ABSTRACT

The Effect of Polycyclic Aromatic Hydrocarbons on Human A549 Cells

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Asthma is a chronic respiratory disease affecting people of all ages and most studied in children and the elderly. This disease causes swelling of the lung's airways leading to wheezing, shortness of breath, chest tightness, and coughing. Many different factors can trigger an asthma attack including, but not limited to, tobacco smoke, mold, weather changes and outdoor air pollution. Polycyclic aromatic hydrocarbons (PAHs) comprise a large portion of air pollution. They are a class of chemicals formed during incomplete combustion reactions, and found in the ambient environment. In this experiment human A549 lung cells were exposed to 5 PAHs, Benzo[a]pyrene, Pyrene, Fluoranthene, Acenaphthylene, and Phenanthrene, and evaluated for viability, proliferation, and inflammatory response. Results indicate that PAHs have a detrimental effect on cell growth and viability. Overall viability results indicated cells were more damaged than solvent controls throughout the treatments and for certain dosing regimens the proliferation was inhibited.

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THE EFFECT OF POLYCYCLIC AROMATIC HYDROCARBONS ON HUMAN A549 CELLS

A Thesis Submitted to the Faculty of

Baylor University

In Partial Fulfillment of the Requirements for the

Honors Program

By

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CHAPTER ONE

Literature Review

Asthma is a respiratory disease that affects people of all ages, although it is most commonly studied in children and older adults (CDC 2012). This chronic lung disease causes the airways of the lungs to swell and narrow, resulting in wheezing, shortness of breath, chest tightness, and coughing. Asthma is induced by inflammation of the airways within the respiratory tract. During an asthma attack, the muscles surrounding the airways contract, causing the lining of air passages to swell and reducing the amount of air that can pass through (Zieve and Hadjiliadis 2012). During an attack it is also common for mucous to further clog the narrowed air passages (CDC 2012). The disease presents as asymptomatic periods with periodic asthma attacks. In some cases individuals may experience shortness of breath over long periods of time. The most common symptoms seen with asthma are cough with or without production of phlegm, intercostal retractions, shortness of breath exacerbated by activity or exercise, and wheezing. Some other symptoms that can be seen are abnormal breathing patterns, breathing stops temporarily, chest pain, and chest tightness. Emergency symptoms include bluish color of the face and lips, decreased alertness during an asthma attack, difficulty breathing, increased or rapid pulse, anxiety caused by shortness of breath, and sweating (Zieve and Hadjiliadis 2012). Asthma attacks are brought on when an asthmatic individual is exposed to his or her triggers (CDC 2012). Triggers vary among individuals but common ones include tobacco smoke, dust mites, outdoor air pollution, cockroach allergen, pets, mold, smoke from burning wood and grass, changes in weather, exercise, pollen, respiratory infections,

toxicants, and stress (CDC 2012; Zieve and Hadjiliadis 2012). Asthma treatment aims to get airway swelling under control and to minimize interaction with an individual's asthma triggers. Two classes of medications, control drugs and quick-relief drugs, are used to treat asthma. Control drugs are long-term medications taken every day to prevent asthmatic symptoms. Inhaled steroids and long-acting beta-agonist inhalers are examples of control drugs. Quick-relief drugs, rescue drugs, are used to quickly control asthma symptoms. They can be taken during an asthma attack or right before exercise to prevent an attack. Inhaled short-acting bronchodilators and oral steroids are examples of quick-relief medications (Zieve and Hadjiliadis 2012). Although asthma can be controlled by taking medications and avoiding triggers, there is currently no cure (CDC 2012; Zieve and Hadjiliadis 2012).

Polycyclic aromatic hydrocarbons (PAHs) are a group of over 100 chemicals that are formed during incomplete combustion of substances such as coal, oil, gas, wood or tobacco (ATSDR 1995). They can be found in nature as a mixture of several chemicals, although they may also be manufactured individually for use in research (ATSDR 1995; EPA 2008). Other common uses for these chemicals include the production of dyes, plastic, pesticides, and medicines (EPA 2008). The purest form of these chemicals are solids ranging from colorless to white or pale yellow-green in color and some may have a faint odor (ATSDR 1995; EPA 2008). Polycyclic aromatic hydrocarbons are persistent in the environment. They generally do not dissolve in water and it takes days or weeks before microorganisms can break them down (ATSDR 1995; EPA 2008). Human exposures to polycyclic aromatic hydrocarbons are primarily due to inhalation of tobacco smoke, wood smoke, ambient air, and consumption of contaminated food. Exposure may

also occur through contaminated soil, drinking water, or workplace exposures (ATSDR 1995). Once they enter the body these chemicals are primarily stored in the kidneys, liver, and adipose tissue. Lower levels of polycyclic aromatic hydrocarbons can be found in the spleen, adrenal glands, and ovaries. The body will biotransform these substances and in some cases the products may be more harmful than the original chemical. Currently there are tests to detect the presence of polycyclic aromatic hydrocarbons in blood, urine, and tissues but these tests do not predict exposure outcomes (ATSDR 1995).

Polycyclic aromatic hydrocarbons account for a large proportion of outdoor air pollution. Dejean et al. 2009 sampled three areas, traffic, urban, and industrial for PAHs in the air. The main PAHs emitted from the traffic site were Phenanthrene, Fluoranthene, Pyrene, and Acenaphtylene. The compounds Phenanthrene, Fluoranthene, Pyrene, Benzo(a)pyrene, Benzo(g,h,i)perylene, and Indenol(1,2,3-cd)pyrene are characteristic of car exhaust and were found to make up 47% of the total PAH concentration in the traffic area with Acenaphtylene contributing 14%. The PAHs most found in the urban site were Acenaphtylene, Acenaphtene, Fluorene, Phenanthrene, Fluoranthene, and Pyrene. They accounted for 65% of the total PAH concentration present. In the urban site the PAHs found most were Acenaphtylene, Acenaphtene, Fluoranthene, and Pyrene and accounted for more than half the total PAH concentration. Some of these compounds are classified as human carcinogens and have been associated with increased lung and bladder cancers among the occupationally exposed. (Dejean et al. 2009) They also may exhibit mutagenic effects and are though responsible for an array of health effects associated with ambient air pollution. In addition, derivatives of polycyclic aromatic hydrocarbons,

formed either in the body or the environment, have been shown to cause oxidative stress and endocrine disruption (Bekki et al. 2013). There are many different factors that induce asthmatic symptoms. It is thought that one environmental factor that could contribute to the exacerbation of asthma is exposure to polycyclic aromatic hydrocarbons.

Suresh et al. 2009 studied the association between polycyclic aromatic hydrocarbons exposure and bronchial asthma and oxidative stress in children. Researchers hypothesized that PAH exposure in children would cause oxidative stress in the respiratory tract, in turn stimulating the redox cycle and generating reactive oxygen species. This pathway would ultimately result in the exacerbation of inflamed airways and present as asthma (Suresh et al. 2009). The study was conducted in a hospital in India over the course of 11 months focusing on children ages 1-14. The study found elevated blood levels of the PAH Phenanthrene in children with bronchial asthma as compared with controls. Decreased blood levels of glutathione and malondialdehyde were also found in these individuals and were indicative of oxidative stress. Although 10 PAHs were studied only a positive association with bronchial asthma and oxidative stress was demonstrated with Phenanthrene exposure. The study aimed to detect specific PAHs that were present in at least 50% of bronchial asthma cases and 15% of controls. Because of this sample size limitation an association cannot be made between PAHs and asthma and oxidative stress where the PAHs are present in more than 15% of controls. A study with a larger sample size is needed to confirm the association (Suresh et al. 2009).

In Miller et al. 2004, researchers hypothesized that prenatal exposure to PAHs in the air were associated with respiratory symptoms in young children. They

also predicted exposure to environmental tobacco smoke (ETS) may worsen symptoms compared with those only exposed to PAH. The study followed 303 pregnant women, and later their children, in Manhattan who were at high risk for exposure to PAH and ETS. In their third trimester of pregnancy, mothers wore a 48-hour monitor that collected air samples and particles. The samples were analyzed for eight different PAHs. Children's exposure to ETS was determined by questionnaires when the child was 6, 12, and 24 months. Plasma cotinine was also collected from maternal and cord blood at birth to determine ETS exposure. Results indicated that exposure to PAH and ETS occurring at the same time was associated with adverse respiratory symptoms and probable asthma by age 12-24 months. (Miller et al. 2004)

Leem et al. 2005 looked at the association between pulmonary inflammation as seen in asthma and exposure to polycyclic aromatic hydrocarbons and environmental tobacco smoke and asthma. The study included 16 asthmatic individuals and 16 control subjects. Polycyclic aromatic hydrocarbon and environmental tobacco smoke exposures were measured using urinary 1-OHPG and cotinine. Researchers noted a significant increase in exposure to both PAH and ETS during asthma attacks. They also found elevated levels of malondialdehyde during asthma attacks, a marker for oxidative stress (Leem et al. 2005).

