The Prevalence of Occupational Asthma and Rhinitis among Animal Laboratory Workers at Health Sciences Laboratory Animal Services (HSLAS), University of Alberta;

A cross-sectional Study

by

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Abstract

Introduction: Laboratory animal allergy (LAA) is a recognised occupational health problem that can cause significant morbidity among exposed workers and imposes a burden on employers and industry. Since first described, many laboratories have introduced preventive measures to reduce the risk of allergy occurring, and the types of animals kept within many laboratories have also changed. Consequently, there is a need for up-to-date data regarding the epidemiology of LAA.

Objectives: To study the prevalence of sensitization, occupational asthma and rhinitis to laboratory animal and to evaluate the association of potential work place risk factors in developing laboratory animal allergy at Health Sciences Laboratory Animal Services **(**HSLAS), University of Alberta.

Methods: Animal husbandry staff (Group 1) and researchers (Group 2) were recruited from HSLAS together with graduate students not working with animals (Group 3). Sensitization was evaluated using skin prick tests (SPT) to laboratory animal allergens. Information on respiratory symptoms, atopy, current job tasks, job history and demographic information were recorded using a standardized questionnaire. A skin prick test was considered positive if it caused a wheal ≥3mm diameter. Work-related asthma or rhinitis was defined as relevant symptoms reported to be worse at work or better on vacation plus positive SPT to a relevant laboratory animal allergen.

Results: The three Groups comprised of 57 ; 57 and 50 subjects. Among Group 1, 86% and 82% were working with mice and rats at the time of study and of those 27% and 57% were sensitized to mice and rats respectively. In Group 2 the number of exposed was lower with 13% and 54% sensitized to mice and rats while no one in Group 3 was sensitized to either mice or rat. Overall prevalence of occupational asthma and rhinitis to mice or rats was 15% and 28% respectively among the currently exposed Group. Atopy and several job tasks including animal sacrifice, shaving fur, injection and manual cage-cleaning were significantly associated with sensitization, occupational asthma or rhinitis.

Conclusion: In spite of all the control measures and preventive modifications implemented in laboratory environments in the last several decades, laboratory animal allergy remained prevalent among exposed laboratory workers at HSLAS at the University of Alberta. Greater attention should be paid to those exposures occuring during the tasks identified as high risk in this study so as to prevent future health problems in laboratory animal workers.

Preface

This thesis is an original work by Neda Dianati Maleki. The research project, of which this thesis is a part, received research ethics approval from the University of Alberta Research Ethics Board, Project Name: “The Prevalence of occupational Asthma and Rhinitis among Animal Laboratory Workers at HSLAS, University of Alberta; A cross-sectional Study”,

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Dedication

To Farangis and Mozafar, my lovely parents,

Navid and Vafa, my dear brothers

and, to

Arash, my amazing husband and best friend.

Acknowlegement

I would like to express my deepest gratitude to my supervisor Dr. Jeremy Beach for his support and wise mentorship as well as his patience and understanding throughout this project.

I would also like to extend my sincere thanks to the members of supervisory committee, Drs. Nicola Cherry, Harissios Vliagoftis as well as the external examiner Dr. Ambikaipakan (Sentil) Senthilselvan, for their valuable guidance and insightful comments.

And finally I would like to thank all the HSLAS staff and students who helped me by participating in this study.

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Abbreviations

ATS-DLD-78	American Thoracic Society- Division of Lung Disease questionnaire
FEV1	Forced expiratory volume in 1 second
HMW	High molecular weight allergen
HSLAS	Health Sciences Laboratory Animal Services
ICF	Informed consent form
IgE	Immunoglobulin E
IUATLD	International Union against Tuberculosis and Lung Disease questionnaire
LAA	Laboratory animal allergy
LMWA	Low molecular weight allergen
MOUSE_OA	Occupational asthma due to mouse exposure
MOUSE_OR	Occupational rhinitis due to mouse exposure
MSA	Mouse serum albumin
MUP	Mouse urine protein
NPV	Negative predictive value
OA	Occupational Asthma
OR	Occupational Rhinitis
PPE	Personal protective equipment
PPV	Positive predictive value
RAT_OA	Occupational asthma due to rat exposure
RAT_OR	Occupational rhinitis due to rat exposure
SPT	Skin prick test
STATA	A statitical Software package
SOB	Shortness of breath
WRCS	Work-related chest symptoms

Chapter One: Background and Formulation of Objectives

1.1 Introduction

Laboratory animals particularly rodents are an important component of biomedical and pharmaceutical research. In US alone, more than 25 million vertebrate animals are estimated to be used in research, testing and education annually. (1) Globally, estimates range from tens of millions to 100 million vertebrates or more per year. (2) Approximately 97% of all animals used in research are mice and rats. (3) Not long after the use of laboratory animals became an integral part of biomedical research, the first reports of allergy and asthma in research technicians and scientists were reported. Laboratory animal allergy (LAA) is now a well-known and common occupational allergic condition seen among laboratory animal workers. Exposure to laboratory animals occurs in two primary settings: Animal husbandry facilities and research laboratories. All workers exposed to laboratory animal allergens in these two settings, including animal husbandry technicians, researchers, cleaning staff, veterinarians and even administrative staff working within the facilities, are at risk of developing LAA.

LAA is caused by the activation of an immune response against animal allergens, which leads to production of specific immunoglobulin E (IgE). Laboratory animal allergens are high-molecular weight molecules, mainly proteins and glycoproteins found in animal

saliva, dander and urine. Exposure to these molecules occurs mainly through inhalation of the animal allergens. These allergens are carried on small particles that are capable of remaining airborne for extended periods and therefore have the potential of penetrating exposed laboratory personnel's lower airways. Inhalation of the allergens then leads to activation of immune system and development of allergic symptoms including allergic asthma and rhinitis.

Occupational allergy and asthma can cause significant morbidity among workers at risk and may pose a financial burden on employers and industry. During decades, several control measures and preventive modifications has been proposed or made obligatory in laboratory environments. These include exposure control methods, equipment performance testing and education plus significant changes in facility designs and operations to minimize the exposure to animal allergens. In addition, health surveillance systems have been established as a means of secondary prevention. Considering all these actions, there is a quite apparent need for up-to-date data regarding LAA in the new settings to re-assess the epidemiology of LAA in comparison to available literature and to evaluate the efficiency of implemented work-place modifications.

1.2 History of Occupational Medicine and Laboratory Animal Allergy

It is worthwhile to briefly review the history of occupational medicine as well as laboratory animal asthma. Many know Bernardino Ramazzinni as the founder and the father of occupational medicine. An Italian physician who made the greatest contribution to the occupational medicine in the 17th century by describing and characterizing the common conditions workers were suffering from. He regularly asked his patients about the kind of work they did and suggested that all physicians do the same. He collected his observations from visiting work places and observing worker's activities in *De Morbis Artificum Diatriba* or Diseases of Workers, which was published in 1700 in Italy. De Morbis Artificum Diatriba is a comprehensive work on occupational diseases outlining the health hazards of chemicals, dust, metals, and other agents encountered by workers in 52 occupations. Each chapter contains a description of the disease associated with a particular work activity followed by a literature analysis, workplace description, disease description, possible remedies, and advice. (4, 5) His emphasizing the importance of collecting a work history in every patient paved the way for recognizing many occupational diseases, including LAA.

Similarly, Jack Pepys is recognized by many in the world of occupational medicine and allergy as the father of Occupational Asthma. Pepys was a British allergist who had himself suffered from severe atopic eczema since infancy and thus he developed an interest in clinical allergy and occupational allergic conditions. During his career he

discovered much about the causes and pathophysiology of farmers' lung. He also documented clinical features, natural history and immunology of allergic broncho-pulmonary aspergillosis known as ABPA. He then pioneered allergen challenge tests of the skin and the lung and for the first time was able to provoke asthma under controlled conditions in his clinical laboratory. (6) Finally he identified numerous causes of occupational asthma and eventually his work led to recognition of occupational asthma as a compensable condition. (7, 8)

Laboratory animal allergy was first recognized in United Kingdom more than 40 years ago (9). In 1976, the British Society for Allergy and Clinical Immunology published preliminary survey results indicating that 23% of 474 participating animal workers experienced one or more symptoms consistent with LAA. (10) However the percentages were noted to be greater than the contemporary reports from United States where the prevalence of LAA was estimated to be about 15%. (11) Later in the 1980s, detailed reports from cross-sectional studies of pharmaceutical companies' laboratory workers in the UK confirmed a prevalence of 19 – 30 % of LAA and about 10% of occupational asthma among the workers. (12-15) Several decades have passed after the first recognition of LAA as a common occupational condition. Today, with increased awareness among both employers and workers and introduction of relevant legislation in different countries to prevent the occurrence of LAA, it is still unclear how much impact there has been on the prevalence and incidence of LAA.

1.3 Definition of Occupational Asthma

The definition of occupational asthma (OA), much like the definition of asthma itself, has changed over the years but the current consensus definition is as follows:

> "Occupational asthma" is a disease characterized by variable airflow limitation and/or airway hyper-responsiveness due to causes and conditions attributable to a particular occupational environment and not to stimuli encountered outside the workplace. (16)

Two types of occupational asthma are distinguished by whether or not they appear after a latency period: (17-19)

1- Immunologic OA, which is characterized by the presence of a latency period before the onset of symptoms. This type of OA can occur in reaction to either: (a) high- or low molecular weight allergens for which an IgE-related immunologic mechanism has been identified. Or (b) agents (e.g., western red cedar) for which a specific immune mechanism has not been identified.

2- Non-immunologic OA also known as irritant-induced asthma or reactive airways dysfunction syndrome (RADS), which may occur after single or multiple exposures to nonspecific irritants at high concentrations.

Work-aggravated asthma is the terminology often used to refer to pre-existing or concurrent asthma that subjectively worsens in the workplace. This can include an increase in frequency or severity of symptoms, an increase in medication required to control symptoms, or clinical improvement when exposures are reduced or eliminated.

While this form of asthma is considered work-related, it is not considered true occupational asthma by many, since by definition it is a reactivation or exacerbation of a pre-existing non-occupational asthma. It can be very challenging to differentiate work-aggravated-asthma from true occupational asthma.

1.4 Animal allergens:

Occupational asthma and allergy can be caused by various high molecular-weight (HMW) and low molecular-weight (LMW) allergens found in work places. HMW allergens are complex mixtures of polypeptides including, animal proteins, flour, latex, etc. The LMW category includes isocyanates, acrylates, amines, wood dust, and metals. In this manuscript the focus will be on high molecular weight animal-derived allergens encountered in animal laboratories.

Allergens have diverse biologic functions and may be enzymes, enzyme-inhibitors, lipid-binding proteins, lipocalins or regulatory or structural proteins.

Lipocalins represent the most important Group of animal inhalant allergens. Except for cats, the majority of all mammalian–derived major allergens including major allergens of mice, rats, dogs and even horses and cows, belong to the lipocalin Group of proteins. (20-22) Lipocalin allergens have also been detected in insects. Although not detected in skin or secreted in salivary glands, at least three minor lipocalin allergens have been described recently in cats by Smith et al. (23)

Lipocalins are small extracellular proteins found in dander and secretions of the majority of mammals as well as other vertebrates, plants and bacteria. (24) They are characterized by a specific three-dimension protein structure however their function is still unknown. (25) It is also unclear how they interact and bias the immune system to an allergic response.

The most common causes of laboratory animal allergy are mouse and rat allergens not because other animals are necessarily less allergenic but primarily because mice and rats are used most commonly in animal laboratories. In fact the results of a large epidemiologic study by Aoyama et al. in Japan suggested animal workers reported a higher incident rate of allergic symptoms to rabbits and guinea pigs than mice and rats after correcting for numbers exposed. (26)

1.4.1 Mouse

Mouse allergens were first identified over 2 decades ago. To date three major allergens have been identified in Mouse skin, serum, and urine (27-29):

(1) A 19 kilo-Dalton (kDa) molecule known as mouse urinary protein (MUP) or Mus_m_1, is a lipocalin found in mouse urine as well as hair follicle and dander. Mus_m_1 is a pre-albumin produced in mouse liver, circulates in the bloodstream, and is cleared by the kidneys.

(2) A 17 kDa protein molecule known as Mus_m_2 that is found in mouse hair and dander but not urine.

(3) A 67 kDa protein, identical to mouse serum albumin (MSA)

Hair and epithelial fragments also carry allergenic molecules, which are primarily derived from urine and saliva. Some individuals get sensitized predominantly to MSA, some to the smaller allergen MUP, while some other may react to both allergens.

(29)Approximately 30% of mouse-sensitized individuals are sensitized to MSA. (30) The concentration of the major allergens from mouse, including MSA and MUP, vary in urine, serum, and pelts of mice. (31) Male rodents excrete higher levels of urinary allergens, about 4 times higher than female rodents, mainly because the gene expression is testosterone dependent. (22) As rodents have continuous dribbling of urine with persistent proteinuria, the allergen is constantly present in their urine. They spray urine on their surroundings, where the proteins dry up and become airborne on dust particles. It is therefore not surprising that several observational studies have shown working with male rodents to be associated with higher risk of sensitization. (32)

1.4.2 Rat

As described by Bayard et al. two major rat allergens have been identified. (33)

(1) Rat_n_1A, a 20 kDa molecule initially thought to be a pre-albumin, but later found to be an isoform of the Alfa2-euglobulin family of rat proteins synthesized in the liver through an androgen-dependent pathway.

(2) Rat_n_1B, a 16 kDa molecule, a member of the lipocalin superfamily, as well as another isoform of alfa2-euglobulin molecule. It is known as major rat urinary protein since it is quantitatively the major protein in the urine of a fertile male rat. It constitutes approximately 30% of the total protein content excreted in the urine and has been reported to be the most allergenic rat protein. It is produced not only in the liver but the salivary and other exocrine glands where its production is not androgen-dependent.

As in mice, rat albumin also possesses some allergenic activity, with about 24% of rat-allergenic individuals manifesting sensitivity to albumin. (30)

1.4.3 Rabbits and Guinea pigs

The antigenic allergens in rabbits have not been fully characterized but at least two antigens, Ory_c_1 and Ory_c_2 have been described and are found in saliva, hair and dander (34-36). Ory_c_1 is a glycoprotein with molecular mass of 17-18kDa and is absent from rabbit urine(36). Ory_c_2 has a molecular mass of 21kDa and has not been completely characterized. (37)

Similarly two guinea pig antigens, Cav_p_1 and Cav_p_2 from lipocalin family have been identified and detected in animal's urine, hair and dander. (34, 38, 39)

1.4.4 Cats and Dogs

Although cats and dogs are more often kept as domestic pets, occupational exposure and hence allergies to theses animals is not uncommon among laboratory animal workers. Among 12 allergens detected in cats, the Fel_d_1, a 38 kDa protein, is the most allergenic one. Fel_d_1 is produced primarily in cat sebaceous glands and then secreted onto the skin and fur. It is also produced to a lesser extent in salivary glands and thereby can be detected in cat saliva. Similar to Mouse Mus_m_1, Fel_d _1 production appears to be androgen dependent since male cats excrete more of the antigen and castration has been shown to decrease the production of the protein. Cat albumin is the second major

allergen and reportedly 20 percent of individuals allergic to cats, react to cat albumin extracts.(40-44)

Two immunologically distinct major dog antigens include:

(1) Can_f_1, a 25 kDa molecule and

(2) Can_f_2 a 19 kDa protein molecule

Although unclear whether through primary sensitization or cross-reactivity, at least a quarter of dog-allergic individuals, have been shown to exhibit sensitivity to dog albumin molecule. (45)

1.5 Prevalence and Incidence of Laboratory Animal Allergy:

From the 1960s and 1970s onwards, numerous reports of laboratory animal workers suffering from allergic respiratory conditions have been published. Today, half a century later, occupational asthma is considered the most common occupational lung disease in the industrialized world and it is thought to account for 9-15% of asthma in adults of working age (46).

Although still controversial, it is generally thought that over the past 40 years, the prevalence of asthma and atopic diseases in general have been increasing but have reached a plateau in recent years. In contrast, data regarding the trend of occupational asthma and rhinitis over the same period has been scarce, controversial and at times even seemed contradicting. In the last couple decades, several studies showed the exposure to laboratory animals could be reduced (47) but despite this, estimates of prevalence and incidence indicate a mixed pattern. While some believe there is no definite evidence to indicate that the incidence of LAA (including OA and OR) is falling (48), several others observed that reduced exposure helped lower the prevalence (49, 50) and incidence (51).

Multiple factors may have impacted estimates of incidence including different reporting criteria, changing exposures, increased awareness and implementation of protective measures at work places. However, while they have been suggested, it remains unclear if new work practices or changing technologies have affected the exposure level and the prevalence and incidence of LAA (52).

In a comprehensive review of literature in 2008, Folletti et al attempted to answer the question regarding the trend of the prevalence of LAA in the last 2-3 decades. They summarized the result of 15 cross-sectional and 4 longitudinal studies. Across the 15 prevalence studies reviewed by Folletti et al, the response rate was between 61-100%, limiting the generalizability of the data. (52) Overall 82 and 70% of the participants were exposed to rats and mice respectively. It was found that the prevalence of OA when defined as work-related chest symptoms (WRCS) ranged from 2.2 to 11.7% and when defined as WRCS and positive SPT to at least one laboratory animal, it was 1.4 to 9.5%. The prevalence of OR defined as work-related nasal symptoms (WRNS) ranged from 6.7 to 41.7% and when defined as WRNS plus positive SPT, it was 2.9 to 18.8%.

The four longitudinal studies included in the Folletti et al review, had evaluated the incidence of OA and OR between 1983 and 2005, of which two were carried out in the UK.

In a one-year follow up of 148 exposed LA workers, published by Davies et al, the incidence of symptoms of OA and OR per 100 person-years was 2.0 and 10.1 respectively. The incidence rates dropped to 1.4 and 4.7/100 person-years based on symptoms and positive serum IgE to laboratory animals, respectively (53).

Cullinan et al. studied the incidence of OA in a cohort of 342 laboratory animal workers with participation rate of 80% with prior exposure to rats from one month to four years. They reported an incidence of WRCS of 3.5/100 person-years, and 1.6 /100 person-years when based on symptoms and SPT (54).

Elliot et al followed 495 exposed laboratory animal workers for 12 years and found an estimated incidence rate of LAA of 2.3/ 100 person-year. Of these 87.8% had developed OR (2.0/100 person-years) and 18.3% had developed OA (0.4/100 person-years). They had defined OR and OA as symptoms plus positive SPT to laboratory animals. They also noted that the incidence e of LAA increased with hours of exposure to laboratory animals or cages (55).

Finally a study of 373 Canadian apprentices in animal health technology estimated that the incidence of probable OA was 2.7/ 100 person-year. However, OA was defined as a positive SPT to one or more laboratory animal allergens and a positive methcholine challenge test i.e. >3.2-fold decrease in the provocative concentration causing a 20% reduction in FEV1 (PC20) (56). Folletti et al. concluded that despite prior reports indicating a mixed pattern, according to their review, the prevalence of OA was declining at a rate of -1.6% per 10 years. Their results on the trend of prevalence of OR was inconclusive.

The review by Folletti et al was not a systematic review and therefore did not include all the published reports to date. Numerous other studies have looked into the prevalence and incidence rates of LAA (including asthma and other allergic symptoms) in different populations and have reported a wide range of prevalence and or incidence rates. It appears that the prevalence of LAA varies geographically from rates reported as low as 6% in parts of Northern Europe (57), 15% in the USA (58) , 23.1% in Japan (50) and as high as 44% in the UK (59). Even within each particular geographical area, it would be

reasonable to expect different prevalence rates when studies are carried out in different time periods and within different work-place settings.

The incidence of LAA has been reported to be as low as 2.26% to as high as 30% with studies showing incidence rates of 9-15% in US (54, 60) and 12 to 30% in UK (51, 53-56, 58, 60-64).

The reported incidence and prevalence of LAA varies according to Geography, the population under study, the specific design of each individual study, definition of LAA and whether or not the presence of LAA was determined based on self-reports alone (i.e. through questionnaires) or by means of additional specific skin and blood tests. Some of the variability in rates of LAA may also stem from the difficulty in diagnosing occupational and rhinitis generally. It has been suggested that when skin reactivity tests are used alone to detect LAA as opposed to screening for allergic symptoms through questionnaires, higher prevalence rates are reported since a number of subjects will have positive SPT or blood tests but no symptoms. (65, 66) The term "laboratory animal allergy" has also been defined vaguely in the literature. It seems that while in some reports LAA included any work-related allergic symptoms, in others it has been limited to OA and OR alone.

The reported estimates of the population at risk for developing LAA also vary according to the geographical area and the time the reports were published. Bland and colleagues (1987) estimated that 90,000 individuals were exposed to laboratory animals in the United States, and 32,000 workers were similarly exposed in the United Kingdom. (67)

12 years later in 1998, the US National Institute for Occupational Safety and Health (NIOSH) estimated that "about one third of the two million people who constantly work with laboratory animals may develop occupational allergies and about 10% are at risk of developing occupational asthma". (68) However one year later in 1999 Seward reported that 40,000 to 125,000 individuals were exposed to laboratory animals in the United States. (64) Irrespective of the different estimates of the population at risk, all these reports convey one single message; Exposure to laboratory animals poses a significant risk to the health and well-being of the workers and continues to be a health challenge despite all the implemented protective measures.

In summary, occupational asthma is considered the most common occupational lung disease in the industrialized world. It is thought to account for 9-15% of asthma in adults of working age (46) with an incidence rate of 2.2 per 1000 person-years. (69) While some diagnoses used in published data are made solely on the basis of symptoms, others require additional testing often including evidence of physiological changes of asthma and evidence of sensitization such as either a positive skin prick test or an antigen specific IgE to a relevant workplace allergen. This is not always possible for all causative agents and therefore there is inevitably variation in the estimated incidence arising from this.

1.6 Pathophysiology of Hypersensitivity Reactions

Like non-occupational asthma and allergy, LAA is also the result of interactions between multiple environmental and genetic factors that lead to activation of the immune system in susceptible individuals and development of allergic reactions. In order to study LAA, it is important to understand the basics and types of allergic reactions. According to the Gell and Coombs classification (1963) hypersensitivity reactions can be classified into 4 categories (70) :

Type I – Immediate in onset and mediated by immunoglobulin E (IgE) antibodies and mast cells and/or basophils.

Type II – Delayed in onset and caused by antibody (usually immunoglobulin G, IgG antibody) mediated cell destruction.

Type III – Delayed in onset and caused by IgG /antigen immune complex deposition and complement system activation.

Type IV – Delayed in onset and T-cell mediated.

Laboratory animal allergy is considered to be a Type I immediate hypersensitivity reaction, also called an "allergic reaction". (71) The initial step in development of LAA consists of production of immunoglobulin E (IgE) antibodies in response to animal antigens. Upon exposure to animal protein and glycoprotein antigens, the allergen molecules are taken up by a Group of cells known as antigen-presenting cells (APC). (72) APCs include monocytes (blood), alveolar macrophages (lungs), dendritic (major

APC in most tissues) and Langerhans cells (skin). These cells are in charge of internalizing and processing the allergen molecule into smaller fragments. APCs then migrate to draining lymph nodes where the fragmented allergen is displayed on the APC membrane in association with MHC class II molecules and then presented to T-lymphocytes. Naïve T-cells recognize the complex of antigen and the MHC class II molecules and become activated. The activated T lymphocyte can then differentiate into one of at least two types of T- cells; Th1 and Th2, each with the potential to secrete a Group of selective but very different cytokines and therefore capable of generating two different types of immune responses. The typical feature of type I hypersensitivity or allergic response is seen when a Th2 response has been elicited. (73) The particular type of immune response depends on multiple factors including the type and dose of antigen in addition to environmental and genetic factors. Atopy, defined by presence of antigen-specific IgE antibodies, is a genetic predisposition factor for allergic reactions and will be discussed later. (74)

Once the T lymphocyte has differentiated to become a Th2 cell, it can secrete a number of mediators and cytokines including IL4, IL5, and IL-9, among others. IL4 induces isotype switching of B-lymphocytes and therefore acts as a signal to B-lymphocytes to induce synthesis and secretion of immunoglobulin E (IgE), while IL5 appears to be important to eosinophil function in type I responses. Some Th2 cells can turn into memory T-cells and circulate in the body for long periods from several years to decades. Subsequent exposure to the initial sensitizing animal antigen elicits a vigorous and rapid response from memory T-cells. (75)

The term sensitization refers to the production of allergen-specific IgE. Sensitization to an allergen is not synonymous with being allergic to that allergen, because individuals may produce IgE to animal allergens, but not develop symptoms upon exposure to that animal. It is unclear why some individuals demonstrate only sensitization while others have active allergic disease.

Once formed, antigen-specific IgE occupies surface receptors on mast cells and basophils throughout the body. Basophils are granulated blood cells and can be recruited out of the blood into the tissues. Mast cells however, do not usually circulate in the blood stream but are found in abundance in tissues that are the site of allergic reactions. These sites include skin, conjunctiva, respiratory system and gastrointestinal tract. Although Mast cells and basophils are from two different cell lines, both cells contain histamine and other biological mediators in their granules.

If the antigen (animal protein) is encountered again, it (or its metabolite) may bind to these IgE molecules, causing crosslinking of the receptors and activation of the cells. This interaction leads to release of preformed biochemical mediators such as histamine as well as production of new inflammatory mediators such as prostaglandins and leukotrienes through the Arachidonic acid cascade.

The chemical mediators in turn result in vasodilation, smooth-muscle constriction, increased mucus secretion, and stimulation of nerve endings resulting in pruritus. These effects occur within minutes of exposure to the allergen, thus the label immediate hypersensitivity. In addition, a delayed second inflammatory component to the IgE-

mediated reaction is well established. This response typically occurs at 3 to 5 hours, reaches a peak between 6 and 12 hours, and resolves within 24 hours and therefore has been termed the late-phase response. (76) This reaction is characterized by the influx of basophils and eosinophils as well as other inflammatory cells into the site. The presence of eosinophils in tissues is a hallmark of allergic inflammation and the eosinophil is probably the key effector cell in airway inflammation that occurs in allergic asthma. It has been observed in the skin, nose, lung, and in systemic anaphylactic reactions. (77)

In summary, as discussed above production of antigen-specific IgE is integral to the pathogenesis of allergic disorders. However, the practical utility of measuring antigen-specific IgE for the purpose of diagnosing laboratory animal allergy is variable. It is important to recognize that the presence of IgE to a specific allergen does not necessarily equate to a clinically meaningful allergic response to that allergen. On the other hand no studies have been conducted to suggest that low or normal levels of serum IgE can be used to exclude the presence of sensitization in animal workers.

1.7 Allergic symptoms:

Similar to any allergic occupational condition, typical symptoms of laboratory animal allergy include:

- Allergic rhinitis: Itchy throat, runny nose, nasal congestion

- Allergic conjunctivitis: Itchy and watery eyes

- Allergic Asthma: Wheezing, dry cough and shortness of breath

- Allergic dermatitis: Eczema (itchy skin rash)

- Anaphylaxis: A serious rapid-onset allergic reaction involving skin and mucosal tissues presenting with respiratory compromise and sudden drop in blood pressure.

The nature and intensity of the symptoms are to an extent dependent on the level of exposure to the laboratory animal allergy by the individual. These symptoms can range from mild skin reaction to severe and life-threatening airway compromise and asthma. The most common symptoms however are those relating to the involvement of the nose and eyes. Time from the first exposure to sensitization and development of symptoms is variable but generally occurs within 3 years of beginning of employment. This period of time is called the latency period. (78)

1.8 Atopic Disease

Atopy is defined by a genetic predisposition to develop allergic disease as a result of contact with or ingestion, injection or inhalation of allergens, the majority of people do not usually react to. In spite of extensive investigations, the genetic basis of atopy is not completely understood. It is believed that when a genetically susceptible subject gets exposed to an environmental allergen, allergen-specific IgE is produced. With further exposures, the circulating IgE starts the cascade of immunologic reactions that eventually leads to mast cell degranulation and release of histamine, which is responsible for the majority of allergic symptoms. In 1975 Pepys developed criteria to define atopy objectively: positive SPT to one or more of the common aeroallergens including: grass mixture, house dust mite, Aspergillus Fumigatus and tree mixture which is used widely in epidemiological studies of LAA. (79)

Atopy is a major risk factor for developing LAA. Multiple studies have shown a strong correlation between atopy and either the development of lab animal sensitization or clinical manifestations of LAA. (13, 57, 66, 67, 80, 81) It is estimated that atopic personnel have a risk of developing sensitization that is 3 to 10 times greater than that of non-atopic individuals. (80) Moreover when animal house staff develops LAA, the disease is often clinically more severe in atopic subjects than in non-atopic subject. (82)

The association between atopy and LAA is sufficiently strong that is has been suggested atopy could be used as a discriminant for employment involving allergenic substances

such as found in animal laboratories. Although a significant association has been shown between positive SPT and OA, it is usually considered that the practice of excluding atopic subjects from employment in animal laboratories should not become practice. Atopy defined either as personal or familial history of allergies or positive SPT to common allergens lacks adequate sensitivity, specificity and positive predictive value for developing LAA, and it would very likely be considered discriminatory to exclude all atopic individuals form such employment. It can be argued that such exclusion may only be rational in workplaces with high rates of specific forms of occupational asthma, which are closely associated with atopy. (15, 83, 84)

Recent evidence indicates that for individuals who are non-atopic, the risk of sensitization to rat urinary proteins increase with increasing intensity and duration of exposure, whereas for atopic subjects, the dose-response relation is less steep. (57) This may be due to the fact that minimal exposure is unlikely to cause sensitization among non-atopics, whereas similar level of exposure leads to earlier and higher rates of sensitization among atopics. Therefore the higher intercept among atopics leads to a less steep dose-response relationship compared to non-atopics.

