PARTICULATE DISTRIBUTION AND RELATIONSHIP TO ENDOTOXIN IN POULTRY PRODUCTION OPERATIONS

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ABSTRACT

This thesis dissertation assessed workers who work in poultry barns and their occupational environment in relation to the type of bird housing in which they were exposed (cage-housed birds (CH) or floor-housed birds (FH)) and examined the environmental variables including dust and endotoxin and potential relationships to respiratory symptoms of workers.

A cross sectional study was undertaken to assess the environmental exposure levels and respiratory health effects of workers who worked in CH and FH poultry operations. The respiratory results suggested an asthma-like syndrome in these workers. Workers who worked in CH facilities reported greater current and chronic respiratory symptoms and significantly greater current and chronic phlegm as compared to workers from FH facilities. Workers from CH poultry facilities were exposed to greater endotoxin load than workers from FH facilities, but workers from FH operations were exposed to greater levels of total dust. It was found that endotoxin load (EU/mg) was a significant predictor of chronic phlegm for all poultry workers.

The effects on dust and endotoxin measurements when utilizing a Marple impactor with greased or ungreased impaction surfaces when sampling in an agricultural environment were unknown, and the potential for effects was tested. There were no significant differences in the aerosol mass median aerodynamic diameters between the greased and ungreased Marple impactors. Endotoxin analysis results appeared to be influenced by impaction grease particularly when very low amounts of endotoxin were present.

Size fractioning the dust and endotoxin using Marple impactors in CH and FH poultry operations showed that endotoxin load (EU/mg) was significantly higher in the respirable fraction of area samples in CH poultry operations as compared to FH operations. There were no differences in endotoxin load in the non-respirable size fractions for area samples between CH and FH operations. FH poultry operations had significantly greater dust mass and dust concentration in both respirable and non-respirable fractions for FH operations. There was significantly greater endotoxin load (EU/mg) in the 3.5-6.0 micron size fraction for the CH poultry operations as compared to the FH operations.

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LIST OF ABBREVIATIONS

ACGIH	American Conference of Governmental Industrial Hygienists
AED	Aerodynamic equivalent diameter
APF	approved protection factor
CH	Cage-housed
cm^3	cubic centimeter
CO_2	carbon dioxide
d_a	aerodynamic diameter
D_{50}	effective cut-off diameter
DGGE	denaturing gradient gel electrophoresis
EDC	Effective cut-off diameter
EU/mg	Endotoxin load in endotoxin units per milligram of dust
EU/m3	Endotoxin concentration in endotoxin units per cubic meter
FEF_{25-75}	Maximal Mid-expiratory Flow Rate
FEV_1	Forced Expired Volume in One Second
FH	Floor-housed
FVC	Forced Vital Capacity
g	gram
GC-MS	gas chromatography-mass spectrometry
GM	geometric mean
GSD	geometric standard deviation
IPM	Inhalable Particulate Matter
Kg	Kilogram
l/min	litres per minute
LPS	lipopolysaccaride
LAL	*Limulus* amebocyte lysate
m^3	cubic meter
mg	milligram
mm	millimeter
MMAD	mass median aerodynamic diameter
ng	nanogram
NH_3	ammonia
PCR	polymerase chain reaction
PEF	Peak Expiratory Flow
PM_{10}	particulate matter with diameter < 10 μm
ppm	parts per million (parts of substance per million parts of air)
PVC	Polyvinyl Chloride
rFC	Recombinant Factor C
RPM	Respirable Particulate Matter
TLR4	Toll-like receptor 4
TLV	Threshold Limit Values
TPM	Thoracic Particulate Matter
μm	micron
Wks	weeks
WPF	workplace protection factor

1. INTRODUCTION

1.1 DISSERTATION ORGANIZATION

The dissertation is comprised of three distinct papers plus appendices. The introduction outlines the broad research questions addressed during the research program as well as an overview of the background and methods which contributed to the research decisions and processes. Where appropriate the background and methods sections are described as pertinent to one of the three papers or appendixes in the dissertation.

Paper 1 "Total Dust and Endotoxin in Poultry Operations: Comparison Between Cage and Floor Housing and Respiratory health effects in Workers" assesses the respiratory outcomes and environmental exposure levels of workers in cage-housed (CH) and floor-housed (FH) poultry operations. Personal total dust and endotoxin levels, across work-shift respiratory symptoms and lung function tests, and current and chronic respiratory symptoms were assessed on 120 poultry workers. This work indicated that the workers from the floor-housed poultry operations had significantly greater exposures to total dust and ammonia, whereas workers from cage-housed poultry operations reported greater frequency of current and chronic symptoms overall and significantly greater current and chronic phlegm. For all workers, endotoxin load (EU/mg) was a significant predictor of chronic phlegm.

The results from the first paper assisted in the methodology for the second paper. The first paper concluded that the greater endotoxin in the presence of lower dust levels coupled with the greater symptoms in the workers in the cage-housed operations could be related to differences in levels of exposures.

Paper 2 "Levels of Endotoxin and Dust at Respirable and Non-respirable Particle Sizes are not Consistent Between Cage and Floor-Housed Poultry Operations" attempted to further the industrial hygiene understanding of the differences in the environments between cage-housed and floor-housed poultry operations. The first paper assessed the poultry work environment

utilizing total dust and total endotoxin measures. For the second paper, dust and endotoxin measures were fractionated utilizing a Marple cascade impactor. The Marple sampler contained 6 stages to represent cut-points of 0.52, 0.93, 1.55, 3.50, 6.0 and 9.8μm. Measures were condensed and further classified into non-respirable fractions and respirable fractions. Personal and area work exposures were measured in both CH and FH operations. Dust and endotoxin were compared between types of operations at the total level, stage level, respirable and non-respirable level, and by mass median aerodynamic diameter (MMAD).

The second paper furthered the findings of Paper 1 by studying: a) the fractionated dust and associated endotoxin levels in both CH and FH operations to determine if differences in dust and endotoxin existed at the different fraction levels, b) if there were differences between respirable and non-respirable dust and endotoxin fractions for the two types of operations, and c) if the mass median aerodynamic diameter of the dust and endotoxin differed between the CH and FH operations. Differences in the dust and endotoxin outcomes of Paper 2 could potentially assist in understanding differences in respiratory outcomes from Paper 1.

The findings from Paper 2 illustrate significantly greater endotoxin per milligram of dust (EU/mg) in the respirable fractions of the area dust samples in the CH poultry operations as compared to the FH operations even though the FH poultry operations consistently had significantly greater levels of dust. Dust by Marple stage tended to follow a pattern of being greater in the FH poultry operations. For endotoxin load (EU/mg) in area samples, stage 5 at 3.5-6.0μm range had the greatest differences between the two types of operations with CH poultry operations having significantly greater endotoxin load. The trend for greater endotoxin load in the CH poultry operations as compared to the FH operations followed through to the smaller stages, although these differences were not significant. The mass median aerodynamic diameter (MMAD) for area and personal dust measures were significantly greater in the FH poultry operations as compared to the CH operations and for both types of operations the MMAD were in the thoracic fraction with an aerodynamic diameter (d_{50}) of >10 μm, a diameter at which particulates would typically deposit anywhere within the lung airways. The MMAD for endotoxin load was similar for the two types of operations but a much smaller MMAD than that of the dust at approximately 3μm.

Paper 3 "'It's a sticky issue': Differences in Particle Bounce and Endotoxin Levels in Marple Cascade Samplers with Greased and Ungreased Filters" describes a project undertaken prior to Paper 2. This paper describes the effects of particle redistribution and associated endotoxin levels in a Marple cascade sampler with and without the use of impaction grease on polyvinyl chloride filters (PVC) when sampling poultry dust in a wind tunnel. Comparisons were made between Marple samplers which contained filters which had been treated with impaction grease to Marple samplers with filters which had not been treated. The results from this paper assisted in determining if the filters utilized in the Marple samplers for Paper 2 would be treated with impaction grease prior to sampling. There were no significant differences in the dust MMAD when impaction grease was applied versus when impaction grease was not applied. The effect of silicone grease on endotoxin analysis was less clear. It appeared that endotoxin readings had the potential to be influenced by impaction grease, particularly when very low amounts of endotoxin were anticipated. As endotoxin was a key element to understanding the research questions for Paper 2 it was decided that the effect of the impaction grease on the endotoxin levels was significant enough to warrant not utilizing the grease for the study reported in Paper 2.

The most widely referenced assay for endotoxin analysis is the *Limulus* ameobocyte lysate assay (LAL) in which the lysate is prepared from the ameobocytes of *Limulus* blood of the horseshoe crab. There are several limitations to the LAL assay including the possibility of enhancement of endotoxin readings due to the interference of (1-3)-beta-D-glucans. Glucans, a component of fungi, could potentially be high in poultry operations. A new assay on the market, the Recombinant Factor C (rFC) assay removed the glucan pathway from the endotoxin assay thereby removing the potential enhancement effect of glucans. A limited number of CH and FH poultry dust samples from Paper 2 were simultaneously analyzed with both LAL and rFC assays to determine if there were differences between the endotoxin levels. The results displayed in Appendix 1 form part of the overall conclusions. The results indicated that there was strong correlation in the endotoxin units per milliliter between the LAL and rFC samples. There was more discrepancy between the differences (LAL- rFC) and the LAL at the lower endotoxin load (EU/mg) for the CH operations suggesting that perhaps there may be lower levels of glucans in

this environment. For the FH operations, there was more discrepancy between the differences (LAL- rFC) and the rFC at the higher endotoxin load (EU/mg) and the FH operations correlated more strongly with the rFC assays suggesting that perhaps there were greater fungi content in the FH operations. Only assumptions can be made from the data as the poultry operations were not analyzed for fungi content. The results though, do suggest that there was strong correlation between the two types of assays for measurement of endotoxin, but there were differences in the endotoxin load results between the rFC and LAL assays when analyzing poultry dust samples from the two different types of operations, particularly at the extremes of the endotoxin measures.

Respiratory measures of workers were undertaken for Paper 2, but due to a small sample size, comparisons between workers from CH and FH operations could not be made. There was a difference in the type of pulmonary function assessment equipment utilized between Papers 1 and 2. In Paper 1 a dry rolling seal spirometer was used to measure pulmonary function. In Paper 2 a Piko-1 electronic peak flow meter was used for assessment of pulmonary function. The Piko-1 electronic flow meter was chosen for Paper 2 as this device is very small and portable. The dry rolling seal spirometer is very large and is computer driven so there is extensive large bulky equipment to transport between farms. Additionally, poultry farms were becoming increasingly concerned about biosecurity and any equipment which was entering a facility needed to be thoroughly disinfected between barn visits. Ensuring good disinfection of the dry rolling seal spirometer and computer between barn visits was not possible. Appendix 2 outlines a small pilot study which was undertaken after the data was collected for Paper 2. The objective of Appendix 2 was to assess the differences in forced expired volume in 1 second (FEV_1) in normal healthy subjects between the dry rolling seal spirometer used in Paper 1 and the peak flow meter used in Paper 2. The variability in the results between the two different methods for measuring FEV_1 were wide enough to be considered of clinical significance if the Piko-1 and dry rolling seal spirometer pulmonary results were to be directly compared. The average individual results, however, correlated strongly between the two different instruments. Pulmonary results from Paper 1 to Paper 2 were not directly compared. The pulmonary results from Paper 2 were utilized to indicate that the same trends that were observed in Paper 1 were also observed in Paper 2. Pulmonary observations from Paper 2 were not utilized in predicting

outcomes related to environmental exposures nor were they directly compared to the pulmonary results obtained from Paper 1.

The same respiratory questions, which were adapted from American Thoracic Society Standardized Questionnaire and previous poultry studies, were utilized for both Papers 1 and 2. Copies of the questionnaires and standard operating procedures are found in Appendix 3.

1.2 OVERVIEW AND RATIONALE

In 2005, there were 4,668 poultry and egg producers in Canada producing just over 1.1 billion kilograms of chicken and turkey meat and over 517 million dozen eggs (Table 1).[1] Poultry and egg farm cash receipts totaled $2.6 billion, making up 7.1% of total agricultural receipts in Canada.[1] Industry associations estimated the meat-processing plants employed about 17,500 people; the egg industry estimated its total employment at 4,000[1]

Numerous studies have indicated that dust, endotoxin and ammonia are the major airborne contaminants in poultry housing units and furthermore, that these contaminants may be primarily responsible for the health effects experienced by workers occupationally exposed to this environment.[2-36] Furthermore, the type of poultry production (cage-housed (CH) or floor-housed (FH)) may result in different environmental contaminant concentrations and therefore differences in worker health responses.[24, 33, 35, 36]

The rationale for differences in total dust and endotoxin and the related worker health responses between the two types of poultry production is not well understood.[12, 36] The hypotheses addressed in this research program address differences in the particulate distribution in these two types of poultry operations, and in particular, if there may be greater levels of particulate in the respirable fractions in the CH operations as compared to the FH operations. It was further hypothesized that endotoxin may be more highly associated with the respirable fractions of particulates as compared to the non-respirable fractions. The composition of the

greater respirable particle fractions in the CH poultry operations could assist in explaining the respiratory differences observed between workers in the two types of operations.

Table 1: Number of Poultry and Egg Producers in Canada – 2005

Province	Chicken	Eggs	Total
BC	337	125	462
AB	285	167	452
NT	-	2	2
SK	**92**	**64**	**156**
MB	118	168	286
ON	1,079	375	1,454
QC	740	105	845
NB	36	17	53
NS	85	23	108
PE	7	12	19
NL	7	11	18
Canada	2,786	1,069	3,855

Size characteristics of inhaled particles can assist in revealing patterns of deposition within an exposed worker's respiratory tract and therefore the potential impact on the respiratory system. Dust particles ranging from 0.1 to 10 µm can be deposited and retained in the lung,[37-39] and if entities such as endotoxin are bound to these particles, the combination may induce a respiratory reaction.

Influencing the relationships between worker respiratory response, type of poultry production, and aerobiological contaminants are factors such as the type of bird, age and size of bird, and length of production cycle. This program included characterization of the work environment and assessed age of the birds, litter/bedding type, flooring type, housekeeping routine, type of feed, type of feeders, type of watering system, manure management practices,

number of birds, area of the room/barn, indoor and outdoor temperature and relative humidity, bird breed, wind speed and direction on the day of testing and the barn/room floor plan.

This program assessed differences in total and particle size fraction levels for two types of poultry production (cage-housed and floor-housed) to try to better understand if there were differences in dust and endotoxin levels between the two types of poultry production methods. Both area and personal exposure measures were undertaken. The effects of impaction grease on particle bounce and endotoxin levels using polyvinyl chloride filters in Marple cascade samplers was assessed. Differences in endotoxin levels in fractionated poultry dust samples were compared using both *Limulus* ameboecyte lysate (LAL) and Recombinant Factor C (rFC) endotoxin assays.

1.3 **RESEARCH QUESTIONS**

The following research questions and hypotheses formed the basis of the research program:

1. Are worker respiratory symptoms related to measured amounts of dust and endotoxin in cage and floor-housed poultry facilities?
2. What are the particle size distributions in floor-housed and cage-housed poultry facilities?
3. What are the distributions of endotoxin among particle size fractions in floor-housed and cage-housed poultry facilities?
4. Does utilizing impaction grease on polyvinyl chloride filters in Marple Cascade impactors influence the particle size distribution or endotoxin levels?
5. Do *Limulus* ameboecyte lysate (LAL) and Recombinant Factor C (rFC) endotoxin assays give different results for samples from poultry operations?

H_o1: There will be no association between respiratory symptoms and environmental measures from workers in the floor-housed and cage-housed poultry facilities.

H_o2: There will no impact on particle size distribution or endotoxin levels when impaction grease is utilized on polyvinyl chloride filters.

H_o3: There will be no association between particulate size distributions with endotoxin equally distributed in the floor-housed and cage-housed poultry facilities.

H_o4: There will be no difference in the particulate size distributions between floor-housed and cage-housed poultry facilities.

H_o5: There will be no difference in endotoxin levels within particle size fractions between floor-housed and cage-housed poultry facilities.

H_o6: There will be no difference in measured endotoxin levels between LAL and rFC endotoxin assays.

BACKGROUND AND METHODS DEVELOPMENT

Information available on factors which would have an impact on understanding the questions in the research program as well as methods development relevant to the three papers and appendixes are reviewed. Sections 1.4-1.7 describe this background information.

Characteristics of particulates and attached endotoxin which would be important to understanding the potential effects on a poultry workers' respiratory system are reviewed. Worker respiratory responses, dust and endotoxin levels in the cage and floor-housed poultry operations, and particulate deposition characteristics are discussed (PARTICULATE AND ENDOTOXIN: CHARACTERISTICS).

An overview of the common practices for assessing worker exposures and protecting a workers respiratory system from particulate and endotoxin exposures is provided (PARTICULATE AND ENDOTOXIN: ASSESSMENT AND PROTECTION STRATEGIES). Strategies for sampling the poultry barn environment and characteristics which could impact sampling strategies are reviewed (ENVIRONMENTAL SAMPLING CHARACTERISTICS AND STRATEGIES). An overview of the endotoxin molecule as well as relevant characteristics

of the molecule are reviewed and provide a background to understanding the potential role of endotoxin in the respiratory health effects experienced by exposed workers. Endotoxin analysis methods are described (ENDOTOXIN AND ENDOTOXIN ANALYSIS).

Characteristics of the worker population are reviewed (WORKER POPULATION CHARACTERISTICS). As the study populations were drawn from a previous sampled population, the relationships to the original sampled population are addressed. Methods for assessing the workers respiratory health are outlined including questionnaire methods and respiratory function measurement methods (WORKER RESPIRATORY HEALTH ASSESSMENTS).

Common characteristics of the cage-housed and floor-housed poultry operations are reviewed under the poultry operation characteristics (POULTRY OPERATION CHARACTERISTICS) in order to provide a better understanding of the poultry operations and to assist in determining the appropriate environmental sampling time for Paper 2.

Variables of interest which could have the potential to influence the levels of particulate and endotoxin in poultry barns were analyzed from the data collected for Paper 2 (ASSESSMENT OF POTENTIAL CONFOUNDING). This information is provided in the background section as it is important to better understanding the environmental differences between cage-housed and floor-housed poultry operations as well as the potential differences related to measurements collected by area and personal sampling methods.

1.4 **PARTICULATE AND ENDOTOXIN: CHARACTERISTICS**

1.4.1 Respiratory Exposures and Worker Health Responses

As early as 1555, Ramazinni indicated that the respiratory tract of farmers was at risk for occupational diseases.[40] Individuals engaged in poultry production are exposed to varying concentrations of airborne contaminants including organic dusts, gases, fungi, bacteria and bacterial constituents such as endotoxin and other biologically active materials. Numerous

studies have indicated that dust, endotoxin and ammonia are the major airborne contaminants in poultry housing units and furthermore, that these contaminants may be primarily responsible for the health effects experienced by workers exposed to this work environment.[2-33, 41] Simpson et al.[42] studied workers in nine different industries and demonstrated that the highest prevalence of work-related lower respiratory tract symptoms (38%), upper respiratory tract symptoms (45%), and chronic bronchitis (15%) were present among poultry handlers, and personal exposure to dust or endotoxin were predictive of symptoms. A European study indicated that 24% of poultry farmers had work-related symptoms (wheezing, breathlessness and cough without phlegm)[32] and compared to swine farmers had lower baseline lung function.[28] In a study conducted in the U.S. 53% of workers who had worked greater than 10 years in turkey operations had cough, 40% had phlegm and 27% wheezed during the winter season.[4] In an investigation of poultry workers the baseline value for FEV_1 was significantly lower among poultry workers as compared to controls as was the average decrease in FEV_1 after methacholine challenge.[36] Symptoms of dry cough, cough with phlegm and shortness of breath were more common among poultry workers as compared to controls.[36] Exposure to the work environment appears to relate to respiratory health effects and significant dose-response relationships for pulmonary function decrements have been shown.[12]

Asthma-like-syndrome has been proposed as one of the outcomes of exposures to endotoxin.[43, 44] Symptoms associated with asthma-like-syndrome are chest tightness, wheeze, dyspnoea, and across-shift decline in FEV_1 of usually less than 10%. In contrast to allergic asthma, previously unexposed subjects with asthma-like-syndrome can develop symptoms and (reversible) airflow obstruction without any prior sensitization or latency period.

Endotoxin is thought to be a primary agent in the respiratory reaction experienced by workers in livestock industries.[12, 45-47] Classically, endotoxin is a structural component of bacteria which is released mainly during rapid growth phases or when bacteria are lysed. A prototypical example of endotoxin is lipopolysaccharide (LPS) found in the outer membrane of Gram-negative bacteria.[48] In agricultural studies the level of exposure is often represented by the endotoxin present in the collected particle sample and often expressed as endotoxin in the volume of air sampled (EU/m^3 or ng/m^3). Studies often utilize EU/m^3 to establish exposure

relationships with respiratory outcomes.[12, 15, 36, 45, 47] Compared to the swine, grain and animal feed industries, the poultry industry has been shown to have the highest endotoxin concentrations.[15, 49, 50] Significant prevalence rate ratios for upper respiratory tract symptoms were found with increasing endotoxin and dust exposure in workers in poultry confinement units.[15] A suggested threshold dose at which poultry workers risk respiratory health effects is 100 EU/m^3.[12]

Studies have shown differing correlations between the levels of dust in livestock operations with the bacteria and endotoxin present in these environments.[49] A review of studies looking at (1-3)-β-D-glucan exposure, airway inflammation and symptoms showed mixed results so that specific symptoms and potential underlying inflammatory mechanisms associated with exposure could not be identified.[51] Exposure to several non-microbial agents have been shown to be associated with acute work-related symptoms of the eyes and nose as well as with cough in farmers.[52] After adjusting for other exposures, exposure to fungal spores was found to be significantly associated with cough in farmers.[52] In a study comparing cowsheds, pig houses and poultry houses, Gram- negative bacteria constituted only 2.6% of the average amount of bacteria in poultry houses.[49] The highest microbial contamination was observed in the poultry houses, where both the average amount of microbes and the amount of Gram- negative bacteria were respectively 2.5 and 1.3 times higher in poultry houses than in pig houses.[49] Endotoxin concentrations were also significantly higher in the poultry houses (3 times) than in the pig houses, with concentrations varying from 800-12 800 EU/m^3.[49] There was a positive correlation (r=0.64) between the average amount of bacteria and the concentration of endotoxin, with a moderate correlation (r=0.59) between levels of Gram-negative bacteria and endotoxin concentration in poultry houses.[49]

A review of the literature has shown that endotoxin are one of the relevant exposures associated with non-atopic asthma or asthma-like-syndrome.[32, 53-56] In contrast to atopic asthma the syndrome is associated with neutrophilic inflammation of the airways rather than eosinophils.[53,57,58, 59]Although the exact pathophysiology is not clear, it is well established that non-allergic occupational asthma is mediated by an acute inflammatory response involving a number of cytokines, including IL-1, IL-6, IL-8 and tumour necrosis factor (TNF)-α, and the

subsequent massive infiltration and activation of neutrophils in the lower and upper airways. Macrophages carry specific endotoxin binding receptors (CD14, TLR4) that appear to play a crucial role in the activation of these cells and the subsequent inflammatory reactions.[60-62] Studies have indicated that different types of organic dusts caused a variety of effects in the lungs, and that each ranged in intensity of their elicited immune responses.[63, 64] Poultry dust exposures were shown to cause over a 100-fold increase in IL-8 production by A549 cells.[35]The greater the dust concentration the greater the IL-8 response, particularly for poultry dust samples, suggesting that the dust samples contained different substances and amounts of substances that incited an inflammatory response.[35] Previous studies have suggested that the immune response elicited after dust exposure is primarily due to endotoxin levels in the dust itself,[64-66] although this correlation was not as strong in other studies.[35] Studies have shown a strong correlation between the bacterial DNA in dust and endotoxin for farm barns.[67] Similarly, farm barn bacterial DNA significantly potentiated IL-10 and IL-12 but not TNF release.[67] Study data strongly suggest that an environment rich in microbial structures, such as a farming environment, may protect against the development of allergies.[68] Differences in airway responsiveness to inhaled endotoxin also exist in healthy non-allergic subjects suggesting that there may be some susceptibility or genetic predisposition associated with individuals at risk.[69]

1.4.2 Environmental Contaminant Levels in CH and FH Poultry Operations

There are different types of poultry operations including housing birds in cages and housing birds on litter on the floor. In the poultry industry, type of production, i.e. floor-housed versus cage-housed birds, may influence the levels of various environmental contaminants including dust and types and levels of Gram-negative and Gram-positive bacteria. Total and inhalable dust levels have consistently been higher in poultry operations in which birds are housed on the floor compared to operations in which birds are housed in cages. For floor-housed operations in the U.S, geometric mean inhalable dust levels were 24 mg/m^3 [70] and in Iran 21 mg/m^3.[22] Dust measurements in floor facilities in Europe ranged from 8-9 mg/m^3 inhalable dust.[71] In Finland, floor levels ranged from 2-9 mg/m^3.[72] In the U.S. total dust levels in floor-housed bird operations were 9 mg/m^3 [23]and in turkey barns in the United States levels ranged from 7-10 mg/m^3.[73] In facilities where birds are housed in cages total dust levels have typically

been considerably lower than those from floor-housed facilities with levels in Europe ranging from 1-4 mg/m^3.[18, 74] In a Swedish study, caged layers had much lower total dust levels compared to birds raised on litter on the floor (2-7 mg/m^3 and 12-17 mg/m^3 respectively).[75] In the United Kingdom respirable and inhalable dust concentrations were significantly higher in broiler operations (floor-housed) as compared to cage operations.[76] In a study from Canada which looked at particles less than 5 μm in diameter, the opposite was true, facilities which housed birds in cages had higher levels (40 particles/ml)[10] than did facilities which housed birds on the floor (7 particles/ml and 27 particles/ml).[9, 77]

Endotoxin levels have been shown to be similar or higher in operations housing birds in cages as compared to the floor-housed poultry operations. Inhalable endotoxin levels in floor-housed U.S. broiler grower operations have been measured at 20-60 ng/m^3;[23]and at a geometric mean of 210 ng/m^3;[70]and between 1440-16,512 EU/m^3 respirable endotoxin in turkey production in the United States in winter.[73] Endotoxin levels for cage-housed operations have typically been higher than that of floor-housed operations at 130-500 ng/m^3,[18] and a United Kingdom study indicated similar inhalable endotoxin levels but higher respirable endotoxin fractions in cage-housed operations as compared to the floor-housed operations.[76]

The type of poultry production (cage-housed (CH) or floor-housed (FH)) may result in different environmental contaminant concentrations and therefore differences in worker health response.[24, 33, 35, 36] Size characteristics of inhaled particles can assist in revealing patterns of deposition within an exposed worker's respiratory tract and therefore the potential impact on the respiratory system. Dust particles ranging from 0.1 to 10 μm can be deposited and retained in the lung,[37-39] and if entities such as endotoxin are bound to these dust particles, the combination may induce an inflammatory respiratory reaction.[24, 35, 46, 78-82] In swine production units it has been found that there is a significant enrichment of endotoxin in the dust particle size fraction 3.5-8.5 μm which includes the size fraction of lower respiratory deposition.[83] Significantly higher endotoxin concentration has been found in the inhalable fraction of livestock houses as compared to the respirable fraction.[84] The size distribution in animal houses was shown to be dominated by particles less than 3 μm and the concentration of particles was higher on the poultry farm than on other types of animal confinement operations.[41] A high fraction (up to

37%) of particles between 2-10 μm was found to be fungal spores.[41]and the highest concentration of actinomycetes were found on the poultry farm.[41] The aerodynamic size of actinomycete spores have been shown to range from 0.57-1.28 μm.[85] The highest airborne fungal isolates have been shown to be *Aspergillus spp.*[41, 86] or *Penicillium spp.*[41, 86]with an aerodynamic size range of 2.6-4.8 μm.[41] Ascospores (3.7-7.5 μm), Basidiospores (5.2-8.3 μm) and *Cladosporium spp*.(5.2-10.9 μm) were the next most predominant spores respectively.[41] The aerobic bacteria common in poultry confinement operations have been shown to be *Bacillus, Micrococcus, Proteus, Pseudomonas, Staphylococcus spp*, and *Escherichia coli,* with the most common anaerobic bacteria being *Clostridia.*[86] The aerodynamic sizes of most bacteria and fungal spores are between 0.7 and 10 μm.[87] Changes in the levels of bacteria and fungi occurred with pH increases in the litter.[86]

In a recent study in Germany investigating dairy, beef, swine, poultry and turkey operations it was found that endotoxin levels in the inhalable fraction of dust exceeded the concentrations in the respirable fraction. For layer operations the median endotoxin concentration (EU/m^3) in the respirable fraction comprised 11% of endotoxin concentrations in the inhalable fraction, whereas in turkey operations the median concentration was 20 percent.[50] This study utilized separate samplers for inhalable and respiratory measures and then compared the two measurements.[50]

As noted, while prior studies have measured total and inhalable dust and endotoxin levels in poultry housing operations the relationships between particulates and endotoxin in CH and FH poultry operations is not yet fully known. Understanding how particulates and endotoxin are distributed in the CH and FH poultry operations may assist in better understanding the differences in health effects experienced by exposed workers.

1.4.3 Particle Deposition and Particle Fractionation

The size of an airborne particle determines the length of time it remains suspended and, when inhaled, the site at which it deposits in the respiratory tract. The most important parameter describing the behavior of particles is particle aerodynamic diameter (d_a). The rate at which an

aerosol particle settles in still air as a result of gravity depends upon the particle's shape, size, and density, as well as viscous resistance arising from moving through the air. The aerodynamic equivalent diameter (AED) of a particle is a concept based on the assumption that such a particle velocity can be measured. The AED is the diameter of a sphere of unit density (1 g per cm^3) which has the same settling velocity in the same gas as the particle in question. Particles with the same AED behave dynamically identically.[37, 38] Accurate determination of deposition in the respiratory tract for a given particle size distribution is very difficult given the variability of parameters among individuals in a population such as health status, age, gender, and ethnicity.[37]

When large particles (>10-20 μm) are airborne, they can be inhaled. These large particles usually impact on the walls of the nose and pharynx .[35] The nose, the pharynx and lower airways are very effective in clearing the inhaled air of suspended particles, so that the volume of large particles to be cleared by the alveoli is minimal. The great majority of inhaled particles in the 5-10 μm diameter range are thought to deposit on the tracheobronchial surface. Deposition at the alveolar level occurs mainly for particles between 0.5-5 μm and approximately 50% of 0.5 μm particles are retained in the alveoli with the remainder being exhaled.[37] The nose represents a narrow airway passage and the high mass inertia of the largest particles prevents them from passing the nasal passage without impacting against the mucosal lining. Even small hygroscopic particles may get trapped in the nasal passage since humidification within the nose can result in these particles swelling to greater size and mass. The rate of particle impaction increases with the velocity of the particle, the angle of deflection, and the square of the (aerodynamic) diameter of the particle. Short-circuiting the nose by mouth breathing, such as during exercise, will increase the particle load to the intrathoracic airways. Particles deposited in intrathoracic airways are removed by mucociliary activity or by phagocytosis.[37]

Particle deposition within airways is governed by three primary mechanisms: impaction, sedimentation and diffusion. The largest particles have the greatest chance of impacting in the larger airways.[35] The term impaction implies that particles, due to their speed and mass inertia, collide with the wall of the airways. The larger the particle's mass, the larger it's mass inertia. Therefore only the smallest particles with the smallest mass will be capable of following the main stream of airflow, while the larger particles impact into the airway walls as the flow is deflected in branching airways.[38] Hence the concentration per unit area of any substance

delivered to the airway wall is likely to be larger in the central than in the more peripheral airways. The further down the airway, the lower the linear velocity of airflow containing the particle. Sedimentation, the process in which the particle falls down due to the effect of gravity, now becomes an increasingly important determinant of deposition onto the airway wall. The speed at which a particle drops is proportional to its density and to the square of its diameter. In the most distal airways and alveoli, motion due to kinetic energy of the particle (diffusion), becomes the primary mechanism contributing to particle deposition due to the linear velocity and mass flow approaching zero.[37]

Non-inert substances which impact on the airway walls and enter the alveoli have the capacity to interact with their host and elicit a response. The nature of the response to the non-inert substance is variable. Even if the particle is inert, it may be the carrier for other substances, such as endotoxin, which adhere to its surface and elicit a response.[37]

Understanding particle behavior at different size fractions and the potential impact on the respiratory system can be further applied to the poultry work environment. Fractionating the particulate from the CH and FH poultry operations and analyzing components such as microorganisms and associated endotoxin may assist in better understanding the related exposures and therefore the health effects experienced by exposed workers.

1.5 PARTICULATE AND ENDOTOXIN: ASSESSMENT AND PROTECTION STRATEGIES

1.5.1 *Particle Size Selective Sampling for Airborne Particulate Matter*

The human respiratory tract is an aerodynamic classifying system for airborne particles. Size and shape characteristics of the particle combined with the size and dimensional characteristics of the respiratory tract influence the deposition of particles. A sampling device such as the Marple sampler is used as a collector of airborne particles to predict the impact of particles on the respiratory tract. The sampler is meant to reproduce to a reasonable degree the

human respiratory system so that lung penetration by airborne particles can be predicted from sampling data.

Impactors operate under the principle that when a stream of particle laden air is directed at a surface, particles of sufficient inertia will impact upon the surface and be collected. In cascade impactors, particle laden air enters and passes through a series of progressively smaller jets, where progressively smaller particles are collected on each stage. The theory of impaction is well developed and has been generally confirmed.[88, 89]

Impactors are most often utilized to assist in assessing the respiratory impact of particles. It is thought that since the lung penetrability of unit density particles is known, and the impactor collects particle sizes on each stage of a calibrated sampler, then if an impactor is used according to standard operating procedure, the stage distribution of collected material should indicate the extent to which the sample would penetrate the respiratory system.[90] Along with this information and the knowledge of the chemical and/or biological properties of the collected particulate, the nature of the health hazard could be estimated.[90] This theoretical approach is consistent until particle related effects are introduced such as particle bounce, re-entrainment and stage loss. If particles bounce or blow-off subsequent to collection, particles penetrate their appropriate stage and contribute to the collection at smaller stages, thereby distorting the size distribution.[91, 92] The hardness of collected particles appears to be a significant factor influencing the collection characteristics of surfaces.[92] Soft materials appear to deform more readily on impact than hard material; consequently, it is expected that bouncing is minimized for soft materials.[92] This leads to the suggestion that particle bounce will be dependent on the nature of the particle and the type of collection surface.[92] It is suggested that an adhesive impaction surface be utilized for collection of particles on certain substrates as a means of reducing loss.[92-101] The use of an adhesive may present additional difficulties including loss of adhesive in the gravimetric analysis and possible interference of the adhesive during chemical analysis.[95, 102] As Hinds et al.[92] indicated, “this underscores the need for field evaluation of impaction surfaces prior to sampling”.

1.5.2 *Marple Impactor:* A *Particle Size Selective Sampler*

The total dust measurements for Paper 1 used a closed-faced cassette for sample collection. Comparison of this type of sample to respiratory deposition and related respiratory health effects is not possible, but did provide an overview of the differences in dust and endotoxin levels between CH and FH poultry operations.

For Paper 2, Marple samplers were utilized. The Marple Impactor is a precision cascade impactor which provides aerodynamic particle size distributions of a sample. The Marple sampler contains a series of impaction stages to separate particles into different size fractions based on their aerodynamic diameters (Figure 1). Particles are collected according to cutoff sizes, which are determined by the flow rate of the pump. The air stream flows around the impaction plates and particles greater than a specified cutoff aerodynamic size hit the first impaction plate, and smaller particles follow the path of the air stream to the next impaction plate. Most if not all of the particles are impacted on the available stages.

The Marple sampler utilized for Paper 2 contained 6 stages to represent cut-points of 0.52, 0.93, 1.55, 3.50, 6.0 and 9.8µm. For analysis purposes stages 3 and 4 of the Marple sampler (>6µm) were chosen to represent the non-respirable fraction and stages 5-final (6.0-<0.5 µm) were chosen to represent the respirable fraction (Figure 1).

