3.0 METHODS

3.1 Participants

There were initially twenty participants studied in this experiment. The age range was between 18-50 years of age. The nine workers came from a private landscape company with the remaining participants from a public institution. The private company did not require their employees to wear personal protective equipment (PPE), but they were provided dust masks by the employer. The public establishment provided, but did not require respirators for their employees. The population that did complete the study was only from the private company. This group was mainly career employees, not seasonal workers, and also all of the workers were tobacco users, male, and Caucasian. The mean age for the participants was 28 years old with a standard deviation of 10.23; and the mean number of years that the eight smokers had been smoking was 10.25 years with a standard deviation of 5.37.

3.2 Hypothesis

The hypothesis was that there will be an acute respiratory response to the organic hazards associated with wood mulch used by landscaper workers. A decrease in forced expiratory volume in one second observed in the lung function of exposed workers is expected and determined with the use of a spirometer. The use of PPE will either eliminate or reduce the decrease in forced expiratory volume or reduce the decrease.

In a study that was published in 1997 in the Journal of Occupational and Environmental Medicine, six volunteers were exposed to low levels of wood mulch for a short period of time. The levels of exposure varied among the participants and the levels were determined from the dust samples based on the size of the particle in their dust monitor. Two of the participants inhaled "not quantifiable" dust particles, meaning that the Marple Cascade Impactor that was used to measure and quantify the dust particles was not able to detect the dust that was in the participant's breathable space. The particles were too small to be measured. As for the remaining four, one participant did not have a dust monitor, but the other three were exposed to quantifiable dust particles. The range of the three quantifiable exposures is from 0.3-3.6 mg/m^3. The exposure time

ranged from 60 to 120 minutes, that is to say that two of the participants were exposed for 60 minutes and the other four for 120 minutes.

Among other tests that were performed, a pulmonary function test was administered before and after the exposure using a spirometer. There were no clinically relevant or statistically significant changes in pulmonary function between pre and post exposure. Post spirometer testing was executed at three different increments of time, two, seven, and 21 hours after exposure. This variety in post exposure follow-up pulmonary function screenings along with the lack of standardization in procedures and small sample size may have led to the non-significant findings.

In this current study in contrast to this 1997 study, a larger sample size will be used and procedures will be closely scrutinized for standardization. Also, the occupational setting will produce results with higher validity for the landscaping population. There is an expected finding that exposed employees will have a significant change in pulmonary function from pre-shift exam to the post-shift exam.

3.3 Location

This study will take place in southwest Virginia during the months of March and April. More specifically, landscaping companies in Montgomery County, Virginia, were solicited for participation. Companies are encouraged by the idea of obtaining information about the safety of the mulching task in the landscaping field. Also, companies should be interested in improving their industry and understanding the risks regardless of the results. This is a study of the landscaping industry, not a specific company.

3.4 Experimental Design

The lung capacity and function of each participating employee was tested before and after each shift. Each employee was only tested for one shift. During the shift, employees wore a dust monitor in order to measure the level of relevant toxins in the breathing air of the employees. Half of the participating employees were selected to wear disposable respirators during the shift in addition to the air monitor. The air samples that were collected from the monitors were sent off to be analyzed for levels of endotoxin, gram negative bacteria, and total fungal spores.

Once the participants were gathered the study began on the job-site. Not only did the field study reduce the inconvenience to the worker, but also increased the validity of the data that was collected. Prior to the date of collection the experimenter: 1)visited the participants and gathered necessary information to setup the experiment design; and 2) showed a video to help minimize variability in pulmonary function testing (PFT). The PFT was administered by different technicians on the day of data collection so by standardizing the initial instruction, the variability in performance among participants was minimized. The information that was gathered at the preliminary meeting was number of males versus females and the number of smokers versus non-smokers.

At the preliminary meeting, workers were asked to complete two questionnaires; one questionnaire collected demographic information and the second was an altered published respiratory questionnaire (Appendix A). The respiratory questionnaire that was used was actually presented in two parts (the first part was administered at the preliminary meeting with the remaining section introduced after the first shift of data collection) to the participants and was from a 1990 article, "Questionnaire Evaluating Organic Dust Exposure". Most respiratory questionnaires used presently in research are based on the original British Medical Research Council and or the American Thoracic Society questionnaire. It is also true that most respiratory questionnaires are published and used as standards; do concentrate more on chronic effects rather than acute response of the respiratory system to a toxin. Rylander et al determined that there should be a modified questionnaire looking for acute effects. Rylander et al came to this conclusion after a swine confinement building study when the data was compiled and there was incomplete information from the standard study. The proposed organic dust questionnaire consists of forty-one questions ranging from previous work and on-the-job symptoms in the past and present, to smoking habits. This study on the landscaping field was particularly interested in acute effects of endotoxin, gram negative bacteria and total fungal spores, resulting in the proposed organic dusts questionnaire being more applicable (Rylander et al., 1990).

