DETECTION OF LATEX AEROALLERGENS IN DENTAL SCHOOLS

Dikeledi Onnicah Mabe

Dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, in fulfilment of the requirements for the degree of Master of Science in Medicine

Johannesburg, 2008

DECLARATION

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

……………………………… (Signature of candidate)

…………… day of………………………….. (month), 2008

DEDICATION

In loving memory of my late grandmother Mamoruang Elizabeth Mabe and with love to my son Mpho, fiancée Lesemang, mom and siblings.

PUBLICATIONS AND PRESENTATIONS

Oral presentations

O.Mabe. Detection of clinically relevant latex aeroallergens: proposal. National Institute for Occupational Health Webster Memorial Day-strategic planning meeting. 23 November 2004.

O. Mabe. Detection of latex aeroallergens in dental schools: protocol. National Institute for Occupational Health Research Forum. 19 October 2005.

O.Mabe. Latex allergens in dental settings: Results. National Institute for Occupational Health Research Forum. 31 October 2007.

Poster presentation

O. Mabe and T.Singh. Estimation of protein concentration in latex products used in dentistry. National Institute for Occupational Health Research Day. 25 October 2006. Award winning poster.

O.Mabe, T.Singh, B.Bello, A.lopata, A.Wadee and A.Davies. Latex aeroallergens in dental schools in South Africa.XXVIIth Congress of European Academy of Allergology and Clinical Immunology. Barcelona, Spain, 7-11 June 2008.

ABSTRACT

Introduction: Exposure to airborne natural rubber latex proteins has become an important occupational health concern, particularly among healthcare workers. The main purpose of this study was to investigate the levels of latex aeroallergens in South African dental schools.

Methods: Area (n=95) and personal (n=369) samples as well as rubber containing gloves and dental devices (n=19) were collected in 5 dental schools. The air samples were collected at a flow rate of 2.5L/min using polycarbonate (PC) filters. Latex allergens (hev b 1, hev b 3, hev b 5 and hev b 6.02) were quantified in filters and rubber extracts by a capture enzyme immunoassay. Data was analysed using STATA 9 computer software (StataCorp, 1984-2007, Texas, USA). Non parametric tests were applied as the data was skewed. The data was interpreted as 'low' with less than 10ng/m^3; 'moderate' with levels between 10-50ng/m^3 and 'high' with greater than 50ng/m^3.

Results: Aeroallergen concentrations varied among institutions in our study, ranging from 1.84 to 46.1ng/m^3 for personal and 1.33 to 14.97ng/m^3 for area samples. Hev b 6.02 was below the detection limit for 86.5% of air samples. This study also found that exposure levels differed by departments and job type. Powdered latex products showed higher allergen concentrations compared to the non-powdered products (p=0.035) and also differed significantly by the type of brands (p=0.022). Hev b 6.02 was the most prominent allergen in powdered gloves and dams.

Conclusion: The air sampling method and capture enzyme immunoassay used in this study offer means for evaluation of airborne allergen concentrations. The initiative to use non-powdered low protein latex gloves and dams should be implemented as a preventive measure.

ACKNOWLEDGEMENTS

It is with appreciation that the following persons are acknowledged:

Professor A.A Wadee and Ms T.S Singh, my supervisors for their support, guidance and constructive criticism.

Professor M. Ross, the former Director of the NIOH for granting me the opportunity to do this study.

Dr A. Lopata for his advice, beneficial comments and constructive criticism.

Professor J.C.A. Davies for co-supervising the project and editorial assistance.

Management of various dental institutions for providing permission to conduct the study.

Staff and students of various dental institutions who contributed to the study.

The Allergy Society of South Africa (ALLSA-UCB) and Medical Research Council of South Africa (MRC) for funding the project.

The study team and collaborators for their efforts and commitment which made this work possible.

Mr B. Bello (NIOH, Epidemiology Section) for his valuable input with statistical analyses.

A special word of thanks to all my colleagues and friends for their encouragement and support throughout the study.

TABLE OF CONTENTS

DECLARATION .... **ii**
DEDICATION .... **iii**
PUBLICATIONS AND PRESENTATIONS .... **iv**
ABSTRACT .... **v**
ACKNOWLEDGEMENTS .... **vi**
TABLE OF CONTENTS .... **vii**
LIST OF FIGURES .... **ix**
LIST OF TABLES .... **x**
NOMENCLATURE .... **xii**
PREFACE .... **xiii**
CHAPTER 1 .... **1**
1.0 INTRODUCTION 1
CHAPTER 2 .... **7**
2.0 LITERATURE REVIEW 7
2.1 Natural Rubber Latex **7**
2.1.1 Latex allergens for *Hevea Brasiliensis* **8**
2.1.2 Sampling for latex aeroallergens **9**
2.2 Prevalence of latex allergy caused by NRL **14**
2.3 Risk groups from exposure to latex allergens **16**
2.4 Sources of exposure to latex allergens **18**
2.5 Health effects associated with latex aeroallergens **19**
2.6 Recommended Occupational Exposure Limits **20**
2.7 Legal implications of latex allergy **23**
2.8 Recommendations for controlling exposure to latex aeroallergens **23**
CHAPTER 3 27
3.0 MATERIALS AND METHODS 27
3.1. STUDY DESIGN **27**
3.2 SAMPLING POPULATION **27**
3.3 BUILDING INSPECTION **27**
3.4 SAMPLE COLLECTION **28**
3.4.1 Area sampling 28
3.4.2 Personal sampling 29
3.4.3 Rubber products 30
3.5 SAMPLE ANALYSIS **30**
3.5.1 Extraction of filter samples **30**
3.5.2 Extraction of latex rubber products **31**
3.5.3 Estimation of total extractable proteins in latex products **31**
3.5.4 Quantification of latex aeroallergens **32**
3.6 STATISTICAL ANALYSIS **34**
3.7 ETHICAL CLEARANCE (see attached appendices) **34**
CHAPTER 4 .... **36**
4.0 RESULTS 36
4.1 Personal samples **36**
4.1.1 Detection limits (DL) of the enzyme immunoassay **38**
4.1.2 Summary description for hev b 5 and hev b 6.02 for personal air samples 39
4.1.3 Hev b 5 and hev b 6.02 classified by institution **39**

4.1.4 Concentrations of hev b 5 and hev b 6.02 classified by departments ……………………………………………………………………………..41
4.1.5 Concentrations of hev b 5 classified by the type of tasks 43
4.2 Area samples **45**
4.2.1 Summary description for hev b 5 and hev b 6.02 for area samples 45
4.2.2 Hev b 5 concentrations classified by institutions for area samples 45
4.3 Latex rubber products **47**
4.3.1 Quantification of hevein allergens and total protein 47
4.3.2 Correlation between total allergen and total protein of latex rubber products……………………………………………………………………... 49
4.3.3 Comparison between different latex products 49
4.3.4 Comparison between total allergen content of different brands of latex rubber products 51
CHAPTER 5 .... **52**
5.0 DISCUSSION 52
5.1 CONCLUSION 66
APPENDICES **67**
REFERENCES **68**

LIST OF FIGURES

Figure **Page**

4.1 A chart showing percentages of participants from various institutions 37

4.2 Graphical illustrations of hev b 5 concentrations by institution 39

4.3 Personal exposures levels to hev b 5 by departments 42

4.4 Hev b 5 concentrations for area samples of various institutions 46

4.5 Correlation between the total allergen and total protein expressed as µg/g of latex extracts in 19 samples 49

4.6 Comparison between the total allergen (µg/g) in gloves and dental dams 50

4.7 Comparison between the total allergen (µg/g) in powdered and non-powdered samples 50

4.8 Comparison between the total allergen (µg/g) in different brands of latex products 51

LIST OF TABLES

Table **Page**

1.1 Allergenicity classifications of latex gloves 5

2.1 Overview of characterised NRL allergens and their significance 13

4.1 Number of participants classified by department for different institutions 38

4.2 Summary description for hev b 5 and hev 6.02 detection levels 38

4.3 Comparison of means of the log transformed values for hev b 5 by institution 40

4.4 Proportion of exposed and unexposed category for hev b 6.02 by institution 41

4.5 The proportion of exposed and unexposed category for hev b 6.02 by department 43

4.6 Hev b 5 concentrations for personal samples hev b 5 by job caregory 43

4.7 The proportion of the exposed and unexposed group for hev b 6.02 by job category 44

4.8 Summary description for hev b 5 and hev 6.02 detection levels for area samples 45

4.9 Comparison of four latex allergens and total extractable protein concentrations in 19 latex rubber extracts 48

NOMENCLATURE

HBAs:	Hazardous biological agents
OHSA:	Occupational health and safety act
HCSs:	Hazardous chemical substances
NIOH:	National institute for occupational health
NHLS:	National health laboratory services
NRL:	Natural rubber latex
SORDSA:	Surveillance of occupational respiratory diseases in South Africa
HCWs:	Healthcare workers
FDA:	Food and drug administration
WHO:	World health organisation
ELISA:	Enzyme-linked immunosorbent assay
LEAP:	Latex ELISA for antigenic proteins
RAST:	Radioallergosorbent test
CHAPS:	3-([3-cholamidopropyl]dimethylammonio)-1-propane sulfonate
Hev b 1:	Rubber elongation factor
Hev b 3:	Small rubber particle protein
Hev b 5:	Acidic structural protein
Hev b 6.02:	Hevein
IUIS:	International Union of Immunological Societies
PTFE:	Polytetrafluoroethylene
PBS:	Phosphate buffered saline
OEL:	Occupational exposure limits
HEPA:	High efficiency particulate air

PC:	Polycarbonate
LAL:	Limulus amoebocyte lysate
DC:	Detergent compatible
EIA:	Enzyme immunoassay
SDS-PAGE:	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
RT:	Room temperature
W/V:	Weight per volume
BSA:	Bovine serum albumin
R^2:	Correlation coefficient
CV:	Coefficient of variation
DL:	Detection limit
MLE:	Maximum likelihood estimation
IQR:	Interquartile range

PREFACE

Occupational exposure to hazardous biological agents (HBAs) has gained momentum recently. Much interest has focused on airborne biological agents (bioaerosols) and the health effects it causes to exposed workers. Bioaerosol hazards arise from exposure to agents of biological origin which could lead to allergenic reactions, respiratory sensitisation and toxicological reactions.

The South African Occupational Health and Safety Act (OHSA), 1993 (Act NO 85 of 1993) requires the employer to create a safe working environment and without risk to the health of workers, as far as reasonably practicable. The OHSA Act includes the regulation for HBA's, in addition to the regulation for hazardous chemical substances (HCS's).

The National Institute for Occupational Health (NIOH) is an institute within the National Health Laboratory Service (NHLS), committed to improving occupational health in South Africa and also to supporting the development of occupational health services in the neighbouring countries (Southern African Development Community). This is achieved through occupational health research, training and specialised services. The findings from this study will contribute to a commitment of reducing exposures and hence creating a safer environment for HCWs.

CHAPTER 1

1.0 INTRODUCTION

Exposure to airborne natural rubber latex (NRL) has lately become a concern worldwide and has emerged as the major cause of respiratory diseases among exposed workers, latex allergy being one of them. It has also been reported as the fourth most common cause of occupational asthma (Heilman et al., 1996; Sussman et al., 1998; Baur et al., 1998; Liss and Sussman, 1999; Vandenplas et al., 2002), and first in South Africa (Hnizdo et al., 2001). Powdered latex gloves have been identified as a major source of occupational exposure to allergens because they contain soluble proteins responsible for sensitisation (Lopes et al., 2004). However not all powdered gloves have high allergen content presumably due to variations in their production (Hunt et al., 2002). When the gloves are snapped on and off, latex proteins bound to the cornstarch powder become airborne and may be inhaled and come into contact with body membranes (SORDSA, 1998; De Beer et al., 1999; Swanson et al., 2001; Reiter, 2002; Jones et al., 2004, Lopes et al., 2004). A case of latex allergy due to airborne latex exposure was first reported in 1990 (De Beer and Cilliers, 2001) Although it is well known that glove powder act as a carrier for latex allergens, a very limited number of studies of latex allergy have included the evaluation of workplace exposures to latex aeroallergens.

Allergy due to NRL has become an important occupational health concern recently. Health Care Workers (HCWs) are at increased risk of latex allergy from frequent use of disposable latex gloves, particularly those that are powdered and of high protein content (Hunt et al., 2002; Swanson et al., 2001). Dentists are considered a high risk-group among the clinical specialities due to prolonged usage of latex gloves during working days (Jones

et al., 2004). Most studies have reported prevalence of latex sensitivity in dental schools using a questionnaire, skin prick testing (SPT) and serological testing. However not many studies have reported on airborne levels of latex allergens in dentistry which contribute to sensitisation (Katelaris et al., 1996; Allmers et al., 1998; Saary et al., 2002; Jones et al., 2004).

The inhalation of allergen particles dispersed in the air is capable of triggering nasal, ocular and respiratory symptoms that may lead to rhinitis, conjunctivitis, asthma and anaphylaxis in sensitised individuals (Lopes et al., 2004). Allmers and colleagues reported upper and lower respiratory tract symptoms and NRL specific IgE antibodies HCWs working in rooms with a detectable latex aeroallergen load (Allmers et al., 1998).

Repeated exposure to natural latex protein resulting in latex allergy is determined by factors such as dose, duration and the route of exposure. It is therefore essential to prevent or minimise further individual sensitisation by controlling exposure to allergenic proteins to ensure safety. The latter cannot be accomplished without accurate and reliable methods for quantifying allergens. Efforts to develop these methods have been hindered by limited knowledge of the identity of individual allergenic proteins; although there has been significant progress in this area recently with more than 16 allergens being identified (Tomazic-Jesic and Lucas, 2002).

However, there is currently no standardised method for determining allergenic latex proteins mainly because relevant latex allergens are not accurately identified. Measurements of these allergens are needed to identify latex products with high levels of extractable allergens; and to examine the effects of various interventions to reduce the

levels of latex aeroallergens (Hunt et al., 2002). Total protein measurements currently serve as a useful indicator of the exposure of concern. These include the modified Lowry method; amino acid analysis; latex ELISA for antigenic protein (LEAP); ASTM6499 and ELISA inhibition assay. However, these methods are not sensitive or specific enough, and are subject to error which may be due to interference with chemicals found in latex products, resulting in inconsistent and unreliable results.

The Food and Drug Administration (FDA) has introduced the modified Lowry method (ASTM D5712) to measure the total soluble protein content of gloves. However, the true allergen content is not determined by this method because not all proteins are allergenic. In addition the procedure is labour intensive and time consuming as it takes a full day to analyse 8 –10 samples and cannot identify or quantify specific proteins that may cause serious allergic reactions. The amino acid analysis measures the amino acid content of proteins after hydrolysis of hydrochloric acid. This method is technically demanding and requires experienced personnel; cannot differentiate relevant allergens from other proteins and requires expensive and advanced equipment. The LEAP assay and ASTM D6499 use serum from rabbits immunised with latex proteins. This method consists of many steps to perform and therefore cannot be standardised; nor do the test results correlate with the allergen content of the end product. The RAST method uses serum from patients diagnosed with latex allergy and has shown the difficulty of standardisation due to variable antibody reactivity of the patient pool and also the scarcity of latex allergic patients.

Measuring total protein content does not provide reliable information regarding the allergenicity of a product as the method cannot differentiate between allergenic and non-allergenic proteins. NRL proteins are known to induce immediate type latex allergies, it is

therefore necessary to improve methods to measure these sensitising agents quickly and accurately. Other conventional methods of measuring the total mass of particulates collected on polyvinyl or mixed cellulose ester filter medium are of little value. Counting of particles is also of little value as numerous small latex particles may be attached to large particles (Reiter, 2002).

FIT Biotech (Finland, Tampere) has developed the FITkit, a commercial latex allergen analyzing tool used to identify four major allergens (individually) out of 13 latex allergens listed by the World Health Organisation (WHO). These tests are sensitive and accurate immunological tests that can identify individual clinically relevant latex proteins in latex gloves. The kit overcomes the significant limitations of previous methods by using highly purified and characterised allergens and specific monoclonal antibodies developed against four relevant allergens (hev b 1, hev b 3, hev b 5, hev b 6.02). These allergens have been demonstrated to resist glove manufacturing processes and have been unequivocally shown to be present in medical glove extracts (Palosuo et al., 2002; Palosuo et al., 2007; Poulos et al., 2002; Mitakakis et al., 2002). The use of highly purified and characterised allergens and monoclonal antibodies enables standardisation and quantification of individual allergens. A significant correlation has also been identified between the IgE based method and the capture EIA (sum of four allergens) in gloves (Palosuo et al., 2002; Palosuo et al,. 2007).

