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UNIVERSITY OF CALIFORNIA, SAN DIEGO

How the October 2007 San Diego Fires Affected Asthmatics

in

A thesis submitted in partial satisfaction of the

requirements for the degree Master of Science

in

Biology

by

Chirag Harshad Vora

Committee in charge:

Joe Ramsdell, Co-Chair
Tracy Johnson, Co-Chair
Kathy French

2008

The Thesis of Chirag Harshad Vora is approved and it is acceptable in quality and form for publication on microfilm:

Co-Chair

Co-Chair

University of California, San Diego

2008

This is dedicated to the San Diego Fire Fighters. They are the heroes that risk their lives everyday so that we can enjoy ours.

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LIST OF ABBREVIATIONS

Abbreviation:	**Stands For:**
PM	Particulate Matter
$PM_{2.5}$	Particulate Matter, diameter is 2.5 microns or less in size
PM_{10}	Particulate Matter, diameter is 10 microns or less in size
FEV_1	Forced Expiratory Volume in One Second
PEFR/PEF/Peak Flow	Peak Expiratory Flow Rate
PFT	Pulmonary Function Test
ED	Emergency Department
IgE	Immunoglobulin E
IRB	Institutional Review Board
ACRN	Asthma Clinical Research Network
MIA	Macrolides in Asthma
ROBOT	Run in of Basalt or Talc
eNO	Exhaled Nitric Oxide

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ABSTRACT OF THE THESIS

How the October 2007 San Diego Fires Affected Asthmatics

by

Chirag Harshad Vora

Master of Science in Biology

University of California, San Diego

Professor Joe Ramsdell, Co-Chair
Professor Tracy Johnson, Co-Chair

This study investigates how the October, 2007 wildfires in San Diego produced poor air quality. It then investigates how this poor air quality affects the respiratory health of eight asthmatic subjects in real time.

It was found that the wildfires caused a statistical significant increase in both morning and evening Particulate Matter ($PM_{2.5}$) values ($p<0.0001$). It was then found that during the wildfires, two of two subjects showed increases in eosinophil counts in the

lower airways, indicating increased inflammation during the wildfires compared to before the wildfires. It should be noted that this was the first study to monitor eosinophil counts during a wildfire. For the eight subjects, it was found that morning and night Peak Flow (PEFR or PEF) values and morning and night Forced Expiratory Volume in One Second (FEV_1) values showed no drastic decreases during the wildfires compared to before and after the wildfires. Finally, it was found that there was a significant increase in rescue medication usage during the wildfires compared to before ($p = 0.04$).

This paper suggests that the wildfires produced poor air quality that resulted in an increase in inflammation in the asthmatic subjects. Yet, the Pulmonary Function Tests (Peak Flow and FEV_1) indicated no significant differences as the effects caused by the inflammation were masked by increased rescue medication usage.

INTRODUCTION

The aim of this study is to investigate how the wildfires in San Diego during the month of October, 2007 affected a cohort of asthmatics involved as subjects in research in real time. Wildfires have been known to produce poor air quality in the environment. This paper studies how the wildfires may have caused poor air quality by monitoring environmental $PM_{2.5}$ concentrations (Particulate Matter of sizes 2.5 microns in diameter). It then investigates how this poor air quality may have affected specific physiological responses in the asthmatic subjects by monitoring a particular biomarker that is correlated to respiratory health. Finally, this paper identifies the amount of subjects' use of a specific rescue medication.

During the month of October, 2007, Southern California was adversely affected by wildfires. Governor Arnold Schwarzenegger declared a state of emergency for seven Southern California counties.[1] The most catastrophic wildfires were located in San Diego County, including the two largest and most destructive wildfires: the Witch Creek Fire and the Harris Fire, both of which began on October 21, 2007.[2] The San Diego fires accounted for over 1,500 destroyed homes, over a billion dollars of damage, at least five deaths, and many more injuries.[3] Furthermore, the wildfires were thought to be responsible for producing poor air quality that may have compromised the general health of civilians in the area. For example, Chancellor Marye Anne Fox of the University of California, San Diego sent letters to all students to inform them that school would be closed during the week of the wildfires because "the air quality continues to threaten the health of all in this general area".[4]

Four years earlier, in October of 2003, wildfires had devastated San Diego County. These fires, including the Cedar Fire, consumed an area of over 390,000 acres,

destroyed 5597 buildings, and directly caused sixteen deaths. During these fires, the air quality was monitored; it was found that the Cedar Fire released about 300,150 tons of Particle Matter (PM) and other pollutants into the environment. These types of pollutants are known to cause serious health problems in the human respiratory system.[5] Furthermore, it was found that this poor air quality correlated to increased adult Emergency Department (ED) visits for selected respiratory-related conditions and asthma related medical visits and inquiries.[6] The 2003 wildfire smoke also led to increased acute exacerbations of the respiratory and eye symptoms and increased demand for health services for both asthmatic and non-asthmatic children.[7,8]

In particular, during the 2003 fires the concentrations of small particles known as Particulate Matter 2.5 ($PM_{2.5}$) directly correlated with the significant increase in ED visits for asthma, other respiratory illnesses with no fever, eye irritation, and smoke inhalation.[6] $PM_{2.5}$ values refer to Particulate Matter that is smaller than 2.5 micrometers in diameter. Increases in Particulate Matter (including $PM_{2.5}$) values are associated with illnesses including short-term effects on mortality,[9] increased plasma viscosity,[10] increased risks of heart rate,[11] electrocardiographic changes in human subjects,[12,13,14] triggering of myocardial infarction,[15] and increased concentrations of plasma fibrinogen.[16]

It is also well documented that increased $PM_{2.5}$values are associated with respiratory illnesses in subjects who suffer from asthma. Asthma is a prevalent chronic lung disease with marked impact on both individuals and society; people of all ages, from childhood until late adulthood, suffer from asthma.[17,18,19,20] Significant associations were found between ED visits for asthma in children and fine PM values in the environment.[21] Similar results of increased asthma admissions were found for both children, adults, and

the elderly.[22] Increased small PM values have also been associated with increased bronchitic symptoms in children with asthma, shown by a study that took place in Southern California.[23] Overall, it is understood that the concentration of small Particulate Matter is positively correlated with respiratory irritation and illness in human asthmatic subjects.

One reason that subjects may suffer from increased Particulate Matter exposure is because these small particles may trigger an asthmatic episode. During an asthmatic episode, subjects' airways become inflamed and smooth muscles in the airways become constricted, causing a decrease in area for which air can travel. Because of this, subjects suffering from asthma show symptoms of wheezing, chest tightness, bronchial hyper-responsiveness, and labored breathing.[24] An asthmatic episode can be triggered by a variety of factors, including exercise, emotional stress, allergens, respiratory infections, and/or environmental irritants such as $PM_{2.5}$.[25] Increased concentrations of $PM_{2.5}$ and other small irritants in the environment may travel into subjects' lower airways, causing inflammation and smooth muscle restriction. It is thought that the PM trigger may bind to IgE (immunoglobulin E) receptors of mast cells, resulting in a complex sequence of biochemical events that cause cellular activation, arachidonic acid metabolism, and mediator release, inclusive of eosinophil chemotactic factors.[26]

A direct way to measure the severity of an asthmatic episode is to monitor the amount of airway inflammation. Many studies have shown that eosinophil granulocytes (eosinophil) from induced sputum that is derived from subjects' airways represent a good biological marker for determining the severity of the asthmatic episode and thus the overall respiratory health. Eosinophils are white blood cells of the immune system that

are both mobile and phagocytic; they have surface receptors for IgE and are involved in phagocytosis of immune complexes.[27] It has been found that the distribution of eosinophils in induced sputum from children can be used to directly monitor airway inflammation.[28] The same results have been found with adult subjects.[29] From Veen et. al, it was concluded that the distribution of eosinophil values from induced sputum samples that excluded samples with more than eighty percent squamous cells represented a reliable measure for determining airway inflammation. [30] Further, eosinophil values from sputum samples have been used in many studies to determine the amount of inflammation in subjects' airways. For example, in a previous study, it was shown that for a 1 ug/m3 increase in coarse PM, there was a .16% increase in circulating eosinophil values, indicating increased airway inflammation.[31]

Although using the distribution of eosinophil values from induced sputum is a direct and reliable way to measure a subjects' asthma, this process has a drawback: the subjects must be available in the laboratory for sputum inductions. The sputum induction itself is a simple, non-invasive procedure that usually takes less than thirty minutes to perform. However, subjects must come into the laboratory where the sputum induction apparatus is located. During real time emergencies, such as wildfires, asking subjects to come into the laboratory for sputum inductions is both unreasonable and irresponsible as there are many inherent risks that come from travelling times of natural disasters. Because of this, other measurements must be used during real time disasters to measure the respiratory health of subjects.

A direct way to measure the breathing capacity and overall respiratory health, during a real time natural disaster, is by having the subjects breathe through a take-home

peak flow meter that determines the subjects' peak expiratory flow rate (PEFR or PEF) and Forced Expiratory Volume in One Second (FEV_1) values. Both the PEFR and FEV_1 values are measures that are used to indirectly determine the subjects' lung function at any given time; for both measures, a higher value usually indicates improved breathing while a lower value usually indicates a difficulty in breathing, possibly caused by constriction of the airways. The inherent advantage in using a take home peak flow meter is that it allows researchers to determine how a subject is breathing without having to bring the subject into the laboratory. This is an especially useful tool during real time disasters, such as the wildfires. The disadvantage to using these peak flow monitors over the sputum induction apparatus is that the PEFR and FEV_1 measures are only indirect measures of airway constriction. This is because these values are dependent on both the subjects' effort and technique, vary due to external factors including time of day and/or illnesses, and are not overseen by a researcher as the subjects use the peak flow monitors at their own homes. Yet, it is common that large decreases in both PEFR and FEV_1 values indicate an asthmatic episode.

When a subject is suffering from an asthmatic episode, he or she may be instructed by a physician to use pharmacological therapy to ameliorate the exacerbation. There are many types of medications available to asthmatics, depending on their individual cases and on what their physicians recommend. Medications include, but are not limited to, anti-inflammatory medications and ß-adrenergic agonists. In a previous study that took place in Southern California, it was shown that increased PM_{10} values affected asthmatics on anti-inflammatory medications to a lesser extent than those not on

it. This shows that anti-inflammatory medications helped ameliorate respiratory health in asthmatics, but not completely.[32]

To our knowledge, few studies look at the direct consequence of the use of albuterol (salbutamol) on asthmatic health during real time wildfires. Albuterol is a specific type of ß-adrenergic agonist; in particular, it is a $ß_2$-adrenergic agonist bronchodilator with an onset of action time of five to fifteen minutes and a duration of four to six hours.[26] It is known to have three biological reactions:

1) Actuation of cell membrane's adenylcyclase enzyme, which converts adenosine triphosphate to cyclic adenosine monophophate with its inherent ability to relax smooth muscle. More specifically, cAMP stimulates protein kinases, which increases the formation of MLCK-P (inactive), causing a reduction in enzyme affinity for calmodulin-Ca^{+2} complex, which leads to a decrease formation of active MLCK. When cAMP stimulates protein kinases, it also leads to an incease in efflux of Ca^{+2} from the cell, an increase in binding of Ca^{+2} to intracellular sites, and decrease in Ca^{+2} entry into the cell; all three mechanisms lead to a decrease of free intracellular Ca^{+2}, which is responsible for a reduced amount concentration of calmodulin-Ca^{+2} complex formation, accounting for an additional decrease in active MLCK formation. The reduction in concentration of active MLCK results in a decrease in concentration of myosin-P, which leads to a decrease in actin-myosin coupling. All of this results in the relaxation of the smooth muscle that constricts during an asthmatic episode (Figure 19).