During the time between the preliminary meeting with the participants and the data collection day, the experimenter determined whether random pairing was necessary for post analysis statistics. The experimental design for this study was initially a

covariance block design with two treatments. For both the university and the company there was a block with the associated population divided into two equal groups for each treatment, respirator and no respirator. Even though both establishments make some type of respiratory personal protective equipment (PPE) available to the employees, the experimenter provided NIOSH approved half-face disposable respirators for the protected half of the participants. An N-100 respirator was used for each of the participants in the randomly assigned "protected" group. This was to help minimize discrepancies in manufacture, fit, use, and style PPE provided to the workers. In addition to providing respirators to minimize error and increase validity, the gender of participants and smoking preference was taken into consideration with assigning individuals to treatments (respirator versus no respirator). If possible there should be an equal number of males and females in each of the treatment groups and an equal number of smokers. The following was an initial representation of the experimental design:

Table 1: Experimental Design

	University Population	Company Population
Respirator Use	5	5
No Respirator Use	5	5

The covariance was a result of the relationship between the pre and post PFT and the air sample levels. The levels of endotoxin, gram-negative bacteria, and total fungal spores should have been directly related to the shift in pre to post PFT.

The day of data collection started on site with the experimenter and additional PFT technicians performing the spirometer testing and equipping each participant with a dust collector monitor. After the lung capacity was recorded and the dust monitor was functioning, the experimenter did not leave the site for any long periods of time. The experimenter, volunteers, and PFT technician returned after the shift was completed to collect the dust samples from the monitors and also to execute a post shift spirometer test. It was at this time that the remaining section of the adapted Rylander's questionnaire was administered and the respirator design questionnaire (Appendix B) was also completed by the participants. The dust samples were sent to a lab to be developed and results to be

generated. Again the lab determined the levels of endotoxin and total fungal spores that were present in the employee's breathing zone.

3.5 Equipment Administration

A Renaissance Spirometer was used to determine if there was any sign of acute pulmonary problems associated with the mulching tasks (shoveling, spreading, and transporting) of the landscapers. This also established a base line for follow-up to identify whether there was an association between the tasks and chronic pulmonary disease among these workers. The tasks performed by landscaper workers involve disturbing natural endotoxin in the wood mulch environment that may have an effect of the respiratory system of workers.

3.6 Spirometer Evaluation

Spirometery can identify if there was the presence of restrictive lung disease, obstructive lung disease, or both by the measures forced vital capacity (FVC), forced expiration volume in one second (FEV_1), and FEV_1/FVC. The following is a table reproduced from page 2-11 of the NIOSH-Approved (#043) Spirometery Training manual that relates these measures to the disease that is expressed in the spirometer results.

Table 2: Lung Diseases and Spirometry Results (NIOSH, 2001)

Interpretation	FVC	FEV_1	FEV_1/FVC%
Normal Spirometery	Normal	Normal	Normal
Airway Obstruction	Low or Normal	**Low**	**Low**
Lung Restriction	**Low**	**Low**	Normal
Combination	**Low**	**Low**	**Low**

The Normal Spirometery readings referred to in this table are based on the patient's gender, age and ethnicity. The values are based on extensive studies by Knudson that considered individual differences and how differences can affect lung

capacity and lung function. Knudson's values are not the only published values, but are the values dictated by OSHA and are used in mandatory spirometery industries. Knudson values are published in the American Review of Respiratory Disease from 1976. The study that Knudson executed in 1976 used the American Thoracic Society approved standards. The population studied was an urban Caucasian population that had never smoked. He revised his values in 1983 resulting in a slight shift in numbers, but it remains the cornerstone for industries with exposure to coal, asbestos, and cotton. Other tables were compiled by the following: Kory (1961); Morris (1971); Cherniack (1972); Crapo (1981)(NIOSH, 2001).

