

**(1-3)- $\beta$ -D GLUCAN EXPOSURE ASSESSMENT IN POULTRY  
FARMS IN SOUTH AFRICA**

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## **DECLARATION**

I, Payal Dayal declare that this dissertation is my own work. It is being submitted for the degree Master of Science in Medicine in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other institution.

..... (Signature of candidate)

..... day of.....(month), 2014

## **DEDICATION**

I dedicate this work to my mentor & friend,

Dr Tanusha Singh. You have imparted a wealth of knowledge, skills and belief in me.

To Prof Ahmed Wadee for his supervision and mentorship.

To my parents – your never-ending faith in me has fuelled me with determination and the courage to conquer life's challenges.

To my brother and husband – thank you for your support and encouragement.

# **PUBLICATIONS AND PRESENTATIONS**

## **Oral presentations**

Part of this dissertation has been presented at national conferences or scientific meetings as listed below:

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## ABSTRACT

**Introduction:** Poultry workers have an increased risk of respiratory symptoms associated with various irritant and allergenic exposures causing airway inflammation. This study investigated the levels of (1-3)- $\beta$ -D glucan exposure in several poultry farming processes. The objectives involved categorising the different tasks undertaken in the poultry industry. After which a method was established and validated to detect and quantify the levels of (1-3)- $\beta$ -D glucan using the GlucateLL assay. This assay was used to measure the amount of (1-3)- $\beta$ -D glucan poultry farm workers were exposed to using personal sampling. Thereafter, general respiratory symptoms were described briefly via the administration of a respiratory questionnaire. **Method:** A total of 308 personal air samples were collected from several poultry farming processes (rearing, laying, hatchery, broilers, catching) of a large poultry farm in the North West Province. A walkthrough checklist was used to obtain information on various exposure determinants such as farm size, number of chickens, ventilation system, bedding material used and poultry feed used. The GlucateLL assay (Associates of Cape Cod, East Falmouth, MA, USA) was used to quantify the concentration of (1-3)- $\beta$ -D glucans in the air samples.

**Results:** The geometric mean concentrations of (1-3)- $\beta$ -D glucans ranged from 24.38 to 645.98 ng/m<sup>3</sup> across the various poultry farming processes investigated. Workers in the broiler farms were exposed to two times higher levels of (1-3)- $\beta$ -D glucans compared to those in the breeding farms. The sizes of the broiler farm houses as well as the age of the chickens were among the main determinants of exposure. The larger broiler farm houses (GM=5.2 ng/m<sup>3</sup>, GSD=3.74) had significantly ( $p < 0.05$ ) lower levels than the smaller broiler farm houses (GM=6.4 ng/m<sup>3</sup>, GSD=2.14) whilst houses with older chickens had higher (1-3)- $\beta$ -D glucan levels (G=5.8 ng/m<sup>3</sup>,

GSD=3.48) than the baby chick houses (GM=5.2 ng/m<sup>3</sup>, GSD=3.56). The prevalence of work-related cough (45%) and wheeze (61.8%) in this study were greater than rates reported in the literature.

**Conclusion:** Elevated levels of (1-3)- $\beta$ -D glucans were demonstrated in most of the poultry farming processes with catching crew being the highest and hatchery being the lowest. The size of the broiler houses and the age of the chickens also impacts on the risk of exposure. Work related respiratory symptoms were found to be higher in this study compared to international studies.

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## **NOMENCLATURE**

ANOVA:	Analysis of variance
CV:	Coefficient of variation
DECOS:	Dutch Expert Committee on Occupational Safety
ECRHS:	European Community Respiratory Health Survey
EIA:	Enzyme immunoassay
ELISA:	Enzyme linked immunosorbant assay
ESCS:	Electrostatic space charge system
EU:	Endotoxin units
GM:	Geometric mean
GSD:	Geometric standard deviation
IOM:	Institute of Occupational Medicine
KZN:	Kwa-Zulu Natal
LAL:	Limulus amebocyte lysate assay
LOD:	Limit of detection
LOQ:	Limit of quantification
NIOH:	National Institute for Occupational Health
NHLS:	National Health Laboratory Service
ng/m <sup>3</sup> :	Nanogram per meter cube
OEL:	Occupational exposure limit
OHSA:	Occupational Health and Safety Act
PAg:	Poultry related antigens
PPE:	Personal protective equipment
SAPA:	South African Poultry Association
TNF- $\alpha$ :	Tumor necrosis factor alpha
W Cape:	Western Cape

## **PREFACE**

Numerous studies conducted over the past decade indicate that exposure to bioaerosols can result in a direct increase in respiratory and related illnesses. Due to the increasing evidence of the risk of occupational exposure to bioaerosols and in particular (1-3)  $\beta$ -D-glucans internationally, there is an urgent need to identify and appropriately quantify these agents in South Africa so that suitable control methods and preventative actions may be developed and implemented.

(1-3)  $\beta$ -D-glucans may be found in high levels in the agricultural sector, including poultry farming. The poultry industry continues to dominate the South African livestock sector as chicken remains an affordable protein source compared to other meat sources. This industry remains an important contributor of employment opportunities both in the formal and informal sector with 80% of the industry comprising small, medium and micro enterprises which employ approximately 77 000 people. Therefore, characterising the occupational risk to (1-3)  $\beta$ -D-glucans is important to maintain a healthy workforce.

The National Institute for Occupational Health (NIOH) a branch of the National Health Laboratory Service (NHLS) is committed to improving occupational health in South Africa. The findings from this study will contribute to the commitment of reducing exposures and hence creating a safer environment for poultry farm workers.

# **CHAPTER 1**

## **1.0 INTRODUCTION**

### **1.1 BACKGROUND**

The poultry industry continues to dominate the South African livestock sector as chicken remains an affordable protein source compared to other meat products. The South African poultry industry mainly includes broiler, egg and chick producers. This industry remains an important contributor to employment opportunities both in the formal and informal sector with 80% of the industry comprising small, medium and micro enterprises which employ approximately 77 000 people (i.e. 60 000 broiler, 10 000 egg and 7 000 chick producer) (SAPA, 2008).

Previous studies have demonstrated that poultry workers have an increased incidence of respiratory symptoms due to exposure to organic dust (Zuskin et al., 1995; Rees et al., 1998; Kurychuk et al., 2003; Rylander and Carvalheiro, 2006; Faria et al., 2006; Mirabelli et al., 2012). Direct contact with live poultry and poultry litter is a major cause of sensitisation in some poultry workers. Asthma symptom prevalence in farmers has been found to be higher for poultry farmers. Up to 17.4% of poultry farmers reported symptoms of asthma (Kimbell-dunn et al., 1999). Bar-sela et al (1984) reported rhinitis and asthma in approximately 16 of 44 symptomatic poultry workers after exposure to poultry. In most of these workers the symptoms occurred within a few minutes after entering the poultry house. The findings from this study suggested

that the symptoms related to poultry exposure were IgE mediated and were related to sensitisation to poultry-related antigens (PAg) (Bar-sela et al., 1984).

A study conducted in poultry houses in Switzerland demonstrated that chicken catchers were at high risk of exposure to bioaerosols (inhalable dust, total bacteria and endotoxin) with levels above the Swiss occupational recommended limits (Oppliger et al., 2008). One attempt by Donham in 1990 and 2000 was done to ascertain the dose at which poultry house dust exposure is associated with significant impairment of pulmonary function. This was estimated at 2.4 mg/m<sup>3</sup> total dust, 0.16 mg/m<sup>3</sup> respirable dust, 614 EU/m<sup>3</sup> endotoxin and 12 ppm ammonia (Donham et al., 1999; Donham et al., 2000). This study also demonstrated high levels of bioaerosols during the fattening period of chickens, since the biomass of faeces and feather dandruff increases as chickens grow generating aerosols during catching and transporting activities (Calvet et al., 2009). A fairly recent study conducted in the Free State province of South Africa investigated the microbial composition of air in a chicken slaughtering facility (Lues et al., 2007). This study found the highest counts of airborne microorganisms in the initial stages of processing, involving the receiving, killing and de-feathering areas, which decreased toward the evisceration, air-chilling, packaging and dispatch areas (Lues et al., 2007). However, the latter study did not focus on the occupational risk to workers in the different areas.

Previous studies have also demonstrated that organic dusts and by-products from the decomposition of organic matter can cause respiratory symptoms through airway inflammation (Douwes, 2005; Rylander and Carvalheiro, 2006). Bioaerosols in poultry dust may be derived from soil, and dust from feed and bedding material (Morris et al., 1991; Crook et al., 2008; Just

et al., 2009). Poultry farm workers are exposed to a wide variety of agents which might include both organic and inorganic material derived from litter, feathers, dander (skin material), feed, excreta and microorganisms and their by-products which could cause respiratory disease. The organic dust may contain several inflammatory agents including (1-3)- $\beta$ -D-glucan (Thorn et al., 2001), which are water insoluble glucose polymers commonly found in the cell walls of most (60%) moulds, some soil bacteria and plants (Douwes, 2005; Lossifova et al., 2007). (1-3)- $\beta$ -D-glucan has been used as a surrogate marker for mould exposure in airborne studies (Rylander, 1997; Rylander, 1999; Chew et al., 2001; Lossifova et al., 2007). Concentrations of (1-3)- $\beta$ -D-glucan in poultry houses from previous studies ranged from 4-870 ng/m<sup>3</sup> (Rylander and Carvalheiro, 2006) and a more recent study by Sander et al (2008) found similar levels (2-972ng/m<sup>3</sup>) in chicken houses.

Recently (1-3)- $\beta$ -D-glucan has been recognised for its potent ability to induce pro-inflammatory reactions, similar to endotoxins (a by-product of Gram negative bacteria) in occupational settings (Douwes et al., 2003; Young et al., 2011). Other studies involving animal models have reported an interaction between (1-3)- $\beta$ -D-glucan and endotoxin in causing airway inflammation (Rylander and Fogelmark, 1994). Another study related (1-3)- $\beta$ -D-glucan to the increased prevalence of symptoms and airway responsiveness in workers employed in poultry houses (Rylander and Carvalheiro, 2006). More general symptoms as a result of (1-3)- $\beta$ -D-glucan exposure include fatigue and joint pains, headache, nose and throat irritation, accompanying respiratory symptoms due to airway inflammation and lung function changes (Rylander and Carvalheiro, 2006; Lossifova et al., 2007).

Rubel and Freeman (1998) showed that farmers handling chicken feed were exposed to a variety of chemicals. It was found that exposure to a preservative known as ethoxyquin added to the chicken feed was responsible for allergic contact dermatitis experienced by a farm worker. Furthermore, Burrows and colleagues (1975) reported of airborne contact dermatitis in an animal feed mill worker initially exhibiting facial lesions and subsequent distribution throughout the body. While general chemical exposure is well documented, the focus of the current study was to characterise (1-3)- $\beta$ -D-glucan exposure as this has not been assessed previously in South Africa.

There is considerable evidence that the risk of developing occupational allergy and asthma is correlated with increasing exposure to organic dust. Therefore there is a particular need to understand the relationship between exposures to components of organic dust, in particular (1-3)- $\beta$ -D glucans, in the poultry industry and respiratory symptoms. A comprehensive exposure assessment needs to be done in occupational settings such as the poultry industry with multiple exposures.

## **1.2 MOTIVATION**

Over a decade ago, a study conducted at the NIOH investigating work-related respiratory symptoms in poultry workers found a high prevalence of exposure-related symptoms associated with organic dust exposure (Rees et al., 1998). However specific causative agents were not identified which is important as it will assist in management of affected workers. A further

limitation of this study was the lack of objective exposure assessments and the inability to demonstrate an association between allergic sensitisation and respiratory symptoms. More recently (2002-2006), the NIOH allergy unit tested several workers from poultry farms with respiratory symptoms and asthma. Although 43% were positive to allergens tested from the workplace, 57% were negative to the aeroallergens and poultry specific allergens (chicken feathers, poultry feed, shavings and poultry litter) pointing to the possibility of a non-IgE mediated inflammatory response. These findings are consistent with that of other studies which suggest that at most only 50% of asthma cases are attributable to “allergic asthma” (Douwes et al., 2002).

In light of such studies on the occupational hazards of exposure to bioaerosols in the poultry industry, there is a need to identify and appropriately quantify the agents and their health impact in the South African poultry setting. This also has implications for asthma diagnosis and clinical management of affected workers or provides the possibility of introducing control measures and preventative actions.

This project enabled the development, optimisation and establishment of an assay for (1-3)- $\beta$ -D-glucan in the Immunology and Microbiology Section of NIOH and forged international research collaborative. Occupational exposure assessments on (1-3)- $\beta$ -D-glucan have not previously been reported in South Africa and were not available in the country.

### 1.3 HYPOTHESIS AND OBJECTIVES

There is limited information available on occupational respiratory diseases in the South African poultry industry. Poultry dust contains multiple biological agents and includes (1-3)- $\beta$ -D-glucan that may be related to respiratory symptoms including asthma among chicken farm workers. The study undertook to:

- Categorise the different tasks performed within the poultry farm.
- Develop sampling methods that may be used to confirm occupational exposure to (1-3)- $\beta$ -D-glucan by establishing and validating a method for the detection and quantification of (1-3)- $\beta$ -D-glucans in environmental samples using the GlucateLL assay.
- Document exposure of poultry workers by measuring (1-3)- $\beta$ -D-glucan using personal air sampling.
- Describe general respiratory symptoms experienced by workers by task.

## CHAPTER 2

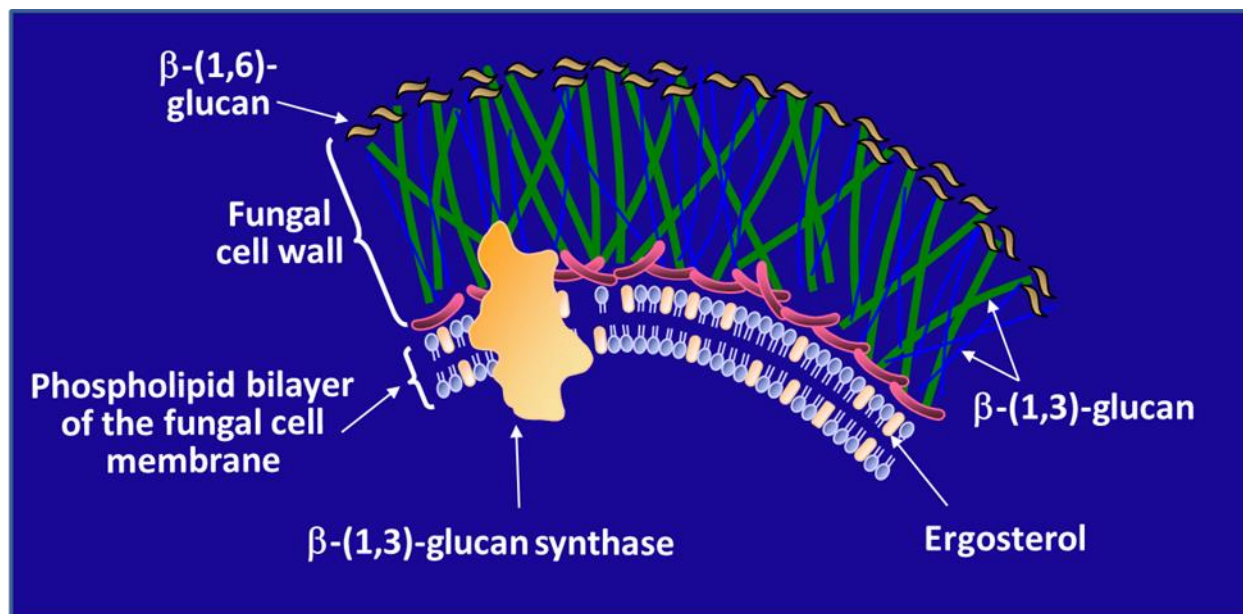
### 2.0 LITERATURE REVIEW

There is limited information available on occupational respiratory diseases in the South African poultry industry. Poultry dust contains multiple biological agents and includes (1-3)- $\beta$ -D-glucan that may be related to respiratory symptoms including asthma among chicken farm workers. To date similar research has not been documented in South Africa as a result No occupational exposure limits are available for (1-3)- $\beta$ -D-glucan. Currently several research groups are investigating levels of exposure in different industries. The associated health effects of (1-3)- $\beta$ -D glucan exposure have been described by various researchers. The literature gathered in this review has been accessed from scientific resources such as Pubmed and Science Direct

### 2.1 WHAT ARE (1-3)- $\beta$ -D-GLUCANS?

There are various types of glucans that originate from different fungal species, which include laminaran, curdlan and grifolan (Chen and Seviour, 2007). (1-3)- $\beta$ -D-glucans are non-allergenic, water insoluble structural cell wall components of most fungi. **Figure 2.1** illustrates the structure of a fungal cell wall containing (1-3)- $\beta$ -D glucan molecules (Richardson, cited: 16 September 2008). These glucan molecules has been considered as playing a pivotal role in the development of respiratory symptoms which have been associated with indoor and occupational fungal exposure in environments with high humidity and temperature (Foto et al., 2004; Douwes et al., 2005; Ghasemkhani et al., 2006; Samadi et al., 2009);. In addition, these molecules have been recognised for their potent ability to induce inflammatory reactions, similar to endotoxins in

occupational and non-occupational settings (Rylander, 1999; Douwes et al., 2003; Rylander et al., 2006).



**Figure 2.1** Structure of fungal cell wall containing (1-3)- $\beta$ -D glucan molecules. (Richardson, cited: 16 September 2008).

## 2.2 SOURCES OF EXPOSURE TO (1-3)- $\beta$ -D-GLUCANS

(1-3)- $\beta$ -D-glucans may originate from a large variety of sources including moulds, some bacteria and most plants (Rylander, 1999; Douwes et al., 2005; Cherid et al., 2011). Fungal growth is predominant in conditions of high humidity and temperature. These climatic conditions favour the proliferation of fungal hyphae which become aerosolised and are thus inhaled by the worker. In addition, settled dust may also possess a large quantity of fungal material and its by-products. Personal exposure via this source is dependant on the extent of room activity which results in considerable temporal variability in airborne concentrations of fungal matter (Foto et al., 2004; Hansen et al., 2011).

The large scale production of a variety of livestock has resulted in high density of animals kept within confined spaces in buildings which has led to a source of human exposure to organic dust. One component of such organic dust is (1-3)- $\beta$ -D-glucans (Oppliger et al., 2008; Samadi et al., 2009).

### **2.3 OCCUPATIONAL SETTINGS AT RISK**

Workers in several industries are exposed to increased levels of microorganisms and their by-products associated with respiratory symptoms and/or airway inflammation (Bar-sela et al., 1984; Thelin et al., 1984; Hagmar et al., 1990; Morris et al., 1991; Douwes et al., 2003; Kirychuk et al., 2003; Rylander et al., 2006; Lossifova et al., 2007). Workplaces that reported an association between occupational exposure to (1-3)- $\beta$ -D-glucans and symptoms include woodwork and the paper industry (Rylander et al., 1999; Mandryk et al., 2000), household waste collection, poultry farms, composting and sewage treatment (Douwes et al., 2003; Rylander et al., 2006).

Rylander and colleagues (1999) showed an increase in the prevalence of subjective respiratory symptoms and an increase in airway responsiveness among workers at particular worksites in the paper and pulp industry owing to airborne (1-3)- $\beta$ -D-glucan exposure. A study conducted by Mandryk et al., (2000) further reinforced this finding where woodworkers with a high prevalence of respiratory and nasal symptoms had significantly higher personal airborne dust exposure levels. In another study symptomology occurred when the level of (1-3)- $\beta$ -D-glucans was detected at low levels (i.e. 10 ng/m<sup>3</sup>) (Rylander, 1996). This was corroborated by studies on sick

building syndrome where dose-response relationships were found between low (1-3)- $\beta$ -D-glucans exposures with eye and throat irritation, dry cough and itchy skin (Rylander, 1996). This may be indicative of the priming effect similar to endotoxins which modify the airway in asthmatic subjects enhancing allergen challenge (Alexis et al., 2003). It can therefore be argued that (1-3)- $\beta$ -D-glucan may act on their own or as adjuvants to other substances in the workplace.

Similarly, Rylander in 2006 showed that there was significantly higher airway responsiveness among poultry workers than in controls. A higher prevalence with respect to toxic pneumonitis, airway inflammation and chronic bronchitis was demonstrated in this study possibly attributed to significant levels of (1-3)- $\beta$ -D-glucans in the environment (Rylander et al., 2006). A study conducted in 2011 by Cyprowski and Sowiak of Poland confirmed these findings where the average content of soluble (1-3)- $\beta$ -D-glucans in the metal and composting industries was found to be 90%.

In addition, studies conducted in the waste recycling industries clearly demonstrate that an increase in the duration of exposure to (1-3)- $\beta$ -D-glucans is associated with a range of work-related symptoms and an increase in the intensity and severity of various respiratory and gastro intestinal symptoms (Gladding et al., 2003). Due to their presence in the fungal cell wall, (1-3)- $\beta$ -D-glucans are likely to be present in compost and potentially airborne dust associated with compost (Douwes et al., 2000); in metal plants where the use of metal working fluids are applied; waste water treatment plants and waste composting plants. Varying levels of (1-3)- $\beta$ -D-glucan in different agricultural industries have been reported (**Table 2.1**). This variability could

be attributed to difference in climate, sampling procedures, seasonal variability as well as type of agricultural process (Tendal et al., 2011).

**Table 2.1** Levels of (1-3)- $\beta$ -D glucan in different agricultural industries.

<b>Industry</b>	<b>Sample population size</b>	<b>(1-3)-<math>\beta</math>-D glucan levels ng/m<sup>3</sup></b>	<b>Respiratory health effects</b>	<b>Reference</b>
Agriculture (strawberry farm)	57	57.3	Not reported	Tendal et al., 2011
Composting	117	0.98	Not reported	Sykes et al., 2011
Greenhouse workers	60	10–100	Prevalence of respiratory symptoms higher in workers than in controls, however not significant due to low statistical power	Adhikari et al., 2011
Horse stables	90	9500	Not reported	Samadi et al., 2009
Poultry	42	4-870	Increased prevalence of toxic pneumonitis, airways inflammation and chronic bronchitis	Rylander & Carvalheiro, 2006
	9	2-972	Not reported	Sander et al., 2008
Vegetable growers	142	50 and 1500	Not reported	Hansen et al., 2011

## 2.4 HEALTH EFFECTS ASSOCIATED WITH EXPOSURE TO (1-3)- $\beta$ -D-GLUCANS

Occupational exposure to (1-3)- $\beta$ -D-glucans has become increasingly evident in a number of different industries as listed in Table 2.1. The most common general and respiratory symptoms related to (1-3)- $\beta$ -D-glucans exposure include tiredness, headache, nose and throat irritation, cough, airways inflammation and lung function impairment (Rylander and Fogelmark, 1994; Rylander and Carneiro, 2006; Lossifova et al., 2007; Adhikari et al., 2011). Although exposure has been measured in several industries, not many have conducted health hazards evaluations (Douwes, 2005). (1-3)- $\beta$ -D-glucans have been implicated as causative agents in 'sick building syndrome' where dose-response relationships existed between low (1-3)- $\beta$ -D-glucans exposure and eye and throat irritation, as well as dry cough and itchy skin (Rylander, 1996; Ronald et al., 2003).