Figure 1: Marple Sampler Cut-off Diameters and designations

Stage	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*
Cut-off diameter, µm	21.3	14.8	9.81	6.00	3.50	1.55	0.93	0.52
			Non-respirable		Respirable			

Information adapted from: Anderson-Instruments. Marple personal cascade impactors 290 series operators manual. Smyrna, GA, USA; 1999[91]

The Marple sampler was chosen for Paper 2 rather than inhalable or respirable samplers to more accurately answer the research questions and hypotheses of the research program. One

of the hypotheses was that there would be greater endotoxin present in the smaller fractions of particulate. The staging of the Marple sampler allowed for a more defined analysis of particle size fractionation and endotoxin concentration comparisons. For analysis purposes, the staging of the Marple was defined to represent the respirable and non-respirable fractions of the sample (Figure 2). The American Conference of Governmental Industrial Hygienists (ACGIH) has defined particle size-selective threshold limit values (TLVs®) in three forms.[103]

1. Inhalable Particulate Matter TLVs (IPM-TLVs) for those materials that are hazardous when deposited anywhere in the respiratory tract.
2. Thoracic Particulate Matter TLVs® (TPM-TLVs) for those materials that are hazardous when deposited anywhere within the lung airways and the gas-exchange region.
3. Respirable Particulate Matter TLVs (RPM-TLVs) for those materials that are hazardous when deposited in the gas-exchange region.

The definition of respirable for Paper 2 is not the same 50% diameter cut-off (d_{50}) as defined by ACGIH respirable fractions (Table 2) which is set at a d_{50} of 4µm.[103] The Marple sampler does not contain a cut-point at 4µm. The cut-point range choices for the Marple were 1.55-3.5µm or 3.5 to 6.0µm (Figure 4). Stages 5 to the final stage were chosen to represent the respirable fraction for Paper 2. This designation allowed for a small representation of non-respirable particles to be included in the respirable designation, as the particle fractionation at stage 5 actually collects particles to the 6µm size (d_{17}). This was in contrast to choosing the lower end of 3.5µm (stage 6) which would have under-represented the respirable fraction. Choosing the higher end (stage 4) may have over-represented the non-respirable fraction. The designation of thoracic particulate probably more accurately reflects the categorizations of the staging for the Marple.

Table 2: Collection efficiencies of inhalable, thoracic and respirable fractions

Particle Aerodynamic Diameter (µm)	Inhalable Particulate Matter Fraction	Thoracic Particulate Matter Fraction	Respirable Particulate Matter Fraction
4	0.89	0.89	0.50
10	0.77	0.50	0.01
80	0.50	0.00	0.00
100	0.50	0.00	0.00

1.5.3 *Mass median Aerodynamic Diameter*

Particle diameter, density and concentration impact how long a particle remains airborne as well as its ability to be inhaled into the upper respiratory tract and lung airways. Since most particles are irregularly shaped and not amenable to direct measurement of diameter an operational definition of particle diameter, aerodynamic diameter (AD), is often used to characterize the effective sizes of particles in an aerosol.[37,38,52] AD is based on a particle's inertial and gravitational motion in air. A particle falling through air under the force of gravity (gravitational sedimentation) accelerates until it reaches a velocity at which the force of gravity is just balanced by the viscous resistive force exerted by the air (Stokes Law). This velocity is known as the terminal settling velocity. [37,38] Thus, the AD of a particle, however shaped, is taken as the diameter of a unit density sphere that would have the identical terminal settling velocity. [37,38] AD is used to predict where in the respiratory tract particles will deposit.

The AD of the particles, the geometry of the airways, and the depth and pattern of respiration help determine the pattern of particle deposition. As described earlier, the nose, the pharynx and lower airways are very effective in clearing the inhaled air of suspended particles, so that the volume of large particles to be cleared by the alveoli is minimal. The great majority of inhaled particles in the 5-10 µm diameter range are thought to deposit on the tracheobronchial surface. Deposition at the alveolar level occurs mainly for particles between 0.5-5 µm and approximately 50% of 0.5 µm particles are retained in the alveoli with the remainder being exhaled.[37] Determining if there is a difference in the AD of particles from the two different poultry environments would assist in determining if AD of the aerosols may be a contributing factor in the respiratory response experienced by workers.

Half the particles of an aerosol have diameters smaller than the median physical diameter (count median diameter, CMD) but because particle mass is proportional to the cube of the diameter, the collective mass of the particles smaller than the CMD may constitute only a small fraction of the aerosol's total mass. As the amount of a toxic material a particle contains is proportional to its mass rather than its diameter, mass median diameter (MMD) is often specified. MMD is the particle diameter for a particle whose mass falls at the median of the particle mass distribution of the aerosol.

The Marple sampler used in this research program is an aerodynamic separation device that separates the mass of the collected sample and expresses calibration sizes in terms of aerodynamic diameter. Each stage of the Marple sampler is characterized by an effective cut-off diameter (EDC or D_{50}), the aerodynamic particle size of the cut-off for an equivalent ideal impactor. The ECD values are given by the manufacturer. An ideal impactor has a sharp cut-off with all particles larger than the cut-off collected and all particles smaller passing through.[104] The ECD, D_{50} represents the aerodynamic diameter for which 50% of that size of particle is collected and 50% pass through.[104] Mass median aerodynamic diameter (MMAD) corresponds to the median unit density equivalent aerodynamic diameter calculated from the Marple sampler MMD and ECD data.[52,104]

Most aerosol distributions have a wide range and a skewed shape with a long tail at the larger sizes. A normal distribution does not typically fit an aerosol measurement as an aerosol typically has a distribution of particle diameters and the width of this distribution, is usually lognormally distributed, so geometric mean and standard deviation are often used to describe their distribution. The MMAD (D_{50}) replaces the geometric mean of the mass distribution. If the distribution is lognormal, the MMAD (GM) and the GSD can be read directly from a cumulative plot of the Marple data. With normal distribution one standard deviation is equal to the difference between the 84 percentile and the 50 percentile (median). Because the lognormal distribution is based on the logarithm of the particle size the GSD is equal to the ratio of the 84 percentile size to the 50 percentile (median) size.

Particle penetration is often described by MMAD as being respirable, thoracic or inspirable. The designations include respirable particles (mass median aerodynamic diameter smaller than 4 μm), thoracic particles (mass median aerodynamic diameter smaller than 10 μm), and inspirable particles (mass median aerodynamic diameter smaller than 100 μm) as described in Table 2.

The MMAD is used to describe the aerosol characteristics for both Papers 2 and 3. The MMAD provides interesting additional information to the dust mass and concentration. The MMAD provides a summary measure to compare the dust and endotoxin in the environments.

1.5.4 E*ndotoxin and Endotoxin Analysis*

1.5.4.1 Endotoxin Characteristics

Endotoxin is a broad category of heat-stable, lipopolysaccharide (LPS)-protein complexes in the outer membranes of Gram-negative bacteria. The term endotoxin actually describes a broad category of biomolecules, as opposed to a singular molecular entity. Wide interspecies variations can exist in the Gram-negative bacterial carbohydrate content of endotoxin, as well as intraspecies heterogeneity.[37] Endotoxin usually refers to the biologically active toxin present in the bacterial cell wall, while LPS is the chemically purified molecule with no other cell wall components present.[37] There are three distinct regions of endotoxin molecules: 1) O-specific polysaccharide; 2) core polysaccharide (outer and inner cores); and 3) Lipid A. The polysaccharide portion of the molecule represents the antigenic surface and the lipid A portion confers toxicological properties to the molecule.[105] The core polysaccharide and the lipid A of endotoxin are conserved within bacterial species, but vary in structure and composition among species and, to a greater extent, among genera.[105]

Because LPS is not a uniform molecule, analysis of endotoxin in a given sample can be tenuous; as structural heterogeneity causes variations in molecular potency.[106, 107] The work of Bang in the 1950's set the "benchmark" for today's endotoxin analysis.[108] The lysate prepared from the ameboecytes of *Limulus* blood was found to be a sensitive indicator for endotoxin,[109]

which led to the development of *Limulus* Amebocyte lysate (LAL) assays for measurement of endotoxin in aqueous samples. The LAL assay has been the most widely used assay for endotoxin analysis. The assay measures LPS potency, which is dependent on such factors as the fatty acid content of the Lipid A portion, polysaccharide content and LPS aggregational properties.[107] There can be wide variability of airborne endotoxin concentrations due to levels of contamination of the air as well as collection media, sampling[82, 110-113] and extraction methods,[110, 112] inhibition and enhancement including enhancement due to (1-3)-beta-D-glucans[114-116] and the variability inherent in the bioassays. The interpretation of endotoxin from bioassays tends to be problematic because of the variability in composition of biological material and the variations in sampling and analysis. The two basic reagents needed for LAL assays, purified endotoxin for the preparation of standard solutions and *Limulus* lysate for endotoxin detection, are subject to variations in compositions.[111] There are manufacturer dependent processes and preparations, and lot-to-lot variations, which do not allow for a common basis for comparison of LAL data. Interlaboratory comparisons on common samples, following standardized methods, still allows for wide variations in results.[82, 117-119]

1.5.4.2 Endotoxin Analysis

1.5.4.2.1 Kinetic-QCL *Limulus* Amebocyte Lysate Assay

Kinetic-QCL is a quantitative assay for the detection of Gram-negative bacterial endotoxin. Factor C is activated by endotoxin binding and Factor B is activated by Factor C. An alternate pathway, the Factor G pathway, can be activated by glucan binding. A sample is mixed with the LAL/substrate reagent, placed in an incubating plate reader, and automatically monitored (OD 405 nm) over time for the appearance of a yellow colour. Using the initial absorbance reading of each well as its own blank, the reader determines the time required for the absorbance to increase to 0.200 absorbance units and this time is considered the reaction time. The reaction time is inversely proportional to the amount of endotoxin present. In the presence of a large amount of endotoxin the reaction occurs rapidly, whereas in the presence of a smaller amount of endotoxin the reaction time is increased. The concentration of endotoxin in unknown samples is referenced to a standard curve which is prepared on the same plate as the unknown

samples. Log/log linear correlations are used to compute endotoxin concentrations in unknowns. The Kinetic-QCL assay is optimized to be linear from 0.005 EU/ml to 50.0 EU/ml.

Glass fiber filters containing the total dust samples (Paper 1) and PVC filters from each stage of the Marple sampler (Paper 2) were individually extracted in 10ml of sterile, non-pyrogenic water (LAL reagent water; BioWittaker, Walkersville, MD) in the original 50 milliliter centrifuge tube and rocked at room temperature for sixty minutes (Labquake shaker; Labindustries, Berkeley, CA). Dilutions of the supernatant fluids were analyzed by the Kinetic LAL assay. (Cambrex BioScience Walkersville Inc, Walkersville, MD). The concentrations of endotoxin in dust were reported (EU/mg) as well as converted to concentrations of endotoxin in air and reported as endotoxin units (EU)/m^3. Endotoxin samples were referenced to the RSE: EC-6 (*E Coli*:O55:B5).

1.5.4.2.2 Recombinant Factor C (rFC) Endotoxin Assay

A duplicate subsample of the dust samples from Paper 2 were analyzed utilizing a new Recombinant Factor C (rFC) endotoxin assay (Appendix 1). The samples were drawn at the same time from the same supernatant fluids as described in the LAL methods. The initial preparation for the rFC assay is carried out in exactly the same manner as the LAL assay. Samples were handled in exactly the same manner until plating. Both LAL and rFC were plated in the same manner except that the LAL was plated in a 96 well top-read plate and the rFC was plated in a 96 well bottom-read plate.

The rFC assay was created as an endotoxin specific assay. Factor C was purified and the gene for this was cloned. The activation of rFC is determined by the fluorescence generated by the enzymatic cleavage of a peptide-coumarin substrate. When activated by endotoxin binding, recombinant Factor C acts upon a fluorogenic substrate in the assay mixture to produce a fluorescent signal in proportion to the endotoxin concentration in the sample. Fluorescence was measured at time zero and after one hour incubation at 37^{o}C in a fluorescence microplate reader using excitation/emission wavelengths of 380/440nm. The difference between the one hour reading and the time zero reading were corrected for blank fluorescence. The log net

fluorescence is proportional to the log endotoxin concentration and is linear in the 0.1-10 EU/ml range. The concentration of endotoxin in unknown samples is referenced to a standard curve which is prepared on the same plate as the unknown samples. Log/log linear correlations were used to compute endotoxin concentrations in unknowns. The minimum detection of endotoxin is ~0.01 EU/ml (which is equivalent to 0.1 EU in the sample). The rFC assay has been found to detect no (1,3)- β-D-glucan activity, an improvement in specificity compared to the LAL assay.

1.5.5 *Exposure Assessment*

Concentrations measured at fixed locations (area samples) can be dramatically different from those incorporated by personal exposure assessments made in the same space.[50, 121] The risk paradigm shown in Figure 2 is often utilized to describe the concept that measurements of concentration do not necessarily accurately quantify actual exposure.[121] The sources produce emissions that result in environmental concentrations that result in an exposure. The exposure can be dependent on many influencing factors, the most notable being the time spent in proximity to the emission. The exposure produces a dose which is dependent on many uptake factors of the individual, and for respiratory exposures one of the most notable being inhalation rate. The result is an adverse health effect in the susceptible population.[121]

Figure 2: Risk Paradigm

source→emission→concentrations→exposure→dose→effect

While concentrations are produced by emissions from sources, exposures only occur if an individual is close enough to the source for a sufficient period of time to result in a significant exposure. Area monitors do not account for proximity or time relationships to the source, although they are very useful for categorizing the types and levels of exposures that could occur, as well as the maximal level that could occur at a location given a fixed proximity to a source. Personal exposure measures provide the most integrated picture of an individual exposure.[121]

Although it is dose that is the cause of the biological effect in the individual, typically the airborne concentration is what is measured, and it is the corresponding estimate of exposure

which is then used as a correlate to health effects or predictors of risk.[121] Dose is often difficult to measure directly. Most studies use exposure as a surrogate for dose and the exposure is typically the personal or area measurement of the pollutant. Individuals with the same exposure might receive a very different dose dependent on mouth breathing versus nose breathing, breathing rate, and other physiological variables. Since work environments are so variable both spatially and temporally, one time measurements or single day measurements are often not entirely reflective of potential exposure.[121] Therefore the use of measurements of airborne concentrations as a basis for estimating or comparing dose needs to be done cautiously.

A meta-analytic approach was utilized to look at the patterns of exposure variability and found that the day-to-day variation in exposures generally exceeded the variation between workers; that aggregating workers on the basis of job title and location did not necessarily yield homogeneously exposed groups; gaseous exposures were more homogenous than exposures to aerosols or dermal agents; and groups with longer monitoring periods were characterized by significantly higher levels of within-worker variability.[122]

Understanding the limitations of environmental measurement parameters assists in understanding the limitations of the dataset.

1.5.6 *Personal versus Area Environmental Monitoring*

Studies comparing personal versus stationary monitoring for particulate and other contaminants has shown mixed reviews with one indicating an inhalable sampler adequately assessed personal exposure from a static stationary mode[123] whereas another indicated higher particle mass and fungal counts in personal monitors than in stationary monitors.[124] Due to the known variability of poultry production environments and the worker time spent in direct contact with the birds both personal and area environmental measurements were undertaken simultaneously in the same barns for Paper 2. This type of sampling has been undertaken by other authors.[50]

Personal sampling provides the best indicator of a workers' exposure. It is well understood that generalizing results from personal sampling is difficult due to the known variability between and within samples. Personal sampling however, provides a better indication of the true exposures for the worker as compared to area sampling. An area sample provides an indication of the general work environment and is useful for describing the general exposures to which workers could be subjected. Generalizations to health effects can be made, although these findings would tend to be more general than findings from personal measurements and less specific to the individual. One difficulty with personal monitoring for sampling the workers from the CH and FH poultry operations for Paper 2 was getting a long enough sampling time to attain reliable environmental results. It had been established from the original poultry studies (Paper 1) that workers from FH poultry facilities were spending on average two hours per day in the poultry facility. Workers from CH facilities had more variable amounts of time spent in the facilities, depending on the number of layer birds, with a mean time of three hours spent in the barn (Paper 1, Table 2). Knowing the environmental variability of poultry operations and understanding that the fractionated dust samples (Paper 2) were going to require a longer sampling period than a total dust sample (Paper 1) in order to attain sufficient dust and endotoxin on the lower stages of the sampler, it was decided to undertake area sampling in conjunction with personal sampling for Paper 2 to assure that there would be sufficient dust and endotoxin in the lower stages of the Marple sampler for analysis.

The worker who spent the greatest length of time in the facility was asked to wear a Marple personal sampler for the duration of their work-shift for both FH and CH facilities. Because of the variability within workers and between workers in airborne contaminant exposures within an occupational setting, two area Marple samplers were also placed in the middle of the barn equal distances from the outside ends of the barn at the height of the average breathing zone (1.5 meters) and out of the reach of the birds. Area samples were collected for a four hour period. A set of samples for any one barn included a personal sample (6-stage Marple); two area samples (6 stage Marple with four hour collection), one personal ammonia and carbon dioxide sampler and two area ammonia and carbon dioxide samplers with the same sampling period as the Marple samplers.

1.5.7 *Particulate and Gas Sampling Methods*

1.5.7.1 Paper 1

Before beginning work, workers were fit with an environmental sampling backpack that measured total dust, ammonia (NH_3), carbon dioxide (CO_2), temperature and relative humidity over the work-shift. Measurements were recorded every sixty seconds over the range of 0-50 ppm ± 5% for ammonia using an electrochemical system (Biosystems Inc., Middletown, CT). Total dust and endotoxin were collected using a Sensidyne constant airflow pump (GilAir-3, Clearwater, Florida) run at 2 litres per minute with pre-weighed glass fiber filter (1.0 µm binder free, type AE, SKC Inc., Eighty Four, PA) in a closed-faced 37mm cassette. The cassette with filter was attached at the workers' breathing zone. The filter was gravimetrically analyzed for total dust (milligrams of dust/m^3 of air, mg/m^3) and with endpoint Limulus Amebocyte Lysate assay (*E. coli* O55:B5; Cambrex BioScience Walkersville Inc, Walkersville, MD) for airborne endotoxin and endotoxin concentration (endotoxin units/m^3 of air, EU/m^3 and endotoxin units/mg of dust, EU/mg).

1.5.7.2 Paper 2

A Marple 6-stage sampler was utilized for fractionating the dust and endotoxin air samples (Thermo Electron Corp., Waltham, MA). The Marple sampler contained 6 stages to represent cut-points of 0.52, 0.93, 1.55, 3.50, 6.0 and 9.8µm. Polyvinyl chloride (PVC) 5 micron, 34mm filters with radial slits was used as the filter media for collection of dust and endotoxin in the Marple samplers (Thermo Electron Corp., Waltham, MA). The Marple sampler was connected to a SKC constant airflow pump (Universal 224-PCXR4, Eighty Four, PA) and run at 2 liters per minute over the sampling period. For analysis purposes stages 3 and 4 of the Marple sampler (>6µm) were chosen to represent the non-respirable fraction and stages 5-final (6.0-<0.5 µm) were chosen to represent the respirable fraction (Figure 4). Laboratory and field blanks were included as components of the study. Filters were individually analyzed for dust [milligrams of dust (mg) and milligrams of dust per m^3 of air (mg/m^3)] (MX5 microbalance, Mettler-Toledo, Greifensee, Switzerland) and with Kinetic-QCL *Limulus* Amebocyte Lysate assay (*E. coli* O55:B5; Cambrex BioScience Walkersville Inc, Walkersville, MD) for airborne

endotoxin concentration [endotoxin units per m^3 of air (EU/m^3) and endotoxin units per mg of dust (EU/mg)].

Temperature and relative humidity were measured twice during the 4 hour sampling period utilizing a VelociCalc (8347A-M-G, TSI Inc., Shoreview, MN). Temperature and relative humidity can impact the levels of endotoxin with higher average temperatures yielding higher levels of endotoxin and decreases in relative humidity associated with increased endotoxin levels.[125]

Measurements for NH_3 and CO_2 were collected with passive colorimetric gas diffusion tubes (NH_3 2.5-1500 ppm-hours, CO_2 0.13-30 vol%; Gastec, Kanagawa, Japan).

With pre-weighed filters in place, the Marple was calibrated pre and post measurement utilizing an electronic calibrator (Bios DryCal DC-Lite, Butler, NJ). The Marple sampler was attached by tygon tubing to the air sampling pump and calibrated inline to 2 liters/minute (lpm). During personal sampling, the Marple was hung at the worker's breathing zone. The breathing zone, as defined by the Environmental Protection Agency of the United States, is the area of air in which an organism inhales. The sampling pump for the personal sample was hung from a belt at the worker's waist. The worker carried the pump, the Marple, and ammonia and a carbon dioxide diffusion tubes over the entire work-shift. The area samples were set-up in the exact same manner as the personal samples with the exception that the sampling units were hung in the middle of the barn equal distance from the outside ends of the barn at the height of the average breathing zone (1.5 meters) and out of the reach of the birds. The sampling start and stop times were recorded on the field flow sheet.

The PVC filters were desiccated for a minimum of 24 hours pre- and post weighing. Pre and post filter weights were recorded on the lab flow sheet. The post-weighed filters from each sampler were placed in 50 milliliter centrifuge tubes, labeled and refrigerated at 4^oC until endotoxin analysis was undertaken.

1.5.8 *Occupational Exposure Limits for Endotoxin and Organic Dust*

Occupational exposure limits (OELs) or threshold limit values (TLVs) for endotoxin are tenuous due to a lack of consensus on standardized procedures for the sampling and quantitative analysis of endotoxin.[111] Numerous studies have recommended exposure levels. Rylander calculated an endotoxin threshold of 33ng/m^3,[126] Donham et al recommended an exposure limit in poultry barns of 614 EU/m^3 for total endotoxin and 7.15 EU/m^3 for respirable endotoxin.[12] In 1998, the Dutch Expert Committee on Occupational standards proposed a health-based occupational exposure limit of 50 endotoxin units/m^3 or 5ng/m^3 based on personal inhalable dust measured as an 8-hour time weighted average. The National Health Council of the Netherlands (DECOS) has proposed a health-based recommended threshold value for endotoxin of 50 EU/m^3.[127] Relative exposure limits for endotoxin have been discussed in the United States, which would incorporate a comparison of the measured endotoxin levels to a background measure with proposed action levels which differ dependent on presence or absence of symptoms.[128]

That there are to date no standards for endotoxin exposures indicates the complexity in measuring, analyzing and understanding this agent in different work environments and therefore underscoring the need to better understand the environments in which endotoxin is thought to be a primary agent in worker health effects.

1.5.9 *Respiratory Protection*

Respiratory protection such as an N95 respirator is the most often utilized personal protection for poultry workers. N95 filtering facepiece respirators have the filtration efficiency of at least 95% for the particle size 0.3 μm. With the aerodynamic sizes of most bacteria and fungal spores between 0.7 and 10 μm[87], the filtration efficiency by an N95 respirator should be an effective barrier. A study of the workplace protection factors (WPF) for dust and microorganisms in agricultural farms showed that WPFs increased with increasing particle size and ranged from 21 for 0.7-1 μm particles up to 270 for 5-10 μm particles.[129] The WPFs differed by type of contaminant and were significantly greater for total culturable fungi (WPF 35) than

for culturable bacteria (WPF 9).[129] The WPFs for bioaerosols were found more frequently below 10, which is the recommended assigned protection factor (APF) for an N95 respirator.[129] More than 50% of the WPFs for microorganisms (mean aerodynamic diameter <5um) were less than the proposed APF of 10, indicating that these N95 respirators may not adequately protect against microorganisms.[129]

Understanding, assessing and controlling for workplace exposures are primary functions in industrial hygiene. Respiratory protection is one of the controls by which poultry workers protect themselves from workplace exposures. It appears microorganisms may not be effectively protected against by an N95 respirator. Endotoxin, an associate of microorganisms, may have the capacity to penetrate an N95 respirator. Fractioning the particulate in the poultry barn environment and analyzing the endotoxin concentration of the fractionated samples will assist in understanding the potential for insult on a worker protected by an N95 respirator.

1.6 WORKER AND BARN POPULATION CHARACTERISTICS

1.6.1 Worker and Barn Populations

The worker population for Paper 1 was taken from the same population base as the subjects from an initial cross-sectional cohort of 303 poultry producers.[14] Forty six poultry workers in the provinces of Saskatchewan and Manitoba were studied during the winters of 1998-2000 and 74 workers were studied during the winters of 2002-2004, for a total of 120 workers studied (Table 3).

There were nine workers from poultry operations who were not included in the analysis because their operations included mixed methods of poultry housing. The floor-housed poultry operations studied were raising birds for human consumption. The cage-housed poultry operations studied were producing table eggs for human consumption.

Table 3: Poultry Operation Study Population from Paper 1

	Manitoba	Saskatchewan	Total
Floor-housed			
1998-2000	15	12	27
2000-2004	50	3	53
Total	**65**	**15**	**80**
Cage-housed			
1998-2000	16	2	18
2000-2004	7	6	13
Total	**23**	**8**	**31**
Mixed housing			
1998-2000	1	0	1
2000-2004	4	4	8
Total	**5**	**4**	**9**
Overall Total	**93**	**27**	**120**

Workers were classified according to the type of poultry housing in which they worked:

Floor-housed (FH): *Broiler/Breeder Operations;*
Broiler Operations;
Turkey Operations
Cage-housed (CH): *Egg/Pullet Operations*
Mixed: *A combination of floor and cage-housed operations*

Definitions:

Breeder - A bird that is utilized to produce hatching eggs for producing offspring.

Broiler - Chicken, sometimes called fryers, reared primarily for meat production. Age to market weight is typically 6 to 8 weeks (2.3 to 3.6 kilograms).

Hatchery - Eggs are typically collected from breeder farms, taken to a hatchery and stored from 0 to 10 days prior to being set in an incubator. These eggs will be stored at temperatures between 13-20° C, depending on when they are to be incubated. When the eggs are placed in incubators, embryonic development begins. Different species of birds require different incubation times. Chickens hatch in 21 days while turkeys and ducks need 28 days. The hatchlings (chicks, poults, or ducklings) are processed (vaccinated, gender sorted, and/or other procedures) then transported to commercial grow-out facilities.

Pullet - A laying hen before it lays its first egg.

For Paper 2, participation was by invitation and sites were chosen from the available Saskatchewan population (Table 4).

Table 4: Registered Chicken and Egg Producers in Saskatchewan and Total Number Studied for Paper 2

		Total Studied Paper 2
Caged Operations		
Layers	64	15
Floor Operations		
Broiler	92	15
Total Operations	156	

The sites were chosen to most closely represent the most typical CH and FH operations which were studied as part of Paper 1. Of the 64 CH poultry operations in Saskatchewan, 23% took part in the study while 16% of the FH operations (of 92) took part (Table 4). Operations which took part closely represented the Saskatchewan/Manitoba poultry industry (flock size, manure management system, age of barn, poultry breed) as identified from Paper 1. Sites were not chosen if producers had more than one type of poultry operation (both cage and floor-housed poultry operations). For Paper 2, one barn and one worker on each site were studied during the winters of 2005 and 2006. Sites were all located in the province of Saskatchewan.

1.6.2 *Worker Respiratory Health Assessments*

1.6.2.1 Questionnaires

Before the first measurement for each of the participating barns, a general questionnaire regarding the facilities was completed by the barn manager under the direction of the research technician. The questionnaire included a building floor plan and information on number of rooms and room areas; number and placement of fans; type, placement and number of feeders; type, placement and number of watering stations; type of feed; type of litter; flooring type; manure management system; type and number of cages, and other barn related information. A blank general questionnaire is included as appendix 3.

A previously administered and piloted general health questionnaire was administered to each worker prior to the beginning of the work-shift for both Papers 1 and 2. General respiratory health questions included current and chronic respiratory symptoms that were modified from the American Thoracic Society standardized questionnaire. General questions included occupational

history; work related respiratory symptoms, principal health conditions, current medication use and smoking history. A blank general questionnaire is included as appendix 3.

1.6.2.2 Pulmonary Function

For Paper 1, pulmonary function indices of Forced Vital Capacity (FVC), Forced Expired Volume in one second (FEV_1), ratio of forced expiratory volume in one second to forced vital capacity (FEV_1/FVC), and maximal mid-expiratory flow rate (FEF_{25-75}) were measured using a SensorMedics volume displacement spirometer (SensorMedics, Anaheim, CA). Pulmonary function tests were performed as an exhaled maneuver with the testing procedures conducted according to American Thoracic Society guidelines. Pulmonary function tests and an acute respiratory symptom questionnaire were administered before beginning work and repeated again at the end of the work-shift (Appendix 3). Across-shift differences were calculated by subtracting the post shift measurement from the pre-shift measurement and dividing by the pre-shift measurement. Results are presented by volume, volume/time or percent change.

For Paper 2, pulmonary function indices of peak expiratory flow (PEF) and forced expired volume in one second (FEV_1) were measured using the Piko-1 electronic peak flow/FEV_1 meter (Ferraris, Louisville, CO). Pulmonary function tests were performed as an exhaled maneuver with the testing procedures conducted according to American Thoracic Society guidelines. Pulmonary function tests and an acute respiratory symptom questionnaire were administered before beginning work and repeated again at the end of the work-shift (Appendix 3). Across-shift differences were calculated by subtracting the post shift measurement from the pre-shift measurement and dividing by the pre-shift measurement. Results are given as the volume, volume/time or as the percent change. The worker from each barn that carried the personal sampler had an acute symptom questionnaire and pulmonary function measurements performed.

Results of two small pilot studies looking at the comparison of thePiko-1 meter to the dry rolling seal spirometer for FEV_1 and peak expiratory flow (PEF) can be found in Appendix 2. We chose to use the Piko-1 electronic flow meter for Paper 2 as this device is very small and

portable. The dry rolling seal spirometer is very large and is computer driven so there is extensive large bulky equipment to transport between farms. Additionally, poultry farms were becoming increasingly concerned about biosecurity and any equipment which was entering a facility needed to be thoroughly disinfected between barn visits. Ensuring good disinfection of the dry rolling seal spirometer between barn visits was not possible. The results indicate that the dry rolling seal spirometer had significantly higher results than thePiko-1 electronic flow meters. The variability in the results is wide enough to be considered of clinical significance if the Piko-1 and dry rolling seal spirometer pulmonary results were to be directly compared. We are not directly comparing the pulmonary results from Paper 1 to Paper 2. Due to the small population sample size for Paper 2, the pulmonary results were utilized to indicate that the same trends that were observed in Paper 1 were also observed in Paper 2. Pulmonary observations from Paper 2 were not utilized in predicting outcomes related to environmental exposures nor were they directly compared to the pulmonary results obtained from Paper 1.

1.7 **POULTRY OPERATION CHARACTERISTICS**

Due to the diversity of poultry production in general, a number of variables were examined prior to sampling in an attempt to sample during what we believed to be less environmentally variable time frames in the growth cycles of the cage (CH) and floor-housed (FH) poultry. Timelines for production, previous environmental data related to growth cycles, growth charts of the common poultry breeds, and the average production times for the CH and FH poultry operations in Western Canada (Manitoba/Saskatchewan) were reviewed.

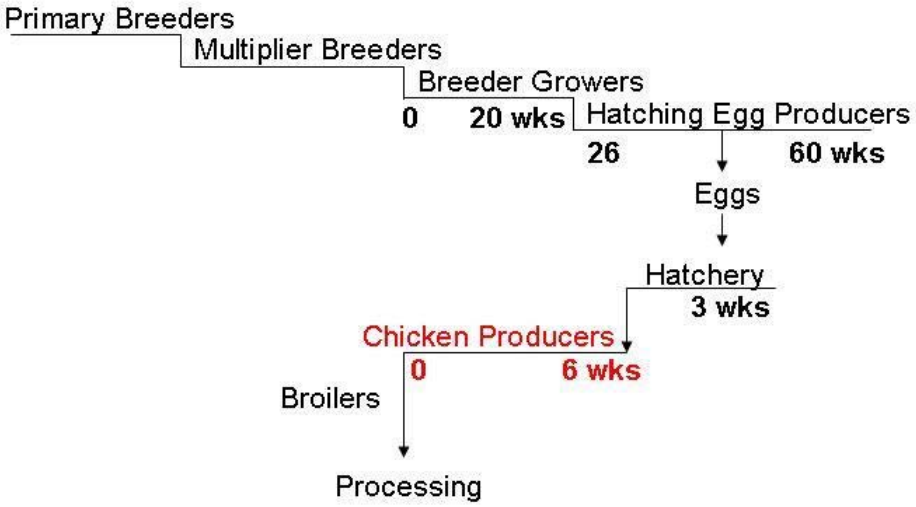
1.7.1 *Common Production Timelines for Cage and Floor Housed Poultry*

The common production timelines for CH and FH poultry are shown in Figures 3 and 4.[1] The timeline for chicken production in Saskatchewan is shown in Figure 3.[1] Highlighted in red is the portion of the production timeline which corresponds to the time in the production cycle for which barns were chosen for the FH poultry operations for Paper 1 and Paper 2. In general

the timeline indicates a maximum six week growth period for broiler chickens. In Saskatchewan, the cycle is slightly shorter with average growth time closer to five weeks.

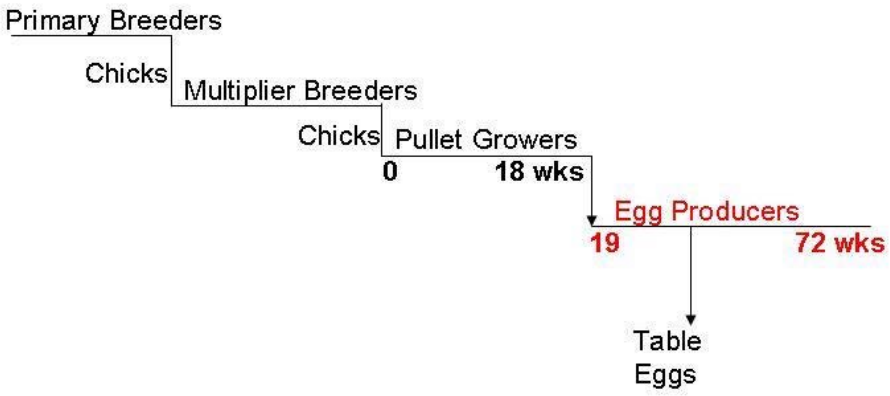
The egg production timeline is shown in Figure 4.[1] Highlighted in red is the portion of the timeline which corresponds to the time in the production cycle for which barns were chosen for the CH poultry operations for Paper 1 and Paper 2. The laying hen begins laying eggs at approximately 18 weeks of age and by the end of her first year, she may have produced upwards of 200 eggs. The hen reaches peak egg production (95 + %) within 4 to 6 weeks after she begins to lay eggs.

Figure 3: Chicken Production Timeline (Floor-Housed)



Primary breeders produce genetically improved multiplier breeders, which in turn produce approximately 140 eggs during their laying period. Hatcheries incubate these fertilized chicken eggs for 3 weeks producing chicks that are placed on farms of chicken producers for approximately 6 weeks before processing.
Reproduced from National Farm Products Council Report 2006 p.21

Figure 4: Egg Production Timeline (Cage-housed)



Primary breeders maintain and expand pure bloodlines and develop cross-bred bloodlines. The eggs they produce are hatched into multiplier breeders. Multiplier breeders produce eggs which, when hatched, are grown to the age of 18 weeks by puller growers. At 19 weeks, the pullets are placed in egg producers' barns where they begin to produce eggs for the retail and processed markets.
Reproduced from National Farm Products Council Report 2006 p.41

1.7.2 *Growth Cycle Relationships to Total Dust and Endotoxin in a FH Poultry Operation*

The data given in Figures 5 and 6 assisted in understanding the potential trends for environmental variables over the growth cycles of the birds in cage and floor-housed poultry operations. A small pilot project was undertaken in the University of Saskatchewan FH poultry production barn in the winter of 1999 to get a general idea of the variability of dust and endotoxin over a production cycle in this barn. Figure 5 indicates the variability in the total dust concentration over a 39 week growth cycle. There is a very strong positive linear trend (r^2=0.97) for total dust concentration to increase over the production cycle. Figure 6 indicates there is a less strong trend for endotoxin concentration. Endotoxin concentration appears to start high at the beginning of the production cycle and to slightly increase over the entire growth cycle. Data is included for 39 days of growth which is a slightly longer growth period than the average Saskatchewan industry growth period. Thirty three to thirty five days growth was more typical of the average Saskatchewan period during our studies.

