LAB ANIMAL ALLERGY SURVEILLANCE AT A PRIVATE MEDICAL SCHOOL IN THREE DISSIMILAR ANIMAL RESEARCH FACILITIES

by

JEFFREY P. FEINBERG, B.S.

APPROVED:

TOMMY DOUGLAS, PH.D.

REBECCA LANGER, PH.D.

JACQUELYN SLOMKA, PH.D.

THE UNIVERSITY OF TEXAS SCHOOL OF PUBLIC HEALTH
Houston, Texas
November, 2007

DEDICATION

To my wife Shelby, for always doing more than your sixty-percent, being a constant source of encouragement and support (which was needed on more than one occasion), having immeasurable patience which I dearly love to test, being a wonderful cook, a great mother to all of the critters in the zoo, and a beautiful person in all senses of the word. This clearly defines how, “I got the better deal”.

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THESIS

Presented to the Faculty of The University of Texas

School of Public Health

in Partial Fulfillment

of the Requirements

for the Degree of

MASTER OF PUBLIC HEALTH

THE UNIVERSITY OF TEXAS SCHOOL OF PUBLIC HEALTH
Houston, Texas
November, 2007

UMI Number: 1447190

ProQuest Information and Learning Company
300 North Zeeb Road
P.O. Box 1346
Ann Arbor, MI 48106-1346

PREFACE

Funding for all laboratory supplies, laboratory analysis, and air sampling media was provided by the Office of Environmental Safety at MS (the private medical school in which the study was performed) and the University of Texas-Health Science Center School of Public Health, Southwest Center for Occupational and Environmental Health Research Training Program, NIOSH ERC Pilot Projects – "Proactive Laboratory Animal Allergy Surveillance and Education (ProLASE)". Equipment used was provided by the Office of Environmental Safety at MS and from the Environmental and Occupational Health division at the University Of Texas-Health Science Center School of Public Health.

ACKNOWLEDGEMENTS

I WOULD LIKE TO THANK DR. REBECCA "BECKY" LANGER FOR HER ASSISTANCE IN OBTAINING APPROVAL FROM THE OFFICE OF RISK MANAGEMENT, OFFICE OF GENERAL COUNSEL, AND THE INSTITUTIONAL REVIEW BOARD AT MS TO PROCEED WITH THE STUDY. IN ADDITION I WOULD LIKE THANK DR. LANGER FOR HER GUIDANCE, ADVICE, OCCASIONAL MOTIVATION, AND MOST IMPORTANTLY FOR THE TIME SHE DEDICATED TO ME AND OVERSIGHT FOR THIS STUDY.

Thesis submitted to the MPH Committee on October 01, 2007

LAB ANIMAL ALLERGY SUVEILLANCE AT A PRIVATE MEDICAL SCHOOL IN THREE DISSIMILAR ANIMAL RESEARCH FACILITIES

Jeffrey P. Feinberg, B.S., M.P.H.
The University of Texas School of Public Health, 2007

Thesis Advisor: Rebecca Langer

Very few studies have described MUP-1 concentrations and measured prevalence of Laboratory Animal Allergy (LAA) at such a diverse institution as the private medical school (MS) that is the focus of this study. Air sampling was performed in three dissimilar animal research facilities at MS and quantitated using a commercially available ELISA. Descriptive data was obtained from an anonymous laboratory animal allergy survey given to both animal facility employees and the researchers who utilize these facilities alike. Logistic regression analysis was then implemented to investigate specific factors that may be predictive of developing LAA as well as factors influencing the reporting of LAA symptoms to the occupational health program. Concentrations of MUP-1 detected ranged from below detectable levels (BDL) to a peak of 22.64 ng/m^3. Overall, 68 employees with symptoms claimed they improved while away from work and only 25 employees reported their symptoms to occupational health. Being Vietnamese, a smoker, not wearing a mask, and working in any facility longer than one year were all significant predictors of having LAA symptoms. This study suggests a LAA monitoring system that relies on self-reporting can be inadequate in estimating LAA problems. In addition, efforts need to be made to target training and educational materials for non-native English speaking employees to overcome language and cultural barriers and address their specific needs.

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INTRODUCTION

Mouse urinary proteins (MUPs) are pre-albumins belonging to the family of lipocalin proteins and have a molecular weight of approximately 19-20kD (Ferrari, 2004; Lucke, 1999; Virtanen, 1999). Mus m 1 (MUP-1), one of the many MUP isoforms, is the major mouse allergen which is produced in the liver and can be excreted via hair, dander, and urine of mice (Ferrari, Tsay, Eggleston, Spisni, & Chapman, 2004). MUP is excreted in the highest concentration, up to 5 ng/mL, in the urine and can be up to 100-fold more concentrated in urine from male mice because the gene expression is testosterone dependent (Ferrari, 1997; Renstrom, 2001; Wood, 2001). The pathology associated with a MUP exposure is due to the fact that the MUP protein attaches to relatively small (3.3 – 10 μm) particles which allow the MUP to deposit in the tracheobronchial and nasopharyngeal region of the respiratory tract when respired (Bush, 1998; DiNardi, 2003). MUPs have been demonstrated to be a causative agent in the development of the serious allergic disease called Laboratory Animal Allergy (LAA). LAA is an occupational disease that has been recognized by the National Institute for Occupational Safety and Health (NIOSH) as well as the National Institutes of Health (NIH) as being a serious problem (NIH, 2003; NIOSH, 1998). Further, NIOSH recommends health monitoring and subsequent counseling for workers who have developed LAA (Gordon, Kiernan, Nieuwenhuijsen, Cook, Tee & Taylor, 1997).

Studies encompassing varying settings and occupations have documented between 11 to 44% of individuals that work with laboratory animals develop symptoms associated with LAA (Bush & Stave, 2003). Given their job duties, animal technicians and cage cleaners are

consistently exposed to the highest MUP concentrations and are therefore more likely to be at risk for the development of the occupational LAA (Cullinan, 1999; Gordon, 1997). Sensitization to MUP typically occurs at airborne levels of 3 ng/m^3 and this risk greatly increases along with the development of LAA symptoms at airborne levels of 5 ng/m^3 or above (Cullinan, 1997; Gordon, 2001). The symptoms associated with LAA include rashes, sneezing spells, watery/itchy eyes, hives, and eczema. Occupational asthma, a more serious manifestation of LAA, can produce coughing, wheezing, and shortness of breath in up to 38% of people who have demonstrated sensitivity to MUP or other animal allergens (Bush, 2001). Development of LAA associated symptoms can be further complicated by several risk factors. Smoking and atopy have both been associated with increased risk of the development of LAA symptoms (Cullinan, et al., 1999).

Past studies estimate that 90,000 – 125,000 individuals are exposed to laboratory animals during their workday in the United States (Bush & Stave, 2003). However, intensity of individual exposure can vary greatly within a given facility depending on the particular job assignment, engineering controls and personal protective equipment utilized. Engineering controls which implement modern technology have significantly reduced airborne MUP levels within animal research facilities. Some design elements that can help abate MUP levels include pressurized rooms, directional airflow, and high air exchange rates. In addition individually ventilated cage systems, vacuum dump stations, robotic cage cleaners and the use of biological safety cabinets can reduce the levels of MUP especially when used during activities that tend to produce particles (Gordon, Fisher, & Raymond, 2001). Since

the majority of MUP attaches to particles in the range of 3.3-10µm activities such as cage changing and cleaning have the potential to produce large numbers of MUP laden particles (Bush, 1998; Ohman, 1994). New research suggests that the risk for LAA extends beyond the workplace (Krop, Doekes, Stone, Aalberse, & Van der Zee, 2007). MUP as well as other laboratory animal allergens have been detected in the mattresses and pillows of laboratory animal workers. This suggests a route of indirect exposure for family that might be living with individuals who work with laboratory animals and stresses the importance of wearing and proper use of personal protection equipment (PPE) to prevent the transfer of MUP outside of the research facility. NIOSH recommends leaving work clothes at the workplace to prevent the possibility of exposing people outside of the facility to allergens. This new research suggests that employees of animal research facilities should shower, including the washing of hair before leaving work to prevent the spread of allergens outside the facility (Krop, 2007; NIOSH, 1998). However, if shower out facilities are not available the transfer of MUP can be greatly reduced by wearing disposable hair caps, surgical gowns, and shoe covers.