(1-3)- $\beta$ -D-glucans are able to initiate a wide range of biological responses in vertebrates such as the induction of cytokine release (Sigsgaard et al., 2000), respiratory and gastrointestinal symptoms (Gladding et al., 2003) and an increase in peak expiratory flow (Douwes et al., 2000). However, inhalation studies which involve direct nasal deposition of pure (1-3)- $\beta$ -D-glucan preparations demonstrate no inflammatory responses suggesting that (1-3)- $\beta$ -D-glucan acts synergistically with other airborne bioaerosols such as endotoxin to produce an inflammatory response (Rylander et al., 1995; Fogelmark et al., 2001).

Inhalation of (1-3)- $\beta$ -D-glucans by healthy individuals caused an increase in eosinophilic cationic protein, TNF- $\alpha$  and a reduction of peripheral blood eosinophil numbers (Thorn et al.,

2001). Similar observations have been reported in experimental exposure assessment studies in mice and guinea pigs sensitised to oval albumin (Fogelmark et al., 2001). Such studies have demonstrated that (1-3)- $\beta$ -D-glucans could enhance allergen induced airway inflammation by increasing eosinophilic infiltration and specific IgE (Fogelmark et al., 2001; Douwes., 2005; Lossifova et al., 2007), further supporting the theory of an additive or synergistic role played by (1-3)- $\beta$ -D-glucans. In addition, blood leukocytes from healthy volunteers and patients allergic to house dust glucans enhance the release of IgE and histamine *in vitro* (Holck et al., 2007), suggesting that the effects of inhaled (1-3)- $\beta$ -D-glucans may be different in eosinophilic pneumonia compared to healthy and allergic individuals.

## **2.5 SAMPLING METHODS FOR (1-3)- $\beta$ -D-GLUCANS**

Three of the most frequently used sampling methods include settled dust sampling, stationary air sampling and personal air sampling. Each of these has advantages and limitations. In the case of settled dust sampling, the sampling is variable and the amount of fungal (1-3)- $\beta$ -D-glucans deposited on the surface over the sampling can be calculated. However, this is a crude measurement of inhalable dust (Douwes et al., 2005). Large particles settle quickly whilst smaller particles remain airborne for longer periods. Depending on the sampling time and air movement, a proportion of the agent in question may well be missed. On the other hand, stationary dust sampling allows for inhalable dust particles to be sampled but requires a large volume of sample to be obtained for accurate assessment. This is due to the temporal variation in airborne concentrations (Douwes et al., 2005). Personal air sampling permits direct sampling of

inhalable dust and is therefore seen as a true measure of dust that could be inhaled by the worker (MDHS, 1999).

The method employed for sampling should not only include a true measure of personal inhalable dust but should also provide an appropriate substrate that would allow for the precise capture of the offending agent, by use of an appropriate size filter, as well as a medium from which maximum extraction of the fungal toxin (1-3)- $\beta$ -D glucan can be achieved. Depending on the sampling type, a recommended flow rate of 2 L/min is used for sampling (Douwes et al., 2000; Gladding et al., 2003; Sander et al., 2008; Samadi et al., 2009) for 6-8 hours (a full work day shift) using a sampling pump and fibre glass filter of 1  $\mu$ m (SKC Ltd, USA). Douwes, (2000) and Samadi, (2009) collected personal airborne samples with the Gilian air sampling pump with 25 mm glass fiber filters at a flow rate of 2 L/min. Sampling was conducted over a full day work shift (8 hours) as well as half day shift work for Douwes, (2000) and Samadii, (2009) respectively. Ronald et al. (2003) utilised personal sampling pumps (SKC model 224-44XR) with 25 mm diameter Teflon filters of pore size 0.45  $\mu$ m at a flow rate of 3.5 L/min. (1-3)- $\beta$ -D glucan exposure levels were found to be 9.5  $\mu$ g/m<sup>3</sup> and 92.5  $\mu$ g/m<sup>3</sup> for Douwes and Samadii respectively. Samples in both these studies were extracted using the heat extraction method as described by Douwes in 1996. However the, assay used to determine the levels of (1-3)- $\beta$ -D glucan differed. Samadi employed the specific enzyme immunoassay as described by Douwes in 1996 whereas Douwes used the kinetic chromogenic Limulus amoebocyte assay lysate (LAL) method. Douwes employed the use of this assay as he recognised the drawbacks of the specific enzyme immunoassay. These drawbacks include reactivity with other polysaccharides and relatively high costs of the reagents required for analysis. This could explain the difference in (1-

3)- $\beta$ -D glucan levels obtained from both these studies. In addition, the sampling method used in both these studies differed which could have contributed to the differences as well.

## **2.6 EXTRACTION PROCEDURES FOR (1-3)- $\beta$ -D-GLUCAN**

Two extraction methods available for (1-3)- $\beta$ -D-glucan from environmental samples are an alkaline extraction method which uses sodium hydroxide (Rylander et al., 1999; Lossifova et al., 2007; Young et al., 2011) and a heat extraction technique that is accomplished by autoclaving samples at a temperature of 120 °C for approximately one hour (Douwes et al., 2000; Ronald et al., 2003; Wouters et al., 2006; Sander et al., 2008). The amount of (1-3)- $\beta$ -D-glucan extracted in each of these methods varies particularly when employing the Limulus Amebocyte Lysate (LAL) assay (Douwes et al., 2003). It has been noted that an alkaline extraction method be employed when conducting the LAL assay for (1-3)- $\beta$ -D-glucan analysis as this would produce an optimum yield sufficient for statistically significant results (Young et al., 2011). This is due to the fact that an alkaline treatment allows for the unwinding of the triple helical formation of (1-3)- $\beta$ -D-glucans to a single helix or random coil form which is thus correlated to increased factor G activation (Douwes et al., 2005). Factor G is a horseshoe crab coagulation factor which coagulates in the presence of (1-3)- $\beta$ -D-glucan (Douwes et al., 2005).

The GlucateLL assay has been a recent advent for (1-3)- $\beta$ -D-glucan quantification and therefore not much research has been reported on which technique would be useful. Extraction of the (1-3)- $\beta$ -D-glucan by autoclaving was the preferred choice of extraction as noted by Sander et al. (2008) because the alkaline extraction method could potentially inhibit the antibody binding in

samples with low (1-3)- $\beta$ -D-glucan content that could not be highly diluted. Therefore, for the purposes of this study, the heat extraction method (i.e. use of the autoclave) was selected as the method of choice for the extraction of filter samples.

## **2.7 QUANTIFICATION OF (1-3)- $\beta$ -D-GLUCAN**

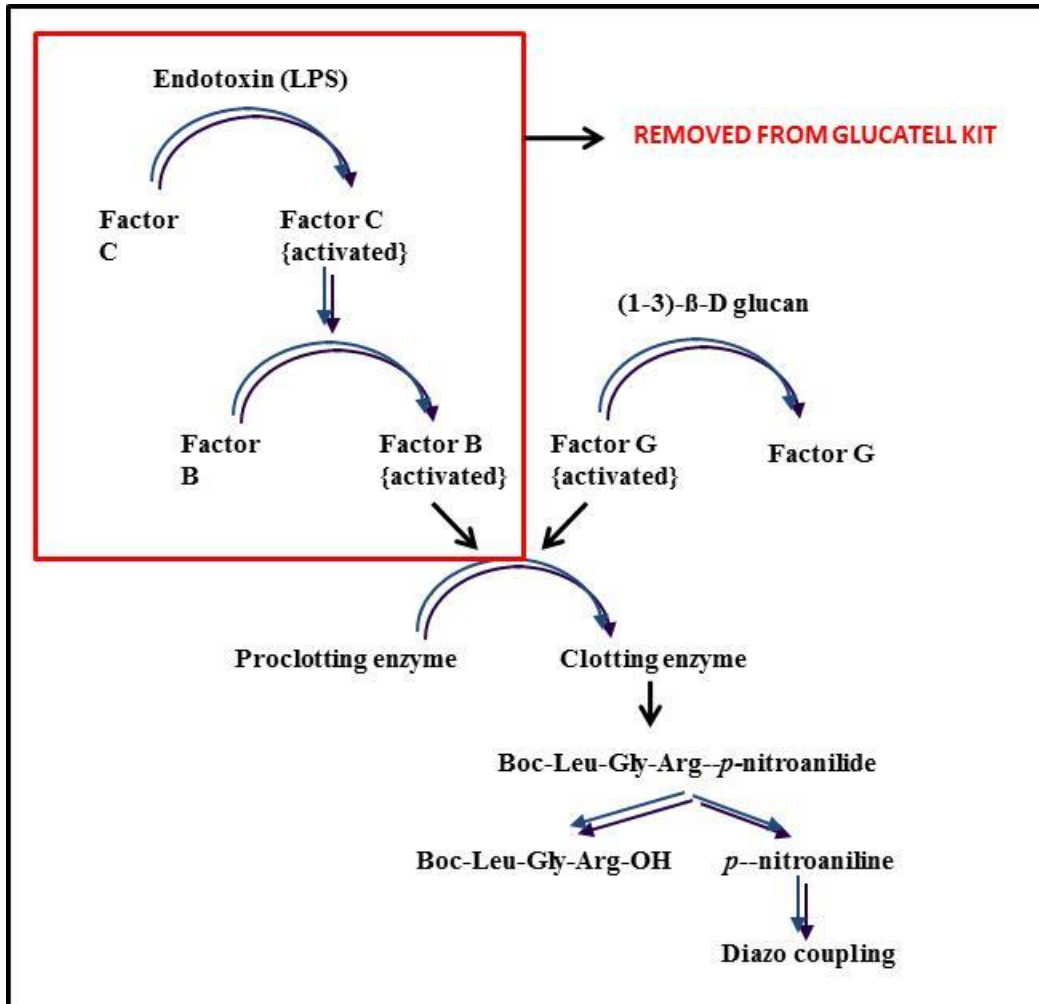
The conventional approach involves an Enzyme Linked ImmunoSorbant Assay (ELISA) and the utilisation of antibodies against (1-3)- $\beta$ -D-glucan by manipulation of the LAL assay used for endotoxin analysis (Douwes et al., 2003; Foto et al., 2004; Sander et al., 2008; Adhikari et al., 2011). Although these two methods are sensitive, the ELISA has proven to be laborious and time consuming.

Douwes and colleagues (1996) developed an inhibition enzyme immunoassay (EIA) to measure (1-3)- $\beta$ -D-glucan. This method was able to detect heat-extractable (1-3)- $\beta$ -D-glucan in dust samples and proved to have high sensitivity toward the glucan laminarin (40-3000 ng/ml). However, less sensitivity was shown toward curdlan. This could be due to purity, chain length and conformation of the glucans which may vary and significantly influence the detection limit expressed on a weight basis (Douwes et al., 1996).

Milton and colleagues (2001) later developed an ELISA using a high affinity receptor and specific monoclonal antibody for the capture and detection of (1-3)- $\beta$ -D-glucan. The method was found to be more sensitive in detection (0.8 ng/ml) than the method developed by Douwes in 1996 (40 ng/ml). Due to the extreme specificity, sensitivity and reproducibility of the test it was

suggested as a method of choice in the detection for (1-3)- $\beta$ -D-glucans. However, more recently, Sander and co-workers (2008) developed a two-site Enzyme Immunoassay (EIA) based on monoclonal antibodies against laminarin. The method proved to have a low detection limit (1.1 ng/m<sup>3</sup>) and was highly sensitive.

The LAL based method is capable of detecting both endotoxin and glucan with a possibility of underestimation of the levels of (1-3)- $\beta$ -D-glucan. In addition, the LAL assay is not specific for mould as pollen and certain vegetable fibers also contain (1-3)- $\beta$ -D-glucan. This could result in false positives. The glucan-specific LAL assay (Associates of Cape Cod, Falmouth, MA, USA), commonly known as the GlucateLL assay is the most recently developed assay used at the time of the study for the detection of (1-3)- $\beta$ -D-glucan. This method is based on the same principal as the LAL assay which has been described for endotoxin measurements (Thorne et al., 2003). However, instead of activating factor C, glucans activate factor G which initiates a cascade of enzymatic reactions, finally producing a colour or turbidimetric response. The glucans-specific LAL eliminates the possibility of cross reactivity with endotoxin as factor C has been completely removed or disabled from the LAL preparation (**Figure 2.2**) (Douwes et al., 2005; Sander et al., 2008). It is a sensitive, quick (~ 1.5 hours) and specific method and has therefore been chosen as the method of choice for the purposes of this study.



**Figure 2.2** Diagram illustrating the Limulus protease activation pathway. (Richardson, accessed 16 September 2008)

## 2.8 OCCUPATIONAL EXPOSURE LIMIT FOR (1-3)-β-D-GLUCANS

Occupational exposure limits (OELs) provide a valuable tool in evaluating workplace safety. A statutory OEL can be defined as a legally enforceable limit on the amount or concentration of a substance in the work environment to which employees/workers may be exposed to during a specified period. These limits are established by taking into consideration factors such as

exposure evaluation and assessment using animal models, extrapolating from human clinical data using models and the determinants that affect acceptable daily intake of the component and the application of risk assessment methodologies. In addition, the relevance and applicability of developing such an OEL should be explored. Statutory OEL's may also incur a cost where job losses may occur if levels of exposure are found to be higher than those recommended.

Due to the lack of a standardised quantification methods for (1-3)- $\beta$ -D-glucans and inconsistent environmental conditions in a variety of industries, an OEL for (1-3)- $\beta$ -D-glucans has not been established (Bonlokke et al., 2006; Samadi et al., 2009). Therefore (1-3)- $\beta$ -D-glucan concentration comparisons between different studies may be hindered. It is also interesting to note that different studies report varying degrees of symptoms at different (1-3)- $\beta$ -D-glucan concentrations (Mandryk et al., 2000). It has been suggested that the properties of dust may change the dose-response relationship as other constituents of dust may play a role in the severity and effect of (1-3)- $\beta$ -D-glucan (Rylander et al., 2006; Bonlokke et al., 2006). For this reason and many other health effects reported with (1-3)- $\beta$ -D-glucan exposure, it can be presumed relevant in establishing standard extraction and quantification methods such that an OEL for (1-3)- $\beta$ -D-glucan be established.

An OEL for (1-3)- $\beta$ -D-glucans does not exist for use in South Africa. This study therefore aims to determine the exposure levels in the poultry industry which will form the basis for further research to establish minimum acceptable levels that may not result in adverse health effects.

## **2.9 EXPOSURE VARIABILITY**

Variable exposure within the same work environment may be considered as a plausible outcome. This could be due to factors such as job process, job task, distance from source of exposure, and seasonal variability. Other factors which play a key role in increased (1-3)- $\beta$ -D-glucans exposure include but are not limited to: 1) increased generation of organic dust as a result of over production in agricultural sectors due to the increasing demand on sustainable resources; 2) poor ventilation systems and machinery; and 3) the increase in global temperatures and humidity which favour the proliferation of microbes, in this instance those of fungal origin (Wouters et al., 2006).

These factors could result in within worker and between worker variability. (1-3)- $\beta$ -D-glucan is predominantly derived from fungal material and as a result of its ease of growth in any environment, exposure variability is higher than that of chemical exposure. Therefore measurements are only suitable to describe (1-3)- $\beta$ -D-glucan exposure in clearly circumscribed areas and with regard to the actual working activity (Wouters et al., 2006).

## **2.10 CONTROLLING EXPOSURE TO (1-3)- $\beta$ -D-GLUCANS**

Occupational exposure to (1-3)- $\beta$ -D-glucans has raised serious health concerns which suggest that exposure control is crucial (Rylander 1999; Douwes et al., 2005; Wouters et al., 2006). Since these fungal cell wall components form a substantial proportion of bioaerosols, avoidance of exposure poses a challenge as fungi are ubiquitous in the environment (Douwes et al., 2005).

The Occupational Health and Safety Act (OHSA) (No. 85 of 1993) of South Africa protects the rights of employees and stipulates that the employer is obliged to provide a safe working environment as far as reasonably practicable, and without risk to the health of workers. Therefore, it is critical for workplaces to control levels of exposure to (1-3)- $\beta$ -D-glucans.

The use of PPE such as N-95 masks for respiratory protection, impermeable overalls with rubber gloves and boots for skin protection and a helmet and visor for dirty work has been suggested for use in work environments that have been found to have a high risk of exposure (Robert-Sauve Research Institute for Occupational Safety and Health (IRSST), 2001). However, enforcing this policy has been challenging as not all workers are diligent in practicing good health and safety control measures and, those who do wear PPE only do so for approximately 50% of the time (Mandryk et al., 1999). In addition, many companies utilise low cost PPE which does not offer suitable protection for the employee against inhalable agents. This is mainly evident in under developed countries with reduced financial resources for purchasing products of a higher quality. Furthermore, it has been suggested that the development and dissemination of exposure guidelines, such as public guidelines, legislation and education and training, and enforcement of legislation be established to improve compliance with PPE usage (Mandryk et al., 1999).

Also, the levels of exposure vary with regard to the type of job performed by the worker and shift undertaken (Samadi et al., 2009; O'Shaughnessy et al., 2010). It has been shown that cleaning tasks such as sweeping result in increased exposure by a factor of 4.4 or 4.9 (Samadi et al., 2009). Due to the variability in (1-3)- $\beta$ -D-glucans exposure between the different job tasks

noted by Samadi et al., (2009) and O'Shaughnessy et al., (2010), control measures should be tailored according to the specific working process in order to maximise efforts to minimise exposure. Engineering control measures have played a substantial role in the efforts made to reduce bioaerosol exposure in the work environment. These methods include the installation of local exhaust ventilation systems (Jacobs and Smith, 1988).

To gain a holistic view of workplace exposures, exposure measurement studies should include personal as apposed to stationary sample measurements. This should be coupled with a task-related exposure assessment accompanied by the administration of an in-depth health questionnaire. In doing so, the investigator is able to link the quantity of exposure to a specific job task. Furthermore, workplace control measures (both engineering and personal) that are currently in place should be recorded and updated to help explain varying levels of exposure that may occur with workers. By implementing these simple measures, investigators are able to provide a more concise and accurate recommendation for controlling bioaerosol exposure in the work environment.

## **CHAPTER 3**

### **3.0 MATERIALS AND METHODS**

#### **3.1. STUDY DESIGN**

This was a cross sectional study of 191 workers working on a commercial poultry farm in the North West Province, South Africa.

##### **3.1.1 THE POULTRY FARM**

Securing permission to conduct this study was challenging thereby affecting the project timeline. A poultry farm in the North West of South Africa agreed to participate in the study and was used as the study site. Several major poultry farms refused to participate. The poultry farm in the North West was considered as a large commercial enterprise and complies with local poultry farm guidelines and is thus representative of other similarly sized farms in the country. Although only one farm enterprise was included in the study it comprised of different farm sites at different locations in the North West province and will be referred to as “farm sites” throughout the text. The farm produces both broilers (chickens) and eggs. It has 15 different farm sites comprising the following processes rearing, laying (manual and automated), hatchery (old and new), broiler (housed – big/small and free range – big/small) and catching crew. The washing and cleaning of the houses are an outsourced service and were not included in the study. The farm houses between 40 000 to 750 000 chickens depending on the number of houses that are filled at any one time. The size of broiler house was identified based on the number of chickens it housed. Broiler houses that held  $\leq 20\ 000$  chickens per house were classified as small broilers whereas big broilers were classified as those that housed  $> 20\ 000$  chickens. The factory where

slaughtering is conducted was not included in this study as the exposure was different and the processes included wet work which is not within the scope of this study.

### **3.1.2 STUDY POPULATION**

The total staff complement at the farm was approximately 1 315. This included permanent (90%) and casual labourers (10%). Of these, the majority of the employees worked in the factory (n=639) followed by the farms (n=596) and lastly the administrative staff (n=80). Of the 596 farm workers, 523 were permanent and 73 were casual workers. For the purposes of the environmental exposure assessment a random stratified sample of a minimum of 10 workers was chosen from each farm site comprising a total of 191 farm workers for all the farm sites. The selection also included a representative sample size within each task which was sampled twice to determine the variability between exposures on different sampling days. A total of 338 filter samples were collected, which comprised 30 field blanks and 308 personal inhalable filter samples including repeats. There were eight main job types as listed in **Table 4.1**. Job tasks conducted throughout the poultry farming process were also identified.

### **3.1.3 FARM SITE INSPECTION**

A walkthrough of the selected farm sites was conducted by a qualified occupational hygienist from an approved inspection authority using a checklist (see Appendix C) in order to obtain information on the workforce, specific tasks and the control measures in place from direct observation. Information on the estimated number of chickens bred, bedding material, type of chicken feed and frequency of cleaning procedures was sought.

### **3.1.4 ENVIRONMENTAL SAMPLING**

A total of 308 personal inhalable air samples including repeat samples were collected. In addition, 30 field blanks and 30 lab blanks were collected as controls to rule out possible contamination, equating to 368 samples in total. Organic dust samples were collected by an Occupational Hygienist [Occupational Hygiene Section, NIOH] using the Gilair battery operated personal sampling pumps (SKC Ltd, USA). Sampling pumps were calibrated daily pre and post sampling using a Gilibrator bubble flow meter (Gilian Instrument Corp., Wayne, N). Sampling was conducted at a flow rate of 2 L/min using 1 µm pore size glass fibre filters housed in Institute of Occupational Medicine (IOM) inhalable dust sampler cassettes (Gladding et al., 2003) for a minimum of 4 to 8 hours (Mandryk et al., 2000; Gladding, et al., 2003). The filter cassettes were placed in the breathing zone of each participant and sampling was performed during their work activity for the entire shift. The workers were categorised into exposure groups (rearing, laying, hatchery, broiler and catching) based on the site inspections. A random sample of approximately 20% of all workers from the various exposure groups was chosen based on the number of chickens housed on the farm site. Small farms housed  $\leq 20\ 000$  chickens whereas big farms housed  $> 20\ 000$  chickens. Workers were sampled twice for a full work shift to enable for a statistically significant result and to gather knowledge and the variation in distribution of (1-3)- $\beta$ -D glucan. Post sampling, filters were sealed within its IOM cassette and stored at room temperature before being transported back to the laboratory at the NIOH where they were post weighed and subsequently extracted.

### **3.1.5 LABORATORY ANALYSIS**

#### **3.1.5.1 Extraction**

Filters were extracted at room temperature in 4 ml pyrogen free water with 0.05% Tween 20 (Douwes et al., 1995). Tubes were shaken vigorously for 15 minutes at room temperature and then autoclaved for 60 minutes at 120 °C. Thereafter, tubes were shaken for a further 15 minutes at room temperature and subsequently centrifuged at 1000g for 15 minutes. Extracts were aliquoted for the (1-3)- $\beta$ -D-Glucan test and stored at -20 °C for later use.