For those with atopy, a history of respiratory symptoms in the pollen season and the number of hours in contact with rodents also are determining factors for the risk of sensitization to laboratory animals. (56) Further investigations indicate that pre-existing lung function, airway hyper-responsiveness and sensitivity to pets are all associated with an increased risk for the development of occupationally related asthma.

1.9 Diagnosis of Occupational Asthma and Rhinitis

As an initial step in the diagnosis of occupational asthma and allergy, obtaining a detailed occupational history is important in establishing a link between symptoms and potential work place exposures.

Ideally a “Specific inhalational challenge” test may be performed when there is high suspicion for occupational asthma. This highly specific test is considered the gold standard test for clinical diagnosis of OA. It is the best way to assess airway responsiveness to a specific occupational allergen by measuring some parameter of airway function, typical forced expiratory volume in 1 second (FEV1), before and for a period after exposure to the "sensitizing" agent. During the procedure, subjects are exposed to a suspected occupational agent in a controlled clinical setting and under close observation in a hospital laboratory. A positive response is usually defined as a decrease in baseline FEV1 of 15 to 20 percent. In many parts of the world this test is rarely done, primarily because of the high costs and the potential risk to patients.

More often, a “non-specific inhalation challenge” test with either histamine or methacholine can be used to confirm airway hyper-responsiveness in workers with typical symptoms of asthma. Although a negative challenge test while a worker is still exposed is usually considered enough to rule out work-related asthma, false negative results can commonly occur after worker has left the work place environment and thus has avoided the allergen for some time. The provocative concentration of methacholine that causes a 20% reduction in FEV1 is called the PC20. A three-fold improvement in

PC20 after at least 14 days of exposure avoidance is considered significant for the diagnosis of OA (85). However failure of improvement of PC20 does not rule out OA.

Other less invasive measure includes serial peak expiratory flow (PEF) self-monitoring with the subject at work and away from work during the period PEF is monitored. This is useful in obtaining objective information for the confirmation of OA.

Skin prick testing is another less invasive test widely used to prove presence of an immediate IgE related reaction to specific allergens and is more frequently used in diagnosing occupational allergy rather than OA. A negative skin test may be useful in excluding the diagnosis of an allergic reaction to animal proteins. In a review of seven published studies (12, 14, 31, 59, 86-88), Bush compared the relationship between SPT to laboratory animals and work-related symptoms (WRS). The rate of concordance between SPT and WRS was different among the seven studies but compiling the data from all seven studies, Bush concluded that the overall concordance between skin tests and symptoms was 81%. However, 13% had a positive skin test but were asymptomatic and another 6% had symptoms suggestive of allergic reactions to animal proteins but did not have a positive skin test (89). The inter-study variation in the findings regarding the relationship of SPT to laboratory animals and work-related symptoms among the studies may be the result of inadequate standardization of the allergens and the testing methodology. It is also likely that some individuals may become sensitized to proteins other than those used in the skin prick test solutions available commercially.

Radioallergosorbent test (RAST) and Enzyme-liked immunosorbent assay (ELISA) are tests to detect specific IgE antibodies to suspected or known allergens. Both tests have been used by the occupational health specialists for the purpose of guiding a diagnosis of allergy. As discussed earlier IgE is the antibody associated with Type I allergic reactions and a positive blood test exhibiting a high level of IgE directed against a specific allergen may indicate the person is sensitized to it. However a person who has outgrown an allergy may still have positive IgE years after exposure. Although RAST is done "in vitro" in contrast to SPT which is "in vivo", they are similar in terms of sensitivity and specificity and RAST or ELISA has the advantage that it can still be used in the case of severe skin involvement (such as eczema). It is not necessary to remove workers from antihistamine therapy before RAST or ELISA. However allergic reactivity is a complex phenomenon and is not simply explicable on the basis of presence or absence of IgE antibodies. It has been reported that despite extensive use of RAST in detection of antigen specific IgE in studies of both occupational and non-occupational asthma and allergy, RAST results do not correlate as well with clinical findings and allergic symptoms as skin prick test results. (87, 90, 91)

1.10 Treatment

Eliminating the exposure is the treatment of choice for laboratory animal allergy. An exposed worker who has developed asthma symptoms from allergy to animal often improves and may recover completely if he or she immediately stops being exposed to animal allergens. However, recurrent or continuous exposure to the sensitizing agent is associated with airway injury and damage and can potentially lead to persistent and irreversible airway obstruction through airway remodeling (92). It is therefore recommended that employees with suspected LAA be assigned other duties to avoid animal exposure for a trial period until the diagnosis can be confirmed. This may, of course make diagnosis more difficult if a long time elapses between removal from normal duties and investigations. Other than environmental control, the management of OA symptoms is no different than that for non-occupational asthma.

It is important to note that pharmacologic treatment is not considered effective in preventing deterioration of lung function in immunologic allergen-induced OA when subjects remain exposed to the causal agent. In contrast, patients with irritant-induced OA without concurrent sensitization can usually return to the workplace if they have adequate pharmacologic control of their asthma and if there are appropriate occupational hygiene controls in place to prevent the likelihood of a repeat high-level respiratory irritant exposure. (78)

1.11 Study Objectives

In summary, laboratory animal allergy (LAA) is a recognised occupational health problem that can cause significant morbidity among exposed workers and imposes a burden on employers and industry. Since first described over four decades ago, many laboratories have introduced preventive measures to reduce the risk of allergy occurring, and the types of animals kept within many laboratories have also changed. Consequently, there is a need for up-to-date data regarding the epidemiology of LAA.

The objectives of this present study were:

1. To estimate the prevalence of LAA including occupational asthma (OA), occupational rhinitis (OR) and sensitization to laboratory animal allergens among Health Sciences Laboratory Animal Services (HSLAS) workers and researchers, as well as a Group of unexposed researchers, at the University of Alberta.
2. To estimate the prevalence of relevant work exposures and personal risk factors for development of occupational asthma and rhinitis due to exposure to laboratory animals, among the same population.
3. To identify associations between the potential risk factors and the health outcomes of interest (sensitization, OA, OR) in this population, as well as assessing the factors that might confound an association between the studied exposures and health outcomes, identifying particularly any modifiable risk factors that might provide opportunities for prevention.

Chapter Two: Methods

This chapter outlines the methods and procedures by which the data were gathered and analyzed for this study.

2.1 Study design and Sampling

A cross-sectional design was used. All animal care staff and research staff (students and academics) working with laboratory animals at HSLAS at the time of the study were eligible for inclusion. In addition, a sample of 50 graduate students or staff from the School of Public Health without laboratory animal exposure were recruited.

After presenting information about the study by a variety of methods to the target population (see section 3.4. Enrolment process), interested workers contacted the investigators voluntarily to participate in the study.

2.2 HSLAS

Health Sciences Laboratory Animal Services (HSLAS) is the University of Alberta's main animal house located in the university's north campus. The technical services provided by HSLAS include laboratory animal ordering, husbandry, breeding, surgical procedures and euthanasia.

2.3 Participants

Healthy adult volunteers were enrolled in the study from June 2012 to April 2013.The participants were recruited in 3 different categories:

1. HSLAS staff **(Group 1)**
2. Graduate students and researchers from the Department of Medicine who were exposed to lab animals **(Group 2)**
3. Graduate and under graduate students from the Departments of Medicine, Education and the School of Public Health without exposure to lab animals (control Group or **Group 3**)

2.4 Enrolment process

2.4.1 HSLAS staff

In a briefing session in June 2012, information about this study was presented in both written and oral forms to the HSLAS staff. HSLAS subsequently forwarded a recruitment package including an introductory letter from the investigators, the study information sheet and consent form (Appendix 1), and a reply paid envelope to all staff on behalf of the study investigators. To protect the confidentiality of the HSLAS staff, contact details for HSLAS staff were not released to the investigators. Interested HSLAS personnel were asked to return the signed consent forms in the pre-addressed reply paid envelopes included in the recruitment package via university mail. In September 2012 a

flyer briefly describing the study, was forwarded to the HSLAS staff as a reminder of the study.

2.4.2 Research staff

No central electronic register of all researchers undertaking work in HSLAS was available in the University, only a listing of principal investigators. Consequently, contact with the majority of researcher staff was only possible through the principal investigators. An animal Care and Use Committee (ACUC) coordinator from the research ethics office was asked to send an introductory package on behalf of the investigators to a confidential list of university of Alberta's principal investigators whose laboratory research entailed laboratory animal handling. In the letter included in this package the principal investigators were asked to share the LAA study information sheet with their graduate students, laboratory technicians, and other research staff and to ask the interested individuals to contact the investigators.

All the graduate coordinators from different departments of the faculty of medicine and dentistry were also contacted by the investigators and asked to forward an introductory email regarding the LAA study to their graduate students working with laboratory animals.

2.4.3 Unexposed students/researchers

To enroll volunteers for the unexposed Group, the graduate coordinator of the University of Alberta's School of Public Health forwarded an introductory email including the study information sheet and consent form to all the graduate students and staff of the School. The interested individuals were asked to contact the investigators via email or phone.

In addition, to increase awareness of the study among all Groups of potential participants information about the study was included in the faculty of graduate studies and research (FGSR) weekly newsletter and also in the School of Public Health information digest. Finally, study posters were hung in designated spaces within a number of university buildings with contact details for the investigators.

Volunteers were offered a $20 Tim Horton's gift card in appreciation of their participation in the study.

2.5 Skin Prick Test

Allergy skin prick tests were carried out in accordance with international guidelines. (93) Prick tests are commonly used in epidemiologic studies because they are safe, inexpensive and there is a high degree of correlation with symptoms. If performed correctly, skin prick tests also have a high sensitivity and specificity for the diagnosis of sensitization to inhalant allergens.(94, 95) We used commercially available extracts that are also used for clinical purposes (see section 3.5.) for the test and included positive

(histamine) and negative (glycerosaline) controls. Testing was performed on normal skin of the volar surface of the forearm and the medications used by the participants prior to the test were recorded.

Duotip plastic lancets (Lincoln Diagnostics INC, Decatur, Illinois) were used to administer skin tests in this study. The duotip lancet is a sterile disposable, plastic bifurcated needle that when employed with allergenic extracts, provides a convenient and standardized procedure. According to the manufacturer the lancet retains approximately 40 microliters of the extract in the meniscus between the two points of the lancet.

Eleven allergenic extracts were used in this study (Table 2.6.1) The points of the duotip lancet were immersed into test solutions and picked up test doses via capillary attraction. A rotation technique was used to administer the test. The shaft of the lancet was held between index finger and thumb and the points were pressed vertically against the skin with enough pressure on the skin while rotating the shaft clockwise or counter-clockwise 360 degrees. Separate lancets were used for each allergen so as not to mix the solutions.

In general, there are no known absolute contraindications to allergy skin testing. However, study participants were asked about any pre-existing medical conditions and prescription medication as a part of the questionnaire.

2.6 Allergens

The standardized allergens and test equipment were ordered from OMEGA laboratories LTD, an authorized Canadian distributor of Hollister-Stier laboratories. The allergens were supplied in dropper vials containing in addition to the extract allergens, 50% (volume/volume) glycerin as preservative, 0.5% sodium chloride and 0.275% sodium bicarbonate. The strength of these extracts was expressed in terms of:

1. Weight to volume ratio (w/v)
2. Protein Nitrogen Unit/ mL (PNU/ mL)
3. Allergy Units/mL (AU/mL)
4. Bioequivalent Allergy Units/mL (BAU/mL)

The allergenic extracts were expected to produce erythema or erythema and wheal reactions in patients with significant IgE-mediated sensitivity to relevant allergens if used in scratch, prick or puncture testing. Table 2.6.1 presents the list of allergens used in the SPT. It was suggested by HSLAS that in addition to the laboratory allergens listed below, the staff were also commonly exposed to ducks and aspen. Allergenic extracts for duck and aspen were not available.

Table 2.6.1 List of Allergens		
Allergen	**Concentration**	**Content:**
Mouse	1/20 w/v	Epithelium *
Rat	1/10	Epithelium *
Rabbit	1/10	Epithelium *
Guinea Pig	1/10	Epithelium *
Cat	10,000 BAU/mL**	Pelt *
Dog	1/10	Epithelium *
Timothy Grass	100,000 BAU/mL**	Timothy Grass pollen *
Mold mix	1/10	Mold mix *
Mite (1) [Dermatiophagoides Pteronyssinus]	30,000 AU/mL †	D. Pteronyssinus *
Mite (2) [Dermatiophagoides Farinae]	5k PNU/mL‡	D. Farinae *
Tree mix [6 trees]	1/20	6 Trees *
Negative Control	-	Saline *
Positive Control	1mg/mL	2.75 mg/ml Histamine Phosphate + 50% v/v Glycerin+ 0.4 % phenol
* in 50% Glycerin +0.5 % Phenol ** Bioequivalent Allergy Unit, † Standardized allergy unit, ‡ Protein Nitrogen units		

2.7 Questionnaire

A comprehensive questionnaire was designed in 11 sections with questions gleaned from American Thoracic Society- Division of Lung Disease (ATS-DLD-78 Adult) and International Union against Tuberculosis and Lung Disease (IUATLD) questionnaires. Vandenplas et al assessed the key items from an occupational asthma questionnaire to identify the significant predictors of OA. They concluded that patients should directly be asked about wheezing, rhinitis and conjunctival symptoms at work. Runny nose and nasal/ocular itching were specifically associated with OA due to high molecular weight agents. (96) All these items were included in the questionnaire.

Our questionnaire consisted of 75 questions in 11 sections from A to K. (Appendix 2) The sections included questions about personal demographic information (date of birth, gender, education), environmental data (history of living in a farm at early age), occupational data (past and current jobs), nature of occupational exposure to laboratory animals, use of personal protective equipment, allergic upper and lower respiratory symptoms, smoking history, family history and use of antihistamine medications. Participants were asked to complete section D (Laboratory Animal Information sheet) separately for each of the laboratory animals they were exposed to at their current job. Section D consisted of questions about the nature of occupational exposure to laboratory animals. Estimates of exposure to laboratory animals in general were derived from the following question: “How many hours per week on average do you work with lab animals?” Workers were asked to answer this question separately for each laboratory

animal. Exposure to laboratory animals in general was reported as a continuous variable, in hours per week.

2.8 Study Subject Visits

The interested participants who had either sent back their signed informed consent forms or had contacted us via email, were scheduled for a 30-minute appointment at their convenience to do the skin prick tests and to complete the questionnaire. The participants were invited to the test room in the Heritage Medical Research Center (HMRC) building in the northern campus of University of Albert. During the appointment, the participants were asked to review the information sheet (Appendix 1) and given an opportunity to ask any questions. After applying the skin prick test to the volar aspect of the arm, the questionnaire was administered by the interviewer while waiting for the result of the skin test. After completing the questionnaire, the results of the SPT were read and wheal size was recorded in the SPT report form. The size of the wheal was measured using the two greatest perpendicular diameters. A copy of the SPT report form was given to the participants upon their request. The signed ICFs, questionnaires and SPT report forms were kept in a locked cabinet throughout the study.

2.9 Data Analysis

2.9.1 Data preparation

Data from the questionnaires and SPT results were de-identified and transferred to an encrypted Microsoft excel sheet. More than 331 variables derived from questionnaire questions were defined in the format of nominal, dichotomous, categorical and time (dates). Dates were entered in a dd/mm/yyyy format. In case of partial dates where the date was incomplete, the missing day and month were entered as July 1 (mid-year) when both day and month were missing, and 15th of the month when only day was missing. To calculate the difference between two dates, for example to calculate the participants' age, the date of birth and the test date were subtracted and the difference (days) was then divided by 365.25.

The main focus of this study was on mouse and rat outcomes including mouse and rat sensitization, asthma and rhinitis.

As described below, multiple logistic regression models were developed to evaluate the importance of exposures as predictors of each outcome (positive SPT result, occupational asthma and rhinitis for mice and rats).

STATA software version 13.1 was used to perform the analyses of the dataset. (StataCorp, 4905 Lake way Dr., College Station, TX 77845, USA)

2.9.2 Definition of Independent Variables

A list of all exposure variables including baseline characteristics of the study participants, as well as occupational exposures, use of PPE and other protective measures were used during occupational exposure to rats and mice can be found in Appendix 13. As discussed in more details below, these were used both in bivariate analysis of exposure variables and health outcomes and as independent variables in building multiple logistic regression models.

2.9.3 Definition of Outcome Variables

Variables "RAT" and "Mouse" representing the skin prick test wheal diameters for rat and Mouse ranged from 0 to 10 mm. In addition two binary variables, "RAT_POSITIVE" and "MOUSE_POSITIVE", were defined with values "1" for wheal diameters greater than or equal to 3mm as per standard definition of a positive response or sensitization to an allergen in SPT, and "0" for wheal diameter less than 3mm or a negative response or a negative test to the allergen to rat and mouse respectively.

Other outcome variables including RAT_OA, RAT_OR, MOUSE_OA and MOUSE_OR representing occupational asthma (OA) and occupational rhinitis (OR) for rat and mouse respectively were defined as below:

Participants were considered as having Occupational Asthma to Rats (RAT_OA) if:

They reported having undue cough, wheezing/ chest whistling or shortness of breath in the last year

AND,

Their symptoms (either cough, wheezing or shortness of breath) was worse at work OR better off work or on vacation

AND

They had a positive SPT to Rat.

Also they were considered as having occupational rhinitis to Rats (RAT_OR) if they reported having rhinitis and nasal congestion in the last year

AND,

Symptoms worse at work OR better off work or on vacation

AND,

They had a positive SPT to Rat.

STATA syntheses used to create RAT_OA and RAT_OR variables based on above definitions can be found in Appendix 9.

Similar definitions were applied to mouse asthma and rhinitis. Participants were considered as having occupational asthma to mice (MOUSE_OA) if they reported

having undue cough, wheezing or chest whistling or shortness of breath in the last year AND symptoms worse at work and better off work or on vacation AND they had a positive SPT to mice.

They were also considered as having occupational rhinitis to mice (MOUSE_OR) if they reported having rhinitis and nasal congestion in the last year AND symptoms worse at work and better off work or on vacation AND they had a positive SPT to mice.

2.9.4 Statistical Methods and Construction of Logistic Regression Models

One-way ANOVA, Chi-squared (Chi^2) and Fisher's exact test were used to compare the baseline characteristics of the study participants across the HSLAS workers (Group 1), students and academics working with laboratory animals (Group 2) and unexposed students (Group 3).

To meet objectives one and two (section 1.11), the prevalence of the health outcomes as well as the prevalence of relevant work exposures and personal risk factors were calculated using the following formula:

Prevalence = (Number of cases at a given point in time /Total number of persons in the population) x 100

To meet objective three, first bivariate analyses were undertaken comparing all exposure related variables and potential confounders for which we had collected data with the skin prick test results and other health outcomes as defined above. Chi-squared and Fisher's Exact tests were used to test for significant associations in these bivariate comparisons.

We then tried to build multiple logistic regression models to determine the factors associated with the six binary outcome variables defined above including: RAT_POSITIVE, MOUSE_POSITIVE, RAT_OA, RAT_OR, MOUSE_OA and MOUSE_OR.

Since the outcomes were binary categorical variables, logistic regression analysis was applied. "Purposeful Selection of Covariates" method "(Appendix 10) was used to build the logistic regression model. (97) Two major STATA commands for logistic regression, LOGIT and LOGISTIC were used to obtain the parameter estimates or coefficients (i.e. the natural log of odds), as well as the odds ratio for each potential independent variable, respectively. A list of independent variables used in the analysis can be found in Appendix 12. Data regarding use or personal protective equipment (PPE) and detailed information about specific animal husbandry tasks for each animal **was gathered only from study subjects who were in contact with animals at the time of administration of the questionnaire** including 74 study subjects identified as "currently exposed" to rats and 80 subjects "currently exposed" to mice at HSLAS. Therefore we included data only for those currently working with rats/mice in the multiple logistic regression analysis.

2.10 Ethics Approval

The study protocol (Appendix 5) was approved by the University of Alberta, Health Research Ethics Board on May 3, 2012. Study ID= Pro: 00028614. (Appendix 6)

Chapter Three: Results

3.1 Descriptive Analysis

3.1.1 Baseline Characteristics:

164 individuals signed the informed consent form and participated in the study. 57 in Groups 1 and 2 each and 50 in Group 3. One of the participants in Group 1 was pregnant at the time of the study and only completed the questionnaire without doing the skin prick tests.

Table 3.1.1.1 summarizes and compares the baseline characteristics of all participants across the three Groups. Participants from Group 3 were on average younger than the other two Groups. Participants from Group 1 (HSLAS staff) were more likely to be female, atopic and current smokers. Also the likelihood of having been born or raised in a livestock farm was higher in Group 1. None of the participants in Group 3 were current smokers.

Among the Groups with ever exposure, those in Group 2 were more likely to have graduate level education and less likely to be born on animal or livestock farms compared to Group 1.

Table 3.1.1.1 Baseline characteristics of study participants across the three Groups				
Variables	**Group 1 (n=57)**	**Group 2 (n=57)**	**Group 3 (n=50)**	**P-value**
Age, mean (SD), year	35.9 (9.7)	35.2 (9.8)	29.6 (7.0)	< 0.001 *
Gender, female	45 (79.0 %)	35 (61.4 %)	36 (72.0 %)	NS
Atopy	39 (68.4 %)	35 (61.4 %)	244 (48.0 %)	NS
Currently Smoking tobacco	5 (8.8 %)	3 (5.3 %)	0	NS †
Ever smoked Tobacco	13 (22.8 %)	10 (17.5 %)	4 (8.0 %)	NS †
Graduate level Education	6 (10.5 %)	30 (52.6 %)	16 (32.0 %)	< 0.001
Family History of allergic conditions	45 (79.0 %)	38 (66.7 %)	41 (82.0 %)	NS
Born or lived in Livestock Farm	18 (31.5 %)	9 (15.8 %)	3 (6.0 %)	0.002 †
Pets at home	45 (79.0 %)	39 (68.4 %)	31 (62.0 %)	NS
Chi-squared test used except when indicated otherwise. * One-way ANOVA, † Fisher's exact test NS: Not significant i.e. p-value > 0.05				

In total 45 (79%), 39 (68%) and 31 (62%) participants in Groups 1, 2 and 3 respectively had pets at home at the time of the study (P-value > 0.1) and 8, 9 and 6 respectively attributed their allergic symptoms at least to some extent to allergy to their pets.

3.1.2 Occupational Data and Laboratory Animal Exposure

Table 3.1.2.1 shows the list of job titles of all participants. "Master's degree student" followed by "Animal health technician" were the two most common job titles.

Table 3.1.2.1 Job titles of study participants	
Job Title (All participants)	**Frequency**
MSc student	38
Animal health technician	31
PhD student	13
Post-doctorate fellow	11
Lab assistant, Cage Wash	10
Investigator services technician	4
Lab manager	4
Lab technologist	3
Principal Investigator	7
Research assistant	7
Research coordinator	3
Surgical technologist	2
Undergraduate student	3
Acquisitions assistant	3
Operations supervisor	2
Other	23
Total	164

The following tables summarize and compare the occupational data including duration of work with laboratory animals, type of exposure and job tasks at HSLAS across the two exposed Groups. Table 3.1.2.2 summarizes the mean duration of working of participants under their current job titles across the three Groups, in years. Mean duration of work was lowest for Group 3.

Table 3.1.2.2 Mean duration of working (year) under current job title

	Mean duration *	Standard deviation	Minimum duration	Maximum duration
Group 1 (n=57)	4.27	6.87	0.03	31.52
Group 2 (n=57)	5.09	7.20	0.26	38.28
Group 3 (n=50)	2.17	2.67	0.08	14.59
* One-way ANOVA P-value= 0.72				

Table 3.1.2.3 shows the working hours/week for all study participants across the three Groups. The majority of study participants were working full time i.e. 30-40 hours /week at the time of the study.

Table 3.1.2.3 Number of hours/week working under current job title

	Group 1	Group 2	Group 3	Total
10-20 hrs/week	0	0	1	1
20-30 hrs/week	1	0	2	3
30-40 hrs/week	56	56	47	159
>40 hrs/week	0	1	0	1
Total	57	57	50	164

Table 3.1.2.4 demonstrates that there was no difference in the duration of working with laboratory animal in four Groups (< 1yr, 1-3 yrs., >3-5 yrs. and >5 years), between the HSLAS staff and the researchers. No subjects from Group 3 reported working with laboratory animals.

Table 3.1.2.4 Duration of work with laboratory animal among HSLAS staff					
	< 1 year	**1 to 3 years**	**>3 to 5 years**	**>5 years**	**Total**
Group 1	20, (35.1 %)	17, (29.8 %)	10, (17.5 %)	10, (17.5 %)	57
Group 2	17, (29.8 %)	12, (21.1 %)	15, (26.3 %)	13, (22.8 %)	57
Total	37	29	25	23	114
Chi^2 P-value > 0.1					

Table 3.1.2.5 shows the number of study participants exposed to specific laboratory animals at work at the time of study. Also among other animals, 10 participants reported exposure to swine (7 from Group 1 and 3 from Group 2) and 5 reported working with frogs (All from Group 1).

Table 3.1.2.5 Number (%) of study participants in each Group exposed to laboratory animals at the time of study			
Animals	**Group 1 (n=57)**	**Group 2 (n=57)**	**P-value**
Mouse	49 (86.5 %)	32 (56.1 %)	< 0.001
Rat	47 (82.5 %)	27 (47.4 %)	< 0.001
Guinea Pig	30 (52.6 %)	1 (1.8 %)	< 0.001
Rabbit	30 (52.6 %)	3 (5.3 %)	< 0.001

Cat	25 (43.9 %)	2 (3.5 %)	< 0.001
Dog	20 (35.1 %)	1 (1.8 %)	< 0.001

The following 4 tables summarize the data regarding participants' exposure to mice and rats and different job tasks while handling each animal in the animal laboratories and facilities, across the two exposed Groups. Exposure to mouse urine was significantly more frequent among Group 1 participants whereas in Group 2 contact with mouse blood/serum and internal organs were more common.

Table 3.1.2.6 Exposure to different animal tissues while working with mice.

Exposure to:	**Group 1 (n=49)**	**Group 2 (n=32)**	**Chi^2 P-value**
Mouse Skin	35 (71.4 %)	27 (84.4 %)	NS
Mouse Fur/dander	44 (89.8 %)	27 (84.4 %)	NS
Mouse Serum/Blood	12 (24.5 %)	17 (53.1 %)	**< 0.01**
Mouse Urine	39 (79.6 %)	15 (46.9 %)	**< 0.01**
Mouse Saliva	15 (30.6 %)	7 (21.9 %)	NS
Mouse Organs/Tissues	11 (22.5 %)	23 (71.9 %)	**< 0.001**
Mouse Carcass	19 (38.8 %)	15 (46.9 %)	NS
Mouse Cages/Wastes	41 (83.7 %)	24 (75.0 %)	NS
NS: Not significant i.e. p-value > 0.05			

As noted in table 3.1.2.7, exposure to rat internal organs was significantly more common in Group 2 participants compared to Group 1.