These readings are standards published in "Tables of Predicted Values". Currently the set of tables used by OSHA is Knudson's 1983 tables. In this study the Knudson 1983 predicted values were used in the analysis of the participant's spirographs. The tables only contain values for Caucasian male and females, but there is a standard correction factor for other ethnicities. Research has shown that non-Caucasian individuals have 85 percent of the lung function and capacity of Caucasian patients. Once the value is obtained from the table, if the patient is not Caucasian the value must be multiplied by a factor of 0.85 (NIOSH, 2001).

Restrictive lung disease refers to any disease that affects the tissue of the lungs, i.e. asbestosis. Spirometery is not a conclusive test for restrictive pulmonary disease, but based on the results of spirometery it can be determined if there should be more specific tests in order to identify if and what is causing the pulmonary difficulty. Primary identifier is a low FVC value, since technically the FEV_1 may show up normal in a spirograph of an individual with restrictive pulmonary disease. This makes sense since the effect of restrictive disease is the lungs inability to expand, so the forced vital capacity would suffer, where the FEV_1 for that individual may be normal because the lungs can collapse, or expire, in one second as normal (NIOSH, 2001).

Unlike restrictive pulmonary disease, spirometery is a conclusive test for obstructive pulmonary disease. Obstructive pulmonary disease is any disease of the airways, i.e. asthma. The most important indicator of obstructive pulmonary disease is the resulting low FEV_1/FVC% value, though it is intuitive that in order to have a low FEV_1/FVC% the FEV_1 value is also low. Again, this is logical considering obstructive

disease affects the flow in the airway, so it will take an individual more time to exhale (due to the obstruction in the airway) the air out of the airway into the spirometer in the first second. One with obstructive disease may be able to have a normal FVC it will just take longer to reach. The resulting interpretation is if all three measures are lower than normal, then the individual suffers from a combination of the two diseases (NIOSH, 2001).

Spirometers measure the rate at which the individual can exhale (and some spirometer models will also measure inhalation). Patients are instructed to take a deep breath and then blow out as hard as possible until the experimenter tells the patient to stop. Stopping the exhalation will occur after six seconds at the earliest. Less than six seconds will result in an incomplete spirometery test and will need to be redone. All patients should complete three valid attempts for each spirometer test. The maximum number of attempts is eight, but after the participant has attempted five times to produce three valid spirograms, the participant will be asked if they feel that they can continue, then after eight if they are still not able to produce three valid curves reschedule their PFT for another time. A few other elements that can ruin a spirometer attempt, for example, are coughing, glottis closure, and tight clothing (NIOSH, 2001).

The spirometer maps out a tracing of the patient's information. The tracing is referred to as a spirograms. From the spirogram measurements can be determined. The measurements of interest for this study are the forced vital capacity (FVC), forced expiration volume at one second (FEV_1), forced expiration volume at one second as a percentage of forced vital capacity (FEV_1/FVC), and forced mid-expiratory flow ($FEF_{25-75\%}$). Many spirometers will output this information, but analog machines and for verification purposes it is vital to understand how to generate and interpret these numbers (NIOSH, 2001).

Once the spirographs are produced by the participants, the spirographs will be analyzed by the experimenter. For each individual, the three pre shift PFTs and three valid post PFTs must be reduced into one valid pre shift and one valid post shift PFT for each individual. The three spirographs will be reduced using the following method. This method was demonstrated in the NIOSH-approved (#043) Spirometery Training course in May 2002 at the University of Medicine and Dentistry of New Jersey by Vincent Scoles.

The resulting values that will be measured from the spirographs by the experimenter will first be reported in the following table. The three spirographes will arbitrarily be assigned an identifier of A, B, or C.

Table 3: Spirometry Evaluation - Empty

	Curve 'A'	Curve 'B'	Curve 'C'
FVC			
FEV_1			
Total			

Once the table is full of values like the example below (twenty-three year old, Caucasian, female that is 173 cm tall) the "best" curve can be identified.

Table 4: Spirometery Evaluation - Full

	Curve 'A'	Curve 'B'	Curve 'C'
FVC (Liters)	**5.39**	5.31*	5.12
FEV_1 (Liters)	3.94	**4.11**	3.79
Total (Liters)	9.33	*9.42*	8.91

These readings are valid because the difference in the two highest FVCs is less than 200 milliliters. The "best" curve for this example is curve 'B' with a total value of 9.42 liters. (* Note that Curve 'B' does not have the maximum FVC value.)

Next the values from this table help to fill and determine the values for the next table.