2) Increased ciliary movement. This should be responsible for improved secretion clearance.

3) Modifies mast cells to decrease mediator release (i.e. eosinophil).

Because of these three important biological roles, albuterol should have profound impact on asthmatic respiratory health. [26,33]

The purpose of this study is to investigate how the wildfires in San Diego affected the respiratory health of asthmatic individuals through a biological, basic science perspective. Specifically, this study aims to determine how the increased environmental $PM_{2.5}$ values may have affected subjects' airways by monitoring the environmental $PM_{2.5}$ values, by measuring subjects' PEFR, FEV_1, and eosinophil values, and by evaluating subjects' use of their rescue albuterol medication during the wildfires. It should be noted that this is the first study, to our knowledge, that investigates eosinophil values of asthmatics during a real-time wildfire. Many previous studies have shown that increased $PM_{2.5}$ values are associated with negative health, including respiratory illnesses.[5-16, 21,22,23,31] In particular, one study shows that increased PM values were associated with reductions in PEF (PEFR).[34] However, there is evidence that during a Sydney bushfire in 1994 that resulted in large increased environmental PM values, asthmatic human subjects showed no significant differences in PEFR values.[35] This data seems unexpected because of how drastically different it is from previous studies. This is the first paper, to our knowledge, that attempts to bridge the gap between this seemingly contradiction with the use of human asthmatic subjects during the real time, unannounced (and thus unprepared for), catastrophic wildfires in San Diego in October, 2007.

METHODS

Subjects: Eight asthmatic human subjects from concurrent Asthma Clinical Research Network (ACRN) studies at the UCSD Clinical Trials Center participated in this study. Only subjects who showed compliant activity of their peak flow monitor during the period of the October, 2007 wildfires in San Diego were chosen to participate in this study. For all subjects, a complete health history and physical examination were completed, and informed consent was obtained according to UCSD Institutional Review Board (IRB) requirements. Two of the subjects were enrolled in the ACRN Macrolides in Asthma (MIA) study and six were enrolled in the ACRN Run in of BASALT or TALC (ROBOT) study. Prior to this study, all subjects were assessed as asthmatics from inclusive criteria provided by the ACRN. It should be noted that the two MIA subjects were mild asthmatics on placebo medications and the six ROBOT subjects were more severe asthmatics on a low dose of anti-inflammatory medication. The physical characteristics of the subjects are presented in Table 1.

Monitoring of Air Quality: The air quality was measured by the County of San Diego - Air Pollution Control District. Specifically, the $PM_{2.5}$ values were collected at the Downtown San Diego station. This station was used because it is the closest station to the UCSD Clinical Trials Center (located in Hillcrest) and because all subjects reside within twenty-five miles of it. The $PM_{2.5}$ values for before the wildfires, during the wildfires, and after the wildfires were retrospectively collected through the County of San Diego - Air Pollution Control District's website at http://www.sdapcd.org/air/air_quality.html as all data is archived. $PM_{2.5}$ values were collected for each day at 7:00AM and 10:00PM as these were the times at which the subjects were instructed to use their peak flow monitors.

Eosinophil Values: Eosinophil values were collected for two subjects. Only two subjects were included because they were the only subjects to have 1) a previous, consented for, non-wildfire data point while being only on placebo medications and 2) were able to come into the UCSD Clinical Trials Center on the Monday after the wildfires were contained. The second point is important because inflammation from an asthmatic episode is reversed relatively quickly. Thus, eosinophil values from subjects after a week or longer after the wildfires were contained may not accurately reflect changes caused by the wildfires. Eosinophil values were not obtained for subjects during the period of the wildfires because all subjects were asked not to come in during the dangerous conditions.

Eosinophil values were gathered from the two subjects using the ACRN MIA and ACRN ROBOT protocols. The protocol included three steps: 1) sputum induction; 2) slide processing of the sputum; 3) reading of the slides. First, a sputum induction was performed on each subject two times, once sometime before the wildfires and once on the Monday after. According to the ACRN, "Sputum Induction is a relatively simple, repeatable and noninvasive method of collecting airway secretions" (ACRN MIA Appendix III). It is an in-lab procedure in which subjects breathe a hypertonic saline solution from a sputum induction apparatus. This causes sputum to move from the subjects' respiratory system (hypotonic solution) toward the hypertonic saline solution by osmosis. The subjects were instructed to gargle out sputum that they perceived to be from their throats and to collect sputum that they perceived to be from their lower airways. It was later verified that the sputum did come from the lower respiratory system and not from the throat by observing the squamous cell count. Because sputum

inductions with a squamous count of over eighty percent are thought to be collected from the upper respiratory tract and not from the lower, samples with over eighty percent squamous cells were excluded from the study.

Once the sputum was collected, it was processed and read at the bench. A replica of the ACRN protocol that was used follows:

Processing Induced Sputum

A. Induced Sputum Processing

1. Determine the weight of the sputum sample collected. Tare the balance with an empty 50 mL conical polypropylene tube (or a 15 ml tube if a small volume is collected), transfer the collected sputum sample into this tube and record its weight.

2. To the sputum sample, add a volume (mL) of 10% Sputolysin** equal to the weight (gms) of the sputum sample. (SS: Sputum + 10% Sputolysin)
**10% Sputolysin (1 part Sputolysin and 9 parts normal saline, Caldon Biotech Inc.). This solution should be discarded after 48 hours.

3. Mix the sputum and 10% Sputolysin sample (SS) with a serological pipette. To ensure complete homogenization, aspirate and dispense the sample through the serological pipette several times. Do not vortex the sample.

4. Place the SS sample in a 37^0 C shaking water bath for 15 minutes.

5. Set Shaker at 160 shakes/min.

6. At 5, 10, and 15 minute intervals briefly remove the SS sample from the shaking water bath, mix the sample well with the serological pipette (aspirate and dispense the sample several times) and return it to the shaking water bath.

B. Cell Count

1. Aliquot 0.5 to 1 ml (or more depending on the sample volume) cell count/cell differential designated SS sample and use this to do cell count and to make slides for differential count.

2. Mix 100 microliters of the SS sample with 100 microliters of Turks* Solution. (SST sample: SS sample + Turks solution)
*Turks Solution:

10 mg Crystal Violet
3 mL Glacial Acetic Acid
Bring total volume up to 100 mL with distilled water

3. Dispense 10 microliters of the SST sample into the well on one side of the cover slipped hemocytometer.

4. Count the number of cells bordered within the 4 large corner squares of the hemocytometer.

Calculate the total cell count per ml:

Total cell count per ml = (total # of cells in 4 large squares / 4) x 10,000 x 2* x 2**
*Dilution with 10% Sputolysin
**Dilution with Turks solution

C. Slide Preparation for Cell Differential Count

1. Divide the total cell count per mL by 160,000 cells per mL to obtain an approximate dilution factor to prepare cell differential slides.

2. Dilute the cell count/cell differentiated designated SS aliquot (not the whole SS sample but the aliquot designated for cell count as step 1) according to the dilution factor obtained from Step 1 with normal saline if needed. Although a minimum volume of 1 mL is required for 4 slides, prepare at least 1.5 mL aliquot.

3. Assemble cytofunnels, filter papers, slides, and slide clips for 4 slides.

4. Place 250 microliters of the sample obtained from step 2 in each cytofunnel.

5. Centrifuge at 500 rpm for 5 minutes.

6. Check the cytospun slides to make sure that they are not too crowded or too sparse. If they are too crowded, further dilute the cell count/cell differentiated designated SS aliquot and make new slides. If they are too sparse, one may concentrate, re suspend and prepare another set of slides.

7. Stain slides with KWIK-DIFF staining kit (Shandon) or any other quick staining kits following the manufacturer's guidelines. Check the staining quality of the slides and re strain as necessary.

8. When the slides are dry, mount the slides with the cover slips.

9. Count at least 500 non-squamous cells. A sputum sample is considered unacceptable if it is comprised of more than or equal to 80% squamous cells.

Peak Flow and FEV_1 Values: All eight subjects were provided with a take-home electronic peak flow meter and used it consistently before, during, and after the wildfires. The subjects were given the Jaeger the Asthma Monitor AM1 peak flow meters. These meters electronically measure and save PEF and FEV_1 measures. The Jaeger the Monitor AM1 consistently monitored both peak flow and FEV_1 values, both measures that help identify how asthmatics pulmonary functioning (refer to Introduction). Subjects were instructed to use the monitor two times each day: once in morning and once at night. Only subjects that were compliant were included in the study. These peak flow meters provided us with an easy, non-invasive way to measure subjects' pulmonary functions during the real-time periods of the wildfires. Although all values were electronically stored on the Jaeger the Monitor AM1 peak flow meters and were later easily retrieved, the subjects were also instructed to record the values in a diary.

Intake of Pharmaceutical Medications: For the periods of before, during, and after the wildfires, subjects were instructed to record which pharmaceutical medications they used and how many times they used them in a diary. The subjects were provided with and instructed to use albuterol as their rescue medication. After the study, subjects were instructed to turn in the diaries and we analyzed the number of puffs of albuterol each subject used.

Statistical Analysis: Wilcoxon signed-rank test analyses, a non-parametric alternative to the paired t-test, were computed using a commercially available software

package (SYSTAT Software). Variables were considered significantly different when the p-value was 0.05 or less.

RESULTS

Air Quality: Environmental $PM_{2.5}$ values were highest during the wildfires as compared to before and after the wildfires. This is true for $PM_{2.5}$ values evaluated at 7:00AM (Figure 1) and at 10:00PM (Figure 2). Specifically, there was a statistical significant increase in $PM_{2.5}$ values during the wildfires compared to before the wildfires for both times of the day (Table 3, $p<0.0001$ for both times).

Inflammation in Lower Airways: For the two subjects monitored, there was a drastic increase in eosinophil values (Table 4). There was an average of 315 percent increase in cell count percentage for eosinophils during the wildfires compared to before. This increase suggests that there was increased inflammation in the lower respiratory system of subjects during the wildfires as compared to before.

Morning Pulmonary Function Tests: There were no significant changes in morning Pulmonary Function Tests during the wildfires compared to before and after. Morning Peak Flow values remained consistent for before, during, and after the fires (Figure 3, $R^2 = 0.027$). This is also shown by observing individual subjects' morning Peak Flow values (Figure 4). There was also no significant change in morning Peak Flow values during the wildfires as compared to before (Table 3, $p = 0.40$). Further, there was no correlation found between morning $PM_{2.5}$ values and morning Peak Flow values (Figure 11, $R^2 = .007$).

