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Work-related asthma associated with endotoxin exposure in dental workers in South Africa

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WORK-RELATED ASTHMA ASSOCIATED WITH ENDOTOXIN EXPOSURE IN DENTAL WORKERS IN SOUTH AFRICA

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A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, in fulfillment of the requirements for the degree

of

Doctor of Philosophy

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http://www.general-anaesthesia.com/images.dentistry.html

Dentistry *circa* 1880,

….Dental unit waterlines may be contaminated with high levels of microorganisms and their by-products, particularly endotoxin, a cell wall component of Gram-negative bacteria first discovered by Richard Pfeiffer (1858-1945). A consequence of endotoxin exposure …. is the development or exacerbation of asthma[1]….The lack of definitive evidence may reflect the difficulty of establishing epidemiological links between infections with extended incubation times and antecedent dental procedures and therefore go unrecognised or unreported[2]…

([1]Pankhurst C et al., 1998 & [2]Barbeau, JC et al., 1998)

DECLARATION

I, Tanusha Soogreem Singh, declare that this thesis is my own work. It is being submitted for the degree of Doctor of Philosophy in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

I further declare that the work presented was approved by the Committee for Research on Human Subjects of the University of the Witwatersrand, and granted the clearance number M040403.



_____Signature of candidate

T S SINGH

_____1st _____day of _____March_____(month), 2010

DEDICATION

In my quest for knowledge I dedicate this work to

my parents, who sacrificed their dreams so I could live mine,

my loving husband for his unconditional love

and for being my pillar of strength,

my adorable son, my greatest reward,

and my siblings for their support and encouragement….

PUBLICATIONS

Part of this thesis has been published or has been submitted for publication in national and international journals as listed below:

T S Singh, B Bello, O D Mabe, K Renton, M F Jeebhay. 2009. Workplace determinants of endotoxin exposure in dental health care facilities in South Africa. Annals of Occupational Hygiene, doi:10.1093/annhyg/mep095: 1-10.

T S Singh & O D Mabe. 2009. Occupational exposure to endotoxin from contaminated dental unit waterlines. South African Dental Journal, 64 (1): 8-14.

T S Singh & O D Mabe. 2008. Work-related asthma: current concepts and management approaches to various phenotypes. Occupational Health Southern Africa, 9: 12-19.

PRESENTATIONS

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

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T S Singh. Biomarker profiling used in assessing asthma subtypes. NIOH Research Form, 23 July 2008, NIOH Johannesburg.

T S Singh, O D Mabe, B Bello, M F Jeebhay. Workplace determinants of endotoxin exposure in dental health care settings. XXVII Congress of the European Academy of Allergology and Clinical Immunology (EAACI), Barcelona, Spain. 7-11 June 2008.

T S Singh. Endotoxins in dental schools: the highs and the lows. NIOH Research Forum. 31 October 2007, NIOH, Johannesburg.

T S Singh and O D Mabe. Endotoxins in dental unit waterlines: a snap shot. NIOH Research Day. 23 November 2006, NIOH, Johannesburg. Award winning poster.

T S Singh. Work-related asthma associated with endotoxin exposure in dental workers in South Africa: problems encountered. NIOH Research Forum, 19 October 2005, NIOH, Johannesburg.
T S Singh. Work-related asthma associated with endotoxin exposure in dental workers in South Africa: progress report. Fogarty International Centre: Environmental & Occupational Research in Southern Africa Conference. 10-11 September 2005, Sunnyside Park Hotel, Johannesburg.

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ABSTRACT

Background: Dental procedures associated with dental unit waterlines (DUWLs) in dental health care settings generate aerosolised droplets that have the potential to cause adverse health effects in exposed workers. **Aim:** The aim of this study was to evaluate the risk of work-related asthma associated with endotoxin exposure in dental health care workers. **Materials and methods:** The study population included dental personnel (n = 454) from 5 academic dental institutions in South Africa. Personal air samples (n = 413) in various dental jobs as well as airborne area and water samples from dental handpieces and basin taps were collected. A self-administered modified European Community Respiratory Health Survey questionnaire was used to obtain information on respiratory symptoms and the occupational history. Serum samples were collected to determine atopic status, specific IgE to composite latex (k82) extract and 8 recombinant latex proteins, myeloperoxidase (MPO), eosinophilic cationic protein (ECP), inflammatory cytokines, and endotoxin levels. Spirometry including pre- and post-bronchodilator testing was conducted according to ATS/ERS guidelines. Multivariate linear and logistic regression and factor analysis was used in the data analysis. **Results:** Airborne endotoxin levels were variable across departments with administration having the lowest and laboratories the highest mean exposures (geometric mean: 2.38 versus 5.63 EU/m^3). Job status as a student (compared to staff member) and dental unit characteristics (age, model type, number of units) were important predictors of airborne endotoxin. The most common asthma phenotypes were atopic asthma (6.9%), non-atopic asthma (5.9%) and work-aggravated asthma (4%). Four inflammatory groups related to eosinophilic versus neutrophilic inflammation and chronicity of the response were identified. Acute neutrophilic response was associated with work-related chest symptoms (OR = 4.99, 95% CI: 1.32 - 18.92). Cumulative endotoxin exposures (>51.12 EU/m^3-year) was an important predictor of work-related ocular-nasal symptoms (OR = 3.82, 95% CI: 1.01 – 14.41) in non-atopic workers. Borderline significant associations were also observed between current airborne endotoxin concentrations (>5.83

EU/m^3) and asthma-related symptoms (OR = 2.24, 95% CI: 0.97 – 5.17) as well as suboptimal lung function (FEV_1<80% predicted) (OR = 8.02, 95% CI: 0.94 – 68.35) in non-atopic workers. Dental workers using latex gloves and concurrently exposed to low-grade (> 5.83 EU/m^3) elevated endotoxin levels were at increased risk (OR = 2.59, 95% CI: 1.20 – 5.60) of presenting with latex sensitisation. **Conclusion:** This study demonstrated that endotoxin exposures from DUWLs play an important role in the manifestation of non-atopic asthma through the neutrophilic-response mechanism. Neutrophilic inflammatory cell asthma phenotypes coexist with eosinophilic inflammatory cell asthma phenotypes in this group of workers. Furthermore, low-grade elevated endotoxin levels increase the risk of sensitisation to latex among dental workers using latex gloves. This is the first study to demonstrate airway effects associated with low-grade elevated endotoxin exposures in dental settings.

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NOMENCLATURE

BHR:	Bronchial hyperresponsiveness
CCDs:	Cross-reacting carbohydrate determinants
CFU:	Colony forming unit
CI:	Confidence interval
CO_2:	Carbon dioxide
CV:	Coefficient of variation
DUWLs:	Dental unit waterlines
ECCS:	European Community for Coal and Steel
ECP:	Eosinophilic cationic protein
ECRHS:	European Community Respiratory Health Survey
EDTA:	Ethylene-diamine-tetra-acetic acid
EU:	Endotoxin units
GM-CSF:	Granulocyte macrophage-colony stimulating factor
HBA:	Hazardous biological agent
HBROEL:	Health based recommended occupational exposure limit
HCW:	Health care worker
HEV:	Hevein
HMW:	High molecular weight
HRP:	Horse radish peroxidase
IFN-γ:	Interferon gamma
IL:	Interleukin
LAL:	Limulus amoebocyte lysate
LMW:	Low molecular weight
LN:	Logarithm
LOD:	Limit of detection
LOQ:	Limit of quantification
LPS:	Lipopolysaccharide
LBP:	Lipid binding protein
MBP:	Maltose binding protein
MPO:	Myeloperoxidase
MUXF3:	Bromelain
NRL:	Natural rubber latex
OA:	Occupational asthma
OR:	Odds ratio
pNPP:	ρ-nitrophenyl phosphate
PAMPs:	Pathogen associated molecular patterns
PC:	Polycarbonate
PCA:	Principal component factor analysis
PFA:	Principal factor analysis
PPE:	Personal protective equipment
PRR:	Pathogen recognition receptors
TLR:	Toll-like receptor
TNF-α:	Tumour necrosis factor alpha
TWA:	Time-weighted average
WAA:	Work-aggravated asthma
WRA:	Work-related asthma
%RH:	Percent relative humidity

PREFACE

A considerable number of people work in dental health care facilities where endotoxin exposure might pose a potential occupational health risk due to, among others, the quality of the water used for dental procedures. Therefore it would be very informative to see the results of a study on occupational endotoxin exposure in this field. Occupational exposure particularly to allergens account for 45-50% of the population attributable risk for adult asthma. However these estimates do not account for the risk from irritant exposure (e.g. endotoxin). Endotoxin has been shown to elicit a non-allergic neutrophilic inflammatory response among workers in some occupational settings. However neutrophilic asthma has not been investigated in the dental industry. It is hypothesised that a proportion of asthma is based on a neutrophilic airway inflammation, possibly triggered by exposure to endotoxin among dental workers. Therefore, this study focused on exposure to endotoxin among workers in dental health care facilities in South Africa and the association with work-related asthma. The work described in this thesis was carried out between September 2004 and December 2008 at the National Institute for Occupational Health. Each of the chapters that follow is intended as a "stand-alone" component of this thesis, reporting on specific aims of the current study.

CHAPTER 1

1.0 LITERATURE REVIEW

1.1 OCCUPATIONAL EXPOSURE TO ENDOTOXIN IN DENTAL HEALTH CARE SETTINGS

Occupational exposure to endotoxin has been assessed in many environments and is especially associated with livestock and agricultural farming and processing industries. Endotoxin exposures in these occupational settings are highly variable ranging from low levels (<100 EU/m^3) in the woodmaking industry to high levels (6000 EU/m^3) in agricultural settings. It is only in a few working facilities such as livestock farms that continuous high airborne exposure are measurable (Oppliger et al., 2005; Liebers et al., 2008). Endotoxin exposure assessments in various occupational settings have been extensively investigated (Smid et al., 1992; Post et al., 1998; Thorn et al., 2002; Thorne et al., 2003; Rongo et al., 2004; Portengen et al., 2005; Schulze et al., 2006; Visser et al., 2006; Eduard, 2007; Oldenburg et al., 2007). However little is known about the dental industry (Pankhurst et al., 2005; Huntington et al., 2007).

Bacterial contamination was identified as a problem in the 1960's and has become a growing concern for clinical dentistry in recent times (Miller et al., 1971; Bennett et al., 2000b; Mills, 2000; Lindstrom et al., 2002; Szymanska, 2005b; Pankhurst and Coulter, 2007; Szymanska, 2007). Although infection control has assumed great importance, the waterlines of dental units

remain a potential weakness in the control of infection, as they can easily become contaminated with both patient-derived and municipal-water impurities. Reports of waterline contamination by potential pathogens such as Gram-negative bacteria and their by-products (e.g. endotoxin) have highlighted the seriousness of the contamination (Cobb et al., 2002; Franco et al., 2005). The generation of aerosols from dental unit waterlines (DUWLs) is a potential source of exposure to microorganisms and endotoxin for both the health care provider and the patient. Despite the advances made in modern dentistry, biological hazards still put considerable strain on infection control procedures (Leggat et al., 2007; Szymanska, 2007). Since Gram-negative bacteria predominate in dental environments, investigations into the role of these organisms as an occupational hazard should be prioritised (Milton et al., 1992). The aim of the current review is to provide an exposé of endotoxin exposure emanating primarily from DUWLs and the risk that such exposures pose to dental workers.

1.1.1 SOURCES OF BIOAEROSOLS

1.1.1.1 Dental units

Dental units are the focal point of dental clinics. The units are equipped with a network of thin, plastic tubes known as DUWLs. The tubing can contain approximately 6 metres of narrow bore flexible polyurethane or polyvinyl chloride tubing. Although DUWLs are usually supplied by well maintained municipal water, they become heavily contaminated over time as microorganisms adhere to the luminal surface of the waterlines, coalescing into a film containing multiple microbiological species called a biofilm (Szymanska, 2005d). Previous research has shown extensive growth of biofilm in DUWLs using scanning electron microscopy (Szymanska, 2005a). Although microorganisms consistent with oral flora are recovered from biofilm communities, the majority of microbes are Gram-negative water bacteria or endotoxin-containing

microvesicles, protozoa cysts and mineral deposits. These impurities are usually found in small numbers in municipal water systems and may also enter the dental unit during repair and maintenance (Mills, 2000; Singh and Coogan, 2005).

Previous studies have shown that the water used as a coolant and irrigant during dental procedures can be heavily contaminated with microorganisms, leading to potentially dangerous levels and can reach colony forming units (CFUs) of one million per ml (Cobb et al., 2002). Large multi-chair dental clinics such as those associated with schools of dentistry, military installations and public health clinics are confronted with unique problems with regard to control of contaminated DUWLs. Some dental units may remain unused for frequent quiescent periods, thereby allowing undisturbed biofilm to increase in mass due to the lack of shearing force from laminar water flow (Cobb et al., 2002; Szymanska, 2003a). Unacceptable levels can also result from periods of water stagnation due to the rhythm of work during the day and during the night. Investigations have demonstrated that biofilms can form within 8 hours of connecting a new dental unit to mains water supply developing into a rich climax community of microcolonies embedded in a protective extracellular matrix. With time, individual microorganisms, as well as pieces of the biofilm, can dislodge, contributing to the microbial load in the water as it exits the waterlines (Szymanska, 2003b). Szymanska has reported on a number of studies demonstrating the widespread and high levels of contamination in dental surgeries a short period after new dental units were installed (Szymanska, 2003a).

1.1.1.2 Dental procedures

Many dental procedures produce fine aerosol and splatter composed of various combinations of water, organic particles (tissue and tooth dust) and organic fluids (blood and saliva). These may

be inhaled by the attending dental personnel, as they splash off the surface of the patient's mouth. Aerosol production is an inescapable effect of the rapidly vibrating tips of dental handpieces. Fitted transducer elements create vibrations from 25000 Hertz to 40000 Hertz which are then transmitted as mechanical energy to the tips (Harrel et al., 1998). To prevent heat production from the high energy levels, water is supplied to the handpieces, serving as a coolant as well as to lavage the working area in order to increase the health care provider's vision. The aerosolised water comprises particles of varying size ranging from 0.001 μm to 100 μm including both aerosol and splatter. While most of the water in the spray is removed by high-volume aspiration, particles greater than 100 μm in diameter disperse and deposit quickly on the surface of objects due to gravitational forces. Splatter particles, moving along trajectories, can reach the dental personnel as the range of splatter is from 15 to 120 cm (Szymanska, 2007). Splatter of aerosol onto mucous membranes or intact skin was the second (26.9%) most frequent type of occupational exposure reported in a recent South African study (Nemutandani et al., 2007). Particles less than 50 μm are small enough to stay airborne for an extended period before they settle on environmental surfaces or enter the respiratory tract. The smaller particles of an aerosol (0.5 to 10 μm in diameter) have the potential to penetrate and lodge in the smaller passages of the lungs and are thought to carry the greatest potential for transmitting infections and other health effects on the airways (Harrel and Molinari, 2004).

1.1.1.3 Other sources

The potential routes for the spread of infection in a dental office apart from dental units and procedures are direct contact with body fluids (e.g. saliva and respiratory fluids) of an infected patient or other sources in the dental clinic such as air-conditioning systems (Harrel and Molinari,

2004). Body fluids are unlikely to be contaminated with endotoxin unless mixed with contaminated dental water during procedures. However, poorly maintained ventilation and air-conditioning systems can be a potential source of fungal and other microbial organisms. The air-conditioning system could therefore act as a vehicle for the transmission of such microorganisms in the dental clinic (Leggat and Kedjarune, 2001; Shivakumar et al., 2007).

1.1.2 CONSTITUENTS OF BIOAEROSOLS PRODUCED IN DENTISTRY

Many reports in the literature demonstrate increased numbers of airborne bacteria during various aerosol-producing dental procedures. The airborne bacterial load resulting from high-speed turbines may reach up to 300 cfu/m^3, and a 250% increase over background counts can be reached during a 50 minute dental procedure. Thirty minutes of ultrasonic scaler use can increase background bacterial counts by approximately 5000 cfu/m^3 and has been reported to be the greatest producer of contaminated aerosol and splatter (Barbeau et al., 1998; Harrel et al., 1998; Szymanska, 2005c).

Infectious agents such as viruses (e.g. hepatitis B virus, hepatitis C virus), bacteria and fungi are spread by aerosols from contaminated dental units (Mills, 2000; Leggat et al., 2007). Gram-negative rods producing endotoxin are important risk factors among the potentially toxic microorganisms (Szymanska, 2005a).

1.1.2.1 Physical and biochemical properties of endotoxin

Environmental endotoxin is heterogeneous containing variable amounts of lipopolysaccharide (LPS), phospholipid and membrane protein. LPS are stable water soluble molecules composed of lipid and polysaccharide (**Figure 1.1**). The lipid moiety of LPS is termed 'lipid A' and is

responsible for the toxic properties of LPS (Technical Committee and CEN/TC 137, 2003). In addition the chemical structure of the polysaccharide and, to a small degree, the lipid A portions of LPS varies between serotypes and species of Gram-negative bacteria, and within a single type of bacteria when grown under different conditions (Milton et al., 1992). Endotoxins are easily released in large quantities in the form of discoid particles (microvesicles) 30-50 nm in diameter with a characteristic triple-tracked membrane (Milton et al., 1992). They are components of particulate matter less than 10 µm in aerodynamic diameter fraction (PM_{10}) (Mueller-Anneling et al., 2004; Huntington et al., 2007). Differentiating between particle size is important as not all particles pose a health risk.

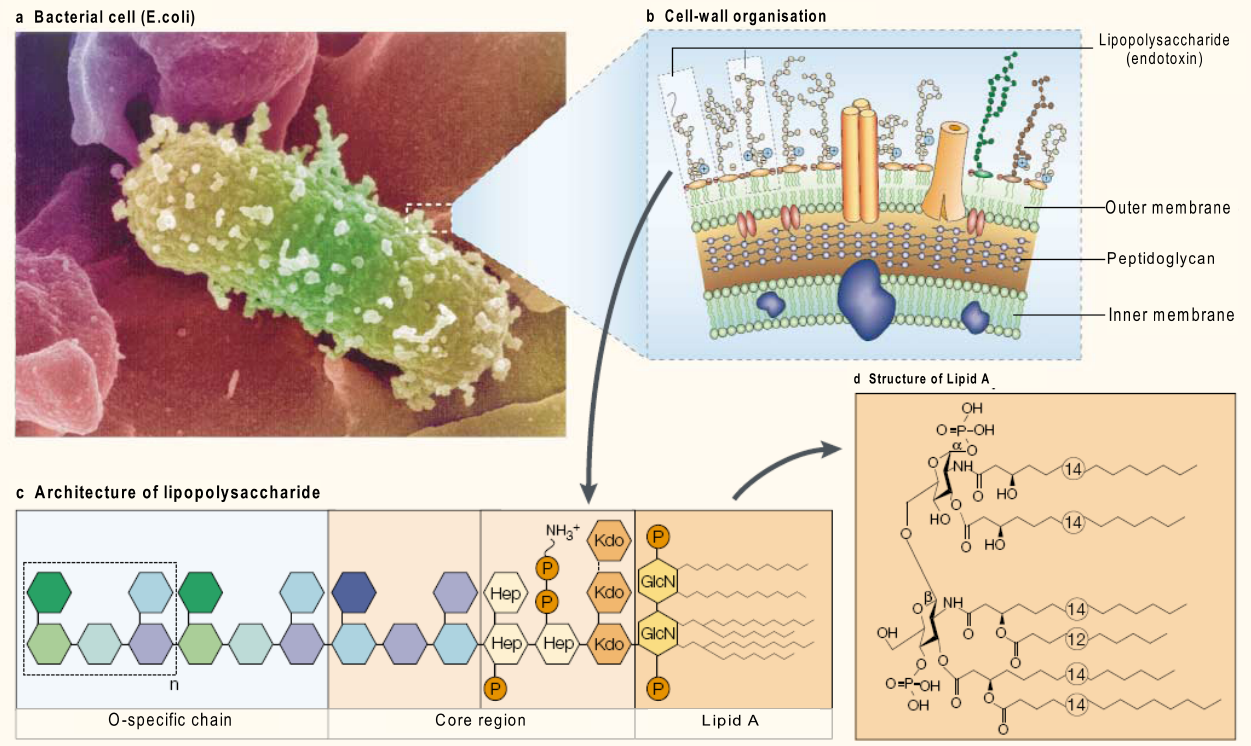


Figure 1.1 A Gram-negative bacterium. Electron micrograph of *Escherichia coli* (a), together with a schematic representation of the location of lipopolysaccharide, (endotoxin, LPS) in the bacterial cell wall (b) and the architecture of LPS (c). Also shown is the primary structure of the toxic centre of LPS, the lipid A component (d). GlcN, D-glucosamine; Hep, L-glycero-D-manno-heptose; Kdo, 2-keto-3-deoxy-octulosonic acid; P, phosphate (Beutler and Rietschel, 2003).

1.1.3 OCCUPATIONAL EXPOSURE RISK IN DENTAL PERSONNEL

Occupational health complaints such as dematoses, eye, respiratory and systemic complaints, allergies, infections and musculoskeletal disorders are not unusual to dentistry (Leggat et al., 2007; Nemutandani et al., 2007) and the prevalence of exposures (e.g. aerosol / splatter containing blood or bodily fluid) ranged from 3.8 – 65.3% in a South African study (Nemutandani et al., 2007). However, the latter study did not identify the microbiological content of the aerosols or splatter. Dentists are exposed to a number of occupational hazards which can be biological (e.g. bacteria, viruses), chemical (e.g. acrylates, metals such as mercury), physical (e.g. noise, radiation), psycho-social (e.g. shift work and stress) and ergonomic (work station design) (Leggat et al., 2007). These hazards result in the manifestation of various occupational diseases over the duration of employment. These diseases and syndromes range from stress-related issues, musculoskeletal disorders, asthma and allergies (Szymanska, 1999; Tosic, 2004). To date the epidemiological evidence of significant health problems associated with the use of contaminated DUWLs is still scanty (Cobb et al., 2002; Franco et al., 2005). Only three epidemiologic studies suggesting occupational exposure to contaminated DUWLs have been reported (Clark, 1974). In 1974, Clark recovered the same species of Gram-negative bacteria (*Pseudomonas* species) from dental units and the nasal flora of 14 of 30 dentists he evaluated. While no clinical symptoms were reported, seeding of the respiratory tract with Gram-negative bacteria has been identified as an antecedent event in the development of Gram-negative pneumonia in hospitalised patients (Clark, 1974). Studies have demonstrated that dental health care workers (HCWs) show evidence of increased exposure to *Legionella* bacteria as evidenced by elevated antibody titres when compared with demographically similar control populations. Although these studies suggest a possible increased risk of experiencing illness

among dental HCWs, no clinical cases of legionellosis were reported (Fotos et al., 1985; Reinthaler et al., 1988).

Clinical dental teams ar at increased risk of respiratory infections (e.g. Hepatitis B, influenza A, respiratory syncytial virus) compared to the general population or other health care professionals (Radi et al., 2002; Jaakkola et al., 2003; Le Moual et al., 2004; Scannapieco et al., 2004; Pankhurst et al., 2005). A decline in lung function in 15% of 57 children aged 6 to 18 years who underwent dental treatment, has been suggestive of bacteria-laden aerosols or aeroallergens from the dental clinic environment (Mathew et al., 1998). Endotoxin, a major predictor of the development and progression of occupational airways disease in a variety of occupational settings (Schwartz, 2001; Douwes et al., 2002b; Pankhurst et al., 2005) has recently been investigated in dental settings (Fulford et al., 2004; Pankhurst et al., 2005; Szymanska, 2005b; Huntington et al., 2007; Puttaiah and Cederberg, 2008). Prolonged daily exposure to endotoxin contaminated DUWLs has been implicated as a risk factor for the development of asthma among dental workers (Pankhurst et al., 2005). There is therefore a need for epidemiological and clinical studies investigating the link between endotoxin exposure and health effects in dental personnel.

1.1.4 CURRENT EXPOSURE LIMITS FOR ENDOTOXIN

Despite the reported health effects associated with endotoxin, a permissible exposure limit has not been promulgated, partly because of weaknesses in epidemiological evidence arising from problems with quantifying endotoxin concentrations (Thorne et al., 1997) as well as exposure variability (Liebers et al., 2008).

1.1.4.1 Exposure variability

It has to be considered that within a single workplace setting exposure to endotoxin also varies with working activity (e.g. production process, job or task), distance to the source, individual operating procedure and climatic circumstances as well as the presence or absence of adequate ventilation. These factors can vary from day to day and from person to person, and consequently, the exposure levels between and within workers can vary considerably. Since endotoxin derives from bacteria, a source that can grow by itself the exposure variability is thought to be much larger than for chemical exposure. Gram-negative bacteria have a heterogeneous source and their growth is dependent on many environmental factors, which enlarges exposure variability (Spaan et al., 2007b). Therefore measurements are only suitable to describe endotoxin exposure in clearly circumscribed areas and with regard to the actual working activity. A lack of consideration of these factors would result in discrepancies in the dose-response relationships between endotoxin exposure and health effects observed by different groups (Douwes et al., 1995; Liebers et al., 2008).

1.1.4.2 Endotoxin levels in water

The quality of dental water is of considerable importance since the regular exposure to it and its aerosols constitutes an occupational hazard in the dental team's work. Water that does not meet potable-water standards is inappropriate for use in dentistry and violates basic principles of clinical infection control (Anderson et al., 2002). However, a permissible exposure limit for endotoxin in drinking water or recreational waters has not been promulgated.

A consensus opinion on prevention measures is that the water used for dental procedures should be the same quality "as for drinking water" and sterile water should be used for surgical

procedures (Franco et al., 2005). The United States Pharmacopeia, have set a limit for endotoxin in sterile water for irrigation at 0.25 EU/ml (United States Pharmacopeial Convention, 1997). This limit could be used as a bench-mark for dental procedures requiring sterile water. Another attempt to decrease the medical risk of DUWL contamination is to reduce the bacterial contamination of water to a lower limit suggested by the American Dental Association to less than 200 cfu/ml (American Dental Association, 1996; Dutkiewicz, 1997).

1.1.4.3 Endotoxin levels in air

The Dutch Expert Committee on Occupational Standards (DECOS) recommends a health-based recommended occupational exposure limit (HBROEL) for airborne endotoxin of 50 endotoxin units (EU)/m^3 based on personal inhalable dust exposure measured as 8-hour time-weighted average (TWA) (Heederik and Douwes, 1997). However, a recent revised exposure standard proposes two HBROEL limits depending on the version of the limulus amoebocyte lysate (LAL) assay used for the exposure assessment. A limit of 90 EU/m^3 based on personal inhalable dust exposure, measured as an 8-hour TWA was recommended if an older version of the assay is used and 135 EU/m^3 if the recent assay version is used (Dutch Expert Committee on Occupational Safety and Nordic Expert Committee, 2009). The Robert-Sauve Research Institute for Occupational Safety and Health proposed an action criterion if field measurements exceed >30 times background levels (Robert-Sauve Research Institute for Occupational Safety and Health (IRSST), 2001). IRSST is a non-profit scientific research organisation established in Quebec since 1980. The organization contributes through research, to the prevention of industrial accidents and occupational diseases. However, in South Africa no legally binding limit has yet been established.

1.1.5 APPROACHES TO ENDOTOXIN EXPOSURE REDUCTION

Owing to the multiple ports of entry of microorganisms to the DUWL system, no single method or device but rather combinations of procedures and equipment should provide water of a standard higher than that of drinking water. However, all systems require strict adherence to maintenance protocols to perform to their full potential (Franco et al., 2005). A number of measures (e.g. flushing, chemical treatment, filtration, ventilation) are highlighted in a recent review which can assist in reducing the levels of contamination within the DUWLs and clinic environment (Dutil et al., 2009; Singh and Mabe, 2009).

Since dental personnel and the increasing numbers of people with diminished resistance to overt and opportunistic pathogens who are seeking dental treatment may be susceptible to infection as a result of exposure to contaminated dental unit water, every effort should be made to ensure that water within the waterlines is of an acceptable quality. The ultimate decision to take measures to improve and maintain the quality of water used in dental practice lies with the employer / dentist (Shearer, 1996; Mills, 2000).

1.2 ENDOTOXIN PROPERTIES AND EXPOSURE-RESPONSE RELATIONSHIPS

The individual immune response to endotoxin is the result of a complex interaction between dose, location, route, time and duration of exposure, additive or synergistic effects from co-exposure (e.g. tobacco smoke (Liebers et al., 2008), (1-3)-β-glucans (Douwes, 2005), nanoparticles (Inoue et al., 2007)), as well as genetic disposition and sex. Endotoxin effects

differ between acute singular or repeated exposures and are known to cause acute and chronic effects depending on the context of exposure, as well as adjuvant effects for allergy (Liebers et al., 2008).

1.2.1 ADVERSE HEALTH EFFECTS

Adverse health effects in respect of endotoxin exposure have been investigated in several occupational settings such as waste collecting and textile industry (Liebers et al., 2008). Acute effects such as headache, fever, fatigue, or respiratory complaints arise 6 hours after starting exposure and last for approximately 6 hours (Williams and Halsey, 1997). Endotoxin has also been shown to elicit chronic respiratory health effects such as wheezing, shortness of breath, cough, airway obstruction, accelerated lung function decline (Post et al., 1998), bronchial hyperresponsiveness, organic dust toxic syndrome (Smid et al., 2008), exacerbation of pre-existing asthma (Technical Committee and CEN/TC 137, 2003; Howell et al., 2004; Min et al., 2007; Liebers et al., 2008), dose-dependent influx of neutrophil granulocytes into the nose or the lung and subsequent increase in microvascular permeability (Williams and Halsey, 1997), and is an important factor in the aetiology of non-allergic asthma (Dutkiewicz, 1997). Threshold levels below 200 EU/m^3 have been suggested for both acute and chronic effects (Yang, 2008). Paradoxically, repeated endotoxin exposure may lead to endotoxin tolerance. This is supported by the findings with byssinosis where elevated levels of endotoxin are associated with symptoms that are worst on Monday morning (after having recovered during the weekend), and are characterised by an adaptive process that occurs during the working week (Medvedev et al., 2000; Robinson et al., 2005; Raulf-Heimsoth et al., 2007). Furthermore, studies have shown that human monocytes and macrophages can be induced to become tolerant, showing altered tumour

necrosis factor-alpha (TNF-α), interleukin (IL) -1 and IL-6 production due to endotoxin stimulation (Liebers et al., 2008).

1.2.1.1 Asthma

Work environments can trigger or induce asthma, resulting in several conditions under the umbrella term work-related asthma (WRA). The categorisation of these conditions is based on the strength of the causal relationship, clinical features and/or pathobiology. The WRA spectrum comprises the following conditions: 1) sensitiser-induced occupational asthma (OA); 2) irritant-induced OA; 3) work-aggravated asthma (WAA) or work-exacerbated asthma (WEA); and 4) variant asthma syndromes (Youakim, 2001; Vandenplas and Malo, 2003; Vandenplas et al., 2003; Nemery, 2004; Mapp et al., 2005; Yang and Holloway, 2007). Asthma caused by factors in the workplace is a common occupational disease, both in developing and industrialised countries, underscoring the importance of efforts aimed at understanding and reducing morbidity related to work. Aside from the economic implications, the disease is debilitating and can be fatal (Jeebhay and Quirce, 2007). Asthma is a chronic inflammatory disorder of the respiratory tract that is characterised by reversible airflow obstruction, bronchial hyperresponsiveness (BHR), airway inflammation and airway remodelling. The symptoms commonly include wheezing, breathlessness, chest tightness and coughing. The increasing prevalence and heterogeneity of the disease may be attributed to genetic and other host determinants as well as environmental triggers, including workplace exposures (Oryszczyn et al., 2007).

The reported prevalence of sensitiser-induced (IgE-mediated) asthma ranges from about 5% for high molecular weight (HMW) agents and 5% to 10% for low molecular weight (LMW) agents (Mapp et al., 2005). Identification of irritant-induced OA on the other hand, often relies on

historical data or certain modified diagnostic criteria, which include low or moderate workplace irritant exposures (Tarlo, 2000; Balmes, 2002; Mapp et al., 2005; Pechter et al., 2005). Hence estimates of 11% to 22% of all WRA cases were reported as irritant-induced OA in several studies (Kogevinas et al., 1999; Mapp et al., 2005; Pechter et al., 2005; Kogevinas et al., 2007). In South Africa, large-scale epidemiological studies on irritant-induced (non-IgE mediated) OA are few, despite the presence of large industries where high-risk exposures may be more prevalent (Jeebhay and Quirce, 2007).

1.2.1.2 Endotoxin exposure and work-related asthma and related symptoms

Epidemiological studies in occupationally exposed populations have shown exposure-response relationships between current endotoxin exposure and chronic respiratory health effects; however large variations in the levels of endotoxin associated with respiratory symptoms have been reported. Collectively, however, they show that lung function decline and respiratory symptoms are common in endotoxin-exposed settings (**Table 1.1**). It is the most frequently reported occupational lung disease in a number of industrialised countries (Caldeira et al., 2006). In developing countries like South Africa, OA has been ranked the second most common occupational lung disease reported after pneumoconioses (Hnizdo et al., 2001; Jeebhay and Quirce, 2007). A recent study in farmers and agricultural industry workers demonstrated that adjusted odds ratios (OR) for an interquartile range increase in endotoxin level were elevated for respiratory symptoms such as wheezing (OR: 1.41, 95% confidence interval (CI): 1.16-1.72), wheezing with shortness of breath (OR: 1.50, 95% CI: 1.18-1.90) and daily cough (OR: 1.29, 95% CI: 1.03-1.62) (Smit et al., 2009). In a cross-sectional multicentre DUWL survey of 265 dentists in general practice, the temporal onset of asthma was associated with occupational exposure to contaminated DUWLs. Dentists who were exposed in their dental surgery to

bacterial counts ≥ 200 cfu/ml were more likely to report symptoms of asthma "since starting dentistry" (Pankhurst et al., 2005). Although endotoxin was not directly measured in the latter study, correlation between endotoxin and bacterial load within DUWLs counts is consistent with published data (Huntington et al., 2007) demonstrating the potential risk for asthma in this occupational setting.

Table 1.1 Studies showing significant dose-response relationships between endotoxin and respiratory health effects.

Industry	Sample population size	Endotoxin levels	Respiratory health effects	Reference
Cotton	443	0.002 - 0.55 $\mu g/m^3$	Dose-response trend between current endotoxin and FEV_1	(Kennedy et al., 1987)
Pig farm	183	31 – 343 ng/m^3	Dose-response relationship between endotoxin exposure and FEV_1	(Heederik et al., 1991)
Textile	409	9.0 – 126 ng/m^3	Association between cumulative endotoxin exposure and FEV_1% and FVC%	(Sigsgaard et al., 1992)
Animal Feed	265	0.2 – 470 ng/m^3	Across-week change detected between endotoxin and FEV_1	(Smid et al., 1994)
Animal feed	315	0.2 – 470 ng/m^3	Endotoxin exposure and cumulative endotoxin exposure related to decreases in lung function	(Smid et al., 1992)
Swine farm	207	202 EU/m^3		(Donham et al., 1995)
Organic dust	1032	72 ng/m^3	Endotoxin predictive of upper and lower respiratory tract symptoms	(Simpson et al., 1998)
Potato processing	61	14 - 60 EU/m^3	8-10% across-shift decreases in lung function associated with endotoxin levels > 53 EU/m^3	(Zock et al., 1998)
Poultry	257	614 EU/m^3	Dose-response relationship observed between endotoxin and pulmonary function decrement	(Donham et al., 2000)
Wastewater treatment	468	0.6 – 2093 EU/m^3	Causal relationship observed between endotoxin and lower respiratory and skin symptoms	(Smit et al., 2005)

FEV_1 – forced expiratory volume in 1 second, FVC – forced vital capacity.

1.2.2 PROTECTIVE EFFECTS FROM ENDOTOXIN EXPOSURE

Traditionally, endotoxin was thought to reduce the risk of allergic sensitisation by inducing a shift from allergic T_H2 responses to T_H1 dominated responses through stimulation of the innate immune system and regulatory T-cells. The T_H1/T_H2 paradigm, is supported by two occupational studies of farmers which showed negative associations between airborne endotoxin exposure and atopic asthma and atopy (Eduard et al., 2004; Portengen et al., 2005) possibly due to the much debated hygiene effect. Opposing views suggest that a simple change in the ratio of T_H1 and T_H2 cytokines does not account for the ability of pathogens to protect against the progression of asthma as an inverse exposure-response relationship exist regardless of childhood farming exposures (Smid et al., 2008).

1.2.3 SYNERGISTIC ROLE OF ENDOTOXIN EXPOSURE

The modulatory role of endotoxin in the development of allergic disease and increased levels of non-specific airway hyperresponsiveness has been the subject of considerable interest as a result of mixed exposures in the workplace and also because of its ability to alter the T_H1/T_H2 balance (Howell et al., 2004). Studies have shown that allergens known to induce allergic rhinitis are not always the sole trigger of the inflammatory reaction. It appears that nasal exposure to endotoxin in combination with allergen has the ability to induce a subclinical pulmonary inflammation. This type of low-grade inflammation is known to occur without any accompanying symptoms. The result is a priming effect of allergen on nasal responsiveness to endotoxin (Bachar et al., 2007).

Endotoxin acts synergistically with allergen resulting in a magnification of specific allergen-induced mediator release (Williams and Halsey, 1997; Mathew et al., 1998) and has also been

described as an enhancer of the severity in asthmatics sensitised and exposed to high allergen levels (Blease et al., 1998; Murakami et al., 2006; Bachar et al., 2007). Patients with asthma showed an increase in eosinophilic nasal inflammation after nasal exposure to allergen and endotoxin; however the degree to which allergic inflammation is modified is unknown (Blease et al., 1998; Schaumann et al., 2008). Furthermore, synergistic interaction between endotoxin and nanoparticles (particles < 0.1 μm in mass median aerodynamic diameter) resulted in increased levels of chemotactic molecules for effector leukocytes compared to levels from exposure to nanoparticles or endotoxin alone (Inoue et al., 2007). Previous research has shown that airway allergen sensitisation by differential doses of endotoxin induces different forms of asthma that are type 1 (polarising cytokines IL-12 and IL-18) and type 2 asthma phenotypes by high and low doses of endotoxin, respectively. The latter effect implies that allergen sensitisation with low dose endotoxin induces the T_H2 asthma phenotypes associated with the up-regulation of TNF-α which plays a critical role in the development of T_H2 response to allergens (Kim et al., 2007).

1.2.4 IMMUNOLOGICAL CHARACTERISTICS OF ENDOTOXIN

Endotoxins are part of the pathogen associated molecular patterns (PAMPs) recognised by pathogen recognition receptors (PRR). Upon inhalation, endotoxin binds to the LPS binding protein (LBP) forming the LPS-LBP complex which subsequently binds to CD14 located on the cell surface of macrophages, coupling to the trans-membrane toll-like receptor 4 (TLR-4). This binding initiates a complex signal transduction cascade resulting in a systemic inflammatory reaction. The TLR interacts with various cytoplasmic proteins inducing the release of transcription factor NF-κB. NF-κB migrates into the nucleus and induces gene activation and consequently the expression, production and release of a variety of inflammatory mediators, particularly IL-1β, TNF-α and IL-6 (Raulf-Heimsoth et al., 2007).

1.2.5 AREAS FOR FUTURE RESEARCH

Despite a strong correlation between endotoxin exposure and respiratory health outcomes in certain occupational settings (Smid et al., 1992; Rongo et al., 2004; Liebers et al., 2006; Oldenburg et al., 2007), its relevance in dental facilities is uncertain due to the ubiquitous nature of endotoxins in this environment. Where endotoxin is found, so too are large numbers of other agents which may have allergenic and toxic properties. Further research clarifying the role of endotoxin exposure and health effects, particularly WRA in dental workers requires in-depth evaluation. One of the challenges facing clinical diagnosis is to be able to distinguish between the various phenotypes and forms of WRA so as to ensure optimal clinical management of the affected worker in a workforce where multiple exposures are common. In addition, future research on various asthma phenotypes needs to explore further the synergistic effects between endotoxin and other allergens encountered during dental procedures.

1.3 SUMMARY

The literature review has discussed several aspects and mechanisms that are involved and observed among workers with exposure to endotoxin. It has demonstrated the protective health effects as well as the detrimental effects following exposure to endotoxin, particularly its role in neutrophilic asthma. Endotoxin as a major predictor of the development and progression of occupational airways disease is dependent on several host and occupational determinants (Schwartz, 2001; Douwes et al., 2002b; Pankhurst et al., 2005). The delineation of the asthma phenotypes based on inflammatory mediators was reviewed. In addition, the possible synergistic effects with other agents augmenting the inflammatory reaction have been explored as well as

allergen-endotoxin induced asthma that is differentially regulated according to endotoxin levels at the time of exposure to aeroallergens. If dental workers are exposed to endotoxin and other agents, including latex allergens, what inflammatory response prevails? Does a synergistic relation exist between these agents and how does this affect exposed workers?

Owing to the paradoxical effects of endotoxin the study aimed at exploring the association between WRA and endotoxin exposure among dental workers in South Africa. The mechanisms leading to apparent differences in airway inflammation are not known in this occupational group and need to be understood for effective clinical management of exposed dental workers. This was achieved by investigating the level of endotoxin exposure in five public sector dental health care facilities in South Africa through personal air sampling. In addition, the prevalence of asthma among dental workers was determined through a self-administered respiratory health questionnaire and spirometry measurements. Cytokine profiling allowed for the differentiation of asthma phenotypes. True latex sensitisation was identified through specific IgE measurements. These measurements enabled the investigation of possible exposure-response relationships as well as the interaction between latex and endotoxin.

The specific objectives of this study were:

1.3.1 to conduct a detailed characterisation of endotoxin exposures in public sector dental health care facilities in South Africa,

1.3.2 investigate the determinants of exposure variability in endotoxin levels in dental health care facilities,

1.3.3 determine the prevalence of various asthma phenotypes that could be identified in dental HCWs based on environmental and work-related triggers and clinical presentation,

1.3.4 determine the host risk factors (e.g. atopy, smoking, sex, previous history of lung disease) and work-related factors (e.g. professional status: staff vs student, employment duration, proximity to aerosol source, self-perceived intensity of exposure, glove use) associated with these various asthma phenotypes,

1.3.5 identify the inflammatory pathways of asthma in dental HCWs,

1.3.6 define the exposure-response relationships in respect of the endotoxin exposures and the clinical endpoints in dental HCWs while controlling for potential confounders (age, sex, smoking),

1.3.7 investigate the relationship between serum endotoxin and personal airborne endotoxin exposure,

1.3.8 determine whether serum endotoxin is a predictor of clinical endpoints of interest.

Among the central chapters of this thesis, **Chapter 2** describes the occupational exposure levels of endotoxin in dental health care settings. The emphasis of this chapter is placed on the determinants of exposure. The prevalence, as well as potential predictors, of three broad asthma phenotypes among dental personnel is described in **Chapter 3**. Owing to the mixed nature of exposures in the dental setting, which are key determinants of the inflammatory response in

asthmatics, **Chapter 4** explored the inflammatory cell phenotypes based on the cytokine response to identify the major pathophysiological mechanisms that underlie the asthmatic manifestations.

In **Chapter 5**, the association between endotoxin exposure and the major asthma phenotypes and clinical endpoints of interest are presented. Details of the exposure-response relationships and interaction between endotoxin and latex are also addressed in this chapter. **Chapter 6** explores the utility of using serum endotoxin as an alternative to endotoxin exposure measurements in predicting asthma in a dynamic workforce such as dentistry.

Chapter 7 deliberates on the overall findings of the study taking cognisance of the heterogeneity of asthma and the role of endotoxin in a workplace with multiple asthmagenic agents. The final conclusions are highlighted and recommendations are proposed regarding the reduction of endotoxin exposure and prevention of asthma in dental workers.

1.4 SUMMARY OF METHODS

The overall methodology of the entire study is presented here. Details of the various methods are described in the respective chapters.

1.4.1 STUDY DESIGN AND SAMPLING STRATEGY

A cross-sectional study of approximately 1816 currently employed dental workers and registered students was conducted in five academic dental institutions in South Africa. One of the institutions had two campuses and the second campus was therefore considered as a sixth sampling site in this study. These included 1) University of KwaZulu-Natal: Westville,

KwaZulu-Natal Province; 2) University of Limpopo: Medunsa Campus, North West Province; 3) University of Pretoria, Gauteng Province; 4) University of the Western Cape: Mitchells Plain campus and Tygerberg campus, Western Cape Province and 5) University of the Witwatersrand, Gauteng Province. The institutions are designated as A, B, C, D, E and F to maintain anonymity. The participants included dental staff (clinical, administration, laboratory and auxiliary) and clinical dental students. Ethical clearance was obtained from the University of Kwa-Zulu-Natal, University of Michigan (funding organisation), University of Pretoria and the University of the Witwatersrand prior to the study being conducted. Permission was also obtained from the University of Limpopo: Medunsa Campus and University of the Western Cape where the project was conducted, as well as from the Provincial Department of Health offices.