Table 3.1.2.7 Exposure to different animal tissues while working with rats.			
Exposure to:	**Group 1 (n=47)**	**Group 2 (27)**	**Chi^2 P-value**
Rat Skin	31 (65.9 %)	20 (74.1 %)	NS
Rat Fur/dander	42 (89.4 %)	26 (96.3 %)	NS *
Rat Serum/Blood	13 (27.7 %)	19 (70.4 %)	NS
Rat Urine	35 (74.5 %)	16 (59.3 %)	NS
Rat Saliva	14 (29.8 %)	5 (18.5 %)	NS
Rat Organs/Tissues	8 (17.0 %)	22 (81.5 %)	< 0.001
Rat Carcass	19 (40.4 %)	13 (48.1 %)	NS
Rat Cages/Wastes	41 (87.2 %)	20 (74.1 %)	NS
* Fisher’ s exact test P-value, NS: Not significant i.e. p-value > 0.05			

Job tasks including mouse feeding, box changing, manual cage cleaning and unit cleaning were more common among Group 1 participants working with mice (see table 3.1.2.8).

Table 3.1.2.8 Number of participants involved in different job tasks at least several days/ week while working with mice, across the two Groups.			
Job Tasks with Mice	**Group 1 (n= 49)**	**Group 2 (n=32)**	**Chi^2 P-value**
Handling	36 (73.5%)	19 (59.4%)	NS
Feeding	24 (49.0 %)	6 (18.7 %)	0.006
Injections/Procedures	9 (18.4 %)	11 (34.4 %)	NS
Shaving	1 (2.0 %)	4 (12.5 %)	NS *
Animal Sacrifice	16 (32.6 %)	9 (28.1 %)	NS
Box changing	23 (46.9 %)	4 (12.5 %)	0.002 *
Disposal of Soiled litter	21 (42.9 %)	4 (12.5 %)	0.006 *
Handling multiple animals	22 (44.9 %)	14 (43.7 %)	NS
Manual Cage-cleaning	13 (26.5 %)	0 (0.0 %)	0.001 *
Automated Cage-cleaning	7 (14.3 %)	1 (3.1 %)	NS *
Indirect contact	35 (71.4 %)	12 (37.5 %)	0.002
Cleaning within animal unit	30 (61.2 %)	1 (3.1%)	0.000 *
* Fisher’ s exact test P-value, NS: Not significant i.e. p-value > 0.05			

Table 3.1.2.9 shows that among study participants exposed to rats, shaving and injecting was more common in Group 2 whereas disposal of soiled litter, manual and automated cage cleaning and cleaning within the unit were more common among Group 1 participants.

Table 3.1.2.9 Number of participants involved in different job tasks at least several days/week while working with rats, across the two Groups.			
Job Tasks with Rats	**Group 1 (n= 47)**	**Group 2 (n=27)**	**Chi^2 P-value**
Handling	30 (63.8 %)	20 (74.1 %)	NS
Feeding	19 (40.4 %)	8 (29.6 %)	NS
Injections/Procedures	6 (12.8 %)	12 (44.4 %)	< 0.05 *
Shaving	1 (2.1 %)	8 (29.6 %)	< 0.05 *
Animal Sacrifice	12 (25.5 %)	7 (25.9 %)	NS
Box changing	18 (38.3 %)	7 (25.9 %)	NS
Disposal of Soiled litter	21 (44.7 %)	4 (14.8 %)	< 0.05 *
Handling multiple animals	19 (40.4 %)	12 (44.4 %)	NS
Manual Cage-cleaning	12 (25.5 %)	1 (3.7 %)	< 0.05 *
Automated Cage-cleaning	7 (14.9 %)	0 (0.0 %)	< 0.05 *
Indirect contact	30 (63.8 %)	12 (44.4 %)	NS
Cleaning within animal unit	26 (55.3 %)	3 (11.1 %)	< 0.001 *
* Fisher’ s exact test P-value, NS: Not significant i.e. p-value > 0.05			

3.1.3 Use of Personal Protective Equipment

Tables 3.1.3.1 and 3.1.3.2 compare the rate of use of personal protective equipment (PPE) and preventive measures by study participants while working in the animal laboratory across the two Groups. Use of protective gowns, facial masks, IVCs female/juvenile rats were all significantly more common in Group 1.

Table 3.1.3.1 Regular use of PPE among participants while exposed to mice, across the two Groups.

Use of PPE:	Group 1 (n= 49)	Group 2 (n=32)	Chi^2 P-value
Protective Gloves	47 (95.9 %)	31 (96.9 %)	NS *
Protective Gowns	46 (93.9 %)	23 (71.9 %)	< 0.05 *
Safety Glasses/Shields	15 (30.6%)	6 (18.7 %)	NS
Surgical type face mask	43 (87.8 %)	18 (56.2 %)	< 0.05
Protective particle filter respirator	4 (8.2 %)	1 (3.1 %)	NS *
Use of Filter top Cages	37 (75.5 %)	18 (56.2 %)	NS
Use of individually ventilated cages (IVC)	35 (71.4 %)	11 (34.4 %)	< 0.05
Use of Female / Juvenile mice	19 (38.8 %)	4 (12.5 %)	< 0.05
Biosafety cabinets/ Extracted work station	12 (24.5 %)	12 (37.5 %)	NS

* Fisher's exact test P-value, NS: Not significant i.e. p-value > 0.05

Table 3.1.3.2 shows that use of protective gowns, facial masks, filter-top cages and IVC were significantly more common among Group 1 participants while handling rats

Table 3.1.3.2 Regular use of PPE among participants while exposed to rats, across the two Groups.			
Use of PPE:	**Group 1 (n= 47)**	**Group 2 (n=27)**	**Chi^2 P-value**
Protective Gloves	46 (97.9 %)	24 (88.9 %)	NS *
Protective Gowns	44 (93.6 %)	20 (74.1 %)	< 0.05 *
Safety Glasses/Shields	3 (6.4 %)	4 (14.8 %)	NS *
Surgical type face mask	40 (85.1 %)	17 (63.0 %)	< 0.05
Protective particle filter respirator	9 (19.1 %)	6 (22.2 %)	NS
Use of Filter top Cages	32 (68.1 %)	12 (44.4 %)	< 0.05
Use of individually ventilated cages (IVC)	28 (59.6 %)	8 (29.6 %)	< 0.05
Use of Female / Juvenile rats	18 (38.3 %)	7 (25.9 %)	NS
Biosafety cabinets/ Extracted work station	10 (21.3 %)	7 (25.9 %)	NS
* Fisher's exact test P-value, NS: Not significant i.e. p-value > 0.05			

3.1.4 Occupational Respiratory and Rhinitis Symptoms

Participants were asked to report any pre-existing allergic conditions they had. According to participants' self-reports, hay fever /allergic rhinitis was the most common allergic condition among all participants across the three Groups, followed by skin allergies.

Table 3.1.4.1 Self-reported history of allergic conditions (at least 3 months).

	Group 1 (n=57)	Group 2 (n=57)	Group 3 (n=50)	Chi^2 P-value
Hay fever/ Allergic rhinitis	26 (45.6 %)	37 (64.9 %)	17 (34.0 %)	0.005
Asthma	8 (14.0 %)	9 (15.8 %)	8 (16.0 %)	NS
Eczema/ Skin allergies	23 (40.3 %)	23 (40.3 %)	15 (30.0 %)	NS
Allergic conjunctivitis	25 (43.9 %)	23 (40.3 %)	7 (14.0 %)	0.002
No allergic symptoms	14 (36.8 %)	5 (13.2 %)	19 (30.5%)	0.001 *

* Fisher's exact test, NS: Not significant i.e. p-value > 0.05

When directly asked to name specific allergies, 23 (40.4%), 24 (42.1%) and 19 (38.0%) of study subjects from Groups 1, 2 and 3 respectively reported history of various allergies to various agents, including but not limited to antibiotics (penicillin family), Pollen, Trees, fruits (strawberry), metals (nickel), latex, animals (cat), etc.

Tables 3.1.4.2 to 3.1.4.5 compare the rate of self-reported work-related allergic symptoms and health conditions across the three exposed Groups.

As shown in table 3.1.4.2 seven, five and one participants in Groups 1, 2 and 3 respectively reported cough symptoms in the last 12 months of which of which four and four in Groups 1 and 2 were work-related. No participant in Group 3 reported work-related cough.

Table 3.1.4.2 Number of participants reporting "Cough".

	Group 1 (n=57)	**Group 2 (n=57)**	**Group 3 (n=50)**
History of waking up at night by attack of coughing in the last 12 months	5 (8.8 %)	4 (7.0 %)	1 (2.0 %)
Morning coughing in the last 12 months	4 (7.0 %)	2 (3.5 %)	1 (2.0 %)
If answered yes to either of above questions:			
Work-related cough	3/7	4/5	0/1
Coughing improves off work or on vacation	4 /7	4/5	0/1

As shown below in table 3.1.4.3, nine, eleven and two participants in Groups 1, 2 and 3 respectively reported symptoms of wheezing in the last 12 months, of which six, eight and one were work-related.

Table 3.1.4.3 Number of participants reporting "Wheezing".			
	Group 1 (n=57)	**Group 2 (n=57)**	**Group 3 (n=50)**
History of wheezing or chest whistling in the last 12 months	9 (15.8 %)	10 (17.5 %)	2 (4.0 %)
History of attacks of SOB with wheezing in the last 12 months	6 (10.5 %)	8 (14.0 %)	2 (4.0 %)
If answered yes to either of above questions:			
Wheezing only with common cold?	0/9	0/11	0/2
Wheezing associated with exercise	3/9	4/11	2/2
Work-related wheezing	6/9	8/11	1/2
Wheezing improves off work or on vacation	6/9	8/11	1/2

Similarly, table 3.1.4.4 shows that eight, six and three participants in Groups 1, 2 and 3 respectively reported shortness of breath (SOB) in the last 12 months of which seven and two were work-related. No work-related SOB was reported in Group 3.

Table 3.1.4.4 Number of participants reporting "Undue shortness of breath (SOB)"			
	Group 1 (n=57)	**Group 2 (n=57)**	**Group 3 (n=50)**
History of waking up at night by attack of shortness of breath in the last 12 moths	5 (8.8 %)	4 (7.0 %)	3 (6.0 %)
History of SOB during the day or night in the last 12 months	3 (5.3 %)	6 (10.5 %)	3 (6.0 %)
If answered yes to either of above questions:			
Work-related SOB	7/8	2/6	0/3
SOB improves off work or on vacation	4/8	1/6	0/3

Table 3.1.4.5 below shows the number of participants with pre-existing physician-diagnosed non-occupational asthma.

Table 3.1.4.5 Number of participants reporting "Asthma" *			
	Group 1 (n = 57)	**Group 2 (n = 57)**	**Group 3 (n =50)**
History of asthma diagnosed by a physician	4 (7.0 %)	8 (14.0 %)	8 (16.0 %)
History of asthma attack	3 (5.3 %)	8 (14.0 %)	5 (10.0 %)
Childhood asthma that went away	0	0	2
Childhood asthma that went away and is now back again	1	3	0
Childhood asthma that is still active	1	4	3
Adult onset asthma	1	1	1
History of asthma before starting current HSLA job	2	4	0
* Asthma refers to Physician-diagnosed asthma			

Among study subjects who indicated experiencing any respiratory symptoms, the frequency of symptoms was different among participants. While some had symptoms on a daily basis, others were not symptomatic except for few days per year. However, the most common frequency chosen by the participants was "few days each week". Also of all symptomatic individuals, 11 (68%) and 6 (33.33%) in Groups 1 and 2 respectively were able to identify changes in work processes and job duties in the week preceding the onset of their lower respiratory symptoms. The most common exposure identified was "entering rat rooms".

Four and one individuals in Groups 1 and 2 respectively were able to identify an unusual work exposure within 24 hours before the onset of their initial asthma symptoms. These included: changing the HEPA filters on an animal cage, construction dust, entering the room of diabetic rats, and changing rat cages. Table 3.1.4.6 shows that Group 1 and 2 participants were more likely to suffer from upper respiratory tract symptoms at the time of study.

Table 3.1.4.6 Rhinitis symptoms across the three Groups.

	Group 1 (n = 57)	**Group 2 (n = 57)**	**Group 3 (n = 50)**
Watery nasal discharge	30 (52.6 %)	38 (66.7 %)	17 (34.0 %)
Nasal congestion	23 (40.4 %)	23 (40.4 %)	9 (18.0 %)
Attacks of sneezing	23 (40.4 %)	27 (47.4 %)	7 (14.0 %)
Nasal itching	22 (38.6 %)	28 (49.1 %)	9 (18:0 %)
Watery/itchy eyes	25 (43.9 %)	23 (40.4 %)	7 (14.0 %)

The mean age of onset of rhinitis symptoms was 23.0 and 21.1 years between Group 1 and 2 participants, whereas the mean age was as low as 15.1 years in Group 3.

Table 3.1.4.7 The frequency of rhinitis symptoms among participants reporting having rhinitis across the Groups.			
Frequency	**Group 1 (n=37)**	**Group 2 (n=40)**	**Group 3 (n=18)**
Once/ Few days ever	2	2	1
Few days/ year	7	3	3
Few days/ month	8	11	7
Few days/ week	11	17	3
At least once every day or night	9	7	4

Also of all symptomatic individuals, 12 (32.4%) and 8 (20.5%) in Groups 1 and 2 respectively were able to identify changes in work processes and job duties in the *week* preceding the onset of their rhinitis symptoms. The most common event reported was "Initiation of work with animals (at HSLAS)".

One and three individuals in Groups 1 and 2 respectively were able to identify a specific work exposure within *24 hours* before the onset of their initial rhinitis symptoms. These included: rat exposure, changing rat cages (reported twice) and use of cleaning products.

Eighteen (48.7 %) and 18 (46.2 %) of participants with rhinitis symptoms in Groups 1 and 2 thought their symptoms were caused by something they breathed in at work. 18 (48.7 %) and 22 (56.4 %) respectively reported their symptoms improved while off work or on vacation.

17 (29.8 %), 21 (36.8 %) and 18 (36.0 %) of all patients in Groups 1, 2 and 3 respectively said they rhinitis symptoms were seasonal and thought they had seasonal allergies.

Only 4 (10.5 %), 0, and 3 (15.0 %) in gs 1, 2 and 3 said they experienced rhinitis symptoms only when they had cold.

3.1.5 Sensitisation

Table 3.1.5.1 compares the rate of sensitization to mouse, rat, guinea pig, rabbit, cat and dog in all participants across the three Groups. Between Group 1 and 2 participants, sensitization was more commonly seen to cat, rat and guinea pig. Group 3 study subjects had no occupational exposures and some were only exposed and/ or sensitized to cats and dogs.

Table 3.1.5.1 Number (%) of positive SPTs to laboratory animals across the three groups

Variables	**Group 1 (n = 57)**	**Group 2 (n = 57)**	**Group 3 (n = 50)**
Mouse	16 (28.1 %)	7 (12.3 %)	0 (0.0 %)
Rat	29 (50.9 %)	18 (31.6 %)	0 (0.0 %)
Guinea Pig	29 (50.9c%)	14 (24.6 %)	0 (0.0 %)
Rabbit	22 (38.6 %)	13 (22.8 %)	0 (0.0 %)
Cat	31 (54.4 %)	25 (43.9 %)	9 (18.0 %)
Dog	23 (40.4 %)	14 (24.6 %)	1 (2.0 %)

Table 3.1.5.2 compares the rate of sensitization to mouse, rat, guinea pig, rabbit, cat and dog among participants with direct contact with laboratory animals in Groups 1 and 2.

Table 3.1.5.2 Number (%) of positive SPTs among workers currently exposed to laboratory animals, across the two groups.

Variables	Group 1	Group 2	Chi^2 P-value
Mouse*	13/49 (26.5 %)	4/ 31 (12.9 %)	NS **
Rat	27/47 (57.5 %)	14/26 (53.9 %)	NS
Guinea Pig	22/30 (73 %)	1/1 (100 %)	NS
Rabbit	18/30 (60 %)	2/3 (66.7 %)	NS
Cat	18/25 (72 %)	1/2 (50 %)	NS
Dog	15/20 (75 %)	0/1 (0.0 %)	NS

* n/ total # of workers exposed, (%), ** Fisher's exact test,
NS: Not significant i.e. p-value > 0.05

A number of laboratory workers not directly working with lab animals at the time of study, also showed positive SPT. All these workers either had remote history of direct contact or were working within the facility with unquantifiable level of indirect exposure to the laboratory animals (i.e. the administrative personnel)

Table 3.1.5.3 Number (%) of positive SPTs among workers not directly in contact with laboratory animals at the time of the study.			
Variables	**Group 1**	**Group 2**	**Group 3**
Mouse*	3/ 8 (37.5 %)	3/25 (12.0 %)	0/50
Rat	2/10 (20 %)	4/30 (13.3 %)	0/50
Guinea Pig	7/27 (25.9 %)	13/55 (23.6 %)	0/50
Rabbit	4/27 (14.8 %)	11/53 (20.7 %)	0/50
Cat	13/32 (40.6 %)	24/54 (44.4 %)	9/50 (18 %)
Dog	8/37 (21.6 %)	14/55 % (25.4 %)	1/50 (2 %)
* n/ total # of workers not working directly with lab animal, (%)			

Table 3.1.5.4 compares the number of laboratory workers in contact with mouse, rat, guinea pig, rabbit, cat and dog at the time of the study among all individuals with positive SPT to these animals. For those with positive skin tests to dog and cat, particularly in Group 2, it appeared that exposure leading to sensitization may have occurred outside the workplace.

Table 3.1.5.4 Number (%) of workers currently exposed to laboratory animals among those with positive SPT, across the three Groups.			
Variables	**Group 1**	**Group 2**	**Group 3**
Mouse	13/16 (81.2 %)	4/7 (57.1 %)	0 (0.0 %)
Rat	27/29 (93.1 %)	14/18 (77.8 %)	0 (0.0 %)
Guinea Pig	22/29 (75.9 %)	1/14 (7.1 %)	0 (0.0 %)
Rabbit	18/22 (81.8 %)	2/13 (15.4 %)	0 (0.0 %)
Cat	18/31 (58.1 %)	1/25 (4.0 %)	0/9 (0.0 %)
Dog	15/23 (65.2 %)	0/14 (0.0 %)	0/1 (0.0 %)

Table 3.1.5.5 compares the rate of sensitization to common aeroallergens.

Table 3.1.5.5 Number of positive SPTs to common aeroallergens across the three Groups.				
Variables	**Group 1**	**Group 2**	**Group 3**	**Chi^2 P-value**
Grass Pollen	20 (35.1 %)	17 (30.4 %)	11 (22 %)	NS
Mold mix	22 (38.6 %)	5 (8.9 %)	1 (2.0 %)	0.000 *
Mite D. Pteronyssinus	20 (35.1 %)	15 (26.8 %)	11 (22.0 %)	NS
Mite D. Farinae	23 (40.3 %)	12 (21.4 %)	7 (14.0 %)	< 0.01
Trees mix	22 (38.6 %)	4 (7.1 %)	0 (0.0 %)	0.000 *
* Fishers exact test. NS: Not significant i.e. p-value > 0.05				

Tables 3.1.5.6 and 3.1.5.7 examine the association of duration of work with laboratory animals and sensitization to rat and mouse. It was noted that, sensitization to mice and rats was not significantly associated with the duration of work with laboratory animals.

Table 3.1.5.6 Sensitization to rat and duration of work with laboratory animal.

	Positive SPT	**Negative SPT**	**Total**
< 1 year	12 (29.3 %)	12 (37.5 %)	24
1 to 3 years	16 (39.0 %)	5 (15.6 %)	21
3 to 5years	5 (12.2 %)	10 (37.5 %)	15
> 5 years	8 (19.5 %)	5 (15.6 %)	13
Total	41 (100 %)	32 (100 %)	73
Chi^2 P-value > 0.05			

Table 3.1.5.7 Sensitization to mouse and duration of work with laboratory animal.

	Positive SPT	**Negative SPT**	**Total**
< 1 year	6 (27.3 %)	22 (72.7 %)	28
1 to 3 years	6 (28.6 %)	15 (71.4 %)	21
3 to 5years	1 (5.9 %)	16 (94.1 %)	17
> 5 years	4 (28.6 %)	10 (71.4 %)	14
Total	17	63	80
Chi^2 P-value > 0.1			

We also examined the relationship between sensitization to mouse and rat (Table 3.1.5.9). There was a significant association between sensitization to rat and mouse. Almost three quarters of those sensitized to mouse also being sensitized to rat. It was noted that the majority of positive rat-senstisation was not associated with mouse-sensitization whereas most cases of positive mouse-sensitization were associated with rat-sensitization.

Table 3.1.5.9 Relationship between sensitization to mouse and rat.

	Rat_positive	**Rat_negative**
Mouse_positive	17	6
Mouse_negative	30	110
Chi^2 P-value < 0.001		

Tables 3.1.5.10 to 3.1.5.71 (Appendix 11) summarize the results of cross-tabulations to examine the possible association of sensitization to a) rat and b) mouse AND different occupational animal exposures, job tasks and use of PPE at HSLAS. In summary: none of the rat tissue exposures were associated with sensitization. Among job tasks, shaving rats was associated with rat sensitization (P-value < 0.05). Among PPEs and preventive measures, use of respirators was associated with rat sensitization (P-value < 0.05). Regarding mouse tissue exposures, contact with mouse urine (P-value < 0.05) and saliva (P-value < 0.05) were associated with mouse sensitization. None of the job tasks and PPE and preventive measure was associated with mouse sensitization. Table 3.1.5.72 compares the overall rate of sensitization to laboratory animals (rat, mouse or both)

across the two Groups. A significant difference was noted in the rate of overall sensitization between the two Groups.

Table 3.1.5.72 Prevalence of sensitisation to either Mouse or Rat (overall and across the two Groups.)			
	Group1 (n=57)	**Group2 (n=57)**	**Overall prevalence (n=114)**
Positive SPT to Rat or Mouse, (n) % [95% CI]	(33) 57.9 % [44.1 – 70.8]	(21) 36.8 % [24.4 – 50.6]	(54) 47.4 % [37.9 – 56.9]
Negative SPT to both Rat and Mouse, (n) % [95% CI]	(24) 42.1 % [29.1 – 55.9]	(36) 63.2 % [49.3 – 75.5]	(60) 52.6 % [43.1 – 62.1]
Total	(57) 100%	(57) 100%	(114) 100%
Chi ^{2} P-value < 0.05			

Table 3.1.5.73 summarizes the rate of sensitisation to common allergens across the three Groups.

Table 3.1.5.73 Number of positive SPTs to common allergens across the three Groups.				
	Group 1 (n= 57)	**Group 2 (n= 57)**	**Group 3 (n= 50)**	**P-value**
Timothy Grass, %	20 (35.1 %)	17 (30.4 %)	11 (22 %)	NS
Mold mix, %	22 (38.6 %)	5 (8.9 %)	1 (2:0 %)	< 0.001 *
Mite D. Pteronyssinus, %	20 (35.1 %)	15 (26.8 %)	11 (22:0 %)	NS
Trees mix, %	22 (38.6 %)	4 (7.1 %)	0 (0.0 %)	< 0.001 *

Table 3.1.5.74 presents the prevalence of atopy across the two Groups. Atopy was numerically less prevalent in Group3 but the difference did not reach statistical significance

Table 3.1.5.74 Prevalence of atopy across the three Groups

	Group 1 (n= 57)	**Group 2 (n= 57)**	**Group 3 (n= 50)**	**Total**
Atopy	39 (68.4 %)	35 (61.4 %)	24 (48 %)	98 (59.8 %)

Chi ^{2} test, not significant.

3.1.6 Occupational Asthma and Occupational Rhinitis

Table 3.1.6.1 shows the overall prevalence of occupational asthma and rhinitis among laboratory workers currently exposed to laboratory animals. Overall 15 (15.1 %) and 28 (28.3 %) of the exposed study population had developed OA and OR respectively.

Table 3.1.6.1 Overall prevalence of OA and OR across the two Groups among workers currently exposed to mouse or rat.

	Group 1 (n=51)	Group 2 (n=48)	Total (n=99)	Chi^2 P-value
OA	6 (11.8 %)	9 (18.7 %)	15 (15.1 %)	> 0.1
OR	15 (29.4 %)	13 (27.1 %)	28 (28.3 %)	> 0.1

Table 3.1.6.2 shows the prevalence of rat occupational asthma (RAT_OA) and rhinitis (RAT-OR) among laboratory workers who self-identified as being exposed to laboratory rats at the time of the study. We found that 14 (18.9 %) and 25 (33.8 %) participants had RAT_OA and RAT_OR respectively.

Table 3.1.6.2 Prevalence of RAT_OA and OR across the two Groups among those with current exposure to rat.

	Group 1 (n= 47)	Group 2 (n= 27)	Total (n= 74)	Chi^2 P-value
RAT_OA	6 (12.8 %)	8 (29.6 %)	14 (18.9 %)	> 0.1
RAT_OR	12 (25.5 %)	13 (48.1 %)	25 (33.8 %)	< 0.05

Table 3.1.6.3 shows the prevalence of mouse occupational asthma (MOUSE_OA) and rhinitis (MOUSE_OR) among laboratory workers exposed to laboratory mice at the time of the study. We found that 5 (6.2 %) and 18 (22.2%) participants had MOUSE_OA and MOUSE_OR respectively, which were less prevalent than rat asthma and rhinitis.

Table 3.1.6.3 Prevalence of Mouse_OA and OR across the two Groups among those with <u>current</u> exposure to mouse.

	Group 1 (n=49)	**Group 2 (n= 32)**	**Total (n=81)**	**Chi^2 P-value**
MOUSE_OA	4 (8.2 %)	1 (3.1 %)	5 (6.2 %)	> 0.1*
MOUSE_OR	12 (24.5 %)	6 (18.7 %)	18 (22.2 %)	> 0.1
* Fisher's exact test				

Table 3.1.6.4 summarizes the overall prevalence of OA and OR across the two groups.

Table 3.1.6.4 Overall prevalence of OA and OR

	Group 1 (n= 51)	**Group 2 (n= 48)**	**Total (n= 99)**	**P-value**
OA (n) % [95% CI]	(6) 11.8 % [4.4 – 23.8]	(9) 18.7% [8.9 – 32.6]	(15) 15.1 % [8.7 – 23.7]	NS
OR (n) % [95% CI]	(15) 29.4% [17.5 – 43.8]	(13) 27.1% [15.2 – 41.8]	(28) 28.3% [19.6 – 38.2]	NS

Tables 3.1.6.5 and 3.1.6.6 cross-tabulate rat and mouse occupational asthma and occupational rhinitis respectively. Four individuals were noted to have both rat and mouse occupational asthma. Similarly, 15 participants were found to have both rat and mouse occupational rhinitis. There seemed to be a significant association between rat-OA and mouse_OA. Similar association was noted between rat- and mouse-OR.

Table 3.1.6.5 Cross tabulation of RAT_OA and MOUSE_OA among participants currently exposed to rat and mouse. (n=99)

	RAT_OA (+)	**RAT_OA (-)**	**Total**
MOUSE_OA (+)	4	1	5
MOUSE_OA (-)	10	84	94
Total	14	85	99
Fisher's exact test p-value < 0.001			

Table 3.1.6.6 Cross tabulation of RAT_OR and MOUSE_OR among participants currently exposed to rat and mouse. (n=99)

	RAT_OR (+)	**RAT_OR (-)**	**Total**
Mouse_OR (+)	15	3	18
Mouse_OR (-)	10	71	81
Total	25	74	99
Fisher's exact p-value < 0.001			

Table 3.1.6.7 below shows the association between sensitisation to rat among individuals with and without self-reported asthma symptoms (possibly due to exposure to rats). Of those with asthma symptoms, only one was not sensitized to rats, while among those without asthma symptoms less than 50% were sensitized to rat. Of 41 individuals sensitized to rats, 14 had asthma symptoms, whereas only 1 of 33 non sensitized individuals reported asthma symptoms. There was a statistically significant relationship between asthma symptoms and sensitization to rats (Fisher's exact < 0.001). Considering the presence of asthma symptoms as the positive outcome, sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) for sensitization to rats could be calculated. Sensitization to rats appears to have high sensitivity (93.33 %) and NPV (96.9 %) for asthma symptoms. Presence of rat asthma symptoms was significantly associated with rat sensitization. (P-value <0.001)

Table 3.1.6.7 Cross tabulation of asthma symptoms and sensitization in participants <u>currently</u> exposed to rat. (n=74)

	Asthma symptoms	**No asthma symptoms**	**Total**
Sensitized	14	27	41
Not Sensitized	1	32	33
Total	15	59	74
Fisher's exact < 0.001			

Sensitivity: 14/ (14+1) = 93.3 %

Specificity: 32/ (32+27) = 54.2 %

PPV: 14 /(14+27) = 34.1%

NPV: 32/ (1+32) = 96.9 %

Similarly, Table 3.1.6.8 below shows the association between sensitization to rat and rhinitis symptoms. Out of 30 participants with rhinitis symptoms (possibly due to exposure to rats), only 5 were not sensitized to rats. Of 41 individuals sensitized to rats, 25 had rhinitis symptoms. Presence of rat rhinitis symptoms was significantly associated with sensitization to rats. (P-value < 0.001)

Sensitisation to rats had a sensitivity of > 80% and a specificity of > 60 % for having rhinitis symptoms.

