, R. Altmaier and K. M. Kelly, *J Occup Environ Hyg*, 2010, **7**, 7-13.

47 B. Nehme, V. Letourneau, R. J. Forster, M. Veillette and C. Duchaine, *Environ Microbiol*, 2008, **10**, 665-675.

48 J. Seedorf, J. Hartung, M. Schroder, K. H. Linkert, S. Pedersen, H. Takai, J. O. Johnsen, J. H. M. Metz, P. W. G. G. Koerkamp, G. H. Uenk, V. R. Phillips, M. R. Holden, R. W. Sneath, J. L. Short, R. P. White and C. M. Wathes, *Journal of Agricultural Engineering Research*, 1998, **70**, 39-47.

49 A. Senthilselvan, J. Beach, J. Feddes, N. Cherry and I. Wenger, *Occup Environ Med*, 2011, **68**, 102-107.

50 A. Oppliger, N. Charriere, P. O. Droz and T. Rinsoz, *Ann Occup Hyg*, 2008, **52**, 405-412.

51 N. Just, C. Duchaine and B. Singh, *J Occup Med Toxicol*, 2009, **4**, 13.

52 H. Kromhout, E. Symanski and S. M. Rappaport, *Ann Occup Hyg*, 1993, **37**, 253-270.

53 K. Liu, J. Stamler, A. Dyer, J. McKeever and P. McKeever, *J Chronic Dis*, 1978, **31**, 399-418.

Online appendix to:

Title:

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

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Table A1 Distribution of full-time farmers per type of specialization and geographical area in the SUS12 cohort population (n=1239).

Farm type	**Geographical area, n (%)**			
	Denmark	**Jutland**	**Zealand**	**Funen**
Cattle, dairy	106 (25.1)	91 (28.0)	11 (16.9)	4 (12.1)
Cattle, beef	18 (4.3)	16 (4.9)	1 (1.5)	1 (3.0)
Pig	206 (48.7)	159 (48.9)	28 (43.1)	19 (57.6)
Mink	16 (3.8)	15 (4.6)	0 (0)	1 (3.0)
Field/crop	52 (12.3)	25 (7.7)	22 (33.8)	5 (15.2)
Poultry	2 (0.4)	2 (0.7)	0 (0)	0 (0)
Other, mixed	23 (5.4)	17 (5.2)	3 (4.6)	3 (9.1)
Total	**423**	**325**	**65**	**33**

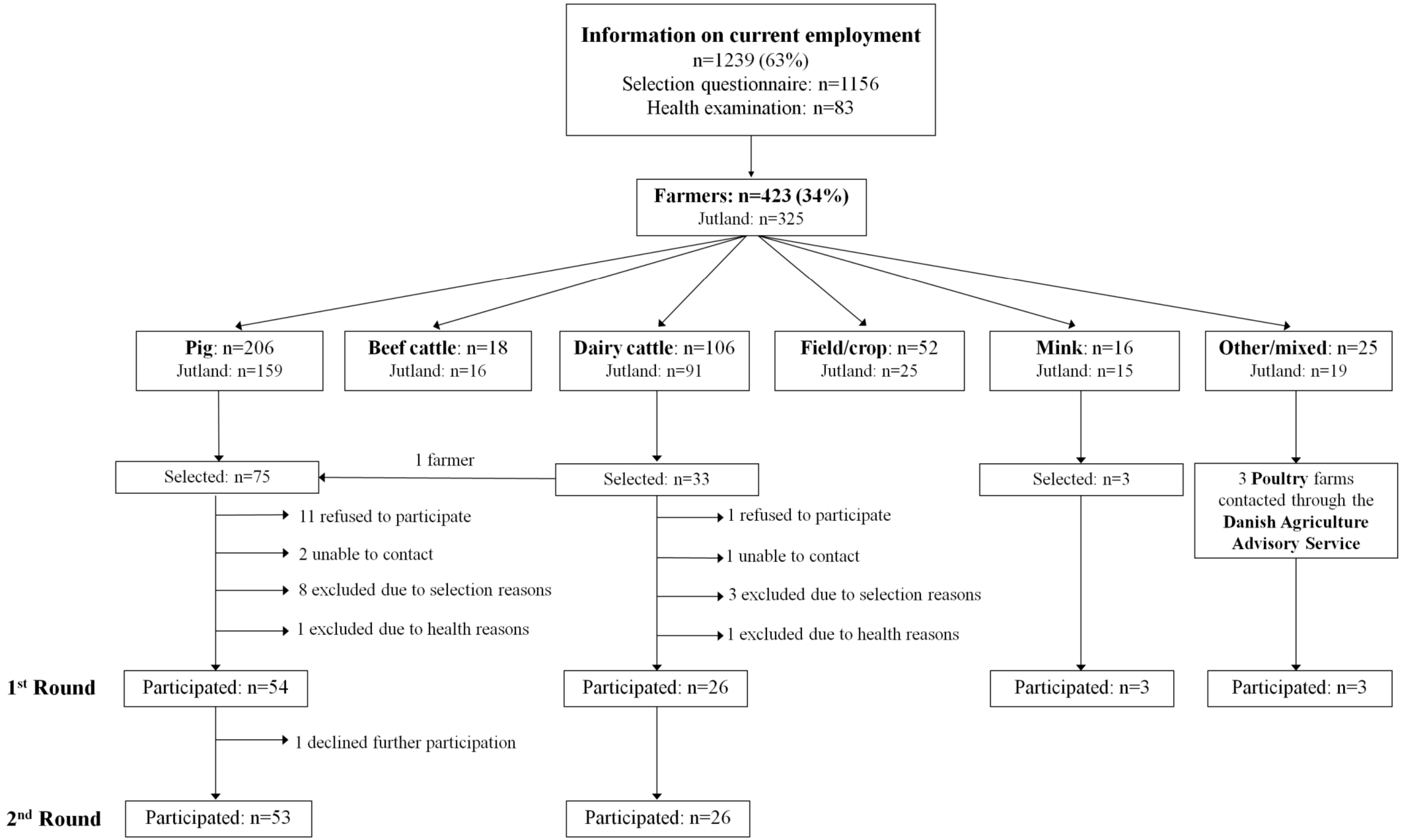

Figure A1 Flow chart describing the recruitment of farmers within the study.

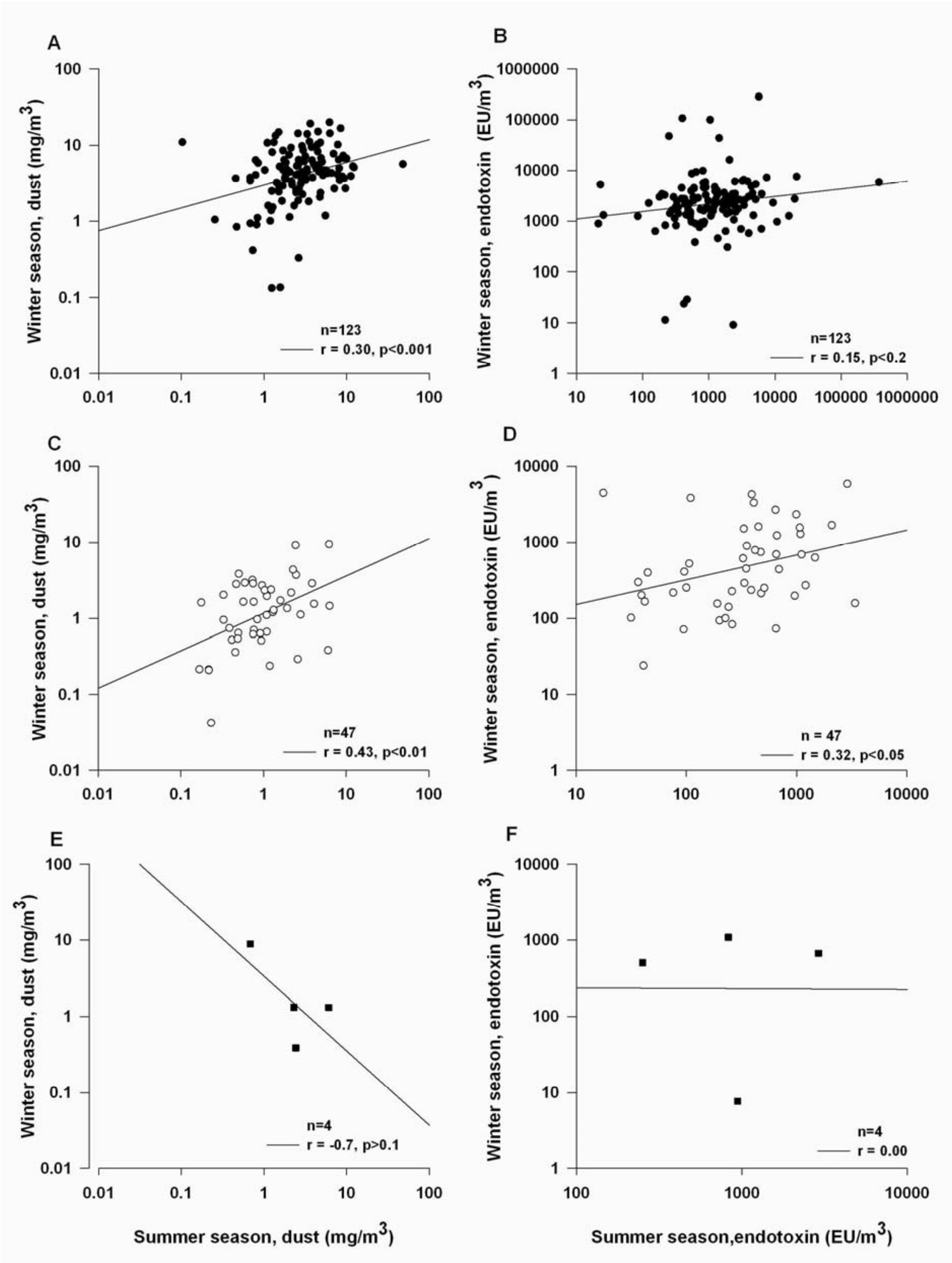

Figure A2 Correlations between repeated measurements for measured dust (A, C and E) and endotoxin (B, D and F) concentrations in pig (black dots, n=123), dairy cattle (white dots, n=47), and mixed farmers (black squares, n=4), respectively.

Manuscript II

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Title:

Sensitization to common allergens and respiratory symptoms in endotoxin exposed workers: a pooled analysis

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Keywords: Occupational endotoxin exposure; Pooled study; Farm childhood; Respiration disorders; Allergy.

ABSTRACT

Objective: To test the hypotheses that current endotoxin exposure is inversely associated with allergic sensitization and positively with non-allergic respiratory diseases in four occupationally exposed populations using a standardised analytical approach.

Methods: Data were pooled from four epidemiological studies including 3883 Dutch and Danish employees in veterinary medicine, agriculture, and power plants using biofuel. Endotoxin exposure was estimated by quantitative job-exposure matrices specific for the study populations. Dose-response relationships between exposure, IgE-mediated sensitization to common allergens and self-reported health symptoms were assessed using logistic regression and generalized additive modelling. Adjustments were made for study, age, sex, atopic predisposition, smoking habit, and farm childhood. Heterogeneity was assessed by analysis stratified by study.

Results: Current endotoxin exposure was dose-dependently associated with a reduced prevalence of allergic sensitization (ORs of 0.92, 0.81 and 0.66 for low mediate, high mediate and high exposure) and hay fever (ORs of 1.16, 0.81 and 0.58). Endotoxin exposure was a risk factor for organic dust toxic syndrome (ODTS), and levels above 100 EU/m^3 significantly increased the risk of chronic bronchitis ($p<0.0001$). Stratification by farm childhood showed no effect modification except for allergic sensitization. Only among workers without a farm childhood endotoxin exposure was inversely associated with allergic sensitization. Heterogeneity was primarily present for biofuel workers.

Conclusions: Occupational endotoxin exposure has a protective effect on allergic sensitization and hay fever but increases the risk for ODTS and chronic bronchitis. Endotoxins' protective effects are most clearly observed among agricultural workers.

What this paper adds

- Adult-onset endotoxin exposure is a well-known risk factor for respiratory disorders, but recent studies on farming populations suggest that it may also protect from allergic disease.
- Evidence on this dual effect of endotoxin exposure is limited, and whether the protective effects apply to other populations than farmers remains unclear.
- Occupational endotoxin exposure significantly increased the risk of chronic bronchitis and ODTS symptoms.
- Inverse relationships between endotoxin and atopy and hay fever were found most clearly among workers with agricultural related exposures.

INTRODUCTION

Several adult population studies show that early life exposure to farming decreases the risk of allergic sensitization and asthma throughout life.[1-4] This association is commonly attributed to high exposure of farm children to microbial agents of bacterial and fungal origin like endotoxin and β(1→3)-glucans.[5] In particular, exposure to such agents is speculated to stimulate the innate immune system, either by suppression of the atopic Th2 responses or by the induction of an increase in the shift from Th2 to the Th1 phenotype.[5] Evidence exists that this protective effect may not be limited to exposure during childhood. Studies in adult populations have indicated that farmers[6] and rural dwellers[1] have a lower risk of asthma and allergic sensitization. These results were confirmed by studies using animal contact as a proxy for microbial exposure, demonstrating the lowest risk of allergic sensitization in subjects combining farm childhood and farm animal contact in adulthood.[2, 4, 7] In addition, inverse associations between measured endotoxin exposure and atopy or allergic asthma, were found in studies among Norwegian[8] and Dutch farmers,[9] and among agricultural industry workers.[10]

Beside its protective effects, bacterial endotoxin is well-known for its pro-inflammatory capability. High occupational exposure to airborne endotoxin has long been associated with a number of acute and chronic health effects like organic dust toxic syndrome (ODTS), bronchial hyper-responsiveness, asthma-like symptoms, chest tightness, cough, shortness of breath, wheezing, chronic bronchitis, inflammation in the airways, and accelerated lung function decline.[10-17] Interestingly, these adverse health effects were also found by studies in which inverse dose-response relationships between endotoxin exposure and allergic asthma or atopy were reported.[8-10, 14] These intriguing findings indicate both a positive and negative role of endotoxin on the development of health effects in humans. Nevertheless, the information on this dual effect of endotoxin remains limited as half of the studies that reported it,[8, 9] did not consider farm exposures that occurred during childhood and were exclusively performed in farming populations where selection out of farming might also explain the observed inverse associations between endotoxin exposure and atopy.

In the present study, we pooled data from four studies of employees including veterinary students, farm apprentices, and biofuel and agricultural industry workers exposed to microbial exposures at work. We performed combined analysis and explored relationships between endotoxin exposure and allergic sensitization, asthma and other respiratory diseases, taking early-life exposures to farming into consideration. Furthermore, we investigated whether dose-response relationships differed between the four subpopulations in order to explore the hypothesised extension of the effects of endotoxin exposure in other occupational groups than farmers.[10] The study was performed in the framework of the GABRIEL project (www.gabriel-fp6.org).

MATERIALS AND METHODS

Study Design

The present study is a pooled analysis of the baseline data from four studies from Denmark and the Netherlands: i) a cross-sectional investigation that explored relationships between current endotoxin exposure and respiratory and allergic outcomes in a population of 525 farmers (participation rate 61%) and 376 workers in 23 agricultural processing companies (participation rate 90%) in the Netherlands.[10] ii) A Danish prospective cohort study (SUS) including 1964 farm apprentices (participation rate 79%) that explored the role of farm exposures on the development of atopy and respiratory diseases in young farmers.[3, 18, 19] iii) A cross-sectional study among Danish power plant workers which assessed relationships between bio-aerosol exposure, allergy and respiratory health.[17] The latter study included 94 power plant workers using straw (participation rate 75%) and 138 power plant workers using wood chip (participation rate 74%). iv) A cross-sectional study that addressed the effect of bio-aerosol exposure on the development of allergic and non-allergic respiratory diseases in veterinary students (the veterinarians' health study). The study population consisted of veterinary students at Utrecht University participating in the study from June to October 2006 (n=901, participation rate 65%). More details on the design and methodology of the specific study are given in the online supplement (supplement A).

All four studies used detailed questionnaires on asthma, atopy, familial history of asthma and/or allergy, smoking and occupational history. The comparability between questionnaires was assessed based on the meaning and timing of questions referring to the same airway or atopic symptom or personal characteristic (see supplement B, Table S1 for details).