The different dental clinics within the abovementioned institutions surveyed included Maxillo-facial & Oral Surgery clinic, Restorative Dentistry or Conservation clinic, Periodontology and Oral Medicine clinic, Prosthetics & Prosthodontics clinic, Orthodontics clinic and Dental Therapy & Oral Hygiene clinic. A total of 14 different job titles were identified and participants were proportionally sampled so that each job type was adequately represented in each exposure group. The curriculum for dental students begins in January of each year, and is divided into four semesters with three vacation breaks. On average, staff worked 8 hours (08h00 to 16h00) a day, while the student's day is divided into lectures and clinic sessions, for which the timetable varies for each institution. However, the average clinic session is 2 ½ hours a day. Students are expected to work in the clinics for an average of 35 hours per semester in the third, 39 hours in the fourth and 42 hours in the fifth years of study.

1.4.2 ENVIRONMENTAL OUTCOMES

1.4.2.1 Air sampling

Inhalable personal air sampling (n = 413) was done on volunteers during the working day on dental workers performing clinic sessions during 2006, using 37 mm polycarbonate (PC) filters and an average flow rate of 2.5 l/min (Thorne et al., 1997; Technical Committee and CEN/TC 137, 2003). Personal sampling was conducted for approximately 150 minutes depending on the duration of the clinic session. After sampling, the filters were sealed and stored in plastic bags in a fridge (~4˚C) for a week until transported to the National Institute for Occupational Health (NIOH) laboratory for immediate extraction and subsequent endotoxin analyses.

Area samples (n = 116) were also taken to identify possible sources of exposure and to describe characteristics of airborne endotoxin that could not be determined by personal sampling. Samples were collected using PC filters at an average flow rate of 2.5 l/min (Thorne et al., 1997; Technical Committee and CEN/TC 137, 2003), over a period of 3.4 to 8.9 hours in locations where worker activities were concentrated. Measurements were taken approximately 1.5 m above the floor.

1.4.2.2 Water sampling

Water samples (5-10 ml volume) were aseptically collected in pyrogen-free 10 ml glass bottles (Lonza, Walkersville, USA) from 3-in-1 dental handpieces and basin taps. A total of 403 water samples were collected. Samples were kept in a fridge at 4˚C and were analysed within 24 hours of receipt by the laboratory.

1.4.2.3 Determination of endotoxin concentration

Extracted air filter samples and water samples were analysed using the chromogenic-1000 LAL assay (Lonza, Walkersville, USA). Throughout the analytical process, pyrogen-free products including water were used. Endotoxin analysis was done according to the manufacturer's instructions. Pyrogen-free water was analysed as the negative controls and 0.4 EU/ml *E. coli* for the positive controls. Assays in which the standard curve had a correlation coefficient ≥ 0.98 and samples with a coefficient of variation (CV) ≤ 10% were accepted.

1.4.3 HEALTH OUTCOMES

1.4.3.1 Health and employment questionnaire

Important demographic factors were obtained through a modified questionnaire (see Appendix C) specifically designed for the investigation of asthma contained in the Protocol for the European Community Respiratory Health Survey (European Community Respiratory Health Survey, 1993) and recent study of seafood-processing workers (Jeebhay et al., 2008).

1.4.3.2 Determination of phadiatop, recombinant latex and latex-specific IgE levels

Blood samples for serum analysis for common inhalants using Phadiatop® test and latex-specific IgE (k82) (*Hevea brasiliensis*) determinations from participants (n = 421) were obtained. Specific IgE antibody tests to individual recombinant latex allergens (n = 85) (Hev b 1, Hev b 3, Hev b 5, Hev b 6.01, Hev b 6.02, Hev b 8, Hev b 9, Hev b 11) as well as cross-reacting carbohydrate determinants (CCDs) (horse radish peroxidase (HRP) and bromelain (MUXF3)) were performed on workers (n = 41). Tests were done according to the manufacturer's instructions using the ImmunoCAP system 100 (Phadia AB, Uppsala, Sweden). A value of ≥ 0.35 kU/l was considered positive.

1.4.3.3 Measurement of eosinophilic cationic protein (ECP)

Serum ECP was measured using the Phadia ImmunoCAP system ECP FEIA (Phadia AB, Uppsala, Sweden), according to the manufacturer's instructions.

1.4.3.4 Measurement of myeloperoxidase (MPO)

MPO concentration in serum was measured using the MPO enzyme immunoassay (MPO-EIA) (MPO-EIA, Oxford Biomedical Research). The assay was performed according to the manufacturer's instructions (Oxford Biomedical Research).

1.4.3.5 Measurement of non-allergic inflammatory mediators (cytokines)

The BD^{TM} CBA Human Inflammation kit was used to quantitatively measure non-allergic inflammatory cytokines (IL-8, IL-1β, IL-6, IL-10, TNF-α and IL-12p70) in each serum sample. Six bead populations with distinct fluorescence intensities coated with the specific ILs were multiplexed and resolved in the red channel of the flow cytometer (BD FacsArray^{TM}) (Becton Dickson, Oxford, UK). The determination of the serum inflammatory ILs was done according to the manufacturer's instructions.

1.4.3.6 Measurement of allergy-related inflammatory mediators (cytokines)

The allergy-related inflammatory ILs (IL-7, IL-3, IL-4, IL-5, eotaxin and GM-CSF) were measured using the BD^{TM} CBA Human Soluble Protein Flex Set assay which allow for multiplexed analysis of multiple proteins from a single sample. The multiplexed bead populations were resolved in the near-infrared (NIR) and red channels of the flow cytometer (BD FacsArray^{TM}). The procedure was done according to the manufacturer's instructions.

1.4.3.7 Serum endotoxin measurements

Blood was collected from study participants in sterile 6 ml vacutainer tubes containing ethylene-diamine-tetra-acetic acid (EDTA) (K2E) (BD Vacutainer Systems, United Kingdom). Endotoxin analyses using the LAL assay were conducted according to the manufacturer's instructions similarly to the method described above for airborne endotoxin determination.

1.4.3.8 Spirometry

Spirometry was conducted according to the American Thoracic Society (ATS) guidelines (American Thoracic Society (ATS), 1995). A Jaeger Masterscope spirometer (Höchberg, Germany) was used, by trained technologists. Each participant performed up to eight trials to produce three acceptable curves, in a sitting position without nose clips. The lung function indices of primary interest included FEV_1 and FVC.

Post-bronchodilator tests were done to determine whether airway obstruction was reversible with inhaled beta-2 agonists (salbutamol, 400 μg). An improvement of at least 12% and 200 ml after administration of salbutamol was regarded as a significant response. The post-bronchodilator test was done at least 10 minutes after administration of the bronchodilator.

1.4.4 STATISTICAL ANALYSIS

Statistical analysis was performed using Stata9 computer software (StataCorp, 2007, Texas, USA). Descriptive statistics were generated for the total sample and for subgroups (institution and exposure groups) using geometric means (GM) and geometric standard deviations (GSD). Analysis of variance was used to explore the between- and within-variance components by institution, department and exposure groups. Univariate and multivariate linear regression was carried out to explore the determinants of variability in endotoxin exposure using the natural logarithm (Ln) of measured endotoxin levels as the dependent variable.

Multiple logistic regression analysis was carried out to assess the association between risk factors (host and occupational risk factors) and the definitions of asthma, adjusting for potential confounders, including age, smoking habits and sex. The key disease outcome variables of interest were WAA, atopic asthma and non-atopic asthma. Factor analysis was used to determine inflammatory patterns based on 12 cytokines (Rodrigo and Rodrigo, 1993; Lapperre et al., 2004). To further determine the association between the cluster groupings and cytokine patterns in relation to health outcomes, multivariate logistic regression analysis was applied.

Exposure assessment was carried out using a combination of work history information and personal exposure measurements. Exploratory bivariate analysis was used to assess the nature of the association between exposures, covariates and health outcomes. Logistic regression was conducted to assess the association between exposure risk factors and the health outcomes defined.

CHAPTER 2

WORKPLACE DETERMINANTS OF ENDOTOXIN EXPOSURE IN DENTAL HEALTH CARE FACILITIES IN SOUTH AFRICA

2.0 INTRODUCTION

Dental unit waterlines (DUWLs) are commonly contaminated with Gram-negative bacteria, which release endotoxin during bacterial growth phase and upon lysis (Fulford et al., 2004; Dutil et al., 2009). A previous study done in DUWLs in South Africa showed high levels of *Legionella*, a Gram-negative bacterium (Singh and Coogan, 2005). Despite numerous reports of Gram-negative bacteria in dental water, there are few published reports investigating endotoxin in the DUWLs and indoor air of dental clinics (Putnins et al., 2001; Fulford et al., 2004; Szymanska, 2005c; Szymanska, 2005b; Huntington et al., 2007; Puttaiah and Cederberg, 2008; Dutil et al., 2009).

During dental procedures with high-speed dental instruments, bioaerosols composed of particles varying in size are produced. Some particles evaporate forming droplet nuclei smaller than 5 μm in diameter which remain airborne for many hours (Szymanska, 2005c). Detectable levels of endotoxin have been reported in air samples obtained from dental institutions with some as high as 625 EU/m^3 (Szymanska, 2005b). However, in the study by Huntington et al. (2007) the airborne endotoxin levels did not exceed 2.7 EU/m^3 despite the endotoxin levels in the DUWLs being high (15 000 EU/ml). Similarly, low personal exposure levels (0.63 EU/m^3) in a simulated dental treatment room were recently reported by Dutil and colleagues (Dutil et al., 2009).

Of potentially greater significance than the occasional endotoxin exposure of patients undergoing dental health care is the continual exposure of those involved in the dental profession. Chronic endotoxin inhalation represents an occupational respiratory hazard to this group of professionals (Huntington et al., 2007). Exposure-response relationships between endotoxin and lung function decline have been reported in a number of studies. The risk estimates ranged from an odds ratio of 1.79 – 3.3 for endotoxin levels greater than 200 EU/m^3 (Kennedy et al., 1987; Sigsgaard et al., 1992; Post et al., 1998; Zock et al., 1998; Smit et al., 2005). However only a few studies in farming (Preller et al., 1995; Vogelzang et al., 1998) and the domestic environment (Park et al., 2001) have investigated determinants of endotoxin exposure, for application in the development of control measures. Farming characteristics such as feeding and cleaning activities were predominant predictors of endotoxin exposure for pig farms (Preller et al., 1995; Vogelzang et al., 1998) while pet ownership, moisture sources and increased amounts of settled dust were key determinants in the domestic setting (Park et al., 2001). Workplace determinants of endotoxin exposure in the dental setting which could be used to set priorities for the reduction of endotoxin exposure have not been previously investigated.

The aim of this study was to conduct a detailed characterisation of endotoxin exposures in public sector dental health care facilities in South Africa. This was achieved by:

- determining the levels of endotoxin in DUWLs in various dental health care facilities
- determining the levels of endotoxin in ambient air through personal and area environmental sampling
- examining the correlation between endotoxin levels in water and in air
- investigating the determinants of exposure variability in endotoxin levels in dental health care facilities.

2.1 MATERIALS AND METHODS

2.1.1 SAMPLING STRATEGY

A cross-sectional study was conducted on 413 dental personnel in five academic dental institutions (comprising six sampling sites) situated in four provinces in South Africa. Participation was voluntary and participants completed a consent form before being interviewed.

The different dental clinics within the abovementioned institutions surveyed included Maxillo-facial & Oral Surgery clinic, Restorative Dentistry or Conservation clinic, Periodontology and Oral Medicine clinic, Prosthetics & Prosthodontics clinic, Orthodontics clinic and Dental Therapy & Oral Hygiene clinic. A total of 14 different job titles were identified. The job titles included the Bachelor of Dental Therapy students (x 3 categories), Bachelor of Dental Science students (x 3 categories), dental assistants, dental technicians, dental nurses, oral hygienists, dentists, administration, security and cleaning staff. Participants were proportionally sampled so that each job type was adequately represented in each exposure group (**Table 2.1**). The

curriculum for dental students begins in January of each year, and is divided into four semesters with three vacation breaks. The clinics experience quiescent periods during these breaks where major servicing and cleaning of the dental units occurs.

Table 2.1 Description of jobs in relation to the assigned exposure group in different departments.

Departments	Exposure group	Description of jobs within exposure group	Main task/s
Clinical	Maxillo-facial and Oral Surgery	Diagnosis, surgical and related treatment of diseases, injuries and defects involving the head, mouth, teeth, gums, jaws and neck.	Extractions
	Restorative / Conservation	Restoration of natural teeth that have been damaged, decayed or lost through fillings, crowns and bridges.	Fillings
	Periodontology and Oral Medicine	Treatment of diseases of the periodontium (supporting structures of the teeth) as a result of bacterial infection of the gums. Treatment includes the placing of dental implants to replace missing teeth.	Treatment and implants
	Prosthodontics / Prosthetics	Restoration and maintenance of oral function by the replacement of missing teeth and structures by artificial devices or prostheses (dentures).	Root canals, grinding and fillings
	Orthodontics	Treatment of malocclusions (improper bites), which may be a result of tooth irregularity, disproportionate jaw, or both and is treated by the insertion of dental braces.	Braces
	Dental Therapy and Oral Hygiene	Dental therapy encompasses the treatment of diseases and conditions that affect the teeth and gums, while oral hygiene is the maintenance of clean and healthy teeth.	Cleaning and polishing
	Sterilisation	Decontamination of instruments using autoclaves, sterilisers and chemical solutions.	Decontaminating
Laboratory	Technician lab	Making dental prostheses from wax or ceramic models for bridges, crowns, dentures using porcelain and metal. The procedure involves grinding and baking.	Moulding, waxing, ceramics, grinding and baking
Administration	Clerical	Computers and filing and cash transactions.	Record handling and payments
	Reception	Answering telephones and patient bookings.	Bookings
	Security	Guarding security points.	Security checks
	Stores	Distribution of stock items.	Stock control
Auxiliary (non-classifiable)	Cleaning	Cleaning clinics and offices, washing linen and laboratory coats and ironing.	Cleaning
	General	Delivery of mail and other items and transporting of patients between clinics.	Deliveries and maintenance

2.1.2 WALKTHROUGH INSPECTION

An experienced occupational hygienist performed a walkthrough inspection of clinics at each institution. Information regarding the age of the building, recent renovations to the building, model of the dental units used, age of dental units, number of floors per dental clinic, type of water supply to the dental unit (municipal or reservoir) and the presence of a ventilation system were obtained.

2.1.3 MICROCLIMATIC PARAMETERS

Ambient daily temperatures (measured at a height of 1.8 m), percent relative humidity (%RH) values and carbon dioxide (CO_2) levels were measured using the TSI Q TRAK Model 8551 (SKC Inc. Pittsburg, Pa.). Air velocity in the work areas was measured using the TSI Velocicalc plus model 8388-M-GB (SKC Inc. Pittsburg, Pa.).

2.1.4 AIR SAMPLING

Inhalable personal air sampling (n = 413) was done on volunteers during the working day on dental workers performing clinic sessions during 2006. Samples were collected using preloaded three-piece 37 mm clear styrene closed faced cassettes with pre-sterilised 0.4 µm polycarbonate (PC) filters and endotoxin-free backing pads. A sampling train was set up with the filter cassette fixed to the lapel of the participant's laboratory coat or collar. Gillian (SKC Inc. Pittsburg, Pa.) or Casella Apex (Casella, USA) battery-operated air-sampling pumps running at an average flow rate of 2.5 l/min were used (Thorne et al., 1997; Technical Committee and CEN/TC 137, 2003). Personal sampling was conducted for approximately 150 minutes depending on the duration of the clinic session. During the sampling period any unusual occurrences were noted. After sampling, the filters were sealed and stored in plastic bags in a fridge (~4˚C) for a week until

transported to the National Institute for Occupational Health (NIOH) laboratory for immediate extraction and subsequent endotoxin analyses. At least one field blank was taken on each field sampling day and was included in the analyses to take into consideration any contamination of the filter cassettes used. Sampling pumps were calibrated before and after sampling using a Gillibrator bubble flow meter (Gillian Instrument Corp., Wayne, N.) to verify that the air sampling rate had been constant. If the flow rate was more than 5% below the initial flow rate the sample was not included in the analysis (NIOSH, 1998).

Area samples (n = 116) were also taken to identify possible sources of exposure and to describe characteristics of airborne endotoxin that could not be determined by personal sampling. Samples were collected using preloaded three-piece 37 mm pre-sterilised cassettes with 0.4 µm PC filters. Gillian (SKC Inc. Pittsburg, Pa.) or Casella Apex (Casella, USA) battery-operated air-sampling pumps running at an average flow rate of 2.5 l/min were used (Thorne et al., 1997; Technical Committee and CEN/TC 137, 2003), over a period of 3.4 to 8.9 hours in locations where worker activities were concentrated. Measurements were taken approximately 1.5 m above the floor.

2.1.5 WATER SAMPLING

Water samples (5-10 ml volume) were aseptically collected in pyrogen-free 10 ml glass bottles (Lonza, Walkersville, USA) from 3-in-1 dental handpieces and basin taps. A total of 403 water samples were collected. Water samples were not collected from participants performing dental procedures that did not require water at the time of sampling. Samples were kept in a fridge at 4˚C and were analysed within 24 hours of receipt by the laboratory.

2.1.6 LABORATORY ANALYSIS OF SAMPLES

2.1.6.1 Extraction procedure

Air filter samples were extracted in 6 ml pyrogen-free water (Lonza, Walkersville, USA) and 0.05% Tween 20 for 3 hours at room temperature with gentle agitation. The samples were filtered using 0.45 µm Acrodisc syringe filters (Pall Corporation, USA). The extracts were then concentrated (Baur, 2002) using Cryodos -50˚C freeze dryer (Telstar Cryodos, Spain) and reconstituted in 1 ml LAL water. The procedure was done according to the method described by Baur (Baur, 2002). The suspension was stored at -70˚C for a period of approximately 30 days before analysis. According to Spaan et al. prolonged duration of specimen storage does not appear to have a major impact on the integrity of the specimen (Spaan et al., 2007a). To test the effectiveness of the extraction procedure, sterile filters were spiked with known concentrations of endotoxin, extracted as described above and then assayed. The results were comparable to the spiked concentration.

2.1.6.2 Determination of endotoxin concentration

The chromogenic-1000 LAL assay (Lonza, Walkersville, USA) was used. Throughout the analytical process, pyrogen-free products including water were used. Endotoxin analysis was done according to the manufacturer's instructions. Pyrogen-free water was analysed as the negative controls and 0.4 EU/ml *E. coli* for the positive controls. The standard curve included 0.10, 0.25, 0.50 and 1.00 EU/ml and was prepared with the *E. coli* provided in the kit. The plate was read at 405 nm. Change in absorbance relative to the assay reagent blank was calculated, and a standard curve of delta absorbance versus endotoxin activity was generated. Assays in which the standard curve had a correlation coefficient ≥ 0.98 and samples with a coefficient of variation (CV) $\leq 10\%$ were accepted. Using the calculated volume of air sampled and endotoxin

concentrations, the endotoxin units per cubic meter (EU/m^3) were calculated. The formula used was:

[EU/ml **X** sample volume (ml)] / [time (min) **X** (rate (L/min) **X** 1 m^3/1000 L] (Lonzo, 2001)

The limit of detection (LOD) (0.03 EU/ml) was computed by calculating the mean of the field blanks. The LOD denotes the lowest level above which endotoxin had been detected during sampling. The limit of quantification (LOQ) (0.08 EU/ml) was calculated by adding the mean plus 3 times the standard deviation of the field blanks. LOQ denotes the limit above which there is confidence in the value measured. For data analyses, values for exposure measurements below the LOD were replaced by the analytic LOD divided by the square root of two (LOD/$\sqrt{2}$) (Finkelstein and Verma, 2001).

The detection method was validated in the laboratory to ensure reproducibility and repeatability. Interference of the samples was assessed by spike recovery of endotoxin in samples that were diluted 10-fold. No interference was noted. Since no local laboratory performing endotoxin analyses using the chromogenic assay could be identified at the time, the method recommended by Bland and Altman, 1986 was used to test the level of agreement of the standard curves of two repeated test runs from independent days (Bland and Altman, 1986). The mean difference was -0.03 with a range from 0.29 to 0.42, and was considered acceptable. The intra-assay (within plate) CV was $\leq$ 10% and the regression coefficient (R^2) for the two test runs was $\geq$ 0.98.

2.1.7 STATISTICAL ANALYSIS

Statistical analysis was performed using Stata9 computer software (StataCorp, 2007, Texas, USA). A test for normality indicated that endotoxin concentrations for personal air samples and water samples were not normally distributed. The data was log transformed to meet the assumptions of normality. One sample had a value below the LOD (0.03 EU/ml) and was censored. Descriptive statistics were generated for the total sample and for subgroups (institution and exposure groups) using geometric means (GM) and geometric standard deviations (GSD).

For the statistical analysis, job titles in each department were grouped into similarly exposed groups. The groups included: Dental Therapy students, third year to fifth year Bachelor of Dental Science students, clinical staff (dental assistants, dental nurses, dental therapists, oral hygienists, dentists and technicians), administration and security and cleaning staff. Each exposure group contained a number of job titles that were deemed by the study team to represent similar tasks and involve similar exposures based on information from self-administered questionnaires. Analysis of variance (with Bonferroni correction) was used to test for the difference between exposure groups.

Analysis of variance was used to explore the between- and within-variance components by institution, department and exposure groups. Univariate and multivariate linear regression was carried out to explore the determinants of variability in endotoxin exposure using the natural logarithm (Ln) of measured endotoxin levels as the dependent variable. For multivariate linear regression, variables were considered for inclusion into the model using criteria such as plausibility of relationship, results from univariate explorations and automatic variable selection. Independent variables considered for inclusion into the models included microclimatic

parameters (temperature, air velocity, humidity, CO_2, season and day of sampling) and occupational factors (institution, age of building, department, task, job type, dental unit model, number of dental units, dental unit age, number of patients seen, source of water and endotoxin levels in water). Although not significant, endotoxin level in water was factored into the model as theoretically it could be a strong predictor of airborne endotoxin exposure (Szymanska, 2005c).

2.2 RESULTS

2.2.1 CHARACTERISTICS OF DATA

The total number of individuals from each institution that participated in the study was as follows: institution A – 78; institution B – 105; institution C – 57; institution D – 71; institution E - 91 and institution F – 52, giving a total of 454 (**Figure 2.1**). Among these, 164 were dental students and 290 were clinical and non-clinical staff. On average this represented 25% of the individuals at these institutions. A total of 413 personal air sampling measurements were collected from the six sampling sites. Four samples were rejected as the sampling process was interrupted, yielding spurious results. In addition, 403 water samples were collected. Ten participants working on prosthetics procedures did not use water during their clinic session and as a result water samples were not collected from these participants' work areas.

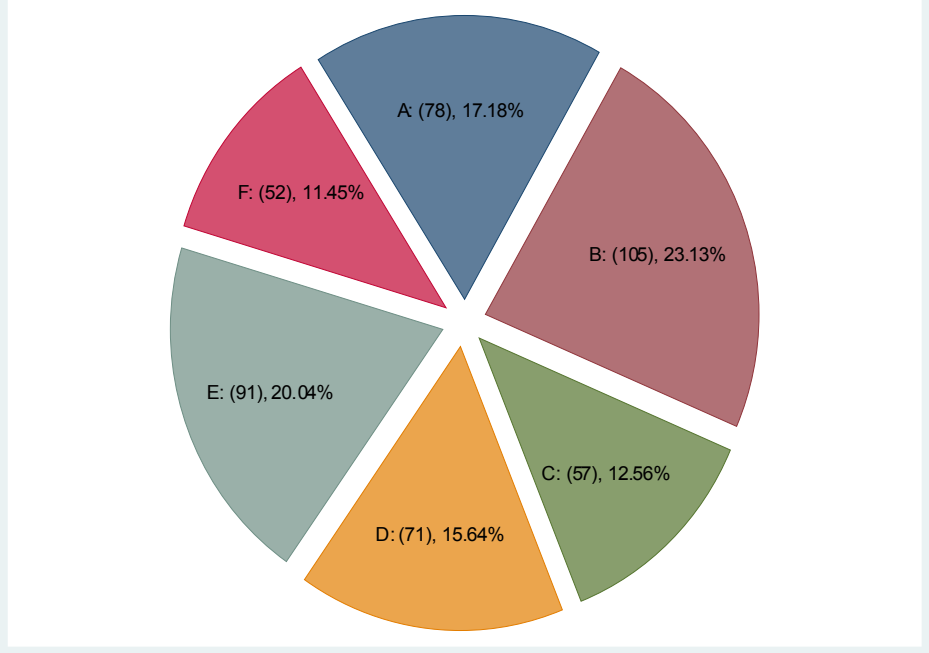


Figure 2.1 Distribution of participants from each institution that were sampled in the study (n = 454).

2.2.2 BUILDING CHARACTERISTICS

The structural characteristics of the dental institutions are presented in **Table 2.2**. It is evident that the majority of the dental facilities were relatively old.

Table 2.2 Structural characteristics of dental institutions.

Description	Institution					
	A	**B**	**C**	**D**	**E**	**F**
Building age (years)	5	22	20	40	36	20
Number of levels in building	1	3	6	2	3	2
Building location	urban	sub-urban	urban	urban	urban	sub-urban
Ventilation system	defective*	defective*	absent	defective*	present	present
Last renovation (years)	-	-	-	5	-	-
Number of clinical areas	3	4	6	2	5	5
Clinic types:						
Maxillo-facial & Oral Surgery	+	+	+	-	+	+
Restorative / Conservation Dentistry	+	+	+	-	+	+
Periodontology & Oral Medicine	+	-	+	-	+	+
Orthodontics	+	+	+	-	+	+
Prosthetics / Prosthodontics	+	+	+	-	+	+
Dental Therapy & Oral Hygiene	+	-	+	+	-	-

* The installed ventilation system was not mechanically operational at the time of sampling

2.2.3 INDOOR AIR QUALITY ASSESSMENT

The measurements for microclimatic factors during the sampling periods for the different institutions are presented in **Table 2.3**. The sampling for Institution A was conducted in summer, Institution B in autumn, Institutions C and D in winter and Institutions E and F in the spring season, hence the variation in temperature and humidity observed among the institutions. CO_2 measurements were taken; however, they did not appear to show any effect.

Table 2.3 Assessment of endotoxin levels in air and water samples and microclimatic quality in dental clinics during the sampling period.

Institution	Sampling period	Mean endotoxin levels, GM (GSD)			Ambient parameter: Mean (SD)		
		Personal Air (EU/m^3) n = 413	Area Air (EU/m^3) n = 116	Water (EU/ml) n = 403	Temp ˚C n = 183	Humidity % n = 183	Air velocity m/s n = 307
A	Summer	1.5 (2.2)	1.0 (2.6)	222 (10.7)	23 (0.96)	48 (1.86)	0.07 (0.02)
B	Autumn	6.5 (4.0)	1.7 (6.2)	1.7 (6.2)	21 (2.10)	47 (4.56)	0.06 (0.02)
C	Winter	2.9 (2.3)	0.8 (5.6)	320 (2.7)	12 (2.89)	15 (3.85)	0.01 (0.003)
D	Winter	3.3 (2.5)	0.5 (3.7)	44 (7.1)	11 (0.92)	22 (1.85)	0.04 (0.01)
E	Spring	8.9 (3.1)	0.5 (3.0)	41 (1.7)	12 (0.32)	17 (1.17)	0.04 (0.01)
F	Spring	4.5 (2.1)	0.6 (2.7)	15 (4.3)	12 (0.52)	21 (1.04)	0.08 (0.04)

GM: Geometric mean; GSD: Geometric standard deviation; SD: Standard deviation

2.2.4 ENDOTOXIN EXPOSURE METRICS

The overall GM and GSD for all participants were 4.11 EU/m^3 and 3.42 EU/m^3, respectively.

Table 2.4 outlines endotoxin levels by department and exposure group for all institutions.

Highest mean levels were found in the laboratory compared to the other departments. The mean endotoxin concentration in personal samples was five times higher than area samples. The overall GM and GSD for endotoxin concentration in water samples were 101 EU/ml and 6.03 EU/ml, respectively.

The log transformed endotoxin exposures varied across institutions in respect of personal air samples ($p < 0.001$), area samples ($p < 0.05$) and water samples ($p < 0.001$). Personal endotoxin levels differed significantly by institution ($p < 0.001$) and were highest for Institution E (GM: 8.9 EU/m^3, GSD: 3.5 EU/m^3). However since the value for the Bartlett's test of equal variance was relatively small for the area samples, the results should be treated with circumspection. Personal air samples differed by as much as ten times among institutions whereas area samples were less variable as indicated by the Bartlett's test. Log differences in endotoxin concentration in water samples were noted among institutions (**Table 2.4**).

Table 2.4 Characteristics of mean exposure levels of endotoxin in air (EU/m^3) and water (EU/ml) for the various institutions, departments and exposure groups.

Exposure Matrix	Personal Samples (EU/m^3)					Area Samples (EU/m^3)					Water Samples (EU/ml)				
	n	AM	GM	GSD	Range	n	AM	GM	GSD	Range	n	AM	GM	GSD	Range
Institution A	77	2.3	1.5	2.2	0.2-27.8	29	1.7	1.0	2.6	0.3-6.1	78	8491	222	10.7	6-100000
Clinical	60	2.5	1.6	2.3	0.2-27.8	25	1.7	1.0	2.5	0.3-5.8	61	9592	258	11.8	6-100000
Maxillo-facial & Oral Surgery	13	1.6	1.4	1.9	0.3-3.2	9	0.7	0.7	1.3	0.4-0.9	13	11359	365	13.3	17-97000
Restorative / Conservation	30	3.0	1.9	2.1	0.9-27.8	8	1.4	0.9	2.1	0.7-5.7	31	6636	183	10.9	6-97000
Periodontology	1	0.8	0.8	-	-	1	0.3	0.3	-	-	1	45700	45700	-	-
Prosthodontics / Prosthetics	1	2.4	2.4	-	-	1	1.8	1.8	-	-	1	63	63	-	-
Orthodontics	2	1.6	1.4	2.2	0.8-2.4	1	0.6	0.6	-	-	2	50040	2828	155	80-100000
Dental Therapy & Hygiene	8	3.3	2.2	2.2	1.4-14.3	3	5.7	5.7	1.2	5.6-5.8	8	10656	325	10.2	60-84000
Sterilisation	5	1.2	0.7	3.3	0.2-2.4	2	2.7	1.6	4.7	0.5-4.8	5	126	111	1.8	60-210
Laboratory	6	1.4	1.1	2.0	0.4-2.7	1	6.1	6.1	-	-	6	159	119	2.7	18-310
Technicians lab	6	1.4	1.1	2.0	0.4-2.7	1	6.1	6.1	-	-	6	159	119	2.7	18-310
Administration	4	1.0	0.7	2.1	0.3-1.7	1	0.4	0.4	-	-	4	125	63	3.5	22-390
Clerical	3	1.0	0.8	2.5	0.3-1.7	1	0.4	0.4	-	-	3	36	34	1.5	22-50
Reception / switchboard	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Stores	1	0.5	0.5	-	-	-	-	-	-	-	1	390	390	-	-
Auxiliary	7	1.6	1.4	1.7	1.0-2.7	2	0.6	0.6	1.1	0.6-0.7	7	10818	210	17.1	17-74800
Security	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cleaning	7	1.6	1.4	1.7	1.0-2.7	2	0.6	0.6	1.1	0.6-0.7	7	10818	210	17.1	17-74800
Institution B	91	21.6	6.5	4.0	0.3-555.9	20	5.24	1.7	6.2	0.1-24.7	104	314	171	2.65	16-6804
Clinical	63	16.5	7.2	3.7	0.3-117	18	4.8	1.7	5.8	6.1-24.8	72	357	175	2.9	16-6804
Maxillo-facial & Oral Surgery	17	8.5	5.4	2.6	0.8-42	7	8.3	2.8	8.8	0.1-24.7	23	209	134	2.6	16-908
Restorative / Conservation	25	23.4	10.9	3.8	1.2-117	6	2.8	0.8	6.9	0.1-10.4	28	248	178	2.3	55-761
Periodontology	1	6.6	6.6	-	-	-	-	-	-	-	-	-	-	-	-
Prosthodontics / Prosthetics	2	2.2	2.1	1.6	1.5-2.8	-	-	-	-	-	2	360	139	9.7	28-693
Orthodontics	2	12.9	8.7	3.8	3.4-22.4	-	-	-	-	-	2	3498	1146	12.4	193-6804
Dental Therapy & Hygiene	10	22.7	9.0	5.3	0.3-94.9	5	2.3	2.0	1.7	1.3-4.9	10	239	147	2.9	30-779
Sterilisation	6	7.2	3.0	3.7	0.8-32.2	-	-	-	-	-	7	547	320	3.0	70-2061
Laboratory	4	199.3	43.7	11.8	3.9-555.9	1	18.3	18.3	-	-	4	257	190	2.3	101-638
Technicians lab	4	199.3	43.7	11.8	3.9-555.9	1	18.3	18.3	-	-	4	257	190	2.3	101-638
Administration	9	4.3	2.3	3.1	0.4-20.2	1	0.2	0.2	-	-	9	311	234	2.2	97-816
Clerical	7	4.8	2.5	3.3	0.4-20.2	1	0.2	0.2	-	-	7	269	221	2.0	108-545
Reception / switchboard	2	2.6	1.7	3.9	0.7-4.5	-	-	-	-	-	2	457	282	4.5	97-816
Stores	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Auxiliary	15	6.2	4.7	2.2	1.3-20.9	-	-	-	-	-	19	163	131	2.0	57-430
Security	2	14.3	12.7	2.0	7.7-20.9	-	-	-	-	-	3	87	86	1.2	70-96
Cleaning	13	4.9	4.0	2.0	1.3-13.8	-	-	-	-	-	16	197	141	2.0	57-430

n, number of measurements; AM, arithmetic mean; GM, geometric mean; GSD, geometric standard deviation; where one sample was collected the standard deviation was not calculated.

Table 2.4 *Continued*

Exposure Matrix	Personal Samples (EU/m^3)					Area Samples (EU/m^3)					Water Samples (EU/ml)				
	n	AM	GM	GSD	Range	n	AM	GM	GSD	Range	n	AM	GM	GSD	Range
Institution C	49	4.1	2.9	2.3	0.4-19.3	16	3.5	0.9	5.6	0.1-20.6	56	509	320	2.7	16-5135
Clinical	38	3.3	2.4	2.2	0.4-19.3	14	3.7	0.8	5.8	0.1-20.6	46	534	317	2.9	16-5135
Maxillo-facial & Oral Surgery	8	4.5	2.7	2.7	0.9-19.3	6	4.0	0.8	6.5	0.1-20.6	11	405	352	1.9	74-687
Restorative / Conservation	19	3.3	2.6	2.2	0.4-7.1	5	1.3	1.1	2.3	0.3-2.4	21	773	416	3.1	33-5135
Periodontology	1	0.9	0.9	-	-	1	20.5	20.5	-	-	1	427	427	-	-
Prosthodontics / Prosthetics	1	3.5	3.5	-	-	-	-	-	-	-	2	298	236	2.7	116-479
Orthodontics	2	1.4	1.3	2.0	0.8-2.0	1	0.1	0.1	-	-	2	395	392	1.2	349-441
Dental Therapy & Hygiene	5	3.1	2.9	1.5	1.6-4.9	1	0.1	0.1	-	-	7	234	171	2.5	38-469
Sterilisation	2	2.0	1.7	2.3	0.9-3.1	-	-	-	-	-	2	210	81	9.6	16-403
Laboratory	2	9.7	7.7	2.7	3.8-15.6	1	3.8	3.8	-	-	2	453	452	1.0	432-474
Technicians lab	2	9.7	7.7	2.7	3.8-15.6	1	3.8	3.8	-	-	2	453	452	1.0	432-474
Administration	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Clerical	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Reception / switchboard	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Stores	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Auxiliary	9	6.0	4.6	2.3	1.6-15.0	1	0.2	0.2	-	-	8	376	309	2.5	34-446
Security	-	-	-	-	-	-	-	-	-	-	0	-	-	-	-
Cleaning	9	6.0	4.6	2.3	1.6-15.0	1	0.2	0.2	-	-	8	376	309	2.5	34-446
Institution D	63	5.8	3.3	2.7	0.3-53.3	13	0.8	0.5	3.7	0.1-6.4	63	177	44	7.11	1-2075
Clinical	47	6.7	3.6	2.7	0.3-53.3	9	0.6	0.4	3.1	0.1-1.4	52	210	61	6.6	2-2075
Maxillo-facial & Oral Surgery	10	7.6	2.7	2.2	0.5-53.3	1	0.8	0.8	-	-	12	109	48	5.7	2-463
Restorative / Conservation	3	7.5	6.1	2.3	2.5-12.6	3	0.8	0.5	4.3	0.1-1.4	2	57	57	1.0	-
Periodontology	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Prosthodontics / Prosthetics	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Orthodontics	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dental Therapy & Hygiene	31	6.9	4.2	2.7	0.3-47.6	3	0.4	0.2	4.0	0.1-1.0	35	270	78	7.1	2-2075
Sterilisation	3	1.7	1.4	2.1	0.7-2.9	2	0.7	0.6	1.9	0.4-1.0	3	16	8	4.1	2-38
Laboratory	2	4.2	4.2	1.2	3.7-4.8	-	-	-	-	-	2	2	2	1.6	1-3
Technicians lab	2	4.2	4.2	1.2	3.7-4.8	-	-	-	-	-	2	2	2	1.6	1-3
Administration	5	3.5	2.6	2.2	1.3-9.1	3	2.5	1.2	4.7	0.3-6.4	4	27	17	4.2	2-36
Clerical	4	3.9	2.9	2.4	1.3-9.1	3	2.5	1.2	4.7	0.3-6.4	4	27	17	4.2	2-36
Reception / switchboard	1	1.9	1.9	-	-	-	-	-	-	-	-	-	-	-	-
Stores	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Auxiliary	9	2.4	2.1	1.8	0.9-4.9	1	0.1	0.1	-	-	5	25	11	6.1	2-41
Security	2	2.6	2.4	1.7	1.6-3.5				-	-	-	-	-	-	-
Cleaning	7	2.4	2.0	1.9	0.9-4.9	1	0.1	0.1	-	-	5	25	11	6.1	2-41

n, number of measurements; AM, arithmetic mean; GM, geometric mean; GSD, geometric standard deviation; where one sample was collected the standard deviation was not calculated.

Table 2.4 *Continued*

Exposure Matrix	Personal Samples (EU/m^3)					Area Samples (EU/m^3)					Water Samples (EU/ml)				
	n	AM	GM	GSD	Range	n	AM	GM	GSD	Range	n	AM	GM	GSD	Range
Institution E	82	15.3	8.9	3.5	0.1-73.6	14	0.8	0.5	3.0	0.1-3.2	66	46	41	1.7	3-95
Clinical	72	16.2	9.3	3.1	0.1-73.6	13	0.9	0.6	2.6	0.1-3.2	57	48	44	1.7	3-95
Maxillo-facial & Oral Surgery	18	10.7	4.9	3.4	0.1-31.5	6	0.9	0.6	3.1	0.1-3.2	15	49	47	1.4	19-95
Restorative / Conservation	27	21.2	15.2	2.6	2.1-62.7	3	0.8	0.8	1.1	0.8-0.9	24	46	40	2.0	3-81
Periodontology	6	9.7	6.1	3.0	1.8-24.3	4	0.8	0.6	3.1	0.1-2.0	7	48	46	1.4	22-62
Prosthodontics / Prosthetics	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Orthodontics	5	17.5	10.8	4.5	1.8-24.3	-	-	-	-	-	5	54	54	1.0	54-56
Dental Therapy & Hygiene	7	18.4	14.3	2.7	1.6-28.4	-	-	-	-	-	3	52	51	1.1	46-55
Sterilisation	9	13.7	6.8	3.1	1.4-73.6	-	-	-	-	-	3	44	42	1.5	27-53
Laboratory	3	8.1	6.3	2.3	3.1-16.2	-	-	-	-	-	3	19	19	1.0	-
Technicians lab	3	8.1	6.3	2.3	3.1-16.2	-	-	-	-	-	3	19	19	1.0	-
Administration	3	4.3	3.5	2.3	1.5-8.1	1	0.1	0.1	-	-	2	54	54	1.2	48-60
Clerical	2	5.7	5.2	1.9	3.4-8.1	1	0.1	0.1	-	-	2	54	54	1.2	48-60
Reception / switchboard	1	1.5	1.5	-	-	-	-	-	-	-	-	-	-	-	-
Stores	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Auxiliary	4	13.5	9.6	2.4	5.3-34.5	-	-	-	-	-	4	26	26	1.1	23-26
Security	1	8.8	8.8	-	-	-	-	-	-	-	1	23	23	-	-
Cleaning	3	15.1	10.0	2.9	5.3-34.5	-	-	-	-	-	3	26	26	-	-
Institution F	51	6.2	4.5	2.2	1.5-35.5	24	0.9	0.6	2.7	0.1-2.2	36	29	15	4.3	1-70
Clinical	36	5.7	4.3	2.1	1.5-17.5	21	0.9	0.6	2.5	0.1-2.2	26	30	14	4.7	1-70
Maxillo-facial & Oral Surgery	5	3.4	3.3	1.4	2.4-17.4	3	1.0	1.0	1.1	0.9-1.1	4	25	14	4.9	1.4-50
Restorative / Conservation	13	4.5	3.5	2.0	1.5-16	9	0.9	0.6	2.7	0.1-2.2	11	45	36	2.4	3-70
Periodontology	4	3.4	3.2	1.5	2.3-5.5	2	1.2	1.2	1.1	1.2-1.3	4	15	3.0	6.4	1-56
Prosthodontics / Prosthetics	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Orthodontics	2	11.5	10	2.1	5.9-17	2	1.0	1.0	1.4	0.8-1.2	1	29	29	-	-
Dental Therapy & Hygiene	6	10	8.2	2.1	2.5-17.5	2	0.2	0.2	2.5	0.1-0.4	1	50	50	-	-
Sterilisation	6	5.7	4.1	2.5	1.6-10.9	3	0.8	0.5	3.9	0.1-1.4	5	8	4	3.9	2-26
Laboratory	5	10.8	6.6	2.7	2.8-35.5	1	1.5	1.5	-	-	2	41	39	1.7	27-55
Technicians lab	5	10.8	6.6	2.7	2.8-35.5	1	1.5	1.5	-	-	2	41	39	1.7	27-55
Administration	7	5.0	4.0	2.0	1.9-14.8	1	0.1	0.1	-	-	7	19	11	3.6	3-51
Clerical	5	3.7	3.5	2.7	2.8-35.5	1	0.1	0.1	-	-	5	26	19	3.0	3-51
Reception / switchboard	2	8.3	5.2	1.4	2.5-6.1	-	-	-	-	-	2	3	3	-	-
Stores	-	-	-	-	-	-	-	-	-	-	0	-	-	-	-
Auxiliary	3	7.7	4.9	3.1	2.5-18.1	1	1.3	1.3	-	-	1	32	32	-	-
Security	2	10.3	6.8	4.0	2.5-18.1	0	-	-	-	-	-	-	-	-	-
Cleaning	1	2.6	2.6	-	-	1	1.3	1.3	-	-	1	32	32	-	-

n, number of measurements; AM, arithmetic mean; GM, geometric mean; GSD, geometric standard deviation; where one sample was collected the standard deviation was not calculated.

2.2.5 CORRELATION BETWEEN EXPOSURE METRICS

Pearson correlations between water samples with personal and area air samples were done for the overall clinic samples. An inverse correlation was found between the water samples from the DUWLs and the personal air samples collected from the clinical department ($r = -0.39$, $p < 0.001$, $n = 335$) (**Figure 2.2**). No correlation was found between the area endotoxin measurements and the endotoxin in the DUWLs ($r = 0.07$, $p > 0.05$, $n = 226$).

No correlation was observed between the air samples and the endotoxin levels from tap water samples collected from the laboratory, administration and auxiliary departments.