Table 3.1.6.8 Cross tabulation of Rhinitis symptoms and Sensitization in participants currently exposed to rat. (n=74)

	Rhinitis symptoms	**No Rhinitis symptoms**	**Total**
Sensitized	25	16	41
Not Sensitized	5	28	33
Total	30	44	74
Chi^2 p-value < 0.001			

Sensitivity: 25/ (25+5) = 83.3 %

Specificity: 28/ (16+28) = 63.6 %

PPV: 25 /(25+16) = 60.9 %

NPV: 28/ (5+28) = 84.8 %

Table 3.1.6.9 shows the association between asthma symptoms and sensitization to mouse. Out of 10 individuals with asthma symptoms (possibly due to exposure to mice), 5 were not sensitized to mice. Of 17 individuals sensitized to mice, only 5 had asthma symptoms. Presence of mouse asthma symptoms was significantly associated with sensitization to mice. (P-value < 0.05)

According to the table, sensitisation to mice had a sensitivity of about 50 % but a specificity of > 80 % for having asthma symptoms.

3.1.6.9 Cross tabulation of Asthma symptoms and Sensitization in participants <u>currently</u> exposed to mouse. (n=80)			
	Asthma symptoms	**No asthma symptoms**	**Total**
Sensitized	5	12	17
Not Sensitized	5	58	63
Total	10	70	80
Chi^2 P-value < 0.05			

Sensitivity: 5/ (5+5) = 50.0 %

Specificity: 58/ (12+58) = 82.8 %

PPV: 5 /(5+12) = 29.4 %

NPV: 58/ (5+58) = 92.1 %

Finally, Table 3.1.6.10 shows the association between rhinitis symptoms and mouse sensitization. Out of 23 participants with rhinitis symptoms (possibly due to exposure to mice), 14 were not sensitized to mice. Of 17 individuals sensitized to rats, almost half, i.e. 9 had rhinitis symptoms. Presence of mouse rhinitis symptoms was significantly associated with sensitization to mice. (P-value < 0.05)

Sensitisation to mice had a sensitivity of < 40% but a specificity of > 80 % for having rhinitis symptoms.

Table 3.1.6.10 Cross tabulation of Rhinitis symptoms and Sensitization in participants <u>currently</u> exposed to mouse. (n=80)

	Rhinitis symptoms	**No Rhinitis symptoms**	**Total**
Sensitized	9	8	17
Not Sensitized	14	49	63
Total	23	57	80
Chi^2 p-value < 0.05			

Sensitivity: 9/ (9+14) = 39.13 %

Specificity: 49/ (8+49) = 85.96 %

PPV: 9/ (9+8) = 52.94 %

NPV: 49/ (49+14) = 77.77 %

Tables 3.1.6.11 and 3.1.6.12 examine the association of duration of work at HSLAS and rat asthma and rhinitis. A significant association between rat asthma and duration of work with laboratory animals was noted. (P-value = 0.005) It seems that the majority of exposed workers in each of the "work duration" Groups had developed RAT_OA. There seemed to be a trend indicating higher prevalence of RAT_OA with shorter period of work at HSLAS.

Table 3.1.6.11 Cross-tabulation of Rat asthma and duration of work with laboratory animal among currently exposed workers.

	RAT_OA	**No RAT_OA**	**Total**
< 1 year	2 (8.3 %)	22 (91.7 %)	24 (100 %)
1 to 3 years	8 (38.1%)	13 (61.9 %)	21 (100 %)
3 to 5years	0 (0.0 %)	16 (100 %)	16 (100 %)
> 5 years	4 (30.8 %)	9 (69.2 %)	13 (100 %)
Total	14	60	74
Fischer's exact P-value= 0.005			

Similarly, as seen in the table below, there was a statistically significant relationship between rat rhinitis and duration of work with laboratory animals. (P-value < 0.05) Shorter duration of work with animals at HSLAS was associated with higher prevalence of RAT_OR.

Table 3.1.6.12 Cross-tabulation of Rat rhinitis and duration of work with laboratory animal among currently exposed workers.			
	RAT_OR	No RAT_OR	Total
< 1 year	6 (58.3 %)	18 (41.7 %)	24
1 to 3 years	10 (47.6 %)	11 (52.4 %)	21
3 to 5years	2 (12.5 %)	14 (87.5 %)	16
> 5 years	7 (53.8 %)	6 (46.2 %)	13
Total	25	49	74
Fischer’ s exact P-value < 0.05			

Tables 3.1.6.13 and 3.1.6.14 examine the association between duration of work at HSLAS and mouse asthma and rhinitis. There was no relationship between mouse asthma and duration of work with laboratory animals. (P-value > 0.1)

Table 3.1.6.13 Cross-tabulation of Mouse asthma and duration of work with laboratory animal among currently exposed workers.			
	MOUSE_OA	**No MOUSE_OA**	**Total**
< 1 year	1 (3.6 %)	27 (96.4 %)	28 (100 %)
1 to 3 years	3 (14.3 %)	18 (85.7 %)	21 (100 %)
3 to 5years	0 (0.0 %)	17 (100 %)	17 (100 %)
> 5 years	1 (7.2 %)	13 (92.8 %)	14 (100 %)
Total	5	75	80
Fischer’s exact P-value > 0.1			

There was also no relationship between mouse rhinitis and duration of work with laboratory animals (P-value > 0.1)

Table 3.1.6.14 Cross-tabulation of Mouse rhinitis and duration of work with laboratory animal among currently exposed workers.			
	MOUSE_OR	**No MOUSE_OR**	**Total**
< 1 year	5 (17.9%)	23 (82.1 %)	28 (100 %)
1 to 3 years	7 (33.3 %)	14 (66.6 %)	21 (100 %)
3 to 5years	1 (5.9 %)	16 (94.1 %)	17 (100 %)
> 5 years	5 (35.7 %)	9 (64.3 %)	14 (100 %)
Total	18	62	80
Fischer's exact P-value > 0.1			

And finally tables 3.1.6.15 to 3.1.6.118 (Appendix 12) summarize the results of cross tabulations to examine the possible association of occupational asthma and rhinitis to a) rat and b) mouse AND different occupational animal exposures, job tasks and use of PPE at HSLAS. In summary: RAT_OA, RAT_OR and MOUSE_OR were significantly associated with presence of atopy in the study participants. Among all job tasks, shaving animals was the single task to be significantly associated with all four major outcomes i.e. RAT_OA, RAT_OR, MOUSE_OA and MOUSE_OR. Animal sacrifice was associated with asthma and rhinitis to rats. Among PPEs, use of respirator was significantly associated with three of the four major outcomes i.e. RAT_OA, RAT_OR and MOUSE_OR.

3.2 Logistic Regression Model Building

3.2.1 Multivariate Logistic Regression Model; Sensitization to Rat

As discussed in the methods section, we used the "Purposeful Selection of Covariates" method to build multivariate logistic regression models to identify specific baseline characteristics, exposures, job tasks and preventive measures significantly associated with rat sensitization, asthma and rhinitis. Appendix 13 shows the list of all independent variables used in the analysis for RAT and MOUSE.

The method and details steps of model building were discussed in the previous chapter under the methods section. (Appendix 10) Table (3.2.1.1) summarizes the result of univariate logistic regression analysis of all included independent variables for outcome of rat sensitization (RAT_POSITIVE).

Table 3.2.1.1 Univariate Analysis of Independent Variables for RAT_POSITIVE *

Variable	Odds ratio, [95% CI]	P-value
Atopy	39, [7.9, 192.1]	0.000
FARM	3.2, [1.1, 9.4]	0.029
FH	2.4, [0.8, 7.2]	0.105
PET	2.0, [0.8, 5.3]	0.159
R_SERUM	2.1, [0.8, 5.4]	0.120
R_GOWN_BINARY	3.4, [0.8, 14.4]	0.081
R_RESPIRATOR_BINARY	4.1, [1.1, 16.1]	0.026
R_FEEDIND_BINARY	0.5, [0.2, 1.3]	0.151
R_INJECTION_BINARY	2.6, [0.8, 8.3]	0.093
R_SHAVING_BINARY	7.8, [0.9, 65.6]	0.052

R_SACRIFICE_BINARY	2.1, [0.7, 6.3]	0.181
R_DISPOSAL_BINARY	0.4, [0.1, 1.0]	0.056
R_MANUALCC_BINARY	0.4, [0.1, 1.5]	0.176
R_FEMALE_BINARY	0.5, [.2, 1.3]	0.159
* Only variables with p-value < 0.2 in the univariate analysis are listed.		

14 variables out of 35 had p values less than 0.2 and were therefore considered in the next step and included in the multivariable analysis. Using the 14 selected variables, a multiple logistic regression model was fitted. The overall Likelihood ratio Chi^2 test for the fitted logistic regression model is significant (Likelihood Ratio $Chi^2 = 59.26$ and P value < 0.0001), which means the fitted model significantly predicts the occurrence of binary outcome (i.e. RAT_POSITIVE). The STATA output 3.2.1.1 in Appendix 14 shows the coefficients and the respective WALD test P values. Then variables with insignificant coefficients were dropped one at a time while the changes in the coefficients of the remaining variables were closely monitored to detect potential confounding effects. After excluding the insignificant variables, preliminary main effects model for RAT_POSITIVE was created (STATA output 3.2.1.2 in Appendix 14) with ATOPY, PET, R_FEEDING, R_SACRIFICE and R_MANNUALCC significantly predicting RAT_POSITIVE i.e. sensitization to Rat. After dropping variable R_FEEDING, the parameter estimate for variable R_SACRIFICE was decreased > 20%. Therefore, R_FEEDING was kept in the model as the confounding term for R_SACRIFICE despite the P value of 0.074.

Change in the coefficient estimates for R_SACRIFICE_BINARY:

| (2.12 - 2.54) | / 2.12 > 20%

At this point pairwise multiplicative interaction terms were created using "gen" command and added to the model one at a time. But none were significant. Therefore, no interaction term was added to the final model. (Appendix 14)

Table 3.2.1.2 shows the odds ratios for each of the variables in the logistic regression model (STATA output 3.2.1.3 in Appendix 14). Atopy, having pets and animal sacrifice were associated with associated with higher risk of sensitization to rats. Whereas feeding animals and manual cage-cleaning were associated seem to be protective.

Table 3.2.1.2 Odds ratios for independent variables in final RAT_POSITIVE model

Variables	**Odds ratio, [95% CI]**
Atopy	355.3, [20.7- 6078]
Pet	7.2, [1.2- 43.6]
Feeding	0.2, [0.04- 1.1]
Sacrifice	12.7, [1.1- 144.9]
Manual cage cleaning	0.1, [0.007- 0.5]

Goodness of fit tests (Chi-squared and Hosmer–Lemeshow) were performed and both were insignificant. Therefore, it can be concluded that the final model significantly predicts the outcome variable (STATA output 3.2.1.4 in Appendix 14, final model in Appendix 15).

3.2.2 Multivariate Logistic Regression Model; Occupational Asthma to Rat

Based on the definition of occupational asthma (OA) in this study, out of 74 participants exposed to rats at work at the time of conducting the stud, 14 had OA. The prevalence of OA was then calculated as 18.91%. Similar to the previous section, after the univariate analysis of all independent variables, significant variables with alpha level of <0.2 were use to build a multivariate logistic regression model to identify the baseline characteristics, exposures and job tasks associated with the occurrence of occupational asthma to rats; RAT_OA (STATA output 3.2.2.1 in Appendix 14, final model in Appendix 15)

Table 3.2.2.1 Univariate analysis of independent variables for RAT_OA *		
Variables	**Odds ratio, [95% CI]**	**P-value**
Atopy	8.1, [1.0- 66]	0.051
R_Saliva	0.2, [0.02-1.5]	0.110
R_SACRIFICE_BINARY	5.9, [1.7-20.6]	0.005
R_INJECTION_BINARY	4.4, [1.3-15.3]	0.018
R_SHAVING_BINARY	7.7, [1.7-34.6]	0.007
R_MASK_BINARY	0.3, [0.09- 1.04]	0.057
R_RESPIRATOR_BINARY	6.5, [1.8, 23.5]	0.004
R_FILTERTOP_BINARY	0.2, [0.1-0.7]	0.014
R_IVC_BINARY	0.2, [0.1-0.9	0.032
* Only variables with p value < 0.2 in the univariate analysis are listed.		

The goodness of fit test confirmed the good fit of the model (STATA output 3.2.2.2 in Appendix 14)

The odds ratios (Table 3.2.1.2) were calculated using LOGISTIC command (STATA output 3.2.2.3 in Appendix 14). Animal sacrifice and use of respirators were associated with higher risk of rat asthma but use of individually ventilated cages was protective. (Final model in Appendix 15)

Table 3.2.2.2 Odds ratios for independent variables in final RAT_OA model

Variables	**Odds Ratio, [95% CI]**
Sacrifice	42.87, [5.0- 366.2]
Respirator	32.20, [4.0- 255.9]
IVC	0.05, [0.006-0.341]

3.2.3 Multivariate Logistic Regression Model; Occupational Rhinitis to Rat

25 out of 74 subjects exposed to rats at time of conducting the study were diagnosed with occupational rhinitis to rats. The prevalence of RAT_OR was 33.78 %. After univariate logistic regression analysis, 11 variables were selected and used to build a multivariate logistic regression model.

Table 3.2.3.1 Univariate analysis of independent variables for RAT_OR

Variable	Odds Ratio, [95% CI]	P value
Atopy	9.4, [2.0- 44.1]	0.005
Smoke	0.3, [0.05-1.3]	0.104
R_SKIN	3.3, [1.0 - 11.1]	0.052
R_CARCASS	2.2, [0.8- 5.8]	0.117
R_RESPIRATOR_BINARY	3.3, [0.9- 9.0]	0.080
R_INJECTION_BINARY	4.7, [1.5, 14.5]	0.007
R_SHAVING_BINARY	9.1, [1.7- 48.2]	0.009
R_SACRIFICE_BINARY	5.5, [1.8, 17.0]	0.003
R_DISPOSAL_BINARY	0.4, [0.1- 1.1]	0.079
R_MANUALCC_BINARY	0.3, [0.06-1.5]	0.139
R_BIOSAFETYCAB_BINARY	0.2, [0.04-0.94]	0.042

* Only variables with p-value < 0.2 in the univariate analysis are listed.

In the final model, atopy, animal sacrifice and manual cage cleaning were significantly associated with RAT_OR and the goodness of fit tests were insignificant (STATA output 3.2.3.1 and 3.2.3.2, Appendix 14, final model in Appendix 15).

Table 3.2.3.2 shows the Odds ratios for the significant variables in the final model (STATA output 3.2.3.3, Appendix 14). Atopy and animal sacrifice were associated with higher risk of rat rhinitis whereas manual cage cleaning seemed to be protective.

Table 3.2.3.2 Odds ratios for independent variables in RAT_OR model

Variables	**Odds Ratio, [95% CI]**
Atopy	42.5, [3.7- 487.8]
Animal Sacrifice	27.4, [3.1- 240.2]
Manual cage cleaning	0.07, [0.006- 0.9]

3.2.4 Multivariate Logistic Regression Model; Sensitization to Mouse

17 out of 80 participants exposed to Mice at work were sensitized to mice in Skin prick test (21.25%). To meet specific objective 24 as discussed above, purposeful model building method was used to build a multiple variable logistic regression model for "sensitization to mouse" (MOUSE_POSITIVE). All 35 variables from Table 3.2.1.1 were used in the univariate analysis to identify those significantly associated with MOUSE_POSITIVE. Table 3.2.4.1 shows variables significantly associated with MOUSE_POSITIVE at an alpha level of 0.2.

Table 3.2.4.1 Univariate analysis of independent variables for MOUSE_POSITIVE

Variable	Odds Ratio, [95% CI]	P-value
AGE	1.0, [0.9-1.1]	0.162
SEX	0.2, [0.04-0.96]	0.045
FARM	2.7, [0.9- 8.4]	0.089
m_urine	4.9, [1.0- 23.5]	0.045
m_saliva	4.3, [1.4-13.4]	0.011
m_carcass	2.5, [0.8- 7.4]	0.103
m_injection_binary	2.9, [0.9-9.4]	0.064
m_unitcleaning_binary	2.1,[0.7-0.4]	0.181

* Only variables with p-value < 0.2 in the univariate analysis are listed.

Only two variables including mouse saliva and injection were significantly associated with sensitization to mice. The goodness of fit tests for the final model were insignificant (STATA output 3.2.4.2 in Appendix 14, final model in Appendix 15).

Table 3.2.4.2 shows the odds ratios for the variables included in the logistic regression model for sensitization to Mouse (STATA output 3.2.4.3 in Appendix 14). Contact with animal saliva and animal injection both were associated with higher risk of sensitization to mice.

Table 3.2.4.2 Odds ratios for independent variables in MOUSE_POSITIVE model

Variables	**Odds Ratio, [95% CI]**
Saliva	4.9, [1.5- 15.9]
Injection	3.5, [1.0- 12.1]

3.2.5 Multivariate Logistic Regression Model; Occupational Asthma to Mouse

6.25% i.e. 5 out of 80 participants exposed to mice at work at the time of study were identified as having occupational asthma to mice. Purposeful model building method was used to build a multiple variable logistic regression model for occupational asthma to Mice (MOUSE_OA). Of all the 35 independent variables used in uni-variate analysis only two were significant enough to enter multivariate logistic regression analysis. (Table 3.2.5.1).

Table 3.2.5.1 Independent variables associated with MOUSE_OA in univariate analysis

Variable	Odds Ratio, [95% CI]	P-value
m_CARCASS	6.3, [0.7- 59.6]	0.106
m_MASK_BINARY	0.2, [0.03, 1.3]	0.087
* Only variables with p-value < 0.2 in the univariate analysis are listed.		

After multivariate logistic regression analysis both variables were included in the final model. Although the overall model is significant, the individual independent variables did not reach the desired significance level of 0.05 (STATA output 3.2.5.1, Appendix 14).

Goodness of fit test for the multivariate model was insignificant (STATA output 3.2.5.2, Appendix 14, final model in Appendix 15).

Table 3.2.5.2 shows the odds ratios for variables in logistic regression multivariate analysis for MOUSE_OA. The result indicates contact with mouse carcass at work is associated with 7 times higher risk of developing MOUSE_OA where use of surgical masks seems to be protective (STATA output 3.2.5.3, Appendix 14).

Table 3.2.6.2 Odds ratios for independent variables in MOUSE_OA model.	
Variables	**Odds Ratio, [95% CI]**
Carcass	7.16, [0.7- 70.9]
Mask	0.17, [0.02- 1.2]

3.2.6 Multivariate Logistic Regression Model; Occupational Rhinitis to Mouse

18 (22.5%) out of 80 participants exposed to mice at the time of conducting the study were identified as having occupational rhinitis to Mice. To build a multivariate logistic regression model for MOUSE_OR, a univariate analysis was performed using all 35 independent variables separately. 7 variables were significant enough and were entered in the multivariate analysis.

Table 3.2.6.1 Independent variables associated with MOUSE_OR in univariate analysis

Variable	Odds Ratio, [95% CI]	P value
ATOPY	16.0, [2.0- 127.2]	0.003
M_URINE	3.1, [0.82- 12.1]	0.100
M_GLOVES_BINARY	0.1, [0.01- 1.5]	0.103
M_GLASSES_BINARY	2.2, [0.7- 6.7]	0.161
M_RESPIRATOR_BINARY	6.1, [0.9- 39.2]	0.059
M_SHAVING_BINARY	6.0, [0.9- 39.2]	0.059
M_FILTERTOP_BINARY	2.9, [0.03- 0.43]	0.123

* Only variables with p-value < 0.2 in the univariate analysis are listed.

After excluding variable M_URINE, the parameter estimate (coefficient) for M_SHAVING_BINARY changed significantly in indicating the confounding effect of variable M_URINE (STATA output 3.2.6.1, Appendix 14):

| (1.9- 2.4)| / 1.9 * 100= 28.1 > 20%

Therefore, M_URINE was kept in the model as a confounding factor. The goodness of fit tests were desirably insignificant (STATA output 3.2.6.2, Appendix 14, final model in Appendix 15).

Table 3.2.6.2 shows the odds ratios (STATA output 3.2.6.3, Appendix 14). While atopy, contact with mouse urine, mouse shaving and use of filter-top cages were all associated with higher risk of developing mouse rhinitis, the use of gloves while working with mice, seemed to be protective.

Table 3.2.5.2 Odds ratios for independent variables in MOUSE_OA model.

Variables	**Odds Ratio, [95% CI]**
Atopy	22.8, [2.06- 252.9]
Urine	6.4, [0.9- 45.6]
Gloves	0.02, [0.0005- 0.6]
Shaving	11.4, [1.1- 109.1]
Filter-top cages	6.2, [0.001- 2.3]

3.2.7 Summary of Logistic Regression Models

The table below shows a summary of the independent variables associated with each of the six outcomes in the multivariate logistic regression models (Full models for all six major outcomes in Appendix 15):

Table 3.2.7.1 Summary of independent variables associated with outcomes	
Rat Sensitization	Atopy, pet, animal sacrifice, feeding and manual cage cleaning
Rat Asthma	Animal sacrifice, use of respirators and IVC
Rat Rhinitis	Atopy, animal sacrifice, manual cage cleaning
Mouse sensitization	Mouse saliva, injection.
Mouse Asthma	Mouse carcass, use of facial masks
Mouse Rhinitis	Atopy, mouse urine, shaving, use of gloves and filter-top cages

Chapter Four: Discussion

4.1. Discussion:

In this cross-sectional study, we studied the prevalence of sensitization, occupational rhinitis (OR) and asthma (OA) to common laboratory animals among a Group of animal laboratory husbandry workers (Group 1) and researchers (Group 2) exposed to laboratory animals in facilities associated with Health Sciences Laboratory Animal Services (HSLAS) at university of Alberta, Edmonton, AB, Canada. We also included a comparison Group comprised of students from the University of Alberta with no prior occupational exposure to laboratory animals (Group 3).

Compared to HSLAS workers (Group 1), the researchers were of similar age range but the students were significantly younger. The researchers and students were more likely to have graduate level education and were less likely to have previous environmental exposures to animals including history of living in livestock farms as compared to the HSLAS workers.

A detailed review of all the occupational exposures and job tasks among the study participants revealed that activities such as changing soiled litter, cage-cleaning and cleaning within the animal units were significantly more common among the HSLAS workers (Group 1) compared to the researchers (Group 2). Such activities are known to cause significant exposure to laboratory animal urinary allergens and have been studied

extensively in the literature. In fact, according to several studies, cage-cleaning alone has been identified as the most important determinant of personal exposure to mouse and rat urinary allergens. (98) In another study of quantitative measurement of murine urinary allergens in an animal facility, highest personnel allergen exposure was detected during cage change and emptying of soiled cages. (99)

The researchers (Group 2) on the other hand were more likely to engage in job tasks exposing them to mouse and rat serum, blood and internal tissues. It is unclear whether exposure to animal blood or internal organs affects the risk of developing LAA and there is no evidence suggesting such work-place exposures would lead to asthma or rhinitis symptoms.

As a notable observation, we found that the rate of PPE use was significantly higher among laboratory animal workers compared to the researchers. The HSLAS workers were especially more likely to use protective gowns and surgical masks. This could be due to HSLAS institutional policies requiring the staff to use certain PPE while exposed to laboratory animals within the animal husbandry facilities. The researchers however, would often take the animals out from the HSLAS facilities into research laboratories within different departments of Faculties of Medicine and Dentistry or Pharmacy and Pharmaceutical Sciences, where the use of protective measures was probably not enforced as strictly as within HSLAS.

Similarly, filter-top and individually ventilated cages (IVC) were more commonly used by HSLAS workers compared to the researchers. IVCs and filter-top cages have been

shown to reduce the exposure level. According to a study comparing the efficacy of five different types of cages used in laboratory animal facilities, allergen concentrations were lowest in rooms with sealed IVC under positive or negative pressure and with unsealed IVC under negative pressure. The study showed that the use of sealed IVC, significantly minimized allergen exposure among the animal husbandry staff. (99)

We looked at the rate of sensitization to laboratory animals. Based on our findings, more than half of HSLAS workers were sensitized to rats (50.9%) and more than a quarter of them were sensitized to mice (28.1%). The overall rate of sensitization to mouse and/or rats was 47.4% [37.9-56.9] but across the two groups, animal laboratory workers had significantly higher rates of sensitization in comparison to the researchers, that is 57.9 [44.1-70.8] 95% CI, versus 36.8 [24.4-50.6] 95% CI, respectively. None of the students in Group 3 showed sensitization to laboratory animal allergens but 18% were sensitized to cats. These findings represent a significant level of sensitization to laboratory animals among HSLAS workers and researchers. Previous studies have shown overall rodent sensitization rates ranging between 10- 46% (59, 100-103) and animal-specific sensitization rates of 18-19% and 14% to rat and mouse respectively (104, 105). Our findings could suggest that either 1) the efforts to control exposure to rat and mouse allergens in animal laboratories to date, at least at the University of Alberta, have not been successful, or that 2) sensitization does not invariably correlate with the level of exposure and a clear dose-response relationship probably does not exist or at least is not completely linear.

The highest rate of sensitization across the three Groups was seen to cats as 54.4 % and 44.6% in Groups 1 and 2 respectively in addition to 18% of the control Group were sensitized to cats. But it was also noted that HSLAS workers and researchers were significantly more likely to keep cats as pets at home compared to those in Group 3.

The rate of sensitization to common aeroallergens and therefore the prevalence of atopy (defined as positive SPT to at least one common aeroallergen) were numerically higher among exposed participants (Groups 1 and 2) compared to Group 3 but the difference did not reach statistical significance. Within the exposed participants, we found that the animal workers had significantly higher rates of positive SPT to certain common aeroallergens compared to the researchers. These included mold mix, trees mix and mite allergens. Given the fact that the overall prevalence of atopy was comparable between HSLAS workers and researchers the importance of such findings is unclear. While some still question whether individuals with atopy and pre-existing non-occupational allergies to agents from outside the laboratory have an increased risk of developing sensitization, the majority of prior studies suggest it is an important risk factor. (56, 57, 81)

The overall prevalence of atopy was close to 60% in our study, which is almost double that of the average of general population however rates as low as 16% in Brazil and as high as 63 % in certain areas of China and Japan have been previously reported in the literature. (65, 106, 107) It is important to note that different definitions have been used to define the concept of atopy in various studies. Atopy has been defined as presence of at least one positive SPT to common aeroallergens (used in our study), two or more positive SPTs to common aeroallergens, serum IgE levels > 100 U/ml, positive RAST

test against common allergens or merely the presence of personal or family history of allergic conditions (hay fever, asthma or eczema) (56, 108-111). Use of different definitions for atopy has complicated the comparison between the rates reported by different studies.

We found a very strong association between atopy and sensitization to rat especially and mouse. One potential explanation for this observation was the possibility of direct cross-reactivity between laboratory animal allergens and the common aeroallergens. However, there was no significant difference between the rates of atopy between the animal laboratory workers (i.e. HSLAS staff and researchers) with the students. As an alternative explanation, it is important to note that several studies have shown an association between sensitization to common aeroallergens including house dust mites and LAA. Laboratory animal workers seem to be at high risk of exposure and sensitization to mite-derived allergens.(112, 113) We did not categorize atopy based on the type of the common aeroallergen and therefore we were unable to examine this possible explanation.

While the possible association of atopy and sensitization to laboratory animals is still under investigation, multiple published reports provide evidence that atopy increases the risk of developing occupational asthma due to laboratory animal allergens through yet unknown mechanisms. (49, 51, 56) In fact, this has been studied extensively and it is widely accepted that atopy increases the risk of developing occupational asthma caused by exposure to HMW agents that induce the production of IgE antibodies. (49, 51, 84,

114, 115) A similar pattern was not detected among asthmatics due to low molecular weight agents including but not limited to red western cedar allergens (116, 117).