This study focused on three dissimilar animal research facilities at a large private medical school, hereafter referred to as "MS", which currently trains more than 3,000 graduate level students, post-doctoral fellows, and medical residents. MS has a total of 8 animal research facilities. The three focused on for this study, referred to as Facility "A", "B", and "C", were chosen for being representative of animal research facilities and research activities that occur at MS and therefore there was a great deal of variance among them.

Facility “A” is a 65,000 sq.ft. mouse only facility that houses approximately 25,000 mouse cages making it one of the larger facilities of its kind in the nation (Figure 1). The facility is located in a basement where it is isolated from all other parts of the building that it is located within. Facility “A” is a clean facility meaning PPE must be put on when entering and removed when exiting the facility to minimize potential contamination. When entering the facility shoe covers, surgical gown, hair cap and gloves are required. All of the cages in Facility A are stored in positive pressurized cage racks within their rooms, the rooms are then positive pressurized to the suite hallway which is negative pressurized to the main corridor (Figure 1). Cage changes are performed on a rotating basis so that the cages in each suite get changed every two weeks. Clean cages are transported from the clean side of the cage wash room to the individual rooms within a suite. The mice are then removed from the dirty cages and placed into the new clean cage within a class I hood. The dirty cages are placed onto carts and transported to the dirty side of the cage wash room where they are processed. The dirty cages are processed by having the soiled bedding dumped and then the empty cages are passed through a washer and steam sterilizer. The soiled cages can be dumped one of three ways: by an automated robotic arm, manually into a vacuum dump station and manually into a large portable dumpster. The robotic arm is designed to minimize the production of particles and dumps the cages into a vacuum dump station. However, the robotic arm is limited by its speed and can not keep up with the load of dirty cages. Due to the limits of the robotic arm some cages must be manually dumped. These cages are dumped into either a vacuum dump station or simply into a large dumpster and sometimes both.

Facility “B” is a multi-species facility and is one of the smallest of the facilities on the grounds of MS. Facility “B” is around 9,600 square feet (Figure 2). Facility “B” houses approximately 700 mouse cages and is located in a research/clinical building completely separated from Facility “A” and “C”. The mouse cages are similar in design to those used in the other two facilities but are housed in non pressurized racks within the rooms. Cage changes are performed on a rotating basis similar to Facility “A”. However, the dirty cage wash area in Facility “B” is about ¼ of the size of the Facility “A” dirty cage wash area and therefore does not contain a robot for cage dumping. All dirty cages are dumped into a non-vacuumed dump station. This non-vacuumed dump station is then periodically emptied when full.

Facility “C” (Figure 3) is also a multi-species facility in between Facility “A” and “B” in both physical size and number of cages housed. The facility is 32,133 sq.ft. and includes a biohazard area for BSL-2 work. The facility houses a wide range of animals including mice. Like the other two facilities cage changes are performed on a rotating basis every two weeks. All dirty cages in Facility “C” are manually dumped into a large dumpster. The large dumpster is then periodically transported to a dock where its contents are transferred to a larger dumpster for later pickup by a contracted entity. All cages coming from BSL-2 level rooms are autoclaved prior to being manually dumped.

A significant portion of the animal husbandry employees surveyed (approx. 50%) in all three facilities studied are of non-native English speaking origin, mainly Vietnamese and Hispanic. English is a second language for some of these workers and a significant percentage of those do not speak any English. Most of the employees work a regular daytime schedule Monday through Friday. Employees in all three of the research facilities studied are provided all PPE and work clothing including: disposable lab coats, gowns, gloves, head covers, shoe covers, and respiratory protection when appropriate. Employees change into scrubs upon arriving at work and then change out of the scrubs prior to leaving work.

The current LAA monitoring system at MS only includes a brief interview at time of hire but no subsequent monitoring. The Occupational Health Program (OHP) maintains that the main emphasis of treatment is on avoidance and reducing exposure and the system relies solely on the self reporting of symptoms. The OHP has both verbally and in writing suggested there are fewer than five diagnosed new or worsening cases of LAA reported each year.

The OHP program relies on these non-native English speaking employees to report any LAA symptoms they may be experiencing. These language barriers can lead to the inability of the employees to effectively communicate any symptoms they may be experiencing. This inability to communicate can lead to a lack of cultural specific training and ultimately a disproportionate number of injuries and sickness to non English speaking workers (Flory, 2001). This suggests the inability to communicate could mean potential allergies are going underreported at MS. In addition, cultural differences could also be leading to

underreporting due to the fear of an employee losing his/her job because of reporting the allergy. There has been little to no research investigating the effect of the specific factors such as language and cultural barriers on the reporting of LAA symptoms in this type of setting.

The specific aims of this study were to quantitate the levels of MUP-1 in three dissimilar laboratory animal research facilities at MS, administer a Lab Animal Allergy Survey (Appendix A) to assess the level of LAA and associated symptoms, and investigate if factors are present that may lead to a disproportionate amount as well as underreporting of LAA symptoms. In addition, an initial qualitative job hazard analysis was performed for the animal husbandry staff who work in these facilities to obtain a broad view of the risks associated with the job other than LAA that may be going unrecognized. The goal of this work was to better characterize LAA at MS, to investigate the current monitoring and reporting system, and to serve as a pilot study to spur future research endeavors into this topic at MS. This study was approved by the Institutional Review Board at both MS and The University of Texas School of Public Health-Houston.

METHODS

This study utilized a cross-sectional design to examine the concentrations of MUP-1 in the three representative research facilities at MS. In addition, the study utilized an anonymous Lab Animal Allergy survey (Appendix A) to characterize the current prevalence of symptoms associated with LAA with respect to the facility where exposure takes place as well as demographics, information on possible confounding factors and relevant general

allergy history of the study population. Historical data was obtained from the Occupational Health Program on the number of reports of lab animal allergies which provided a baseline for comparison with the current prevalence of symptoms among workers obtained through the Lab Animal Allergy survey. Symptoms were considered related to the exposure to MUP-1 if the symptoms improve while away from work and if the levels of MUP-1 were present in the facility represented by the survey.

