EFFECT OF BISPHENOL A ON STRESS-INDUCED PREMATURE SENESCENCE

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ABSTRACT

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Plastics are used for a wide variety of commercial applications all over the world, but recently a number of health risks have been linked to plastics and plastic products. For example, bisphenol A (BPA) is a plasticizer used in the production of plastics such as storage containers, plastic bottles, adhesives, and many other everyday objects. BPA-containing plastics are subject to degradation, resulting in the ingestion of BPA from many different sources. BPA has been shown to cause negative side-effects once ingested due to its ability to act as an estrogen mimic, along with being implicated in the formation of cancers and the metabolic syndrome. BPA has also been linked to inducing oxidative stress, which can lead to damaged DNA, promote tumorigenesis, and can cause cell death in extreme cases. On the other hand, little is known about the effect of BPA on cellular senescence, a state of irreversible cell cycle arrest and a recognized barrier to cancer progression. Because of its pro-oxidizing role, it was hypothesized that BPA could increase the likelihood of cells becoming senescent. To test this, cells were exposed to a variety of experimental conditions for varying lengths of time; they were then stained for β-galactosidase, a widely used biomarker of senescence, and interleukin-6, a pro-inflammatory cytokine linked to senescence. It was found that, although BPA may not have a direct effect on senescence induction in a dose dependent manner, dimethyl sulfoxide (DMSO), the vehicle used to dissolve BPA in this experiment, could have an effect on senescence induction at very low doses.

KEY WORDS: Stress-induced premature senescence, Bisphenol A, β-galactosidase, Interleukin-6, Senescence-associated secretory profile

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CHAPTER I

Introduction

Background

Plastics are constantly used due to their durability, widespread applicability, accessibility, and low cost. However, the overzealous use of these materials has been accompanied with unanticipated problems. Plasticizers, such as Bisphenol A, were discovered to be endocrine disruptors, resulting in reproductive disorders and cancers in humans, and even feminization in aquatic species. In recent years, Bisphenol A has also been linked to metabolic syndrome, diabetes, obesity, and heart disease. Although this chemical has been thoroughly researched as an endocrine disruptor, its effects on senescence, a cellular process with ties to neoplasia, has not yet been examined (Thompson *et al*, 2009; Levy *et al*, 2004; Bhandari *et al*, 2015).

Bisphenol A

Bisphenol A (BPA) is a chemical that is widely used as a commercial plasticizer in the production of polycarbonate plastics. BPA was first synthesized in 1891 by Russian chemist Alexander Dianin (Rubin & Soto, 2009), but it wasn't until the 1950s that its industrial role took off. Initially it was used as an epoxy resin to provide linings and protective coatings on metals such as pipes, the interior of food cans, adhesives for laying floors, as well as dental sealants. In 1957, it was found that polymerized BPA formed a very durable, strong, and clear plastic, and was used in automobiles, food storage containers, water bottles, electronics, and safety equipment (Vogel, 2009).

Unfortunately, BPA-containing plastics can degrade due to incomplete polymerization and unusually high temperatures can lead to the BPA leaching from the

parent material. Consequently, BPA is commonly ingested from various sources, such as contaminated food or water from packaging, or via contact with receipts or other BPA-containing products (Fenichel *et al,* 2013). In a reference population in the United States, BPA was found at concentrations ≥ 0.1 μg/L urine in 95% of the urine samples tested, which suggests that Americans are exposed to this chemical on a regular basis (Calafat *et al*, 2005).

BPA is well established as an endocrine disruptor due to its role as an estrogen mimic, which was first established in the 1930s (Rubin & Soto, 2009). The United States Environmental Protection Agency (EPA) defines endocrine disruptors as substances that interfere with the production, elimination, transport, metabolism, or action of hormones that are responsible for reproduction, homeostasis, and development in humans. Endocrine disruptors can act through nuclear receptors, nonnuclear steroid receptors such as membrane estrogen receptors, non-steroid receptors such as neurotransmitter receptors, orphan receptors, and many other pathways that converge on endocrine and reproductive systems (Diamanti-Kandarakis *et al*, 2009). Even at low concentrations, BPA demonstrates a potency similar to estradiol in endocrine-receptor-dependent signaling pathways, due to the phenol in its structure (Figure 1) (Konieczna *et al*, 2015).

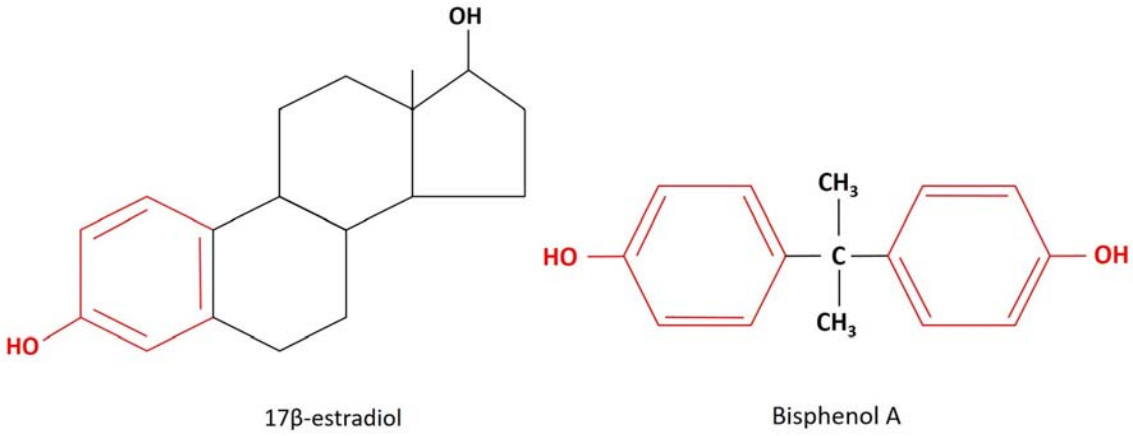

Figure 1. Structures of estradiol and bisphenol A. The phenolic structures are highlighted in red for clarity. (Adapted from Konieczna et al, 2015).