Regarding the prevalence of OA and OR, numerous reports have been published in the past four decades. While the overall prevalence of occupational asthma in previous studies has been about 10%, prevalence of OA due to laboratory animal exposure when defined as work-related chest symptoms (WRCS) was found to range from 2.2% (118) to 11.7% (15) and when defined as WRCS plus positive SPT to laboratory animals, it was reported to be between 1.4 and 9.5% . Our study suggests a relatively higher prevalence of OA with an overall prevalence of 15.1 % [8.7-23.7] 95% CI, among all exposed laboratory workers at HSLAS. Based on our results 12.8% [4.8 -25.7] 95% CI, and 29.6 % [13.7-50.2] 95% CI, of study subjects in Groups 1 and 2 had occupational asthma secondary to rat exposure (Rat-OA) and 8.2% [2.3-19.6] 95% CI, and 3.1% [0.1-16.2] 95% CI, in Groups 1 and 2 respectively were found to have occupational asthma secondary to mouse exposure (Mouse-OA) at the time of study.

Overall over the last 40 years the prevalence of asthma and related atopic disease has been increasing. Whereas the prevalence of the atopic diseases now seems to have reached a plateau in many western countries, they are still on the rise in the developing world (119). With regards to occupational asthma, several longitudinal studies have reported a trend towards a progressive decline in the prevalence of OA due to laboratory animal exposure whereas others suggested there is no evidence that prevalence of OA is falling (52) In our study however, due to the cross-sectional nature of our study and lack of prior prevalence studies in our population of workers, we are unable to comment on

the trend of the prevalence of LAA at HSLAS, although it was found to be higher than many previous reports.

Susceptibility to development of occupational asthma is complex. The high prevalence of OA among our study population is likely multifactorial, but could be due to higher rates of occupational exposure, different work practices and / or inadequate implementation of preventive measures at the animal facilities.

The hygiene hypothesis is another possible contributor to the observed rates of OA in our study. According to the hygiene hypothesis microbial exposures early in life interact with the host genetic background to modify the risk for developing asthma and allergic diseases. Childhood infections and exposure to microbial antigens were found to be inversely associated with the likelihood of developing asthma and allergies later in life (120, 121). There is convincing evidence that atopy increases the risk of developing occupational asthma due to high molecular weight allergens (i.e. laboratory animal allergens) through yet unknown mechanisms (51). Higher rates of OA observed in our study population may be, as well, associated with higher rates of atopy in our population. Let us remember that the rate of atopy among our study population was twice as high as that of the general population. This together with the fact that our study was conducted in an urban area in a developed country suggests the hygiene hypothesis as a potential explanation at least in part for the higher prevalence of OA in this population.

Use of different criteria to detect OA secondary to laboratory animal exposure across the prior studies, is another potential explanation for the observed differences in the rate of OA. While we considered subjective reporting of work-related asthma symptoms plus positive SPT as evidence of presence of occupational asthma, many studies have used more specific tests including objective measurement of lung volumes via pulmonary function testing (PFT) or even more specific challenge tests to define occupational asthma to certain allergens. While work-related asthma symptoms (i.e. respiratory symptoms that improve on days away from work or during holidays) are considered as a fairly sensitive screening method for detection of possible occupational asthma, a positive response is not specific, as occupational asthma can only be confirmed in 50% of workers with cough or wheeze that improves on days away from work (122). Evidently the use of more sensitive tests provides with lower rates of false negative diagnoses at the cost of the specificity and results in higher estimates. It is possible that our definition of OA was more sensitive than other studies' criteria, which as stated above would make it a potentially suitable screening test but as a result, its use led to higher estimates of OA prevalence.

Occupational rhinitis is generally more prevalent than asthma and reportedly has been detected in up to 30% (with reports from 6.7% to 41.7%) of exposed animal laboratory workers (52). Our study revealed similar results among HSLAS staff with an overall prevalence of OR of 28.3% [19.6-38.2] 95% CI. There was no significant differences between the two groups in the overall prevalence of OR. Among those workers exposed to rats, RAT-OR was more common among researchers that is 25.5 [13.9-40.3] 95% CI,

versus 48.1% [28.7-68.1] 95% CI, in Groups 1 and 2 respectively. No notable difference was detected in Mouse-OR across the two groups as 24.5 [13.3-38.9] 95% CI and 18.7% [7.2-36.4] 95% CI had Mouse-OR in the two Groups respectively.

Finally, we found certain work exposures and job tasks to be associated with development of LAA. Most notably, engaging in animal shaving was a significant risk factor for development of both asthma and rhinitis due to exposure to rats and mice. Animal sacrifice was another task identified as a risk factor for rat asthma and rhinitis. Animal injection and litter changing were also found to be significantly associated with rat asthma and rhinitis respectively. These findings are of paramount importance since they help in identification of job tasks where workers would benefit from implementation of more efficient control measures.

In the multivariate analyses it seemed that use of respirators and filter-top cages were associated with increased risk of LAA. However, the likely explanation is that the laboratory animal staff who had developed allergic symptoms had started using PPE including respirators more frequently and more consistently compared to their asymptomatic co-workers. Similarly, it seemed that cage-cleaning had a protective effect on developing LAA (i.e. OR<1) while it is known that cage-cleaning is associated with high levels of allergen exposure, that is probably more than any other task at the animal house. We explained this finding by considering the possibility that the symptomatic laboratory animal staff were removed and assigned to do tasks other than cage-cleaning to avoid excessive exposure (survivor bias).

4.2. Implications:

While there was no significant difference in the overall rate of OA and OR across the two Groups, there were important differences between the two Groups in terms of the rate of animal-specific LAA. Laboratory animal workers had numerically higher rates of Mouse-OA and Mouse-OR compared to the researchers. They also had higher rates of sensitization to both rat and mouse. Group 1 participants were more likely to engage in activities known for producing high allergen concentration including changing soiled litters and cage-cleaning while using PPE more consistently and IVCs more frequently. To decrease the rate of mouse allergy, this Group would likely benefit from better inhalation exposure control, including but not limited to the use of masks and respirators, as well as use of biosafety cabinets and standardized work-stations especially while handling mice. The researchers (Group 2) on the other hand had higher rates of Rat-OA and Rat-OR and were less likely to develop sensitization to laboratory animals. Contrary to the HSLAS workers, the researchers were more frequently involved in job tasks entailing direct contact with animal serum, blood and internal organs. This Group would likely benefit from more consistent use of PPE including gloves and gowns among others especially when handling rats.

According to the logistic regression models, rat allergy (including sensitization, asthma and rhinitis) was significantly associated with job tasks including rat sacrifice or manual cage cleaning whereas development of mouse allergy was associated with contact with mouse saliva, urine and carcass and job tasks including injection and shaving fur. Since the risk of sensitization and OA is increased by higher exposure to workplace agents,

these findings suggest opportunities for better prevention of animal-specific LAA by controlling exposures during these high-risk activities.

Finally, we did not observe an association between the duration of work with laboratory animals and the rate of sensitization, but we noted a relationship between duration of working with animals and development of OA and OR among exposed individuals, which has been reported previously in the literature (52). Longer duration of employment and therefore exposure to laboratory animal allergens is a known risk factor for developing LAA, suggesting a cumulative dose‑response relationship between the exposure to laboratory animals and the rate of developing allergic symptoms (123). Therefore long-term surveillance strategies are recommended as informative prognostic parameters and should be continued as long as workers are exposed to laboratory animals.

4.3. Strengths and Limitations:

This study had a cross-sectional design and was therefore carried out in a relatively short period of time. It was a relatively inexpensive study, which provided with information regarding the prevalence of sensitization, OA and OR to laboratory animals between two different exposed populations under study. Our study also indicated association between potential risk factors, job tasks and protective measures at work place that may exist and are therefore useful in hypothesis generation for future research.

Due to its cross-sectional nature, our study was unable to give any indication of the sequence of events or temporal relationship between outcomes and exposure-whether the work exposures/use of PPE occurred before, after or during the onset of the asthma or rhinitis symptoms. Therefore, it is very difficult to make causal inference based on our data. Secondly, our study was only able to evaluate prevalent rather than incident outcomes and thus people who developed the outcome but had to quit their job due to severity of symptoms were not included in the study. In other words this study was prone to prevalence-incidence bias (also known as Neyman bias or selective survival bias) where any risk factor that resulted in severe respiratory symptoms leading to study subjects quitting their job would have been potentially under-represented among those remaining in the study (124, 125).

Moreover as in all observational research studies our study involved direct observation of individuals in their natural settings and as such the differences in baseline characteristics and variation in the prevalence of study outcomes (sensitization, OA,

OR) is determined by individuals characteristics. It is therefore very important to always consider the potential of alternative explanations (confounding) for study results. And therefore as in all observational research, confounding remains the major challenge in this study (126).

Other potential biases include possible prevalent recruitment of workers with work-related or non-work-related respiratory symptoms who were more interested in participating in the study or on the contrary prevalent recruitment of healthy-workers, because symptomatic workers did not participate in the study due to a fear of job loss.

Our study was also limited due to a relatively small sample size in spite of a participation rate of about 75% among HSLAS staff. The response rate among the exposed researchers, i.e. Group 2, however was more limited. We found atopy to be a strong risk factor for LAA especially rat sensitisation. Although we defined atopy as a positive SPT to at least one of the common aeroallergens for the sake of consistency with the majority of prior reports, this definition is neither sensitive nor specific for this broad and not fully understood genetic predisposition to allergies. Despite the well-established relationship of atopy and LAA and the great interest in studying the possible mechanisms of such association, it seems more practical to prioritize and focus on identification of modifiable risk factors of LAA.

Finally and concerning the external validity of the study, the participants were recruited from a single animal house at university of Alberta in Edmonton and therefore our

results may not be generalizable to other animal houses where the number/ type of animals and occupational conditions are different.

4.4. Conclusion:

Our study suggests that in spite of implementation of extensive preventive and protective measures in HSLAS animal house over the last decade, the prevalence of sensitization, occupational asthma and rhinitis remains relatively high. The use of personal protective equipment and preventive measures especially while conducting activities entailing close contact with animals and their tissues should be encouraged. Prevention of the development of LAA should be the aim of all facilities engaged in the use of laboratory animals and exposure elimination should be the preferred primary prevention approach in all such occupational environments.

Atopy appears to be highly associated with development of LAA and therefore pre-placement screening of hired workers for atopy and allergy to other antigens such as pollens, molds, and animal dander could help in identifying individuals at higher risk for developing LAA. Such individuals could potentially benefit from closer health monitoring, appropriate counseling and in case of developing symptoms of, regular medical follow-ups. Also assigning employees at risk to specific jobs with lower levels of exposure can be considered, in an effort to reduce risks for development of laboratory animal sensitivity, but might be considered discriminatory and so would need to be considered very carefully. Comprehensive surveillance protocols for detecting and monitoring workers at increased risk for sensitization may reduce the incidence of

occupational asthma and rhinitis due to laboratory animal exposure or prevent its progression.

However, even though atopy appears to be a strong risk factor for high-molecular-weight antigen-induced asthma, this finding should not be used to exclude workers from employment in high-risk occupations. The low positive predictive value (PPV) of atopy for occupational asthma precludes its use in hiring and placement practices [23,27,28]. But these employees should be informed of their increased risk, educated to use appropriate measures to minimize their exposure to high-molecular-weight antigens, and monitored for possible signs of asthma.

Finally, despite well-established relationship of atopy and LAA and the great interest in studying the possible mechanisms of such association, it seems more practical to prioritize and focus on identification of modifiable risk factors of LAA as opposed to the non-modifiable genetic predispositions for development of allergies. We found that animal shaving and sacrifice were most strongly associated with development of occupational asthma and rhinitis. The animal husbandry staff are most likely to benefit from more efficient use of PPE and stricter control measures while engaging in such activities.

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Appendix 1: Participant Information Sheet and Informed Consent Form

UNIVERSITY OF ALBERTA

DIVISION OF PREVENTIVE MEDICINE
DEPARTMENT OF MEDICINE
Faculty of Medicine & Dentistry

5-30 University Terrace
8303 – 112 St
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Tel: 780.492.6291
Fax: 780.492.9677
www.medicine.med.ualberta.ca/AboutUs/Divisions/PMED

PARTICIPANT INFORMATION SHEET

Study Title: The prevalence of Occupational Asthma and Rhinitis among animal laboratory workers at Health Sciences Laboratory Animal Services (HSLAS), University of Alberta; A cross-sectional study

Principal Investigator: Jeremy Beach
Associate Professor, Department of Medicine
Faculty of Medicine and Dentistry
Telephone: 780 492-8175

Co-Investigator: Neda Dianati Maleki
Graduate student at School of Public Health
Telephone: 780 246-4697

BACKGROUND AND PURPOSE:

Historically, there is considerable evidence that workers with exposure to allergens at work developed laboratory animal allergy (LAA). Studies suggested that the prevalence of LAA could be as high as 44% in certain groups. Most workers who developed allergies presented with symptoms within 3-4 years of exposure, the peak incidence occurring at 1-2 years, with the allergy often taking the form of either occupational rhinitis (OR) or occupational asthma (OA). Animal allergens are found in fur, dander, skin epithelium and body fluids (saliva, serum, urine) of mice, rats, guinea pigs, rabbits, and other animals. Exposure of laboratory workers can occur either through direct contact or by contact with airborne particles. Researchers and workers in charge of activities such as feeding, cleaning, sampling, sacrifice, cage handling, as well as maintenance and waste disposal personnel were all thought to be at risk.

In the last decade or so there have been substantial changes in the way that laboratory animals are housed and used in research. The proportion of different animal types has changed, with mice now often predominating, and laboratories now routinely using a number of control measures to that place barriers between the animal and the worker, so reducing the potential for exposure In light of these changes there is a need for up-to-date data regarding LAA to re-assess the incidence and prevalence of LAA in the modern animal house setting, to evaluate the efficiency of implemented work-place modifications, and if necessary to highlight where further preventive measures might be usefully implemented.

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OBJECTIVES:

Our goal is to estimate the prevalence of LAA, including both occupational asthma and occupational rhinitis among Health Sciences Laboratory Animal Services (HSLAS) workers and employees. We are also interested in studying the association of the potential risk factors and laboratory animal allergy symptoms, in this population.

PROCEDURES:

If you choose to participate in this research study, please sign and return the consent form accompanying this letter. We will then make an appointment to meet with you at HSLAS where you will be asked to complete a questionnaire about your health and the nature of your work at HSLAS. We will then ask you to undergo skin prick testing to identify if you are sensitised to a number of laboratory animal allergens. This is a simple skin test where a few drops of the relevant purified allergens are put onto the skin of your forearm and then a 1mm tipped sterile lancet used to prick through the drop into the skin surface. Several allergens can be tested at the same time. We will use 8 different allergens that we relevant for HSLAS. If you are sensitized to one of the allergens a wheal of 3 mm or more will occur after 10-15 minutes, and then quickly disappear.

The skin prick test will take 15 minutes maximum. Filling the questionnaire will also take another 15 minutes, but to save time you can also fill it while waiting for the results of the skin prick test. The total time needed for the test and the questionnaire will not exceed 30 minutes. Since the test will be done during working hours at the main complex of HSLAS, if for any reason you are not comfortable doing the test at HSLAS, we can arrange to meet you either at Occupational Health office, University Terrace or at Garneau Lung Laboratory, College Plaza, both very closely located to HSLAS.

BENEFITS:

The results of your own testing will be immediately available to you which may be of help to you as an individual. If the study identifies preventable risk-factors for LAA then additional control measures could be considered within HSLAS to reduce the risk of LAA for all workers.

RISKS:

Skin Prick Tests are considered very safe and carry only a low risk. They are routinely carried out in many doctors' offices and clinical laboratories. A positive reaction to one of the allergens

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is similar to localized hives, or an insect sting, and usually disappears within about half an hour after the test. In rare cases they can persist for slightly. Any positive responses may be itchy and are best treated by applying an over the counter antihistamine cream which will be available at the time we test you. There is a very rare possibility of a more widespread allergic reaction which would need to be treated the same as any other allergic reaction if it occurred.

CONFIDENTIALITY:

All information will be kept confidential and used only for the purposes of this study. It will be available only to the study investigators, and not to your managers or anybody else at HSLAS. Your details will not be disclosed in any report published as a result of this study. By signing the consent form you give permission to the study staff to access and use the personal health information you have provided during the study. The data from this study will be used by the co-investigator, Neda Dianati Maleki in her MSc degree thesis.

In addition to the study team, the University of Alberta's Health Research Ethics Board may have access to your personal health records to monitor the research and verify the accuracy of study data. The study data will be stored for a minimum of 5 years at the occupational health office by the principle investigator.

VOLUNTARY PARTICIPATION AND FREEDOM TO WITHDRAW:

Participation in the study is voluntary. If you choose to participate in the study, you can withdraw from the study at any time. You do not have to give a reason for withdrawing.

COSTS:

The study-related tests and procedures will be provided to you at no cost to you or your insurance company. Your Skin Prick Test (SPT) may be performed at HSLAS at your convenience.

ADDITIONAL CONTACTS:

If you have any concerns about any aspect of this study you may contact the Health Research Ethics Office 780-492-2615. This office has no affiliation with study investigators. If you have any questions or concerns about the study activities please contact:

Dr. Jeremy Beach	Office 780 492-6291
Neda Dianati Maleki	Cellular 780 246-4697

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CONSENT FORM

Study Title: The prevalence of Occupational Asthma and Rhinitis among animal laboratory workers at Health Sciences Laboratory Animal Services (HSLAS), University of Alberta; A cross-sectional study

Investigator: Dr. Jeremy Beach Telephone: 780 492-6291

- Do you understand that you have been asked to be in a research study? Yes ☐ No ☐
- Have you read and received a copy of the attached Information Sheet? Yes ☐ No ☐
- Do you understand the benefits and risks involved in your taking part in this research study? Yes ☐ No ☐
- Have you had an opportunity to ask questions and discuss this study? Yes ☐ No ☐
- Do you understand that you are free to refuse to participate in the study or withdraw from the study at any time? You do not have to give a reason. Yes ☐ No ☐
- Has the issue of confidentiality been explained to you? Do you understand who will have access to your provided health information? Yes ☐ No ☐

This study was explained to me by: _____

I agree to take part in this study. Yes ☐ No ☐

_____ _____
Signature of the Participant Date mm/dd/yyyy

_____
Printed Name

I believe that the person signing this form understands what is involved in the study and voluntarily agrees to participate.

_____ _____
Signature of Investigator or Designee Date

Appendix 2: Laboratory Animal Allergy Study Questionnaire

Division of Preventive Medicine
Department of Medicine
Faculty of Medicine and Dentistry

The Questionnaire

Study Title:

The prevalence of Occupational Asthma and Rhinitis among animal laboratory workers at Health Sciences Laboratory Animal Services (HSLAS), University of Alberta; A cross- sectional study

Principal Investigator: Jeremy Beach
Associate Professor, Department of Medicine
Faculty of Medicine and Dentistry
Telephone: 780 492-8175

Co-Investigator: Neda Dianati Maleki
Graduate student at School of Public Health
Telephone: 780 246-4697

Occupational Asthma and Rhinitis Symptoms Questionnaire

Date:_____ ID number:_____

A. Personal data:

1. DOB: ___ ___/___ ___ ___/___ ___
2. Gender: ☐ M ☐ F
3. Highest degree or high school grade completed:

   Grade school or Junior High: 1 2 3 4 5 6 7 8
   High School: 9 10 11 12
   Post High School- Technical School: 1 2

   College: 1 2 3 4 Bachelor's Degree ☐ Advanced Degree ☐_____

B. Environmental data

1. At the time when you were born, was your family living on a Farm?

   If Yes, was it a: ☐grains/ vegetables farm

   ☐Livestock farm. Please name the animals_____

2. Did you live on a farm for a period of three months or longer before the age of 5?

   If Yes, was it a: ☐grains/ vegetables farm

   ☐Livestock farm. Please name the animals_____

C. Occupational data

1. Current job title:_____
2. When did you first start working under your current job title? Year_____
3. Duration of work per week under the same job title:

   Less than 10 h/week ☐ 10-20 h/week☐ 20-30 h/week ☐ 30-40h/week ☐ 40< h/week ☐

4. When did you first start working at HSLAS? Year_____

5. When did you first start working with laboratory animals? Year_____

6. Are you still working with laboratory animals? Yes ☐ No ☐

If No, a. What date did you stop? _____

b. Why did you stop? _____

7. Please fill the following table regarding all your <u>previous</u> occupations.

No	Occupation	Industry	Start date (Month/Year)	End date (Month/Year)	Were you exposed to Lab animals ? (Y/N)	Please name the animals.
1.						
2.						
3.						
4.						
5.						

8. Do you handle or deal with lab animals (dead or alive) or come into contact with any animal products or waste in your current job at HSLAS?

No□ if No, please go to **section E**

Yes□ if yes, please continue with question 9

9. What type of animals or animal products/waste do you handle or deal with in your current job at HSLAS? Choose all that apply.

1. Mice ☐ 2. Rats ☐ 3. Guinea pigs ☐ 4. Rabbits ☐

5. Primates ☐ 6. Birds ☐ 7. Cats ☐ 8. dogs ☐

9. other☐ (please specify_____)

10. For <u>each</u> animal type that you have chosen in the above question, please fill a "Laboratory Animal information sheet". One sheet is included in this questionnaire in **section C**. You may ask the interviewer for more sheets if you have chosen <u>more than one</u> animal type.

D. Laboratory Animal information sheet

Participant ID Number:_____

For each animal type that you have chosen in question 9 from section C, please fill a separate "Laboratory Animal Information Sheet". You may ask the interviewer for more sheets if you have chosen more than one animal type.

1. Animal type:_____

2. How many hours per week on average do you deal with this animal type? _____ hours per week

3. Please indicate with which part, organ or tissue of this animal body you are mostly in contact? Choose all that apply.

   Skin☐ Fur & dander ☐ Serum☐ Urine☐ Saliva☐

   Internal organs and tissues ☐ Carcass/ carrion ☐ Cages/ wastes/ beddings ☐

   Other☐ (please specify_____)

4. Please indicate if you use any of the following personal protective equipment (PPE) while working with this animal type in the laboratory:

	Never	Rarely	Sometimes	Often	Always
Protective Gloves					
Protective clothing/Gowns					
Safety Glasses/shields					
Surgical type Facial Masks					
Respiratory protective equipment such as Particle filter respirator (SPf2)					

5. Please indicate if you are involved in any of the following tasks related to this animal.

6

	Never	Few days per month	Few days per week	everyday
1.Handling and working with animals (dead or alive)				
2. Feeding animals				
3.Injections or other invasive procedures				
4.Shaving fur				
5.Animal Sacrifice				
6. Box changing				
7.Disposal of soiled litter				
8.Procedures involving More than one animal				
9.Manual Cage cleaning				
10.Automated Cage cleaning				
11.Indirect contact in animal room				
13.Cleaning within animal unit				

6. Please indicate if any of the following measures or equipment is in use in your lab while working with this Animal?

	Never	Rarely	Sometimes	Often	Always
Filter-top cages instead of Open-top cages					
Individually ventilated cages (IVC)					
Female or juvenile rats (predominantly)					
biosafety cabinets/ extracted work station					

E. General allergic symptoms

1. Please indicate if you have ever experienced any of the following conditions for a period of at least 3 months. Please choose all that apply. If you answered YES to any of the symptoms, please indicate when you first and last noticed it.

	No	Yes	At what age did you first experienced the symptoms?	At what age did you last experience the symptoms?	Did this condition go away for a period of several months or years?
Hay fever/ Allergic Rhinitis					
Asthma					
Eczema/ Skin Allergies					
Itchy or watery eyes (Allergic Conjunctivitis)					

2. Do you have any known allergies? No□ Yes□

   If Yes, to what? Please name: _____

F. Lower Respiratory symptoms

I. Cough	
1. Have you at any time in the last 12 months been woken up at night by an attack of coughing?	No□ Yes□
2. In the last 12 months, do you usually cough on getting up or first thing in the morning?	No□ Yes□
If you answered Yes to either of the above questions, continue with question 3. If you answered No to both, go to table **II. Wheezing.**	
3. Do you think that your cough has been caused by something you breathed in at work?	No□ Yes□
4. Do you think your cough gets better when you are off work or on vacation?	No□ Yes□

II. Wheezing:	
1. Have you at any time in the last 12 months experienced chest wheezing or whistling?	No□ Yes□
2. In the last 12 months have you ever had attacks of shortness of breath with wheezing?	No□ Yes□
If you answered Yes to either of the above questions, continue with question 3. If you answered No to Both, go to table **III. Undue shortness of breath.**	
3. Do you get this only when you have a cold?	No□ Yes□
4. Do you get this when exercising or shortly after you have stopped exercising?	No□ Yes□
5. Do you get this most days or nights each week?	No□ Yes□
6. Do you think that this wheezing or whistling experience in your chest has been caused by something you breathed in at work?	No□ Yes□
7. Do you think this wheezing gets better when you are off work or on vacation?	No□ Yes□

Lower Respiratory symptoms (Continued...)

III.	**Undue shortness of breath**
1.	Have you at any time in the last 12 months been woken up with a feeling of shortness of breath? No□ Yes□
2.	In the last 12 months, do you usually have a feeling of shortness of breath during the day or at night? No□ Yes□
	If you answered Yes to either of the above questions, continue with question 3. If you answered No to both, go to table **IV. Asthma**
3.	Do you think that this feeling of shortness of breath have been caused by something you breathed in at work? No□ Yes□
4.	Do you think your shortness of breath gets better when you are off work or on vacation? No□ Yes□

IV.	**Asthma:**
1.	Have you ever had an asthma attack? No□ Yes□
2.	Have you ever been told by a doctor that you have asthma? No□ Yes□
	If you answered Yes to either of the questions above, please continue with question 3. If you answered No to both questions above, go to **V. General questions about Lower respiratory symptoms**
3.	Please indicate if: □ You had childhood asthma which went away and now you are asymptomatic □ You had childhood asthma which went away for several years, but is now back again □ You had childhood asthma which you still have at this age. □ You have adult onset asthma □ Others, please specify_____
4.	When was the onset of your asthma symptoms? (How old were you?)_____ years
5.	Did you have asthma before starting your current type of work at HSLAS? No□ Yes□
6.	In the last 12 months have your asthma symptoms got worse or remained the same? _____ _____

Lower Respiratory symptoms (Continued...)

If you had any of the four symptoms/conditions above, please continue with this section. If you did not have any of them, go to Section G. Rhinitis.

V. General questions about Lower respiratory symptoms:

1. **What has been the most troublesome chest symptom or symptoms?**

   ☐Wheezing or whistling ☐Attacks of shortness of breath

   ☐ Chest tightness ☐Attacks of cough ☐ Other(specify:_____)

2. **In general how often have you had these respiratory symptoms?**

   ☐Only once ☐ Only a few days ever ☐ A few days each year

   ☐ A few days each month ☐ A few days each week ☐ Usually at least once each day or night

3. **Were there changes in work processes in the week preceding the onset of your symptoms?**

   ☐No ☐Yes, Please specify_____

4. **Was there an unusual work exposure within 24h before the onset of the initial asthma symptoms?**

   ☐No ☐Yes, Please specify_____

5. **Have you noticed any specific exposures (including pets, pollens, work exposures, etc.) were associated with your symptoms?**

   ☐No ☐Yes, Please specify_____

G. Rhinitis

I. Have you experienced any of the following symptoms in the last 12 months?		
1. Clear watery nasal discharge	No☐	Yes☐
2. Chronic nasal congestion or stuffy nose	No☐	Yes☐
3. Attacks of several vigorous sneezing	No☐	Yes☐
4. Nasal itchy sensation	No☐	Yes☐
5. Frequent red, watery and itchy eyes	No☐	Yes☐
If you answered Yes to any of the questions above, continue with next questions. If you answered No to all 5 questions, go to section H.		

1. When did you first notice the Rhinitis symptoms? _____

2. In general how often have you had rhinitis symptoms?

   ☐Only once ☐ Only a few days ever ☐ A few days each year

   ☐ A few days each month ☐ A few days each week ☐ Usually at least once each day or night

3. Were there changes in work processes in the week preceding the onset of your symptoms?

   ☐No ☐Yes, Please specify_____

4. Was there an unusual work exposure within 24h before the onset of the initial rhinitis symptoms?

   ☐No ☐Yes, Please specify_____

5. Do you think that your Rhinitis symptoms have been caused by something you breathed in at work?

   ☐No ☐Yes

6. Have you ever felt that your Rhinitis symptoms get better when you are off work or on vacation?

   ☐No ☐Yes

7. Are/were your symptoms worse during a particular season of the year?

   ☐No ☐Yes, Please specify_____

8. Are/were you experiencing these symptoms only when you had a cold?

   ☐No ☐Yes

9. Have you noticed any specific exposures (including pets, pollens, work exposures, etc.) were associated with your rhinitis symptoms?
   If yes, please specify:_____

H. Smoking

1. Have you ever smoked cigarettes regularly (at least one pack per day for a year)?

   ☐No ☐Yes

2. How old were you when you started smoking regularly? _____

3. Do you still smoke cigarettes?

   ☐No ☐Yes

4. If you answered No to 3, how old were you when you last gave up smoking? _____

5. How many years have you been smoking regularly? _____ Years.

6. Over the years that you smoked, on the average approximately how many cigarettes per day did you smoke?
   _____ Cigarettes per day.