These major protein allergens have been categorised as low, moderate and high as shown in Table 1.1, based on the correlation of FITkit^{TM} allergens to the true total allergen levels measured by the ELISA inhibition test (Palosuo et al., 2002; NAM, 2003; FITkit, 2002).

Table 1.1 Allergenicity classifications of latex gloves (NAM, 2003; FITkit, 2002)

Allergenicity Classifications		
Classification	ELISA IgE Inhibition	Sum of Hev b 1 , Hev b 3, Hev b 5, Hev b 6.02
Low	<10AU/ml	≤ 1µg/g
Moderate	10-100AU/ml	1-5 µg/g
High	>100AU/ml	>5µg/g

Hev b 5 and hev b 6.02 are considered the most important allergens for HCWs (Palosuo, 2002, Palosuo et al., 2007). Hev b 1 and hev b 3 have been reported in spina bifida. Adding to the list, Bernstein et al (2003) described hev b 13 (43kDa) as another relevant allergen among HCWs using SPT. Lee et al (2006) also identified a 30kDa, hevamine (hev b 14, at least 50% according to WHO/IUIS), a candidate for a major *H. brasiliensis* allergen as a predominant protein in the latex gloves used by medical workers in Taiwan.

Research on allergenic proteins in rubber gloves has been done for a long time but the means to measure clinically relevant allergens have been lacking until recently. The capture ELISA assay allows manufacturers and authorities to measure allergen levels in gloves and thus provide a means of measuring allergenicity for safer use of latex gloves among HCWs. However it has not been applied to measure airborne latex allergens. Airborne latex allergens are unavoidable, thus making it difficult to control exposure or prevent inhalation of these allergens. Thus far only a few publications have reported the measurement of latex allergens in glove powder and in airborne particulates (Baur, 2002; Kujala et al., 2002). There have been no reports on measuring the levels of latex allergens in the environment in South Africa, thus more detailed research is needed to establish these methods for workplace evaluations and assessments of risks of exposure locally.

For the purpose of this study enzyme immunoassay (FITkit Biotech, Tampere, Finland) will be adapted for determining the levels of clinically relevant latex allergens in air samples collected from various dental institutions. It has been selected as the method of choice due to its suitability that allows for the direct measurement of relevant individual allergens and short assay time (~2 hours) when compared to other methods. The assay is currently available only for research purposes as stated on the kit's inserts, therefore a promising ELISA assay for latex allergen quantification using monoclonal antibodies.

The purpose of this study was to investigate the levels of airborne latex allergens in work areas of five dental schools of South Africa.

The main objectives of this study are:

- To validate the FITkit and establish a reliable technique at the National Institute for Occupational Health (NIOH) for detecting latex aeroallergens from air samples collected in dental schools.
- To identify the type of latex products used in dental schools which may be contributing to aerosols in dental schools.
- To estimate the total protein content and measure the total allergen content (sum of hev b 1, hev b 3, hev b 5, hev b 6.02) in latex products e.g. gloves and rubber dams used in South African dental schools.
- To detect and measure the levels of latex aeroallergen in various dental work areas.

These objectives were addressed in this study and are highlighted in three sections of this dissertation; chapter 3 which will cover materials and methods, chapter 4 which will cover the results and chapter 5 which will be the discussion and conclusion.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Natural Rubber Latex

Natural rubber latex (NRL) comes from the milky sap derived from a rubber plant known as *Hevea brasiliensis* found predominantly in South East Asia (Fish, 2002; Mc Fadden, 2002). The constituents of latex are proteins, lipids, nucleotides and cofactors (De Beer et al., 1999). The use of latex dates back to as early as 1600 BC; however, its use in the medical setting was only adopted by surgeons between 1890 and 1910. Currently, NRL is widely used in the workplace and plays an important role in providing effective barrier protection against transmission of bloodborne pathogens and other infectious agents such as the human immunodeficiency virus (HIV) and Hepatitis B for HCWs (De Beer et al., 1999; Mc Fadden, 2002). Because of its unique advantages in elasticity, flexibility, barrier properties, comfort and cost effectiveness, latex is still chosen over synthetic polymers (e.g. polyvinyl chloride and nitrile) as essential for accomplishing barrier protection (Charous, 1998; Mc Fadden, 2002).

This increased demand for latex gloves brought about changes in the processing and manufacturing of gloves, which have resulted in poor quality and highly allergenic products. Changes in manufacturing include short washing processes and shelf lives, which increased the amount of extractable latex protein antigens in gloves associated with the increase in prevalence and exposure to latex (De Beer et al., 1999). NRL is a component of various medical devices and domestic products and is used for protective and hygiene purposes (Koh et al., 2005). Besides the healthcare settings, latex products including gloves are commonly used in other industries such as food and beverage,

manufacturing, and glove factories (Heilman et al., 1996; Poulos et al., 2002; Swanson et al., 1994, Koh et al., 2005). The growing use of NRL products, particularly in the health profession has led to the unfortunate trade-off between protection offered by these gloves and adverse reactions associated with an increase in allergic diseases and contact dermatitis from rubber additives related to the use of these devices (Saary et al., 2002). Latex gloves have therefore introduced health hazards associated with the use thereof (Wrangsjo et al., 2001).

2.1.1 Latex allergens for *Hevea Brasiliensis*

Latex allergens are proteins found in latex products and on powders present in latex products in the form of aeroallergen particles (Reiter, 2002). Healthcare settings using powdered gloves are likely to have a measurable concentration of latex allergens in the air (Swanson et al., 2001) and represent a health hazard to healthcare workers who are inadvertently exposed to them on a regular basis (Potter et al., 2001).

Latex allergen settles rapidly from the air and very little, if any, airborne latex allergens remain in an unused room after 24 hours (Hunt et al., 2002), until resuspended by movement. Latex allergens may be present in the air as individual particles or may adhere to the surface of other particulates (Reiter, 2002). Natural rubber proteins from latex are allergenic (Swanson et al., 1994) and commonly found in medical and dental office buildings (Swanson and Ramalingam, 2002). It is not the powder that the person becomes allergic to, rather the protein sticking to the powder (Amr and Bollinger, 2004).

Research efforts have resulted in identification of several purified latex allergens. The World Health Organization and the International Union of Immunological Societies

(WHO/IUIS) now lists 13 NRL allergens characterised at the molecular level (Rihs and Raulf-Heimsoth, 2003; Bernstein et al., 2003; Yeang, 2004; Wagner and Breiteneder, 2005). Eight of these allergens have been reported to be the most significant latex allergens because they are the allergenic proteins that yield the highest prevalence of IgE specificities from serum and by skin prick test (Yeang et al., 2006). Table 2.1 shows an overview of the current status of characterised NRL allergens with their immunological and clinical properties.

2.1.2 Sampling for latex aeroallergens

Sampling for airborne allergens in the occupational setting usually involves collection of airborne dust on filters using pumps. Sampling can either be static (area) or personal (breathing zone) depending on the needs of the individuals. Collection of inhalable dust using personal sampling pumps has clear relevance to personal exposure compared to static sampling. Several methods have been developed to assess exposure to protein allergens, both enabling and necessitating a choice between different methods (Renstrom, 2002).

The sampling method used for latex aeroallergens must be capable of collecting submicron sized particles efficiently on a medium from which the allergens can be extracted completely. The volume of air sampled must be adequate to provide enough allergens for precision analysis with sensitivity in the nanogram per cubic meter (ng/m^3) range. The recommended flow rate is 180L/ for 2 to 4 hours min (21 600 to 43 200 L) using a high volume sampler and polytetrafluoroethylene (PTFE) filter. Latex allergen sampling can also be achieved by surface sampling using either wipe sampling or surface vacuuming. The technique indicates the presence and absence of latex allergens (qualitative) and may

also be semi quantitative by indicating the levels of latex allergen per square surface area. The results are expressed as detected versus undetected; nanogram per sample; nanogram per gram of dust or nanogram per square surface area. The shortfall of these techniques is inconsistency in wiping efficiency and possibility of filter media destruction during sampling (Reiter, 2002).

Swanson et al (1994) used area samplers and personal breathing zone samplers to collect air samples from various areas in the medical centre. Area samplers were operated at a flow rate of 3L/sec for 4 hours and personal samplers at a flow rate of 5L/min (Swanson et al., 1994; Poulos et al., 2002; Hunt et al., 2002). A portable vacuum with PTFE filter connected to the hose was used to collect particles from used laboratory coats and surgical suits at a flow rate of 1L/sec for 1 hour. Air particles were collected onto PTFE filters. Airborne allergen concentrations for area samples varied from 13 to 121ng/m^3 while for personal samples varied from 8 to 978ng/m^3 in areas where latex gloves were frequently used (Swanson et al., 1994). Using the same flow rates as Swanson et al (1994), a cross sectional study done at the Mayo medical centre (Rochester, Minnesota) reported latex aeroallergen levels ranging from 13 to 208ng/m^3 in 11 areas using powdered latex gloves and levels between 0.3 to 1.8ng/m^3 in 4 areas where powder free or synthetic gloves were used (Hunt et al., 2002).

In a Canadian epidemiological study, 12 personal and 8 area samples were collected in different wards and operating rooms during use of powdered gloves. The number of pairs of gloves used during the day of sampling ranged from 3-14. Latex aeroallergen were quantified using indirect ELISA method and personal exposure ranged from 5-616µg/m^3 (Sri-akajunt et al., 2000).

A study by Page et al (2000) collected area air samples using 2 high volume samplers operated at 5.7L/sec and 6.1L/sec. The sampling time was 8 hours and 17 minutes. Latex particles were collected on bilaminate glass fibre and PTFE membrane filters. The concentrations of latex ranged from non-detected to 3.33ng/m^3 with geometric mean concentration of 0.52ng/m^3.

Swanson and co-workers (2001) have collected air particles onto PTFE filters by a volumetric filtration method using a static air sampler at a flow rate of 3l/sec. The NRL allergens were extracted from filter samples in PBS and quantified by an immunoassay using pooled sera containing latex specific human IgE. The concentrations of latex allergens during working hours in areas where latex gloves were frequently used ranged from 5 to 26ng/m^3. The highest concentration of latex allergen (26ng/m^3) was found in a gynaecology examination room. Areas that seldomly used latex or powder free gloves demonstrated concentrations below the detection limit of less than 5ng/m^3. Data was interpreted as: low (less than 10ng/m^3); moderate 10-50ng/m^3; and high greater than 50ng/m^3. It was found that the levels of latex allergens captured were dependent on the number of gloves used (Swanson et al., 2001).

Baur (2002) collected airborne latex particles using two different types of area samplers operated for 20 hours with cellulose membrane filters. The VC 25 area sampler was operated at a flow rate of 22.5m^3/h for rooms larger than 200m^3 and WAZAU area sampler at a flow rate of 2.8m^3/h for rooms smaller than 200m^3. The concentration of latex aeroallergen ranged from less than 0.4 to 240ng/m^3 (Baur, 2002).

Another study was done in ambulances to quantify concentrations of NRL bioaerosols. Latex allergens were captured on 0.3µm PTFE filters with an area sampler operated at a flow rate of 2.5L/s for 1hour. Personal air sampling pumps were worn by paramedics during their work shift inside the ambulance at a flow rate of 3L/min for 5 to 6 hours. The detection limit was approximately 1ng/m^3 for area samples and 5ng/m^3 for personal samples, the differences related to collection volumes. The latex aeroallergen levels in so-called latex-free ambulances were undetectable with an area air sampler. The results from the study have shown that ambulances where powdered latex gloves are used, show moderate (10-50ng/m^3), high (>50ng/m^3) and even very high latex aeroallergen concentrations. The recommendation made was that by using powder free latex gloves and cleaning vehicle surfaces thoroughly, latex allergen concentrations can be decreased to acceptable levels (Quirce et al., 2004).

Air sampling for latex allergens differs between laboratories, therefore the suitability and shortcomings should be taken into consideration when planning the sampling strategy. Some factors have to be considered when sampling the dental population and this includes: short sampling times due to the type of procedures (clinic sessions that last a maximum of 2 hours 30 minutes), variety of work, effort to minimise disruption between patient and dental staff performing the procedure, ensuring well serviced pumps are used to reduce the noise levels and pumps that are cumbersome to wear in the presence of patients.

Table 2.1 Overview of characterised NRL allergens and their significance.

Latex allergen	Molecular mass	Protein name	Significance as latex allergen	Is recombinant protein available?
Hev b 1	14.6	Rubber elongation factor (REF)	High, (spina bifida (SB) patients	Yes
Hev b2	34-36	ß-1,3-glucanase	Medium	Yes
Hev b3	24-27	Small rubber particle protein	High (SB patients)	Yes
Hev b4	50-57	Beta-glucosidase (microhelix component)	Not determined	No
Hev b5	16-24	Acidic structural protein	High-all risk groups. HCW, SB, atopics	Yes
Hev b6.01	20	Prohevein (hevein precursor)	High-all risk groups. HCW, SB, atopics	Yes
Hev b6.02	4.7	Hevein	High-all risk groups. HCW, SB, atopics	Yes
Hev b6.03	14	C-domaine of prohevein	High in context with Hev b6.01	Yes
Hev b7.01	42	Patatin-like protein (esterase) from latex B- serum	Low-medium	Yes
Hev b7.02	44	Patatin-like protein (esterase) from latex C- serum	Medium only in SB	Yes
Hev b8	14	Profilin (actin-binding protein	Low	Yes
Hev b9	51	Enolase	Low	Yes
Hev b10	26	Manganese superoxide dismutase (MnSOD)	Low	Yes
Hev b11	32	Class I chitinase	Low	Yes
Hev b12	9	Lipid transfer protein	Low	Yes
Hev b13	42	Esterase	High	Yes

Rihs&Raulf-Heimsoth, 2003; Yeang, 2004; Wagner&Breiteneder, 2005

2.2 Prevalence of latex allergy caused by NRL

NRL is a natural product and, like other plant based material, it has the potential for sensitisation (Kalman, 2005). Allergy due to NRL has become an important occupational health concern particularly among healthcare workers and the source of workplace exposure is the use of powdered latex gloves (Liss and Tarlo, 2001). Most studies have reported an increased prevalence of latex allergy internationally but this has not been widely documented in South Africa (Brathwaite et al., 2001).

Currently, latex allergy is recognised as a serious worldwide health problem with 5-17% prevalence in HCWs (depending on the occupational group and country of origin), 1-6% in the general population; and 8.8-13% of dentists being allergic to latex. A high rate of sensitisation to NRL has been documented in dental students and staff members in Ontario, Canada and the prevalence was found to be 10 % in a total sample size of 131, with students developing sensitivity as early as the second year of substantial glove use, that is third year students (Tarlo et al., 1997; Saary et al., 2002). There have been increasing percentages of positive skin tests to latex with increasing years of study among the students, 0% of first and second year; 6% third year and 10% of fourth year students (Tarlo et al., 1997). Thirty nine of 226 (17.3%) dental students had a positive skin prick test to at least 1 of the 6 latex substances tested (Schmid et al., 2002).

Previous research indicated that latex allergy is a problem in South African hospitals (Pretorius et al., 2001) with Potter et al (2001) reporting a prevalence of 9-20% of latex sensitivity in three South African hospitals using powdered latex gloves.

South Africa's first case of latex allergy was diagnosed in 1993 in a nursing sister at Groote Schuur hospital in Cape Town and this led to a survey, which identified 23 more cases of latex allergy. This study showed the possibility that latex allergy problem may be widespread in South Africa (De Beer et al., 1999; Potter et al., 2001).

Several other studies have been done in South Africa: one in the Red Cross War Memorial Children's Hospital (RXH) and the other at Groote Schuur hospital. Latex allergy was found to be a significant occupational health risk at RXH, with 48.5% (146/302) staff (medical, nursing and auxiliary) showing one or more allergic symptoms and 7% (10/142) positive for IgE mediated hypersensitivity to latex (Braithwaite et al., 2001). The prevalence of symptomatic sensitisation to latex among all hospital workers was found to be between 9.2% and 11.2% at Groote Schuur hospital. At Tygerberg, also in the Cape, 20.8% of a high risk group of HCWs (ICU and operating theatres) were also found to be latex sensitive (Potter et al., 2001).