Figure 5: Average Dust (mg/m3) in a FH Poultry Barn over a 39 Week Growth

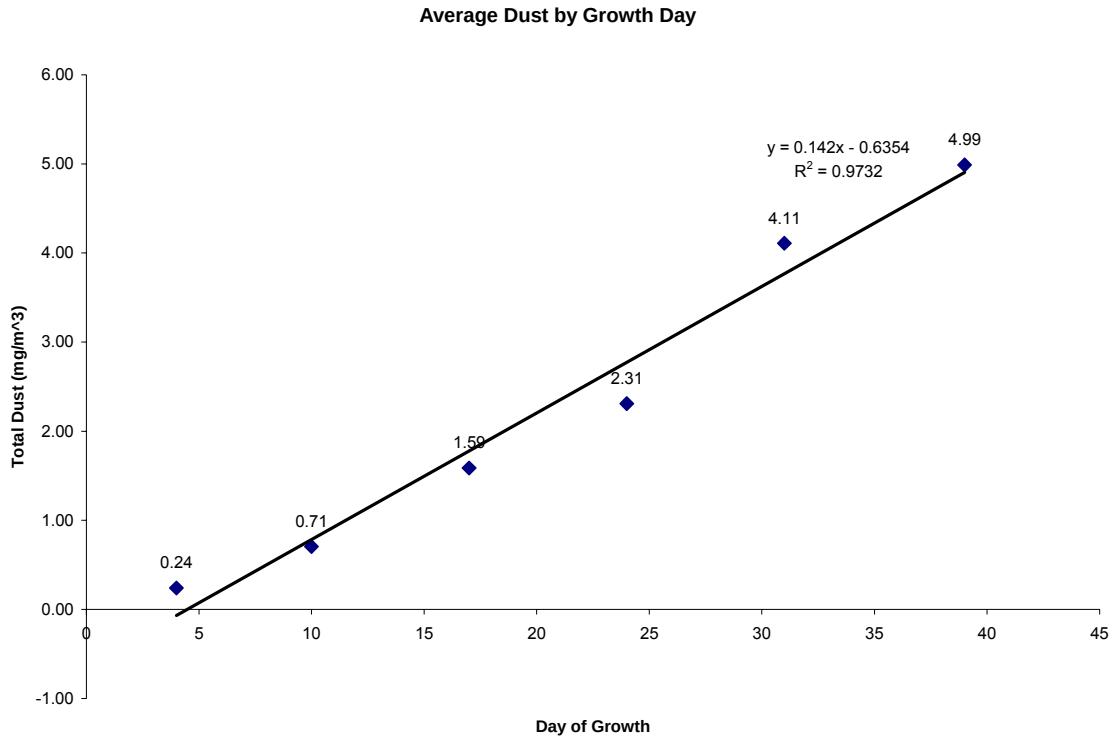
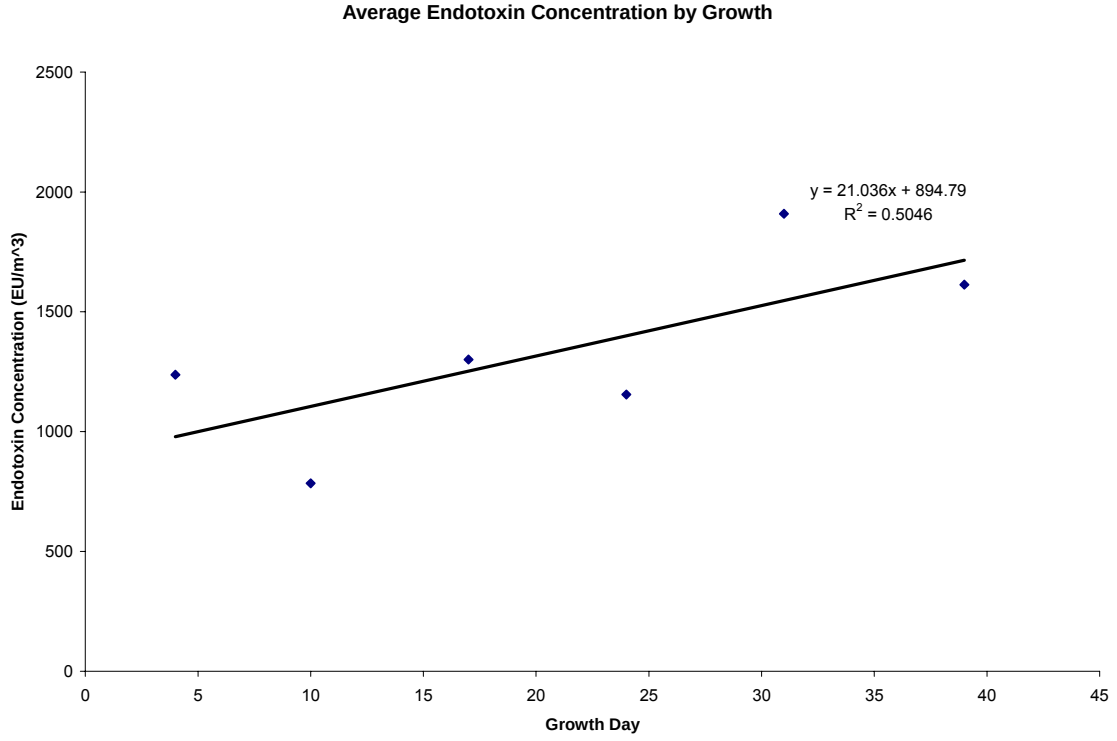


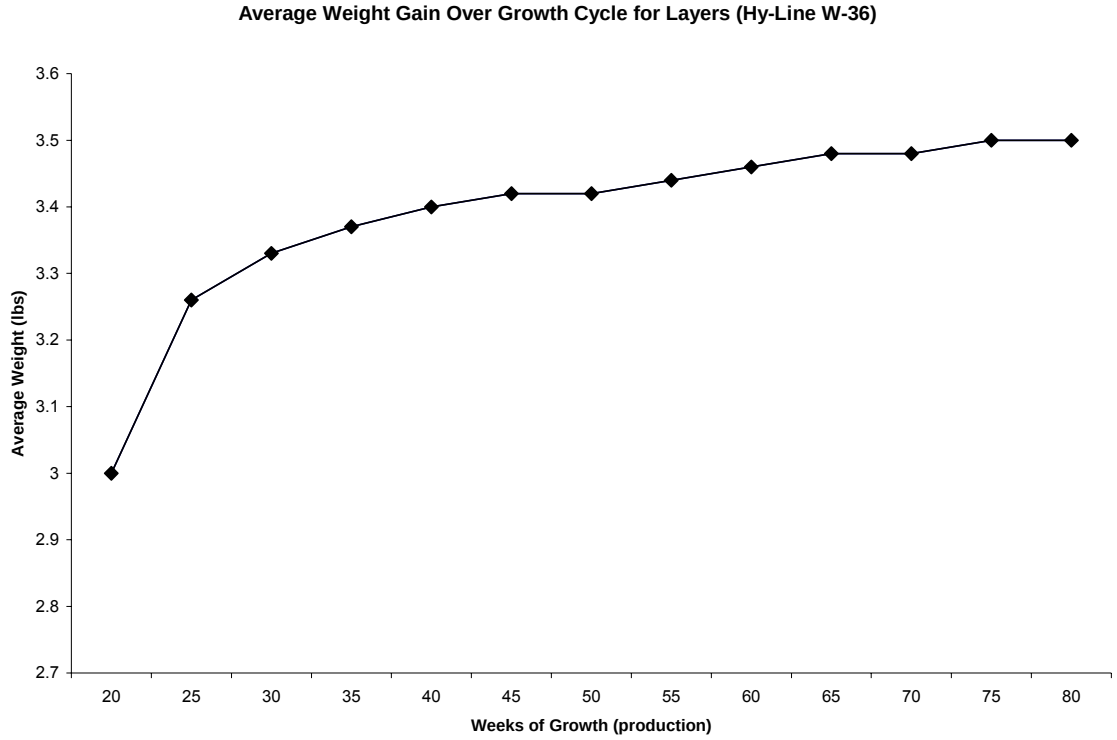
Figure 6: Average Endotoxin Concentration (EU/m3) in a FH Poultry Barn over a 39 Week Growth Cycle



1.7.3 *Average Growth Rates for Common Poultry Breeds*

From Paper 1, the most common poultry breeds for the CH and FH operations were identified. The most common breed in the CH operations was the Hy-line variety. Thirteen of the fifteen barns studied for Paper 2 had Hy-line variety as the sole poultry variety in their layer operation. Two of the operations studied had Hy-line mixed with Bovan variety or had only Bovan variety of poultry. To better understand the rate of growth of the layers over the 72 week growth cycle, body weight charts for the Hy-line variety were assessed to determine if/when there may be stable or accelerated periods of growth during the cycle. Sampling for Paper 2 was undertaken during what was considered the most stable periods in the growth cycle. Figure 7 outlines the average body weight over the growth cycle for Hy-line W-36 layers.[130] Figure 7 indicates a very slow growth rate for the Hy-line variety between 35-80 weeks. Therefore, in terms of growth, the layers gain only about 0.06 kgms from 32-70 week of age, so there would be little change in stocking density due to bird weight between these weeks. Therefore it was felt that layer could be studied almost anywhere between 32-70 weeks.

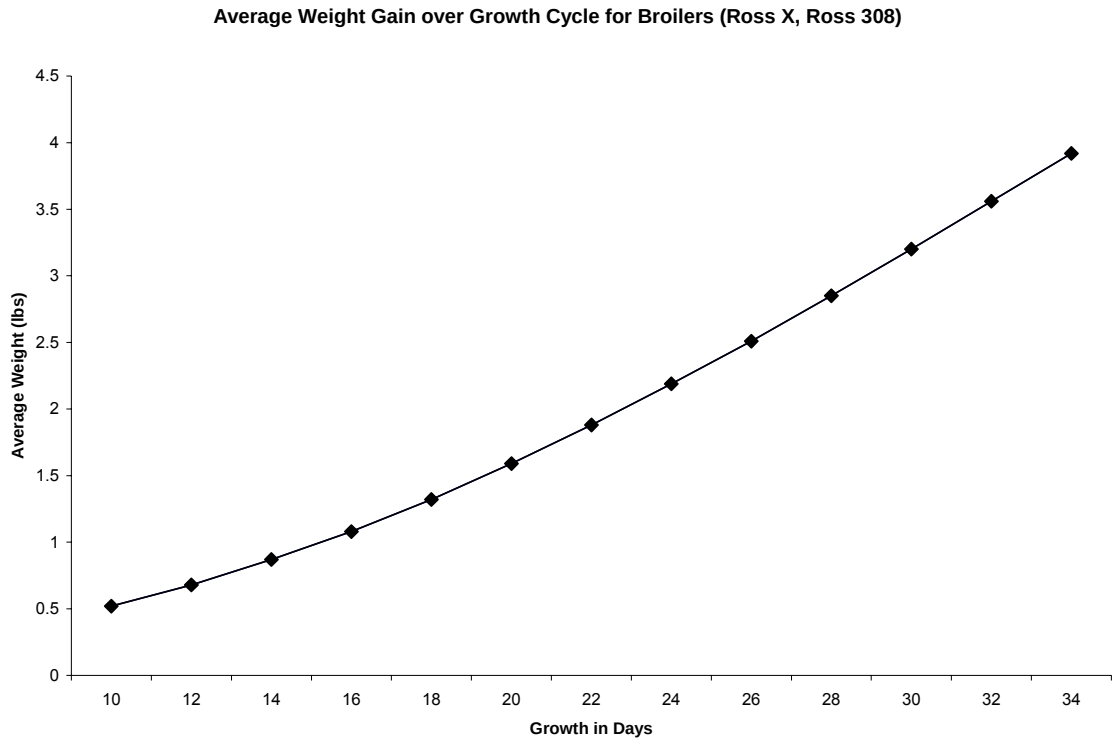
Figure 7: Average Body Weight over the Growth Cycle for Layers



The most common poultry breed for the FH operations was the Ross variety. Thirteen of the fifteen barns studied for Paper 2 had Ross variety as the sole poultry variety in their broiler operation. Two of the operations studied had Ross mixed with Cobb variety of broilers. To better understand the rate of growth of the broilers over the 5-6 week growth cycle, growth charts for the Ross variety were assessed to determine if/when there may be stable or accelerated periods of growth during the cycle. Sampling for Paper 2 was undertaken during the end of the production cycle as the birds continued to grow over the cycle. Figure 8 outlines the average weight gain over the growth cycle for Ross x Ross 308 broilers.[131]

For the Ross varieties in the FH operations there is continuous growth and weight gain over the entire cycle. As the birds grow the bird density (pounds/metre^2) is going to increase over the growth cycle. This increased density may have a relationship to the environmental changes in total dust observed in Figure 5.

Figure 8: Average Weight Gain over the Growth Cycle for Broilers



1.7.4 *Determination of Sample Timing*

The information from the small pilot project on dust and endotoxin along with the knowledge of the average production cycle together with the growth charts for the common poultry breeds assisted in determining when sampling would occur in the CH and FH operations for Paper 2.

It was determined that the CH operations would be studied any time during the production cycle. This was not decided because of lack of variability, as we were uncertain of how the environment would vary over the growth cycle. The modest weight changes over the growth cycle coupled with the length of the housing (~ 1year), and the variability in timing of placement of birds in the different facilities in Saskatchewan led to the decision to sample the CH operations at anytime during the cycle. Of the 15 barns studied, the minimum age of birds studied was 22 weeks and the maximum age was 72 weeks (mean 47.0 weeks ± SD 15.43 weeks).

Due to the variability in the total dust levels from the pilot study and the continued growth of the birds over the growth cycle for the FH poultry operations, it was decided that the FH poultry operations would be studied between days 21-25 of growth.

1.8 **SUMMARY**

The background section described information that was important in the development of the hypotheses, the methods and the conclusions for the Papers and Appendixes presented in the dissertation. The background described that workers in poultry operations are subjected to endotoxin, dust, ammonia, fungi and other microbes at levels, which in a large number of workers, induce respiratory symptoms of cough, phlegm, wheeze, shortness of breath and decreases in FEV_1, often referred to as asthma-like-syndrome. It appears that there may be some individual susceptibility associated with reactions to this type of work environment, and endotoxin appears to be a major component related to the respiratory reaction experienced in

these workers. Additionally, workers who work in CH poultry operations report greater cough and phlegm in response to their work environment as compared to workers who work in FH poultry operations. A comparative review of the literature indicates that there may be differences in the environmental contaminant levels in these two types of poultry operations. FH poultry operations tend to have higher levels of dust and lower levels of endotoxin as compared to CH operations. There were no studies that directly compared the dust and endotoxin levels between CH and FH poultry environments. Additionally, there was only one study that looked at the health effects of workers exposed to the two types of poultry barn environments.

Paper 2 of this dissertation undertook to understand if there were differences in the particulate fractions from CH and FH poultry operations. An overview of particle deposition and particle fractionation theory were provided as a background to understanding the methods and conclusions for Papers 2 and 3. The theory of particle size selective sampling provided the basis for understanding the Marple sampler utilized for Paper 2. Additionally exposure assessment theory and methods were described to provide insight into the decisions for measuring both personal and area environmental levels and to provide a background to the risk paradigm for exposed workers. How to protect a worker from exposures was also reviewed as controlling worker exposures is a key element of industrial hygiene principles.

Worker population characteristics were described to provide a better understanding of how populations were determined for Papers 1 and 2. Methods for attaining worker measurements and data were reviewed. Similarly poultry operation characteristics were provided as background information to assist in explaining how poultry operations were selected and how the timing of exposure sampling for CH and FH poultry operations was determined for Paper 2.

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2. PAPER ONE: TOTAL DUST AND ENDOTOXIN IN POULTRY OPERATIONS: COMPARISON BETWEEN CAGE AND FLOOR HOUSING AND RESPIRATORY HEALTH EFFECTS IN WORKERS

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Total Dust and Endotoxin in Poultry Operations: Comparison Between Cage and Floor Housing and Respiratory Health Effects in Workers

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Running Title: Total dust and endotoxin in poultry operations

2.1 ABSTRACT

Objective: To assess respiratory outcomes and exposure levels of workers exposed to cage and floor-housed poultry.

Methods: Airborne contaminant levels in 120 poultry operations in western Canada were evaluated and companion respiratory symptoms and lung function of workers were conducted.

Results: Those working with floor-housed poultry had significantly greater personal exposures to total dust and ammonia.

Workers from cage-housed poultry operations reported greater frequency of current and chronic symptoms overall and significantly greater current and chronic phlegm (39% versus 18%, p=0.02 and 40% versus 11%, p=0.001 respectively) as compared to workers from floor-housed poultry operations.

Endotoxin load (EU/mg) was a significant predictor (p=0.05) of chronic phlegm for poultry workers.

Conclusions: Greater endotoxin load in the presence of significantly lower total dust, in conjunction with greater respiratory symptoms in workers from cage-housed poultry operations, as compared to workers from floor-housed poultry operations, appears to indicate that differences in exposures may impact respiratory outcomes.

2.2 INTRODUCTION

Individuals engaged in poultry production are exposed to varying concentrations of airborne contaminants including organic dusts, gases, endotoxin, fungi, bacteria and bacterial constituents. Long-term exposure to this environment may put the worker at risk for developing respiratory dysfunction. Simpson et al.[1] studied workers in nine different industries and demonstrated that the highest prevalence of work-related lower respiratory tract symptoms (38%), upper respiratory tract symptoms (45%), and chronic bronchitis (15%) were present

among poultry handlers, and personal exposure to dust or endotoxin were predictive of symptoms. A European study indicated that 24% of poultry farmers had work-related symptoms (wheezing, breathlessness and cough without phlegm)[2] and compared to swine farmers had lower baseline lung function.[3] In a study conducted in the U.S., 53% of workers who had worked greater than 10 years in turkey operations had cough, 40% had phlegm and 27% wheezed during the winter season.[4]

Although poultry dust is a combination of feed and fecal particles, feathers, skin, fungal constituents, bacteria, viruses, and litter particles;[5] dust, endotoxin and ammonia are the most frequently reported environmental contaminants in poultry operations and also the contaminants most frequently associated with respiratory health effects experienced by workers, The aerobic bacteria common in poultry confinement operations are *Bacillus, Micrococcus, Proteus, Pseudomonas, Staphylococcus spp*, and *Escherichia coli,* while the most common anaerobic bacteria was *Clostridia,* and the highest fungi airborne isolates are either Aspergillus or Penicillium with changes in the levels of bacteria and fungi occurring with pH increases in the litter.[6] Endotoxin are lipopolysaccharide containing fragments of the cell wall of Gram-negative bacteria and are often reported as levels in relation to the measured dust levels. Endotoxin may be primarily responsible for the respiratory health effects experienced by workers in livestock confinement operations.[7-11]

There are different types of poultry operations including housing birds in cages and housing birds on litter on the floor. In the poultry industry, type of production, i.e. floor-housed versus cage-housed birds, may influence the levels of various environmental contaminants. In particular, total and inhalable dust levels have consistently been higher in poultry operations in which birds are housed on the floor compared to operations in which birds are housed in cages. For floor-housed operations in the U.S, geometric mean inhalable dust levels were 24 mg/m^3 [12] and in Iran 21 mg/m^3.[13] Total dust measurements in floor facilities in Europe ranged from 8-9 mg/m^3 inhalable dust.[14] In Finland, floor levels ranged from 2-9 mg/m^3.[15] In the U.S. total dust levels in floor-housed bird operations were 9 mg/m^3 [16]and in turkey barns in the United States levels ranged from 7-10 mg/m^3.[17] In facilities where birds are housed in cages total dust levels have typically been considerably lower than those from floor-housed facilities with levels in

Europe ranging from 1-4 mg/m^3.[18,19] In a Swedish study, caged layers had much lower total dust levels compared to birds raised on litter on the floor (2-7 mg/m^3 and 12-17 mg/m^3 respectively).[20] In the United Kingdom respirable and inhalable dust concentrations were significantly higher in broiler operations (floor-housed) as compared to cage operations.[21] In a study from Canada which looked at particles less than 5 μm in diameter, the opposite was true, facilities which housed birds in cages had higher levels (40 particles/ml)[22] than did facilities which housed birds on the floor (7 particles/ml and 27 particles/ml).[23,24]

Endotoxin levels have been shown to be similar or higher in operations housing birds in cages as compared to the floor-housed poultry operations. Inhalable endotoxin levels in floor-housed U.S. broiler grower operations have been measured at 20-60 ng/m^3;[16]and at a geometric mean of 210 ng/m^3;[12]and between 1440-16,512 EU/m^3 respirable endotoxin in turkey production in the United States in winter.[17] Endotoxin levels for cage-housed operations have typically been higher than that of floor-housed operations at 130-500 ng/m^3,[18] and a United Kingdom study indicated similar inhalable endotoxin levels but higher respirable endotoxin fractions in cage-housed operations as compared to the floor-housed operations.[21]

For poultry workers in general, exposure to the work environment appears to relate to respiratory health effects, and significant dose-response relationships for pulmonary function decrements have been shown.[10] The difference in respiratory responses based on the type of poultry operation and related work exposures are not well understood. In the study reported here-in, differences in total dust, airborne endotoxin (EU/m^3), endotoxin load (EU/mg), and respiratory symptoms between workers from cage-housed and floor-housed poultry production operations in Western Canada, were evaluated.

2.3 MATERIALS AND METHODS

2.3.1 *Study Population*

An initial cross sectional study was conducted on 303 poultry workers during the winters of 1998-1999 involving a respiratory health questionnaire and pulmonary function tests in which

subject recruitment has been described.[25] During data collection for the cross-sectional study, workers were asked if they would be willing to have their poultry barn environment measured and have lung function tests conducted over their work-shift. From the cross-sectional cohort, 74 poultry workers in the provinces of Saskatchewan and Alberta were studied during the winters of 1998-2000 and 46 workers from the provinces of Saskatchewan and Manitoba were studied during the winters of 2002-2004, for a total of 120 workers studied from the original 303 workers in the cross-sectional cohort. There were nine workers from poultry operations who were not included in the analysis because their operations included mixed methods of poultry housing.

Workers were classified according to the type of poultry housing in which they worked:

Floor-housed: *Broiler/Breeder Operations;*
Broiler/Roaster Operations;
Turkey Operations

Cage-housed: *Egg/Pullet Operations*

Mixed: *A combination of floor and cage-housed operations*

The study was approved by the ethics committees of the Universities of Saskatchewan, Manitoba and Alberta and informed consent was received from participants prior to data collection.

2.3.2 *Environmental Measures*

Before beginning work, workers were fit with an environmental sampling backpack that measured total dust, ammonia (NH_3), carbon dioxide (CO_2), temperature and relative humidity over the work-shift.[26] Measurements were recorded every sixty seconds over the range of 0-50 ppm ± 5% for ammonia using an electrochemical system (Biosystems Inc., Middletown, CT). Total dust and endotoxin were collected using a Sensidyne constant airflow pump (GilAir-3, Clearwater, Florida) run at 2 litres per minute with pre-weighed glass fiber filter (1.0 µm binder free, type AE, SKC Inc., Eighty Four, PA) in a closed-faced 37mm cassette. The cassette with filter was attached at the workers' breathing zone. The filter was gravimetrically analyzed for

total dust (milligrams of dust/m^3 of air, mg/m^3) and with endpoint Limulus Amebocyte Lysate assay (*E. coli* O55:B5; Cambrex BioScience Walkersville Inc, Walkersville, MD) for airborne endotoxin concentration and endotoxin load (endotoxin units/m^3 of air, EU/m^3 and endotoxin units/mg of dust, EU/mg).

2.3.3 *Questionnaires and pulmonary function*

A previously administered and piloted general health questionnaire was administered to each worker prior to the beginning of the work-shift. General respiratory health questions including current and chronic respiratory symptoms were modified from the American Thoracic Society standardized questionnaire.[27] General questions included an overview of the poultry operation, personal occupational history, work related respiratory symptoms, principal health conditions, current medication use and smoking history. Pulmonary function tests and an acute respiratory symptom questionnaire were administered before beginning work and repeated again at the end of the work-shift. Across-shift differences were calculated by subtracting the post shift measurement from the pre-shift measurement and dividing by the pre-shift measurement.

2.3.4 *Statistical Analysis*

Analyses were completed using SPSS version 13. Arithmetic means and standard error or standard deviation were used to describe continuous variables, including age, years worked in the poultry barn, time spent in the barn, height, weight, total dust, endotoxin, carbon dioxide and ammonia. Categorical variables, including respiratory symptoms, gender and smoking status were described using frequencies and percentages. Data in tables and figures are displayed in the original scale of measurement. However, because the environmental variables (total dust, airborne endotoxin concentration EU/m^3), endotoxin load (EU/mg) and ammonia) were not normally distributed, logarithmic transformations ($\log^e$) were applied to the environmental variables, which normalized the data, prior to analyses. The differences in the means of continuous variables between the study groups were tested using one-way analyses of variance and t-tests. Multivariate logistic regression analyses were used to examine the association between current and chronic respiratory symptoms and environmental variables after adjusting

for age, gender, smoking status, number of years worked in the poultry barn, poultry housing method, and worker time spent in the barn. Due to co-linearity, individual logistic regression models were fit for each of the environmental variables.

2.4 RESULTS

Table 1 indicates the number of workers studied from each of the types of operations for the original cohort study. The workers studied for this paper were drawn from the registered lists of all poultry producers for Manitoba and Saskatchewan, some of whom may have been studied in the original cohort. The restudied workers for this paper included floor-housed operations which comprised 67% (n=80), cage-housed operations 26% (n=31), and mixed operations 7% (n=9) of the study population. Mixed operations were not included in the analysis of effects due to differences in work environments and exposures.

Figure 1 outlines the environmental results for the cage and floor-housed poultry operations. After log transforming the data, personal total dust exposures in floor-housed operations were significantly ($p=0.01$) greater than were the personal total dust exposures in the cage-housed poultry operations. Similarly, ammonia levels in the floor-housed operations were significantly greater than in the cage-housed poultry operations ($p=0.02$). Personal airborne endotoxin concentration (EU/m^3) and endotoxin load per milligram of dust (EU/mg) were not significantly different between the cage-housed and floor-housed poultry operations, although there was a trend towards higher levels of endotoxin load (EU/mg) in cage-housed poultry operations. Furthermore, when looking at the high and low endotoxin load (EU/mg) by caged and floor-housed poultry operations 56% of workers from the cage-housed operations were categorized in the high endotoxin load (> 578 GM EU/mg) compared to only 48% of workers from the floor-housed operations, although this difference in proportions was not statistically significant.

As indicated in Table 2, workers from cage-housed poultry facilities were, on average, significantly shorter ($p=0.02$) and spent more time in the poultry barns ($p=0.001$) as compared to the floor-housed poultry barn workers. There were no differences in age, smoking status or

across-shift values for lung function tests between workers from cage and floor-housed poultry operations.

There were significant differences in current phlegm (p=0.02) and chronic phlegm (p=0.001) between workers from floor and cage-housed poultry operations (Table 2). Both current and chronic phlegm were reported more frequently in workers from cage-housed poultry operations compared to workers from floor-housed poultry operations (current: 39% vs. 18%; chronic: 40% vs. 11%, respectively). Although there were some large differences in the prevalence of other respiratory symptoms between groups, with workers from cage-housed operations typically experiencing greater symptoms, there were no statistically significant differences between the two groups for current cough, wheeze, shortness of breath, or chronic wheeze or cough. Overall, the most common symptom reported by poultry workers was current cough (25%), followed by current phlegm (24%) and shortness of breath when hurrying on the level (17%). The most common symptom occurring chronically for all poultry workers was phlegm (19%) followed by wheeze (16%) and cough (13%).

As shown in Table 3, endotoxin load (EU/mg) was a significant predictor of chronic phlegm (OR=1.69, 95% CI=1.01-2.83, p=0.05) after controlling for gender, age, years in the poultry industry, time spent in the barn, type of poultry production (cage-housed or floor-housed) and smoking status.

After categorizing the log transformed endotoxin load (EU/mg) into low (< 578 EU/mg) and high (> 590 EU/mg) levels using the 50^{th} percentile, it was found that high endotoxin load was a significant predictor of chronic phlegm for all workers (OR=5.49, 95% CI= 1.23-24.63, p=0.03).

Table 1: Study Population from Original Cohort Study and those Restudied

	Alberta	Manitoba	Saskatchewan	Total
Original Cohort				
Floor-housed	98	39	44	181
Cage-housed	26	34	62	122
Total	**130**	**81**	**113**	**303**
Mixed housing	6	8	7	21
Re-studied				
Floor-housed				
1998-2000	0	15	12	27
2000-2004	0	50	3	53
Total	**0**	**65**	**15**	**80**
Cage-housed				
1998-2000	0	16	2	18
2000-2004	0	7	6	13
Total	**0**	**23**	**8**	**31**
Mixed housing				
1998-2000	0	1	0	1
2000-2004	0	4	4	8
Total	**0**	**5**	**4**	**9**

Table 2: Demographics, Pulmonary Function, and Environmental Measurements of Workers from Floor and Cage-housed Poultry Operations

	Floor Housed	Cage Housed
Number	80	31
Age, years (mean ± SD)	42.61±11.50	45.74±12.92
Height, cm (mean ± SD)	177.18±7.18	173.41±8.94*
Weight, kg (mean ± SD)	85.08±14.72	81.96±16.87
Time worked in barn on sampling day, minutes (mean ± SD)	95.38±51.83	160.97±146.09†
Gender, n (%)		
Male	75 (93.7)	29 (93.5)
Female	5 (6.3)	2 (6.5)
Smoking Status, n (%)		
Non-smoker	54 (67.5)	20 (64.5)
Ex-smoker	21 (26.2)	5 (16.1)
Current smoker	5 (6.3)	6 (19.4)
Across-shift pulmonary function, (mean ± SD)		
Forced expired volume in 1 second, FEV_1	0.17±5.11	0.23±5.45
Forced vital capacity, FVC	0.81±4.24	1.80±4.43
Forced Expired flow at 25%-75%, FEF_{25-75}	-1.70±15.96	-0.86±12.80
Environmental Measurements, (mean ± SD)		
Total Dust, mg/m^3	9.56±7.95	7.57±8.99‡
Endotoxin Load, EU/mg	7483.79±9020.41	9544.02±14189.62
Airborne Endotoxin, EU/m^3	1106.40±1420.30 ($110.64±142.03$ ng/m^3)	1291.47±1349.74 ($129.15±134.97 ng/m^3$)
Ammonia, ppm	17.2±18.2	10.5±11.2*

Statistical difference: * p=0.02, † p=0.001, ‡ p=0.01

Figure 1: Ammonia (ppm), Total Dust (mg/m^3), Endotoxin Load (EU/mg) and Airborne Endotoxin (EU/m^3) for Floor and Cage- housed Poultry Operations (mean ± SE)

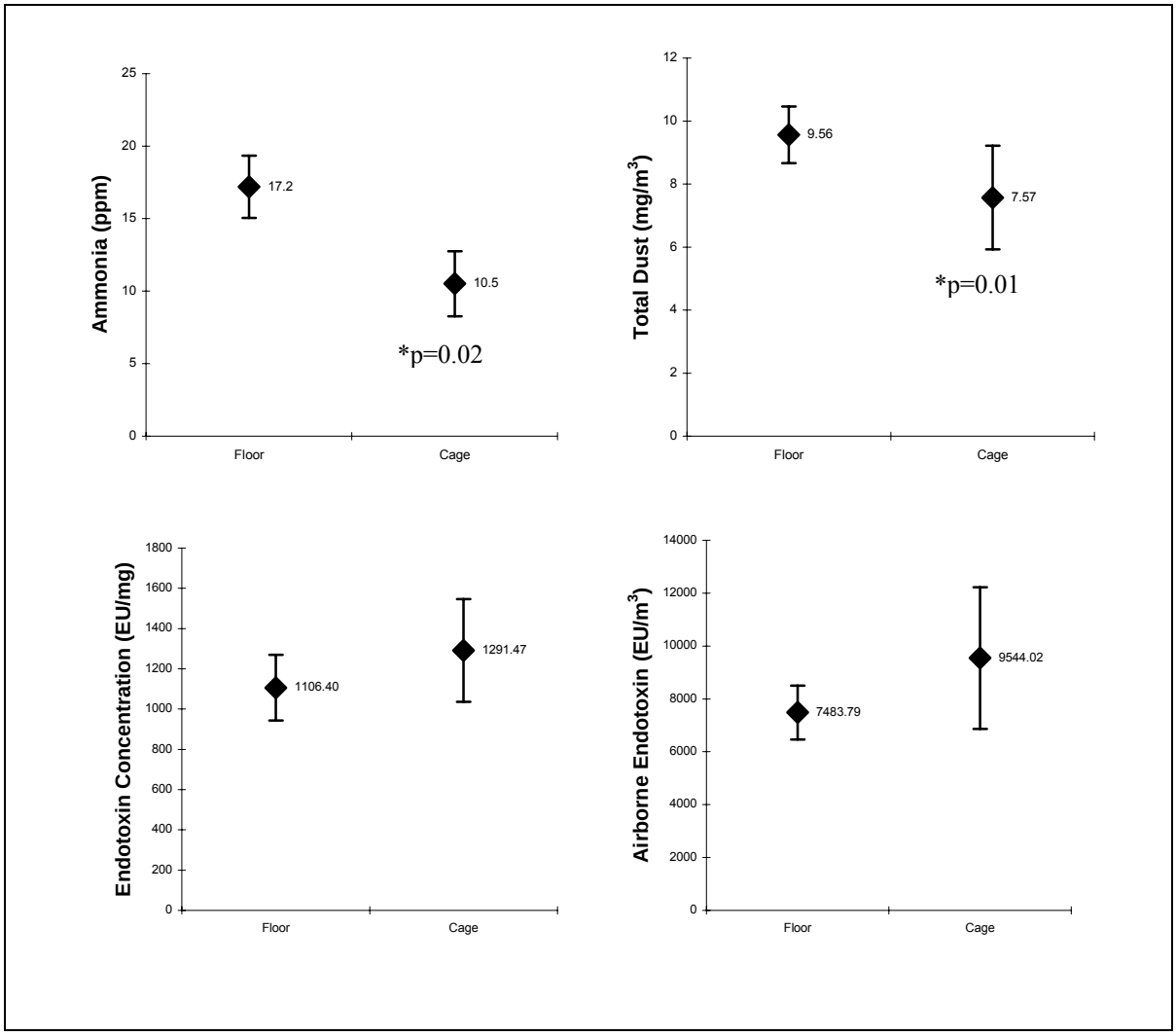

Table 3: Current and Chronic Respiratory Symptoms of Poultry Workers

	Floor Housed	Cage Housed	Overall
Current Symptoms, n (%)			
Cough	17 (21.5)	11 (35.5)	28 (25.5)
Phlegm	14 (17.7)	12 (38.7)*	26 (23.6)
Wheeze	3 (3.8)	3 (9.7)	6 (5.1)
Shortness of breath	11 (13.9)	8 (25.8)	19 (17.3)
Chronic Symptoms, n (%)			
Cough	8 (10.1)	6 (19.4)	14 (12.7)
Phlegm	9 (11.4)	12 (40.0)†	21 (19.3)
Wheeze	12 (15.6)	5 (16.1)	17 (15.7)

Statistical difference between cage and floor housed: * p=0.02, † p=0.001

Table 4: Multivariate Logistic Regression Model of Risk Factors for Chronic Phlegm Production in Poultry Workers

	OR (95% CI)	p-value
Age	1.00 (0.94,1.06)	0.96
Years worked in the poultry barn	1.00 (0.93, 1.05)	0.76
Time spent in barn on sampling day	1.00 (1.00, 1.01)	0.19
Type of poultry production		
Floor housed	1.00	
Cage housed	0.28 (0.07, 1.09)	0.07
Smoking status		
Non-smoker	1.00	
Ex-smoker	2.05 (0.31, 13.58)	0.45
Current smoker	0.08 (0.01, 0.55)	0.01
Endotoxin Load, ln(Eu/mg)	1.69 (1.01, 2.83)	0.05

results were adjusted for gender (ns)

2.5 DISCUSSION

Although poultry workers are exposed to a mixture of contaminants in the work environment, endotoxin is thought to be a primary agent responsible for inflammatory reactions experienced by livestock workers.[7] Compared to controls and workers from layer operations (in which birds are housed in cages), broiler growers (who work with birds grown on the floor)

have shown a greater across-shift decline in forced expired volume in one second.[28] Higher dust and airborne endotoxin have been correlated with changes in lung function[29] and significant dose-response relationships for pulmonary function decrements in poultry workers have been suggested at thresholds of 2.4 mg/m^3 total dust, 0.16 mg/m^3 respirable dust, 614 EU/m^3 endotoxin and 12 ppm ammonia.[10] A study looking at airway hyper-responsiveness in naïve subjects exposed to cage and floor-housed poultry systems found that inhalable endotoxin concentration was similar (100 ng/m^3) between the two types of operations but there was twice as much inhalable dust in the floor-housed systems, yet bronchial responsiveness was slightly higher in the persons exposed to the cage-housed environment.[30]

This study reconfirms results from previous studies that poultry workers experience high rates of respiratory symptoms. The results from this study are generally lower than those reported by other studies[1,2,4] but similar to Swedish results[29] and studies of poultry producers in Canada.[25]

Particle size appears to be important in respiratory and inflammatory health effects and may be a factor in the results presented herein. Fine particles can represent a substantial component of particle numbers in total dust and in particulate matter with a diameter of < 10 μm (PM_{10}), although they would represent only a small fraction of the total mass.[31] Finer particles have a larger surface area than larger particles, and if fine particles are more toxic than larger particles, adverse effects would be expected at lower mass concentrations because the fine particles would contribute very little to the overall particle mass.[32-36] At low ambient particle mass, concentrations of smaller particles can be relatively persistent whereas at higher concentrations aggregation to larger particle sizes occurs more rapidly.[33] Factors which suggest that finer particles may be more toxic than larger particles are related to (1) the dosimetric aspects of deposition and disposition of particles; (2) the larger surface area per mass of finer particles may act as a catalyst for reactions; (3) the increased surface area could act as a carrier for co-pollutants.[33] One study of poultry confinement operations indicated that respirable suspended particles constituted 4-6% of the total suspended particles but the respirable fraction of endotoxin constituted more (11-30%) of the total airborne endotoxin.[16] The average endotoxin concentrations in total dust were between 6-16 ng/mg with endotoxin concentration of

the respirable fraction considerably higher, ranging from 20-40 ng/mg, with the majority of the respirable fraction being < 3.5 μm in size.[16] This suggests that endotoxin is considerably enriched in the smaller particles. The role of smaller fine particles is yet to be delineated in the poultry work environment, as are the differences in the particle concentrations between the two types of poultry operations, and the potential impact on worker health. Ultrafine particles have been associated with increased morbidity and mortality in relation to urban air pollution,[37-42] and it is possible that in the poultry work environment fine or ultrafine particles and attached co-pollutants, such as ammonia and endotoxin, could act alone or synergistically to potentiate respiratory health effects in workers.