Air Sampling

To characterize the levels of MUP-1 in the facilities area air samples were collected using SKC® SURE-SEAL, Leak Free, 37 mm, 2 piece air sampling cassettes, SKC® 37 mm; 1.0um PTFE filters, and SKC universal PCXR8 air pumps. All sampling cassettes were assembled at MS within a class II biological safety cabinet and the filters handled with sterilized forceps to prevent possible contamination of the filters. Stands were used during sampling to position the filters and pumps at the breathing zone level at an estimated average height of 66 inches. This was done in attempt to accurately represent the types of exposures the employees may be receiving. Air samples were then collected at a rate of 2 L/min for a period of 120 minutes in each location (Gordon, 2001; Krop, 2007) yielding at total sample volume of 240 L of air. A field blank was also included for analysis for each day of sampling. All pumps were pre and post calibrated. Any samples in which the post calibration flow rate varied 10% from the starting flow rate were dismissed from analysis. Sampling locations within each of the three facilities are represented by stars on Figures 1, 2,

and 3 respectively. The dirty cage wash areas within all three of the facilities were sampled due to the likelihood of this area having the highest potential for MUP laden aerosols. Facility sampling locations as well as the days and time intervals when sampling occurred were chosen in attempt to get an accurate representation of the levels of exposure the employees experience; therefore the days and times varied depending on the schedule of the individual facility. The attempt was always made to position the pump and filter in the area of each location where the employees were most likely to be spending the majority of their time without hindering their ability to perform any job functions. On days when the dirty cage wash rooms were sampled, observations of work practices and counts of the number of cages being processed during the 120 minute interval were conducted in an attempt to correlate levels of activity to concentrations of MUP-1. Such work practice observations included: cage dumping technique, efficiency of employee at dumping cages, and any other activity which could have added to or reduced the production of aerosols containing MUP.

Analysis of Air Samples - ELISA

After collecting the air samples the actual concentrations of MUP-1 were determined by using a commercially available ELISA kit (product code EL-MM1) produced by Indoor Biotechnologies, Incorporated, Manchester, U.K. and using statistical forecasting. The Indoor Biotechnologies developed ELISA displays no known cross-reactivity with any other animal allergens and displays a high sensitivity for Mus m 1 (MUP-1), detectable down to 0.2 ng/mL. These two factors made the ELISA suitable for this study analyzing occupational exposure to MUP-1 in the animal research facilities. The MUP-1 was eluted from the filters

after air sampling by incubating the filter for 2 hours in one ml of PBS-0.5% Tween 20. The specific ELISA protocol followed was provided by Indoor Biotechnologies and is listed below.

ELISA Protocol for MUP-1

1. Diluted the Anti-Mus m 1 Polyclonal Ab (PA-MM1), supplied in the Indoor Biotechnologies Mus m 1 ELISA Kit, 1:1000 in 50 mM carbonate-bicarbonate buffer, pH 9.6 (Sigma C3041). Coated the ELISA plate wells with 100 µl/well of the diluted PA-MM1 per well. Incubated overnight at 4°C.

2. Washed wells 3X with PBS-0.05% Tween 20 (Sigma P7949), pH 7.4 (PBS-T). Incubated for 30 min at room temperature with 100 µl/well 1% BSA PBS-T. Washed 3X with PBS-T.

3. Added 100 µl/well of samples (diluted 1:2) from the air filters. Incubated for 1 hour at room temperature. Samples were diluted in 1% BSA-PBS-T.

4. Diluted the biotinylated anti-Mus m 1 Ab 1:1000 in 1% BSA-PBS-T. Washed wells 3X with PBS-T and added 100 µl/well diluted biotinylated anti-Mus m 1 Ab (BI-MM1) supplied with the Indoor Biotechnologies ELISA kit. Incubated for 1 hour at room temperature.

5. Reconstituted 0.25 mg of Streptavidin-Peroxidase (Sigma S5512) in 1 ml of distilled water and diluted 1:1000 (i.e. 10 µl / 10 ml) in 1% BSA PBS-T. Washed wells 3X with PBS-T and added 100 µl/well diluted Streptavidin-Peroxidase. Incubated for 30 minutes at room temperature.

6. Washed wells 3X with PBS-T and developed the assays by adding 100 µl/well 1 mM ABTS (Kirkegaard & Perry Laboratories). The plate was then read using an ELISA plate reader at 405 nm to get an Optical Density (OD) value for each sample.

The MUP concentration (ng/m^3) of each sample was then determined by using the following progression of calculations:

a. OD to ng/ml
    i. Microsoft Excel Forecast function predicted the unknown ng/ml value of the air samples by using linear regression based on the known values from the MUP-1 standard curve.*

b. ng/ml to ng
    i. ng/ml values were multiplied by 2 (the dilution factor) and 1 ml (the total volume from the elution of the air samples)

c. ng to ng/m^3
    i. Air samples were collected for 120 minutes at a flow rate of 2 L/min = 240 L.
    ii. 240 L = 0.240 m^3
    iii. ng values of air samples were divided by 0.240 m^3 =ng/m^3

*Actual concentrations of MUP were determined by using forecasting in Microsoft Excel. Forecasting predicted the MUP-1 concentrations from the unknowns by using linear regression based on known values from the MUP-1 standard created with MUP-1 concentrate provided in the Indoor Biotechnology ELISA kit.

Lab Animal Allergy Survey

The anonymous LAA medical survey, void of any personal identifiers, was made available to all employees of the Center for Comparative Medicine (CCM) as well as researchers on IACUC protocols who utilize the animal research facilities in order to assess the prevalence of the various symptoms associated with laboratory animal allergy, allergy history, and confounding factors. The survey was offered in three languages; English, Spanish, and Vietnamese to accurately represent the broad array of languages spoken in the animal facilities. The survey was translated from English to Spanish and Vietnamese by the professionals at Translations Services USA, Inc. Employees were allowed to pick the survey

in the language in which they felt most comfortable. Prior to distributing the surveys it was explained to employees that the survey was completely voluntary and in no way was an evaluation of their job performance nor would anybody other than the researcher view the completed surveys. The survey was then made available to all employees in each of the respective facilities for a period of one week and consent was implied by completion of the survey. Employees were allowed the time to complete the survey during normal work hours in the break room of each facility. Once completed, employees returned the survey in an unmarked campus mail envelope to the principal investigator via campus mail. The researchers were emailed the survey, asked to print it out, complete it, and then return it in an unmarked campus mail envelope in a similar fashion to the animal facility staff.

Analysis of Survey

The results of the survey were coded and entered into a Microsoft Excel database for statistical analysis. Results were then summarized using basic descriptive statistics and epidemiological descriptors. In addition, using the statistical software XLSTAT logistic regression analysis and *t*-tests were used to compare and predict the variables that are important in the development of LAA as well as the reporting of LAA and associated symptoms. Using logistic regression analysis and different binary outcomes from the survey as response variables allowed for the determination of factors that have a significant influence on the response variable. This type of analysis determined the important factors in the development and reporting of symptoms for the study population at MS.

Historical information on reports of LAA was requested and received both verbally and in writing from the Director of the Occupational Health Program at MS. The LAA information obtained from the Occupational Health Program was on the incidence of new or post-hire worsening cases of LAA symptoms over a period of the past 5 years. This data provided a useful comparison to the results of the survey and was important in determining if LAA symptoms are going underreported or misdiagnosed.

Job Hazard Analysis

To identify other potential hazards that could be unnoticed or unreported and as a baseline for future studies, a qualitative job hazard analysis was performed. This analysis focused on the job classification of animal husbandry staff in each facility. Observations were made on a single representative work day for each facility. Potential chemical, physical and ergonomic hazards were noted and compiled to form an initial job hazard analysis (Appendix B).