Another small study was conducted among 146 laboratory workers at the South African Institute for Medical Research (SAIMR), now the branch of the National Health Laboratory services (NHLS) in Johannesburg using CAP RAST test and a prevalence of 2.7% was reported (Potter et al., 2001). In 2002, the National Centre for Occupational Health (NCOH), now the NIOH, conducted a small study on latex allergy at the Johannesburg Hospital (NJH) where employees were tested using the UNICAP and skin prick tests. The results from this study have shown a prevalence of 28.2% (22/78) IgE mediated hypersensitivity (Z. Kirsten, personal communication, 2006). An epidemiological study done recently in South Africa at

Pretoria academic hospital and Medforum reported 38.2% dermatological reactions to latex among participating nursing healthcare professionals (Pretorius et al., 2001).

2.3 Risk groups from exposure to latex allergens

Latex gloves are commonly used in various occupations including manufacturing and healthcare (Swanson et al., 1994; Heilman et al., 1996; Poulos et al., 2002) and are regarded as the most common source of latex exposure (Lindberg and Silverdahl, 2000; Jones et al., 2004). In addition to hand eczema and acrylate allergy, latex allergy has been an occupational health issue in the dental profession as in other fields of heath care (Lindberg and Silverdahl, 2000; Saary et al., 2002). Type 1 sensitivity to natural rubber latex has been recognised more frequently among healthcare workers, including physicians and dentists (Hunt et al., 1995). It has been demonstrated that dental students with increasing exposure to latex have an increasing incidence of latex allergy (Saary, 2002). Studies have indicated that atopics have an increased risk of sensitisation to workplace allergens, particularly high molecular weight agents like latex (Poulos et al., 2002; Petsonk, 2002).

Healthcare workers are at risk of latex allergy from frequent use of disposable latex gloves, particularly those that are powdered and of high protein content (Hunt et al., 2002). Among this group, dentists are considered a high risk-group due to prolonged usage of latex gloves during working days (Jones et al., 2004). Due to the high latex component of many products associated with dental practice (e.g. dental dams, suction tubing and impression casts) many dental practitioners had to quit their profession due to the development of latex allergy from the wearing of gloves (Jones et al., 2004). Although many latex allergic people are able to

continue practising by switching to non-latex gloves, others develop severe allergic responses such as asthma and anaphylaxis such that they are forced to change their careers (Saary et al., 2002). Up to 40 % of dental personnel reported dermatological reactions due to latex gloves. Studies have reported that dental students using powder free gloves have shown no sensitisation while 5-15% of those using powdered gloves were sensitised (Liss & Tarlo, 2001; Allmers et al., 2004). The general population is also exposed to latex with the use of natural rubber products in normal life (Crippa et al., 2006). Other distinct groups that are at risk include rubber plantation workers, glove manufacturing (Sri-Akajunt et al., 2000), hairdressers (Crippa et al., 2006), housekeeping personnel (Koh et al., 2005) and food processors.

A recent study conducted in South Africa by Lopata et al (2007) demonstrated severe occupational sensitisation to latex among workers in the textile industry. Occupational asthma was caused by excessive dust originating from elastic material, not initially suspected to contain latex, utilising specialised allergological testing. This is one of few international studies to demonstrate sensitising latex allergens in material other than gloves.

Furthermore, latex sensitisation was also demonstrated among seafood processing workers in the Western Cape, and the significance of non-specific carbohydrate determinants is highlighted too, relevant for correct diagnosis (Raulf-Heimsoth, In press).

2.4 Sources of exposure to latex allergens

Exposure routes to latex proteins vary from skin contact by wearing latex gloves to inhalation of latex protein bound to glove powder that becomes airborne (De Beer, 1999). Studies have shown that high concentrations of latex aeroallergen have been found in areas where large numbers of gloves are routinely donned and discarded, and increased latex aeroallergen exposure using personal breathing zone sampling has also been documented (Hunt et al., 1995; Heilman et al., 1996; Vandenplas et al., 1996; Poulos et al., 2002).

The cornstarch powder used to coat gloves poses a serious threat because proteins bind to the powder and become aerosolised and inhaled (De Beer et al., 1999). Aerosolised glove powder is the most common source of latex protein inhalation (Heilman et al., 1996). This powder or bioaerosols feature as a carrier for natural rubber latex aeroallergen (Swanson and Ramalingam, 2002). Heilman et al (1996) suggested that aeroallergen levels found by air sampling on high allergen glove days were associated with the number of high allergen gloves used and not with the total number of gloves used on low allergen days. In contrast, a study done in air samples from different areas at the Mayo clinic has shown that the amount of latex allergen measured correlated with the frequency of glove use and glove changes (Poley and Slater, 2000).

The amounts of latex proteins carried by glove powder are sufficient to generate levels of aeroallergen capable of triggering allergic reactions, thus promoting atopic sensitisation indirectly (Charous, 1998). Allergen carried on the powder is not the only source of exposure. The high levels of aeroallergen were reported at a plant producing chlorinated gloves during

the outstripping process when no workers were present. The concentrations of airborne allergen in many areas of glove manufacturing plants where workers are present are similar to those in medical care facilities where the levels are high (Hunt et al., 2002). Aeroallergens have also been reported in plantations when the sap is being harvested from rubber trees (Sri-akajunt et al., 2000).

Not all airborne latex allergens arise from gloves (Hunt et al., 2002). Although the risk of exposure is greatest during donning or removal of gloves, another concern regarding airborne latex allergens is that the particles may also be deposited throughout the environment. These particles may be resuspended from surfaces e.g. surgical suits, coats or other clothing, furniture (Swanson et al., 2001; Poulos et al., 2002; Hunt et al., 2002) by human activity, air movement or ventilation, creating a secondary source of exposure causing respiratory diseases when inhaled (Reiter, 2002). Previous research has revealed that avoiding direct contact with latex gloves was not enough to reduce sensitisation in allergic individuals when other workers use powdered latex gloves in the same enclosed environment (Hunt et al., 1995; Vandenplas et al., 2002; Beezhold, 1996; Poulos et al., 2002; Mitakakis et al., 2002; Lopes et al., 2004).

2.5 Health effects associated with latex aeroallergens

Several studies have shown that inhalation of latex aeroallergens carried on cornstarch powder of latex gloves causes cutaneous, conjunctival and/or respiratory responses to latex. Studies by Tarlo and colleagues were among the first to suggest that rhinoconjuctivitis and asthma were caused by airborne cornstarch particles bearing latex allergens (Fish, 2002). Symptoms associated with exposure to aerosolised NRL reactions include urticaria, rhinitis, hoarseness,

wheezing, shortness of breath, conjunctivitis, chest tightness, asthma and life threatening anaphylaxis. Other indications of latex allergy include sneezing and a runny nose. Many other workers have also reported eye symptoms that appeared to be associated with aerosolised allergen (Liss and Sussman, 1999; Vandenplas et al., 1996; Hunt et al., 1995; Fish, 2002; Mitakakis et al., 2002; Swanson et al., 2001; Liss and Tarlo, 2001; Swanson and Ramalingam, 2002; Reiter, 2002).

Asthma due to latex may result from indirect exposure to airborne latex in medical settings and latex proteins are believed to be the potent causative agents (Vandenplas et al., 1996). Latex has become one of the main causative agents of occupational asthma (Baur, 2002) and was the most frequently reported agent by SORDSA between 1996 and 1998 (Hnizdo et al., 2001). The route of particles from ambient air into the lower respiratory tract is complicated. In general, particles <5μm diameter can penetrate the lungs while those >10μm are unable to penetrate below the glottis. Thus the nose and the eyes have greater exposure to airborne allergens than the lungs (Fish, 2002).

2.6 Recommended Occupational Exposure Limits (OEL)

Exposure limits can be defined as the levels that can be expected to cause allergic reactions in individuals who are exposed to a particular allergen (Poley and Slater, 2000). The safe occupational exposure limits for latex allergens causing sensitisation and symptom provocation are currently not documented and comparison of sample results with the OEL to interpret data is therefore impossible (Reiter, 2002; Baur, 1998; Heilman et al., 1996). Since methods for sampling and analysis of airborne allergens vary between laboratories,

determining the occupational exposure limits is not feasible and general standardised methods are needed.

Presently, exposure criteria for latex allergens are based on research and toxicological data (Reiter, 2002) by measuring the concentration of allergens in the work area and comparing this with symptoms (Hunt et al., 2002). This approach makes the interpretation more difficult and no conclusion can be drawn about exposure (Reiter, 2002). NRL is a mixture of potent allergens of which each has different stability and bioavailability characteristics and is likely to occur at different levels in various environments depending on the source of exposure. An average of exposure limit values may mask biologically significant specific allergen limits (Poley and Slater, 2000).

Two approaches have been adopted in an attempt to determine how much airborne allergen is required to cause respiratory symptoms and what permissible exposure limits should be for NRL. The first approach is to measure the levels in the work area and compare it with symptoms. Using this approach it was estimated that a few symptoms occurred at levels below $10ng/m^3$ (Hunt et al., 2002). Several other studies demonstrated that latex levels of $0.6ng/m^3$ or greater induced occupational respiratory responses (e.g. conjunctivitis, rhinitis and asthma) and the development of latex specific antibodies (Baur, 1998; Swanson and Ramalingam, 2002). This figure is 100 to 1000 fold less than the levels measured in areas where latex gloves are routinely used, suggesting that avoidance of aeroallergen exposure is the only way to prevent latex allergy in the workplace (Poley and Slater, 2000).

The second approach was to challenge patients under controlled exposure conditions which indicated that a higher value of 100 to greater than 1000ng/m^3 is required to provoke mild reactions (Hunt et al., 2002). An industrial hygiene study reported air sample results exceeding 50ng/m^3 as having a high risk potential for triggering allergic reactions; 10-50 ng/m^3 with moderate risk potential particularly to atopics and less than 10ng/m^3 have shown low risk of allergic reaction depending on an individual's immune system (Reiter, 2002; Hunt et al., 2002). These values refer to the risk of exposure by skin contact but can also be used to evaluate surface or dust samples (Reiter, 2002).

Researchers who determined the levels of allergen using immunoassays concluded that lower allergen levels were necessary to induce symptoms than researchers who measured the dust content and have set higher limits of allergen detection in the dust. OELs do not provide information on the incremental degree of sensitisation that occurs with increasing exposure and/or the reduction in sensitisation and reaction rates associated with a decrease in exposure (Poley and Slater, 2000). Starch powder is regarded as a carrier for NRL aeroallergen and the FDA proposed a limit of 120 mg of powder per glove. The FDA did not choose elimination of powdered gloves but preferred to limit the protein content of gloves and the powder mass (Swanson and Ramalingam, 2002). Previous studies have reported a decrease in number of cases of latex induced occupational asthma in Ontario, Canada eith the use gloves with reduced powder and/or protein (Allmers et al., 2004).

2.7 Legal implications of latex allergy

In South Africa, the rights of employees are protected by the Occupational Health and Safety Act (No 85 of 1993) under which the employer is obliged to provide a safe working environment as far as reasonably practical, and without risk to the health of workers (Potter et al., 2001; Pretorius et al., 2001). If powdered gloves are worn, latex allergens may be airborne and inhaled, thus representing a health hazard (Potter, 1998).

Sensitisation of workers to latex may involve many high costs which includes workers' compensation (Horwitz and Kammeyer-Mueller, 2002). Natural rubber latex is legally a substance hazardous to health under regulation 7 of Control of Substance Hazardous to Health (COSHH) Regulations 2002 in the United Kingdom, and exposure must be prevented or adequately controlled (Kalman, 2005).

2.8 Recommendations for controlling exposure to latex aeroallergens

Latex allergic diseases due to exposure to airborne latex allergens are a recognisable problem not only for exposed workers but also for the general population. Preventive measures are required to limit the number of new cases of latex sensitivity and avoid clinical manifestations in previously sensitised people.

Avoidance of exposure to latex allergens is difficult to implement as the allergens are readily distributed via their binding onto cornstarch powder (Vandenplas et al., 2002). However, a recent longitudinal study has shown a decreased prevalence of work-related symptoms in HCWs in 5 years after a recommendation to avoid natural rubber latex in 2000. This was

achieved using skin prick testing and latex specific Immunoglobulin E. Despite avoidance and reduced symptoms, the study also found out that most HCWs continue to retain percutaneous reactivity to non-ammoniated latex (NAL). This finding confirms that avoidance of NRL decreases but does not eliminate *in vivo* sensitisation to NAL (Smith et al., 2007).

Studies have demonstrated that powdered gloves contain more proteins than non-powdered gloves and therefore pose a risk factor by dispersing antigenic proteins into the work environment (Lopes et al., 2004). Several studies involving latex sensitive healthcare workers suggest that symptoms can be reduced with environmental modification, which involves removal of powdered latex gloves from the environment to prevent symptoms from latex aeroallergen (Hunt et al., 2002; Reiter, 2002; Mc Fadden, 2002).

Results from a few studies have suggested that the use of low protein, non-powdered latex examination gloves are effective in reducing the release of airborne protein levels (Heilman et al., 1996; Charous, 1998; Swanson et al., 2001; Schmid et al., 2002; Vandenplas et al., 2002), thus leading to a decrease of sensitisation and asthma in those persons not already sensitised to NRL (Liss and Tarlo, 2001; Wrangsjo et al., 2001; Saary et al., 2002; Jones et al., 2004). Vandenplas et al (2002) reported an improvement in asthma and rhinitis symptoms and nonspecific bronchial hyperresponsiveness in the subjects who minimised their exposure to latex. Tarlo et al (1994) demonstrated that a latex sensitive laboratory technician had no symptoms when co-workers changed from powdered latex gloves to powder free gloves and latex aeroallergen levels were not detectable without any other environmental modification (Hunt et al., 2002).

Personal use of non-latex gloves has been associated with a decrease in sensitisation and serum specific IgE levels; however this has shown not to be sufficient to prevent inhalation of latex antigens and the development of asthma if a co-worker continued to use powdered latex gloves (Vandenplas et al., 2002). Another study indicated a decrease of aeroallergen levels from up to 49.9ng/m^3 to below the detection limit within 24 hours in areas using synthetic or powder free latex gloves after replacing powdered NRL gloves. This demonstrated that removal of the source reduced the aeroallergen levels below the detection limit thus decreasing the risk of NRL sensitisation and permitting sensitised personnel to remain in their careers (Allmers et al., 1998).

The most effective way to manage sensitisation in workplaces is to adopt a glove selection policy for South Africa as it has been recommended internationally (UK, Canada, Germany and Scandinavia) and by Potter (Potter, 2001). Although the investment in adopting a latex free environment may be initially costly, the long-term savings such as loss of staff and productivity, the legal and financial implications for employers and healthcare institutions far outweigh the short-term benefit of purchasing cheap products of low quality (Charous, 1998; Potter et al., 2001; Lopes et al., 2004).

Other control measures include engineering controls that reduce the amount of latex allergen that becomes airborne. Such controls include the installation of local exhaust ventilation systems. Common industrial measures that are more applicable to industry than in healthcare are installation and use of automated systems, construction of enclosures and latex glove manufacturing refinements. Use of personal protective equipment (PPE) such as skin and

respiratory protection has been recommended as a control measure in industry where NRL is used. Remediation is another method to minimize latex allergen particulates. Remediation includes vacuuming surfaces that may contain latex particles with a high efficiency particulate air (HEPA) filtered vacuum and wet wiping using isopropyl alcohol as a wiping agent. The success of remediation was illustrated in a latex free dental office and a hospital administration office, which had led to sensitisation of workers. Air and surface sampling results following remediation was conducted indicating that the concentrations were below the level of detection (Reiter, 2002).

Interventions that involved education programmes, establishment of latex committees, surveillance and changes in gloves were implemented at different times in various hospitals in Ontario, Canada (Liss and Tarlo, 2001). Banning of powdered latex gloves in the workplace and substitution with latex free material seems to be a sufficient prevention strategy for latex sensitisation (Allmer et al., 1998). In addition to this measure, workers who complain of dermal or respiratory symptoms that are strongly linked with the use of latex gloves should be provided non latex gloves such as vinyl and synthetic surgical gloves (Lopes et al., 2004).

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1. STUDY DESIGN

This was a cross sectional (descriptive) study investigating the levels of latex aeroallergens in dental schools situated in various provinces.

3.2 SAMPLING POPULATION

The study workplaces were five dental schools from various provinces which included the Universities of Kwa-Zulu Natal (Kwa-Zulu Natal), the Western Cape (Western Cape), Limpopo-MEDUNSA campus (North West), Pretoria and Witwatersrand (Gauteng). The study was voluntary and was presented to dental staff and students working at various institutions. The study was approved by the ethics committee of the University of the Witwatersrand as described in 3.5.6. Interested participants completed a consent form. The study participants included dental staff (clinical, administration, laboratory and auxiliary) and dental students (excludes first years who are not working in the clinics). The study participants were asked to wear a sampling pump for the duration of one clinic session.