There is evidence from the swine industry that decreasing airborne dust and endotoxin levels results in significant decreases in total and inhalable dust levels but concomitant increases in the proportion of the diminutive dust (0.3-0.5 μm); with consequential increases in the endotoxin load (EU/mg).[8] Amongst the poultry workers studied herein, the personal total dust levels were significantly lower amongst the workers exposed to the cage-housed poultry as compared to those exposed to the floor-housed poultry, yet there was a trend towards greater endotoxin load (EU/mg) for the cage-housed poultry operations. It is possible that the lower total dust in the cage-housed poultry operations could relate to a greater proportion of diminutive particulates present in the work atmosphere, and that these smaller particles, with a lower mass but larger surface area, could carry a greater portion of endotoxin. These smaller particles with higher levels of endotoxin, with potential to penetrate deeper into the lung, might contribute to the greater respiratory health effects experienced by the exposed workers in the cage-housed poultry operations.

In a study on floor-housed poultry aged 2-6 weeks housed in clean rooms, the greatest number of respirable particles were in the size range 1-2 μm followed by 2-3 μm.[43] A study of respirable aerosol concentrations in broiler houses (floor-housed) indicated that particles in the size range 1-2 μm were consistently greater than particles of the 0.7-1μm size range over a twenty-four hour period.[44] A study in Canadian broiler barns indicated that for particles greater than 5 μm there was a 10-fold increase in mean particle concentration over a seven week growth cycle versus a 1000-fold increase for the size fractions less than 5 μm.[23] Studies from laying

houses indicate similar trends, if not to a greater proportion, for particles of a smaller size range. In a U.S. study of particle size distribution in laying houses only 2.4% of particles were larger than 5 μm and particles of 0.3-0.5 μm in diameter accounted for 43.6% of the total number of particles.[16]

The small sample size in this study limits the results. A larger sample size, particularly for the cage-housed poultry operations, would assist in further delineating the results. Secondly, environmental data collection included only total dust and not inhalable or fractionated dust levels, and these would assist in furthering the hypotheses presented. Differences in operations and work practices between the two types of operations including worker time spent in direct contact with birds, predominance of female poultry in cage-housed poultry operations, age of the birds, length of time birds have been in housing, and the housing management practices could result in different dust fractionations and different exposure profiles of endotoxin or other substances that were not studied here, including other bioaerosols, mold and fungi, all of which could contribute to the respiratory health effects experienced by workers.

The study presented herein has found significantly higher total dust and ammonia in facilities in which poultry are housed on the floor as compared to facilities which house poultry in cages, along with trends for higher endotoxin load (EU/mg) in the cage housing poultry facilities as compared to the floor housing facilities. In addition, workers from the cage housing facilities reported significantly greater frequency of current and chronic phlegm and greater current cough, wheeze, and shortness of breath; and greater chronic cough and wheeze as compared to workers from the floor-housed poultry facilities. Furthermore, high endotoxin load (EU/mg) was a significant predictor of chronic phlegm in poultry workers.

2.6 CONCLUSIONS

Despite higher total dust and ammonia exposures in the floor-housed poultry operations, the workers from the cage-housed poultry operations indicated the greater respiratory symptoms. This may be a function of exposure to endotoxin load (EU/mg), which was a significant predictor of chronic phlegm, as there was a trend for greater endotoxin loads in the cage-housed poultry

operations as compared to the floor-housed poultry operations. This study is only able to present the total dust levels, but it is possible that the measured dust from the cage-housed operations represents smaller particle sizes with a larger surface area, and therefore the resultant lower total dust concentration, as compared to the floor-housed poultry operation environment. Furthermore, these smaller particles in the cage-housed poultry operations may be enhanced with endotoxin as indicated by the trend to greater endotoxin load (EU/mg), and these factors may be important influences on the presence of symptoms in workers. Although the comparison between high and low exposure to endotoxin loads between floor and cage-housed operation workers was not statistically significant, there was a trend for the higher endotoxin load to be present in the cage-housed poultry operations, and this finding may become clearer with a larger sample size.

Dust size fractionation and associated endotoxin load, in the cage and floor-housed poultry operations and related respiratory health effects and immune system indicators in workers would assist in further elucidating the relationships between exposures and respiratory outcomes in workers in the industry.

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3. PAPER TWO: LEVELS OF ENDOTOXIN AND DUST AT RESPIRABLE AND NON-RESPIRABLE PARTICLE SIZES ARE NOT CONSISTENT BETWEEN CAGE AND FLOOR-HOUSED POULTRY OPERATIONS

Levels of Endotoxin and Dust at Respirable and Non-respirable Particle Sizes are not Consistent between Cage and Floor-housed Poultry Operations

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Keywords: poultry, endotoxin load, dust, mass median aerodynamic diameter,

Running Title: Endotoxin in poultry dust

3.1 ABSTRACT

An earlier study on poultry workers in Western Canada had indicated that the rate of respiratory symptoms differed between workers exposed to two different methods of poultry production. Workers who worked with poultry housed in cages (CH) had greater respiratory symptoms and significantly greater current and chronic phlegm as compared to workers who worked with poultry reared on the floor (FH). Endotoxin load (EU/mg) was a significant predictor of chronic phlegm.

The work presented herein furthered the above findings by assessing both CH and FH poultry operations utilizing Marple cascade impactors to fraction dust and endotoxin. The results illustrate workers from the FH poultry operations spent significantly more time in direct contact with the birds, although workers from CH operations spent additional time sorting eggs. The FH poultry operations housed significantly greater number of birds and on average the CH birds were significantly older than the FH birds. Workers from the FH poultry operations had a statistically significant loss in FEV_1 over the work-shift as compared to almost no FEV_1 change for workers from CH poultry operations. There was significantly greater endotoxin per milligram of dust (EU/mg) in the area respirable fractions in the CH poultry operations as compared to the FH operations even though the FH poultry operations had significantly greater levels of dust. The mean mass aerodynamic diameters for dust were significantly greater in the floor-housed poultry operations (16.52 μm) as compared to the cage-housed operations (12.65 μm). The MMAD for endotoxin mass was smaller than for dust at approximately 3μm, which was similar for the two types of poultry operations. These results may assist in explaining differences in respiratory response experienced by workers exposed to these two types of work environments.

3.2 INTRODUCTION

Individuals engaged in work in the poultry production industry have the greatest prevalence of respiratory symptoms compared to workers from other industries.[1] Workers in nine different industries in the US were studied including poultry, swine, cotton and animal feed

workers and the highest prevalence of work related lower respiratory tract (cough, phlegm, wheeze and shortness of breath)symptoms were recorded in poultry handlers. Chronic bronchitis and upper respiratory tract (eye and nose) symptoms were most common in poultry handlers.[1] Classifying poultry industry workers according to bird housing (cage-housed or floor-housed birds) indicated that workers in cage-housed poultry operations reported overall greater frequency of current and chronic upper respiratory symptoms with significantly greater current and chronic phlegm.[2]

Endotoxin is thought to be a primary agent in the inflammatory reaction experienced by workers in livestock industries.[3-6] Classically, an endotoxin is a structural component in bacteria which is released mainly when bacteria are lysed. A prototypical example of endotoxin is lipopolysaccharide (LPS) found in the outer membrane of gram-negative bacteria.[7] In agricultural studies the level of exposure is often represented by the endotoxin present in the collected particle sample and often expressed as endotoxin in the volume of air sampled (EU/m^3 or ng/m^3). Often studies utilize EU/m^3 to establish exposure relationships with respiratory outcomes.[1, 3, 4, 6] As compared to the swine, grain and animal feed industries, the poultry industry has been shown to have the highest endotoxin concentrations and significant prevalence rate ratios for upper respiratory tract symptoms were found with increasing endotoxin and dust exposure.[1] For poultry workers dose-response respiratory relationships have been suggested at 100 EU/m^3.[4]

Workers in the poultry industry have diverse air quality exposures related to the type of bird housing, manure management, age and number of birds, type of feed and litter, rate and type of ventilation, size of building, amount of worker time spent in direct contact with birds, and general housekeeping practices. Assessing particulate levels in the poultry industry according to bird housing indicates a consistent trend for birds housed on the floor to have lower endotoxin levels (EU/m^3) in the presence of greater dust concentrations as compared to operations in which birds are housed in cages.[2, 8-10] Evaluating endotoxin load in relation to the mass of dust collected (EU/mg) may help assess the relationship between endotoxin and respiratory outcomes of workers. Airborne endotoxin (EU/m^3) is an indication of the concentration of endotoxin in the sampled air whereas endotoxin load (EU/mg) is an indication of the endotoxin present in the

mass of particulate sampled. Endotoxin load, when coupled with the type of sampling (inhalable, respirable, non-respirable) could provide insight into dose and deposition in the respiratory tract and might further aid in understanding the respiratory consequences for workers. In a recent study endotoxin load (EU/mg) was a significant predictor of chronic phlegm.[2] The study described herein further examined the relationship of higher endotoxin in the presence of lower dust in cage-housed poultry operations by fractioning total dust in the two types of poultry operations and measuring the endotoxin levels associated with the various particle sizes. We hypothesized that there would be greater endotoxin on lesser dust mass in the smaller particle fractions in the cage-housed operations as compared to the floor-housed operations.

3.3 **METHODS**

3.3.1 *Study Population*

Ethics approval for the project was granted by the University of Saskatchewan. Poultry operations were classified according to the manner in which the poultry were housed. Operations in which poultry were housed on the floor were classified as floor-housed (FH) and operations in which poultry were housed in cages were classified as cage-housed (CH). Participation was by invitation and sites were chosen from the available Saskatchewan population. Of the 78 CH poultry operations in Saskatchewan, 19% took part in the study while 20% of the FH operations (of 73) took part. Operations who took part closely represented the western Canadian poultry industry (flock size, manure management system, age of barn, poultry breed). Sites were not chosen if producers had more than one type of operation (both cage and floor-housed poultry operations). One barn and one worker on each site were studied during the winters of 2005 and 2006. Sites were all located in the province of Saskatchewan.

3.3.2 *Environmental Measures*

Two area measurements and one personal measurement were carried out in the thirty barns. Area measurements occurred over a 4-hour sampling period. Two area measurements per barn were collected along the middle of the barn and equal distance from all 4 walls. The area

sampling pack included a Marple cascade sampler as well as ammonia (NH_3) and carbon dioxide (CO_2) diffusion tubes that were placed approximately 1.5m above the floor and out of the reach of the birds. Personal measurements were performed on one worker in each barn. Before beginning work, workers were fit with an environmental sampling backpack that contained the Marple cascade sampler, NH_3, and CO_2 diffusion tubes. The Marple sampler was attached near the workers' breathing zone and worn during the entire work-shift.

Temperature and relative humidity were measured twice during the 4 hour sampling period utilizing a VelociCalc (8347A-M-G, TSI Inc., Shoreview, MN). Measurements for NH_3 and CO_2 were collected with passive colorimetric gas diffusion tubes (NH_3 2.5-1500 ppm-hours, CO_2 0.13-30 vol%; Gastec, Kanagawa, Japan).

Fractioned dust and endotoxin were collected using a Marple cascade impactor (Thermo Electron Corp., Waltham, MA) connected to a SKC constant airflow pump (Universal 224-PCXR4, Eighty Four, PA) run at 2 liters per minute with pre-weighed radial slit polyvinyl chloride (PVC) filters (5µm, Thermo Electron Corp., Waltham, MA). The Marple sampler contained 6 stages to represent cut-points of 0.52, 0.93, 1.55, 3.50, 6.0 and 9.8µm. Filters were individually analyzed for dust [milligrams of dust (mg) and milligrams of dust per m^3 of air (mg/m^3)] (MX5 microbalance, Mettler-Toledo, Greifensee, Switzerland) and with Kinetic-QCL Limulus Amebocyte Lysate assay (*E. coli* O55:B5; Cambrex BioScience Walkersville Inc, Walkersville, MD) for airborne endotoxin and endotoxin concentration [endotoxin units per m^3 of air (EU/m^3) and endotoxin units per mg of dust (EU/mg)]. Endotoxin samples were referenced to the RSE: EC-6. For analysis purposes stages 3 and 4 of the Marple sampler (>6µm) were chosen to represent the non-respirable fraction and stages 5-final (<0.5 µm-6 µm) represent the respirable fraction.

3.3.3 *Questionnaires and Pulmonary Tests*

A general poultry operation questionnaire was administered and completed by each poultry operation manager during the sampling period and the information collected included breed, number and age of birds, size and age of barn, and other operation related information.

The same worker who wore the personal sampling backpack also completed a respiratory health questionnaire prior to beginning the work-shift. The general respiratory health questions were modified from the American Thoracic Society standardized questionnaire[11]and included current and chronic respiratory symptoms. Pulmonary tests for peak expiratory flow (PEF) and forced expired volume in 1 second (FEV_1) and an acute respiratory symptom questionnaire were administered before beginning work and repeated again at the end of the work-shift. Pulmonary tests were performed using the Piko-1® electronic peak flow/FEV_1 meter (Ferraris, Louisville, CO). Across-shift differences for PEF and FEV_1 were calculated by subtracting the pre-shift measurement from the post-shift measurement and dividing by the pre-shift measurement.

3.3.4 *Statistical Analysis*

Analyses were completed using SPSS version 13 and SAS version 8.2. Arithmetic means and standard error or standard deviation were used to describe continuous variables, including age, years worked in the poultry barn, time spent in the barn, number of birds and age of birds. Geometric mean and geometric standard deviation were used to describe the environmental data including dust, endotoxin, carbon dioxide and ammonia. Comparisons between housing types were completed using all the Marple stages as well as after stratification by cut-point into respirable and non-respirable fractions. Categorical variables including respiratory symptoms were described using frequencies and percentages. Prior to the regression analysis, environmental variables (dust, endotoxin, carbon dioxide and ammonia) were log-transformed ($\log^e$) to obtain approximate normal distributions for the variables. The differences in the means of continuous variables between the study groups for personal measures were tested using univariate analysis of variance to test the difference between poultry barns and Marple samplers with geometric means and confidence intervals reported to describe the differences. The generalized estimation equations (GEE) for general linear model were used to adjust for the correlation between repeated area environmental measurements taken in the same barn for testing the differences between the cage and floor based operations.

3.4 RESULTS

The study population comprised 15 floor-housed poultry operations and 15 cage-housed poultry operations. There were no significant differences between workers from FH and CH poultry operations for mean age and mean length of time worked in the industry (Table 1). Workers from the FH poultry operations spent significantly more time in direct contact with the birds as compared to workers from the CH operations, although workers from CH operations spent additional time sorting eggs.

Table 1: Demographics, Symptoms and Pulmonary Results of Workers

	Cage-housed	**Floor-housed**	**p-value**
n	15	15	
Age of workers, years (mean±SD)	42.27±17.21	43.47±13.16	p=0.83
Years worked in industry, (mean±SD)	14.65±14.81	16.73±17.38	p=0.73
Time spent in direct contact with birds, hours (mean±SD)	**1.1±1.3**	**2.4±1.5**	**p=0.002**
Time spent sorting eggs, hours (mean±SD)	4.2±8.5	0.0±0.0	
Symptoms: (n, %)			
Current			
Cough	4 (27%)	5 (33%)	p=0.50
Phlegm	6 (40%)	7 (47%)	p=0.50
Chronic			
Cough	4 (27%)	6 (40%)	p=0.34
Phlegm	7 (47%)	7 (47%)	p=0.64
Wheeze	1 (7%)	1 (7%)	p=0.76
Nasal	6 (40%)	5 (33%)	p=0.44
Eye	4 (27%)	6 (40%)	p=0.35
Pulmonary: (mean±SD)			
Across-shift FEV_1, L	**0.04±0.50**	**-0.29±0.26**	**p=0.03**
Across-shift PEF, LPM	-15.0±37.0	-9.6±43.0	p=0.72

No significant differences were observed in the proportions of current and chronic cough and phlegm, current wheeze, nasal and eye symptoms between the two groups of workers. Workers from the FH poultry operations had a statistically significant, loss in FEV_1 over the work-shift as compared to almost no FEV_1 change for workers from CH poultry operations.

Workers from both types of operations had losses in PEF over the work-shift with no significant difference in PEF loss between the workers from the two types of operations.

Table 2 outlines poultry barn characteristics. The poultry barns were of similar age with the FH poultry operations housing significantly greater number of birds as compared to the CH operations. On the study day the age of the birds were significantly different by type of operation with the CH birds being significantly older than the FH birds. Ammonia levels were similar between the two types of operations and carbon dioxide was significantly higher in the FH poultry operations.

Table 2: Poultry Operation Characteristics

	Cage-housed	**Floor-housed**	**p-value**
n	15	15	
Bird age, weeks (mean±SD)	**47.0±15.4**	**3.2±0.3**	**p<0.001**
Barn age, years (mean±SD)	19.7±12.2	15.3±12.3	p=0.33
Number of birds, 1000's (mean±SD)	**12.7±12.9**	**23.4±12.8**	**p=0.03**
Ammonia, ppm (GM±GSD)	7.44±2.46	7.07±3.38	p=0.90
Carbon dioxide, ppm (GM±GSD)	**3070.43±1.39**	**4140.95±1.33**	**p=0.01**

Dust and endotoxin area measures for CH and FH poultry operations are shown in Table 3. There was significantly greater total dust mass (mg) and total dust concentration (mg/m^3) in the FH poultry operations as compared to the CH operations. Total endotoxin concentration and total endotoxin load were not significantly different between the two types of operations although total endotoxin load (EU/mg) was borderline significantly higher in the CH poultry operations.

Area measures were further classified utilizing the cut-point diameters of the samples into non-respirable (stages 3 and 4, >6.0µm) and respirable (stages 5-final, 6.0 - <0.5µm) size fractions (Table 3). Both non-respirable and respirable dust mass (mg) and dust concentration (mg/m^3) were significantly higher in the FH poultry operations. Endotoxin load on respirable particles (EU/mg) was significantly higher in the CH poultry operations yet there was no difference in the non-respirable endotoxin load between the two types of operations. Interestingly there was significantly greater non-respirable endotoxin concentration (EU/m^3) in

the FH operations as compared to the CH operations and no difference in the respirable endotoxin concentration between the two types of operations.

Table 3: Area Measures: Total, Respirable and Non-respirable Dust and Endotoxin

	Cage-housed GM (95%CI)	**Floor-housed** GM (95%CI)	**p-value**
n	30	30	
Dust mass, mg			
Total	**0.86** (0.66,1.12)	**2.15** (1.76, 2.64)	p<0.001
Non-respirable	**0.51** (0.33, 0.78)	**1.38** (0.84, 2.27)	p=0.003
Respirable	**0.24** (0.17, 0.36)	**0.40** (0.32, 0.52)	p=0.04
Dust concentration, mg/m^3			
Total	**1.86** (1.41, 2.46)	**4.70** (4.02, 5.50)	p<0.001
Non-respirable	**1.08** (0.69, 1.71)	**3.00** (1.83, 4.92)	p=0.003
Respirable	**0.53** (0.36, 0.78)	**0.88** (0.68, 1.13)	p=0.04
Endotoxin load, EU/mg			
Total	832.91 (604.36, 1148.15)	530.64 (376.18, 748.51)	p=0.06
Non-respirable	1044.48 (374.71, 1190.42)	728.78 (419.56, 1265.90)	p=0.24
Respirable	**667.88** (450.82, 1028.02)	**313.47** (201.28, 488.09)	p=0.04
Endotoxin concentration, EU/m^3			
Total	1567.83 (988.55, 2486.56)	2489.43 (1854.81, 3341.18)	p=0.10
Non-respirable	**1155.85** (708.11, 1886.25)	**2199.88** (1639.83, 2951.21)	p=0.03
Respirable	363.50 (223.82, 590.34)	274.16 (195.93, 383.62)	p=0.35

Personal measures for dust and endotoxin for CH and FH poultry operations are shown in Table 4. Total dust mass (mg) and total dust concentration (mg/m^3) were significantly higher in the FH poultry operations as compared to the CH operations. Total endotoxin load (EU/mg) was not significantly different between the two types of operations. Due to interaction between the Marple samplers, endotoxin load was stratified by Marple and only Marple 3 showed a significant difference between CH and FH operations (p=0.01) although the sample size was very small (n=8 and n=6 respectively). Total endotoxin concentration was significantly higher in the FH operations.

Personal dust and endotoxin measures were further classified into non-respirable and respirable size fractions (Table 4). Non-respirable dust mass (mg) and dust concentration are significantly higher in the FH poultry operations as compared to the CH operations. Personal respirable dust mass and concentration are not significantly different between the two types of

operations. Non-respirable and respirable endotoxin load (EU/mg) means were higher in the CH operations as compared to the FH operations but the difference does not reach statistical significance. Non-respirable airborne endotoxin concentration (EU/m^3) was significantly higher in the FH operations as compared to the CH operations, whereas respirable concentration was not significantly different between the operation types.

Table 4: Personal Measures: Total, Respirable and Non-respirable Dust and Endotoxin

	Cage-housed GM (95%CI)	**Floor-housed** GM (95%CI)	**p-value**
n	15	15	
Dust mass, mg			
Total	**0.24** (0.14,0.42)	**0.76** (0.45, 1.28)	p=0.004
Non-respirable	**0.15** (0.08, 0.29)	**0.60** (0.32, 1.11)	p=0.004
Respirable	0.07 (0.04, 0.13)	0.12 (0.07, 0.20)	p=0.23
Dust concentration, mg/m^3			
Total	**1.59** (0.90, 2.83)	**5.24** (2.99, 9.14)	p=0.005
Non-respirable	**1.01** (0.52, 1.94)	**4.39** (2.32, 8.34)	p=0.003
Respirable	0.50 (0.29, 0.89)	0.73 (0.42, 1.27)	p=0.34
Endotoxin load, EU/mg			
Total	633.87 (358.10, 1122.02)	628.06 (383.71, 1028.02)	p=0.98
Non-respirable	937.56 (495.45, 1770.12)	625.17 (342.77, 1140.25)	p=0.35
Respirable	642.69 (317.69, 1303.17)	508.16 (261.22, 990.83)	p=0.62
Endotoxin Concentration, EU/m^3			
Total	**1312.20** (809.10, 2128.14)	**3250.87** (2032.36, 5199.96)	p=0.01
Non-respirable	**946.24** (571.48, 1566.75)	**2710.19** (1659.59, 4425.88)	p=0.005
Respirable	312.61 (180.30, 540.75)	424.62 (248.89, 722.77)	p=0.42

Table 5: Area and Personal Dust and Endotoxin Measures by Marple Impactor Stage

	Area (n=30)		
	Cage-housed GM (CI)	**Floor-housed** GM (CI)	**Sig.**
Stage 3			
Dust, mg	0.28 (0.15,0.50)	0.96 (0.58,1.59)	0.002
Endotoxin, mg	1065.86 (625.89,1814.68)	798.36 (450.19,1415.79)	0.47
Stage 4			
Dust, mg	0.16 (0.10,0.25)	0.35 (0.18,0.69)	0.06
Endotoxin, mg	1127.20 (860.40,1476.73)	648.78 (335.12,1256.03)	0.13
Stage 5			
Dust, mg	0.06 (0.03,0.12)	0.19 (0.14,0.27)	0.002
Endotoxin, mg	1388.35 (672.05,2867.48)	404.67 (261.40,626.61)	0.004
Stage 6			
Dust, mg	0.06 (0.03,0.10)	0.05 (0.03,0.09)	0.79
Endotoxin, mg	772.50 (408.41,1461.50)	545.51 (288.34,1031.81)	0.45
Stage 7			
Dust, mg	0.006 (0.003,0.01)	0.003 (0.002,0.006)	0.18
Endotoxin, mg	562.47 (209.46,1510.43)	912.85 (338.45,2462.07)	0.50
Stage 8			
Dust, mg	0.003 (0.002,0.007)	0.003 (0.002,0.005)	0.70
Endotoxin, mg	632.99 (214.58,1867.67)	803.34 (358.34,1801.36)	0.73
Final stage			
Dust, mg	0.01 (0.007,0.03)	0.02 (0.01,0.02)	0.83
Endotoxin, mg	56.95 (17.80,182.18)	115.32 (62.29,213.45)	0.29
	Personal (n=15)		
	Cage-housed GM (CI)	**Floor-housed** GM (CI)	**Sig.**
Stage 3			
Dust, mg	0.08 (0.04, 0.17)	0.40 (0.21, 0.74)	0.002
Endotoxin, mg	1202.26 (698.23,2074.91)	741.31 (442.59,1238.80)	0.19
Stage 4			
Dust, mg	0.04 (0.02,0.11)	0.15 (0.06,0.38)	0.05
Endotoxin, mg	833.68 (308.32,2254.24)	465.59 (181.97,1191.24)	0.39
Stage 5			
Dust, mg	0.01 (0.003,0.03)	0.03 (0.01,0.09)	0.09
Endotoxin, mg	1548.82 (504.66, 4742.42)	959.40 (332.66,2766.94)	0.53
Stage 6			
Dust, mg	0.009 (0.003,0.03)	0.01 (0.004,0.03)	0.80
Endotoxin, mg	1230.27 (417.83,3614.10)	972.75 (351.56,269.53)	0.75
Stage 7			
Dust, mg	0.005 (0.002,0.01)	0.002 (0.001,0.005)	0.21
Endotoxin, mg	716.14 (187.93,2728.98)	615.18 (191.42, 1976.97)	0.86
Stage 8			
Dust, mg	0.003 (0.001,0.008)	0.003 (0.001,0.006)	0.76
Endotoxin, mg	1091.44 (246.60, 4841.72)	792.50 (216.27,2904.02)	0.74

When analyzing the data by individual stages of the Marple the same trends persist for area and personal measures (Table 5). The dust mass was similar or significantly greater for both personal and area measures in the FH poultry operations and the endotoxin load tends to be higher in the CH poultry operations for both area and personal measures. Calculating the mass median aerodynamic diameter (MMAD) for dust and endotoxin in both area and personal samples indicates that the dust MMAD for both area and personal measures was significantly greater in the FH poultry operations as compared to the CH operations (Table 6). The MMAD for endotoxin was not significantly different between the two types of poultry operations.

Table 6: Mass median Aerodynamic Diameters for Area and Personal Dust and Endotoxin

	Cage-housed GM (95%CI)	**Floor-housed** GM (95%CI)	**p-value**
Area, (n=30)			
Dust MMAD, µm	12.64 (10.23, 15.08)	16.56 (14.19, 18.85)	0.02
Endotoxin MMAD, µm	3.32 (2.34, 4.17)	3.44 (2.50, 4.26)	0.85
Personal, (n=14)			
Dust MMAD, µm	11.86 (7.54, 15.76)	18.72 (14.69, 15.76)	0.01
Endotoxin MMAD, µm	3.78 (2.43, 4.67)	3.01 (1.75, 3.91)	0.32

Factors such as age of birds, number of birds, gas levels, feed type, litter type, stocking density (number of birds/m^2) and antibiotic use can differ by poultry housing type. These variables can potentially have an influence on the dust and endotoxin levels in CH and FH poultry operations resulting in spurious associations between type of housing and environmental variables.

Descriptive characteristics of the barn and handling variables which could potentially influence the particulate and endotoxin levels in the CH and FH poultry operations are presented in Table 7 for both types of operations. The influence of barn and handling characteristics (bird age, stocking density, feed type, antibiotic use) on the association between environmental variables [endotoxin concentration (EU/m^3), endotoxin load (EU/mg) and dust concentration (mg/m^3)] were evaluated by bird housing type (CH or FH). These evaluations were undertaken separately for personal and area environmental measures.

Table 7: Descriptive characteristics of barn variables by type of operation

	Cage-housed (mean±SD) or n (%)	Floor-housed (mean±SD) or (n) %
Age of Worker, years	42.27±17.21	43.47±13.16
Number of birds on farm	22026.20±17994.44	83860.00±102535.77
Number of birds in barn	12755.53±12915.92	23413.33±12853.51
Bird Age, (weeks and days)	47.00±15.43 (weeks)	22.47±2.23 (days)
Barn Age, years	19.73±12.221	15.27±12.35
Stocking Density, #birds/m^2	19.25±18.99	16.99±4.24
Barn temperature, celcius	19.80±2.42	26.23±2.58
Barn relative humidity	48.14±6.09	63.10±11.15
Ammonia Level, ppm	9.0±7.8	12.0±20.4
Carbon Dioxide Level, ppm	3233.3±1085.5	4316.7±1388.6
Outdoor Carbon Dioxide, ppm	506.73±168.43	527.63±189.31
Egg Collection Type		
Conveyor belt	15 (100%)	*
Cage Type		
Double-tier	5 (33.0%)	*
Triple -tier	7 (46.6%)	
Four-tier	2 (13.3%)	
Six -tier	1 (6.7%)	
Egg Collection Type		
Conveyor belt	15 (100%)	*
Liter Type		
Straw	*	9 (60.0%)
Paper		5 (33.3%)
Sawdust		1 (6.7%)
Feeding Mechanism Type		
Automatic	15 (100%)	15 (100%)
Feed Type		
Ground meal/mash	5 (33.3%)	2 (13.3%)
Pellets/crumb	10 (66.7%)	13 (86.7%)
Floor Type		
Concrete	14 (93.3%)	10 (66.7%)
Clay	1 (6.7%)	0 (0.0%)
Soil	0 (0.0%)	2 (13.3%)
Concrete/Soil	0 (0.0%)	3 (20.0%)
Antibiotic Use		
In water and/or feed	*	6 (40.0%)
None		9 (60.0%)

* not applicable

Spearman's correlations (non-parametric) between personal dust concentration (mg/m^3), endotoxin concentration (EU/m^3) and endotoxin load (EU/mg) and stocking density and number of birds in the barn (bird number) were computed separately for CH and FH operations. The correlation between stocking density and the environmental variables were not statistically significant. There was a significant negative correlation (r = -0.51, p=0.05) between the number of birds in the barn and the endotoxin concentration among CH.

Using Mann-Whitney tests, personal endotoxin concentration (EU/m^3) was significantly different in the FH operations for feed types comparing pellets/crumb feed (mean 3.56±0.19 EU/m^3) to ground meal/mash feed (mean 3.14±0.20 EU/m^3, p=0.04). There was no significant difference in endotoxin concentration between feed types in the CH operations (p=0.89).

Antibiotics were only utilized in the FH operations. Mann-Whitney tests were administered using data from the FH operations comparing those operations which utilized antibiotics in their feed or water against those operations which were not using antibiotics. Those FH operations which utilized antibiotics had significantly higher levels of endotoxin concentration and endotoxin load as compared to those FH operations which did not utilize antibiotics (Table 8).

Table 8: Distribution of endotoxin concentration and load by antibiotic use in FH operations

	N	Mean	SE	Sig.
EU/m^3				
Used antibiotics	6	3.68	0.06	
Did not use antibiotics	8	3.37	0.07	0.02
EU/mg				
Used antibiotics	6	2.99	0.09	
Did not use antibiotics	9	2.63	0.07	0.01

Separate linear regression models were fitted with the outcomes of dust (mg/m^3), endotoxin concentration (EU/m^3) and endotoxin load (EU/mg). Firstly, a crude model was fitted that included only type of operation (model 1). Secondly, an adjusted model was fitted which

included type of operation and the potential confounder (model 2). The beta coefficients for the difference between the types of operation were compared between the two models to assess confounding where confounding was considered to be present if there was greater than 15% difference in the beta coefficient between the models.

When comparing the beta coefficients for the crude and adjusted models when feed type was considered, there was not an important difference that would change the interpretation of results (Table 9). Thus, confounding was unlikely to have occurred.

Table 9: Crude and adjusted models in the assessment of confounding by feed type in the association between type of operation and personal environmental variables

	β	Std Error	Sig.
Regression models for EU/m^3			
Model 1 – CH and FH	0.35	0.14	0.02
Model 2 – CH and FH	0.33	0.14	0.03
Feed Type	0.12	0.16	0.47
Regression models for EU/mg			
Model 1 – CH and FH	-0.17	0.16	0.28
Model 2 – CH and FH	-0.22	0.16	0.16
Feed Type	0.27	0.18	0.16
Regression models for mg/m^3			
Model 1 – CH and FH	0.50	0.16	0.004
Model 2 – CH and FH	0.53	0.16	0.003
Feed Type	-0.16	0.19	0.42

* β is the regression coefficient for the difference between FH and CH operations

Bird age confounded the relationship between the operation type (CH or FH) and total dust, endotoxin load and endotoxin concentration (Table 10). There were large differences between the crude model and the adjusted model beta coefficients for type of operation when using either outcome of endotoxin load or endotoxin concentration. Thus bird age influenced the associations between type of operation and endotoxin and dust levels. This assessment of

confounding should be interpreted cautiously, however, due to the small number of measurements.