Morning FEV_1 values were consistent with the morning Peak Flow trends. Morning FEV_1 values remained consistent for before, during, and after the wildfires (Figure 7, $R^2 = 5 \times 10^{-7}$). This is also shown by individual subjects' morning FEV_1 values (Figure 8). There was also no significant change in morning FEV_1 during the

wildfires compared to before (Table 3, $p = 0.35$). Additionally, there was no correlation between morning $PM_{2.5}$ values and morning FEV_1 values (Figure 13, $R^2 = 0.0262$).

Night Pulmonary Function Tests: There were no drastic decreases in night Pulmonary Function Tests during the wildfires compared to before and after. There was a small increase in night Peak Flow values during and after the wildfires compared to before (Figure 5, $R^2 = 0.1894$). This is somewhat shown as a trend between individual subjects (Figure 6). However, the increase in night Peak Flow values was not significant for during the wildfires compared to before the wildfires (Table 3, $p = 0.50$). Further, there was a positive correlation between night $PM_{2.5}$ values and night Peak Flow values (Figure 12, $R^2 = 0.2284$). It should be noted that this correlation indicates that during higher $PM_{2.5}$ values (presumably worst air quality), subjects showed increased Peak Flow values.

Night FEV_1 values were consistent for before, during, and after the wildfires (Figure 9, $R^2 = 0.0003$). This is also shown by individual subjects' night FEV_1 values (Figure 10). There was no significant change in night FEV_1 values for during the wildfires compared to before (Table 3, $p = 0.74$). Additionally, there was no correlation between night $PM_{2.5}$ values and night FEV_1 values (Figure 14, $R^2 = 0.0577$).

Daily Albuterol Usage: There was a correlation between the averaged daily $PM_{2.5}$ values and averaged daily albuterol (Rescue Medication) usage (Figure 15, $R^2 = 0.251$). There was a statistically significant increase in daily albuterol usage during the wildfires compared to before (Table 3, $p = 0.04$). This increase can be seen by individual subjects' usage of albuterol (Figure 16). However, the subject's reported usage declined after the wildfires as there was no significant difference between usage after the wildfires

and before (Table 3, $p = 0.14$). Finally, there was only a small correlation between night Peak Flow values and albuterol usage (Figure 12, $R^2 = 0.1088$).

DISCUSSION

This study suggests that the increases in environmental $PM_{2.5}$ values caused by the October, 2007 San Diego wildfires may have led to an increase in inflammation in asthmatics, although PEFR (Peak Flow) and FEV_1 measures in real time remained masked by drastic increases in albuterol usage by the human subjects.

The air quality was monitored for $PM_{2.5}$ values for one month in Downtown San Diego. During this month, $PM_{2.5}$ was monitored immediately before the wildfires, during the wildfires, and immediately after the wildfires. It was found that the $PM_{2.5}$ values were significantly higher during the wildfires compared to both before and after, suggesting that the wildfires caused the increase. It is probable that the smoke emitted by the wildfires was responsible for the increase in $PM_{2.5}$ values. It is important to note that although all subjects resided within a 25 mile radius of the Downtown monitoring station, some lived closer to the station while some lived further (Appendix, Table 1). This is important because not all areas experienced the same increases in $PM_{2.5}$ values. Thus, some subjects experiences more drastic environmental changes than others. Along the same logic, some subjects were outdoors, in the poor environment, for longer periods of time than others. Thus, these subjects may have exposed their airways to a higher concentration of Particulate Matter than others. However, since all asthmatic subjects were exposed to wildfires in real time, the data they presented represents important data in understanding how respiratory physiology changes with real time environmental changes.

The wildfires did have a negative effect on the respiratory health of the asthmatics. From interviews with the subjects, it was found that the subjects did complain about asthmatic symptoms, including perceived tightness of the chest and

difficulty of breathing. By investigating the eosinophil values of two subjects, it was found that there was an increase in inflammation (Appendix, Table 4). Eosinophil values represent a sound biological marker for indicating the severity of an asthmatic episode because of the basic sciences involved. When mast cells in airway tissues are triggered, they release chemicals, including eosinophil chemotactic factors. These factors attract eosinophils to the airways in an inflammatory response. During this response, there is a large increase in eosinophils in the airways. Thus, a large increase in the eosinophil count from induced sputum signifies inflammation in the airways, which may be caused by an asthmatic response.

Although there was a substantial increase in eosinophils in the induced sputum, this data can at most be seen as suggestive as there was sample size of only two subjects. The reason only two subjects were used is because the eosinophil values needed to be determined during or soon after the wildfires. If eosinophil values were determined a few days or more post wildfires, the changes in inflammation would not be caused by the wildfires as a few days may enough for the body's compensation and repair mechanisms to reverse the effects of the wildfires. Thus, only eosinophil values from during the wildfire or less then a week after the wildfires could be used in determining the wildfire's actual effect on the airways. However, all subjects were asked not to come into the lab (where the sputum induction apparatus is located) during the wildfires because of the inherent risks involved. After the wildfires were contained, only two subjects were able to come in the lab for the sputum induction. Although this data is satisfactory for a case series, it is important for future studies to monitor inflammation, either through

eosinophil values or other measures such as exhaled Nitric Oxide (eNO) levels, of more subjects.

It should be noted that the baseline eosinophil values for the two subjects differed by ten fold (Table 4). Subject 16-09-102 had a low baseline eosinophil value of 0.20 percent. This baseline value was taken on 02/02/2007 when the subject was in the run-in period of the ACRN MIA study (thus, subject was on only placebo medications). As this baseline is much lower than the eosinophil values taken during the fires (10/29/2007 – eosinophil count of 7.8 percent), another eosinophil count from a different, non-wildfire period was used to confirm that the subject did indeed have a low baseline eosinophil count. On 09/20/2007, while the subject was still involved in the ACRN MIA study, the subject showed an eosinophil count of 0.0 percent, verifying that the subject regularly shows a low eosinophil count in the lower airways. Although both subjects showed increases in eosinophil values during the wildfires as compared to before the wildfires, the baseline values may account for the magnitude of the differences. In future studies, it would be important to monitor eosinophil values of subjects with similar baseline values to better assess how the wildfires altered inflammation in asthmatics.

The subjects in this study did not show substantial real time changes in indirect breathing measures, specifically the PEFR and FEV_1 measures. This data may seem counterintuitive as inflammation in the airways results in decreased area for air to move, making it less likely for subjects to maintain consistent PEFR and FEV_1 measures. Furthermore, increased incidence of respiratory illnesses in asthmatics during wildfires should indicate that asthmatics suffer from decreased breathing capabilities, which are shown by reduced PEFR and FEV_1 measure. Yet, the data of no changes in PEFR during

the wildfires compared to before and after the wildfires has been shown in previous research.[35]

Surprisingly, night PEFR values actually showed a positive correlation to night $PM_{2.5}$ values (Appendix, Figure 12). Additionally, night PEFR values were higher during the times of the wildfire compared to before the wildfires (Appendix, Figure 5 and 6). This data is surprising because subjects showed improvements in breathing during times of poorer environmental air quality. There are a few reasons why subjects showed improved breathing during poorer air quality. First, there are additional environmental factors other than $PM_{2.5}$ that contribute to the subjects' respiratory health. Secondly, subjects were measuring their night PEFR after the using of their asthma medications throughout the day. There was a small correlation between albuterol usage and night PEFR values (Figure 17, $R^2 = 0.1088$). The reasons this R^2 value was small may be attributed to the small sample size and the variability of albuterol usage between subjects. Furthermore, during the night time, subjects may have been outside to a lesser extent (e.g. the general population travels to work during the day but remains indoors at night). Thus, since subjects may not have been as exposed to the poor air quality, it is possible that they showed better breathing values even though environmental air quality was at its worst.

I believe that real time PEFR and FEV_1 measures did not decrease during the wildfires because of significant increases in albuterol usage. This study shows that the subjects showed a significant increase in rescue medication (albuterol) usage during the wildfire compared to before the wildfire and after the wildfire (Table 3, $p = 0.04$). Albuterol affects the bronchospasm element of an asthmatic episode by relaxing the

smooth muscle that surrounds the airways. As described in the introduction, this pharmaceutical works by stimulating protein kinases in a cAMP-dependent process that decrease actin-myposin coupling. In this process, albuterol has two major effects: first, it stimulates the removal of phosphate ions from myosin, which decreases myosin ATPase activity. This decreases myosin-actin coupling and the overall contraction of the smooth muscle (Figure 18). Also, albuterol assists in unbinding Calmodulin from Calcium ions. This change in free Calcium ions in the cytoplasm causes a decrease of Calcium ions into the cell, increases Calcium ion efflux from the cell to out of the cell, and increases Calcium ion diffusion back into the sacroplasmic reticulum; the decrease of overall Calcium is also involved in the relaxation of the smooth muscle (Figure 19).

Certainly, albuterol is involved in the relaxation of the smooth muscle in the airways. When human subjects take a significant amount of the albuterol, there may be more relaxation than in normal, non-wildfire conditions. In normal settings, a proportion of the smooth muscle may be slightly contracted by calcium-dependent pathway; however, with excess albuterol usage, this proportion may be overcompensated for. Thus, there may be enough relaxation of the smooth muscles that cancels the effects of the decreased airway area caused by inflammation, resulting in no net change - thus causing no observable PEFR or FEV_1 changes. Therefore, the data in this paper suggests that the excessive usage of albuterol during the wildfires was enough to mask the changes in the PEFR and FEV_1.

Because no control group was used in this study, the results are only suggestive that increases in environmental $PM_{2.5}$ concentrations caused pulmonary health problems and that an increased usage of albuterol was enough to mask the differences in PEFR and

FEV_1. In a future study, it would be important to use a control group of asthmatics situated in non-wildfires areas (with lower environmental $PM_{2.5}$ concentrations) that individually have similar baseline breathing values and eosinophil values as the subjects used in this study. This control group should be divided into two groups: control group A would consist of asthmatics that would not use albuterol or any other rescue medication while control group B would consist of asthmatics that would use similar amounts of albuterol as the subjects of this study. Because it is hypothesized that the increase in $PM_{2.5}$ values (caused by the wildfires) was responsible for decrease pulmonary functioning and that an increase usage of albuterol resulted in masking differences in pulmonary function values, it would be expected that control group A would show similar pulmonary function test values as subjects in this study (as control group has no pollutant irritants that may cause an asthmatic episode), but would show decreased eosinophil values compared to subjects as little inflammation is expected in humans in areas lacking wildfires (as long as other factors are held constant). On the other hand, it would be expected that control group B would show decreased eosinophil values compared to subjects and would show modest increases in breathing values because of increased albuterol usage. Any deviation from these expectations may suggest that another mechanism may be at play.