3.3 BUILDING INSPECTION

A walk-through inspection was performed by a qualified occupational hygienist at each dental institution prior to sampling. The aim was to identify the type of gloves used, storage facilities, disposal procedures, house keeping; and to obtain information on control measures in place by direct observation.

3.4 SAMPLE COLLECTION

3.4.1 Area sampling

Air samples were collected by an Occupational Hygienist (Occupational Hygiene Section, National Institute for Occupational Health) and a trained technician in the clinics. Two types of air sampling pumps were used due to a large number of samples being collected over a short sampling period (two weeks on average). These pumps function the same and will therefore have no negative effect on the sampling procedure. The Gillian (SKC Ltd, USA) or Casella Apex (Casella, USA) battery operated air sampling pumps were used to collect airborne particles in work areas of various dental clinics. Airborne particles were collected on 0.4µm, 37 mm sterile polycarbonate (PC) filters at a flow rate of 2.5l/min for approximately 6 hours. This method has been adapted from Quirce et al., 2004 and was slightly modified by replacing the PTFE filters with PC filters and by adjusting the flow rate from 2L/min to 2.5L/min. Prior to application, both filters were validated in the laboratory under the same conditions and they yielded similar results. The PC filter was chosen because it was used in another study which took place simultaneously with this study, therefore minimizing the costs. Quirce et al (2004) used this method for personal sampling for 5-6 hours. Due to the unavailability of high volume area air samplers, the latter method was adapted for area sampling in our study for ~6hours. A total of 95 area samples for all five institutions were collected in areas where latex gloves were commonly used as well as in areas presumed to be unexposed (administration). After sampling, the filters were sealed and stored in zipped plastic bags until analysis at the National Institute for Occupational Health (NIOH) laboratories.

Environmental parameters such as relative humidity (RH) and temperature that may influence the findings were also monitored during the sampling period. Calibration of pumps was performed daily before and after sampling using a Gillibrator bubble flow meter (Gillian Instrument Corp., Wayne, N). The flow rate of pumps was checked before and after sampling to determine the stability of the flow rate over the sampling period. Using the flow rate and the sampling time, the volume of the air sampled was calculated. . The concentration of latex allergens was obtained by dividing the measured concentration (µg/L) in air filters extracts with the sampled air volume; the final aeroallergen concentration was expressed as ng/m^3.

3.4.2 Personal sampling

For personal sampling, air sampling pumps were attached to sampling belts which were worn around the waist of the participants. Airborne particles were collected on 0.4µm, 37 mm preloaded sterile polycarbonate (PC) filters at a flow rate of 2.5l/min on the breathing of participants breathing zone during one clinic session. Sampling times ranged from 1 h to 3 hours depending on duration of the dental procedure or session. A total of 369 personal samples were collected in all five institutions where latex gloves were commonly used. Of the 369 personal samples, 28 (7.6%) were negative controls i.e people not handling powdered latex products (administration). After sampling, the filters were sealed and stored in zipped plastic bags before analysis.

Field blanks were included during sampling for quality control (QC) purposes. Filters were handled in the same way as the samples by exposing them to the same environment but not withdrawing air as with the true samples. The blanks and controls were also analysed in the

same batch as the samples. Samples were randomly selected and sent to BGFA in Germany and FITkit Ltd in Finland for interlaboratory comparison.

3.4.3 Rubber products

A total of 19 latex rubber products (14 gloves and 5 dental dams) representing six brands were collected from the various dental schools. These included 13 powdered and 6 non-powdered latex products. Information for each rubber product was collected and recorded in a walkthrough checklist. These included sample type, powdered or non-powdered, brand and specification of protein levels. The brand names of the rubber products could not be disclosed for ethical reasons, and were coded brand 1 to 6. The products were sealed and stored in labelled zipped plastic bags before analysis. Three rubber samples (2 gloves and 1 rubber dam) were sent to BGFA (Germany) and FITkit (Finland) for external quality control.

3.5 SAMPLE ANALYSIS

Among the healthcare workers, hev b 5 and hev b 6.02 have been shown to be the two most important latex allergens (Koh et al., 2005), thus these two allergens were selected for the analysis of airborne filter samples for this study.

3.5.1 Extraction of filter samples

Filter membranes were aseptically removed from the filter cassettes using forceps. The membranes were eluted in 6 ml limulus amoebocyte lysate (LAL) water with 0.1% Tween 20 by stirring for 3 hours. The extracts (6ml) were freeze dried using a Crodos

-50°C freeze dryer (Labotec, Spain) and reconstituted in 1ml of 0.1% LAL-Tween. The supernatant was stored in micro centrifuge tubes at -70°C until analysis.

3.5.2 Extraction of latex rubber products

Gloves and dental dams from various institutions were cut into small pieces and weighed. One gram of rubber sample was extracted in 5ml phosphate buffered saline (PBS) with 0.1 % Tween 20 (1:5 w/v) at pH 7.4. The samples were shaken at 160 rpm for 2 hours at 28°C±2 in a shaking incubator. After shaking, the rubber solids were removed and the extracts were transferred into 1.5 ml centrifuge tubes and centrifuged (Hettich Mikro22R, Germany) at 12000 rcf for 15 minutes at 25°C. The supernatant was filtered using 25mm, 0.45µm Acrodisc® filter membranes (Life Sciences, Pall Corporation, USA) and stored at -20°C prior to analysis.

3.5.3 Estimation of total extractable proteins in latex products

Filter and rubber extracts were analysed for total protein content using the detergent compatible (DC) protein assay kit (BIO-RAD, Hercules, CA). The protein assay kit is a colometric assay for protein concentration following detergent solubilisation and is available commercially as a ready-to-use total protein assay kit. The assay is based on the reaction of protein with an alkaline copper tartrate solution (Reagent A) and folin reagent (Reagent B). The copper treated protein reduces folin reagent thereby producing a blue colour with maximum absorbance at 750 nm and minimum absorbance 405nm. The reaction is similar to the well documented Lowry assay but with colour development in a shorter period of time (15 min). The total protein content in this study refers to the total extractable protein.

The assay was performed according to the manufacturer's instructions. In summary, 20 µl of reagent S (surfactant solution) was added to 1ml of reagent A needed for the run depending on the volume needed for samples (reagent A can be used directly if samples do not contain detergent). A two-fold serial dilution ranging from 0.2 to 1.2mg/ml of protein standard was prepared for the standard curve. Standards were prepared using bovine serum albumin (BSA) of concentration 1.44mg/ml (BIORAD, Hercules, CA) and PBS containing 0.1% Tween 20 as the diluent. Five µl of standards and extracts were dispensed into a sterile microtiter plate. Working reagent A (25µl) was added into each well followed by reagent B (200µl). The plate was gently agitated for approximately 5 seconds to mix the reagents, avoiding bubble formation. After 15 minutes incubation, the optical density (OD) was read using a microplate reader (BIOTEK instruments, USA) at a wavelength of 750nm. A linear regression using KC4 ELx808 software (BIOTEK instruments, USA) was used to determine the concentrations. The results were expressed as µg/g for rubber extracts. The detection limit (DL) of the method was 0.15mg/ml and the reporting limit (limit adjusted to the weight of rubber sample) was 700µg/g. Results were acceptable if the correlation coefficient (R^2) ≥ 0.98 and coefficient of variation (CV) was ≤ 10%.

3.5.4 Quantification of latex aeroallergens

The latex allergen content of filter and rubber extracts was determined using the FITkit ™ (FIT Biotech, Tampere, Finland). FITkit is an immunological test based on the capture-enzyme immunoassay (EIA) principle for direct determination of clinically relevant latex allergens (hev b 1, hev b 3, hev b 5 and hev b 6.02) in latex products. These kits use specific monoclonal antibodies developed against these four individual allergens with each allergen

providing results separately. For the purpose of this study, the two major allergens that have been reported in healthcare settings i.e. hev b 5 and hev b 6.02 were selected for the analysis of filter extracts. The rubber extracts were tested for all four allergens to estimate the total allergen content. The total allergen in this study refers to the sum of four clinically relevant allergens (hev b 1, hev b 3, hev b 5 and hev b 6.02).

The microwell strips provided with the kit are coated with a monoclonal antibody that binds specific allergens from the sample extracts. The assay was performed according to the manufacture's instructions. All reagents were brought to room temperature prior to testing. The test control was reconstituted with 500µl distilled water and was allowed to stand for 30 minutes. The wash concentrate (50ml) was diluted with 450ml of distilled water to make up 500ml washing solution. 100µl of the assay buffer was added to the plate, followed by 25µl of standards, control and product extracts, respectively. All tests were performed in duplicate. The microwell plates were incubated for 1 hour at RT on a Stuart microtitre plate shaker (Labex, USA) at 200 rpm. The microwell plates were washed four times using an automated PW 40 microplate washer (BIO-RAD, France). After washing, 100µl of enzyme conjugate was added per well and incubated for 30 minutes at RT on a plate shaker. The plates were washed again and 100µl substrate was added to start the reaction and the plates incubated for 15 minutes. The reaction was terminated by adding 100µl of the stopping solution. The plates were shaken for 2 minutes and read on a microplate reader (BIOTEK instruments, USA) at 405nm. The allergen concentrations of extracts were calculated by log-log regression and spline curve fitting using software that fits the calibrator curve (KC4-ELX 808; BIOTEK instruments, USA). The results were expressed as µg/l and the final results as mass protein per cubic meter of air (ng/m^3). For QC purposes, each FITkit contains a test control which

should record results within a specified range provided in the certificate of analysis enclosed in the kit.

3.6 STATISTICAL ANALYSIS

Descriptive statistics were used and the airborne concentrations are reported by institution. Data analysis was first recorded and cleaned in MS Excel 2003 and imported to STATA 9 computer software (StataCorp, 1984-2007, Texas, USA). As the data of the allergens showed a skewed distribution, non parametric tests were applied and parametric tests (log transformed) was used were necessary. ANOVA was used to test for significant difference between the means of the log-transformed values. Two group comparisons were done using Wilcoxon rank-sum (Mann-Whitney) while multiple group comparison were done using Kruskal Wallis test. Data below the detection limit were censored using the Maximum Likelihood estimation (MLE) method (Hornung and Reed, 1990; Finkelstein and Verma, 2001). These methods involve the substitution of $L/2$ and $L/\sqrt{2}$ for each non-detectable value where L is the limit of detection. The latter was chosen for this analysis because it is reported to be more accurate for the estimation of mean and standard deviation when the data are not highly skewed. Spearman's rank correlation coefficient was used to assess the relationship between total protein levels and total allergen levels. A P-value of less than 0.05 was considered statistically significant.

3.7 ETHICAL CLEARANCE

Ethical clearance (M050512) was obtained from the University of Witwatersrand Ethics Committee. Permission was also granted by various dental schools to conduct the study and consent was obtained from each participant (see attached appendices).

Some institutions required that ethical clearance be obtained from their respective institutions (See appendices D-F). Permission was also granted from the Gauteng Department of Health (Provincial) to conduct the study.

CHAPTER 4

4.0 RESULTS

Inspection of dental clinics revealed that latex gloves (powdered and non powdered) and rubber dams were the most common latex products used in dentistry. The work practices (e.g. storage and disposal of latex products; and ventilation system) differed by institution. Of the four institutions (A, B, D, E) that have the ventilation systems, 3 (A, B, D,) were defective during the sampling period. Institution C did not have the ventilation system in place. The handling of latex products was good for some institutions and poor for the others.

4.1 Personal samples

The study population consisted of 454 participants who originally consented to participate in the study. However due to some participants withdrawing from the study and financial constraints, a total of 369 personal aerosol samples from all five institutions were analysed for the two latex allergens (hev b 5 and hev b 6.02) which have been reported in HCWs. The proportion of participants from each institution is shown in Figure 4.1.

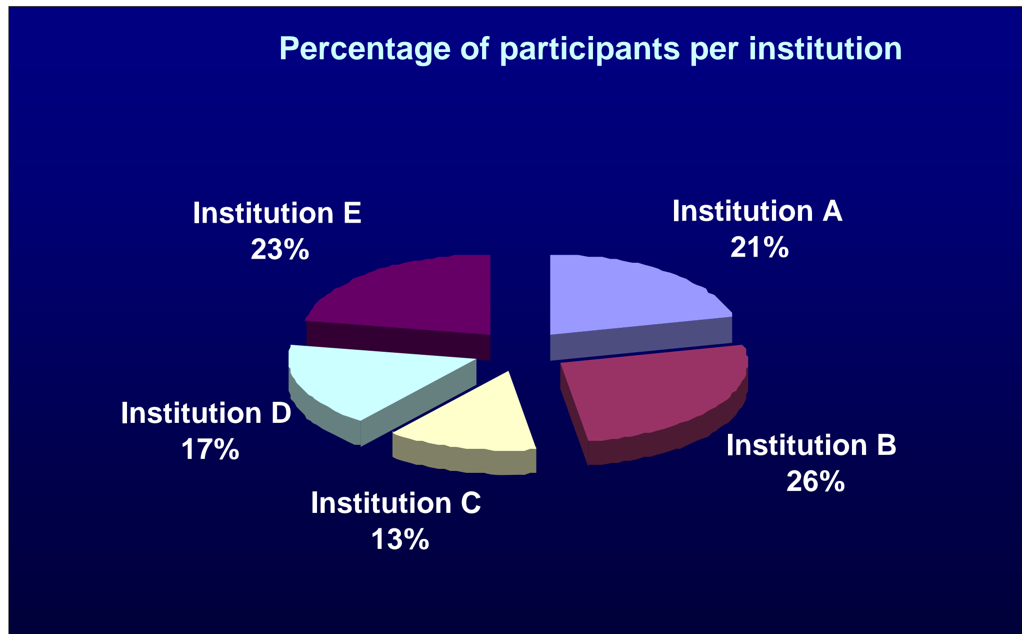

Figure 4.1 A chart showing percentages of participants for various institutions.

Of 369 personal samples, 28 (7.6%) were negative controls i.e administration staff not handling powdered latex products. Table 4.1 shows the proportion of staff from various departments found in various dental institutions. The participants included dental staff (clinical, laboratory, administration and auxiliary) and dental students (excluding first year students as they do not perform practical work). All staff and students working in the clinics were categorised as clinical. Laboratory staff included all technicians and technologists. Administrative staff included switchboard operators, secretaries and receptionists whereas auxiliary staff comprised of security, cleaners, porters, messengers and laundry personnel. Job types were categorised into 7 groups which included administration, laboratory technicians, dental assistants, students (DTOH and BDS), dentists and general assistants (auxiliary).

Table 4.1 Number of participants classified by department for different institutions

Institution	Departments: n (%)					
	Clinical	**Laboratory**	**Administration**	**Auxiliary**	**Other***	**Total**
A	61 (78.2)	6 (7.69)	3 (3.85)	8 (10.26)	0 (0.0)	78
B	66 (70.2)	2 (2.1)	6 (6.4)	16 (17)	4 (4.3)	94
C	37 (75.51)	2 (4.08)	1 (2.04)	8 (16.33)	1 (2.04)	49
D	47 (74.6)	3 (4.8)	5 (7.9)	8 (12.7)	0 (0.0)	63
E	63 (74.1)	2 (2.4)	13 (15.3)	7 (8.2)	0 (0.0)	85
Total	274 (74.3)	15 (4.1)	28 (7.6)	47 (12.7)	5 (1.4)	369

*not classified by department

4.1.1 Detection limits (DL) of the enzyme immunoassay

Approximately 50% of air samples demonstrated the presence of hev b 5, while hev b 6.02 accounted for 14.6 % samples. The remaining proportion was below the detection limit for both allergens. In addition, for some specimens, the lowest calibrator (5µg/l) was diluted further to 2.5µg/l for quantifying hev b 5 and hev b 6.02 latex allergens. This step was necessary to broaden the detection range because several samples could not be detected. The descriptive analysis for hev b 5 and hev b 6.02 allergen concentrations in personal air samples is presented in Table 4.2. All observations below DL were censored as described in chapter 3.