Asthma is a respiratory disease that is exacerbated by many environmental factors. In the studies mentioned above an increase in asthma symptoms was seen when individuals were exposed to polycyclic aromatic hydrocarbons. Because of the negative effects seen with exposure to polycyclic aromatic hydrocarbons, it is imperative to

continue research on these compounds and prevent exposure to them in an effort to mitigate their negative effects on human health.

CHAPTER TWO

Materials and Methods

Materials

Polycyclic aromatic hydrocarbons tested included: Benzo[a]pyrene, Pyrene, Fluoranthene, Phenanthrene, and Acenaphthylene. These compounds as well as the Janus Green stock solution were purchased from Sigma-Aldrich, USA.

Cell Culture

Human A549 cells, alveolar adenocarcinoma cells, were used to evaluate cell viability and proliferation when exposed to polycyclic aromatic hydrocarbons (PAHs). The alveolar adenocarcinoma cell line is well characterized and widely used in molecular biology. A549 cells have been tested and approved by the FDA. One clinical application for this cell line is studying how viruses cause asthma in order to develop better treatments for the disease (OTT 2009). A549 cells were cultured in T-75 flasks in an incubator kept at 37°C. They were allowed to grow until 80-90% confluence in the T-75 flask. Media was replaced as it began to change pH and color from dark pink to yellow, approximately every 2 days. Old media was first aspirated from the flask. 20mls of fresh media, warmed for 30 minutes in the incubator, was then added to the T-75 flask. Once the cells reached confluence, they were split. This process began with aspirating the cell media. The cells were then rinsed with 20 mls of warm PBS free. PBS free was then aspirated from the flask and replaced with 3mls of warm trypsin. The flask was then whacked to detach

cells. The trypsin was not left on the cells longer than 2-3 minutes to prevent cell lysis. Fresh warm media (7mls) was then added to the flask containing cells and trypsin. A 20 microliter aliquot of this solution was taken for cell counting. The solution was then transferred into a vial and centrifuged for 3 minutes. The media in the vial was aspirated and replaced so that the ratio of media to cells 1mls for 1 million cells. The cells were then transferred into 96 well plates. This was done by placing 0.5mls of cell containing media into approximately 9.5mls of fresh warm media. A micropipette was then used to add 0.1mls per well. These cells were allowed to grow in the incubator for 24 hours before they were dosed with PAHs.

Dosing

The A549 cells were dosed with media containing an increasing dosage of a particular PAH. The first well line, the control line, contained only media. The second well line was the solvent control containing media and 0.5% DMSO. Cell lines were treated with the following amounts of PAH: 1ppm, 2ppm, 5ppm, 10ppm, and 20ppm to determine cellular responses at different chemical exposure concentrations. The treatment solutions were made up of serial dilutions from the stock PAH solution. Both viability and proliferation assays were then performed on each treatment to evaluate the cells response to toxin exposure. New A549 cells were dosed with media, DMSO containing media, or PAH containing media in the concentrations outlined above. After 24 hours the viability and proliferation assays were run.

Viability and Proliferation Assay

The Janus Green Assay was used on cells to test for viability and proliferation. The protocol followed is outlined in detail in Reich et al. 1993. Media was first aspirated from all wells in the plate using a multichannel pipet. All wells were then rinsed with 100 microliters of PBS free two times. It was important to remove all PBS free from the wells at this time. To all wells undergoing the proliferation assay, 100 microliters of absolute ethanol was added to the wells for 90 seconds. Ethanol was then removed and the plate allowed to dry until all ethanol had evaporated. 100 microliters of Janus Green solution was then added to all wells. They dye was removed after it was allowed to sit on the cells for 60 seconds. PBS free was then used to rinse the cells; this process was repeated two times. 100 microliters of absolute ethanol and 100 microliters of nano-pure water were then added to each well. The 96-well plates were placed in a spectrophotometer and absorbance measured at 630nm.

Cyto Tox-one homogeneous membrane integrity assay

A549 cells were exposed to control, solvent control, 1ppm or 20ppm of a PAH for 24 hours. The plate was then taken out of the incubator and allowed to sit for 20 minutes until it reached a temperature of 22 degrees Celsius. 2 microliters of lysis solution were then added to the Max LDH controls. 100 microliters of Cyto-Tox-ONE (CTO) reagent was then added to each well and the plate was shaken for 30 seconds. After the plate sat for ten minutes at 22 degrees Celsius, 50 microliters

of CTO stop solution was added to all wells. The plate was shaken for 10 seconds. Pictures of the plate were taken on a microscope using a Cy 3.5 filter.

Statistical Analysis

The data collected was averaged for each treatment (n=8) and graphed to show the cells' response to polycyclic aromatic hydrocarbon exposure. The student's t-test was used to determine statistically significant differences between solvent control and treated cells. Viability average optic densities were divided by proliferation average optic densities to determine the percent damage caused by each compound.

CHAPTER THREE

Specific Aims and Objectives

Asthma is a chronic disease of the lungs known to be exacerbated by many factors. Both toxicological and epidemiological studies have shown air pollutants to be associated with increased wheezing, respiratory symptoms, the use of rescue medications, hospital visits and acute asthma attacks. It has also been seen that the prevalence of asthma has increased most notably in highly industrialized urban areas. (Arbex 2012) Polycyclic aromatic hydrocarbons are aromatic compounds formed from incomplete combustion of fuel and other substances. They are prevalent in ambient air and may exacerbate asthma by causing bronchial inflammation and bronchoconstriction. A study performed in Fresno, California showed that PAHs having between 4 and 6 membered aromatic rings as well as phenanthrene cause an increase in wheezing in asthmatic individuals. (Gale 2012) Repeated pyrene exposures while in the womb or in early childhood have also been correlated with non-atopic asthma, asthma not caused by an allergen. (Jung 2012) Because of the evidence linking polycyclic aromatic hydrocarbons to asthma exacerbation, we hypothesize that exposure to polycyclic aromatic hydrocarbons causes a toxic and inflammatory response in human A549 cells.

The specific aims that will be used to test the hypothesis are 1) evaluate changes in viability and proliferation of human lung cells from exposure to polycyclic aromatic hydrocarbons and 2) evaluate inflammatory response of human lung cells following exposure to polycyclic aromatic hydrocarbons.

To evaluate changes in viability and proliferation of the cells a Janus Green assay will be performed. The human A549 cells will be exposed to one of five different compounds, benzo[a]pyrene, fluoranthene, acenaphthelene, pyrene, or phenanthrene, at a time. Each 96 well plate will consist of 1 control column, 1 solvent control column, and two columns of each dosing treatment. The dosing treatments will contain a compound in 1ppm, 2ppm, 5ppm, 10ppm, or 20ppm. The viability assay will provide data on approximate cell damage following exposure. The proliferation assay provides data on the cells ability to recover and continue dividing following exposure. Data obtained from these experiments will help determine overall tissue health.

To evaluate the inflammatory response of the cells following exposure a Cyto Tox-one homogeneous membrane integrity assay will be run. This assay uses fluorescence to measure the release of lactate dehydrogenase from cells with damaged cell membranes. (Promega 2009) Data from this experiment will provide insight into the cytotoxicity and inflammation response from exposures to the five polycyclic aromatic hydrocarbons mentioned above.

CHAPTER FOUR

Results

Quantification of Data

In this experiment cell viability and proliferation were quantified using an ELx 800 microplate reader (Bio Tek Instruments, Inc., Winooski, VT). The microplate reader measures absorbance and the data generated are optic densities. The optic densities used to calculate percent damage are directly proportional to the thickness of the sample in the well, in this case proportional to amount of damaged cells. Standard error was calculated and represented using the error bars in each figure. A student's t-test was also performed and the p value resulting from this was used to determine significance as follows: one "*" indicates a p-value less than 0.05, two "**" indicates a p-value less than 0.01 and three "***" indicates a p-value less than 0.001.