The questionnaire was followed by a comprehensive health investigation in all four studies. Allergic sensitization against common inhalant allergens (pollen, house dust mite, cat, dog) was assessed by means of skin prick tests in the Danish studies, and by serological testing of specific IgE using enzyme immunoassays [20] in the Dutch studies.

Ethics approval was provided for the original studies by the Ethical Committees of Aarhus County and University of Utrecht for Danish and Dutch studies, respectively.

Pooled population

Questionnaire information was available for all 3998 subjects in the four studies. In addition, information on IgE-mediated sensitization was available for 434 (342 processing workers, 92 famers) agricultural industry workers, 641 veterinary students, 1959 farm apprentices and 200 (120 wood chip workers, 80 straw workers) biofuel workers. Twenty-one farm apprentices, 4 veterinary students and 46 biofuel workers without data on exposure as well as one biofuel and 15 agricultural industry workers aged >65 years were excluded from the analysis. A further 28 workers (10 farm apprentices, 9 agricultural industry and 9 biofuel workers) with incomplete data on potential confounders (farm childhood, age, familial history of allergic diseases or smoking status) were also removed resulting in 3170 and 3883 subjects available for statistical analysis with sensitization and symptoms, respectively. Supplement C, Figure S1 represents a schematic overview of the pooling process.

Health Outcome Definitions

Asthma was defined as a positive response to any of the following questions: “Are you currently taking any medicine for asthma?”, “Have you ever had asthma?”, “Have you sometimes had wheezing in the chest, during the last year?” and “During the last 12 months have you, sometimes been woken up with a feeling of tightness in your chest?”. Chronic bronchitis was defined as “coughing up phlegm almost daily, for 3 months in a row during the last year" and wheezing as “at least one attack of wheezing during the last year”. A combination of self-reported pollen allergy accompanied by eye (itching or watery eyes) or nose (sneezing) symptoms or a positive answers to the question “Have you ever had hay fever?” was used to define hay fever. Atopy was determined as elevated serum IgE levels or positive skin-prick tests (the mean of the longest diameter and the midpoint orthogonal diameter of the weal >3 mm) to one or more of the following common allergens: pollen (grass or birch), house-dust mites, cat and dog (details in supplement B, Table S1). Self-reported allergy was defined as self-reported allergic reactions (lung, nose and/or eye symptoms) against pollen, animal or house dust allergens. An affirmative answer to the question “Have you, during the past 12 months, had sudden episodes of flu-like symptoms such as fever, chills, malaise, muscle- or joint pains, and felt completely well within 1-2 days?” was used as a proxy for ODTS episodes.

Exposure Assessment

Information on the participant’s occupational and exposure history including information on specializations, tasks, areas and the duration of exposure (e.g. hours per day or weeks per year) were available from the study questionnaires. In addition, endotoxin levels were available from more than 1200 personal and stationary measurements performed within the investigations of the Danish biofuel and the Dutch agricultural and veterinary studies and during the 15th year follow-up of the Danish SUS study.[10, 17, 18, 21, 22] The current personal endotoxin exposure was estimated for every worker by means of quantitative Job-Exposure Matrices (JEMs) developed from measurements and the available questionnaire information in each of the participating studies (for details, see the supplement D). For 535 farm apprentices, who had complete information on exposure but considered non-exposed in the corresponding JEM, a background exposure concentration of 1 EU/m^3 was assumed based on levels reported in non-industrial occupational[23, 24] and residential[25] indoor environments.

Data Analysis

Data analysis was performed using SAS statistical software (version 9.2; SAS Institute Inc, Cary, NC, USA), for Windows. The pooled study population was divided into four exposure categories (low, low mediate, high mediate and highly exposed). Earlier proposed exposure standards of 50 and 200 EU/m^3 for endotoxin exposure by the National Health Council and the Social and Economical council of the Netherlands [12, 26] along with the suggested "no-effect level" of 100 ng/m^3 (~1000 EU/m^3) [27] were used as cut-off points. Differences in prevalence of diseases and characteristics between sub-groups of the pooled study population were assessed with chi-square tests. Kruskal-Wallis and Mann-Whitney tests were used for comparison of continuous variables. Associations between endotoxin exposure and health outcomes were assessed with logistic regression analysis (PROC LOGISTIC), using the lowest exposure group as reference. Potential confounders (atopic predisposition, gender, smoking, age and exposure to farming during childhood) were considered in the analysis. The reliability of the demonstrated associations and the heterogeneity across populations were investigated by adjustment for study and by stratified analyses. Effect modification by farm childhood and atopic status was assessed using interaction terms in the multiple regression models and by stratified analyses.

The linearity and the shape of the estimated associations in the main analysis was further tested (a) by using endotoxin exposure as a continuous variable in the final multivariate logistic regression models and (b) by generalized additive modelling (smoothing).[28] All analyses with endotoxin exposure as a continuous variable were performed on log-transformed endotoxin exposure concentrations. The statistical significance level was set at 5% (two-sided) for all the applied tests and models.

RESULTS

Basic characteristics of the four studies and the pooled population are shown in table 1. Overall, the median estimated current average endotoxin exposure was 219 EU/m^3 (range: 0.01-10645 EU/m^3). Exposure differed significantly between study populations ($p<0.0001$) and the estimated levels ranged (median) from between 14-10645 EU/m^3 (219 EU/m^3) for Dutch farmers and agricultural industry workers to 1-1495 (215 EU/m^3), 3.2-749 (309 EU/m^3) and 0.01-294 EU/m^3 (3.4 EU/m^3) for farm apprentices, veterinary students and biofuel workers, respectively. In addition, the study populations differed significantly also in gender, farm childhood, smoking habits and atopic predisposition. Farm apprentices were younger and reported less frequently allergic and respiratory symptoms than other occupational groups. Most of the participants (75%) who reported ODTS symptoms were Dutch farmers and agricultural industry workers. The prevalence of atopy was highest among veterinary students.

Table 1 Basic characteristics of 3883 workers in the pooled population and by participating study.

	Netherlands		Denmark		
	Agricultural industry workers (n=877)*	**Veterinary students (n=897)***	**Farm apprentices (n=1933)***	**Biofuel workers (n=176)***	**Pooled population (N=3883)***
Demographics					
Age, years	43.6 ±10.5	23.7±3.5	19.2 ±2.6	47.6±8.6	27.0 ±12.2
Gender, female	155 (17.7)	722 (80.5)	229 (11.9)	7 (4.0)	1113 (28.7)
Farming environment during childhood †	509 (58.0)	90 (10.0)	854 (44.2)	63 (35.8)	1516 (39.0)
Atopic predisposition					
Asthma or allergy in parents or siblings	273 (31.1)	674 (75.1)	587 (30.4)	30 (17.1)	1564 (40.3)
Asthma or allergy in parents	147 (16.8)	496 (55.3)	350(18.1)	16 (9.1)	1009 (26.0)
Asthma or allergy in siblings	181 (20.7)	509 (56.7)	346(17.9)	16 (9.1)	1052 (27.1)
Smoking habits,					
Past smoker	292 (33.3)	79 (8.8)	91 (4.7)	53 (30.1)	515 (13.3)
Current smoker	199 (22.7)	94 (10.5)	568 (29.4)	54 (30.7)	915 (23.6)
Smoking history, pack-years *	6.9 ±11.4	0.6 ±1.8	1.1 ±2.6	14.4 ±21.2	2.9 ±8.1
Endotoxin exposure					
EU/m^3, median (range)	219 (14-10645)	309 (3-749)	215 (1-1495)	3 (0.01-294)	219 (0.01-10645)
<50 EU/m^3	68 (7.8)	263 (29.3)	535 (27.7)	134 (76.1)	1000 (25.8)
50-200 EU/m^3	172 (19.6)	45 (5.0)	153 (7.9)	30 (17.1)	400 (10.3)
200-1000 EU/m^3	506 (57.7)	589 (65.7)	1076 (55.7)	12 (6.8)	2183 (56.2)
>1000 EU/m^3	131 (14.9)	0 (0)	169 (8.7)	0 (0)	300 (7.7)
Health symptoms					
Chronic bronchitis§	64 (7.3)	51 (5.7)	62 (3.2)	11 (6.3)	188 (4.9)
Wheezing§	101 (11.5)	100 (11.2)	172 (9.2)	30 (17.2)	403 (10.5)
Asthma§§	156 (17.9)	187 (20.9)	245 (12.8)	40 (23.3)	628 (16.3)
ODTS§	161 (18.6)	17 (1.9)	11 (0.6)	26 (14.9)	215 (5.6)
Hay fever§§	94 (10.7)	177 (19.7)	186 (9.7)	36 (20.8)	493 (12.7)
Self reported allergy§§	155 (17.7)	252 (28.1)	151 (7.8)	34 (19.9)	592 (15.3)
Atopy‡	83 (19.4) #	157 (24.6)$	345 (17.9)	30 (17.4)	615 (19.4)

Demographic data and data on health symptoms are presented as n (%) or as mean ±SD; EU, endotoxin unit.
*Numbers may vary due to missing values in the health outcome variables.
§Self-reported symptoms during the last 12 months.
§§Self-reported symptoms at any point in life.
‡Determined by serum IgE (the Netherlands) or skin-prick tests (Denmark) against pollen, house-dust mites, and cat and dog allergens.
#Based on information for 429 (91 famers, 338 agricultural processing workers) participants.
$Based on information from 639 participants. †Born, raised or lived in a farm for at least a year till the age of 5.

Table 2 summarizes the results of univariate and multiple logistic regression analysis between occupational endotoxin exposure and health outcomes of interest. Significant positive associations between endotoxin exposure, chronic bronchitis and ODTS were found. In contrast, endotoxin exposure was associated, in a dose dependent manner with a decreased prevalence of atopy, self-reported allergy and hay fever. Exposure to high levels of endotoxin was significantly associated with asthma and wheezing symptoms. Adjustment for confounders did not substantially change the associations between endotoxin exposure and the health outcomes (0% to 12% difference between crude and adjusted ORs), except for

ODTS where the association became less prominent but remained statistically significant (OR [95%CI] = 2.07 [1.24-3.46], 1.77 [1.16-2.70], and 3.20 [1.87-5.49] for the low mediate, high mediate and high exposure groups; respectively). Further analysis with adjustment for participating study barely attenuated the estimated ORs for most of the health outcomes.

Table 2 Univariate and multiple logistic regression results describing the association between occupational endotoxin exposure and respiratory and allergic disorders.

Symptom and Exposure group	**Univariate model**		**Adjusted model***		**P value for trend***
	OR	**95% CI**	**OR**	**95% CI**	
Chronic bronchitis§					
Low	1.00		1.00		
Low mediate	1.34	0.75 to 2.40	1.38	0.74 to 2.54	< 0.0001
High mediate	1.40	0.94 to 2.07	1.49	0.97 to 2.31	
High	3.66	2.23 to 6.00	4.11	2.36 to 7.15	
Wheezing§					
Low	1.00		1.00		
Low mediate	0.74	0.49 to 1.13	0.80	0.51 to 1.24	0.2040
High mediate	1.01	0.79 to 1.29	1.10	0.83 to 1.45	
High	1.69	1.16 to 2.45	1.72	1.14 to 2.60	
Asthma§§					
Low	1.00		1.00		
Low mediate	0.76	0.55 to 1.07	0.80	0.56 to 1.14	0.5676
High mediate	0.93	0.76 to 1.14	0.96	0.77 to 1.20	
High	1.42	1.03 to 1.96	1.52	1.07 to 2.15	
ODTS§					
Low	1.00		1.00		
Low mediate	3.30	2.02 to 5.39	1.68	0.95 to 2.96	0.0078
High mediate	1.71	1.14 to 2.56	1.80	1.07 to 3.02	
High	3.89	2.34 to 6.46	2.44	1.30 to 4.60	
Hay fever§§					
Low	1.00		1.00		
Low mediate	0.95	0.69 to 1.32	1.16	0.82 to 1.63	0.0177
High mediate	0.71	0.57 to 0.88	0.81	0.64 to 1.02	
High	0.45	0.29 to 0.71	0.58	0.36 to 0.93	
Self reported allergy§§					
Low	1.00		1.00		
Low mediate	0.88	0.65 to 1.20	0.90	0.64 to 1.25	0.0018
High mediate	0.71	0.58 to 0.87	0.70	0.56 to 0.87	
High	0.60	0.41 to 0.88	0.72	0.47 to 1.08	
Atopy‡					
Low	1.00		1.00		
Low mediate	0.90	0.64 to 1.26	0.92	0.65 to 1.30	0.0299
High mediate	0.83	0.68 to 1.01	0.81	0.66 to 1.00	
High	0.66	0.45 to 0.97	0.66	0.44 to 0.99	

*Results adjusted for gender, age, farm childhood, atopic predisposition, smoking habits, and participating study

§Self-reported symptoms during the last 12 months.

§§Self-reported symptoms at any point in life.

‡Determined by serum IgE or skin-prick tests against pollen, house-dust mites, and cat and dog allergens.

Analysis of the data with non-parametric methods (smoothing) confirmed the demonstrated pooled associations for chronic bronchitis and hay fever (Figure 1). A clear rise (Table 2; p value for trend <0.0001) in risk for chronic bronchitis from an exposure of 100 EU/m^3 and higher was seen, whereas for hay fever, the prevalence steadily decreased with exposure levels exceeding the 50 EU/m^3. In addition, endotoxin levels above 20 EU/m3 tended to decrease the risk of atopic sensitization (supplement E, Figure S2). A similar but steeper association was found for self-reported allergy. Despite the overall low frequency of ODTS, its prevalence tended to increase gradually with elevated levels of endotoxin exposure. A weak increasing trend was seen for symptoms of wheeze (supplement E, figure S2).

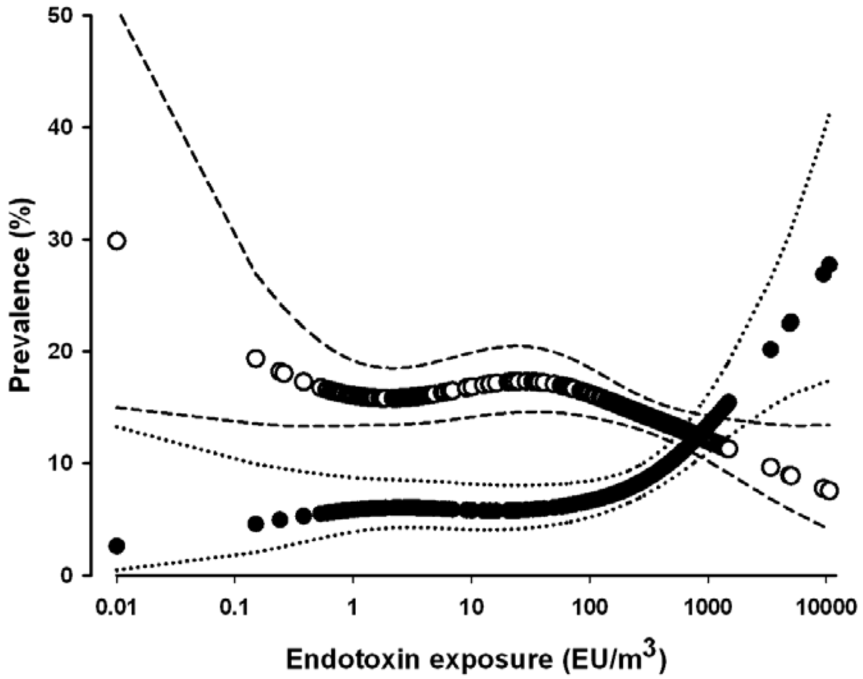

Figure 1 Smoothed relationships between endotoxin exposure, chronic bronchitis and hay fever for the pooled study population. ●: Chronic bronchitis; ○: Hay fever; ········ : ± 95% confidence intervals for chronic bronchitis. ———: ± 95% confidence intervals for hay fever. Results are adjusted for gender, age, farm childhood, atopic predisposition, smoking habits and participating study.

Study stratified analyses were undertaken to investigate possible heterogeneity between the involved populations (Table 3). Overall, there were no major differences in the estimated relationships across the three sub-populations of agricultural industry workers, veterinary and farm apprentices for most of the health outcomes under investigation. However, the protective effects of endotoxin exposure on atopy and hay fever appeared to be more prominent in the Dutch agricultural study. Similarly, stronger dose-response relationships between endotoxin exposure, wheeze and asthma were seen among Dutch agricultural industry workers compared to veterinary students and farm apprentices. The sub-population of biofuel workers showed biggest differences compared to the other studies in the estimated associations for ODTS, atopy and hay fever. Nevertheless, sensitivity analysis by excluding Danish biofuel workers hardly changed associations (Table 2) for any of the health outcomes under investigation (not shown).