Table 4.2 Summary description for hev b 5 and hev 6.02 detection levels

Allergen (µg/l)	Detection limit (DL)	n (%)
Hev b5 (n=367)	Below DL <2.5*	109 (29.7)
	Below DL <5	75 (20.4)
	Detected	183 (49.9)
Hev b6.02 (n=369)	Below DL <2.5	319 (86.5)
	Detected	50 (13.5)

* 5 µg/l calibrator diluted further to 2.5 µg/l to broaden the detection range

4.1.2 Summary description for hev b 5 and hev b 6.02 for personal air samples

Overall, the median level for hev b 5 was 9.36ng/m^3 with an interquartile range (IQR) of 7.71ng/m^3. The concentrations for hev b 5 ranged from 1.84 to 46.1ng/m^3 for all institutions. Since hev b 6.02 had a high proportion of measurements below the DL, the data was analysed differently as a categorical variable.

4.1.3 Hev b 5 and hev b 6.02 classified by institution

Variations between institutions for hev b 5 allergen levels were assessed (Figure 4.2). Median (ng/m^3) levels are indicated in the graph.

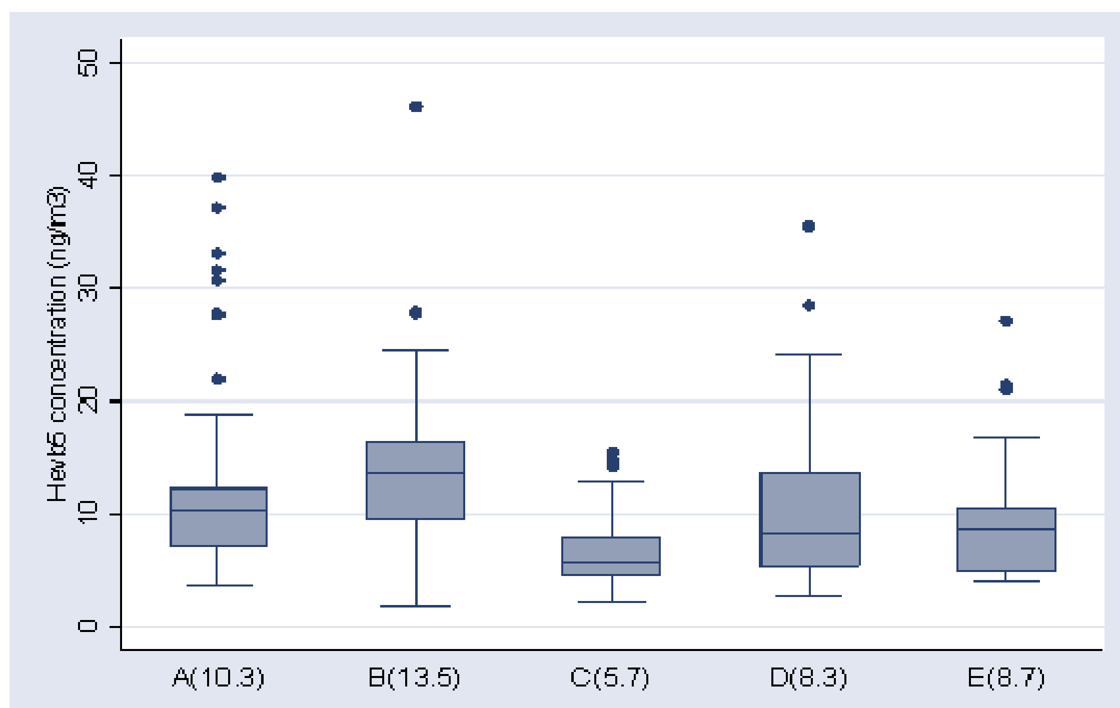

Figure 4.2 Graphical illustrations of hev b 5 concentrations by institution. Median values in parentheses.

Figure 4.2 shows differences in the concentrations of hev b 5 between institutions. Institution B showed the highest median level (13.5ng/m^3) and institution C the lowest median level (5.7ng/m^3). Kruskal Wallis test for the equality of population medians showed that the exposure levels differed significantly by institutions in terms of hev b 5 latex allergen ($p \leq 0.001$). To further test for significant difference between respective institutions, an ANOVA analysis (including Bonferroni comparison) was done on the log-transformed values of hev b 5 (Table 4.3).

Table 4.3 Comparison of means of log-transformed values for hev b 5 by institution

Institution	**Arithmetic mean**	**Comparison difference (p-value)**			
		A	**B**	**C**	**D**
A	2.30	-	-	-	-
B	2.49	0.19 (0.095)	-	-	-
C	1.78	0.51 (0.000)	0.71 (0.000)	-	-
D	2.15	0.14 (1.000)	0.34 (0.000)	0.38 (0.000)	-
E	2.06	0.23 (0.03)	0.43 (0.000)	0.28 (0.022)	0.09 (1.000)

The results of the log-transformed values demonstrated that personal exposure levels for institution B were significantly higher than all the other institutions except institution A ($p<0.001$). Institution C was again significantly lower than all institutions which confirm the results obtained in Figure 4.2. There was also a significant difference between institution A and E ($p<0.05$) as shown in Table 4.3.

Participants with detectable exposure levels (13.6 %) for hev b 6.02 were categorised as exposed and those below detectable levels (86.4 %) as unexposed as indicated in Table 4.4 (Braimoh Bello, personal communication, 2007). The proportion of the exposed category to hev b 6.02 differed significantly by institution ($p<0.001$).

Table 4.4 Proportion of exposed and unexposed category for hev b 6.02 by institution

	Hev b 6.02 allergen category (µg/l)		
Institution	**Exposed** n (%)	**Unexposed n** (%)	**Total** n (%)
A	32 (41.03)	46 (58.97)	78 (100.00)
B	0 (0.00)	94 (100.00)	94 (100.00)
C	1 (2.04)	48 (97.96)	49 (100.00)
D	7 (11.11)	56 (88.89)	63 (100.00)
E	10 (11.76)	75 (88.24)	85 (100.00)
Total	50 (13.55)	319 (86.45)	369 (100.00)

Table 4.4 shows that Institution A has a higher proportion of hev b 6.02 personal exposure and forms part of the 13.6% of the detectable levels for the allergen suggesting that more participants were exposed to hev b 6.02 than they were for hev b 5. Theses allergens are two individual allergens analysed using two separate kits, therefore one allergen can be detected while the other cannot from the same sample.

4.1.4 Concentrations of hev b 5 and hev b 6.02 by departments

The differences in exposure levels of hev b 5 and hev b 6.02 between four departments (clinical, laboratory, administration and auxiliary) were assessed. The medians levels (ng/m^3) for hev b 5 are indicated in Figure 4.3.

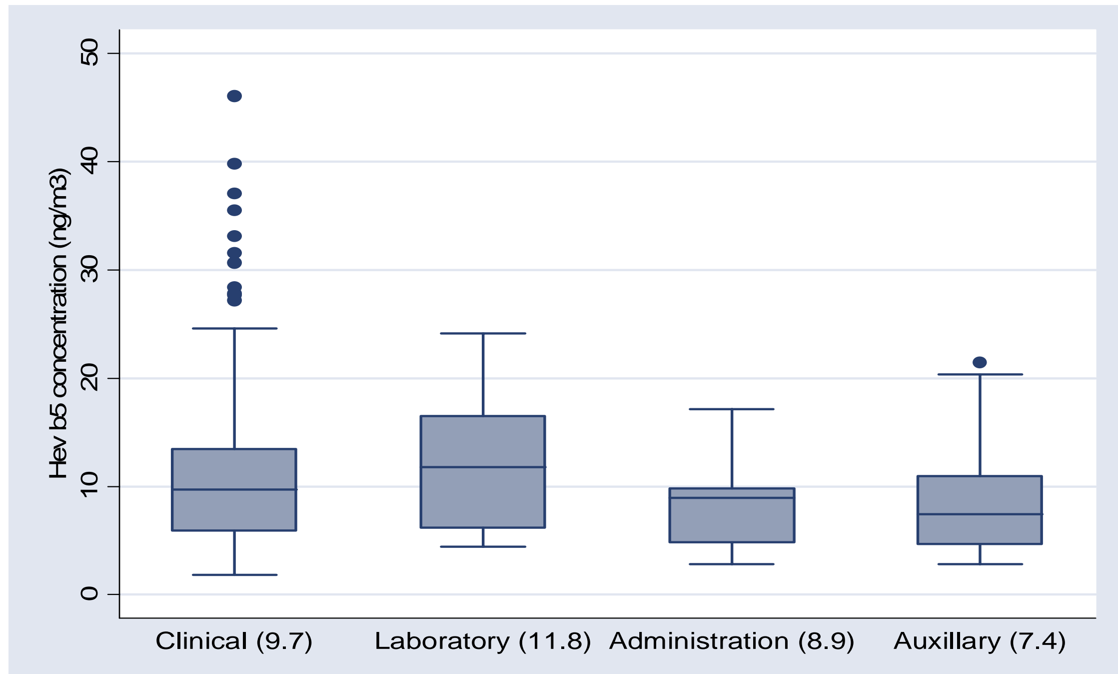

Figure 4.3 Personal exposures levels of hev b 5 by departments.

The results illustrate a difference in the levels for hev b 5 (Figure 4.3) by departments. The clinical and laboratory departments showed higher concentrations of hev b 5 than the administration and auxiliary departments. Kruskal Wallis test showed that the exposure levels differed significantly by departments in terms of hev b 5 latex allergen (p=0.0113). An ANOVA analysis (including bonferroni comparison) on the log-transformed values of hev b 5 showed further significant differences between the clinical and auxiliary departments (p=0.029) . Table 4.5 illustrate the proportions of hev b 6.02 by department which is significantly different (p=0.027).

Table 4.5 Proportion of exposed and unexposed category for hev b 6.02 by departments

	Hev b 6.02 allergen category (µg/l)		
Institution	**Exposed** n (%)	**Unexposed n** (%)	**Total** n (%)
Clinical	46 (16.79)	228 (83.21)	274 (100)
Laboratory	1 (6.67)	14 (93.33)	15 (100)
Administration	2 (7.14)	26 (92.86)	28 (100)
Auxiliary	1 (2.13	46 (97.87)	47 (100)
Total	50 (13.74)	314 (86.26)	364 (100)

4.1.5 Concentrations of hev b 5 classified by the type of tasks

The differences in exposure levels for hev b 5 between tasks are presented in Table 4.6. Although the median values for various jobs were similar, the maximum values differed markedly. It is evident that the clinical staff and students are exposed to higher levels of hev b 5.

Table 4.6 Hev b 5 concentrations for personal samples by job category

Job tasks	Median	Lower and upper quartiles	Min and Max range
Laboratory (n=14)	10.6	6.2, 14.2	4.4, 19.3
Administration(n=27)	9	4.8, 9.8	2.8, 17.1
DTOH* (n=41)	9.4	5.9, 12.5	3.9, 35.5
BDS** (n=78)	11.5	8.4, 15.3	1.84, 46.1
Assistants (n=118)	9	5.2, 12.7	3.6, 20.1
Dental staff (n=34)	9.6	6, 14	3.6, 27.2
Auxiliary (n=47)	7.4	4.7, 11	2.8, 21.4

*Dental therapy and oral hygiene students, **Bachelor of Dental Science

Kruskal Wallis test showed significant difference between the job tasks in terms of hev b 5 latex allergen (p=0.0035). Further test (Bonferroni) on the log-transformed values of hev b 5

demonstrated that the BDS students differed significantly with the administration (p=0.046); assistants (p=0.032); and auxiliary (p=0.001).

The proportions of hev b 6.02 by the type of job was also significantly different (p<0.001) as indicated in Table 4.7.

Table 4.7 The Proportion of the exposed and unexposed group for hev b 6.02 by job category

Job tasks	**Exposed** n (%)	**Unexposed** n (%)	**Total** n (%
Laboratory	1 (7.1)	13 (92.9)	14 (100)
Administration	2 (7.1)	26(92.9)	28 (100)
DTOH*	4 (9.8)	37 (90.2)	41 (100)
BDS**	26 (32.9)	53 (67.1)	79 (100)
Assistants	9 (7.6)	110 (92.4)	119 (100)
Dental staff	7 (19.4)	29 (80.6)	36 (100)
Auxiliary	1 (2.1)	46 (97.9)	47 (100)
Total	50 (13.7)	314 (86.3)	364 (100)

*Dental therapy and oral hygiene students, **Bachelor of Dental Science

4.2 Area samples

A total of 95 area sample extracts were analysed for the presence of two latex allergens. Table 4.8 summarizes the detection levels for both allergens using the FITkit assay.

Table 4.8 Summary description for hev b5 and hev 6.02 detection levels for area samples

Allergen (μg/l)	Detection limit (DL)	n (%)
Hev b 5 (n=95)	Detectable	45 (47.4)
	Below DL <2.5*	30 (31.6)
	Below DL <5	20 (21)
Hev b 6.02 (n=93)	Detectable	4 (4.3)
	Below DL <2.5*	89 (95.7)

* 5 μg/l calibrator diluted further to 2.5 μg/l to broaden the detection range

4.2.1 Summary description for hev b 5 and hev b 6.02 for area samples

The median detection level of area samples was 2.96ng/m^3 with an interquartile range (IQR) of 2.68ng/m^3. The concentrations for area samples for all institutions ranged from 1.33 to 14.97ng/m^3. The presence of hev b 6.02 could not be detected in a high proportion of area samples assessed (Table 4.8). The presence of this allergen was therefore not analysed further.

4.2.2 Hev b 5 concentrations classified by institutions for area samples

Figure 4.4 illustrates the difference in concentrations of hev b 5 between institutions. The concentrations of hev b 5 allergen differed significantly by institution (p=0.002). The concentration was highest for institution B with a median value of 5.6 ng/m^3 and lowest for institution D, with a median value of 1.6ng/m^3.

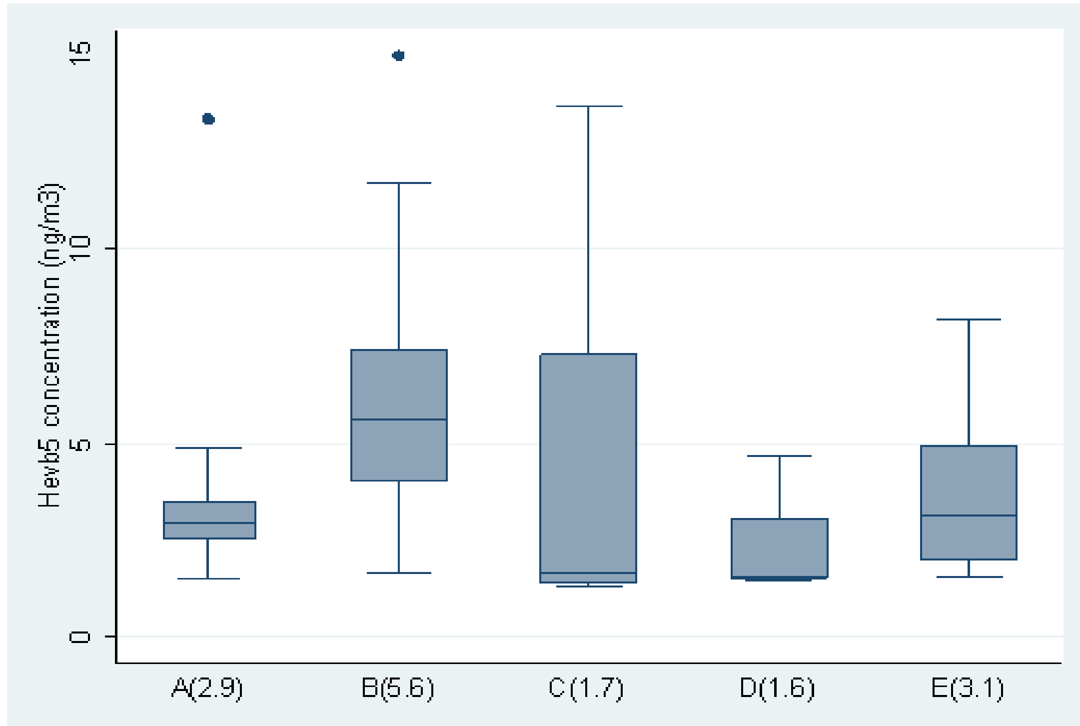

Figure 4.4 Hev b 5 concentrations for area samples of various institutions. Median values in parentheses.

The log-transformed values of hev b 5 were used to compare institutions. The results showed that the exposure levels for institution B were significantly higher than all other institutions ($p<0.001$). However, there was no significant difference between the remaining institutions.