This case series shows that subjects most likely suffered from pulmonary irritation and not pulmonary obstruction. Poor air quality can cause pulmonary irritation, which is when there is increased inflammation and decreased Pulmonary Function Test values that can be immediately reversed by proper pharmaceutical treatment. From the results, it was found that there was an increase in eosinophil values (indicating increase

inflammation) and no drastic drops in Pulmonary Function Test values after albuterol usage. On the other hand, pulmonary obstruction refers to a significant increase in inflammation and swelling on the smooth muscles surrounding the airways, causing a decrease in Pulmonary Function Test values that cannot be immediately reversed by pharmaceutical treatment. This was not the case as albuterol immediately reversed effects caused by the poor air quality.

The largest drawback to this study is that there is little statistical power in my evidence because the study only includes a sample size of eight subjects. This study is a retrospective study that looks at how an unpredicted wildfire affects asthmatics. Because this study is a study in nature and was unpredicted, a large sample group was not recruited. Instead, only subjects that were participating in other studies with the proper take home equipment were used. Thus, although this study is important in its principle, future studies with a larger sample size need to be performed.

When considering the results of this study, it is important to note that other asthmatic episode triggers, other than $PM_{2.5}$ values, need to be considered. One of these triggers includes allergies in wildfires. During a wildfire, it is possible that the burning of specific fuels may cause an environmental increase in various allergens that are known to cause asthmatic episodes.[36] The biological mechanism of how allergens cause asthmatic episodes is thought to be similar to how I have identified how the $PM_{2.5}$ causes them: allergens react with mast cells which causes an increase in eosinophil chemotactic factors in the lower airways. In future studies, it is recommended that environmental allergen levels be monitored, if possible, and divide subjects into groups of those who are atopic

for specific allergens and those who are non-atopic. This would help identify the role of allergy-mediated asthmatic episodes during wildfires.

Another factor that must be considered during this study is stress. During the San Diego wildfires, many community members lost their homes, were separated temporarily from their families, were forced to live in unfamiliar shelters, and were dealing with respiratory and other illnesses. It would be unlikely for people that faced these types of real-life situations to not worry or stress. Stress is known to increase hormonal levels; for example, the level of stress in a human subject is correlated to cortisol levels in saliva and in the bloodstream. Stress and the elevation of the related hormones are known to cause asthmatic symptoms.[25] In future studies, researchers could monitor cortisol levels of subjects from before the wildfire until after the wildfire. They could do this by simply performing an ELISA assay on the saliva of the subjects to indirectly monitor subjects real-time stress levels. This would help identify the role of stress in asthmatic episodes caused by wildfires.

As this study identifies how real-time $PM_{2.5}$ may be involved in triggering asthmatic episodes, there are real implications that follow. First, it would be instrumental for physicians to consider prescribing β2-agonist pharmaceutical treatment to asthmatics in wildfire-prone areas, such as San Diego, who do not already have the treatment already available. This is because if a wildfire were to begin, the asthmatic would have proper treatment available without having to seek medical attention at a hospital. Also, it would be recommended that asthmatics not expose themselves to environments where there may be high amount of small particles floating around that may travel into their lower

airways. These settings would include social gatherings where there is a high amount of cigarette smoke, industrial areas, and hookah bars, along other areas.

Ultimately, it is important for researchers and the general public to understand the basic biology behind asthma so that we can better the general health asthmatics.

APPENDIX

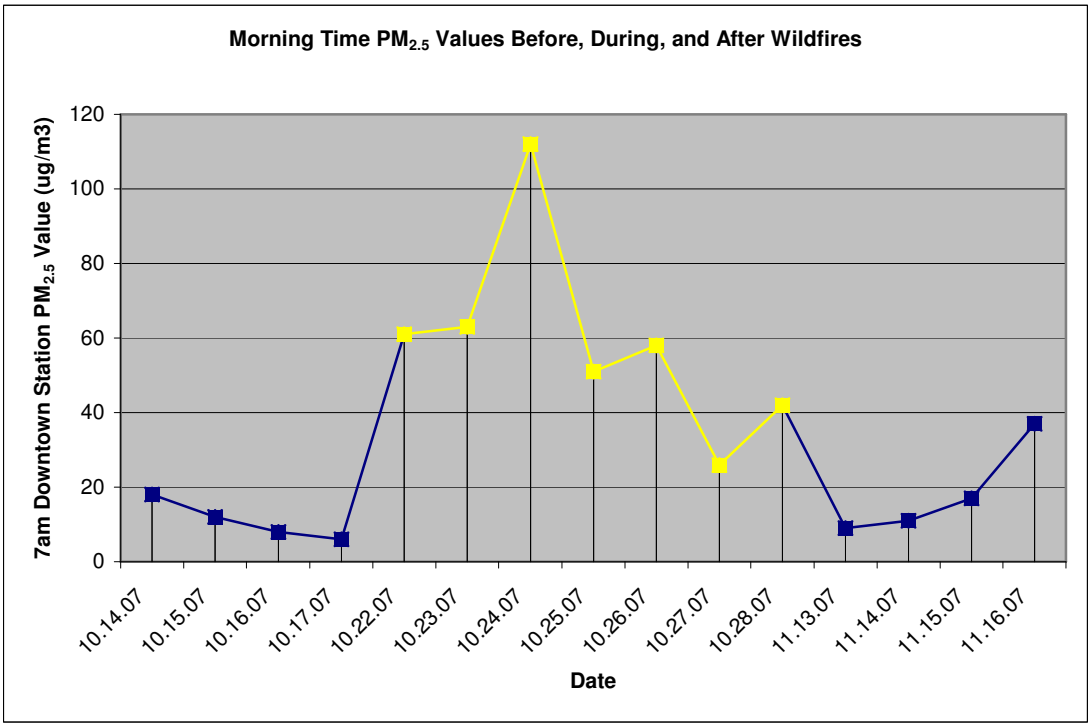

Figure 1: Day Time Values of $PM_{2.5}$ Before, During, and After the Wildfires

Note: ug/m3 stands for Micrograms / Cubic Meter of Air
Yellow Line indicates values during wildfire

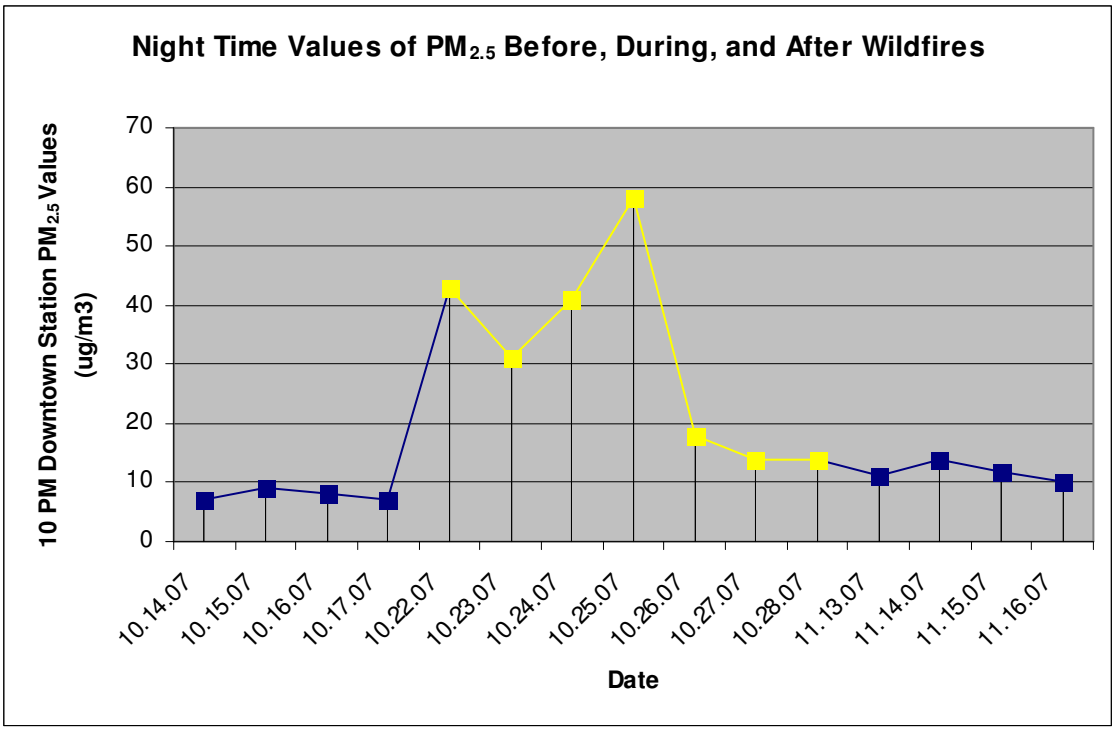

Figure 2: Night Time Values of $PM_{2.5}$ Before, During, and After the Wildfires

Note: ug/m3 stands for Micrograms / Cubic Meter of Air
Yellow Line indicates values during wildfire

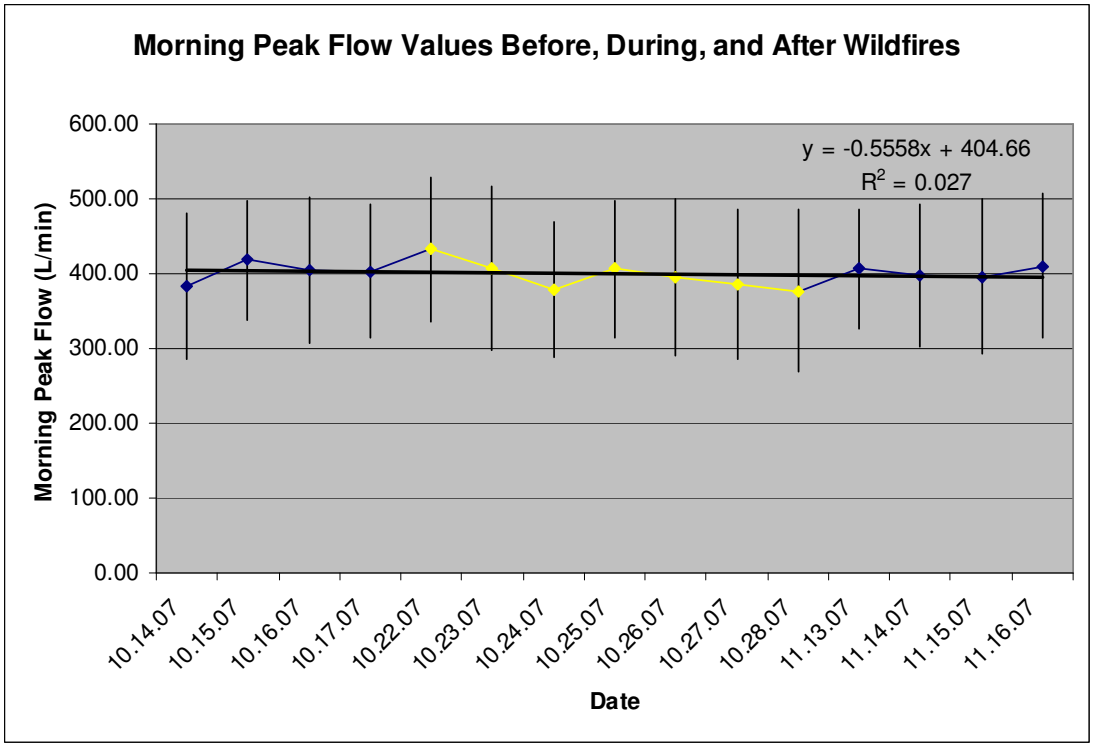

Figure 3: Morning Peak Flow Values (Average) for Before, During, and After the Wildfires

Note: Yellow Line indicates values during wildfire
Error bars represent one standard deviation above and below the average mean.