RESULTS

In total 79 air samples were collected from the three animal facilities: 55 from Facility "A", 18 from Facility "B", and 6 from Facility "C". Overall 88% of the samples were above the detection limit of the ELISA. Locations of the samples can be seen on Figures 1, 2, and 3. All samples were collected at a rate of 2 L/min for a period of 120 minutes. All of the sampling locations in each facility were sampled on at least 3 different days in attempt to accurately characterize the levels of MUP-1 in each of the locations. The Facility "A" dirty cage wash section was sampled on 7 different days. The four extra days of sampling were

performed in the dirty cage wash of Facility "A" due to its large size and highly variable schedule. The days and times in which sampling occurred were dictated from the animal facility managers. The animal facility managers were asked to suggest days and times that would be most representative of the typical load of cage changes and dirty cages being processed. Locations of the samples were then chosen to be in close proximity to where employees would be at the highest risk of being exposed to MUP-1 aerosols as well as some of the immediate surrounding areas. The results of the MUP-1 ELISA analysis are completely summarized in Table 1. MUP-1 concentrations ranged from below detectable levels in several of the animal suites and corridors in both Facility "A" and Facility "B" up to a peak of 22.64 ng/m^3, detected in the dirty cage wash section of Facility "C" on the first day of sampling. In general the highest levels were found to be in the dirty cage wash sections of the three Facilities with the lowest levels being found in the corridors of Facility "A" and "B". In each of the three facilities cages are changed every two weeks on a rotating schedule so that each day a different group of cages are being changed. This provides a constant flow of dirty cages; however depending on which group of cages is being changed can affect the overall number of dirty cages changed that day. Numbers varied from approximately 800 dirty cages on a slower day to a peak of approximately 1300 for a two hour sampling period in Facility "A" and "C". The much smaller Facility "B" averaged between approximately 75-175 dirty cages processed for a given two hour sampling period.

A total of 233 recordable Laboratory Animal Allergy surveys were completed and returned by employees and researchers that work in 7 different facilities at MS. All of the surveys returned were eligible for inclusion in the study. More than 70% of the surveys that

were returned were from the 3 facilities focused on in this study. The other 30% were from employees/researchers that utilize one of the other 5 facilities at MS. Of the 233 surveys returned 94 (40%) were completed by animal facility staff, 124 (53%) by researchers, 13 (6%) by other job classes, and 2 (1%) from undisclosed job classes. Overall, 146 (62.4%) employees reported having at least one symptom related to LAA. The most common symptoms reported in order were runny/stuffy nose, sneezing/coughing, and watery/itchy eyes. Stratified by job class: 61 (64.9%) were animal facility employees, 77 (62.1%) were researchers and 8 (61.5%) were other job classes. A *t*-test revealed there was no significant difference between the proportions of animal facility employees and researchers, $p>0.05$, 95% CI, in terms of presence of symptoms. A summary of the LAA survey population is in Table 2.

Of the 146 surveys that reported having at least one LAA symptom 68 stated their symptoms were worst while at work. Stratified, 47.5% of animal facility staff and 49.4% of researchers stated their allergies were worst while at work. Again, a *t*-test revealed no significant difference between animal facility staff and researchers, $p>0.05$, 95% CI.

Only 25 (17%) of the 146 employees with LAA symptoms reported their problem to occupational health. There was a significant difference between animal facility employees and researchers, $p=0.05$, with 24.6% of animal facility employees claiming to have reported their problem to occupational health while only 11.7% of researchers did.

Binary logistic regression analysis allowed investigation into independent variables that could be predictive of LAA symptoms. Using the category, "reported allergies worst at work", as the response or dependent variable, being Vietnamese ($p=0.035$), not wearing a

mask (p=0.008), being a smoker (p=0.010) and working in any facility more than one year (p=0.016) were statistically significant predictors of having at least one symptom of LAA and that symptom(s) being worst at work, p<0.05, 95% CI. A similar binary logistic regression analysis was performed in attempt to predict independent variables that could be predictive of, "reporting symptoms to occupational health", but there were no statistically independent variables that were predictive of this behavior. A summary of this model parameter analysis is below in Table 3. Eight of the surveys were not included in the XLSTAT logistic regression analysis due to unanswered questions in the surveys.

The results of the job hazard analysis performed on the animal husbandry staff that spend the majority of their work hours in the dirty side of the cage wash is represented in Appendix B. Other than the topic of this study, being exposed to MUP-1, it is estimated the greatest potential hazard present was the likelihood of a slip, trip, or fall. The nature of the work that occurs in the dirty cage wash areas creates a constant damp/wet floor. This situation was particularly noticeable in Facility "B" and "C" where they frequently utilize a water hose to clean the floors. This is due to the fact that those two facilities strictly utilize a rolling dumpster to empty the soiled bedding into. This method inherently produces a large amount of soiled bedding on the floor which must be periodically washed away. Another potential significant hazard is the constant presence of noise. This is predominantly a problem in the two larger facilities, "A" and "C". The source of the noise in these facilities is the constant running of the pass through sterilizers as well as the knocking of the plastic animal cages to empty out the soiled bedding. The two dirty cage wash areas of both of those facilities are under an OSHA dictated hearing conservation program. All employees working within these

areas are supposed to wear some form of hearing protection while in the dirty cage wash. Most of the employees were noted as doing so but many did not wear them in a manner that provided them the level of protection the hearing protection advertised. Other noteworthy potential hazards present and listed on the Job Hazard Assessment form are the risk of back injury due to moving heavy bags of bedding and feed up to 75lbs, and ergonomic stressors in the form of cumulative trauma disorders that originate from the repetitive nature of the cage emptying process.

Figure 1: Map and Sampling Locations of Facility "A"

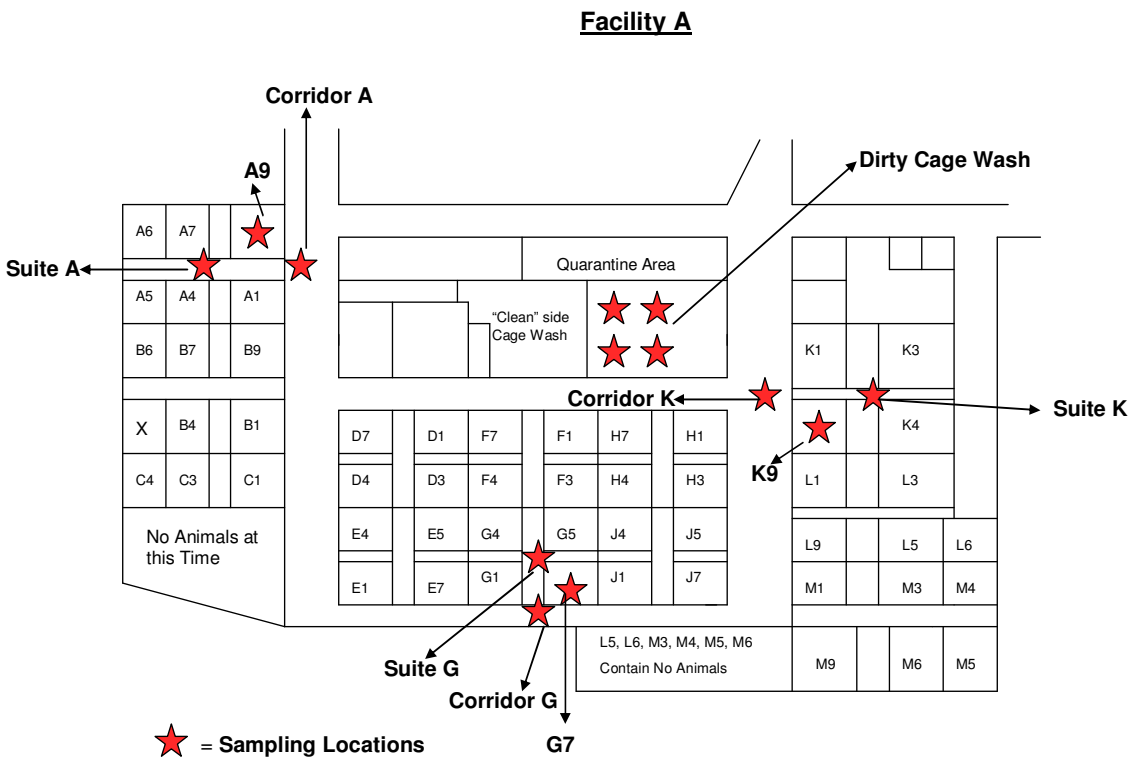

Figure 2: Map and Sampling Locations of Facility "B"