4.3 Latex rubber products

4.3.1 Quantification of hevein allergens and total protein

Of the 19 samples of rubber products (14 gloves; and 5 dental dams) representing six brands, 13 were powdered and 6 were non-powdered. Hev b 1 was detected in 80% (4/5) of dental dams but not in gloves, however hev b 3 was found in 89% (17/19) of gloves and all dental dams, hev b 5 in 93% (13/14) of gloves and all dental dams. All rubber samples of gloves and dental dams analysed demonstrated the presence of hev b 6.02. The concentration of the allergenic proteins in relation to their total protein content is summarised in Table 4.9. Hev b 1 concentrations were the lowest of the four allergens, ranging from <0.05 (noted as 0) to 7.9µg/g whereas the concentration of hev b 6.02 detected was markedly higher ranging from <0.025 to 61.5µg/g. The concentration of hev b 3 found ranged from <0.05 to 30.12µg/g and hev b 5 from <0.025µg/g to 9.2 µg/g. The total protein content could only be measured only in 12 samples and was below the detection limit (700µg/g) for 7/19 (37%) extracts (Table 4.9). Regarding total allergenic protein content of the extracts (also referred to as total allergenicity), 14/20 fell in the high category, 4 tested as moderate and 1 was low.

Table 4.9 Comparison of four latex allergens and total extractable protein concentrations in 19 latex rubber extracts.

Sample ID	Sample type	Powdered/Non -powdered	Allergen concentrations in extracts (μg/g of glove)				Total allergen (μg/g)	Total protein (μg/g)	Allergenicity
			hev b1	**hev b3**	**hev b5**	**hev b6.02**			
1	glove	powdered	0	0.1	9.2	38.39	47.69	3130	high
2	glove	powdered	0	0	0.27	46.09	46.36	2740	high
3	glove	powdered	0	2.69	6.87	15.46	25.02	3180	high
4	glove	powdered	0	0.05	3.62	20.12	23.79	3380	high
5	glove	powdered	0	0.22	4.1	11.64	15.96	2080	high
6	glove	powdered	0	1.25	4.74	0.3	6.29	<700	high
7#	glove	powdered	0	0.45	0.3	2.75	3.5	<700	moderate
8	glove	powdered	0	0	2.84	0.56	2.99	770	moderate
9	glove	non-powdered	0	24.71	0.24	11.09	36.04	2150	high
10	glove	non-powdered	0	17.43	1.96	0.09	19.48	<700	high
11	glove	non-powdered	0	2.13	4.23	0.36	6.72	<700	high
12	glove	non-powdered	0	3.67	2.32	0.23	3.9	<700	moderate
13	glove	non-powdered	0	3.59	2.45	0.15	2.81	<700	moderate
14	glove	non-powdered	0	0.58	0	0.09	0.67	<700	low
15	dam	powdered	7.9	18.31	5.26	61.5	92.97	2790	high
16	dam	powdered	0.31	30.12	4.44	55.53	90.4	3130	high
17	dam	powdered	0.12	25.69	3.26	51.84	80.91	3230	high
18	dam	powdered	0	1.53	4.52	54.3	60.35	2610	high
19	dam	powdered	0.72	0.1	2.15	37.57	40.54	3360	high

Detection limits (DL): **hev b 1**=0.05 μg/g, **hev b 3**=0.05 μg/g, **hev b 5**=0.025 μg/g, **hev b 6.02**=0.025 μg/g, **Total protein**=700 μg/g
Values below DL for allergens are recorded as zero.
reduced protein

4.3.2 Correlation between total allergen and total protein of latex rubber products

As shown in Figure 4.5, statistical analysis revealed that the total allergen in 19 latex rubber products correlated well with the total protein content (Spearman's r=0.74, p<0.001, n=19). The graph includes data for all latex products analysed.

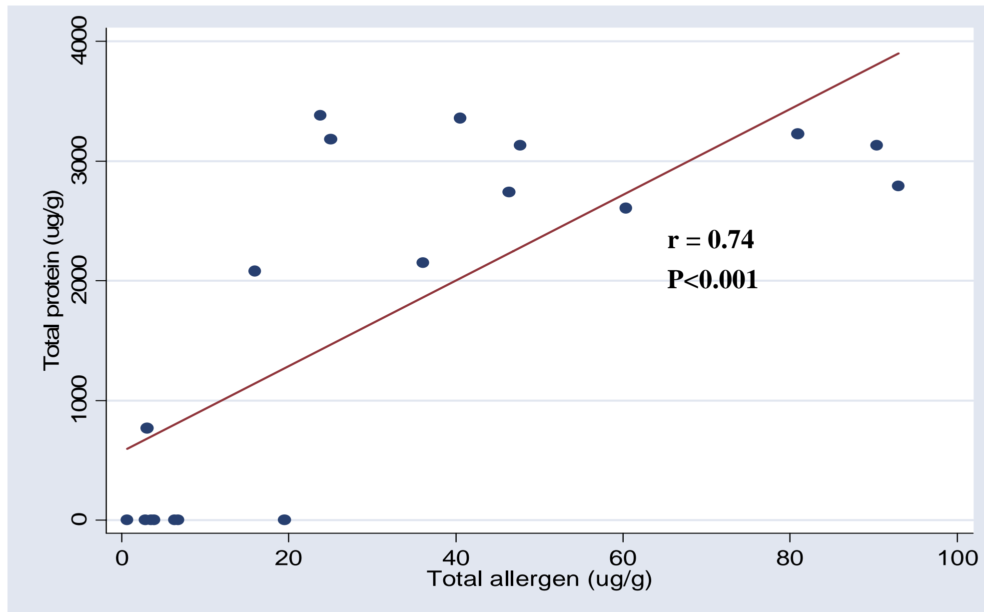

Figure 4.5 Correlation between the total allergen and total protein expressed as µg/g of latex extracts in 19 samples.

4.3.3 Comparison between different latex products

A two-sample (Mann-Whitney) test showed a significant difference (p=0.002) between the latex concentrations of gloves and dental dams. Hev b 1 and hev b 3 are more common allergens in the dental dams as compared to the gloves (Figure 4.6). Similarly there was a significant difference (p=0.035) between powdered and non-powdered samples (Figure 4.7). Powdered latex products showed higher concentrations of total allergen content compared to the non-powdered products. A comparison of the latex concentrations of the various branded latex products also showed a significant difference (p=0.022) using the Kruskal Wallis test (Figure 4.8).

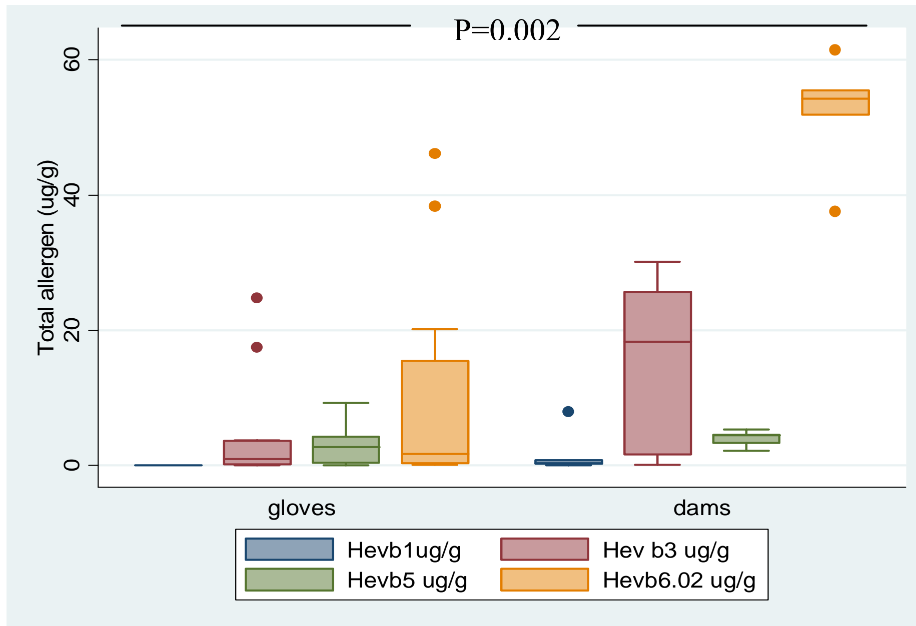

Figure 4.6 Comparison between the four hevein allergens (µg/g) in gloves and dental dams.

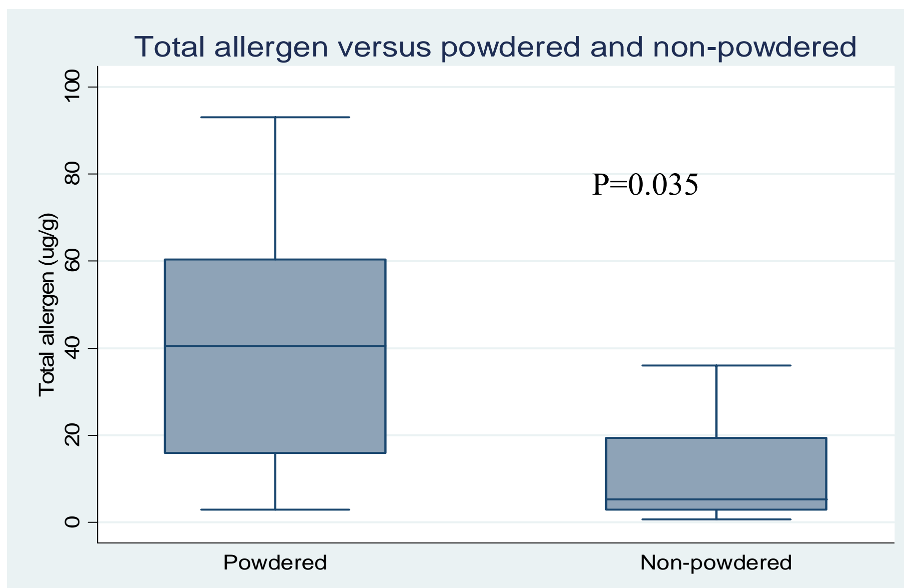

Figure 4.7 Comparison between the total allergen (µg/g) in powdered and non-powdered samples. Medians (powdered=40.54µg/g, non-powdered = 5.31µg/g).

4.3.4 Comparison between total allergen content of different brands of latex rubber products

In Figure 4.8, brand 3 which is dental dams (median: 80.91µg/g) showed higher concentrations followed by brand 1 (median: 24.40µg/g) and brand 2 (median: 11.34µg/g). Brand 4 and 6 were not statistically represented (one sample per brand). They are however included herein for the purpose of demonstrating the difference in allergen concentrations of the different brands. Brand 5 was found to be present in much higher concentrations (median: 36.04µg/g) than most glove brands whereas brand 6 showed the lowest concentration (median: 6.29µg/g).

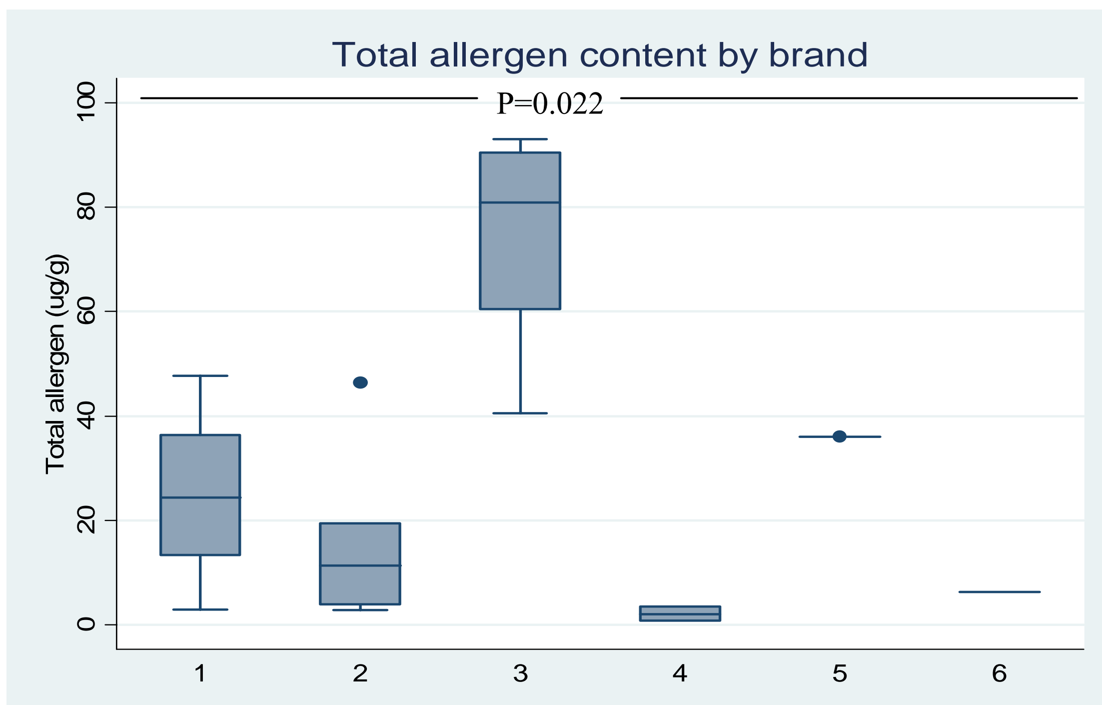

Figure **4.8** Comparison between the total allergen (µg/g) in different brands (1-6) of latex products.

CHAPTER 5

5.0 DISCUSSION

This study used a combination of air sampling, immunological and statistical techniques to determine the concentrations of clinically relevant allergens in 5 dental schools within South Africa. The present study has shown that airborne allergen concentrations vary considerably among the five participating dental schools. The concentration of latex allergens for personal samples in this study were 2.8 to 46.1ng/m^3 for hev b 5 and 1.1 to 46.2 ng/m^3 for hev b 6.02. These levels were lower than those reported by Swanson et al (1994), which varied from 8 to 978ng/m^3. The differences may be attributed to the varying sampling methodology. In our study we used a similar sampling principle at a flow rate of 2.5L/min, whilst Swanson et al used a flow rate of 5L/min for personal sampling which may have contributed to the measurement difference. Furthermore the detection technique we utilised was based on ELISA principle rather than inhibition assay.

In this study, differences were also observed when comparing different personnel categories, referred to as departments in the previous chapter i.e clinical, laboratory, administration and auxiliary. Individuals based in the clinical department were exposed to the highest levels of hev b 5 (37.1ng/m^3) compared to those in the other departments. It is possible that the high levels of exposure maybe due to various dental procedures done in this department. Each clinic session requires change of gloves, most of which were powdered; hence the risk of exposure increases with each change of gloves. In addition, procedures which require the use of dental dams which were also powdered increase the risk of exposure. Analysis of exposure to hev b 5 also showed higher concentration of this allergen in the laboratory department compared to the auxiliary department (Figure 4.3).

In the current study, the administration department was used as a control group as the risk of exposure to latex allergens was thought to be minimal in this category. The auxiliary department on the other hand was exposed to latex allergens very rarely because the majority of personnel in this category did not wear gloves nor use the dental dams. It was therefore somewhat surprising to note that the median levels of exposure in the administration department (median: 8.9ng/m^3) was slightly higher than the one observed in the auxiliary department (median: 7.4ng/m^3). However the standard deviation between the two categories overlap and these median differences are minor. Nevertheless the allergen level in both departments was markedly lower than observed in the clinical and laboratory departments. During our surveys, we observed that both the dental staff and students wore their labcoats in common areas (canteens, rest rooms and offices) which may have lead to allergens being transferred to other areas. The trace levels of latex aeroallergens may also be transported to the offices on equipment and footwear of workers moving between the work area and offices (Sri-Akajunt et al., 2000). Thus workers not directly involved with latex products may also be exposed to latex aeroallergens. Moreover, the administration departments of most of the dental institutions are in close proximity to the clinics thus resulting in transfer of latex aerosols. This finding suggests that the frequent use of latex products in the dental clinics does contribute to airborne latex particles.

Furthermore, our study has demonstrated that exposure to latex allergens differ by the type of work performed. The Bachelor of Dental Science (BDS) students have shown higher latex concentration than permanently employed personnel. The difference is significant for assistants, auxiliary and administration and not significant for dental staff although the median and maximum levels are higher for BDS students than the dental staff. This difference could be attributed to the fact that students come into contact with patients

throughout the day in the clinics and are directly and more frequently exposed to latex allergens compared to other staff; and dentists who only supervise the dental processes and occasionally wear gloves.