#### **3.1.5.2 Measurement of (1-3) $\beta$ -D-Glucan**

The aliquot obtained after extraction was diluted two-fold in pyrogen free water containing 0.05% Tween 20 and heat treated by autoclaving at 120°C for one hour before testing. (1-3)- $\beta$ -D-glucan present in the extracts was measured using the GlucateLL assay (Associates of Cape Cod, East Falmouth, MA, USA). The analysis was done as per manufacturer's instructions. Briefly, the method involves the addition of the unknown sample and glucateLL reagent into respective microplate wells. The microplate was incubated for 30 minutes at 37 °C on a heating block. Thereafter, a stopping solution (sodium nitrite), activating (ammonium sulfamate) and lastly a colour detection reagent (N-(1-Naphthyl) ethylenediamine dihydrochloride (NEDA)) were added. The microplate was read between 540-550 nm using a spectrophotometer (Biotek Instruments, USA). The kit's detection range for (1-3)- $\beta$ -D-glucan is between 5 and 40 pg/ml. The final concentration of (1-3)- $\beta$ -D-glucan in the air was calculated using the flow rate and time of the sampling period and reported as ng/m<sup>3</sup>. All personal samples that exceeded detection limit of the

kit were diluted in order to broaden the detection range, which was adjusted for in the final calculation.

### **3.1.5.3 Assay validation**

The GlucateLL assay that detects (1-3)- $\beta$ -D glucan was established at the NIOH by first validating the method for routine analysis. A sample of a known standard concentration of 25 pg/ml was used. This was plated seven times in duplicate in three separate runs to test for reproducibility and repeatability, respectively. The coefficient of variation (CV) and the regression coefficient ( $R^2$ ) were calculated from the above test runs. A CV of <10% and a  $R^2 \geq 0.98$  was considered acceptable. A mean and standard deviation was determined for each data set, thereafter a grand mean and standard deviation was calculated. Subsequently, a Levey-Jennings graph was plotted and an upper and lower limit was established by using the grand average plus or minus three times the standard deviation, respectively. All subsequent test runs which did not fall within the limits were rejected.

### **3.1.5.4 Quality control**

Field and laboratory blanks were routinely analysed to assess filter contamination. For the laboratory analysis, the limit of detection (LOD), which is the lowest level of detecting the agent, was computed by calculating the mean of the field blanks. The limit of quantification (LOQ), is the limit above which there is confidence of the agent measured and was calculated by adding the mean plus three times the standard deviation of the field blanks. The co-efficient of variation (CV) between two measurements was  $\leq 30\%$  and  $R^2 \geq 0.98$  for all assay runs was accepted.

### **3.1.6 RESPIRATORY QUESTIONNAIRE**

The study collected information on important demographic factors (Appendix D). Each participant answered a modified questionnaire specifically designed for investigation of asthma from the European Community Respiratory Health survey (ECRHS II) and supermarket bakery workers study (Baatjies et al., 2009). The questionnaire was administered by myself with the assistance of the study team members (i.e. two medical intern scientists). The questionnaire was administered in English which was the preferred language of the workers as established during presentations to them prior to the study. Interpreters' for Afrikaans, Tswana and Zulu were available if required. The questionnaire addressed work-related respiratory symptoms and a history of previous medical illnesses. Other questions included employment history, degree of exposure to aerosols and smoking status.

### **3.1.7 DATA MANAGEMENT AND ANALYSIS**

Smoking status was classified into three categories namely: non-smoker for lifelong abstinence from smoking; ex-smoker if the subject ceased smoking completely more than one month before the survey; and current smoker. Symptom variables that were evaluated for its relationship to work included: respiratory (wheeze and / or tight chest); ocular (itchy eyes, red eyes); and nasal (runny nose, blocked nose / stuffy nose) symptoms. Work-related asthma symptoms were defined as an affirmative response to the question: "Does being at work ever make your chest tight or wheezy?". However, the case definition for self-reported asthma was a positive response to one or a combination of the following: "Have you had an attack of asthma in the last 12 months?", and "Are you currently taking any medication for asthma?".

STATA statistical package (version 9, StataCorp, 2007, Texas, USA) was used to analyze the environmental exposure data and respiratory questionnaire data. Exposure metrics were developed on the basis of individually measured exposures and mean levels within each job category. Descriptive statistics was used to summarise the distribution of each measured variable. The raw data of the (1-3)- $\beta$ -D glucan concentrations was shown to be a skewed distribution and therefore the data was log-transformed before commencing with analysis. This is one of the key assumptions for conducting ANOVA. A P-value of less than 0.05 was considered statistically significant.

## **CHAPTER 4**

### **4.0 RESULTS**

#### **4.1 CATEGORISATION OF TASKS UNDERTAKEN WITHIN THE POULTRY FARMING PROCESS**

**Table 4.1** illustrates a list of the different job types identified in the poultry farm studied and the specific job tasks undertaken. This information was gathered during the walkthrough of the poultry farm process. Information was supplied by the farm managers.

**Table 4.1** List of job types and specific job tasks for the poultry farm workers.

<b>Job type</b>	<b>Job tasks</b>
<b>General worker (househand) broiler/ free range</b>	<ul style="list-style-type: none"> <li>• General maintenance of buildings, broiler houses and farm yard</li> <li>• Keep water boiler in working condition</li> <li>• Offloading of chickens</li> <li>• Welfare, medication and feeding of chickens</li> <li>• Keep houses clean from mortality and wet bedding</li> <li>• Monitor chickens while roaming during the day (only for free range)</li> <li>• Get houses ready for catching</li> <li>• Empty the feed from bulk feed tank, bag feed and weigh it</li> <li>• Maintain biosecurity</li> </ul>
<b>Night watch</b>	<ul style="list-style-type: none"> <li>• Check boiler temperature and ventilation</li> <li>• Ensure that the water &amp; feeders are clean</li> <li>• Report all problems to management</li> <li>• React on all alarms</li> </ul>
<b>Breeder layer site supervisor</b>	<ul style="list-style-type: none"> <li>• Ensuring the correct quality of hatching eggs by means of careful grading procedures</li> <li>• Checking and recoding of all eggs produced daily and verification of egg stocks on site at the close of the day</li> <li>• Ensuring that the correct egg collection times as indicated on site control sheet are strictly adhered to</li> <li>• Check flock health and the immediate reporting of apparent health problems or sick birds</li> <li>• Weak cocks to be placed in the hospital pens &amp; must be rotated when they have recovered</li> <li>• Cock grading at the required ages, ensuring the correct cock mating ratios</li> <li>• Detection of parasites, red mite, worms and rodents</li> <li>• Day to day management of the separate sex feeding system, kilograms fed, the level of the feeding pans, and monitoring to see that the hens do not eat the cocks food</li> </ul>
<b>Site egg collector</b>	<ul style="list-style-type: none"> <li>• Collection of mortalities</li> <li>• Maintaining nest boxes conditions at all times</li> <li>• Carrying out of hatching and floor egg collections</li> <li>• The accurate and thorough grading of all eggs collected, ensuring that all rejects are removed, including dirties, cracks, undersized, oversized, misshapen, or any other egg that does not qualify as a hatching egg</li> <li>• Proper traying up of the eggs, i.e. round side up and transfer to cold room</li> <li>• General maintenance of house and yard</li> </ul>
<b>Maintenance aid rearing farms</b>	<ul style="list-style-type: none"> <li>• Assist with any aspects pertaining to the running of the live stock, equipment, general maintenance or farm processes relevant to the chubby operation</li> <li>• Aid supervisor in maintenance on farm of farm property</li> </ul>
<b>Chick grader</b>	<ul style="list-style-type: none"> <li>• Count and grade all the day old chicks</li> <li>• All chicks that is not suitable for placing on the farms must be culled</li> <li>• When not grading chicks workers must do vaccination</li> </ul>
<b>Catching crew</b>	<ul style="list-style-type: none"> <li>• Catch and load of chickens</li> <li>• Loading off crates and ensure that crates are tied</li> </ul>
<b>Catchers</b>	<ul style="list-style-type: none"> <li>• Catch chickens and put in modules, 27 in each drawer</li> <li>• Put in barricade and modules in correct place in the chicken house</li> </ul>

#### **4.1.1 REARING**

Rearing is a process where chickens are bred for the purposes of farming for meat or eggs (Cilliers, 2009). Male and female chickens are procured and reared until 20 weeks old where healthy chickens are transferred to the laying farm. At the rearing farm, workers are involved in the general upkeep of the poultry house (e.g. placing the shavings in chicken houses, feeding, maintaining the water boiler, cleaning the farm site). Their routine tasks also involve checking the functionality of the ventilation system, weighing and inoculating the chickens, trimming the chicken beaks, removing chicken mortalities from the houses, taking blood from chickens and collecting bedding samples for disease and pest identification, respectively.

#### **4.1.2 LAYING**

Based on observations, chickens are received from the rearing farm at the beginning of week 21 and are kept for 60 weeks where they are transported to an external supplier to be sold. At the laying farm, hens and cocks are mated and fertile hens go on to lay their eggs. There are two types of laying farms, manual and automated. Egg collection is one of the primary job tasks in this type of farming process. The workers main task is to collect eggs from nests five times per day thereby spending more than half their day inside the chicken house. Workers based in the automated chicken house spend approximately three hours inside the house. Eggs are laid by hens on a conveyer belt that transports them to the sorting and packing station. Workers however, do make routine inspections through the chicken house to check for mortality and the presence of any eggs that might be have been laid on the floor instead of the conveyor belt. Eggs that meet the grading criteria are placed on racks and sent to the hatchery.

### **4.1.3 HATCHERY**

Hatchery is a process where eggs are hatched under controlled environmental conditions that aid the hatching process (Cilliers, 2009). These environmental conditions include adjusted temperature and humidity levels. The first task of the day at the hatchery involves a process known as take off where chicks are counted, graded and vaccinated. One hundred chicks, which have been inspected by the chick counters for abnormalities, are placed per crate, loaded onto a truck and transported to the broiler farms. This task takes between 30 minutes to four hours depending on the number of chicks to be processed. The second process at the hatchery involves transfer where eggs are removed from the trays that arrive from the laying farm and are placed in crates which are then stacked and placed in incubators. Thereafter, these crates are transferred from one incubator to another that is set at a lower temperature (36°C - 37.7°C). Parallel to this process, eggs are graded in a dark room to check for infertility. Infertile eggs are discarded whilst fertile eggs are placed into the incubator. Two types of hatchery's were identified and sampled. The one was classified as the old hatchery which was the first hatchery before the construction of the new and more modern hatchery site due to increasing productivity demands. The new site is also relatively bigger in comparison with better ventilation systems.

### **4.1.4 BROILER**

At the broiler farms, chickens are raised for the purposes of meat production. There are two types of broilers. There are ordinary broiler houses and free range houses (Cilliers., 2009). Both are equipped with drinking and feeding facilities, temperature control and ventilation systems. Broiler houses are washed and cleaned before bedding is placed on the floor and one day old chicks from hatchery are introduced to the house, where they are grown to 32-35 days, and

thereafter sent to the factory for slaughtering. Tasks performed in broiler farms include feeding the chickens, ensuring automated feed systems and water systems are functional, removal of mortalities from the broiler house to ensure the prevention of disease outbreak and general cleaning and maintenance of the site. The latter include cutting of grass and painting. The free range farm operates similarly to the ordinary broiler farm except that the chickens are allowed to roam freely for a period of the day and are confined to the broiler house in the evening. Workers do not frequent the houses as much as they do for the ordinary broiler houses.

#### **4.1.5 CATCHING CREW**

In all sectors of the poultry industry, chickens have to be caught and placed into some form of container by hand. Catching is a physically demanding job, where workers often work long and anti-social hours. These hours range between 10pm to 6am. The night is dark and is ideal for catching as the chickens are more settled and create less dust formation (Calvet et al., 2009) and the effects of hot weather conditions is also minimised during transportation. This takes place at the end of the chicken growth cycle which is every 32-35 days per site for broiler farms. At the end of 20 and 60 weeks chickens from the rearing and laying farms, respectively are also caught using the same process for transfer. The poultry house is cleared of any obstructions such as the feed and water systems. Metal cases containing plastic drawers are brought into the poultry house via a forklift. The chicken catchers catch the chickens and place them into plastic crates which are transported to the factory where they will be slaughtered. In one “catch” a worker catches between 6 and 13 chickens with a combined weight of 22–25 kgs. The number of chickens that a catcher grabs at a time depends on the size of the chicken.

## 4.2 VALIDATION

### 4.2.1 PRECISION AND ACCURACY

As precision often varies with analyte concentration, repeatability and in-house reproducibility were calculated by using a known concentration of (1-3)- $\beta$ -D-glucan (25 pg/ml) plated seven times in duplicate in three separate assay runs (**Table 4.2**). Reproducibility is the relative standard deviation on results obtained under reproducibility conditions, with the same method on the same sample by different operators within a larger period of time (USEPA. 2000). For this method we used in-house reproducibility as only one operator/analyst performed the test in the lab. The calibration curve was determined using the following known standard concentrations, i.e. 5, 10, 20 and 40 pg/ml. The calibration curves were best fitted to a linear regression. For the validation of (1-3)- $\beta$ -D glucans to be accepted, the following criteria for precision and accuracy must be fulfilled. The correlation coefficient ( $R^2$ ) had to be  $\geq 0.99$  (Associates of Cape Cod Inc., 2007) for the assay standard curve and the coefficient of variation (CV) had to be  $\leq 10\%$  and sample results less than the limit of quantification (LOQ) should be repeated. All of the correlation coefficients ( $R^2$ ) for the validation test runs were  $\geq 0.99$ . The coefficient of variation for each duplicate set from each test run was  $\leq 10\%$ . The average mean and standard deviation for test run 1, 2 and 3 were ( $\mu_1 = 24.456$ ,  $SD_1 = 1.154$ ), ( $\mu_2 = 24.831$ ,  $SD_2 = 1.033$ ) and ( $\mu_3 = 24.348$ ,  $SD_3 = 0.911$ ), respectively. The overall average and standard deviation of all three test runs was  $\mu$ -average = 24.545,  $SD_3 = 1.033$ .

**Table 4.2** Results for repeatability and with-in laboratory reproducibility.

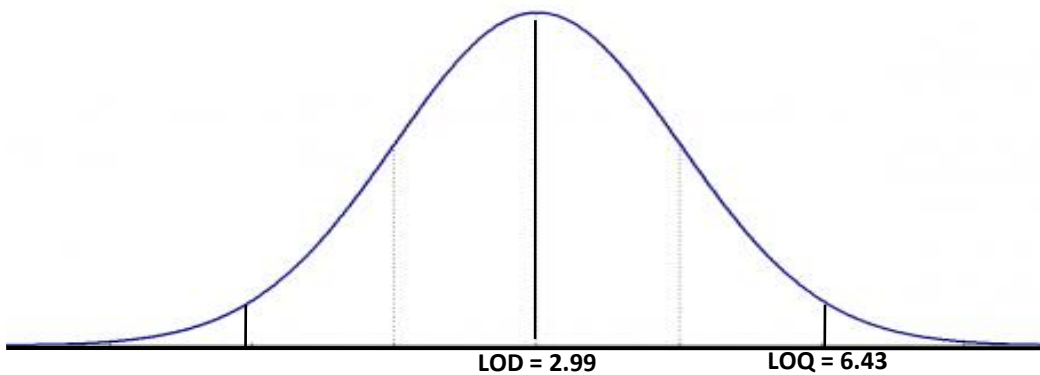
	Test run 1					Test run 2					Test run 3				
	Rep. 1 pg/ml	Rep. 2 pg/ml	Mean pg/ml	STD Dev	CV %	Rep. 1 pg/ml	Rep. 2 pg/ml	Mean pg/ml	STD Dev	CV %	Rep. 1 pg/ml	Rep. 2 pg/ml	Mean pg/ml	STD Dev	CV %
<b>Known concentration (25 pg/ml)</b>	24.051	24.982	24.516	0.658	2.685	24.994	24.065	24.53	0.658	2.680	24.656	27.108	25.882	1.733	6.697
	22.104	22.95	22.527	0.599	2.657	22.459	23.558	23.008	0.777	3.377	24.396	25.856	25.126	1.033	4.110
	23.204	25.32	24.262	1.496	6.167	22.712	24.318	23.515	1.136	4.829	24.604	23.874	24.239	0.516	2.130
	23.373	24.897	24.135	1.077	4.464	26.093	24.825	25.459	0.897	3.522	23.874	24.5	24.187	0.443	1.830
	24.897	25.828	25.362	0.658	2.596	25.248	26.347	25.798	0.777	3.012	23.666	24.552	24.109	0.627	2.600
	23.543	26.42	24.982	2.035	8.146	28.122	25.586	26.854	1.793	6.677	22.883	24.865	23.874	1.401	5.870
	24.304	26.505	25.405	1.556	6.125	23.811	25.502	24.656	1.195	4.848	22.57	23.457	23.014	0.627	2.724
<b>Blank:</b>	<3.250	<3.250	<3.250	0	0	<3.250	<3.250	<3.250	0	0	<3.250	<3.250	<3.250	0	0

#### 4.2.2 LIMITS OF DETECTION AND QUANTIFICATION

Since the blank reading from the assay runs resulted in a non-detect, it was recommended to assign non-detect residue values to avoid underestimating exposure to a potentially sensitive or highly exposed group. Therefore, the calculation of the limit of detection (LOD) was based on the mean result of the replicates lowest standard, divided by 2 (USEPA, 2000). The quotient was added three times the standard deviation of the blanks to establish a LOQ (Finkelstein and Verma, 2001). Results that were less than the LOQ were repeated.

The mean LOD was 5.981 pg/ml with a standard deviation of 1.147.

$$\begin{aligned} \text{LOQ} &= \text{LOD}/2 + 3 \times \text{standard deviation} \\ &= (5.981/2) + 3 (1.147) \\ &= 6.432 \end{aligned}$$



**Figure 4.1** Graph illustrating the limit of detection (LOD) and limit of quantification (LOQ)

### 4.3 PROCESS CLASSIFICATION

Based on the information retrieved during the building walkthrough inspection, the following farming processes were identified: rearing, laying, hatchery, broiler and catching crew. Samples were collected at both the manual and automated laying farms as well as the old and new hatchery to establish differences in (1-3)- $\beta$ -D glucan levels. Broiler processing samples were collected from big ( $> 20\,000$  chickens) and small ( $\leq 20\,000$  chickens) farm sites that had young ( $\leq 14$  days old) and old ( $> 14$  days old) chickens. In addition, free range farm sites were also sampled when chickens were young and old. A stratified random sample of a minimum of 10 workers was selected from each exposure category is shown in **Table 4.3**. A total of 338 filter samples were collected, which comprised 30 field blanks and 308 personal inhalable filter samples including repeats. A representative number of workers from each farm site were sampled twice to determine the variability between exposures on different sampling days.

**Table 4.3** Number of personal inhalable air samples collected based on exposure category.

<b>Category:</b>	<b>Size:</b>	<b>Age of Chickens:</b>	<b>Total number of workers (k)</b>	<b>Personal samples (n)</b>
Rearing	n/a	n/a	76	24
Laying: Manual	n/a	n/a	201	24
Laying: Automated	n/a	n/a		24
Hatchery: Old	n/a	n/a	76	24
Hatchery: New	n/a	n/a		48
Broiler	small	young	220	20
Broiler	small	old		10
Broiler	big	young		35
Broiler	big	old		24
Broiler free range	n/a	young		15
Broiler free range	n/a	old		18
Catching crew	n/a	n/a	23	42
<b>Total:</b>			<b>596</b>	<b>308</b>

#### **4.4 DEMOGRAPHIC CHARACTERISTICS**

A total of 191 workers was interviewed and completed a respiratory health questionnaire from which health outcomes were deduced. The mean age of study participants was 35 years and the majority of participants was male (**Table 4.4**). Sixty one percent (61%) of the subjects were non-smokers and 37% were current smokers and 2% ex-smokers. Among the smokers, the average age when they started smoking was 21 years.

**Table 4.4** Demographic characteristics of poultry farm workers in the North West Province, South Africa.

Demographic characteristics	Overall	Exposure categories				
	N = 191	Rearing n = 18	Laying n = 68	Hatchery n = 54	Broiler n = 41	Catching n = 10
Age (years)	35 ± 10.4	32 ± 9.6	35 ± 9.1	39 ± 13.1	32 ± 7.9	38 ± 8.6
Average Age: female : male	36:35	0:33	36:33	37:41	24:33	0:38
Sex: female: male (%)	34:66	0:100	47:53	59:41	2:98	0:100
Smoking:						
– Current	70 (37%)	12 (67%)	15 (22%)	13 (24%)	25 (61%)	5 (50%)
– Ex-smoker	4 (2%)	–	–	2 (4%)	2 (5%)	–
– Non-smoker	117 (61%)	6 (33%)	53 (78%)	39 (72%)	14 (34%)	5 (50%)
– Average number of years smoked	13	13	9	20	12	9
– Average age when started smoking (years)	21	19	24	21	20	28

**Note:** Continuous variables- mean ± SD; Categorical variables- number (%)

#### **4.5 (1-3)-B-D GLUCAN CONCENTRATION IN PERSONAL AIR SAMPLES**

The participants of the study ranged from general workers, cleaners, egg collectors, chick counters, supervisors, drivers, security and catchers. The job types were classified into 6 job categories based on similar job tasks (**Table 4.5**). In addition, a further job classification was established for hatchery to look at the possible varying levels of (1-3)- $\beta$ -D glucan exposure as their working processes were very specific and well defined.

Elevated levels of (1-3)- $\beta$ -D glucans were demonstrated in most of the poultry farming processes with catching crew being the highest and hatchery being the lowest. Variability between air samples collected on day 1 and day 2 for the different processes are tabulated in **Table 4.6**. No significant variability was found in laying, hatchery and the broiler houses (**Table 4.6**). Significant variability was noted in rearing which could be attributed to less observable work being conducted on day 1 of sampling as the team prepared for vaccination of chickens that were scheduled for day 2 of sampling. The vaccination process creates a lot of dust as the chickens flap their wings when avoiding being caught by the workers. Therefore, lower mean levels of exposure were observed in sample 1 when compared to sample 2. However, since this was a once-off sample, this was included in the analysis. Significant variability was observed for the catching activity which was also a very dusty procedure as chickens avoid being caught by the workers.