Table 10: Crude and adjusted models in the assessment of confounding by bird age in the association between type of operation and personal environmental variables

	β	Std Error	Sig.
Regression models for EU/m^3			
Model 1 – CH and FH	0.35	0.14	0.02
Model 2 – CH and FH	0.53	0.31	0.10
Bird Age	0.004	0.006	0.53
Regression models for EU/mg			
Model 1 – CH and FH	-0.17	0.16	0.28
Model 2 – CH and FH	-0.59	0.35	0.11
Bird Age	-0.01	0.007	0.20
Regression models for mg/m^3			
Model 1 – CH and FH	0.50	0.16	0.004
Model 2 – CH and FH	1.10	0.34	0.004
Bird Age	0.01	0.007	0.06

* β is the regression coefficient for the difference between FH and CH operations

Statistical testing of the differences for the area measures was completed using generalized estimating equations to account for the clustering of the area samples, i.e. two samples per barn. Spearman's correlations (non-parametric) between area dust concentration (mg/m^3), endotoxin concentration (EU/m^3) and endotoxin load (EU/mg) and stocking density and bird number were computed separately for CH and FH operations. Stocking density was significantly correlated with endotoxin concentration (r =-0.44, p=0.02) and endotoxin load (r =-0.63, p=<0.001) for the CH operations but not the FH operations (r = 0.05, p=0.80, r = 0.16, p=0.42 respectively). Bird number was significantly correlated with endotoxin load (r =-0.54, p=0.002) and endotoxin concentration (r =-0.52, p=0.004) for the CH operations and significantly correlated with endotoxin concentration (r =-0.39, p=0.04) for the FH operations.

Area endotoxin concentration (EU/m^3) was significantly different between feed types among the FH operations. Endotoxin concentration levels were higher when using pellets/crumb feed (mean 3.44±0.26 EU/m^3) compared to ground meal/mash feed (mean 3.13±0.15 EU/m^3, $p<0.05$) in the FH operations. Endotoxin load levels were higher when using pellets/crumb feed (mean 2.77±0.31 EU/mg) compared to ground meal/mash feed (mean 2.45±0.11 EU/mg, $p<0.05$) in the FH operations. These relationships are similar and show the same trends as the relationships seen with the personal measurements. These differences were not seen in the CH operations.

Antibiotics were only utilized in the FH operations (Table 11). Those operations which utilized antibiotics had significantly higher levels of area endotoxin concentration and endotoxin load as compared to those operations which did not utilize antibiotics. This is the same relationship as was seen with the personal measurements.

Table 11: Endotoxin associations with antibiotic use in FH operations

	N	Mean	SE	Sig.
EU/m^3				
Using antibiotics	6	3.58	0.07	
Do not use antibiotics	8	3.27	0.05	<0.05
EU/mg				
Using antibiotics	6	2.97	0.08	
Do not use antibiotics	9	2.56	0.05	<0.05

Separate linear regression models were fitted for the area measures with the outcomes of dust (mg/m^3), endotoxin concentration (EU/m^3) and endotoxin load (EU/mg). Firstly, a crude model was fitted that included only type of operation (model 1). Secondly, an adjusted model was fitted which included type of operation and the potential confounder (model 2). The beta coefficients for type of operation were compared between the two models to assess confounding where confounding was considered to be present if there was greater than a 15% difference between the models.

There appears to be confounding present after adjusting for feed type (Table 12). Feed type is confounding the association between endotoxin concentration and type of poultry operation. This is also true for endotoxin load, but not to the same extent. Feed type does not appear to confound the association between dust concentration and housing type.

Table 12: Crude and adjusted models in the assessment of confounding by feed type in the association between type of operation and area environmental variables

	β	Std Error	Sig.
Regression models for EU/m^3			
Model 1 – CH and FH	-0.20		0.098
Model 2 – CH and FH	-0.13	0.10	0.19
Feed Type	-0.35	0.15	0.02
Regression models for EU/mg			
Model 1 – CH and FH	0.20	0.10	0.06
Model 2 – CH and FH	0.25	0.09	0.004
Feed Type	-0.27	0.11	0.01
Regression models for mg/m^3			
Model 1 – CH and FH	-0.40	0.07	<0.001
Model 2 – CH and FH	-0.39	0.06	<0.001
Feed Type	-0.07	0.09	0.41

* β is the regression coefficient for the difference between FH and CH operations

Bird age appears to confound the association between the operation type (CH or FH) and the endotoxin concentration and endotoxin load for the area measures (Table 13). The crude and adjusted models are very similar for dust concentration, suggesting that bird age does not confound the association between dust levels and operation type. There are however large variations between the crude and adjusted models for endotoxin concentration and endotoxin load, suggesting that bird age confounds the association between endotoxin levels and poultry operation type.

Bird number is also confounding the association between operation type and the environmental variables for area measures (Table 14). The crude and adjusted models are very similar for dust concentration, suggesting that bird number does not confound the association between dust concentration and poultry operation type. However, the crude and adjusted models are quite different for endotoxin concentration and endotoxin load, suggesting that bird number confounds the association.

Table 13: Crude and adjusted models in the assessment of confounding by bird age in the association between type of operation and area environmental variables

	β	Std Error	Sig.
Regression models for EU/m^3			
Model 1 – CH and FH	-0.20		0.098
Model 2 – CH and FH	-0.03	0.38	0.94
Bird Age	-0.004	0.009	0.67
Regression models for EU/mg			
Model 1 – CH and FH	0.20	0.10	0.06
Model 2 – CH and FH	0.32	0.27	0.23
Bird Age	-0.003	0.006	0.66
Regression models for mg/m^3			
Model 1 – CH and FH	-0.40	0.07	<0.001
Model 2 – CH and FH	-0.40	0.20	0.07
Bird Age	-0.0007	0.004	0.86

* β is the regression coefficient for the difference between FH and CH operations

Table 14: Crude and adjusted models in the assessment of confounding by bird number in the association between type of operation and area environmental variables

	β	Std Error	Sig.
Regression models for EU/m^3			
Model 1 – CH and FH	-0.20	0.12	0.098
Model 2 – CH and FH	-0.32	0.14	0.03
Bird Number	-0.000	0.000	0.06
Regression models for EU/mg			
Model 1 – CH and FH	0.20	0.10	0.06
Model 2 – CH and FH	0.13	0.11	0.26
Bird Number	-0.00	0.000	0.23
Regression models for mg/m^3			
Model 1 – CH and FH	-0.40	0.07	<0.001
Model 2 – CH and FH	-0.45	0.08	<0.001
Bird Number	-0.00	0.00	0.10

* β is the regression coefficient for the difference between FH and CH operations

The significant and biologically relevant variables (feed type, bird age and bird number) were included in a linear regression model with the type of poultry housing (Table 15). The reference category was FH operations. After controlling for feed type, bird number and bird age there was a significant negative association between dust concentration and type of operation. That is, there was less dust concentration in the CH operations as compared to the FH operations. The relationships for endotoxin are not statistically significant. Although not statistically significant, the associations between endotoxin concentration and endotoxin load with type of poultry operation are in opposite directions. There is a negative relationship for endotoxin concentration and a positive relationship for endotoxin load. However, due to the small sample size, interpretations should be made cautiously.

Table 15: Multiple regression analyses of endotoxin and dust measurement with feed type, bird number and bird age

	β	Std Error	Sig.
Endotoxin Concentration, EU/m^3			
Bird Age	0.002	0.006	0.76
Bird Number	-0.000	0.000	0.01
Feed Type	-0.35	0.11	0.002
Type of Housing (FH reference)	-0.33	0.28	0.24
Endotoxin Load, EU/mg			
Bird Age	0.001	0.005	0.83
Bird Number	-0.000	0.000	0.19
Feed Type	-0.27	0.08	0.001
Type of Housing (FH reference)	0.14	0.23	0.54
Dust Concentration, mg/m^3			
Bird Age	0.001	0.004	0.76
Bird Number	-0.000	0.000	0.07
Feed Type	-0.07	0.08	0.35
Type of Housing (FH reference)	-0.49	0.20	0.02

* β is the regression coefficient for the difference between FH and CH operations

3.5 DISCUSSION

The findings illustrate significantly greater endotoxin per milligram of dust (EU/mg) in the respirable fractions of the area dust samples in the CH poultry operations as compared to the floor housed operations even though the FH poultry operations consistently had significantly greater levels of dust. Personal measures were included in the results but due to the short sampling time and small sample size, it was difficult to draw inferences from the personal measurements. The personal measures were therefore included to support the findings from the area measures in terms of similar exposure patterns. Overall, the results indicate that barn and handling characteristics may influence the association between type of housing and environmental variables. The results show the same trends utilizing either personal or area

environmental measurement values. The negative correlations between stocking density, bird number and endotoxin levels for the CH operations. These negative correlations could be related to the environmental samples being point estimates. These point estimates occurred over a wide period of the production cycle (47.0±15.4 weeks of age). It is possible that the endotoxin levels over the production cycle in CH operations are not linear relationships over time. It is possible that the endotoxin levels could begin high and reduce over time, or that there could be more spurious variations over time for endotoxin. A study which measures the endotoxin levels over the growth cycle in CH operations would be a better indicator of the correlations in environmental variables with stocking density and bird number. The results augment the need to measure and report potential variables of influence such as feed type, bird age and bird number when reporting environmental measures from CH and FH poultry operations.

The MMAD for area and personal dust measures were significantly greater in the FH poultry operations as compared to the CH operations (16.56 and 18.72 versus 12.64 and 11.86μm respectively). For both types of operations the MMAD were in the thoracic fraction with an aerodynamic diameter (d_{50}) of >10 μm, and where particulates would typically deposit anywhere within the lung airways.[12] The MMAD for endotoxin load at approximately 3μm is similar for the two types of operations but a much smaller MMAD than that of the dust. The MMAD for endotoxin is consistent with the typical size of enteric Gram-negative bacteria[13] and at 3μm is at a size fraction considered to be respirable (4um d_{50}). Particles of a respirable aerodynamic diameter would have a tendency to deposit in the gas-exchange regions of the lung.[12] The mass median aerodynamic diameter results (MMAD) from the CH and FH poultry operations provided additional insight into the deposition properties of the dust and endotoxin from the two different types of poultry housing. The dust MMADs were very similar between the area and personal measurements. FH poultry operations had a greater MMAD than the CH operations although all calculations had an aerodynamic diameter (d_{50}) of greater than 10μm, with greatest potential respiratory health effects in the thoracic region. This size fraction and related level of deposition could assist in explaining the greater cough and phlegm experienced by poultry workers in general. These findings could assist in explaining the greater cough and phlegm symptoms which are consistent with upper or lower respiratory insults, and wheeze, a lower respiratory symptom, which are common symptoms experienced by workers from the

poultry operations. The differences in the MMADs between the FH and CH operations, although significant, both were still considered of a thoracic nature. Therefore, other factors are likely contributing to the different respiratory health effects experienced by the CH and FH poultry operation workers.

Similar variations in aerodynamic diameter between dust and endotoxin in which there is a much greater aerodynamic diameter for dust as compared to endotoxin have been shown for corn farms and swine operations,[14, 15] with a three fold enrichment of endotoxin in the aerodynamic size fractions less ≤8.5µm in swine operations.[15] A study of poultry barns in the United States where dust and endotoxin were fractionated showed a MMAD of 15µm for dust with most of the dust mass represented by non-respirable particles and endotoxin load highest in the respirable size fraction less than 3.5µm.[16] The poultry samples were collected in three broiler poultry barns on a cassette impactor with four cut-off diameters between 20 and 3.5µm with the back-up filter collecting the size fractions <3.5µm. Although the sample size is very small and the impactor design allowed for wide variations in cut-size, the differences in dust and endotoxin load between the respirable and non-respirable fractions supports our findings.

The sample size from this study is too small to make inferences regarding respiratory health effects. Although the results showed a 0.29 liter loss in FEV_1 over the work-shift for the FH workers, which was significantly different from the CH workers, this loss in FEV_1 needs to be interpreted with caution as there were only 15 subjects per group. Additionally, subjects with pre-existing respiratory illness were not excluded from the analysis. Our previous study utilizing workers from the same cohort showed that workers from CH operations appeared to suffer from greater chronic cough and phlegm as compared to workers from FH poultry operations,[2] and poultry workers have been shown to suffer from some of the highest prevalence of respiratory symptoms.[1] The literature indicates that endotoxin may be a prime agent responsible for the respiratory health effects experienced by workers,[3, 4, 10, 17] and endotoxin has been shown to be a significant predictor of chronic phlegm production for poultry workers.[2]

Fractionation of dust and endotoxin was undertaken with both personal and area sampling. Many of the personal samples were of shorter duration and there were low dust and

endotoxin levels on the lower stages of the samples due to the amount of worker time, and therefore the amount of sampling time, in the barns. The low levels of dust and endotoxin limited the generalizability of the results for the personal samples. In general, the personal measures followed similar patterns for dust and endotoxin as the area measurements. The similar trend between personal and area measurements provided some reassurance in the conclusions that were drawn from the area measurement results and the potential relationships to worker respiratory outcomes. Fractionating the dust provided a framework for understanding the nature of the dust in the two types of poultry production operations and provided a justifiable foundation for future respirable or inhalable sampling. Respirable or inhalable sampling is more compressed sampling as compared to the Marple sampler and numerous Marple stages could be collected as a complete sample with the respirable or inhalable samplers and may therefore be more practical for achieving reliable personal sampling in shorter sampling durations.

The different dust MMAD for the two types of poultry operations may play some part in explaining the differences in respiratory health effects experienced by workers.[2] Although the dust MMADs were of a thoracic fraction for both the CH and FH operations, the deposition and inflammatory capabilities of the dust in the CH poultry operations may be different from those of the FH poultry operations, and coupled with the endotoxin size fraction effects, may induce a different reaction in the lungs.

The MMADs for the particles containing the endotoxin were very similar for area and personal measures and very similar between the CH and FH poultry operations with an overall average aerodynamic diameter of approximately 3.4 μm. The MMAD for endotoxin is consistent with the typical size of enteric Gram-negative bacteria and at 3 μm would be considered in the respirable size fraction. Particles of a respirable aerodynamic diameter would have the tendency to deposit in the gas-exchange regions of the lung. As the endotoxin MMADs do not differ by poultry housing type the effect of deposition alone from the endotoxin does not assist in explaining the respiratory symptom differences experienced by the workers from the two different poultry housing environments. The MMAD deposition may however help explain why poultry workers in general may experience greater respiratory health effects than other industrial workers and other workers exposed to organic dusts. The deposition characteristics of

the endotoxin on the smaller respirable dust particles may be producing the greater respiratory reactions.

The similar MMAD but differing levels of respirable endotoxin load between the two types of poultry operations, and the different respiratory response experienced by workers[2] would indicate there may be other factors at play in this environment. The two types of poultry operations may differ in the types of bacterial species present in the environment and/or the bacteria present in these environments may be of different chemical composition with differing endotoxin potency. Endotoxin analysis by Marple stage allowed for some distinctions to be made by finer size classifications than respirable and non-respirable. Dust by stage tended to follow a pattern of being greater in the FH poultry operations. For endotoxin load (EU/mg), stage 5 at 3.5-6.0µm cut-point has the greatest differences between the two types of operations with CH poultry operations having significantly greater endotoxin load. The trend for greater endotoxin load in the CH poultry operations as compared to the FH operations followed through to the smaller stages. The differences in endotoxin may relate to housing management differences which may have an impact on the differences in bacteria and fungi present in these two types of operations.

The findings could also indicate differences not only in levels of common bacteria but perhaps also differences in the chemical composition or potency of the endotoxin present in the two types of operations. The LAL assay, utilized in this study, is a measure of endotoxin potency and mainly detects biologically active endotoxin. The biological activity of endotoxin is dependent on the bacterial species and may differ between cell-bound and free endotoxin.[19, 20] Measures of 3-hydroxy fatty acids using gas chromatography-mass spectrometry (GC-MS) may have provided additional information on the differences in endotoxin present in these two environments as the GC-MS assay detects the 3-hydroxy (OH) fatty acids (3-OH) as chemical markers of endotoxin and can be quantified from both biologically-active and inactive endotoxin.[19] The lipid A component of endotoxin, which is highly conserved among Gram-negative bacteria, is thought to mediate the physiologic effects of endotoxin. It has been suggested that differences in the chemical composition of the endotoxin could relate to differences in the pulmonary toxicity of endotoxin. Specifically, 3-OH-14:0 fatty acid has been

associated with respiratory health effects in workers and animals.[21, 22] Helander et al[22] found that endotoxin of Enterobacteriacaeae, composed of predominantly 3-OH-14:0 fatty acid, had higher biological activity than endotoxin of bacteria which included other 3-OH fatty acids. If bacterial species and chemical composition of LPS differ by poultry housing type this may assist in explaining the differences in not only endotoxin levels between the two types of operations but also the respiratory response experienced by workers.[1, 2]

There were a number of limitations to our study the first of which is the sample size for the personal measures. Due to the shorter work period of the workers, there is less personal sampling time for the FH poultry operation workers resulting in lower levels of particulate collection. As well, only one personal sample was collected per barn and a larger personal sample size might assist in further delineating the results. A much larger study involving personal sample collection and respiratory assessment of workers would be required to assess respiratory health effects and relationships to endotoxin load. Second, endotoxin levels were measured using the LAL method, which has been shown to have variations and is a measure of only biologically active endotoxin. Although associations between total endotoxin measures and respiratory health effects have not yet been established, measuring total endotoxin with GC-MS may have provided additional information in further delineating these results.

Future research into the types and chemical nature of bacteria present at the different size stages of particulate for the two types of poultry production would assist in determining if the differences in endotoxin load relate to the presence of bacteria, levels of similar bacteria, and/or composition of the endotoxin. Cell stimulation looking at resultant inflammatory responses utilizing the varying size fractions of particulates from the two types of operations would further assist in understanding the work environment and respiratory responses.

3.6 CONCLUSIONS

Poultry operations differ in the levels of dust and endotoxin present in the environment in relation to the type of poultry housing. Operations in which poultry are housed on the floor appear to have much greater levels of respirable and non-respirable dust as compared to

operations in which the poultry are housed in cages. In the presence of the significantly lower dust levels in the area samples of operations in which the poultry are housed in cages, there is significant enrichment of endotoxin in the respirable fractions of these lower dust levels. The MMAD of the dust from both types of poultry operations is greater than 10µm. The MMAD for the endotoxin is similar for both types of operations at 3µm and would be considered a respirable aerodynamic diameter. Although the dust MMADs were of a thoracic fraction for both the CH and FH operations, the deposition and inflammatory capabilities of the dust in the CH poultry operations may be different from those of the FH poultry operations and may have the ability to induce a different reaction in the lungs of exposed workers.

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4. PAPER THREE: "IT'S A STICKY ISSUE": DIFFERENCES IN PARTICLE BOUNCE AND ENDOTOXIN LEVELS IN MARPLE CASCADE SAMPLERS WITH GREASED AND UNGREASED FILTERS

"It's a sticky issue": Differences in Particle Bounce and Endotoxin Levels in Marple Cascade Samplers with Greased and Ungreased Filters

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4.1 ABSTRACT

Objectives: The effect of particle bounce with polyvinyl chloride (PVC) filters in Marple cascade impactors when sampling with poultry barn dust was unknown. Similarly, the effect of impaction grease on endotoxin levels in the measured particulate was unknown. Methods: A pilot study was undertaken utilizing six-stage Marple cascade impactors and PVC filters to estimate the particulate size distribution distortion and impaction grease effects on endotoxin levels in sampled poultry dust in a wind tunnel. Results: The mass median aerodynamic diameter (MMAD) results indicate that there was greater than 50% decrease in MMAD when grease was not utilized in the impactor and there was no significant difference in the overall dust concentration. There was no significant difference in the MMAD for endotoxin between the greased and ungreased filters. There was no difference in airborne endotoxin concentration between filters treated with impaction grease and those not treated. Conclusions: The results indicate that although the differences were not significant, particle bounce appears to be present in the Marple sampler with PVC substrates when not utilizing impaction grease when sampling poultry barn dust. The differences appear to be greatest in the lower cut-points.

4.2 INTRODUCTION

Impactors operate under the principle that when a stream of particle laden air is directed at a surface, particles of sufficient inertia will impact upon the surface and be collected. In cascade impactors, particle laden air enters and passes through a series of progressively smaller jets, where progressively smaller particles are collected on each stage. The theory of impaction is well developed and has been generally confirmed.[1-3] Impactors are most often utilized to assist in assessing the respiratory impact of particles. It is thought that since the lung penetrability of unit density particles is known, and the impactor collects particle sizes on each stage of a calibrated sampler, then if an impactor is used according to standard operating procedure, the stage distribution of collected material should indicate the extent to which the sample would penetrate the respiratory system.[4] Along with this information and the knowledge of the chemical and/or biological properties of the collected particulate, the nature of the health hazard could be estimated.[4] This theoretical approach is consistent until particle related effects are

introduced such as particle bounce, re-entrainment and stage loss. If particles bounce or blow-off subsequent to collection, particles penetrate their appropriate stage and contribute to the collection at smaller stages, thereby distorting the size distribution.[5, 6] The hardness of collected particles appears to be a significant factor influencing the collection characteristics of surfaces.[6] Soft materials appear to deform more readily on impact than hard material; consequently, it is expected that bouncing is minimized for soft materials.[6] This leads to the suggestion that particle bounce will be dependent on the nature of the particle and the type of collection surface.[6] It is suggested that an adhesive impaction surface be utilized for collection of particles on certain substrates as a means of reducing loss.[6-15] The use of an adhesive may present additional difficulties including loss of adhesive in the gravimetric analysis and possible interference of the adhesive during chemical analysis.[9, 16] As Hinds et al.[6] indicated, " underscoring the need for field evaluation of impaction surfaces prior to sampling".

The bounce effects when sampling poultry barn air with Marple samplers utilizing polyvinyl chloride (PVC) filters were unknown. The larger experimental design required post analysis of samples for endotoxin and the effects of impaction grease on this process were unknown. The objectives of the study were to: (1) test the difference in bounce between six stage Marple samplers with PVC filters and no impaction grease to those treated with impaction grease; and (2) to test the differences in endotoxin levels between treated and untreated filters.

4.3 **METHODS**

4.3.1 *Wind Tunnel*

A bulk sample of poultry dust (30 grams) was run through a wind tunnel in which four six-stage Marple samplers were stationed. For each of three wind tunnel runs that were carried out, the filters of two of the Marple samplers were treated with impaction grease (greased, n=6) and two of the Marple samplers were not treated (ungreased, n=6). Temperature, relative humidity and wind speed were measured at the beginning of each of the three wind tunnel sampling runs utilizing a VelociCalc (8347A-M-G, TSI Inc., Shoreview, MN). Aerosols were produced from agricultural dusts collected in the field, sieved to remove particles larger than 420μm using a 42 mesh sieve, and ground with a ball mill (Glen Mills Inc., Clifton, NJ). A wind

tunnel with 1-m^2 cross sectional area was used for each of the sample runs. Samplers were placed in the middle of the wind tunnel attached to stationary rods. For each sampling run, 30 grams of poultry dust was generated into the wind tunnel using an NBS dust feeder.

4.3.2 *Impaction Grease*

Dow Corning 316 silicone release spray (Dow Corning, Midland, MI) was administered to each of the treated filters. A greasing template (Model 290-IGT, Anderson Instruments, Smyrna, GA) was utilized for application of the impaction grease. Filters were placed on the bottom plate of the greasing template with two locating pins through opposite perforations of the filter. The top plate of the greasing template, located by the two placing pins, was placed on top. A one-pass thin layer of grease was sprayed within the six slots of the template. Manufacturers suggested drying time was three minutes, although a fifteen minute drying time was given for each filter, after which the filter was weighed.

4.3.3 *Dust and Endotoxin Levels*

Dust and endotoxin were collected using Marple cascade impactors (Anderson Instruments, Smyrna, GA) connected to SKC constant airflow pumps (Universal 224-PCXR4, Eighty Four, PA) run at 2 liters per minute (lpm) with radial slit polyvinyl chloride (PVC) filters (5µm, Thermo Electron Corp., Waltham, MA) with a PVC backup filter (0.8 µm, 37mm SKC, Eighty Four, PA). The Marple sampler contained 6 stages to represent cut-points of 0.52, 0.93, 1.55, 3.50, 6.0 and 9.8µm. Impactors were calibrated pre and post sampling utilizing a calibration adapter (Anderson Instruments, Smyrna, GA) and DC-Lite primary flow meter (DCL-M, Bios International, Butler NJ). All filters were dessicated for 12 hours prior to initial weighing and grease application and after post sampling. Filters were individually analyzed for dust [milligrams of dust (mg) and milligrams of dust per m^3 of air (mg/m^3)] (MX5 microbalance, Mettler-Toledo, Greifensee, Switzerland) and with Kinetic-QCL Limulus Amebocyte Lysate assay (*E. coli* O55:B5; Cambrex BioScience Walkersville Inc, Walkersville, MD) for airborne endotoxin and endotoxin concentration [endotoxin units per m^3 of air (EU/m^3) and endotoxin units per mg of dust (EU/mg)]. Endotoxin samples were referenced to the RSE: EC-6.

4.3.4 *Statistical Analyses*

Analyses were completed using SPSS version 13. To normalize data, environmental variables were log transformed prior to analyses. Geometric means, geometric standard deviations and medians describe environmental variables including dust mass (mg), dust concentration (mg/m^3), endotoxin load (EU/mg) and endotoxin concentration (EU/m^3). Means and standard deviations describe wind tunnel measurements. For Marple stage data, differences in means of continuous variables between study groups were tested using independent sample t-tests. For total samples, differences in means of continuous variables between study groups were tested using Mann-Whitney tests.

4.4 **RESULTS**

The average wind tunnel speeds for the three runs were 0.20±0.02 meters/second. For the three runs, the average run time to circulate the 30g of poultry dust was 35.3±12.6 minutes, with an average relative humidity in the wind tunnel of 41.7±3.2% and an average temperature of 19.6±0.5 degrees Celsius.

Assessing the Marple data by individual stage (Table 1) there were no significant differences between filters which were treated with impaction grease and those not treated with impaction grease for dust mass, dust concentration, endotoxin load or endotoxin concentration.

Table 1: Dust and Endotoxin by Marple Stage

	Grease GM±GSD n=3	**No Grease** GM±GSD n=3	**Sig.**
Stage 3			
Dust, mg	0.45±1.23	0.28±1.91	0.09
Endotoxin, EU/mg	761.33±1.35	962.52±1.36	0.39
Dust, mg/m^3	6.51±1.61	4.18±2.33	0.31
Endotoxin, EU/m^3	4956.26±1.47	4021.99±2.16	0.39
Stage 4			
Dust, mg	0.09±1.90	0.15±1.35	0.24
Endotoxin, EU/mg	1549.97±1.87	1028.32±1.23	0.13
Dust, mg/m^3	1.28±1.73	2.13±1.68	0.18
Endotoxin, EU/m^3	1984.51±1.74	2194.61±1.65	0.59
Stage 5			
Dust, mg	0.01±10.37	0.05±8.04	0.18
Endotoxin, EU/mg	3464.59±8.88	1617.50±10.58	0.39
Dust, mg/m^3	0.20±11.50	0.71±6.80	0.18
Endotoxin, EU/m^3	676.48±2.07	1146.77±1.74	0.24
Stage 6			
Dust, mg	0.02±4.52	0.04±1.71	0.39
Endotoxin, EU/mg	2939.36±4.83	964.37±2.18	0.24
Dust, mg/m^3	0.25±5.83	0.60±2.25	0.70
Endotoxin, EU/m^3	739.06±1.75	582.78±1.19	0.59
Stage 7			
Dust, mg	0.01±3.35	0.01±8.94	0.70
Endotoxin, EU/mg	308.23±3.23	843.91±6.15	0.39
Dust, mg/m^3	0.14±4.32	0.097±10.08	0.94
Endotoxin, EU/m^3	42.93±1.83	77.23±1.94	0.18
Stage 8			
Dust, mg	0.01±6.00	0.01±3.09	0.31
Endotoxin, EU/mg	143.78±5.74	196.04±2.55	1.00
Dust, mg/m^3	0.07±7.99	0.13±3.62	0.70
Endotoxin, EU/m^3	9.73±2.45	25.24±1.68	0.06
Final stage			
Dust, mg	0.07±30.94	0.15±5.80	0.94
Endotoxin, EU/mg	20.70±45.92	20.83±6.12	0.79
Dust, mg/m^3	1.07±42.22	2.25±7.17	0.94
Endotoxin, EU/m^3	17.45±2.54	46.79±2.34	0.18

Assessing the Marple data by total sample (Table 2), i.e. all stages together, shows there were no significant differences between impactors treated with impaction grease as compared to impactors with no treatment in dust mass, dust concentration, endotoxin load or endotoxin concentration.

Table 2: Dust and Endotoxin

	Grease n=3		No Grease n=3		
	GM±GSD	Median	GM±GSD	Median	Sig.
Dust Mass, mg	1.07±2.01	0.78	1.01±1.66	0.81	1.00
Dust Concentration, mg/m^3	15.61±2.80	10.73	14.92±2.27	9.96	0.94
Endotoxin load, EU/mg	548.78±1.95	572.65	560.53±1.41	653.66	0.82
Endotoxin Concentration, EU/m^3	8568.41±1.56	7329.31	8363.73±1.82	6204.38	0.82

Differences in mass median aerodynamic diameter (MMAD) between filters treated with impaction grease and those not treated are given in Table 3. The difference in MMAD for dust or endotoxin between treated and untreated Marple samplers is not significant, although the treated filters showed a 52% decrease in MMAD. Figure 1 graphs the differences in the mass fraction percentages for the dust at the different Marple stages. Table 4 indicates there were no significant differences in the mass fraction percentage by stage between greased and ungreased samplers. Figure 2 indicates there is no difference in the mass fraction percent difference of endotoxin load between treated and untreated filters. There were no differences in the above dust mass or endotoxin load relationships after correcting for the effect of interstage loss.

Table 3: Dust and Endotoxin Mass Median Aerodynamic Diameter

	Grease n=3	No Grease n=3	Sig.
	GM±GSD	GM±GSD	
Dust MMAD, µm	21.38±13.41	10.22±5.59	0.12
Endotoxin MMAD, µm	3.91±1.26	3.70±0.60	0.71

Table 4: Dust Mass Fraction Percent by Stage

	Grease	No Grease	%	Sig.
	Mean±SD	Mean±SD	difference	
Stage 3 (>9.8µm), %	47.2±23.5	32.8±17.0	30.5	0.24
Stage 4 (6.0-9.8µm), %	12.0±9.1	15.4±6.5	22.1	0.48
Stage 5 (3.5-6.0µm), %	4.6±6.7	17.1±25.4	73.1	0.18
Stage 6 (1.54-3.5µm), %	2.5±2.2	4.6±2.3	45.6	0.24
Stage 7 (0.91-1.54µm), %	1.2±0.9	2.2±3.0	45.4	0.82
Stage 8 (0.53-0.91µm), %	0.8±1.0	1.3±1.0	38.5	0.39
Back-up (< 0.53µm), %	31.6±29.5	26.5±24.1	16.1	0.94

Figure 1: Endotoxin load Fraction Percent by Marple Stage

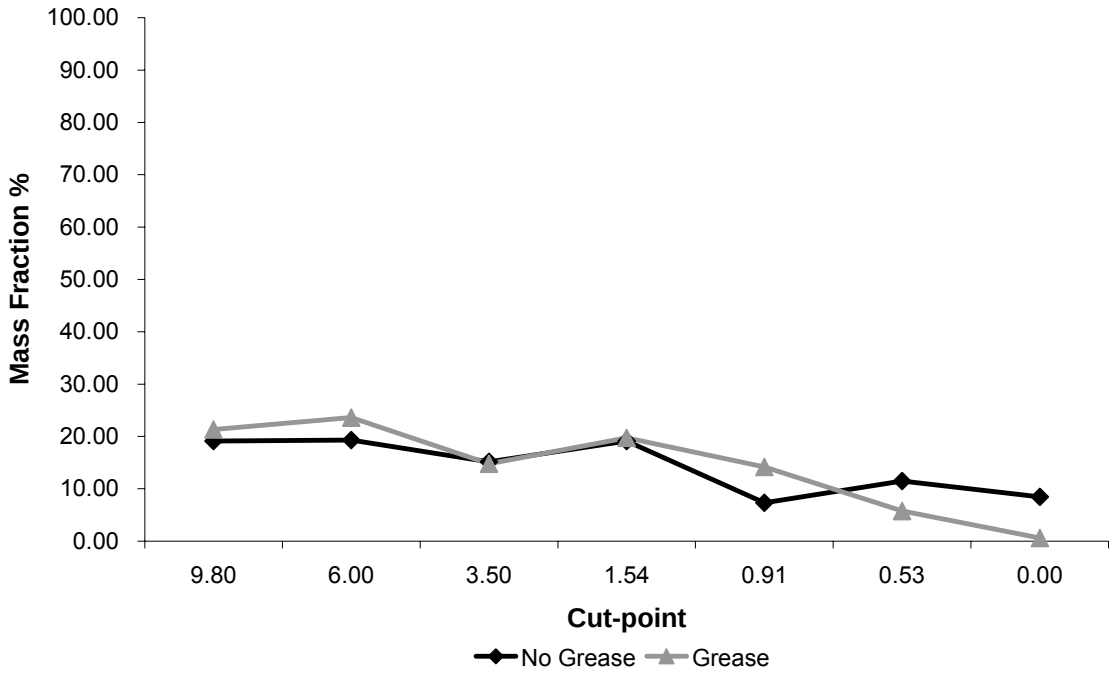
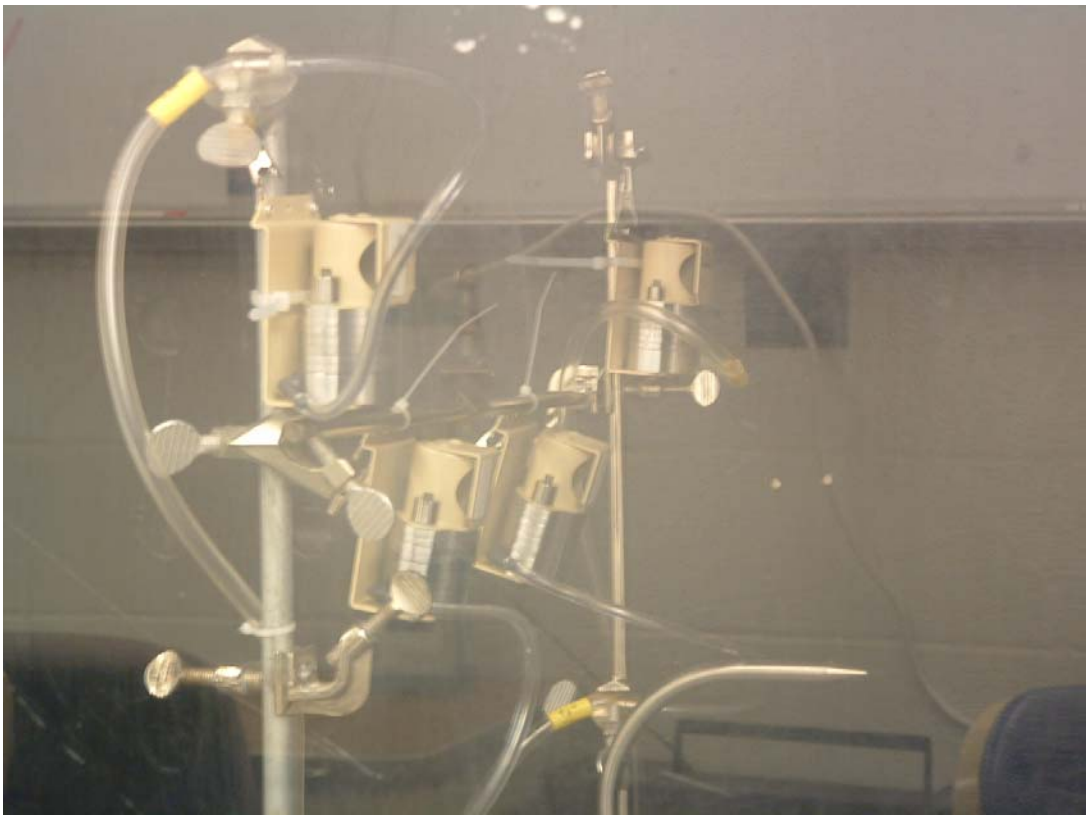


Figure 2: Greased and Ungreased Marple Samplers in the wind tunnel



4.5 DISCUSSION

Our results suggest there was no significant difference in dust or endotoxin levels for Marple samplers run at 2 liters per minute sampling poultry dust with polyvinyl chloride filters which were coated with impaction grease compared to filters not coated with impaction grease. Although there was a 52% decrease in dust MMAD between greased and ungreased filters, there was also large variation, resulting in no significant difference in the mass median aerodynamic diameter (MMAD) when grease was not applied to the filters as compared to greased filters. Similar mass was collected between the greased and ungreased impactors suggesting that the total mass of dust was likely collected during sampling.