The occupational exposure limits for latex allergens causing sensitisation and symptom provocation are currently not documented in South Africa. An industrial hygiene study reported a high risk potential for triggering allergic reactions when latex levels exceeded 50ng/m^3, with moderate risk potential of 10-50 ng/m^3 particularly to atopics and low risk of allergic reaction at levels less than 10ng/m^3 depending on an individual's immune system (Reiter, 2002; Hunt et al., 2002; Mc Fadden, 2002). Adopting this recommended OEL, the ranges were classified as low (<10ng/m^3), moderate (10-50ng/m^3) and high risk (>50ng/m^3). Our results are similar to those reported by Quirce et al (2004) who also showed a range of moderate to high latex aeroallergen concentrations in ambulances where personnel used powdered latex gloves. Several other studies have demonstrated that a latex level of 0.6ng/m^3 or greater induced occupational respiratory responses (e.g. conjunctivitis, rhinitis and asthma) as well as the development of latex specific antibodies (Baur, 1998; Baur, 2002; Swanson and Ramalingam, 2002). If we were to adopt this cut-off for our study, the risk of personal exposure to latex allergens will be increased 2 to 77 times higher (1.1 to 46.2ng/m^3) than what our current cut-off allows. The study presented here therefore suggests that for individuals such as those in the dental care profession, the latter cut-off which would result in lower limits be applied, thus providing less exposure to latex allergens by these individuals, particularly atopics.

A second aspect of the study examined 95 air samples from work areas of five dental schools for their content of latex allergens. The results of our study show that latex aeroallergens vary considerably among the dental schools (Figure 4.4). The overall latex concentration levels for area samples taken from all participating institutions ranged between below the detection level to 14.97ng/m^3 for hev b 5 allergen. These exposure levels were considerably lower than those reported by Allmers et al., 1998 (0.86 to 49.93 ng/m^3) Swanson et al., 2001 (13 to 121ng/m^3), Baur et al., 2002 (0.4 to 240 ng/m^3).

In general, although the exact number of individuals exposed could not be identified (not all students and personnel participated in the study), the dental clinics of institution A, B and E were identified as having high numbers of staff and students. Institution C and D on the other had reflected lower numbers of staff and students. It is therefore interesting to note that the latex levels of these three institutions were higher with the most significantly raised levels found in Institution B (Figure 4.4). Consequently, the constant and obviously more frequent donning and removal of gloves by a higher number of cohorts could result in the possibility of greater exposure as evidenced in figure 4.4; although the most significant was only observed in institution B. This corroborated with Swanson et al., 1994.

Institution B, the largest of the institutions showed higher exposure levels than institution D which was the lowest. Institution B clinics were also busier during the sampling periods, thus more procedures were performed resulting in more glove changes which may have led to high levels of latex allergens. In addition the ventilation system of institution B was defective during the sampling period which could also explain the high allergen concentrations. Institution E was the only institution with functioning ventilation but did

not show the lowest exposure to aeroallergens. This finding suggest that the ventilation system for Institution E was either not well maintained, however it was difficult to reach a conclusion as the service records were not available during the walkthrough nor were they available at a later stage. It is also possible that the ventilation system was not effective in diluting the particles as the systems were centralized and the dental procedures were performed in cubicles in Institution E. In addition except for the ventilation system, there are other possible determinants of exposure such as work practices (surface cleaning, disposal of gloves) and the frequency of glove usage. The current study aimed to determine if airborne latex particles could be detected in dental schools, and to record the presence and absence of ventilation equipment and the working condition thereof at the time of sampling. In that case the administrative department was included as the control instead of an institution with a functioning ventilation system, as the administration department was expected to have low latex levels since the participants did not use latex products.

In this study an association between aeroallergen levels and the number of gloves used was not investigated as reported by (Baur et al., 2002) and (Swanson et al., 2001). However, our findings suggest that the use of powdered latex products resulted in the presence of detectable allergen levels as most dental schools used powdered products. The recording of gloves would have been ideal and was considered at the initial stage of the study but was not easy to execute due to the minimum number of the study team members who were responsible for setting up the equipment, monitoring the equipment during work operations, recording the sampling data before and after sampling, performing a walkthrough. In addition, the clinic sessions took place in different clinics at different times.with 15-30 students performing tasks per clinic, depending on the number of patients per session. The other reason is that one student or staff changed gloves per patient if

deemed necessary which would complicate the process more. Another option would have been to wait until the session is finished and count the gloves; however this was a risk of exposure to biological hazards as the gloves will be mixed with other potential hazardous substances. In addition, with the infection control awareness programmes at the institutions, most clinics were cleaned after each session and sometimes biological waste was discarded. The whole academic setup in dental clinics was not easy to manage as a result of student groupings, the timetables (sessions versus lectures) and locating participants who gave consent for sampling, thus making it difficult to count the gloves used per participant.

In general, area samples showed lower concentration of hev b 5 allergen than personal samples in our study. It has been reported that area (static) sampling usually collects significantly less allergen than personal sampling (Renstrom, 2002) due to a dilution effect of the environment. This is in contrast to a possible higher recording of allergens in close proximity to individuals handling latex in personal sampling. This may then explain the lower levels found for area sampling in our study.

It is also possible that the capture ELISA kit is not sensitive enough for air samples as the allergen are at too low concentration for detection by this assay. It has also been postulated that latex allergens could adhere to particles >10µm that settles rapidly, which could also influence the detection levels (Brown et al., 2004). The latter however was not proven in our study. The other possible explanation could be that the extraction method used in our study did perhaps not wash out all the latex allergens, and therefore the levels found do not compare well with other studies.

To our knowledge, our study is the first nationally and one of a few globally to adapt the FITkit assay for quantification of airborne latex allergens. Thus the sensitivity of this kit for air samples is not readily available. However, the Health and Safety Laboratory (HSL) in the UK quantified latex allergens from air samples using FITkit in a braiding factory. This study detected hev b 6.02 in 9/14 personal air samples ranging from below DL to 3.1μg/m^3 (equivalent to 3100ng/m^3), which is 67 fold of the results we found. Hev b 1, hev b 3 and hev b 5 were undetectable in this study (Elms et al., 2005). This difference may be explained by the type of setting from where the samples were collected, the source of latex used and the levels of the allergen present in the latex source that could be aerosolised.

The estimation of latex aeroallergens in work areas is a necessary step in the evaluation of latex sensitivity of workers. Information on the levels of airborne latex allergen is necessary to confirm the existence and extent of distribution of the concentrations of allergens. This could develop into determining interventions which would alleviate the indoor aeroallergen exposure, thus reducing sensitisation and improving symptoms in workers who are allergic to latex (Swanson et al., 2001). Air sampling and analysis of the allergens provide an objective means of evaluation for this purpose. Changing to non-powdered or low allergen latex products appears to be the most effective method to control exposure to airborne allergens (Baur, 2002).

Several investigations have demonstrated that powdered latex gloves are the major contributor to atmospheric latex aeroallergens found in many areas of medical centres, especially in dental schools (Allmers et al., 1998; Swanson et al., 2001; Hunt et al., 2002; Schmid et al., 2002). Our findings confirm these previous reports by demonstrating that

powdered gloves are a source of latex in the workplace. In addition, our study has also shown that the dental dams contain much more allergens as compared to the gloves. The dams contain hev b 1 allergen which could not be detected in the gloves; and also higher levels of hev b 3 than hev b 5 as compared to the gloves. The dental dams used in the dental settings are powdered and therefore provide a source of aerosolised latex allergen. However, one limitation of our study is that hev b 1 and hev b 3 allergens were not included in the analysis of air samples due to budget constraints.

There is currently no standardised method for determining the allergenic content of latex materials mainly because the allergens have not been accurately identified (Vandenplas, 1995). Total protein measurements currently serve as a useful indicator of the exposure to latex allergenic proteins (Beezhold, 1996; Audo, 2004). For evaluation of allergic respiratory responses to occupational latex exposure, the information regarding allergen content in medical gloves in use is useful, in addition to quantification of latex aeroallergens (Kujala et al., 2002). Previous research has documented that the total protein levels does not necessarily correlate with allergen content (Lee et al., 2006). However our results have shown a significant correlation (Spearman's, $r = 0.74$, $p<0.001$) between the total protein content and total allergen (Figure 4.5). Nevertheless, there are limitations to the protein assay; and the fact that this method measures all extractable proteins and cannot differentiate between allergenic and non allergenic proteins (Audo et al., 2004) is recognised in the present study.

Previous research has shown that some latex gloves have high total protein content but low NRL allergen concentration and *vice versa* (Palosuo et al., 2002). Other allergens such as cow's milk casein, which is used as stabilisers during the glove manufacturing process,

have been identified in glove extracts (Ylitalo et al., 1999). This casein content was almost half of the total protein (400/1000μg/g) suggesting that the total protein content is not a good measurement for latex allergen content of rubber products (Ylitalo et al., 1999). Our results illustrates that all the samples that were below the DL for total protein (<700μg/g) had total allergen content albeit low, compared to those with high protein content. The finding confirms that measuring protein alone is not useful for latex products with <700μg/g protein per gram of sample. However the results of the present study show a good correlation of total protein content and total allergen content (Fig 4.5). The present study therefore suggests a place for the use of correlating total protein and allergen content in evaluating latex allergen exposure. Alternatively, due to socio-economic reasons the developing countries using latex products could use the protein assay as a screening tool to estimate allergens in the products.

The Food and Drug administration (FDA) have introduced the modified Lowry method (ASTM D5712) to measure the total soluble protein content of gloves and has recommended the cut-off limit of 50μg protein per gram of glove (Charous et al., 2002; Audo et al., 2004). This cut-off value was a consequence of discussions between various regulatory groups (Audo et al., 2004). In the present study, a modification of the Lowry method was used with reduced processing steps thereby saving valuable time and preventing the loss of detectable proteins. The results show that, 7/19 (37%) samples demonstrated proteins <700 μg/g and they fall above 50μg/g range. Future protein studies and technology could look at the current detection limit of 50μg/g. This could make a significant difference in the allergenicity of the product.

In the present study, hev b 1 was not detectable in most of the latex rubber extracts. Similarly, (Palosuo et al., 2007) detected hev b 1 only in a few glove samples in their study. Our results contrast those by (Lee et al., 2006) who demonstrated that hev b 1 was predominant in latex gloves used by medical personnel in Taiwan. The two allergens (hev b 5 and hev b 6.02) that have been previously reported to be the most abundant allergens in gloves (Palosuo et al., 2002; Sutherland, 2002) were present in all the samples in the current study.

Palosuo et al., 2002 reported positive skin test reaction to gloves in latex allergic patients when the sum of four allergens exceeded 1µg protein/g of glove (Palosuo et al., 2002). In the present study all the samples assessed were above this limit with the exception of one (sample 14) which contained 0.67µg/g and this was categorised as low allergenicity in the present study (Table 4.9). Total protein content for the latter was also below the limit of detection. Latex products in the low allergenicity category contain such low levels of the natural rubber allergens that they are suitable not only for non allergenic individuals but also for most of the sensitised users (Wagner et al., 2005).

Sample 7 which demonstrated reduced protein levels also had total protein below the DL. However the total allergen content was 3.5µg/g for this sample which is above the 1µg/g limit, indicative of moderate allergenicity (Palosuo, 2002). This observation confirms previous reports that some products have low extractable proteins but high allergenic proteins (Palosuo et al., 2002).

It is common knowledge that the latex allergen content seems to be higher in powdered gloves than in non-powdered gloves (Kujala et al., 2002). The present study is in

agreement with this by reaffirming that powdered latex samples have high allergen levels than non-powdered latex samples (Figure 4.7). Although non-powdered gloves have lower allergens, they are nevertheless high enough to cause sensitisation with 50% of sample tested reflecting high allergenicity and 33% demonstrating moderate allergenicity (Table 4.9).

Most latex products currently used in dental schools in South Africa are powdered and may contribute to latex aeroallergen load in the dental clinics and may consequently trigger allergic reactions, not only in workers but patients as well. The information gathered during the survey showed that all institutions used both powdered and non-powdered latex gloves simultaneously except institute A which only used powdered latex products at the time of sampling. All the rubber dams were powdered for all institutions. The rubber samples were randomly collected from the working stations were both powdered and non powdered products were stored. It is therefore difficult to state whether powdered or non-powdered gloves were used more often in a specific clinic in order to justify the levels in the air. In addition work practices (e.g. how gloves are put on and removed) also has an impact on the aerosolised latex concentrations. The type of procedure performed may also play a role in airborne latex levels e.g dental rubber dams which showed high allergen content than latex gloves are only used for certain procedures. If the dental dams are not used, not much protein will be released into the air thus low latex levels will be detected at the time and vice versa. It can therefore be concluded that the type of latex product used and whether it is powdered or non powdered do somehow contribute to the aerosolised latex protein, taking into consideration all the other factors such as ventilation, work practices, number of patients seen , number of students or staff, duration of clinic session, size of the clinic etc.

Results from a few studies done in dental schools demonstrated a significant reduction in latex allergy in dental students after switching from relatively high protein, powdered latex gloves to low protein, non -powdered NRL gloves (Liss and Tarlo, 2001; Wrangsjo et al., 2001; Saary et al., 2002; Jones et al., 2004). Another study showed that the use of non-powdered, low protein latex products resulted in reduced levels of airborne latex allergens and was effective in allowing a laboratory worker with latex induced OA to return to work (Liss and Tarlo, 2001).

Generally researchers agree that there may be considerable differences between different brands of gloves, manufacturers and even between gloves of different batches from the same manufacturer (Kujala et al., 2002; Palosuo et al., 2002). The present study has shown the difference between brands of latex products used (Figure 4.8). Brand 3 (dental dams) demonstrated the highest concentration of total allergen content and brand 4 the lowest. Interestingly, sample 14 which was below the limit of 1µg/g for the sum of four allergens is brand 4 which had the lowest concentration and is collected from institution C which had lower airborne latex levels (pg 39 and 46). Sample 7 which declared reduced protein and also showed extractable proteins below the DL is also brand 4. All of these indicate that brand 4 would contain the lowest levels of allergen and extractable protein and is therefore the brand of choice from this study. It has been suggested that powdered latex products are acceptable if the protein content is low, however assessment for development of allergic reactions should be maintained (Charous et al., 2002). These products are often provided to a minority of personnel diagnosed with latex allergy by a healthcare practitioner.

Our study demonstrated that measuring individual allergens using capture ELISA assay is a reliable method for evaluating the allergenicity of latex products which will assist in setting up occupational exposure limits (OELs) for safety purposes which could become a guideline to authorities, manufacturers, industries and consumers in South Africa. Our study also shows that this method is more sensitive than the currently used total protein measurement. This is also supported by other studies (Palosuo et al, 2002; Ylitalo, 1999). Our study has also proven that NRL allergens in the latex materials used in dental schools of South Africa are present at levels high enough to cause NRL allergy.

Adopting a powder-free, low protein policy will provide healthcare workers with a strategy for preventing occupational exposures to latex allergens. NIOSH and OSHA are supporting this recommendation (Lopes et al., 2004). Our findings serve as evidence for a possible requirement to adopt a glove selection and/or powder free policy for South Africa as it has been recommended in other countries such as UK, Canada and Scandinavia and Germany (Potter, 2001). It is suggested that a requirement be that manufacturers should produce latex products with low allergen levels and at least declare the allergen levels on the package inserts. The challenge is for healthcare administrators to purchase low allergen products thus decreasing the overall rates of sensitisation in workers using latex products.

Research data has also indicated that changing to low allergen gloves alone will not be sufficient to create a latex safe environment because of other sources of latex, such as transfer of allergen from less restricted sites, either on clothing, footwear, ventilation ducts, or from equipment moved from one room to another (Sri-Akajunt et al., 2000; Hunt et al., 2002; Heilman et al., 1996; Mitakakis et al., 2002). Therefore, workers should be educated

on the safety, care and clinical practices. Management should also be aware of the importance of maintenance of the ventilation systems.

Previous studies suggested methods for measuring NRL allergen levels in air samples and latex rubber products. However, most methods are based on human IgE based reagents which cannot be standardised due to variable antibody reactivity of given pool sera and/or lack of standardised allergens (Palosuo et al., 2002; Koh et al., 2005). The IgE based assays includes RAST inhibition assay, CAP system and SDS-PAGE immunoblotting (Swanson et al., 1994; Swanson et al., 2001; Baur 2002). Using these techniques is also limiting because human serum containing IgE antibodies to hev b 1 and hev b 3 is scarce (Palosuo et al., 2002). Several clinically relevant allergens can now be measured by immunoassays using monoclonal antibodies and recombinant allergens. These methods are specific, sensitive and can be standardised (Palosuo et al., 2002). It should be noted that, except for hev b 6.02, little is known about the molecular forms of proteins that can resist the glove manufacturing process and retain their allergenicity (Palosuo et al., 2002; Tomazic-Jezic and Lucas, 2002).