**Table 4.5** Number of samples collected per job category including repeats.

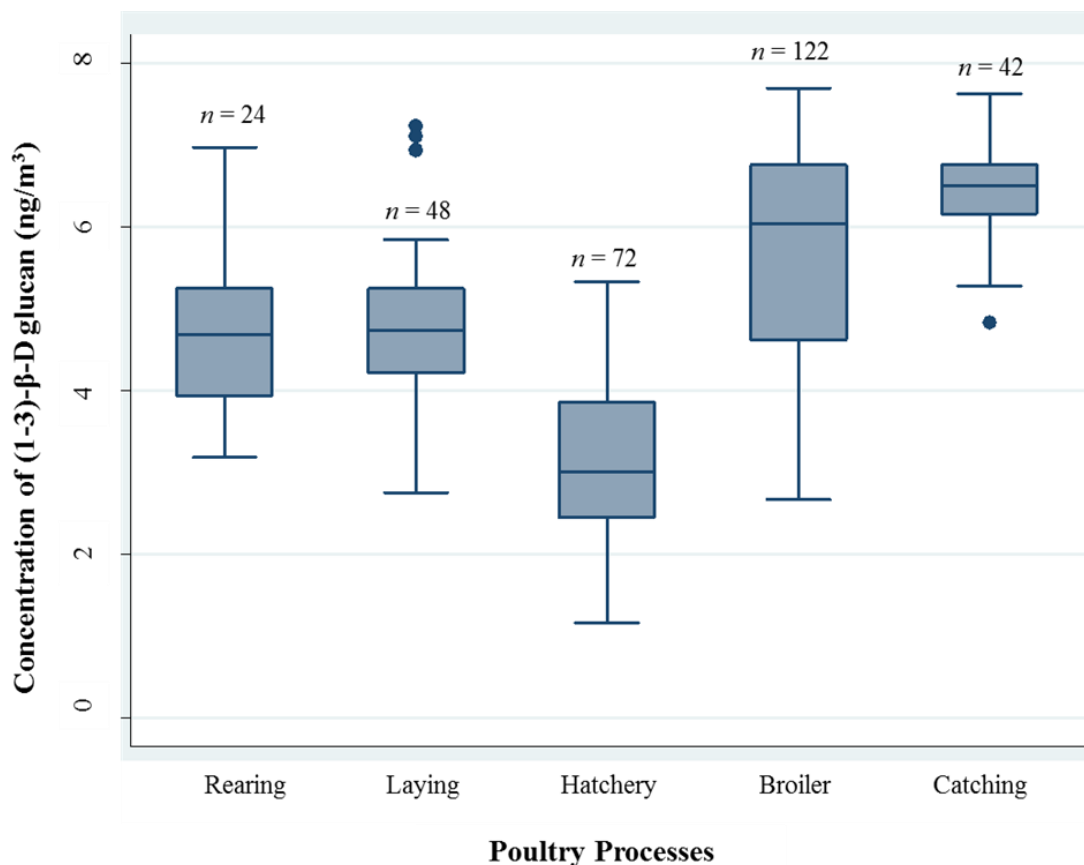
<b>Exposure group</b>	<b>Job category</b>	<b>n</b>
Rearing	General workers/househand	24
Laying	General workers	48
	Take-off	27
	Transfer	5
	Egg grading + take-off	6
	Mangers + Supervisors	5
	General worker	23
	Cleaners	6
Broiler	General workers	122
Catching	Catchers	42
Total		308

**Table 4.6** Variability of mean  $\beta$ -glucan levels concentration in  $\text{ng/m}^3$  between air samples collected on day 1 and day 2.

<b>Processes</b>	<b>1<sup>st</sup> sample Mean <math>\beta</math>-glucan</b>	<b>2<sup>nd</sup> sample Mean <math>\beta</math>-glucan</b>	<b>Variability score</b>	<b>p-value</b>
Rearing	4.1	5.08	9.6	0.0052
Laying	4.6	4.74	0.6	0.4422
Hatchery	3.08	3.03	0.04	0.8413
Broiler	5.57	5.38	0.29	0.5906
Catching	6.67	6.22	5.51	0.0239

Overall, the mean level of exposure for (1-3)- $\beta$ -D glucan was  $2.11 \text{ ng/m}^3$ . The concentration of (1-3)- $\beta$ -D glucan ranged between  $0.51 \text{ ng/m}^3$  and  $3.34 \text{ ng/m}^3$  across all exposure categories. The data was not normally distributed and therefore was log transformed for advanced analysis. Graphs that follow are an illustration of log transformed data.

Figure 4.2 illustrates the varying levels of (1-3)- $\beta$ -D glucan exposure for the various exposure categories at the poultry farm. Specimens that were obtained from the “catching” procedure demonstrated highest mean levels (6.51 ng/m<sup>3</sup>) and specimens that were obtained from the “hatchery” showed the lowest mean levels (3.01 ng/m<sup>3</sup>). An addition, the ANOVA test showed that the (1-3)- $\beta$ -D glucan exposure levels differed significantly by process ( $p < 0.05$ ).



**Figure 4.2** Box plot illustrating varying (1-3)- $\beta$ -D glucan exposure levels for the different exposure categories.

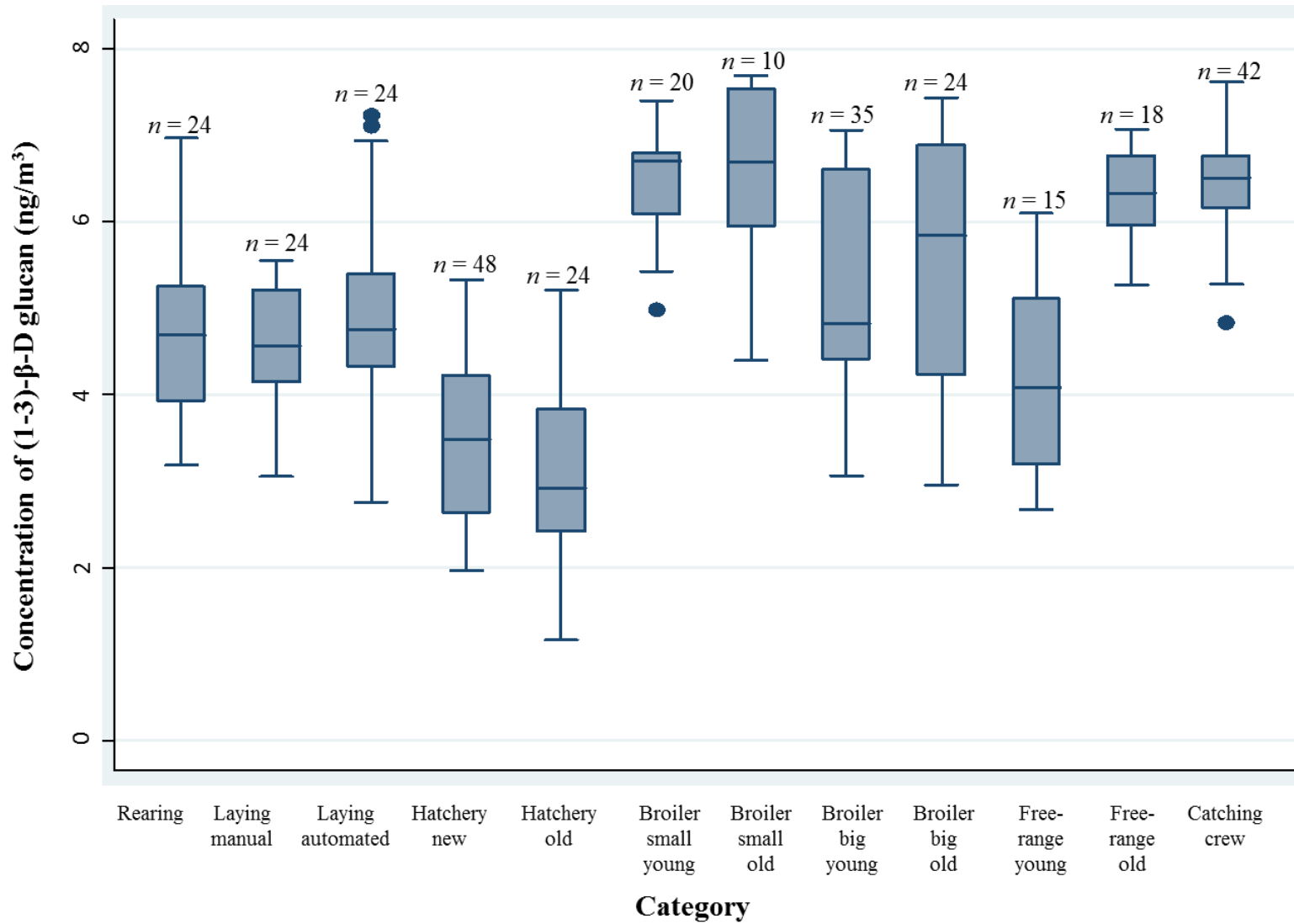
The differences in mean levels of log-transformed data are presented in **Table 4.7**. It is evident that the workers at the hatchery are exposed to the least levels of (1-3)- $\beta$ -D glucan (1.17 – 5.33 ng/m<sup>3</sup>) whilst the catching crew were exposed to the highest levels (4.83 – 7.62 ng/m<sup>3</sup>).

**Table 4.7** Mean levels of log-transformed (1-3)- $\beta$ -D glucan concentrations by farming process.

Process	Mean (ng/m <sup>3</sup> )	Min and max range (ng/m <sup>3</sup> )
Rearing	4.65	3.19 - 6.97
Laying	4.75	2.76 - 7.23
Hatchery	3.19	1.17 - 5.33
Broiler	5.64	2.68 - 7.70
Catching	6.47	4.83 - 7.62

The broiler farming process were further categorised by the size of the farm and the age of the chickens, type of laying farm and type of hatchery (**Figure 4.3**). Mean levels of (1-3)- $\beta$ -D glucan exposure found for rearing was 4.69 ng/m<sup>3</sup>. This was very similar to the levels found at laying. (1-3)- $\beta$ -D glucan exposure levels were found to be similar in the automated (4.76 ng/m<sup>3</sup>) and manual (4.57 ng/m<sup>3</sup>) laying process. However, this difference was not shown to be significant when using the analysis of variance (ANOVA) test ( $p \geq 0.05$ ). Similarly, the old hatchery showed higher mean levels of (1-3)- $\beta$ -D glucan exposure (3.49 ng/m<sup>3</sup>) than the new hatchery (2.92 ng/m<sup>3</sup>). However, this difference was also not significant ( $p \geq 0.05$ ).

With respect to broiler farms, the highest mean (1-3)- $\beta$ -D glucan exposure levels were observed in small broilers that had old chickens (6.49 ng/m<sup>3</sup>). Also, higher levels of exposure were noted in big broiler farms that had older chickens (5.56 ng/m<sup>3</sup>) than big broilers with younger chickens (5.31 ng/m<sup>3</sup>), however, this was not found to be significant ( $p \geq 0.05$ ). Free range broiler farms showed a significant difference in the (1-3)- $\beta$ -D glucan level of exposure ( $p \leq 0.05$ ) for older chickens (6.28 ng/m<sup>3</sup>) than younger ones (4.14 ng/m<sup>3</sup>). The catching crew exhibited high mean levels of exposure (6.47 ng/m<sup>3</sup>).



**Figure 4.3** Comparison of (1-3)-β-D glucan exposure level by process – Taking into consideration the size of farm and age of chickens

The overall geometric mean (GM) and geometric standard deviation (GSD) for all the participants were 4.70 ng/m<sup>3</sup> and 4.62 ng/m<sup>3</sup>, respectively. **Table 4.8** outlines (1-3)- $\beta$ -D glucan levels for personal air samples by farming process. Catching crew exhibited higher levels of exposure (6.47 ng/m<sup>3</sup>), followed closely by small broiler farms with young and old chickens (6.41 ng/m<sup>3</sup>). The lowest levels were shown by the new hatchery (2.95 ng/m<sup>3</sup>).

**Table 4.8** Personal air sample concentration for (1-3)- $\beta$ -D glucan by exposure group.

Farm Process	Type	Age of chicken	Personal air samples (ng m <sup>-3</sup> )			
			n	AM	GM	GSD
Rearing	n/a	n/a	24	4.65	4.57	2.47
Laying	Manual	n/a	24	4.60	4.54	2.05
	Automated	n/a	24	4.91	4.80	2.94
Hatchery	Old	n/a	24	3.43	3.33	2.61
	New	n/a	48	3.08	2.95	2.45
Broiler	Small	young	20	6.43	6.41	1.80
		old	10	6.49	6.41	2.89
	Big	young	35	5.31	5.19	3.16
		old	24	5.56	5.33	4.67
	free range	young	15	4.14	4.02	2.72
		old	18	6.28	6.26	1.79
Catching crew	n/a	n/a	42	6.47	6.44	1.89

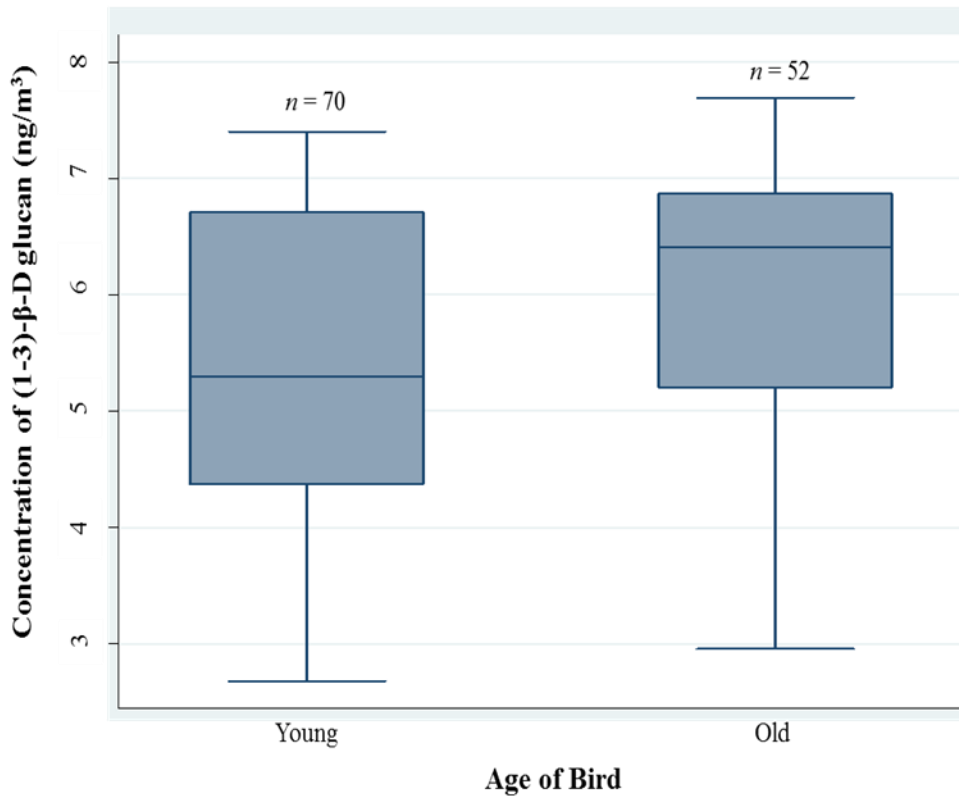
n, number of measurements; AM, arithmetic mean; GM, geometric mean; GSD, geometric standard deviation.

The box plot below showed higher mean levels of (1-3)- $\beta$ -D glucan exposure in smaller boiler houses (6.45 ng/m<sup>3</sup>) than in bigger boiler houses (5.41 ng/m<sup>3</sup>) This was shown to be statistically significant by the ANOVA test ( $p < 0.05$ ) (**Figure 4.4**).



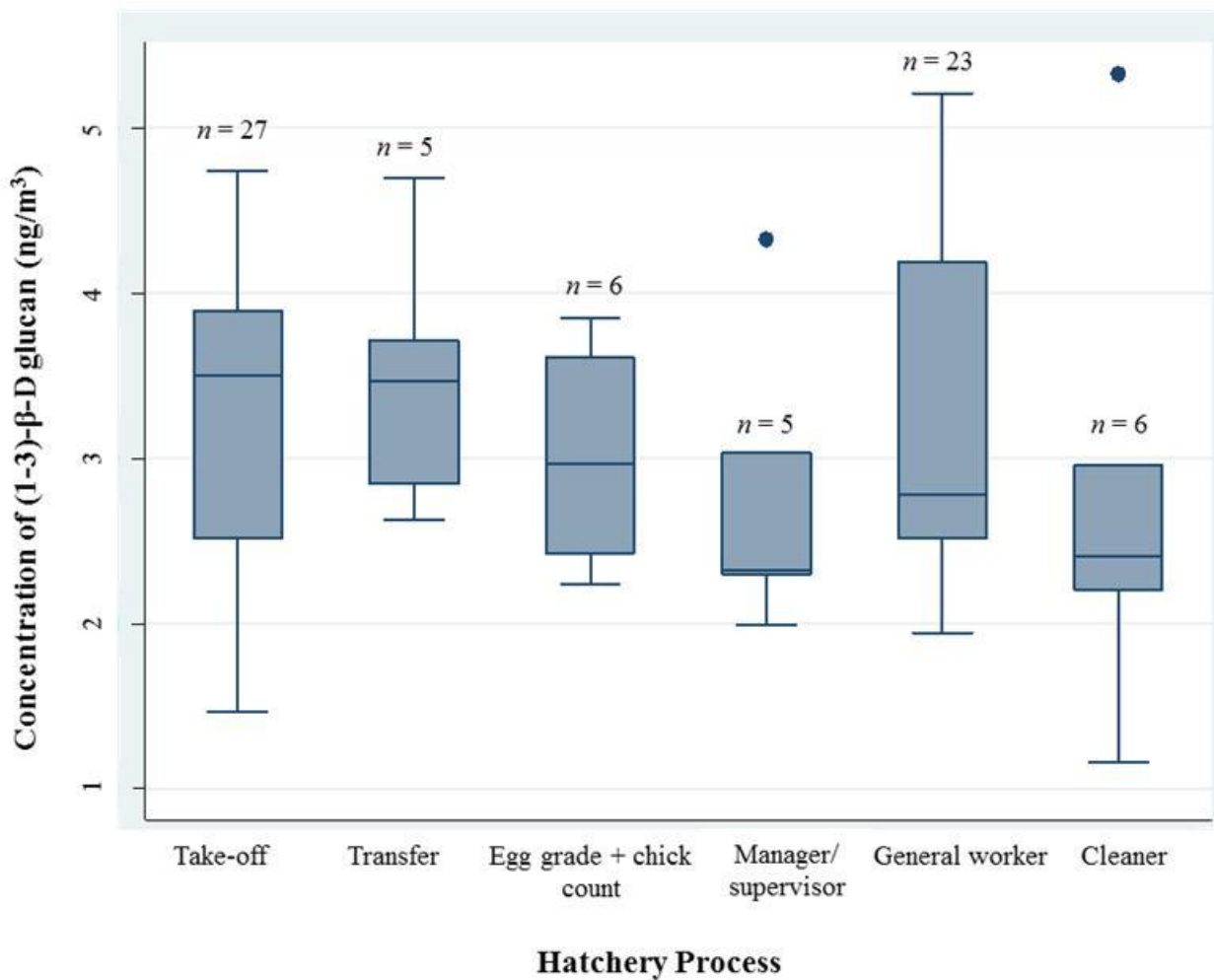
**Figure 4.4** Comparison of (1-3)- $\beta$ -D glucan exposure level by size of broiler house.

Moderately but not significantly higher mean levels of (1-3)- $\beta$ -D glucan was observed in boiler houses that had older chickens (5.99 ng/m<sup>3</sup>) than in broiler houses with young chickens (5.38 ng/m<sup>3</sup>) (Figure 4.5). The ANOVA test showed the difference to be significant (p<0.05).



**Figure 4.5** Comparison of (1-3)- $\beta$ -D glucan exposure level by age of chicken.

Varying levels of (1-3)- $\beta$ -D glucan exposure based on the job type of workers present in the hatchery is shown in Figure 4.6. Similar mean levels of exposure, 3.35 ng/m<sup>3</sup> and 3.47 ng/m<sup>3</sup> was noted for workers in the take-off and transfer process, respectively, and although not significant ( $p > 0.05$ ). Cleaners exhibited the lowest levels of (1-3)- $\beta$ -D glucan exposure (2.74 ng/m<sup>3</sup>) followed closely by managers and supervisors (2.79 ng/m<sup>3</sup>). The ANOVA test showed no significant difference between job tasks ( $p > 0.05$ ).

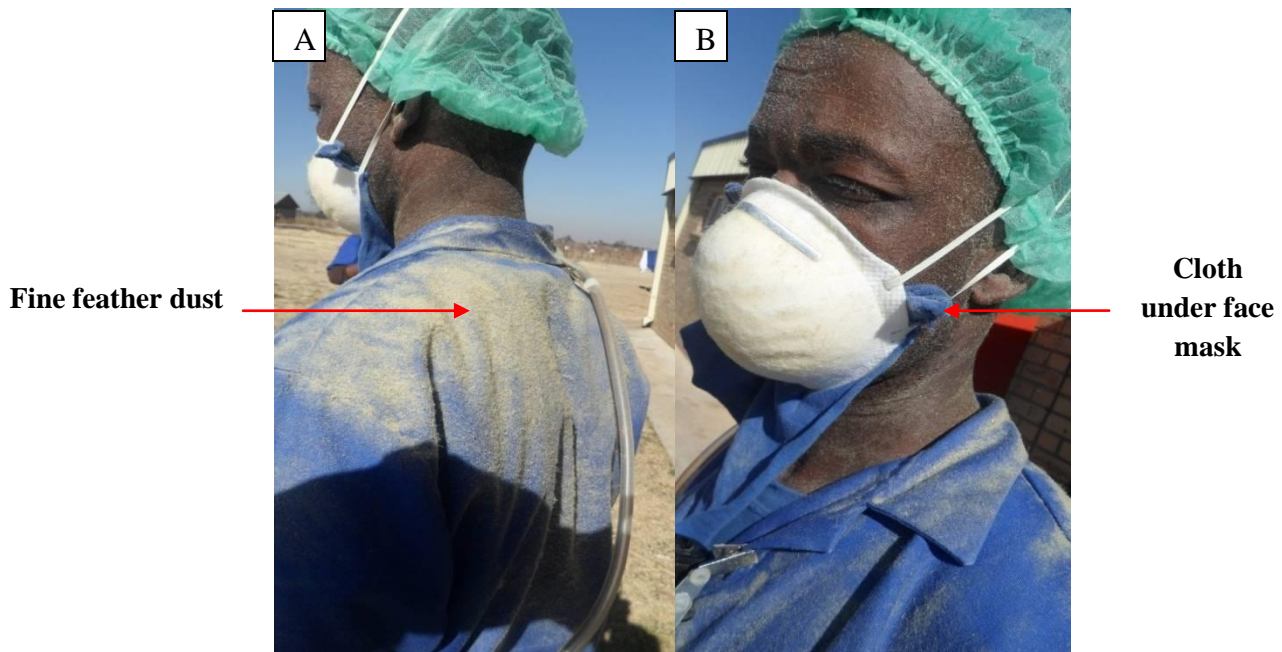


**Figure 4.6** Comparison of (1-3)- $\beta$ -D glucan exposure level by job type in the hatchery process.

The take-off process being the highest (3.51 ng/m<sup>3</sup>) followed closely by transfer (3.47 ng/m<sup>3</sup>). Take-off involves the physical removal of hatched chicks from crates and placing them into clean ones once they have been checked for abnormalities and they have been vaccinated. A large amount of fine feather dust as shown in Figure 4.7 and Figure 4.8 is released during this process.



**Figure 4.7** Photographs A and B showing the process known as take-off conducted at Hatchery.



**Figure 4.8** Photographs A showing the fine feather dust settled on worker's overall after the take-off procedure at Hatchery, and photograph B showing a worker who has put a clothe under his face mask for 'double protection'.

#### **4.6 SELF-REPORTED ASTHMA AND RESPIRATORY SYMPTOMS IN POULTRY FARM WORKERS**

Doctor-diagnosed asthma (self-reported) was reported in 2% of the study participants (**Table 4.9**). More than half these subjects (60%) developed asthma after the age of 17 years. Asthma-related symptoms were commonly reported in this group (17%) when compared to doctor-diagnosed asthma. Sixty two percent (62%) of subjects interviewed reported chest tightness when at work. A greater proportion of workers experienced work-related skin symptoms (61%) than ocular and nasal related work symptoms (47%). Whereas work-related chest tightness was experienced the least (24%).

**Table 4.10** shows the prevalence of asthma related symptoms based on the job tasks identified in the poultry farming process. The chi square test showed a significant difference for wheezing in the absence of cold and flu across the different job tasks ( $p < 0.05$ ).