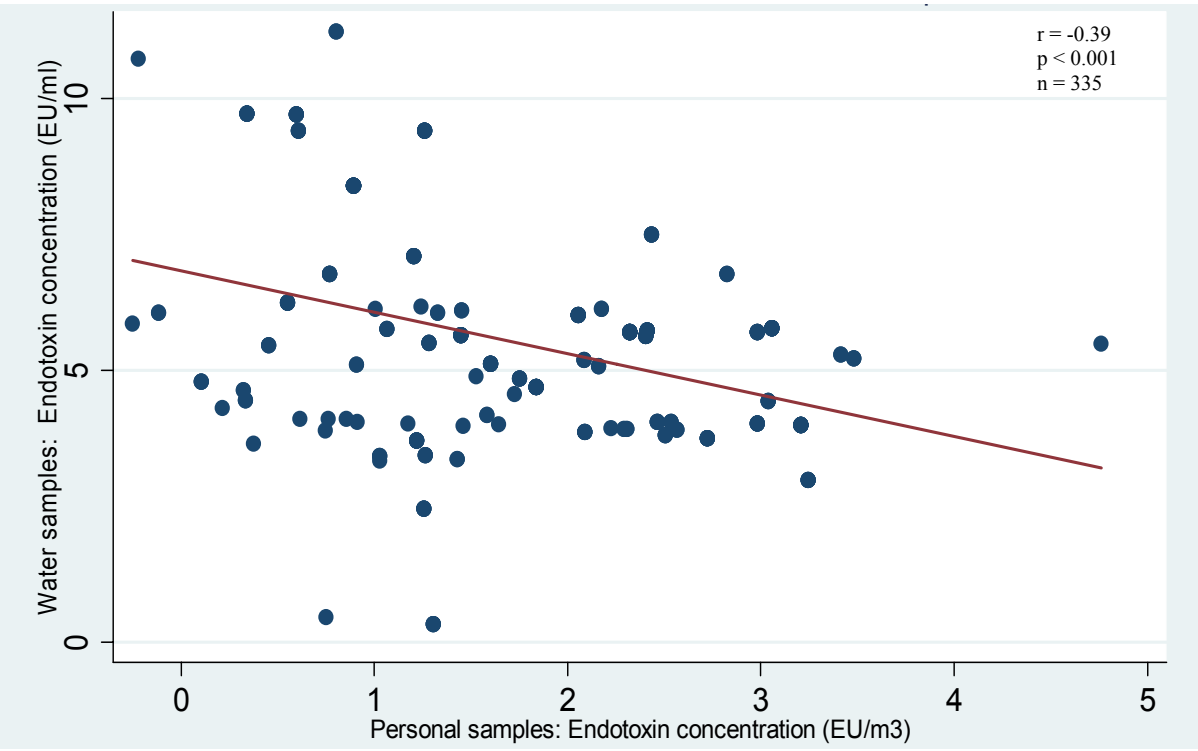


Figure 2.2 Correlation between personal air endotoxin samples and endotoxin in water samples collected from DUWLs for the clinical department.

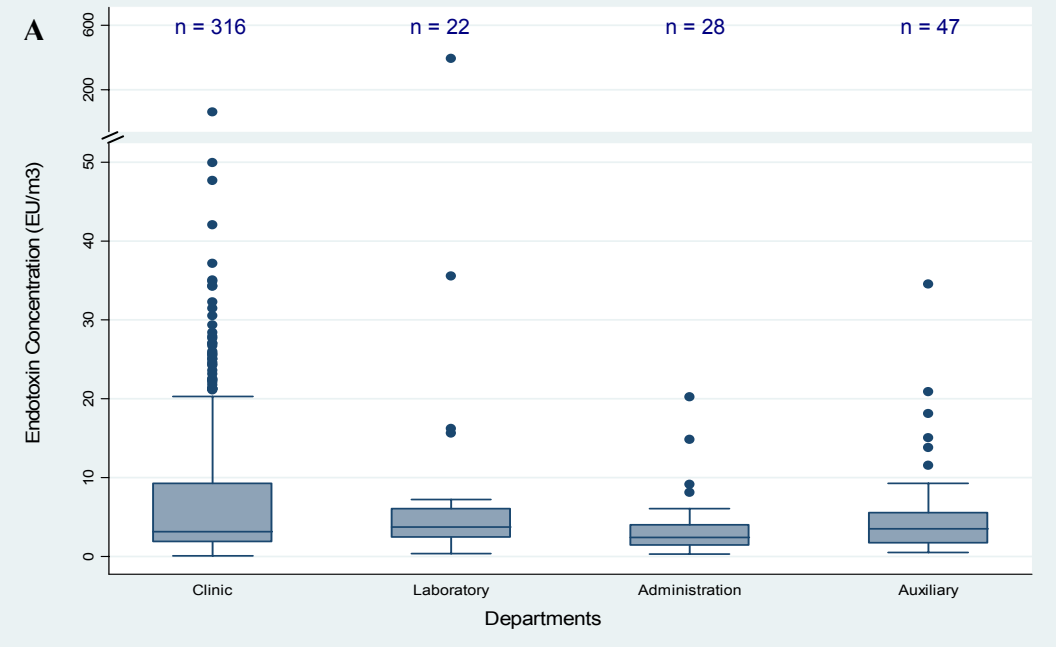
2.2.6 VARIANCE COMPONENTS

Table 2.5 shows the variance components of endotoxin levels, namely between-group (BG) and within-group (WG) by institution and other variables. The p-value (< 0.05) indicates that there is a difference between and within the variable; however it does not demonstrate where the difference is. Overall, the BG variability was lower than the WG variability for departments as is evident in **Figure 2.3**. Endotoxin air levels were significantly lower in the administration and auxiliary departments as compared to the clinical department ($p < 0.05$). The endotoxin air levels were also significantly lower in the administration department compared to the laboratory. However, the endotoxin levels were not significantly different between the clinic and laboratory ($p > 0.05$), as well as the administration and auxiliary departments. Overall, the average endotoxin levels in water and air samples were higher for the clinical workers (clinical staff and students) than the non-clinical workers.

Table 2.5 Between-group and within-group variance components based on log transformed, ln data (n = 413).

	Endotoxin levels, Ln (EU/m^3)		
	BG	**WG**	**p-value**
Institution	0.46	1.06	<0.001
Department	0.05	1.44	0.036
Exposure group	0.05	1.42	0.016
Job type	0.16	1.30	<0.001
Tasks	0.04	1.42	0.034

BG, between-group variance components
WG, within-group variance components by institution and other variables



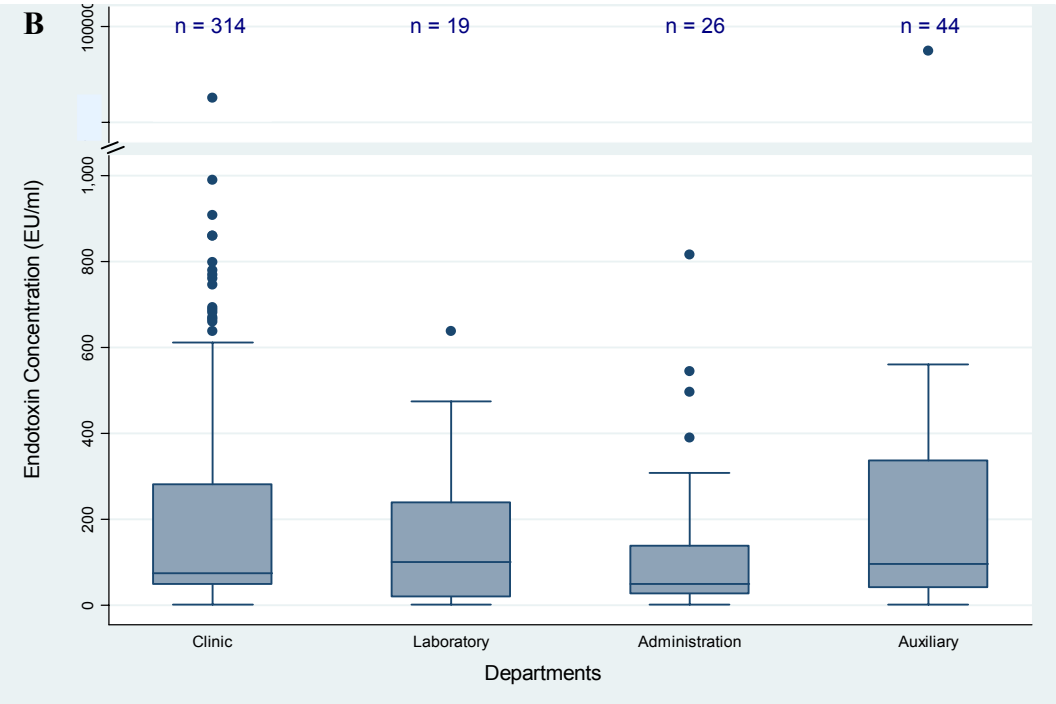


Figure 2.3 Endotoxin concentration in a) personal air samples and b) water samples by department. A box-and-whisker plot was used to display the endotoxin data. The box encompasses the 25th to the 75th percentile of the data, with the median marked as an internal line. The whiskers show the range of the data excluding the outliers, which are represented by the dots. Values greater than 1.5 times the interquartile ranges above the 75th or below the 25th percentile are plotted as dots.

2.2.7 DETERMINANTS OF EXPOSURE AND EXPOSURE VARIABILITY

Among the occupational factors investigated in univariate regression analyses, the age of the dental units explained the most variability observed in the personal air samples ($R^2 = 0.20$, $p < 0.001$), followed by season of the year ($R^2 = 0.11$, $p < 0.001$) (**Table 2.6**). Institution and total number of dental units per institution also explained a modest degree of variability in airborne endotoxin exposure. Based on the variance difference between various job types, the 14 job titles were collapsed into four similarly exposed groups (SEGs); students, clinical staff, administrative related work and auxiliary services. The job titles explained 3% of the variance in endotoxin levels. Other explanatory variables such as tap water explained a much lower variability in endotoxin concentration.

Table 2.6 Specific exposure determinants of personal airborne endotoxin concentrations (log transformed, ln) among dental HCWs in univariate regression models.

Variable	Personal endotoxin level, Ln (EU/m^3)		
	β (SE)	R^2	p-value
Microclimatic parameters (n = 116)			
Temperature (˚C)	-0.05(0.03)	0.02	0.051
Humidity (%)	-0.03(0.01)	0.05	0.006
Air velocity (m/s)	-8.41 (2.14)	0.05	< 0.001
CO_2 (ppm)	0.001 (0.001)	0.02	0.155
Season	0.25 (0.04)	0.11	< 0.001
Sampling day	0.06 (0.05)	0.01	0.298
Occupational factors (n = 413)			
Institution	0.20 (0.03)	0.08	< 0.001
Building age (years)	0.01 (0.01)	0.03	< 0.001
Departments	-0.10 (0.06)	0.01	0.078
Exposure group	-0.02 (0.02)	0.01	0.159
Job type[1]	0.24 (0.07)	0.03	< 0.001
Tasks	-0.04 (0.02)	0.01	0.043
Dental student versus clinical staff[2]	0.30 (0.13)	0.01	0.020
Dental unit model	0.20 (0.05)	0.06	< 0.001
Total number of dental units per institution	0.01 (0.001)	0.08	< 0.001
Age of dental units (years)	0.06 (0.01)	0.20	< 0.001
Number of patients seen	-0.01 (0.01)	0.03	0.009
Municipal vs reservoir water source	-0.09 (0.15)	0.01	0.567
DUWL vs tap water source	0.25 (0.14)	0.01	0.073
Endotoxin in water (EU/ml)	-0.05 (0.03)	0.01	0.111

The value in each cell represents a separate regression model.
[1] Fourteen job types were classified into one of four similarly exposed groups (clinical staff, students, administration and auxiliary)
[2] Clinical staff used as baseline for comparison

The multivariate model explained 40% of the variability in the level of endotoxin in the personal air samples (model: adjusted $R^2 = 0.40$, $p < 0.001$, $n = 100$) (**Table 2.7**). Variables which explained the greatest variability of endotoxin in the air included: the age of institution buildings, total number of dental units per institution, air velocity, temperature, job category (staff versus student), age of the dental unit and the dental unit model. Apart from job category, dental (e.g. age of dental unit and certain dental unit models) and institutional characteristics (e.g. working in older buildings, number of dental units per institution) were identified as important determinants of airborne endotoxin exposure. Measurements with missing data for a specific factor were omitted from the analysis resulting in a reduction of the total number of measurements in the model. Since job types which contain non-clinical functions were included in the model it reduced the sample size as these subjects were not assigned to a dental unit.

The final model satisfied post-regression diagnostic tests (linearity, normality, $p = 0.24$; constant variance, $p = 0.73$; omitted variables, $p = 0.08$). The model can be expressed as:

Log (endotoxin personal concentration) = $\beta 0$ + $\beta 1$ building age + $\beta 2$ total number of dental units + $\beta 3$ air velocity in work areas + $\beta 4$ ambient temperature in work areas + $\beta 5$ log (endotoxin in water) + $\beta 6$ job type + $\beta 7$ dental unit model + $\beta 8$ age of dental unit

Table 2.7 Multivariate linear regression for endotoxin levels in personal air samples (log transformed, ln) in dental clinics.

Variable	Personal endotoxin level, Ln (EU/m^3), n = 100	
	β (SE)	p-value
Intercept	-25.39 (9.00)*	0.001
Building age (years)	0.15 (0.06)*	0.002
Total number of dental units (no.)	0.05 (0.02)**	<0.001
Air velocity (m/s)	-22.65 (7.80)*	0.012
Temperature (˚C)	0.36 (0.17)*	0.010
Log (endotoxin in water) (EU/ml)	-0.06 (0.06)	0.488
Dental student versus clinical staff [1]	1.04 (0.29)**	<0.001
Dental unit model: A-dec [2]	8.25 (2.32)**	<0.001
Dental unit model: Promas [2]	7.20 (2.30)**	<0.001
Dental unit model: Ritters [2]	-15.34 (4.38)**	<0.001
Dental unit age	0.88 (0.24)**	<0.001

[1] Clinical staff used as baseline for comparison
[2] Dental unit model: Pelton used as base of comparison (lowest endotoxin levels in DUWLs)
* $p<0.05$; ** $p<0.001$

2.3 DISCUSSION

The findings of this study demonstrated considerably high concentrations (100 000 EU/ml) of endotoxin in the DUWLs as well as high levels (555.9 EU/m^3) of endotoxin aerosolised in the dental clinic environments compared to previous studies (Huntington et al., 2007; Dutil et al., 2009). The high levels may be attributed to a number of factors (e.g. stagnation, surface area, flow rates, inadequate disinfection, and presence of biofilm) but most importantly indicates the occurrence of occasional high exposure values during dental procedures. The average endotoxin level for the administration group was 3.84 EU/m^3 which corresponds with the mean endotoxin background level of 0.063-3.600 EU/m^3 reported for background environments (Madsen, 2006). It is clear that the administration department (unexposed grouping descriptor) demonstrated less exposure which confirms that DUWLs are a potential source of endotoxin exposure. These results are consistent with other studies that demonstrate that DUWLs are an important source of bacterial endotoxin

contaminating the dental clinic environment (Fulford et al., 2004; Huntington et al., 2007). Factors that have been identified for the increased levels within the DUWLs include quiescent periods experienced during weekends, study breaks and vacations, increased temperatures, lack of disinfection procedures, poor water quality, small bore size of DUWLs and low flow rates (< 60 ml/minute) (Putnins et al., 2001; Franco et al., 2005). These factors have been extensively studied and for this reason were excluded from the current study. Factors that have not been studied or rarely reported were a focus in the current study.

Although the majority (97%) of personal air endotoxin concentrations found in this study were below the recommended limit of 50 EU/m^3, considered to be associated with no health effects (Heederik and Douwes, 1997), there were a small number of samples that were above this level. It is conceivable that the low-grade concentrations of endotoxin measured in this study may still be significant as chronic endotoxin inhalation has been reported to pose an occupational hazard in different settings (Reed and Milton, 2001; Liu, 2002; Huntington et al., 2007). Airborne endotoxin as low as 28 EU/m^3 have been reported to result in pulmonary function damage (Rose et al., 1998). Endotoxins have not only been implicated in organic dust toxic syndrome but may also act as an adjuvant facilitating sensitisation to other allergens or they may increase the severity of allergic disease (Boehlecke et al., 2003; Szymanska, 2005b; Huntington et al., 2007). Furthermore there were 11 samples in the current study with levels above the recommended level of 50 EU/m^3 (Heederik and Douwes, 1997) of which 5 samples were above 90 EU/m^3, the revised HBROEL that has been proposed recently (Dutch Expert Committee on Occupational Safety and Nordic Expert Committee, 2009). It is also the level suggested as a threshold for acute mucous membrane irritation and pulmonary change among cotton workers (Haglind and Rylander, 1984). These elevated measurements reflect a potential for intermittent peak exposure of dental personnel to

levels that may induce acute endotoxin-related effects (Liebers et al., 2008). While low-grade endotoxin levels predominated, the variability observed is suggestive of the fact that endotoxin are components of growing microorganisms in a dynamic ecosystem which explains the variability of endotoxin concentrations (Spaan et al., 2008). A study by Putin and colleagues reported differences in contamination within and between dental units indicating the variability of exposure in this setting (Putnins et al., 2001). The levels within DUWLs may decrease during flushing and decontamination treatments; however, the waterlines are conducive to the promulgation of microorganisms during favourable conditions, therefore endotoxin is expected to reach levels above the recommended limits. For this reason, the relationship between chronic inhalation exposure and respiratory illness in this group of professionals needs further exploration.

One of the interesting findings of this study was the inverse correlation ($r = -0.39$, $p < 0.001$, $n = 335$) observed between endotoxin levels in DUWLs and in personal air samples collected from dental personnel during clinic sessions. This was similar to the study of Szymanska et al. in which a significant inverse correlation ($r = -0.65$, $p < 0.001$, $n = 25$) between these indices was demonstrated (Szymanska, 2005c). In another study, while detectable levels (2.7 EU/m^3) of airborne endotoxin were found, Huntington M.K. and colleagues (personal communication, December 06, 2007) did not find a correlation between these two parameters. The inverse relationship found in this study may be partially attributable to particle size. Large aerosols (> 15 μm) tend not to disperse very far and settle out quickly whereas smaller particles (< 5 μm) may have the potential for a greater degree of dispersion (Bennett et al., 2000a). It is likely that only a small component of the endotoxin adherent particles that exit the waterlines are captured by the sampling device (Bennett et al., 2000b). The inverse correlation may also be attributable to other factors that could influence the levels in the air and waterlines such as ventilation, duration of clinical session, decontamination of waterlines,

aspiration of water and time of sampling as is evident in the multivariate regression model compared to the univariate model. According to Rylander, endotoxin concentrations depend on its physical state in samples. Endotoxin concentration in dust samples may be lower than in water solution because the LAL assay may underestimate the total amount of bioactive material as the endotoxins may still be part of fragments of an intact bacterial cell wall. A ratio of 1:10 between the concentration of endotoxin in dust samples and bioactive material has been suggested (Rylander, 2002).

An important finding of this study is the identification of factors that determine increasing endotoxin levels in the dental setting. In this study, a number of exposure determinants were significantly associated with endotoxin levels in dental clinic settings, with the identified factors explaining 40% of variability in airborne endotoxin levels. Determinants of endotoxin levels that have practical implications for the dental clinics are the dental unit characteristics such as the model of the dental units, the age of the dental units and the total number of dental units per institution, as well as microclimatic factors such as temperature. One of the three dental unit models most commonly used within the dental institutions showed a protective effect. Although the age of dental units is an important determinant of endotoxin exposure, the age of the dental unit in question was similar to the other dental unit models in use (~25 years old). Therefore it is likely that other factors regarding the dental unit model (e.g. type of materials used in the tubings, bore size of the tubing and the frequency of use) (Challacombe and Fernandes, 1995) may influence the level of endotoxin levels.

Another factor that has implications for dental practice involves dental students' practicals in dental clinics. Students were shown to be exposed on average to higher levels of endotoxin during their practical sessions then other jobs types irrespective of whether the latter were clinical or non-clinical

in nature. During clinic inspections, it was evident that the students were supplied with inappropriate respiratory protective equipment as in **Figure 2.4**. Students usually work in pairs, and the assisting student who is also working in close proximity to the DUWL is not protected with suitable personal protective equipment (PPE). However, there were considerable fluctuations in the levels of endotoxin among the dental students of different years of study. This may be related to the type of dental procedure being performed at the time of sampling, as is the case with increased volumes of water being used for certain procedures such as restorative dentistry (Harrel, 2003; Agostinho et al., 2004). It is possible that because clinical staff perform supervisory roles and are not sedentary for most of the clinic session they may be exposed to lower levels of endotoxin. These findings highlight the need for adequate measures to reduce exposure levels to training students. PPE and training on good hygiene practices should be provided to improve worker behaviour.



Figure 2.4 Photograph illustrating dental students with poor PPE compliance.

Another concern for academic settings, public and military dental clinics is the large numbers of dental units per clinic, as increasing number of units appears to increase the endotoxin levels. Previous studies have demonstrated increases in bacterial air contamination following dental treatment (Miller et al., 1971; Grenier, 1995). It follows that increasing the number of dental units per clinic would equate to increasing dental treatments and subsequent increase in airborne microbial load if infection control procedures are not adhered to. In addition, aerosols generated during dental procedures have the capacity to spread throughout multichair dental clinic environments, even into areas where there is no dental activity (Grenier, 1995), thus posing a potential risk to both clinical as well as non-clinical staff.

A microclimatic factor such as indoor temperature was shown to be a good predictor of airborne endotoxin levels in dental settings, while air velocity can be a key element in a reduction strategy to control endotoxin levels. These findings suggest that increasing ventilation would assist in reducing the endotoxin load in dental setting environments.

In this study ageing dental units and ageing buildings were shown to increase the endotoxin levels. This implies that the dental units provide an environment conducive to the rapid proliferation of microorganisms. In the absence of an adequate infection control regimen the microorganisms would increase to unacceptable levels (Szymanska, 2003b). In contrast, age of a building may be a proxy for many other variables, for example older buildings may have less efficient ventilation systems, contaminated air-conditioning systems which recirculate microorganisms in the air or they may have old water distribution pipes which may be colonised with microorganisms.

Since all dental schools in South Africa were selected for this study, the exposure conditions described in this study are reasonably representative of public sector dental clinics in the country. To our knowledge, this is the first study to report important dental institutional characteristics that determine endotoxin exposure levels in dental clinic settings. The findings of this study provide a basis for designing dental clinics taking cognisance of the relative importance of certain factors over others in reducing endotoxin exposure. One of the limitations of the study was not being able to do repeat measurements to ascertain the variability within individuals. However this study provides a good basis for determining inter-group predictors of endotoxin exposure.

In conclusion, this study identified several workplace determinants that predict endotoxin exposure in dental settings which can be manipulated to reduce exposure. The ability of low-grade endotoxin exposure to produce ill health effects warrants further investigation. Future studies should also assess the value of the identified predictors of exposure in reducing the endotoxin levels within dental settings.

CHAPTER 3

WORK-RELATED ASTHMA PHENOTYPES IN DENTAL HEALTH CARE WORKERS IN SOUTH AFRICA

3.0 INTRODUCTION

Asthma is a common disease of the airways and has been described in the recent literature as a spectrum of phenotypes, resulting from the interaction between genetic makeup and the environment (Wardlaw et al., 2005; Hargreave and Parameswaran, 2006; Oryszczyn et al., 2007). Asthma presents itself as a complex of multiple, separate syndromes that overlap. Three broad categories with substantial overlaps have been reported in the literature; phenotypes defined by clinical or physiological criteria; phenotypes related to environmental triggers; and phenotypes defined by their pathobiology on the basis of patterns of inflammation, specifically the presence or absence of particular inflammatory cell types (Wenzel, 2006) (**Table 3.1**).

The OA phenotype accounts for up to 18% of adult-onset asthma (Toren and Blanc, 2009). OA has several sub-phenotypes, which result from: 1) development of an immunologically mediated sensitisation to the causal agent, usually HMW agents, causing an IgE-mediated response similar to allergic asthma; 2) development of an immunologically mediated response to LMW agents, in which an IgE response is not consistently seen; and 3) development of a non-immunological, rapid-onset irritant response after exposure to chemicals (Wenzel, 2006; Jeebhay and Quirce, 2007).

Table 3.1 Potential phenotypic categories of asthma.

Phenotypes	Description
Clinical or Physiological[1]	
Severity-defined[2]	Asthma defined by severity on the basis of lung function, symptoms and use of medication.
Exacerbation-prone	Asthmatics predisposed to frequent exacerbations which can be very severe.
Defined by chronic obstruction	Asthma present with marked airflow obstruction but patients have only moderately symptomatic or exacerbation-prone disease.
Treatment resistant[2]	Asthma that is resistant to treatment, generally corticosteroids, and common in severe asthmatics, probably due to a different type of inflammation.
Defined by age of onset	The age at which a patient develops asthma could identify features specific to their disease. Early-onset asthma has a greater likelihood of allergic sensitisation and clinical response to triggers than late-onset disease.
Triggers[1]	
Allergic sensitisation[3]	Allergic sensitisation to common inhalant allergens is a common trigger of asthma. Family history of asthma has been shown to be associated with allergic asthma.
Anti-inflammatory drugs	Aspirin and other non-steroidal anti-inflammatory drugs are common triggers of asthma.
Occupational allergens or irritants	Exposure to allergens or irritants in the work environment has been implicated in asthma.
Menses[4, 5]	Exacerbations of many medical disorders (e.g. asthma) are associated with the luteal phase of the menstrual cycle or with the onset of menses itself. However the hormonal mechanisms are not known.
Exercise[3]	Exercise-induced asthma is common. The mechanisms seem to involve acute inflammatory, epithelial, and vasoactive responses.
Pathobiological[1, 6]	
Eosinophilic	Asthma characterised by a high proportion of eosinophils in sputum.
Neutrophilic	Asthma characterised by a high proportion of neutrophils in sputum.
Granulocytic	Asthma characterised by increased levels of both neutrophils and eosinophils in sputum.
Pauci-granulocytic	Asthma characterised by normal levels of both neutrophils and eosinophils in sputum.

Adapted from: [1](Wenzel, 2006); [2](Diamant et al., 2007); [3](Nieves et al., 2005); [4](Ensom et al., 1999); [5](Real et al., 2008); [6](Simpson et al., 2006)

Various studies have suggested that exposure in the dental environment, in particular dental workplace aerosols, increases the risk of respiratory disease in dental HCWs and patients (Scannapieco et al., 2004). Dental personnel also use or come into contact with many potential allergens and irritants in the course of their work that may have deleterious effects on their health (Gawkrodger, 2005). Common causative agents implicated in asthma in this group of professionals include natural rubber latex (NRL) (Henriks-Eckerman et al., 2001), methacrylates (Quirce et al., 2001; Jaakkola et al., 2007) and more recently endotoxin (Szymanska, 2005b; Huntington et al., 2007; Dutil et al., 2009).

The incidence of asthma was evaluated in a population-based case-control study from Finland that looked at the association between different occupations and new adult-onset asthma. The risk of asthma was increased among dental workers (including dentists and dental assistants) with an OR of 4.74 (95% CI: 0.48 – 46.5) (Jaakkola et al., 2003). Similarly, a cross-sectional analysis of 14151 adults from a French air pollution study also showed a slightly increased risk of asthma (OR: 1.45, 95% CI: 0.57 - 3.66) among dentists (Le Moual et al., 2004). In another cross-sectional study from France, dental technicians had increased prevalence of respiratory symptoms but the actual causes were not investigated (Radi et al., 2002). A cross-sectional multicentre survey of 265 dentists using DUWLs in general practice found that 14% of the dentists enrolled in the study reported "ever having suffered" from asthma either in childhood or as an adult, although there was no significant association with any microbial variables evaluated (Pankhurst et al., 2005). In a sub-population of this study, dentists who were exposed to aerobic counts at a concentration ≥ 200 cfu/ml in their dental surgery were more likely to report symptoms of asthma since commencement of employment. The study demonstrated that the temporal onset of asthma may be associated with occupational exposure to contaminated DUWLs. A detailed study of different asthma phenotypes among South African dental workers has to our knowledge not been conducted.

The aims of this study were to:

- determine the prevalence of various asthma phenotypes that could be identified in dental HCWs based on environmental and work-related triggers and clinical presentation,
- determine the host risk factors (e.g. atopy, smoking, sex, previous history of lung disease) and work-related factors (e.g. professional status: staff vs student, employment duration, proximity to aerosol source, self-perceived intensity of exposure, glove use) associated with these various asthma phenotypes.

3.1 MATERIALS AND METHODS

3.1.1 STUDY DESIGN AND SAMPLING

A cross-sectional study was conducted on 454 dental personnel in five academic dental institutions (comprising six sampling sites) situated in four provinces in South Africa. Participation was voluntary and participants completed a consent form before being interviewed.

3.1.2 HEALTH AND EMPLOYMENT QUESTIONNAIRE

The study collected information on important demographic factors. Each participant answered a modified questionnaire (see Appendix C) specifically designed for the investigation of asthma contained in the Protocol for the European Community Respiratory Health Survey (European Community Respiratory Health Survey, 1993) and recent study of seafood-processing workers (Jeebhay et al., 2008). It 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.

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 their 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?".

3.1.3 SERUM ANALYSIS FOR PHADIATOP, RECOMBINANT LATEX AND LATEX-SPECIFIC IGE LEVELS

A 6 ml blood sample was taken from workers using a Becton Dickinson Vacutainer CAT tube (with clot activator). Samples were left to coagulate for 3-6 hours at room temperature and were then centrifuged at 1500 g for 10 minutes. The serum was transferred into 5 ml plastic cryotubes (Nunc, Denmark) and placed in storage at -20°C until transported to the laboratory and then stored at -70°C until analysis. Blood samples for serum analysis for common inhalants using Phadiatop® test and latex-specific IgE (k82) (*Hevea brasiliensis*) determinations from participants (n = 421) were obtained. Data from 33 subjects were not included in the analysis for various reasons including haemolysed samples, insufficient serum samples and refusal to provide sample for religious purposes. Specific IgE antibody tests to individual recombinant latex allergens (n = 85) (Hev b 1, Hev b 3, Hev b 5, Hev b 6.01, Hev b 6.02, Hev b 8, Hev b 9, Hev b 11) as well as cross-reacting carbohydrate determinants (CCDs) (horse radish peroxidase (HRP) and bromelain (MUXF3)) were

performed on workers (n = 41) with a positive response to latex (k82) as well as those who reported latex-related symptoms (n = 35) and healthy controls (n = 9). Maltose-binding protein (MBP) was also included in the analysis as a negative control. Tests were done according to the manufacturer's instructions using the ImmunoCAP system 100 (Phadia AB, Uppsala, Sweden). A value of $\geq$ 0.35 kU/l was considered positive.

3.1.4 SPIROMETRY

Spirometry was conducted according to the American Thoracic Society (ATS) guidelines (American Thoracic Society (ATS), 1995). A Jaeger Masterscope spirometer (Höchberg, Germany), calibrated at least twice a day with a 3 L syringe, was used. The tests were conducted by trained technologists. Each participant performed up to eight trials to produce three acceptable curves, in a sitting position without nose clips.

Test reproducibility was used as a guide to whether further attempts were necessary. Reproducibility criteria included the two best tracings for both forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC) varying by no more than 0.2 L (van Schalkwyk et al., 2004). However, failure to meet reproducibility criteria did not result in exclusion of the spirogram results from the statistical analysis. Poor reproducibility may also be an independent marker of airway dysfunction (Becklake and White, 1993). The lung function indices of primary interest included FEV_1 and FVC. The best FEV_1 and FVC readings were used regardless of whether they belonged to the same tracing. Measurements obtained by spirometry were adjusted according to the temperature and atmospheric pressure measured throughout the day.

Spirometry was conducted during the working day, depending on when the subject was scheduled. Owing to logistical reasons these tests were scheduled throughout the working week, rather than towards the end of the working week, which would have been preferable. Special instructions were given to workers to ensure that they did not smoke tobacco (at least one hour) and did not use any anti-asthmatic inhalers (4 hours) or take oral asthma medications (8 hours) prior to the test. Workers' heights were recorded for calculating predicted lung function indices using reference values of the European Community for Coal and Steel (ECCS) with lower limits corresponding to the 95th percentile (Quanjer et al., 1993). Exclusion criteria were conditions that might expose the participant to increased risk or discomfort, such as pregnancy, nursing mothers, epilepsy, recent (3 months prior) stroke or heart attack, recent (3 weeks prior) respiratory tract infection, TB diagnosis, high blood pressure and inability to perform acceptable quality spirometry. Nine participants were excluded from doing spirometry due to these health reasons.

Post-bronchodilator tests were conducted on the majority of subjects who participated in the baseline test with the exception of 18 subjects where this was not logistically possible. The bronchodilator test was done to determine whether airway obstruction was reversible with inhaled beta-2 agonists (salbutamol, 400 μg). An improvement of at least 12% and 200 ml after administration of salbutamol was regarded as a significant response. The post-bronchodilator test was done at least 10 minutes after administration of the bronchodilator. Both pre- and post-bronchodilator FEV_1 had to be reproducible for calculating the degree of the bronchodilator response.

3.1.5 STATISTICAL ANALYSIS

The general approach involved univariate, bivariate and multivariate analyses of the outcomes in relation to the predictors of interest. Univariate analyses summarised the distribution of each measured variable. Exploratory bivariate analyses were used to assess the nature of the associations between outcomes and covariates. Multiple logistic regression analysis was carried out to assess the association between risk factors (host and occupational risk factors) and the definitions of asthma. Odds ratios were used as the measure of effect and were estimated along with 95% CIs. In this study we adjusted for potential confounders, including age, smoking habits and sex. The key disease outcome variables of interest were WAA, atopic asthma and non-atopic asthma. Definitions of the various asthma phenotypes are described in **Table 3.2**. Statistical analyses were performed using STATA9 computer software (StataCorp, 2007, Texas, USA).

Table 3.2 Definitions of asthma phenotypes based on questionnaire and clinical assessment.

Variable	Criterion
Atopic asthma	Defined as either having an asthma attack **or** use of asthma medication in the past 12 months **or** positive bronchodilator test (increase in FEV_1 and / or FVC $\geq$ 12% and $\geq$ 200 ml increase compared with baseline); **and** presence of atopy*
Non-atopic asthma	Defined as either having an asthma attack **or** use of asthma medication in the past 12 months **or** positive bronchodilator test (increase in FEV_1 and / or FVC $\geq$ 12% and $\geq$ 200 ml increase compared with baseline); **and** absence of atopy
Work-aggravated asthma	Defined as either having an asthma attack **or** use of asthma medication in the past 12 months **or** positive bronchodilator test (increase in FEV_1 and / or FVC $\geq$ 12% and $\geq$ 200 ml increase compared with baseline); **and** work-related chest symptoms (tight chest or wheezing when at work *or* job change due to work-related symptoms *or* improvement after changing jobs)

Adapted from: (Jeebhay et al., 2008; Baatjies et al., 2009)
* Defined as positive response to Phadiatop test

3.2 RESULTS

3.2.1 DEMOGRAPHIC CHARACTERISTICS

The mean age of study participants was 36 years and 74% of study participants were female (**Table 3.3**). The average body mass index (kg/m^2) for the participants was 27 which is the overweight category. The majority of the subjects were non-smokers (79%), 13% were current smokers and 8% ex-smokers. Among the smokers, the average age when they started smoking was 20 years. Smoking was more prevalent in staff from institutions D, E and F.

3.2.2 OCCUPATIONAL CHARACTERISTICS AND EXPOSURE PROFILE

The average employment duration in the current job was 10 years (**Table 3.4**). A large proportion (~50%) of the study population reported being exposed to sprays in the work environment and worked close (< 1 m) to the source of exposure. There were 22% who reported excessive exposure (i.e. exposure exceeding that thought to be normal or usual) and 18% experienced chest symptoms due to peak exposures (i.e. the largest amount of exposure that a person is exposed to at one time). There were 65% of participants who reported using PPE, which included gloves, face masks, goggles and laboratory coats. Most clinic staff and students (95%) in the dental clinics and the laboratories used PPE whereas only 78% of cleaning staff wore PPE. Almost half of the subjects reported wearing powdered latex gloves while only 5% used non-latex gloves.

Table 3.3 Demographic characteristics of dental HCWs in South Africa.

Demographic characteristics	Overall N = 454	INSTITUTION A n = 78	B n = 105	C n = 57	D n = 71	E n = 91	F n = 52
Age (years)	**36 ± 13.3**	33 ± 14.1	39 ± 13.3	41 ± 12.8	36 ± 13.0	31 ± 12.0	38 ± 11.1
Sex: female : male (%)	**74 : 26**	73 : 27	80 : 20	67 : 33	77 : 23	69 : 31	75 : 25
Height (cm)	**163 ± 9.0**	162 ± 8.8	162 ± 8.3	167 ± 8.5	161 ± 9.8	164 ± 9.2	163 ± 8.6
- Female	**160 ± 7.3**	159 ± 6.5	160 ± 7.0	165 ± 7.7	159 ± 7.9	161 ± 7.4	160 ± 6.0
- Male	**171 ± 8.6**	171 ± 8.6	170 ± 8.1	173 ± 7.7	170 ± 10.8	171 ± 8.7	172 ± 8.4
Weight (kg)	**71 ± 16.8**	68 ± 17.2	72 ± 16.4	77 ± 15.7	67 ± 18.7	71 ± 16.2	76 ± 14.0
- Female	**69 ± 17.0**	66 ± 17.2	72 ± 16.4	75 ± 16.0	63 ± 18.2	69 ± 17.4	73 ± 13.5
- Male	**77 ± 14.7**	74 ± 16.0	76 ± 16.4	80 ± 15.1	80 ± 14.6	74 ± 12.6	84 ± 13.1
Body mass index[1]	**27 ± 6.4**	26 ± 6.8	28 ± 6.7	28 ± 5.9	26 ± 6.5	26 ± 6.0	29 ± 5.3
Smoking history: no. (%)							
- Current	**53 (13%)**	6 (8%)	7 (8%)	6 (11%)	10 (15%)	14 (20%)	10 (21%)
- Ex-smoker	**31 (8%)**	5 (7%)	9 (10%)	7 (12%)	1 (2%)	6 (9%)	3 (7%)
- Non-smoker	**319 (79%)**	63 (85%)	76 (82%)	43 (77%)	-	50 (71%)	34 (72%)
- Average number of years smoked	**12 ± 10.0**	21 ± 12.6	9 ± 5.8	8 ± 5.9	15 ± 17.1	11 ± 9.9	3 -
- Average age when started smoking (years)	**20 ± 6.8**	21 ± 8.9	22 ± 10.0	18 ± 6.8	22 ± 7.1	18 ± 2.9	19 ± 2.9

Note: Continuous variables- mean ± SD; Categorical variables- number (%); where one sample was collected the standard deviation was not calculated

[1] Body mass index: underweight (< 18.5), normal (18.5 - 24.9), overweight (25.0 - 29.9), obese (> 30.0)

Table 3.4 Occupational characteristics and self-reported exposure profile of dental HCWs in South Africa.

Occupational characteristics and self-reported exposures	Mean (± SD) / Prevalence (%) (N = 454)
Employment duration in current job (years)	10 ± 9.1
Employment duration in current clinic (years)	9 ± 8.3
Employment duration prior to current employment (years) (n = 129)	7 ± 6.5
Number of patients consulted per day	16 ± 20
Staff clinical working hours per day	8 ± 2.3
Occupational exposure to	
- Spray	240 (53%)
- Mist	26 (6%)
- Steam	14 (3%)
- Dust	5 (1%)
Extent of occupational exposure	
- Excessive (most of the day)	98 (22%)
- Regular (thrice a week)	124 (27%)
- Minimal (once a week)	90 (20%)
- Nil	142 (31%)
Distance from source of exposure	
- Right next to the source (<1 m)	229 (50%)
- About 1-2 m	44 (10%)
- More than 2 m	34 (40%)
Episode of peak exposure causing tight chest, wheeze or cough	83 (18%)
Regular use of PPE	297 (65%)
- Goggles	180 (40%)
- Gloves	311 (69%)
- Masks	280 (62%)
- Laboratory coats	252 (56%)
Type of gloves used (n = 311)	
- Powdered latex	150 (48%)
- Powder-free low-protein latex	65 (21%)
- Non-latex	14 (5%)
- Combination of > 1 type	82 (26%)
Contact with latex containing products at work	311 (83%)

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

3.2.3 SELF-REPORTED MEDICAL HISTORY

Twenty percent of the study population reported recent chest infections with 7% reporting pneumonia (**Table 3.5**). A history of skin conditions was the most common allergic disease among this group based on the high percentage of reported allergic conditions (e.g. contact dermatitis, eczema, urticaria), followed by ocular-nasal and chest symptoms as a result of environmental triggers in almost a third of the participants. Among family history of allergies, hay fever (21%) was the most prevalent followed by asthma (19%). Tomato, banana and kiwi were the most common food allergens reported.

Table 3.5 Self-reported medical history among dental HCWs in South Africa, (N = 454).

Characteristics	Prevalence (%)
Past medical history	
- Recent chest infections	93 (20%)
- Pneumonia	31 (7%)
- Tuberculosis	10 (2%)
- Chronic bronchitis	5 (1%)
History of allergic disease	
- Skin rash	201 (44%)
- Ocular-nasal symptoms (due to environmental triggers)	160 (35%)
- Chest symptoms (due to environmental triggers)	159 (35%)
- Hay fever	95 (21%)
- Drug allergies	41 (9%)
- Insect allergies	15 (3%)
Family history of allergy	
- Hay fever	95 (21%)
- Asthma	84 (19%)
- Eczema	58 (13%)
Environmental triggers of asthma attack	
- Tobacco smoke	40 (9%)
- Changes in the weather	34 (7%)
- Strenuous exercise	28 (6%)
- Grass or flowers	26 (6%)
- Contact with animals	23 (5%)
- Breathing in cold air	18 (4%)
Food allergies	
- Tomato	9 (2%)
- Banana	8 (2%)
- Kiwi	7 (2%)
- Avocado	5 (1%)
- Chestnut	2 (0.4%)
- Papaya	1 (0.2%)
- Other (e.g. raw potato, stone fruits)	14 (3%)

Note: Categorical variables- number (%)

3.2.4 SELF-REPORTED ASTHMA AND LATEX-RELATED SYMPTOMS AMONG DENTAL HCWS

Doctor-diagnosed asthma (self-reported) was reported in 12% of the study participants (**Table 3.6**). Among these subjects approximately one third developed asthma after the age of 17 years and only half reported being on asthma medication at the time. Asthma-related symptoms for the purpose of this study was relatively high (30%) compared to doctor-diagnosed asthma. Interestingly, 71% of those with self-reported asthma-related symptoms developed symptoms after the age of 17 years. There were 4% of subjects who reported chest tightness when at work. Among the 311 latex-exposed individuals 30% experienced skin symptoms and approximately 10% experienced upper respiratory tract symptoms, while 7% reported lower respiratory tract symptoms. However, only 3% reported latex allergy. Symptoms associated with domestic exposure to latex products were reported in 6-11% of subjects.

Table 3.6 Self-reported asthma and latex-related symptoms among dental HCWs in South Africa.

Respiratory symptoms	Prevalence (%) (N = 454)
Asthma history	
Doctor-diagnosed asthma[1]	56 (12%)
- Before 17 years	36 (8%)
- After 17 years	20 (4%)
Current use of asthma medication	25 (6%)
Asthma-related symptoms (in the last 12 months)	
- Wheezing in the absence of flu or cold	57 (13%)
- Asthma attack in last 12 months	23 (5%)
- Woken up by cough	103 (23%)
- Woken up by tight chest	67 (15%)
- Woken up by shortness of breath	34 (7%)
- Asthma-related symptoms[2]	135 (30%)
Work-related asthma symptom experience	
- Tight chest or wheezing when at work	17 (4%)
- Tight chest or wheezing after peak exposure	83 (18%)
- Job change due to work-related chest symptoms	7 (2%)
- Improvement after changing jobs	6 (1%)
Symptoms associated with work-related latex exposure	
- Self-reported latex allergy	13 (2.9%)
- Skin (itchy, cracked, rash)	138 (30%)
- Ocular (itchy eyes)	48 (11%)
- Nasal (runny or blocked nose)	46 (10%)
- Chest (cough, wheeze, difficulty breathing)	33 (7%)
Symptoms associated with domestic latex exposure	
- Rubber sporting equipment (n = 312)	48 (11%)
- Balloons (n = 337)	29 (6%)

Note: Categorical variables- number (%)

[1] **Doctor-diagnosed asthma:** yes to both: "Have you ever had asthma?" and "Was this confirmed by a doctor?"

[2] **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?"