The percent mass differences varied across the stages suggesting that perhaps similar particle distributions were not collected by the different Marples in the wind tunnel, or that the dust was not homogenously distributed in the wind tunnel resulting in different size fractions for the different Marples. The largest particles (stage 3, >9.8μm) were more efficiently collected on the greased filters. This would be highly plausible as the larger the particle, or the greater the particle velocity, the more likely particles will collect on a surface other than the intended surface.[17] Coating with grease increases adhesion energy, deformation, and the dissipative energy and greatly reduces a bounce effect.[17] The mass fraction which was not collected on the initial stages of the ungreased impactor stages could potentially have been redistributed throughout the lower stages as indicated by the different mass percentages in the lower stages between the greased and ungreased filters. A potential redistribution of the mass could lead to an underestimation of the MMAD. This effect has been identified previously for both high volume samplers[8-10] and low volume samplers.[6, 15]

Distortion of the aerosol size distribution becomes important when references of the deposition patters to the human respiratory tract are to be inferred from the data. A significantly lower MMAD would lead to inferences of potential effects to the lower airways of the human respiratory tract when in reality the MMAD and resultant effects may be much different if redistribution were not present.[13] Redistribution would hinder an accurate calculation of the inhalable, thoracic and respirable fractions of the sample.

A limitation to utilizing a coating in cascade samplers has been the prohibitive effects of non-inert coatings on chemical and toxicological characterization of collected particles.[9, 16] The effect of Dow Corning silicone release spray on an endotoxin assay was unknown. The Limulus Amebocyte Lysate (LAL) reaction is an enzyme mediated reaction and as such has an optimal pH range and specific salt and divalent cation requirements. The effect of the coating on the LAL reaction was unknown. Inhibition of the LAL would result in longer reaction times and therefore indicate lower levels of endotoxin than may be present. There were no significant differences in the endotoxin concentrations between the greased and ungreased filters indicating a small likelihood of inhibition of the LAL assay from the impaction grease. Although the differences were not significant, there were divergences between the greased and ungreased filters at the lower cut-point sizes. These lower cut-point sizes were anticipated as being very important to the major hypotheses of the research program, and any potential effects in the smaller cut-points needed to be avoided. According to the manufacturer, the silicone spray is insoluble in water and it therefore may have had a reduced impact on the LAL assay from our methodology. If additional or other agents other than water were utilized for particle release from the filters, the inhibition results of the LAL assay may significantly vary.

There were a number of limitations to utilizing grease with PVC filters. Although a greasing template was utilized, the filters had a tendency to tear upon removal from the greasing template. Additionally, achieving a similar grease application for each filter was difficult to attain. Nozzle pressure, nozzle occlusion and pass speed varied between filters resulting in variable grease application rates. The manufacturer recommended a three minute drying time, although we utilized a fifteen minute drying time. Other studies had utilized no drying time[8] up to a twenty-four hour drying time.[10]

4.6 CONCLUSIONS

Sampling poultry dust with Marple cascade impactors run at 2 liters per minute with PVC filters and no impaction grease versus Marple cascade impactors with impaction grease showed no significant difference between the two methods. The percent mass differences varied across

the stages suggesting that perhaps similar particle distributions were not collected by the different Marples in the wind tunnel, or that the dust was not homogenously distributed in the wind tunnel resulting in different size fractions for the different Marples.

The effect of impaction grease on endotoxin analysis was less clear. It appeared that endotoxin readings had the potential to be influenced by impaction grease, particularly when very low amounts of endotoxin were anticipated. The specific outcomes to be analyzed would need to be weighed against the potential influence of the impaction grease on the chemical analysis to determine grease application. Other methods for particle dissolution from filters treated with silicone release spray may have differing effects on the endotoxin assay which would need to be further investigated.

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5.0 DISCUSSION

5.1 **OVERALL DISCUSSION**

This research program took an industrial hygiene approach to further understanding the work environment and respiratory health effects for those individuals who work in cage-housed (CH) and floor-housed (FH) poultry operations.

In an original 2003 study, separate from the work presented herein, workers from poultry operations in Western Canada were studied along with matching grain farmer and non-farmer control subjects.[1] Results indicated that workers from CH poultry operations had greater cough, phlegm, wheeze and shortness of breath than workers from FH operations, grain farmers and non-farmers.[1] Workers from CH operations had significantly lower mean values for forced expired volume in the first second (FEV_1) compared to workers from FH poultry operations.[1] The symptoms and pulmonary function results suggested an asthma-like syndrome.[1] This research did not however undertake to examine workplace environmental factors. Levels of contaminants such as dust and endotoxin could be important in explaining differences in worker respiratory responses.

This thesis program expanded on the above study[1] and the literature by investigating poultry workers in relation to the type of bird housing in which they worked (CH or FH) and examining more in-depth the environmental variables of dust and endotoxin and potential relationships to respiratory symptoms.

A cross sectional study was performed to assess the environmental exposure levels and respiratory health effects of workers who worked in CH and FH poultry operations (Paper 1). Personal total dust and endotoxin measurements combined with respiratory symptoms and across-shift lung function tests were undertaken on poultry workers. The respiratory results for all poultry workers were similar to the results obtained in the original study (Paper 1, Table 1) suggesting an asthma-like syndrome in these workers. The respiratory symptom results suggest that working in the poultry industry can induce an asthma-like syndrome, at least in some

workers and methods to reduce exposure, such as wearing respiratory protection, should be encouraged.

There were differences in the respiratory response of the workers depending on the type of bird housing in which the workers worked. Workers who worked in poultry facilities in which the birds were raised in cages (CH) reported greater current and chronic respiratory symptoms and significantly greater current and chronic phlegm as compared to workers from poultry facilities in which birds were raised on the floor (FH) (Paper 1, Table 3). Environmental analysis indicated that workers from the CH poultry facilities were exposed to greater levels of endotoxin concentration than workers from FH poultry facilities, but that the workers from the FH poultry operations were exposed to greater levels of total dust (Paper 1, Table 2 and Figure 1). It was found that endotoxin load (EU/mg) was a significant predictor of chronic phlegm for all poultry workers (Paper 1, Table 4). Endotoxin may be important in the respiratory response experienced by workers and levels of endotoxin or potency of endotoxin may differ by poultry housing type and this difference may be reflected in the difference in respiratory symptoms reported by workers.

Paper 1 helped to further the hypothesis that endotoxin may be important in the respiratory health effects experienced by poultry workers, and in particular the relationship to phlegm. Dichotomizing the poultry work environment by type of bird housing provided insight into the complexity of work environments and how the primary occupant (bird in these cases) of the work space may be similar yet the environmental influences and resultant health effects may differ.

From the literature and Paper 1, respiratory health effects related to the poultry work environment had been most closely associated with endotoxin. Differences in particle size fractions for dust and endotoxin would impact respiratory deposition characteristics and therefore could potentially assist in explaining the differences in respiratory outcomes observed. The approach taken for Paper 2 was to further assess the complex of dust and endotoxin in the work environment and to measure fractionated dust and endotoxin in these two different poultry

housing operations. The intent was to understand if there were particulate size differences for the dust and endotoxin by type of poultry operation.

Marple cascade impactors were chosen as the instrument for measuring the dust and endotoxin size fractions. Although these samplers had been utilized in industry for several years and had been field tested with numerous contaminants, there was no literature available on the potential for particle size misclassification which may occur in the Marple sampler while sampling with polyvinyl chloride filters in an agricultural environment. To reduce the potential for misclassification of particle sizes, the manufacturer recommended that impaction grease be utilized on the filters during sampling. The literature, however, also indicated that impaction grease had the potential to influence chemical analysis of collected agents. Collected samples for this research program were to be further analyzed for endotoxin and understanding the effect of impaction grease on both dust and endotoxin analysis was important. The results of the study (Paper 3) indicated that when impaction grease was not utilized the mass median aerodynamic diameter results were reduced but results were not significantly different between the greased and ungreased Marple samplers (Paper 3, Table 3). Endotoxin readings had the potential to be influenced by impaction grease, particularly for very low amounts of endotoxin (Paper 3, Figure 1). The effect of endotoxin for the smaller particle size fractions was of particular interest in this research project. As endotoxin was considered to be of primary interest in the research project it was decided that reducing any potential influences on this outcome variable was important. It was therefore decided that impaction grease would not be utilized on the Marple samplers for Paper 2. The results would however suggest that impaction grease does have the potential to minimize particle size misclassification and could be beneficial when measuring agricultural dusts, particularly poultry dust, if additional chemical analyses are not going to be undertaken. Additionally, the results suggest the importance of investigating the impact of impaction grease and filter selection prior to sampling Marple samplers.

Size fractioning the dust and endotoxin using area and personal monitoring in CH and FH poultry operations for Paper 2 showed that endotoxin load (EU/mg) was significantly higher in the respirable fraction of area samples in CH poultry operations as compared to FH operations (Paper 2, Table 3). The differences in endotoxin occurred in spite of greater dust mass for all FH

operation measurements (area, personal, respirable, non-respirable, and by individual stage) (Paper 2, Tables 3, 4 and 5). The endotoxin results support the hypothesis that there could be differences in the environment between CH and FH operations, particularly in respirable particulate. The respirable endotoxin load results could assist in explaining differences in respiratory responses of exposed workers. Endotoxin load, particularly for area respirable fractions, was significantly greater in the CH operations. From the literature and Paper 1 (Table 3) it was the workers from the CH operations that experienced the greater symptoms. It is possible that this greater endotoxin load in the respirable mass of particles in the CH poultry operations may play a role in the greater respiratory symptoms experienced by workers. Other agents may also contribute to these symptoms and can not be ruled out from this research.

The mass median aerodynamic diameter results (MMAD) from the CH and FH poultry operations provided additional insight into the deposition properties of the dust and endotoxin from the two different types of poultry housing. The dust MMADs were very similar between area and personal measurements (Paper 2, Table 6). FH poultry operations had a greater MMAD than the CH operations (Paper 2, Table 6). Both CH and FH MMADs were of an aerodynamic diameter (d_{50}) of greater than 10μm and at this size fraction, the greatest potential respiratory health effects are typically in the thoracic region of the lung. The MMADs for the particles containing the endotoxin were very similar for area and personal measures and very similar between the CH and FH poultry operations with an overall average aerodynamic diameter of approximately 3.4 μm (Paper 2, Table 6). The smaller endotoxin MMAD suggests that endotoxin was more highly concentrated in the smaller size fractions of the particulate for both CH and FH poultry operations. The smaller MMAD of endotoxin would also indicate that the higher endotoxin concentrated particles have the potential to penetrate further into the lung, as 3 μm would be considered of a respirable size fraction, as compared to the dust MMADs thoracic tendencies. The concentration of endotoxin in the smaller median size fractions may be important in explaining the greater respiratory symptoms reported in the literature by poultry workers as compared to other industries. It is possible that in other industries the concentration of endotoxin may differ by size fraction and in particular, in other industries with a lesser degree of symptoms endotoxin may be more concentrated in the larger size fractions of particulates.

Similar variations in aerodynamic diameter between dust and endotoxin in which there is a much greater aerodynamic diameter for dust as compared to endotoxin have been shown for poultry barns in the United States where dust showed a MMAD of 15µm with most of the dust mass represented by non-respirable particles and endotoxin load highest in the respirable size fraction less than 3.5µm.[2] These samples were collected in three broiler poultry barns on a cassette impactor with four cut-off diameters between 20 and 3.5µm with the back-up filter collecting the size fractions <3.5µm. Although the sample size is very small and the impactor design allowed for wide variations in cut-size, the differences in dust and endotoxin load between the respirable and non-respirable fractions supports the findings in this dissertation. Similar differentials between dust and endotoxin have been shown for other agricultural exposures including corn farms and swine operations,[3,4] with a three fold enrichment of endotoxin in the aerodynamic size fractions less ≤8.5µm in swine operations.[4] The size fraction for endotoxin in swine operations appears to be greater than that found in the poultry operations, which may help explain differences in respiratory symptoms of workers. It is possible that poultry operations may have a tendency for enrichment of endotoxin in the smaller size fractions as compared to other industries and this enrichment of endotoxin in the smaller and more respirable size fractions may be contributing to the increased respiratory response experienced by exposed workers.

Subjecting the endotoxin data to further interpretation may provide additional insights into potential explanations for respiratory differences in workers. The MMAD provides an indication of the aerodynamic diameter at which fifty percent of the mass of particles is larger and fifty percent of the mass is smaller. It takes many smaller particles to make the same mass as a larger particle and if endotoxin is more concentrated on the smaller particles the potential for respiratory insult is stronger due to the greater surface area per mass unit for binding. Furthermore, the CH poultry operations had significantly greater respirable endotoxin load further strengthening the possibility that the greater prevalence of symptoms that were reported may be due to greater endotoxin effects in the lower respiratory system for workers from CH poultry operations as compared to workers from FH operations.

Due to the high relative humidity, the number of live animals and the presence of feed, water and feces in the building, fungi may be an important component in the work airspace of poultry operations. The respiratory health effects of fungi were not a component of this research program, but the enhancement effect of fungi on the endotoxin assay and results were of interest. In the *limulus* amebocyte lysate assay (LAL) the Factor G pathway can be activated by glucan (from fungi) which would activate a proclotting enzyme into a clotting enzyme resulting in an erroneous increase in the endotoxin level detected. A new to the market endotoxin assay was made available shortly into this research program. The new endotoxin assay marketed as Recombinant Factor C (rFC) selectively only recognizes the Factor C pathway, thereby negating the enhancement effect of glucans from fungi. As LAL has historically been the endotoxin assay of choice and is the methodological assay of choice in the literature, LAL was utilized and reported in this research program. However, a small pilot study was undertaken to compare the LAL assay to the rFC assay to assess if there would be differences between the two assays for the poultry dust samples (Appendix 1). The results indicated that there was strong correlation in the endotoxin units between the LAL and rFC samples from the poultry operations (Appendix 1, Figures 1, 2 and 3). Although the results were strongly correlated in this research study the rFC assay is probably the assay of choice as the rFC assay has been found to detect no (1,3)- β-D-glucan activity, an improvement in specificity compared to the LAL assay. Additionally, the rFC assay is a recombinant assay and as such there should be less lot to lot variability in comparison to the LAL assay.

5.2 **LIMITATIONS**

Certain limitations reduce the generalizability of the results from this research program including the collection period and sample size for the personal measures. Due to the shorter work period of the workers, there is less personal sampling time for the FH poultry operation workers resulting in lower levels of particulate collection (Paper 2). As well, only one personal sample was collected per barn for Paper 2 and a larger personal sample size might assist in further delineating results. Secondly, the worker population studied with the Marple sampler for Paper 2 was too small to utilize the respiratory results for statistical assessment. The respiratory results from the Paper 2 assist in confirming the same nature and extent of symptoms as Paper 1,

but the results can not be utilized for statistical purposes to determine relationships between environmental contaminants and respiratory outcomes. A larger study involving personal sample collection and respiratory assessment of workers would be required to assess respiratory health effects and relationships to endotoxin load.

5.3 FUTURE RESEARCH

Future research into the types and chemical nature of bacteria present at the different size stages of particulate for the two types of poultry production would assist in determining if the differences in endotoxin load relate to the presence of bacteria, levels of similar bacteria, and/or composition of the endotoxin. Endotoxin levels were measured using the LAL assay method, which has been shown to have variability and is a measure of only biologically active endotoxin and not cell-bound endotoxin. Although associations between total endotoxin measures (biologically active and cell-bound endotoxin) and respiratory health effects have not yet been established, assessing total endotoxin with GC-MS may have provided additional important information. Differences in the endotoxin loads between the two types of poultry operations may relate to the types of bacterial species present in the environment and/or the bacteria present in these environments may be of different chemical composition with differing endotoxin potency. The LAL assay utilized in this project, is a measure of endotoxin potency and mainly detects biologically active endotoxin. The biological activity of endotoxin is dependent on the bacterial species and may differ between cell-bound and free endotoxin.[5,6] Assessing 3-hydroxy fatty acids (3-OHFAs) using gas chromatography-mass spectrometry (GC-MS) would provide additional information on the differences in levels of total endotoxin present in these two environments. The GC-MS assay detects the 3-OHFAs as chemical markers of endotoxin and can be quantified from both biologically-active and inactive (cell-bound) endotoxin.[5] The lipid A component of endotoxin, which is highly conserved among Gram-negative bacteria, is thought to mediate the physiologic effects of endotoxin. It has been suggested that differences in the chemical composition of the endotoxin could relate to differences in the pulmonary toxicity of endotoxin. Specifically, 3-OH-14:0 fatty acid have been associated with respiratory health effects in workers and animals.[7,8] Helander et al[8] found that endotoxin of Enterobacteriacaeae, composed of predominantly 3-OH-14:0 fatty acid, had higher biological activity than endotoxin

of bacteria which included other 3-OH fatty acids. The aerodynamic size of Enterobacteriacaeae is of the size fractions which would fit well within the MMAD for endotoxin identified in Paper 2. If bacterial species and chemical composition of LPS differ by poultry housing type this may assist in explaining the differences in not only endotoxin levels between the two types of operations but also perhaps the respiratory response experienced by workers.

Analyzing the dust samples for identification and quantification of bacterial species may assist in explaining the differences between the poultry operations. The microbial content of the different size fractions of the dust could be evaluated using polymerase chain reaction (PCR) with denaturing gradient gel electrophoresis (PCR_DGGE) and. Quantitative Real Time PCR could be used to quantify the microbial load of the various bacteria. A comparison between the two poultry housing types bacterial make-up would further assist in explaining potential health related effects of exposures.

Stimulating cell lines, such as alveolar macrophages, with the varying size fractions of particulates from the two types of operations and looking at the resultant inflammatory responses would further assist in understanding the inflammatory response in relation to the two work environments.

The above methods along with a larger population based study looking at across the work-shift respiratory symptoms and environmental exposures including fractionated dust and endotoxin would further the understanding of worker response to the different poultry operation environments.

5.4 **RECOMMENDATIONS**

The environmental exposures in this environment appear to put workers at risk for current and chronic respiratory symptoms. The incidence of long-term respiratory health effects such as chronic bronchitis and chronic obstructive pulmonary disease from these types of work exposures are not well understood. Utilizing procedures that would reduce direct worker exposure to dusts and endotoxin may assist in reducing respiratory symptoms. Such procedures

could include automatic versus manual egg handling, direct removal of manure from CH operations, automatic feeders versus manual feeders, adequate ventilation associated with stocking density, utilizing low dust litter and floor coverings, and good housekeeping practices to reduce settled dust levels. Wearing respiratory protection such as an N95 respirator would assist in reducing respirable insult for the worker. For the rather short time duration the workers from the FH operations are spending in direct contact with the animals, the use of N95 respirators would be an economically feasible control measure for reducing personal exposures. Generally, it is recommended that poultry operations take steps to reduce dust concentrations and worker exposures in the work environment.

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6.0 CONCLUSIONS

Poultry operations differ in the levels of dust and endotoxin present in the environment in relation to the type of poultry housing. Despite higher dust and ammonia exposures in the floor-housed poultry operations, the workers from the cage-housed poultry operations reported the greater respiratory symptoms particularly significantly greater current and chronic phlegm. The greater respiratory symptoms could relate to a higher endotoxin load on particles. Endotoxin load (EU/mg), was a significant predictor of chronic phlegm for poultry workers. The respirable particles in the CH poultry operations had a greater endotoxin load than the same fraction of particles in the FH operations. It is possible that this greater endotoxin load in the respirable mass of particles in the CH poultry operations may play a role in the greater respiratory symptoms experienced by workers. Other agents may also contribute to these symptoms and can not be ruled out from this research.

The deposition and inflammatory capabilities of the dust and endotoxin in the CH poultry operations may be different from those of the FH poultry operations and may have the ability to induce a different reaction in the lungs of exposed workers. Additionally, the types of microorganisms may differ by poultry operation type. It may be possible that microorganisms which produce more potent endotoxin are present in the CH operations as compared to the FH operations. Understanding the types and chemical nature of bacteria present at the different size fractions of particulate for the two types of poultry production would assist in determining if the differences in endotoxin load relate to the presence of bacteria, levels of similar bacteria, and/or composition of the endotoxin. It is also possible that there may be differences in the levels of cell-bound versus biologically available endotoxin between the CH and FH poultry operations. The assay from this research describes only the biologically available endotoxin. It is possible that cell-bound endotoxin may also be important and could be an immune system stimulant. Determining the levels of total endotoxin present in these two environments would further assist in delineating differences in respiratory effects of CH and FH poultry workers.

Differences in housing characteristics and management practices between CH and FH poultry operations may play an important role in environmental contaminant levels and related worker respiratory response and these variables should be measured and considered. Important characteristics include time spent in the poultry barns including the time spent in direct bird contact as well as time spent sorting eggs. Additionally, the age and number of birds or stocking density should be reported. These variables may be important in the types and levels of endotoxin present in the work environment.

Housekeeping and bird rearing practices which reduce the levels of dust and endotoxin in the work space should be encouraged. Additionally, workers should be encouraged to wear respiratory protection during poultry barn work.

APPENDIX 1: DIFFERENCES IN ENDOTOXIN LEVELS FROM POULTRY BARN DUST: COMPARISON OF RECOMBINANT FACTOR C AND LIMULUS AMEBOCYCTE LYSATE ASSAYS

Figure 1: Correlation between LAL and rFC EU/ml for all samples (CH and FH)

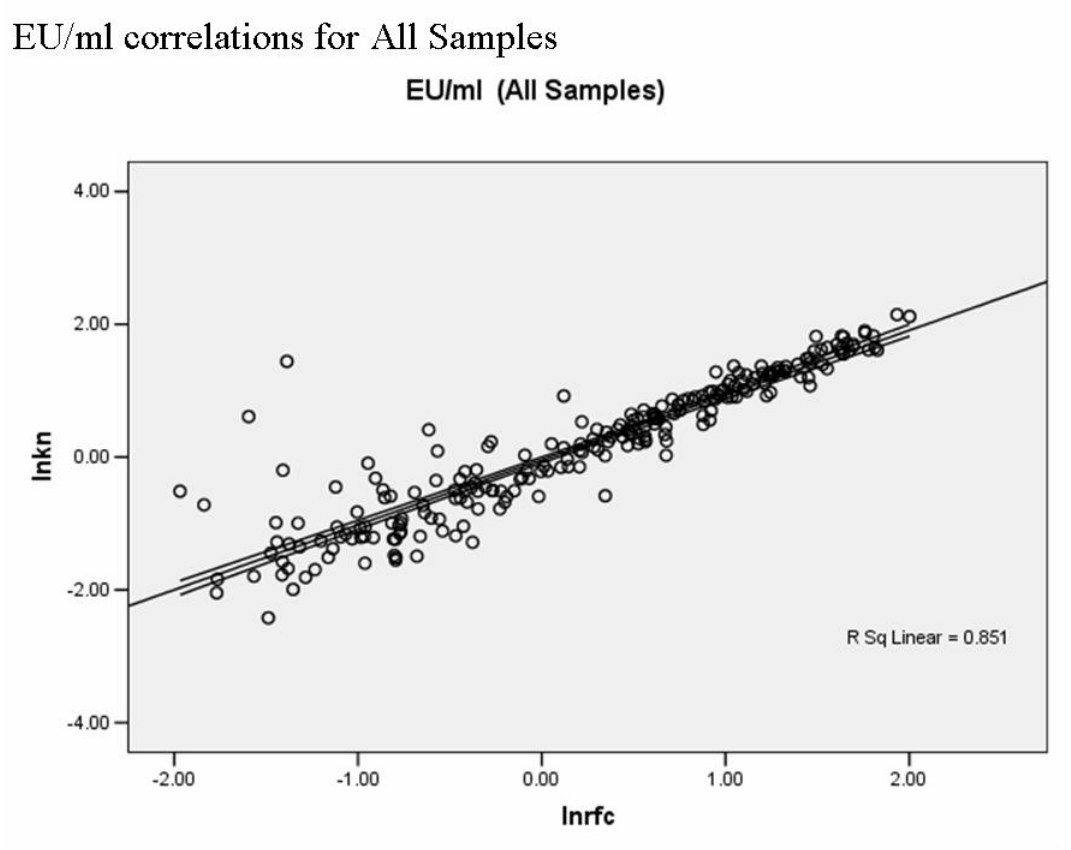


Figure 2: Correlation between LAL and rFC EU/ml for cage-housed operation samples

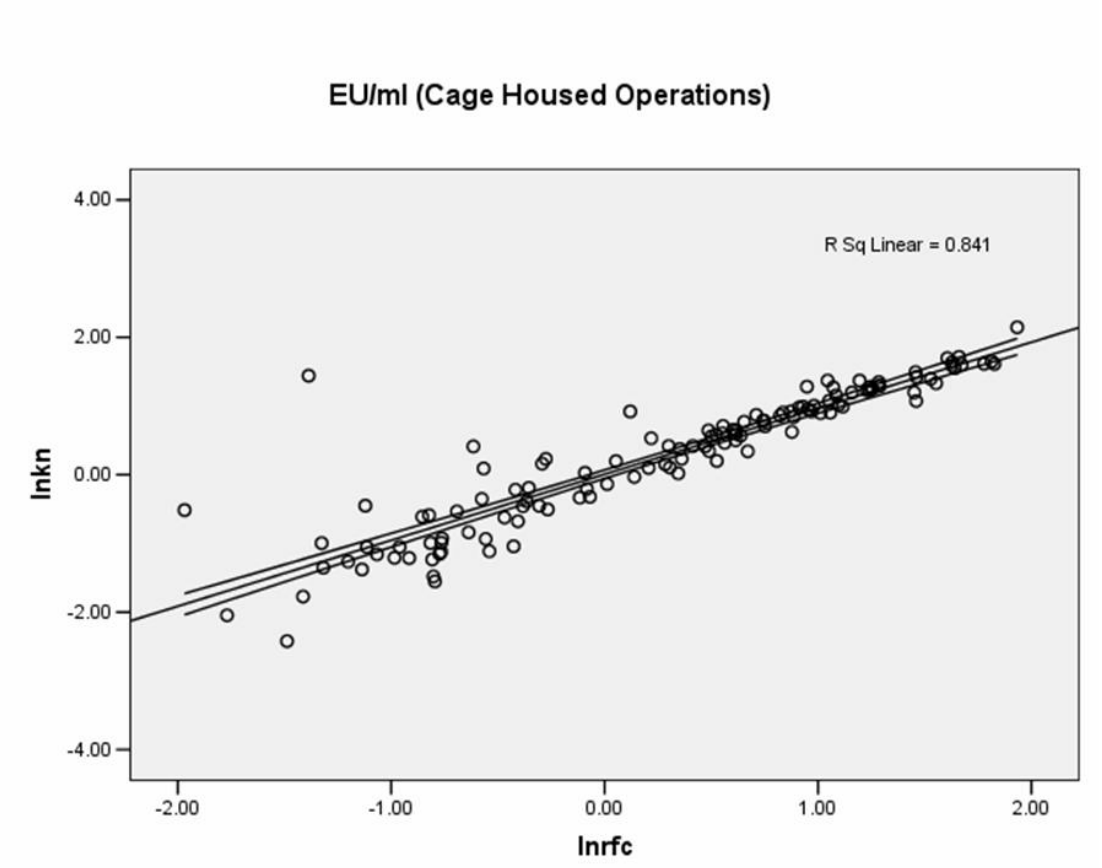


Figure 3 Correlation between LAL and rFC EU/ml for floor-housed operation samples

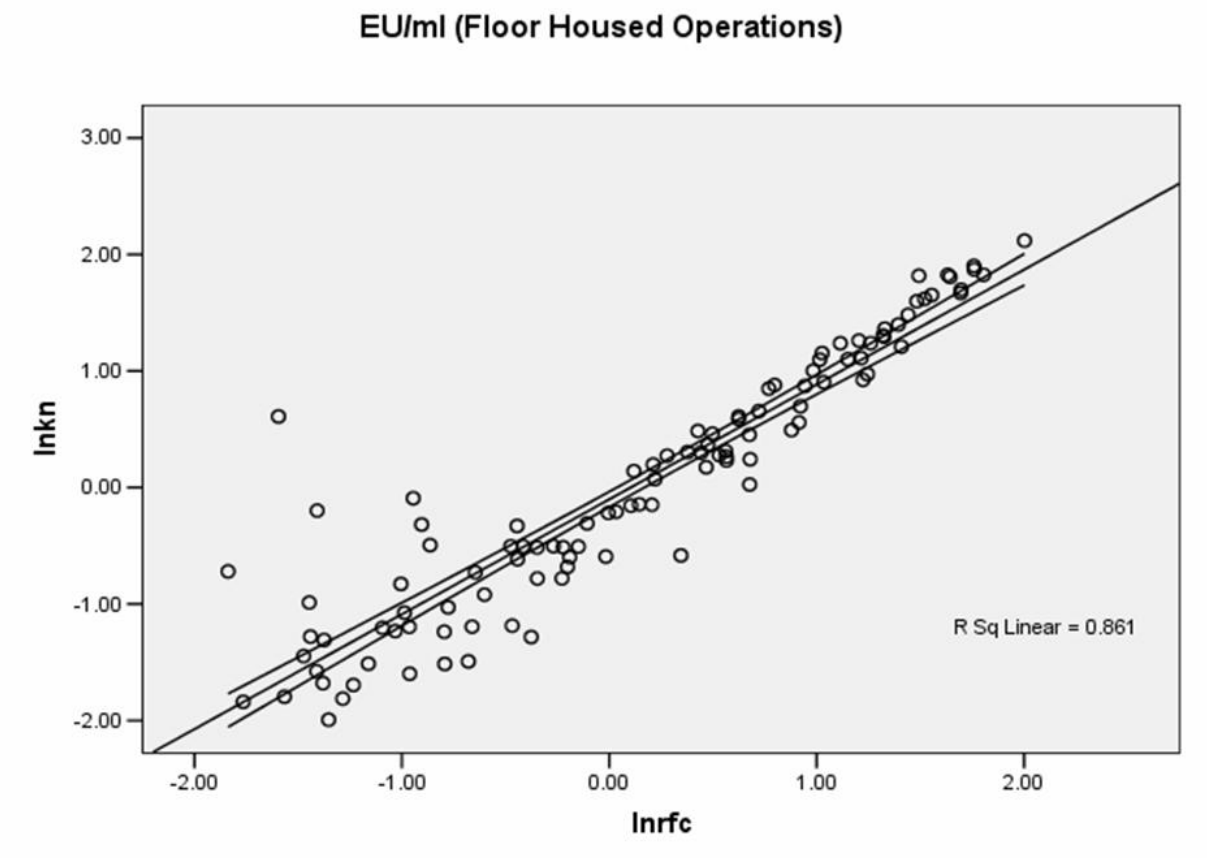


Table 1: Spearman's correlations between EU/mg LAL measurement and the overall difference in EU/mg between the LAL and rFC assays for all poultry operations

	Kinetic LAL	Difference LAL-rFC[t]
All stages (n=233)		
Kinetic LAL		-0.20*
rFC	0.82*	0.24*

[t] Difference between EU/mg determined by LAL and rFC correlated with LAL and rFC estimates
***p < 0.05**

Table 2: Spearman's correlations between EU/mg LAL measurement and the overall difference in EU/mg between the LAL and rFC assays for cage-housed operations

	Kinetic LAL	Difference LAL-rFC[t]
All stages (n=125)		
Kinetic LAL		-0.32*
rFC	0.80*	0.17

[t] Difference between EU/mg determined by LAL and rFC correlated with LAL and rFC estimates
***p < 0.05**

Table 3: Spearman's correlations between EU/mg LAL measurement and the overall difference in EU/mg between the LAL and rFC assays for floor-housed operations

	Kinetic LAL	Difference LAL-rFC[t]
All stages (n=108)		
Kinetic LAL		-0.12
rFC	0.75*	0.36*

[t] Difference between EU/mg determined by LAL and rFC correlated with LAL and rFC estimates
***p < 0.05**

Table 4: T-test comparison of Kinetic and rFC endotoxin values

n=232	LAL GM±GSD	rFC GM±GSD	p-value
Total, EU/ml	1.50±1.04	1.80±0.98	0.06
Total, EU/mg	376.53±0.72	459.09±0.65	0.03

DISCUSSION

As LAL has historically been the endotoxin assay of choice and is the methodological assay of choice in the literature, LAL was utilized and reported in this research program. However, a small pilot study was undertaken to compare the LAL assay to the rFC assay to assess if there would be differences between the two assays for the poultry dust samples (Appendix 1). The results indicated that there was strong correlation in the endotoxin units between the LAL and rFC samples (Appendix 1, Figures 1, 2 and 3). There were inverse correlations for the differences in endotoxin load (EU/mg) with the rFC and LAL assays between the CH and FH operations. The correlation was negative and significant between the difference

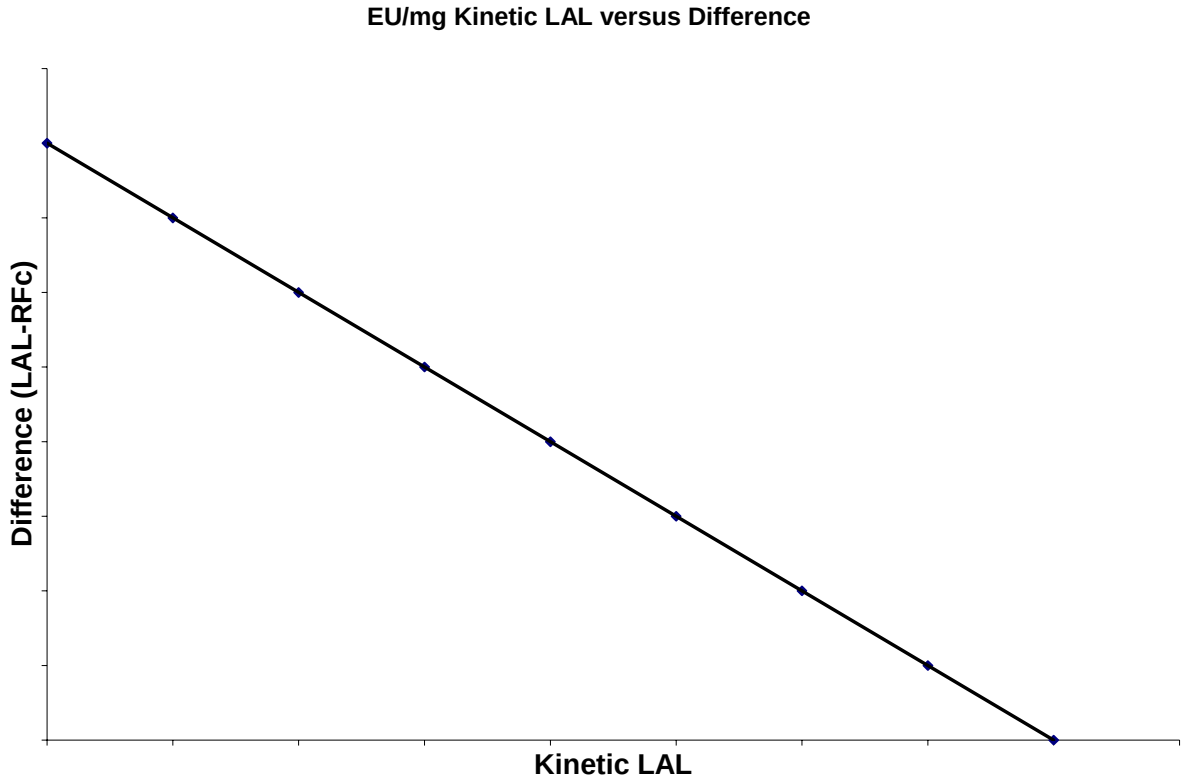
(LAL- rFC) and the kinetic LAL for the CH operations, whereas there was a significant positive correlation between the difference (LAL- rFC) and the rFC assay for the FH operations. This suggests that the differences in the assays were greatest at the extremes of the measures. There was more discrepancy between the differences and the LAL at the lower endotoxin load (EU/mg) or vice versa, the differences and the LAL were more alike as the EU/mg got higher suggesting that the LAL was a more general measure of endotoxin. That the kinetic LAL and differences correlation was stronger for the CH operations suggests that there may be lower levels of glucans in this environment. The inverse was true for the rFC assay. There was a larger discrepancy between the differences and the rFC at the higher endotoxin load (EU/mg) or vice versa, the differences and the rFC were more alike at the lower levels of EU/mg suggesting that the rFC was a more specific measure for EU/mg. If this is true, the FH operations correlated more strongly with the rFC assays suggesting that perhaps there was greater fungi content in the FH operations. Only assumptions can be made from the data as the poultry operations were not analyzed for fungi content. The results though, do suggest that there were differences in the results between the rFC and LAL assays when analyzing poultry dust samples, particularly at the extremes of the endotoxin measures.