Table 3 Multiple logistic regression results describing the association between endotoxin exposure and health outcome on each of the involved studies (results are adjusted for farm childhood, gender, age (continuous), atopic predisposition and smoking habits).

Symptom and Exposure group	Agricultural industry workers		Veterinary students		Farm apprentices		Biofuel workers	
	OR	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**
Chronic bronchitis§								
Low	1.00		1.00		1.00		1.00	
Low mediate	1.80	0.37 to 8.82	1.66	0.51 to 5.44	1.15	0.31 to 4.21	11.05[a]	1.27 to 96.35[a]
High mediate	2.65	0.61 to 11.43	1.09	0.56 to 2.15	1.37	0.69 to 2.72	8.44[a]	0.49 to 145.09[a]
High	6.04	1.35 to 27.14			3.34	1.48 to 7.53		
Wheezing§								
Low	1.00		1.00		1.00		1.00	
Low mediate	1.81	0.48 to 6.81	0.31	0.07 to 1.37	1.02	0.50 to 2.08	1.78	0.62 to 5.09
High mediate	3.32	0.98 to 11.25	0.79	0.50 to 1.24	1.01	0.68 to 1.49	5.09	1.28 to 20.24
High	5.92	1.67 to 20.99			1.14	0.64 to 2.05		
Asthma§§								
Low	1.00		1.00		1.00		1.00	
Low mediate	0.84	0.37 to 1.87	0.96	0.43 to 2.14	0.80	0.44 to 1.48	1.32	0.50 to 3.49
High mediate	1.26	0.62 to 2.56	0.91	0.64 to 1.31	0.87	0.63 to 1.20	3.60	0.93 to 13.94
High	1.91	0.88 to 4.14			1.24	0.76 to 2.01		
ODTS§								
Low	1.00		1.00		1.00		1.00	
Low mediate	1.45	0.64 to 3.30	x[b]		7.31[c]	0.64 to 83.09[c]	2.59	0.95 to 7.05
High mediate	1.80	0.85 to 3.82	1.45[c]	0.46 to 4.61[c]	2.21[c]	0.26 to 19.14[c]	0.77	0.09 to 6.82
High	2.17	0.95 to 4.95			6.28[c]	0.61 to 64.82[c]		
Hay fever§§								
Low	1.00		1.00		1.00		1.00	
Low mediate	0.94	0.45 to 1.96	0.98	0.44 to 2.20	1.19	0.66 to 2.12	0.68	0.21 to 2.24
High mediate	0.44	0.22 to 0.88	0.92	0.64 to 1.33	0.84	0.59 to 1.20	1.61	0.36 to 7.08
High	0.26	0.10 to 0.67			0.66	0.36 to 1.22		
Self reported allergy§§								
Low	1.00		1.00		1.00		1.00	
Low mediate	1.10	0.56 to 2.18	0.63	0.30 to 1.32	1.11	0.58 to 2.11	0.34	0.09 to 1.30
High mediate	0.71	0.38 to 1.34	0.68	0.49 to 0.94	0.78	0.53 to 1.15	0.55	0.06 to 5.07
High	0.72	0.34 to 1.50			0.74	0.39 to 1.41		
Atopy‡								
Low	1.00		1.00		1.00		1.00	
Low mediate	0.73	0.32 to 1.70	0.71	0.27 to 1.88	0.86	0.54 to 1.39	0.83	0.25 to 2.82
High mediate	0.35	0.17 to 0.69	0.78	0.52 to 1.17	0.90	0.69 to 1.18	1.36	0.24 to 7.79
High	0.25	0.10 to 0.61			0.75	0.46 to 1.20		

§Self-reported symptoms during the last 12 months.

§§Self-reported symptoms at any point in life.

‡Determined by serum IgE or skin-prick tests against pollen, house-dust mites, and cat and dog allergens.

[a]calculated only for males

[b]not calculated due to infinitive likelihood

[c]calculated only for current and non-smokers

We investigated whether associations between endotoxin exposure and asthma, wheeze and hay fever were modified by atopic status. Atopic and non-atopic subjects had approximately the same age, gender distribution, smoking habits, and exposure distribution. But atopic workers were less likely to be exposed to farming during childhood (28.3 vs. 39.3%) and had significantly more asthma (30.3 vs. 12.1%), wheezing (21.1 vs. 7.7%), and hay fever (43.0 vs.5.3%) symptoms than non-atopic workers.

Interestingly, in stratified multivariate analysis the prevalence of wheeze and asthma increased with exposure above 200 EU/m^3 among non-atopic workers and not in atopic workers (Table 4). In addition, the previously demonstrated inverse association between endotoxin exposure and hay fever was present only among atopic subjects. However, all p values for the interaction between endotoxin exposure and atopy were >0.05 (i.e. there were no formal interactions). Atopic status was available for only 18% (91 subjects) of the sub-population of farmers in the Dutch agricultural industry study. A sensitivity analysis excluding the Dutch farmers' population from the analysis had only a small influence on the estimated relationships for both atopic and non atopic subjects (not shown).

Table 4 Logistic regression analysis stratified by atopic status describing the associations between endotoxin exposure and the health symptoms of interest.

Symptom and Exposure group	Non-atopic workers (n=2555)		Atopic workers (n=615)	
	OR	**95% CI**	**OR**	**95% CI**
Wheezing§				
Low	1.00		1.00	
Low mediate	0.96	0.49 to 1.88	1.45	0.69 to 3.05
High mediate	1.25	0.83 to 1.87	1.03	0.64 to 1.66
High	2.04	1.17 to 3.55	0.80	0.30 to 2.11
Asthma§§				
Low	1.00		1.00	
Low mediate	0.75	0.43 to 1.30	1.52	0.79 to 2.95
High mediate	1.03	0.76 to 1.41	0.99	0.65 to 1.50
High	1.99	1.26 to 3.12	0.84	0.37 to 1.94
Hay fever§§				
Low	1.00		1.00	
Low mediate	1.37	0.72 to 2.62	1.28	0.68 to 2.41
High mediate	1.01	0.65 to 1.56	0.76	0.52 to 1.11
High	1.00	0.46 to 2.20	0.31	0.13 to 0.74

ORs are adjusted for gender, age, farm childhood, atopic predisposition and smoking habits and participating study.
§Self-reported symptoms during the last 12 months.
§§Self-reported symptoms at any point in life.

Univariate analysis showed a clear protective effect of farming exposure in childhood for atopy, self-reported allergy, hay fever, asthma, and wheeze, with ORs [95%CI] of 0.61[0.50-0.74], 0.49[0.40-0.59], 0.50[0.40-0.62], 0.61[0.51-0.73], and 0.62[0.50-0.78]; respectively. Adjustment for potential confounders and study did not considerably affect the estimated relationships for farm childhood and the respective health endpoints which remained strong and significant. Associations were not confounded by current exposure to endotoxin either (see supplement F, Table S2 for details).

Stratification of the population by farm childhood showed no evidence of effect modification for asthma, wheeze, self-reported allergy, hay fever and chronic bronchitis (p value for interaction >0.2). However, the relationship between endotoxin exposure and atopy was dependent on the presence of farm exposures in early life (p value for interaction =0.0086). In workers with a farm childhood no association was found between endotoxin exposure and atopy (OR [95%CI] = 0.90 [0.47-1.75], 1.31 [0.86-2.00], and 1.49 [0.73-3.05] for the low mediate, high mediate and high exposure groups; respectively). In contrast, a negative dose dependent trend between endotoxin exposure and atopy was found in workers without a farm childhood (OR [95%CI] = 0.98 [0.65-1.49], 0.68 [0.53-0.87], and 0.48 [0.30-0.79] for the low mediate, high mediate, and high exposure groups; respectively).

DISCUSSION

In this study we used pooled health data from four epidemiological studies (veterinary students, farm apprentices, and biofuel and agricultural industry workers) and exposure estimates based on measurements to investigate whether and how endotoxin exposure predicts the likelihood of allergic sensitization and airway disease. We found significant, inverse dose-response relationships between endotoxin exposure, hay fever and atopy, while current endotoxin exposure was associated with an increased prevalence of chronic bronchitis and ODTS. A farm childhood was a protective factor for hay fever and asthma symptoms, independent of current exposure to endotoxin. However, current endotoxin exposure showed an inverse dose-response relationship with allergic sensitization only in workers without a farm childhood.

Endotoxin exposure, farm childhood and atopic sensitization

Our results confirm the previously demonstrated[9, 10, 14] protective effect of current endotoxin exposure in adulthood by showing clear inverse relationships between occupational endotoxin exposure and atopy and hay fever. Like previous studies suggesting that the protective effect of early childhood exposure to farming on atopic diseases is persistent in adulthood,[1-4] we found significantly less atopy and asthma symptoms in workers with than without a farm childhood. These associations were not confounded by the level of current exposure, and farm childhood did not significantly modify the effect of current endotoxin exposure on hay fever. On the other hand, the prevalence of atopic sensitization was only inversely related to current endotoxin exposure in workers who did not live on a farm during childhood. These findings further support the hypothesised protective effect of adult-onset endotoxin exposure on allergic disorders. The absence of an association among workers with a farm childhood may be due to the very low prevalence of atopic sensitization in this particular group.[14] Yet the use of farm childhood as surrogate for early life exposures can be prone to misclassification and is short in ability to classify intensity and type of exposure. A recent multicenter European study on children showed that atopic and asthma responses can vary depending on type of farming and applied farming practices.[29] Therefore, studies with additional measured early life exposures and with follow-up of health and exposure of workers since commencement of current exposures are of particular interest in revealing whether early-life or current-onset exposure is of highest relevance.

Similar trends were found for study-specific endotoxin-atopy and endotoxin-hay fever associations in the subpopulations of Dutch farmers, veterinary students and farm apprentices. These three populations are all closely related to agriculture (veterinary students handle farm animals as well and those are the ones with the highest exposure levels) suggesting that agricultural related exposures is of importance. However, our population of biofuel workers, the only non-agricultural related, is relatively small and lower exposed compared to the other occupational groups. Further studies with larger non-agricultural related populations and with more comparable exposure levels to those found in agricultural environments are needed in order to confirm this finding.

Endotoxin exposure, chronic bronchitis and ODTS

We found endotoxin exposure to be a dose-dependent risk factor for both ODTS and chronic bronchitis from exposure levels of 100 EU/m^3 and higher. Smit and colleagues in two recent studies among Dutch agriculture seed[15] and wastewater[30] workers showed high occupational exposure to endotoxin to significantly induce ODTS-like symptoms. Elevated endotoxin exposure was found to significantly increase the likelihood of chronic bronchitis and cough with phlegm symptoms among Norwegian[16] and Dutch[10] farmers, respectively.

Approximately 46% of the participants with self-reported chronic bronchitis also reported asthma symptoms. The prevalence of smoking was relatively similar (39.9 vs. 33.6%) in persons with chronic bronchitis and persons with asthma symptoms; but asthmatic persons with chronic bronchitis were more likely to be smokers than persons with only chronic bronchitis or asthma symptoms (53 vs. 28 vs. 30%, respectively). Asthmatic subjects with chronic bronchitis had a longer (7 vs. 3.6 pack-years) smoking history than asthmatic subjects without chronic phlegm symptoms. Exclusion of asthmatic cases from the analysis did not affect the relationship between endotoxin exposure and chronic bronchitis which remained positive and significant (OR [95%CI] = 1.21 [1.08-1.35], p value for trend 0.0007). Thus, the dose-dependent association between endotoxin exposure and chronic bronchitis is unlikely to be due to misclassification between cases of asthma and chronic bronchitis.

Endotoxin exposure, atopic and non-atopic asthma

A Norwegian study in farmers that used personal exposure measurements [8] showed no association between asthma *per se* and endotoxin exposure, but after stratification for atopic status endotoxin exposure was positively associated with non-atopic asthma and negatively with atopic asthma. In our analysis, we were also unable to find clear patterns in the relationships between endotoxin exposure, asthma *per se*, and wheezing; but when trying to confirm the presence of the two asthma phenotypes current endotoxin levels above 1000 EU/m^3 appeared to increase the prevalence of both asthma and wheezing in non-atopic workers. Nevertheless, there were no clear inverse dose-response relationships between endotoxin and atopic wheeze, and the patterns were similar even in further analysis using doctor-diagnosed asthma (at any point in life) as an endpoint (not shown). This lack of association in atopic wheezing participants is difficult to understand. It could be due to differences in the nature (i.e. type of endotoxin, other organic dust exposures) and level of exposure, or it could be a result of methodological and analytical differences (i.e. differences in the exposure assessment strategies and cut-off levels and in the asthma and atopy definitions) between the two studies. The combined skin-prick and blood test definition of atopy that we used may also have an impact on the result. The lower sensitivity of the IgE test, compared with the skin-prick test,[20] could have underestimated the prevalence of atopic sensitization, and thereby attenuated the endotoxin-wheeze relationships among atopic workers. Nevertheless, a major effect of such bias seems unlikely; when we stratified by method of atopy assessment, we found no systematic differences in the exposure-response relationships for hay fever and self-reported allergy (see supplement G, Table S3).

Heterogeneity across studies

A major concern in pooled analysis is the heterogeneity of the estimates between the involved studies.[31] Indications for heterogeneity existed mainly for Danish biofuel workers. However, the pooled estimates were not considerably affected by the exclusion of the specific study population and adjustment for study had only a small influence on the estimated ORs for the pooled population (Table 2). Furthermore, the results were similar in separate analyses with adjustment and stratification by country (not shown). Thus, although present, heterogeneity between the involved studies is unlikely to have substantially influenced the results.

Exposure misclassification

A previous exposure assessment study in biofuel plants showed that personal exposure levels are higher than levels measured by stationary sampling.[32] The use of stationary levels in the exposure estimations for biofuel workers most likely resulted in an underestimation of their personal exposure levels. This, probably, also reflects to the lower than background exposure levels (0.1 vs. 1 EU/m^3) estimated within the specific population. However, biofuel workers accounting for only 5% of the total population of the present study, and, as discussed in the results section, their exclusion did not considerably affect the results of the main analysis.

It could be argued that the estimation of exposure based on endotoxin levels obtained from recent measurements does not accurately represent the exposure of the baseline SUS population at the early 90’s. The Danish primary agriculture sector underwent major structural and technological changes through the last 20 years. However, the currently measured endotoxin concentrations for pig, poultry and dairy farmers in the SUS study are comparable to the personal levels reported from studies in Dutch pig [33] and American poultry [34] and dairy cattle [35] farmers performed during the early 90’s. In order to check for the possibility of misclassification in the specific study we re-analysed the data. We used the livestock endotoxin exposure levels obtained from stationary measurements within the Danish part of a large European exposure assessment study [36] conducted in the same time period as the "SUS" study along with the external field work measurements from the Dutch agricultural industry study.[10] The estimated trends in the exposure response relationships for the pooled and the SUS study populations remained essentially similar to the currently presented ones (not shown) despite that the use of external exposure levels resulted in differences in the exposure estimates. Although the possibility of exposure misclassification cannot be completely excluded we have no reason to believe that it has been differential to the health outcome.

Healthy worker effect

Because of its cross-sectional design the present study is prone to the occurrence of both hire and survivor healthy worker effects.[37] However, the protective effect of current endotoxin exposure on hay fever was independent of the occurrence of allergic diseases in the family suggesting a limited impact of hire selection effects through multiple generations. The occurrence of survivor effects among Dutch agriculture industry workers has been addressed in an earlier publication.[14] Inverse associations between endotoxin exposure and IgE-mediated sensitization against grass pollen were found also among persons without self-reported allergic symptoms indicating the absence of self-selection on the specific population. In addition, the subpopulations of farm apprentices and veterinary students consisted of individuals who only recently started their working careers. These young populations accounted for 73% of our study population and most of them (67%) were not exposed to farming during childhood. Exposure-dependent selection or survivor effects are unlikely to have occurred in these subpopulations, due to the short time interval, that they have been exposed to endotoxin. The homogeneity in the estimated associations between endotoxin exposure and hay fever on these subpopulations further supports the reliability of the pooled estimates. Thus, a healthy-worker effect is unlikely to have a major impact on the results of the present study.