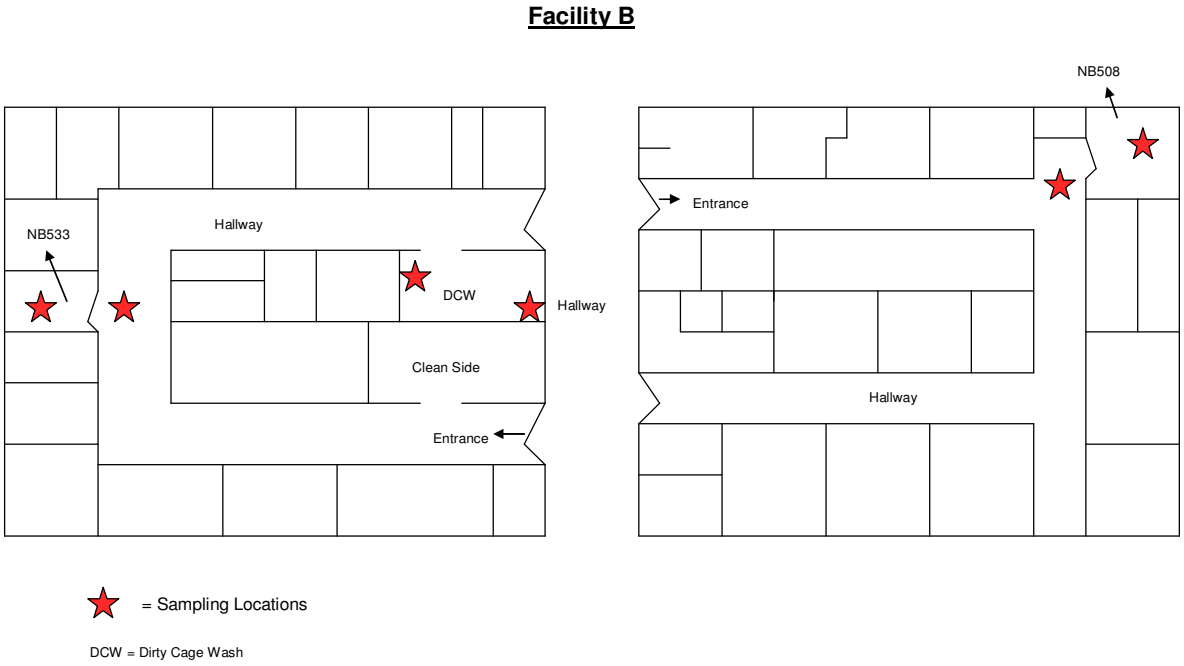

Figure 3: Map and Sampling Locations of Facility "C" (East Wing)

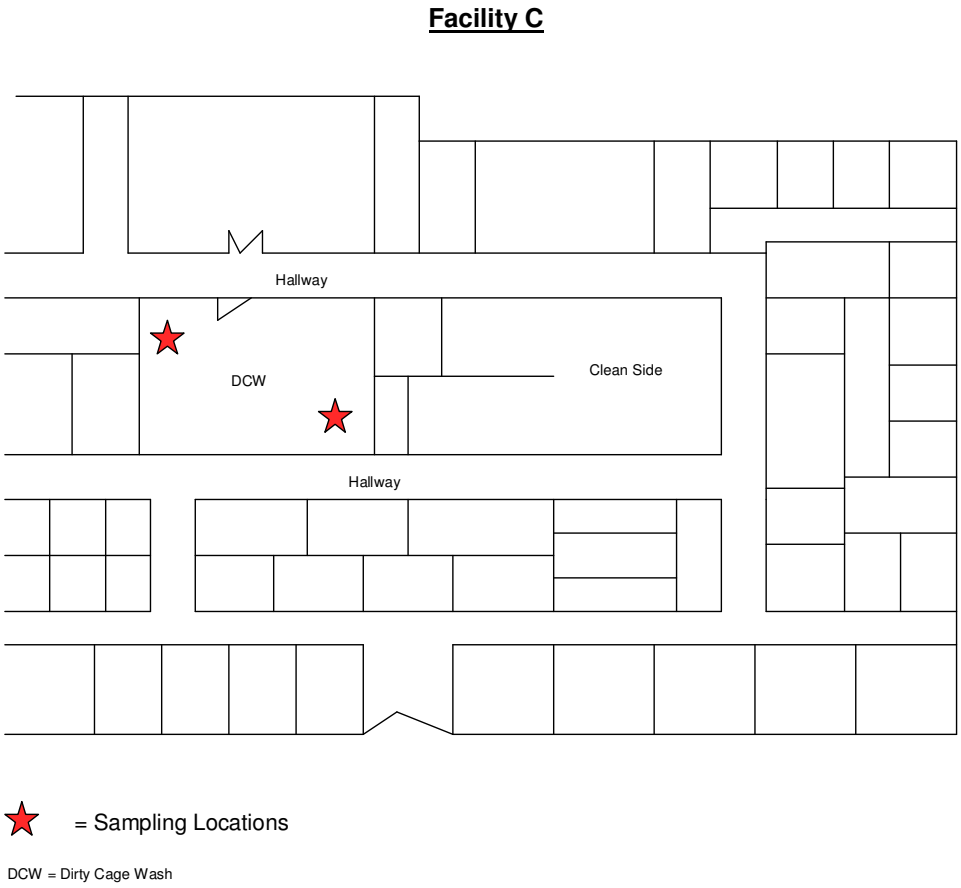

Table 1: MUP-1 Airborne Concentrations (in units of ng / m^3)

Location	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Average
Facility A-Cage Dump	22.64	6.9	2.24	13.53	5.67	0.935	4.25	8.0
Facility A-Cage Stack	4.95	0.6	0.47	1.43	0.347	0.378	0.632	1.3
Facility A-Outside Robot	8.84	5.23	0.38	1.16	0.66	0.06	1.09	2.5
Facility A-Inside Robot	2.02	1.44	0.31	1.63	0.64	0.02	0.01	0.86
Facility B-Cage Dump	3.29	0.70	BDL*					1.33
Facility B-Cage Stack	0.05	BDL	BDL					0.02
Facility C-Cage Dump	21.28	0.001	0.63					7.30
Facility C-Cage Stack	0.87	0.002	BDL					0.29
Facility B-Inside RM 508	0.28	1.08	BDL					0.45
Facility B-Outside RM 508	0.09	0.66	BDL					0.25
Facility B-Inside RM 533	BDL	0.70	0.06					0.25
Facility B-Outside RM 533	3.32	0.27	0.16					1.25
Facility A-Corridor A	0.21	0.13	0.31					0.22
Facility A-Suite A	0.22	0.18	0.11					0.17
Facility A-Room A	0.48	3.17	0.41					1.35
Facility A-Corridor G	0.09	0.07	0.1					0.06
Facility A-Suite G	0.625	0.07	0.47					0.39
Facility A-Room G	1.06	1.73	3.29					2.03
Facility A-Corridor K	0.02	0.02	BDL					0.01
Facility A-Suite K	0.18	0.32	BDL					0.17
Facility A-Room K	1.1	0.25	0.03					0.46

*BDL = Below Detectible Limits; given value of zero for calculating average.