I. Family History

1. Indicate any of the blood relatives that whoever had any of the following:

	Parents	Grand parents	Brother/Sister	Children
Hay fever/ Allergic Rhinitis				
Eczema (Skin Allergies)				
Asthma				
Itchy or watery eyes (Allergic Conjunctivitis)				
Other Allergies				

J. Previous medical illnesses

1. Do you currently have any clinical conditions, illnesses, or major health problems:

Condition	Year started
1	
2	
3	
4	

2. Do you currently take any medications including anti-histamines?

Medication	For condition: (number above)	Year started	Date& time of the last dose (Anti-histamines)
1			
2			
3			
4			

K. Pets

1. Do you or have you ever kept a pet (an animal or a bird) at home?

☐No ☐Yes, please specify the animal type_____

2. If you answered yes, do you think that you have had allergic symptoms caused or exacerbated by exposure To your pet(s)?

☐No ☐Yes

Questionnaire Administrator:_____ Date:_____

Appendix 3: Skin Prick Test Repost Form

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Skin Prick Test Report Form:

Participant ID: _____ **Date:** _____/_____/_____

#	Allergen/Concentration	Wheal (mm)	Flare (mm)
	Workplace Allergens		
1	Mouse		
2	Rat		
3	Rabbit		
4	Guinea pig		
5	Cat		
6	Dog		
7	Latex		
	Common Aeroallergens		
8	Grass [Timothy]		
9	Mold mix [Alternariatenuis, Aspergilus, Hormodendrumherbarum, Penicillium]		
10	Mite [D. Pteronyssinus]		
11	Mite [D. Farinae]		
12	Trees mix [6 trees]		
13	Negative control [Saline in glycerin]		
14	Positive control [Histamine]		

Antihistamine taken within 4 days?

Yes ☐ **No** ☐

Name: _____

Date of last dose? _____

* Wheals >= 3mm in diameter are considered as positive test results.

Test administered by:

_____

The above table shows the results of your Skin Prick Test. If you have any concerns or questions you can ask your family doctor to refer you to See Doctor Jeremy Beach, Occupational Medicine Specialist, at:

Occupational Medicine Clinic
2E2, Walter C. Mackenzie Health Sciences Centre.

Extract Manufacturer: OMEGA

version April 16, 2012

Appendix 4: Introductory Letter to Graduate Coordinators

UNIVERSITY OF ALBERTA

DIVISION OF PREVENTIVE MEDICINE
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Dear Colleague,

Your graduate coordinator is forwarding this letter to you on my behalf in order to protect your confidentiality. Myself and a group of co-investigators from the Department of Medicine at the University of Alberta (Dr Nicola Cherry, Dr Eugene Waclawski, and Dr Harissios Vliagoftis) are looking to investigate the prevalence of sensitization to laboratory animals and respiratory symptoms among laboratory animal workers. Even if you have never worked with laboratory animals you may receive this as we are also hoping to recruit some individuals who have never been exposed. We would like to invite you to participate in this study. Your contact details cannot be released directly to me for confidentiality reasons and so if you do not contact me yourself you will not be able to be included in the study.

Along with this letter an information and consent form should be attached to this e-mail. You may either print that and return the consent form to me, or if you prefer contact me and I will send a paper copy of both to you so that you may complete and return the consent form. Simply contact me via e-mail at jeremy.beach@ualberta.ca or by phone at (780) 492-6291 and I can arrange the follow up that suits you best. Alternatively, you may contact the graduate student working on this project, Neda Dianati Maleki via e-mail at diantim@ualberta.ca. To thank you for participating in the study we will offer you a $20 Tim Horton's card.

If you have any concerns or comments about this planned study please do let me know. Ethical approval for the study has been received from HREB. If you would like to arrange a time to meet up so I can explain the study in more detail please let me know and I would be happy to meet up at a time convenient to you and your research staff and students.

Many thanks for your help

Jeremy Beach, Principal Investigator.
Associate Professor and Residency Program Director,
Occupational Medicine Program
Division of Preventive Medicine,
Department of Medicine
University of Alberta.

Appendix 5: Study Proposal

Proposal

Title: Asthma and allergy symptoms among animal laboratory workers.

Introduction:

Historically, there is considerable evidence that workers with consistent exposure to high or low molecular weight inhaled substances in workplaces develop allergic symptoms. Among several different workplace settings, animal laboratories have been at the center of attention for decades. Today, laboratory animal allergy (LAA) is a well-known clinical entity commonly seen among workers exposed to lab animals (1). According to several cross-sectional studies, the prevalence of LAA has been reported up to 44% in certain groups but longitudinal studies estimating the incidence are still scant (2),(3), (10). Most sensitized workers present allergic symptoms within 3-4 years of exposure with the peak incidence said to occur at 1-2 years (11), (13). Symptoms can be classified as occupational rhinitis (OR) and occupational asthma (OA). According to previous studies the prevalence of OA and OR among animal lab workers has been estimated to be about 10 % and 30 % respectively. OA alone constitutes 10-15 % of adult asthma and can be categorized to Allergen-induced asthma or asthma with latency (90%) and Irritant-induced asthma or asthma without latency (10%) (5), (8), (9). Allergen-induced asthma and sensitization is a type 1 immunologic hypersensitivity reaction where specific Immunoglobulin E (Ig E) is produced in response to and external allergen inhalation (2), (7). Irritant-induced asthma usually develops after a single massive exposure to an irritating substance. In the latter –which is also called Reactive airway dysfunction syndrome (RADS) - the immune system is not involved and symptoms present quickly within a few hours after exposure, i.e. without latency (4). Based on another classification, OA can be divided into two distinct groups; Work induced asthma (WIA) which refers to newly developed asthma symptoms and Work aggravated asthma (WAA) which refers to pre-existing asthma worsened by workplace exposures (6).

Animal allergens are found in fur, dander, skin epithelium and body fluids (saliva, serum, urine) of mice, rats, guinea pigs, rabbits, etc. contamination can occur either through direct contact or by airborne high molecular weight allergens. There is convincing evidence that a certain dose-response relationship exist between the level of exposure and allergic symptoms. Numerous research studies conducted by occupational health experts have studied the possible risk-factors which increase the chance of sensitization among animal laboratory workers, and several potential risk factors have been identified. Researchers and workers in charge of activities and procedures such as feeding, cleaning, sampling, sacrifice, cage handling, as well as maintenance and waste disposal personnel are especially at risk (12).

Occupational allergy and asthma can cause significant morbidity among workers at risk and may pose a financial burden on employers and industry. During decades, several control measures and preventive modifications has been proposed or made obligatory in laboratory environments. These include exposure control methods, equipment performance testing and education plus significant changes in facility designs and operations. In addition, health surveillance systems have been established as means of secondary prevention.

Considering all these actions, there is a quite apparent need for up-to-date data regarding LAA in the new settings to re-assess the epidemiology of LAA in comparison to available literature and to evaluate the efficiency of implemented work-place modifications.

Objectives:

This study is designed:

- To estimate the prevalence of LAA (OA, OR) and sensitization among Health Sciences Laboratory Animal Services (HSLAS) workers and employees,
- To study the association of the potential risk factors and outcomes of interest (sensitization, OA, OR), in this population.

Initially a cross sectional study will collect information on all current workers and researchers working in HSLAS. This will include newly recruited staff and researchers who have not previously been exposed to laboratory animals. This group is of particular interest and will be recruited to an ongoing cohort, along with staff and researchers joining HSLAS in subsequent years for a longitudinal follow up.

Methods and Population:

According to HSLAS, 150 trainees and employees have been trained for animal handling from July 2010 to July 2011. Based on the presumption that the trainees will need 3 years on average to complete their research, the total number of individuals handling animals can be estimated to be about 400-500.

Individuals working in HSLAS would be identified through a record of having completed their training to work with laboratory animals. All individuals working with laboratory animals are required to undergo mandatory training in animal care before starting work in HSLAS. This listing will not be made available to the researchers directly but rather HSLAS will send a recruitment package on behalf of the researchers to all individuals with current access to HSLAS. Potential participants may respond by mail, e-mail or phone to the researchers, and in addition the researchers will make themselves available within HSLAS at pre-advertised times so that potential participants can also approach them directly there. A number of briefing sessions which individuals working in HSLAS will be invited to attend will be organized at the time the study is commencing to provide information about the study to potential participants and to answer any questions arising.

In the initial phase, a cross-sectional study design would describe many features of the study population and would provide an estimate of the prevalence of the outcomes of interest and different exposure types. In addition a cross-sectional study can support inferences of cause and effect between the proposed potential risk factors and outcomes (OR, OA and sensitization).

As mentioned above in objectives section, of the whole study population, the newly recruited animal-naïve personnel will form a cohort for a longitudinal study in the next phase of the research. Newly

employed or enrolled staff during the follow up periods will also be recruited and added to the existing cohort.

Inclusion criteria:

1. All personnel and staff (including researchers, workers, lab technicians, animal care specialists and support and maintenance staff) who work in the HSLAS animal house and are in direct or indirect contact with lab animals or their tissues, organs, saliva, serum, urine, dander, fur, skin, wastes, beddings, carrions, cages and contaminated equipment.

Exclusion criteria:

None.

At the time of recruitment, study subjects will first be asked to read and sign an informed consent form (ICF) approved by the ethical committee. The participants will then, undergo a Skin Prick Test (SPT) and will complete an interviewer-administered questionnaire. In addition to general demographic data, the questionnaire will explore allergic symptoms of different end organs including skin, eyes, nose and lungs. The questions more specifically aim to detect rhinitis and asthma symptoms. The questionnaire has been specifically designed for use in this study but assembled from components of pre-validated questionnaires where available.

The questionnaire also includes detailed questions about potential risk factors comprising:

1. Type of exposure (activities and procedures the participant is involved in);
2. level of exposure (total number of hours per week the participant is exposed);
3. Duration of time from the first exposure;
4. Animal exposure data (type, gender, age and number of animals the worker deals with per day or per week)
5. Part of the animal body the worker in mostly in contact with (fur, dander, skin epithelium, saliva, serum, urine, etc.);
6. Safety measures and preventive precautions in use (gowns, gloves, facial masks, working under ventilation or negative pressure, protocols for decontamination and disinfection, and handling wastes, beddings and carcasses).

Potential confounders including, age, gender, ethnicity, atopy, smoking, previous allergic conditions, etc. will also be addressed and considered in the final analysis.

Outcomes of interest:

1. Positive SPT as an indicator of sensitization and /or atopy
2. Doctor diagnosed asthma occurring after start of exposure
3. Occupational Asthma symptoms, particularly wheeze not associated with a cold, occurring after start of exposure
4. Occupational Rhinitis symptoms occurring after start of exposure

References:

1. Goodno L E, Stave G M. Primary and Secondary Allergies to Laboratory Animals. *JOEM*. 2002; 12, Vol 44.1143-1152

2. Meijer E, Grobbee D E, Heederik D. Detection of workers sensitized to high molecular weight allergens: a diagnostic study in laboratory animal workers. *Occup Environ Med* 2002;59:189–195

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4. American Thoracic Society Workshop. Proceedings of the First Jack Pepys Occupational Asthma Symposium

5. Tarlo S M, Balmes J, Balkissoon R, Beach J. Asthma: American College of Chest Diagnosis and Management of Work-Related Physicians Consensus Statement. *Chest* 2008; 134 ;1S-41S.

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10. Acton D, McCauley L. Laboratory Animal Allergy ‚An Occupational Hazard. *AAOHN Journal, 2007; vol. 55, no. 6*

11. Gordon S, Preece R. Prevention of laboratory animal allergy. *Occupational Medicine*. 2003;53: 371-377

12. Elliot L, Heederik D, et al. Incidence of allergy and allergy symptoms among workers exposed to laboratory animals. *Occup Environ Med*2005;62: 766-771

13. Folletti A, Forcina A, et al. Have the prevalence and incidence of occupational asthma and rhinitis because of laboratory animals declined in the last 25 years? *Allergy* 2008;63: 834-841

Appendix 6: Letter of Approval from Research Ethics Board

Approval

Date:	May 3, 2012
Study ID:	Pro00028614
Principal Investigator:	Jeremy Beach
Study Title:	The Prevalence of Occupational Asthma and Rhinitis among animal laboratory workers at HSLAS, University of Alberta; A Cross-sectional study.
Approval Expiry Date:	May 2, 2013

Thank you for submitting the above study to the Health Research Ethics Board - Health Panel . Your application, including revisions received May 2 & 3, 2012, has been reviewed and approved on behalf of the committee.

A renewal report must be submitted next year prior to the expiry of this approval if your study still requires ethics approval. If you do not renew on or before the renewal expiry date, you will have to re-submit an ethics application.

Approval by the Health Research Ethics Board does not encompass authorization to access the patients, staff or resources of Alberta Health Services or other local health care institutions for the purposes of the research. Enquiries regarding Alberta Health Services approvals should be directed to (780) 407-6041. Enquiries regarding Covenant Health should be directed to (780) 735-2274.

Sincerely,

Doug Gross, Ph.D.
Associate Chair, Health Research Ethics Board - Health Panel

Note: This correspondence includes an electronic signature (validation and approval via an online system).

Appendix 7: Study Poster

UNIVERSITY OF ALBERTA

Laboratory Animal Allergy study

seeking research participants

An ideal participant will be either:

- An HSLAS staff member,
- A lab technician,
- A research associate or
- A graduate student / post-doc fellow,

Who has exposure to laboratory animals including mice, rats, rabbits, guinea pigs, cats or dogs.

Participate in our study & Receive a **20$ Tim Hortons gift card**!

Participants will be scheduled for a 20-min appointment, where they will have a Skin Prick Test (Allergy test) and will complete a questionnaire.

If you are interested in participating Please contact us at:
dianatim@ualberta.ca

dianatim@ualberta.ca Lab Animal Allergy study

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Appendix 8: Cover Letter and follow up Questionnaire

UNIVERSITY OF ALBERTA

DIVISION OF PREVENTIVE MEDICINE
DEPARTMENT OF MEDICINE
Faculty of Medicine & Dentistry

5-30 University Terrace
8303 – 112 St
Edmonton, Alberta, Canada T6G 2T4
Tel: 780.492.6291
Fax: 780.492.9677
www.medicine.med.ualberta.ca/AboutUs/Divisions/PMED

December 10, 2012

Dear HSLAS staff member,

You may have previously been invited to participate in a study about Laboratory Animal Allergy, which is a research project being undertaken by myself and colleagues from Occupational Medicine at the University of Alberta. This letter is being distributed to you regarding this project by HSLAS on my behalf as the university cannot pass on your contact details to me for privacy reasons.

If this is your first time hearing about this study and you are interested to learn more about the study, you may either contact me at Jeremy.beach@ualberta.ca or Neda, the graduate student on this project, at dianatim@ualberta.ca.

If you have previously heard about this research study and to date have preferred not to participate, we do understand and respect your decision. However, we would like to invite you one last time to participate, and if you would prefer not to participate fully ask if you might at least consider completing and returning the short questionnaire on the back of this letter. This will provide us with at least a minimum of information to help us understand if there has been any bias in terms of recruitment of people participating in the study. With your answers, the final results of the study will be more accurate and we appreciate your help.

Please note that your answers will remain confidential.

Yours Sincerely

Dr. Jeremy Beach
Associate Professor and Residency Program Director,
Occupational Medicine,
Division of Preventive Medicine,
Department of Medicine,
University of Alberta

UNIVERSITY OF ALBERTA

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Questionnaire:

Please answer the following questions and return this questionnaire using the pre-addressed envelope.

1. When did you first start working with lab animals? Year_____

2. Have you experienced any rhinitis symptoms (including clear watery nasal discharge, chronic nasal congestion, attacks of several sneezing or nasal itchy sensation) in the last 12 months?
   Yes □ No □

3. If you answered yes to question number 2, do you think your symptoms have been caused by something you breathed in at work?
   Yes □ No □

4. Have you experienced any lower respiratory symptoms (including undue shortness of breath, coughing, chest wheezing or whistling or asthma) in the last 12 months?
   Yes □ No □

5. If you answered yes to question number 4, do you think your symptoms have been caused by something you breathed in at work?
   Yes □ No □

Name: _____ (Optional)

Email: _____ (Optional)

Thank you.

Appendix 9: STATA syntheses to create RAT_OA and RAT_OR variables

STATA syntheses for creating RAT_OA variable:

```
generate RAT_OA=0
replace RAT_OA=1 if  COUGH_1+C_VAC==2 & RAT_POSITIVE==1
replace RAT_OA=1 if  WHEEZE1_OR_WHEEZE2_BINARY+ W_VAC==2 & RAT_POSITIVE==1
replace RAT_OA=1 if  SOB1_OR_SOB2_BIANARY+SOB_VAC==2  & RAT_POSITIVE==1
```

STATA syntheses for creating RAT_OR variable:

```
. gen RAT_OR=0
. replace RAT_OR=1 if  NASAL_D + URT6==2 & RAT_POSITIVE==1
```

Similar method was used to create MOUSE_OA and MOUSE_OR outcomes.

Appendix 10: Logistic Regression Model Building; Purposeful Selection of Covariates

As explained in section 2.9.3, we used the 'Purposeful selection of covariates' method to build the multivariable logistic regression models for each outcome variable

- Step 1: First a univariate analysis of each variable was performed. Any variable having a significant univariate test at an arbitrary p value (here <0.2) was selected as a candidate for the next step that is the multivariate analysis. More traditional levels such as =<0.05 may fail in identifying variables known to be important and are not usually used.(127, 128)
- Step 2: At this step a multivariable analysis was performed using the selected variables from the previous step. This was based on the WALD test from the logistic regression and the traditional p-value cut-off point of =<0.05. In the iterative process of variable selection, covariates were removed from the model if they were non-significant and not a confounder. Significance was evaluated at the 0.05 alpha level and confounding as a change in any remaining parameter estimate greater than 20% as compared to full model. A change in parameter estimate or coefficient (natural log of odds) above the specified level indicated that the excluded variable was important in the sense of providing a needed adjustment for one or more of the variable remaining in the model.

  $\Delta\beta = |\beta_{\text{with C}} - \beta_{\text{without C}}| / \beta_{\text{with C}} * 100 > 20\%$

  $\beta_{\text{with C}}$ was the parameter estimate with the confounding variable in the model

  $\beta_{\text{without C}}$ was the parameter estimate without the confounding variable

- Step 3: At the end of this iterative process, the model contained significant covariates and confounders. At that point any variables not selected for the original multivariate model were added back one at a time, with significant covariates and confounders retained earlier. This step can be potentially helpful in identifying variables that were not significantly related to the outcome but could make a potential contribution to the prediction in the presence of other variables. Any variables that were significant at the 0.05 level were included in the model and the model was iteratively reduced as before but only for the variables that were additionally added. This resulted in the preliminary main effects model.
- Step 4: To for interaction/ effects modification, pairwise multiplicative interaction terms were created using all remaining variables and with STATA command "gen". Interaction terms were then added to the model one at a time and were kept in the final model if significant.
- Step 5: Performing diagnostics. The *"estat gof"* command was used to compute goodness-of-fit test statistics (using two methods: Pearson Chi^2 and Hosmer-Lemeshow) for logit/ logistic. Goodness of fit tests the pairwise difference between the observed and expected values based on the proposed model. When performing goodness of fit tests, the desired test result was a non-significant test, which showed the difference between observed, and expected values were non-significant. This means that the proposed regression model significantly predicted the outcome.

Eventually the multivariate regression model was created as below where Y is the dependent or outcome variable and Var_1 to Var_n are the independent variables kept in the final model. β_0 is the intercept and β_1 to β_n are the parameter estimates.

$$Y= \beta_0 +\beta_1.Var_1+ \beta_2.Var_2\ldots+\beta_n.Var_n$$

The H_0 will be:

$$H_0: \ \beta_1= \beta_2=\ldots=\beta_n=0$$

Finally the Odds ratios were calculated using "*Logistic*" command.

Appendix 11. Association of rat/mouse sensitization AND occupational exposures, job tasks and use of PPE across the two Groups.

Rat Sensitisation

Table 3.1.5.10 Association of rat sensitisation and atopy		
	Atopy (+)	Atopy (-)
RAT_POSITIVE **(+)**	39	2
RAT_POSITIVE **(-)**	11	22
Fischer's exact P-value < 0.001		

Table 3.1.5.11 Association of rat sensitisation and rat skin exposure		
	Rat skin (+)	Rat skin (-)
RAT_POSITIVE **(+)**	30	11
RAT_POSITIVE **(-)**	21	12
Chi^2 P-value > 0.1		

Table 3.1.5.12 Association of rat sensitisation and rat fur exposure		
	Rat fur (+)	Rat fur (-)
RAT_POSITIVE **(+)**	37	4
RAT_POSITIVE **(-)**	31	2
Fischer's exact P-value > 0.1		

Table 3.1.5.13 Association of rat sensitisation and rat serum exposure		
	Rat Serum (+)	Rat Serum (-)
RAT_POSITIVE **(+)**	21	20
RAT_POSITIVE **(-)**	11	22
Chi^2 P-value > 0.1		

Table 3.1.5.14 Association of rat sensitisation and rat urine exposure		
	Rat urine (+)	Rat urine (-)
RAT_POSITIVE **(+)**	28	13
RAT_POSITIVE **(-)**	23	10
Chi^2 P-value > 0.1		

Table 3.1.5.15 Association of rat sensitisation and rat saliva exposure		
	Rat saliva (+)	Rat saliva (-)
RAT_POSITIVE **(+)**	12	29
RAT_POSITIVE **(-)**	7	26
Chi^2 P-value > 0.1		

Table 3.1.5.16 Association of rat sensitisation and rat internal tissue exposure		
	Rat tissue (+)	Rat tissue (-)
RAT_POSITIVE **(+)**	18	23
RAT_POSITIVE **(-)**	12	21
Chi^2 P-value > 0.1		

Table 3.1.5.17 Association of rat sensitisation and rat carcass exposure		
	Rat carcass (+)	Rat carcass (-)
RAT_POSITIVE **(+)**	19	22
RAT_POSITIVE **(-)**	13	20
Chi^2 P-value > 0.1		

Table 3.1.5.18 Association of rat sensitisation and rat cage exposure		
	Rat cage (+)	Rat cage (-)
RAT_POSITIVE **(+)**	33	8
RAT_POSITIVE **(-)**	28	5
Chi^2 P-value > 0.1		

Table 3.1.5.19 Association of rat sensitisation and rat handling		
	Handling (+)	Handling (-)
RAT_POSITIVE **(+)**	28	13
RAT_POSITIVE **(-)**	22	11
Chi^2 P-value > 0.1		

Table 3.1.5.20 Association of rat sensitisation and rat feeding		
	Feeding (+)	Feeding (-)
RAT_POSITIVE **(+)**	12	29
RAT_POSITIVE **(-)**	15	18
Chi^2 P-value > 0.1		

Table 3.1.5.21 Association of rat sensitisation and rat injection		
	Injection (+)	Injection (-)
RAT_POSITIVE **(+)**	13	28
RAT_POSITIVE **(-)**	5	28
Chi^2 P-value > 0.1		

Table 3.1.5.22 Association of rat sensitisation and rat shaving		
	Shaving (+)	Shaving (-)
RAT_POSITIVE **(+)**	8	33
RAT_POSITIVE **(-)**	1	32
Chi^2 P-value < 0.05		

Table 3.1.5.23 Association of rat sensitisation and rat sacrifice		
	Animal sacrifice (+)	Animal sacrifice (-)
RAT_POSITIVE **(+)**	13	28
RAT_POSITIVE **(-)**	6	27
Chi^2 P-value > 0.1		

Table 3.1.5.24 Association of rat sensitisation and rat box-changing		
	Box changing (+)	Box changing (-)
RAT_POSITIVE **(+)**	12	29
RAT_POSITIVE **(-)**	13	20
Chi^2 P-value > 0.1		

Table 3.1.5.25 Association of rat sensitisation and disposal of rat litter		
	Litter disposal (+)	Litter disposal (-)
RAT_POSITIVE **(+)**	10	31
RAT_POSITIVE **(-)**	15	18
Chi^2 P-value > 0.1		

Table 3.1.5.26 Association of rat sensitisation and rat manual cage cleaning		
	Cage cleaning (+)	Cage cleaning (-)
RAT_POSITIVE **(+)**	5	36
RAT_POSITIVE **(-)**	8	25
Chi^2 P-value > 0.1		

Table 3.1.5.27 Association of rat sensitisation and rat unit cleaning		
	Unit cleaning (+)	Unit cleaning (-)
RAT_POSITIVE **(+)**	17	24
RAT_POSITIVE **(-)**	12	21
Chi^2 P-value > 0.1		

Table 3.1.5.28 Association of rat sensitisation and use of gloves		
	Gloves (+)	Gloves (-)
RAT_POSITIVE **(+)**	39	2
RAT_POSITIVE **(-)**	31	2
Fisher's exact P-value > 0.1		

Table 3.1.5.29 Association of rat sensitisation and use of gowns		
	Gowns (+)	Gowns (-)
RAT_POSITIVE **(+)**	38	3
RAT_POSITIVE **(-)**	26	7
Fisher's exact P-value > 0.1		

Table 3.1.5.30 Association of rat sensitisation and use of masks		
	Masks (+)	Masks (-)
RAT_POSITIVE **(+)**	31	10
RAT_POSITIVE **(-)**	26	7
Fisher's exact P-value > 0.1		

Table 3.1.5.31 Association of rat sensitisation and use of respirator		
	Respirator (+)	Respirator (-)
RAT_POSITIVE **(+)**	12	29
RAT_POSITIVE **(-)**	1	30
Fisher's exact P-value < 0.05		

Table 3.1.5.32 Association of rat sensitisation and use of filter-top cage		
	Filter-top (+)	Filter-top (-)
RAT_POSITIVE **(+)**	22	12
RAT_POSITIVE **(-)**	22	11
Chi^2 P-value > 0.1		

Table 3.1.5.33 Association of rat sensitisation and use of IVCs		
	IVC (+)	IVC (-)
RAT_POSITIVE **(+)**	19	22
RAT_POSITIVE **(-)**	17	16
Chi^2 P-value > 0.1		

Table 3.1.5.34 Association of rat sensitisation and use of female rats		
	Female rat (+)	Female rat (-)
RAT_POSITIVE **(+)**	11	30
RAT_POSITIVE **(-)**	14	19
Chi^2 P-value > 0.1		

Table 3.1.5.35 Association of rat sensitisation and use of biosafety cabinets.		
	Biosafety cabinets (+)	Biosafety cabinets (-)
RAT_POSITIVE **(+)**	11	30
RAT_POSITIVE **(-)**	14	19
Chi^2 P-value > 0.1		

Mouse Sensitisation:

Table 3.1.5.36 Association of mouse sensitisation and atopy		
	Atopy (+)	Atopy (-)
MOUSE_POSITIVE **(+)**	17	0
MOUSE _ POSITIVE **(-)**	32	31
Fisher's exact P-value < 0.001		

Table 3.1.5.37 Association of mouse sensitisation and mouse skin exposure		
	Rat skin (+)	Rat skin (-)
MOUSE _ POSITIVE **(+)**	14	3
MOUSE _ POSITIVE **(-)**	47	16
Fisher's exact P-value > 0.1		

Table 3.1.5.38 Association of mouse sensitisation and mouse fur exposure		
	Rat fur (+)	Rat fur (-)
MOUSE _ POSITIVE **(+)**	15	2
MOUSE _ POSITIVE **(-)**	55	8
Fisher's exact P-value > 0.1		

Table 3.1.5.39 Association of mouse sensitisation and mouse serum exposure		
	Rat Serum (+)	Rat Serum (-)
MOUSE _ POSITIVE **(+)**	7	10
MOUSE _ POSITIVE **(-)**	21	42
Chi^2 P-value > 0.1		

Table 3.1.5.40 Association of mouse sensitisation and mouse urine exposure		
	Rat urine (+)	Rat urine (-)
MOUSE _ POSITIVE **(+)**	15	2
MOUSE _ POSITIVE **(-)**	38	25
Fisher's exact P-value < 0.05		