Benzo[a]pyrene

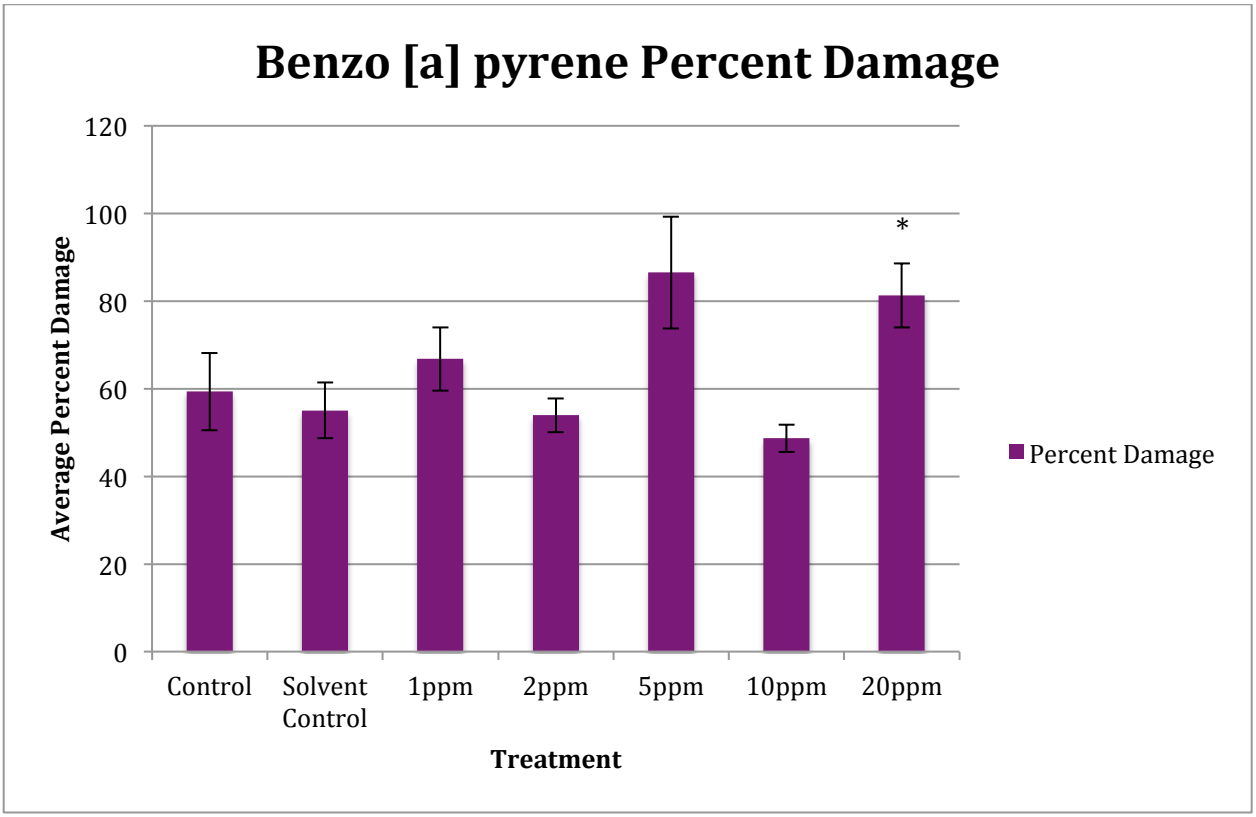

Figure 1: Bars represent the percent damage caused by a polycyclic aromatic hydrocarbon. . Error bars were calculated using standard error. Treatments yielding a result significantly different from the control are denoted with stars. One "*" indicates a student's t-test p-value less than 0.05, two "**" indicates a p-value less than 0.01, three "***" indicates a p-value less than 0.001.

Percent damage was calculated by dividing optic densities for cell viability by optic densities for proliferation and multiplying by 100. The results for each treatment were then averaged to obtain the graphs in this section. The average percent damage values calculated for Benzo[a]pyrene were 59.36, 55.07, 66.77, 53.93, 86.49, 48.67, and 81.25. These values correspond to the control, solvent control, 1ppm, 2ppm, 5ppm, 10ppm, and 20ppm treatments.

Pyrene

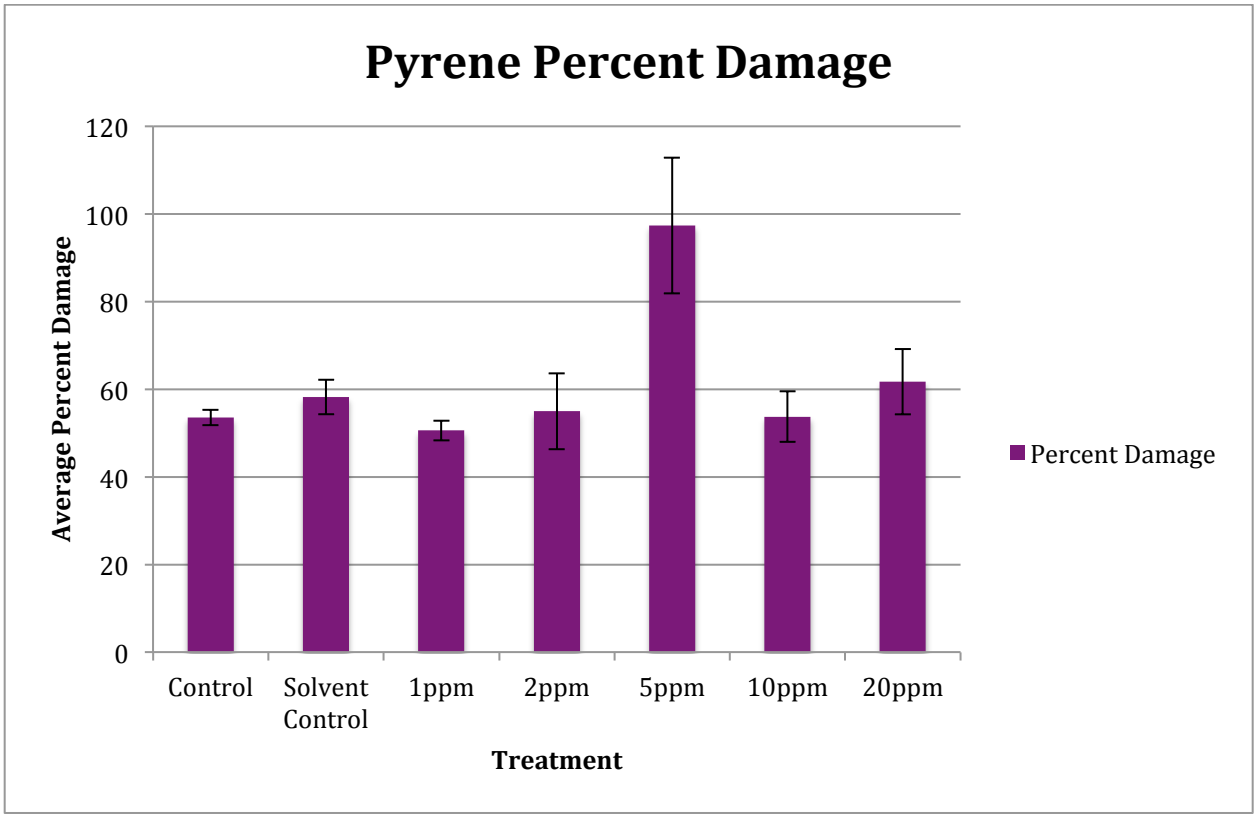

Figure 2: Bars represent the percent damage caused by a polycyclic aromatic hydrocarbon. Error bars were calculated using standard error. Treatments yielding a result significantly different from the control are denoted with stars. One "*" indicates a student's t-test p-value less than 0.05, two "**" indicates a p-value less than 0.01, three "***" indicates a p-value less than 0.001.

The average percent damage values calculated for Pyrene were 53.52, 58.26, 50.56, 54.99, 97.31, 53.74, and 61.71.