Table 5: Individual's Results Compared to Predicted Values

	ATPS	BTPS	Predicted Value	% Predicted Value
FVC (Liters)	5.39	5.82	4.09	142.3
FEV_1 (Liters)	4.11	4.44	3.48	127.6
FEV_1/FVC (%)	76.3	N/A	N/A	N/A
FEF_{25-75}	4.05	4.37	N/A	N/A

The FVC value in the "ATPS" column is the maximum FVC value among all three curves (not necessarily the 'best' curve), same with the FEV_1 value. The first column labeled "ATPS" refers to the measured value which is at Ambient Temperature Pressure Saturation. Temperature can affect the measured volume, so since there is usually (unless ATPS equals 99°F or 37°C) a difference in the ambient temperature and one's body temperature the measured value must be converted into means of body temperature. This must be done when using the Knudson predicted values tables, since those published values are reported in body temperature pressure saturation (BTPS). Either the experimenter needs to measure the air temperature, or the spirometer may have a built in thermometer (most do) that will measure the temperature. The experimenter should verify that the temperature is being correctly measured by the spirometer before data collection. The conversion factors for converting ATPS to BTPS that are going to be used in this study are published in the NIOSH-Approved (#043) Spirometery Training manual (NIOSH, 2001).

After the conversion is made the resulting value is filled in the second column of data. Then the predicted value can be located in the Knudson 1983 set of tables for FVC and FEV_1. To fill the final column, simply divide the BTPS value by the predicted to determine the percent difference. If the resulting percent is between 80 and 120, then the patient has normal pulmonary function (NIOSH, 2001).

The only values not mentioned yet, are the values in the rows labeled FEV_1/FVC (%) and FEF_{25-75}. The FEV_1/FVC is the first row and column divided by the value in the second row and first column. In this case the percentage is 76.3%, which is considered

normal, that is, anything percentage about 70 percent is normal. This ratio is conclusive for obstructive pulmonary disease. In this example it is clear that the patient has no presence of obstructive pulmonary disease (NIOSH, 2001).

The last set of data points in the FEF_{25-75} row are calculated using values from the 'best curve'. Forced Mid-Expiratory Flow Rate is an optional measurement that will be calculated in this study, but will not be considered in identifying a shift in pulmonary function. It is calculated by taking the FVC values associated with the 'best' curve, in this case 5.31 and multiplying it by 0.25 and 0.75.

→ $FVC_B = 5.31$

(0.25)(5.31) = 1.33 Liters

(0.75)(5.31) = 3.98 Liters

5.82 – 1.77 = 4.05 ATPS

The points 5.82 and 1.77 liters are measured from the 'best' curve by plotting 1.33 and 3.98 and drawing a line between these two points. The points at which that line crosses the time lines (one second apart) are the 5.82 and 1.77 values.

For this study, a significant shift in pulmonary function will be expressed by a ten percent decrease in FEV_1 between pre and post shift PFTs. This ten percent change in FEV_1 for this study is derived from the NIOSH recommendation. NIOSH considers this ten percent window of change or more to be significant for pre/post shift comparison in valid pulmonary function tests (NIOSH, 2001).

3.7 Exposure Sampling Methods

There are several methods that can be used to take air samples. Air samples for bioaerosol are no exception, "impaction, impingement, filtration, and electric precipitation" are all possible methods (Aizenberg et al.). For this study, the measurement of interest was a personal exposure measurement, not a general area measurement. Filtration was the method of choice for this study, because of it was compatible with using a personal sampling device and the outdoor nature of the study. That is, given the physical activity level involved in the mulching work task, the closed-face filter cassette minimized particle loss that would occur from any other available sampling device.

The method for collecting samples in the workers' breathable space used SKC Universal PCXR Personal Sampling Pumps^{TM} and SKC Aerosol Samplers^{TM}. The samplers contained 25mm Mixed Cellulose Ester filters that were analyzed by means of epifluorescence microscopy and Limulus Amoebocyte Lysate (LAL) Assay. The first analysis, epifluorescence microscopy, measured the amount of fungal spores that were in the breathable space; and the second method, LAL, allowed for the amount of endotoxin to be measured (Aizenberg et al.).

In a recent study performed by the Aerosol Research and Exposure Assessment Laboratory at the University of Cincinnati, Button Aersol Samplers performed well with almost 100 percent collection efficiency a tested particle size range (Aizenberg et al.)