3.2.5 PATTERNS OF SENSITISATION TO INDIVIDUAL LATEX ALLERGENS AND CCDS AMONG DENTAL HCWS

Almost half of the study population (46%) was atopic as demonstrated by positive Phadiatop test (**Table 3.7**). Latex sensitisation to composite latex (k82) extract was demonstrated in 10% of the subjects however, only 7% demonstrated true latex sensitisation after those with positive reactions to CCDs were excluded. This implies that 3% of workers with reactivity to composite latex extract (k82) demonstrated a false-positive reaction to latex. Fourteen percent of dental workers were sensitised to Hev b 8 followed by 12% sensitised to Hev b 6.01. The prevalence of sensitisation to Hev b 5 was demonstrated in 4% of workers. However, the prevalence of probable occupational latex asthma in this group of workers was low (0.9%).

Table 3.7 Patterns of sensitisation to individual latex allergens and CCDs.

Allergic sensitisation	Prevalence (%)
Atopy (Phadiatop positive) (n = 421)	193 (46%)
Latex (k82) (n = 421)	41 (10%)
Recombinant latex allergens (n = 85)	
- Hev b 1	0 (0%)
- Hev b 3	0 (0%)
- Hev b 5	3 (4%)
- Hev b 6.01	10 (12%)
- Hev b 6.02	6 (7%)
- Hev b 8	12 (14%)
- Hev b 9	1 (1%)
- Hev b 11	2 (2%)
CCD markers	
- MUXF3	13 (15%)
- HRP	18 (21%)
True latex sensitisation[1]	29 (7%)
Chloramine T (n = 69)[2]	2 (0.5%)
Probable occupational latex asthma[3]	4 (0.9%)

CCD – cross-reacting carbohydrate determinants; MUXF3 – Bromelain; HRP – horse radish peroxidase

[1] True latex sensitisation refers to positive response to latex (k82) and negative response to CCDs.

[2] Subjects who answered yes to "Do you think your work environment is causing you to develop allergic symptoms?" were tested for chloramine T.

[3] Defined as either having an asthma attack, or use of asthma medication in the past 12 months, or positive bronchodilator test (increase in FEV_1 and / or FVC ≥ 12% and ≥ 200ml increase compared with baseline) and true sensitisation to latex.

3.2.6 PULMONARY FUNCTION OUTCOMES OF DENTAL HCWS

The mean FEV_1 of the entire group was 2.76 L, with the mean FEV_1 being higher in males than females (**Table 3.8**). Nineteen percent of subjects had a baseline FEV_1 less than 80% predicted. A slightly higher proportion (8%) demonstrated a 12% increase in FEV_1 and /or FVC accompanied by a 200 ml increase post administration of a bronchodilator.

Table 3.8 Pulmonary function outcomes of dental HCWs in South Africa.

Pulmonary function indices	Overall (N = 412)
Entire group	
FEV_1 (litres)	2.76 ± 0.7
FVC (litres)	3.26 ± 0.9
FEV_1 % predicted	93 ± 16.4
FVC % predicted	94 ± 17.0
FEV_1 / FVC %	85 ± 8.4
No. with initial FEV_1 < 80% predicted	84 (19%)
Proportion with FEV_1 / FVC ≤ 0.7	17 (4%)
Proportion with FEV_1 post-bronchodilator increase ≥ 12% + 200 ml (n = 394)	21 (5%)
Proportion with FEV_1 and / or FVC post-bronchodilator increase ≥ 12% + 200 ml (n = 394)	32 (8%)
Males (n = 102)	
FEV_1 (litres)	3.39 ± 0.6
FVC (litres)	4.08 ± 0.8
FEV_1 % predicted	91 ± 15.0
FVC % predicted	92 ± 16.0
FEV_1 / FVC %	84 ± 7.6
Females (n = 310)	
FEV_1 (litres)	2.55 ± 0.6
FVC (litres)	2.99 ± 0.7
FEV_1 % predicted	94 ± 16.8
FVC % predicted	95 ± 17.2
FEV_1 / FVC %	86 ± 8.6

Continuous variables-mean ± SD; Categorical variables-number (%); Reference values are from the ECCS, 1993.

3.2.7 Relationship Between Asthma Phenotypes And Host-Associated Risk Factors In Dental HCWs

The prevalence of atopic asthma and non-atopic asthma among dental HCWs was 6.9% and 5.9%, respectively. A lower prevalence (4.0%) was demonstrated for WAA. In the unadjusted logistic regression models, dental HCWs with atopic asthma were four times more likely to have ocular-nasal symptoms (**Table 3.9**). In addition, environmental triggers such as grass or flowers were significantly associated with atopic asthma. Interestingly, workers with WAA were less likely to have had childhood-onset asthma. A history of work-related ocular-nasal symptoms was also significantly associated with WAA. Inhalation of cold air was identified as a significant trigger associated with WAA.

3.2.8 Relationship Between Asthma Phenotypes And Occupational Risk Factors In Dental HCWs In South Africa

In the unadjusted logistic regression models non-atopic asthma phenotype was associated with increasing duration of employment in the dental clinic (> 20 years) (**Table 3.10**). Wearing of PPE had a protective effect on workers with non-atopic asthma.

In the multivariate logistic regression models, after adjusting for age, sex and smoking status, a history of pneumonia was significantly associated with atopic asthma while ocular-nasal symptoms also remained a significant predictor of this phenotype as well as WAA (**Table 3.11**). Strenuous exercise was a significant predictor of atopic asthma in the adjusted model. While childhood-onset asthma associated with WAA in the adjusted models did not predict this phenotype, atopy demonstrated an elevated odds ratio (OR = 3.40, 95% CI: 1.07 – 10.83). Tuberculosis infections and latex sensitisation were significant predictors of non-atopic asthma. The use of all types of PPE

was protective for non-atopic asthma while glove use was protective for atopic asthma. Although not significant, students were more likely to present with non-atopic asthma than staff. Adjusting for age, sex, smoking and latex sensitisation did not change the associations observed in the unadjusted models.

Table 3.9 Association between asthma phenotypes and host-associated risk factors in dental HCWs in South Africa (unadjusted OR).

Characteristics	Asthma Phenotype, OR (95% CI)		
	Atopic asthma	Non-atopic asthma	Work-aggravated asthma
Prevalence (N = 421) (%)	n = 29 (6.9%)	n = 25 (5.9%)	n = 17 (4.0%)
Age (years)	0.98 (0.95 - 1.01)	**1.04 (1.00 - 1.07)***	1.03 (0.99 - 1.06)
Sex (%), baseline: male	0.55 (0.25 - 1.20)	1.12 (0.44 - 2.87)	0.84 (0.29 - 2.42)
Atopy[1]	n/a	n/a	2.02 (0.72 - 5.67)
Obesity[2]	1.00 (0.91 - 1.04)	1.05 (0.98 - 1.11)	1.06 (0.98 - 1.14)
Asthma history			
- Family history of asthma	1.84 (0.73 - 4.66)	0.84 (0.20 - 3.63)	1.45 (0.45 - 4.66)
- Childhood-onset asthma (< 17 years)	1.03 (0.34 - 3.13)	-	**0.18 (0.05 - 0.62)***
- Number of asthma attacks in past 12 months	0.71 (0.28 - 1.81)	1.88 (0.38 - 9.27)	0.71 (0.31 - 1.64)
Smoking history (%), baseline: non-smoker			
- Current	1.48 (0.53 - 4.11)	2.11 (0.73 - 6.07)	2.89 (0.70 - 7.46)
- Ex-smoker	1.52 (0.43 - 5.42)	1.40 (0.30 - 6.41)	1.93 (0.41 - 9.13)
Past medical history			
- Pneumonia	2.60 (0.91 - 7.45)	2.81 (0.88 - 9.02)	**15.95 (5.61 - 45.41)****
- Tuberculosis	1.81 (0.22 - 14.96)	-	2.91 (0.34 - 24.60)
- Recent chest infections	0.67 (0.22 - 2.03)	0.96 (0.34 - 2.67)	1.19 (0.37 - 3.84)
Allergic disease			
- Ocular-nasal symptoms (due to environmental triggers)	**4.21 (1.93 - 9.17)****	0.89 (0.36 - 2.19)	**5.99 (2.07 - 17.35)****
- Work-related ocular-nasal symptoms	1.62 (0.63 - 4.14)	1.54 (0.56 - 4.25)	**7.65 (2.84 - 20.66)****
Self-reported environmental triggers of recent asthma attack			
- Contact with animals	1.32 (0.38 - 4.58)	0.39 (0.03 - 4.63)	0.60 (0.15 - 2.40)
- Grass or flowers	**5.44 (1.26 - 23.59)***	0.30 (0.03 - 3.60)	1.72 (0.43 - 6.85)
- Strenuous exercise	6.92 (1.30 - 36.82)	1.00 (0.08 - 12.07)	1.74 (0.39 - 7.81)
- Breathing in cold air	3.75 (0.89 - 15.81)	0.50 (0.04 - 6.05)	**10.63 (1.87 - 60.25)***
- Spray, mist or steam at work	1.35 (0.37 - 4.92)	3.56 (0.34 - 37.69)	1.75 (0.44 - 6.93)
- Tobacco smoke	0.43 (0.09 - 2.00)	1.00 (0.10 - 9.93)	1.62 (0.29 - 9.09)
- Changes in the weather	2.89 (0.68 - 12.28)	2.24 (0.24 - 21.15)	4.20 (0.81 - 21.77)
Baseline pulmonary function indices (n = 412)			
- FEV_1 / FVC < 0.7	0.98 (0.12 - 7.73)	-	-
- FEV_1 < 80% predicted	2.92 (0.39 - 22.01)	2.54 (0.33 - 19.23)	1.66 (0.21 - 12.81)

Each OR is derived from a separate unadjusted model. * < 0.05; ** < 0.001

[1] Atopy defined as a positive Phadiatop result

[2] Obesity defined as a body mass index > 30

Table 3.10 Association between asthma phenotypes and occupational risk factors in dental HCWs in South Africa (unadjusted OR).

Occupational characteristics and exposures	Asthma Phenotype, OR (95% CI)		
	Atopic asthma	Non-atopic asthma	Work-aggravated asthma
Prevalence (N = 421) (%)	n = 29 (6.9%)	n = 25 (5.9%)	n = 17 (4.0%)
Employment duration in current clinic (years)	1.01 (0.95 - 1.06)	**1.06 (1.01 - 1.11)***	1.04 (0.99 - 1.10)
Employment duration in dental institute (baseline ≤ 10 years)			
- 11 - 20 years	1.06 (0.38 – 2.91)	0.55 (0.12 – 2.47)	0.84 (0.18 – 3.92)
- > 20 years	1..02 (0.34 – 3.10)	**2.98 (1.21 – 7.38)***	2.69 (0.89 – 8.17)
Age at employment in dental clinic (baseline ≤ 20 years)			
- 21 - 30 years	1.58 (0.47 - 5.29)	1.77 (0.54 - 5.83)	1.63 (0.41 - 6.48)
- > 30 years	0.88 (0.23 - 3.36)	1.44 (0.42 - 4.94)	0.70 (0.14 - 3.54)
Employment duration prior to current employment (years)	0.91 (0.77 - 1.06)	1.01 (0.92 - 1.11)	0.93 (0.78 - 1.12)
Professional status (staff (baseline) versus student)	1.30 (0.60 - 2.81)	0.45 (0.17 - 1.24)	0.54 (0.17 - 1.69)
Average number of patients seen per day	0.99 (0.96 - 1.02)	1.01 (0.99 - 1.04)	0.99 (0.96 - 1.03)
Occupational exposure to spray, mist, steam, dust	2.11 (0.61 - 7.29)	0.45 (0.19 - 1.07)	0.99 (0.31 - 3.12)
Extent of occupational exposure (self-reported), baseline (low)			
- Moderate	0.56 (0.18 - 1.72)	2.46 (0.50 - 12.16)	0.66 (0.16 - 2.74)
- High	0.89 (0.30 - 2.68)	1.85 (0.33 - 10.39)	0.90 (0.22 - 3.73)
Distance from source of exposure, baseline (> 1 m away)			
- Right next to the source (< 1 m)	1.10 (0.39 - 3.10)	0.40 (0.15 - 1.05)	0.93 (0.29 - 3.02)
Work-related chest symptoms (peak exposure)	0.69 (0.21 - 2.24)	2.35 (0.82 - 6.71)	2.01 (0.63 - 6.45)
Use of all types of PPE	1.00 (0.28 - 3.53)	**0.24 (0.09 - 0.65)***	0.41 (0.13 - 1.36)
Glove versus no glove use	0.74 (0.34 - 1.61)	0.67 (0.30 - 1.54)	0.84 (0.30 - 2.31)
Latex versus non-latex gloves	0.90 (0.42 - 1.92)	0.61 (0.26 - 1.41)	1.62 (0.60 - 4.32)
Use of face mask versus no mask	0.74 (0.34 - 1.61)	0.67 (0.30 - 1.54)	0.84 (0.30 - 2.31)
Allergic sensitisation			
- Sensitisation to composite latex (k82) extract	1.54 (0.51 - 4.65)	0.37 (0.05 - 2.81)	0.61 (0.08 - 4.73)
- True latex sensitisation[1]	1.62 (0.46 - 5.72)	0.55 (0.07 - 4.20)	0.90 (0.11 - 7.04)

Each OR is derived from a separate unadjusted model. * < 0.05; ** < 0.001

[1] True latex sensitisation refers to positive response to latex (k82) and negative response to CCDs.

Table 3.11 Association between host and occupational risk factors associated with asthma phenotypes in dental HCWs in South Africa (adjusted OR).

Characteristics	Prevalence (N = 421) (%)	Asthma Phenotypes, OR (95% CI)		
		Atopic asthma n = 29 (6.9%)	Non-atopic asthma n = 25 (5.9%)	Work-aggravated asthma n = 17 (4.0%)
Atopy		n/a	n/a	**3.40 (1.07 - 10.83)***
Obesity		0.97 (0.90 - 1.05)	1.02 (0.94 - 1.10)	1.04 (0.95 - 1.14)
Asthma history				
- Family history of asthma		1.89 (0.68 - 5.24)	1.20 (0.24 - 5.99)	1.94 (0.54 - 7.03)
- Childhood-onset asthma (< 17 years)		0.63 (0.17 - 2.34)	1.11 (0.98 - 1.26)	0.26 (0.06 - 1.09)
- Number of asthma attacks in past 12 months		0.39 (0.11 - 1.30)	6.43 (0.57 - 72.05)	0.70 (0.23 - 2.16)
Past medical history				
- Pneumonia		**3.47 (1.17 - 10.33)***	2.42 (0.64 - 9.17)	**15.86 (5.14 - 48.94)****
- Tuberculosis		2.19 (0.249 - 19.30)	**1.05 (1.01 - 1.09)***	2.02 (0.23 - 17.99)
- Recent chest infections		0.71 (0.23 - 2.15)	0.94 (0.30 - 2.99)	1.26 (0.38 - 4.12)
History of allergic disease (self-reported)				
- Ocular-nasal symptoms		**4.43 (1.92 - 10.25)****	0.58 (0.19 - 1.79)	**5.88 (1.94 - 17.81)***
- Work-related ocular-nasal symptoms		1.85 (0.70 - 4.89)	0.99 (0.28 - 3.54)	**6.70 (2.35 - 19.13)****
Environmental triggers of recent asthma attack				
- Contact with animals		0.83 (0.20 - 3.41)	0.89 (0.04 - 22.06)	0.81 (0.16 - 4.19)
- Grass or flowers		3.74 (0.75 - 18.53)	0.70 (0.03 - 14.41)	2.60 (0.46 - 14.58)
- Strenuous exercise		**6.85 (1.11 - 42.44)***	6.42 (0.15 – 280)	4.14 (0.59 - 28.88)
- Breathing in cold air		5.03 (0.91 - 27.65)	0.47 (0.02 - 9.02)	**14.36 (1.36 – 151)***
- Spray, mist or steam at work		5.11 (0.52 - 50.23)	9.57 (0.21 – 426)	4.48 (0.44 - 45.31)
- Tobacco smoke		0.26 (0.04 - 1.47)	2.05 (0.07 - 59.63)	1.88 (0.23 - 15.15)
- Changes in the weather		**9.78 (1.24 - 77.04)***	0.83 (0.04 - 19.39)	2.42 (0.43 - 16.08)
Occupational exposures				
Professional status (staff (baseline) versus student)		0.62 (0.19 - 2.01)	1.73 (0.37 - 8.16)	0.84 (0.16 - 4.39)
Use of all types of PPE		0.73 (0.19 – 2.76)	**0.23 (0.08 - 0.68)***	0.45 (0.13 - 1.56)
Glove versus no glove use		**0.39 (0.17 - 0.89)***	0.61 (0.23 - 1.61)	0.74 (0.25 - 2.22)
Latex versus non-latex gloves		0.72 (0.32 - 1.60)	0.78 (0.31 - 1.96)	1.68 (0.59 - 4.81)
Use of face mask versus no face mask		**0.39 (0.17 – 0.89)***	0.61 (0.23 – 1.61)	0.74 (0.25 – 2.22)
Employment duration (≤ 10 years)				
- 11 - 20 years		1.70 (0.48 – 5.96)	0.35 (0.07 – 1.82)	0.61 (0.11 – 3.37)
- > 20 years		1.92 (0.45 – 8.17)	1.53 (0.43 – 5.39)	1.99 (0.46 – 8.70)
Age at current employment (≤ 10 years)				
- 11 - 20 years		0.36 (0.10 – 1.27)	-	1.09 (0.20 – 5.82)
- > 20 years		0.43 (0.14 – 1.30)	-	0.34 (0.07 – 1.71)
Allergic disease				
- Sensitisation to composite latex (k82) extract		1.49 (0.47 - 4.69)	**1.04 (1.01 - 1.08)***	0.78 (0.10 - 6.39)
- True latex sensitisation		1.58 (0.43 - 5.81)	**1.04 (1.01 - 1.08)***	1.22 (0.15 - 10.10)
Baseline pulmonary function indices (n = 412)				
- FEV_1 / FVC < 0.7		1.34 (0.16 – 11.09)	1.05 (1.01 - 1.08)	1.03 (0.99 - 1.07)
- FEV_1 < 80% predicted		2.51 (0.31 - 20.04)		1.88 (0.23 - 15.68)

Each OR is derived from a separate model adjusted for age, sex, smoking. * < 0.05; ** < 0.001.

3.3 DISCUSSION

In this study of dental HCWs the prevalence of atopic asthma (6.9%) was similar to non-atopic asthma (5.9%). These findings are consistent with other studies which demonstrated that only 50% of asthma is attributable to eosinophilic inflammation as is found in atopic asthma (Douwes et al., 2002a). The findings suggest that other agents (Fahy et al., 1995) aside from common inhalant allergens play a role in the development of asthma in dental HCWs. Aerosolised products and materials that can irritate the respiratory tract include acrylic dusts, filling composites, disinfectant agents as well as biological agents such as endotoxin (Robert-Sauve Research Institute for Occupational Safety and Health (IRSST), 2001; Pankhurst et al., 2005).

The prevalence of WAA was slightly lower (4.0%) in this study population. Among the work-aggravated asthmatics ten were atopic (59%) and six were non-atopic (35%). This is consistent with previous studies which have shown the occurrence of exacerbations of asthma without an increase in eosinophilic inflammation (Bernstein et al., 1999). The results of previous studies showed that up to 34% of asthmatics reported that their symptoms were exacerbated by workplace exposures (Henneberger et al., 2002). The prevalence of WRA is in the lower range of estimates (5 - 19%) for adult asthma attributable to occupation with a median of 9% (Tarlo, 2008). The prevalence of asthma attributable to occupation in the current study is also lower than that found in the cohort of private dentists with self-reported asthma (14%) since starting training (Pankhurst et al., 2005). HCWs including dental workers accounted for 16% of 1879 confirmed WRA cases from a surveillance study in four American states. The distribution of exposures for this group included cleaning products, latex, gluteraldehyde, formaldehyde and acrylates (Pechter et al., 2005). Only one study has been reported on occupational exposures among dental assistants in South Africa;

however, the focus was primarily on infectious material from needlestick injuries (Nemutandani et al., 2007). The prevalence of probable occupational latex asthma in the current study was similar to a study of WRA in two hospitals in Canada which was 1.4% (Lemiere et al., 2007).

Various host factor attributes have been associated with various asthma phenotypes. These include age (Miranda et al., 2004; Caldeira et al., 2006), atopy (Mapp et al., 2005), cigarette smoking (Mapp et al., 2005; Haahtela et al., 2006; Haldar and Pavord, 2007; Jeebhay and Quirce, 2007), sex (Bakke and Gulsvik, 2000; Haahtela et al., 2006; Jeebhay and Quirce, 2007) and obesity (Bousquet et al., 2008). In this current study sex and obesity did not play a significant role in asthma. However, atopy was shown to be a significant predisposing factor for WAA in the multivariate regression model (OR = 3.4, 95% CI: 1.07 – 10.83). Previous studies have shown that atopy is an important risk factor for WRA due to HMW agents (Cartier, 1998; Caldeira et al., 2006). This may be as a result of pre-existing asthma in subjects who are more likely to react to triggers in the workplace.

Ocular-nasal symptoms were strongly associated with the atopic asthma (OR = 4.43, 95% CI: 1.92 – 10.25) and WAA (OR = 5.88, 95% CI: 1.94 – 17.81) phenotypes. An even stronger association was observed between work-related ocular-nasal symptoms and WAA (OR = 6.70, 95% CI: 2.35 – 19.13). Possible agents in this setting that could contribute to these symptoms include methacrylates (Toren, 2007), rubber products, metals, resins and latex (Gawkrodger, 2005). Previous studies have reported the association between rhinitis and asthma in both atopic and non-atopic adults (Guerra et al., 2002). Allergic rhinitis and conjunctivitis were also shown to be significant predictors of WRA among florist workers (Akpinar-Elci et al., 2004). Rhinoconjunctivitis symptoms often precede the onset of asthma symptoms among workers exposed to HMW agents (Arif et al., 2003). Although it is usually considered a mild disease, these workers should be considered a high-risk group

(Karjalainen et al., 2003) because of the likelihood of developing OA (Malo et al., 1997). Recent studies have concluded that rhinitis is a significant and independent risk factor for adult-onset asthma (Guerra et al., 2002) and work-related ocular-nasal symptoms can aggravate pre-existing asthma (Arif et al., 2003; Fishwick et al., 2008). In addition, the risk of developing OA is highest in the year after the onset of occupational rhinitis (Nicholson et al., 2005).

In this current study, one third of dental HCWs used powdered latex gloves, while only 3% had self-reported latex allergy. Although 10% were sensitised to the composite latex extract, only 7% demonstrated true latex sensitisation. The differentiation between true latex sensitivity and cross-reactivity was based on the concentration of IgE antibodies to latex and the CCD markers. The prevalence of latex allergy among dental professionals in a previously published study ranged from 8.8 to 13% (Saary et al., 2002), however whether these percentages reflect true latex allergy is questionnable. In the current study 65 workers (14.3%) had latex-allergic ocular-nasal symptoms and four workers (0.9%) had probable latex-allergic asthma. This is lower than the prevalence (1.2% - 16.3%) for rhinoconjunctivitis in NRL exposed HCW populations (Fish, 2002). The prevalence of probable OA is also lower than the prevalence of 2.5% among hospital workers exposed to NRL (Vandenplas et al., 1995). A questionnaire-based study evaluating the prevalence of respiratory illness, including asthma, among dental students in North America and Canada reported an asthma prevalence of 1.7-5.5% (Scannapieco et al., 2004). This low prevalence of latex-related asthma observed indicates that exposures to other workplace agents aside from latex need to be considered in WRA. It is also likely that sensitised or allergic workers have relocated to unexposed jobs. Interestingly latex sensitisation appeared to be more relevant for non-atopic asthmatics in this study. This merits further investigation.

In the current study WAA displayed an association with breathing in cold air whereas exercise and weather changes were important predictors of atopic asthma. The ambient temperature measured in the dental clinics in the summer season was 23˚C ± 0.96 and in the winter season was 12˚C ± 2.89 as discussed in **Chapter 2**. These levels were on the borderline (for summer) and below (for winter) the recommended acceptable thermal conditions for summer (23-26˚C) and winter (20-24˚C) suggested by the American Society of Heating, Refrigeration and Air-conditioning Engineers (American Society of Heating Refrigeration and Air-conditioning Engineers (ASHRAE), 1992). It is likely that the thermal discomfort experienced by dental workers is an important trigger of WAA. In a recent review of six epidemiologic studies, it was demonstrated that physical factors (e.g. extreme temperatures, exercise), among others, are common occupational triggers for work-exacerbated asthma (Henneberger, 2007). Inciters such as exercise or exposure to cold air, can trigger short-lasting asthma symptoms and transient airflow obstruction, but these stimuli do not generally cause airway inflammation (Vandenplas and Malo, 2003; Tarlo et al., 2008).

Pneumonia was a significant predictor of atopic asthma (OR = 3.47, 95% CI: 1.17 – 10.33) and WAA (OR = 15.86, 95% CI: 5.14 – 48.94). Various infectious diseases such as *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* are important factors in the occurrence of asthma and may have a bi-directional effect on the development of asthma and allergy (Chen et al., 2000; Douwes et al., 2002a). However the reason for the relation between respiratory infections and respiratory allergic diseases is not known (Chen et al., 2000). A significant determinant of non-atopic asthma (OR = 1.05, 95% CI: 1.01 – 1.09) was previous TB infection. This may be due to a switch in the T_H1/T_H2 balance from T_H1 which was initially stimulated by bacterial infection but suppressed by disease progression to T_H2 mechanism. Other studies have shown that long-term exposure to microbial agents from the spray/mist is associated with an increased risk of non-atopic

asthma and WRA (Howell et al., 2004). Over half the study participants (53%) were exposed to sprays at work with 50 – 60% working in close proximity to the source of exposure. Among these workers 22% complained of excessive exposure to these sprays. However, the extent of exposure was not significantly associated with any of the asthma phenotypes.

In this study duration of employment was significantly associated with non-atopic asthma. Dental HCWs with non-atopic asthma were twice as likely (OR = 2.98, 95% CI: 1.21 - 7.38) to be working for long periods (> 20 years). This finding is supported by a recent study which showed that risk factors and lung outcomes differ in various age groups (Caldeira et al., 2006). However, these findings are at odds with a previous study in which the prevalence of respiratory disease (including asthma) among dental students did not demonstrate an association with the length of time students had worked in the dental school (Scannapieco et al., 2004).

The current study also demonstrated that the use of PPE was protective for non-atopic asthma. This demonstrates the likelihood of occupational exposure, possibly to endotoxin playing a role in non-atopic asthma if poor compliance to PPE usage. PPE compliance may result in decreased endotoxin inhalation which adjusts the T_H1/T_H2 model towards T_H2 as opposed to high levels switching to T_H1 effect hence protecting against non-atopic asthma (Vercelli, 2003). However, the findings from the current study may be suggestive of the incorrect use of PPE or the use of inappropriate PPE, particularly the type of face masks used (e.g. surgical mask versus N95 respiratory face mask) (see **Chapter 2**, **Figure 2.4**), which may explain the prevalence of WAA observed in the study apart from the possibility of thermal conditions being a possible predictor. 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.

Some of the limitations identified in this study are outlined: Firstly, because of the relatively low prevalence of asthma observed for the various phenotypes, we were unable to demonstrate significant associations due to lack of power, for example the association between latex allergy and atopic asthma, which has been demonstrated in previously (Vandenplas et al., 2009). Added to this were the limitations of the study design such as survivor bias, which may result from exclusion of workers who left the workplace before the study was conducted (Caldeira et al., 2006). The response rate in the current study was 25%; therefore selection bias may also have influenced the findings. An investigation into non-participation was done and revealed that time was a limiting factor in their consideration to participate. Furthermore, the comparison of results with previous studies posed some challenges because of differing definitions of asthma and work-relatedness of asthma. In addition, differences in exposures across sites in this study as demonstrated in **Chapter 2**, suggest considerable differences in exposure profiles, which is likely to affect outcomes.

In conclusion, the results of this study have demonstrated that several factors determine the manifestation of the various asthma phenotypes. Closer focus on these factors may improve our understanding of the manifestation of the specific asthmatic phenotype, which will improve the management of dental HCWs presenting with asthma. The identification of various phenotypes of asthma is particularly important in the dental setting as workers are exposed to multiple agents; this suggests that different mechanisms of inflammation may be involved in the development of asthma. Although a high proportion of dental workers were sensitised to latex, the prevalence of probable

occupational latex asthma was low, suggesting other triggers may play a role. The pathophysiological basis that forms the foundation for these different clinical phenotypes based on trigger factors needs to be explored further so as to identify specific causes for the asthma observed in this group of workers.

CHAPTER 4

CHARACTERISING INFLAMMATORY CELL ASTHMA PHENOTYPES IN DENTAL WORKERS USING CYTOKINE PROFILING

4.0 INTRODUCTION

Cytokines play an important role in the inflammatory process of asthmatic airways since they are capable of inducing many of the pro-inflammatory effects characteristic of this disease (Chung and Barnes, 1999). These molecules can modify the airway microenvironment directly or by activating resident cells (Haley and Drazen, 1998). Although two major groups of cytokines have been identified based on the pathogenesis of asthma (**Table 4.1**) (Chung and Barnes, 1999), these cytokines cannot be easily categorised in acute and chronic asthma due to their pleiotropic nature and overlapping properties (Doshi et al., 2002). Single cytokine levels are therefore not useful discriminators in asthma, but instead an expression pattern consisting of several distinct cytokines to ensure reproducibility appears to be a more useful approach (Brasier et al., 2008).

Table 4.1 Cytokines involved in the pathogenesis of asthma in relation to specific cell types.

Cell types	List of cytokines
Eosinophil	IL-3, IL-4, IL-5, IL-7, IL-9, IL-13, Eotaxin and GM-CSF
Neutrophil	IL-1β, IL-6, IL-8, IL-10, IL-12p70, IL-18, TNF-α

IL – interleukin; GM-CSF – granulocyte macrophage-colony stimulating factor; TNF-α – tumour necrosis factor alpha
Adapted from (Chung and Barnes, 1999; Jatakanon et al., 1999; Kips, 2001; Doshi et al., 2002; Mahajan and Mehta, 2006).

Asthma has historically been regarded as entirely an atopic disease involving allergic (IgE-mediated) sensitisation resulting in IL-5 mediated eosinophilic airways inflammation. In earlier studies IL-5 was thought to be a cardinal feature in asthma (de Magalhaes Simoes et al., 2005). However, the role of IL-5 in asthma has in recent times been under much scrutiny. Studies in the past decade have demonstrated that only 50% of asthma cases can be attributable to "allergic asthma" (Douwes et al., 2002b). This conclusion is based on inconsistent associations between the prevalence of asthma and atopy. In addition, treatment regimens aimed at neutralising IgE (anti-IgE) have only been partially effective in asthma cases. The phenomenon of non-eosinophilic inflammation (also referred to as neutrophilic inflammation) is prevalent in certain occupational populations (Douwes et al., 2002b). These populations include those with exposure to metal-working fluid (Leigh and Hargreave, 1999), LMW agents such as isocyanates and cobalt (Anees et al., 2002) and endotoxin exposure in farming (Portengen et al., 2005). Although not demonstrated in working populations, recently bakery flour dust has been shown to elicit a neutrophilic inflammation in mouse models (Marraccini et al., 2008). Asthma phenotypes can therefore be classified based on predominant inflammatory cell infiltrates. The resulting phenotypes include neutrophilic asthma, eosinophilic asthma, granulocytic asthma with concomitant eosinophilic and neutrophilic inflammation and a group, paucigranulocytic asthma, in which levels of both neutrophils and eosinophils are normal (Douwes et al., 2002b; Simpson et al., 2006; Wenzel, 2006).

Eosinophilic inflammation is a common feature in OA due to HMW sensitisers (Bochner, 2004; Cicutto and Downey, 2004). These cells contain and release toxic pro-inflammatory mediators (e.g. major basic protein and eosinophil cationic protein [ECP]), reactive oxygen species, lipid mediators and cytokines (Bochner, 2004). The measurement of sputum ECP has been suggested as a predictive marker for the presence of eosinophilic inflammation in the lung (Watt et al., 2005). The pathobiological effects of eosinophils occur through a myriad of mediators (**Table 4.1**) including GM-CSF, IL-3, IL-5 and certain chemokines such as eotaxin (Doshi et al., 2002; Alam and Busse, 2004; Mahajan and Mehta, 2006).

Neutrophils also play a role in the development of well-defined clinical sub-phenotypes (Wenzel et al., 1999; Ordonez et al., 2000; Mapp et al., 2005; Mann and Chung, 2006; Diamant et al., 2007; Haldar and Pavord, 2007). Some studies have shown that neutrophilia may be characteristic of non-atopic asthma and OA due to LMW agents (Wenzel et al., 1999; Ordonez et al., 2000; Anees et al., 2002; Mapp et al., 2005; Mann and Chung, 2006; Tsoumakidou et al., 2006; Diamant et al., 2007; Haldar and Pavord, 2007; Turato et al., 2007). Neutrophils are a potential source of inflammatory mediators, enzymes (e.g. neutrophil elastase, myeloperoxidase [MPO]), reactive oxygen species and cytokines (**Table 4.1**) such as IL-8 and TNF-α. IL-8 is considered as a marker of severe asthma and it has also been linked to allergic rhinitis (Chung and Barnes, 1999; Mann and Chung, 2006).

The use of biomarkers in the assessment of airway inflammation is important for exploring the underlying mechanisms of asthma and may reveal diseased airways that are not detectable by parameters commonly used in current practice (e.g. symptom experience, lung function, airway hyperresponsiveness) (Cicutto and Downey, 2004). Some studies have shown a strong relationship

between the presence of inflammatory cell markers and enhanced airway responsiveness, whereas others have not been able to demonstrate such a relationship (Haley and Drazen, 1998; Alam and Busse, 2004; Miranda et al., 2004; Lemiere et al., 2006; Wenzel, 2006; Haldar and Pavord, 2007; Brasier et al., 2008). Airway-infiltrating cells (e.g. eosinophils) modify airway responses and have profound biologic effects in the micro-environment of their release as well as at distant sites. However, they may no longer be present at the time the effects of the cytokine are apparent; this has contributed to the discordance in previous studies. The discordance may also be attributed to the heterogeneity of asthma (Lemiere et al., 2006; Wenzel, 2006; Brasier et al., 2008). Inferences about the relationship between airway hyperresponsiveness and airway inflammation therefore need to be made with circumspection (Brusasco et al., 1998). One of the potential advantages of the use of inflammatory cell markers is that subjects with similar clinical characteristics may comprise heterogeneous subtypes which can only be further distinguished on the basis of serial measurement of cytokines (Brasier et al., 2008). This study hypothesised that different cytokine groupings could be used to recognise different inflammatory cell phenotypes in asthmatics which could be used to examine the heterogeneity of airway inflammation in dental HCWs.

The aim of this study was therefore to identify the inflammatory pathways of asthma in dental HCWs through:

- identifying subject groupings on the basis of common patterns in inflammatory responses,
- determining the relationship between these identified cytokine patterns, inflammatory mediators (ECP and MPO) and asthma outcomes to assess the robustness of this approach in characterising the asthma phenotypes.

4.1 MATERIALS AND METHODS

4.1.1 STUDY POPULATION

The study population included 76 subjects with asthma, who had previously been diagnosed with asthma by a doctor, as well as subjects with probable asthma and those with asthma-related symptoms. Probable asthma included subjects who reported either an attack of shortness of breath, attack of asthma, or use of asthma medication. Those with current asthma-related symptoms included those who had a positive response to one or more of the following symptoms: shortness of breath, chest tightness, coughing, and wheezing in the last 12 months.

Health outcome information was obtained from the self-administered health questionnaire (described in Chapter 3). Control subjects (n = 9) included those with a negative response to doctor-diagnosed asthma, probable asthma or asthma-related symptoms. The number of controls is low and calculations demonstrated poor power however comparisons between subjects and controls will be based on previous literature.

4.1.2 SERUM SAMPLES

A 6 ml blood sample was taken from all workers using a Becton Dickinson Vacutainer CAT tube (with clot activator). Samples were left to coagulate for 3-6 hours at room temperature and were then centrifuged at 1500 g for 10 minutes. The serum was transferred into 5 ml plastic cryotubes (Nunc, Denmark) and placed in storage at -20°C until transported to the laboratory, and then stored at -70°C until analysis.

4.1.3 MEASUREMENT OF ATOPY AND ALLERGEN SPECIFIC IGE

The serum was analysed for IgE to common inhalants using Phadiatop® test for atopy and latex-specific IgE (k82) determinations. Tests were done according to the manufacturer's instructions using the ImmunoCAP system 100 (Phadia AB, Uppsala, Sweden). Specific IgE antibodies to CCDs, HRP and MUXF3-bromelain were also performed. A result of > 0.35 kU/l was regarded as positive for allergen specific IgE determinations.

4.1.4 MEASUREMENT OF EOSINOPHILIC CATIONIC PROTEIN (ECP)

Serum ECP was measured using the Phadia ImmunoCAP system ECP FEIA (Phadia AB, Uppsala, Sweden), according to the manufacturer's instructions. Briefly, anti-ECP covalently coupled to ImmunoCAP, binds with the ECP in the patient's serum. After washing, enzyme labelled antibodies against ECP were added to form a complex. After incubation, unbound enzyme-anti-ECP was washed away and the bound complex was then incubated with a developing solution. The reaction was stopped, the fluorescence of the eluate was measured and the response transformed to concentration levels using a calibration curve. The normal range for serum ECP on the patient population that was established in the laboratory was 2.00 – 15.7 μg/l. Therefore values above this range were considered elevated for the study population. The maximum value was similar to that of a previous study (13.3 μg/l) (Arm and Lee, 1992). To ensure quality control the laboratory participated in an external quality assessment program (Phadia: Quality Club ECP (52-5213-99/05)).

4.1.5 MEASUREMENT OF MYELOPEROXIDASE (MPO)

MPO concentration in serum was measured using the MPO enzyme immunoassay (MPO-EIA) (MPO-EIA, Oxford Biomedical Research). The assay was performed according to the manufacturer's instructions (Oxford Biomedical Research). Briefly, antigen captured by a solid

phase monoclonal antibody was detected with a biotin-labelled goat polyclonal anti-MPO. An avidin alkaline phosphatase conjugate was bound to the biotinylated antibody. The alkaline phosphatase substrate ρ-nitrophenyl phosphate (pNPP) together with the yellow product (ρ-nitrophenol) was read at 405 nm using a spectrophotometer (Biotek Instruments, USA). A standard curve was obtained by plotting the absorbance values as a function of the standard MPO concentrations. Standard curves were fitted using a 4 parameter data reduction method. Assays in which the standard curve had a correlation coefficient (R^2) $\geq$ 0.98 and samples with a CV $\leq$ 10% were accepted. The normal range for serum in the patient population that was established in the laboratory was 8.43 - 77.35 ng/ml. Therefore values above this range were considered elevated for the study population.

4.1.6 MEASUREMENT OF NON-ALLERGIC INFLAMMATORY MEDIATORS (CYTOKINES)

The BD™ CBA Human Inflammation kit was used to quantitatively measure non-allergic inflammatory cytokines (IL-8, IL-1β, IL-6, IL-10, TNF-α and IL-12p70) in each serum sample. Six bead populations with distinct fluorescence intensities coated with the specific ILs were multiplexed and resolved in the red channel of the flow cytometer (BD FacsArray™) (Becton Dickson, Oxford, UK). The determination of the serum inflammatory ILs was done according to the manufacturer's instructions. In summary, 10 µl of each human inflammation capture bead suspension was mixed together. The mixed suspension was centrifuged at 200 g for 5 minutes and the supernatant carefully aspirated. The bead populations were resuspended in serum enhancement buffer (equal volume to the amount aspirated) and vortexed briefly. The suspension was incubated at room temperature, protected from light. After incubation, 50 µl of the mixed capture bead suspension was added to the appropriate assay tubes. Aliquots of 50 µl of the reconstituted human inflammation standard were added to the control tubes and 50 µl of test sample to the test assay tubes. The assay

tubes were incubated for 1.5 hours, protected from direct light. After incubation, samples were washed with 1 ml wash buffer and centrifuged at 200 g for 5 minutes. The supernatant was carefully aspirated leaving 100 µl of liquid in each assay tube. To this 50 µl of Human Inflammation PE detection Reagent was added and the tubes were gently agitated to resuspend the bead pellet. The assay tubes were once again incubated for 1.5 hours at room temperature, protected from light. After incubation, the samples were washed with 1 ml wash buffer and centrifuged at 200 g for 5 minutes. The supernatant was carefully aspirated from each tube and 300 µl wash buffer was added to resuspend the bead pellet. The samples were vortexed for 3-5 seconds immediately before pipetting into a microtitre plate and analysing on the flow cytometer (BD FacsArray™). A $R^2 \geq 0.98$ for each IL standard curve was accepted. The results were interpreted by the level of expression of the cytokine with categories of low expression to highly expressed cytokines (0-2.9, >2.9-4.5, >4.5-6.5, >6.5-9.4, >9.4-13.2, >13.2-28, >28-80.4, >80.4-429 pg/ml).

4.1.7 Measurement Of Allergy-Related Inflammatory Mediators (cytokines)

The allergy-related inflammatory ILs (IL-7, IL-3, IL-4, IL-5, eotaxin and GM-CSF) were measured using the BD™ CBA Human Soluble Protein Flex Set assay which allow for multiplexed analysis of multiple proteins from a single sample. The multiplexed bead populations were resolved in the near-infrared (NIR) and red channels of the flow cytometer (BD FacsArray™). The procedure was done according to the manufacturer's instructions. In brief, 10 µl of each IL capture bead suspension was mixed together and the suspension was centrifuged at 200 g for 5 minutes and the supernatant carefully aspirated. The bead populations were resuspended in capture bead diluent to a final volume of 50 µl and incubated for 15 minutes at room temperature prior to use. A volume of 50 µl of standard or sample was added to the assay tubes and incubated for 1 hour at room temperature. After incubation, 50 µl of mixed PE detection reagent was added to each assay tube

and incubated for a further 2 hours. After incubation, samples were washed with 1 ml wash buffer and centrifuged at 200 g for 5 minutes. The supernatant was carefully aspirated and 300 µl of wash buffer added to resuspend the bead pellet. The samples were vortexed for 3-5 seconds immediately before pipetting into a microtitre plate and analysing on the flow cytometer (BD FacsArray™) (Becton Dickson, Oxford, UK). The results were interpreted as described in section 4.1.6.

4.1.8 SPIROMETRY

Spirometry was conducted according to the ATS guidelines (American Thoracic Society (ATS), 1995) as described in Chapter 3 (section 3.1.4).

4.1.9 STATISTICAL ANALYSIS

Statistical analysis was performed using STATA9 computer software (StataCorp, 2007, Texas, USA). The statistical approach used partitional and hierarchical clustering and factor analysis. The results of the continuous variables were expressed as means and standard deviation. Variables were log transformed to meet the model assumptions of normally distributed variables with equal variances and parametric analyses were then applied. However, where certain variables followed a non-Guassian distribution after transformation, non-parametric analyses were used. Chi-squared statistics were applied for binary comparisons. The grouping of subjects on the basis of their cytokine profiles was achieved by both agglomerative hierarchical complete linkage clustering (Nriagu et al., 1999) and partitional clustering to compare how well the algorithms performed on the cytokine measurements. For the former technique the identification was based on the largest stopping rule index (Calinski/Harabasz pseudo-F = 118.67). The study sample was also divided into four clusters using partition clustering (kmedians), based on previously known inflammatory cell asthma phenotypes (neutrophilic, eosinophilic, granulocytic and paucigranulocytic) (Simpson et al.,

2006). Factor analysis was used to determine inflammatory patterns based on 12 cytokines (Rodrigo and Rodrigo, 1993; Lapperre et al., 2004). Principal factor analysis (PFA) as well as principal component factor analysis (PCA) was done to determine the inflammatory cell phenotypes. The appropriateness of the correlation matrix (0.015) for factor analysis was assessed by the Bartlett's test of sphericity ($p< 0.001$) and the Kaiser-Meyer-Olkin (0.75). The number of factors was determined by the Eigenvalue criterion, whose magnitude should be ≥ 1, as well as the percentage of total variance explained by the factors. To increase the interpretability, the factors were rotated orthogonally through the Varimax rotation procedure. The following criteria were used in labelling the factors: i) a factor loading had to be > 0.45; ii) variables that loaded > 0.45 on more than one factor were excluded; and iii) single factor loadings were not accepted (Riekert and Eakin 2008). The naming of factors was based on the general association between the various cytokines and severity of asthma cited in the literature (Lichtman et al., 2005; Rogerio et al., 2007; Kasahara et al., 2008). To test for the difference between cluster groupings by phenotypic characteristics, analysis of variance (ANOVA) (with Bonferroni correction) was used. This method is similar to bi-clustering where not only the objects are clustered but also the features of the objects (i.e. the rows and columns are clustered simultaneously) (IPAM Group UCLA, 2008). To further determine the association between the cluster groupings and cytokine patterns in relation to health outcomes, multivariate logistic regression analysis was applied.