Fluoranthene

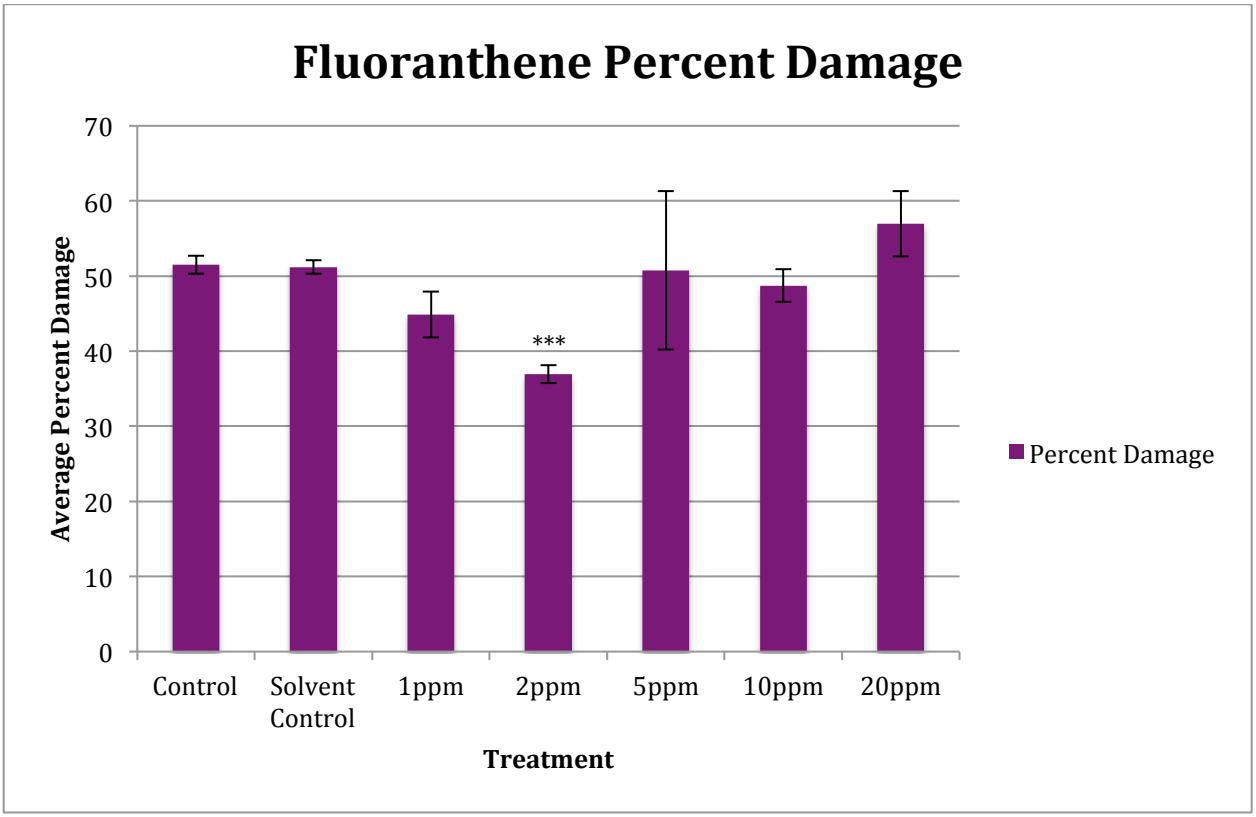

Figure 3: Bars represent the percent damage caused by a polycyclic aromatic hydrocarbon. . Error bars were calculated using standard error. Treatments yielding a result significantly different from the control are denoted with stars. One "*" indicates a student's t-test p-value less than 0.05, two "**" indicates a p-value less than 0.01, three "***" indicates a p-value less than 0.001.

The percent damage values calculated for Fluoranthene were 51.50, 51.19, 44.89, 36.94, 50.77, 48.72, and 56.97.

Phenanthrene

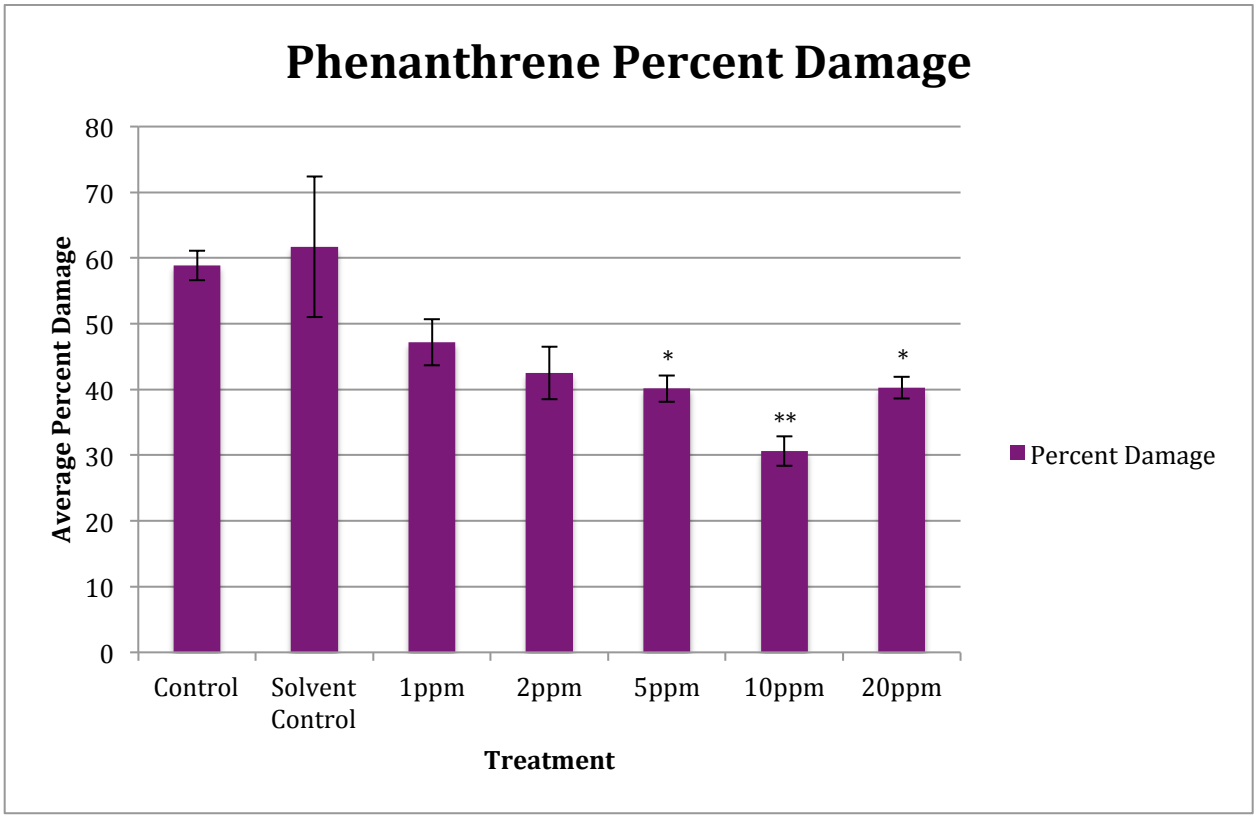

Figure 4: Bars represent the percent damage caused by a polycyclic aromatic hydrocarbon. . Error bars were calculated using standard error. Treatments yielding a result significantly different from the control are denoted with stars. One "*" indicates a student's t-test p-value less than 0.05, two "**" indicates a p-value less than 0.01, three "***" indicates a p-value less than 0.001.

The percent damage values calculated for Phenanthrene were 58.86, 61.73, 47.15, 42.49, 40.13, 30.63, and 40.27.

Acenaphthylene

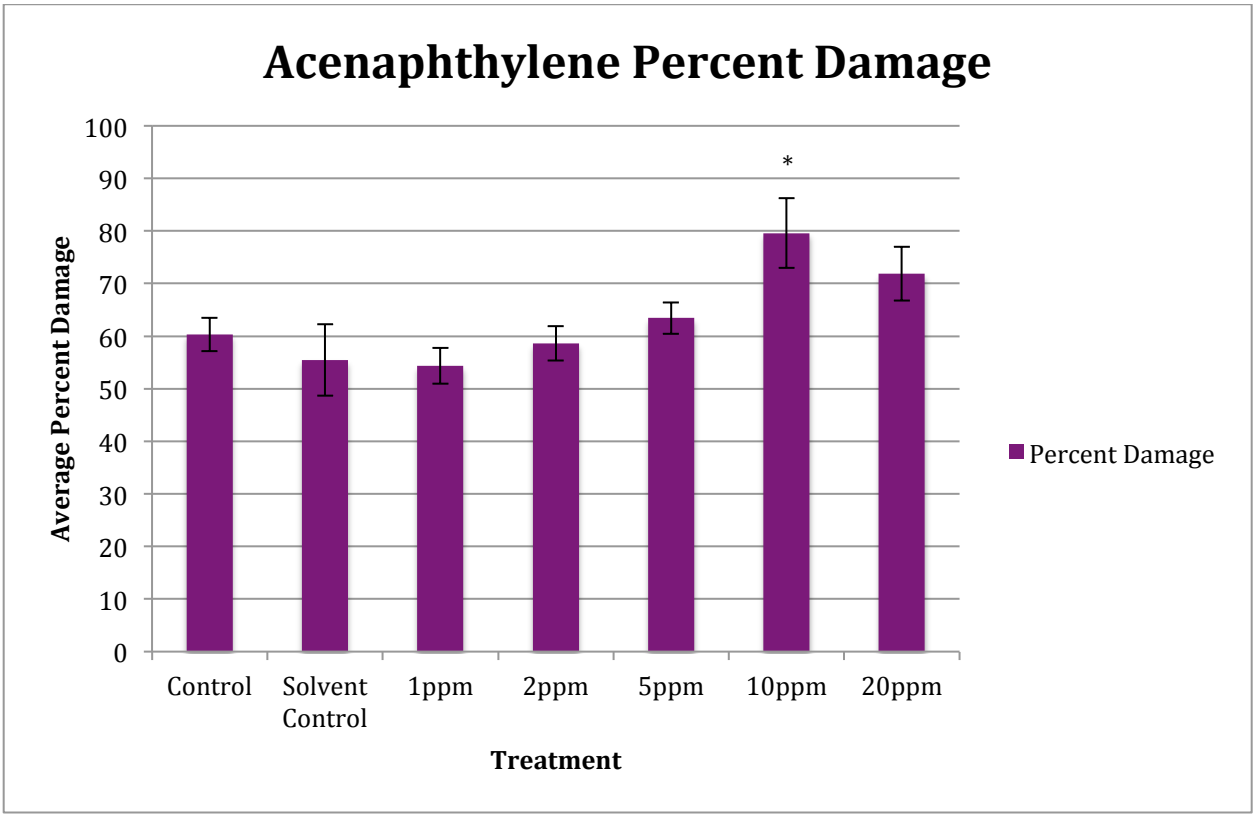

Figure 5: Bars represent the percent damage caused by a polycyclic aromatic hydrocarbon. . Error bars were calculated using standard error. Treatments yielding a result significantly different from the control are denoted with stars. One "*" indicates a student's t-test p-value less than 0.05, two "**" indicates a p-value less than 0.01, three "***" indicates a p-value less than 0.001.