Table 3.1.5.41 Association of mouse sensitisation and mouse saliva exposure		
	Rat saliva (+)	Rat saliva (-)
MOUSE _ POSITIVE **(+)**	9	8
MOUSE _ POSITIVE **(-)**	13	50
Chi^2 P-value < 0.05		

Table 3.1.5.42 Association of mouse sensitisation and mouse internal tissue exposure		
	Rat tissue (+)	Rat tissue (-)
MOUSE _ POSITIVE **(+)**	7	10
MOUSE _ POSITIVE **(-)**	26	37
Chi^2 P-value > 0.1		

Table 3.1.5.43 Association of mouse sensitisation and mouse carcass exposure		
	Rat carcass (+)	Rat carcass (-)
MOUSE _ POSITIVE **(+)**	10	7
MOUSE _ POSITIVE **(-)**	23	40
Chi^2 P-value > 0.1		

Table 3.1.5.44 Association of mouse sensitisation and mouse cage exposure		
	Rat cage (+)	Rat cage (-)
MOUSE _ POSITIVE **(+)**	13	4
MOUSE _ POSITIVE **(-)**	51	12
Fisher's exact P-value > 0.1		

Table 3.1.5.45 Association of mouse sensitisation and mouse handling		
	Handling (+)	Handling (-)
MOUSE _ POSITIVE **(+)**	11	6
MOUSE _ POSITIVE **(-)**	20	43
Chi^2 P-value > 0.1		

Table 3.1.5.46 Association of mouse sensitisation and mouse feeding		
	Feeding (+)	Feeding (-)
MOUSE _ POSITIVE **(+)**	6	11
MOUSE _ POSITIVE **(-)**	24	39
Chi^2 P-value > 0.1		

Table 3.1.5.47 Association of mouse sensitisation and mouse injection		
	Injection (+)	Injection (-)
MOUSE _ POSITIVE **(+)**	7	10
MOUSE _ POSITIVE **(-)**	12	51
Chi^2 P-value > 0.05		

Table 3.1.5.48 Association of mouse sensitisation and mouse shaving		
	Shaving (+)	Shaving (-)
MOUSE _ POSITIVE **(+)**	2	15
MOUSE _ POSITIVE **(-)**	3	60
Fisher's exact P-value > 0.1		

Table 3.1.5.59 Association of mouse sensitisation and mouse sacrifice		
	Animal sacrifice (+)	Animal sacrifice (-)
MOUSE _ POSITIVE **(+)**	5	12
MOUSE _ POSITIVE **(-)**	19	44
Chi^2 P-value > 0.1		

Table 3.1.5.60 Association of mouse sensitisation and mouse box-changing		
	Box changing (+)	Box changing (-)
MOUSE _ POSITIVE **(+)**	7	10
MOUSE _ POSITIVE **(-)**	19	44
Fisher's exact P-value > 0.1		

Table 3.1.5.61 Association of mouse sensitisation and disposal of mouse litter		
	Litter disposal (+)	Litter disposal (-)
MOUSE _ POSITIVE **(+)**	5	12
MOUSE _ POSITIVE **(-)**	19	44
Fisher's exact P-value > 0.1		

Table 3.1.5.62 Association of mouse sensitisation and mouse manual cage cleaning		
	Cage cleaning (+)	Cage cleaning (-)
MOUSE _ POSITIVE **(+)**	4	13
MOUSE _ POSITIVE **(-)**	9	54
Fisher's exact P-value > 0.1		

Table 3.1.5.63 Association of mouse sensitisation and mouse unit cleaning		
	Unit cleaning (+)	Unit cleaning (-)
MOUSE _ POSITIVE **(+)**	9	8
MOUSE _ POSITIVE **(-)**	22	41
Chi^2 P-value > 0.1		

Table 3.1.5.64 Association of mouse sensitisation and use of gloves		
	Gloves (+)	Gloves (-)
MOUSE _ POSITIVE **(+)**	17	0
MOUSE _ POSITIVE **(-)**	60	3
Fisher's exact P-value > 0.1		

Table 3.1.5.65 Association of mouse sensitisation and use of gowns		
	Gowns (+)	Gowns (-)
MOUSE _ POSITIVE **(+)**	16	1
MOUSE _ POSITIVE **(-)**	53	10
Fisher's exact P-value > 0.1		

Table 3.1.5.66 Association of mouse sensitisation and use of masks		
	Masks (+)	Masks (-)
MOUSE _ POSITIVE **(+)**	11	6
MOUSE _ POSITIVE **(-)**	49	14
Chi^2 P-value > 0.1		

Table 3.1.5.67 Association of mouse sensitisation and use of respirator		
	Respirator (+)	Respirator (-)
MOUSE _ POSITIVE **(+)**	2	15
MOUSE _ POSITIVE **(-)**	3	60
Fisher's exact P-value > 0.1		

Table 3.1.5.68 Association of mouse sensitisation and use of filter-top cage		
	Filter-top (+)	Filter-top (-)
MOUSE _ POSITIVE **(+)**	13	4
MOUSE _ POSITIVE **(-)**	41	22
Fisher's exact P-value > 0.1		

Table 3.1.5.69 Association of mouse sensitisation and use of individually ventilated cages (IVCs)		
	IVC (+)	IVC (-)
MOUSE _ POSITIVE **(+)**	8	9
MOUSE _ POSITIVE **(-)**	38	25
Chi^2 P-value > 0.1		

Table 3.1.5.70 Association of mouse sensitisation and use of female mice		
	Female rat (+)	Female rat (-)
MOUSE _ POSITIVE **(+)**	3	14
MOUSE _ POSITIVE **(-)**	20	43
Fisher's exact P-value > 0.1		

Table 3.1.5.71 Association of mouse sensitisation and use of biosafety cabinets.		
	Biosafety cabinets (+)	Biosafety cabinets (-)
MOUSE _ POSITIVE **(+)**	5	12
MOUSE _ POSITIVE **(-)**	19	44
Chi^2 P-value > 0.1		

Appendix 12: Association of rat/mouse asthma and rhinitis AND occupational exposures, job tasks and use of PPE across the two Groups.

Rat Occupational Asthma:

Table 3.1.6.15 Association of rat asthma and atopy		
	Atopy (+)	Atopy (-)
RAT_OA **(+)**	13	1
RAT_ OA **(-)**	37	23
Fischer's exact P-value < 0.05		

Table 3.1.6.16 Association of rat asthma and rat skin exposure		
	Rat skin (+)	Rat skin (-)
RAT_ OA **(+)**	10	4
RAT_ OA **(-)**	41	19
Chi^2 P-value > 0.1		

Table 3.1.6.17 Association of rat asthma and rat fur exposure		
	Rat fur (+)	Rat fur (-)
RAT_ OA **(+)**	13	1
RAT_ OA **(-)**	55	5
Fisher's exact P-value > 0.1		

Table 3.1.6.18 Association of rat asthma and rat serum exposure		
	Rat Serum (+)	Rat Serum (-)
RAT_ OA **(+)**	7	7
RAT_ OA **(-)**	25	35
Chi^2 P-value > 0.1		

Table 3.1.6.19 Association of rat asthma and rat urine exposure		
	Rat urine (+)	Rat urine (-)
RAT_ OA **(+)**	10	4
RAT_ OA **(-)**	41	19
Fisher's exact P-value > 0.1		

Table 3.1.6.20 Association of rat asthma and rat saliva exposure		
	Rat saliva (+)	Rat saliva (-)
RAT_ OA **(+)**	1	13
RAT_ OA **(-)**	18	42
Chi^2 P-value > 0.1		

Table 3.1.6.21 Association of rat asthma and rat internal tissue exposure		
	Rat tissue (+)	Rat tissue (-)
RAT_ OA **(+)**	5	9
RAT_ OA **(-)**	25	35
Chi^2 P-value > 0.1		

Table 3.1.6.22 Association of rat asthma and rat carcass exposure		
	Rat carcass (+)	Rat carcass (-)
RAT_ OA **(+)**	8	6
RAT_ OA **(-)**	24	36
Chi^2 P-value > 0.1		

Table 3.1.6.23 Association of rat asthma and rat cage exposure		
	Rat cage (+)	Rat cage (-)
RAT_ OA **(+)**	13	1
RAT_ OA **(-)**	48	12
Fisher's exact P-value > 0.1		

Table 3.1.6.24 Association of rat asthma and rat handling		
	Handling (+)	Handling (-)
RAT_ OA **(+)**	11	3
RAT_OA **(-)**	39	21
Fisher's exact P-value > 0.1		

Table 3.1.6.25 Association of rat asthma and rat feeding		
	Feeding (+)	Feeding (-)
RAT_ OA **(+)**	5	9
RAT_ OA **(-)**	22	38
Chi^2 P-value > 0.1		

Table 3.1.6.26 Association of rat asthma and rat injection		
	Injection (+)	Injection (-)
RAT_ OA **(+)**	7	7
RAT_ OA **(-)**	11	49
Chi^2 P-value < 0.05		

Table 3.1.6.27 Association of rat asthma and rat shaving		
	Shaving (+)	Shaving (-)
RAT_ OA **(+)**	5	9
RAT_ OA **(-)**	4	56
Fisher's exact P-value < 0.05		

Table 3.1.6.28 Association of rat asthma and rat sacrifice		
	Animal sacrifice (+)	Animal sacrifice (-)
RAT_ OA **(+)**	8	6
RAT_ OA **(-)**	11	49
Chi^2 P-value < 0.05		

Table 3.1.6.29 Association of rat asthma and rat box-changing		
	Box changing (+)	Box changing (-)
RAT_ OA **(+)**	4	10
RAT_ OA **(-)**	21	39
Fisher's exact P-value > 0.1		

Table 3.1.6.30 Association of rat asthma and disposal of rat litter		
	Litter disposal (+)	Litter disposal (-)
RAT_ OA **(+)**	3	11
RAT_ OA **(-)**	38	22
Fisher's exact P-value > 0.1		

Table 3.1.6.31 Association of rat asthma and rat manual cage cleaning		
	Cage cleaning (+)	Cage cleaning (-)
RAT_ OA **(+)**	1	13
RAT_ OA **(-)**	12	48
Fisher's exact P-value > 0.1		

Table 3.1.6.32 Association of rat asthma and rat unit cleaning		
	Unit cleaning (+)	Unit cleaning (-)
RAT_ OA **(+)**	4	10
RAT_ OA **(-)**	25	35
Fisher's exact P-value > 0.1		

Table 3.1.6.33 Association of rat asthma and use of gloves		
	Gloves (+)	Gloves (-)
RAT_ OA **(+)**	13	1
RAT_ OA **(-)**	57	3
Fisher's exact P-value > 0.1		

Table 3.1.6.34 Association of rat asthma and use of gowns		
	Gowns (+)	Gowns (-)
RAT_ OA **(+)**	12	2
RAT_ OA **(-)**	52	8
Fisher's exact P-value > 0.1		

Table 3.1.6.35 Association of rat asthma and use of masks		
	Masks (+)	Masks (-)
RAT_ OA **(+)**	1	13
RAT_ OA **(-)**	6	54
Fisher's exact P-value > 0.1		

Table 3.1.6.36 Association of rat asthma and use of respirator		
	Respirator (+)	Respirator (-)
RAT_ OA **(+)**	7	7
RAT_ OA **(-)**	8	52
Fisher's exact P-value < 0.05		

Table 3.1.6.37 Association of rat asthma and use of filter-top cage		
	Filter-top (+)	Filter-top (-)
RAT_ OA **(+)**	4	10
RAT_ OA **(-)**	40	20
Fisher's exact P-value < 0.05		

Table 3.1.6.38 Association of rat asthma and use of individually ventilated cages (IVCs)		
	IVC (+)	IVC (-)
RAT_ OA **(+)**	3	11
RAT_ OA **(-)**	33	27
Fisher's exact P-value < 0.05		

Table 3.1.6.39 Association of rat asthma and use of female rats		
	Female rat (+)	Female rat (-)
RAT_ OA **(+)**	3	11
RAT_ OA **(-)**	22	38
Fisher's exact P-value > 0.1		

Table 3.1.6.40 Association of rat asthma and use of biosafety cabinets.		
	Biosafety cabinets (+)	Biosafety cabinets (-)
RAT_ OA **(+)**	2	12
RAT_ OA **(-)**	15	45
Fisher's exact P-value > 0.1		

Rat Occupational Rhinitis:

Table 3.1.6.41 Association of rat rhinitis and atopy		
	Atopy (+)	Atopy (-)
RAT_OR **(+)**	23	2
RAT_OR **(-)**	27	22
Fisher's exact P-value = 0.001		

Table 3.1.6.42 Association of rat rhinitis and rat skin exposure		
	Rat skin (+)	Rat skin (-)
RAT_OR **(+)**	21	4
RAT_ OR **(-)**	30	19
Fisher's exact P-value > 0.05		

Table 3.1.6.43 Association of rat rhinitis and rat fur exposure		
	Rat fur (+)	Rat fur (-)
RAT_ OR **(+)**	24	1
RAT_ OR **(-)**	44	5
Fisher's exact P-value > 0.1		

Table 3.1.6.44 Association of rat rhinitis and rat serum exposure		
	Rat Serum (+)	Rat Serum (-)
RAT_ OR **(+)**	13	12
RAT_ OR **(-)**	19	30
Chi^2 P-value > 0.1		

Table 3.1.6.45 Association of rat rhinitis and rat urine exposure		
	Rat urine (+)	Rat urine (-)
RAT_ OR **(+)**	19	6
RAT_ OR **(-)**	32	17
Chi^2 P-value > 0.1		

Table 3.1.6.46 Association of rat rhinitis and rat saliva exposure		
	Rat saliva (+)	Rat saliva (-)
RAT_ OR **(+)**	6	19
RAT_ OR **(-)**	13	36
Chi^2 P-value > 0.1		

Table 3.1.6.47 Association of rat rhinitis and rat internal tissue exposure		
	Rat tissue (+)	Rat tissue (-)
RAT_ OR **(+)**	12	13
RAT_ OR **(-)**	18	31
Chi^2 P-value > 0.1		

Table 3.1.6.48 Association of rat rhinitis and rat carcass exposure		
	Rat carcass (+)	Rat carcass (-)
RAT_ OR **(+)**	14	11
RAT_ OR **(-)**	18	31
Chi^2 P-value > 0.1		

Table 3.1.6.49 Association of rat rhinitis and rat cage exposure		
	Rat cage (+)	Rat cage (-)
RAT_ OR **(+)**	21	4
RAT_ OR **(-)**	40	9
Fisher's exact P-value > 0.1		

Table 3.1.6.50 Association of rat rhinitis and rat handling		
	Handling (+)	Handling (-)
RAT_ OR **(+)**	18	7
RAT_ OR **(-)**	32	17
Chi^2 P-value > 0.1		

Table 3.1.51 Association of rat rhinitis and rat feeding		
	Feeding (+)	Feeding (-)
RAT_ OR **(+)**	9	16
RAT_ OR **(-)**	18	31
Chi^2 P-value > 0.1		

Table 3.1.6.52 Association of rat rhinitis and rat injection		
	Injection (+)	Injection (-)
RAT_ OR **(+)**	11	14
RAT_ OR **(-)**	7	42
Chi^2 P-value < 0.01		

Table 3.1.6.53 Association of rat rhinitis and rat shaving		
	Shaving (+)	Shaving (-)
RAT_ OR **(+)**	7	18
RAT_ OR **(-)**	2	47
Fisher's exact P-value < 0.05		

Table 3.1.6.54 Association of rat rhinitis and rat sacrifice		
	Animal sacrifice (+)	Animal sacrifice (-)
RAT_ OR **(+)**	12	13
RAT_ OR **(-)**	7	42
Chi^2 P-value < 0.05		

Table 3.1.6.55 Association of rat rhinitis and rat box-changing		
	Box changing (+)	Box changing (-)
RAT_ OR **(+)**	9	16
RAT_ OR **(-)**	16	33
Chi^2 P-value > 0.1		

Table 3.1.6.56 Association of rat rhinitis and disposal of rat litter		
	Litter disposal (+)	Litter disposal (-)
RAT_ OR **(+)**	5	20
RAT_ OR **(-)**	20	29
Fisher's exact P-value > 0.05		

Table 3.1.6.57 Association of rat rhinitis and rat manual cage cleaning		
	Cage cleaning (+)	Cage cleaning (-)
RAT_ OR **(+)**	2	23
RAT_ OR **(-)**	11	38
Fisher's exact P-value > 0.1		

Table 3.1.6.58 Association of rat rhinitis and rat unit cleaning		
	Unit cleaning (+)	Unit cleaning (-)
RAT_ OR **(+)**	10	15
RAT_ OR **(-)**	19	30
Fisher's exact P-value > 0.1		

Table 3.1.6.59 Association of rat rhinitis and use of gloves		
	Gloves (+)	Gloves (-)
RAT_ OR **(+)**	23	2
RAT_ OR **(-)**	47	2
Fisher's exact P-value > 0.1		

Table 3.1.6.60 Association of rat rhinitis and use of gowns		
	Gowns (+)	Gowns (-)
RAT_ OR **(+)**	22	3
RAT_ OR **(-)**	42	7
Fisher's exact P-value > 0.1		

Table 3.1.6.61 Association of rat rhinitis and use of masks		
	Masks (+)	Masks (-)
RAT_ OR **(+)**	19	6
RAT_ OR **(-)**	38	11
Chi^2 P-value > 0.1		

Table 3.1.6.62 Association of rat rhinitis and use of respirator		
	Respirator (+)	Respirator (-)
RAT_ OR **(+)**	8	17
RAT_ OR **(-)**	7	42
Chi^2 P-value > 0.05		

Table 3.1.6.63 Association of rat rhinitis and use of filter-top cage		
	Filter-top (+)	Filter-top (-)
RAT_ OR **(+)**	13	12
RAT_ OR **(-)**	18	31
Chi^2 P-value > 0.1		

Table 3.1.6.64 Association of rat rhinitis and use of individually ventilated cages (IVCs)		
	IVC (+)	IVC (-)
RAT_ OR **(+)**	11	14
RAT_ OR **(-)**	25	24
Chi^2 P-value > 0.1		

Table 3.1.6.65 Association of rat rhinitis and use of female rats		
	Female rat (+)	Female rat (-)
RAT_ OR **(+)**	6	19
RAT_ OR **(-)**	19	30
Chi^2 P-value > 0.1		

Table 3.1.6.66 Association of rat rhinitis and use of biosafety cabinets.		
	Biosafety cabinets (+)	Biosafety cabinets (-)
RAT_ OR **(+)**	2	23
RAT_ OR **(-)**	15	34
Fisher's exact P-value > 0.05		

Mouse Occupational Asthma:

Table 3.1.6.67 Association of mouse asthma and atopy		
	Atopy (+)	Atopy (-)
MOUSE_OA **(+)**	5	0
MOUSE _ OA **(-)**	44	31
Fisher's exact P-value > 0.1		

Table 3.1.6.68 Association of mouse asthma and mouse skin exposure		
	Rat skin (+)	Rat skin (-)
MOUSE _ OA **(+)**	4	1
MOUSE _ OA **(-)**	57	18
Fisher's exact P-value > 0.1		

Table 3.1.6.69 Association of mouse asthma and mouse fur exposure		
	Rat fur (+)	Rat fur (-)
MOUSE _ OA **(+)**	4	1
MOUSE _ OA **(-)**	66	9
Fisher's exact P-value > 0.1		

Table 3.1.6.70 Association of mouse asthma and mouse serum exposure		
	Rat Serum (+)	Rat Serum (-)
MOUSE _ OA **(+)**	3	2
MOUSE _ OA **(-)**	25	50
Chi^2 P-value > 0.1		

Table 3.1.6.71 Association of mouse asthma and mouse urine exposure		
	Rat urine (+)	Rat urine (-)
MOUSE _ OA **(+)**	5	0
MOUSE _ OA **(-)**	48	27
Fisher's exact P-value > 0.1		

Table 3.1.6.72 Association of mouse asthma and mouse saliva exposure		
	Rat saliva (+)	Rat saliva (-)
MOUSE _ OA **(+)**	4	1
MOUSE _ OA **(-)**	21	54
Fisher's exact P-value > 0.1		

Table 3.1.6.73 Association of mouse asthma and mouse internal tissue exposure		
	Rat tissue (+)	Rat tissue (-)
MOUSE _ OA **(+)**	2	3
MOUSE _ OA **(-)**	31	44
Fisher's exact P-value > 0.1		

Table 3.1.6.74 Association of mouse asthma and mouse carcass exposure		
	Rat carcass (+)	Rat carcass (-)
MOUSE _ OA **(+)**	4	1
MOUSE _ OA **(-)**	29	46
Fisher's exact P-value > 0.1		

Table 3.1.6.75 Association of mouse asthma and mouse cage exposure		
	Rat cage (+)	Rat cage (-)
MOUSE _ OA **(+)**	5	0
MOUSE _ OA **(-)**	59	16
Fisher's exact P-value > 0.1		

Table 3.1.6.76 Association of mouse asthma and mouse handling		
	Handling (+)	Handling (-)
MOUSE _ OA **(+)**	3	2
MOUSE _ OA **(-)**	51	24
Fisher's exact P-value > 0.1		

Table 3.1.6.77 Association of mouse asthma and mouse feeding		
	Feeding (+)	Feeding (-)
MOUSE _ OA **(+)**	2	3
MOUSE _ OA **(-)**	28	47
Fisher's exact P-value > 0.1		

Table 3.1.6.78 Association of mouse asthma and mouse injection		
	Injection (+)	Injection (-)
MOUSE _ OA **(+)**	2	3
MOUSE _ OA **(-)**	17	58
Fisher's exact P-value > 0.1		

Table 3.1.6.79 Association of mouse asthma and mouse shaving		
	Shaving (+)	Shaving (-)
MOUSE _ OA **(+)**	0	5
MOUSE _ OA **(-)**	5	70
Chi^2 P-value < 0.05		

Table 3.1.6.80 Association of mouse asthma and mouse sacrifice		
	Animal sacrifice (+)	Animal sacrifice (-)
MOUSE _ OA **(+)**	2	3
MOUSE _ OA **(-)**	22	53
Fisher's exact P-value > 0.1		

Table 3.1.6.81 Association of mouse asthma and mouse box-changing		
	Box changing (+)	Box changing (-)
MOUSE _ OA **(+)**	2	3
MOUSE _ OA **(-)**	24	51
Fisher's exact P-value > 0.1		

Table 3.1.6.82 Association of mouse asthma and disposal of mouse litter		
	Litter disposal (+)	Litter disposal (-)
MOUSE _ OA **(+)**	2	3
MOUSE _ OA **(-)**	22	53
Fisher's exact P-value > 0.1		

Table 3.1.6.83 Association of mouse asthma and mouse manual cage cleaning		
	Cage cleaning (+)	Cage cleaning (-)
MOUSE _ OA **(+)**	0	5
MOUSE _ OA **(-)**	13	62
Fisher's exact P-value > 0.1		

Table 3.1.6.84 Association of mouse asthma and mouse unit cleaning		
	Unit cleaning (+)	Unit cleaning (-)
MOUSE _ OA **(+)**	2	3
MOUSE _ OA **(-)**	29	46
Chi^2 P-value > 0.1		

Table 3.1.6.85 Association of mouse asthma and use of gloves		
	Gloves (+)	Gloves (-)
MOUSE _ OA **(+)**	5	0
MOUSE _ OA **(-)**	72	3
Fisher's exact P-value > 0.1		

Table 3.1.6.86 Association of mouse asthma and use of gowns		
	Gowns (+)	Gowns (-)
MOUSE _ OA **(+)**	5	0
MOUSE _ OA **(-)**	64	11
Fisher's exact P-value > 0.1		

Table 3.1.6.87 Association of mouse asthma and use of masks		
	Masks (+)	Masks (-)
MOUSE _ OA **(+)**	2	3
MOUSE _ OA **(-)**	58	17
Fisher's exact P-value > 0.1		

Table 3.1.6.88 Association of mouse asthma and use of respirator		
	Respirator (+)	Respirator (-)
MOUSE _ OA **(+)**	1	4
MOUSE _ OA **(-)**	4	71
Fisher's exact P-value > 0.1		

Table 3.1.6.89 Association of mouse asthma and use of filter-top cage		
	Filter-top (+)	Filter-top (-)
MOUSE _ OA **(+)**	3	2
MOUSE _ OA **(-)**	51	24
Fisher's exact P-value > 0.1		

Table 3.1.6.90 Association of mouse asthma and use of individually ventilated cages (IVCs)		
	IVC (+)	IVC (-)
MOUSE _ OA **(+)**	2	3
MOUSE _ OA **(-)**	44	31
Fisher's exact P-value > 0.1		

Table 3.1.6.91 Association of mouse asthma and use of female mice		
	Female rat (+)	Female rat (-)
MOUSE _ OA **(+)**	0	5
MOUSE _ OA **(-)**	23	52
Fisher's exact P-value > 0.1		

Table 3.1.6.92 Association of mouse asthma and use of biosafety cabinets.		
	Biosafety cabinets (+)	Biosafety cabinets (-)
MOUSE _ OA **(+)**	2	3
MOUSE _ OA **(-)**	22	53
Fisher's exact P-value > 0.1		

Mouse Occupational Rhinitis:

Table 3.1.6.93 Association of mouse rhinitis and atopy		
	Atopy (+)	Atopy (-)
MOUSE_OR **(+)**	17	1
MOUSE _OR **(-)**	32	30
Fisher's exact P-value = 0.001		

Table 3.1.6.94 Association of mouse rhinitis and mouse skin exposure		
	Rat skin (+)	Rat skin (-)
MOUSE _OR **(+)**	15	3
MOUSE _ OR **(-)**	46	16
Fisher's exact P-value > 0.1		

Table 3.1.6.95 Association of mouse rhinitis and mouse fur exposure		
	Rat fur (+)	Rat fur (-)
MOUSE _ OR **(+)**	16	2
MOUSE _ OR **(-)**	54	8
Fisher's exact P-value > 0.1		

Table 3.1.6.96 Association of mouse rhinitis and mouse serum exposure		
	Rat Serum (+)	Rat Serum (-)
MOUSE _ OR **(+)**	7	11
MOUSE _ OR **(-)**	21	41
Chi^2 P-value > 0.1		

Table 3.1.6.97 Association of mouse rhinitis and mouse urine exposure		
	Rat urine (+)	Rat urine (-)
MOUSE _ OR **(+)**	3	15
MOUSE _ OR **(-)**	24	38
Fisher's exact P-value > 0.1		

Table 3.1.6.98 Association of mouse rhinitis and mouse saliva exposure		
	Rat saliva (+)	Rat saliva (-)
MOUSE _ OR **(+)**	4	14
MOUSE _ OR **(-)**	18	44
Chi^2 P-value > 0.1		

Table 3.1.6.99 Association of mouse rhinitis and mouse internal tissue exposure		
	Rat tissue (+)	Rat tissue (-)
MOUSE _ OR **(+)**	6	12
MOUSE _ OR **(-)**	27	35
Chi^2 P-value > 0.1		

Table 3.1.6.100 Association of mouse rhinitis and mouse carcass exposure		
	Rat carcass (+)	Rat carcass (-)
MOUSE _ OR **(+)**	8	10
MOUSE _ OR **(-)**	25	37
Chi^2 P-value > 0.1		

Table 3.1.6.101 Association of mouse rhinitis and mouse cage exposure		
	Rat cage (+)	Rat cage (-)
MOUSE _ OR **(+)**	14	4
MOUSE _ OR **(-)**	50	12
Fisher's exact P-value > 0.1		

Table 3.1.6.102 Association of mouse rhinitis and mouse handling		
	Handling (+)	Handling (-)
MOUSE _ OR **(+)**	12	6
MOUSE _ OR **(-)**	42	20
Chi^2 P-value > 0.1		

Table 3.1.6.103 Association of mouse rhinitis and mouse feeding		
	Feeding (+)	Feeding (-)
MOUSE _ OR **(+)**	7	11
MOUSE _ OR **(-)**	23	39
Chi^2 P-value > 0.1		

Table 3.1.6.104 Association of mouse rhinitis and mouse injection		
	Injection (+)	Injection (-)
MOUSE _ OR **(+)**	6	12
MOUSE _ OR **(-)**	13	49
Chi^2 P-value > 0.1		

Table 3.1.6.105 Association of mouse rhinitis and mouse shaving		
	Shaving (+)	Shaving (-)
MOUSE _ OR **(+)**	3	15
MOUSE _ OR **(-)**	2	60
Chi^2 P-value < 0.05		

Table 3.1.6.106 Association of mouse rhinitis and mouse sacrifice		
	Animal sacrifice (+)	Animal sacrifice (-)
MOUSE _ OR **(+)**	6	12
MOUSE _ OR **(-)**	18	44
Chi^2 P-value > 0.1		