4.2 RESULTS

4.2.1 DEMOGRAPHIC CHARACTERISTICS

The age distribution between the asthmatic subjects and controls was similar (**Table 4.2**). There was a larger proportion of females than males in both groups. The asthmatic workers had a marginally higher body mass index, placing them in the overweight category, compared to the control group in which the body mass index was borderline normal. The majority of subjects from both groups were non-smokers. The control group had increased lung function readings compared to the asthmatic group, although not significant based on power calculations. Nonetheless, the findings concur with previous studies showing a decline in lung function in dental workers as opposed to non-exposed subjects. Both serum inflammatory biomarkers (ECP, MPO) were higher in the asthmatic group whereas the cytokine levels were variable among both groups.

Table 4.2 Demographic characteristics of study population.

Demographic characteristics	Asthma subjects n = 76	Controls n = 9
Clinical characteristics		
Age (years)	34 ± 13.00	33 ± 12.67
Sex		
- Male	26 (34%)	1 (11%)
- Female	50 (66%)	8 (89%)
Height (cm)	164 ± 10.86	160 ± 6.34
Weight (kg)	69 ± 16.90	61 ± 13.18
Body mass index[1]	26 ± 6.13	24 ± 4.23
Smoking history		
- Current	12 (17%)	1 (12%)
- Ex-smoker	4 (6%)	N/A
- Non-smoker	55 (77%)	7 (88%)
Atopy	44 (59%)	N/A
Upper respiratory symptoms		
- Ocular-nasal symptoms	32 (42.11%)	N/A
- Work-related ocular-nasal symptoms	7 (9.21%)	N/A
Lower respiratory symptoms		
- Asthma-related symptoms	28 (36.84%)	N/A
- Work-related chest symptoms	12 (15.79%)	N/A
Lung function		
- FEV_1 predicted (%)	86.18 ± 17.21	91.53 ± 14.49
- FVC predicted (%)	90.77 ± 14.21	93.13 ± 9.23
- FEV_1/FVC (%)	98.88 ± 13.85	104.99 ± 11.38
Serum inflammatory biomarkers		
- ECP (kU/L)	10.57 ± 13.40	4.20 ± 4.50
- MPO (μg/L)	48.96 ± 38.27	29.76 ± 23.24
Cytokines		
- IL-1β	4.92 ± 9.69	3.57 ± 5.89
- IL-3	3.32 ± 7.37	4.03 ± 5.16
- IL-4	3.08 ± 3.91	4.39 ± 3.96
- IL-5	1.99 ± 3.39	2.10 ± 2.15
- IL-6	5.57 ± 9.51	4.23 ± 4.17
- IL-7	11.77 ± 9.92	12.20 ± 8.61
- IL-8	12.94 ± 21.86	4.41 ± 5.50
- IL-10	3.93 ± 3.12	5.47 ± 3.20
- IL-12p70	9.31 ± 5.90	11.21 ± 6.64
- TNF-α	7.46 ± 5.91	9.86 ± 5.41
- Eotaxin	22.53 ± 34.75	38.57 ± 35.19
- GM-CSF	9.22 ± 49.17	10.94 ± 23.58

Note: Continuous variables- mean ± SD; Categorical variables- number (%), N/A - not applicable

[1] Underweight (<18.5), normal (18.5-24.9), overweight (25.0-29.9), obese (>30.0)

4.2.2 CYTOKINE GROUPINGS DEFINED BY CLUSTER ANALYSIS

The expressions of 12 cytokines are shown in the heat map which illustrates the variability of the measured cytokines among the asthmatic study population (n = 76) (**Figure 4.1**). The agglomerative clustering analysis procedure identified four potential clusters within the population. However, the agglomerative clustering produced background noise making it difficult to distinguish between groups. The population was therefore partitioned into four discernable groups using partition clustering namely cluster-1, cluster-2, cluster-3 and cluster-4. This provided some optimality criterion and therefore the clusters identified by this method were used for further analysis in this investigation.

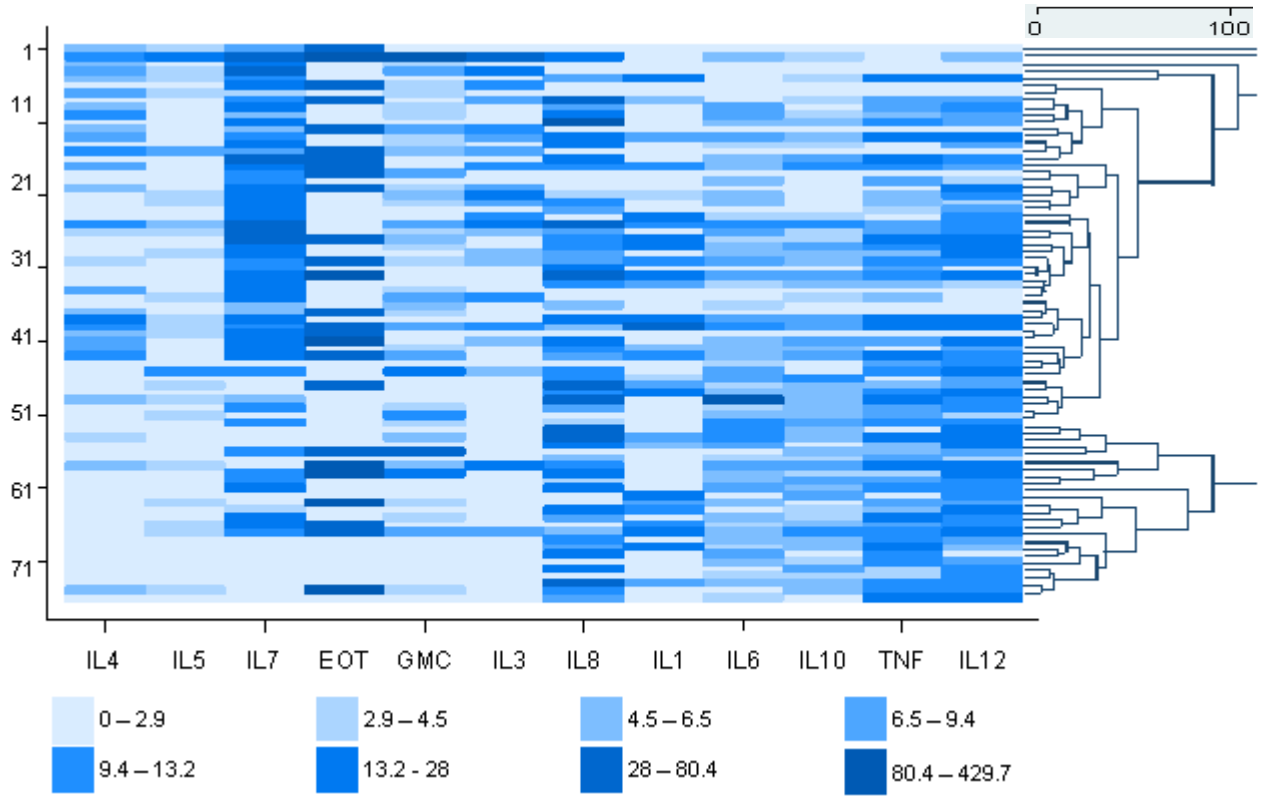


Figure 4.1 Heat map showing molecular profile of 12 inflammatory markers concentrations. Each value is represented by a coloured rectangular block. The high expressing cytokines (pg/ml) are depicted by the darker colour shades and the low expressing cytokines by the lighter shades. Each row is an individual subject and the individual cytokine values are shown in each column. To the right of the heatmap, is the dendrogram showing the distinction between groups.

4.2.3 IDENTIFICATION OF INFLAMMATORY PATTERNS

The correlation matrix of cytokines was first subjected to PFA of the 76 subjects (**Figure 4.2**). A 2-factor model was confirmed based on the Eigenvalues for factor-1 (F1) and factor-2 (F2) with 2.83 and 1.95, respectively. The following cytokines met the factor selection (correlation (ρ) values >0.45), F1: IL-5, eotaxin, GM-CSF, IL-3 and F2: IL-1β, IL-10, TNF-α, IL-12p70. The exception was eotaxin which featured in F1 but did not meet the selection criteria when Varimax rotation was applied ($\rho = 0.43$). Further analysis was done to extract the highest possible variance in the dataset using PCA and Varimax rotation. A four-factor (F1, F2, F3 and F4) solution was found to represent the data based on Eigenvalue criteria **(Figure 4.3)**, explaining approximately 67% of variance in the data set ($p < 0.001$). The Varimax rotation demonstrated unique factor loadings in F3 for IL-4 and IL-7 which was excluded from the initial PFA (**Table 4.3**). The labelling of the four factors identified by Varimax rotation was based on the factor selection criteria and the relation of the inflammatory patterns in asthma (**Table 4.3**).

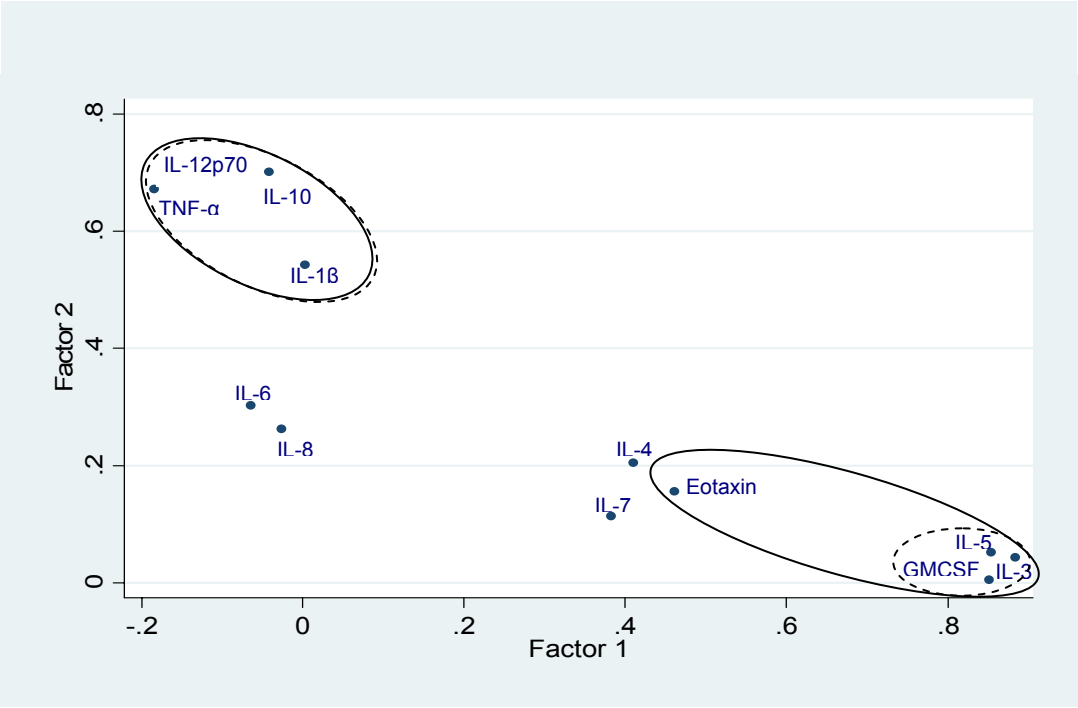


Figure 4.2 Factor loading plot for principal factor analysis showing factor groupings with and without Varimax rotation. Solid line denotes variable loadings included in factors without rotations and dashed lines denote variable loadings included in factors with Varimax rotation.

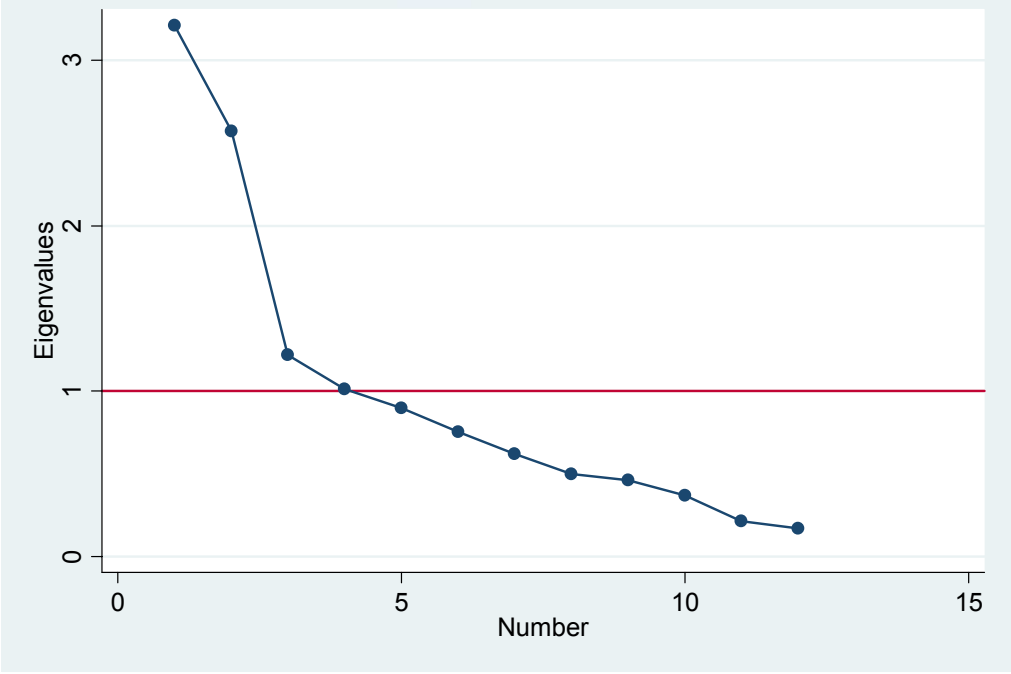


Figure 4.3 A scree test, performed by plotting the Eigenvalues of each extraction in descending order after Varimax rotation, confirming four factors.

Table 4.3 Principal component factor loading with Varimax rotation and factor labelling according to the inflammatory response of the cytokine groupings.

Variable	F1: Chronic eosinophilia	F2: Acute neutrophilia	F3: Acute eosinophilia	F4: Chronic neutrophilia
IL-5	**0.89**	-0.04	0.15	0.04
Eotaxin	**0.55**	0.23	0.11	-0.34
GMCSF	**0.93**	-0.07	0.01	0.03
IL-3	**0.88**	-0.05	0.22	-0.03
IL-4	0.29	0.17	**0.67**	-0.14
IL-7	0.21	-0.04	**0.81**	0.13
IL-8	-0.01	0.13	0.16	**0.76**
IL-6	0.06	0.26	-0.15	**0.63**
IL-1β	-0.03	**0.71**	0.22	-0.29
IL-10	0.01	**0.75**	0.11	0.22
TNF-α	-0.14	**0.75**	0.04	0.21
IL-12p70	-0.03	**0.80**	-0.19	0.12

4.2.4 Relationship Between Cluster Groupings And Inflammatory Patterns

To determine whether there were differences between the four inflammatory patterns and the four cluster groupings an ANOVA was conducted (**Table 4.4**). All the cytokines which scored high in F1 (chronic eosinophilia) demonstrated the highest mean levels in subjects from cluster-1. IL-6 and IL-8 which were highly expressed in F4 (chronic neutrophilia) showed the highest mean levels for subjects in cluster-3. There was a significant variance in the cytokine measurements featured in F2 and F4 and the cluster groupings. The majority of cytokines which scored high in F2 (acute neutrophilia) were highly expressed in cluster-4. These results demonstrate that there are distinct differences between the clusters and the majority of the factors.

Table 4.4 Analysis of variance between cytokine measurements of the identified factors, by the four cluster groupings.

Clusters / Factors	Cluster-1 n = 26	Cluster-2 n = 31	Cluster-3 n = 2	Cluster-4 n = 17	p value
F1: Chronic eosinophilia					
IL-5	**3.1 ± 4.70**	1.7 ± 2.61	2.6 ± 0.58	0.7 ± 1.61	0.139
Eotaxin	**65.9 ± 25.57**	0	0	0	<0.001
GM-CSF	**21.6 ± 83.52**	3.9 ± 4.97	1.3 ± 1.85	1.0 ± 2.32	0.474
IL-3	**5.9 ± 10.76**	2.5 ± 4.76	0	1.2 ± 3.60	0.149
F2: Acute neutrophilia					
IL-1β	7.7 ± 14.50	1.4 ± 3.81	0	**7.6 ± 6.11**	0.044
IL-10	4.1 ± 3.45	3.0 ± 2.56	5.4 ± 0.78	**5.3 ± 3.24**	0.061
TNF-α	7.3 ± 6.73	5.9 ± 4.32	**12.2 ± 1.77**	10.0 ± 6.61	0.076
IL-12p70	9.2 ± 6.74	7.6 ± 5.03	9.4 ± 4.03	**12.6 ± 5.16**	0.004
F3: Acute eosinophilia					
IL-4	**4.5 ± 3.85**	2.2 ± 3.40	2.7 ± 3.77	2.5 ± 4.52	0.468
IL-7	13.6 ± 9.29	**14.2 ± 9.43**	14.3 ± 11.85	4.4 ± 8.74	0.002
F4: Chronic neutrophilia					
IL-8	10.6 ± 15.17	5.9 ± 5.45	**103.5 ± 88.59**	18.7 ± 13.01	<0.001
IL-6	3.7 ± 2.98	4.3 ± 2.65	**43.9 ± 55.50**	6.1 ± 3.45	<0.003

IL-7, IL-10 and TNF-α were not normally distributed after transformation hence non-parametric tests were used. Arithmetic means and standard deviations are displayed.

4.2.5 Relationship Between Serum Inflammatory Markers And Individual Cytokines

The relationship between serum inflammatory markers (ECP and MPO) and individual cytokines was determined (**Table 4.5**). A significant correlation was demonstrated between IL-8 and both ECP and MPO. A modest correlation was found between both allergic inflammatory cytokines (IL-5, IL-7) and MPO. An inverse correlation was demonstrated between IL-1β and MPO.

Table 4.5 Correlation between log transformed serum inflammatory markers (ECP and MPO) and cytokine concentrations for the various factors.

	Pearson correlation coefficient (r) (n)	
Factors	**ECP (kU/l)**	**MPO (ng/ml)**
F1: Chronic eosinophilia		
IL-5	-0.01 (40)	**0.30 (42)***
Eotaxin	0.03 (30)	0.29 (32)
GM-CSF	-0.02 (50)	0.17 (52)
IL-3	0.01 (24)	0.15 (26)
F2: Acute neutrophilia		
IL-1β	-0.25 (30)	**-0.40 (31)***
IL-10	-0.08 (59)	0.08 (60)
TNF-α	-0.13 (62)	-0.24 (63)
IL-12p70	-0.19 (70)	-0.12 (71)
F3: Acute eosinophilia		
IL-4	0.18 (42)	0.11 (43)
IL-7	0.12 (62)	**0.25 (62)***
F4: Chronic neutrophilia		
IL-8	**0.42 (57)****	**0.47 (58)***
IL-6	0.16 (66)	0.13 (67)

* $p<0.05$, ** $p<0.001$

4.2.6 Relation Between Inflammatory Patterns And Health Outcomes

Examination of the health outcomes and cytokine inflammatory patterns revealed a significant association between WAA symptoms and all four inflammatory patterns (**Table 4.6**). Subjects with adverse pulmonary obstruction were more likely to have a mixed cellular infiltrate. The odds of having increased MPO are higher in subjects with both acute and chronic neutrophilic inflammation, although not significant. Frequency of asthma attacks appeared to be more common in subjects with acute and chronic eosinophilia. The odds of having atopic asthma were higher in subjects with a chronic eosinophilic response and appeared to be similar in both acute eosinophilic and neutrophilic responses. Conversely, the odds of WAA were significantly higher in subjects with chronic neutrophilia. Similar observations were demonstrated in the adjusted multivariate models.

Table 4.6 Association of health outcomes with the identified factors defining the inflammatory phenotypes in dental HCWs (n = 76) were obtained using logistic regression.

Health outcomes	Inflammatory Phenotype (OR, CI)			
	F1 ***Chronic Eosinophilia*** **OR (95% CI)**	**F2** ***Acute Neutrophilia*** **OR (95% CI)**	**F3** ***Acute Eosinophilia*** **OR (95% CI)**	**F4** ***Chronic Neutrophilia*** **OR (95% CI)**
Upper respiratory symptoms				
- Ocular-nasal symptoms	1.24 (0.54 - 2.86)	2.12 (0.80 - 5.62)	2.01 (0.96 - 4.20)	2.32 (0.14 - 37.42)
- Work-related ocular-nasal symptoms	0.23 (0.03 - 1.71)	0.79 (0.18 - 3.54)	0.62 (0.18 - 2.11)	6.06 (0.37 - 98.15)
Lower respiratory symptoms				
- Asthma-related symptoms[1]	1.80 (0.80 - 4.02)	0.98 (0.34 - 2.85)	1.54 (0.73 - 3.27)	-
- Work-related chest symptoms	**4.40 (1.36 - 14.17)***	**4.99 (1.32 - 18.92)***	**3.54 (1.11 - 11.25)***	**21.6 (1.30 - 357.99)***
- Number of asthma attacks[2]	**9.49 (3.49 - 25.79)****	**2.64 (0.57 - 12.31)**	**7.42 (2.79 - 19.75)****	**-**
Lung function				
- FEV_1 / FVC < 0.7	4.01 (0.82 - 19.64)	2.44 (0.29 - 20.43)	**5.25 (1.35 - 20.47)***	**25 (1.49 - 421.80)***
- FEV_1 < 80% predicted	3.17 (0.84 - 11.96)	2.74 (0.56 - 13.33)	**6.11 (2.15 - 17.39)****	12.18 (0.74 - 199.96)
Atopy	1.84 (0.81 - 4.19)	1.54 (0.56 - 4.22)	1.97 (0.93 - 4.16)	1.18 (0.07 - 19.03)
Latex sensitisation				
- Composite latex (k82) extract	0.83 (0.19 - 3.65)	0.62 (0.08 - 4.80)	1.55 (0.51 - 4.66)	-
- True latex sensitisation	-	0.90 (0.11 - 7.04)	1.50 (0.43 - 5.26)	-
Serum inflammatory biomarkers				
- ECP[3]	1.53 (0.59 - 3.99)	0.95 (0.27 - 3.41)	1.33 (0.55 - 3.12)	4.51 (0.28 - 72.87)
- MPO[4]	1.74 (0.63 - 4.82)	2.25 (0.70 - 7.17)	0.48 (0.11 - 2.08)	7.12 (0.44 - 115.49)
Asthma phenotypes				
- Atopic asthma	**10.77 (4.28 - 27.15)****	**5.06 (1.54 - 16.65)***	**5.30 (2.06 - 13.64)****	-
- Non – atopic asthma	-	1.08 (0.14 - 8.45)	1.95 (0.55 - 6.92)	17.83 (1.08 - 293.89)
- Work-aggravated asthma	**5.80 (1.75 - 19.27)***	3.75 (0.77 - 17.90)	**4.67 (1.43 - 15.30)***	**27.25 (1.63 - 455.49)***

* $p<0.05$, ** $p<0.001$

[1] 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 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?"

[2] Frequency of asthma attack in past 12 months defined as "frequent" if more than one attack per month and "none" if no attack per month.

[3] ECP categorical variable: Positive response based on values above the normal range of the patient population.

[4] MPO categorical variable: Positive response based on values above the normal range of the patient population.

4.3 DISCUSSION

The results of this study demonstrated four cluster groupings based on the varying cytokine measurements between study subjects and defined important inflammatory phenotypes in assessing allergy and asthma in this group of workers. A classification of asthma subtypes based on cytokine measurements using factor analysis (Riekert and Eakin, 2008) also produced four distinct inflammatory groups (factors). Two factors were more clearly related to an eosinophilic type of inflammation whereas the other two related to a neutrophilic response. Each cellular response appeared to be related further to severity of inflammation (i.e. acute or chronic) as identified by the particular cytokine expressions per factor. Similar results were shown in the study by Brasier and colleagues in which at least four phenotypically distinct subgroups based on bronchoalveolar lavage-derived cytokines of severe asthma were identified (Brasier et al., 2008). Shared variance among clusters and cross-loadings of factors were also evident, indicating that some cytokines may overlap between groups, or represent aetiological pathways that have smaller effects (Pillai et al., 2008). The results found in this study are suggestive of the robustness of the factor analysis (Riekert and Eakin, 2008) as a tool for identifying inflammatory patterns in asthma among dental HCWs.

Inference of these cellular types was based on the predictive markers of these cells and the cytokines that were directly or indirectly involved. Soluble markers such as ECP and MPO are generally used to assess the presence of eosinophils and neutrophils, respectively (Monteseirin et al., 2001; Cicutto and Downey, 2004). A combination of cellular types (Jatakanon et al., 1999) in some groups generally reflects an inflammatory response to multiple agents. The findings from the current study showed a correlation between cytokines associated with chronic neutrophilia and both ECP and MPO (DunnGalvin et al., 2006). In addition, a correlation was also found between acute and

chronic eosinophilic cytokines and MPO. These findings suggest the role of dual pathways resulting from mixed exposures in this group. Although this study demonstrated distinct subtypes based on the cytokine measurements, it is clear that a complex network exists between cytokines and cellular markers (Simpson et al., 2006). The dual response may be suggestive of the synergistic effects between exposures in this setting which augment the inflammatory response. The primary agent inducing these inflammatory responses is believed to be bacterial endotoxin (Douwes et al., 2002b), which has recently been shown to act as an adjuvant for certain allergens (Liu, 2002; Liebers et al., 2006). Furthermore, neutrophils can also be a source of ECP in IgE-mediated responses (Monteseirin et al., 2007) which could explain the discordance between the serum inflammatory cells and cytokines associated with the various inflammatory patterns. The mutual interaction between the cytokines needs to be borne in mind when trying to assess the functional role of an individual cytokine in a complex disease process such as asthma (Brasier et al., 2008).

In this study ECP and MPO did not demonstrate any significant association with any of the asthma phenotypes. This lack of association suggests that these two biomarkers in serum are not useful as predictive markers in serum since they may not be present when the effects of these cytokines are manifest (Lemiere et al., 2006; Wenzel, 2006; Brasier et al., 2008). It would also appear that cytokine production, rather than the influx of eosinophils or production of IgE, is the main cause of bronchial hyperresponsiveness (Ordonez et al., 2000; Monteseirin et al., 2007).

The cytokines associated with the neutrophilic responses in this study included IL-1β, IL-10, TNF-α, IL-12p70 and IL-8, IL-6. The results also demonstrated that both acute and chronic neutrophilic groups are more likely to be caused by work-related factors in the absence of latex playing a major role. In addition, subjects with chronic neutrophilic inflammation were more likely to have WAA,

although an association was also demonstrated with eosinophilic inflammation. The findings of the current study are consistent with other studies that non-allergic asthma associated with endotoxin exposure is mediated by an inflammatory response involving a number of cytokines, including IL-1, IL-6, IL-8, and TNF-α, IL-12 (Haldar and Pavord, 2007; Smit et al., 2009). IL-6 and IL-8 are also commonly associated with neutrophilic inflammation in severe asthma (Fahy et al., 1995; Mahajan and Mehta, 2006; Tsoumakidou et al., 2006; Brasier et al., 2008). The role that exposure to multiple workplace agents plays in the manifestation of different inflammatory types of asthma is also emphasised (Tarlo, 2000; Singh and Mabe, 2008).

Cytokines commonly associated with eosinophilic inflammation in asthma include IL-3, IL-5, eotaxin and GM-CSF (Doshi et al., 2002; Alam and Busse, 2004; Mahajan and Mehta, 2006). These cytokines were prominent in a particular factor which was called chronic eosinophilic inflammation. This is further supported by the fact that the odds of having atopic asthma were increased in subjects displaying chronic eosinophilia when compared to the others. The other group of cytokines associated with an eosinophilic inflammation in the acute inflammatory response involves IL-4 and IL-7. These cytokines have been reported to synergise with each other to induce human IgE and IgG4 production in a T-cell dependent manner (Jeannin et al., 1998). Overexpression of IL-4 in the lungs leads to a lymphocytic and eosinophilic inflammation, without causing airway hyperreactivity (Renauld, 2001; Mahajan and Mehta, 2006). In addition, there is evidence to suggest that IL-4 is associated with atopic diseases (e.g. allergic rhinitis and atopic dermatitis) (Holberg et al., 2001). This was confirmed in the current study, as the odds of being atopic were increased in this group relative to the other groups, although not significantly.

The results of this study clearly illustrate the presence of a complex cytokine network and the existence of various inflammatory subtypes in asthmatic dental workers especially acute/chronic eosinophilia and acute neutrophilia. This suggests that asthma is not exclusively due to an eosinophilic inflammatory response in this group. The mixed inflammatory profile may be the result of synergistic effects between allergens and other agents such as endotoxin causing asthma. The multiple expressions of the different cytokine patterns may also be related to the level of exposure to various agents which may overwhelm the immune system, resulting in a more severe immune response. This study has highlighted the use of these biomarkers in subjects with similar clinical characteristics of asthma to ascertain the pathophysiological basis for the symptoms observed in this group. Previous studies have used factor analysis to identify distinct asthma phenotypes with specific focus on clinical measures and quality of life (Juniper et al., 2004), duration of attack and steroid use (Rodrigo and Rodrigo, 1993). This current study has shown that factors expressed as quantitative traits in the form of cytokine inflammatory patterns to characterise asthma phenotypes are useful in the exploration of asthma causation in working populations.

In conclusion, the results demonstrated the pathological mechanisms manifesting in the different phenotypes. WRA, encompassing WAA and probable latex OA and airway inflammation in dental HCWs can be characterised by both eosinophilic and neutrophilic phenotypes. The most important determinants for the eosinophilic subtype are IL-3, IL-4, IL-5, IL-7, eotaxin and GM-CSF, and for the neutrophilic subtype IL-1β, IL-6, IL-8, IL-10, TNF-α and IL-12p70. Subjects with similar clinical characteristics can have heterogeneous subtypes suggesting that inflammatory patterns can assist in improved diagnosis. Furthermore, the clinical management of asthma could potentially be significantly enhanced by the identification of objective and clinically feasible biomarkers through

which clinicians could adjust therapy and improve asthma control. With this in mind appropriate action can also be taken towards developing effective prevention strategies at a population level if the causative agents can be identified. It should be borne in mind that, the broad definition for probable asthma and asthma-related symptoms may have resulted in disease misclassification and should be investigated in future studies.

CHAPTER 5

ENDOTOXIN EXPOSURE-RESPONSE RELATIONSHIPS FOR ASTHMA PHENOTYPES IN DENTAL WORKERS

5.0 INTRODUCTION

There is increasing evidence that exposure to endotoxin has an ambiguous relationship with allergy and asthma. It has been reported that airways reactive to allergens can enhance the response to inhaled endotoxin, and that endotoxin can enhance the airway response to allergens (Williams and Halsey, 1997; Reed and Milton, 2001; Schwartz, 2001). Although it is plausible that bacterial endotoxin may protect against the development of asthma, the evidence for this relationship is mixed. Firstly, a protective effect has only been established for atopy (and mainly specific pollen sensitisation) and for hay fever. Secondly, the prevalence of asthma in children with frequent contact with animals is only marginally reduced. Thirdly, it is well established that endotoxin exposure itself may induce respiratory symptoms including (non-allergic) asthma symptoms. While endotoxin exposure may prevent the primary causation of allergic asthma, it may be both a primary and secondary cause of occupational respiratory disease including non-allergic asthma (Douwes et al., 2002a; Heederik and Wouters, 2007).

In this bimodal effect of endotoxin on the T_H1/ T_H2 balance, occupational studies suggest that exposure to endotoxin more likely induces asthma rather than protects against it (Radon, 2006). There is also evidence to suggest that endotoxin exposure may both exacerbate pre-existing asthma and induce new asthma in adults (Douwes et al., 2002b). Endotoxin studies suggest that the patterns

of airway inflammation may vary under different conditions. Furthermore, occupational exposure to endotoxin causes a greater fall in FEV_1 in atopic than in non-atopic workers (Sigsgaard et al., 1992). Chronic exposure to endotoxin has also been associated with the development of asthma, chronic bronchitis, emphysema and chronic obstructive lung disease (Reed and Milton, 2001; Douwes et al., 2002b; Singh and Schwartz, 2005).

Various studies suggest that the adverse health effects are dose dependent in that the non-allergic respiratory symptoms caused by endotoxin may occur only at higher endotoxin levels. Exposure to low-dose endotoxin may increase allergen-induced bronchial inflammation in asthmatics through a priming effect (Sohy et al., 2006) whereas high doses may result in an increased risk of chronic bronchitis and non-atopic asthma (Douwes et al., 2004). However, some studies have shown that high endotoxin levels in the occupational and general environment may in fact protect from sensitisation to allergens (Liu, 2002; Delayre-Orthez et al., 2004; Radon, 2006). *In vitro* studies have shown that low-level endotoxin exposure primes macrophages to release cytokines (i.e. TNF-α and IL-12) and enhances neutrophil immune responsiveness to stimuli. Therefore, T_H1-promoting immune responses can also occur with lower and more frequent endotoxin doses, circumventing the inflammatory or toxic immune responses seen at higher doses (Liu, 2002).

Endotoxin has been shown to be an important predictor of the development and progression of occupational airways disease in a variety of occupational settings (Schwartz, 2001; Douwes et al., 2002b; Pankhurst et al., 2005). In dental health care settings health teams are exposed to varied levels of endotoxin concentrations (Pankhurst et al., 2005; Huntington et al., 2007). However, studies of exposure-response relationships are scanty, with only one study that has evaluated the possible role of contaminated DUWLs on the prevalence and timing of onset of asthma in dentists.

In that study dentists who were exposed to high bacterial counts from DUWLs were more likely to develop asthma since starting training as a dentist (Pankhurst et al., 2005). Studies of endotoxin among dental workers who are subjected to prolonged daily exposure to contaminated DUWLs and their risk of developing asthma have however not been conducted.

The aims of this study were to:

1 Characterise the exposures of dental HCWs using the following indices:

- employment duration
- airborne endotoxin concentration in current job
- cumulative exposure (employment duration x concentration),

2 Characterise the clinical endpoints of interest:

- general asthma-related symptoms (chest tightness, wheeze)
- work-related symptoms (ocular-nasal, chest)
- lung function (FEV_1 < 80% predicted, FEV_1 / FVC < 0.7)
- latex sensitivity (latex specific IgE reactivity)
- early response inflammatory biomarker (ECP, MPO)
- asthma phenotypes based on inflammatory cell type: eosinophilic, neutrophilic
- asthma phenotypes based on environmental and occupational triggers: atopic asthma, non-atopic asthma, work-aggravated asthma,

3 Define the exposure-response relationships in respect of the endotoxin exposures and the clinical endpoints in dental HCWs while controlling for potential confounders (age, sex, smoking).

5.1 MATERIALS AND METHODS

5.1.1 STUDY POPULATION

A cross-sectional study of 454 dental HCWs from five public sector academic health care institutions comprising six sampling sites was conducted. The participants included dental staff (clinical, laboratory, administration and auxiliary) and students (performing clinical sessions).

5.1.2 ENVIRONMENTAL EXPOSURE ASSESSMENT

Exposure assessment was carried out using a combination of work history information and personal exposure measurements. Work history information obtained from a self-administered questionnaire was assessed by: workers' description of job; distance from aerosol source; subjective categorisation according to the participants' interpretation of exposure to aerosols into none, low or high categories; use of PPE, e.g. face masks, gloves, goggles. Exposure assessments were conducted on study volunteers within each of 13 identified 'exposure groups' (EGs) nested within four departments in the academic institutions. Inhalable personal air samples were conducted with a pocket sampler and analysed for endotoxin. Exposure metrics were developed on the basis of individually measured exposures with an average (arithmetic mean) computed for each EG (see **Chapter 2**). Several indices of exposure were constructed using the endotoxin exposure intensities to develop models for exposure histories relevant to the disease onset process:

Current and historical endotoxin exposure levels

Current exposure levels to endotoxin were calculated as the average concentration within each EG and assigned to each member of the group. Group average exposure levels can be superior to individual exposure levels when limited data are available about the individual (Heederik and

Douwes, 1997). Modelled endotoxin exposure showed a good correlation with measured exposure ($r = 0.61$, $p < 0.001$, $n = 413$) in the subjects with available measurement data. The parameters used for modelling included exposure group and the day of sampling. Since no exposure measurements had been done in the past, exposure concentrations obtained during environmental sampling of each EG were used as a proxy for historical exposure concentrations. This was considered to be reasonable since none of the institutions had undergone any major structural changes to work processes or engineering controls in at least the past 5 years.

Cumulative exposure

Cumulative exposure for endotoxin was expressed as the sum of the product of the average concentrations in each EG and the employment duration in each EG:

$$CE = \Sigma(X_{EG} \times D_{EG})$$

Where X_{EG} is the average exposure in an exposure group and D_{EG} is the duration of work in that group.

5.1.3 ASSESSMENT OF CLINICAL ENDPOINTS

The health outcome measurements obtained are described in detail in **Chapter 3**. Briefly, a self-administered health and work history questionnaire adapted from the European Community Respiratory Health Survey (ECRHS) questionnaire was used (European Community Respiratory Health Survey, 1993). Questions relating to workers' general health, smoking and work history were included. Blood samples were obtained for the determination of allergic and inflammatory

biomarkers in sera of subjects. Phadiatop® test, latex-specific IgE for composite latex (k82), as well as CCDs (HRP and MUXF3-bromelain) were performed, using the ImmunoCAP System (Phadia AB, Uppsala, Sweden). A result of > 0.35 kU/l was regarded as positive. ECP concentrations were determined using the ImmunoCAP ECP FEIA, according to the manufacturer's instructions. The normal range for serum ECP on the patient population established in the laboratory was 2.00 – 15.7 µg/l. Determination of serum MPO concentration was done using the MPO-EIA, sandwich ELISA method (MPO-EIA, Oxford Biomedical research). The normal MPO range established in the laboratory was 8.43 - 77.35 ng/ml. Therefore values above this range were considered elevated for the study population. The BD^{TM} CBA human inflammation kit was used to quantitatively measure non-allergic related IL (IL-8, IL-1β, IL-6, IL-10, IL-12p70, TNF-α) levels in each serum sample. The allergic ILs (IL-7, IL-3, IL-4, IL-5, eotaxin and GM-CSF) were measured using the BD^{TM} CBA human soluble protein flex set. The samples were read with a flow cytometer (BD FacsArray^{TM}) (Becton Dickson, Oxford, UK). Detailed methodology of serum IL determinations is described in **Chapter 4**. Lung function spirometry was done (pre- and post-bronchodilator) on all participants as described in **Chapter 3**.

5.1.4 STATISTICAL ANALYSIS

The general statistical approach involved bivariate and multivariate analyses of the outcomes in relation to the predictors of interest. Exploratory bivariate analysis was used to assess the nature of the association between exposures, covariates and outcomes. Key associations of interest involved investigating the relationships between endotoxin exposure (current endotoxin concentration, duration of employment, cumulative endotoxin exposure) and health outcomes. Health outcome variables included general ocular-nasal and asthma-related symptoms, and work-related ocular-nasal and asthma symptoms, the pattern of baseline spirometry (primarily FEV_1/ FVC ratio, FEV_1 % of

predicted) and allergic sensitisation to latex. The key disease outcome variables of interest included atopic asthma, non-atopic asthma and WAA. These were defined *a priori* based on the criteria outlined in **Chapter 3**. Asthma-related symptoms were defined as: 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 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?" ECP and MPO categorical variables were developed based on positive response values above the normal range of the patient population established in the laboratory.

Logistic regression was conducted to assess the association between exposure risk factors and the health outcomes defined. Initial logistic regression modelling of endotoxin as the explanatory variable was performed on data grouped into tertiles, where the data was divided into three approximately equally sized groups. All models were formulated using the saturated model approach when adjusting for confounders (age, sex and smoking status [current, ex-smokers, never smoked]) to enable comparison across various exposure metrics. Data were analysed using STATA9 computer software (StataCorp, 2007, Texas, USA).

5.2 RESULTS

5.2.1 PREVALENCE OF CLINICAL ENDPOINTS IN RELATION TO VARIOUS EXPOSURE INDICES

Associations between various clinical endpoints and airborne endotoxin exposure indices were explored in this study.

5.2.1.1 Airborne endotoxin exposure in current job

In the bivariate models, ocular-nasal and asthma symptoms did not appear to be significantly different between the various endotoxin exposure categories (**Table 5.1**). Latex sensitisation to composite latex (k82) was significantly ($p < 0.05$) higher among subjects exposed to higher levels of low-grade endotoxin. An increasing trend across exposure tertiles was observed between true latex sensitisation, ECP and endotoxin exposure, although this was not significant.

Table 5.1 Prevalence of clinical endpoints according to different categories of endotoxin exposure (tertiles) in current job among dental HCWs (n = 454).

Characteristics	Endotoxin in air (EU/m^3)			
	Lower tertile < 2.25 n = 137	Middle tertile 2.25 – 5.83 n = 137	Upper tertile > 5.83 n = 136	Chi-square p-value
Ocular-nasal and asthma symptoms (in last 12 months)				
- Ocular-nasal	42 (30%)	43 (31%)	42 (30%)	0.991
- Wheezing without flu or cold	14 (10%)	22 (16%)	13 (9%)	0.190
- Woken up by cough	30 (21%)	35 (25%)	31 (22%)	0.759
- Woken up by tight chest	18 (13%)	21 (15%)	22 (16%)	0.767
- Woken up by shortness of breath	11 (8%)	11 (8%)	9 (6%)	0.878
- Asthma-related symptoms[1]	37 (27%)	46 (33%)	44 (32%)	0.458
- Asthma attacks	8 (6%)	8 (6%)	6(4%)	0.410
- Current asthma treatment	5 (4%)	8 (6%)	8 (6%)	0.808
Work-related symptoms				
- Work-related chest symptoms	3 (2%)	6 (4%)	7 (5%)	0.424
- Work-related ocular-nasal symptoms	15 (10%)	23 (16%)	19 (13%)	0.377
- Chest symptoms associated with peak exposure	33 (24%)	24 (17%)	21 (15%)	0.164
Baseline pulmonary function indices (n = 412)				
- FEV_1 / FVC < 0.7	3 (23%)	6 (46%)	4 (31%)	0.401
- FEV_1 < 80% predicted	121 (88%)	125 (91%)	129 (94%)	0.510
Allergic sensitisation to latex				
- Sensitisation to latex (k82) (n = 421)	9 (6%)	9 (6%)	19 (13%)	**0.048**
- True sensitisation to latex (n = 41)[2]	5 (3%)	8 (5%)	13 (9%)	0.093
Serum inflammatory biomarkers				
- ECP	18 (13%)	19 (14%)	29 (21%)	0.093
- MPO	14 (10%)	16 (12%)	20 (15%)	0.442
Asthma phenotypes (n = 421)				
- Atopic asthma	7 (5%)	9 (6%)	8 (5%)	0.876
- Non-atopic asthma	10 (7%)	9 (6%)	4 (2%)	0.246
- Work-aggravated asthma	3 (2%)	7 (5%)	5 (3%)	0.437

Note: Categorical variables- number (%)

[1] 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 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?"

[2] True latex sensitisation refers to positive response to latex (k82) and negative response to CCDs.

5.2.1.2 Employment duration

The prevalence of ocular-nasal symptoms was significantly ($p < 0.05$) higher among dental workers with shorter employment duration **(Table 5.2)**. Decreased lung function ($FEV_1 < 80\%$ predicted) was slightly more common among staff who worked for shorter periods in the dental clinics. High MPO levels were more prevalent among workers with long service, with borderline significance.

Table 5.2 Prevalence of clinical endpoints according to different categories of employment duration of dental HCWs (n = 454).