CONCLUSION

In conclusion, the present pooled study is one of very few studies that used quantified exposure estimates in order to assess dose-response relationships between current endotoxin exposure, atopy and respiratory symptoms and diseases among adults. Furthermore it enabled the assessment of differences in exposure-response relationships across a variety of occupationally exposed populations using a standardised approach that minimised existing methodological differences. Its results confirm earlier published associations and further suggest that the protective effects of endotoxin might be stronger when the exposure is agricultural related.

COMPETING INTERESTS:

None declared.

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REFERENCE LIST

1 **Kauffmann F,** Oryszczyn MP, Maccario J. The protective role of country living on skin prick tests, immunoglobulin E and asthma in adults from the Epidemiological study on the Genetics and Environment of Asthma, bronchial hyper-responsiveness and atopy. *Clin Exp Allergy* 2002;**32** (3):379-86.

2 **Koskela HO,** Happonen KK, Remes ST*, et al.* Effect of farming environment on sensitisation to allergens continues after childhood. *Occup Environ Med* 2005;**62** (9):607-11.

3 **Portengen L,** Sigsgaard T, Omland O*, et al.* Low prevalence of atopy in young Danish farmers and farming students born and raised on a farm. *Clin Exp Allergy* 2002;**32** (2):247-53.

4 **Radon K,** Schulze A, Nowak D. Inverse association between farm animal contact and respiratory allergies in adulthood: protection, underreporting or selection? *Allergy* 2006;**61** (4):443-6.

5 **Heederik D,** Sigsgaard T. Respiratory allergy in agricultural workers: recent developments. *Curr Opin Allergy Clin Immunol* 2005;**5** (2):129-34.

6 **Filipiak B,** Heinrich J, Schafer T*, et al.* Farming, rural lifestyle and atopy in adults from southern Germany--results from the MONICA/KORA study Augsburg. *Clin Exp Allergy* 2001;**31** (12):1829-38.

7 **Smit LA,** Zuurbier M, Doekes G*, et al.* Hay fever and asthma symptoms in conventional and organic farmers in The Netherlands. *Occup Environ Med* 2007;**64** (2):101-7.

8 **Eduard W,** Douwes J, Omenaas E*, et al.* Do farming exposures cause or prevent asthma? Results from a study of adult Norwegian farmers. *Thorax* 2004;**59** (5):381-6.

9 **Portengen L,** Preller L, Tielen M*, et al.* Endotoxin exposure and atopic sensitization in adult pig farmers. *J Allergy Clin Immunol* 2005;**115** (4):797-802.

10 **Smit LA,** Heederik D, Doekes G*, et al.* Exposure-response analysis of allergy and respiratory symptoms in endotoxin-exposed adults. *Eur Respir J* 2008;**31** (6):1241-8.

11 **Douwes J,** Thorne P, Pearce N*, et al.* Bioaerosol health effects and exposure assessment: progress and prospects. *Ann Occup Hyg* 2003;**47** (3):187-200.

12 **Liebers V,** Bruning T, Raulf-Heimsoth M. Occupational endotoxin-exposure and possible health effects on humans. *Am J Ind Med* 2006;**49** (6):474-91.

13 **Rylander R.** Endotoxin and occupational airway disease. *Curr Opin Allergy Clin Immunol* 2006;**6** (1):62-6.

14 **Smit LA,** Heederik D, Doekes G*, et al.* Occupational endotoxin exposure reduces the risk of atopic sensitization but increases the risk of bronchial hyperresponsiveness. *Int Arch Allergy Immunol* 2010;**152** (2):151-8.

15 **Smit LA,** Wouters IM, Hobo MM*, et al.* Agricultural seed dust as a potential cause of organic dust toxic syndrome. *Occup Environ Med* 2006;**63** (1):59-67.

16 **Eduard W,** Pearce N, Douwes J. Chronic bronchitis, COPD, and lung function in farmers: the role of biological agents. *Chest* 2009;**136** (3):716-25.

17 **Schlunssen V,** Madsen AM, Skov S*, et al.* Does the use of biofuels affect respiratory health among male Danish energy plant workers? *Occup Environ Med* Published Online First: 23 November 2010 doi:10.1136/oem.2009.054403.

18 **Elholm G,** Omland O, Schlunssen V*, et al.* The cohort of young Danish farmers - A longitudinal study of the health effects of farming exposure. *Clin Epidemiol* 2010;**2**:45-50.

19 **Omland O,** Sigsgaard T, Hjort C*, et al.* Lung status in young Danish rurals: the effect of farming exposure on asthma-like symptoms and lung function. *Eur Respir J* 1999;**13** (1):31-7.

20 **Doekes G,** Douwes J, Wouters I*, et al.* Enzyme immunoassays for total and allergen specific IgE in population studies. *Occup Environ Med* 1996;**53** (1):63-70.

21 **Samadi S,** Wouters IM, Houben R*, et al.* Exposure to inhalable dust, endotoxins, beta(1->3)-glucans, and airborne microorganisms in horse stables. *Ann Occup Hyg* 2009;**53** (6):595-603.

22 **Samadi S,** Heederik DJ, Krop EJ*, et al.* Allergen and endotoxin exposure in a companion animal hospital. *Occup Environ Med* 2010;**67** (7):486-92.

23 **Reynolds SJ,** Black DW, Borin SS*, et al.* Indoor environmental quality in six commercial office buildings in the midwest United States. *Appl Occup Environ Hyg* 2001;**16** (11):1065-77.

24 **Rylander R,** Persson K, Goto H*, et al.* Airborne Beta-1,3-Glucan May Be Related to Symptoms in Sick Buildings. *Indoor Environ* 1992;**1**:263-7.

25 **Noss I,** Wouters IM, Visser M*, et al.* Evaluation of a low-cost electrostatic dust fall collector for indoor air endotoxin exposure assessment. *Appl Environ Microbiol* 2008;**74** (18):5621-7.

26 **The Dutch Expert Committee on Occupational Standards (DECOS).** Health based recommended Occupational Exposure Limit for Endotoxins. Health Council for the Netherlands. Report 1998/03 WGD. 1998.

27 **Rylander R,** Jacobs RR. Endotoxins in the environment: A criteria document. *J Occup Environ Health* 1997;**3** (1):S1-S48.

28 **Portengen L,** de Meer G, Doekes G*, et al.* Immunoglobulin G4 antibodies to rat urinary allergens, sensitization and symptomatic allergy in laboratory animal workers. *Clin Exp Allergy* 2004;**34** (8):1243-50.

29 **Ege MJ,** Frei R, Bieli C*, et al.* Not all farming environments protect against the development of asthma and wheeze in children. *J Allergy Clin Immunol* 2007;**119** (5):1140-7.

30 **Smit LA,** Spaan S, Heederik D. Endotoxin exposure and symptoms in wastewater treatment workers. *Am J Ind Med* 2005;**48** (1):30-9.

31 **Ressing M,** Blettner M, Klug SJ. Systematic literature reviews and meta-analyses: part 6 of a series on evaluation of scientific publications. *Dtsch Arztebl Int* 2009;**106** (27):456-63.

32 **Madsen AM.** Exposure to airborne microbial components in autumn and spring during work at Danish biofuel plants. *Ann Occup Hyg* 2006;**50** (8):821-31.

33 **Preller L,** Heederik D, Boleij JS*, et al.* Lung function and chronic respiratory symptoms of pig farmers: focus on exposure to endotoxins and ammonia and use of disinfectants. *Occup Environ Med* 1995;**52** (10):654-60.

34 **Donham KJ,** Cumro D, Reynolds SJ*, et al.* Dose-response relationships between occupational aerosol exposures and cross-shift declines of lung function in poultry workers: recommendations for exposure limits. *J Occup Environ Med* 2000;**42** (3):260-9.

35 **Kullman GJ,** Thorne PS, Waldron PF*, et al.* Organic dust exposures from work in dairy barns. *Am Ind Hyg Assoc J* 1998;**59** (6):403-13.

36 **Seedorf J,** Hartung J, Schroder M*, et al.* Concentrations and emissions of airborne endotoxins and microorganisms in livestock buildings in Northern Europe. *J Agri Eng Res* 1998;**70** (1):97-109.

37 **Le Moual N,** Kauffmann F, Eisen EA*, et al.* The healthy worker effect in asthma: work may cause asthma, but asthma may also influence work. *Am J Respir Crit Care Med* 2008;**177** (1):4-10.

Online Supplement

Title:

Sensitization to common allergens and respiratory symptoms in endotoxin exposed workers: a pooled analysis

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Supplement A. Description of the Veterinarians Health study.

The Veterinarians health study contains both a cross-sectional and a prospective component. It aims to assess the effect of occupational exposures on the development of allergic and non-allergic respiratory diseases in veterinary students. In the current study only data from the cross-sectional part collected between June 16 and October 4, 2006 have been included. All 1400 veterinary students at the University of Utrecht during 2006 were invited to participate in this investigation and in the specific period 901 students (participation rate 65%) filled in a detailed questionnaire on allergic and non-allergic respiratory symptoms, zoonotic diseases, medication usage, smoking status, family history, job-history, and previous and current contact with animals besides their studies. 641 of the included participants (71% of the eligible) provided a blood sample. The blood samples were used to determine specific IgE against common (house dust mite, grass pollen, tree, cat and dog) and animal allergens (pig, cow, horse, goat, chicken, guinea pig, Budgerigar), as well as to measure total serum IgE using enzyme immunoassays as previously described.[1] Quantitative measurements of exposure were conducted using personal, stationary, and passive dust collection methods at various locations within the different animal clinics associated with the veterinary educational program.[2, 3] Personal dust measurements were collected during typical practical activities of the students. The collected dust samples were extracted and analysed for endotoxin, β(1→3)-glucan, as well as common and specific animal allergens.[2, 3]

Supplement B. Availability, comparability and usage of information within the study

Table S1 Summary of the available and comparable information from questionnaires and health investigations of the different participating study populations and definitions used in the pooled study.

Symptom/ characteristic and definition used	Agricultural industry workers	Veterinary medicine students	Farming school students	Bio-fuel plant workers
Basic characteristics				
Farm childhood, defined as Born, raised or lived in a farm for at least a year till the age of 5.	Did you grow up on a farm/agricultural company?	Have you ever lived in an operative or hobby farm? Answers: No, Yes-period	Extracted from a broader exposure anamneses table. Assessing work before(age: 1-5 years), during and after basic education. Information available on years, type and amount (%) of farming.	Are you born in the countryside? Answers: yes on a farm, yes, but not in a farm, no
Smoking status	Have you smoked more than 100 cigarettes/50 cigars/500 g of pipe tobacco in your whole life? Answers: No, Yes but I quit smoking, Yes I am a current smoker.	Have you smoked more than 100 cigarettes/50 cigars/500 g of pipe tobacco in your whole life? Answers: No, Yes but I quit smoking, Yes I am a current smoker.	Extracted from 2 questions: 1) Have you ever smoked one or more cigarettes per day for a period longer than 14 days? Answers: Yes, No; 2) Do you still Smoke? Answers: Yes, No	Extracted from 2 questions: 1) Have you ever smoked? *(This means at least one cigarette, cigar or pipe a day for at least a year)*? Answers: Yes, No; 2) Do you still Smoke? Answers: Yes, No
Smoking amount	Can you indicate the average number of cigarettes, cigars and g of pipe tobacco you are smoking per day (or smoked, when you used to smoke)? (1 pack of shag = 40 cigarettes)	How many cigarettes, cigars and g of pipe tobacco you are smoking per day? (1 pack of shag = 40 cigarettes	Extracted from 3 questions: 1) How many cigarettes did you on average smoke per day?; 2) How many cigars did you on average smoke per day?; 3) On average, how many grams of tobacco did you smoke per month?	Extracted from 3 questions: 1) How many cigarettes did you on average smoke per day?; 2) How many cigars did you on average smoke per day?; 3) On average, how many grams of tobacco did you smoke per month?
Smoking period	Extracted from 2 questions: 1) At what age did you start smoking?; 2) If you used to smoke, how old were you when you stopped or quit smoking?	Extracted from 2 questions: 1) At what age did you start smoking?; 2) If you used to smoke, how old were you when you stopped or quit smoking?	Extracted from 2 questions: 1) If you stopped smoking, when did you stop?; 2) For how many years did you smoke a) cigarettes, b) cigars (cheroots), c) pipe?	Extracted from 2 questions: 1) If you used to smoke, how old were you when you stopped or quit smoking?; 2) How many years have you been smoking in total?
Asthma and/or allergy in parents	Extracted from 2 questions: 1) Did your father or mother ever have asthma?; 2) Did your father or mother ever have hay fever or other nose allergies?	Extracted from 2 questions: 1) Did your father or mother ever have asthma?; 2) Did your father or mother ever have hay fever or other nose allergies?	Did any of your parents have: Asthma, Allergic hay-fever, Atopic eczema? Answers: mother, father, both for every symptom	Have anyone of your relatives (parents, grandparents, children or siblings) atopic eczema, hay fever or asthma? Answers: 1) Father, 2) Mother, 3) Grandparents, 4) Children, 5) Siblings
Asthma and/or allergy in siblings	2 individual questions: 1) Did any of your brothers or sisters ever have asthma?; 2) Did any of your brothers or sisters ever have “hay fever” or any other nose allergy?	Extracted from 3 questions: 1) Did any of your brothers or sisters ever have asthma?; 2) Did any of your brothers or sisters ever have “hay fever” or any other nose allergy?; 3) Did 1 or more of your brothers or sisters ever have eczema?	Did any of your siblings have: Asthma, Allergic hay-fever, Atopic eczema? Answers: None, one, two for every symptom	Have anyone of your relatives (parents, grandparents, children or siblings) atopic eczema, hay fever or asthma? Answers: 1) Father, 2) Mother, 3) Grandparents, 4) Children, 5) Siblings

Table 1. Continued

Symptom/ characteristic and definition used	Agricultural industry workers	Veterinary medicine students	Farming school students	Bio-fuel plant workers
Airway symptoms				
Chronic bronchitis defined as "Coughing up phlegm almost daily, for 3 months in a row during the last year"	Did you cough up phlegm almost daily, for 3 months in a row in the last 12 months?	Did you cough up phlegm sometimes almost daily, for 3 months in a row in the last 12 months?	Do you usually bring up any phlegm from your chest on most days for as much as 3 months a year?	Do you usually bring up any phlegm from your chest on most days for as much as 3 months a year?
Chest tightness, defined as "During the last 12 months have you, sometimes been woken up with a feeling of tightness in your chest?"	During the last 12 months have you, sometimes been woken up with a feeling of tightness in your chest?	During the last 12 months have you, sometimes been woken up with a feeling of tightness in your chest?	Extracted from 2 questions: 1) Do you wake in the morning with chest tightness?; 2) How often are you disturbed by this? Answers : 1-2 times per year, 1-2 times per month, 1-2 times per week, Daily, Irrelevant	Have you been woken up with a feeling of tightness in your chest, during the last 12 months?
Wheeze, defined as "Have you sometimes had wheezing in the chest, during the last year?"	Have you sometimes had wheezing in the chest, during the last year?	Have you had an attack of wheezing in the chess during the last 12 months?	How often are you troubled from whistling / wheezing breathing? Answers : 1-2 times per year, 1-2 times per month, 1-2 times per week, Daily, Irrelevant	How often are you bothered by wheezing? Answers : daily, daily in periods, 1-2 times a week, 1-2 times a month, 1-2 times a year, <2 times a year
Ever asthma, defined as "Have you ever had asthma?"	Have you ever had asthma?	Have you ever had asthma?	Have you ever had asthma?	Have you ever had asthma?
Asthma medication, defined as "Are you currently taking any medicine for asthma?"	Are you currently taking any medicines (e.g. inhalers, aerosols, tablets) for asthma?	Did you use any medicines for asthma within the last 12 months?	Do you take medicines for your asthma? If yes, how often you take the medicines?	Are you currently taking any medicines (e.g. inhalers, aerosols, tablets) for asthma?
ODTS, defined as "Have you, during the past 12 months, had sudden episodes of flu-like symptoms such as fever, chills, malaise, muscle- or joint pains, and felt completely well within 1-2 days?"	Have you, during the past 12 months, had sudden episodes of flu-like symptoms such as fever, chills, malaise, muscle- or joint pains, and felt completely well within 1-2 days?) How many times did this occur during the last 12 months? Answer: …..times	How frequently during the last 12 months have you had sudden attacks of flu-like symptoms such as fever, shivers, malaise, joined muscle pains, in which you get completely better after 1-2 days. Answers: Never, 1-2/motnh, 1-2/week, daily/almost daily	Extracted from 2 questions; 1) Have you ever, in connection with your work, had attacks of fever, shivering or chills or sensations of influenza (common cold or influenza are not considered here)?; 2) How many attacks did you have per year during the last 2 years? Answers: 1-2, 2-5, 6-10, > 10.	Have you, during the past 12 months, had sudden episodes of flu-like symptoms such as fever, chills, malaise, muscle- or joint pains, and felt completely well within 1-2 days?) How many times did this occur during the last 12 months? Answer: …..times
Allergy				
Self-reported allergy, defined as "self-reported, positive, allergic reactions with airway, nose and/or eye symptoms against pollen, animal or house dust allergens"	Extracted from the following question: Are you sensitive or allergic to one or more substances / materials? If yes, please tick which of the following materials you are sensitized or allergic to and indicate the symptoms that you have? materials: house dust, certain food, house animals, plant or grass pollen; symptoms: nose (sneezing, runny nose), airway (asthma, tightness), skin (blushing, itching), eye (watering, itching)	Extracted from the following question: Are you sensitive or allergic to one or more substances / materials? If yes, please tick which of the following materials you are sensitized or allergic to and indicate the symptoms that you have? materials: house dust, certain food, house animals, plant or grass pollen; symptoms: nose (sneezing, runny nose), airway (asthma, tightness), skin (blushing, itching), eye (watering, itching)	Extracted from 3 identical questions, one for every type of symptoms: Have you experienced that some of the following things have worsened your a) eye , b) nose, c) chest tightness problems: "Something" in your home, Cold, Exercise (cycling, running, hard work), Pollen *(e.g. birch, grass or artemisia)*, Animal *(e.g. dog, cat, horse),* Other. Eye & nose symptoms during the last 12 months. Chest tightness see note 1 below	Extracted from 3 identical questions, one for every type of symptoms: Have you experienced that some of the following things have worsened your a) eye , b) nose, c) chest tightness problems: "Something" in your home, Cold, Exercise (cycling, running, hard work), Pollen *(e.g. birch, grass or artemisia)*, Animal *(e.g. dog, cat, horse),*Tobacco smoke, strong smells, Other. All questions refer to symptoms within the last 12 months.