Table 2: Summary of LAA Survey Population

Population	Number	At Least 1 LAA Symptom	Symptoms that are Worst at Work	Symptoms Reported to Occupational Health
Animal Facility Staff	94	61/94 = 64.9%	29/61 = 47.5%	15/61 = 24.6%
Researchers	124	77/124 = 62.1%	38/77 = 49.4%	9/77 = 11.7%
Other	13	8/13 = 61.5%	1/8 = 12.5%	1/8 = 12.5%
Unidentified	2	0/2 = 0%	0/0 = 0%	0/0 = 0%
Total (N =)	233	146/233 = 62.7%	68/146 = 46.6%	25/146 = 17.1%

Table 3: Summary Statistics and Model Parameter Analysis

Variable	Category	Frequency	Percent	*p*-value
Allergies Worst at Work	Yes	65	47.10	
	No	73	52.90	
Age	18-24	11	7.97	-
	25-34	38	27.54	0.081
	35-44	33	23.91	0.053
	45-54	26	18.84	0.194
	55+	30	21.74	0.878
Job Position	Animal Facility Staff	56	40.58	-
	Researcher	79	57.25	0.817
	Other	3	2.15	0.992
Facility	Facility A	61	44.20	0.991
	Facility B	13	9.42	0.991
	Facility C	27	19.57	0.991
	Facility D	4	2.90	-
	Facility E	9	6.52	0.990
	Facility F	8	5.80	0.991
	Facility G	6	4.35	0.991
	Satellites	3	2.17	0.991
	None	7	5.07	0.991
Race / Ethnicity	Caucasian	52	37.68	-
	African American	19	13.77	0.415
	Hispanic	24	17.39	0.078
	Vietnamese	22	15.94	0.035
	Other	21	15.23	0.117
Worked in Dirty Cage Wash	Yes	45	32.61	0.789
	No	93	67.40	-
Worked with Animals Before	Yes	55	39.85	-
	No	83	60.15	0.169
Wear a Mask	Yes	69	50	-
	No	68	49.28	0.008
	No Response	1	0.725	0.995
Smoker	Yes	26	18.84	0.010
	No	112	81.16	-
Pets	Yes	82	59.42	0.986
	No	56	40.58	-
Years in Current Facility	0-1	24	17.39	-
	1-3	40	28.99	0.016
	3-5	24	17.39	0.005
	5+	50	36.23	0.017

DISCUSSION

The results of the MUP-1 air monitoring samples indicated that in several areas of the animal research facilities studied the levels of the allergen are present in concentrations at times up to more than 4X the amount known to elicit serious LAA symptoms (Cullinan, 1999; Gordon, 2001). As expected the samples with the highest concentrations of MUP-1 were collected in the dirty cage wash sections. The two samples with the highest concentrations came from the dirty side of the cage wash in Facility "A" and Facility "C" and were 21.28 and 22.64 ng/m^3 respectively. Other studies have found that the highest concentrations of MUP-1 were typically collected from the animal husbandry staff, particularly those in charge of dumping the soiled bedding from cages, and in addition typically have the highest incidence of LAA symptoms (Bush, 2003; Cullinan, 1999; Gordon, 2001; Hollander, 1997). These samples collected within the dirty cage wash were believed to have the potential to produce the highest MUP-1 concentrations because they were collected closest to the area where the dumping of the soiled corncob bedding takes place. The process of dumping the soiled corncob bedding was observed to generate large amounts of particulates regardless of the receptacle or technique used. Facility "C" is an older facility and the dirty cage wash is not equipped with the same level of ventilation or a vacuum line dump station in which to dump the soiled bedding. This undoubtedly contributed to the highest concentration sample of the study. Although Facility "A" is a newer facility and the dirty cage wash is equipped with a vacuum line dump station and a partially enclosed robotic cage dumper these devices were not utilized consistently. It is theorized after making observations on 7 different days in

Facility “A”, the lack of fully utilizing the vacuum line dump station and the robotic cage dumper was due to their lack of efficiency. It was determined the dirty cage wash employees were able to process cages faster by using the rolling dumpster versus the vacuum line dump station. In addition on 4 of the 7 days sampling was conducted in Facility “A” the partially enclosed robotic cage dumper was out of service. After speaking to employees in the facility it was discovered this service interruption was a regular occurrence. The partially enclosed cage along with the vacuum line dump station are intended to help minimize the generation of aerosols and therefore the concentrations of MUP-1 in the immediate environment. The days the two peak samples occurred were also the some of the highest volume days in terms of dirty cages processed during the sampling schedule for Facilities “A” and “C”. This combination of factors mentioned above is most likely the cause of these elevated samples. Although these two samples were significantly higher than all of the other samples collected from other areas during this study results, these are dwarfed by levels found in other studies collected by similar methods which have yielded results ranging up to 244.3 ng / m^3 (Bush, 2003; Gordon, 2001; Hollander, 1997; Lucke, 1999). However, there were other days of sampling in Facility “A” and “C” where the number of processed dirty cages was similar in volume to the days that produced the two highest samples. This large gap between MUP-1 levels is believed to be caused to one of several factors. All of the facilities house mice from a broad range of studies. Some of these studies may involve the use of medications that lead to increased urination and therefore a greater potential for MUP-1 concentrations. Studies that utilize strictly or a predominantly male population would also experience greater concentrations of MUP-1 (Ferrari, 1997; Renstrom, 2001). Studies of this nature could be

responsible for the days where elevated levels were collected since the cage changes are on a rotating schedule.

The LAA survey revealed that 62.7% of the 233 employees who participated in the survey reported having at least one LAA symptom. More importantly 46.6% of those with at least one LAA symptom claimed their symptoms improved while they were away from work. This result would put MS near the top of the estimated 44% of animal workers who develop LAA symptoms (Bush, 2003). Like other LAA studies the most common symptoms were rhinitis, sneezing/coughing, and watery/itchy eyes (Cullinan, 1999; Ferrari, 1997; Gordon, 2001). Further investigation into the survey data leads to the conclusion that being Vietnamese, a smoker, not wearing a mask and working in any of the studied facilities 1 year or longer put employees disproportionately at risk for developing LAA symptoms. These results were not a surprise but are concerning. Vietnamese employees make up a large percentage of the dirty cage wash staff in the three facilities studied. Combined with the fact all of them have been working in these facilities longer than one year and usually were observed wearing dust masks instead of N95 respirators it is no surprise that are at a disproportionate risk of developing LAA symptoms. Many of these employees are Vietnamese with English as a second language and among whom a significant percentage speaking little or no English. MS currently offers no training in Vietnamese and makes no accommodations for these employees. It is feasible they do not understand the significance of LAA, the importance of proper PPE, dumping techniques, and reporting to occupational health. Of the 17.1% of employees who claimed to have reported their symptoms to the

Occupational Health Program only 4 of those individuals were Vietnamese. Yet Vietnamese made up a disproportionate amount of the employees with LAA symptoms.

These data suggest the current OHP program for monitoring LAA among the employees, especially those who are not native English speakers, in the animal research facilities is inadequate. While there are no specific legal requirements for occupational health programs for animal facilities, MS is covered by the general duty clause of the Occupational Safety and Health Administration which requires employers to maintain a workplace free of recognized hazards (OSHA, 1970). In addition, MS receives funds from the National Institutes of Health (NIH) and therefore is obligated to comply with the requirements of the National Research Council's, Guide for the Care and Use of Laboratory Animals, which clearly describes the necessity of an occupational health program (National Research Council, 1996). The NIH recommends instituting a broad combination of prevention measures to control animal allergens including engineering controls, administrative controls, and personal protective equipment. In addition NIH recommends a comprehensive medical evaluation including clinically indicated medical testing. Any employees or researchers that have or develop allergic reactions to animal allergens are recommended to utilize N95 respirators (NIH, 2003).