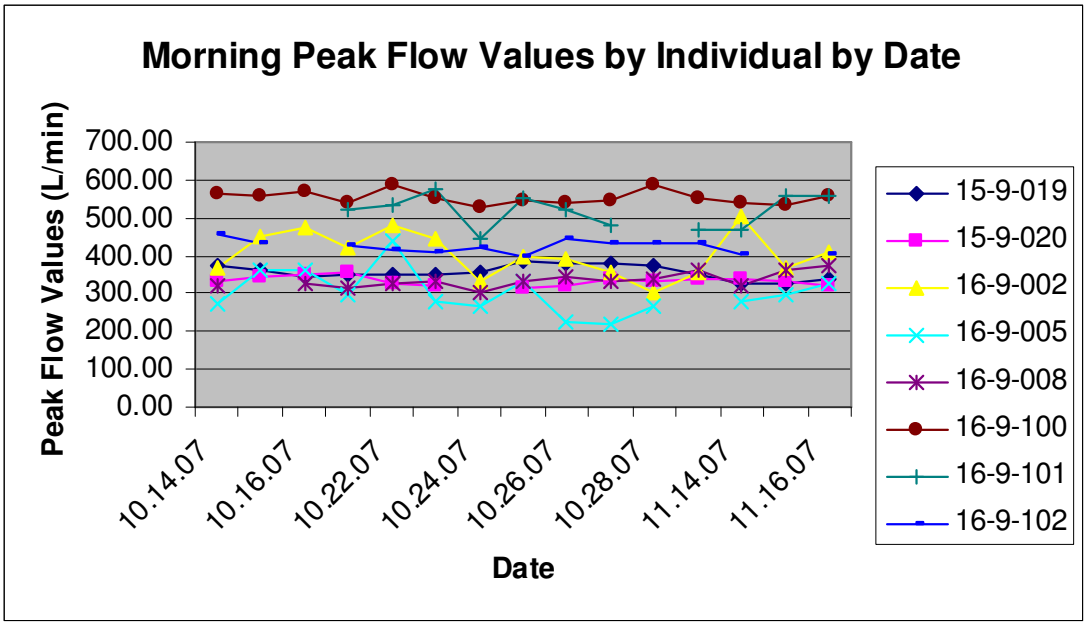

Figure 4: Morning Peak Flow Values Shown for Each Subject for Before, During, and After the Wildfires

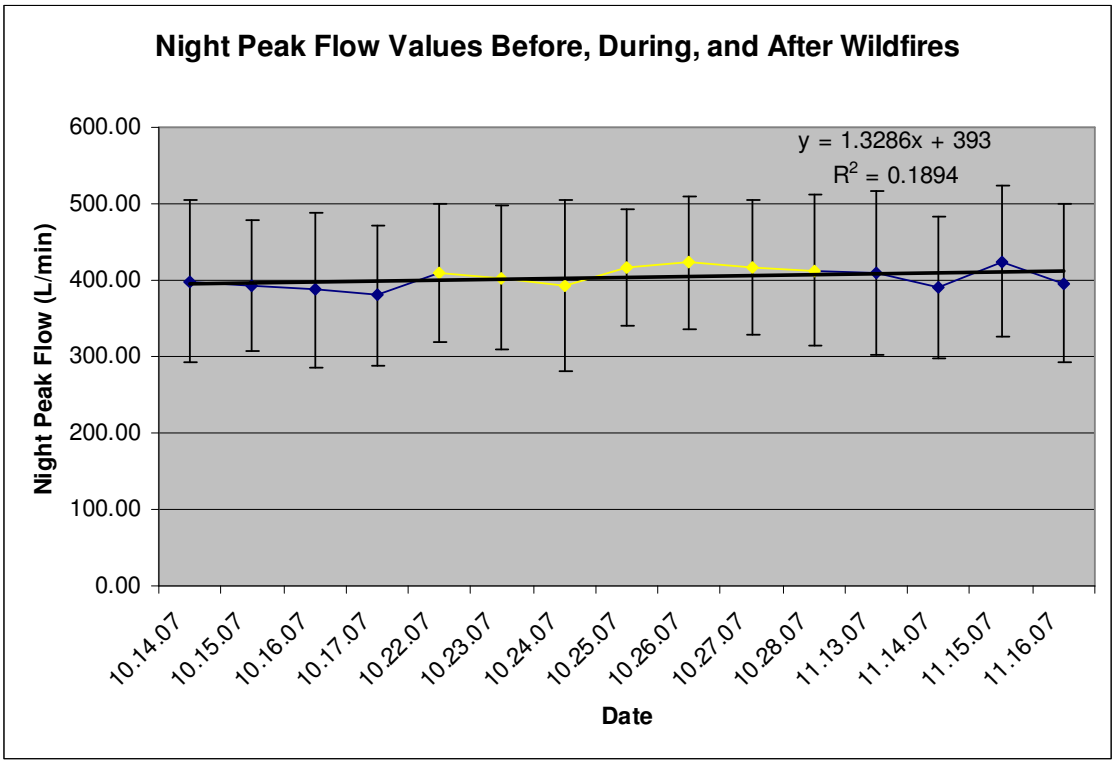

Figure 5: Night Peak Flow Values for Before, During, and After the Wildfires

Note: Yellow Line indicates values during wildfire
Error bars represent one standard deviation above and below the average mean.

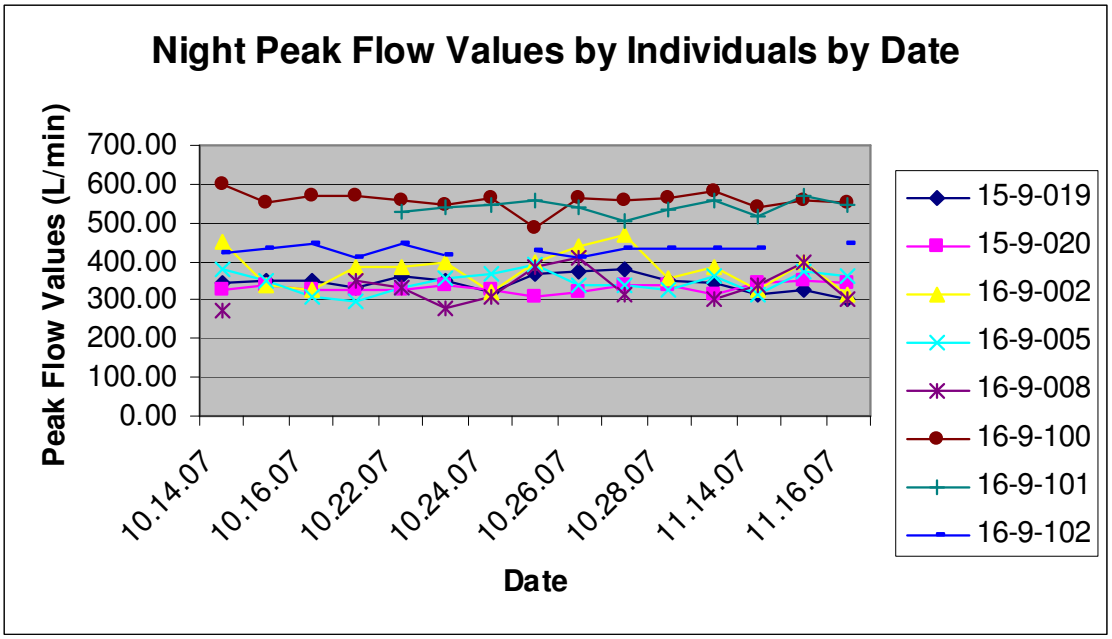

Figure 6: Night Peak Flow Values Shown for Each Subject for Before, During, and After the Wildfires

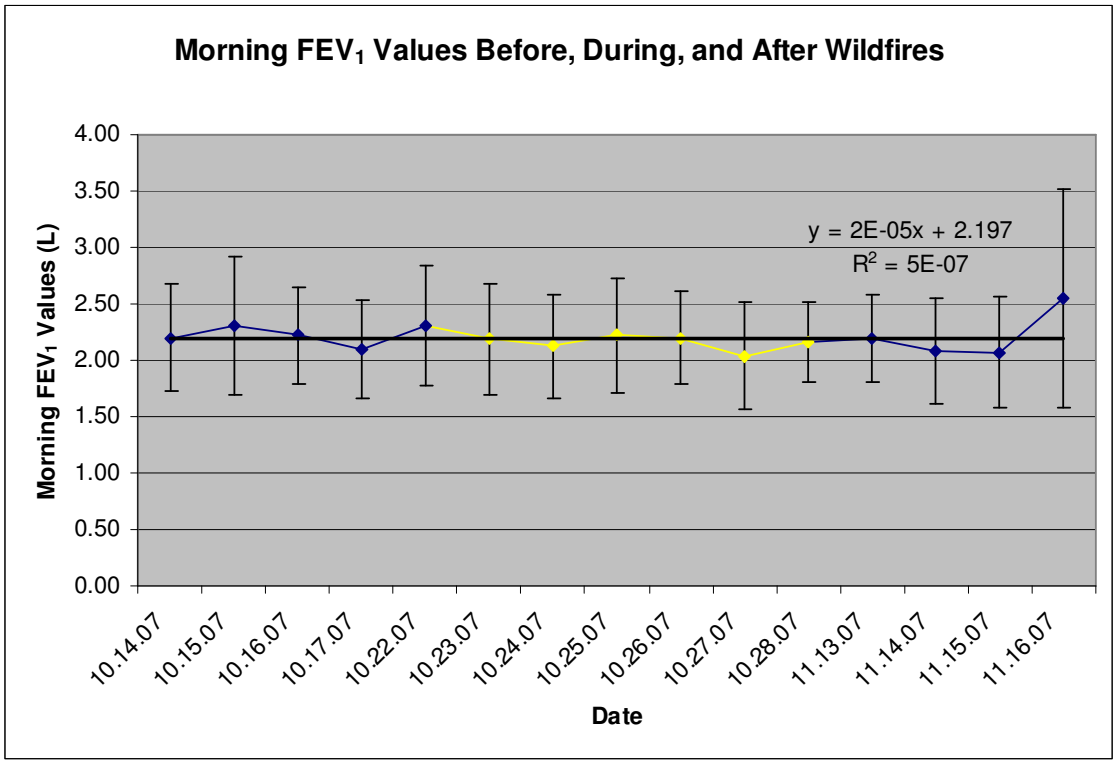

Figure 7: Morning FEV_1 Values for Before, During and After the Wildfires

Note: Yellow Line indicates values during wildfire
Error bars represent one standard deviation above and below the average mean.