Table 1. Continued

Symptom/ characteristic and definition used	Agricultural industry workers	Veterinary medicine students	Farming school students	Bio-fuel plant workers
Hay fever, defined as "Have you ever had hay fever?"	Extracted from the question for self-reported allergy (see above). The answers for eye and nose symptoms on the section for plant and grass pollen were used	Extracted from the question for self-reported allergy (see above). The answers for eye and nose symptoms on the section for plant and grass pollen were used	Have you ever had hay fever?	Have you or have you had hay fever: *(Please fill in every line)*? Answers: 1) Before I went to school, 2) During school age, 3) As grown up, 4) During the last 12 months
Atopy, defined as elevated serum IgE levels or a positive skin-prick test against at least one allergen.	In vitro tested, IgE levels against grass pollen (mix of timothy and perennial ryegrass) or house-dust mite or cat or dog allergens	In vitro tested, IgE levels against house dust mite, grass pollen (mix of timothy and perennial ryegrass), birch, cat, dog	In vivo tested, skin-prick test against house dust mite, grass pollen (mix of five species), birch, cat, dog	In vivo tested, skin-prick tests against house dust mite, grass pollen (mix of five species), birch, cat, dog

1= The following questions used: "Do you ever have chest tightness?" and "Do you wake in the morning with chest tightness?"

Supplement C. Description of the pooling process

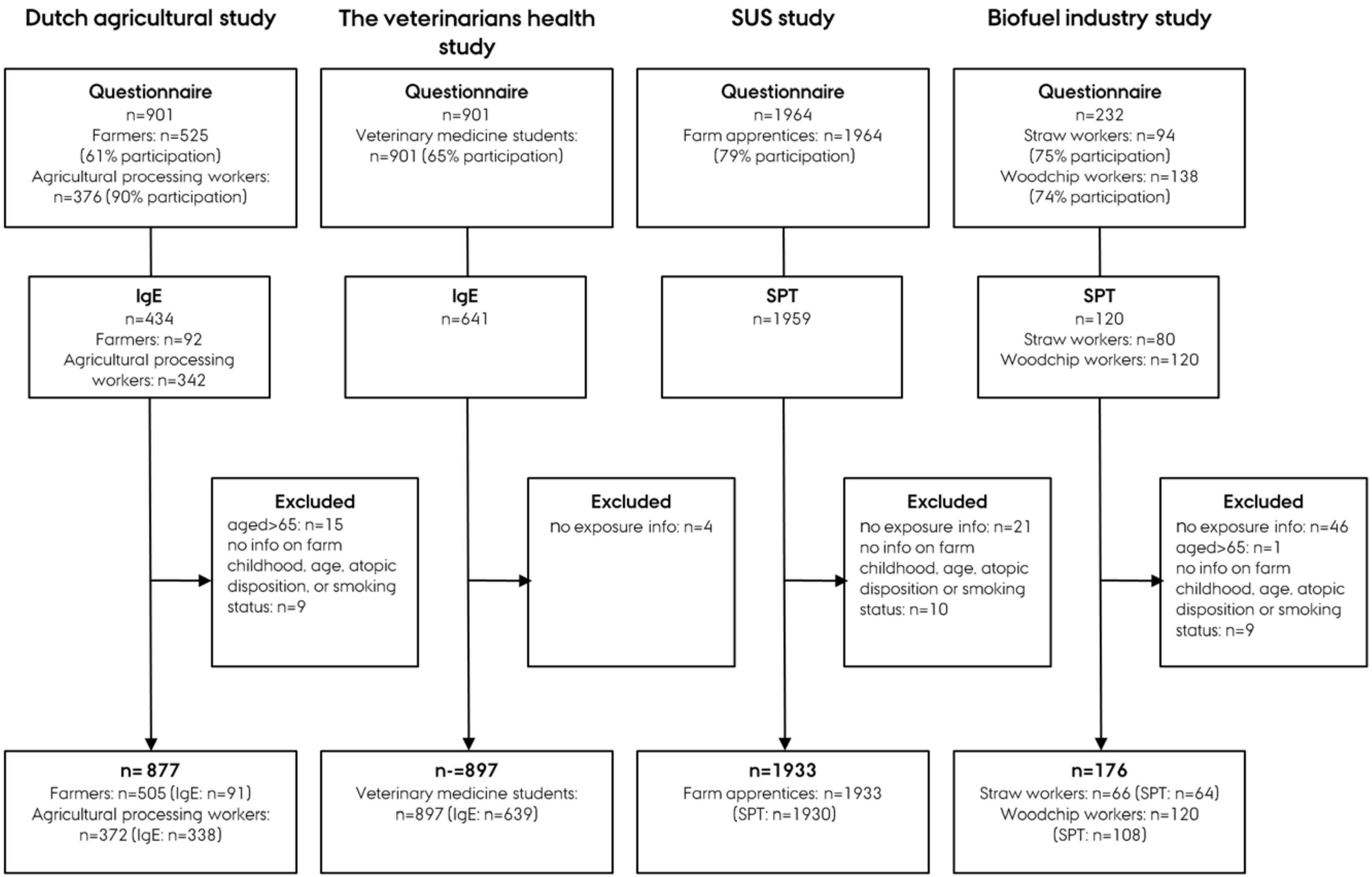

Figure S1 Schematic description of the pooling process in the study

Supplement D. Description of the developed Job-exposure matrices (JEMs).

The job-exposure matrices (JEM) that were used to estimate the current personal endotoxin exposure level for each of the participating studies are described below. The descriptions are given per study population and in an alphabetical order.

Agricultural process industry workers (The Netherlands)

Details on the modelling of exposure on the specific study have been published elsewhere.[5] Briefly, exposure assessment was based on 249 personal full-shift inhalable dust measurements collected in a sample of 82 farmers and 116 workers throughout the study. Mixed effects models, stratified by sector with worker identity as a random effect and job-title as a fixed effect were used to estimate exposure for different job-titles. The resulting equations were subsequently used along with the job-title of all subjects to assign personal levels of endotoxin exposure.

Biofuel process industry workers (Denmark)

Details on the exposure assessment in the biofuel process industry study have been published elsewhere.[6] Briefly, the current personal endotoxin exposure level was estimated based on information on the time spent on each working task or area from one week exposure diaries and endotoxin levels obtained from 181 stationary dust samples collected in all main working areas of the participating workers.

Farming school students, SUS study (Denmark)

Information about the student's farming work history was collected at the baseline interview questionnaire. There was information available on amount (years, weeks and hours/week) and type of farm work (% with pig, cattle, poultry and crop-field) that they performed before (1-6 years), during (7-16 years) and after (>16 years) basic school education. Mean livestock personal inhalable endotoxin exposure levels were pooled from 507 personal full-shift measurements in farmers performed during the 15th year follow-up of the study. The measurements were performed between 2008-2009 using 37 mm glass-fiber filters on GSP sampling heads[7] with a flow rate of 3.5 L/min. The collected samples were sequentially extracted and analysed for endotoxin as described previously.[4] For field-crop work the endotoxin results of 21 task-specific measurements obtained within the previously mentioned measurement series were used. The current exposure to endotoxin for every student was calculated by merging the measurements with the questionnaire information on the type of work (%) after basic school education.

Veterinary medicine students, The Veterinarians health study (The Netherlands)

Students were classified into categories according to their specialization (companion animals, farm animals and horses) and study phase. Personal exposure levels were assigned to students based on these categories and endotoxin levels obtained from personal inhalable dust measurements performed within the study. In particular, for specializations on horses and companion animals endotoxin exposure was assessed using endotoxin levels obtained from 38 measurements in workers in horse stables[2] and 55 measurements in veterinary medicine students working in a companion animal hospital,[3] respectively. For the farm animal specialization, endotoxin exposure levels were available from 160 study phase-specific measurements in students working with ruminants and poultry within the study and 18 time-weighted measurements on students attending pigs from a preceding pilot study. Exposures to endotoxin through part or full time jobs with regular animal contact outside the veterinary medicine study were added to the assigned specialization levels based on the same exposure data as described above, and the total estimate was regarded as the current endotoxin exposure for each subject.

Reference List

1 **Doekes G,** Douwes J, Wouters I, *et al.* Enzyme immunoassays for total and allergen specific IgE in population studies. *Occup Environ Med* 1996;**53** (1):63-70.

2 **Samadi S,** Wouters IM, Houben R, *et al.* Exposure to inhalable dust, endotoxins, beta(1->3)-glucans, and airborne microorganisms in horse stables. *Ann Occup Hyg* 2009;**53** (6):595-603.

3 **Samadi S,** Heederik DJ, Krop EJ, *et al.* Allergen and endotoxin exposure in a companion animal hospital. *Occup Environ Med* 2010;**67** (7):486-92.

4 **Spaan S,** Doekes G, Heederik D, *et al.* Effect of extraction and assay media on analysis of airborne endotoxin. *Appl Environ Microbiol* 2008;**74** (12):3804-11.

5 **Smit LA,** Heederik D, Doekes G, *et al.* Exposure-response analysis of allergy and respiratory symptoms in endotoxin-exposed adults. *Eur Respir J* 2008;**31** (6):1241-8.

6 **Schlünssen V,** Madsen AM, Skov S*, et al.* Does the use of biofuels affect respiratory health among male Danish energy plant workers? *Occup Environ Med* Published Online First: 23 November 2010 doi:10.1136/oem.2009.054403

7 **Kenny LC,** Aitken R, Chalmers C*, et al.* A collaborative European study of personal inhalable aerosol sampler performance. *Ann Occup Hyg* 1997;**41** (2):135-53.

Supplement E. Results from Generalized additive modelling (smoothing).

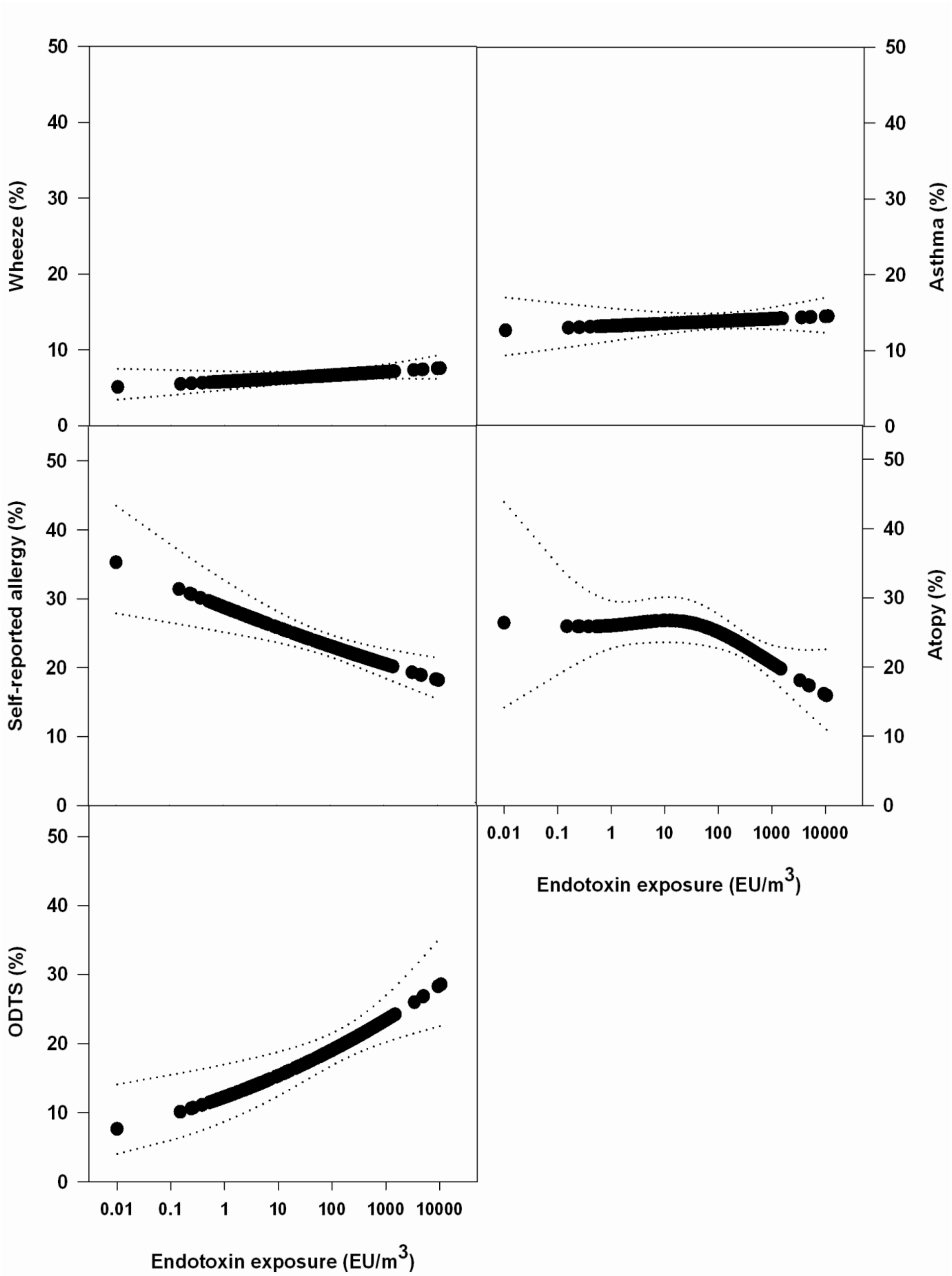

Figure S2 Smoothed relationships between endotoxin exposure, wheeze, asthma, self-reported allergy, atopy and ODTS for the pooled study population.. ········ : ± 95% confidence intervals for the symptom under investigation. Results are adjusted for gender, age, farm childhood, atopic predisposition and smoking habits and participating study.

Supplement F. Results from the analysis with farm childhood as a main independent variable.

TABLE S2 Univariate and multiple logistic regression analysis describing associations between farm childhood, and the health symptoms of interest.