Currently Facility "A" is the only of the 3 facilities studied that has proper engineering controls other than general ventilation. While they are not consistently utilized, Facility "A" is equipped with a partially enclosed automated robotic cage dumper, a vacuum lined dump station, and the animal suites are equipped with individually ventilated cage racks which have been proven to reduce airborne concentrations of MUP-1 (Gordon et al., 2001).

However, the dirty cage wash in Facility "B" does have a HEPA (High Efficiency Particulate Air Filter) filtered disposal station. Bedding is dumped into a garbage can through a hole inside this HEPA filtered disposal station. This undoubtedly helps with the control of aerosols during the actual dumping process but the soiled bedding is still falling below into a garbage can, potentially generating aerosols under the cabinet and when the garbage fills and needs to be removed for emptying. While the goal should be to move towards implementing similar engineering controls in the other facilities, in the interim administrative controls, technique adjustments, and PPE should be utilized to help mitigate the potential for the development of LAA. Employees who have demonstrated animal allergies in the past or currently should discontinue use of the dust "cone" style mask for a N95 respirator. Using a P2 respirator (a European designation similar to that of N95) has been demonstrated to reduce the amount of inhaled animal allergen by 90% in research animal workers (Renstrom, Karlsson, & Tovey, 2002). Cage dumping into the rolling dumpsters should cease in Facility "A" and the vacuum lined dump station should be used exclusively in addition to the robot. In Facility "B" and "C" which lack these devices, wetting down the bedding prior to dumping should be considered to help reduce MUP laden aerosols. NIOSH recommends additional administrative controls including: decreasing animal density, leaving work clothes at work, when feasible keeping female mice since they excrete less MUP, providing training to educate the workers about LAA, and provide health monitoring and counseling with follow-up for those employees who currently exhibit or have exhibited allergies (NIOSH, 1998). Showering prior to leaving work should also be considered for the employees of the

facilities to prevent the possible sensitization of family or other household members (Krop et al., 2007).

CONCLUSIONS

Concentrations of MUP-1 capable of eliciting lab animal allergy were discovered in all three facilities studied. The dirty cage wash of Facility "A" and "C" exhibited levels at greater than 4X the level know to elicit serious LAA. Facility "A" is the only facility that has the engineering controls in place to help mitigate the problem although as the samples indicated they were not being fully utilized. The long term goal for the other two facilities should be to move towards implementing engineering controls mechanisms while in the interim using administrative controls in conjunction with PPE to help prevent LAA from occurring or worsening in those individuals who staff these dirty cage wash areas. It is recommended immediately that all individuals who have ever or currently experienced LAA symptoms be trained, fitted and utilize N95 respirators. In addition, in Facility "A" where the engineering controls are in place to help mitigate the levels of MUP-1 dumping of cages into an open rolling garbage container should cease and only the vacuum lined dump station should be utilized for disposal of soiled bedding. Dumping cages into non-ventilated open garbage containers is generating large amounts of potentially MUP-1 laden particulates. Consistently utilizing the vacuum lined dump station could greatly reduce the potential of elevated MUP-1 airborne concentrations. The sampling results indicate these individuals that staff the dirty

cage wash of all three facilities are at the greatest risk of developing LAA symptoms due to the high levels of MUP-1 that result from the nature of the work performed there.

The data from the LAA survey showed that 62.7% of employees that either work in or utilize the animal facilities have at least one symptom of LAA with the most common reported symptoms being runny/stuffy nose, sneezing/coughing, and water/itchy eyes. Statistical testing revealed that both animal facility staff and researchers were equally likely to have symptoms. An alarming 46.6% of those employees who stated having at least one LAA symptom reported there symptoms improved while away from work. Once again there was no difference between animal facility staff and researchers. It is strongly advised that researchers like animal facility staff that have ever or currently exhibit LAA symptoms utilize a N95 respirator while working in any of the animal research facilities. The survey also revealed only 25 (17.1%) of the employees who reported their symptoms are worst at work have visited occupational health. This is in agreement with what was stated by the director of the occupational health program who estimated 5 or fewer cases of LAA being reported and diagnosed per year over the past 5 years.

Logistic regression analysis revealed that being Vietnamese, a smoker, not wearing a mask, and working in any of the facilities studied for longer than one year were predictors of having at least one LAA symptom and that symptom(s) improving while away from MS. In the facilities studied Vietnamese employees make up a disproportionate percentage of the dirty cage wash staff. Only 4 employees of Vietnamese origin reported symptoms to the occupational health program. All of this indicates the need for ethnicity targeted training and education. Currently there are no training or educational materials that are in Vietnamese.

Training materials should be provided in multiple languages utilizing pictures that demonstrate the appropriate cage dumping techniques as well as the required PPE. In addition educational materials (posters, pamphlets) in multiple languages with pictures should be made available on site to all employees that point out the importance of LAA, the most common symptoms of LAA, the need for the correct PPE, and the importance of reporting symptoms to occupational health emphasizing that having LAA symptoms is not punishable.

All of the results of this study point to there being a problem with LAA at MS. Symptoms likely related to lab animal exposure are going underreported, which suggests that employees are suffering unnecessarily and potentially experiencing a lower quality of life. This problem is likely due to the fact the occupational health program provides no annual follow up of employees who began work at MS with a history of LAA or have reported having LAA. Relying of a system of self-reporting is not appropriate for an institution that employs a large number of non native English speaking people. Coming from different cultural backgrounds and not speaking English fluently may pose enough of a barrier that LAA is going underreported. However, this pilot study was only intended to raise the issue and some of the complex relationships. This study did not perform rigorous, thorough sampling and had a relatively small sample size. Further research, sampling and MUP specific allergen skin testing are needed to confirm LAA cases and to fully describe and understand the observed relationships.

APPENDICES

Appendix A: Lab Animal Allergy Survey

Laboratory Animal Allergy Survey

Please do not put your name on this questionnaire!

The following information is being collected only for informational purposes with regards to a study that will help determine if there is an increased prevalence of allergies among workers in research animal facilities. Participation in the survey is completely voluntary and all responses will be kept anonymous.

Demographic Information

1. What is your position with MS?

   CCM Administration CCM staff Researcher Other

2. Which CCM facility do you spend the most time in? (Circle One)

   A B C D E F G

3. Have you ever worked in the dirty cage portion of an animal facility?
   Yes No

4. Approximately how many hours do you spend in the facility each week? (Circle One)

   0-10 hrs 11-20 hrs 21-30 hrs 31-40 hrs

5. How long have you worked in the above facility? (Circle One)

   Less than 1 yr 1-3 yrs 3-5 yrs 5 yrs+

6. What is your race/ethnicity?

   Caucasian African-American Hispanic Vietnamese Other

7. What is your age? (Circle One)

   18-24 yrs 25-34 yrs 35-44 yrs 45-54 yrs 55 yrs +

Current Allergic Symptoms/Medical History

8. Have you experienced any of the following symptoms on a regular basis?

Watery/itchy eyes	Yes	No
Runny or stuffy nose	Yes	No
Sneezing/coughing	Yes	No
Difficulty swallowing/chest tightness	Yes	No
Excessive mucous production	Yes	No
Frequent colds	Yes	No
Skin problems	Yes	No

(Itchy skin, eczema, dry skin, etc)