Characteristics	Employment duration (years)			
	< 10 n = 206	10 – 20 n = 87	>20 n = 161	Chi-square p-value
Ocular-nasal and asthma symptoms (in last 12 months)				
- Ocular-nasal	75 (36%)	30 (34%)	32 (19%)	**0.002**
- Wheezing without flu or cold	26 (12%)	10 (11%)	21 (13%)	0.939
- Woken up by cough	49 (23%)	19 (21%)	35 (21%)	0.878
- Woken up by tight chest	29 (14%)	10 (4%)	28 (17%)	0.427
- Woken up by shortness of breath	12 (5%)	5 (2%)	17 (10%)	0.183
- Asthma-related symptoms[1]	58 (28%)	27 (31%)	50 (31%)	0.798
- Asthma attacks	8 (6%)	2 (1%)	13 (10%)	0.86
- Current asthma treatment	8 (6%)	3 (2%)	14 (10%)	0.135
Work-related symptoms				
- Work-related chest symptoms	8 (3%)	1 (1%)	8 (4%)	0.316
- Work-related ocular-nasal	33 (16%)	16 (18%)	16 (9%)	0.124
- Chest symptoms associated with peak exposure	45 (21%)	18 (20%)	20 (12%)	0.055
Baseline pulmonary function indices (n = 412)				
- $FEV_1 / FVC < 0.7$	8 (4%)	1 (1%)	6 (4%)	0.400
- $FEV_1 < 80\%$ predicted	193 (93%)	80 (91%)	139 (86%)	**0.050**
Allergic sensitisation to latex				
- Sensitisation to latex (k82) (n = 421)	22 (10%)	6 (6%)	13 (8%)	0.511
- True sensitisation to latex (n = 41)[2]	16 (7%)	5 (5%)	8 (4%)	0.595
Serum inflammatory biomarkers				
- ECP	35 (16%)	13 (14%)	28 (17%)	0.749
- MPO	19 (9%)	8 (9%)	26 (16%)	0.056
Asthma phenotypes (n = 421)				
- Atopic asthma	11 (5%)	5 (5%)	13 (8%)	0.537
- Non-atopic asthma	13 (6%)	2 (2%)	10 (6%)	0.345
- Work-aggravated asthma	6 (2%)	2 (2%)	9 (5%)	0.298

Note: Categorical variables- number (%)

[1] 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 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?"

[2] True latex sensitisation refers to positive response to latex (k82) and negative response to CCDs.

5.2.1.3 Cumulative endotoxin exposure

A significant positive trend ($p < 0.05$) was observed between chest tightness and asthma-related symptoms with increasing levels of cumulative endotoxin exposure **(Table 5.3)**. Both inflammatory and asthma phenotypes did not show any significant differences across the cumulative endotoxin exposure categories.

Table 5.3 Prevalence of clinical endpoints according to different categories of cumulative endotoxin exposure (tertiles) in dental HCWs (n = 454).

Characteristics	Cumulative endotoxin exposure (EU/m^3-year)			
	Lower tertile < 11.78 n = 113	Middle tertile 11.85 – 50.64 n = 113	Upper tertile > 51.12 n = 112	Chi-square p-value
Ocular-nasal and asthma symptoms (in last 12 months)				
- Ocular-nasal	35 (30%)	49 (43%)	36 (32%)	0.100
- Wheezing without flu or cold	18 (15%)	12 (10%)	17 (15%)	0.459
- Woken up by cough	25 (22%)	30 (26%)	32 (28%)	0.527
- Woken up by tight chest	11 (9%)	22 (19%)	24 (21%)	**0.043**
- Woken up by shortness of breath	6 (5%)	12 (10%)	12 (10%)	0.263
- Asthma-related symptoms[1]	30 (26%)	35 (30%)	48 (42%)	**0.028**
- Asthma attacks	5 (4%)	5 (4%)	9 (7%)	0.638
- Current asthma treatment	6 (4%)	4 (3%)	9 (7%)	0.420
Work-related symptoms				
- Work-related chest symptoms	5 (4%)	4 (3%)	7 (6%)	0.621
- Work-related ocular-nasal	14 (12%)	18 (15%)	24 (21%)	0.185
- Chest symptoms associated with peak exposure	27 (23%)	23 (20%)	23 (20%)	0.767
Baseline pulmonary function indices (n = 412)				
- FEV_1 / FVC < 0.7	2 (2%)	6 (5%)	3 (3%)	0.767
- FEV_1 < 80% predicted	102 (90%)	103 (91%)	106 (94%)	0.441
Allergic sensitisation to latex				
- Sensitisation to latex (k82) (n = 421)	9 (7%)	5 (4%)	13 (11%)	0.139
- True sensitisation to latex (n = 41)[2]	8 (7%)	3 (2%)	9 (8%)	0.177
Serum inflammatory biomarkers				
- ECP	15 (13%)	20 (17%)	18 (16%)	0.692
- MPO	12 (10%)	14 (12%)	12 (10%)	0.915
Asthma phenotypes (n = 421)				
- Atopic asthma	7 (6%)	5 (4%)	8 (7%)	0.679
- Non-atopic asthma	10 (8%)	5 (4%)	7 (6%)	0.399
- Work-aggravated asthma	5 (4%)	4 (3%)	7 (6%)	0.621

Note: Categorical variables- number (%)

[1] 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 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?"

[2] True latex sensitisation refers to positive response to latex (k82) and negative response to CCDs.

5.2.2 Relationship Between Clinical Endpoints And Various Exposure Indices Using Unconditional Logistic Regression

In the unadjusted logistic regression model between current endotoxin exposure and health outcomes, workers sensitised to latex (k82) were more likely to be exposed to higher endotoxin levels (OR = 2.46, 95% CI: 1.07 – 5.66; p = 0.035) (**Table 5.4**). The odds of developing true sensitisation were also stronger in subjects exposed to higher endotoxin exposure. A borderline association was also observed with decreased lung function (FEV_1 < 80% predicted) and ECP with increasing endotoxin concentration.

Table 5.4 Association between current endotoxin exposure concentration categories and clinical endpoints in unconditional logistic regression models.

Outcome variables	Endotoxin in air (EU/m^3)			
	Middle tertile 2.25 – 5.83		Upper tertile > 5.83	
	OR (95% CI) n = 137	p-value	OR (95% CI) n = 136	p-value
Ocular-nasal and asthma symptoms (in last 12 months)				
- Ocular-nasal	0.80 (0.48 - 1.31)	0.372	1.04 (0.64 - 1.71)	0.864
- Wheezing without flu or cold	2.51 (0.89 - 7.08)	0.081	0.68 (0.25 - 1.82)	0.438
- Woken up by cough	1.22 (0.70 - 2.14)	0.478	1.05 (0.60 - 1.86)	0.859
- Woken up by tight chest	1.20 (0.61 - 2.36)	0.604	1.28 (0.65 - 2.50)	0.479
- Woken up by shortness of breath	1.00 (0.42 - 2.39)	1.000	0.81 (0.33 - 2.03)	0.655
- Asthma-related symptoms[1]	1.53 (0.90 - 2.59)	0.113	1.59 (0.93 - 2.70)	0.090
- Asthma attacks	0.67 (0.18 - 2.51)	0.549	0.40 (0.10 - 1.56)	0.188
- Current asthma treatment	1.60 (0.38 - 6.82)	0.525	1.20 (0.29 - 4.93)	0.800
Work-related symptoms				
- Work-related chest symptoms	1.85 (0.38 - 9.08)	0.451	2.33 (0.48 - 11.23)	0.291
- Work-related ocular-nasal	1.42 (0.44 - 4.59)	0.557	2.10 (0.70 - 6.30)	0.188
- Chest symptoms associated with peak exposure	0.67 (0.37 - 1.21)	0.182	0.58 (0.31 - 1.06)	0.075
Baseline pulmonary function indices (n = 412)				
- FEV_1 / FVC < 0.7	2.16 (0.53 – 8.85)	0.286	1.30 (0.28 – 5.93)	0.737
- FEV_1 < 80% predicted	1.38 (0.63 - 3.03)	0.426	**2.44 (0.97 - 6.13)**	**0.058**
Allergic sensitisation to latex				
- Sensitisation to latex (k82) (n = 421)	0.97 (0.37 - 2.52)	0.947	**2.46 (1.07 - 5.66)**	**0.035**
- True sensitisation to latex (n = 41)[2]	1.59 (0.51 - 4.99)	0.429	**2.95 (1.02 - 8.55)**	**0.046**
Serum inflammatory biomarkers				
- ECP	1.06 (0.53 - 2.12)	0.879	**1.87 (0.97 - 3.57)**	**0.060**
- MPO	1.12 (0.52 - 2.41)	0.764	1.56 (0.75 - 3.25)	0.232
Asthma phenotypes				
- Atopic asthma	1.31 (0.47 - 3.61)	0.607	1.17 (0.41 - 3.32)	0.768
- Non-atopic asthma	0.89 (0.35 - 2.27)	0.812	0.38 (0.12 - 1.26)	0.114
- Work-aggravated asthma	2.41 (0.61 - 9.50)	0.211	1.70 (0.40 - 7.28)	0.471

Each OR is derived from a separate model

Lower tertile of airborne endotoxin exposure (< 2.25 EU/m^3) was used as baseline for comparison (n = 137)

[1] 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 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?"

[2] True latex sensitisation refers to positive response to latex (k82) and negative response to CCDs.

It was evident from this study that ocular-nasal symptoms were less likely (OR = 0.43, 95% CI: 0.27 - 0.70; $p < 0.001$) to occur in workers who had worked for longer than 20 years in the dental environment (**Table 5.5**). Chest symptoms as a result of peak exposures were also less common among this group of individuals. Suboptimal lung function was less likely (OR = 0.43, 95% CI: 0.21 - 0.87, $p < 0.05$) to occur among workers with long service history. Interestingly the odds of having high MPO levels were higher (OR = 1.97, 95% CI: 1.04 - 3.73, $p < 0.05$) in workers who worked for more than 20 years in dentistry.

While none of the exposure metrics were found to predict the presence of any work-related symptoms, lower levels of cumulative exposure was associated with an increased odds ratio (OR = 1.97, CI: 1.14 – 3.39, $p = 0.015$) for developing ocular-nasal symptoms (**Table 5.6**). The odds of being woken up by tightness in the chest were significantly increased with increasing cumulative exposure (OR = 2.53, 95% CI: 1.17 – 5.45, $p < 0.05$). Furthermore, workers reporting asthma-related symptoms were twice as likely to have higher cumulative exposure (OR = 2.08, 95% CI: 1.18 - 3.64, $p < 0.05$).

Table 5.5 Association between employment duration categories and clinical endpoints in unconditional logistic regression models.

Outcome variables	Employment duration (years)			
	10 – 20		> 20	
	OR (95% CI) n = 87	p-value	OR (95% CI) n = 161	p-value
Ocular-nasal and asthma symptoms (in last 12 months)				
- Ocular-nasal	0.92 (0.54 - 1.55)	0.754	**0.43 (0.27 - 0.70)**	**0.001**
- Wheezing without flu or cold	0.90 (0.41 - 1.95)	0.788	1.04 (0.56 - 1.92)	0.904
- Woken up by cough	0.90 (0.49 - 1.63)	0.718	0.89 (0.54 - 1.46)	0.643
- Woken up by tight chest	0.79 (0.37 - 1.71)	0.553	1.28 (0.73 - 2.26)	0.385
- Woken up by shortness of breath	0.99 (0.34 - 2.89)	0.979	1.91 (0.88 - 4.12)	0.100
- Asthma-related symptoms[1]	1.15 (0.66 - 1.98)	0.620	1.15 (0.73 - 1.80)	0.545
- Asthma attacks	0.92 (0.15 - 5.51)	0.924	**3.25 (1.04 - 10.16)**	**0.043**
- Current asthma treatment	2.12 (0.35 - 12.95)	0.414	2.98 (0.93 - 9.57)	0.067
Work-related symptoms				
- Work-related chest symptoms	0.29 (0.04 - 2.34)	0.244	1.29 (0.47 - 3.53)	0.614
- Work-related ocular-nasal	1.18 (0.61 - 2.28)	0.619	0.58 (0.31 - 1.09)	0.092
- Chest symptoms associated with peak exposure	0.93 (0.50 - 1.73)	0.826	**0.51 (0.29 - 0.90)**	**0.020**
Baseline pulmonary function indices (n = 412)				
- FEV_1 / FVC < 0.7	0.29 (0.04 - 2.34)	0.244	1.14 (0.38 - 3.36)	0.819
- FEV_1 < 80% predicted	0.77 (0.30 - 2.00)	0.591	**0.43 (0.21 - 0.87)**	**0.020**
Allergic sensitisation to latex				
- Sensitisation to latex (k82) (n = 421)	0.62 (0.24 - 1.59)	0.318	0.73 (0.36 - 1.51)	0.401
- True sensitisation to latex (n = 41)[2]	0.69 (0.24 - 1.94)	0.478	0.67 (0.28 - 1.61)	0.370
Serum inflammatory biomarkers				
- ECP	0.82 (0.41 - 1.65)	0.582	1.09 (0.63 - 1.89)	0.766
- MPO	0.93 (0.39 - 2.23)	0.877	**1.97 (1.04 - 3.73)**	**0.036**
Asthma phenotypes				
- Atopic asthma	1.08 (0.36 - 3.21)	0.889	1.57 (0.68 - 3.60)	0.289
- Non-atopic asthma	0.35 (0.08 - 1.58)	0.172	0.98 (0.42 - 2.30)	0.969
- Work-aggravated asthma	0.78 (0.16 - 3.96)	0.769	1.97 (0.69 - 5.66)	0.206

Each OR is derived from a separate model

Employment duration (< 10 years) was used as baseline for comparison (n = 206)

[1] 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 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?"

[2] True latex sensitisation refers to positive response to latex (k82) and negative response to CCDs.

Table 5.6 Association between cumulative endotoxin exposure categories and clinical endpoints in unconditional logistic regression models.

Outcome variables	Cumulative endotoxin exposure (EU/m^3–year)			
	Middle tertile 11.85 – 50.64		Upper tertile > 51.12	
	OR (95% CI) n = 113	p-value	OR (95% CI) n = 112	p-value
Ocular-nasal and asthma symptoms (in last 12 months)				
- Ocular-nasal	**1.97 (1.14 - 3.39)**	**0.015**	1.44 (0.83 - 2.50)	0.192
- Wheezing without flu or cold	0.63 (0.29 - 1.37)	0.242	0.94 (0.46 - 1.94)	0.877
- Woken up by cough	1.27 (0.69 - 2.34)	0.439	1.41 (0.77 - 2.58)	0.267
- Woken up by tight chest	**2.24 (1.03 - 4.88)**	**0.042**	**2.53 (1.17 - 5.45)**	**0.018**
- Woken up by shortness of breath	2.12 (0.77 - 5.86)	0.148	2.14 (0.77 - 5.92)	0.143
- Asthma-related symptoms[1]	1.24 (0.70 - 2.21)	0.463	**2.08 (1.18 - 3.64)**	**0.011**
- Asthma attacks	0.92 (0.21 - 4.05)	0.909	1.65 (0.42 - 6.46)	0.472
- Current asthma treatment	0.42 (0.09 - 2.06)	0.288	1.05 (0.26 - 4.32)	0.946
Work-related symptoms				
- Work-related chest symptoms	0.79 (0.21 - 3.03)	0.734	1.44 (0.44 - 4.68)	0.544
- Work-related ocular-nasal	1.34 (0.63 - 2.85)	0.446	1.93 (0.94 - 3.96)	0.073
- Chest symptoms associated with peak exposure	0.81 (0.43 - 1.53)	0.522	0.82 (0.44 - 1.55)	0.545
Baseline pulmonary function indices (n = 412)				
- FEV_1 / FVC < 0.7	3.07 (0.60 - 15.61)	0.177	1.52 (0.25 - 9.29)	0.652
- FEV_1 < 80% predicted	1.11 (0.45 - 2.73)	0.819	1.91(0.68 - 5.34)	0.221
Allergic sensitisation to latex				
- Sensitisation to latex (k82) (n = 421)	0.53 (0.17 - 1.65)	0.276	1.52 (0.62 - 3.71)	0.360
- True sensitisation to latex (n = 41)[2]	0.35 (0.09 - 1.37)	0.133	1.16 (0.43 - 3.13)	0.770
Serum inflammatory biomarkers				
- ECP	1.36 (0.66 - 2.83)	0.406	1.27 (0.60 - 2.68)	0.530
- MPO	1.18 (0.52 - 2.68)	0.695	1.03 (0.44 - 2.41)	0.942
Asthma phenotypes				
- Atopic asthma	0.69 (0.21 - 2.26)	0.544	1.15 0.40 - 3.30)	0.789
- Non-atopic asthma	0.48 (0.16 - 1.44)	0.190	0.69 (0.25 - 1.87)	0.463
- Work-aggravated asthma	0.79 (0.21 - 3.03)	0.734	1.44 (0.44 - 4.68)	0.544

Each OR is derived from a separate model

Cumulative endotoxin exposure (< 11.78 EU/m^3-year) was used as baseline for comparison (n = 113)

[1] 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 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?"

[2] True latex sensitisation refers to positive response to latex (k82) and negative response to CCDs.

5.2.3 Exposure-response Relationships In Respect Of The Endotoxin Exposures And Clinical Endpoints Stratified By Atopy In Multivariate Conditional Models

Exposure-response relationships in respect of the endotoxin exposures and clinical endpoints of interest in dental HCWs were assessed while controlling for potential confounders (age, sex, smoking) and stratifying by atopic status. No significant associations were demonstrated between endotoxin exposure in current job and any of the clinical endpoints in atopic workers (**Table 5.7**). However, there was a borderline association (OR = 2.24, 95% CI: 0.97 - 5.17) between increasing endotoxin exposure and asthma-related symptoms among non-atopic workers. Furthermore, a borderline association (OR = 8.02, 95% CI: 0.94 - 68.35) was also found between adverse pulmonary function (FEV_1 < 80% predicted) and high endotoxin exposure among the non-atopic workers. Another borderline association (OR = 3.08, 95% CI: 0.90 – 10.52) was observed between increasing endotoxin exposure and true latex sensitisation among atopic dental workers.

Ocular-nasal symptoms were less likely to occur in atopic workers working for more than 20 years (**Table 5.8**). However, long employment duration (> 20 years) was a good predictor (OR = 2.32, 95% CI: 1.08 - 5.00) of asthma-related symptoms in this group of workers. In addition, the odds of having atopic asthma were 2-3 times more likely among these workers. Conversely, the odds of having non-atopic asthma were less likely in workers with duration of employment less than 20 years. Being woken up by shortness of breath was 3 times more likely among non-atopic workers with long service history.

Cumulative exposure based on airborne endotoxin concentrations was found to predict general and work-related ocular-nasal symptoms among non-atopic workers (**Table 5.9**). Non-atopic workers with asthma-related symptoms were 3 times more likely to have high cumulative endotoxin

exposure. Being woken by chest tightness was associated with increasing cumulative airborne endotoxin exposure among non-atopic workers. A borderline association (OR = 2.88, 95% CI: 0.92 – 8.99) was observed between sensitisation to composite latex and cumulative endotoxin among atopic workers.

Table 5.7 Association between current endotoxin exposure concentration categories and clinical endpoints in conditional logistic regression models in dental HCWs stratified by atopy.

Outcome variables	Personal endotoxin exposure (EU/m^3)							
	Atopic				Non-atopic			
	Middle tertile 2.25 – 5.83		Upper tertile > 5.83		Middle tertile 2.25 – 5.83		Upper tertile > 5.83	
	OR (95% CI) n = 87	p-value	OR (95% CI) n = 161	p-value	OR (95% CI) n = 87	p-value	OR (95% CI) n = 161	p-value
Ocular-nasal and asthma symptoms (in last 12 months)								
- Ocular-nasal	1.08 (0.48 - 2.41)	0.853	1.24 (0.55 - 2.78)	0.604	0.96 (0.39 - 2.35)	0.927	1.24 (0.48 - 3.20)	0.655
- Wheezing without flu or cold	2.00 (0.67 - 5.95)	0.214	0.82 (0.25 - 2.71)	0.745	0.92 (0.29 - 2.96)	0.892	0.94 (0.25 - 3.51)	0.924
- Woken up by cough	1.06 (0.43 - 2.59)	0.902	1.23 (0.50 - 3.05)	0.648	1.41 (0.62 - 3.19)	0.409	1.39 (0.57 - 3.37)	0.467
- Woken up by tight chest	1.30 (0.45 - 3.72)	0.624	0.90 (0.29 - 2.78)	0.854	0.82 (0.28 - 2.41)	0.714	2.09 (0.75 - 5.87)	0.160
- Woken up by shortness of breath	1.35 (0.29 - 6.26)	0.700	1.09 (0.22 - 5.37)	0.912	0.78 (0.23 - 2.64)	0.686	0.95 (0.26 - 3.56)	0.945
- Asthma-related symptoms[1]	0.95 (0.41 - 2.16)	0.895	1.00 (0.43 - 2.31)	0.996	1.76 (0.80 - 3.85)	0.158	**2.24 (0.97 - 5.17)**	**0.058**
- Asthma attacks	0.28 (0.03 - 2.51)	0.255	0.18 (0.02 - 1.29)	0.088	0.15 (0.01 - 4.88)	0.284	0.45 (0.02 - 13.59)	0.648
- Current asthma treatment	0.37 (0.03 - 4.21)	0.426	0.54 (0.08 - 3.82)	0.535	-		-	
Work-related symptoms								
- Work-related chest symptoms	-		-		0.61 (0.09 - 4.17)	0.611	1.69 (0.29 - 9.94)	0.561
- Work-related ocular-nasal	2.70 (0.79 - 9.28)	0.114	2.53 (0.71 - 8.99)	0.151	1.07 (0.38 - 3.01)	0.901	1.40 (0.47 - 4.18)	0.551
- Chest symptoms associated with peak exposure	0.62 (0.25 - 1.56)	0.310	0.46 (0.18 - 1.20)	0.112	0.61 (0.25 - 1.51)	0.287	0.86 (0.35 - 2.13)	0.747
Baseline pulmonary function indices (n = 412)								
- FEV_1 / FVC < 0.7	-		-		1.43 (0.28 - 7.31)	0.671	0.86 (0.08 - 9.35)	0.903
- FEV_1 < 80% predicted	1.46 (0.33 - 6.39)	0.618	3.04 (0.48 - 19.05)	0.235	2.88 (0.73 - 11.32)	0.131	**8.02 (0.94 - 68.35)**	**0.057**
Allergic sensitisation to latex								
- Sensitisation to latex (k82) (n = 421)	0.72 (0.24 - 2.18)	0.557	2.29 (0.86 - 6.09)	0.097	-		-	
- True sensitisation to latex (n = 41)[2]	1.42 (0.37 - 5.37)	0.609	**3.08 (0.90 - 10.52)**	**0.073**	-		-	
Serum inflammatory biomarkers								
- ECP	1.16 90.40 - 3.36)	0.786	1.86 (0.67 - 5.15)	0.235	0.91 (0.33 - 2.48)	0.853	0.75 (0.26 - 2.19)	0.602
- MPO	2.09 (0.58 - 7.55)	0.260	1.55 (0.42 - 5.66)	0.510	1.27 (0.41 - 3.90)	0.680	0.70 (0.19 - 2.63)	0.597
Asthma phenotypes								
- Atopic asthma	1.05 (0.35 - 3.21)	0.928	0.82 (0.26 - 2.56)	0.928	-		-	
- Non-atopic asthma	-		-		1.35 (0.45 - 4.06)	0.598	0.51 (0.12 - 2.21)	0.371
- Work-aggravated asthma	4.90 (0.51 - 46.80)	0.168	1.64 (0.13 - 20.15)	0.697	0.39 (0.03 - 5.134)	0.474	1.89 (0.22 - 16.08)	0.561

Each OR is derived from a separate model
ORs were adjusted for age, sex and smoking
Lower tertile of airborne endotoxin exposure (< 2.25 EU/m^3) was used as baseline for comparison (n = 137)

[1] 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 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?"

[2] True latex sensitisation refers to positive response to latex (k82) and negative response to CCDs.

Table 5.8 Association between employment duration categories and clinical endpoints in conditional logistic regression models in dental HCWs stratified by atopy.

Outcome variables	Employment duration (years)							
	Atopic				Non-atopic			
	10 – 20		> 20		10 – 20		> 20	
	OR (95% CI) n = 87	p-value	OR (95% CI) n = 161	p-value	OR (95% CI) n = 87	p-value	OR (95% CI) n = 161	p-value
Ocular-nasal and asthma symptoms (in last 12 months)								
- Ocular-nasal	1.14 (0.43 - 3.00)	0.792	**0.35 (0.16 - 0.75)**	**0.007**	1.21 (0.46 - 3.20)	0.704	0.83 (0.32 - 2.12)	0.691
- Wheezing without flu or cold	0.73 (0.21 - 2.57)	0.628	0.92 (0.36 - 2.36)	0.867	1.01 (0.25 - 4.13)	0.991	2.89 (0.91 - 9.15)	0.072
- Woken up by cough	1.30 (0.47 - 3.60)	0.616	1.33 (0.59 - 2.97)	0.488	0.53 (0.20 - 1.39)	0.195	1.61 (0.72 - 3.59)	0.245
- Woken up by tight chest	0.44 (0.12 - 1.68)	0.232	1.88 (0.75 - 4.71)	0.179	0.61 (0.19 - 1.95)	0.409	1.35 (0.50 - 3.67)	0.558
- Woken up by shortness of breath	0.98 (0.15 - 6.43)	0.983	2.20 (0.61 - 7.95)	0.230	0.86 (0.18 - 4.13)	0.852	**3.30 (0.99 - 10.99)**	**0.052**
- Asthma-related symptoms[1]	1.59 (0.60 - 4.25)	0.352	**2.32 (1.08 - 5.00)**	**0.031**	0.66 (0.28 - 1.57)	0.345	1.48 (0.69 - 3.20)	0.316
- Asthma attacks	0.51 (0.03 - 8.46)	0.636	1.63 (0.34 - 7.89)	0.542	-		2.96 (0.09 - 95.06)	0.540
- Current asthma treatment	-		3.23 (0.60 - 17.48)	0.174	-		-	
Work-related symptoms								
- Work-related chest symptoms	-		1.24 (0.24 - 6.37)	0.795	0.30 (0.03 - 3.32)	0.328	0.93 (0.17 - 5.14)	0.938
- Work-related ocular-nasal	0.68 (0.21 - 2.14)	0.506	0.51 (0.18 - 1.43)	0.201	1.86 (0.60 - 5.76)	0.284	1.46 (0.50 - 4.26)	0.492
- Chest symptoms associated with peak exposure	0.60 (0.19 - 1.87)	0.379	0.44 (0.17 - 1.13)	0.086	1.68 (0.62 - 4.54)	0.308	1.21 (0.48 - 3.06)	0.682
Baseline pulmonary function indices (n = 412)								
- FEV_1 / FVC < 0.7	-		-		0.17 (0.02 - 1.55)	0.115	0.52 (0.08 - 3.22)	0.479
- FEV_1 < 80% predicted	0.66 (0.09 - 4.75)	0.679	0.33 (0.08 - 1.41)	0.135	0.71 (0.17 - 2.93)	0.635	0.83 (0.20 - 3.39)	0.798
Allergic sensitisation to latex								
- Sensitisation to latex (k82) (n = 421)	1.15 (0.34 - 3.93)	0.818	1.00 (0.42 - 2.38)	0.995	-		-	
- True sensitisation to latex (n = 41)[2]	1.79 (0.44 - 7.23)	0.415	0.86 (0.31 - 2.42)	0.782	-		-	
Serum inflammatory biomarkers								
- ECP	1.43 (0.44 - 4.61)	0.551	0.89 (0.36 - 2.17)	0.791	0.74 (0.24 - 2.29)	0.605	0.96 (0.36 - 2.56)	0.934
- MPO	1.90 (0.47 - 7.61)	0.365	1.73 (0.61 - 4.92)	0.308	0.67 (0.15 - 2.97)	0.602	1.06 (0.32 - 3.46)	0.923
Asthma phenotypes								
- Atopic asthma	1.43 (0.38 - 5.39)	0.597	**2.86 (1.08 - 7.57)**	**0.034**	-		-	
- Non-atopic asthma	-		-		**0.18 (0.03 - 0.94)**	**0.042**	0.88 (0.29 - 2.69)	0.824
- Work-aggravated asthma	0.67 (0.10 - 4.60)	0.686	1.66 (0.36 - 7.67)	0.514	-		1.68 (0.22 - 12.57)	0.614

Each OR is derived from a separate model
ORs were adjusted for age, sex and smoking
Employment duration (<10 years) was used as baseline for comparison (n = 206)

[1] 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 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?"

[2] True latex sensitisation refers to positive response to latex (k82) and negative response to CCDs.

Table 5.9 Association between cumulative endotoxin exposure categories and clinical endpoints in conditional logistic regression models in dental HCWs stratified by atopy.

Outcome variables	Cumulative endotoxin exposure (EU/m^3-year)							
	Atopic				Non-atopic			
	Middle tertile 11.85 – 50.64		Upper tertile > 51.12		Middle tertile 11.85 – 50.64		Upper tertile > 51.12	
	OR (95% CI) n = 113	p-value	OR (95% CI) n = 112	p-value	OR (95% CI) n = 113	p-value	OR (95% CI) n = 112	p-value
Ocular-nasal and asthma symptoms (in last 12 months)								
- Ocular-nasal	1.66 (0.68 - 4.05)	0.262	0.96 (0.40 - 2.27)	0.921	**3.56 (1.26 - 10.05)**	**0.016**	1.82 (0.57 - 5.76)	0.310
- Wheezing without flu or cold	0.49 (0.15 - 1.57)	0.231	0.61 (0.20 - 1.84)	0.385	0.81 (0.21 - 3.13)	0.754	1.33 (0.36 - 4.97)	0.672
- Woken up by cough	0.57 (0.21 - 1.53)	0.262	1.10 (0.44 - 2.75)	0.833	1.93 (0.75 - 4.97)	0.171	1.47 (0.54 - 4.02)	0.455
- Woken up by tight chest	0.89 (0.29 - 2.71)	0.834	1.02 (0.35 - 3.02)	0.966	**5.98 (1.21 - 29.64)**	**0.029**	**8.35 (1.68 - 41.46)**	**0.009**
- Woken up by shortness of breath	0.17 (0.02 - 1.57)	0.117	0.80 (0.20 - 3.25)	0.753	-		-	
- Asthma-related symptoms[1]	0.64 (0.26 - 1.59)	0.335	1.13 (0.47 - 2.72)	0.777	1.73 (0.69 - 4.33)	0.244	**3.03 (1.20 - 7.66)**	**0.019**
- Asthma attacks	0.45 (0.06 - 3.37)	0.434	0.11 (0.01 - 2.23)	0.152	-		-	
- Current asthma treatment	0.18 (0.01 - 2.71)	0.213	1.09 (0.07 - 16.38)	0.952	-		-	
Work-related symptoms								
- Work-related chest symptoms	3.13 (0.30 - 33.27)	0.343	1.67 (0.13 - 20.66)	0.691	0.33 (0.03 - 3.77)	0.375	1.64 (0.27 - 10.02)	0.595
- Work-related ocular-nasal	0.51 (0.15 - 1.74)	0.279	1.46 (0.52 - 4.10)	0.478	3.14 (0.87 - 11.35)	0.080	**3.82 (1.01 - 14.41)**	**0.048**
- Chest symptoms associated with peak exposure	0.63 (0.23 - 1.72)	0.370	0.57 (0.21 - 1.57)	0.278	0.89 (0.35 - 2.31)	0.817	0.77 (0.28 - 2.13)	0.612
Baseline pulmonary function indices (n = 412)								
- FEV_1 / FVC < 0.7	-		-		1.71 (0.28 - 10.25)	0.559	0.64 (0.05 - 8.22)	0.730
- FEV_1 < 80% predicted	0.74 (0.14 - 3.85)	0.724	2.50 (0.35 - 17.64)	0.358	0.87 (0.21 - 3.62)	0.847	3.01 (0.46 - 19.84)	0.252
Allergic sensitisation to latex								
- Sensitisation to latex (k82) (n = 421)	0.76 (0.20 - 2.84)	0.687	**2.88 (0.92 - 8.99)**	**0.068**	-		-	
- True sensitisation to latex (n = 41)[2]	0.56 (0.12 - 2.58)	0.461	2.24 (0.65 - 7.68)	0.199	-		-	
Serum inflammatory biomarkers								
- ECP	1.70 (0.56 - 5.13)	0.348	2.28 (0.78 - 6.71)	0.134	1.32 (0.44 - 3.95)	0.625	0.56 (0.15 - 2.08)	0.387
- MPO	1.65 (0.42 - 6.52)	0.476	2.76 (0.76 - 10.02)	0.122	0.66 (0.19 - 2.23)	0.499	0.21 (0.04 - 1.17)	0.074
Asthma phenotypes								
- Atopic asthma	0.54 (0.15 - 1.97)	0.350	0.79 (0.24 - 2.61)	0.702	-		-	
- Non-atopic asthma	-		-		0.30 (0.08 - 1.17)	0.083	0.42 (0.11 - 1.56)	0.197
- Work-aggravated asthma	0.69 (0.12 - 4.02)	0.683	0.57 (0.09 - 3.39)	0.534	0.98 (0.05 - 18.84)	0.989	3.04 (0.23 - 39.90)	0.398

Each OR is derived from a separate model

ORs were adjusted for age, sex and smoking

Cumulative endotoxin exposure (< 11.78 EU/m^3-year) was used as baseline for comparison (n = 113)

[1] 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 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?"

[2] True latex sensitisation refers to positive response to latex (k82) and negative response to CCDs.

5.2.4 EXPOSURE-RESPONSE RELATIONSHIP BETWEEN ENDOTOXIN AND LATEX GLOVE USE AND RISK OF LATEX SENSITISATION

A combination of latex exposure (powdered or non-powdered latex gloves) and airborne endotoxin (tertiles) was a significant predictor of composite latex sensitisation in the high exposure group (OR = 2.59, 95% CI: 1.20 – 5.60) (**Table 5.10**). A borderline association (OR = 2.50, 95% CI: 0.99 - 6.28) was found between the abovementioned exposure and true latex sensitisation. A positive increasing trend was observed for latex sensitisation for both outcomes.

Table 5.10 Relationship between endotoxin exposure and latex glove use and the risk of latex sensitisation.

Latex glove use and endotoxin exposure	Latex sensitisation (k82)			True latex sensitisation[1]		
	Yes (n = 41)	No (n = 413)	OR, 95% CI	Yes (n = 29)	No (n = 392)	OR, 95% CI
None	21 (51%)	231 (56%)	1.00	14 (48%)	214 (55%)	1.00
Low	2 (5%)	72 (17%)	0.31 (0.07 - 1.33) p = 0.115	1 (3%)	71 (18%)	0.22 (0.03 - 1.67) p = 0.141
Medium	6 (15%)	59 (14%)	1.12 (0.43 - 2.90) p = 0.817	6 (21%)	58 (15%)	1.58 (0.58 - 4.30) p = 0.369
High	12 (29%)	51 (12%)	**2.59 (1.20 - 5.60) p = 0.016**	8 (28%)	49 (13%)	**2.50 (0.99 - 6.28) p = 0.052**
Fishers exact	**0.012**			**0.027**		

[1] True latex sensitisation refers to positive response to latex (k82) and negative response to CCDs.
None: endotoxin exposure categorised by lower endotoxin tertile (0 - 2.24 EU/m^3) and no use of latex gloves
Low: endotoxin exposure categorised by lower endotoxin tertile (0 - 2.24 EU/m^3)
Medium: endotoxin exposure categorised by middle endotoxin tertile (2.25 – 5.83 EU/m^3)
High: endotoxin exposure categorised by high endotoxin tertile (> 5.83 EU/m^3)

5.3 DISCUSSION

The impact of endotoxin exposure is diverse and depends on the context of exposure (Liebers et al., 2008). The individual immune response to endotoxins is the result of a complex interaction between dose and timing of exposure, additive or synergistic effects with allergens and genetic predisposition (Vandenbulcke et al., 2006). It is evident from this study that the timing of exposure played a significant role in the presentation of the respiratory outcomes of interest.

Acute endotoxin exposure in the current job was associated with asthma-related symptoms and adverse lung function among non-atopic dental workers in the current study. There is extensive evidence to indicate that repeated exposure to airborne endotoxin is one of the factors responsible for the presence of neutrophils and the development of airway obstruction (Reed and Milton, 2001). However, there is conflicting evidence as to whether occupational exposure to endotoxin causes a greater fall in FEV_1 in atopic or non-atopic workers (Nieves et al., 2005; Singh and Schwartz, 2005; Lemiere et al., 2006; Mann and Chung, 2006). From the current study it was shown that adverse lung function was more prominent with increasing low-grade endotoxin exposure among non-atopic workers. The study by Pankhurst et al. (2005) demonstrated an association between temporal onset of asthma in a group of dentists with high aerobic bacterial counts (OR = 6.00, 95% CI: 1.26 – 28.50, $p < 0.05$) in the DUWLs. The likely cause was attributed to endotoxin exposure (Pankhurst et al., 2005). The assumption was based on the fact that bacterial loads from contaminated DUWLs are highly correlated ($r = 0.94$, $p < 0.05$) with endotoxin levels (Huntington et al., 2007).

In the current study a strong association was shown between latex sensitisation and increasing low-grade endotoxin exposure in the atopic group. This could depend on whether allergen sensitivity exists prior to the endotoxin exposure or not (Ormstad et al., 2003). Sensitivity to endotoxin varies considerably with human subjects being very sensitive with even low-level endotoxin exposure enhancing airway disease in susceptible groups, such as subjects with atopic asthma (Eldridge and Peden, 2000; Michel et al., 2001). Individuals without classic atopic asthma but who are reactive to endotoxin manifest with recurrent asthma symptoms with chronic exposure; and develop irreversible airway obstruction (Reed and Milton, 2001; Liu, 2002). It is well established that not all individuals respond in the same physiological manner to endotoxin and approximately 10-15% develop airflow

obstruction after inhaled challenge with endotoxin (Scannapieco et al., 2004; Pankhurst et al., 2005). There is considerable inter-individual variability of the lung function response to endotoxin, related to the airway inflammatory status as well as the level of nonspecific bronchial hyperresponsiveness (Michel, 2000).

In the current study increasing employment duration as a surrogate for exposure to endotoxin appeared to play a significant role in the manifestation of atopic asthma. These findings are consistent with a previous study which demonstrated that the risk of asthma was 6.72 times more likely (OR = 6.72, 95% CI: 1.15 – 39.24) among dental apprentices exposed to contaminated DUWLs since commencing dental training (Pankhurst et al., 2005). In the current study significant associations were found between several clinical endpoints (asthma-related symptoms in atopic workers and atopic asthma and employment duration between 10 and 20 years (mean: 15.84 years, SD: 2.96) similar to the duration of employment of qualified dental workers. Similarly, in the study by Pankhurst et al. (2005) the study group included principal and senior dentist professionals with an average duration of employment of 10.8 years. These findings suggest a long latency period between exposure and development of obstructive airway disease among dental workers. Furthermore, Lindstrom et al. showed that a dental worker exposed to methacrylate developed respiratory symptoms after 10 years of exposure (Lindstrom et al., 2002). Findings from both the current study and that of Pankhurst et al. (2005) demonstrate a long latency period between exposure and onset of respiratory disease. Alternatively, a low-dose irritant-induced OA may also exist (Francis et al., 2007); however this is unlikely since the current study showed an association with atopic asthma. It must be highlighted that in cases of multiple exposures, the absence of a latency period should no longer be regarded as a distinctive feature of irritant-induced asthma (Vandenplas and Malo, 2003).

In the current study exposure dose also played a significant role in the exposure-response relationship. Chronic endotoxin exposure among non-atopic workers was a strong predictor of several clinical endpoints (ocular-nasal, work-related ocular-nasal and asthma-related symptoms). Atopic status played a key role as upper and lower airway symptoms were associated with an increasing cumulative exposure only in non-atopic workers. Dose-response relationships between endotoxin exposure and health effects have been observed by different groups (Douwes et al., 1995; Liebers et al., 2008). The pathophysiological mechanism may be based on the bimodal relationship between environmental endotoxin and immune response (i.e. T_H2-like at low levels and T_H1-like at high levels) as suggested by Vercelli (Vercelli, 2003).

In the current study latex sensitisation is a risk factor for the development of allergy and asthma and was stronger with increasing low-grade endotoxin exposure in the atopic group. This association may be explained by a synergistic or adjuvant response, which has been previously reported to augment the allergen response in those already sensitised (Bardana, 2008). As a natural adjuvant, endotoxin can augment asthma and atopic inflammation or may act on its own, causing adverse effects on lung function and inflammatory responses (Liu, 2002; Liebers et al., 2006; Raulf-Heimsoth et al., 2007). The dichotomous relationship between endotoxin and the T_H1 response and allergen and the T_H2 response implies that exposure to endotoxin early in life would protect against a T_H2 response to allergens. On the other hand, after allergic sensitisation has occurred, the two pathways of inflammation seem to act together synergistically. The airway response to the combination of endotoxin and allergen is greater than either agent on its own (Reed and Milton, 2001).

An important finding of this study is the increased risk of latex sensitisation as a result of increasing airborne endotoxin exposure among latex glove users. This is particularly relevant for clinical dental

workers exposed to endotoxin levels and dust from glove powder used during dental procedures. These data provide further evidence that concomitant exposure to allergen and endotoxin may be an important factor in asthma pathogenesis. Endotoxin may act as an adjuvant facilitating sensitisation to other allergens or may increase the severity of allergic disease (Heederik and Douwes, 1997; Szymanska, 2005b; Huntington et al., 2007). Endotoxin and allergen exposures often co-exist, both causing inflammation through different pathways following inhalation (Liu, 2002; Liebers et al., 2006; Raulf-Heimsoth et al., 2007). The mechanisms involved are not, however, fully understood (Blease et al., 1998). Bacterial exposure may exacerbate an existing hypersensitivity response in an individual previously sensitised to an allergen or initiate an irritant non-allergic *de novo* response (Bernstein et al., 1994). A further explanation may be that low-grade levels of endotoxin may prime or otherwise modify the response of airways in asthmatic subjects on allergen challenge (Alexis et al., 2003). The finding from the current study supports the hypothesis that low endotoxin doses favour a T_H2 response (Kuipers et al., 2003). Furthermore, the study by Preller showed a strong association between respiratory symptoms and the combination of specific IgE sensitisation to common allergens and high endotoxin exposures (> 1010 EU/m^3), but not with these factors independently (Preller et al., 1996).

Since this was a cross-sectional study, the temporal relationship between endotoxin exposure and asthma could not be established with absolute certainty due to the healthy worker effect. In addition, it is difficult to eliminate healthy worker selection effect in such studies. Avoidance of exposure to endotoxin in subjects with an allergic history would have led to an under estimation of the effect of endotoxin. The study did not demonstrate a strong association between endotoxin exposure and WRA phenotypes (e.g. WAA). This may be due to a number of reasons. Firstly, the definition of the variable may not be sensitive enough; secondly, endotoxin may cause its effects at a subtle level with interplay between work and domestic exposures; thirdly, latex may play a role in causing latex sensitisation in

those workers but not developing further into latex asthma due to healthy worker effect; fourthly, because of the small numbers among the various asthma phenotypes the study may lack statistical power.

In conclusion, this is the first study in dental settings to our knowledge which has shown upper and lower airway effects to chronic low-level exposures of endotoxin in non-atopic individuals supporting the hypothesis that endotoxin is associated with non-atopic workers. It also demonstrated an association between endotoxin exposure and upper airway symptoms, a precursor to lower respiratory airways. Chronic endotoxin exposure was a keen predictor of non-atopic dental workers. From this study it has been shown that increasing low-grade endotoxin exposure may increase the risk of sensitisation to latex irrespective of CCD markers among latex glove users, placing them at higher risk of presenting with latex-allergic asthma. Latex on its own is a risk factor for developing allergy and asthma and with endotoxin exposure the risk is increased. Further research is warranted in a cohort study of dentists to assess timing of exposure to endotoxin in those who are not sensitised and those who become sensitised to latex and other agents.

CHAPTER 6

SERUM ENDOTOXIN A POTENTIAL MARKER OF EXPOSURE IN DENTAL HCWS EXPOSED TO AIRBORNE ENDOTOXIN

6.0 INTRODUCTION

Traditionally, the gastrointestinal tract has been considered to be the source of systemic endotoxin appearing in the circulation of critically ill patients (Swank and Deitch, 1996); however, recently bacterial translocation from the lungs into the systemic circulation has been described in pre-injured lungs in animal models (Murphy et al., 2000). The release of endotoxin from Gram-negative bacterial cell walls into the circulation precipitates an acute inflammatory response that often leads to shock and death (Hudgins et al., 2003). The pulmonary-to-systemic translocation of endotoxin after intratracheal administration was suggestive of a possible marker of lung injury in experimental models (Murphy et al., 2000). Only one study to date has reported the detection of endotoxin in plasma of workers exposed to high levels of airborne endotoxin supported by elevated cytokine levels exceeding those of the non-exposed workers (Sikkeland et al., 2008).