Symptom	Univariate analysis		Model 1[a]		Model 1[b]	
	OR	95% CI	OR	95% CI	OR	95% CI
Chronic bronchitis	0.88	0.65 to 1.19	0.97	0.69 to 1.36	1.00	0.71 to 1.40
Wheezing	0.62	0.50 to 0.78	0.69	0.54 to 0.88	0.70	0.54 to 0.89
Asthma	0.61	0.51 to 0.73	0.71	0.58 to 0.88	0.72	0.59 to 0.89
Hay fever	0.50	0.40 to 0.62	0.63	0.50 to 0.79	0.63	0.50 to 0.79
Self-reported allergy	0.49	0.40 to 0.59	0.65	0.52 to 0.81	0.66	0.53 to 0.83
Atopy	0.61	0.50 to 0.74	0.63	0.51 to 0.77	0.62	0.51 to 0.77

[a] results are adjusted for study, gender, age (continuous), atopic predisposition and smoking habits.

[b] results are adjusted for study, gender, age (continuous), atopic predisposition, smoking habits and for current exposure to endotoxin.

Supplement G. Results from stratified analysis by assessment method of atopy

TABLE S3 Logistic regression analysis stratified by method of assessment of atopy describing associations between endotoxin exposure and the health symptoms of interest.

Symptom	IgE test (n=1068)		Skin-prick test (n= 2102)	
	OR	95% CI	OR	95% CI
Hay fever				
Low	1.00		1.00	
Low mediate	1.35	0.73 to 2.49	1.05	0.63 to 1.76
High mediate	0.71	0.48 to 1.03	0.83	0.59 to 1.16
High	0.12	0.03 to 0.53	0.64	0.35 to 1.18
Self reported allergy				
Low	1.00		1.00	
Low mediate	1.11	0.63 to 1.93	0.82	0.46 to 1.46
High mediate	0.64	0.46 to 0.89	0.72	0.50 to 1.04
High	0.42	0.20 to 0.92	0.71	0.38 to 1.33

ORs are adjusted for study, gender, age (continuous), atopic predisposition, smoking habits and farm childhood.

Manuscript III

Basinas I, Schlünssen V, Takai H, Heederik D, Omland Ø, Sigsgaard T, Kromhout H. *Work tasks and stable characteristics associated with the levels of exposure to inhalable dust and endotoxin among Danish pig Farmers.* Draft manuscript.

Title:

Work tasks and stable characteristics associated with the levels of exposure to inhalable dust and endotoxin among Danish pig Farmers

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Abstract

Objective: To examine the effect of working tasks and stable characteristics on the personal level of dust and endotoxin exposure among pig farmers.

Methods: 354 personal full-shift measurements were performed in 231 farmers employed in 53 Danish pig farms. Filters were gravimetrically analysed for inhalable dust and for endotoxin by the Limulus amebocyte lysate assay. Information on working tasks and stable characteristics were collected using self-reported activity diaries and through walk-through surveys performed during the measurement day. Relationships between log-transformed dust and endotoxin exposure and working tasks and stable characteristics were examined using linear mixed effects analysis. Worker and farm identity were treated as random effects in models separate for working tasks and stable characteristics treated as fixed effects. For stable characteristics, analysis was restricted to indoor workers.

Results: Measured concentrations for inhalable dust ranged between <LOD-47.8 mg/m3 and between <LOD – 374,600 EU/m3 for endotoxin. The working environment (indoor vs. outdoor) was seen to play a dominant role on the exposure variability. Indoor working tasks related to intense animal activity or handling of feed material in storage areas increased exposure, which in contrast decreased during field work. High pressure washing was an factor increasing endotoxin exposure.

Stable characteristics related to feeding practices and the ventilation type were determinants of dust exposure. For endotoxin the most important determinants were the use of dry feed and the slatted floor coverage. Feeding practices could solely explain all the between-farms variability in dust and endotoxin exposure.

Conclusion: These findings provide relevant information for the use of personal protection equipment during performance of specific working tasks, and indicate feeding practices as a prospective area for the development of prevention strategies. However, further investigation is needed, especially considering the combined influential role of tasks performed under certain stable characteristics.

INTRODUCTION

The respiratory tract of animal farmers is exposed to various gases and aerosols of chemical, mineral, plant, animal and microbial origin.[1] Of those the aerosols of organic origin are widely accepted as the main and most important influence on the farmers' respiratory health.[2, 3] One of the most active and well investigated constituents of organic dusts is endotoxin.[3] Endotoxins are lipopolysaccharides primarily of gram-negative bacteria origin very common in workplaces involved in plant and animal material processing or with strong presence of human or animal faeces.[2, 4] Endotoxins have strong pro-inflammatory capabilities, and can induce several respiratory and systemic disorders including chronic bronchitis, bronchial hyperresponsiveness, non-atopic wheeze, fever and chills, malaise, and chronic obstructive pulmonary disease (COPD).[2, 5, 6] Animal farmers are well-documented as highly exposed to both dust and endotoxin.[7-11] However, accurate estimation of dust and endotoxin exposure for animal farmers is difficult, because of the large day-to-day variability in their personal levels of exposure.[12] For example, Dutch pig farms were estimated to have their average daily dust and endotoxin concentrations laying within 9- and 21-folds, respectively, while the range between subjects mean personal concentrations did not exceed the 4-folds.[12, 13] Similarly, in an analysis of a database with more than 6000 personal dermal exposure measurements the average daily exposure concentrations of agricultural workers to pesticides were estimated to lie within a range of 10- to 40-folds.[14] Even larger day-to-day variations in daily average concentrations have been reported among American livestock and arable farmers.[10, 12]

Recently, we also reported on the variability in personal dust and endotoxin concentrations among animal farmers.[15] Using personal repeated measurements on 231 pig and 77 dairy cattle farmers we found average daily dust and endotoxin concentrations to range up to 25- and 250-folds, respectively. We observed an up to 30-folds increase in average daily exposure concentrations among stable workers compared to field workers. These findings suggest a potential for error in exposure estimations, stressing the need for improvement in sampling and estimation strategies within prospective exposure assessment attempts.

Knowledge of determinants of exposure (that is factors explaining systematic differences in exposure through time or between individuals) is an essential component in the process of developing exposure prevention and controlling strategies.[16, 17] Moreover, in-depth knowledge of factors affecting workplace exposure can be used to effectively reduce the measurement error and thereby the attenuation in the risk estimates in epidemiological studies in populations with large variability in exposure,[17] such as agricultural workers and farmers.[12] Observational evaluations of determinants of personal exposures in workplaces are vital because they allow the assessment of multiple factors in real working conditions with a great degree of generalizability.[16] However, comprehensive observational investigations of determinants of personal exposure to dust and endotoxin in animal farmers have been sparse, with, to our knowledge, only one study so far examining the effect of both multiple farm characteristics and working tasks influencing the level of personal dust and endotoxin exposure among pig farmers.[18]

In view of the surprisingly high levels of personal exposure to dust and endotoxin that we recently found we initiated the present analysis with the aim to explore factors that determine the level and variability of personal exposure to dust and endotoxin among pig farmers. To identify activity patterns and stable characteristics that predict the level of personal exposure to dust and endotoxin of pig farmers we used data collected from self-reported activity diaries and walk-through surveys along with the results from more than 300 personal repeated exposure measurements in thorough statistical analysis using linear-mixed effect models.

MATERIALS AND METHODS

Study design

The design, selection process and the sampling and analytical methods applied within the SUS12 exposure assessment have been described in details in a previous publication.[15] Briefly, the SUS study was initiated in 1992 with the aims a) to describe the prevalence and incidence of respiratory symptoms in a farming environment and b) to investigate the effect of farming on the development of allergy, asthma and respiratory disease.[19] The study population included all 2458 second year students at all farming schools of Denmark, and a control group of 967 conscripts in the Danish army. Overall 1964 farming school students (80 %) and 592 conscripts (61%) gave consent to participate in the study. The final population sample consisted of the 1964 students and 407 randomly selected conscripts.

The present work is an integrated component of the follow-up of the SUS cohort. In the 15-year follow-up period several changes in the occupational status of the participants were to be expected. Therefore, *a priory* identification of the remaining active farming population of the initial SUS cohort was essential for the development and the design of the exposure assessment. Information on current and previous employment in farming, type and related farm characteristics (location, size, number of animals) for 1,239 participants (66% participation rate) was obtained from a preliminary selection questionnaire and an exposure scheme filled out during the clinical investigations. Of the participants, 433 emerged as still active and full-time employed in farming and 76 pig farmers located in the area of Jutland were selected randomly after stratified by farm size. Of those, 22 were either excluded or refused to participate in the personal measurements. The remaining 54 pig farmers were asked for an interview date.

The interview was performed in person with standardized developed schemes assessing production characteristics (i.e. number of employees, collaborations, number and type of animals, size, unit structure and locations, building infrastructure) and farm practices (i.e. agriculture form, cleaning and disinfection schedules and frequency, manure handling) at the company level. The content of the project was explained, and the measurement visits were scheduled.

Farm visits, measurements and data collection

For all the selected farms two measurement visits were scheduled in randomly chosen working days during summer (1st of May and 1st of October) and winter (17th of November and 3rd of April) 2008 and 2009. The vast majority of workers in the selected farms were included in the measurement series, which were performed throughout working-shifts including both field and stable work. Overall, 233 farmers were monitored resulting in 358 personal measurements. For the present analysis 4 measurements from 2 farmers, representing a whole farm in our population, were excluded due to their involvement in mixed pig and cattle production activities.

The performed tasks were registered by the farmers in structured, farm-type-specific, activity diaries with a 30 minute interval checklists. More than 25 distinct working tasks were included in each diary, which covered one week per season starting from the measurement day.

Farm characteristics, engineering parameters, and the hygienic conditions present in each department of the visited farm were registered through walk-through surveys performed during the visiting days. Notations were kept in pre-fixed inspection sheets designed to allow assessment for more than 120 well-defined characteristics. The outdoor temperature was measured locally, using a portable weather station (OBH Nordica A/S, Taastrup, Denmark) with a measurement accuracy of ±1 ^{0}C.

Dust measurements and endotoxin analysis

All farmers were equipped with a waist belt carrying 2 portable AirChek XR5000 pumps, each connected through a flexible tube to a conical inhalable sampler (CIS; JS Holdings, Stevenage, UK) mounted with a 37mm glass-fibre (GFA) filter (Whatman international Ltd, Maidstone, UK). The samplers were pinned in the farmers' pectoral area, and sampling was performed at airflow of 3.5 l/min. Filters were gravimetrically measured (pre- and post-sampling weighing) in a room with controlled climatic conditions, and then extracted in pyrogen-free water (PFW) with 0.05% (v/v) Tween-20. Analysis for the endotoxin content in the extracts was performed in PFW (1:200 dilution) using a quantitative kinetic chromogenic Limulus Amboecyte Lysate (LAL) test (Kinetic-QCL 50-650U kit, Lonza, Walkersville, Maryland, USA).[20] The limit of detection (LOD) for dust was 0.074 mg/filter and for endotoxin 0.0137 EU/ml; results were expressed in mg/m^3 and EU/m^3, respectively. For 3 samples with measured dust or endotoxin concentration below the limits of detection (LOD) a 2/3 value of the corresponding LOD was used.

Data preparation and management

The time that a farmer spent on the presence of a farm or environmental characteristic was expressed as a portion of his overall working time on the day of the measurements. Estimations were made for all work allocated in areas where animals were present and stable characteristics (e.g. ventilation) were functional. When time was spent on insemination and early handling of piglets (i.e. castration, tail clipping, teeth cutting), as stated in the workers activity dairy, these activities were pre-allocated on the insemination and farrowing departments, respectively. The remaining animal-related working time was allocated on the involved compartments based on the number of animals present. Weighting factors of 10:2:1:1 per animal housed in (a) farrowing, (b) serve or gestation, (c) weaning and (d) finishing stables were used, respectively. These weighting factors were estimated based on the average time needed for daily nursing (excluding insemination and early piglet handling) of an animal in a specific stage of the production as published by the "Expertise Centre for Agriculture" (previously known as "Danish Agricultural Advisory Service") in the 'Hand Book of operation and planning' database.[21] Due to the large amount of collected information on exposure determinants the above exercise was applied to only a number of farm characteristics considered in the literature as potentially influential on the level of dust and endotoxin exposure i.e. the type of accommodation, feeding, ventilation, flooring, heating, and the basic hygienic conditions present.

Data analysis

All data were analysed using the SAS statistical software version 9.2 (SAS Institute Inc, Cary, NC, USA) on the log-scale in order to acquire normality in exposure distributions and in the derived model residuals. Log transformation was preceded by formal tests of the exposure distributions using Kolmogorov–Smirnov tests. Exposure distribution characteristics are therefore summarised as geometric means (GM) with geometric standard deviations (GSD) provided along with arithmetic mean (AM) values. Relationships between exposure, stable characteristics, and working tasks were assessed using mixed effect linear models (PROC MIXED)[22, 23] with farm and worker included as random effects. Fixed effects (tasks and characteristics) were initially introduced as single covariates and those showing a p-value of 0.2 or smaller were included in further stepwise regression. The final model included only covariates with a significance <0.05. Two different models for a) work tasks and environment and b) stable characteristics were established for both dust and endotoxin exposure. For the later the population was restricted to only workers working indoors with time spent on dealing with the stable characteristics of interest. Forty nine measurements from 38 indoor workers involving outdoor work were excluded as well as measurements from further 26 workers who either worked whole days outdoors (n=23) or indoors, but in an environment irrelevant (e.g. high-pressure wet cleaning on empty stables for a whole shift) to the determinants investigated (n=14). Models for tasks were performed on the basis of the time spent by the farmers on performing the task, whereas stable characteristics were used either as continuous (portion of overall time spent on the presence of a characteristic) or dummy variables (Table 1). A compound symmetric covariance structure was assumed, and all estimations of variance components were based on the restricted maximum likelihood (REML) method. Model adequacy was assessed through influence diagnostic and residual plots. Correlations between tasks and stable characteristics were assessed prior to the modelling process, and rechecked when the final model derived. Pearson correlation coefficients were used to describe relationships between endotoxin and dust, and seasons.

Table 1. Outline of the developed database and basic information for working tasks performed by 231 pig farmers employed in 54 Danish pig farms, and stable characteristics for a sub-group of 181 indoor workers including direct animal exposure.

Working tasks and environment[a]	n	Department characteristics (cut-off time level) [b]	n	Coding (Median) ‡
Indoor Environment	353	Outdoor temperature	268	Continuous (12 ^{0}C)
		Housing [a]		
Tasks inside animal areas		Animals in a loose housing system	58	Continuous (15 %)
Controlling	243	Animals housed in batch pens	205	Continuous (39 %)
Weighing	40	Animal housed in crates (including farrowing)	211	Continuous (64 %)
Moving breeding animals	111	Ventilation		
Moving weaners and finishers	139	Mechanical with neutral pressure (>60%)	15	Present (1) or absent (0)
Handling and nursing piglets (ear tagging, castrating, cutting tails)	116	Mixed type(including natural)	19	Present (1) or absent (0)
		Mechanical with negative pressure (>60%)	234	Ref
Inseminating	112	Mechanical with pit exhaust [a]	48	Continuous (10.3%)
Scanning	13	Heating		
Injection or handling sick animals	171	Floor heating (>50%)	168	Present (1) or absent (0)
Handling dead animals	93	Radiator heating (>50%)	63	Present (1) or absent (0)
Feed preparation and manual feeding	181	Floor type		
Automatic feeding (adjusting/inspecting)	138	Full slatted floor (>50%)	22	Present (1) or absent (0)
Bedding preparation and disposition		Mostly slatted (>50%)	101	Present (1) or absent (0)
Removing manure (in pens and stalls)	86	Mostly concrete	145	Ref
Sweeping or scraping corridors	54	Deep litter	38	Continuous (6.8 %)
Washing with high pressure	72	Showering (water) applied [a]	83	Continuous (42.8 %)
Disinfecting pens/stalls/stables	17	Feeding characteristics		
Repair and maintenance of animal buildings/feed room and installations	77	Dry feed (>80%)	121	Present (1) or absent (0)
		Dry and wet feed	50	Present (1) or absent (0)
Tasks outside animal areas		Wet feed (>80%)	97	Ref
Office work	35	Ad-libitum feeding method	157	Continuous (33.7 %)
Handling feed and seeds in barns and work relating to silos or drying plants	37	Hygienic conditions		
		Floor conditions		
Repairing/maintaining machinery & equipment (e.g. tractor, track, harvester)	48	Wet floor (>80%)	85	Present (1) or absent (0)
		Wet floor	83	Present (1) or absent (0)
Handling manure tanks and dunghills	4	Dry floor (>80%)	100	Ref
Work in the fields (working the soil, sowing, harvesting, applying fertilizers etc.)	15	Very dusty feeding path	73	Continuous (11.1 %)
		Very high dung accumulation	104	Continuous (11.3 %)
		Disinfected with bacterial agents (only endotoxin)	202	Continuous (63.6 %)

n, Number of measurements; [a] For all pig farmers included; [b] Only for workers with a full-indoor working shift and time spend dealing with the characteristics, cut-off level indicates the level of stable working time used to consider the characteristic present; ‡ Median value of portion of time spend with the presence of a characteristic for continuous values estimated for positive values.