Oxidative Stress

Oxidative stress is the result of an imbalance between antioxidant defenses and reactive oxygen species (ROS), such as free radicals (Betteridge, 2000). Free radicals are especially reactive due to the presence of unpaired electrons (Betteridge, 2000). Regardless of the species, ROS can damage biological macromolecules due to their high reactivity with -C-H, and other bonds. This can result in carcinogenesis, as well as lipid peroxidation, which in turn can lead to necrotic cell death (Bertram & Hass, 2008; Mylonas & Kouretas, 1999; Betteridge, 2000).

Importantly, BPA itself has been demonstrated to induce oxidative stress. It has been shown to induce the production of nitric oxide (NO), a free radical capable of modifying DNA, proteins, and lipids. Moreover, BPA has been shown to lower antioxidant defenses, such as activity of superoxide dismutase (SOD). Taken together, these results suggest that BPA may have an important effect on oxidant-antioxidant scale tipping, whose net effect is an increase in oxidative stress (Eid *et al*, 2015).

Cellular Senescence

Severely mutated or damaged DNA acts as a stress signal for cells, and if the damage becomes so severe that the cell can no longer function properly, an adaptive response known as physiological senescence may be induced. Senescent cells are still viable and metabolically active; however, they will no longer proliferate under even ideal growth conditions. Phenotypically, senescent cells are characterized by a flattened and enlarged morphology; they also show an upregulation of some proteins responsible for halting the progression of the cell cycle. Due to this, senescent cells are in a state of irreversible cell cycle arrest. Cellular senescence is accepted to be an important barrier to tumorigenesis and cancer progression (Bernardes de Jesus & Blasco, 2012).

From a physiological standpoint, there are two types of senescence: (1) replicative senescence and (2) stress induced premature senescence (SIPS). In cases of replicative senescence, cells will have reached a critical number of population doublings, which acts as a trigger to stop proliferation. First described by Leonard Hayflick in 1961 for human fetal pulmonary fibroblasts, it is now known that all non-pluripotent cell types have this limited capacity for cell division; although the number of divisions varies from one cell type to another. This phenomenon is now known as the Hayflick limit and is governed by the erosion of telomeres at the chromosome ends (Hayflick & Moorehead, 1961).

Telomeres, which are "caps" on the ends of chromosomes, consist of repeated guanine sequences that work to provide stability for the DNA molecule (Ichikawa *et al,* 2015). During DNA replication, the enzyme DNA polymerase synthesizes complementary DNA in a 5' to 3' direction. Although this doesn't cause a problem for replication of the leading strand, it does cause imperfect replication for the lagging

strand. More specifically, RNA primers must attach to the lagging strand to allow DNA polymerase to synthesize short sections of DNA called Okazaki fragments. Since there is no room for an RNA primer to attach at the most distal 5' end of the lagging strand, a section of the telomere is lost. When the telomeres reach a critically short length, cell division is arrested to avoid the loss of coding DNA, which would be detrimental to the cell.

On the other hand, stress-induced premature senescence (SIPS) occurs before the telomeres reach a critically short length. SIPS can result from things such as DNA damage, disrupted heterochromatin structure, or oncogene expression, all of which trigger the DNA damage response (DDR) (Chen *et al*, 2007). The DDR then allows the cell to monitor and repair any damage to the DNA molecule (Liang *et al*, 2008). However, because the presence of damaged DNA may be detrimental to the cell if it were allowed to continue to divide, a senescent phenotype is induced. In particular, damaged DNA has been associated with the development of cancers (Ghosal & Chen, 2013; Liang *et al*, 2008).

Pro-Senescent Markers

It is important to note that although there are many biomarkers of senescence, no one particular biomarker is universally reliable. Rather, integrating various markers will allow for a more reliable identification of senescence (Kuilman *et al*, 2010).

Senescence-associated β-galactosidase. This enzyme is a commonly used biomarker of senescence in both replicatively senescent cells, as well as SIPS cells (Debacq-Chainiaux *et al*, 2009). β-Galactosidase is a lysosomal enzyme whose activity can normally be detected at pH 4, due to the acidic nature of the lysosome. However,

since lysosomal β-galactosidase activity increases significantly in senescent cells due to an increased lysosome content, this allows for β-galactosidase activity to be detected at pH 6. Thus, lysosomal β-galactosidase is the origin of senescence-associated β-galactosidase (SA-β-galactosidase), although the exact role SA-β-galactosidase plays in senescent cells is unknown (Kuilman *et al*, 2010; Lee *et al*, 2006). Importantly, SA-β-galactosidase activity has been described only in senescent cells. Although a second cell type (known as quiescent cells) is dormant, the cells exist in a state of reversible dormancy (Cheung & Rando, 2013); that is, they maintain their proliferative potential and are not positive for SA-β-galactosidase (Bernardes de Jesus & Blasco, 2012).

Nevertheless, SA-β-galactosidase alone is an insufficient marker of senescence. This is due to the fact that contact-inhibited cells or serum-starved cells can also display positive SA-β-galactosidase staining (Kuilman *et al*, 2010).

Senescence-associated secretory phenotype. Although it is thought that the induction of a senescence phenotype is tumor suppressive, recently it has been shown that senescent cells produce a secretory profile, known as the senescence-associated secretory phenotype (SASP), that can be pro-carcinogenic downstream of the action of secreted molecules to alter the tissue microenvironment (Coppé *et al*, 2010).

SASP factors can be divided into three categories: (1) soluble signaling factors, (2) secreted proteases, and (3) secreted insoluble proteins/extracellular matrix (ECM) components. These secretory factors can work by cleaving or degrading signaling molecules, and degrading the ECM. The end result of these actions is a significant change in the tissue microenvironment which can lead to various pathologies such as cancer (Coppé *et al*, 2010).