After deposition of endotoxin in the airways a myriad of cellular mechanisms ensue. Therefore, the bioactive endotoxin molecule in serum may prove to be a valuable marker for monitoring of endotoxin-related effects (Liebers et al., 2006; Sikkeland et al., 2007).

Detecting endotoxin in whole blood, serum and plasma for the diagnosis of endotoxaemia, Gram-negative meningitis, and Gram-negative bacteremia has gained popularity (Duner, 1993) and the concept is advancing into the occupational arena (Sikkeland et al., 2007). In working populations,

systemic endotoxin may provide an alternative means of assessing exposure due to it being more closely related to internal dose and the lesser chance of contamination during air sampling and ease of sampling.

Previous research has demonstrated an association between airborne endotoxin levels and cross-shift decline in FEV_1 (Kennedy et al., 1987; Douwes et al., 2002a). Endotoxin is also a known trigger of respiratory symptoms and asthma (e Silva et al., 2000; Thorne et al., 2005). However, studies have demonstrated weak correlations between physiologic parameters and various inflammatory markers in asthma subjects (Miranda et al., 2004; Haldar and Pavord, 2007). Furthermore, airway inflammation can be present in asthmatic patients who are clinically well-controlled, suggesting that lung function measurements may not be sensitive enough to reflect the extent of airway inflammation (Leuppi et al., 2001). While it is accepted that airborne endotoxin may cause asthma, no marker of inflammation that is both highly sensitive and specific is currently available. Significant differences are found between patients and healthy controls, but the overlap is too great for use with confidence in individual patients (Wilson and Pedersen, 2000). In light of the above shortcomings, systemic endotoxin may provide an alternative means to assessing endotoxin exposure in the workplace.

The aims:

- determine the variability of serum endotoxin among dental HCWs,
- investigate the relationship between serum endotoxin and personal airborne endotoxin exposure,
- determine whether serum endotoxin is a predictor of clinical endpoints of interest,
- investigate the extent to which these relationships are influenced by host-associated factors (sex, atopy, smoking, respiratory diseases such as bronchitis, pneumonia).

6.1 MATERIALS AND METHODS

6.1.1 STUDY POPULATION

The study population included 436 participants from five public sector academic dental institutions as described in previous chapters. The participants included dental staff (clinical, laboratory, administration and auxiliary) and students (performing clinical sessions).

6.1.2 PERSONAL AIRBORNE ENDOTOXIN MEASUREMENTS

Personal air samples were collected from the breathing zone of study participants according to the method described in Chapter 2. After sampling, the filters were sealed and stored in plastic bags in a fridge (~4˚C) for a week until transported to the National Institute for Occupational Health (NIOH) laboratory for immediate extraction and subsequent endotoxin analyses. Filter extracts were analysed using the chromogenic-1000 LAL assay (Lonza, Walkersville, USA). Throughout the analytical process, endotoxin-free products including pyrogen-free water were used. Endotoxin analysis was done according to the manufacturer's instructions. Standards were made using the standard *E. coli* provided in the kit. Assays in which the standard curve had a correlation coefficient ≥ 0.98 and samples with a CV $\leq 10\%$ were accepted. Using the flow rate and time of the sampling period, the volume of air sampled was calculated and the results were expressed as EU/m^3.

6.1.3 SERUM ENDOTOXIN MEASUREMENTS

Blood was collected from study participants in sterile 6 ml vacutainer tubes containing ethylene-diamine-tetra-acetic acid (EDTA) (K2E) (BD Vacutainer Systems, United Kingdom). Blood samples were centrifuged at 1500 g for 10 minutes. The serum was transferred into sterile Nunc CryoTube™ vials (Cat.No. 3753539, Nunc A/S, Denmark). Serum samples were transported to the laboratory at -

20˚C and stored at -70˚C until analysis. Prior to endotoxin quantification, serum samples were diluted 1/10 with pyrogen-free water and heat inactivated at 75˚C for 12 minutes, to control for interfering factors (Hurley et al., 1991; Talwar et al., 2006). Endotoxin analyses were conducted according to the manufacturer's instructions similarly to the method described above for airborne endotoxin determination.

6.1.4 STATISTICAL ANALYSIS

Data were entered and cleaned in Microsoft Office Excel 2003 and all statistical analyses were performed using STATA9 computer software (StataCorp, 2007, Texas, USA). A test for normality indicated that endotoxin concentrations for personal air samples and serum samples were not normally distributed. The data was log transformed to meet the assumptions of normality and equal variances between groups. Descriptive statistics were generated for the total sample and for subgroups (institution and work groups) using GM and GSD.

An analysis of variance was used to explore the between and within variance components by institution and work groups. Pearson correlation was performed to determine the relationship between airborne and serum endotoxin levels. Exploratory bivariate analyses were used to assess the nature of the associations between clinical endpoints, exposure and covariates. Odds ratio (OR) was used as the measure of association and was estimated along with 95% CI in logistic regression analysis. Potential confounders (age, sex, smoking status, bronchitis, pneumonia) were adjusted for in the multivariate logistic regression analysis, and stratified by atopic status.

6.2 RESULTS

6.2.1 CHARACTERISTICS OF PERSONAL AIRBORNE AND SERUM ENDOTOXIN LEVELS

The overall GM and GSD for personal endotoxin levels was 4.11 EU/m^3 and 3.42 EU/m^3 respectively and the GM and GSD for the serum endotoxin concentrations was 0.64 EU/ml and 2.21 EU/ml (**Table 6.1**). The personal airborne endotoxin levels differed significantly ($p = 0.042$) among the various departments with the laboratory having the highest levels followed by the clinical department. The difference in the serum endotoxin levels for dental workers from the various department was borderline ($p = 0.054$). There was a significant difference ($p = 0.022$) between the personal airborne endotoxin levels and the various exposure groups across all departments. The clinical and laboratory departments had on average higher levels than the administration department. However, no significant difference was detected for the serum endotoxin levels.

Table 6.2 shows the GM and GSD of both personal airborne and serum endotoxin levels for the various job types. It is evident that the personal airborne endotoxin levels were higher for the workers performing clinical and technical dental procedures, dental science students, dental technicians and oral hygienists, with the exception of the security personnel whose levels were also high. However, the serum endotoxin levels appeared to be relatively high for the non-clinical workers, security personnel and cleaners, as well as the clinical workers.

Table 6.1 Characteristics of the overall endotoxin exposure levels in personal airborne samples (EU/m^3) and serum samples (EU/ml) for the various departments and exposure groups.

Exposure metrics	Personal Endotoxin Levels (EU/m^3)			Serum Endotoxin Levels (EU/ml)		
	n	GM (GSD)	Range	n	GM (GSD)	Range
Overall	413	4.11 (3.42)	3.65 - 4.63	436	0.64 (2.21)	0.60 – 0.69
Clinical Department	316	4.32 (3.46)	3.76 - 4.95	333	0.62 (2.22)	0.57 – 0.68
Maxillo-facial & Oral Surgery	71	3.32 (3.40)	2.48 – 4.43	81	0.60 (2.36)	0.50 – 0.73
Restorative / Conservation	117	5.17 (3.55)	4.10 – 6.52	119	0.59 (2.70)	0.49 – 0.70
Periodontology	13	3.71 (2.81)	1.98 – 6.92	14	0.57 (2.22)	0.36 – 0.90
Prosthodontics / Prosthetics	4	2.43 (1.42)	1.39 – 4.25	5	0.95 (1.44)	0.60 – 1.49
Orthodontics	13	5.42 (4.13)	2.30 – 12.76	14	0.71 (2.05)	0.47 – 1.07
Dental Therapy & Hygiene	67	5.11 (3.21)	3.85 – 6.80	68	0.69 (1.62)	0.61 – 0.77
Sterilisation	31	2.88 (3.61)	1.80 – 4.62	32	0.62 (1.98)	0.49 – 0.80
Laboratory Department	22	5.63 (5.37)	2.67 - 11.84	22	0.98 (2.11)	0.70 – 1.36
Technicians lab	22	5.63 (5.35)	2.67 – 11.84	22	0.98 (2.11)	0.70 – 1.36
Administration Department	28	2.38 (2.72)	1.61 – 3.51	30	0.58 (1.76)	0.47 – 0.72
Clerical	21	2.52 (2.66)	1.61 – 3.93	22	0.59 (1.80)	0.46 – 0.77
Reception / Switchboard	6	2.49 (2.90)	0.81 – 7.61	7	0.52 (1.66)	0.32 – 0.83
Stores	1	0.53	-	1	0.99	-
Auxiliary Department	47	3.55 (2.46)	2.72 – 4.63	51	0.69 (1.98)	0.57 – 0.84
Security	7	6.25 (2.65)	2.54 – 15.39	8	0.89 (1.69)	0.57 – 1.38
Cleaning	40	3.22 (2.38)	2.44 – 4.25	43	0.66 (2.02)	0.53 – 0.82

n, number of measurements; GM, geometric mean; GSD, geometric standard deviation; where one sample was collected the standard deviation was not calculated; range, natural data

Table 6.2 Characteristics of the overall endotoxin exposure levels in personal airborne samples (EU/m^3) and serum samples (EU/ml) for the various job types.

Exposure metrics	Personal Endotoxin Levels (EU/m^3)			Serum Endotoxin Levels (EU/ml)		
	n	GM (GSD)	Range	n	GM (GSD)	Range
Students (n = 153)						
B. Dental Therapy – 1st year	6	5.61 (2.59)	2.09 – 15.09	6	0.52 (2.18)	0.23 – 1.17
B. Dental Therapy – 2nd year	33	3.68 (3.12)	2.46 – 5.51	32	0.74 (1.67)	0.62 – 0.89
B. Dental Therapy – 3rd year	5	4.59 (5.85)	0.51 – 41.15	6	0.64 (1.52)	0.41 – 0.99
B. Dental Science – 3rd year	50	8.56 (3.63)	5.94 – 12.35	55	0.36 (2.28)	0.23 – 0.58
B. Dental Science – 4th year	20	2.62 (2.24)	1.79 – 3.82	26	0.36 (3.21)	0.23 – 0.58
B. Dental Science – 5th year	29	5.42 (3.40)	3.40 – 8.63	28	0.50 (2.29)	0.37 – 0.69
Staff (n = 283)						
Dental assistant	83	2.97 (2.99)	2.34 – 3.77	86	0.60 (2.25)	0.50 – 0.71
Technician	22	5.63 (5.35)	2.67 – 11.84	22	0.98 (2.11)	0.70 – 1.36
Nurse	45	2.92 (3.54)	2.00 – 4.27	47	0.65 (2.42)	0.50 – 0.85
Oral hygienist	15	7.02 (3.17)	3.70 – 13.31	15	0.70 (1.77)	0.51 – 0.96
Dentist	30	6.85 (3.45)	4.32 – 10.88	32	0.72 (1.88)	0.58 – 0.91
Administration	28	2.38 (2.73)	1.61 – 3.51	30	0.58 (1.76)	0.47 – 0.72
Security	7	6.25 (2.65)	2.54 – 15.39	8	0.89 (1.69)	0.57 – 1.38
Cleaner	40	3.22 (2.38)	2.44 – 4.25	43	0.66 (2.02)	0.53 – 0.82

B., Bachelor of; n, number of measurements; GM, geometric mean; GSD, geometric standard deviation; where one sample was collected the standard deviation was not calculated; range, natural data

6.2.2 VARIANCE COMPONENTS

Table 6.3 shows the variance components of the between-group (BG) and within-group (WG) variation of serum endotoxin levels by institution and other variables. Overall, the WG variability was higher than the BG variability. A significant difference was demonstrated for institution and job type. The p-value (< 0.05) indicates that there is a difference between and within the variable; however it does not demonstrate where the difference is.

Table 6.3 Between-group and within-group variance components based on serum endotoxin data (log-transformed, Ln) (n = 436).

	Serum endotoxin levels (Ln, EU/ml)		
	BG	**WG**	**p-value**
Institution	0.110	0.530	< 0.001
Department	0.020	0.620	0.054
Exposure group	0.001	0.620	0.375
Job type	0.030	0.610	0.005
Tasks	0.008	0.620	0.140

BG, between-group variance components
WG, within-group variance components by institution and variables of interest

6.2.3 CORRELATION BETWEEN PERSONAL ENDOTOXIN AND SERUM ENDOTOXIN LEVELS

A significant correlation was found between personal airborne endotoxin and serum endotoxin levels among dental students (**Table 6.4**). A stronger correlation ($r = 0.66$, $p < 0.001$) was observed between airborne endotoxin and serum endotoxin among students who did not wear face masks as opposed to those who did ($r = 0.40$, $p < 0.001$). No correlation was observed between airborne and serum endotoxin levels among dental staff.

Table 6.4 Correlation between modelled personal airborne endotoxin levels and serum endotoxin levels (log-transformed, ln).

Variable	Pearson correlation					
	Overall		Use of face masks		No face masks used	
	r (n)	p-value	r (n)	p-value	r (n)	p-value
Staff (n = 283)	0.06 (277)	0.316	0.09 (150)	0.264	0.0003 (127)	0.996
Students (n = 153)	**0.46 (150)**	**<0.001**	**0.40 (116)**	**<0.001**	**0.66 (34)**	**<0.001**

6.2.4 Prevalence Of Clinical Endpoints In Relation To Serum Endotoxin Categories (tertiles)

The proportion of dental HCWs reporting wheezing without cold or flu was shown to increase with increasing serum endotoxin levels, although not significantly (**Table 6.5**). The prevalence of work-related ocular-nasal symptoms also demonstrated a positive trend with increasing serum endotoxin levels. Interestingly, a significant positive trend was observed between high ECP levels in dental HCWs and increasing serum endotoxin. The prevalence of WAA also increased with increasing serum endotoxin, although not significantly.

6.2.5 Relationship Between Clinical Endpoints And Serum Endotoxin Levels

In the unadjusted logistic regression model a positive trend was observed between chest tightness due to peak exposure and increasing serum endotoxin levels, although not significant (**Table 6.6**). The odds of having high ECP levels were unlikely in workers with low serum endotoxin levels. Adverse pulmonary function was more likely among dental HCWs with high serum endotoxin levels, although not significantly so.

Table 6.5 Prevalence of clinical endpoints according to different categories of serum endotoxin levels (tertiles).

Characteristics	Endotoxin in serum (EU/ml)			
	Lower tertile < 0.71 n = 146	Middle tertile 0.71 – 0.89 n = 146	Upper tertile > 0.90 n = 144	Chi-square p-value
Ocular-nasal and asthma symptoms (in last 12 months)				
- Ocular-nasal	43 (29%)	45 (31%)	44 (31%)	0.964
- Wheezing without flu or cold	16 (11%)	18 (12%)	23 (16%)	0.425
- Woken up by cough	30 (21%)	37 (25%)	34 (24%)	0.617
- Woken up by tight chest	22 (15%)	23 (16%)	21 (15%)	0.962
- Woken up by shortness of breath	12 (8%)	13 (9%)	9 (6%)	0.682
- Asthma-related symptoms[1]	38 (26%)	49 (34%)	45 (31%)	0.357
- Asthma attacks	5 (3%)	9 (6%)	8 (6%)	0.841
- Current asthma treatment	6 (4%)	10 (7%)	9 (6%)	0.556
Work-related symptoms				
- Work-related chest symptoms	8 (5%)	6 (4%)	7 (5%)	0.861
- Work-related ocular-nasal	19 (13%)	18 (12%)	24 (17%)	0.520
- Chest symptoms associated with peak exposure	8 (5%)	4 (3%)	5 (3%)	0.457
Baseline pulmonary function indices (n = 412)				
- FEV_1 / FVC < 0.7	4 (3%)	5 (4%)	4 (3%)	0.969
- FEV_1 < 80% predicted	9 (8%)	8 (6%)	12 (10%)	0.504
Allergic sensitisation to latex				
- Sensitisation to latex (k82) (n = 421)	15 (10%)	16 (11%)	9 (6%)	0.325
- True sensitisation to latex (n = 41)[2]	10 (7%)	11 (8%)	7 (5%)	0.706
Serum inflammatory biomarkers				
- ECP[3]	**17 (12%)**	**31 (21%)**	**26 (18%)**	**0.054**
- MPO[4]	14 (10%)	19 (13%)	19 (13%)	0.518
Asthma phenotypes (n = 421)				
- Atopic asthma	8 (5%)	12 (8%)	9 (6%)	0.629
- Non-atopic asthma	6 (4%)	9 (6%)	9 (6%)	0.663
- Work-aggravated asthma	5 (3%)	5 (3%)	7 (5%)	0.767

Note: Categorical variables- number (%)

[1] 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 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?"

[2] True latex sensitisation refers to positive response to latex (k82) and negative response to CCDs.

[3] ECP cutoff: positive response based on values above the normal range of the patient population established in the laboratory.

[4] MPO cutoff: positive response based on values above the normal range of the patient population established in the laboratory.

Table 6.6 Association between serum endotoxin concentration categories (tertiles) and clinical endpoints in unconditional logistic regression models.

Outcome variables	Endotoxin in serum (EU/ml)			
	Middle tertile 0.71 – 0.89		Upper tertile > 0.92	
	OR (95% CI) n = 146	p-value	OR (95% CI) n = 144	p-value
Ocular-nasal and asthma symptoms (in last 12 months)				
- Ocular-nasal	1.07 (0.65 - 1.76)	0.799	1.05 (0.64 - 1.74)	0.838
- Wheezing without flu or cold	1.14 (0.56 - 2.34)	0.715	1.54 (0.78 - 3.06)	0.213
- Woken up by cough	1.31 (0.76 - 2.27)	0.331	1.20 (0.69 - 2.08)	0.530
- Woken up by tight chest	1.05 (0.56 - 1.99)	0.871	0.96 (0.50 - 1.84)	0.907
- Woken up by shortness of breath	1.09 (0.48 - 2.48)	0.834	0.74 (0.30 - 1.83)	0.519
- Asthma-related symptoms[1]	1.44 (0.87 - 2.38)	0.160	1.29 (0.78 - 2.15)	0.326
- Asthma attacks	1.41 (0.37 - 5.45)	0.614	1.47 (0.37 - 5.86)	0.588
- Current asthma treatment	1.67 (0.44 - 6.36)	0.455	2.14 (0.52 - 8.81)	0.291
Work-related symptoms				
- Work-related chest symptoms	0.74 (0.25 - 2.19)	0.585	0.88 (0.31 - 2.50)	0.812
- Work-related ocular-nasal	0.94 (0.47 - 1.87)	0.860	1.34 (0.70 - 2.56)	0.382
- Chest symptoms associated with peak exposure	0.86 (0.46 - 1.60)	0.633	1.50 (0.84 - 2.66)	0.171
Baseline pulmonary function indices (n = 412)				
- FEV_1 / FVC < 0.7	1.17 (0.31 - 4.46)	0.821	1.02 (0.25 - 4.17)	0.980
- FEV_1 < 80% predicted	0.84 (0.31 - 2.24)	0.722	1.42 (0.58 - 3.52)	0.445
Allergic sensitisation to latex				
- Sensitisation to latex (k82) (n = 421)	1.07 (0.51 - 2.26)	0.849	0.58 (0.25 - 1.38)	0.218
- True sensitisation to latex (n = 41)[2]	1.09 (0.45 - 2.66)	0.847	0.73 (0.27 - 1.97)	0.532
Serum inflammatory biomarkers				
- ECP[3]	**2.17 (1.14 - 4.14)**	**0.019**	**1.79 (0.92 - 3.47)**	**0.086**
- MPO[4]	1.41 (0.68 - 2.94)	0.356	1.50 (0.72 - 3.12)	0.281
Asthma phenotypes				
- Atopic asthma	1.54 (0.61 - 3.90)	0.357	1.16 (0.43 - 3.09)	0.769
- Non-atopic asthma	1.53 (0.53 - 4.42)	0.429	1.57 (0.54 - 4.49)	0.414
- Work-aggravated asthma	1.00 (0.28 - 3.53)	1.000	1.44 (0.45 - 4.65)	0.541

Each OR is derived from a separate model.

[1] 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 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?"

[2] True latex sensitisation refers to positive response to latex (k82) and negative response to CCDs.

[3] ECP cutoff: positive response based on values above the normal range of the patient population established in the laboratory.

[4] MPO cutoff: positive response based on values above the normal range of the patient population established in the laboratory.

6.2.6 Exposure-response Relationships Between Serum Endotoxin Exposures And clinical Endpoints

Exposure-response relationships in respect of the serum endotoxin exposures and clinical endpoints of interest in dental HCWs were assessed while controlling for potential confounders (age, sex, smoking, bronchitis, pneumonia). **Table 6.7** shows a borderline association between asthma-related symptoms and serum endotoxin levels. The odds of work-related symptoms increased with increasing serum endotoxin levels, although the relation was not significant. The odds of having high serum ECP levels were also elevated among dental HCWs with moderate serum endotoxin levels.

6.2.7 Exposure-response Relationships Between Serum Endotoxin Exposures And clinical Endpoints Stratified By Atopy

In the stratified logistic regression model the following borderline associations were observed. The odds of being woken up by cough were 2 times more likely in non-atopic individuals with moderate serum endotoxin levels (**Table 6.8**), while chest symptoms as a result of peak exposure were more common among atopic individuals with high serum endotoxin levels. Adverse pulmonary function appeared to be more likely to occur among atopic dental HCWs with moderate serum endotoxin levels. Atopic dental HCWs with high serum endotoxin levels were unlikely to have composite latex sensitisation.

Table 6.7 Association between serum endotoxin concentration categories (tertiles) and clinical endpoints in conditional logistic regression models.

Outcome variables	Endotoxin in serum (EU/ml)			
	Middle tertile 0.71 – 0.89		Upper tertile > 0.92	
	OR (95% CI) n = 146	p-value	OR (95% CI) n = 144	p-value
Ocular-nasal and asthma symptoms (in last 12 months)				
- Ocular-nasal	1.32 (0.76 - 2.27)	0.323	1.36 (0.78 - 2.37)	0.283
- Wheezing without flu or cold	1.20 (0.56 - 2.55)	0.644	1.60 (0.76 - 3.37)	0.216
- Woken up by cough	1.42 (0.79 - 2.54)	0.245	1.47 (0.81 - 2.66)	0.209
- Woken up by tight chest	1.19 (0.60 - 2.35)	0.621	1.20 (0.60 - 2.40)	0.613
- Woken up by shortness of breath	1.21 (0.52 - 2.84)	0.655	0.88 (0.35 - 2.21)	0.781
- Asthma-related symptoms[1]	**1.64 (0.95 - 2.81)**	**0.075**	**1.64 (0.94 - 2.87)**	**0.079**
- Asthma attacks	2.04 (0.39 - 10.66)	0.396	2.30 (0.42 - 12.67)	0.339
- Current asthma treatment	2.41 (0.45 - 12.80)	0.302	2.18 (0.36 - 13.31)	0.397
Work-related symptoms				
- Work-related chest symptoms	0.91 (0.28 - 2.98)	0.873	1.08 (0.33 - 3.50)	0.904
- Work-related ocular-nasal	0.93 (0.45 - 1.91)	0.846	1.38 (0.69 - 2.75)	0.359
- Chest symptoms associated with peak exposure	0.87 (0.45 - 1.67)	0.676	1.64 (0.89 - 3.04)	0.115
Baseline pulmonary function indices (n = 412)				
- FEV_1 / FVC < 0.7	0.80 (0.18 - 3.53)	0.770	0.79 (0.16 - 3.77)	0.764
- FEV_1 < 80% predicted	0.75 (0.26 - 2.21)	0.605	1.39 (0.52 - 3.72)	0.514
Allergic sensitisation to latex				
- Sensitisation to latex (k82) (n = 421)	1.14 (0.52 - 2.52)	0.744	0.65 (0.26 - 1.66)	0.371
- True sensitisation to latex (n = 41)[2]	1.31 (0.50 - 3.41)	0.583	0.90 (0.30 - 2.69)	0.846
Serum inflammatory biomarkers				
- ECP[3]	**1.88 (0.96 - 3.68)**	**0.066**	1.48 (0.73 - 3.03)	0.281
- MPO[4]	1.37 (0.61 - 3.08)	0.441	1.31 (0.56 - 3.05)	0.530
Asthma phenotypes				
- Atopic asthma	1.85 (0.68 - 5.02)	0.225	1.38 (0.47 - 4.07)	0.555
- Non-atopic asthma	2.30 (0.63 - 8.34)	0.205	2.16 (0.59 - 7.91)	0.246
- Work-aggravated asthma	1.32 (0.32 - 5.51)	0.700	2.06 (0.52 - 8.15)	0.303

Each OR is derived from a separate model. ORs were adjusted for age, sex, smoking, bronchitis and pneumonia.

[1] 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 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?"

[2] True latex sensitisation refers to positive response to latex (k82) and negative response to CCDs.

[3] ECP cutoff: positive response based on values above the normal range of the patient population established in the laboratory.

[4] MPO cutoff: positive response based on values above the normal range of the patient population established in the laboratory.

Table 6.8 Association between serum endotoxin concentration categories (tertiles) and clinical endpoints in conditional logistic regression models stratified by atopy.

Outcome variables	Serum endotoxin level (EU/ml)							
	Atopic				Non-atopic			
	Middle tertile 0.71 – 0.89		Upper tertile > 0.92		Middle tertile 0.71 – 0.89		Upper tertile > 0.92	
	OR (95% CI) n = 152	p-value	OR (95% CI) n = 148	p-value	OR (95% CI) n = 152	p-value	OR (95% CI) n = 148	p-value
Ocular-nasal and asthma symptoms (in last 12 months)								
- Ocular-nasal	1.13 (0.52 - 2.45)	0.762	1.27 (0.58 - 2.81)	0.553	1.44 (0.60 - 3.48)	0.413	1.01 (0.38 - 2.69)	0.984
- Wheezing without flu or cold	1.44 (0.51 - 4.08)	0.492	1.33 (0.46 - 3.87)	0.603	0.70 (0.20 - 2.44)	0.572	1.08 (0.33 - 3.52)	0.895
- Woken up by cough	1.00 (0.43 - 2.36)	0.995	1.17 (0.50 - 2.78)	0.716	**2.11 (0.87 - 5.09)**	**0.097**	1.95 (0.78 - 4.92)	0.155
- Woken up by tight chest	1.22 (0.45 - 3.30)	0.700	0.83 (0.28 - 2.43)	0.737	0.81 (0.27 - 2.42)	0.704	1.17 (0.42 - 3.30)	0.766
- Woken up by shortness of breath	0.64 (0.17 - 2.44)	0.514	0.51 (0.12 - 2.18)	0.361	1.99 (0.57 - 6.97)	0.281	1.00 (0.24 - 4.21)	0.997
- Asthma-related symptoms[1]	1.50 (0.67 - 3.39)	0.328	1.35 (0.59 - 3.11)	0.475	1.58 (0.71 - 3.51)	0.257	1.59 (0.70 - 3.64)	0.270
- Asthma attacks	1.30 (0.14 - 11.96)	0.819	0.90 (0.08 - 9.91)	0.933	2.25 (0.24 - 20.74)	0.474	1.53 (0.12 - 19.86)	0.743
- Current asthma treatment	2.25 (0.24 - 20.74)	0.474	1.53 (0.12 - 19.86)	0.743	-		-	
Work-related symptoms								
- Work-related chest symptoms	1.51 (0.30 - 7.54)	0.615	0.96 (0.16 - 5.59)	0.961	0.55 (0.05 - 5.93)	0.626	1.40 (0.19 - 10.39)	0.743
- Work-related ocular-nasal	0.90 (0.30 - 2.67)	0.850	1.89 (0.69 - 5.17)	0.213	1.36 (0.48 - 3.84)	0.558	1.23 (0.41 - 3.66)	0.711
- Chest symptoms associated with peak exposure	1.14 (0.42 - 3.08)	0.797	**2.37 (0.92 - 6.07)**	**0.073**	0.66 (0.26 - 1.67)	0.377	1.36 (0.56 - 3.31)	0.501
Baseline pulmonary function indices (n = 412)								
- FEV_1 / FVC < 0.7	-		-		0.30 (0.04 - 2.08)	0.222	0.79 (0.15 - 4.21)	0.780
- FEV_1 < 80% predicted	**6.40 (0.72 - 57.16)**	**0.097**	4.77 (0.51 - 44.35)	0.170	-		0.93 (0.22 - 3.83)	0.915
Allergic sensitisation to latex								
- Sensitisation to latex (k82) (n = 421)	0.83 (0.34 - 2.01)	0.672	**0.41 (0.15 - 1.13)**	**0.086**	-		-	
- True sensitisation to latex (n = 41)[2]	0.91 (0.33 - 2.54)	0.855	0.56 (0.18 - 1.77)	0.323	-		-	
Serum inflammatory biomarkers								
- ECP[3]	1.60 (0.62 - 4.13)	0.335	1.34 (0.50 - 3.57)	0.556	1.79 (0.65 - 4.89)	0.259	1.34 (0.45 - 4.04)	0.598
- MPO[4]	1.20 (0.37 - 3.84)	0.762	1.48 (0.48 - 4.61)	0.498	1.72 (0.52 - 5.69)	0.369	1.02 (0.25 - 4.12)	0.978
Asthma phenotypes								
- Atopic asthma	1.71 (0.60 - 4.90)	0.316	1.15 (0.37 - 3.51)	0.812	-		-	
- Non-atopic asthma	-		-		2.94 (0.72 - 12.07)	0.133	3.34 (0.79 - 14.06)	0.101
- Work-aggravated asthma	1.15 (0.21 - 6.35)	0.873	0.97 (0.16 - 5.83)	0.971	3.43 (0.13 - 88.23)	0.456	11.55 (0.45 - 297)	0.140

Each OR is derived from a separate model. ORs were adjusted for age, sex, smoking, bronchitis and pneumonia.

[1] 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 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?"

[2] True latex sensitisation refers to positive response to latex (k82) and negative response to CCDs.

[3] ECP cutoff: positive response based on values above the normal range of the patient population established in the laboratory.

[4] MPO cutoff: positive response based on values above the normal range of the patient population established in the laboratory.

6.3 DISCUSSION

In this study high serum endotoxin variability within institutions, departments and job type exposure categories was demonstrated. These findings are similar to the within-group variability for airborne endotoxin for these areas as described in Chapter 2. These results highlight the difficulty in characterising biological exposure such as endotoxin in the workplace because a worker's exposure is never constant over time (Burdorf, 2005). Workers within groups with similar tasks and working environments are rarely uniformly exposed, particularly in the academic dental facilities.

Serum endotoxin was detected in dental HCWs with increased levels in workers working in clinics and laboratories which are known to have higher airborne endotoxin levels. To our knowledge this is the first study to demonstrate an association between serum endotoxin and airway obstruction in dental HCWs with atopy. The systemic endotoxin levels found in this study were comparable to the levels found in the study by Sikkeland et al. who detected lipopolysaccharide (endotoxin) in blood samples of BioProtein processing workers exposed to high levels of occupational airborne endotoxin (Sikkeland et al., 2007). The systemic endotoxin levels reported were higher than those found in the study by Tamura et al. who detected endotoxin in whole blood of humans (Tamura et al., 1991).

Airborne endotoxin was significantly correlated with serum endotoxin in this study, suggesting the possibility that airborne endotoxin may be the source of exposure in this group of dental HCWs. Serum endotoxin was modestly correlated with airborne endotoxin levels in students and staff that did not wear face masks. These findings provide further evidence of airborne endotoxin being the likely source of exposure. A modest correlation was also demonstrated among those who wore face masks. This suggests that inappropriate masks were being used which were therefore ineffective in preventing

exposure. It is also possible that the lack of a strong correlation between serum endotoxin and airborne endotoxin may be due to the short half-life of endotoxin in circulating blood which is estimated to be approximately one hour (Goto and Rylander, 1987). It is possible that the time between the airborne endotoxin sample collection and drawing of blood samples may have been more than an hour, resulting in under-detection in serum samples. These results are however at variance with the study of Sikkeland et al. in which a significant correlation between these indices was not observed (Sikkeland et al., 2007).

Animal models have shown that airborne endotoxin settled in the airways may translocate from the airways by penetrating the lung barrier and entering the blood stream (Goto and Rylander, 1987). It is therefore plausible that endotoxin may translocate to the blood stream in humans exposed to high levels of endotoxin. Endotoxin leakage from the inflamed gut into the blood stream in patients with inflammatory bowel disease has also been documented, lending further support to this being biologically plausible (Gonzalez-Quintela et al., 2007).

The current study demonstrated borderline associations between serum endotoxin levels and asthma-related symptoms in non-atopic workers, as well as obstructive lung disease as well as chest tightness due to peak exposure in atopic dental workers in adjusted models. Once endotoxin reaches the airways and the blood stream, activation of monocytes, neutrophils and endothelial cells occurs, resulting in the production of cytokines and subsequent inflammation (Sikkeland et al., 2007). It therefore appears not to be a sensitive marker for estimating endotoxin dose and demonstrating early respiratory health-effects.

A limitation of the study was that the serum endotoxin was not proven to be derived from the lung. However, due to the fact that the dental HCWs working in the clinics and laboratories had on average

higher serum endotoxin levels compared to the administrative staff demonstrates that contamination in these areas was the likely source of endotoxin.

In conclusion, this study has demonstrated the presence of serum endotoxin in dental HCWs exposed to various levels of airborne endotoxin which appeared to be modestly correlated with each other. However, due to the high variability of serum and airborne endotoxin levels the results of this study must be interpreted with caution. Further studies of a similar nature are needed before inferences can be made about the use of serum endotoxin as a biomarker in assessing asthma risk in high endotoxin exposure occupational settings.

CHAPTER 7

7.1 FINAL DISCUSSION AND CONCLUSION

This study investigating the risk of WRA associated with endotoxin exposure among dental HCWs in South Africa has concluded that dental workers are at increased risk of inhaling bioaerosols containing endotoxin and latex. Higher endotoxin levels were found in the clinical (GM: 1.46 EU/m^3) and laboratory (GM: 1.73 EU/m^3) areas compared to other areas. On average the students were exposed to low grade elevated (2.15 EU/m^3) levels of endotoxin compared to lower levels among the administration staff (0.87 EU/m^3). Several determinants of endotoxin exposure that have practical implications for providing a healthy and safe environment in dental clinics (e.g. dental unit model, the age of the dental units and total number of units per institution, and microclimatic factors such as temperature and air velocity) were identified. Students were also an important determinant of endotoxin levels.

The second major conclusion that can be drawn from this study is that dental workers are at increased risk of developing work-related health outcomes. The prevalence of WAA among this group was 4%. In addition, 6.9% had atopic asthma and 5.9% had non-atopic asthma which demonstrates that different mechanisms are involved in the pathogenesis of these different asthma phenotypes. Various host-related factors were identified for the WAA phenotypes including previous pneumonia, general and work-related ocular-nasal symptoms and atopy. Although, the prevalence of latex sensitisation was relatively high (10%), the prevalence of probable occupational latex asthma was only 0.9%, suggesting that other workplace agents (e.g. acrylates and endotoxin) may play a role in the development of WRA in this group of workers.

Thirdly, an attempt to characterise the pathophysiological basis of these asthma phenotypes based on cytokine measurements produced four distinct inflammatory cell phenotypes. Two were more clearly related to an eosinophilic type of inflammation and the other two to a neutrophilic response. Each cellular response appeared to be related further to severity of inflammation (i.e. acute or chronic) as identified by the particular cytokine expressions. Workers with acute neutrophilic response were most likely to have work-related chest symptoms. This phenotype appeared to be as prevalent as the acute and chronic eosinophilic phenotype. WAA was strongly associated with chronic neutrophilia. MPO was significantly correlated with cytokines associated with a chronic neutrophilic response.

The fourth major conclusion drawn from this study was the association between personal airborne endotoxin levels and WRA symptoms. Endotoxin exposure-related effects were dependent on the atopic status of the individual. Exposure-response relationships were prominent in non-atopic workers with elevated acute or chronic endotoxin exposure. Workers were more likely to present with general and work-related ocular-nasal and asthma-related symptoms and decrements in FEV_1. Dental HCWs using latex gloves and concurrently exposed to low-grade elevated endotoxin levels are at increased risk of presenting with latex sensitisation.

Finally, elevated serum endotoxin was strongly associated with asthma-related symptoms and airway obstruction among this group of workers. However, these associations were borderline; therefore further studies need to be conducted to be able to draw any meaningful conclusion regarding the usefulness of serum endotoxin as a marker of airborne endotoxin exposure.

The overall findings of this study suggest that exposure to endotoxins emanating from DUWLs in dental health care settings play a key role in the manifestation of non-allergic asthma through the neutrophilic-response mechanism. Endotoxin exposure also appears to be important in the increasing risk of sensitisation to latex among dental HCWs using latex gloves.

The study provides further evidence that reinforces the need for dental workers and students especially, to adhere to guidelines on waterline management, aerosol control and dental surgery ventilation in order to reduce exposure to aerosols generated by contaminated DUWLs. Based on the adverse health effects as a result of endotoxin exposure and co-exposure with other agents such as latex in the dental setting, it is recommended that preventive measures be undertaken to reduce health risks and related adverse social outcomes (e.g. absenteeism, decreased productivity, job loss) associated with WRA among dental workers.

7.2 RECOMMENDATIONS

7.2.1 REGULATORY EXPOSURE STANDARDS

Currently no occupational exposure limit exists for endotoxin exposure because of the absence of a global standardised method for testing. The Dutch Expert Committee on Occupational Standards initially recommended a health-based occupational exposure limit for airborne endotoxin of 50 EU/m^3 based on personal inhalable dust exposure measured as 8 hour time-weighted average (Heederik and Douwes, 1997). This standard is currently being revised because of methodological difficulties in the field, with a proposal of 90 EU/m^3 and 135 EU/m^3 depending on the method of endotoxin detection used; that is the old method or the new recent method, respectively (Dutch Expert Committee on

Occupational Safety and Nordic Expert Committee, 2009). The ACGIH has also listed endotoxin as a biologically derived agent under study by its bioaerosols committee. The Regulations for Hazardous Biological Agents (HBAs) under the Occupational Health and Safety Act (OHSA) (Act No. 85 of 1993) in South Africa require that any biological agent that constitutes a risk to human health needs to be addressed. Key aspects of this standard that apply to dental settings include:

- establishment of workplace health and safety committee,
- regular biennial risk assessment,
- employees to follow safe procedures for HBAs,
- training for employees,
- regular exposure monitoring and medical surveillance of workers.

7.2.2 Workplace Interventions And Control Measures

Addressing endotoxin exposures will require both engineering controls and administrative controls. From an engineering control perspective the dental unit manufacturer bears the primary responsibility for ensuring that dental units are designed to reduce the problem of DUWL contamination (Christensen, 2001). However, in terms of the OHSA the responsibility lies with the dentist in choosing to purchase new dental units that are capable of delivering water of an acceptable standard or in retrofitting existing equipment (Mills, 2001). In addition, the optimal performance of the units is also subject to strict adherence to maintenance protocols.

Despite technological advances, engineering controls still need to be coupled with administrative controls for optimal reduction of risk of transmission (Harrel, 2003). Personal or task-based control measures, such as well-maintained local exhaust ventilation and decontamination of DUWLs, are also important in lowering exposures. All workers working in the clinical and laboratory areas, including

cleaning staff, must also wear appropriate PPE. It is imperative that N95 cup style face masks that are appropriately fitted be used. Substitution of powdered-latex gloves with low-protein powder-free latex gloves or latex-free gloves is also essential in reducing the risk of sensitisation to latex, particularly with concurrent endotoxin exposure.

7.2.3 MEDICAL SURVEILLANCE

In order for the risk of adverse health effects associated with exposure to endotoxin and associated sensitisers to be minimised, periodical medical surveillance especially in high-exposure areas may be required. Medical surveillance may also be required to assess the effectiveness of the interventions such as the decontamination of DUWLs and replacement of latex gloves with non-powdered latex gloves. Such programmes should include annual symptom screening questionnaires, and skin prick testing with latex extracts or specific IgE to latex. Additional tests (e.g. spirometry) can be done to confirm WRA should the tests demonstrate a work-related association. Targeted surveillance for specific asthma phenotypes such as workers with work-related ocular-nasal symptoms should be monitored carefully as they are a high-risk group for the future development of asthma.

7.2.4 EDUCATION AND TRAINING

Key to the prevention of WRA and asthma symptoms is education of employers and workers. Key issues that must be addressed include knowledge of WRA and allergic diseases, use of appropriate PPE and training on the correct use thereof, as well as other administrative controls to be followed. The training schedule should be ongoing to accommodate new staff members. In addition, education about the risk of occupational exposure in the dental setting should be included in the training programme of dental students.

7.3 FUTURE RESEARCH

This study has raised a number of unanswered questions that need to be addressed in future research studies. Future exposure characterisation studies of dental workers need to identify determinants of within-worker variability and the impact this has on elevated airborne and serum endotoxin exposures through repeat sampling measurements. In addition, particular attention should be given to the timing of sample collection for serum endotoxin analysis. Follow-up of dental apprentices from commencement of training should be done to determine onset of allergic sensitisation, work-related symptoms and progression of asthma. Furthermore, assessment of biomarkers (e.g. ECP and MPO) and cytokines in sputum samples in relation to asthma symptoms needs further exploration in this group of workers. There is also a need for prospective studies to better document the true incidence of occupational allergic sensitisation and asthma phenotypes due to work practices in the dental profession so that the beneficial effect of different interventions (e.g. introduction of low-latex powder-free gloves, rigorous decontamination of DUWLs, use of N95 respiratory face masks) can be evaluated with greater certainty.