Table 3.1.6.107 Association of mouse rhinitis and mouse box-changing		
	Box changing (+)	Box changing (-)
MOUSE _ OR **(+)**	8	10
MOUSE _ OR **(-)**	18	44
Chi^2 P-value > 0.1		

Table 3.1.6.108 Association of mouse rhinitis and disposal of mouse litter		
	Litter disposal (+)	Litter disposal (-)
MOUSE _ OR **(+)**	5	13
MOUSE _ OR **(-)**	19	43
Chi^2 P-value > 0.1		

Table 3.1.6.109 Association of mouse rhinitis and mouse manual cage cleaning		
	Cage cleaning (+)	Cage cleaning (-)
MOUSE _ OR **(+)**	2	16
MOUSE _ OR **(-)**	11	51
Fisher's exact P-value > 0.1		

Table 3.1.6.110 Association of mouse rhinitis and mouse unit cleaning		
	Unit cleaning (+)	Unit cleaning (-)
MOUSE _ OR **(+)**	9	9
MOUSE _ OR **(-)**	22	40
Chi^2 P-value > 0.1		

Table 3.1.6.111 Association of mouse rhinitis and use of gloves		
	Gloves (+)	Gloves (-)
MOUSE _ OR **(+)**	16	2
MOUSE _ OR **(-)**	61	1
Fisher's exact P-value > 0.1		

Table 3.1.6.112 Association of mouse rhinitis and use of gowns		
	Gowns (+)	Gowns (-)
MOUSE _ OR **(+)**	14	4
MOUSE _ OR **(-)**	55	7
Fisher's exact P-value > 0.1		

Table 3.1.6.113 Association of mouse rhinitis and use of masks		
	Masks (+)	Masks (-)
MOUSE _ OR **(+)**	13	5
MOUSE _ OR **(-)**	47	15
Chi^2 P-value > 0.1		

Table 3.1.6.114 Association of mouse rhinitis and use of respirator		
	Respirator (+)	Respirator (-)
MOUSE _ OR **(+)**	3	15
MOUSE _ OR **(-)**	2	60
Fisher's exact P-value > 0.05		

Table 3.1.6.115 Association of mouse rhinitis and use of filter-top cage		
	Filter-top (+)	Filter-top (-)
MOUSE _ OR **(+)**	15	3
MOUSE _ OR **(-)**	39	23
Fisher's exact P-value > 0.1		

Table 3.1.6.116 Association of mouse rhinitis and use of individually ventilated cages (IVCs)		
	IVC (+)	IVC (-)
MOUSE _ OR **(+)**	11	7
MOUSE _ OR **(-)**	35	27
Chi^2 P-value > 0.1		

Table 3.1.6.117 Association of mouse rhinitis and use of female mice		
	Female rat (+)	Female rat (-)
MOUSE _ OR **(+)**	4	14
MOUSE _ OR **(-)**	19	43
Fisher's exact Chi^2 P-value > 0.1		

Table 3.1.6.118 Association of mouse rhinitis and use of biosafety cabinets.		
	Biosafety cabinets (+)	Biosafety cabinets (-)
MOUSE _ OR **(+)**	5	13
MOUSE _ OR **(-)**	19	43
Chi^2 P-value > 0.1		

Appendix 13: Independent Variables in RAT and MOUSE analysis

Table 3.2.1.1 Independent variables in RAT and MOUSE analysis		
Description of the variable	**Type of the variable**	**Name in Dataset**
Age	Discrete Numeric	AGE
Gender	Dichotomous, Nominal Categorical	SEX
Atopy (positive SPT to at least one common aeroallergen)	Dichotomous, Nominal Categorical	ATOPY
Family History of allergies	Dichotomous, Nominal Categorical	FH
Born or raised in a livestock farm	Dichotomous, Nominal Categorical	FARM
History of smoking for at least a year	Dichotomous, Nominal Categorical	SMOKE1
Current smoker	Dichotomous, Nominal Categorical	SMOKE3
Ever keeping a pet	Dichotomous, Nominal Categorical	PET
Handling animal skin	Dichotomous, Nominal Categorical	R_SKIN
Handling animal fur/dander	Dichotomous, Nominal Categorical	R_FUR
Handling animal serum	Dichotomous, Nominal Categorical	R_SERUM
Handling animal urine	Dichotomous, Nominal Categorical	R_URINE
Handling animal saliva	Dichotomous, Nominal Categorical	R_SALIVA
Handling animal organs/tissues	Dichotomous, Nominal Categorical	R_TISSUE
Handling animal carcass	Dichotomous, Nominal Categorical	R_CARCASS
Handling animal cages/ wastes	Dichotomous, Nominal Categorical	R_CAGE
Use of PPE: Gloves	Dichotomous, Nominal Categorical	R_GLOVES_BINARY
Use of PPE: Gowns	Dichotomous, Nominal Categorical	R_GOWN_BINARY
Use of PPE: Glasses	Dichotomous, Nominal Categorical	R_GLASS_BINARY
Use of PPE: Masks	Dichotomous, Nominal Categorical	R_MASK_BINARY
Use of PPE: Respirators	Dichotomous, Nominal Categorical	R_RESPIRATOR_BINARY
Direct handling of animal	Dichotomous, Nominal Categorical	R_HANDLING_BINARY
Feeding animals	Dichotomous, Nominal Categorical	R_FEEDIND_BINARY
Injections/ invasive procedures	Dichotomous, Nominal Categorical	R_INJECTION_BINARY
Shaving Fur	Dichotomous, Nominal Categorical	R_SHAVING_BINARY
Animal sacrifice	Dichotomous, Nominal Categorical	R_SACRIFICE_BINARY
Box changing	Dichotomous, Nominal Categorical	R_BOX_BINARY
Disposal of soiled litter	Dichotomous, Nominal Categorical	R_DISPOSAL_BINARY

Manual cage cleaning	Dichotomous, Nominal Categorical	R_MANUALCC_BINARY
Automatic cage cleaning	Dichotomous, Nominal Categorical	R_AUTOMATEDCC_BINARY
Cleaning in the animal unit	Dichotomous, Nominal Categorical	R_UNITCLEANING_BINARY
Use of filter-top cages	Dichotomous, Nominal Categorical	R_FILTERTOP_BINARY
Use of Individually ventilated cages	Dichotomous, Nominal Categorical	R_IVC_BINARY
Use of female rats	Dichotomous, Nominal Categorical	R_FEMALE_BINARY
Use of Biosafety cabinets	Dichotomous, Nominal Categorical	R_BIOSAFETYCAB_BINARY

Appendix 14: Logistic Regression Analysis STATA outputs

STATA output (3.2.1.1)

```
. logit  rat_positive atopy farm fh pet  r_serum r_gown_binary  r_respirator_binary
r_feeding_binary r_injection_binary r_shaving_binary r_sacrifice_binary
r_disposal_binary r_manualcc_binary r_female_binary
Logit estimates                                   Number of obs   =         74
                                                  LR chi2(14)     =      59.26
                                                  Prob > chi2     =     0.0000
Log likelihood =   -21.2304                       Pseudo R2       =     0.5826
------------------------------------------------------------------------------
rat_positive |      Coef.   Std. Err.       z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       atopy |   5.758134   1.756923     3.28   0.001     2.314629    9.201639
        farm |   1.008865   1.019557     0.99   0.322    -.9894297    3.007161
          fh |   .3904054   1.140168     0.34   0.732    -1.844283    2.625094
         pet |    1.81369   1.138287     1.59   0.111    -.4173119    4.044691
     r_serum |   .1152767   1.134589     0.10   0.919    -2.108476     2.33903
r_gown_bin-y |   1.712444   1.601375     1.07   0.285    -1.426194    4.851082
r_respirat-y |   1.347809   1.234982     1.09   0.275    -1.072712    3.768329
r_feeding_-y |  -1.880931   1.111274    -1.69   0.091    -4.058988    .2971255
r_injectio-y |  -.2605863   1.489416    -0.17   0.861    -3.179788    2.658616
r_shaving_-y |   -.074033   2.178793    -0.03   0.973     -4.34439    4.196324
r_sacrific-y |   3.011876   1.562747     1.93   0.054    -.0510517    6.074803
r_disposal-y |  -.3026182   1.226913    -0.25   0.805    -2.707323    2.102086
r_manualcc-y |  -3.089358   1.678336    -1.84   0.066    -6.378836    .2001198
r_female_b-y |   .0522665   1.018622     0.05   0.959    -1.944196    2.048729
       _cons |  -6.956101   2.487895    -2.80   0.005    -11.83229   -2.079916
------------------------------------------------------------------------------
```

STATA output (3.2.1.2)

```
. logit  rat_positive atopy  pet  r_feeding_binary   r_sacrifice_binary
r_manualcc_binary , nolog
Logit estimates                                   Number of obs   =         74
                                                  LR chi2(5)      =      54.65
                                                  Prob > chi2     =     0.0000
Log likelihood = -23.535282                       Pseudo R2       =     0.5373
------------------------------------------------------------------------------
rat_positive |      Coef.   Std. Err.       z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       atopy |   5.873091   1.448679     4.05   0.000     3.033733     8.71245
         pet |   1.974829   .9189348     2.15   0.032     .1737498    3.775908
r_feeding_-y |   -1.45754   .8155414    -1.79   0.074    -3.055971    .1408923
r_sacrific-y |   2.542016   1.242213     2.05   0.041     .1073232    4.976708
r_manualcc-y |  -2.770631   1.086658    -2.55   0.011     -4.90044   -.6408212
       _cons |  -4.881272   1.470089    -3.32   0.001    -7.762594   -1.99995
------------------------------------------------------------------------------
```

STATA output (3.2.1.3)

```
. logistic  rat_positive atopy  pet  r_feeding_binary   r_sacrifice_binary
r_manualcc_binary

Logistic regression                                     Number of obs   =         74
                                                        LR chi2(5)      =      54.65
                                                        Prob > chi2     =     0.0000
Log likelihood = -23.535282                             Pseudo R2       =     0.5373
------------------------------------------------------------------------------
rat_positive | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       atopy |   355.3458   514.7819     4.05   0.000     20.77464    6078.113
         pet |   7.205386    6.62128     2.15   0.032     1.189758    43.63711
r_feeding_-y |   .2328084   .1898649    -1.79   0.074      .047077    1.151301
r_sacrific-y |   12.70526   15.78263     2.05   0.041     1.113294    144.9963
r_manualcc-y |   .0626225   .0680492    -2.55   0.011     .0074433    .5268596
------------------------------------------------------------------------------
```

STATA output (3.2.1.4)

```
. estat gof

Logistic model for RAT_POSITIVE, goodness-of-fit test
       number of observations =         74
 number of covariate patterns =         21
          Pearson chi2(15) =         3.73
                  Prob > chi2 =         0.9985



. estat gof, Group(10) table


Logistic model for RAT_POSITIVE, goodness-of-fit test
  (Table collapsed on quantiles of estimated probabilities)
  (There are only 9 distinct quantiles because of ties)
  +-------------------------------------------------------+
  | Group |    Prob | Obs_1 | Exp_1 | Obs_0 | Exp_0 | Total |
  |-------+---------+-------+-------+-------+-------+-------|
  |     1 | 0.0075  |     0 |   0.0 |     8 |   8.0 |     8 |
  |     2 | 0.0518  |     0 |   0.3 |    10 |   9.7 |    10 |
  |     3 | 0.1444  |     1 |   0.7 |     4 |   4.3 |     5 |
  |     4 | 0.4099  |     3 |   3.2 |     5 |   4.8 |     8 |
  |     5 | 0.7294  |     9 |   8.0 |     3 |   4.0 |    12 |
  |-------+---------+-------+-------+-------+-------+-------|
  |     6 | 0.7825  |     1 |   1.6 |     1 |   0.4 |     2 |
  |     8 | 0.9510  |    18 |  18.3 |     2 |   1.7 |    20 |
  |     9 | 0.9716  |     3 |   2.9 |     0 |   0.1 |     3 |
  |    10 | 0.9960  |     6 |   5.9 |     0 |   0.1 |     6 |
  +-------------------------------------------------------+
       number of observations =        74
             number of Groups =         9
     Hosmer-Lemeshow chi2(7) =         2.06
                  Prob > chi2 =         0.9567
```

STATA output (3.2.2.1)

```
. logit RAT_OA   R_SACRIFICE_BINARY R_RESPIRATOR_BINARY  R_IVC_BINARY

Logistic regression                                     Number of obs   =         74
                                                        LR chi2(3)      =      32.37
                                                        Prob > chi2     =     0.0000
Log likelihood = -19.707395                             Pseudo R2       =     0.4509
------------------------------------------------------------------------------
             RAT_OA |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
--------------------+---------------------------------------------------------
 R_SACRIFICE_BINARY |   3.758193   1.094421     3.43   0.001     1.613168    5.903218
R_RESPIRATOR_BINARY |   3.471943   1.057515     3.28   0.001     1.399252    5.544634
       R_IVC_BINARY |  -3.040385   1.002178    -3.03   0.002    -5.004618   -1.076153
              _cons |  -2.630011   .7405764    -3.55   0.000    -4.081514   -1.178508
------------------------------------------------------------------------------
```

STATA output (3.2.2.2)

```
. estat gof
Logistic model for RAT_OA, goodness-of-fit test
       number of observations =        74
 number of covariate patterns =         8
             Pearson chi2(4) =         0.90
                 Prob > chi2 =        0.9249


. estat gof, Group(10) table

Logistic model for RAT_OA, goodness-of-fit test
  (Table collapsed on quantiles of estimated probabilities)
  (There are only 6 distinct quantiles because of ties)
  +-------------------------------------------------------+
  | Group |    Prob | Obs_1 | Exp_1 | Obs_0 | Exp_0 | Total |
  |-------+---------+-------+-------+-------+-------+-------|
  |     2 |  0.0034 |     0 |   0.1 |    17 |  16.9 |    17 |
  |     5 |  0.0672 |     2 |   1.7 |    24 |  24.3 |    26 |
  |     6 |  0.0999 |     1 |   0.7 |     6 |   6.3 |     7 |
  |     8 |  0.1287 |     1 |   1.4 |    10 |   9.6 |    11 |
  |     9 |  0.7555 |     7 |   7.3 |     3 |   2.7 |    10 |
  |-------+---------+-------+-------+-------+-------+-------|
  |    10 |  0.9900 |     3 |   2.8 |     0 |   0.2 |     3 |
  +-------------------------------------------------------+

      number of observations =        74
            number of Groups =        6
     Hosmer-Lemeshow chi2(4) =        0.63
                 Prob > chi2 =        0.9602
```

STATA output 3.2.2.3

```
. logistic RAT_OA   R_SACRIFICE_BINARY R_RESPIRATOR_BINARY  R_IVC_BINARY

Logistic regression                               Number of obs   =         74
                                                  LR chi2(3)      =      32.37
                                                  Prob > chi2     =     0.0000
Log likelihood = -19.707395                       Pseudo R2       =     0.4509
------------------------------------------------------------------------------
      RAT_OA | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
 R_SACRIFICE_BINARY |    42.8709    46.9188     3.43   0.001     5.018686    366.2142
R_RESPIRATOR_BINARY |   32.19923   34.05117     3.28   0.001     4.052166    255.8608
       R_IVC_BINARY |   .0478165   .0479206    -3.03   0.002     .0067069    .3409045
              _cons |   .0720777    .053379    -3.55   0.000     .0168819    .3077375
------------------------------------------------------------------------------
```

STATA output 3.2.3.1

```
. logit RAT_OR ATOPY R_SACRIFICE_BINARY  R_MANUALCC_BINARY

Logistic regression                               Number of obs   =         74
                                                  LR chi2(3)      =      31.99
                                                  Prob > chi2     =     0.0000
Log likelihood = -31.333314                       Pseudo R2       =     0.3380
------------------------------------------------------------------------------
      RAT_OR |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       ATOPY |   3.749136   1.245341     3.01   0.003     1.308311     6.18996
R_SACRIFICE_BINARY |   3.311134   1.107431     2.99   0.003     1.140609    5.481659
 R_MANUALCC_BINARY |  -2.606561    1.27464    -2.04   0.041    -5.104809   -.1083128
       _cons |  -4.191647   1.245325    -3.37   0.001    -6.632439   -1.750855
------------------------------------------------------------------------------
```

STATA output 3.2.3.2

```
. estat gof

Logistic model for RAT_OR, goodness-of-fit test
       number of observations =        74
 number of covariate patterns =         8
             Pearson chi2(4) =         1.32
                 Prob > chi2 =        0.8586


. estat gof, Group(10) table

Logistic model for RAT_OR, goodness-of-fit test
  (Table collapsed on quantiles of estimated probabilities)
  (There are only 5 distinct quantiles because of ties)
  +-------------------------------------------------------+
  | Group |   Prob | Obs_1 | Exp_1 | Obs_0 | Exp_0 | Total |
  |-------+--------+-------+-------+-------+-------+-------|
  |     2 | 0.0149 |     0 |   0.2 |    17 |  16.8 |    17 |
  |     3 | 0.0453 |     0 |   0.3 |     7 |   6.7 |     7 |
  |     4 | 0.2931 |     2 |   1.8 |     4 |   4.2 |     6 |
  |     8 | 0.3911 |    13 |  12.5 |    19 |  19.5 |    32 |
  |    10 | 0.9463 |    10 |  10.2 |     2 |   1.8 |    12 |
  +-------------------------------------------------------+
       number of observations =        74
             number of Groups =         5
     Hosmer-Lemeshow chi2(3) =         0.64
                 Prob > chi2 =        0.8880
```

STATA output 3.2.3.3

```
. logistic RAT_OR ATOPY      R_SACRIFICE_BINARY  R_MANUALCC_BINARY


Logistic regression                               Number of obs   =         74
                                                  LR chi2(3)      =      31.99
                                                  Prob > chi2     =     0.0000
Log likelihood = -31.333314                       Pseudo R2       =     0.3380
------------------------------------------------------------------------------
      RAT_OR | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       ATOPY |   42.48434   52.90751     3.01   0.003     3.699921    487.8264
R_SACRIFICE~Y |   27.4162   30.36155     2.99   0.003     3.128672     240.245
R_MANUALCC_~Y |  .0737879    .094053    -2.04   0.041     .0060675    .8973469
       _cons |   .0151214    .018831    -3.37   0.001     .0013169    .1736254
------------------------------------------------------------------------------
```

STATA output 3.2.4.1

```
. logit MOUSE_POSITIVE  M_SALIVA M_INJECTION_BINARY


Logistic regression                                     Number of obs   =         80
                                                        LR chi2(2)      =      10.31
                                                        Prob > chi2     =     0.0058
Log likelihood =  -36.22266                             Pseudo R2       =     0.1246
------------------------------------------------------------------------------
MOUSE_POSIT-E |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
--------------+---------------------------------------------------------------
     M_SALIVA |   1.582368   .6066022     2.61   0.009     .3934499    2.771287
M_INJECTION-Y |   1.251448   .6345486     1.97   0.049     .0077553     2.49514
        _cons |  -2.244596   .4724254    -4.75   0.000    -3.170533   -1.318659
------------------------------------------------------------------------------
```

STATA output 3.2.4.2

```
. estat gof

Logistic model for MOUSE_POSITIVE, goodness-of-fit test
       number of observations =        80
 number of covariate patterns =         4
              Pearson chi2(1) =         1.08
                  Prob > chi2 =         0.2985


. estat gof, Group(10) table


Logistic model for MOUSE_POSITIVE, goodness-of-fit test

  (Table collapsed on quantiles of estimated probabilities)
  (There are only 4 distinct quantiles because of ties)
  +-------------------------------------------------------+
  | Group |    Prob | Obs_1 | Exp_1 | Obs_0 | Exp_0 | Total |
  |-------+---------+-------+-------+-------+-------+-------|
  |     5 | 0.0958 |     5 |   4.2 |    39 |  39.8 |    44 |
  |     7 | 0.2703 |     3 |   3.8 |    11 |  10.2 |    14 |
  |     9 | 0.3402 |     5 |   5.8 |    12 |  11.2 |    17 |
  |    10 | 0.6432 |     4 |   3.2 |     1 |   1.8 |     5 |
  +-------------------------------------------------------+

       number of observations =        80
             number of Groups =         4
     Hosmer-Lemeshow chi2(2) =         1.08
                  Prob > chi2 =         0.5825
```

STATA output 3.2.4.3

```
. logistic MOUSE_POSITIVE  M_SALIVA M_INJECTION_BINARY

Logistic regression                               Number of obs   =         80
                                                  LR chi2(2)      =      10.31
                                                  Prob > chi2     =     0.0058
Log likelihood =  -36.22266                       Pseudo R2       =     0.1246
------------------------------------------------------------------------------
MOUSE_POSITIVE | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
---------------+--------------------------------------------------------------
      M_SALIVA |   4.866468    2.95201     2.61   0.009     1.482085    15.97919
M_INJECTION_BI |     3.4954   2.218001     1.97   0.049     1.007785    12.12343
         _cons |   .1059703   .0500631    -4.75   0.000     .0419812    .2674937
------------------------------------------------------------------------------
```

STATA output 3.2.5.1

```
. logit MOUSE_OA   M_CARCASS M_MASK_BINARY

Logistic regression                               Number of obs   =         80
                                                  LR chi2(2)      =       6.59
                                                  Prob > chi2     =     0.0371
Log likelihood = -15.410422                       Pseudo R2       =     0.1761
------------------------------------------------------------------------------
     MOUSE_OA |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
--------------+---------------------------------------------------------------
    M_CARCASS |   1.968365   1.170162     1.68   0.093    -.3251099     4.26184
M_MASK_BINARY |  -1.764479    .990293    -1.78   0.075    -3.705417      .17646
        _cons |  -2.849512   1.078808    -2.64   0.008    -4.963938   -.7350862
------------------------------------------------------------------------------
```

STATA output 3.2.5.2

```
. estat gof

Logistic model for MOUSE_OA, goodness-of-fit test
       number of observations =        80
 number of covariate patterns =         4
              Pearson chi2(1) =         2.50
                  Prob > chi2 =         0.1138



. estat gof, Group (10) table

Logistic model for MOUSE_OA, goodness-of-fit test
  (Table collapsed on quantiles of estimated probabilities)
  (There are only 4 distinct quantiles because of ties)
  +-------------------------------------------------------+
  | Group |   Prob | Obs_1 | Exp_1 | Obs_0 | Exp_0 | Total |
  |-------+--------+-------+-------+-------+-------+-------|
  |     4 | 0.0098 |     1 |   0.3 |    34 |  34.7 |    35 |
  |     5 | 0.0547 |     0 |   0.7 |    12 |  11.3 |    12 |
  |     9 | 0.1796 |     1 |   1.7 |    24 |  23.3 |    25 |
  |    10 | 0.2929 |     3 |   2.3 |     5 |   5.7 |     8 |
  +-------------------------------------------------------+
       number of observations =        80
             number of Groups =         4
     Hosmer-Lemeshow chi2(2) =         2.50
                  Prob > chi2 =         0.2865
```

STATA output 3.2.5.3

```
. logistic MOUSE_OA   M_CARCASS M_MASK_BINARY

Logistic regression                                     Number of obs   =         80
                                                        LR chi2(2)      =       6.59
                                                        Prob > chi2     =     0.0371
Log likelihood = -15.410422                             Pseudo R2       =     0.1761

------------------------------------------------------------------------------
     MOUSE_OA | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
--------------+---------------------------------------------------------------
    M_CARCASS |   7.158963   8.377145     1.68   0.093      .722448     70.9404
M_MASK_BINARY |   .1712761   .1696135    -1.78   0.075       .02459    1.192987
        _cons |   .0578726   .0624334    -2.64   0.008     .0069854    .4794641
------------------------------------------------------------------------------
```

STATA output 3.2.6.1

```
. logit MOUSE_OR ATOPY  M_URINE M_GLOVES_BINARY M_SHAVING_BINARY M_FILTERTOP_BINARY

Logistic regression                                     Number of obs   =         80
                                                        LR chi2(5)      =      27.94
                                                        Prob > chi2     =     0.0000
Log likelihood = -28.682771                             Pseudo R2       =     0.3275
------------------------------------------------------------------------------
          MOUSE_OR |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------------+----------------------------------------------------------
             ATOPY |   3.128748   1.226675     2.55   0.011     .7245096    5.532987
           M_URINE |   1.852696   1.003488     1.85   0.065    -.1141043    3.819497
   M_GLOVES_BINARY |  -3.999465   1.823847    -2.19   0.028    -7.57414    -.4247909
  M_SHAVING_BINARY |   2.430415   1.154078     2.11   0.035      .168464    4.692366
M_FILTERTOP_BINARY |   1.830808   .9242055     1.98   0.048     .0193982    3.642217
             _cons |  -2.990693   1.957004    -1.53   0.126    -6.82635     .8449646
------------------------------------------------------------------------------
```

STATA output 3.2.6.2

```
. estat gof

Logistic model for MOUSE_OR, goodness-of-fit test

       number of observations =        80
 number of covariate patterns =        15
             Pearson chi2(9) =         3.11
                 Prob > chi2 =         0.9597


. estat gof, Group (10) table

Logistic model for MOUSE_OR, goodness-of-fit test
  (Table collapsed on quantiles of estimated probabilities)
  (There are only 6 distinct quantiles because of ties)
  +-------------------------------------------------------+
  | Group |    Prob | Obs_1 | Exp_1 | Obs_0 | Exp_0 | Total |
  |-------+---------+-------+-------+-------+-------+-------|
  |     1 | 0.0057  |     0 |   0.1 |    14 |  13.9 |    14 |
  |     2 | 0.0058  |     0 |   0.0 |     4 |   4.0 |     4 |
  |     4 | 0.0757  |     1 |   0.5 |    13 |  13.5 |    14 |
  |     6 | 0.1183  |     2 |   2.0 |    15 |  15.0 |    17 |
  |     9 | 0.4556  |    10 |  11.0 |    15 |  14.0 |    25 |
  |-------+---------+-------+-------+-------+-------+-------|
  |    10 | 0.9049  |     5 |   4.5 |     1 |   1.5 |     6 |
  +-------------------------------------------------------+
       number of observations =        80
             number of Groups =         6
     Hosmer-Lemeshow chi2(4) =         1.19
                 Prob > chi2 =         0.8803
```

STATA output 3.2.6.3

```
. logistic MOUSE_OR ATOPY M_URINE M_GLOVES_BINARY M_SHAVING_BINARY
M_FILTERTOP_BINARY

Logistic regression                               Number of obs   =         80
                                                  LR chi2(5)      =      27.94
                                                  Prob > chi2     =     0.0000
Log likelihood = -28.682771                       Pseudo R2       =     0.3275

------------------------------------------------------------------------------
    MOUSE_OR | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       ATOPY |   22.84537   28.02384     2.55   0.011     2.063719    252.8982
     M_URINE |    6.37699   6.399234     1.85   0.065     .8921649    45.58127
M_GLOVES_BINARY |   .0183254   .0334228    -2.19   0.028     .0005136    .6539065
M_SHAVING_BINARY |    11.3636   13.11447     2.11   0.035     1.183486     109.111
M_FILTERTOP_BINARY |   6.238924   5.766048     1.98   0.048     1.019588    38.17639
       _cons |   .0502526   .0983446    -1.53   0.126     .0010848    2.327895
------------------------------------------------------------------------------
```

Appendix 15: The Final Logistic Regression Models for the Six Outcomes.

Table 3.2.7.1 A summary of multivariate logistic regression models	
Rat Sensitization	RAT_POSITIVE = 5.873 *ATOPY + 1.975 *PET + 2.542 * R_SACRIFICE_BINARY - 2.77 * R_MANUALCC_BINARY - 1.458 R_FEEDING_BINARY -4.88
Rat Asthma	RAT_OA=3.758* R_SACRIFICE_BINARY + 3.472* R_RESPIRATOR_BINAR - 3.0404* R_IVC_BINARY -2.630
Rat Rhinitis	RAT_OR=3.749* Atopy + 3.311* R_SACRIFICE_BINARY -2.606 * R_MANUALCC_BINARY -4.191
Mouse sensitization	MOUSE_POSITIVE= 1.582* M_SALIVA + 1.251* M_INJECTION_BINARY- 2.245
Mouse Asthma	MOUSE_OA= 1.968 * M_CARCASS1 -1.764 * M_MASK_BINARY -2.849
Mouse Rhinitis	MOUSE_OR= 3.129 * Atopy + 1.853 * M_URINE -3.999 * M_GLOVES_BINARY + 2.430 * M_SHAVING_BINARY + 1.831 * M_FILTERTOP_BINARY