The percent damage values calculated for Acenaphthylene were 60.29, 55.48, 54.34, 58.64, 63.42, 79.60, and 71.83.

All PAHs

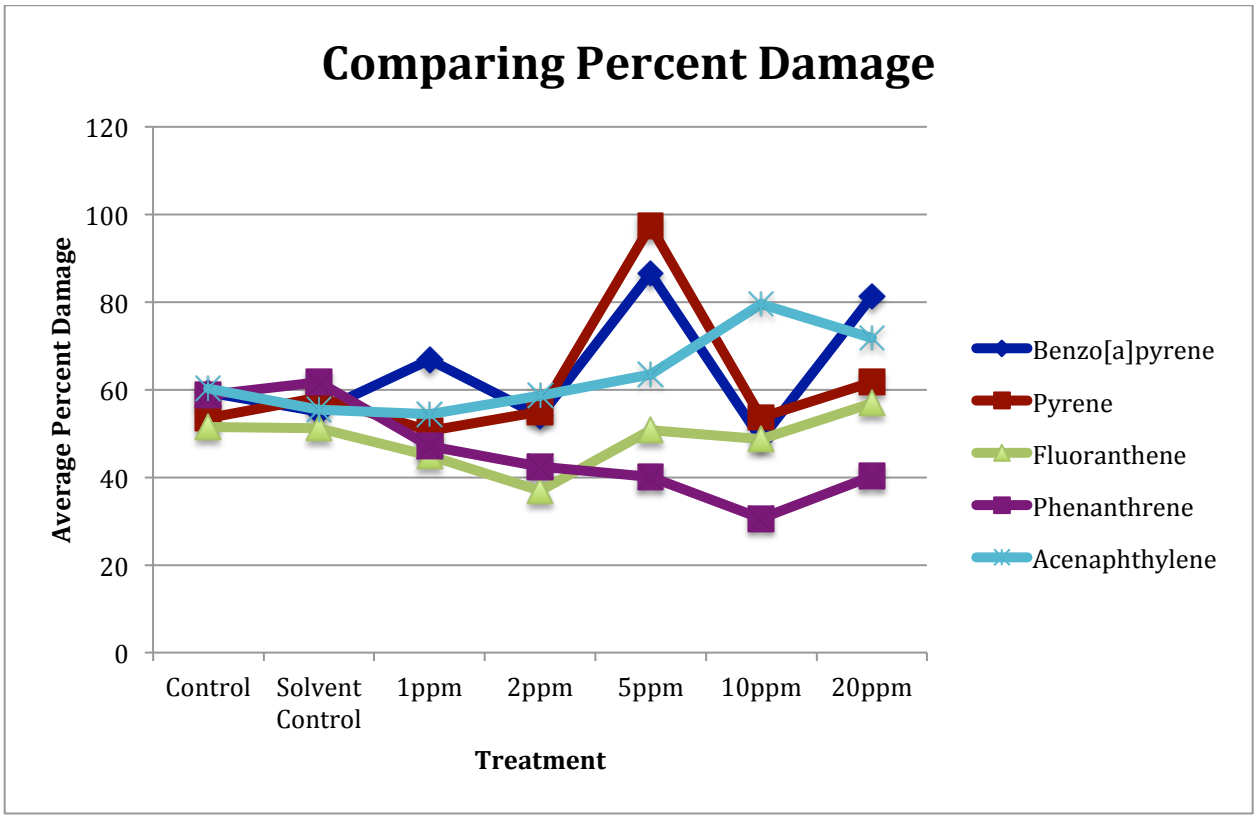

Figure 6: The data from figures 1-5 is represented in this graph. Each line represents percent damage data for a particular compound over the concentration range tested.

Cyto Tox-one homogeneous membrane integrity assay

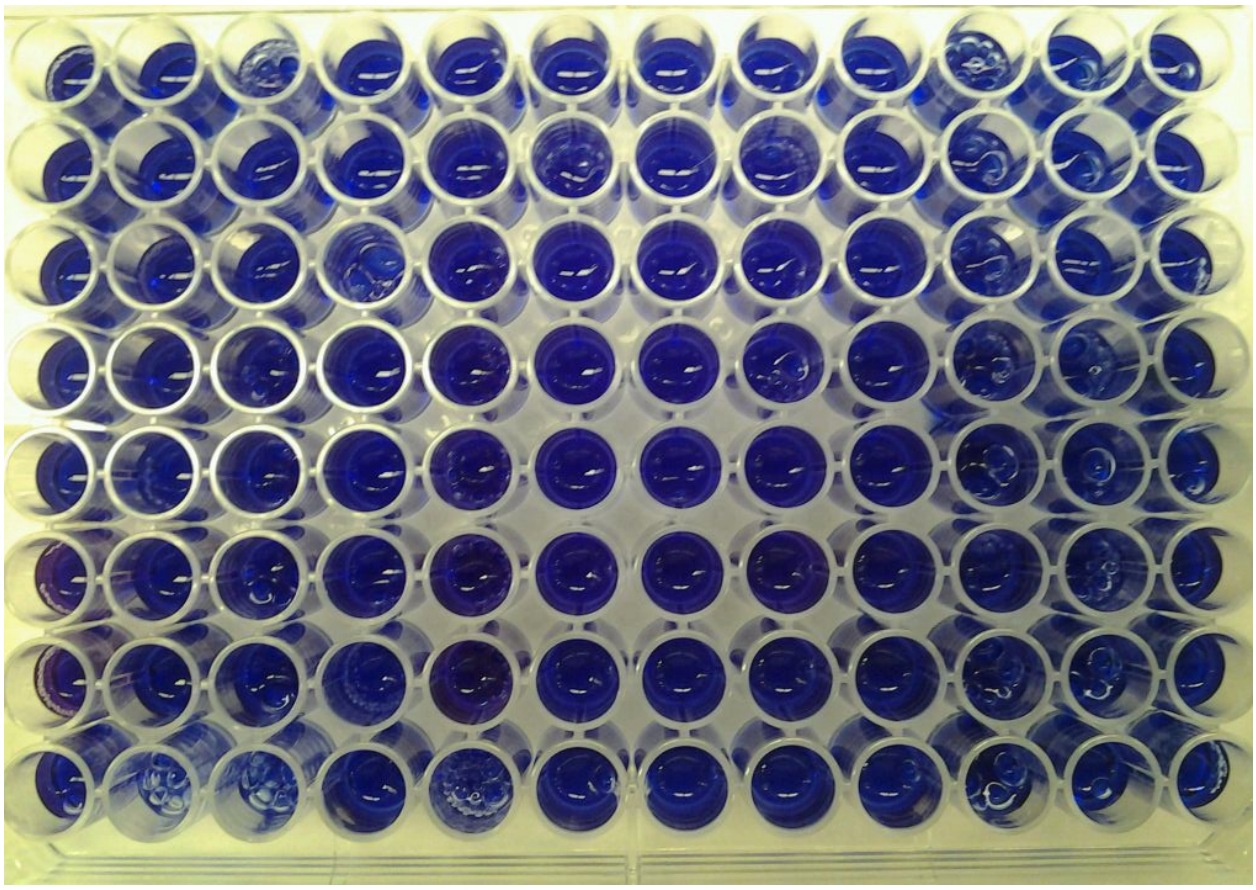

Figure 7: This image shows results for the Cyto tox-one homogeneous membrane assay. The assay uses fluorescence to measure the release of lactate dehydrogenase from cells with damaged cell membranes.