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Appendix A: Building Walkthrough Checklist

CODE				
Date of inspection:				

GENERAL INFORMATION					FOR OFFICE USE ONLY
Name of data collector (s):					
Number of dental clinics:					2
Number of floors:					3
Name of assistant on site:					4
Position of assistant onsite:					5
Age of building:					6
Any recent renovations?			☐ YES	☐ NO	7
If yes, please specify:					7i
Any water features (e.g. fountains, sprays, indoor waterfalls)?			☐ YES	☐ NO	8
History of water damage?			☐ YES	☐ NO	9
Evidence of water damage (stained or discoloured ceiling tiles, walls, floors, carpeting)?			☐ YES	☐ NO	10
Any visible microbial growth?			☐ YES	☐ NO	11
Odours?			☐ YES	☐ NO	12
SURROUNDING AREA					
Type of location:		☐ Urban	☐ Suburban	☐ Rural	13
Surrounding land use:	☐ Business	☐ Residential	☐ Agriculture	☐ Other	14
If other, please specify					14
Any construction activity?			☐ YES	☐ NO	15
Are there cooling towers present?			☐ YES	☐ NO	16
Shallow groundwater areas (e.g. marshes)?			☐ YES	☐ NO	17

HEATING, VENTILATING AND AIR-CONDITIONING SYSTEM			
GENERAL CHARACTERISTICS			
Is there a ventilation system	☐ YES	☐ NO	18
If yes, specify type of ventilation system: (Tick the relevant option)			
• General controlled ventilation		☐	18A
• Local Exhaust ventilation		☐	18B
• Window aircons		☐	18C
• Other:		☐	18D
If other, please specify:			18Di
Is the operation of the ventilation system effective?	☐ YES	☐ NO	19
Is the operation of the ventilation system continuous?	☐ YES	☐ NO	20
If No, state the period of operation:			20i
How often is the ventilation system serviced? ☐ Monthly ☐ Quarterly ☐ Bi-annually ☐ Annually ☐ Other			21
If other, please specify:			21i
Are ventilation service records available?	☐ YES	☐ NO	22
OUTDOOR AIR INTAKE (OAI)			
Is the OAI close to contaminated sources	☐ YES	☐ NO	23
If yes, please specify ☐ Feathers ☐ Bird droppings ☐ Organic matter ☐ Standing water ☐ Other debris			23i
FILTERS			
Are testing or maintenance records for filters available?	☐ YES	☐ NO	24
HEATING AND COOLING COIL AREA (HCCA)			
Does the HCCA have a condensate pan?	☐ YES	☐ NO	25
Does the HCCA have a drain?	☐ YES	☐ NO	26
Are there any odours?	☐ YES	☐ NO	27
SUPPLY SIDE OF AIR-HANDLING UNIT SSAHU			
Where do ducts of the SSAHU enter the building (e.g. ceiling, below floors):			28
Are the ducts lined or unlined?	☐ Lined	☐ Unlined	29
Are there signs of microbial growth?	☐ YES	☐ NO	30
Are there any odours?	☐ YES	☐ NO	31
Is there evidence of water damage?	☐ YES	☐ NO	32
DENTAL CLINICS			
Name of clinic:			33

Size of clinic:					34
Main work activity of the clinic:					35
Average working hours per day:					36
Age of dental units:					37
Model of dental units:					38
Number of dental units:					39
Frequency of servicing/maintenance of dental units:					40
Are records available for maintenance of dental units?			☐ YES	☐ NO	41
Frequency of disinfection of dental units:					42
Type of disinfection:					43
Type of water supply:	☐ Municipal	☐ Reservoir	☐ Other		44
If other, please specify:					**44i**
Frequency of waterline maintenance:					45
Frequency of reservoir maintenance:					46
Waterline flow rates:					47
Which of the following latex products are used in this clinic? (tick the relevant options)					48
• Rubber dams				☐	48A
• Gloves				☐	48B
• Suction tubing				☐	48C
• Impression casts				☐	48D
• Other				☐	48E
If other, please specify:					**48Ei**
Are all latex products designated for specific area?			☐ YES	☐ NO	49
Are all latex products identifiable?			☐ YES	☐ NO	**50**
If no, please specify why					50i
Frequency of cleaning?	Floor surfaces	Benchtops	Other (name)		51

WORK PRACTICES AND PERSONAL PROTECTIVE EQUIPMENT (PPE)					
Type of PPE:	☐ Gloves	☐ Coats	☐ Masks	☐ Goggles	52
Storage of PPE in work areas?	GLOVES	COATS	MASKS	GOGGLES	53
Is PPE dedicated to specific areas? (Cross relevant option)	GLOVES Y N	COATS Y N	MASKS Y N	GOGGLES Y N	**54**

GLOVES:						
Brand name:						55
Glove usage:	☐ Per patient	☐ Per session	☐ Per procedure	☐ Other		56
If other, please specify						56i
Type of gloves:	☐ Powder Latex	☐ Non-powdered latex	☐ Powdered non-latex	☐ Non-powdered non-latex		57
Comment:						58
Total protein content if stated?						59
Latex allergens present if stated?						60
Is there a glove changing station available?				☐ YES	☐ NO	61
If Yes specify:						61i
Is there a procedure for the donning and removal of gloves				☐ YES	☐ NO	62
If Yes specify:						62i
Are **ALL** workers aware of this procedure?				☐ YES	☐ NO	63
If no, specify estimated number						63i
Is care exercised when removing gloves?				☐ YES	☐ NO	64
If no, specify estimated number						64i
Estimated patients seen daily?						65
Estimated gloves used daily?						66
Is the number of gloves used routinely recorded?				☐ YES	☐ NO	67
Are gloves used for all procedures?				☐ YES	☐ NO	68
How are the gloves disposed of?						69
Where are the gloves disposed?						70
COATS:						
How often are coats washed?	☐ Daily	☐ Twice a week	☐ Thrice a week	☐ Weekly	☐ Other	71
If other, please specify						71i
Any other additional information?						72

THANK YOU FOR YOUR TIME

Appendix B: Air Sampling Log Book

Personal / Stationary Samples

Location/Job title	Pump #	Flow rate initial	final	Time on	Time off	LCD reading	Sample #	Comments

TEMPERATURE		HUMIDITY		VELOCITY	
MORNING	AFTERNOON	MORNING	AFTERNOON	MORNING	AFTERNOON
R1:	R1:	R1:	R1:	R1:	R1:
R2:	R2:	R2:	R2:	R2:	R2:
R3:	R3:	R3:	R3:	R3:	R3:

Appendix C: Main Questionnaire

WORK-RELATED ASTHMA ASSOCIATED WITH ENDOTOXIN EXPOSURE IN DENTAL WORKERS IN SOUTH AFRICA

Administered by:

Date of interview | | | | | 2 | 0 | 0 | 5 |
D D M M Y Y Y Y

Code | | | | |
OFFICE USE ONLY

MAIN QUESTIONNAIRE

INSTRUCTIONS:
Please print in block capitals
Please insert cross (x) in box ☐ where relevant
Please cross (x) appropriate options e.g. X or **N OR Y or N**
Please insert answers where required

PERSONAL DETAILS

Surname _____
First name/s _____
Address _____

Home tel. (___ ___ ___) ___ ___ ___ ___ ___ ___ ___
Work tel. (___ ___ ___) ___ ___ ___ ___ ___ ___ ___
Cell no. (___ ___ ___) ___ ___ ___ ___ ___ ___ ___

Date of birth | | | | | | | | |
D D M M Y Y Y Y

Sex Male ☐ Female ☐

Persal no. (WORKER) | | | | | | | | |

Student no. (STUDENT) | | | | | | | | |
YEAR OF STUDY (IN DENTISTRY) _____

Occupation (**CROSS (x)** RELEVANT)

☐ Dentist ☐ Dental surgeon ☐ Dental assistant
☐ Dental technologist ☐ Dental technician ☐ Dental therapist
☐ Dental nurse ☐ Nursing assistant ☐ Oral hygienist
☐ Post graduate ☐ Under graduate ☐ Other

If other, please specify: _____

Wheeze and tightness in the chest

1	Have you had wheezing or whistling in your chest at any time in the last **12 months**?	Y	N
	If YES, continue with Question 1 If NO, skip to Question 2		
1.1	Have you been short of breath when the wheezing noise was present?	Y	N
1.2	Have you had this wheezing or whistling when you **did not** have a cold or flu?	Y	N
2	Have you been woken up at night with a feeling of tightness in your chest at any time in the last **12 months**?	Y	N

Breathing

3	Do you **ever** have trouble with your breathing?	Y	N
	If YES, continue with Questions 3-7 If NO, skip to Question 8		
3.1	Do you have this trouble: Only cross (X) ONE answer		
a	continuously?		
b	repeatedly?		
c	only rarely?		
4	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**?	Y	N
5	Have you had an attack of shortness of breath that came on following running or exercise at any time in the last **12 months**?	Y	N
6	Have you been woken by an attack of shortness of breath at any time in the last **12 months**?	Y	N
7	Are you disabled from walking by a condition other than lung disease?	Y	N
7i	If yes, state the condition _____		
7.1	Are you troubled by shortness of breath when hurrying on level ground or walking up a slight hill?	Y	N
7.2	Do you get short of breath walking with other people of your own age on level ground?	Y	N
7.3	Do you have to stop for breath when walking at your own pace on level ground?	Y	N

Cough and phlegm from the chest

8	Have you been woken by an attack of coughing at any time in the last **12 months**?	Y	N
9	Do you **usually** cough?	Y	N
	USUALLY refers to twice a week If YES, continue to Question 10 If NO, skip to Question 11		
10	When do you **usually** cough: Only cross (X) ONE answer		
a	First thing in the morning		
b	During the day		
c	At night		
d	All the time		
10.1	Do you cough like this **on most** days/nights for as much as three or more months in each of the last two years?	Y	N
	ON MOST refers to more than 3 days a week		

11	Do you **usually** bring up any phlegm from your chest? If YES, continue to Question 12 If NO, skip to Question 13	Y	N
12	When do you **usually** bring up any phlegm from your chest: Only cross (X) ONE answer		
a	First thing in the morning		
b	During the day		
c	At night		
d	All the time		
12.1	Did 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?	Y	N

Asthma

13	Have you ever had asthma? If YES, continue with Question 13 If NO, skip to Question 14	Y	N
13.1	Was this confirmed by a doctor?	Y	N
13.2	How old were you when you were first told you have asthma? Only cross (X) ONE answer		
a	Before the age of 17		
b	At the age of 17 years or older		
	The following references to "attack" of asthma refers to episodes of wheezing, shortness of breath, chest tightness or cough attributed to asthma		
13.3	How old were you when you had your first **attack** of asthma? YEARS OLD		
13.4	How many days/weeks/months/years ago did you have your most recent **attack** of asthma? Only complete ONE option		
a	Days		
b	Weeks		
c	Months		
d	Years		
13.5	Which months of the year do you usually have attacks of asthma?		
a	January/February	Y	N
b	March/April	Y	N
c	May/June	Y	N
d	July/August	Y	N
e	September/October	Y	N
f	November/December	Y	N
13.6	Have you had an attack of asthma in the last **12 months**? If YES, continue with Question 13.6.1 If NO, skip to Question 13.7	Y	N
13.6.1	How often have you had an attack of asthma in the last **12 months**? Only cross (X) ONE answer		
a	Every day		
b	Most days		
c	Once a week		
d	Once a month		
e	Less than once a month		

13.7 Do you think your chest symptoms are caused by, or made worse by any of the following:
Answer all questions

a	Contact with animals/pets	Y	N
b	Grass or flowers	Y	N
c	Heavy exercise	Y	N
d	Breathing cold air	Y	N
e	Sprays, mist, steam or at work	Y	N
f	Tobacco smoke	Y	N
g	Change in the weather	Y	N

13.8 Are you currently taking any medication (including inhalers, aerosols or tablets) for asthma? Y N
If YES, state the medication
If NO, continue with Question 13.9

13.8i _____

13.8ii _____

13.9 What happens to your chest symptoms when you are away from dental school (for example, on weekends and vacations)?
Only cross (X) ONE answer

a	Stay the same	
b	Get better	
c	Get worse	

13.10 Does being at work ever make your chest tight or wheezy? Y N
If YES, continue with Question 13.10
If NO, skip to Question 13.11

a When did you first notice having problems with chest tightness or wheeze at dental school? _____
MONTH & YEAR

b Is there anything that you work with that you think causes you to have these chest symptoms? Y N
If YES, continue with Question 13.10
If NO, skip to Question 13.11

c What do you think is causing these symptoms?
ci Please specify: _____

13.11 Have you ever had to change or leave your work area, either temporarily or permanently, in this clinic or any other clinic because of these chest symptoms? Y N
If YES, continue with Question 13.11
If NO, skip to Question 14

a Where were you working at the time?
Please cross (**x) relevant option**

☐ Maxillo Facial	☐ Periodontology & Oral Medicine	☐ Restorative Dentistry
☐ Oral surgery	☐ Endodontics	☐ Auxillary training
☐ Oral health/hygiene	☐ Radiology	☐ Community dentistry
☐ Orthodontics	☐ Prosthetics	☐ Paediatrics

Other: (please specify): _____

b Did this change happen while at this institution? Y N
bi If NO, please specify _____

c Which clinic did you move to?
Please cross (x**) relevant option**

☐ Maxillo Facial	☐ Periodontology & Oral Medicine	☐ Restorative Dentistry
☐ Oral surgery	☐ Endodontics	☐ Auxillary training
☐ Oral health/hygiene	☐ Radiology	☐ Community dentistry
☐ Orthodontics	☐ Prosthetics	☐ Paediatrics

Other: (please specify): _____

d Did your symptoms improve when you changed work areas? Y N

Atopy and allergies

14 Do you have a history of any of allergic conditions? Y N

If YES, please cross (**x) relevant option/s**
If NO, skip to Question 14.2

a		Anaphylaxis	g		Chronic rhinitis
b		Insect allergies	**h**		Asthma
c		Drug allergies	i		Contact dermatitis
d		Food allergies	j		Eczema
e		Red / itchy eyes	k		Urticaria
f		Seasonal hay fever	l		Swelling of mouth after eating
m		Other occupational allergies (please specify), mi:			

14.1 Does anyone in your immediate family, other than you have a history of?

a Asthma Y N
Specify relation:

b Eczema Y N
Specify relation:

c Hay fever Y N
Specify relation:

14.2 Are you allergic to any fruit or vegetables? Y N

If YES, please cross (**x) relevant option/s**
If NO, skip to Question 14.3

a		Kiwi fruit	f		Papaya
b		Chestnut	g		Tomatoes
c		Bananas	h		Raw potatoes
d		Avocado	i		Other stone fruits
e		Latex			

14.3 When you are near animals (e.g. cats, dogs or horses) or near feathers (e.g. birds, pillows, quilts or duvets) or near grass and flowers or in a dusty part of the house do you ever:

a	start to cough	Y	N
b	start to wheeze	Y	N
c	get a tight chest	Y	N
d	start to feel short of breath	Y	N
e	get a runny/stuffy nose or sneeze	Y	N
f	get itchy or watery eyes	Y	N
g	get itchy skin/rash	Y	N
h	Start to sneeze	Y	N

14.4 Have you ever experienced swelling, itching, hives or other symptoms:

a	after contact with a balloon	Y	N
b	after dental examination or procedure	Y	N
c	after being examined by someone wearing latex/rubber gloves	Y	N
d	following a vaginal or rectal examination or other contact with a diaphragm or condom	Y	N
e	after contact with rubber tubing, injection ampules, anaesthetic apparatus, dental dams, scopes, impression casts	Y	N
f	within one hour after wearing rubber gloves	Y	N
g	after contact with rubber sporting equipment (e.g. tennis grips)	Y	N

14.5	Does your occupation involve contact with products containing latex?	Y	N
	If YES, continue with question 15		
	If NO, skip to question 16		
15	After handling latex/rubber products at work have you experienced any of the following?		
a	Itchy eyes	Y	N
b	Runny or blocked nose	Y	N
c	Coughing	Y	N
d	Wheezing	Y	N
e	Difficulty breathing	Y	N
f	Hives	Y	N
g	Itchy skin	Y	N
h	Skin rashes	Y	N
i	Dizziness	Y	N
j	Cracking/chapping hands	Y	N
k	Swelling	Y	N
l	Redness	Y	N
1mi	Other (Please specify), mi)		
16	Do you think your work environment is causing you to develop allergic symptoms?	Y	N
	If YES, please specify what is the cause in 16.1:		
	If NO, skip to question 17		
16i	Please specify		
17	Have you been diagnosed with any of the following in the past six months:		
a	Pneumonia	Y	N
b	Bronchitis	Y	N
c	Hypersensitivity pneumonitis	Y	N
d	Bacterial infection	Y	N
e	Sepsis	Y	N
f	Rapid heart rate	Y	N
g	Autoimmune disease (e.g. Lupus)	Y	N
h	Other major illness/es:	Y	N
17hi	If other, please specify:		

SMOKING HISTORY

18	Have you **ever smoked** tobacco (cigarettes or pipe) for as long as a year?	Y	N
	YES means at least 7 or 10 cigarettes per day. If YES, continue with Question 18		
	If NO, skip to Question 20		
18.1	How old were you when you started smoking?		
		years old	
18.2	Are you a current smoker?	Y	N
	YES means smoking tobacco in the last month or more. If YES, continue with Question 18		
	If NO, skip to Question 19		
18.3	How much, on average per day, do you currently smoke?		
	Please complete **relevant option**		
a	Number of cigarettes		
b	Pipe tobacco in grams		
c	Other tobacco products		
19	Have you stopped smoking completely?	Y	N
	If YES, continue with Question 19		
	If NO, skip to Question 20		
19.1	How old were you when you stopped smoking completely?		
		years old	
19.2	How many years in total did you smoke cigarettes?		
	(Do not include the years you stopped before you started again)	years	

19.3 In the entire time you smoked, how much did you smoke on average, per day?

a	Number of cigarettes	
b	Pipe tobacco in grams	
c	Other tobacco products	

20 Have you been regularly exposed to tobacco smoke from other people smoking cigarettes or pipe in the last **12 months**? Y N

Regularly means on most days or nights

21 Have you been regularly exposed to smoke **other than** cigarette or pipe in the last 12 months? Y N

If YES, please specify type of smoke

21i _____

WORK HISTORY IN DENTAL FACILITY

22 State your employment history?

Please state in chronological order with most recent first. Include number of years for each job

	Company	Type of work	cross (x) appropriate		# of years
			Private	Public	
1					
2					
3					
4					

23 What type of work do you do in your current job?

Please cross (x) appropriate option

☐	Maxillo Facial	☐	Periodontology & Oral Medicine	☐	Restorative Dentistry
☐	Oral surgery	☐	Endodontics	☐	Auxillary training
☐	Oral health/hygiene	☐	Radiology	☐	Community dentistry
☐	Orthodontics	☐	Prosthetics	☐	Paediatrics
☐	Other: (please specify):				

23.1 What is your main job activity?

Please specify: _____

23.2 When did you start working in your current job? _____ YEARS,MONTHS

23.3 How many hours do you work per day on average? _____ HOURS PER DAY

23.4 How many patients do you see per day on average? _____ PATIENTS PER DAY

24 What dental unit model do you frequently use?

Please specify: _____

24.1 What dental unit model are you currently using?

Please specify: _____

25 Are you exposed to any of the following when working:

If YES, continue with question 25

If NO, skip to question 26

a	Spray	Y	N
b	Mist	Y	N
c	Steam	Y	N
d	Dust	Y	N

25.1 How much spray/mist/steam/dust would you say that job produces:

Insert a cross (X) next to one answer only

b	A little	
c	An average amount	
d	A lot	

25.2 How far do you work from the source of the spray/mist/steam/dust?

Insert a cross (X) next to one answer only

a	Right next to the source (< 1 meter)	
b	About 1-2 metres away	
c	More than 2 metres away	

25.3	Has there ever been an instance when you inhaled a large amount of spray/mist/steam/dust that resulted in you developing a tight chest, wheeze or cough?	Y	N
	If YES, continue with Question 25.3i		
	If NO, skip to Question 26		
25.3i	Please specify what was this type of job: _____		
26	Do you ever do other jobs during your shift on a **regular** basis?	Y	N
	Regularly means on most days or nights		
26i	**If Yes**, please state type of job/s? _____		
27	Do you use any personal protective equipment **regularly** while doing your job?	Y	N
	Regularly means on most days		
	If YES, continue with Question 27		
	If NO, you have come to the end of the questionnaire, thank you for your time		
27.1	Which of the following personal protective equipment do you use on a **regular** basis?		
	Regular means on most days		
a	Goggles:	Y	N
b	Gloves:	Y	N
c	Mask:	Y	N
d	Coat:	Y	N
e	Other	Y	N
27.1ei	Please specify: _____		

29	What type of gloves do you wear?	
	Insert a cross (X) next to one answer only	
a	***Powdered latex gloves***	
b	***Powder-free, low latex gloves***	
c	***Non – latex gloves***	
d	***Other***	
27.2	On average, how many of the following PPE do you wear per day?	
a	Goggles:	_____
b	Gloves:	_____
c	Mask:	_____
d	Coats:	_____
e	Other	_____
27.2ei	Please specify: _____	

27.3	How long have you been wearing the following PPE at work?	
a	Goggles:	_____ # YEARS
b	Gloves:	_____ # YEARS
c	Mask:	_____ # YEARS
d	Coats:	_____ # YEARS
e	Other	_____
27.3ei	Please specify _____	# YEARS

28	Have you always been wearing non-latex gloves?	Y	N
28i	If NO, please specify: _____		
29i	***Please specify brand of gloves worn:*** _____		

THANK YOU FOR ANSWERING THE QUESTIONNAIRE

Appendix D: Lung Function Pre screen Questionnaire

Administered by:

Date of interview

				2	0	0	5
D	D	M	M	Y	Y	Y	Y

Code

OFFICE USE ONLY

LUNG FUNCTION PRE-TEST QUESTIONNAIRE

INSTRUCTIONS:

- Please print in block capitals
- Please insert cross (x) in box ☐ where relevant
- Please cross (x) appropriate options e.g. **Y** or ~~N~~ **OR Y or** ~~N~~
- Please insert answers where required

PERSONAL DETAILS

1 Have you had a heart attack or stroke in the last 3 months? Y N

2 Do you have epilepsy? Y N

3 Have you had any recent operation (in the last 12 months)? Y N

If ***Yes***, what type and how many months ago?

_____ _____ (months)

If YES, to any of the above, indicate to the person that the lung function tests will not be done.
If NO, proceed with the rest of the screening questions

4 **For Women**:

4.1 Are you Pregnant? Y N

4.2 Are you Breastfeeding? Y N

If **Pregnant**, indicate to the person that the Lung Function Test will not be done today.
If **Breastfeeding**, proceed with Lung Function Test with Post-Bronchodilator.
Proceed with the rest of the screening questions.

5 Have you had the flu or lung infection in the past 3 weeks? Y N

If ***Yes***, how many days ago did it end? _____ days

6 Are you being treated for Tuberculosis? Y N

If ***Yes,*** for how long? _____ months _____ weeks

If YES, to either question No. 5 or 6, indicate to the person that the lung function tests will not be done today.
If NO, continue with the rest of the questions.

7 Did you drink coffee, tea or coca-cola in the last 6 hours? Y N

8 Have you smoked in the last hour? Y N

If YES to No. 8, reschedule the Lung Function Test for later the same day (at least one hour since last cigarette) or another date. Other screening procedures can be done first.

9 Have you had asthma in the past? Y N

9.1 Do you have asthma now? Y N

10 Are you taking any medicine/s from a doctor or clinic at the moment for your lungs, any heart condition, or your eyes? Y N

10.1 If ***Yes***, what are you taking and when did you last take them?

Names	**No. of hours since last dose**
_____	_____
_____	_____

If short-acting beta-2-agonist or anti-cholinergic inhalers used in the last **4** hours or long-acting MDI or theo used in last **8** hours, reschedule and counsel accordingly.

11	Have you had any of the following symptoms in the past 12 months (at night, with exercise, exposure to cold air, viral infections, work exposures)		
11.1	chest tightness	Y	N
11.2	shortness of breath	Y	N
11.3	wheezing or whistling in your chest	Y	N
11.4	dry cough	Y	N
12	Do you currently have any of these symptoms?	Y	N
12.1	***If Yes***, which ones?		

_____ _____

_____ _____

HEIGHT:_____ WEIGHT:_____ BP:_____

ID NO. __ __ __ __ __ __ __ __ __ __ __ __ __ __

Appendix E: Information Sheet And Consent Form

INFORMATION SHEET **&** **CONSENT FORM**	**NATIONAL INSTITUTE FOR OCCUPATIONAL HEALTH** **IMMUNOLOGY/MICROBIOLOGY SECTION** **P.O. BOX 4788, JOHANNESBURG, 2000**

Greetings,

I am Tanusha Singh and my colleague is Onnicah Mabe. We are conducting two studies in conjunction the one is "*Work-related asthma associated with endotoxin exposure in the dental workers in South Africa*" and the other is *"Detection of latex aeroallergens in dental schools"*. The studies will be done towards a PhD degree and a MSc degree in the Faculty of Health Sciences, University of the Witwatersrand. We therefore would like to invite you to participate in the study.

PURPOSE OF THE STUDY

Occupational health problems are not uncommon in the dental industry. Therefore monitoring or assessing exposure to hazardous substances is a fundamental step to improving the quality of life for dental personnel. We have identified two substances (namely: **endotoxin**, a by-product of Gram negative bacteria and **latex,** a protein in latex gloves) that we believe may be hazardous to your health and therefore would like to investigate them further.

Asthma rates have been slowly gaining momentum. Until recently asthma was known to be triggered by an allergic response to a particular allergen. Today scientists believe that another mechanism, which is non-allergic, may be involved. The latter response may be triggered by endotoxins. In addition, exposure to airborne latex has now been reported as the fourth most common cause of occupational asthma (allergic response). Studies have shown that high concentrations of latex aeroallergen have been found in areas where large numbers of gloves are routinely donned and discarded. When the gloves are snapped on and off, latex proteins bound to the cornstarch powder become airborne and these can be inhaled causing reaction to persons who are sensitised. Dentists are considered as a high-risk-group within the clinical specialities due to prolonged usage of latex gloves during the working day.

The studies proposes to explore the associations between occupational exposure to endotoxin and asthma mediated through a non-allergic response among dental workers (this includes dental academic staff and students as well as service personnel) in 5 academic institutions in South Africa and to explore the comparison between latex aeroallergen levels and work practices in dental schools. The laboratory work will be undertaken at the National Institute for Occupational Health under the supervision of Prof Adriano Dusé (Department of Clinical Microbiology and Infectious Diseases, Wits), Dr Mohamed Jeebhay (Occupational and Environmental Health Research Unit, School of Public Health and Family Medicine, UCT), Prof Ahmed Wadee (Head of Immunology Department, NHLS) and Ms Tanusha Singh (Immunology & Microbiology Section, NIOH). Permission to undertake this study has been obtained from the Committee for Research on Human Subjects, University of the Witwatersrand.

BENEFITS OF THE STUDY

- The major benefit of the project is create a safer and healthy working environment for you, your fellow colleagues and your patients.
- The key beneficiaries of this project are all individuals exposed to endotoxins and latex, who are at risk of developing asthma and other associated diseases who may benefit from early diagnosis and treatment.
- At an individual level all participants will be given a written copy of their test results along with an interpretation. 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.
- At a group level the study proposes to identify risk factors for acquiring non-allergic asthma and to develop appropriate occupational hygiene monitoring techniques, which will assist in preventing the increasingly high incidence of work-related asthma.
- Identifying the correct mechanism involved will assist in administering the proper medication and asthma management.
- The study will demonstrate the possibilities of being exposed to latex particularly aerosols in workplaces.
- The National Department of Health and Labour will be able to use information obtained through the research to develop appropriate exposure standards and health and safety policies for workers exposed to endotoxins and latex.
- Awareness to employees on exposure to bioaerosols (endotoxin and latex)
- The tests that will be done for the purposes of this study are offered to you at no cost. However the study cannot pay for any additional medical visits or treatments made from the study recommendations.

PROCEDURE TO BE FOLLOWED

If you agree to participate you will be asked to complete the following during working hours:

- Complete a questionnaire, which will provide me with important information on respiratory symptoms and other medical conditions as well as employment history and work practices.
- A building walk through checklist will be completed by an occupational hygienist and your assistance may be required to complete the checklist.
- Donate 20ml (2 tablespoons) blood to be tested for endotoxins, eosinophilic cationic protein (ECP, a biological marker associated with allergic response) and myeloperoxidase (MPO, a biological marker associated with non-allergic response), phadiotops (common aeroallergens), specific IgE for latex and recombinant allergens. No HIV tests will be conducted on the blood.
- Lung function assessments to determine whether you have asthma. The test is routinely done for the diagnosis of lung function impairment and will be conducted by a registered clinical technologist.
- Wear a personal sampling pump for approximately 120 minutes so that we can collect an air sample from your breathing zone which will give us an indication of the amount of endotoxin and or latex that can be inhaled.

RISK AND DISCOMFORT INVOLVED

- Blood tests: occasional temporary bruising may develop around site of the needlestick. This may be prevented as an experienced medical doctor or registered nurse using aseptic techniques will be drawing bloods.
- From breathing tests: There is a small chance that the initial breathing test (baseline spirometry) could cause you to become light-headed or dizzy. Subjects will complete the test in a seated position under the observation of trained personnel to reduce the chance of such problems. Part of the breathing test uses a chemical substance (methacholine) that can cause headache, cough, chest tightness, hoarse voice or a sore throat for a short time in some people. Very rarely it can cause severe breathing problems which can be treated successfully with a different medication (B2-agonist), which subjects breathe in. The subject is given the methacholine only if the spirometry is normal. This greatly reduces the chance of having a serious problem. These tests will be carried out by a trained technologist with a medical practitioner knowledgeable in the treatment of such problems and appropriate equipment immediately available.
- Some discomfort may be experienced from wearing the personal sampling pump however this would not impede on your normal work activities.

CONFIDENTIALITY

Confidentiality and anonymity is assured because data forms will be coded and will not have your name but a coding system that will be used.

NATURE OF PARTICIPATION
Participation is voluntary, no one will be penalised in any way if they do not participate. You may withdraw from the study at any time. The decision to participate (or not) in the study will have no impact on one's employment or student status.

CONTACT PERSON
If you have any concerns or questions about the study you can contact Prof Mary Ross (Director, NIOH). Tel: 011 712 6413, email: mary.ross@nioh.nhls.ac.za
For confidentiality reasons please quote your study number reflected below when communicating with the study team.

Study number: [ ]

We thank you for taking the time to read this information sheet. If you are willing to participate please sign the relevant portion (consent of the participant).

Yours sincerely

....
Tanusha Singh (PhD, Student) Onnicah Mabe (MSc, student)

✂----------

CONSENT OF THE PARTICIPANT

I....., BDS…… (if a student) have read the information in the information sheet or it has been read to me. I fully understand the purpose and conditions of the study and give my consent to participate.

....
Signature of participant Date

Study number: [ ]

Appendix F: Ethics Approval And Institutional Permission

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Singh

CLEARANCE CERTIFICATE	**PROTOCOL NUMBER M040403**
PROJECT among dental workers in SA	Work-related asthma associated with endotoxins
INVESTIGATORS	Ms T Singh
DEPARTMENT	NIOH
DATE CONSIDERED	04.05.07
DECISION OF THE COMMITTEE* withdrawn longer than 24 hours	Approved but no asthma medication may be

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

DATE 04.07.22 **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 10005, 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

Certified a true copy of the original.

Human Research Ethics Committee (Medical)
(formerly Committee for Research on Human Subjects (Medical))
Secretariat: Research Office, Room SH10005, 10th floor, Senate House • Telephone: +27 11 717-1234 • Fax: +27 11 339-5708
Private Bag 3, Wits 2050, South Africa

University
of the Witwatersrand,
Johannesburg



21 April 2005

Ms T Singh
Immunology/Microbiology
National Institute for Occupational Health
University

Dear Ms Tanusha

RE: PROTOCOL M040403

This letter serves to confirm that the Deputy Chair of the Human Research Ethics Committee (Medical) has approved the new consent Form on the abovementioned protocol. Copy attached.

Yours sincerely,

Anisa Keshav (Ms)
Secretary
Human Research Ethics Committee (Medical)

FWA Nr. 0000 2567
IRB Nr. 0000 2235

University of Pretoria
Faculty of Health Sciences Research Ethics Committee
University of Pretoria
Date: 4/07/2005

Soutpansberg Road
MRC-Building
Room 2 - 19

Private Bag x 385
Pretoria
0001

Number	:	32/2005
Title	:	Work related asthma associated with endotoxin exposure in dental workers in S.A.
Investigators	:	Tanusha Sigh; Dept Immunology/Microbiology Section; University of Pretoria Tanusha.singh@tuks.co.za C: 082 706 3744 F: 012-712 6545
Sponsor	:	None
VAT No	:	No
Study Degree	:	PhD

This Protocol has been considered by the Faculty of Health Sciences Research Ethics Committee, University of Pretoria on 29/06/2005 and found to be acceptable.

Advocate AG Nienaber	(female)BA(Hons) (Wits); LLB; LLM (UP); Dipl.Datametrics (UNISA)
Prof S.V. Grey	(female) BSc (Hons); MSc; DSc: Deputy Dean
*Prof V.O.L. Karusseit	MBChB; MFGP (SA); M.Med (Chir); FCS (SA): Surgeon
Dr M E Kenoshi	MB,CHB; DTM & H (Wits); C.E.O. of the Pretoria Academic Hospital
*Prof M Kruger	(female) MB.ChB.(Pret); Mmed.Paed.(Pret); PhDd. (Leuven)
*Dr N K Likibi	MB.BCh.; Med.Adviser (Gauteng Dept.of Health)
Dr F M Mulaudzi	(female) Department of Nursing.
Snr Sr J. Phatoli	(female) BCur (Et.Al) Senior Nursing-Sister
*Dr L Schoeman	(female) Bpharm, BA Hons (Psy), PhD
Prof H.W. Pretorius	MBChB; M.Med (Psych) MD: Psychiatrist
Prof J.R. Snyman	MBChB, M.Pharm.Med: MD: Pharmacologist
*Dr R Sommers	(female) MBChB; M.Med (Int); MPhar.Med;
Prof TJP Swart	BChD, MSc (Odont), MChD (Oral Path) Senior Specialist: Oral Pathology
Prof C W van Staden	MBChB; Mmed (Psych); MD; FTCL; UPLM; Dept of Psychiatry

DR R SOMMERS; MBChB; M.Med (Int); MPhar.Med.
SECRETARIAT of the Faculty of Health Sciences Research Ethics Committee - University of Pretoria

* = Members attended the meeting on 29/06/2005.

UNIVERSITY OF
KWAZULU-NATAL

Research Office
BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
Nelson R Mandela School of Medicine
Private Bag 7, Congella 4013
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604769
Fax: 27 31 2604609
Email: buccas@ukzn.ac.za
Website: www.ukzn.ac.za

19 September 2006

Ms T Singh
NIOH
PO Box 4788
Johannesburg 2000

PROTOCOL: Work related asthma associated with Endotoxin Exposure in Dental Workers in South Africa. Ms T Singh, Wits University, Ref: EXP031/06

EXPEDITED APPLICATION

Dear Ms Singh

This letter serves to notify you that at a full sitting of the Biomedical Research Ethics Committee meeting held on **12 September 2006**, the Committee **RATIFIED** the sub-committee's decision to approve the above study.

Yours sincerely

SURAIYA BUCCAS
Ethics Research Administrator

SB/sn

From: Robert Barrie [rbarrie@uwc.ac.za]
Sent: 23 November 2004 12:32 PM
To: Tanusha Singh
Subject: Permission to conduct study

Dear Ms Singh

Thank you for your letter and protocol for the study on The Relationship between Endotoxin Exposure and Work Related Asthma in Dental Workers, that you sent us some time ago. Sorry that it has taken so long to get back to you.

We have discussed the issue, and are pleased to be able to inform you that we hereby grant you permission to conduct the study in our institution, subject to the following conditions:

1. We are not granting "Ethical Approval" as requested in your letter, as we believe that such approval needs to be granted by Wits who are registering the research project. We are, however, granting you permission to conduct the study in our institution.

2. We would like to be informed of the results of the study before it is published.

3. We would appreciate the minimum disruption to our normal activities, as you have promised. Please liaise with Prof Yusuf Osman (yosman@uwc.ac.za).

Please feel free to contact me if you require any further information.

Kind regards,

Rob

Dr R B Barrie
Deputy Dean: Postgraduate Studies and Research Faculty of Dentistry University of the Western Cape Private Bag X1
7505 Tygerberg
South Africa

Tel: (021) 937 3008
Fax: (021) 931 2287
Cell: 082 444 0911
e-mail: rbarrie@uwc.ac.za

GAUTENG DEPARTMENT OF HEALTH

PROVINCIAL RESEARCH COMMITTEE.

RESEARCH EVALUATION FORM FOR APPROVAL BY THE HEAD OF THE DEPARTMENT.

Title: WORK-RELATED ASTHMA ASSOCIATED WITH ENDOTOXINS.

Principal Investigator: Tanusha Singh.

Institution: National Institute for Occupational Health (NIOH), Wits University.

Background information:

Dental unit waterline contamination has become a concern arising from the fact that bacteria sloughed from established biofilms in the dental unit waterlines increase bacterial counts in water exiting the waterlines.
Gram negative bacteria not only elicit disease by themselves but also produce a lipo-polysaccharide called endotoxin which may cause a non-allergic inflammatory response.
This is a National project by the National Institute for Occupational Health (NIOH) in Johannesburg proposing to explore the association between endotoxin exposure and work-related asthma in dental workers.
In other words, this study will investigate work-related asthma associated with endotoxin exposure from contaminated aerosols generated during dental procedures in various academic institutions in South Africa.
The choice of academic institutions is justified to allow the inclusion of the students in the project so that we have different exposure groups.

Research method:

Objectives:

1. The main objective of the project is:
   1 To determine water quality in dental unit waterlines.
   2 To determine asthma statistics for dental workers in South Africa.
   3 To determine whether endotoxins play a role in non-allergic asthma.

2. The specific objectives are:

   1 To determine the levels of endotoxin in dental unit waterlines of dental clinics/surgeries in various public sector academic institutions.
   2 To determine levels of endotoxin in ambient air of the dental clinic/surgeries conducting dental procedures through environmental sampling.

3 To determine serum levels of endotoxin in dental workers and students.
4 To determine the prevalence of work-related asthma symptoms in dental workers and students using health questionnaires and lung function assessment.
5 To determine whether the asthma symptoms are associated with increased eosinophil cationic protein (ECP) level (allergic response) or myeloperoxidase (MPO) levels (non-allergic response).
6 To determine whether asthmatics exposed to high levels of endotoxins have high MPO levels and low ECP levels.
7 Investigate whether asthmatics exposed to endotoxins have more frequent asthma episodes asthmatics that are not exposed to endotoxins.
8 To characterize the relationship between current exposure to endotoxins and work-related asthma symptoms while controlling for potential confounders such as age, gender and smoking.

Study design

This will be case-control study of currently employed dental personnel as well as students currently registered in the dental faculty of public sector academic institutions.

Study setting:

Five institutions have been identified for the study in the country: University of KwaZulu-Natal, Medunsa, University of Pretoria, University of the Western Cape and Wits University.

Study population

Public sector academic dental institution throughout South African have been identified as possible participate in the project: All dental clinics (Maxillo-Facial, Restorative dentistry, Periodontology, Prosthetic, Orthodontics and Oral Health). The population will include dentists, dental assistants, nurses, oral hygienists and dental students. The dental students will include first year through to fourth year student.

Sampling method:

All participants who have signed consent from will be asked to complete a group-assisted questionnaire, one for staff and one for students.
A total population of 432 will be considered.

Data measurements:

Data measurements for the project would include: self administered questionnaire, collection of water samples from 3-in-1 syringes, collection of air samples and the collection of 15ml blood from participate in the project.

Timeline

The time frame for the study is September 2004- December 2007.

Risks/Benefit for the participants/ Department:

Generally the study will be registered with Wits for a higher degree.

- At an individual level all participants will be given a written copy of their test result along with an interpretation of their meaning. Those with identified work-related asthma problems will also be given the names of specialist for referral.
- Employed personnel with work-related asthma would be entitled to compensation and would be given assistance in applying for it.
- At a group level the study proposes to identify risk factors for acquiring non-allergic asthma and to develop appropriate occupational hygiene monitoring techniques.
- The key beneficiaries of this project are all individuals exposed to endotoxins, who are at risk of developing asthma and other associated diseases who may benefit from early diagnosis and treatment.
- Workers exposed to endotoxins in the workplace will benefit from the results of this study since new monitoring strategies will assist kin preventing the increasingly high incidence of work-related asthma.
- The National Department of Health and Labour will be able to use information obtained through the research to develop appropriate exposure standards and health and safety policies for workers exposed to endotoxins.

Financial implications:

The will be no cost implications to the institutions and disruption of work will be minimized.
The total cost of R 251 685-00 has been applied for through The University of Michigan /Fogarty, the internal center: Southern African Programme in Environmental and Occupational Health. A sum of R100 000-00 has been awarded in support of this research. The remaining budget would be supported by the NIOH (personal communication with Prof David Rees).

ETHICAL CONSIDERATIONS

The study has been submitted for ethical approval at Wit University (see attached letter). Permission is hereby applied to conduct this study in the Gauteng Province, which would oversee the project being done at Wits University and University of Pretoria.

Conclusion:

The project seems to be acceptable; the methodology used is scientifically sound and accurate.

Recommendation:

This study is very interesting. The risks are minimal and the benefits are enormous for the researcher, the participants and the universities.
The study has been conducted for academic purpose. We have no objection to recommend that permission be granted.

Evaluators: Dr M L Likibi

Research and Epidemiology unit.
Tel: 011-355-3310
Fax: 011-355-3338
Cell: 0827819921
E-mail: MupataL@gpg.gov.za

Approved /~~not approved/ approved as amended.~~

Pp HOD

Dr L Rispel 24/12/2004

Cc: MS S. Dumela
Ms T. Chane
Dr M Ahern
Dr A Rahman
Research data base

NORTH WEST DEPARTMENT OF HEALTH

Healthy Living for All

North West Province

Republic of South Africa

1st Floor SABC Building, Left Wing
Mafikeng, 2745
Private Bag X2068
MMABATHO, 2735

KNOWLEDGE MANAGEMENT

Tel: (018) 387 6747
Tel: (018) 387 6774
Tel: (018) 387 6773

Fax: (018) 384 7960

22 February 2005

Ms Tanusha Singh

SUBJECT: Approval for Research- Work-related Asthma Associated with endotoxin exposure in the dental workers in South Africa

Approval is granted to conduct the above studying the North West Province of South Africa. Kindly make arrangements with the relevant management for suitable dates and times. Details at the bottom of this letter has to be completed by you and returned to the Knowledge Management Directorate of this department before your study may commence.

Regards

O. Mongale
Deputy Director General

The NWDoH will be furnished with the final research report by

31 December 2007
Submission date of the final report

28/02/2005
Tanusha Singh

DEPARTMENT OF HEALTH

PROVINCE OF KWAZULU-NATAL

SPECIAL SUPPORT SERVICES

Natalia, 330 Longmarket Street, Pietermaritzburg, 3201
Private Bag X9051, Pietermaritzburg, 3200
Tel : 033 395 3123, Fax : 033 345 4433

ENQURIES : Ms N Sithole
REFERENCE : 9/2/3/R
EXTENSION : 3123

29 SEP 2005

Ms Tanush Singh
National Institute for Occupational Health
Senior Medical Scientist
National Institute for Occupational Health
Immunology/Microbiology Section
P.O. Box 4788
JOHANNESBURG
2000

Dear Ms Singh

APPLICATION TO CONDUCT RESEARCH : WORK-RELATED ASTHMA ASSOCIATED WITH ENDOTOXIN EXPOSURE IN DENTAL WORKERS IN SOUTH AFRICA

Your letter to Prof RW Green-Thompson dated 13 September 2005 refers.

Please be advised that authority is granted for you to conduct research regarding the **"work-related asthma associated with endotoxin exposure in dental workers"** at the Oral and Dental Training Hospital; provided that the following is agreed to:

(a) Prior approval is obtained from Heads of relevant institutions;

(b) Full ethical approval is obtained;

(c) Informed consent from participants is obtained;

(d) Confidentiality is maintained;

(e) The Department is acknowledged; and

(f) The Department receives a copy of the report on completion.

Thank you

SUPERINTENDENT-GENERAL
HEAD : DEPARTMENT OF HEALTH
Nsm/tanush oral dental hospital

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

School of Oral Health Sciences, 7 York Road, Park Town
Johannesburg

Private Bag 3
WITS, 2050, South Africa
+27 11 488 – 4893/4864
Area 385 8th Floor JHB Gen Hospital
Fax: +27 11 488 - 4969
e-mail: deansecrdh@dentistry.wits.ac.za
essop@dentistry.wits.ac.za

20 February 2006

To all HODs and Students (SOHS)

Please assist Ms Tanusha Singh and Ms Onnicah Mabe in conducting their research at the School of Oral Health Sciences for which they have received the necessary permission from the University and Department of health. They propose to collect the data for the duration of their study as approved by their supervisors.

The Topics of the Studies are as follows:

- "Work related Asthma associated with endotoxin exposure in Dental Workers in South Africa."
- "The Detection of Latex Aeroallergens in Dentistry."

Your cooperation in the above is highly appreciated.

Prof Essop ARM
HOS/School of Oral Health Sciences

10/04/2006

University of Pretoria
P O Box 1266 Pretoria 0001 Republic of South Africa
Tel 012-319-2911 Fax 012-329-2508
http://www.up.ac.za

Faculty of Health Sciences
School of Dentistry

Heads of Departments
Support Staff
Students

RESEARCH PROJECTS: NATIONAL INSTITUTE OF OCCUPATIONAL HEALTH

Please assist Ms Tanusha Singh and Ms Onnicah Mabe in conducting their research at the School of Dentistry for which they have received the necessary permission from the University and Department of Health. They propose to collect the data for the duration of their study as approved by their supervisors.

The topics of the Studies are as follows:

- "Work related Asthma associated with endotoxin exposure in Dental Workers in South Africa."
- "The Detection of Latex Aeroallergens in Dentistry".

Your cooperation in the above is highly appreciated.

PROF AJ LIGTHELM
DEAN/MANAGER

UNIVERSITY OF LIMPOPO

FACULTY OF DENTISTRY
DEPARTMENT OF MAXILLOFACIAL & ORAL SURGERY
PO BOX 222
MEDUNSA
0204
SOUTH AFRICA
Tel: (012) 521 4858
Fax: (012) 521 4859
E-mail: michael@ul.ac.za or wlla@ul.ac.za

MEDUNSA CAMPUS

16 March 2006

The Dean and All HOD's
Oral Health Centre
Faculty of Dentistry

Dear Prof Gugushe and HOD's

Please assist Ms Tanusha Singh in conducting her research at the Medunsa Oral Health Centre for which she has received the necessary permission from REPC. She will be collecting data for the duration of her study at Medunsa Oral Health Centre as approved by her supervisors and REPC (UL).

The Topic of her study is:

"Work related Asthma associated with endotoxin exposure in Dental Workers in South Africa"

Your cooperation in the above is highly appreciated.

PROF MMR BOUCKAERT
DEPUTY DEAN: ACADEMIC AFFAIRS
CHAIRPERSON: REPC OF DENTISTRY
HOD: MFOS DEPARTMENT

UNIVERSITY OF
KWAZULU-NATAL

10 AUGUST 2005

MS T SINGH

RE: STUDY PERMISSION

Permission has been granted for you to undertake your research study at the above mentioned university.

Kindly submit all relevant documentation plus your ethical clearance certificate, attested by a notary Public, from your university for our records.

Sorry for the inconvenience.

Many thanks.

DR V NAIKER
DIRECTOR
SCHOOL OF DENTISTRY