The present study acknowledges several limitations which include the inadequate sample size of the latex rubber samples (which was due to budget constraints). However, this will be addressed in a future study which will be conducted in a large healthcare organization of South Africa. Another limitation to the study is that total airborne dust measurements were not done as in the study by Kujala et al., 2002.

5.1 CONCLUSION

The air sampling method and ELISA capture based assay used in this study offer the means of evaluating airborne allergen concentrations. However, the kit is not sensitive at lower concentrations and further research would be required to pursue techniques that will increase the sensitivity of the kit, particularly for air samples. Alternatively, similar methods which are highly sensitive may be developed and the extraction methods be improved. The airborne latex concentrations are dependant on the type of products used, and perhaps the frequency of glove changes in busy clinics during the time of sampling. In addition, the type of set-up and the duration of sampling also determine the airborne latex concentration.

The capture ELISA immunoassay offers a means to reliably assess the allergen content of various latex products and could eventually be used to set up exposure limits for other latex products thus reducing exposure to potentially allergenic proteins. The total protein content determination on the other hand is neither specific nor accurate enough to draw conclusions on the safety of latex products. However, because of the significant correlation yielded between the capture ELISA and total protein assay, the protein assay can be applied as a screening tool for estimating the allergenicity of latex products in the developing countries as it is more cost-effective. It is evident from this study that NRL products containing allergenic levels high enough to cause latex allergy is still being used in the South African market. The initiative to use non-powdered low protein latex gloves, if not synthetic products (non-latex) is therefore poorly supported. Hev b 3 which was found at high levels in dental dams should be considered in future aerosol measurements in dental settings.

APPENDICES

A. Building walk-through checklist

B. Ethical clearance certificate, Human Research Ethics Commiteee (University of the Witwatersrand)

C. Letter of permission to conduct the study by Gauteng Provincial government research committee.

D. Ethical clearance certificate, Faculty of Health Sciences Research Ethics Committee (University of Pretoria)

E. Letter of approval to conduct the study, Biomedical Research Ethics Committee (Nelson R Mandela school of Medicine, University of KwaZulu-Natal)

F. A copy of approval to conduct the study (University of the Western Cape)

REFERENCES

Allmers H, Brehler R, Chen Z, Raulf-Heimsoth M, Fels H, Baur X. Reduction of latex aeroallergens and latex specific IgE antibodies in sensitized workers after removal of powdered natural rubber latex gloves in a hospital. J Allergy Clin Immunol 1998; 102: 841-46.

Allmers H, Schmengler J, John SM. Decreasing incidence of occupational contact urticaria caused by natural rubber latexallergy in German healthcare workers. J allergy Clin Immunol 2004 ; 114: 347-51.

Amr S, Bollinger ME. Latex allergy and occupational asthma in Health Care Workers: Adverse outcomes. Environ Health Perspect. 2004; 112: 378-381.

Audo C, Barbara J, Chabane H, Armange M, Leynadier F. The en 455-3 modified Lowry assay does not yield a reliable estimate of the allergenicity level of latex gloves with low total protein content. Med Sci Monit 2004; 10 (7): 181-186.

Baur X, Chen Z, & Allmers H. Can a threshold limit value for natural rubber latex airborne allergens be defined? J Allergy Clin Immunol 1998; 101: 24-27.

Baur X. Measurement of airborne latex allergens. Methods 2002; 27: 59-62.

Beezhold D, Swanson M, Zehr BD, Kostyal D. Measurement of Natural Rubber proteins in Latex glove extracts: comparison of methods. Ann Allergy Asthma Immunol 1996; 76: 520-526.

Bernstein DI, Biagini RE, Karnani R, Hamilton R, Murphy K, Bernstein C, et al. In vivo sensitization to purified hevea brasiliensis proteins in healthcare workers sensitized to natural rubber latex. J Allergy Clin Immunol 2003; 101:24-27.

Brathwaite N, Motala C, Toerien A, Schinkel M, Potter PC. Latex allergy-The Red cross Children Hospital Experience. SAMJ 2001; 9 (9):750-751.

Charous BL. Latex allergy: a new and common problem. Am fam physician 1998; 57 (1): 42-47.

Crippa M, Belleri L, Mistrello G, Carsana T, Neri G, Alessio L. Prevention of latex allergy among healthcare workers and in general population: latex protein content in devices commonly used in hospitals and general practice. Int Arch of Occ and Envir health 2006 August; 79 (7):550-557.

De Beer C, Cilliers J,, Truter EJ, Potter PC. Latex Gloves: More harm than good. Medical Technology SA 1999; 13 (1): 282-288.

De Beer C, Cilliers J. Identification and management of healthcare personnel affected by latex allergy. Medical Technology SA 2001; 15 (2): 339-344.

Elms J, Fishwick D, Naylor S, Tate P, Bradshaw LM, Henson M, et al. Sensitization and work related respiratory symptoms in workers at a braiding factory. Occupational asthma:

epidemiology and diagnostics 2005 Sep 18, poster presentation. http://www.hsl.gov.uk/cwh/poster4.pdf

Finkelstein MM, Verma DK. Exposure estimation in the presence of nondetectable values: another look. AIHA 2001; 62:195-198.

Fish JE. Occupational asthma and rhinoconjuctivitis induced by natural rubber latex exposure. J. Allergy Clin. Immunol, 2002; 110: S75-81.

FITkit™. Novel tools for detecting protein allergens in latex gloves. The Rubber International Magazine 2002 September; 4 (45):37-42.

Heilman DK, Jones RT, Swanson MC, Yunginger JW. A prospective, controlled study showing that rubber gloves are the major contributor to latex aeroallergen levels in the operating room. J Allergy Clin Immunol 1996; 98: 325-330.

Hnizdo E, Esterhuizen TM, Rees D, Lalloo UG. Occupational asthma as identified by the Surveillance of Work-related and Occupational Respiratory Diseases programme in South Africa. Clin Exper Allergy 2001; 31(1):32-39

Hornung RW, Reed LD. Estimation of average concentration in the presence of nondetectable values. App. Occup. Environ. Hyg 1990; 5: 46-51.

Horwitz IB, Kammeyer-Mueller JD. Natural rubber latex allergy workers compensation claims:Washington state healthcare workers, 1991-1999. App. Occup. Environ. Hyg 2002; 17 (4): 267-275.

Hunt LW, Fransway AF, Reed CE, Miller LK, Jones RT, Swanson MC, et al. An epidemic of occupational allergy to latex involving health care workers. JOEM 1995 October; 37 (10): 1204-1209.

Hunt LW, Kelkar P, Reed CE, Yunginger JW. Management of occupational allergy to natural rubber latex in a medical centre: The importance of quantitative latex allergen measurement and objective follow-up. J Allergy Clin Immunol 2002; 110: S96-106.

Jones KP, Rolf S, Stingl C, Edmunds D, Davies BH. Longitudinal study of sensitization to natural rubber latex among dental school students using powder-free gloves. Ann Occup Hyg 2004; 5: 455-457.

Kalman CJ. The NRL precedent ? Occupational Medicine 2005; 55: 402.

Katelaris CH, Widmer RP, Lazarus RM. Prevalence of latex allergy in a dental school. MJA 1996; 164: 711-714.

Koh D, Ng V, Leow Y-H, Goh CL. A study of natural rubber latex allergens in gloves used by healthcare workers in Singapore. Contact Dermatitis and Allergy 2005; 153: 954-959.

Kujala V, Alenius H, and Palosuo T, Karvonen J, Pfaffli P, Reijula K. Extractable latex allergens in airborne glove powder and in cut glove pieces. Clin. Exp. Allergy 2002; 32: 1077-1081.

Lee M-F, Chen Y-H, Lin H-C, Wang H-L, Hwang G-Y, Wu C-H. Identification of Hevamine and hev b1 as major latex allergens in Taiwan. Int Arch Allergy Immunol 2006; 139: 8-44.

Lindberg M, Silverdahl M. The use of protective gloves and the prevalence of hand eczema, skin complaints and allergy to natural rubber latex among dental personnel in the county of uppsala, Sweden. Contact Dermatitis 2000; 43: 4-8.

Liss GM & Sussman GL. Latex sensitisation: Occupational versus general population prevalence rates. Am.J.Ind.Med 1999; 35: 196-200.

Liss MG., Tarlo SM. Natural rubber latex-related occupational asthma: association with interventions and glove changes over time. American Journal of Industrial medicine 2001; 4: 47-353.

Lopata AL, Kirstein F, Adams S, Jeebhay MF, Raulf-Heimsoth M. Occupational allergy to latex among loom tuners in a textile factory. International Archives of Allergy and Clinical Immunology 2007; 144 (1): 64-68.

Lopes RAM, Benatti MCC, Zollner RL. Occupational exposure of Brazilian neonatal intensive care workers to latex antigens. Allergy 2004 ; 59: 107-110.

Mc Fadden ER. Natural rubber latex sensitivity seminar: conference Summary. J.Allergy Clin Immunol 2002 August: S137-140.

Mitakakis TZ, Tovey ER, Yates DH, Toelle BG, Johnson A, Sutherland MF, et al. Particulate masks and non-powdered gloves reduce latex allergen inhaled by healthcare workers. Clin. Exp. Allergy 2002; 32: 1166-1169.

National Agency for Medicine. Natural Rubber allergen content of latex gloves. A market surveillance study. TLT-INFO1/2003. www.nam.fi/uploads/julkaisut/TLT_info_1_03_engl_04-03-04.pdf

Palosuo T, Alenius H, Turjanmaa K. Quantitation of latex allergens. Method 2002; 27: 52-58.

Palosuo T, Reinikka-Railo H, Kautiainen H, Aleniius H, Kalkkinen N, Kulomaa M, et al. Latex allergy:the sum quantity of four major allergens shows the allergenic potential of medical gloves. Allergy 2007, .62:781-786.

Petsonk EL. Work related asthma and implications for the general public. Environ Health Persect 2002.; 110 (4 suppl): 569-572.

Poley GE, Slater JE. Latex allergy. J Allergy Clin Immunol. 2000; 105: 1054-1062.

Potter PC. Latex allergy and the Law, SAMJ 1998 March; 88(3): 278.

Potter PC. Latex allergy-Time to adopt a powder free policy nationwide. SAMJ 2001 September; 91(9):746-748.

Potter PC, Crombie I, Marian A,Kosheva O, Maqula B, Schinkel M. Latex allergy at Groote Schuur hospital-prevalence, clinical features and outcome, SAMJ 2001; 91(9): 760-765.

Poulos ML, O'Meara TJ, Hamilton RG, Tovey ER. Inhaled latex allergen (Hev b1). J Allergy Clin Immunol 2002; 109:701-706.

Pretorius E, Bester MJ, Becker PJ. Suspected latex allergy within a South African population of health care professionals: a need for guidelines. South Afr J Epidemiol Infect 2001; 16 (2, 3):67-70.

Quirce S, Escudero C, Swanson MC, Martinez-Aramayona MJ, Fernandez-Nieto M, Sastre J. Latex aeroallergen concentrations in ambulances. J Allergy Clin Immunol. 2004. 114 (4,): 978-979.

Raulf-Heimsoth M, Jappe U, Yeang H-J, Arif SAM, Rihs HP, Lopata AL, Jeebhay MF, Brüning TH. Relevance of carbohydrate determinants in differentiating true latex allergy from asymptomatic IgE reactivity. Allergy and Clinical Immunology International (In Press).

Reiter JE. Latex sensitivity:An Industrial hygiene perspective. J Allergy Clin Immunol 2002, 110: S121-128.

Renstrom A. Exposure to airborne allergens: a review of sampling methods. J. Environ. Monit. 2002; 4: 619-622.

Rihs HP, Raulf-Heimsoth M. Natural rubber Latex allergens: characterization and evaluation of their allergenic capacity. New Horizon-Allergy 2003; 13 (1): 282-288.

Saary MJ, Kanani A, Alghadeer H, Holness DL, Tarlo SM. Changes in rates of natural rubber latex sensitivity among dental students and staff members after changes in latex gloves. J Allergy Clin Immunol 2002; 109:131-135.

Schmid K, Broding HC, Niklas D, Drexler H. Latex sensitisation in dental students using powder-free gloves low in latex protein:a cross sectional study. Contact dermatitis 2002; 47: 103-108.

Smith AM, Amin HS, Biagini RE, Hamilton RG, Arif SAM, Yeang HY, et al.. Percutaneous reactivity to natural rubber latex proteins persists in health-care workers following avoidance of natural rubber latex. Clinical and experimental allergy 2007; 37: 1349 -1356.

Sri-Akajunt N, Sadhra S, Jones M, Burge PS. Natural rubber latex aeroallergen exposure in rubber plantation workers and glove manufacturers in Thailand and healthcare workers in a UK hospital. Ann Occup Hyg 2000; 44:79-88.

Surveillance of Occupational Respiratory Diseases in South Africa ALERT. *Latex Allergy: Prevention and management in the workplace.*1998: National Centre for Occupational Health.

Sussman GL, Liss GM, Deal K, Brown S, Cividino M, Siu S, Beezhold DH, et al. Incidence of latex sensitization among latex glove users. J. Allergy Clin. Immunol 1998; 101: 171- 178.

Sutherland MF, Drew A, Rolland JM, Slater JE, Suphioglu C, O'Hehir RE et al. Specific monoclonal antibodies and human immunoglobin E show that hev b 5 is an abundant allergen in high protein powdered latex gloves. Clin. Exp Allergy 2002; 32: 583-589.

Swanson MC, Bubak ME, Hunt LW, Yuginger JW, Warner MA, Reed CF. Quantification of Occupational latex aeroallergens in a medical center. J allergy Clin Immunol 1994; 94: 445 - 451.

Swanson M, Zakharov S, Luss L, Babakhin A, DuBuske L. Latex aeroallergen quantification in hospitals of Moscow, Russia. Ann. Allergy Asthma Immunol 2001 October; 87 (4): 307-310.

Swanson MC, Ramalingam M. Starch and natural rubber allergen interaction in the production of latex gloves: A hand held aerosol. J Allergy Clin Immunol 2002; 110: S15-20.

Tarlo SM, Sussman LG, Holness DL. Latex sensitivity in dental students and staff: A cross-sectional study. J Allergy Clin Immunol 1997; 99: 396-401.

Tomazic-Jezic V, Lucas AD. Protein and allergen assays for natural rubber latex products. J allergy Clin Immunol 2002; 110: S40-46.

Vandenplas O, Delwiche J-P, Sibille Y. Occupational asthma due to latex in a hospital administrative employee. Thorax 1996; 51: 452-453.

Vandenplas O, Jamart J, Delwiche J-P, Evrard G, Larbanois A. Occupational asthma caused by natural rubber latex: Outcome according to cessation or reduction of exposure. J allergy Clin Immunol 2002; 109: 125-130.

Wrangsjo K, Wallenhammar L-M, Ortengren U, Barregard L, Andreasson H, Bjorkner B, et al. Protective gloves in Swedish dentistry: use and side effects. British Journal of DermatologY 2001; 145:32-37.

Wagner S, Breiteneder H. *Hevea brasiliensis* latex allergens: current panel and clinical relevance. Int. Arch. Allergy. Immunol, 2005; 136:90-97.

Yeang H-Y. Natural rubber latex allergens: new developments. Curr Opin Allergy Clin Immunol 2004; 4: 99-104.

Yeang H-Y, Arif SAM, Raulf-Heimsoth M, Loke Y-H, Sander I, Sulong S-H. et al. Hev b 5 and hev b 13 as allergen markers to estimate the allergenic potency of latex gloves. J Allergy Clin Immunol 2004; 114:593-598.

Yeang H-Y, Hamilton RG, Bernstein DI, Arif SAM, Chow K-S, Loke Y-H, et al. Allergen concentration in natural rubber latex. Clinical and Experimental Allergy 2006; 36:1078-1086.

Ylitalo L, Mäkinen-Kiljunen S, Turjanmaa K, Palosuo T, Reunala T. Cow's milk casein, a hidden allergen in natural rubber latex gloves. J Allergy Clin Immunol 1999 July; vol. 104 (1):177-180.

Personal communication references

Ms Zubaydah Kirsten, Immunology Microbiology Section, National Institute for Occupational Health;

Contact: zubaydah.kirsten@nioh.nhls.ac.za; 011 712 6448/6400

Mr Braimoh Bello, Epidemiology and Surveillance Section, National Institute for Occupational Health;

Contact: braimoh.bello@nioh.nhls.ac.za; 011 712 6438/6400