**Table 4.9** Statistics of self-reported asthma and respiratory symptoms among poultry farm workers.

<b>Respiratory symptoms</b>	<b>Prevalence (%)</b> <b>(N = 191)</b>
<b>Asthma history</b>	
Doctor-diagnosed asthma <sup>1</sup>	3 (2%)
– Before 17 years	2 (1%)
– After 17 years	1 (1%)
Current use of asthma medication	11 (6%)
<b>Asthma-related symptoms (in the last 12 months)</b>	
Wheezing in the absence of flu or cold	18 (9%)
Asthma attack in the last 12 months	4 (2%)
Woken up by cough	63 (33%)
Woken up by tight chest	36 (19%)
Woken up by shortness of breath	32 (17%)
Asthma-related symptoms <sup>2</sup>	77 (40%)
<b>Work-related asthma symptom experience</b>	
Tight chest or wheezing after peak exposure	118 (62%)
Tight chest or wheezing when at work	46 (42%)
Job change due to work-related chest symptoms	29 (15%)
Improvement after changing jobs	17 (65%)
<b>Symptoms associated with work-related poultry dust exposure</b>	
Skin (itchy, cracked, rash)	28 (61%)
Ocular (Itchy eyes) / Nasal (runny or blocked nose)	88 (47%)
Chest (cough, wheeze, difficulty breathing)	45 (24%)

Note: Categorical variables- number (%)

<sup>1</sup> **Doctor-diagnosed asthma:** yes to both: “Have you ever had asthma?” and “Was this confirmed by a doctor?”

<sup>2</sup> **Asthma-related symptoms: yes to any of:** “Have you had an attack of asthma in the last 12 months?”; “Have you been woken by an attack of shortness of breath in the last 12 months?”; “Have you been woken up with a feeling of tightness in your chest at any time in the last 12 months?” or “Have you been woken by an attack of coughing at any time in the last 12 months?”

**Table 4.10** Prevalence of asthma related symptoms among various job tasks in the poultry farming process.

Symptoms	Job Task												
	Take-off n = 18	Transfer n = 5	Egg grade + chick count n = 3	Supervisor n = 29	General worker n = 54	Cleaner n = 7	Egg collector n = 28	Maintenance n = 15	Driver n = 6	Houseman n = 10	Chick feeder n = 5	Catching n = 9	Quality Control n = 2
<b>Wheezing in the absence of cold or flu</b>	1 (6%)	1 (20%)	0	1 (3%)	8 (15%)	3 (43%)	1 (4%)	2 (13%)	1 (17%)	0	0	0	0
<b>Asthma attack in the last 12 months</b>	0	0	0	0	1 (2%)	2 (29%)	1 (4%)	0	0	0	0	0	0
<b>Woken up by cough</b>	5 (28%)	3 (60%)	1 (33%)	9 (31%)	22 (41%)	3 (43%)	10 (36%)	4 (27%)	2 (33%)	1 (10%)	1 (20%)	1 (11%)	1 (50%)
<b>Woken up by tight chest</b>	4 (22%)	1 (20%)	0	1 (3%)	7 (13%)	2 (29%)	0	1 (7%)	1 (17%)	1 (10%)	0	0	0
<b>Woken up by shortness of breath</b>	5 (22%)	1 (20%)	0	2 (7%)	9 (17%)	4 (57%)	6 (21%)	1 (7%)	1 (17%)	2 (20%)	2 (40%)	0	0
<b>Asthma related symptoms<sup>1</sup></b>	8 (44%)	3 (60%)	1 (33%)	10 (34%)	24 (44%)	5 (71%)	12 (43%)	5 (33%)	2 (33%)	2 (20%)	3 (60%)	1 (11%)	1 (50%)

<sup>1</sup> **Asthma-related symptoms: yes to any of:** “Have you had an attack of asthma in the last 12 months?”; “Have you been woken by an attack of shortness of breath in the last 12 months?”; “Have you been woken up with a feeling of tightness in your chest at any time in the last 12 months?” or “Have you been woken by an attack of coughing at any time in the last 12 months?”

**Note:** Categorical variables- number (%)

## CHAPTER 5

### 5.0 DISCUSSION

The (1-3)- $\beta$ -D glucan method was validated for 21 samples of known concentration of 25 pg/ml. The test was done in duplicate comprising seven samples per test run on three separate test days. The coefficients of variation (CV) of all samples were  $\leq 10\%$  and the correlation coefficient, ( $R^2$ ) were  $\geq 0.99$  for all assay runs. All samples analysed in this study met the test criteria set for the validation procedure using the GlucateLL assay and the results formed part of the data set on which the statistical analysis was performed. The test method was independently validated by a routine assessment at the NIOH: Immunology and Microbiology section.

Based on an extensive review that was conducted to gather knowledge and information on the poultry industry, it was concluded that this study is the first in South Africa to look at the levels of (1-3)- $\beta$ -D glucan exposure in poultry houses and to utilise the GlucateLL assay for its quantification. A study conducted by Sander (2008) in Germany compared two quantitative methods for (1-3)- $\beta$ -D glucan analysis. A two-site enzyme immunoassay based on monoclonal antibodies was developed and compared to the GlucateLL assay by analysing air filter samples from a chicken shed. Extraction procedures of the filters were the same as in the current study. Values obtain from the two quantitative tests were found to be comparable. However, Sander's results were lower than what was observed in the current study. The difference in levels may be attributed to difference in climate, working procedures, type of chicken house and number of chickens per chicken house.

The findings of this study demonstrate considerably higher levels of (1-3)- $\beta$ -D glucan exposure levels (3.2 – 2199 ng/m<sup>3</sup>) in samples obtained from the various poultry farming processes when compared to other studies (Thorn et al., 1998; Rylander et al., 2006; Sander et al., 2008). Levels of (1-3)- $\beta$ -D glucan exposure identified in this study ranged between 3.21 ng/m<sup>3</sup> and 2199.32 ng/m<sup>3</sup> and exceed levels found in studies conducted in the poultry industry by Rylander and Carneiro (2006) (20-270 ng/m<sup>3</sup>) who used stationary samples and by Sander and colleagues (2008) (2-972 ng/m<sup>3</sup>) who used personal samples. The varying (1-3)- $\beta$ -D glucan levels found between Rylander and Sander could be attributed to the varying sample methodology, difference in sample population and climate. In the current study, the Glucatell assay (Associates of Cape Cod, Falmouth, USA) was used for the quantification of (1-3)- $\beta$ -D glucan, whilst Rylander and Carneiro used the Fungitec assay (Seikagaku Co, Tokyo, Japan). Sander also utilized the Glucatell assay (Pyroquant Mörfeldon, Germany), however difference in levels of (1-3)- $\beta$ -D glucan could be attributed to low statistical power of the study as a result of a small sample population.

Varying levels of (1-3)- $\beta$ -D glucan exposure were observed throughout the poultry farming processes (rearing, laying, hatchery, broiler and catching). The variability could be attributed to job function performed across the different farming processes as described in chapter 3. Both rearing (4.70 ng/m<sup>3</sup>) and laying (4.74 ng/m<sup>3</sup>) exhibited similar levels of average exposure. This could be due to the age of the chickens where they are at a similar developmental stage thus resulting in similar levels of exposure. In addition, the basic or primary job function undertaken by a general worker on both these farming sites is similar.

Rearing entails the breeding of chickens from one day old to 20 weeks old. During this period, the chickens are nurtured and fed until the female reaches reproductive age and is then transferred to the laying farm. Workers ensure that the feeding systems are working, check for mortalities and vaccinate the chickens at specific intervals. These activities increase their risk of dust exposure. One of the labour intensive processes that occur here is weighing and vaccination of every chicken in each of the rearing chicken houses that has a capacity of 60 000 chickens. Albrecht in 2003 found that the vaccination activities that occur during rearing resulted in a tenfold increase in concentration of some airborne microorganisms. These findings show that exposure levels are heightened during the rearing process further reinforcing that in general, concentration of airborne microorganisms depends not only on housing conditions but also on specific work procedures of workers that influence animal activity.

In general, farms where the laying process is conducted has been found to take place in two ways, caged or cage-free. In this study, cage-free laying farms were used as a form of practice. In addition, of the two laying farms investigated in this study one used a manual egg collecting system and the second used an automated egg collecting system. It is expected that lower exposure would be observed in a system that relied on automation. However, the automated laying farms showed slightly higher levels of (1-3)- $\beta$ -D glucan exposure compared to the manually operated system. It is possible that although the automated laying system results in less time being spent within the poultry house itself, workers do make routine walk through inspections to check for mortality, to pick eggs from the floor, ensure the food and water systems are in order and also dust the hens laying enclosure with a feather duster. This latter activity was not noticed on the day of sampling at the manual laying farm and thus could explain the slightly

higher levels of (1-3)- $\beta$ -D glucan exposure found in the automated laying farm. However, the difference in (1-3)- $\beta$ -D glucan exposure levels between the two laying processes (i.e. automated and manual) was not found to be significant ( $p > 0.05$ ).

The (1-3)- $\beta$ -D glucan exposure levels ( $3.01 \text{ ng/m}^3$ ) found in the hatchery area were the lowest of the five farming exposure groups. These findings were expected as the hatchery is considered to be a fairly “clean” process as the eggs are incubated and there is minimal organic dust exposure compared to the farms. A study conducted in 2007 by Skórska suggested that effects of exposure to organic dust in workers in the modern hatchery’s equipped with adequate ventilating mechanisms are less when compared to workers of broiler and laying farms. Based on the two hatchery sites that were sampled (i.e. old and new), higher geometric mean levels of (1-3)- $\beta$ -D glucan exposure was noted in the old hatchery ( $3.33 \text{ ng/m}^3$ ) than in the new ( $2.95 \text{ ng/m}^3$ ). The higher levels could be attributed to the less sophisticated machinery/equipment and ventilation systems in the old hatchery as well as the smaller building size. With the same volume of work being done in both these hatcheries, it is reasonable to conclude that the smaller volume of space that is less equipped and with inefficient ventilation mechanisms contributes to the increased exposure levels.

In this study, differences in exposure levels of (1-3)- $\beta$ -D glucan within the hatchery section, as illustrated in Figure 4.5, varied within the different job tasks. The warm condition within the hatchery are conducive to microbial growth, thus explaining the higher levels of (1-3)- $\beta$ -D glucan exposure observed for this process. Levels of (1-3)- $\beta$ -D glucan exposure during transfer were found to be similar to take-off, albeit not significant. The task of transfer involves the

manual removal of crates containing eggs from one incubator to another. Incubators are set at ~37 °C, thus creating a warm and moist environment suitable for the proliferation of microbial growth. Thus, explaining the higher levels that were found here. It should be noted that although these levels were higher compared to other procedures performed at the hatchery it is among the lowest compared to other exposure groups (Table 4.7). Similarly, a study conducted by Skorska and colleagues in 2007 concluded that the effects of exposure to organic dust in workers of the hatchery were less compared to workers of broiler and egg laying houses.

The job category with the least (1-3)- $\beta$ -D glucan exposure in hatchery was shown to be managers and supervisors (2.32 ng/m<sup>3</sup>). Their primary function is to manage or supervise the process rather than be physically involved in the labour process. They do, however, assist when short staffed or when training new personnel which may explain the outliers in the data. The cleaners based at the hatchery are only employed for the purposes of cleaning at this process and no other. 71% of cleaners exhibited asthma related symptoms. Their microbial risk of exposure to (1-3)- $\beta$ -D glucan is minimal as their work predominantly involves water and chemicals. The detectable levels and asthma related symptoms found could be attributed to the presence of unsettled dust and microorganisms looming in the air after the take-off process have concluded.

Workers involved in broiler house duties, manoeuvre in and out of the broiler house frequently. This causes the chickens' natural state to be disrupted resulting in movement and fluttering of their wings which disrupts layers of biomass found within the bedding. This may explain the significantly higher levels of (1-3)- $\beta$ -D glucan exposure (5.64 ng/m<sup>3</sup>) in the broiler houses in this study (Table 4.7). The (1-3)- $\beta$ -D glucan exposure level found in this study was observed as a

minimum exposure levels in studies conducted by Rylander & Carvalheiro., 2006 as well as Sander and colleagues in 2008 (Table 2.1). Another determinant of (1-3)- $\beta$ -D glucan exposure levels is, the size of broiler houses. Smaller broiler houses showed high mean levels of exposure when chickens were either young or old (6.41 ng/m<sup>3</sup>). Smaller houses would mean a further competition for space by older chickens thus resulting in increased (1-3)- $\beta$ -D glucan exposure (Table 4.8). Similarly, a study conducted in 2013 by Lawniczek-Walczyk showed that workers exposure to airborne microorganisms such as  $\beta$ -glucan and endotoxin, increased with consecutive stages of the chicken production cycle.

In addition, as chickens grow, their physical development would result in greater generation of airborne dust when moving as they compete for space within the house. The chicken faecal biomass and feather dandruff would also increase with age (Oppliger et al., 2008; Calvet et al., 2009). Therefore the age of bird would thus explain why broiler houses with younger chickens had lower (1-3)- $\beta$ -D glucan levels (Figure 4.5) as the contaminant levels within the bedding biomass is less. This was also supported by Calvet in 2009 who showed that dust concentration increased linearly by chicken weight, with the maximum levels at week four of the chicken life cycle. A direct cause-effect relationship between animal activity and dust concentration was demonstrated. In addition, it has been shown that chicken age influences the indoor environmental conditions of poultry houses, and thus may have an effect on workers health (Senthilselvan et al., 2012).

It is important to note that the sampling conducted for environmental analysis was done over the winter period in South Africa. Poultry are sensitive to cold and a decrease in temperature would

result in an increase in chicken mortalities. Therefore, in order to prevent heat loss, poultry houses limit their ventilation (Golbabaie and Islami, 2000). The practice results in a higher concentration of dust within the chicken houses thus increasing the risk of exposure to employees. In addition, to prevent heat loss, chickens inflate their feathers thus adding to the feather dandruff in the air (Golbabaie and Islami, 2000).

The type of bedding material used in the poultry farms in this study was a mixture of sunflower husk and sawdust. With conditions such as moisture and heat present in the chicken house, the bedding material would be an ideal substrate for the proliferation of microbial compounds. A study conducted by Samadi et al., 2012 demonstrated (1-3)- $\beta$ -D glucan in bedding material ranging from 7.84  $\mu\text{g}/\text{m}^3$  to 1.11  $\mu\text{g}/\text{m}^3$  in barns that had compost and sawdust bedding respectively. Bedding material may thus be a significant predictor of bioaerosol exposure.

In this study, employees in the “catching” category were by far the highest exposed group (6.44  $\text{ng}/\text{m}^3$ ). When workers attempt to catch chickens, chickens tend to resist and therefore run, fluttering their wings. This creates the aerosolisation of a mixture of organic poultry dust such as chicken feathers, skin debris, aerosolised feed, broken insect parts, poultry excreta and microbial components such as fungal and bacterial matter (Morris et al., 1991; Just et al., 2009). In addition, the inappropriate use of masks and unavailability of PPE such as overalls and hair nets by catching crew increases the risk of exposure (Oppliger et al., 2008).

Throughout the survey poor compliance with the use of personal protective equipment (PPE) policy was observed (Figure 5.1). In some cases, PPE was not provided and in instances workers

were provided with one face mask per work week. Some workers did not use the PPE correctly questioning the health & safety training of the workers.



**Figure 5.1** Photographs A-E showing the inappropriate use of or lack of PPE.

A study conducted by Chien in 2011 showed that more than a 1000 culturable bacterial colonies can be released from 1 gram of chicken faeces per hour, and approximately 80% of these bioaerosols are respirable. Interestingly, Albrecht reported in 2007 that data from literature showed that inactive or even dead cells can also have the potential to cause adverse health effects. This further heightens the importance of the provision of appropriate training and use of the correct PPE. In addition, it has been pointed out in a previous study (Elliott et al., 2005) that issues related to compliance or of timing of exposures relative to the use of PPE might hinder the assessment of this type of preventive practice which may be the case in the current study. For example, workers might feel pressured to report the use of company-mandated equipment, or workers might be wearing equipment after the onset of symptoms.

The prevalence of tight chest or wheezing when at work was 42% and tight chest or wheezing after peak exposure was even higher with 62% (Table 4.9) in this study was greater than that reported in literature. Simpson et al. in 1998 showed that from the nine different industries that were investigated, the highest prevalence of work-related lower respiratory tract symptoms (38%), upper respiratory tract symptoms (45%) and chronic bronchitis (15%) were found in poultry workers. Rees et al, (1998) found the prevalence of work-related cough (32%) while wheeze (23%) was lower among poultry workers similar to lower respiratory tract symptoms in Simpson's study. To date, this is the first study in South Africa looking at the occupational exposure levels of (1-3)- $\beta$ -D glucan within the poultry industry.

The current study demonstrates self-reported asthma (2%) and respiratory health symptoms (40%) in poultry farm workers that are supported by previous studies that indicated that working

in poultry farm environments can elicit a significant respiratory response (Bar-sela et al., 1984; Thelin et al., 1984; Stewart et al., 1985; Hagmar et al., 1990; Morris et al., 1991; Donham et al., 2000). This also concurred with previous studies where high rates of acute respiratory symptoms was associated with type of work performed (Thelin et al., 1984; Morris et al., 1991; Singh et al., 1999; Kirychuk et al., 2003; Mirabelli et al., 2012).

Singh et al. (1999) conducted an epidemiological survey for respiratory diseases on workers in the agricultural industry (poultry farms, granaries and sugar refinery). Through the administration of a medical questionnaire it was noted that the incidence of respiratory disorders were increased by duration of employment and age of worker. Smoking was found to have a major impact on the incidence of cough and breathlessness. This was corroborated by Mirabelli in 2012 who showed that poultry processing work may affect lung function. In this study, overall, 37% of poultry workers were current smokers.

In comparison to the workers involved in other poultry farming processes (i.e. rearing, laying, hatchery and broiler), the present study shows that duration of exposure is far less when compared to the catching crew thereby increasing their risk to developing respiratory dysfunction. Even though the levels of (1-3)- $\beta$ -D glucan exposure were found to be high in this study, interestingly, catching crew exhibited fairly low levels of asthma related symptoms (11%). In contrast, a study conducted by Morris et al, (1991) demonstrated that chicken catchers reported high rates of chronic respiratory symptoms. In general, chicken catching takes place in a highly contaminated and hazardous work environment. The fast pace of work and low level of control over work intensity all result in high potential for work-related injury and illness,

respiratory effects and musculoskeletal injuries. It would not be feasible to remove low level labour such as catching as no mechanisms have been established to replace this. In most instances, workers employed within this field, especially in the third world countries are dependent on this type of work as their only source of income. More viable solutions would include exploring possible changes in the manner in which job tasks are carried out (Quandt et al., 2012).

Even though the levels of (1-3)- $\beta$ -D glucan exposure were lower in hatchery, work related respiratory health effects were observed and therefore efforts to reduce the airborne fine feather dust levels need to be explored. A customised electrostatic space charge system (ESCS) was developed by Mitchell and Waltman in 2003 which cleans the air within hatchery incubators by transferring a strong negative electrostatic charge to dust and microorganisms that are aerosolised during the hatching process by collecting them on grounded plates or surfaces. The ESCS proved to be a viable treatment method when compared to chemical methods in reducing the levels of airborne pathogens through reduced dust control and containment. In doing so, a reduction of cross contamination and exposure could be achieved between the different job tasks conducted at Hatchery.

Health effects associated with exposure in laying farms found in this study was in contrast to a study conducted by Larson in 1999 who found the environment in the houses for egg laying production induces acute inflammatory reactions in groups exposed in buildings with loose housing for laying hens. Interestingly, Mitloehner and Calvo, (2008) reported that despite the implementation of precautionary measures, workers in concentrated animal feeding

environments such as poultry farming, continue to report work related respiratory health symptoms. Therefore, researchers have suggested that intensive research is required in modelling improved safety strategies that include techniques and protective gear to minimise adverse effects of working in such environments (Douwes, 2005; Mitloehner and Calvo., 2008).

## **5.1. LIMITATIONS**

The limitation of the current study was that the association of (1-3)- $\beta$ -D glucan exposure and health outcomes was not examined. This requires multivariate analysis which was not a requirement for this dissertation however will be addressed in future work investigating the association between exposure and clinical outcomes. This was also a cross sectional study and only provides a snapshot of the exposure levels at the time of sampling. The questionnaires was administered in English by the candidate and two medical intern scientists. English was the preferred language as determined by presentations to workers prior to the study. However, the interviewing team were fluent in Afrikaans, Tswana, Zulu and Sotho and interpreted if required.

## **5.2 RECOMMENDATIONS**

The current study was able to compare (1-3)- $\beta$ -D glucan levels to job tasks. In this way, control measures may be adopted thus allowing for exposure to be managed better. Based on the findings of this study the following recommendations are suggested:

- Refresher awareness training on the correct use and compliance of PPE would assist in the reduction of (1-3)- $\beta$ -D glucan inhalation.
- Introducing the concepts of biological exposure into health and safety training modules.

- The installation of automated faecal removing systems which would avoid the need for manual removal by employees thus reducing the risk of exposure.
- The introduction of more sophisticated ventilation mechanism which further reduces bioaerosol containment within the poultry houses.
- In order to curb the levels of (1-3)- $\beta$ -D glucan exposure in poultry farms, an alternate type of bedding material should be suggested for implementation that would result in decreased bioaerosol exposure. Investigate the use of bedding material which has anti-microbial growth properties.

### **5.3 CONCLUSION**

This study demonstrated high levels of (1-3)- $\beta$ -D glucan within the various farm exposure groups. The highest mean levels of (1-3)- $\beta$ -D glucan exposure was observed for the catching crew whilst the lowest was among hatchery workers. A high prevalence of work-related respiratory chest symptoms was observed among poultry workers. Training on the appropriate use of PPE will aid in the reduced inhalation of dust thereby decreasing the prevalence of associated occupational respiratory health symptoms.

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## **APPENDIX A: PROTOCOL**

### **1. (1-3)- $\beta$ -D-glucan exposure assessment in poultry farms in South Africa.**

## **2. INTRODUCTION**

### **2.1. Background**

The poultry industry continues to dominate the South African livestock sector as chicken remains an affordable protein source compared to other meat products. The South African poultry industry mainly includes broiler, egg and chick producers. This industry remains an important contributor to employment opportunities both in the formal and informal sector with 80% of the industry comprising small, medium and micro enterprises which employ approximately 77 000 people (i.e. 60 000 broiler, 10 000 egg and 7000 chick producer) (SAPA, 2008).

Bioaerosols in the poultry industry may be derived from soil, and dust from feed and bedding material (Crook, B., Easterbrook, A & Stagg, S. 2008). Poultry farm workers are exposed to a wide variety of agents which might include both organic and inorganic material derived from litter, feathers, dander (skin material), feed, excreta and micro-organisms and their by-products which could cause respiratory disease. Organic dust may contain several inflammatory agents including (1-3)- $\beta$ -D-glucan (Thorn, J., Beijer, L & Rylander, R. 2001) which are water insoluble glucose polymers commonly found in the cell walls of most (60%) moulds and some soil bacteria and plants (Douwes, 2005; Lossifova, Y.Y., Reponen, T., Bernstein, D.I., et al. 2007).