Interleukin-6 (IL-6) is a pro-inflammatory cytokine that falls into the category of a soluble signaling factor. IL-6 is associated with DNA damage-induced senescence in various cell types, including mouse fibroblasts (Coppé *et al*, 2010). IL-6 has also been implicated in the pathogenesis of cancers such as breast cancer, prostate cancer, pancreatic cancer, multiple myeloma, and Hodgkin's Lymphoma (Reynaud *et al*, 2011). Since IL-6 is a well-established SASP factor, it can be used as a marker of senescence in conjunction with other markers.

Other markers of senescence. p38, a mitogen-activated (MAP) kinase, is involved in various cellular processes such as the regulation of apoptosis, cell cycle checkpoint regulation, inflammation, and cellular development and differentiation. More recently, it has been implicated in the induction of cellular senescence (Harada *et al*, 2014) via the initiation of a pathway whereby MKK3/MKK6 kinases phosphorylate and activate p38. In turn, p38 then phosphorylates and activates p53 and p21 while alternately activating p16 (Figure 2) (Muñoz-Epsín & Serrano, 2014).

D-type cyclins recognize extracellular growth stimuli, which then promote proliferation (Foster *et al*, 2001). p16INK4a is a tumor suppressor protein that inhibits D-cyclin-Cdk complexes, and works in cellular senescence by arresting the cell in the G_1 phase of the cell cycle (Rodier & Campisi, 2011). In normal proliferating cells, the level of this protein is extremely low; however, there is an overexpression of p16INK4a in senescent cells. The expression is likely linked to stress signaling pathways (Ohanti *et al*, 2004).

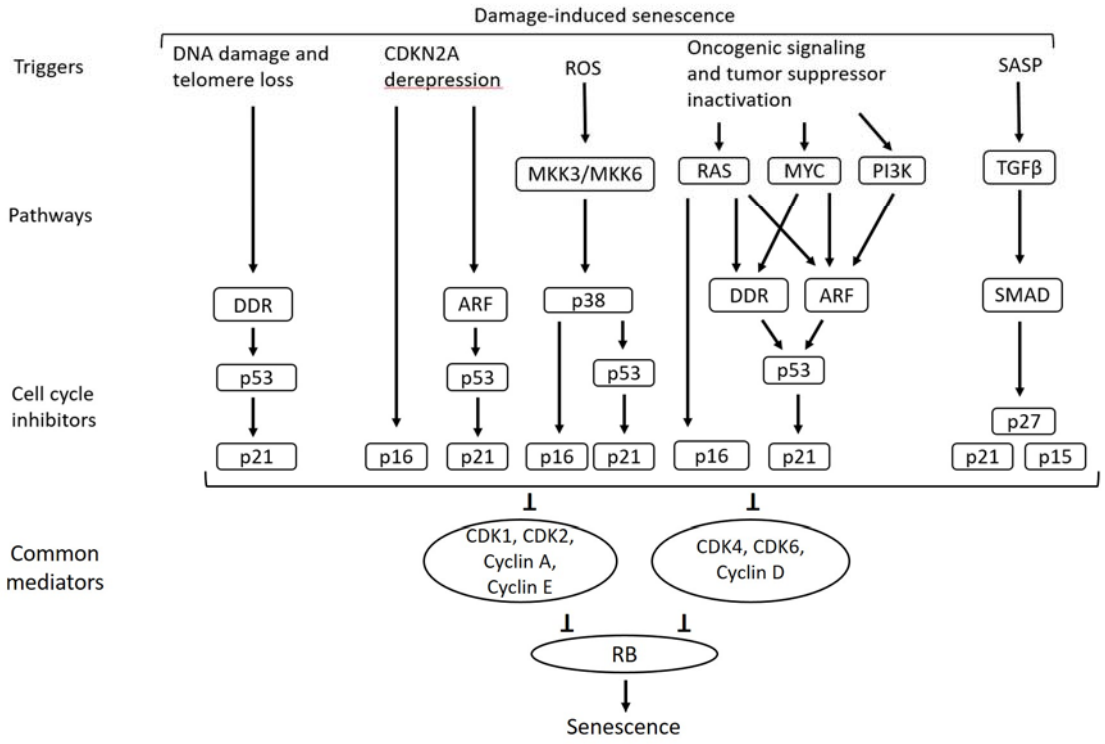

Figure 2. Molecular pathways of senescence. An overlook at the various pathways that induce senescence, converging at various cyclins and cyclin-dependent kinases (CDKs). (Adapted from Muñoz-Epsín & Serrano, 2014).

Hypothesis and Objectives

My null hypothesis was that exposing NIH/3T3 cells to BPA would not affect the induction of SIPS. My specific hypothesis was that exposing these cells to BPA would cause an increase in the number of senescent cells due to the presumed pro-oxidizing effect of BPA (Iso *et al*, 2005). The objectives of this project were: (1) to develop and optimize a protocol to induce SIPS in the NIH/3T3 cell line using serum-free media conditions and Bisphenol A and (2) to determine whether chronic exposure to BPA increases the induction of SIPS by evaluating SA-β-galactosidase staining as well as the concentration of interleukin-6.

CHAPTER II

Materials and Methods

Maintenance of Cell Culture

NIH/3T3 cells (Sigma-Aldrich and the European Collection of Authenticated Cell Cultures) were used for all aspects of this project and were maintained under standard culture conditions. In particular, cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% heat inactivated fetal bovine serum, and anti-microbial agents (penicillin, streptomycin, and amphotericin B). The cells were kept in an incubator at 37°C with 5% CO_2.

Protocol Development

NIH/3T3 cells were seeded at 20,000 cells per well in a 96-well plate, and left to attach overnight in DMEM as described above. The next day, the media was removed, the cells were washed with 1X PBS and growth media was replaced with serum-free DMEM supplemented with 2% bovine serum albumin (BSA) and anti-microbial agents. Serum-free culture conditions prevent continued cellular proliferation (Brunner *et al*, 2010).