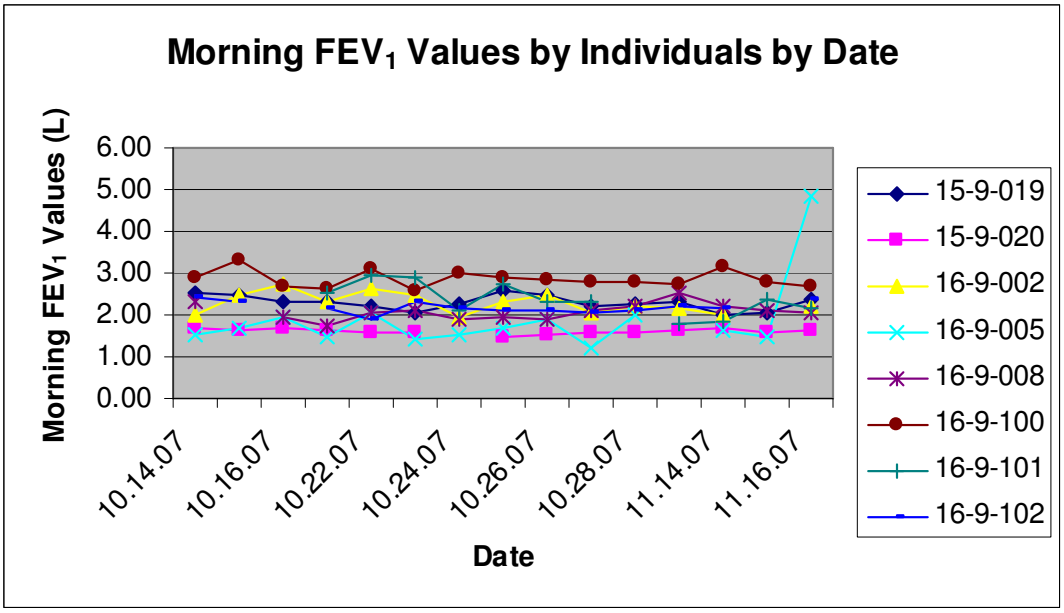

Figure 8: Morning FEV_1 Values Shown for Each Subject for Before, During, and After the Wildfires

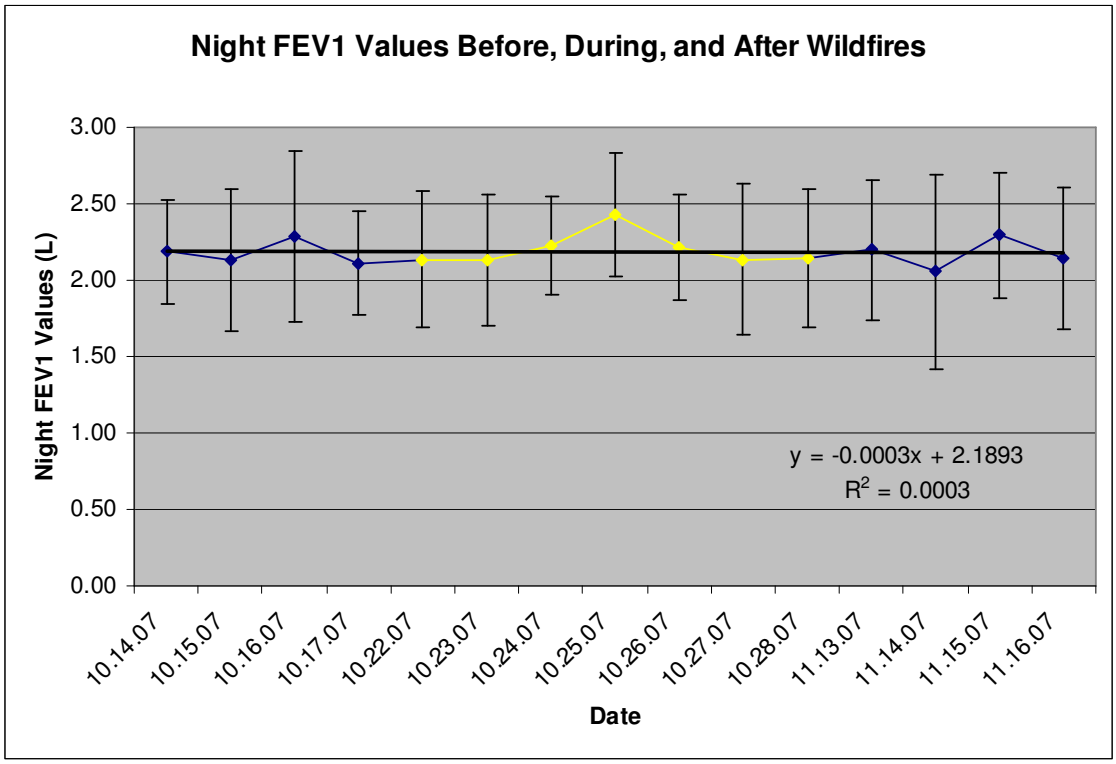

Figure 9: Night FEV_1 Values for Before, During, and After the Wildfires

Note: Yellow Line indicates values during wildfire
Error bars represent one standard deviation above and below the average mean.

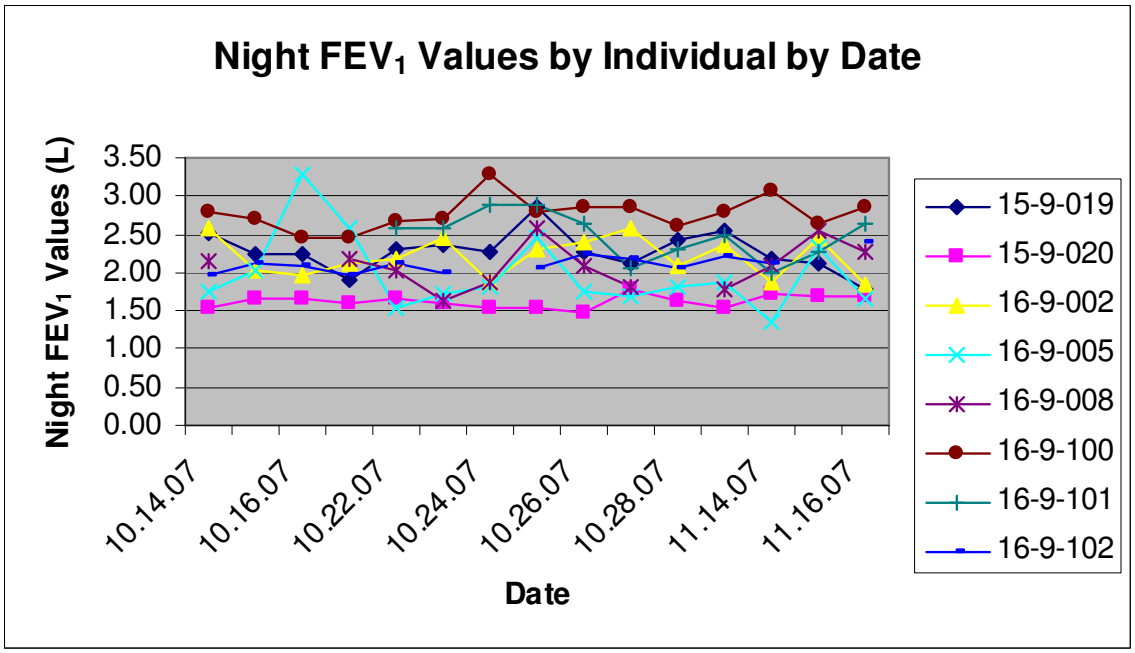

Figure 10: Night FEV_1 Values Shown for Each Subject for Before, During, and After the Wildfires

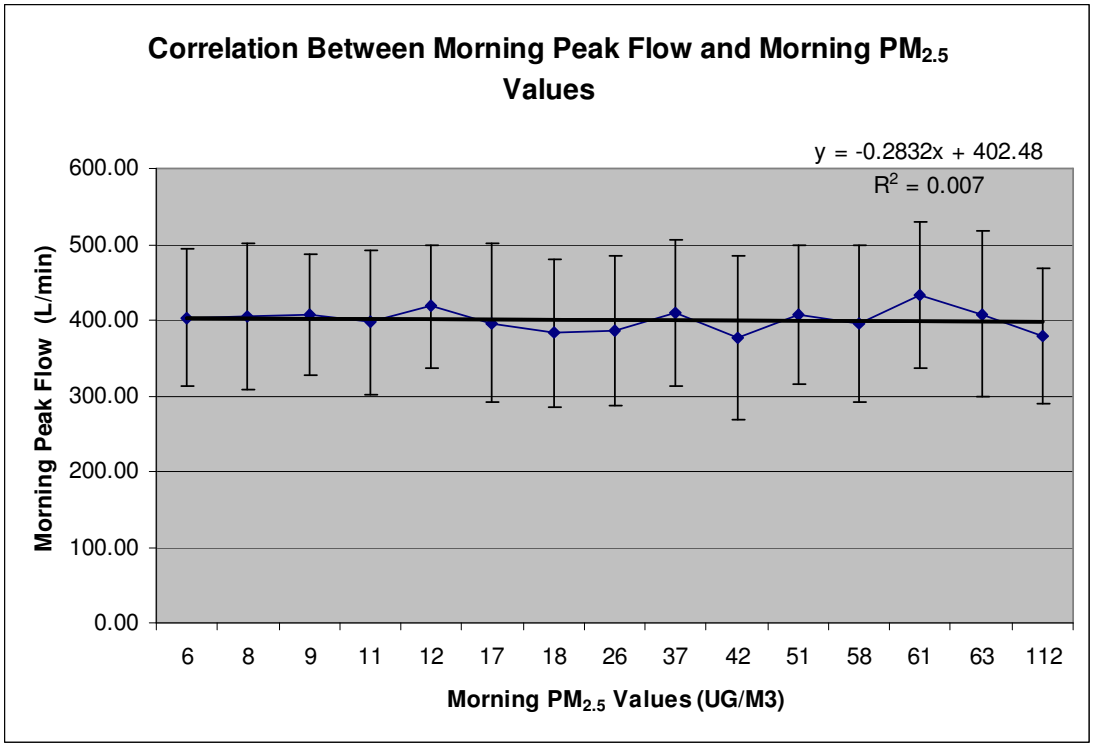

Figure 11: Correlation between Morning Peak Flow (average) to Morning $PM_{2.5}$ Values

Note: ug/m3 stands for Micrograms / Cubic Meter of Air
Error bars represent one standard deviation above and below the average mean.