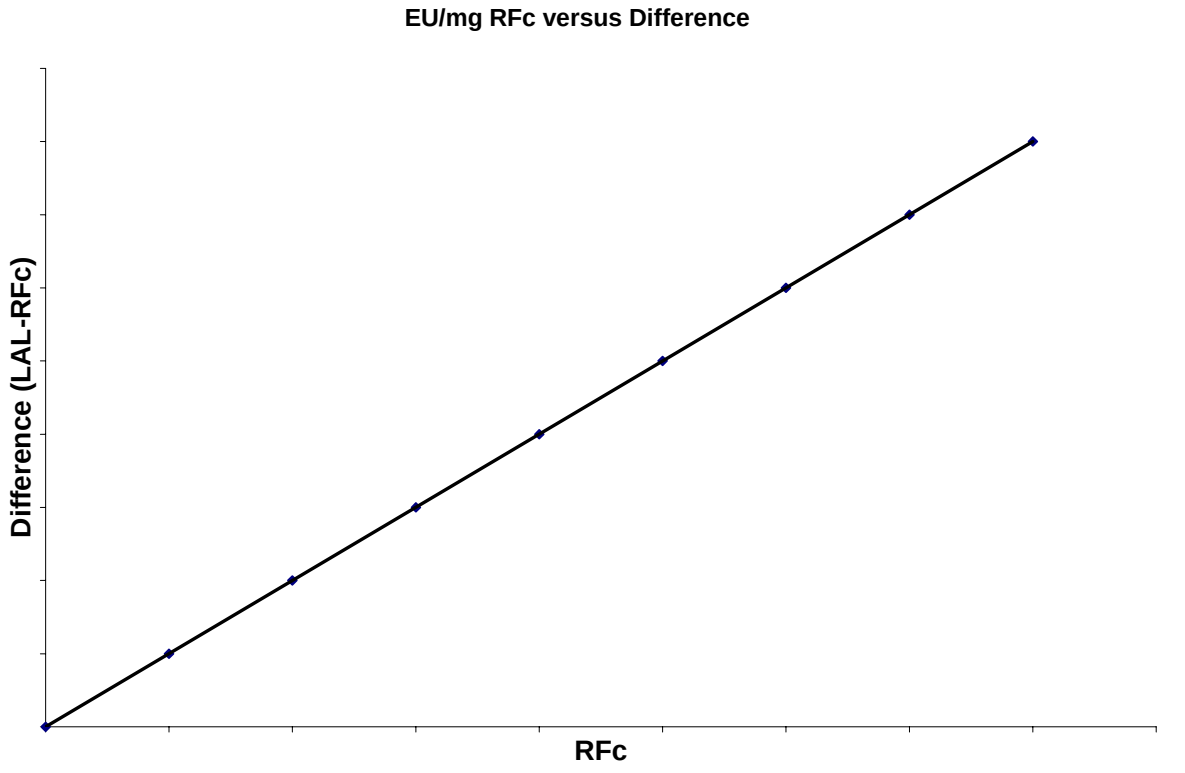
It seems that the 2 tests give more similar results when the EU load is higher, but diverge when the EU load is lower. This would be consistent with a "fixed, small" amount of interference from glucan, as long as overall LAL results indicated more EU than rFC.

An additional explanation for the differences in the LAL and rFC is that the two types of poultry operations may differ in the types of bacterial species present in the environment and/or the bacteria present in these environments may be of different chemical composition with differing endotoxin potency. The LAL assay, utilized in this study, is a measure of endotoxin potency and mainly detects biologically active endotoxin. The biological activity of endotoxin is dependent on the bacterial species and may differ between cell-bound and free endotoxin.[9,10] If we had utilized measures of 3-hydroxy fatty acids using gas chromatography-mass spectrometry (GC-MS) we may have been provided with additional information on the differences in endotoxin present in these two environments. The GC-MS assay detects the 3-hydroxy (OH) fatty acids (3-OH) as chemical markers of endotoxin and can be quantified from both

biologically-active and inactive endotoxin.[38] The lipid A component of endotoxin, which is highly conserved among Gram-negative bacteria, is thought to mediate the physiologic effects of endotoxin. It has been suggested that differences in the chemical composition of the endotoxin could relate to differences in the pulmonary toxicity of endotoxin. Specifically, 3-OH-14:0 fatty acid has been associated with respiratory health effects in workers and animals.[11,12] Helander et al[41] found that endotoxin of Enterobacteriacaeae, composed of predominantly 3-OH-14:0 fatty acid, had higher biological activity than endotoxin of bacteria which included other 3-OH fatty acids. The aerodynamic size of Enterobacteriacaeae is of the size fractions which would fit well within the MMAD for endotoxin identified in Paper 2. If bacterial species and chemical composition of LPS differ by poultry housing type this may assist in explaining the differences in not only endotoxin levels between the two types of operations but also perhaps the respiratory response experienced by workers.

Figure 4: LAL and rFC versus the difference in LAL-rFC





Highlights

1) There tends to be a strong correlation between EU/ml for rFC and LAL assays.
2) There were inverse correlations for the differences in LAL- rFC EU/mg to the absolute EU/mg values suggesting that differences were greatest at the extremes of the measures.
3) Floor-housed operations rFC has a tendency to be higher than the LAL in these same operations.

From the EU/mg correlations, it appears that the rFC assay may be more specific than the LAL assay. As the EU/mg increases the difference between the LAL assay and the rFC assay become greater and they appear to have different values. In the kinetic LAL assay, as the EU/mg gets higher the difference between the rFC and LAL assay lessens suggesting they become more alike.

From the graphs it appears glucans may have an impact on the endotoxin results when reported in LAL. The floor-housed samples would be more greatly impacted as it appears to more generally reflect an influence of glucans as compared to the cage housed operations. The floor housed operations would appear to have greater glucans (fungi) and this is reflected in the assays.

Singh et al, (1996) found predominant fungi were different between floor-housed and cage-housed poultry environments. They found that where poultry were kept indoors, the air had high concentrations of certain fungi. *Candida albicans*, smut, *Scopulariopsis brevicaulis* and *Penicillium nigricans* were found to be characteristic fungi of poultry sheds, while *Aspergillus flavus*, *Aspergillus niger*, *Cladosporium* and *Alternaria* spp. were those of a hatchery.[2]

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APPENDIX 2: COMPARISON OF FEV_1 AND PEF USING THE PIKO-1 ELECTRONIC FLOW METER AND ROLLING SEAL SPIROMETER

This small pilot project was conducted at the Institute of Agricultural Rural and Environmental Health at the University of Saskatchewan. Previous reliability testing of the PIKO-1 electronic peak flow meter had been undertaken by the company.[1,2] This pilot study was conducted since the dry rolling seal spirometer was utilized for Paper 1 and the Piko-1 electronic peak flow meter (Piko-1) was used for Paper 2. The Piko-1 was chosen to be utilized for Paper 2 for biosecurity reasons. For Paper 1, subjects were tested at the residence of the subject. For Paper 2, subjects were tested at the work-site. For biosecurity reasons, any equipment, persons, etc, entering a farm site must follow strict biosecurity policies to avoid the transport of pathogens between farm sites. Outbreaks of avian flu had occurred between the testing periods of Paper 1 and Paper 2 and the biosecurity procedures of our projects needed to ensure that the study team would not and could not be a transporter of pathogens between study sites. It was decided that a portable, more disposable device needed to be utilized for the second study phase. The Piko-1 was not taken to more than one poultry site. After use, the unit was wiped down with an antibacterial solution and then not utilized again in the poultry studies. This type of option was not available if we used the dry rolling seal spirometer.

The seven individuals were randomly assigned to begin with either the dry rolling seal spirometer (volume displacement) or the Piko-1 electronic peak flow meter (pneumotach). Randomization was completed using a coin toss. Heads equaled starting with the dry rolling seal spirometer and tails equaled starting with the peak flow meter. Alternate blows were undertaken with each instrument after the initial randomization was completed.

Peak expiratory flow (PEF) and forced expired volume in 1 second (FEV_1) were recorded from each of the instruments. At least three reproducible tests were required from each of the instruments. Reproducibility was determined from American Thoracic Society standards for spirometry.[3] The tests for FEV_1 and PEF were averaged for each subject for each piece of equipment. Paired t-tests were used to evaluate the results. The results indicate that the dry rolling seal spirometer had significantly higher results than thePiko-1 electronic flow meter for both FEV_1 (0.18 litre mean difference, $p=0.004$) and PEF (1.14 litres/second mean difference, $p=0.007$).

The variability in the results is wide enough to be considered of clinical significance if the Piko-1 and dry rolling seal spirometer pulmonary results were to be directly compared. Fortunately we were not directly comparing the pulmonary results from Paper 1 to Paper 2. Due to the small population sample size for Paper 2, the pulmonary results were utilized to indicate that the same trends that were observed in Paper 1 were also observed in Paper 2. Pulmonary observations from Paper 2 were not utilized in predicting outcomes related to environmental exposures nor were they directly compared to the pulmonary results obtained from Paper 1.

Results from a comparison of 106 school aged children who were tested using both the Piko-1 and the dry rolling seal spirometer support the findings of this small dataset. The children's results showed a 400 milliliter difference in FEV_1 and a 0.4 liter/second peak expiratory flow difference between the two measurement devices. The dry rolling seal spirometer gave the higher FEV_1 and PEF readings.[4]

Table 1: Randomization of PFT for participants

Subno	Gender	Random	Age
1	2	1	49
2	1	2	35
3	2	1	24
4	2	2	37
5	2	1	31
6	1	1	67
7	1	1	30

Variable names:

Mean volume displacement peak expiratory flow (meanvpef)
Mean Piko-1 peak expiratory flow (meanppef2)
Mean volume displacement forced expired volume in 1 second (meanvfev)
Mean Piko-1 forced expired volume in 1 second (meanpfev)

Table 2: Comparison of PEF versus FEV_1 on the PIKO-1 and dry rolling spirometer

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	meanvpef	9.5980	7	2.64775	1.00075
	meanppef2	8.4589	7	2.01029	.75982
Pair 2	meanvfev	3.5702	7	1.00134	.37847
	meanpfev	3.3937	7	.92356	.34907

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1	meanvpef - meanppef2	1.13905	.76123	.28772	.43503	1.84307	3.959	6	.007
Pair 2	meanvfev - meanpfev	.17655	.10428	.03941	.08011	.27299	4.479	6	.004

Correlations

Average Piko-1 FEV_1 readings versus the mean dry rolling seal spirometer FEV_1 were highly correlated $r = 1.00$, $p<0.01$

Average Piko-1 PEF readings versus the mean dry rolling seal spirometer PEF were highly correlated $r = 0.93$, $p=0.003$

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APPENDIX 3: QUESTIONNAIRES AND STANDARD OPERATING PROCEDURES

PAPER 1 QUESTIONNAIRE

POULTRY FARM QUESTIONNAIRE

Institute of Agricultural Rural and Environmental Health
University of Saskatchewan
Saskatoon, Saskatchewan
S7N 0W8
(306) 966-8286

Date(m/d/y):_____ Interviewer: _____

Subject No.:

1. Postal Code:

2. Date of birth (m/d/y):

3. Age:_____ **4**. Sex: Male____ Female_____

5. Highest grade completed in school: _____

POULTRY BARNS

6. Which type of poults form the majority of your operation?
a) Layers/Leghorns
b) Broiler/Roaster
c) Broiler/Breeder

a. Which activities are you associated with?
(If you are not involved in the activity, answer 0 in the blanks.)
1. Feeding/Watering __ hours/day _ days/week
__ weeks/year
__ birds handled in a day

2. Checking Flock (ie. removing dead birds; making adjustments) __ hours/day
_ days/week
__ weeks/year
__ birds handled in a day

3. Cleaning Nests __ hours/day _ days/week
__ weeks/year

4. Collecting Eggs __ hours/day _ days/week
__ weeks/year

5. Cleaning Eggs __ hours/day _ days/week
__ weeks/year

6. Processing __ poults handled in a day
a) Catching crew __ hours/day __ days/week
b) Shackling room __ hours/day __ days/week
c) Slaughtering __ hours/day __ days/week

7. Loading/Transport __ hours/day _ days/week
__ weeks/year
__ birds handled in a day

8. Clean-out _ number of times/year
__ hours/day _ days/year

9. Barn Set-up __ hours/day _ days/year
(ie. spreading litter, hanging feeders/waterers)

b. How old are the birds that you usually handle?
(circle as many as apply)
1. 0-2 weeks old
2. 2-4 weeks old
3. 4-6 weeks old
4. 6-8 weeks old
5. greater than 8 weeks old

c. How many birds are in your barn at this time?
_____ number of birds

d. Approximately how old is your poultry barn?
____ years old

e. What type of housing system is in the barn? (circle)
1. deep litter
2. battery (caged)
3. slatted floor
4. cement and litter floor
5. dirt and litter floor
6. other (please specify)

f. What type of litter is used? (circle type)
1. wood chips/shavings
2. paper
3. straw
4. other (please specify)

g. How often do you spread the litter? (circle)

1. weekly
2. biweekly
3. monthly
4. other (please specify)

h. Do you remove all litter after each flock? (circle)

1. yes 2. no 9. does not apply

i. Do you wash/disinfect walls and equipment? (circle)

1. yes 2. no 9. does not apply

j. Do you disinfect after every flock?(circle)

1. yes 2. no 9. does not apply

k. What type of disinfectant do you use? (circle)

1. soap/water
2. quat. NH_3
3. phenois
4. formaldehyde
5. other (specify)

l. What type of housing clean-out system do you use? (circle)

1. manure scraper
2. belt cleaner
3. deep pit mucking (truck and front end loader)

m. What type of feed is used? (circle type)

1. pellets/crumbs
2. ground meal/mash
3. other, specify

n. What type of ingrediants are likely in the feed? (circle)

1. corn
2. soybean meal
3. minerals
4. salt
5. vitamins
6. other, specify

o. Is oil added to the feed? (circle)

1. yes 2. no

p. What type of feeding mechanism do you use? (circle)

1. hoppers and by hand
2. trolley, filled by auger and pushed by hand
3. automatic chain feeder
4. other, specify

q. What type of drinker do you use? (circle)

1. nipple
2. cup
3. bell
4. other, specify

r. How often are the watering fountains cleaned?

_____ hours/day _____ days/week

s. What type of lighting system is used in the confinement house. (circle)

1. artificial
2. natural
3. combination
4. other, specify

What is the timing of the lighting system used?

___ hours/day

If intermittent lighting is used, please specify age of poult and number of hours of light.

poult age _____ hours of light

poult age _____ hours of light

poult age _____ hours of light

t. What type of ventilation system is used in the barn? (circle)

1. ridge ventilation
2. cross ventilation
3. natural ventilation (curtains and open ridge)
4. other, specify

u. What type of temperature control system is used? (circle)

1. heater (boiler)
2. heat exchanger
3. ventilation controlled

v. Do you usually wear a mask or respirator when working in the barn? (please circle)

1. yes 2. no

w. How frequently do you use a mask while working in the poultry barn? (circle)

1=never (0%)
2=rarely (1-5%)
3=occasionally (6-25%)
4=often (26-50%)
5=very often (51-100%)

x. Do you control dust in the poultry barn? (please circle)

1. yes 2. no

If yes, how?

y. How many hours/day do you spend in the poultry barn? ___ hours/day

z. How many days/week do you spend in the poultry barn? ___ days/week

aa. On average, how many weeks/year do you spend in the poultry barn?

___ weeks/yr

bb. How long have you worked in a poultry barn?

___ years

cc. Please indicate the frequency with which you experience the following when working in the poultry barn: (circle)

1=never, 2=occasional, 3=often, 4=very often

1. headache	1 2 3 4
2. weakness	1 2 3 4
3. dizziness	1 2 3 4
4. fainting or blackout	1 2 3 4
5. muscle aches and pains	1 2 3 4
6. fever	1 2 3 4
7. nausea or vomiting	1 2 3 4
8. plugged, or popping ears	1 2 3 4
9. hearing problems	1 2 3 4
10. burning/watering eyes	1 2 3 4
11. stuffy/runny nose	1 2 3 4
12. scratchy throat	1 2 3 4
13. sputum or phlegm	1 2 3 4
14. cough	1 2 3 4
15. shortness of breath	1 2 3 4
16. wheezing	1 2 3 4
17. tightness in chest	1 2 3 4
18. skin rashes or hives	1 2 3 4
19. other (specify)_____	1 2 3 4

dd. How soon do these symptoms occur after entering the poultry barn? (circle)

1. immediately,
2. within 2 hours
3. within 2-4 hours
4. within 4-8 hours,
5. more than 8 hours later
9. does not apply

ee. How long do these symptoms last after leaving the poultry barn? (circle)

1. within 2 hours,
2. 2-4 hours later,
3. 4-8 hours later,
4. more than 8 hours later
9. does not apply

ff. Do any other farm-related activities cause any of symptoms checked above? (circle)

1. yes 2. no

If yes, please specify the type of activity and symptoms involved:

activity _____ symptoms
activity _____ symptoms

15. GRAIN FARMING

Have you ever raised grain? (circle)

1. yes 2. no 9. does not apply

If yes:

a. Do you currently raise grain? (> 360 acres)(circle)

1. yes 2. no 9. does not apply

(If No, skip to c)

b. How long have you been grain farming? ___ years

If you no longer grow grain:

c. When did you last grow grain? ___ year

d. For how many years were you a grain farmer? __ years

16. a. Do you raise cattle? (> 10 indoor or >50 outdoor)(circle)

1. yes 2. no

b. Do you raise pigs in confinement? (> 50 indoors)(circle)

1. yes 2. no

17. Have you ever been exposed to any of the following in the workplace? (Circle as many as

apply)

1. Mining (specify type)
2. Diesel exhaust
3. Grain dusts
4. Solvent fumes
5. Asbestos
6. Agricultural chemicals(herbicides, insecticides, fungicides)
7. Welding fumes
8. Other (specify)
9. None

18. COUGH (circle)

a. Do you currently have a cough?

1. yes 2. no

b. Do you usually have a cough?

(Count a cough with first smoke or on first going outside. Exclude clearing of throat.)

1. yes 2. no

c. Do you usually cough as much as 4 - 6 times a day, 4 or more days out of the week?

1. yes 2. no 9. does not apply

d. Do you usually cough at all on getting up, or first thing in the morning?

1. yes 2. no 9. does not apply

e. Do you usually cough at all during the rest of the day or at night?

1. yes 2. no 9. does not apply

f. Do you usually cough like this on most days for 3 consecutive months or more during the year?

1. yes 2. no 9. does not apply

g. For how many years have you had this cough?

__ years 9. does not apply

h. Is your cough caused or made worse by exposures to:
(please circle as many as apply)

1. grain dust
2. litter dust
3. cigarette smoke
4. farm chemicals
5. contact with animals
6. plants, pollens, weeds
7. cold air

8. exercise
9. feathers
10. none of the above

19. PHLEGM (circle)

a. Do you currently bring up phlegm from your chest?
1. yes 2. no

b. Do you usually bring up phlegm from your chest?
1. yes 2. no
(Count phlegm with first smoke or on first going outside. Exclude phlegm from the nose, count swallowed phlegm.)

c. Do you usually bring up phlegm like this as much as twice a day, 4 or more days out of the week?
1. yes 2. no 9. does not apply

d. Do you usually bring up phlegm on getting up, or first thing in the morning?
1. yes 2. no 9. does not apply

e. Do you usually bring up phlegm at all during the rest of the day or at night?
1. yes 2. no 9. does not apply

f. Do you bring up phlegm like this on most days for 3 consecutive months or more during the year?
1. yes 2. no 9. does not apply

g. For how many years have you had trouble with phlegm?

___ years 9. does not apply

h. Is this problem caused or made worse by exposure to:
(please circle as many as apply)
1. grain dust
2. litter dust
3. cigarette smoke
4. farm chemicals
5. contact with animals
6. plants, pollens, weeds
7. cold air
8. exercise
9. feathers
10. none of the above

i. In your opinion, which grain dusts are most likely to cause cough and/or phlegm, or make it worse?

(please circle as many as apply)

1. wheat
2. oats
3. barley
4. flax
5. canola
6. mustard
7. other - please specify _____
8. does not apply

20. WHEEZING (circle)

a. Does your chest currently sound wheezy or whistling?

1. yes 2. no

b. Does your chest ever sound wheezy or whistling:

i. when you have a cold ?
1. yes 2. no

ii. occasionally apart from colds?
1. yes 2. no

iii. most days or nights? 1. yes 2. no

c. For how many years has this been present?

___ years 9. does not apply

d. Is your chest wheezing caused or made worse by exposure to:

(please circle as many as apply)

1. grain dust
2. litter dust
3. cigarette smoke
4. farm chemicals
5. contact with animals
6. plants, pollens, weeds
7. cold air
8. exercise
9. feathers
10. none of the above

e. In your opinion, which grain dusts are most likely to cause wheezing or make it worse?

(please circle as many as apply)

1. wheat
2. oats
3. barley
4. flax

5. canola
6. mustard
7. other - please specify
8. does not apply

21. SHORTNESS OF BREATH (circle)

a. Are you troubled by shortness of breath when hurrying on the level or walking up a slight hill?

1. yes 2. no

b. Do you get short of breath during or after exposure to grain/feed dust?

1. yes 2. no 9. does not apply

c. In your opinion, which grain dusts are most likely to cause shortness of breath or make it worse?

(please circle as many as apply)

1. wheat
2. oats
3. barley
4. flax
5. canola
6. mustard
7. other - please specify
8. does not apply

22. NASAL IRRITATION (circle)

a. Do you currently have nasal stuffiness, runny nose, sneezing and/or nasal itchiness?

1. yes 2. no

b. Do these symptoms ever occur:

i. when you have a cold ? 1. yes 2. no

ii. occasionally apart from colds? 1. yes 2. no

iii. most days or nights? 1. yes 2. no

c. For how many years has this been present?

___ years 9. does not apply

d. Is your nasal stuffiness, runny nose, sneezing and/or nasal itchiness caused or made worse by exposure to:

(please circle as many as apply)

1. grain dust
2. litter dust
3. cigarette smoke

4. farm chemicals
5. contact with animals
6. plants, pollens, weeds
7. cold air
8. exercise
9. feathers
10. none of the above

e. In your opinion, which grain dusts are most likely to cause nasal stuffiness, runny nose, sneezing and/or nasal itchiness or make it worse?

(please circle as many as apply)

1. wheat
2. oats
3. barley
4. flax
5. canola
6. mustard
7. other - please specify _____
8. does not apply

23. EYE IRRITATION (circle)

a. Do you have any itching, irritation, tearing and/or redness of the eye(s)? 1. yes 2. no

b. Do you usually get these symptoms for more than 3 months a year? 1. yes 2. no

c. For how many years has this been present?__ years

d. Is your itching, irritation, tearing and/or redness of the eye(s) caused or made worse by exposure to:

1. grain dust
2. litter dust
3. cigarette smoke
4. farm chemicals
5. contact with animals
6. plants, pollens, weeds
7. cold air
8. exercise
9. feathers
10. none of the above

e. In your opinion, which grain dusts are most likely to cause itching, irritation, tearing and/or redness of the eye(s) or make it worse?

(please circle as many as apply)

1. wheat
2. oats

3. barley
4. flax
5. canola
6. mustard
7. other - please specify _____
8. does not apply

IF IN YOUR WORK YOU ARE NOT EXPOSED TO GRAIN OR FEED DUST, PLEASE SKIP TO QUESTION 25

24. FEVER AND/OR CHILLS (circle)

a. Have you ever had fever and/or chills during exposure or after being exposed to grain and/or feed dust?

1. yes 2. no

b. During exposure to grain and/or feed dust have you ever had:

i. burning/watering/or itchy eyes? 1. yes 2. no
ii. stuffy nose? 1. yes 2. no
iii. sore or burning throat? 1. yes 2. no

c. During or immediately after exposure to grain and/or feed mill dust have you ever had itchy skin?

1. yes 2. no

25. PESTICIDES (please circle yes or no)

a. Have you ever been exposed to insecticides?

1. yes 2. no

b. Have you ever been exposed to herbicides?

1. yes 2. no

c. Have you ever been exposed to fungicides?

1. yes 2. no

26. HEADACHES (circle)

a. Do you usually develop a headache during work?

1. yes 2. no

b. Does the headache get better, worse, or stay the same after work?

1. yes 2. no 9. does not apply

27. MEDICAL HISTORY (please circle)

a. Do you currently have a cold? 1. yes 2. no

b. If you get a cold, does it usually go to your chest?
(Usually means more than half the time)
1. yes 2. no 3. don't get colds

c. During the past 3 years, have you had any chest illnesses that have kept you off work, indoors at home or in bed?
1. yes 2. no

If yes, how many? ____ in 3 years

d. Have you ever had any of the following? (please circle as many as apply)
I. Chronic bronchitis?

II. Pneumonia?

III. Emphysema?

IV. Hay fever?

V. Farmer's lung?

VI. Chest operations?

VII. Chest injuries?

VIII. Other chest problems?
Please list

e. **ASTHMA**
Have you ever had asthma?
1. yes 2. no

Do you still have it?
1. yes 2. no 9. does not apply

Was it confirmed by a doctor?
1. yes 2. no 9. does not apply

At what age did it start?
___age in years 9. does not apply

If it has stopped, when?
___age stopped 9. does not apply

f. Has a doctor ever told you that you had any heart trouble?
1. yes 2. no

g. Have you had any treatment for heart trouble in the past 10 years?
1. yes 2. no 9. does not apply

h. Has a doctor ever told you that you have high blood pressure? 1. yes 2. no

i. Have you had any treatment for high blood pressure (hypertension) in the past 10 years?
1. yes 2. no 9. does not apply

j. Are you currently on any medications?
1. yes 2. no

1. inhaler
2. theophyllines
3. cortisone(prednisone)
4. allergy medications
5. heart pills
6. antibiotics
7. blood pressure pills
8. other - please specify _____
9. none

28. SMOKING HISTORY (please circle yes or no)

a. Have you ever smoked cigarettes? 1. yes 2. no
(No, means less than 20 packs, or 400 cigarettes or, 12 oz. of tobacco in a lifetime, or less than 1 cigarette a day for a year)
If NO, SKIP to K

b. Do you currently smoke cigarettes? (as of one month ago) 1. yes 2. no
.

If NO, SKIP to g

CURRENT SMOKERS:
c. How old were you when you first started regular cigarette smoking? _____ years old

d. How many cigarettes do you smoke per day now?
____ cigs/day

e. On average, for the entire time you have smoked, how many cigarettes have you smoked per day? ____ cigs/day

f. Do you inhale the cigarette smoke? (please circle the appropriate number)

1. Not at all
2. Slightly
3. Moderately
4. Deeply

SKIP TO k.

EX-SMOKERS:

g. How old were you when you first started regular cigarette smoking? _____ years old

h. How old were you when you stopped smoking cigarettes completely? _____ years old

i. On the average, for the entire time you smoked, how many cigarettes did you smoke per day? ____ cigs/day

j. Did you inhale the cigarette smoke? (please circle the appropriate number)

1. Not at all
2. Slightly
3. Moderately
4. Deeply

CIGAR/PIPE SMOKERS

k. Have you ever smoked a pipe and/or cigars regularly?
(Yes means more than 12 oz. of tobacco in lifetime or more than 1 cigar a week for 1 year)

1. yes 2. no

IF NO, SKIP to 29.

l. For how long? ____ years

m. Are you currently smoking cigars or pipes?

1. yes 2. no

n. If yes, how much? ___ cigars/week
___ tobacco pouches/week

o. Do you or did you inhale the pipe/cigar smoke?

1. Not at all
2. Slightly
3. Moderately
4. Deeply

YOU ARE NOW FINISHED, THANK YOU.

PAPER 2
QUESTIONNAIRE

POULTRY FARM QUESTIONNAIRE

Current Month (mm):. Current Day (dd): Current Year (xxxx):.

Subject ID: _____

Barn land location:

Address: _____

Postal code:.

Phone: ____

Interviewer initials:

Type of poultry operation: O Layers/Replacement Pullets O Broiler/Roaster O Broiler/Breeder

How many barns do you have? _____

Total number of birds currently on farm: _____

How many birds do you produce/cycle? _____

How many birds do you produce/year ? _____

Age at marketing: weeks

Do you use the "On Farm Food Safety Program" or "Start Clean and Stay Clean Program"? OYes ONo

Sampling Barn

Barn age: Barn years

size:

Barn length: ___ _m Barn width: _m Barn height: .m

Breed:___ Strain:

Age of birds: weeks

Number of birds in this barn:

Flock mortality rate:

Have you had any disease outbreaks? OYes ONo

If yes, what disease:

Date of outbreak:

Equipment and Facilities

What type of cage system to you have?

O Single tier O Double tier O Triple tier O Battery system O DNA O Other.

What type of egg collection system do you have?

O Conveyor Belt OHand O Auger O None O Other-

Type of litter collection system: O Deep pit O Shallow pit O Manure belt O DNA

Do you use litter in your operation? O Yes O No

Type of Litter:

OWoodchips O Straw O Sunflower hulls O DNA

O Shavings O Paper O Woodchips/straw O Other...

Is the litter removed after every flock? Q Yes O No O DNA

Do you rototill your litter? Q Yes O No O DNA

Is the litter wet in winter? O Yes O No O DNA

What type of housing clean out system do you use?

O Front-end Loader O Tractor O Bobcat O Scraper O DNA O Other...

What type of disinfectant do you use?

D Soap & water D Virkon D Peroxigard D Phenols D Formaldehyde Q Other...

What type of feeding mechanism do you use? O Automatic chain O Automatic pans O Hand

O Feed cart O Trolley O Other...

Number of feeders:---------- Feeder diameter: _____

Feed form: O Pellets/crumb O Ground meal/mash O Other...

What is the main type of grain in the feed? O Corn O Wheat O Other...

What is the main type of protein supplement in the feed? O Soybean Meal O Other-

Do you use antibiotics in: O Feed O Water O Feed/water O Neither

What type of drinker is used? O Nipple O Cup O Bell O Other-

Number of drinkers:_____ Water flow rate: _____

How often is the water system cleaned? _____ times/week

What type of flooring is in the barn? O Concrete O Soil O Wood O Other

Do you wash/disinfect walls and equipment? O Yes O No

Do you wash/disinfect after every flock? O Yes O No

What type of lighting system is in this barn? O Incandescent O Florescent O Other...

Are the birds under continual light? Q Yes O No

Is a lighting program used? O Yes O No

If Yes: _____ hours of light/day

What type of ventilation is used in the barn? O Timer O Temperature sensor O Manual O Other...

Number of fans in the barn: ----------

Size of fan(s): _____ Number of fans used: _____

Type of heating system used in the barn?

O Gas box heater/hot water
O Non-vented self-contained natural gas
O Non-vented chimneyless
O Radiant
O Infrared Heaters
O Hot water/forced air/gas brooding
O Forced hot air/gas brooding
O Heat exchange/gas brooding
O Hot water/radiant
O Forced hot air/catalytic heater
O Forced hot air/electric brooding
O Infrared/hot water
O Radiant & brooder
O Heat exchange/electric
O Propane
O Hot water & propane
O Hot water, electric
O Other...

How would you rate the air circulation in the barn?

O Very poor O Poor O Fair O Good O Very good O Excellent

What is the type of inlet control in the barn? O Automatic O Manual O None O Other...

Do you control dust in the barn? O Yes O No O DNA

Do you have a dust control system?

O Foggers O Oil in the feed O Sprinklers O Misters

O Wet the litter O Dampen air/furnace O Canola oil O Other-

How many people work in this poultry operation? _____

Poultry Worker

Date of birth (dd/mm/yyyy): _____

Age: _____

Sex: O Male O Female

How long have you worked in a poultry operation? ----------year(s)

On average, how many weeks/year do you spend in the poultry barn/processing facilities?
_____ weeks/year

On average now many days/week do you spend in the poultry barn/processing facilities?
_____ days/week

How often are you involved in barn set-up (spreading litter, hanging feeders & drinkers) ?

_____ days/year

How many hours do spend each time on barn set-up? _____ hours

How many hours a day do you spend checking the flock, removing deads, adjusting feeders, waters, and ventilation? hours/day

How many hours per day do you spend processing eggs? _____ hours/day

How many hours/week do you spend cleaning or tilling litter? _____ hours/week

Are you involved with loading/transporting the birds? O Yes O No

If yes, how many times per year do your transport birds?_____ /year

How many times per year do you clean out the barn?_____ /year

How many hours do you spend each time cleaning out the barn? _____hours/clean out

How frequently do you use a mask while working in the poultry barn/processing facilities?

O Never (0%) O Occasionally (6-25%) O Very often (51-100%)

O Rarely (1-5%) O Often (26-50%)

What type of mask?

O 2 strap disposable respirator
O 1 strap disposable respirator
O Reusable dust half-mask respirator
O Air helmet
O Half-mask respirator with chemical cartridge
O Two strap with exhaust valve
O Other...

Medical Questions

Please indicate the frequency with which you experience the following when working in the poultry barn?
1 = never, 2 = occasionally, 3 = often, 4 = very often

Headache	O1	O2	O3	O4	Stuffy/runny nose	O1	O2	O3	O4
Weakness	O1	O2	O3	O4	Scratchy throat	O1	O2	O3	O4
Dizziness	O1	O2	O3	O4	Sputum or phlegm	O1	O2	O3	O4
Fainting or blacking out	O1	O2	O3	O4	Cough	O1	O2	O3	O4
Muscle aches and pains	O1	O2	O3	O4	Shortness of breath	O1	O2	O3	O4
Fever	O1	O2	O3	O4	Wheezing	O1	O2	O3	O4
Nausea or vomiting	O1	O2	O3	O4	Tightness in chest	O1	O2	O3	O4
Plugged or popping ears	O1	O2	O3	O4	Skin rash or hives	O1	O2	O3	O4
Burning/watering eyes	O1	O2	O3	O4	Other	O1	O2	O3	O4

How soon do these symptoms occur after entering the poultry barn?

O Disappear immediately O 2-4 hours O > 8 hours later
O 2 hours O 4-8 hours O DNA

How long do these symptoms last after leaving the poultry barn?

O Disappear immediately O 2-4 hours O > 8 hours later
O 2 hours O 4-8 hours O DNA

Do these symptoms improve on weekends and days off? O Yes O No O DNA

Do any other farm-related activities cause any symptoms checked above? OYes ONo

If yes, specify the type of activity:_____

Specify the activity: _____

Other Farming

Have you ever grown grain? O Yes O No

Do you currently grow grain? O Yes O No O DNA

How long have you grain farmed?---------- year(s)

When did you grow grain last? _____ year

How many years were you a grain farmer? _____ year(s)

Do you raise cattle (>10 indoor or >50 outdoor)? *Q* Yes O No

Do you raise pigs in confinement (> 50 indoors)? O Yes O No

Have you ever been exposed to any of the following?

D Mining D Welding fumes D Asbestos D None D Other...