Replicate cell cultures in 96-well plates were exposed to one of five different conditions: (1) only serum-free media, (2) serum-free media supplemented with 0.1% DMSO, (3) 1 μM BPA in serum-free media, (4) 10 μM BPA in serum-free media, and (5) 100 μM BPA in serum-free media. The second condition served as the vehicle control since BPA is not soluble in aqueous solutions. BPA was dissolved in 100% DMSO prior to its dilution to the final working concentration in the DMEM. In addition, individual cultures were maintained under these conditions for varying amounts of time:

(1) fourteen days, (2) ten days, (3) five days, (4) three days or (5) one day. Cells that were subcultured and allowed to attach overnight in complete media only served as a negative control. These cells were not exposed to any of the experimental conditions and were maintained in parallel with the test cultures for no more than 24 hours (Figure 3). For cultures maintained longer than 3 days, the serum-free media was changed every three days for the duration of the experiment. These cells were used for β-galactosidase staining.

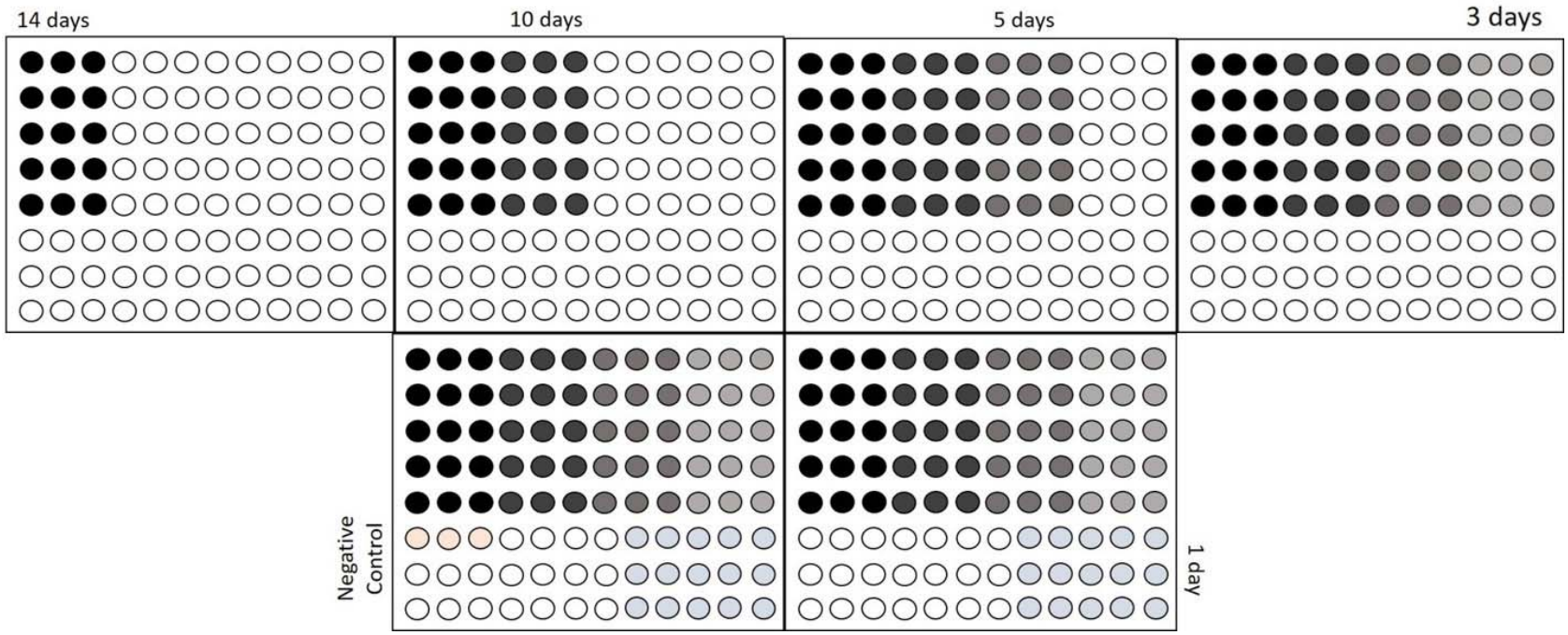

Figure 3. Experimental design. Triplicate cell cultures in a 96-well plate seeded in a staggering fashion. Seeding was staggered so staining could be performed at the conclusion of the fourteen-day experiment.

Lysate Collection

In order to determine the effect of these culture conditions on the SASP, or more specifically IL-6 secretion, parallel cultures of NIH/3T3 cells were seeded into 100mm dishes and left to attach overnight. Cells were treated as described above for the following lengths of time: (1) five days, (2) three days, (3) one day, and (4) overnight (a negative control); afterwards, the cells were lysed using RIPA buffer. These cultures were not maintained for any period greater than five days based on preliminary β-galactosidase staining data showing no appreciable difference in the number of senescent cells after five days. The lysates were stored in microcentrifuge tubes at -80°C until assayed.

β-Galactosidase Staining

β-Galactosidase staining was performed using a staining kit purchased from Cell Signaling Technology. In brief, cells were fixed in the plate using the fixative solution, and a β-galactosidase staining solution was made and brought to a pH between 5.9 and 6.1. The stain was applied to cells and left overnight to develop in an incubator at 37ºC with no CO_2.

In order to quantify the degree of senescence, individual photos were taken from each well of a 96-well plate that was manually divided into four quadrants prior to initiating the experiment. In each case, only 2 of the 4 quadrants (top left and bottom right) were used to capture the image. Afterwards, five individuals objectively scored the number of cells in each photograph as either blue or not blue which was used to estimate the percentage of blue cells in each photograph. Afterwards, the average for each

experimental condition per individual was generated and the median of the five individuals' scores was used for the analysis. The data were analyzed using a general linear model ANOVA with the time in culture and concentration of BPA set as the main effects. Significant main effects were further analyzed using Tukey's post hoc test. All statistical analysis was performed using Minitab 16.