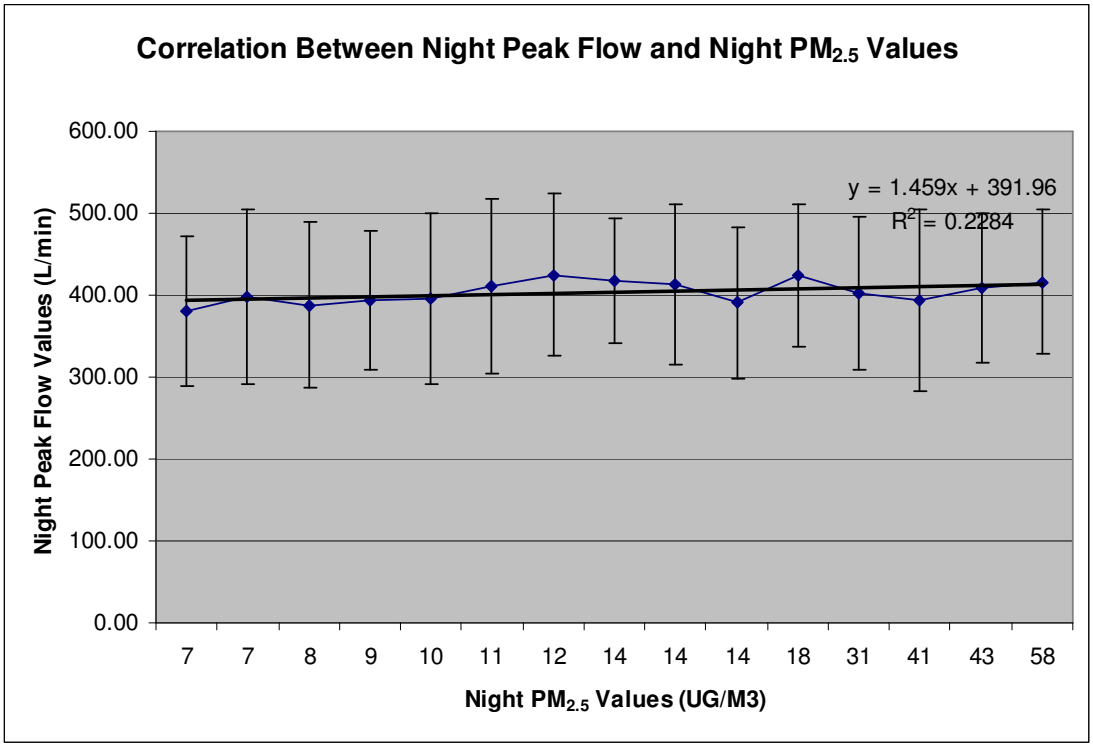

Figure 12: Correlation between Night Peak Flow (average) and Night PM2.5 Values

Note: ug/m3 stands for Micrograms / Cubic Meter of Air
Error bars represent one standard deviation above and below the average mean.

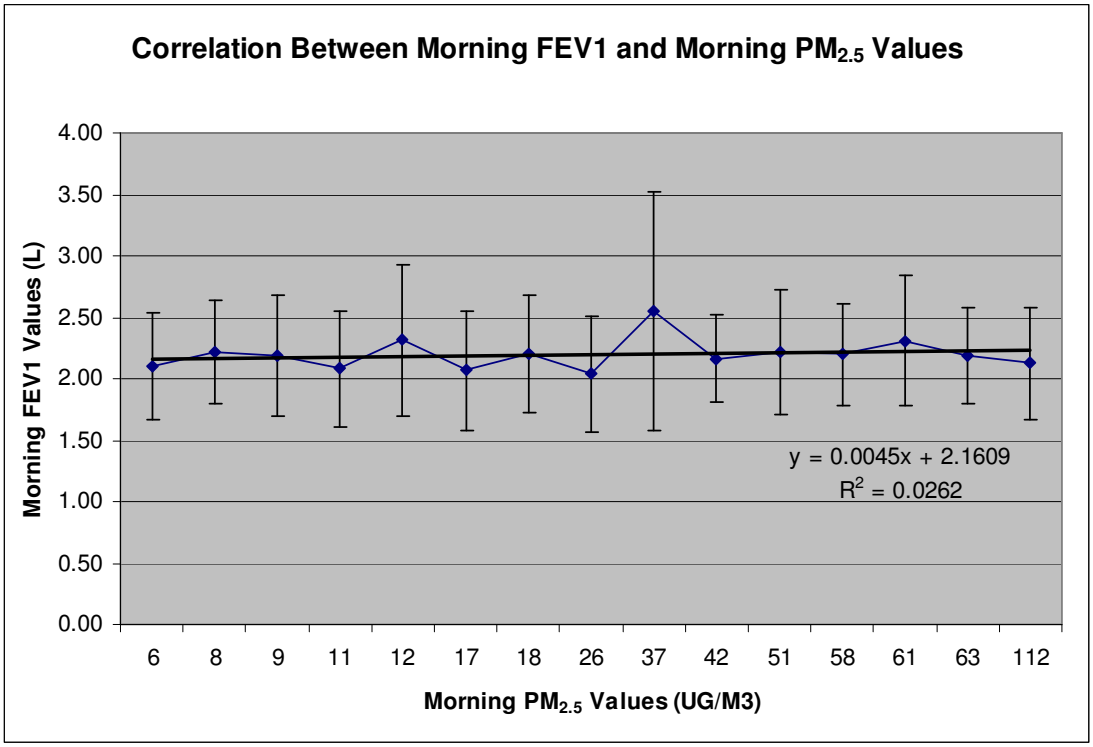

Figure 13: Correlation between Morning FEV_1 (average) and Morning PM2.5 Values

Note: ug/m3 stands for Micrograms / Cubic Meter of Air
Error bars represent one standard deviation above and below the average mean.

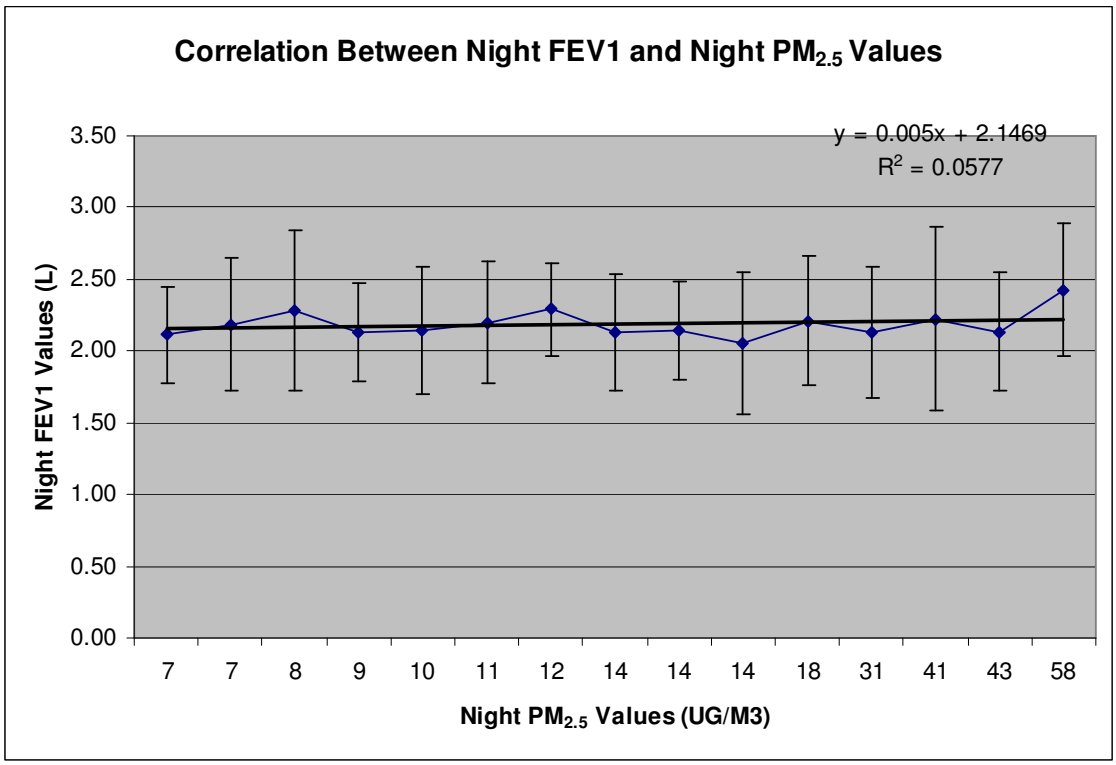

Figure 14: Correlation between Night FEV_1 (average) and Night PM 2.5 Values

Note: ug/m3 stands for Micrograms / Cubic Meter of Air
Error bars represent one standard deviation above and below the average mean.

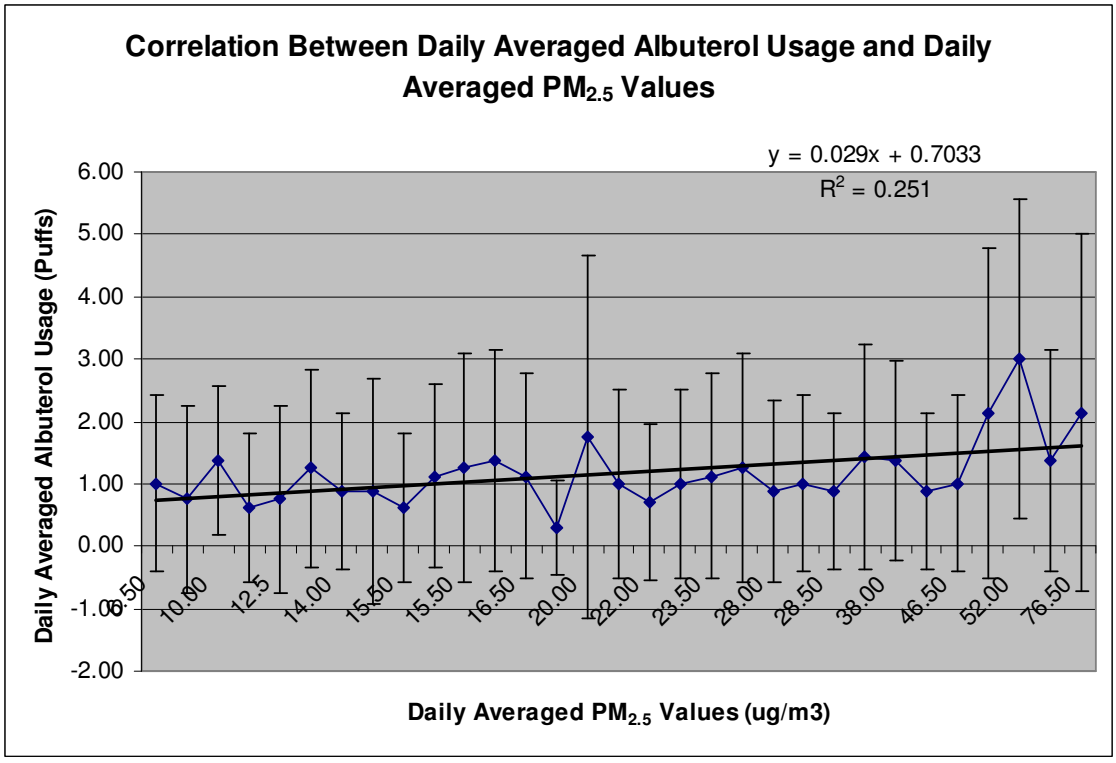

Figure 15: Correlation between Number of Uses of Albuterol (average) and Averaged 2.5 Values

Note: ug/m3 stands for Micrograms / Cubic Meter of Air
Error bars represent one standard deviation above and below the average mean.