The figure above (figure 7) shows results from the cyto tox-one homogeneous membrane, LDH, assay. The first column is the control with the bottom three wells being max LDH controls. The second column is the solvent control. Two columns are allotted for each PAH the first column is the 1ppm treatment and the second is the 20ppm treatment. The figure above can only provide qualitative data where color differences indicate varying degrees of inflammation. We observed significant inflammation for all the PAHs tested at the above noted concentrations. Due to the significant inflammation, at these

concentrations, repeated experiments are being conducted to evaluate PAHs at lower concentrations in an attempt to observe a dose response. The observed response does however confirm that PAHs cause inflammation in human lung cells and could be an indication of exacerbation of asthma.

CHAPTER FIVE

Discussion

At the outset of this experiment it was hypothesized that exposure to polycyclic aromatic hydrocarbons causes a toxic and inflammatory response in human lung (A549) cells. To test this, cells were exposed to varying concentrations of a polycyclic aromatic hydrocarbon (Benzo[a]pyrene, Pyrene, Acenaphtylene, Fluoranthene, or Phenanthrene) and a Janus Green assay was performed for both cell viability and proliferation. In addition, to evaluate the inflammatory response of the cells, as seen in asthma attacks, a Cyto Tox-one homogeneous membrane integrity assay was performed.

Viability and Proliferation

The proliferation portion of the Janus Green assay provides data to quantify the number of cells contained per well. It is performed by fixing the cells with absolute ethanol before staining with Janus Green dye. The viability assay provides an approximation of cell damage following exposure to a toxin (Rieck et al. 1993). Viability and Proliferation data were used to calculate total percent damage caused by individual compounds. The solvent DMSO was used to deliver the polycyclic aromatic hydrocarbons in a 0.5% concentration. The solutions were prepared to contain DMSO in this concentration because it is widely accepted that 0.5% DMSO will not cause additional harm to cells.

Benzo[a]pyrene is classified as a group 1 carcinogen because it is carcinogenic in humans. Its metabolites are highly reactive and form covalent adducts with DNA. In one carcinogenic mechanism of action these adducts induce mutations in the K-RAS oncogene as well as in the TP53 tumor suppressor gene causing lung cancers (IARC 2012). Benzo[a]pyrene has a bay region, space between its aromatic rings. If an epoxide forms in this region it is highly reactive and mutagenic (ATSDR 2009). Cells exposed to Benzo[a]pyrene had the following average percent damages 59.36, 55.07, 66.77, 53.93, 86.49, 48.67, and 81.25 (figure 1). These percent damages correspond to the control, solvent control, 1pm, 2ppm, 5ppm, 10ppm, and 20ppm treatments respectively. There was not a significant difference between the control and solvent control treatments. Because there was no significant difference between these treatments, it shows DMSO did not impact the cells in this experiment. When compared with the solvent control the 20ppm treatment exhibited a "*" statistically significant increase in cellular damage. The damage caused in this treatment can be attributed to Benzo[a]pyrene exposure. It is not surprising to see damage with this treatment because of the known toxicity of the compound.

Pyrene is a four ring PAH. It is unclassifiable as a human carcinogen but does cause mutagenic effects to mammalian somatic cells (MSDS 2005). The following average percent damages were calculated for this compound 53.52, 58.26, 50.56, 54.99, 97.31, 53.74, and 61.71 (figure 2). When compared with the control, the solvent control did not exhibit a statistical difference, meaning DMSO did not impact the cells in this experiment. When compared with the solvent control only the

5ppm and 20ppm treatments exhibited a greater amount of damage than the solvent control. However, none of the data for this experiment was statistically significant. Damage may not be high because cells are dead rather than having a damaged cell membrane. If the cell membrane is not damaged, the Janus Green dye is not able to enter the cell and stain nuclei and cytoplasm this causes a decrease in expected viability data and ultimately a decrease in percent damage.

Fluoranthene is currently unclassifiable as a human carcinogen. It is associated with the formation of DNA adducts in vitro, and tumors in mice. This suggests it may be a cause of some human cancers, but more studies are needed for a classification. It is thought that long-term exposure to this compound in the dust form may cause changes in lung function such as pneumoconiosis (MSDS 2008). Average percent damages calculated for Fluoranthene were 51.50, 51.19, 44.89, 36.94, 50.77, 48.72, and 56.97 (figure 3). DMSO did not affect the cells in the solvent control because there was no significant difference between it and the control treatment. The 2 ppm treatment showed a "***" significance decrease in percent damage when compared to the solvent control. The lack of increase in cellular damage may once again be attributed to cellular death rather than an ineffective compound. As explained above if the cells die rather than have damaged membranes the dye does not adequately portray all damage done by the compound.

Phenanthrene is non classifiable a human carcinogen. It can cause photosensitivity in skin. In some studies it has been shown to exhibit genotoxic effects such as inhibition of cell sheet formation (NTP 2012b). Average percent damages calculated for Phenanthrene were 58.86, 61.73, 47.15, 42.49, 40.13, 30.63,

and 40.27 (figure 4). All percent damage values calculated were less than that of the solvent control with a "*" significance in 5ppm and 10 ppm treatments and a "**" significance in the 10 ppm treatment. These results are surprising and not expected because of the negative respiratory effects seen with Phenanthrene exposure discussed earlier (Gale, 2012; Suresh, 2009). The lack of negative effects seen with this compound may be attributed to wetting loss. When the treatment solutions of PAH were made, only 16 microliters of stock solution was used to make the 20ppm treatment. This solution was serial diluted to make up each of the remaining treatments. It is possible some PAH was sorbed to the pipet tip despite efforts to rinse them thoroughly and deliver all the compound into treatment solutions.

Acenaphthylene is also not classifiable as a human carcinogen. It has been shown to cause irritation to both skin and mucous membranes (NTP 2012a). Percent Damage values calculated for Acenaphthylene were 60.29, 55.48, 54.34, 58.64, 63.42, 79.60, and 71.83 (figure 5). The 2ppm, 5ppm, 10ppm, and 20ppm treatments all showed greater cellular damage than the control and a "*" significance was seen with the 10ppm treatment. Cellular damage can be attributed to Acenaphthylene for the 10ppm treatment. Although not all treatment data for this compound is significant the general increasing trend in damage indicates it may play a role in causing damage to lung tissue and further testing should be done to characterize its toxicity.

Figure 6 shows all percent damage data for Benzo[a]pyrene, Pyrene, Fluoranthene, Phenanthrene, and Acenaphthylene. When compared to each other, Acenaphthylene, Benzo[a]pyrne, and pyrene appear to have caused the most

cellular damage overall. As mentioned above it is surprising Phenanthrene did not exhibit the damage expected because it has been linked to negative respiratory effects.

Cyto Tox-one homogeneous membrane integrity

For this assay, A549 cells were exposed to either the highest or lowest concentration of a polycyclic aromatic hydrocarbon. The assay was then run and evaluated for fluorescence. The assay is designed to measure the release of lactate dehydrogenase from cells and quantify their inflammatory response. However, quantification of the results from this experiment was not possible and only qualitative data was obtainable. The inflammatory response was significant for all the PAHs tested. In fact, the observed fluorescence was at the maximum of the plate reader. To obtain results that could be used to analyze the inflammatory response further testing is being conducted to evaluate lower concentrations of the PAHs. A dose repose curve is needed to evaluate at what concentrations PAHs elicit an inflammatory response. The ultimate goal is to compare these concentrations to those that we see in air concentrations in Texas. Some common PAHs found in air in Texas are Phenanthrene, Fluoranthene, Pyrene, and Acenaphtylene . These PAHs are found at concentrations ranging from 0.06 to 4.57 ng/m^3 (Dejean 2009). Future experimentation needs to be performed to accurately evaluate inflammatory response to the compounds used in this study.