Respiratory Symptoms

Cough

Do you currently have a cough? O Yes O No

Do you usually have a cough (with first smoke or going outside, excluding clearing of throat)?
OYes ONo ODNA

Do you usually cough as much as 4-6 times a day, 4 or more days out of the week? OYes O No O DNA

Do you usually cough at all on getting up, or first thing in the morning? O Yes O No O DNA

Do you usually cough at all during the rest of the day or at night? O Yes O No O DNA

Do you usually cough like this on most days for 3 consecutive months or more during the year?

OYes ONo O DNA

For how many years have you had this cough?_____year(s)

Is your cough caused or made worse by exposure to:

D Grain dust	D Contact with animals	D Feathers
D Litter dust	D Plants, pollens, weeds	D Poultry barn
D Cigarette smoke	D Cold air	D DNA
D Farm chemicals	D Exercise	D Other...

Phlegm

Do you currently bring up phlegm from your chest? O Yes O No

Do you usually bring up phlegm from you chest? O Yes O No

Do you usually bring up phlegm like this as much as twice a day, 4 or more days out of the week?

OYes ONo ODNA

Do you usually bring up phlegm on getting up in the morning? O Yes O No O DNA

Do you usually being up phlegm at all during the rest of the day or at night? O Yes O No O DNA

Do you bring up phlegm like this on most days for 3 consecutive months or more during the year?

OYes ONo O DNA

For how many years have you had phlegm? year(s)

Is this problem caused or made worse by exposure to:

D Grain dust	D Contact with animals	D Feathers
D Litter dust	D Plants, pollens, weeds	D Poultry barn
D Cigarette smoke	D Cold air	D DNA
D Farm chemicals	D Exercise	D Other...

Wheezing

Does your chest currently sound wheezy or whistling? O Yes O No

Does your chest ever sound wheezy or whistling when you have a cold? O Yes O No

Does your chest ever sound wheezy or whistling occasionally apart from colds? O Yes O No

Does your chest ever sound wheezy or whistling most days or nights? Q Yes O No

If yes, for how many years has this been present? _____ year(s)

Is your chest wheezing caused or made worse by exposure to:

D Grain dust	D Farm chemicals	D Cold air	D Poultry barn
D Litter dust	D Contact with animals	D Exercise	D DNA
D Cigarette smoke	D Plants, pollens, weeds	D Feathers	D Other...

Shortness of Breath

Are you troubled by shortness of breath when hurrying on the level or walking up a slight hill?

OYes ONo

Do you get short of breath during or after exposure to the poultry barn? O Yes O No

Nasal Irritation

Do you currently have nasal stuffiness, runny nose, sneezing and/or nasal itchiness? O Yes O No

Do these symptoms ever occur when you have a cold? O Yes O No

Do these symptoms ever occur occasionally apart from colds? O Yes O No

Do these symptoms occur most days or nights? O Yes O No

How many years has this been present? ---------- year(s)

Is your nasal stuffiness, runny nose, sneezing and/or nasal itchiness caused or made worse by exposure to:

D Grain dust D Cigarette smoke D Contact with animals D Cold air D Feathers
D Litter dust D Farm chemicals D Plants, pollens, weeds D Exercise D DNA
D Poultry barn D Other.

Eye Irritation

Do you have itching, irritation, tearing and/or redness of the eye(s)? O Yes O No

Do you usually get these symptoms for more than 3 months a year? O Yes O No

Is your itching, irritation, tearing and/or redness of the eye(s) caused by or made worse by exposure to:

D Grain dust D Farm chemicals D Cold air D Poultry barn
D Litter dust D Contact with animals D Exercise D DNA
D Cigarette smoke D Plants, pollens, weeds D Feathers D Other-

Have you ever had fever and/or chills during exposure or after being exposed to the poultry barn?
OYes ONo

During exposure to the poultry barn have your ever had burning/watering/or itchy eyes? O Yes O No

During exposure to the poultry barn have you ever had a stuffy nose? O Yes O No

During exposure to the poultry barn have you ever had a sore throat or burning throat? O Yes O No

During or immediately after exposure to the poultry barn have you ever had itchy skin? Q Yes O No

Headaches

Do you usually develop a headache during work? O Yes O No

Does the headache get better after work? OYes O No O DNA

Does the headache get worse after work? O Yes O No O DNA

Does the headache stay the same after work? OYes O No O DNA

Medical History

Do you currently have a cold? O Yes O No

If you get a cold, does it usually go to your chest? OYes O No O Don't get colds

During the past 3 years, have you had any chest illnesses that have kept you off work, indoors at home or in bed? OYes O No

If yes, how many times in 3 years?----------times

Have your ever had any of the following?

D Chronic Bronchitis	D Chest operations
D Pneumonia	D Chest injuries
D Emphysema	D Heart disease
D Hay fever	D DNA
D Farmer's lung	D Other...

What type of chest operation? ----------

What type of chest injury? _____

Asthma

Have you ever had asthma? O Yes O No

Do you still have asthma? O Yes O No O DNA

Was it confirmed by a doctor? O Yes O No O DNA

At what age did it start? _____ years old

If it has stopped, when?_____ years old

Cardiovascular Health

Has your doctor ever told you that you had any heart trouble? O Yes O No

Have you had any treatment for heart trouble in the past 10 years? QYes O No

Has your doctor ever told you that you have high blood pressure? QYes O No

Have you had any treatment for high blood pressure in the past 10 years? O Yes O No

Are you currently on any medication? O Yes O No

What medications are you currently taking?

D Inhaler D Cortisone(prednisone) D Heart pills D Blood pressure pills D Other...
D Theophyllines D Allergy medications D antibiotics D DNA

Skin Rashes

Have you ever suffered from skin rashes? O Yes O No

Have you ever suffered from skin rashes lasting longer than 2 weeks? O Yes O No O DNA

Smoking History

Have you ever smoked cigarettes? (No means < 20 packs or 400 cigarettes or less that one cigarette a day for a year?

OYes ONo

Do you currently smoke cigarettes? O Yes O No O DNA

How old were you when you first started regular cigarette smoking?_____ years old

How many cigarettes do you smoke per day now? _____ cigarettes/day

On average, for the entire time you have smoked, how many cigarettes have you smoked per day? _____cigarette/day

Do you or did you inhale the cigarette smoke? O Not at all O Slightly
O Moderately O Deeply O DNA

How old were you when you first started smoking cigarettes regularly?
years old

How old were you when you stopped smoking cigarettes completely? _____ years old

Have you ever smoked a pipe regularly (> 12 oz. of tobacco in a lifetime? O Yes O No

For how long?---------- year(s)

Are your currently smoking a pipe? O Yes O No O DNA

If yes, how much? _____tobacco pouches/week

Do you or did you inhale the pipe smoke? O Not at all O Slightly

O Moderately O Deeply O DNA

How old were you when you first started pipe smoking regularly? _____ years old

How old were you when you stopped smoking a pipe completely? _____years old

On average for the entire time you smoked, how many times /day did you smoke? _____/day

Are you currently smoking cigars? O Yes O No O DNA

Have you ever smoked cigars regularly? O Yes O No

If yes, how much? _____ cigars/week

For how long? _____ years

Do you or did you inhale the cigar smoke? O Not at all O Slightly O Moderately O Deeply O DNA

How old were you when you first started smoking cigars regularly? _____ years old

How old were you when you stopped smoking cigars completely? _____ years old

On average for the entire time you smoked, how many cigars did you smoke/day? _____ *cigars/day*

Field Information

Please indicate intensity of the following symptoms PRIOR to entering the poultry barn (0 = no symptoms, 1 = trivial, 2 = mild, 3 = annoying , 4 = moderate, 5 = severe):

Cough	OO	O1	O2	O3	O4	*O5*
Nasal congestion	OO	O1	O2	O3	O4	*O5*
Eye irritation	OO	O1	O2	O3	O4	*O5*
Shortness of breath	OO	O1	O2	O3	O4	*O5*
Chills	OO	O1	O2	O3	O4	*O5*
Phlegm	OO	O1	O2	O3	O4	*O5*
Headache	OO	O1	O2	O3	O4	*O5*
Chest tightness	OO	O1	O2	O3	O4	*O5*
Wheeze	OO	O1	O2	O3	O4	*O5*

Pre-barn peak flow 1:_____ Pre-barn FEV 1: _____

Pre-barn peak flow 2:_____ Pre-barn FEV2:_____

Please indicate intensity of the following symptoms upon LEAVING the poultry barn (0 = no symptoms, 1 = trivial, 2 = mild, 3 = annoying, 4 = moderate, 5 = severe):

Cough	OO	O1	O2	O3	O4	*O5*
Nasal congestion	OO	O1	O2	O3	O4	O5
Eye irritation	OO	O1	O2	O3	O4	*O5*
Shortness of breath	OO	O1	O2	O3	O4	*OS*
Chills	OO	O1	O2	O3	O4	*O5*
Phlegm	OO	O1	O2	O3	O4	*OS*
Headache	OO	O1	O2	O3	O4	*O5*
Chest tightness	OO	O1	O2	O3	O4	*O5*
Wheeze	OO	O1	O2	O3	O4	*O5*

Post-barn peak flow 1: _____ Post-barn FEV_1 1: _____

Post-barn peak flow 2: _____ Post-barn FEV_1 2:_____

Outdoor temperature-high:	_____	C
Outdoor temperature - low:	_____	C
Wind speed AM:	_____	m/s
Wind speed PM:	_____	m/s
Wind direction:	_____	
Barn temperature:	_____	C
Barn relative humidity:	_____	c
Outdoor $C0_2$1:	_____	ppm
Outdoor CO, 2:		ppm
Outdoor RH:	_____	%

Laboratory measurements

	Pump ID 2:	Pump IDS:
Marplel:	Marple 2:	Marple 3:
Pump start time 1:	Pump start time 2:	Pump start time 3:
Pump stop time 1:	Pump stop time 2:	Pump stop time 3:
Total time 1:	Total time 2:	Total time 3
Pre-calibration 1:	Pre-calibration 2:	Pre-calibration 3:
Post-calibration 1:	Post-calibration 2:	Post-calibration 3:
Avg calibration 1:	Avg calibration 2:	Avg calibration 3:
Volume 1:	Volume 2:	Volume 3:
NH, start 1:	NH_astart 2:	NH, start 3:
NH,stop1:	NH. stop 2:	NH_a stop 3:
NH^I:	NH.2:	NH, 3:
CO. start 1:	$C0_2$ start 2:	CO, start 3:
COj stop 1 :	CO, stop 2:	CO, stop 3:
ClOj1:	CO 2:	CO, 3:
Total dust (mg)F-1:	Total dust (mg) F-2:	Total dust *(ma)* F-3:
Total dust (mg) 8-1:	Total dust (mg) 8-2:	Total dust (mg) 8-3:
Total dust (mg) 7-1:	Total dust (mg) 7-2:	Total dust (mg) 7-3:
Total dust (mg) 6-1:	Total dust (mg) 6-2:	Total dust (mg) 6-3:
Total dust (mq) 5-1:	Total dust (ma) 5-2:	Total dust (mg) 5-3:
Total dust (mg)4-1:	Total dust (mg) 4-2:	Total dust (mg) 4-3:
Total dust (mq) 3-1:	Total dust (mg) 3-2:	Total dust (mg) 3-3:
Endotoxin (mg) F-1:	Endotoxin (mg) F-2:	Endotoxin (mg) F-3:
Endotoxin (mg) 8-1:	Endotoxin (mg) 8-2:	Endotoxin (mg) 8-3:
Endotoxin (mn) 7-1"	Endotoxin (mg) 7-2:	Endotoxin (mg) 7-3:
Endotoxin *(mn*\ fi-"1 '	Endotoxin (mg) 6-2:	Endotoxin (mg) 6-3:
Endotoxin (mn) 5-1"	Endotoxin (mg) 5-2:	Endotoxin (mg) 5-3:
Endotoxin (mg) 4-1:	Endotoxin (mg) 4-2:	Endotoxin (mg) 4-3:
Endotoxin (mg) 3-1:	Endotoxin (mg) 3-2:	Endotoxin (mg) 3-3:
Pre-weight (g) F-1:	Pre-weight (g) F-2:	Pre-weight (g) F-3:
Pre-weight (g) 8-1:	Pre-weight (a) 8-2:	Pre-weight (g) 8-3:
Pre-weight (g) 7-1:	Pre-weight (g) 7-2:	Pre-weight (g) 7-3:
Pre-weiaht(a)6-1:	Pre-weight (g) 6-2:	Pre-weight (g) 6-3:

STANDARD OPERATING PROCEDURE FOR PAPER 2

Test Method for Fractionated Particulate and Endotoxin in Poultry Barn Atmospheres

(Adapted from NIOSH testing standard 0500)

1.0 Summary of Test Method

1.1 Workplace air is drawn into a Marple® 6-stage sampler containing tarred PVC (slit) filters for a specified time period at a rate of 2.0 L/min. The total particulate matter concentration is calculated from the mass gain of the filter and the volume of air sampled.

2.0 Sampling Apparatus

2.1 Pump – A constant flow personal sampling pump capable of a flow rate of 2.0 L/min through the Marple® sampler for a specified time period.
2.2 Marple© Sampler – A six stage Marple© sampler.
2.3 Filters – 37mm slit PVC filters and a PVC final filter
2.4 Field Blank – A filter prepared for sampling that has been taken to the workplace and handled in the same manner as the analytical filters, but which has not had any air drawn through it.
2.5 There will be one field blank for each set of samples.
2.6 Precision Flow Meter for pre- and post-sampling calibration of flow rates.
2.7 Weight room – As set up in SRC laboratory, using anti-static strips for weighing of filters.
2.8 Diffusion tubes – A means of measuring the NH_3 or CO_2 concentration based on diffusion process and the resulting colorimetric change.
2.9 Velocicalc® – an anemometer used to measure velocity, temperature, and humidity.
3.0 PiKo-1 - an instrument to measure PEF and FEV_1.

PRE-BARN VISIT

3.0 SRC Laboratory

3.1 Desiccate filters in the cassettes for a minimum of twenty four hours.
3.2 Calibrate scale daily.
3.3 Place filter on a static neutralizer.
3.4 Weigh each filter and record mass to the nearest 0.001 mg. Record each filter weight (*mg*), barn site ID number (*B# or L#*), stage number (*3→F*), and date (*mm/dd/yy*) on the flow sheet.
3.5 Place tarred filters in the Marple© and close fully. Insert Marple© into CLEAN glass jar transport jar containing desiccant.

4.0 I.ARE.H Laboratory

4.1 Calibrate pumps to 2.0 (±0.02) L/min using the precision flow meter. Ensure the precision flow meter and the Marple® sampler are in line (pump → Marple® → precision flow meter). A minimum of three calibrations should be performed, and the average of the calibrations used in determining Total Weighted Average. Document each calibration on flow sheet. Record pumps' ID (*xxx*) on flow sheet. Include barn site ID (B# or L#) with Marple® ID *(1→3)*. Record this total ID on the flow sheet.

4.2 Install the Marple© with pump onto backpack.

4.3 Install NH_3 and CO_2 diffusion tubes onto backpack.

BARN VISIT – THERE MUST BE MINIMUM 24 HOURS BETWEEN BARN VISITS!

5.0 Barn Site

5.1 Wash hand with hand cleanser.

5.2 Open backpacks and start the flow pumps before entering the barn.

5.3 Dawn protective clothing – coveralls, boots, and head cover. Remove street shoes in the van. Put coveralls and head gear on in the van. Place boots outside the vehicle and put them on as leaving the vehicle.

5.4 Set up instrument to measure outdoor temperature (high and low) and relative humidity. Measure wind speed and wind direction. Measure outdoor CO_2 level.

5.5 Conduct general questionnaire with barn personnel.

5.6 Measure poultry worker's pre-barn PEF and $FEV_{1.}$

5.7 Hang Marple® in barn.

5.8 Attach Marple® sampler to tubing and Hygobaby© once situated in the barn or on the poultry worker. Record sampling start time on flow sheet.

5.9 Break NH_3 and CO_2 diffusion tubes after Marple® sampling has begun.

5.10 Record NH_3 and CO_2 sampling start time on flow sheet.

5.11 Attach NH_3 and CO_2 diffusion tubes to Marple® backpacks and poultry worker using lapel clips.

5.12 Illustrate Marple® locations on barn diagram.

5.13 Locate and then illustrate all operating fans on barn diagram.

5.14 With the Velocicalc®, measure fan velocity in the three sample areas noted in Figure 1. Record the three velocity measurements on the flow sheet.

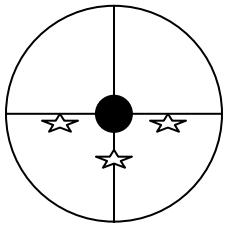


Figure 1. Fan and three sampling areas.

5.15 Remove Marple® backpack and NH_3 and CO_2 diffusion tubes from poultry worker once they vacate the barn. Poultry workers will wear the Marple© and NH_3 and CO_2 diffusion tubes as long as they are in the barn and as a result sample time may vary – BE SURE TO RECORD START AND STOP TIMES!

5.16 Measure poultry worker's post-barn PEF and FEV_1.

5.17 At end of collection period (~5 hours), record sampling stop time on flow sheet. Record NH_3 and CO_2 diffusion tube readings. DO NOT STOP THE PUMP, but remove the Marple© from the backpack. Keep the Marple® upright at all times.
5.18 Outside the barn stop the pump.
5.19 Place Marple® upright in DIRTY transport jar with desiccant. Ensure the Marple® does not tip. Wipe transport jar off with sani-cloth wipes prior to loading in vehicle.
5.20 Place and seal Marple® backpacks and attachments in plastic bags prior to loading in the trunk of the vehicle.
5.21 Remove contaminated barn clothing. Place and seal contaminated clothing in plastic bags prior loading into the trunk of the vehicle.
THE VEHICLE IS CONSIDERED A CLEAN ZONE!
5.22 Wash hands or use antiseptic hand rinse before entering the vehicle.
5.23 Record outdoor temperature, humidity, CO_2, wind speed and direction.
Wipe instrument with sani-cloth wipe.
5.24 Wipe off steering wheel with a sani-cloth.

POST BARN VISIT

6.0 Weighing Procedure

6.1 Wipe the outside of each Marple® with a sani-cloth.
6.2 Post-calibrate the pump with the precision flow meter, precision flow meter and Marple® inline (pump → Marple® → precision flow meter). Calibrate three times and record the average on the flow sheet.
6.3 Desiccate the samples for a minimum of twenty four hours before post-weighing.
6.4 Place the filter on an anti-static strip. Weigh the filter and record the weight on the flow sheet.
6.5 After post-weight, the filter is placed in a 50ml centrifuge tube, and labeled with total ID number (barn site (B# or L#), Marple® *(1→3)*, pump [xxx], stage [*3→F*] and date [*mm/dd/yy*]).
6.6 Wipe the forceps with alcohol swab between each sample.
6.7 Repeat procedure for each filter and field blank.
6.8 Place complete set of Marple® filter tubes in a Ziploc bag together. Place the three sets of Marple® filter tubes from each barn in a larger Ziploc bag. Ensure bags a labeled with complete ID.
6.9 Store centrifuge tubes in lab fridge.

7.0 Cleaning and Disinfecting Procedure

7.1 Dispose of NH_3 and CO_2 diffusion tubes in sharps container. Disinfect lapel tube holders.
7.2 Dispose of Hygobaby© filter and hose attachments.
7.3 Wipe the outside of each pump, the precision flow meter and the Velocicalc® with a sani-cloth.
7.4 Disinfect Marple® backpack attachments with Peroxigard. Soak articles for a minimum 5 minutes. Use glove to remove articles from sink.
7.5 Wash Marple® transport jar exterior.

7.6 Disinfect Marple® in Peroxigard.
7.6 Launder coveralls.
7.7 Launder Marple® backpacks.
7.8 Clean and disinfect rubber boots with Peroxigard.
7.9 Wash vehicle, including the undercarriage between farm visits. Vacuum van where carrying containers were placed.
7.10 Disinfect clipboard and pen.

8.0 Calculations

8.1 Concentration of Total Particulate

$$\text{Concentration of total particulate (mg/m}^3\text{)} = \frac{(W_2 - W_1) - (B_2 - B_1)}{V} \times 10^3$$

W_1 = weight of filter before sampling (mg)
W_2 = weight of filter after sampling (µg)
B_1 = pre-weight of blank filter (mg)
B_2 = post-weight of blank filter (mg)
V = (sampling rate, L/min) (sampling time, min) = Litres

8.2 NH_3 and CO_2 concentration

$$NH_3 \text{ concentration (ppm)} = \frac{\text{detector tube indication}}{\text{duration of measurement (hours)}}$$

$$CO_2 \text{ concentration (ppm)} = \frac{\text{detector tube indication}}{\text{duration of measurement (hours)}}$$

APPENDIX 4: MASS MEDIAN AERODYNMIC DIAMETER CALCULATION EXAMPLE

METHODS FOR MMAD CALCULATIONS:

This spreadsheet was designed specifically for use with the Marple Personal Cascade Impactor (Anderson Series 290) by Patrick T. O'Shaughnessy, The University of Iowa, Department of Occupational and Environmental Health.

BACKGROUND

Two spreadsheets were used:

1. The "Graphical Method" involves the application of the well-known method for determining the MMAD and GSD by plotting on log-probability paper.

Because Excel cannot create a probability axis, probability, in terms of cumulative percent, is transformed into the associated standard deviation value and plotted on a linear scale. Both an "uncorrected" and "corrected" mass percent is computed, where the "correction" involves a correction for sampling inefficiencies that result in the loss of almost 50% of the larger particles, for example (1). These corrected percentages are applied to the Graphical Method.

2. The uncorrected percent is used in the "Inversion Method" in the second spreadsheet. This method determines the MMAD and GSD that minimizes the differences between the measured mass fractions and those computed under the assumption that the aerosol is perfectly UNIMODAL, and LOGNORMALLY distributed. (This version of the method does not have an option to analyze multimodal distributions). The original paper describing this method was given by Raabe (2). A paper by O'Shaughnessy and Raabe (3) describes the application of a spreadsheet to this method where the primary utility of the spreadsheet is to make the method readily available and to take advantage of the powerful root-solving function, "Solver", incorporated in Excel. This is the spreadsheet resulting from that work. Corrections for both inlet efficiency and inner losses are incorporated in the Inversion spreadsheet and, therefore, should be compared with the uncorrected percentages developed in the data table. To explain further: The percentages in the inversion spreadsheet are manipulated to compensate for the

losses etc. that would result in the mass percentages resulting directly from an analysis of the net weights. Therefore, one should not compare "corrected" percentages to those compensated for in the Inversion method

Another point of explanation: The Inversion method utilizes the actual stage efficiency curves in its determination. These curves were first developed by Rubow et al. (1) and later reanalyzed and given numerical approximations by Rader et al. (4), which are used here. The curve functions are based on what Rader calls the "slip-corrected aerodynamic diameter", Dpc, and Raabe (5) calls the "aerodynamic resistance diameter", Dar. Therefore, a column is included to compute Dar, given the aerodynamic diameter, Dae. The "stage effectiveness" values given in the "Graphical Method" sheet are those given by Rader et al. (4). This spreadsheet could be adapted to apply to a different impactor by incorporating either functions or interpolated values to indicate the stage efficiencies of the impactor used. For example, Rader (4) also gives curve functions for the Anderson Mark III impactor.

DISCUSSION

Note that the MMAD and GSD computed by the Inversion will always be smaller than that computed by the Graphical method. The simplest explanation is (for MMAD) that for stage 1, for example, the graphical method assumes that all mass is a result of particles greater than the cut-diameter of 21 microns whereas the Inversion method "realizes" that a good portion of the mass is associated with particles less than 21 microns. Therefore, the Inversion method attributes mass to the correct diameter and determines a more accurate MMAD than the graphical method. The implication is that an MMAD determined by the Graphical method overestimates the MMAD and therefore underestimates the respirable fraction, and, consequently, the ill-health consequences associated with smaller particles. Further explanation is given by O'Shaughnessy and Raabe (3).

To assess the accuracy of the program for calculating MMAD, the first twelve calculations were plotted by hand using the cumulative percent (x-axis) plotted against the cut-off diameter (y-axis) on log-probability paper. A straight line was drawn through the data points.

The MMAD (the particle diameter at 50% cumulative diameter) and the geometric standard deviation (the particle diameter at 84% cumulative diameter/MMAD) were read from the plot. The remainder of the MMADs was calculated utilizing only the spreadsheets.

Table 1: Marple MMAD calculation: Graphical Method

MARPLE IMPACTOR DATA REDUCTION GRAPHICAL METHOD

Trial Name:
Test Date: Flow Rate: 2 lpm
Calibrated: Sample Time: 90 min

STAGE NO.	Cut Diam. µm	Initial Weight, mg	Final Weight, mg	Net Weight, mg	Uncorrected Mass Fraction, %	Stage Effect.	Corrected Net Wt., mg	Corrected Mass Fraction, %	Cum. % >	Cum. % <
1	21.10	149.105	149.152	0.047	4.92	0.52	0.090	8.14	8.14	91.86
2	15.00	149.922	149.970	0.048	5.03	0.61	0.079	7.09	15.23	84.77
3	9.80	150.453	150.567	0.114	11.94	0.78	0.146	13.16	28.39	71.61
4	6.00	151.392	151.578	0.186	19.48	0.89	0.209	18.82	47.21	52.79
5	3.50	150.606	150.919	0.313	32.77	0.95	0.329	29.67	76.88	23.12
6	1.54	150.090	150.304	0.214	22.41	0.96	0.223	20.07	96.95	3.05
7	0.91	150.411	150.435	0.024	2.51	0.97	0.025	2.23	99.18	0.82
8	0.53	150.763	150.771	0.008	0.84	0.99	0.008	0.73	99.91	0.09
FILTER		8.734	8.735	0.001	0.10	1.00	0.001	0.09	100.00	
			Sum	0.96		Sum	1.11	mg		
						Avg. Conc.	6.17	mg/m^3		

MMAD = 6.40 µm
GSD = 2.23 µm

Table 1 is used to determine the uncorrected mass fraction for each stage that is used by the inversion method to determine the MMAD and GSD.

Table 2: Determination of Inverstion Method Initial MMAD and GSD

X-Range Y-Range Regression

Z	Log-Dia.	Log Dia	Dia.
1.396	3.049	2.975	19.586
1.027	2.708	2.679	14.571
0.571	2.282	2.314	10.115
0.070	1.792	1.912	6.768
-0.735	1.253	1.267	3.550
-1.874	0.432	0.354	1.425
-2.401	-0.094	-0.068	0.934
-3.121	-0.635	-0.646	0.524

intercept = 1.856045
slope = 0.801547

The information in Table 2 is used to determine an initial value of the MMAD and GSD (as shown, 6.40 and 2.23 μm) which is used as a starting value for the inversion method.

Figure 1: Log-probit graph used to determine intial MMAD and GSD

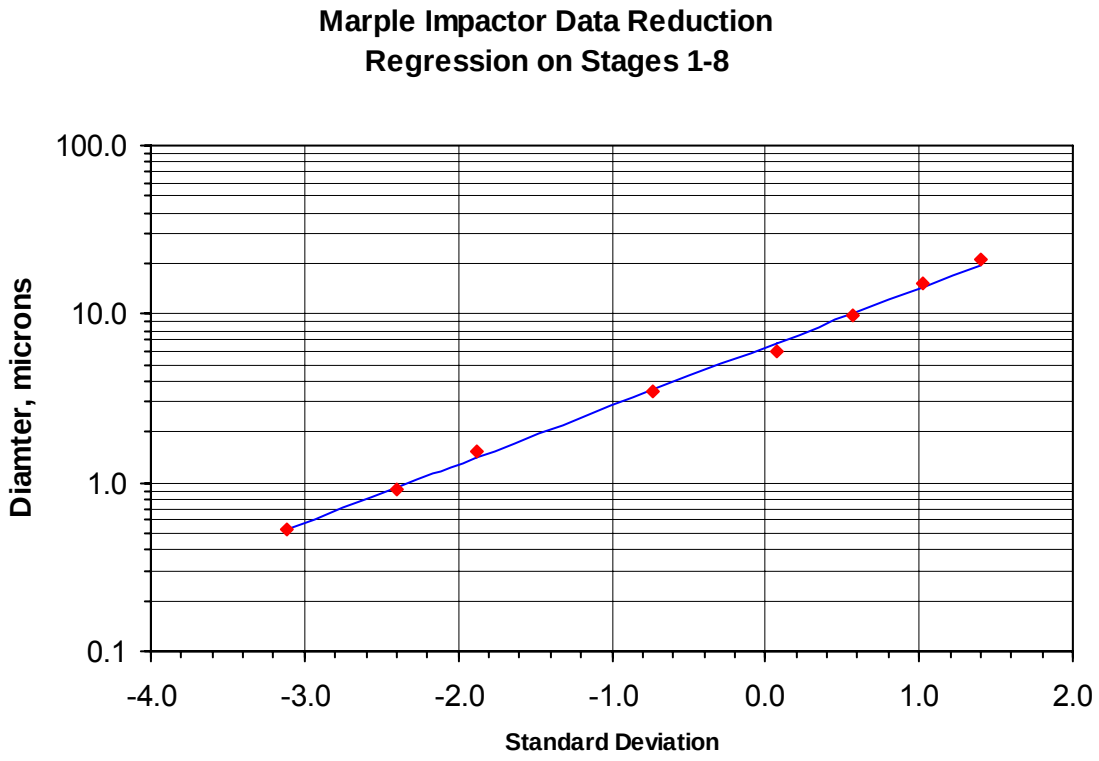


Table 3: MMAD Calculation using the Inversion Method

INVERSION METHOD USING RADER ET AL. EFFICIENCY CURVES
This applies a weight equivalent to the variance of the frequency values defined as f(1-f).
It also applies sampler losses and inlet efficiency corrections

Stage	Mass Percents from Data Reduction	Mass Percents Computed below	Variances	Weighted Squared error	Minimum Error
1	4.921466	3.9394055	0.046793	20.61102	196.7293
2	5.026178	5.8034563	0.047736	12.65643	
3	11.93717	11.441652	0.105122	2.335771	
4	19.47644	22.313514	0.156831	51.32262	
5	32.77487	29.48627	0.220329	49.08505	
6	22.40838	24.107857	0.17387	16.61142	
7	2.513089	2.6044558	0.024499	0.34074	
8	0.837696	0.2800814	0.008307	37.43136	
F	0.104712	0.0233088	0.001046	6.334934	

"Solved" Values		log values
MMAD	5.19 μm	0.7147945
GSD	1.88 μm	0.2730069

MARPLE IMPACTOR DATA REDUCTION MAXIMUM LIKELIHOOD METHOD

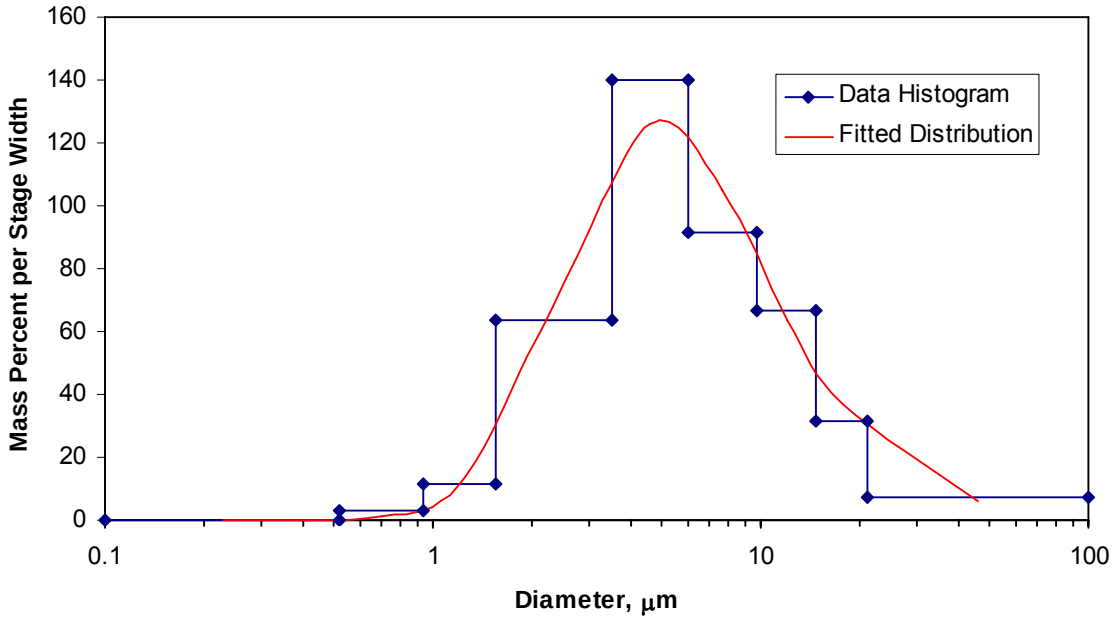
Trial Name: 0
Test Date: 0 Flow Rate: 2 lpm
Calibrated: 0 Sample Time 90 min

STAGE NO.	Cut Diam. mm	Initial Weight, mg	Final Weight, mg	Net Weight, mg	Uncorrected Mass Fraction, %	Stage Effect.	Corrected Net Wt., mg	Corrected Mass Fraction, %
1	21.1	149.105	149.152	0.047	4.921	0.52	0.090	8.140
2	15	149.922	149.97	0.048	5.026	0.61	0.079	7.086
3	9.8	150.453	150.567	0.114	11.937	0.78	0.146	13.162
4	6	151.392	151.578	0.186	19.476	0.89	0.209	18.821
5	3.5	150.606	150.919	0.313	32.775	0.95	0.329	29.671
6	1.54	150.09	150.304	0.214	22.408	0.96	0.223	20.075
7	0.91	150.411	150.435	0.024	2.513	0.97	0.025	2.228
8	0.53	150.763	150.771	0.008	0.838	0.99	0.008	0.728
FILTER		8.734	8.735	0.001	0.105	1.00	0.001	0.090
			Sum	0.955		Sum	1.110	mg
						Avg. Conc.	6.169	mg/m^3

MMAD	5.19 μm	
GSD	1.88 μm	

The values in the "Mass Percents from Data Reduction" column in Table 3 are the same as those in Table 1 "Uncorrected Mass Fraction". The inversion method utilizes the actual collection efficiency curves from each stage to determine an MMAD and GSD that produce mass percent values as close to those values as possible (as given in "Mass Percents computed below" column. A weighted squared error is used to determine a "minimum error" between the two sets of fractions).

Figure 2: Histogram from Inversion Method



INSTRUCTIONS

1. The "Graphical Method" spreadsheet was used to record pre- and post-weights. All other values and the resulting linear regression were automatically computed. Note the computed values of the MMAD and GSD for this method given below the graph. The uncorrected mass fractions are automatically transferred to the "Inversion Method" spreadsheet.

2. Copy and pasted (absolute) the MMAD and GSD values computed in the "Graphical Method" sheet to the appropriate cells in the "Inversion Method" sheet.

3. In the "Inversion Method" spreadsheet:

Used the "Solver" to minimize the sum of the weighted squared error value.

Go to "Tools", "Solver", then in the Solver toolbox: "Set Target Cell:" to G5"Equal to:" check on "min""By Changing Cell::" "Subject to Constraints:" (must be added in using "Add") >= 0.01 >= 1.01Click "OK" to solve

Resulting MMAD and GSD were calculated

4. All changes made to "Graphical Method" sheet were automatically transferred to the inversion sheet.

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(1) Rubow, K. L.; Marple, V. A.; Olin, J.; McCawley, M. A. 1987. A personal cascade impactor: design, evaluation and calibration. Am. Ind. Hyg. Assoc. J. 48(6):532-538.

(2) Raabe O.G. 1978. A general method for fitting size distributions to multicomponent aerosol data using weighted least-squares. Environ. Sci. Technol. 12(10):1162-1167.

(3) O'Shaughnessy, P. T., Raabe, O. G. A Comparison of cascade impactor data reduction methods. Journal of Aerosol Science and Technology. 2001 *American Association for Aerosol Research Conference*, pp. 15-19.

(4) Rader DJ, Mondy LA, Brockmann JE, Lucero DA, Rubow KL. 1991. Stage response calibration of the Mark III and Marple Personal cascade impactors. Aerosol Science and Technology 14:365-379.

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