(1-3)- $\beta$ -D-glucan has been used as a surrogate measure for mould exposure in airborne studies (Lossifova et al, 2007). Concentrations of (1-3)- $\beta$ -D-glucan in poultry farms from previous studies ranged from 4-870ng/m<sup>3</sup> (Rylander and Carvalheiro, 2006). A more recent study by Sander et al (2008) found similar levels of (2-972ng/m<sup>3</sup>) in poultry farms.

Previous studies have demonstrated that poultry workers have increased incidence of respiratory symptoms due to exposure to organic dust (Rylander, et al., 2006; Faria et al., 2006). Direct contact with chicken and poultry litter is a major cause of sensitisation in some poultry workers. Asthma related symptom incidence in farmers has been found to be higher for poultry farmers reporting up to 17.4% prevalence (Kimbell-Dunn, M., Bradshwa, L., Slater, T., et al. 1999). Barsela (1984) reported rhinitis and asthma in all 16 symptomatic poultry workers investigated after exposure to poultry. In most of these workers the symptoms occurred within a few minutes after entering the poultry house. The findings from the latter study suggested that the symptoms related to poultry exposure were IgE mediated sensitisation to poultry-related antigens (PAg) (Barsela, S., Teichtahl, H & Lutsky, I. 1984). Only one study in the South African poultry sector on health outcomes has been reported thus far. This study reported a very high prevalence of exposure-related symptoms associated with organic dust exposure (Rees, D., Nelson, G., Kielkowski, D., et al. 1998).

Studies conducted in the Free State province of South Africa and in Switzerland established that the distribution of airborne microorganisms varies in the different stages in chicken slaughtering facilities with levels exceeding Swiss occupational recommended limits (1000 EU m<sup>-3</sup>)

(Oppliger, A., Charriere, N., Droz P-O., et al. 2008). Initial stages of the process which involves the catching-transporting, receiving-killing, de-feathering and fattening periods of the chicken demonstrated high levels of bioaerosols due to the increase in faecal biomass and feather dandruff (Lues, J.F.R., Theron, M.M., Venter, P., et al. 2007; Oppliger, et al., 2008), which then decreased toward the evisceration, air-chilling, packaging, and dispatch areas (Lues, et al., 2007; Oppliger, et al., 2008).

Previous studies have demonstrated that organic dust and the by-products of the decomposition of the organic matter cause mainly respiratory symptoms, in addition to fatigue and joint pains, headache, nose and throat irritation, through airway inflammation (Wilkins, C.K., Larsen, S.T., Hammer M., et al. 1998; Douwes, 2005; Rylander, et al., 2006; Lossifova, et al., 2007) and sick building syndrome (Sander, I., Fleischer, C., Borowitzki, G., et al. 2008).

There is considerable evidence that the risk of developing occupational allergy and asthma increases with an increase in exposure to organic dust. There is therefore a need to understand the relationship between exposure to agents in the poultry industry and respiratory symptoms. In addition, (1-3)- $\beta$ -D-glucan levels will be determined in order to minimize exposure of workers, thus reducing respiratory symptoms. It is also necessary to establish which job tasks have increases exposure so that proper control measures and/or work practices can be implemented. Particular interest recently has been on the interaction between agents causing an additive or synergistic effect. For example, studies involving animal models have reported an interaction between (1-3)- $\beta$ -D-glucan and endotoxin in causing airway inflammation (Rylander and

Fogelmark, 1994). In addition, (1-3)- $\beta$ -D-glucan has been recognized for its potent ability to induce pro-inflammatory reactions, in occupational settings (Douwes, J., Thorne, P., Pearce, N., et al. 2003). Therefore, an exposure assessment needs to be done in occupational settings such as the poultry industry with multiple exposures.

## **2.2 Motivation**

As previously mentioned, a study conducted a decade ago at the NIOH investigating work-related respiratory symptoms in poultry workers found a very high prevalence of exposure-related symptoms associated with organic dust exposure (Rees, et al., 1998). However specific causative agents were not identified which is important as it will assist in management of affected workers. A further limitation of this study was the lack of objective exposure assessments and the inability to demonstrate an association between allergic sensitisation and respiratory symptoms. In addition, the NIOH allergy unit has tested several workers (2002-2006) from poultry farming with respiratory symptoms and asthma. Although 43% were positive to allergens tested from the workplace 57% were negative to the aeroallergens and poultry specific allergens (chickens feathers, poultry feed, shavings and poultry litter) pointing to the possibility of a non-IgE mediated inflammatory response. These data are consistent with the finding of other studies which suggest that at most only 50% of asthma cases are attributable to “allergic asthma” (Douwes, J., Gibson, P., Pekkanen, J., et al. 2002). These findings suggest the need for further research investigating other potential agents such as (1-3)- $\beta$ -D-glucan, causing non-IgE mediated inflammatory reactions. This has implications for asthma diagnosis and also management of affected workers.

This project will enable the establishment of a new assay for (1-3)- $\beta$ -D-glucan in the Immunology section of NIOH resulting in increased capacity and contribute to international research activities which would facilitate new collaborations. We have also recently received a query to analyse (1-3)- $\beta$ -D-glucans so there is a market for the agent under investigation. Occupational exposure assessments on (1-3)- $\beta$ -D-glucan have not been reported in South Africa and the former test is not available in the country. Therefore, we would be improving our specialised service component in the section.

### **2.3 Hypothesis**

Poultry dust contains multiple biological agents one of which includes (1-3)- $\beta$ -D-glucan that may be associated with respiratory symptoms among workers.

### **3. STUDY OBJECTIVES**

- To establish and validate a method for the detection and quantification of (1-3)- $\beta$ -D-glucans in environmental samples using the GlucateLL assay.
- To investigate environmental exposure of poultry workers by measuring (1-3)- $\beta$ -D-glucan using personal sampling.
- To categorise the different tasks undertaken within the poultry industry and compare this to general respiratory symptoms experienced by workers.

## **4. METHODS**

### **PHASE 1**

#### **Mapping of the industry**

South African Poultry Association (SAPA) will be contacted to obtain information on the number of large, medium and small farms available; to identify the size of each type of farms and the type of farming and also the number of workers employed at various farms. The poultry farmers will be identified and the number of farms to participate in this will be determined in this phase, taking into consideration the sample size. Permission will then be sought from the farmers to participate in the study. Based on the number of sites approved by SAPA in Gauteng, 2 big farms, irrespective of type, i.e broiler or egg producer, will be chosen in order to provide a sufficient sample number for the study.

#### **Building inspection**

A walkthrough will be conducted by a qualified hygienist in order to obtain information on workforce, specific tasks and the control measures in place using a checklist completed from direct observation. Information on the estimated number of chickens bred, bedding material, type of chicken feed, frequency of cleaning procedures will also be sought. The various task will also be assigned a subjective exposure category (none, low, medium, high) after collation of all relevant exposure information.

## **PHASE 2:**

### **Stage 1:**

#### **Environmental:**

Organic dust samples will be collected using personal sampling pumps and 37mm, 1µm pore size sure sealed preloaded weigh checked glass fibre filters at a flow rate of 2L/min (Gladding, T., Thorn, J & Stott D. 2003) for a minimum of 4 to 6 hours (Mandryk, J., Alwis, K.U & Hocking, A.D. 2000; Gladding, et al., 2003). The sampling pumps will be placed at each participants breathing zone during their work activity. Sampling will be conducted during the entire shift. The workers will be categorised into exposure groups (departments) based on the inspections. A stratified random sample of all workers will be chosen from each group. Repeated sampling will be conducted on a subgroup of workers (20%) on at least two further occasions to assess the intra-individual variability. It is estimated that a total of 200 samples will be collected.

#### **Questionnaire**

A modified questionnaire designed for investigation of asthma from the European Community Respiratory Health survey (ECRHS II) and seafood processing workers study (Baatjies, R., Lopata, A. L., Raulf-Heimsoth, M et al) will be used to gather information on general respiratory symptoms experienced by the poultry workers. The modified questionnaire will be administered in English and in the language of the worker if necessary. (Appendix 1)

### **Stage 2:**

## **Laboratory Analysis:**

### ***Extraction***

The two extraction methods for environmental samples present to date for beta glucans are an alkaline extraction which uses sodium hydroxide (Rylander., 1999; Lossifova et al., 2007) and a heat extraction method using autoclaving at 120 °C for approximately 1 hour (Douwes, J., Zuidhof, A., Doekes G et al., 2000; Ronald, L.A., Davies, H.W., Bartlett, K.H et al., 2003; Wouters et al., 2006; Sander et al., 2008). EQA samples will be sent to the Netherlands, whom also uses the heat extraction method.

Filters will be extracted at room temperature in 5 ml pyrogen free water with 0.05% Tween 20 (Douwes, J. P., Verloot, A., Hollander, A., et al. 1995). Extracts will be aliquoted for the (1-3)- $\beta$ -D-Glucan test and stored at -20 °C for later use.

### ***Measurement of (1-3) $\beta$ -D-Glucan***

The conventional approach for the quantification of (1-3)- $\beta$ -D glucan involved the manipulation of the LAL assay capable of detecting endotoxin and (1-3)- $\beta$ -D glucan resulting the under estimation of glucans and/or the ELIZA which is an enzyme immunoassay used to detect an antigen of interest in a particular sample (Douwes et al., 2003; Foto et al., 2004; Sander et al., 2008). Although these two methods are sensitive, the ELIZA has proven to be highly laborious and time consuming. In addition to this, the LAL assay is not specific for mould as pollen and certain vegetable fibers also contain (1-3)- $\beta$ -D glucan, resulting in false positives. The glucan-specific limulus amebocyte lysate (LAL) (available from Associates of Cape Cod, Falmout, MA,

USA), commonly known as the GlucateLL assay is the most recently developed assay used for the detection of (1-3)- $\beta$ -D glucan . This method is based on the same principals as the LAL assay which has been described for endotoxin measurements (Foto et al., 2004). Instead of activating factor C, glucans activate factor G which initiates a cascade of enzymatic reactions, finally producing a color or turbidimetric response. The glucans-specific LAL assay rules out the option for cross reactivity with endotoxin as factor C has been completely removed or disabled from the LAL preparation (Douwes et al., 2005; Sander et al., 2008). It is a sensitive, fairly quick and specific method and it has therefore been chosen as the method of chose for the purposes of this study.

The aliquot obtained after extraction will be diluted two-fold with pyrogen free water with 0.05% Tween 20 and heat treated by autoclaving at 120°C for 1h before testing. (1-3)- $\beta$ -D-Glucan present in extract will be measured using GlucateLL assay (Associates of Cape Cod, East Falmouth, MA, USA). The detection limit for (1-3)- $\beta$ -D-glucan is 20 pg/ml (Rylander, et al., 2006).

### ***Quality control***

Field blanks and laboratory blanks will be included on each day of measurement. Pumps will be calibrated before and after sampling to ensure variance is within 5%. For the laboratory analysis, the limit of detection (LOD), which is the lowest level of detecting the agent, will be computed by calculating the mean of the field blanks. The limit of quantification (LOQ), which is the limit above which there is confidence of the agent measured, will then be calculated by adding the mean plus 3 times the standard deviation of the field blanks. For statistical analyses, values for

exposure measurements below the LOD will be censored using  $L/\sqrt{2}$  (Finkelstein and Verma, 2001). The co-efficient of variation (CV) between two measurements will be  $\leq 10$  and  $R^2 = 0.99$  for all assay runs will be accepted.

### ***Assay validation***

The GlucateLL assay that detects for (1-3)- $\beta$ -D glucan will be established at the NIOH by first validating the method.

A sample of known standard concentration of 25pg/ml will be used. This will be pipetted in 3 sets of 8 to test for reproducibility and repeatability respectively. A mean and standard deviation will be determined for each data set, thereafter a grand mean and standard deviation will be calculated. Subsequently, a levy-jennings graph will be plotted and an upper and lower limit will be established by using the grand average and a 95% confidence interval.

Positive control samples should lie within this established upper and lower limit. This is the first criteria to be met by each assay run for acceptance of results. If the control lies outside these 2 limits, the assay run will be rejected and repeated.

## **5. DATA ANALYSIS**

STATA statistical package (version 9) will be used to analyse the data, questionnaires and laboratory analysis. Exposure metrics will be developed on the basis of individually measured exposures and average levels within each job category. Descriptive statistic will be used to summarize the distribution of each measured variable. The chi-square test and analysis of variance (ANOVA) will be used to test associations between health outcomes and various explanatory variables. Univariate and multivariate linear regression models will be constructed to determine the variance components of exposure. Pearson correlation will be performed to determine the relationship between log transformed total inhalable dust and  $\beta$ -glucan.

## **6. ETHICS**

Ethics approval will be sought through the Wits Human Research Ethics Committee pending permission from poultry farms to conduct study.

## 7. TIMING

Grant Year	2010				2011			
Quarter	1	2	3	4	1	2	3	4
➤ Protocol initiation, approval (NIOH & Wits). Ethics application								
➤ Industry mapping, sampling strategy, method validation								
➤ Develop sampling schedule, order reagents & consumables								
➤ Environmental measurements: - Microclimatic parameters								
- Bioaerosol sampling ( $\beta$ -glucan)								
➤ Laboratory analysis: - Extraction								
- ( $\beta$ -glucan)								
➤ Data management and analysis: - Capturing and validation								
- Cleaning and analysis								
➤ Reporting of Data: - Dissertation write up								
- NIOH Research forum/Research Day								
- Journal publication								

## 8. RESPONSIBILITIES OF INVESTIGATORS

<b>Responsibility</b>	<b>Investigators</b>	<b>Duties</b>
Project manager	Ms Payal Dayal	Conduct & coordinate project & report of data
Project supervisor	Prof Ahmed Wadee (NHLS: Immunology, Wits)	Supervising & advisory
Co-supervisor	Ms Tanusha Singh (NIOH)	Supervising, advisory and assist with data analysis and interpretation
Co-workers	Mr Kevin Renton (Occupational Hygienist: NIOH)	Risk assessment & collection of air samples

## 9. BUDGET

We expect at least 2 sites to be approved by SAPA for sampling. Assuming that there are 12 farm houses on each site and 5 departments within each farm house, the number of samples that will be collected would be 60 (i.e.  $12 \times 5 \times 2 = 120$  samples) In addition, samples will be collected in triplicate (i.e. 1 sample per day, over 3 days) therefore the total number of samples expected is 360 ( $120 \times 3$ ). Based on this the budget was calculated.

<b>BUDGET</b>	<b>Quantity</b>	<b>Est. unit cost</b>	<b>Est. costs (R)</b>
• <b>Pilot study instruments</b> (e.g. questionnaires, data collection forms, method validation)			<b>20 000</b>
• <b>Stationary</b> (e.g. paper, files, labels, stamps)			<b>2 000.00</b>
• <b>Travelling to sites</b>			
✓ Transportation (3 x sites* – depending on study numbers)			<b>10 000.00</b>
✓ B&B Accommodation (x 2 team members)	<b>10 days</b>	<b>300.00</b>	<b>6000.00</b>
✓ Subsistence (x 2 team members)	<b>10 days</b>	<b>60.00</b>	<b>1200.00</b>
• <b>Overheads</b> (rate per hr)			
✓ Occupational hygienist	<b>80</b>	<b>340.00</b>	<b>27 200.00</b>
• <b>Direct consumables</b>			
✓ Reagents			<b>8 000.00</b>
✓ Labware (e.g. Dilution tubes, pipette tips, reagent reservoirs)			<b>5 000.00</b>
✓ Glass fibre preloaded cassettes <sup>†</sup>			
✓ GlucateLL kit (48 tests per kit)	<b>8</b>	<b>18 000.00</b>	<b>144 000.00</b>
• <b>Referred tests or outsourced services</b>			
✓ Interlab proficiency / EQA	<b>10%</b>		<b>26 600.00</b>
• <b>Training &amp; Skills development</b>			
✓ Local conference	<b>1</b>		<b>5500.00</b>
• <b>Contingency</b>			<b>15 000.00</b>
<b>TOTAL</b>			<b>R270 500</b>

\* Pending on number of sites approved by SAPA

† Occupational hygiene section (NIOH) will cover these costs

## 10. FUNDING

An application for funding was submitted to the MRC and ALLSA. Both applications were successful in receiving R130 000 for every year for the next 3 years from the MRC and a once-off amount of R25 000 from ALLSA.

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## APPENDIX B: ETHICS CLEARANCE CERTIFICATE

**UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG**  
**Division of the Deputy Registrar (Research)**

**HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)**  
R14/49 Ms Tanusha Singh

**CLEARANCE CERTIFICATE**

**M10646**

**PROJECT**

Allergic Sensitisation and Work-Related  
Asthma among Poultry Workers in South  
Africa

**INVESTIGATORS**

Ms Tanusha Singh.

**DEPARTMENT**

Department of Immunology

**DATE CONSIDERED**

25/06/2010


**DECISION OF THE COMMITTEE\***

Approved unconditionally

**Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.**

**DATE** 16/08/2010

**CHAIRPERSON**.....

  
(Professor PE Cleaton-Jones)

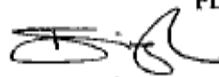

\*Guidelines for written 'informed consent' attached where applicable  
cc: Supervisor :

**DECLARATION OF INVESTIGATOR(S)**

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.

I/We fully understand the conditions under which I/am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

 04/11/2010  
 04/11/2010

# APPENDIX C: WALKTHROUGH CHECKLIST

## POULTRY FARM INSPECTION CHECKLIST

Date of interview: \_\_\_\_\_

Farm ID: \_\_\_\_\_

Location: \_\_\_\_\_

Address: \_\_\_\_\_

Phone: \_\_\_\_\_

Name of Interviewer/observer: \_\_\_\_\_

Name of Assistant on-Site \_\_\_\_\_

### GENERAL INFORMATION

1. Type of poultry operation:

A. Broiler/Roaster

B. Broiler/Breeder

C. Egg layers

D. Other \_\_\_\_\_

2. Age of farm: \_\_\_\_\_

3. Size of farm: \_\_\_\_\_

4. Cage or floor or mixed housing? \_\_\_\_\_

5. Total number of birds currently on farm: \_\_\_\_\_

6. How many birds do you produce/cycle? \_\_\_\_\_

7. How many birds do you produce/year? \_\_\_\_\_

7.1. Age at marketing: \_\_\_\_\_ weeks

8. How many people work in this poultry operation? \_\_\_\_\_

9. Activities on-site? \_\_\_\_\_

10. Exposure control in use: \_\_\_\_\_

## SAMPLING BARN/HOUSES

1. How many barns/houses do you have? \_\_\_\_\_

2. Barn age: \_\_\_\_\_ years

3. Barn size:

3.1 Barn length: \_\_\_\_\_ m

3.2 Barn width: \_\_\_\_\_ m

3.3 Barn height: \_\_\_\_\_ m

4. If floor housing, length of floor housing (if applicable) \_\_\_\_\_

5. Breed: \_\_\_\_\_

6. Age of birds: \_\_\_\_\_ weeks

7. Number of birds/chickens in this barn: \_\_\_\_\_

8. Flock mortality rate: \_\_\_\_\_

9. Have you had any disease outbreaks?

A. Yes

B. No

9.1. If yes, what disease: \_\_\_\_\_

9.2. Date of outbreak: \_\_\_\_\_

10. Any visible microbial growth (*circle the correct answer*)

A. Yes

B. No

11. Any odours excluding chicken droppings (*circle the correct answer*)

A. Yes

B. No

11. Material involved (directly or indirectly) and contaminants: \_\_\_\_\_

**EQUIPMENT AND FACILITIES**

12.1. What type of cage system do you have?

- A. Single tier
- B. Double tier
- C. Triple tier
- D. Battery system
- E. NA
- F. Other \_\_\_\_\_

12.2. Size of cages? \_\_\_\_\_

12.3. What type of egg collection system do you have?

- A. Conveyor belt
- B. Hand
- C. Auger
- D. None
- E. Other

12.4. Type of litter collection system:

- A. Deep pit
- B. Shallow pit
- C. Manure belt
- D. NA

12.5. Do you use litter in your operation?

- A. Yes
- B. No

**LITTER/BEDDING**

13.1. Type of litter used:

- A. Woodchips
- B. Straw
- C. Sunflower hulls

- D. Shavings
- E. Paper
- F. Woodchips/Straw
- G. NA
- H. Other \_\_\_\_\_

13.2. Is the litter removed after every flock?

- A. Yes
- B. No
- C. NA

13.3. Do you rototill your litter?

- A. Yes
- B. No
- C. NA
- D.