RESULTS

The measured levels of dust and endotoxin exposure along with the numbers of participating farms and workers are given in Table 2. The mean sampling time was 368 min (SD=89.4) during summer and 366 min (SD 84.3) during winter. Measured inhalable dust and endotoxin concentration in winter were significantly higher than those in summer. Correlations between dust and endotoxin were moderate (overall r = 0.62) and between seasons low (r = 0.30 for dust and 0.15 for endotoxin).

Table 2. Basic measurement characteristics and personal levels of dust and endotoxin exposure of Danish pig farmers.

Period	n	f	k	Dust			Endotoxin			r
				AM	GM (GSD)	Range	AM	GM (GSD)	Range	
Overall	354	53	231	4.9	3.4 (2.6)	<LOD - 47.8	6241	1494 (4.3)	<LOD - 374579	0.62*
Summer	181	52	181	4.3	2.8 (2.6)	0.1 - 47.8	5949	1088 (4.2)	14.4 - 374579	0.66*
Winter	173	53	173	5.5	4.0 (2.5)	<LOD - 20.0	6546	2085 (4.2)	<LOD - 285264	0.54*

n=number of measurements; f=number of farms; k=number of workers; AM=arithmetic mean; GM=geometrical mean; GSD=geometrical standard deviation; r,=Pearson correlations between measured dust and endotoxin concentrations; *p<0.0001.

Model a (Work tasks and environment)

The results from the applied linear mixed models with working tasks for dust and endotoxin exposure among the overall population are shown in Table 3. The basic characteristics in respect to task occurrence, the average working time needed for each task, and the use of personal protective equipment (PPE) are also given. Overall, the tasks included in the final model along with the working environment explained 29% of the within-workers variability for dust exposure and 20% for endotoxin exposure. In univariate analysis injection and handling of sick animals was the most influential task (β = 0.009, p = 0.002) associated with dust exposure, and high pressure washing the most influential task (β = 0.005, p = 0.002) for endotoxin exposure. Field work alone explained 14% of the within-workers variability for both dust and endotoxin.

The final models consisted of 11 tasks for dust and 6 for endotoxin, which along with the environment explained 38% of the overall variability in dust exposure and 28% in endotoxin exposure. Handling of feeding materials related to silos and barns was the strongest predictor for both dust and endotoxin exposure. Given a time for task performance of 40 min (the average time performed in our population) that specific task will increase the level of dust exposure by 30% and that of endotoxin exposure by 23%. When high pressure washing for 90 min an increase by a factor of 1.5 ($e^{0.0045*90}$) in endotoxin exposure is to be expected. Interestingly the use of personal protection equipment was overall low with prevalence above 10% only for high pressure washing.

Table 3. Effect of working activities (per 1 min) on the log-transformed personal level of exposure to dust (mg/m^3) and endotoxin (EU/m^3) among Danish pig farmers. Results estimated on the basis of 354 measurements performed in 231 farmers employed in 54 farms.

	n	PPE (n)	MDN (min)	Dust			Endotoxin		
				β	***e***	***p***	***β***	***e***	***p***
Naïve Model									
Intercept				1.2114	0.0616	<.0001	7.3074	0.0806	<.0001
$_{bf}\sigma^2$				0.041	0.035	0.1168	0.002	0.049	0.484
$_{bw}\sigma^2$				0.193	0.097	0.0195	0.184	0.218	0.2004
$_{ww}\sigma^2$				0.663	0.088	<.0001	1.972	0.250	<.0001
Model with tasks and environment									
Intercept				0.4213	0.1726	0.0181	5.7989	0.2821	<.0001
Indoor working environment				0.0051	0.0019	0.0105	0.0160	0.0030	<.0001
Moving breeding animals	111	3	55	0.0019	0.0009	0.048			
Moving weaners and finishing pigs	139	4	30	0.0023	0.0007	0.0007	0.0024	0.0010	0.0198
Handling and nursing piglets (ear tagging, castrating, cutting tails etc.)	116	5	90	0.0019	0.0006	0.0028			
Injection or handling sick animals	171	6	45	0.0022	0.0009	0.0209			
Feed preparation and manual feeding	181	10	40	0.0033	0.0012	0.0094			
Washing with high pressure	72	9	90				0.0045	0.0011	<.0001
Disinfection	17	4	30				-0.0178	0.0046	0.0002
Repair and maintenance of animal buildings/feed room and stable installations	48	0	30	0.0020	0.0007	0.0031			
Handling feed and seeds in barns and work relating to silos or drying plants	37	5	40	0.0070	0.0012	<.0001	0.0053	0.0019	0.0062
Work in the fields (working the soil, sowing, harvesting, applying fertilizers)	15	0	210	-0.0049	0.0009	<.0001	-0.006	0.0014	<.0001
Office work	35	0	60	-0.0043	0.001	<.0001	-0.0048	0.0016	0.004
$_{bf}\sigma^2$				0.029	0.0224	0.0952	0.113	0.0638	0.0382
$_{bw}\sigma^2$				0.058	0.0628	0.1763	0		
$_{ww}\sigma^2$				0.490	0.0657	<.0001	1.438	0.1164	<.0001
Explained within worker variability				26%			28%		
Explained total variability				36%			28%		

n=number of observations; PPE=number of cases reported for use of personal protection equipment; MDN=median time spent on an activity estimated only for positive responses on the day of the measurements; *β*=regression coefficient; *e*=standard error; *p*=p-value; $_{bf}\sigma^2$=between-farm variance; $_{bw}\sigma^2$=between-worker (within-farms) variance; $_{ww}\sigma^2$=within-worker (day-to-day) variance.

Model b (Stable characteristics)

Table 4 summarizes the basic characteristics for the measurements used in the mixed effect analysis examining the influence of different pig stable characteristics on the personal exposure concentrations of dust and endotoxin. The average sampling time for these measurements was 367 min (SD 85), and similarly to the overall population significant seasonal differences in dust and endotoxin exposure concentrations were observed. The average (GM) exposure level for these measurements was 4.0 mg/m^3 for dust and 1,837 EU/m^3 for endotoxin. The sole sample with an endotoxin exposure level beneath the LOD value could probably be attributed to the long time interval (240 min) the specific farmer spent on handling and distribution of disinfection substances.

Table 4. Basic characteristics and personal levels of dust and endotoxin exposure for indoor measurements dealing with stable characteristics related to the presence of animals among Danish pig farmers. The given population is a sub-set of an overall population of 232 farmers monitored during 2008-2009.

Period	n	f	k	Dust			Endotoxin		
				AM	GM (GSD)	Range	AM	GM (GSD)	Range
Overall	268	51	181	5.3	4.0 (2.1)	0.46-47.8	5,250	1837 (3.2)	<LOD-374,578
Summer	135	47	135	4.7	3.3 (2.2)	0.46-47.8	5,200	1,412 (3.2)	154-374,578
Winter	133	49	133	5.8	4.8 (1.9)	0.47-20.0	5,300	2,399 (3.1)	<LOD-107,579

n=number of measurements; f=number of farms; k=number of workers; AM=arithmetic mean; GM=geometrical mean; GSD=geometrical standard deviation; r=Pearson correlations between measured dust and endotoxin concentrations; *p<0.05, **p<0.01, ***p<0.0001.

In univariate analysis the type of feed explained all given variability between farms for both dust and endotoxin exposure, whereas the outdoor temperature explained 17% of the within-worker variability for endotoxin. Other factors showing clear associations with endotoxin exposure included batch pen (β = 0.008, p = 0.0006) and crate animal housing (β = -0.008, p = 0.0002), use of ad-libitum feeding system (β = 0.006, p = 0.0014), and the presence of a dusty feeding path (β = 0.008, p = 0.0326). Similar trends were observed for dust exposure. Apart from the above, factors eligible to enter the multivariate model for endotoxin included floor exhaust ventilation, feed path dustiness, dung accumulation, and slatted floor coverage. For dust the eligible factors were the type of ventilation, the heating parameters, the slatted floor coverage, the feed path dustiness, and the level of floor dampness.

The final models are shown in table 5. The model for dust consisted of 5 factors explaining 81% of the variability between farms, but only 23% of the total variability.

For endotoxin only 3 factors remained in the final model that, though, explained all the between-farms variability. The use of dry feed remained a strong determinant of both dust and endotoxin exposure. Farmers with more than 80% of their stable working time spent on stables with dry feeding had a 1.5 to 1.7 factor increased levels of exposure when compared to those exposed for the same time in an environment with wet feeding. Time spent on a department with an ad-libitum feeding installation elevated the level of personal dust. Dust exposure was decreased in workers spending most of their time working under mechanical ventilation with negative pressure. Ventilation did not seem to be associated with the level of endotoxin exposure even in univariate analysis, though increased slatted floor area coverage was related to increased levels of endotoxin. A wet floor decreased dust, but not endotoxin exposure.

Table 5. Mixed effect models results on determinants of log-transformed personal dust (mg/m^3) and endotoxin (EU/m^3) exposure among indoor pig farmers. All characteristics are estimated on the worker level.

	Dust			Endotoxin		
	β	***e***	***p***	***β***	***e***	***p***
Naïve Model						
Intercept	1.3923	0.0584	<.0001	7.5224	0.0801	<.0001
$_{bf}\sigma^2$	0.053	0.0315	0.0469	0.056	0.062	0.1836
$_{bw}\sigma^2$	0.094	0.0673	0.0806	0		
$_{ww}\sigma^2$	0.406	0.0654	<.0001	1.325	0.124	<.0001
Model with determinants						
Intercept	1.3077	0.1109	<.0001	7.4371	0.2014	<.0001
Outdoor temperature	-0.025	0.0048	<.0001	-0.0408	0.0085	<.0001
Ventilation (1/0)						
Mostly neutral pressure	0.3582	0.1849	0.0563			
Mixed type (incl. Natural)	0.2640	0.1641	0.1116			
Mostly negative pressure	Ref					
Feed type (1/0)						
Dry	0.4296	0.113	0.0003	0.5568	0.1693	0.0015
Dry and wet	0.3577	0.1308	0.0077	0.6996	0.2056	0.001
Wet	Ref			Ref		
Ad-libitum feeding system[a]	0.0046	0.0016	0.0054			
Floor type (1/0)						
Full slatted				0.6146	0.2592	0.0201
Mostly slatted				0.2086	0.1507	0.17
Mostly concrete				Ref		
Floor condition (1/0)						
Wet floor	-0.249	0.1107	0.0275			
Mixed floor condition	-0.057	0.093	0.5448			
Dry floor	Ref					
$_{bf}\sigma^2$	0.010	0.0242	0.337	0		
$_{bw}\sigma^2$	0.117	0.0558	0.0179	0.082	0.1566	0.3013
$_{ww}\sigma^2$	0.304	0.0495	<.0001	1.118	0.1779	<.0001
Explained bf variability	81%			100%		
Explained total variability	23%			9%		

[a] per portion (1%) of overall time spend on the presence of a characteristic; *β*=regression coefficient; *e*=standard error; *p*=p-value; $_{bf}\sigma^2$=between-farm variance; $_{bw}\sigma^2$=between-worker (within-farms) variance; $_{ww}\sigma^2$=within-worker (day-to-day) variance.

DISCUSSION

Study approach

In the present study we tried to identify tasks and stable characteristics that potentially influence the level and the variability of personal exposure to dust and endotoxin among Danish pig farmers using an observational approach based on collected repeated measurements of exposure. We applied a "real-life" scenario using a random sampling design by performing measurements on common working days for the farmers without restricting monitoring to indoor environments. This approach enabled a comparative assessment of working tasks performed indoors and outdoors as well as in stables and other enclosed farm areas where work is commonly performed by the farmers. Our results suggest specific tasks and the working environment to influence within workers variability, and they highlight the feed type as the most important determinant of the between farms variability.

Considering the validity of our reported inhalable dust and endotoxin exposure levels, these are in good agreement with the results of previous studies that used comparable sampling[7, 8] and analytical methodologies.[8] The partly systematic selection of farms in our study is unlikely to have biased the representativeness of Danish pig farms in our farm sample. The distribution of farms in Denmark in our initial sampling was similar to the one reported by the Danish authorities, with more than 85% of the farms located in the areas of Jutland and Funen.[24] When we preformed sensitivity analysis to assess whether our selected farms differ in size from our initial sample of farms we observed no statistically significant differences, and analysis of the variance comparing the measured personal dust and endotoxin levels between the different farm size strata gave similar results (not shown).

Influence of field work

Using the farmers self-reported survey information on performed activities and a statistical approach based on unbalanced linear mixed effect analysis, we were able to explain 26% of the given within-workers variability for dust exposure and 28% for endotoxin exposure. Performance of field work was a strong protective factor for both dust and endotoxin exposure. Our measurements were distributed over a long time period, and the field working tasks performed consisted mostly of common tasks related to soil preparation (e.g. ploughing, land rolling, tilling), sowing and post sowing handling (e.g. manure and fertilizer spreading), and less to crop harvesting. All farms in our study were equipped with cabined tractors. The protective effect of field work is generally supported by a previous study among Norwegian farmers that reported lower levels of dust and endotoxin exposure during hay and grain harvesting compared to pig animal tending.[25] Similarly, in an earlier study among Californian farmers,[10] task-based measured endotoxin levels were in general lower for field crop related tasks compared to those related to livestock tending. However, in a large Dutch exposure assessment study on different branches of the primary agricultural production, workers involved in potato cultivation and grain harvesting were exposed to considerable levels of dust and endotoxin exposure.[8] These measurements were worst-case scenarios; given the cyclic nature of field related activities, lower concentrations are generally to be expected.

Influence of tasks requiring near contact with animals

Considering animal related working tasks, the movement of pigs and tasks that included intense animal handling like castration, teeth cutting as well as the injection and handling of sick animals (a very common task for workers in weaning and finishing herds) were significantly associated to an increased exposure to organic dust. These tasks are associated with increased animal activity, which is known to increase the levels of exposure.[26-28] In a recent study among American pig breeders, O'Shaughnessy et al.[29] also reported greater dust concentrations in tasks related to animal movement during the weaning process. A task-based estimation approach based on linear regression models with photometer readings and time-weighted estimates derived from full-shift personal sampling was used. Activities related to intense animal handling (castration, teeth cutting, ear tagging) and movement (re-penning) were also related to higher dust and even endotoxin exposure concentrations in a study among Dutch pig farmers that used a data collection and analysis strategy similar to ours.[18]

Influence of the tasks related to food preparation, feeding and cleaning

The same study of Preller et al.[18] reported increased dust and endotoxin exposure during tasks related to animal feeding and aerial cleaning (e.g. floor sweeping, cleaning of food storage and removal of dry manure). Most of these tasks were included in our investigation and feed preparation and manual feeding as well as the removal of manure were included in our initial models. Their absence from our final models might reflect the different modeling approach that we followed, the inclusion of outdoor work, and the stronger impact on exposure variability in our population by other tasks such as high pressure washing (for endotoxin) and feed handling in barns. The strong effect of the later activity on dust and endotoxin exposure is not unexpected, considering the high exposure levels measured among animal feed and seed processing workers,[8, 30] and among farmers performing farm indoor activities.[31] Moreover, Preller et al.[18] found a strong relationship between tasks related to cleaning of food storage areas and dust exposure.