APPENDIX

Benzo[a]Pyrene

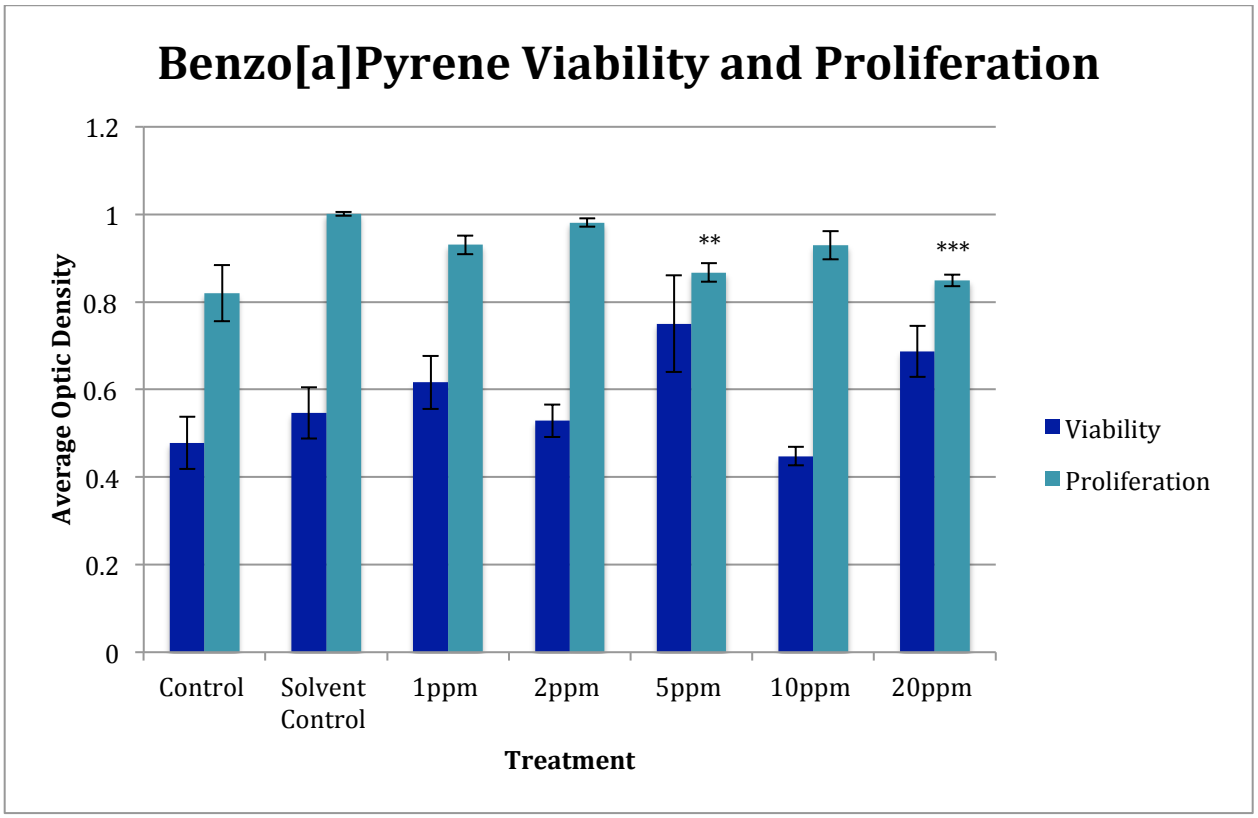

Figure 1.1: Bars represent average optic densities for each treatment measured on an absorbance micro plate reader at a wavelength of 630nm. Error Bars were calculated using standard error. Treatments yielding a result significantly different from the control are denoted with stars. One "*" indicates a student's t-test p-value less than 0.05, two "**" indicates a p-value less than 0.01, three "***" indicates a p-value less than 0.001.

Pyrene

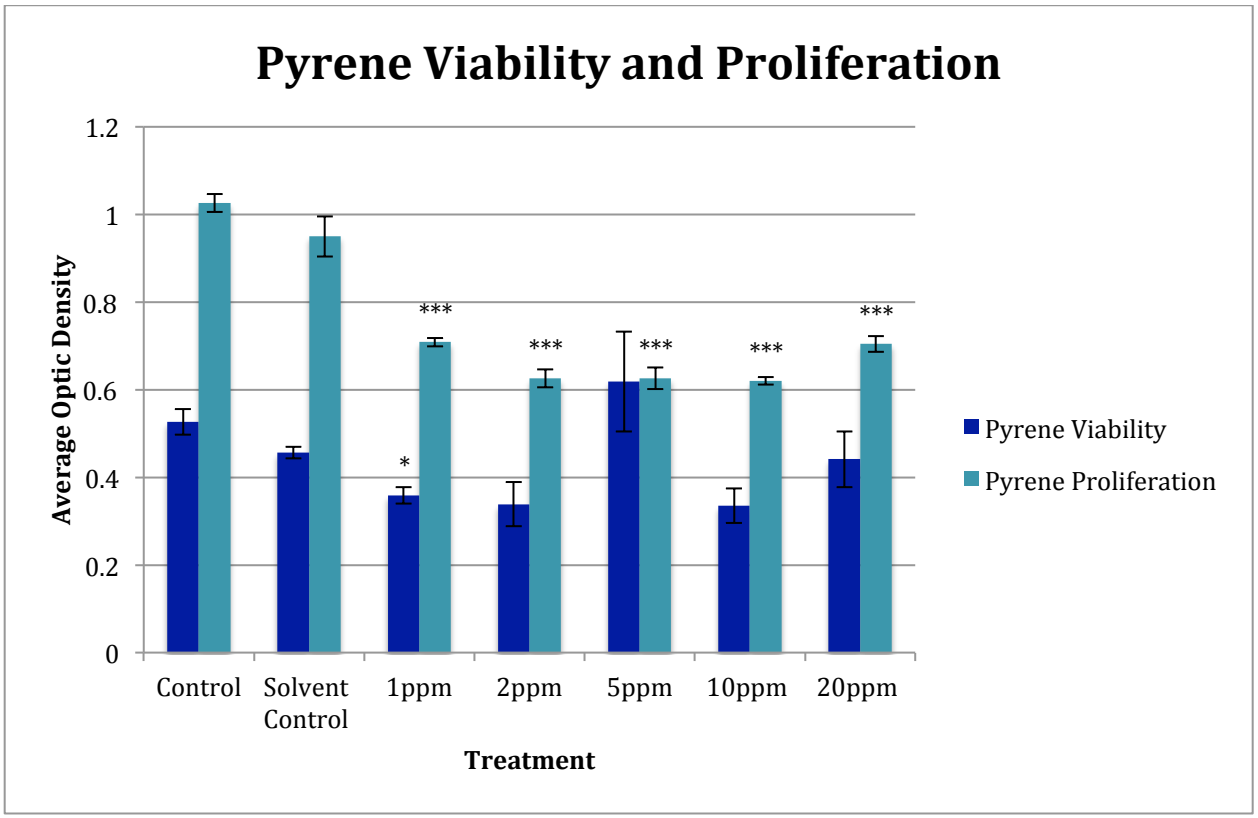

Figure 2.1: Bars represent average optic densities for each treatment measured on an absorbance micro plate reader at a wavelength of 630nm. Error Bars were calculated using standard error. Treatments yielding a result significantly different from the control are denoted with stars. One "*" indicates a student's t-test p-value less than 0.05, two "**" indicates a p-value less than 0.01, three "***" indicates a p-value less than 0.001.

Fluoranthene

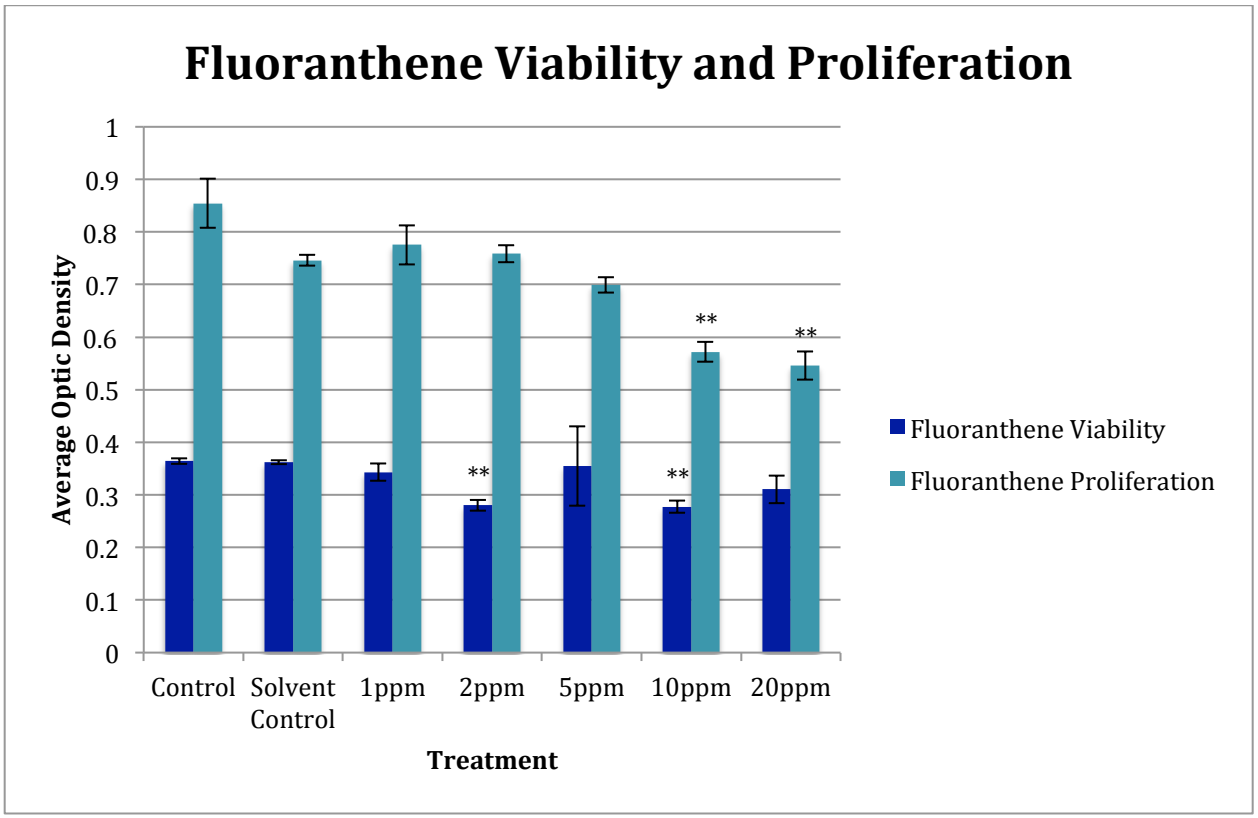

Figure 3.1: Bars represent average optic densities for each treatment measured on an absorbance micro plate reader at a wavelength of 630nm. Error Bars were calculated using standard error. Treatments yielding a result significantly different from the control are denoted with stars. One "*" indicates a student's t-test p-value less than 0.05, two "**" indicates a p-value less than 0.01, three "***" indicates a p-value less than 0.001.