Enzyme-Linked Immunosorbent Assay:

Prior to assay, the total protein content of individual lysates was determined using the bicinchoninic acid (BCA) assay. This was done so that the same amount of total protein was used in every well of the ELISA. Then, IL-6 levels in individual lysates were quantified using an Enzyme-Linked Immunosorbent Assay (ELISA) for mouse from eBioscience (Ready-SET-Go!) according to the manufacturer's instructions. The data were analyzed using a general linear model ANOVA test with the time in culture and concentration of BPA set as the main effects. All statistical analysis was performed using Minitab 16.

A simple linear regression model was generated to see if there was a relationship between the degree of β-galactosidase staining and secreted levels of IL-6 in cultures maintained under identical conditions.

CHAPTER III

Results

Protocol Development

Protocol development initially began by utilizing paraquat and cadmium to induce SIPS due to their well-known ability to cause oxidative stress in cells. Since the experiment was run using growth media, supplemented with fetal bovine serum, the cells continued to divide, even after reaching confluence. Because of this, a separate experiment was run using serum-free media in parallel with the growth media. It was determined that using serum-free media helped to curb this phenomenon; therefore, serum-free media was used for the remainder of the experiments. During these trials, it was found that prolonged culture in the serum-free media created a stressful environment for the cells, even in the absence of paraquat or cadmium; therefore, serum-free media was adopted to induce baseline stress for the experiments.

β-Galactosidase Staining

There was a significant main effect of treatment day on β-galactosidase staining only; exposure to BPA did not affect β-galactosidase staining in this model (*Table 1*). The result of Tukey's post-hoc test indicated that β-galactosidase staining was significantly higher ($p < 0.05$) after fourteen days of exposure (labeled C in Figure 4) relative to all other treatments. In addition, exposure to BPA for either ten or five days resulted in a similar degree of β-galactosidase staining (labeled B in Figure 4) that was significantly greater than that seen after one day of exposure (labeled A in Figure 4).

Table 1

ANOVA results for β-galactosidase Staining

	Degrees of Freedom	F-score	P-value
Treatment Day	3	17.41	< 0.01
Experimental Conditions	4	1.08	0.382
Treatment Day* Experimental Conditions	12	0.47	0.916

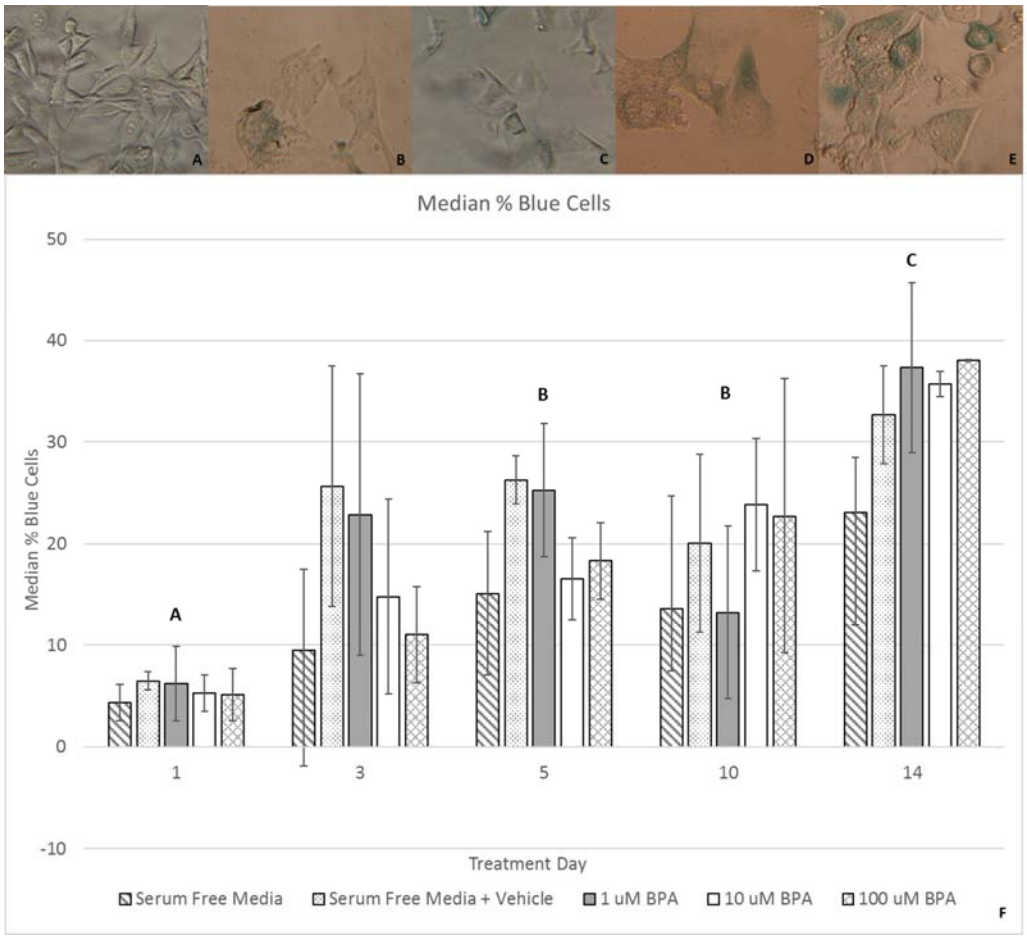

Figure 4. The effect of serum-free media, DMSO, & BPA exposure on β-galactosidase staining on NIH/3T3 cells. Top panel: representative picture of cells at each treatment day (A) one day; (B) three days; (C) five days; (D) ten days; (E) fourteen days; Bottom panel: (F) % mean (+/- SEM) β-galactosidase positive cells under each experimental condition, (n=3). Three day results are present in the graph but not included in the statistical analysis (n=2). Tukey's post hoc test results displayed above each treatment day grouping.