Influence of feed, floor and ventilation

Our analysis showed the type of feed along with the ventilation as the most important determinants for dust exposure, and the type of feed along with the flooring for endotoxin exposure. Feed was the most influential parameter, and in univariate analysis explained all the given between-farms variability. These findings are in agreement with those from the study of Preller et al.,[18] who reported a more than 20% decrease in dust exposure when wet feeding was used, and an increase of 16% when full slatted floor was present. Feed is recognized as a source of dust and endotoxin exposure within pig buildings,[30, 32] and in their exposure assessment study of 171 Dutch pig stables Atwood et al.[33] reported considerably lower dust concentrations in stables using wet feed compared to stables using dry feed. The positive association between the ad-libitum feeding and exposure we demonstrated contradicts previously reported results as summarised by Gustafsson.[26] Considering the strong correlation that we found between ad-libitum feeding and batch pen housing (a system applied primarily in weaning and finishing houses), the effect of ad-libitum could reflect the expected higher animal movement and animal intensity. These are both strong exposure determinants,[26, 33-35] in weaning and finishing houses compared to departments housing sows where restricted feeding is most commonly used. In both our study and the one of Preller et al.[18] an increased outdoor temperature was associated with a decrease in the exposure levels. This can probably be attributed to the higher rate of ventilation used at higher temperatures. The outdoor temperature can be an indirect indicator of the ventilation rate,[26] and to optimize production pigs require temperatures within specific ranges.[36] The observed increase in levels of dust exposure in relation to workers mostly exposed to a neutral ventilation system compared to those mostly exposed to a negative pressure ventilation system is difficult to explain, but it could relate to air movement and distribution within the animal house. Further investigation will be needed to validate this finding.

The strong association between the slatted floor coverage and endotoxin probably reflects the increased exposure to faeces, a known source for endotoxin exposure.[1, 37]

Our univariate analysis results on hygienic conditions, in general, are supported by Preller et al.[18] who showed high levels of overall and feed path dustiness to increase exposure. The protective effect of a damped floor in comparison to a dry floor could be a result of lower dust resuspension due to binding of dust on the floor, including the wasted feeding materials, and probably the lower presence of dry manure.[27]

Variability issues

Our analysis with stable characteristics as fixed effects revealed several potential determinants of personal dust and endotoxin exposure among pig farmers. Though, the final derived statistical models were relatively small with only 4 parameters for dust and 3 for endotoxin, they explained almost all variability between farms in our study population. Most farms in our study comprised of several departments, and in most cases of several units. Expansion of these farms occurred periodically within several years and consequently different stable characteristics (e.g. ventilation, heating, feed system) were installed across departments based on the building recommendations for maximum productivity existing in that specific period. Accordingly, farmers in most cases were exposed to different conditions throughout a working shift, and many characteristics correlate when used directly as portions of time dealing with a stable characteristic (not shown). By examining the distribution of the individual time spent on stable characteristics we tried to maximize contrast and perform comparisons based on characteristics where farmers mostly spent their time. This practice minimized discrepancies in involved stable characteristics between most persons within a farm unit. However, personal working patterns were different between seasons, and workers on the same farm were not always working on the same areas. Thus, given the large discrepancy in stable characteristics within a farm, it is not surprising that the farm characteristics affected the between-workers and the within-workers variability.

Use of personal protection equipment

Our findings on the low frequency of PPE use are comparable to results previously reported among American[38] and Brazilian[39] farmers. Recently, the Agricultural Safety and Health Council of America in a recommendation paper advocated the development of a respiratory protection program based on the use of personal respiratory protection equipment during performance of specific tasks.[40] This recommendation was based on results from experimental studies among subjects previously unexposed to pig farming, which demonstrated a significant decrease in inflammatory reactions among those wearing a respirator compared to those unprotected.[41-43] Our study, apart from showing the need for a similar educational program among Danish farmers, provides suggestions on potential tasks to be subject of PPE usage.

CONCLUSIONS

Overall, the present study suggests activities related to nursing and movement of animals, work related to feed storage areas as well as high pressure water cleaning to increase the level of personal exposure to dust or endotoxin. Several farm characteristics showed a relation to the level of dust and endotoxin exposure, but dry feeding showed the strongest effect, explaining all given variability between-farms. These findings provide information relevant to the use of personal protection equipment during performance of specific working tasks, and indicate feeding practices as a prospective area for the development of prevention strategies. However, further investigation is needed especially considering the combined influential role of tasks performed under certain stable characteristics. We will therefore in the future expand our models by including working tasks and examine the role of more farm characteristics that are available in our databases.

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REFERENCE LIST

1. M. B. Schenker, D. Christiani, Y. Cormier, H. Dimich-Ward, G. Doekes, J. Dosman, J. Douwes, K. Dowling, D. Enarson, F. Green, D. Heederik, K. Husman, S. Kennedy, G. Kullman, Y. Lacasse, B. Lawson, P. Malmberg, J. May, S. McCurdy, J. Merchant, J. Myers, M. Nieuwenhuijsen, S. Olenchock, C. Saiki, D. Schwartz, J. Seiber, P. Thorne, G. Wagner, N. White, X. P. Xu, M. Chan-Yeung and A. E. O. Hlth, *Am J Resp Crit Care*, 1998, **158**, S1-S76.
2. J. Douwes, P. Thorne, N. Pearce and D. Heederik, *Ann Occup Hyg*, 2003, **47**, 187-200.
3. T. Sigsgaard, Ø. Omland and P. S. Thorne, in *Occupational Asthma*, eds. T. Sigsgaard and D. Heederik, Birkhauser Basel, Basel, Switzerland, Editon edn., 2010, pp. 163-183.
4. V. Liebers, T. Bruning and M. Raulf-Heimsoth, *Am J Ind Med*, 2006, **49**, 474-491.
5. R. Rylander, *Curr Opin Allergy Clin Immunol*, 2006, **6**, 62-66.
6. V. Liebers, M. Raulf-Heimsoth and T. Bruning, *Arch Toxicol*, 2008, **82**, 203-210.

7. K. Radon, B. Danuser, M. Iversen, E. Monso, C. Weber, J. Hartung, K. Donham, U. Palmgren and D. Nowak, *Ann Agric Environ Med*, 2002, **9**, 41-48.

8. S. Spaan, I. M. Wouters, I. Oosting, G. Doekes and D. Heederik, *J Environ Monit*, 2006, **8**, 63-72.

9. G. J. Kullman, P. S. Thorne, P. F. Waldron, J. J. Marx, B. Ault, D. M. Lewis, P. D. Siegel, S. A. Olenchock and J. A. Merchant, *Am Ind Hyg Assoc J*, 1998, **59**, 403-413.

10. M. J. Nieuwenhuijsen, K. S. Noderer, M. B. Schenker, V. Vallyathan and S. Olenchock, *Ann Occup Hyg*, 1999, **43**, 35-42.

11. J. C. Simpson, R. M. Niven, C. A. Pickering, L. A. Oldham, A. M. Fletcher and H. C. Francis, *Ann Occup Hyg*, 1999, **43**, 107-115.

12. H. Kromhout and D. Heederik, *Scand J Work Environ Health*, 2005, **31 Suppl 1**, 33-38; discussion 35-37.

13. L. Preller, H. Kromhout, D. Heederik and M. J. Tielen, *Scand J Work Environ Health*, 1995, **21**, 504-512.

14. H. Kromhout and R. Vermeulen, *Ann Occup Hyg*, 2001, **45**, 257-273.

15. I. Basinas, T. Sigsgaard, D. Heederik, H. Takai, Ø. Omland, N. T. Andersen, I. M. Wouters, J. K. Bønløkke, H. Kromhout and V. Schlunssen, Editon edn., 2011.

16. I. Burstyn and K. Teschke, *Am Ind Hyg Assoc J*, 1999, **60**, 57-72.

17. A. Burdorf, *Occup Environ Med*, 2005, **62**, 344-350.

18. L. Preller, D. Heederik, H. Kromhout, J. S. Boleij and M. J. Tielen, *Ann Occup Hyg*, 1995, **39**, 545-557.

19. G. Elholm, O. Omland, V. Schlunssen, C. Hjort, I. Basinas and T. Sigsgaard, *Clin Epidemiol*, 2010, **2**, 45-50.

20. S. Spaan, G. Doekes, D. Heederik, P. S. Thorne and I. M. Wouters, *Appl Environ Microbiol*, 2008, **74**, 3804-3811.

21. Dansk landbrugsrådgivning, *Håndbog til drifts-planlægning. [Expertise Centre for Agriculture. Manual for farm operation].* Landbrugsforlaget. p. 93-101., Aarhus, 2003.

22. C. Peretz, A. Goren, T. Smid and H. Kromhout, *Ann Occup Hyg*, 2002, **46**, 69-77.

23. S. M. Rappaport, M. Weaver, D. Taylor, L. Kupper and P. Susi, *Ann Occup Hyg*, 1999, **43**, 457-469.

24. StatBank Denmark, *Statistics Denmark. BDF07: Farms by region, type, farms and area (2006-2010): HDYR07: Livestock by county, unit and type (2006-2010): PELS2: Furred animals by region, unit and type of farm (2007-2009). Available at http://www.statbank.dk/statbank5a/default.asp?w=1440 (accessed 08 April 2011).*

25. E. Melbostad and W. Eduard, *Am J Ind Med*, 2001, **39**, 209-217.

26. G. Gustafsson, *Journal of Agricultural Engineering Research*, 1999, **74**, 379-390.

27. H. Takai, S. Pedersen, J. O. Johnsen, J. H. M. Metz, P. W. G. G. Koerkamp, G. H. Uenk, V. R. Phillips, M. R. Holden, R. W. Sneath, J. L. Short, R. P. White, J. Hartung, J. Seedorf, M. Schroder, K. H. Linkert and C. M. Wathes, *Journal of Agricultural Engineering Research*, 1998, **70**, 59-77.

28. R. Schierl, A. Heise, U. Egger, F. Schneider, R. Eichelser, S. Neser and D. Nowak, *Ann Agric Environ Med*, 2007, **14**, 129-136.

29. P. T. O'Shaughnessy, K. J. Donham, T. M. Peters, C. Taylor, R. Altmaier and K. M. Kelly, *J Occup Environ Hyg*, 2010, **7**, 7-13.

30. T. Smid, D. Heederik, G. Mensink, R. Houba and J. S. Boleij, *Am Ind Hyg Assoc J*, 1992, **53**, 362-368.

31. A. Molocznik, *Ann Agric Environ Med*, 2002, **9**, 71-78.

32. K. J. Donham, W. Popendorf, U. Palmgren and L. Larsson, *Am J Ind Med*, 1986, **10**, 294-297.

33. P. Attwood, R. Brouwer, P. Ruigewaard, P. Versloot, R. de Wit, D. Heederik and J. S. Boleij, *Am Ind Hyg Assoc J*, 1987, **48**, 745-751.

34. C. Duchaine, Y. Grimard and Y. Cormier, *AIHAJ*, 2000, **61**, 56-63.

35. P. S. Thorne, A. C. Ansley and S. S. Perry, *J Occup Environ Hyg*, 2009, **6**, 211-220.

36. C. Wathes and C. Whittemore, in *Whittemore's Science and Practice of Pig Production*, Blackwell Publishing Ltd, Editon edn., 2007, pp. 533-592.

37. DECOS, *Endotoxins: health based recommended exposure limit. A report of the Health Council of the Netherlands, publication no. 2010/04OSH*, Health Council of the Netherlands, The Hague, 2010.

38. W. S. Carpenter, B. C. Lee, P. D. Gunderson and D. T. Stueland, *American Journal of Industrial Medicine*, 2002, **42**, 236-247.

39. M. Costa, P. J. Teixeira and P. F. Freitas, *J Bras Pneumol*, 2007, **33**, 380-388.

40. S. Von Essen, G. Moore, S. Gibbs and K. L. Larson, *Journal of Agromedicine*, 2010, **15**, 216-225.

41. L. Palmberg, B.-M. Larsson, B.-M. Sundblad and K. Larsson, *American Journal of Industrial Medicine*, 2004, **46**, 363-370.

42. J. A. Dosman, A. Senthilselvan, S. P. Kirychuk, S. Lemay, E. M. Barber, P. Willson, Y. Cormier and T. S. Hurst, *Chest*, 2000, **118**, 852-860.

43. B. M. Sundblad, K. Sahlander, A. Ek, M. Kumlin, M. Olsson, K. Larsson and L. Palmberg, *Scand J Work Environ Health*, 2006, **32**, 145-153.

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Subject: Co-authors declarations

To whom it may concern,

Currently, some of my co-authors are under annual leave (summer holidays). Therefore, my PhD Dissertation titled "Dust and endotoxin exposure in animal farming populations - formulating the basis for a model-based exposure assessment approach" is submitted with declarations that have not been signed by all co-authors. My request has already been forwarded to them. As soon as possible upon their return I will forward a signed declaration by them to the Research Office of the Faculty, both by mail and as a hard copy.

Sincerely Yours,

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Declaration of co-authorship

Full name of the PhD student: Ioannis Basinas

This declaration concerns the following article/manuscript:

Title:	Exposure to inhalable dust and endotoxin among Danish livestock farmers: results from the SUS cohort study.
Authors:	Ioannis Basinas, Torben Sigsgaard, Dick Heederik, Hisamitsu Takai, Øyvind Omland, Nils T. Andersen, Inge M. Wouters, Jakob H. Bønløkke, Hans Kromhout, Vivi Schlünssen

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If published, give full reference:

If accepted or submitted, give journal: Journal of Environmental Monitoring

Has the article/manuscript previously been used in other PhD or doctoral dissertations?

No ☒ Yes ☐ If yes, give details:

The PhD student has contributed to the elements of this article/manuscript as follows:

A. No or little contribution
B. Has contributed (10-30 %)
C. Has contributed considerably (40-60 %)
D. Has done most of the work (70-90 %)
E. Has essentially done all the work

Element	Extent (A-E)
1. Formulation/identification of the scientific problem	D
2. Planning of the experiments and methodology design and development	D
3. Involvement in the experimental work/clinical studies	D
4. Interpretation of the results	D
5. Writing of the first draft of the manuscript	E
6. Finalization of the manuscript and submission	E

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This declaration concerns the following article/manuscript:

Title:	Sensitization to common allergens and respiratory symptoms in endotoxin exposed workers: a pooled analysis
Authors:	Ioannis Basinas, Vivi Schlünssen, Dick Heederik, Torben Sigsgaard, Lidwien A.M. Smit, Sadegh Samadi, Øyvind Omland, Charlotte Hjort, Anne Mette Madsen, Simon Skov, Inge M. Wouters

The article/manuscript is: Published ☐ Accepted ☒ Submitted ☐ In preparation ☐

If published, give full reference:

If accepted or submitted, give journal: Journal of Occupational and Environmental Medicine

Has the article/manuscript previously been used in other PhD or doctoral dissertations?

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4. Interpretation of the results	D
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6. Finalization of the manuscript and submission	E

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Full name of the PhD student: Ioannis Basinas

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Title:	Work tasks and stable characteristics associated with the levels of exposure to inhalable dust and endotoxin among Danish pig Farmers
Authors:	Ioannis Basinas, Vivi Schlünssen, Hisamitsu Takai, Dick Heederik, Øyvind Omland, Torben Sigsgaard, Hans Kromhout

The article/manuscript is: Published ☐ Accepted ☐ Submitted ☐ In preparation ☒

If published, give full reference:

If accepted or submitted, give journal:

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6. Finalization of the manuscript and submission	E

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Vedr. Ph.D-studerende Ioannis Basinas.

Ioannis Basinas har gennemført et Ph.D-projekt omfattende et meget omfattende feltstudie af eksponering for organisk støv i landbruget, se venligst vedlagte projektresumé. Ioannis har arbejdet selvstændigt både med etablering af studiet samt i forbindelse med forskningssamarbejdet med IRAS Utrecht NL.

Ioannis Basinas har ved flere lejligheder fremlagt resultater fra sit projekt på dansk såvel som på engelsk og således arbejdet en del med formidlingen af resultaterne. Kursusprogrammet er gennemført tilfredsstillende og kravene til Ph.D-afhandlingen er således opfyldt.

Med venlig hilsen

Torben Sigsgaard
Hovedvejleder

Research Office
Faculty of Health Sciences
Vennelyst Boulevard 9
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31 July 2011

Dear Sir or Madam,

With the present letter I would like to declare that my PhD Dissertation titled "*Dust and endotoxin exposure in animal farming populations - formulating the basis for a model-based exposure assessment approach*" **has never** previously been assessed, either in the same or in a more or less amended form, with a view to conferral of an academic degree.

Faithfully Yours

Ioannis Basinas, MSc

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