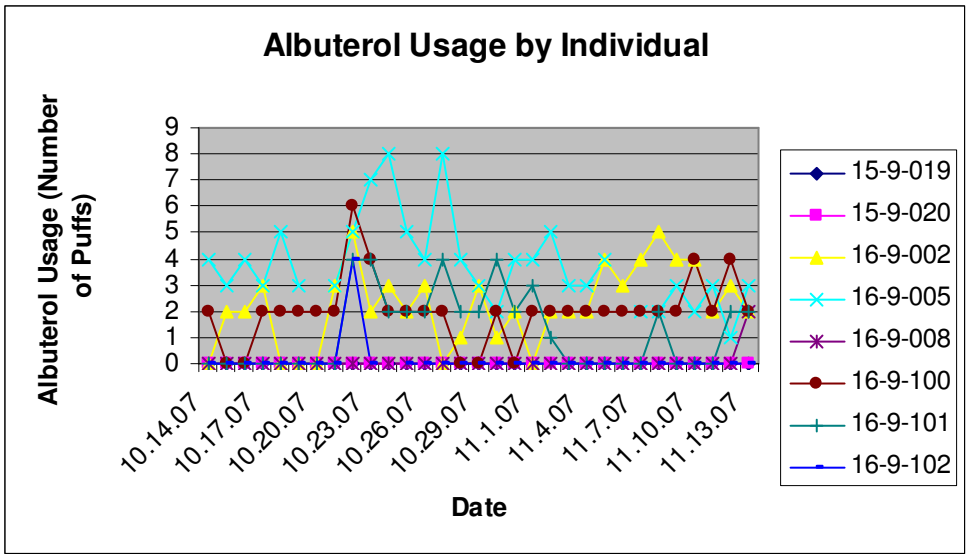

Figure 16: Number of Uses of Albuterol by Each Subject for Before, During, and After the Wildfires.

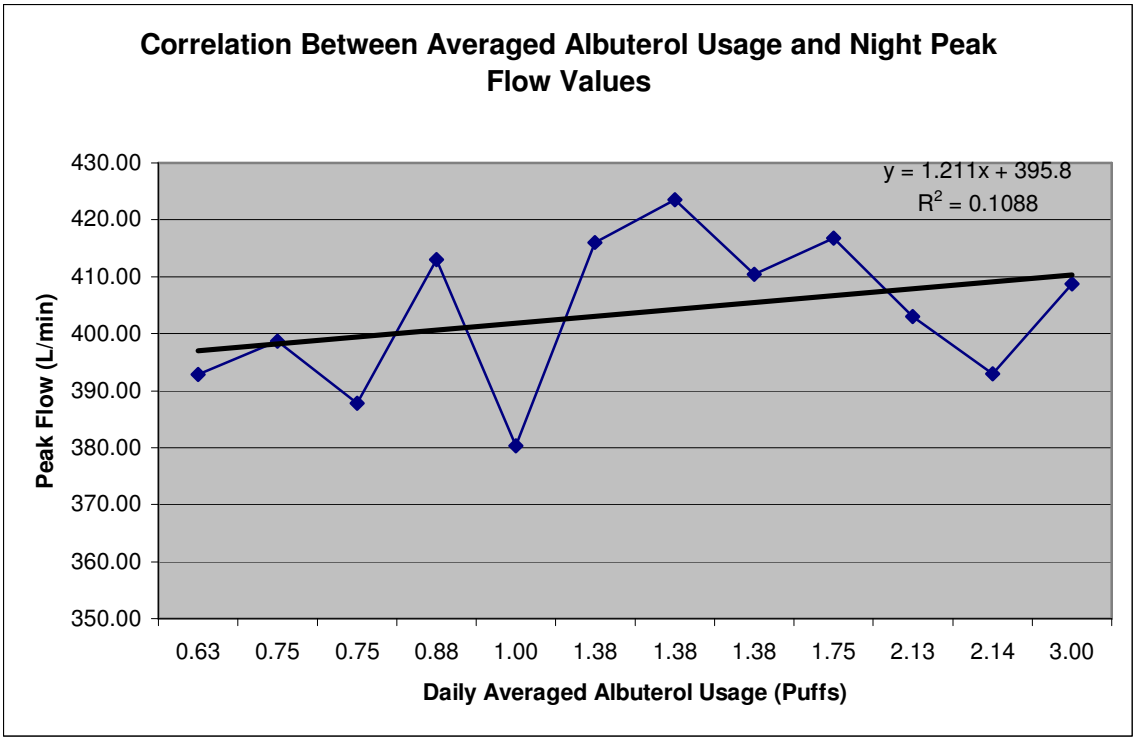

Figure 17: Correlation between Number of Uses of Albuterol (average) and Night Peak Flow Values.

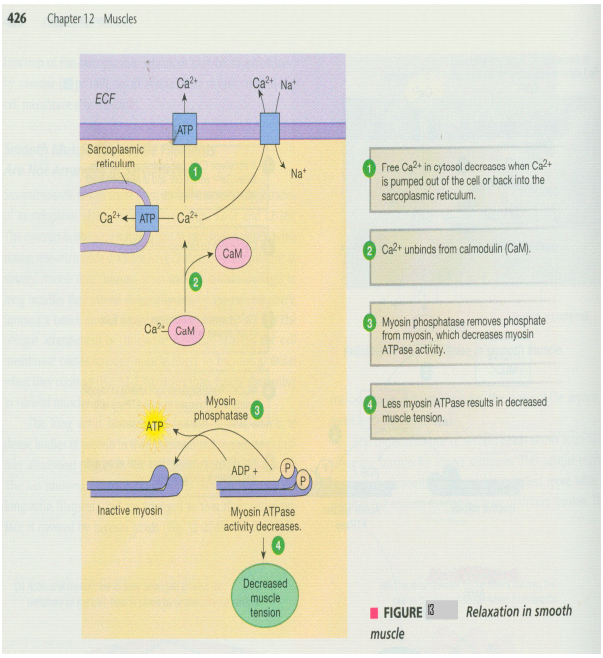

Figure 18: Relaxation in Smooth Muscle[37]

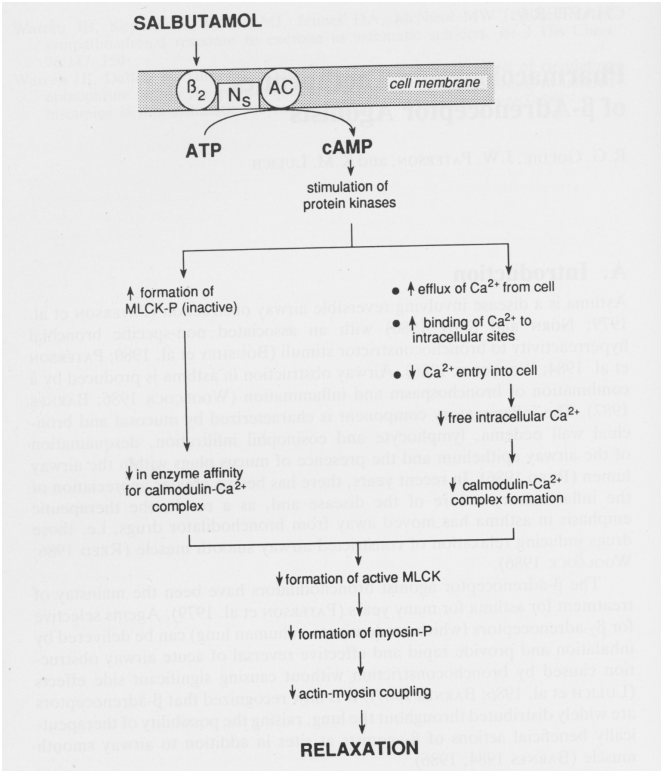

Figure 19: Relaxation in Smooth Muscle as a Result of Albuterol[38]

Table 1: Subject Data

Subject ID	Age (Years)	Height (cm)	Weight (kg)	Distance from Downtown (miles)
15-9-019	27	163	55.5	13.3
15-9-020	60	163	118	4.6
16-9-002	43	168	57.3	14.5
16-9-005	43	169	89.5	13.3
16-9-008	44	156	62	21.1
16-9-100	25	178	111	24.8
16-9-101	29	173	146	8.1
16-9-102	52	164	95.9	6.6
Average	39.5	163.5	75.7	13.3

Table 2: Actual Measurement of Breathing Values (FEV_1 and Peak Flow), Air Quality ($PM_{2.5}$), and Number of Use of Rescue Medication for Before, During, and After the Wildfires

Measurement	Pre-Wildfires	During Wildfires	Post-Wildfires
FEV_1, AM (L)	2.23	2.19	2.24
FEV_1, PM (L)	2.17	2.23	2.17
Peak Flow, AM (L/min)	411.39	404.60	550.26
Peak Flow, PM (L/min)	384.14	406.30	405.59
$PM_{2.5}$, AM (ug/m3)	11.00	59.00	18.50
$PM_{2.5}$, PM (ug/m3)	7.75	31.29	11.75
Rescue Medication (Puffs)	0.71	1.98	1.18

Table 3: Differences in Breathing Values (FEV_1 and Peak Flow), $PM_{2.5}$, and Use of Rescue Medication for During and After the Wildfires Compared to Before Based on Averages

Measure	During - Before, Count Differences	During - Before, z-values	During - Before, p-values
FEV_1 (AM)	2.00	-0.94	0.35
FEV_1 (PM)	4.00	0.34	0.74
Peak Flow (AM)	4.00	-0.84	0.40
Peak Flow (PM)	5.00	0.68	0.50
Rescue Medication	5.00	2.02	**0.04**
PM2.5 (AM)	7.00	9.46	**less than 0.0001**
PM2.5 (PM)	5.00	8.95	**less than 0.0001**
Measure	**After - Before, Count Differences**	**After - Before, z-values**	**After - Before, p-values**
FEV_1 (AM)	2.00	-0.70	0.48
FEV_1 (PM)	4.00	-0.63	0.53
Peak Flow (AM)	1.00	-1.40	0.16
Peak Flow (PM)	4.00	-1.40	0.89
Rescue Medication	4.00	1.48	0.14
PM2.5 (AM)	7.00	9.46	**less than 0.0001**
PM2.5 (PM)	5.00	7.09	**less than 0.0001**

Table 4: Eosinophil Values (Cell Count Percentage) and Differences for Subjects Before and During the Wildfires

Subject	Pre-Fire (% Eosinophil)	During Fires (% Eosinophil)	Percent Difference
16-09-100	0.20	7.80	increase of 3800%
16-09-102	2.40	3.00	increase of 25%
average	1.30	5.40	increase of 315%

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