Enzyme-Linked Immunosorbent Assay

There was a significant main effect of treatment day on interleukin-6 concentrations. There was also a main effect of doses of BPA on interleukin-6 concentrations. The interaction term was not significant, suggesting that the effect of the experimental conditions was the same regardless of the number of days exposed (*Table 2;* Figure 5).

Table 2

ANOVA results for the Interleukin-6 ELISA

	Degrees of Freedom	F-score	P-value
Treatment Day	2	19.22	< 0.01
Experimental Conditions	4	4.61	0.005
Treatment Day* Experimental Conditions	8	1.89	0.100

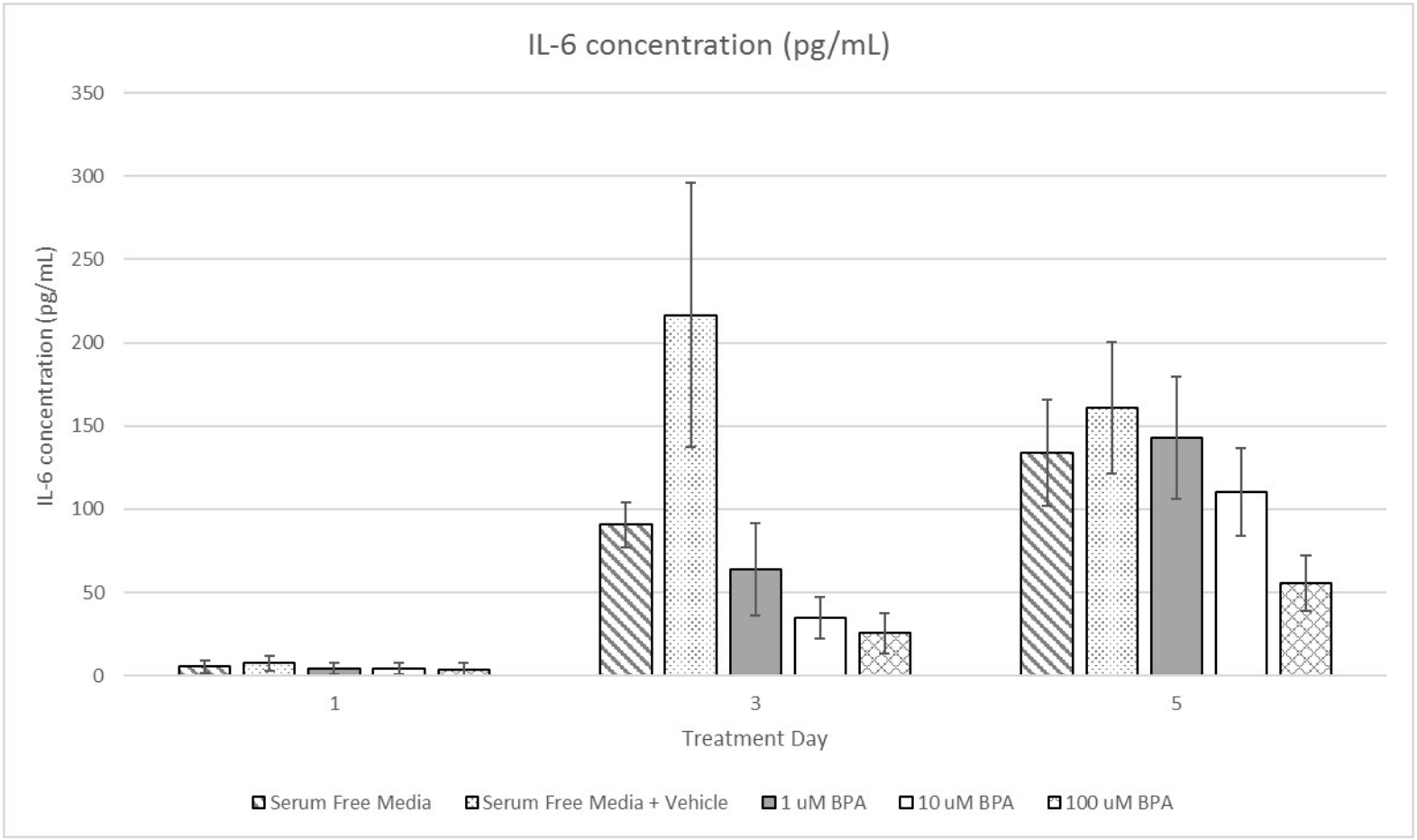

Figure 5. The effect of serum-free media, DMSO, & BPA on IL-6 levels. Mean interleukin-6 levels (+/- standard error mean) in cells treated with serum-free media, serum-free media + vehicle, 1 µM BPA, 10 µM BPA, and 100 µM BPA per each treatment day, (n=3).

Regression Analysis

Using simple linear regression, we found that there was a significant relationship between the degree of β-galactosidase staining and secreted IL-6 levels ($R^2 = 0.69$, $p < 0.01$). More specifically, as the number of cells positive for β-galactosidase staining increased, the level of IL-6 also increased. (Figure 6).