13.4. Is the litter wet in winter?

- 
- A. Yes
  - B. No
  - C. NA

#### **CLEANING**

14.1. Cleaning frequency:

- A. Daily
- B. weekly
- C. monthly
- D. quarterly
- E. 6monthly
- F. Other \_\_\_\_\_

14.2. Who does the cleaning?

- A. Permanent staff
- B. Contractor

14.3. What type of housing clean out system do you use?

- A. Front – end Loader
- B. Tractor

- C. Bobcat
- D. Scraper
- E. NA
- F. Other \_\_\_\_\_

14.4. What type of disinfectant do you use?

- C. Soap and water
- D. Virion
- E. Proximad
- F. Phenols
- G. Formaldehyde
- H. Other \_\_\_\_\_

14.5. What type of flooring is in the barn?

- A. Concrete
- B. Soil
- C. Wood
- D. Other \_\_\_\_\_

---

14.6. Do you wash/disinfect walls and equipment?

- A. Yes
- B. No

14.7. Do you wash/disinfect after every flock?

- A. Yes
- B. No

#### **HEATING, VENTILATION AND LIGHTING SYSTEMS**

15.1. Do you have heating and ventilation and lighting systems?

- A. Yes
- B. No

15.2. What type of lighting system is in the barn?

- A. Incandescent
- B. Fluorescent
- C. Other (specify) \_\_\_\_\_

15.3. Are the birds under continual light?

- C. Yes
- D. No

15.4. Is a lighting program used?

- A. Yes
- B. No

15.4.1. If yes: \_\_\_\_\_ hours of light/day

15.5. What type of ventilation is used in the barn?

- A. Timer
- B. Temperature sensor
- C. Manual
- D. Other \_\_\_\_\_

15.6. Is the ventilation system working?

- A. Yes
- B. No

15.7. Is the ventilation system operated continuously?

- A. Yes
- B. No

15.8. How often is the ventilation system serviced?

- A. 1 month
- B. 3 months
- C. 6 months
- D. 12 months
- E. Other \_\_\_\_\_

15.9. Are the records of service available?

- A. Yes
- B. No

15.10. Number of fans in the barn: \_\_\_\_\_

15.11. Size of the fan(s): \_\_\_\_\_

15.12. Number of fans used: \_\_\_\_\_

15.13. Type of heating system used in the barn?

<input type="radio"/> Gas box heater/hot water	<input type="radio"/> Forced hot air/catalytic heater
<input type="radio"/> Non-vented self-contained natural gas	<input type="radio"/> Forced hot air/electric brooding
<input type="radio"/> Non-vented chimneyless	<input type="radio"/> Infrared/hot water
<input type="radio"/> Radiant	<input type="radio"/> Radiant and brooder
<input type="radio"/> Infrared Heaters	<input type="radio"/> Heat exchange/electric
<input type="radio"/> Hot water/ forced air/gas brooding	<input type="radio"/> Propane
<input type="radio"/> Forced hot air/gas brooding	<input type="radio"/> Hot water and propane
<input type="radio"/> Heat exchange/gas brooding	<input type="radio"/> Hot water, electric
<input type="radio"/> Hot water/radiant	<input type="radio"/> Other _____

15.14. How would you rate the air circulation in the barn?

- A. Very poor
- B. Poor
- C. Good
- D. Very good
- E. Excellent

15.15. What is the type of inlet control in the barn?

- A. Automatic
- B. Manual
- C. None
- D. Other \_\_\_\_\_

15.16. Do you control dust in the barn?

- A. Yes
- B. No

C. NA

15.17. Do you have a dust control system (*tick the correct one*)?

<input type="radio"/> Foggers	<input type="radio"/> Sprinklers
<input type="radio"/> Oil in the feed	<input type="radio"/> Misters
<input type="radio"/> Wet the litter	<input type="radio"/> Canola oil
<input type="radio"/> Dampen air/furnace	<input type="radio"/> Other <i>Specify</i> _____

**FEEDING**

16.1. What type of feeding mechanism do you use?

- A. Automatic chain
- ~~B. Automatic pans~~
- C. Hand
- D. Feed cart
- E. Trolley
- F. Other

16.2. Number of feeders: \_\_\_\_\_

16.3. Feeder diameter: \_\_\_\_\_

16.4. Feed form:

- A. Pellets/crumbs
- B. Ground meal/mash
- C. Other \_\_\_\_\_

16.5. What is the main type of grain in the feed?

- A. Corn
- B. Wheat
- C. Other \_\_\_\_\_

16.6. What is the main type of protein supplement in the feed?

- A. Soybean meal
- B. Other \_\_\_\_\_

16.7. Do you use antibiotics in:

- A. Feed
- B. Water
- C. Feed/water
- D. Neither

16.8. Feed ingredients (check package): \_\_\_\_\_

**DRINKING SYSTEM**

17.1. What type of drinker is used?

- A. Nipple
- B. Cup
- C. Bell
- D. Other \_\_\_\_\_

17.2. Number of drinkers: \_\_\_\_\_

17.3. Water flow rate: \_\_\_\_\_

17.4. How often is the water system cleaned? \_\_\_\_\_ times/week

**OBSERVATIONS**

**APPENDIX D: RESPIRATORY QUESTIONNAIRE**

<b>NIOH OCCUPATIONAL ALLERGY AND ASTHMA STUDY AMONG POULTRY WORKERS OF SOUTH AFRICA - 2010</b>
<b>Appendix C</b>
<b>ENGLISH QUESTIONNAIRE</b>

Survey Number

**A. IDENTIFICATION DATA**

1. Surname	_____
2. First name/s	_____
3. Address	_____ _____ _____ _____ _____
4. Work number	_____
5. Date of birth:	Day____Month____Year____
6. Gender:	Male (1) Female (2)
7. Home Language:	English (1) Afrikaans (2) Setswana (3) Other (4)

8. Interviewer's initials \_\_\_\_\_

9. Date of interview:

Day\_\_\_\_Month\_\_\_\_\_Year\_\_\_\_\_

10. Broiler farm: \_\_\_\_\_

11. Are you a casual or permanent worker?

Casual (1)

Permanent (2)

12.1 Date of last work shift?

Day\_\_\_\_Month\_\_\_\_\_Year\_\_\_\_\_

12.2 Which shift did you work today?

07:00 - 15:00 (1)

15:00 - 23:00 (2)

23:00 - 07:00 (3)

Other: \_\_\_\_\_

## **B.HEALTH PROBLEMS**

### **Wheeze and tightness in the chest**

1. Have you ever had wheezing or whistling in your chest in the past?

Yes (1)

No (2)

If YES, go on to Question

1.1

If NO, skip to Question 2

1.1 If yes, when was the first time you had these symptoms.

Month \_\_\_\_\_ Year

Date: \_\_\_\_\_

2. Have you had wheezing or whistling in your chest at any time in the **last 12 months?**

Yes (1)  
No (2)

If YES, go on to Question  
2.1  
If NO, skip to Question 3

2.1 Have you been short of breath when the wheezing noise was present?

Yes (1)  
No (2)

2.2 Have you had this wheezing or whistling when you did not have a cold or flu?

Yes (1)  
No (2)

2.3 Have you been woken up with a feeling of tightness in your chest at any time in the **last 12 months?**

Yes (1)  
No (2)

### **Shortness of breath**

3. Have you had an attack of shortness of breath that came on during the daytime when you were at rest at any time in the **last 12 months?**

Yes (1)  
No (2)

4. Have you had an attack of shortness of breath that came on following running or exercise at any time in the **last 12 months?**

Yes (1)  
No (2)

5. Have you been woken by an attack of shortness of breath at any time in the **last 12 months?**

Yes (1)

No (2)

**Cough and phlegm from the chest**

6. Have you been woken by an attack of coughing at any time in the **last 12 months?**

Yes (1)

No (2)

7. Do you usually cough first thing in the morning?

Yes (1)

No (2)

8. Do you usually cough during the rest of the day, or at night?

Yes (1)

No (2)

If YES, go on to Question

8.1

If NO, skip to Question 9

8.1 Do you cough like this on most days/nights for as much as three or more months in each of the last two years?

Yes (1)

No (2)

9. Do you usually bring up any phlegm from your chest first thing in the morning?

Yes (1)

No (2)

10. Do you usually bring up any phlegm from your chest during the day, or at night?

Yes (1)

No (2)

If YES, go on to Question  
10.1  
If NO, skip to Question 11

10.1 Do you bring up phlegm like this on most days/  
nights for as much as three or more months in each of  
the last two years?

Yes (1)

No (2)

### **Breathing**

11. Do you ever have trouble with your breathing?

Yes (1)

No (2)

If YES, go on to Question  
11.1  
If NO, skip to Question 12

11.1 Do you have this trouble:

Give all options at once  
Insert a cross (X) next to one answer  
only

- a) continuously so that your breathing is never  
quite right? \_\_\_\_\_
- b) repeatedly, but it goes away completely  
between the times when it troubles you? \_\_\_\_\_
- c) only rarely? \_\_\_\_\_

12. Are you disabled from walking by a condition other  
than heart or lung disease?

Yes (1)

No (2)

If YES, state the condition \_\_\_\_\_

and go on to Question 13  
If NO, go to Question 12.1

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

Yes (1)  
No (2)

If YES, go on to Question 12.1.1  
If NO, skip to Question 13

12.1.1 Do you get short of breath walking with other people of your own age on level ground?

Yes (1)  
No (2)

12.1.2 Do you have to stop for breath when walking at your own pace on level ground?

Yes (1)  
No (2)

### **Asthma**

13. Have you ever had asthma?

Yes (1)  
No (2)

If YES, go on to Question  
13.1  
If NO, skip to Question  
13.8

13.1 If yes, was this confirmed by a doctor?

Yes (1)  
No (2)

13.2 How old were you when you were told you have asthma?

Give all options at once

Insert a cross (X) next to one answer only

- a) Only before you were 17 years old \_\_\_\_\_
- b) Only at the age of 17 years or older \_\_\_\_\_
- c) Both \_\_\_\_\_

The following references to "attack" of asthma refers to episodes of wheezing, shortness of breath, chest tightness or cough attributed to asthma

13.3.1 How old were you when you had your first attack of asthma?

\_\_\_\_\_ years old

13.3.2 How old were you when you had your most recent attack of asthma?

\_\_\_\_\_ years old

13.4.1-6 Which months of the year do you usually have attacks of asthma?

13.4.1 January/February

Yes (1)

No (2)

13.4.2 March/April

Yes (1)

No (2)

13.4.3 May/June

Yes (1)

No (2)

13.4.4 July/August

Yes (1)

No (2)

13.4.5 September/October

Yes (1)  
No (2)

13.4.6 November/December

Yes (1)  
No (2)

13.5 Have you had an attack of asthma in the last **12 months?**

Yes (1)  
No (2)

If YES, go on to Question 13.5.1  
If NO, skip to Question  
13.6

13.5.1 How often have you had an attack of asthma in the **last 12 months?**

Give all options at once  
Insert a cross (X) next to one answer  
only

- a) Every day \_\_\_\_\_
- b) More than 2 times a week \_\_\_\_\_
- c) More than 1 time per month \_\_\_\_\_
- d) 3 to 12 times in the whole year \_\_\_\_\_
- e) 1 to 2 times in the whole year \_\_\_\_\_

13.6 Are your chest symptoms caused by, or made worse by any of the following:

Answer all questions

13.6.1 Contact with animals/pets

Yes (1)  
No (2)

13.6.2 Grass or flowers

Yes (1)  
No (2)

13.6.3 Heavy exercise

Yes (1)  
No (2)

13.6.4 Breathing cold air

Yes (1)  
No (2)

13.6.5 Dusts or sprays at work

Yes (1)  
No (2)

13.6.6 Tobacco smoke

Yes (1)  
No (2)

13.6.7 Change in the weather

Yes (1)  
No (2)

13.7 Does your chest symptoms seem better or worse when you are away from work (for example, on weekends, off-shift and vacations)?

Give all options at once  
Insert a cross (X) next to one answer only

- a) Stay the same \_\_\_\_\_
- b) Get better \_\_\_\_\_
- c) Get worse \_\_\_\_\_

13.8 Does being at work ever make your chest tight or wheezy?

Yes (1)  
No (2)



Yes (1)  
No (2)

If YES, go on to Question 13.9.2.1  
If NO, skip to Question  
13.10

13.9.2.1 What area/section did you move to?  
\_\_\_\_\_

13.9.2.2 What job did you do there?  
\_\_\_\_\_

13.9.2.3 Did your symptoms improve when you changed jobs?

Yes (1)  
No (2)

13.10 Have you ever worked in a job or jobs that exposed you to vapours, gas, dust (e.g. feed, litter, bedding) or fumes?

Yes (1)  
No (2)

If YES, go on to Question 13.10.1.  
List the jobs beginning with the most recent  
If NO, skip to Question  
13.11

13.10.1 What was or is this job? \_\_\_\_\_  
(if current job write 'current job' and specify)

13.10.2 Before that? \_\_\_\_\_

13.10.3 Before that? \_\_\_\_\_

13.11 Has there ever been an instance when you inhaled a large amount of vapour, gas, dust or fumes in any of these jobs that resulted in you developing a tight chest, wheeze or cough?

Yes (1)  
No (2)

If YES, go on to Question 13.11.1.  
If NO, skip to Question  
13.12

13.11.1 What was or is this job? \_\_\_\_\_  
(if current job write 'current job' and specify)

13.12 Are you using any medicines, including inhalers/  
pumps, nebulizers, syrups or tablets, for asthma or  
breathing problems?

Yes (1)  
No (2)

If YES, go on to Question 13.12.1, showing examples of  
each  
If NO, skip to question  
13.13

13.12.1 Which medicines?

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

13.12.2 Do you take these medicines every day even  
when you do not have any trouble breathing?

Yes (1)  
No (2)

13.13 Have you ever been treated for any of the  
following:

Answer all questions

13.13.1 Repeated chest infections as a child

Yes (1)  
No (2)  
UNK (3)

13.13.2 Tuberculosis (TB)

Yes	(1)
No	(2)
UNK	(3)

13.13.3 Chronic bronchitis

Yes	(1)
No	(2)
UNK	(3)

**Nose and eye symptoms**

14. Have you ever had any nose or eye problems or allergies such as hay fever?

Yes	(1)
No	(2)

14.1 How old were you when you first noticed these symptoms?

\_\_\_\_\_ years old

If YES, go on to Question 14.2 Answer all questions

If NO, skip to Question

14.4

14.2 During the past 12 months have you had two or more episodes of:

14.2.1 sneezy, itchy or runny nose when you did not have a cold or flu?

Yes	(1)
No	(2)

14.2.2 red, itchy or watery eyes

Yes	(1)
No	(2)

14.2.3 Do you usually have the nose or eye symptoms at any particular time of the year?

Yes	(1)
-----	-----

No (2)

14.2.3.1 If YES, which is the worst season?

Give all options at once  
Insert a cross (X) next to one answer  
only

- a) Winter \_\_\_\_\_
- b) Spring \_\_\_\_\_
- c) Summer \_\_\_\_\_
- d) Autumn \_\_\_\_\_

If YES to any of the above in question 14.2, go on to Question 14.3  
If NO, skip to Question 14.4

14.3 Do your nose or eye symptoms seem better or worse when you are away from work (for example, on weekends, off-shift and vacations)?

Give all options at once  
Insert a cross (X) next to one answer  
only

- a) Stay the same \_\_\_\_\_
- b) Get better \_\_\_\_\_
- c) Get worse \_\_\_\_\_

14.4 Does being at work ever cause you to have sneezy/itchy/runny nose or red/itchy/watery eyes?

- Yes (1)
- No (2)

If YES to any one of the above, go on to Question 14.4.1  
If NO, skip to Question 14.6

14.4.1 Since when have you been having these symptoms at work?

Date: Month \_\_\_\_ Year \_\_\_\_

14.4.2 Is there anything that you work with that causes you to have these symptoms?

Yes (1)

No (2)

If YES, go on to Question 14.4.3 (**specify feed, bedding, litter, manure &/or premix**) or any other substance

If NO, skip to Question 14.5

14.4.3 What do you think is causing these symptoms?

---

14.5 Are you using any medicines, including nose sprays, drops, tablets or injections, for your nose or eye symptoms at present?

Yes (1)

No (2)

If YES, go on to Question 14.5.1

If NO, go on to Question 14.6

Present a chart with different samples of allergy medicines (N.B. a worker might show you his/her medicines).

14.5.1 Which medicines?

---

---

14.6 Did you have hay fever (itchy or watery eyes/nose) as a child?

Yes (1)

No (2)

### **Skin symptoms**

15. Have you ever had any kind of skin problem either at home or at work?

Yes (1)

No (2)

If YES, go on to Question  
15.1  
If NO, skip to Question  
15.4.4

15.1 How old were you when you **first** noticed this skin problem?

\_\_\_\_\_ years old

15.2 During the past **12 months** have you had any skin problems that occurred **2 or more times**?

Yes (1)

No (2)

If Yes, which of the following problems did you have?

Go through each option in the table below and circle the appropriate response.

	<b>Forearms</b>	<b>Whole</b>
	<b>Hands</b>	<b>Body</b>
15.2.1		
itchy or scratchy skin	Yes/No	Yes/No
15.2.2		
hives ("bommels")	Yes/No	Yes/No
	<b>Forearms</b>	<b>Whole</b>
	<b>Hands</b>	<b>Body</b>
15.2.3		
dry, scaly skin	Yes/No	Yes/No
15.2.4		
redness of the skin	Yes/No	Yes/No
15.2.5		
blisters or weeping skin	Yes/No	Yes/No

15.2.6		
burning skin	Yes/No	Yes/No
15.2.7		
started within		
an hour of	Yes/No	Yes/No
contact with		
a substance (feed, litter, bedding etc)		
or food item (e.g. chicken)		
15.2.8		
Other?	Yes/No	Yes/No
Specify:		

If YES, to any of the above go on to Question 15.3  
 If NO, skip to Question  
 15.4

15.3 Do your skin problems seem better or worse when you are away from work (for example, on weekends, off-shift and vacations)?

Give all options at once  
 Insert a cross (X) next to one answer  
 only

- a) Stay the same \_\_\_\_\_
- b) Get better \_\_\_\_\_
- c) Get worse \_\_\_\_\_

15.4 Does being at work ever cause you to have skin problems?

- Yes (1)
- No (2)

If YES, go on to Question 15.4.1  
 If NO, skip to Question  
 15.4.4

15.4.1 Since when have you been having these skin problems at work?

Date:                      Month \_\_\_\_ Year \_\_\_\_

15.4.2 Is there anything that you work with that makes these skin problems worse?

Yes                      (1)  
No                        (2)

If YES, go on to Question 15.4.3 (**specify feed, bedding, litter, manure &/or premix**) or any other substance

If NO, skip to Question  
15.4.4

15.4.3 What do you think is causing these skin problems?

---

15.4.4 Have you ever bruised or injured your fingers or hands while working in the broiler farm?

Yes                      (1)  
No                        (2)

15.5 How many times do you wash your hands in the course of a day?

Give all options at once  
Insert a cross (X) next to one answer  
only

0                              \_\_\_\_\_  
1 time                      \_\_\_\_\_  
2-3 times                    \_\_\_\_\_  
4-5 times                    \_\_\_\_\_  
6 or more                    \_\_\_\_\_

15.6 Are you using any medicines, including any creams or ointments, for your skin problems at present?

Yes                      (1)  
No                        (2)

If YES, go on to Question 15.6.1

If NO, skip to next question 15.7

15.6.1 Which medicines?

---

---

15.7 Did you have eczema as a child?

Yes (1)  
No (2)

**Other allergic conditions**

16. Are you allergic to insect stings or bites?

Yes (1)  
No (2)

If YES, go on to Question

16.1

If NO, skip to Question 17

16.1.1-3 What kind of reactions do you have?

16.1.1 Breathing difficulty, feeling faint, fever?

Yes (1)  
No (2)

16.1.2 Redness, itching or swelling at the sting site

Yes (1)  
No (2)

16.1.3 Other: \_\_\_\_\_

17. Have you ever had any difficulty with your breathing after taking medications or injections that you did not have before?

Yes (1)  
No (2)

If YES, go on to Question

17.1

If NO, skip to 18.1

17.1 Which medicines?

---

18.1-6 When you are near animals (such as cats, dogs or horses), near feathers (including pillows, quilts or duvets), near grass and flowers, or in a dusty part of the house, do you **ever**

18.1 Start to cough?

Yes (1)  
No (2)

18.2 Start to wheeze?

Yes (1)  
No (2)

18.3 Get a tight chest?

Yes (1)  
No (2)

18.4 Start to feel short of breath?

Yes (1)  
No (2)

18.5 Get a runny/stuffy nose or sneeze?

Yes (1)  
No (2)

18.6 Get itchy or watery eyes?

Yes (1)  
No (2)

18.7 Get itchy skin/rash?

Yes (1)  
No (2)

19. Have you ever had an illness or trouble caused by eating a particular type of food/fruit?

Yes (1)  
No (2)

If YES, go on to Question  
19.1  
If NO, skip to 20

19.1 What type of food/fruit was this?  
\_\_\_\_\_

19.1.1-6 Did this illness or trouble include:

19.1.1 Itchy skin or rash

Yes (1)  
No (2)

19.1.2 Diarrhoea or vomiting

Yes (1)  
No (2)

19.1.3 Runny or stuffy nose

Yes (1)  
No (2)

19.1.4 Severe headaches

Yes (1)  
No (2)

19.1.5 Breathlessness/tight chest/wheeze

Yes (1)  
No (2)

19.1.6 Other:\_\_\_\_\_

19.2 Was the food canned or preserved?

Yes (1)  
No (2)

19.3 Do you experience these problems when you drink  
fizzy drinks also?

Yes (1)  
No (2)

**C. FAMILY HISTORY**

1. Do/did any members of your family (blood relatives) ever have any kind of allergies?

Do not include relatives by marriage  
 If family history is completely unknown (subject is adopted, etc.), mark UNK and do not complete table. Move to next section

Yes (1)  
 No (2)  
 UNK (3)

If YES, complete table below. Insert a cross (X) in the appropriate block for each option

Type of Allergy	NO ONE in family	YES, present in the family			Do Not Know
		Parent	Brother/ Sister	Child	
1.1 Hay fever	1	2	3	4	5
1.2 Eczema	1	2	3	4	5
1.3 Asthma	1	2	3	4	5
1.4 Chicken related Allergy	1	2	3	4	5
1.5 Other allergy	1	2	3	4	5
Specify:					
_____					
_____					
_____					

## D. SMOKING HISTORY

1. Have you ever smoked tobacco (cigarettes or pipe) for as long as a year?

'YES' means at least 20 packs of cigarettes or 360 grams of tobacco in a lifetime or at least one cigarette per day for one year

Yes (1)  
No (2)

If YES, go on to Question  
1.1  
If NO, skip to Question 2

1.1 How old were you when you started smoking?

\_\_\_\_\_ years old

1.2 Do you now smoke?

'YES' means smoking tobacco in the last month or more

Yes (1)  
No (2)

If YES, go on to Question  
1.2.1  
If NO, skip to Question 1.3

1.2.1-2. How much do you now smoke on average?

1.2.1 Number of cigarettes per day \_\_\_\_\_

1.2.2 Pipe tobacco in grams/week \_\_\_\_\_

1.3. Have you stopped smoking completely?

Yes (1)  
No (2)

If YES, go on to Question  
1.3.1  
If NO, skip to Question 1.4

1.3.1. How old were you when you stopped smoking completely?

\_\_\_\_\_ years old

1.3.1.1 How many years in total did you smoke cigarettes? (Do not include the years you stopped before you started again)

\_\_\_\_\_ years

1.3.2.1-2 On average of the entire time you smoked, how much did you smoke?

1.3.2.1 Number of cigarettes per day \_\_\_\_\_

1.3.2.2 Pipe tobacco in grams/week \_\_\_\_\_

1.4 Do you or did you inhale the smoke?

Yes (1)

No (2)

2. Have you been regularly exposed to tobacco smoke from other people smoking cigarettes or pipe in the last 12 months?

'Regularly' means on most days or nights

Yes (1)

No (2)

**E. DIETARY HISTORY/DOMESTIC ACTIVITIES**

1. How often have you eaten the following grain products in the last 12 months?

Go through each chicken product option and insert a cross (X) in the block for each option

Type of chicken product	Daily	1 to 3 times a week	1 to 3 times per month	Never
1. chicken polony	1	2	3	4

2. chicken	1	2	3	4
viennas				
3. Chicken				
stakelets	1	2	3	4
4. Chicken	1	2	3	4
burger				
5. Chicken	1	2	3	4
nuggets				
6. Chicken	1	2	3	4
bites				
7. Chicken	1	2	3	4
canned food				
8. chicken liver				
9.chicken soup	1	2	3	4
10. chicken				
stock tubes				
11. Eggs				
12. Other	1	2	3	4
Specify:				
_____				
_____				

2. Have you changed your diet or avoided certain chicken (eg. chicken/eggs/???) products because they do not agree with you when you eat them?