9. If you experience any of the above symptoms where are they the worst? (Circle One)

Work Home Vacation No Difference

10. If you experience any of the above symptoms have you ever reported them to the following individuals?

Supervisor	Yes	No
Co-worker	Yes	No
Occupational Health	Yes	No

11. Has a physician ever told you that you have allergies or asthma?

Yes No

12. If yes, have you ever taken prescription medication for allergies or asthma?

Yes No

Occupational History

13. Have you worked with laboratory animals before this job? (Circle One)

Yes No

14. If yes, did you experience any allergy symptoms at that job? (Circle One)

Yes No

15. When working with laboratory animals or their cages do you wear a mask/respirator?

Yes No

Home Environment

16. Do you or any other members of your household smoke cigarettes?

Yes No

17. Have you ever owned any house pets? (i.e. dog(s), cat(s), etc.)

Yes No

18. Is there anyone in your household with allergies or asthma?

Yes No

Appendix B: Job Hazard Analysis

JOB HAZARD ANALYSIS (JHA)		Date:	X New JHA Revised JHA
JOB TITLE: **Cage Wash Attendant– Applies to Employees who Primarily Work in the Cage Wash Areas of their Respective Facilities**		JHA Number: **2007-008**	Page ___**1**___of ___**6**__
Job Performed By: **Various**	Analysis By: **Jeffrey Feinberg**	Supervisor:	Reviewed By:
Required Standards:	**29CFR 1910.95 – Occupational Noise Exposure** **29 CFR 1910 Subpart I – Personal Protective Equipment** **9 CFR Subchapter 1 – Subpart A – Animal Welfare Act and Related Amendments, Policies, etc.**		
General Notes:	**Any employee who chooses or is required to wear a N95 respirator must be trained on the proper use and limitations of the respirator as well as being fit tested annually prior to use.**		
Required Personal Protective Equipment:	**Work clothes (scrubs), Hearing Protection, nuisance dust mask/N95 respirator, Latex/Nitrile gloves, leather gloves (task specific), safety goggles, face shield (task specific), slip resistant footwear preferably ANSI approved, hairnet, shoe covers, gown, and waterproof clothing when appropriate**		
Tools and Equipment:	**Cage cart , vacuumed bedding disposal dump, rolling dumpster, pass-through sterilizer, Getinge/Castle Robot**		

Job/Task Step	Potential Hazards/ Injury sources	Control Method/Recommendations/PPE/Training
Various Tasks **Slip, Trips, and Falls**	**Numerous**	1. **Slips, trips, and falls are one of the most common causes of injuries in the workplace.** 2. **Practice "Eyes on the Path" especially in areas where there are known or frequent slip, trip, and fall hazards.** 3. **When working in areas where slip hazards are present wear slip resistant footwear such as rubber boots.** 4. **Always clean-up after yourself if you spill a beverage or other slip hazard.** 5. **Ensure all electrical wiring is either tie-wrapped or tapped down to prevent tripping.** 6. **If you are performing a task that creates a hazardous environment make sure some sort of barrier or obvious warning signs are in place.**

JHA - CONTINUATION SHEET	JHA Number: **2007-008**	Page ___**2**__of ___**6**__
Job/Task Step	Potential Hazards/ Injury sources	Control Method/Recommendations/PPE/Training
Lifting of Heavy Objects (i.e. feed, bedding bags, water bottle crates, etc.)	**Back Injury/Strain, Other Bodily Harm**	**1. Size up the load before attempting lift, if it feels too heavy do not attempt to lift it without mechanical aid or help from another worker. 2. Bend your knees, not your back. Lift straight up letting your legs do the work, not your back. 3. Do not twist or turn your body once you have made the lift. 4. Make sure the path you are carrying the load down is clear. 5. Set the load down properly by bending at the knees not your back. 6. Always push, not pull, the object when possible. 7. If possible avoid lifts below the knees or above the shoulders by using mechanical aids. 8. Remember back injuries are usually cumulative trauma disorders and may take weeks, months, or years to develop.**
Transporting Clean or Dirty Cages to and from Suites and the Cage Wash	**Cages Falling From Cage Cart, Running into Somebody, Various Back Injuries/Ailments**	**1. When loading clean/dirty cages on rolling cart do not stack more than the cart is designed to hold to prevent cages from potentially falling off onto yourself or somebody else. 2. Always push the cart, never pull and always keep two hands on the cart at all times. 3. When available utilize blind-spot mirrors when going around blind corners. 4. Make sure to clean-up any water or other slip hazards that may have been caused by transporting the cages. 5. When done replace rolling cart in its proper location. 6. Always follow good basic hygiene practices.**
Dumping of Dirty Cages	**Exposure to Animal Allergy Laden Particulates, Cumulative Trauma Disorders, Noise, Slips, Trips, Falls, Exposure to Hazardous Chemicals in Bedding**	**1. Always wear proper PPE prior to entering cage wash areas to include: work clothes, slip resistant footwear, hairnet, latex gloves, goggles/eye protection, and hearing protection. 2. When feasible dump soiled bedding into vacuumed dump station to reduce aersolization of animal allergen laden particulates. 3. When transporting the rolling dumpster always push it, not pull and utilize blind spot mirrors where available. 4. Try to minimize the use of water to wash the floor as it creates a slip hazard. 5. Try to Position yourself during cage dumping as to minimize twisting of your torso and un-neutral postures. For further information and training on ergonomics contact OES (x 8-4799). 6. Always ensure both doors are closed and nobody is inside before activating the pass-through sanitizer/washer.**

JHA - CONTINUATION SHEET	JHA Number: **2005-008**	Page __**3**___of ___**6**__
Activity/Sequence of Job Steps	Potential Hazards/ Injury sources	Safe Action or Procedure
Dumping of Dirty Cages, Con't	**Exposure to Animal Allergy Laden Particulates, Cumulative Trauma Disorders, Noise, Slips, Trips, Falls, Exposure to Hazardous Chemicals in Bedding**	**7. When dumping/cleaning cage pans from NHP's take caution to minimize the generation of aerosols from the cage pans and make sure you are wearing a face shield to avoid splashes to the eyes, nose, or mouth. 8. If you experience allergy-like symptoms while working consult with Occupational Health and consider the use of a N95 respirator. Prior to wearing a N95 respirator you must be trained on the proper use and fit-tested by Environmental Safety. For further information contact OES at x8-4799. 9. Always practice good housekeeping by cleaning up spilled bedding and water from the floor, dumping stations and surrounding areas on a regular basis. 10. Always follow good basic hygiene practices to include washing hands upon leaving the cage wash area. 11. To prevent the possible spread of animal allergens to potentially susceptible individuals outside of MS it is strongly recommended that you change out of your work clothes prior to leaving and if possible shower at the end of your shift.**
Using Getinge/Castle Automated Robotic Cage Processor	**Various Types of Blunt Trauma Electrical Shock**	**1. Only employees who are trained on the proper use of the Getinge/Castle machine should attempt to operate it. 2. Follow the manufacturer's instructions with regard to proper operation and maintenance of the Getinge/Castle machine. 3. Never enter the enclosed area while the robotic arm is still moving. If it becomes necessary to enter the enclosed area turn-off the machine and wait for it to come to a stop prior to entering. 4. Do not attempt to repair the unit unless you are trained to do so or are being assisted by an authorized repair representative.**

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VITA

I was born in Tallahassee, Florida to Philip and Judy Feinberg on May 24, 1982 as the second of three children. I grew up what I would describe as a 'normal' childhood spending the first 23 years of my life in Tallahassee including graduating from The Florida State University with a B.S. in Biology in the summer of 2004. Having never been able to decide exactly what I wanted to do with my life and working for one year in a dead-end job I came to two conclusions: I needed to go back to school and I needed to leave Tallahassee to do it. It was not until that epiphany followed by research of what kind of graduate programs interested me that I knew the field of Public Health existed. Having family that lived in Houston and gaining acceptance into the University of Texas School of Public Health made the decision easy, a fifteen hour drive later I ended up in Houston in a seasonably warm December 2005. Shortly thereafter, I met my wife to be Shelby, got married a year and a half later, and began writing this thesis.