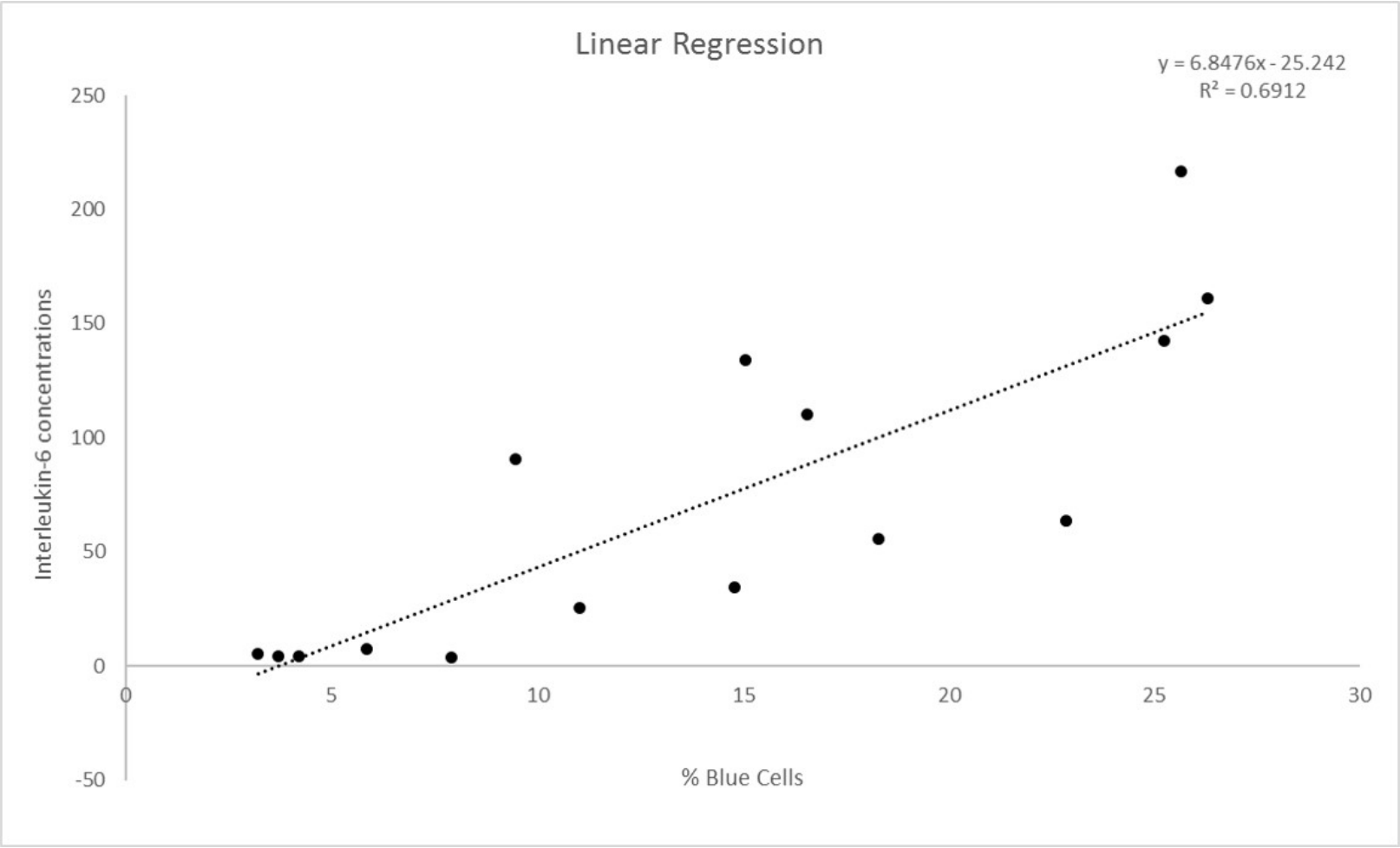

Figure 6. IL-6 levels increase as a function of β-galactosidase staining in senescent NIH/3T3 cells. Data are from cells grown in serum-free media for either 1, 3 or 5 days only in the presence of different doses of BPA. Each point represents the mean value of the secreted IL-6 against the mean value for β-galactosidase staining for each treatment day*experimental condition.

CHAPTER IV

Discussion

General Discussion

I found that the number of β-galactosidase-positive cells and the secretion of interleukin-6 increased as the number of treatment days increased, even in the absence of DMSO and/or BPA. These results were expected due to the stressful environment created from using serum-free media, along with the expected increase in oxidative stress caused by DMSO and BPA. The NIH/3T3 cell line was used for this project. This is a murine embryonic fibroblast cell line, which has been used in other studies on premature senescence (Chen *et al*, 2002; Volonte *et al*, 2002; Dasari *et al*, 2006) as well as studies on effects of BPA (Chen *et al*, 2016); however, it has not been previously used to look at possible connections between BPA and premature senescence.

There was also a strong positive correlation between the results of the two markers used in this project, which has also been observed in other studies (Zurgil *et al*, 2014). However, it should be noted that the interleukin-6 data were generated from parallel cultures and is only exploratory. The level of interleukin-6 in cultures subjected to β-galactosidase staining was never directly assessed. Since both β-galactosidase staining and interleukin-6 levels are used as markers for senescence, it would be expected that the results corroborate each other. Other markers of senescence should be measured in the future, such as those included in the senescence-associated secretory phenotype, to see if the results found here could be further corroborated.

The one thing that did remain consistent across all of the treatment days was that the number of β-galactosidase-positive cells and the concentration of interleukin-6

dramatically increased upon the addition of DMSO. However, there was no additive effect of either BPA or the time spent under serum-free conditions. More specifically, despite the increase in secreted interleukin-6 level with the treatment day, the highest interleukin-6 level was seen after three days, not five days as predicted (Figure 5). This may be due to the high variability and small sample, however. Meanwhile, the number of β-galactosidase positive cells was consistently higher with the duration of exposure to serum-free conditions, especially in the presence of DMSO alone or in conjunction with BPA. BPA itself had no significant effect on β-galactosidase staining (Figure 4).

Interestingly, in cells maintained under serum free conditions for ≤ 5 days, there was a dose-dependent effect of BPA on interleukin-6 secretion in that higher doses of BPA are associated with declining levels of interleukin-6 secretion (Figure 5). This is in contrast to previous studies showing that BPA can lead to dysfunction of inflammatory pathways and increased interleukin-6 levels in cells (Ben-Jonathan *et al*, 2009; Valentino *et al*, 2013). In both of the studies mentioned, ethanol was used as the vehicle to dissolve BPA prior to use in culture. This experiment utilized cell lysates in order to measure intracellular interleukin-6 levels; however, future experiments can utilize secreted levels of interleukin-6 by saving and assaying the media.