Yes (1)

No (2)

If YES, go on to Question

2.1

If NO, skip to next Section F on WORK HISTORY

2.1 What chicken products have you avoided?

\_\_\_\_\_

\_\_\_\_\_

3. Do you chicken at home?

Yes (1)

No (2)

If YES, go on to Question  
3.2  
If NO, go to Question 3.1

3.1 If no, does any one else prepare chicken food at home?

- Yes (1)
- No (2)

3.2 How often do you do prepare chicken food at home?

- a) once a month \_\_\_\_\_
- b) 2-3 times a month \_\_\_\_\_
- c) 2-3 times per week \_\_\_\_\_
- d) once a week \_\_\_\_\_
- e) everyday \_\_\_\_\_

3.3 What do you prepare?

- a)meat \_\_\_\_\_
- b)chicken soup \_\_\_\_\_
- c)chicken lasagne \_\_\_\_\_
- d) Other: \_\_\_\_\_

Specify: \_\_\_\_\_

3.4 How often do you use the chicken products?

- a) once a month
- b) 2-3 times a month
- c) 2-3 times per week
- d) once a week
- e) everyday

**F. HEALTH AND SAFETY EDUCATION AND TRAINING**

1. What are the hazards associated with poultry dust?

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

2. Have you had any health and safety training on how to protect yourself when working with poultry dust?

- Yes (1)
- No (2)

## G. WORK HISTORY IN BROILER INDUSTRY

1. How long have you been working at this broiler farm?

\_\_\_\_\_ years  
\_\_\_\_\_ months

### **Present job**

2. How long have you been working in your current job?

\_\_\_\_\_ years  
\_\_\_\_\_ months

3. In which area/section are you currently working?

\_\_\_\_\_

3.1 What is your job in this area/section?

Job Title \_\_\_\_\_

get a short description of the job

\_\_\_\_\_  
\_\_\_\_\_

3.2 What does your task involve:

a)Catching&Loading	Yes	(1)
	No	(2)
b) Laying litter	Yes	(1)
	No	(2)
c)Flock management	Yes	(1)
	No	(2)
d)House cleaning	Yes	(1)
	No	(2)
e)Catching/depopulatin g	Yes	(1)
	No	(2)

f) Litter removal	Yes	(1)
	No	(2)
G)Disinfection	Yes	(1)
	No	(2)
h) other	Yes	(1)
	No	(2)

Specify:\_\_\_\_\_

3.3 What do you work with?

a)chickens	Yes	(1)
	No	(2)
b) Feed	Yes	(1)
	No	(2)
c)Whole straw bed	Yes	(1)
	No	(2)
d) Chopped straw bed	Yes	(1)
	No	(2)
e)wood shreds/shavings	Yes	(1)
	No	(2)
f) Other	Yes	(1)
	No	(2)

Specify:\_\_\_\_\_

3.4 Do you ever do other jobs during your shift on a regular basis (almost every day)?

Yes	(1)
No	(2)

If **Yes**, which jobs? \_\_\_\_\_  
\_\_\_\_\_

3.5 How much dust would you say your current job produces:

Give all options at once  
Insert a cross (X) next to one answer only

- a) None \_\_\_\_\_
- b) A little \_\_\_\_\_
- c) An average amount \_\_\_\_\_
- d) A lot \_\_\_\_\_

3.5.1. What type of cleaning activities in your daily work are very dusty.

3.5.1.1. clearing dust surfaces

- Yes (1)
- No (2)

3.5.1.2 Sweeping and mopping floors

- Yes (1)
- No (2)

3.5.1.3 Blowing cages

- Yes (1)
- No (2)

3.5.2 How far do you work from the source of the dust?

Give all options at once  
Insert a cross (X) next to one answer only

- a) Right next to the source \_\_\_\_\_
- b) About 1-2 metres away \_\_\_\_\_
- c) More than 3 metres away \_\_\_\_\_
- d) Does not apply \_\_\_\_\_

3.6 Do you use any personal protective equipment on a

regular basis (almost every day) while doing your job?

Yes (1)  
No (2)

If NO, skip to Question 4  
If YES, continue with Question 3.6.1

3.6.1 Which of the following personal protective equipment do you use on a regular basis (almost every day)?

3.6.1.1 Goggles: Yes (1)  
No (2)

3.6.1.2 Gloves: Yes (1)  
No (2)

3.6.1.3 Mask: Yes (1)  
No (2)

3.6.1.4 Aprons: Yes (1)  
No (2)

3.6.1.5 Other: \_\_\_\_\_

If NO to all of the previous questions, skip to Question 4  
If YES to any one of the above questions, continue with Question 3.6.2.1

3.6.2.1 Goggles \_\_\_\_\_ years

3.6.2.2 Gloves: \_\_\_\_\_ years

3.6.2.3 Mask: \_\_\_\_\_ years

3.6.2.4 Aprons: \_\_\_\_\_ years

3.6.2.5 Other: \_\_\_\_\_ years

**Previous jobs in present broiler farm**

4. Before doing this job at this Broiler farm, did you do a different job here?

Yes (1)  
No (2)

If NO, skip to question 5  
If YES, continue with question 4.1

4.1 What other jobs did you do here?

Start with the first job and work forward, getting a one-line description of each job. If casual worker, denote each period of employment as a separate job. For continuous years of seasonal work consider as one job (provided no broken years service)

**Job 1**

4.1.1 Area/section \_\_\_\_\_

4.1.2 Job Title \_\_\_\_\_

get a short description of the job

\_\_\_\_\_

4.1.3 Permanent/casual: \_\_\_\_\_

4.1.4. How long did you work in this job?

\_\_\_\_\_ years  
\_\_\_\_\_ months

4.1.5 Which task did you perform?

a)Catching&Loading	Yes	(1)
	No	(2)
b) Laying litter	Yes	(1)
	No	(2)
c)Flock management	Yes	(1)
	No	(2)
d)House cleaning	Yes	(1)
	No	(2)

e)Catching/depopulating	Yes	(1)
	No	(2)
f) Litter removal	Yes	(1)
	No	(2)
G)Disinfection	Yes	(1)
	No	(2)
h) other	Yes	(1)
	No	(2)

Specify: \_\_\_\_\_

#### 4.1.6 What material did you work with?

a)chickens	Yes	(1)
	No	(2)
b) Feed	Yes	(1)
	No	(2)
c)Whole straw bed	Yes	(1)
	No	(2)
d) Chopped straw bed	Yes	(1)
	No	(2)
e)wood shreds/shavings	Yes	(1)
	No	(2)
f) Other	Yes	(1)
	No	(2)

Specify: \_\_\_\_\_

4.1. 7 How much dust would you say that this job produced:

Give all options at once  
Insert a cross (X) next to one answer only

- a) None \_\_\_\_\_
- b) A little \_\_\_\_\_
- c) An average amount \_\_\_\_\_
- d) A lot \_\_\_\_\_

4.1.8.. What type of cleaning activities in your daily work were very dusty.

4.1.8.1 clearing dust surfaces

- Yes (1)
- No (2)

4.1.8.2 Sweeping and mopping floors

- Yes (1)
- No (2)

4.1.8.3 Blowing cages

- Yes (1)
- No (2)

4.1.9 How far did you work from the source of the dust?

Give all options at once  
Insert a cross (X) next to one answer only

- a) Right next to the source \_\_\_\_\_
- b) About 1-2 metres away \_\_\_\_\_
- c) More than 3 metres away \_\_\_\_\_
- d) Does not apply \_\_\_\_\_

4.1.10 Did you use any personal protective equipment on a regular basis (almost every day) while doing your

job?

Yes (1)  
No (2)

If NO, skip to Question  
4.2.1  
If YES, continue with Question 4.1.10.1

4.1.10.1 Which of the following personal protective equipment did you use on a regular basis (almost every day)?

4.1.10.1.1 Goggles: Yes (1)  
No (2)

4.1.10.2 Gloves: Yes (1)  
No (2)

4.1.10.3 Mask: Yes (1)  
No (2)

4.1.10.4 Aprons: Yes (1)  
No (2)

4.1.10.5 Other: \_\_\_\_\_

If NO to all of the previous questions, skip to Question  
4.2.1  
If YES to any one of the above questions, continue with  
Question 4.1.11.1

4.1.11.1 Goggles \_\_\_\_\_ years

4.1.11.2 Gloves: \_\_\_\_\_ years

4.1.11.3 Mask: \_\_\_\_\_ years

4.1.11.4 Aprons: \_\_\_\_\_ years

4.1.11.5 Other: \_\_\_\_\_ years

**Job 2**

4.2.1 Area/section \_\_\_\_\_

4.2.2 Job Title \_\_\_\_\_

get a short description of the job

4.2.3 Permanent/casual: \_\_\_\_\_

4.2.4. How long did you work in this job?

\_\_\_\_\_ years

\_\_\_\_\_ months

4.2.5 Which task did you perform?

a)Catching&Loading	Yes	(1)
	No	(2)

b) Laying litter	Yes	(1)
	No	(2)

c)Flock management	Yes	(1)
	No	(2)

d)House cleaning	Yes	(1)
	No	(2)

e)Catching/depopulatin g	Yes	(1)
	No	(2)

f) Litter removal	Yes	(1)
	No	(2)

G)Disinfection	Yes	(1)
	No	(2)

h) other	Yes	(1)
	No	(2)

Specify: \_\_\_\_\_

4.2.6 What material did you work with?

a)chickens

Yes (1)  
No (2)

b) Feed

Yes (1)  
No (2)

c)Whole straw bed

Yes (1)  
No (2)

d) Chopped straw bed

Yes (1)  
No (2)

e)wood shreds/shavings

Yes (1)  
No (2)

f) Other

Yes (1)  
No (2)

Specify: \_\_\_\_\_

Specify: \_\_\_\_\_

4.2.7 How much dust would you say that this job produced:

Give all options at once  
Insert a cross (X) next to one answer only

- a) None \_\_\_\_\_
- b) A little \_\_\_\_\_
- c) An average amount \_\_\_\_\_
- d) A lot \_\_\_\_\_

4.2.8 What type of cleaning activities in your daily work were very dusty.

4.2.8.1 clearing dust surfaces

Yes	(1)
No	(2)

4.2.8.2 Sweeping and mopping floors

Yes	(1)
No	(2)

4.1.8.3 Blowing cages

Yes	(1)
No	(2)

4.2.9 How far did you work from the source of the dust?

Give all options at once  
Insert a cross (X) next to one answer only

- a) Right next to the source \_\_\_\_\_
- b) About 1-2 metres away \_\_\_\_\_
- c) More than 3 metres away \_\_\_\_\_
- d) Does not apply \_\_\_\_\_

4.2.10 Did you use any personal protective equipment on a regular basis (almost every day) while doing your job?

Yes	(1)
No	(2)

If NO, skip to Question 4.3.1 or 5 if no other jobs  
If YES, continue with Question 4.2.10.1

4.2.10.1 Which of the following personal protective equipment did you use on a regular basis (almost every day)?

4.2.10.1.1 Goggles:

Yes	(1)
No	(2)



a)Catching&Loading	Yes	(1)
	No	(2)
b) Laying litter	Yes	(1)
	No	(2)
c)Flock management	Yes	(1)
	No	(2)
d)House cleaning	Yes	(1)
	No	(2)
e)Catching/depopulatin g	Yes	(1)
	No	(2)
f) Litter removal	Yes	(1)
	No	(2)
G)Disinfection	Yes	(1)
	No	(2)
h) other	Yes	(1)
	No	(2)

Specify:\_\_\_\_\_

#### 4.3.6 What material did you work with?

a)chickens	Yes	(1)
	No	(2)
b) Feed	Yes	(1)
	No	(2)
c)Whole straw bed	Yes	(1)
	No	(2)
d) Chopped straw bed		

	Yes	(1)
	No	(2)
e) wood shreds/shavings	Yes	(1)
	No	(2)
f) Other	Yes	(1)
	No	(2)

Specify: \_\_\_\_\_

Specify: \_\_\_\_\_

4.3.7 How much dust would you say that this job produced:

Give all options at once  
Insert a cross (X) next to one answer only

- a) None \_\_\_\_\_
- b) A little \_\_\_\_\_
- c) An average amount \_\_\_\_\_
- d) A lot \_\_\_\_\_

4.3.8 What type of cleaning activities in your daily work were very dusty.

4.2.8.1 clearing dust surfaces

- Yes (1)
- No (2)

4.3.8.2 Sweeping and mopping floors

- Yes (1)
- No (2)

4.3.8.3 Blowing cages

- Yes (1)
- No (2)

4.3.9 How far did you work from the source of the dust?

Give all options at once  
Insert a cross (X) next to one answer  
only

- a) Right next to the source \_\_\_\_\_
- b) About 1-2 metres away \_\_\_\_\_
- c) More than 3 metres away \_\_\_\_\_
- d) Does not apply \_\_\_\_\_

4.3.10 Did you use any personal protective equipment on a regular basis (almost every day) while doing your job?

- Yes (1)
- No (2)

If NO, skip to Question 4.4.1 or 5  
If YES, continue with Question 4.3.10.1

4.3.10.1 Which of the following personal protective equipment did you use on a regular basis (almost every day)?

- 4.3.10.1.1 Goggles: Yes (1)  
No (2)

- 4.3.10.2 Gloves: Yes (1)  
No (2)

- 4.3.10.3 Mask: Yes (1)  
No (2)

- 4.3.10.4 Aprons: Yes (1)  
No (2)

4.3.10.5 Other: \_\_\_\_\_

If NO to all of the previous questions, skip to Question 4.4.1 or 5  
If YES to any one of the above questions, continue with  
Question 4.3.11.1



f) Litter removal	Yes	(1)
	No	(2)
G)Disinfection	Yes	(1)
	No	(2)
h) other	Yes	(1)
	No	(2)

Specify:\_\_\_\_\_

4.4.6 What material did you work with?

a)chickens	Yes	(1)
	No	(2)
b) Feed	Yes	(1)
	No	(2)
c)Whole straw bed	Yes	(1)
	No	(2)
d) Chopped straw bed	Yes	(1)
	No	(2)
e)wood shreds/shavings	Yes	(1)
	No	(2)
f) Other	Yes	(1)
	No	(2)

Specify:\_\_\_\_\_

4.4.7 How much dust would you say that this job produced:

Give all options at once  
Insert a cross (X) next to one answer  
only

- a) None \_\_\_\_\_
- b) A little \_\_\_\_\_
- c) An average amount \_\_\_\_\_
- d) A lot \_\_\_\_\_

4.4.8 What type of cleaning activities in your daily work were very dusty.

4.4.8.1 clearing dust surfaces

- Yes (1)
- No (2)

4.4.8.2 Sweeping and mopping floors

- Yes (1)
- No (2)

4.4.8.3 Blowing cages

- Yes (1)
- No (2)

4.4.9 How far did you work from the source of the dust?

Give all options at once  
Insert a cross (X) next to one answer  
only

- a) Right next to the source \_\_\_\_\_
- b) About 1-2 metres away \_\_\_\_\_
- c) More than 3 metres away \_\_\_\_\_
- d) Does not apply \_\_\_\_\_

4.4.10 Did you use any personal protective equipment on a regular basis (almost every day) while doing your job?

- Yes (1)
- No (2)

If NO, skip to Question 5  
If YES, continue with Question 4.4.10.1

4.4.10.1 Which of the following personal protective equipment did you use on a regular basis (almost every day)?

4.4.10.1.1 Goggles:            Yes            (1)  
   No            (2)

4.4.10.2 Gloves:            Yes            (1)  
   No            (2)

4.4.10.3 Mask:            Yes            (1)  
   No            (2)

4.4.10.4 Aprons:            Yes            (1)  
   No            (2)

4.4.10.5 Other:            \_\_\_\_\_

If NO to all of the previous questions, skip to Question 5  
If YES to any one of the above questions, continue with  
Question 4.4.11.1

4.4.11.1 Goggles            \_\_\_\_\_    years

4.4.11.2 Gloves:            \_\_\_\_\_    years

4.4.11.3 Mask:            \_\_\_\_\_    years

4.4.11.4 Apron:            \_\_\_\_\_    years

4.4.11.5 Other:            \_\_\_\_\_    years

**Previous work in other broiler farms**

5. Have you worked in any other broiler farms in the past two years?

Yes            (1)  
No            (2)

If NO, skip to question 6  
 If YES, continue with question 5.1

5.1 Why did you change jobs?

\_\_\_\_\_

\_\_\_\_\_

5.2 What is the total amount of time you have worked in the poultry industry before you started working in this broiler farm?

Years \_\_\_\_\_ Months \_\_\_\_\_

**Previous work experience**

6. Name all the previous workplaces that you have worked in, when not working in this broiler farm or before coming to work in this broiler farm:

Start with the most recent job and work backwards (including all other bakeries and jobs done)

Name of Company	What did you do?	Job Title (what did you do?)	Date start (Year)	Date stop (Year)	Total (yrs)

**THANK YOU FOR ANSWERING THE QUESTIONNAIRE**

## APPENDIX E: LETTER OF CONSENT

Listed in Schedule 2 Part A: National Public Entities – Practice No.5200298



NATIONAL INSTITUTE  
FOR  
OCCUPATIONAL HEALTH  
*A Division of the National Health Laboratory Service*

Immunology & Microbiology Section

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### Information sheet and consent form

Good day,

We, Onnicah Matuka and Tanusha Singh, medical scientists from the National Institute for Occupational Health (NIOH) would like to invite you to consider participating in a research study entitled "Allergic sensitisation and work-related asthma among poultry workers in South Africa". These investigations will allow the NIOH to gain an understanding of non-allergic asthma in poultry workers.

#### Why this study?

Occupational health problems are not uncommon in the workplace. Therefore monitoring or assessing exposure to hazardous biological substances is a fundamental step to improving the quality of life for workers. Previous research studies have shown that poultry farm workers are exposed to a wide range of agents from dust derived from the litter, feathers, feed, excreta and microorganisms which could cause respiratory disease. In South Africa no study has reported associations between exposure to airborne biological agents and respiratory health effects in poultry workers, therefore the (NIOH) would like to investigate these agents. The study will include ~ 346 South African poultry workers (excluding pregnant women) and we anticipate that the collection of data will take 2-3 weeks per farm. The measurements will include personal air sampling, questionnaire, blood tests and lung function tests.

#### What do we expect from the participants in this study?

If you agree to participate in this study you will be asked to complete a questionnaire, which will provide us with important information on respiratory symptoms and other medical conditions as well as employment history. During the course of the study a doctor or nurse will use sterile (free of germs) equipment to take 10ml (~2 teaspoons) of blood to be tested if you have allergy (specific IgE and phadiotops) and inflammation by testing allergic mediators (IL-7, IL-3, IL-4, IL-5, Eotaxin and GM-CSF) and non-allergic mediators (IL-8, IL-15, IL-6, IL-10, TNF- $\alpha$ ) in each blood sample. A registered clinical technologist has the strength of your lungs tested before and after work (1hour) and test for asthma by challenging your lungs with metacholine (1h).



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You will wear a personal sampling pump connected to a filter for a minimum duration of 240 minutes while performing your daily duties so that we can collect the air on your breathing zone to test if you are exposed to hazardous biological agents. The dust collected in the air during your work activity will also be used to determine the effectiveness of the controls in place. Analysis will include total dust, moulds and their by-products (glucan and MVOCs), spores concentration in bulk samples.

**Are there any risks involved for participating in the study?**

Wearing the dust measuring equipment is not a risk to you and it will not interfere with your work. You may feel a little dizzy for a short while and have slight bruising during the blood tests. You may also have mild shortness of breath, cough, chest tightness, wheezing, chest soreness or headache during asthma tests, which last only a few minutes. A doctor or nurse will always be available in case of any emergency.

**The potential benefits for the participants:**

The indirect benefit of the project is creating a safer and healthy working environment for you and your fellow colleagues. All participants will be given a written copy of their laboratory test results and what they mean and at the end of the study (after 4 years) we will present our findings at the farms and answer to all questions. Participants may also benefit from early diagnosis of asthma and treatment thus preventing the increasingly high incidence of work-related asthma. Those with identified work-related asthma problems will also be given the names of specialists for referral. Employed personnel with work-related asthma would be entitled to compensation and would be given assistance in applying for it.

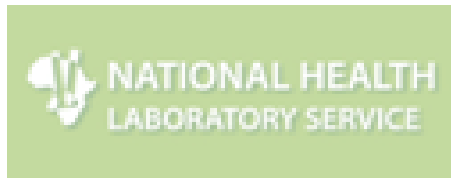
Participants will not be paid for participating in the study.

**May I withdraw from the study?**

Yes, your participation in this study is entirely voluntary; you can decline to participate or withdraw at any time during the course of the study with no penalty.

**Is confidentiality assured?**

Yes, all information and research data obtained during the course of this study will be strictly confidential. Data forms will be coded and will not have your name but a coding system that will be used.



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If you are happy to take part in the study, please read and sign the attached consent form. Address study queries to Onnicah or Tanusha at National Institute for Occupational Health, PO Box 4788, Johannesburg 2000. Email [onnica.matuka@nioh.nhls.ac.za](mailto:onnica.matuka@nioh.nhls.ac.za) or [tanusha.singh@nioh.nhls.ac.za](mailto:tanusha.singh@nioh.nhls.ac.za) , Tel 011 712 6475 or 6487 Fax 011 712 6426

For ethics queries or complaints please contact Anisa Keshav at [keshava@research.wits.ac.za](mailto:keshava@research.wits.ac.za) Tel 011 717 1234 of the Human Research Ethics Committee.

I ..... (Print name) hereby confirm that I have been informed of the nature (including risks and benefits) of the study "Allergic sensitisation and work-related asthma among poultry workers in South Africa" outlined in the information sheet and agree to be part of this study.

PARTICIPANT Survey no.....

.....  
Print Name Signature Date and Time

WITNESS  
.....  
Print Name Signature Date and Time

# APPENDIX F: INFORMATION SHEET AND CONSENT FORM FOR TAKING PHOTOS



NATIONAL INSTITUTE  
for  
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## Appendix A2

### Information sheet and consent form for taking photos

**Title:** Allergic sensitisation and work-related asthma among poultry workers in South Africa.

Hello. We, Onnicah and Tanusha, are from the NIOH of the National Health Laboratory Services in Johannesburg. We are doing a study on exposure to dust found in poultry farms as this has not been investigated in detail in South Africa. We would like to gather information about the work practices and control measures used at your workplace. We invite you to take part in this study. The study will include ~ 346 South African poultry workers (excluding pregnant women) and will last approximately 2-3 weeks per farm.

**What will be done:** In order to better demonstrate normal work practices, we would like to take photos of you when you do certain job tasks at your workstations.

**How will the photos be taken:** The occupational hygienist will be visiting various departments doing different job categories/ tasks at their workstations and making notes of what he observes and taking photos at the same time.

**Where will the photos be kept:** The camera with photos will be kept temporarily with the study team at the sampling site and later be saved on a CD and stored in the NIOH Backup Safe.

**Who will have access to the photos:** Only the study team will have access to these pictures via authorisation of the principal investigator.

**What will happen to the photos at the end of the study:** All information and pictures will be used in future only for illustrating occupational hygiene conditions in your work place (poultry farming). The photographs will be changed in such a way that nobody will be able to recognize you.

#### What do we expect from you as participant?

You will not be directly involved in this process. The process will not disturb you as you will continue with your normal duties.

**Benefits to you:** This study may result in improvements in your work place by reducing dust exposure which will improve your health. This study will contribute to knowledge on best work practices that may help other workers exposed to dust, like you. Participants will not be paid for participating in the study.

**Risks involved:** No risk involved

**Participation is voluntary:** You have a right to refuse to have your photos taken and this will not count against you in any way. You can also change your mind at any time during the study.

**Confidentiality:** Your personal information will be kept confidential and only be accessed by study team. For future training purposes your face will not be shown when demonstrating work practices.

Address study queries to Onnicah or Tanusha at National Institute for Occupational Health, PO Box 4788, Johannesburg 2000. Email [onnica.h.matuka@nioh.nhls.ac.za](mailto:onnica.h.matuka@nioh.nhls.ac.za) or [tanusha.singh@nioh.nhls.ac.za](mailto:tanusha.singh@nioh.nhls.ac.za) , Tel 011 712 6475 or 6487 Fax 011 712 6426


For ethics queries or complaints please contact Prof Cleaton Jones the chairperson of the Human Research Ethics Committee of the University of Witwatersrand.  
Tel 011 717 2301

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X

I Dean Faurie..... (Print name) hereby confirm that I have been informed of the nature of the study and agree to be part of this study.

I do give permission for my photos to be taken while doing my job.

Signature: ..... Survey No..... Date: 28/6/2011

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