Phenanthrene

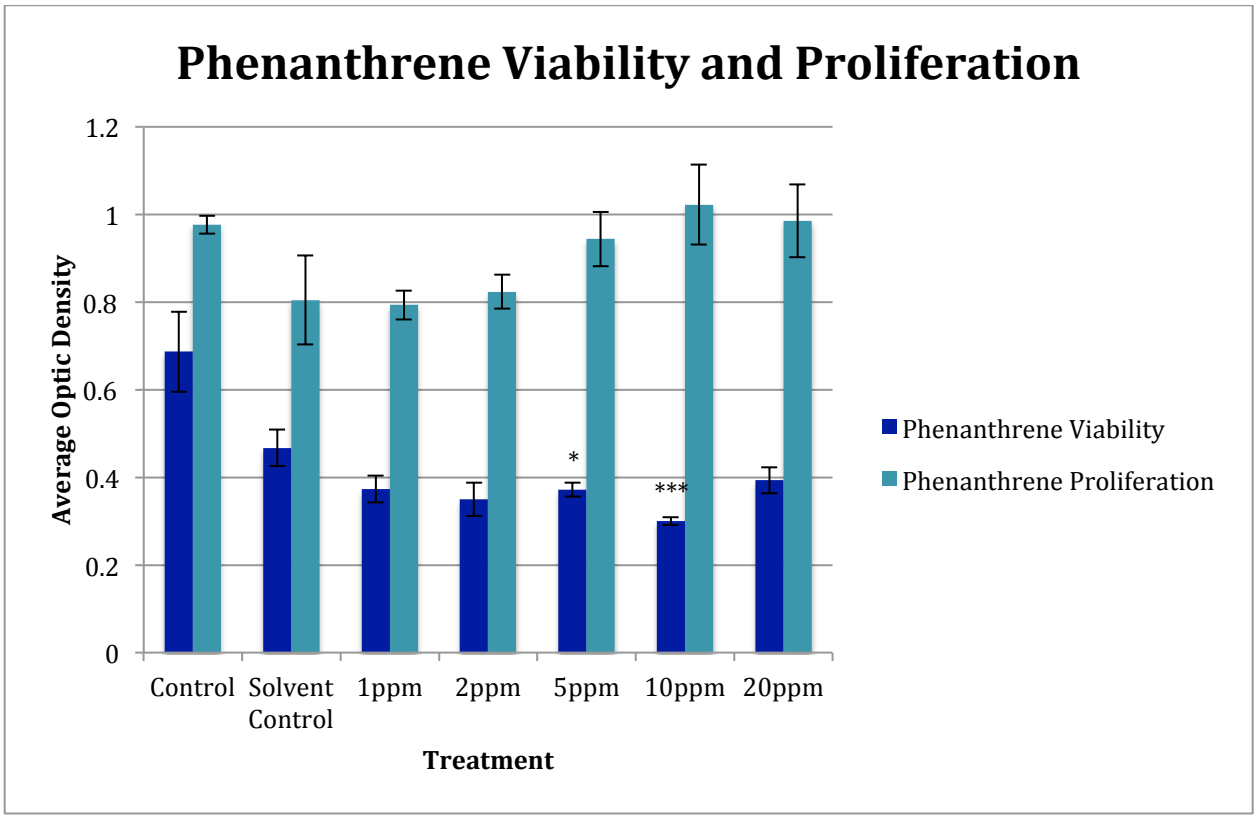

Figure 4.1: Bars represent average optic densities for each treatment measured on an absorbance micro plate reader at a wavelength of 630nm. Error Bars were calculated using standard error. Treatments yielding a result significantly different from the control are denoted with stars. One "*" indicates a student's t-test p-value less than 0.05, two "**" indicates a p-value less than 0.01, three "***" indicates a p-value less than 0.001.

Acenaphthylene

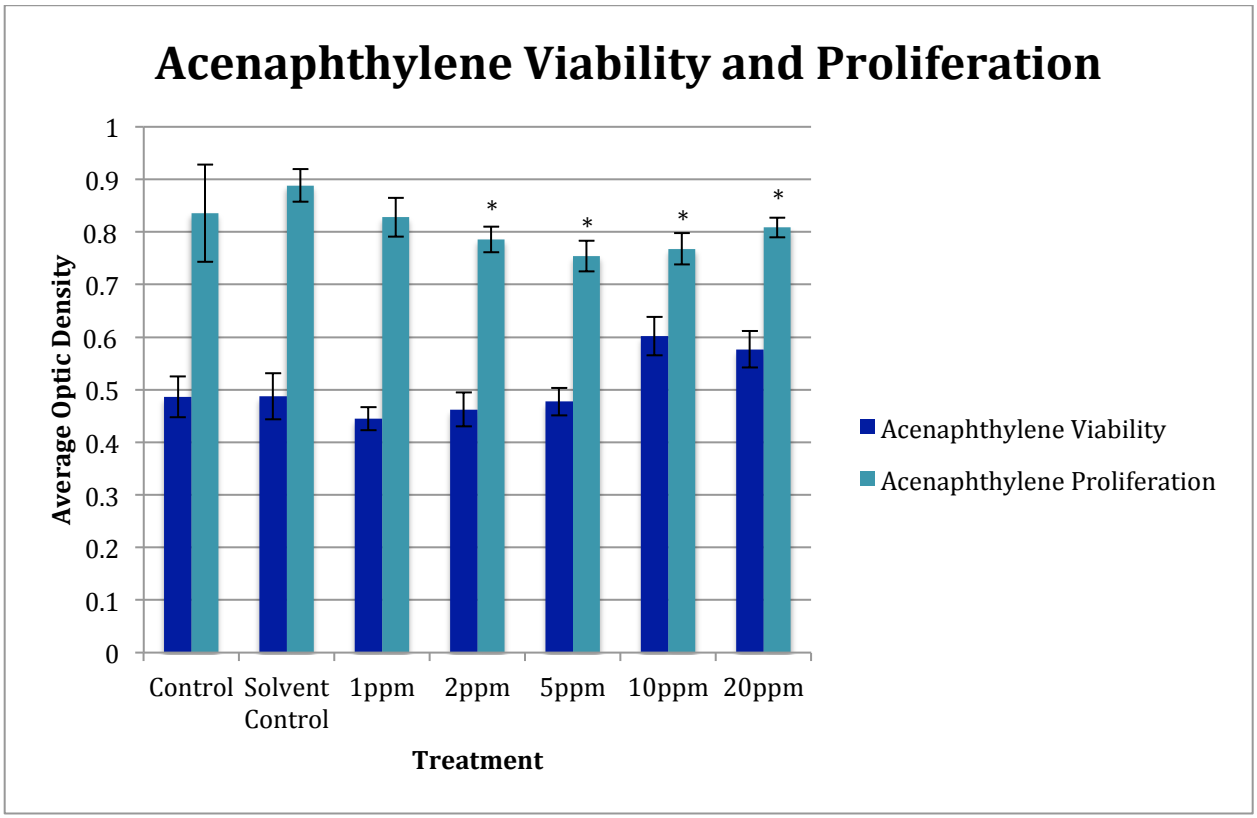

Figure 5.1: Bars represent average optic densities for each treatment measured on an absorbance micro plate reader at a wavelength of 630nm. Error Bars were calculated using standard error. Treatments yielding a result significantly different from the control are denoted with stars. One "*" indicates a student's t-test p-value less than 0.05, two "**" indicates a p-value less than 0.01, three "***" indicates a p-value less than 0.001.

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