Although there was a statistically significant correlation between the interleukin-6 and β-galactosidase markers, individually, the markers revealed differences in how BPA exposure affected the induction of senescence. An increase in BPA caused interleukin-6 levels to decrease, however BPA levels did not have a significant effect on β-galactosidase staining. This discrepancy could have occurred due to human error of counting blue cells as part of the β-galactosidase staining analysis.

Since plastic tubes were used throughout the length of the experiments, there was a chance of potential leaching of BPA. However, the tubes were never heated, each of the experimental conditions was made immediately prior to use, and the tubes were never reused. Because of these precautions, the chance of BPA leaching was very minimal, and was not a concern during this project.

DMSO is commonly used as a solvent for chemical compounds in cell culture studies. Since BPA does not dissolve in aqueous solutions, it was dissolved in 100% DMSO prior to being diluted in serum-free media to the final working concentrations: 1 μM, 10 μM, and 100 μM BPA. This resulted in the highest working concentration of DMSO to be at 0.1% which was adopted as the vehicle control for this study.

My data suggests that DMSO alone was sufficient to cause a dramatic increase in both β-galactosidase staining and interleukin-6 secretion (Figures 4 and 5). Previous studies have found that at concentrations of 0% - 5%, DMSO can induce the generation of intracellular ROS in a dose-dependent manner (Yuan *et al*, 2014). This increase in ROS could account for the results seen here, since ROS generation is closely linked with stress-induced premature senescence (Coleman *et al*, 2010; Betteridge, 2000). Since DMSO seems to influence the results by increasing the number of senescent cells, even in the absence of BPA, a different vehicle, such as ethanol or olive oil, should be used in the future to account for this. These vehicles have been used when dissolving BPA for use in cell culture-based studies, and did not appear to have an effect on the results of the studies (Valentino *et al*, 2013; Ribeiro-Varandas *et al*, 2014; Eid *et al*, 2015).

In conclusion, it seems as though BPA does not have an effect on the induction of stress induced cellular senescence *in vitro* in NIH/3T3 cells as hypothesized. Neither β-

galactosidase staining nor secreted levels of interleukin-6 were significantly different in the presence of BPA in cells grown under chronic serum-free conditions (Figures 4 and 5). However, exposure to a low dose of DMSO (0.1%) alone did result in a senescent phenotype in this cell line. Although DMSO has been used as the vehicle for other studies of senescence induction (Qin *et al*, 2012; Marazita *et al*, 2016), no apparent effect was reported at any concentration of DMSO below 1%, while intracellular ROS was unchanged regardless of the concentration used. The reason for this discrepancy remains unknown, but it may have to do with the specific cell lines used, and/or the methods used to induce senescence. The effect of maintaining cells under chronic serum-free conditions has not been well-studied in the context of SIPS, and should be investigated further in future experiments.

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APPENDIX

Bicinchoninic Acid (BCA) Assay Results

Plate Number	Treatment	Total Protein Content (μg/mL)
1	Serum Free Media, 5 days	1205.60
1	Serum Free Media, 3 days	1110.71
1	Serum Free Media, 1 day	687.35
1	Serum Free Media + 0.1% DMSO, 5 days	919.71
1	Serum Free Media + 0.1% DMSO, 3 days	766.42
1	Serum Free Media + 0.1% DMSO, 1 day	746.96
1	1 μM, 5 days	896.59
1	1 μM, 3 days	796.84
1	1 μM, 1 day	802.92
1	10 μM, 5 days	871.05
1	10 μM, 3 days	874.70
1	10 μM, 1 day	766.42

(continued)

Plate Number	Treatment	Total Protein Content (μg/mL)
1	100 μM, 5 days	811.44
1	100 μM, 3 days	985.40
1	100 μM, 1 day	187.35
2	Serum Free Media, 5 days	857.66
2	Serum Free Media, 3 days	911.19
2	Serum Free Media, 1 day	850.36
2	Serum Free Media + 0.1% DMSO, 5 days	818.73
2	Serum Free Media + 0.1% DMSO, 3 days	895.38
2	Serum Free Media + 0.1% DMSO, 1 day	799.27
2	1 μM, 5 days	832.12
2	1 μM, 3 days	984.18
2	1 μM, 1 day	756.69
2	10 μM, 5 days	763.99

(continued)

Plate Number	Treatment	Total Protein Content (μg/mL)
2	10 μM, 3 days	884.43
2	10 μM, 1 day	804.14
2	100 μM, 5 days	781.02
2	100 μM, 3 days	823.60
2	100 μM, 1 day	875.91
3	Serum Free Media, 5 days	1065.70
3	Serum Free Media, 3 days	822.60
3	Serum Free Media, 1 day	695.14
3	Serum Free Media + 0.1% DMSO, 5 days	946.12
3	Serum Free Media + 0.1% DMSO, 3 days	871.22
3	Serum Free Media + 0.1% DMSO, 1 day	793.69
3	1 μM, 5 days	917.21
3	1 μM, 3 days	848.88

(continued)

Plate Number	Treatment	Total Protein Content (μg/mL)
3	1 μM, 1 day	751.64
3	10 μM, 5 days	939.55
3	10 μM, 3 days	977.66
3	10 μM, 1 day	848.88
3	100 μM, 5 days	981.60
3	100 μM, 3 days	917.21
3	100 μM, 1 day	792.38

VITA

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Education

M.S. Biology
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B.S. Biology with a minor in Chemistry
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Research Experience

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Working with James M. Harper, Ph.D
Thesis focus: Examine the influence of Bisphenol A on stress-induced premature senescence in 3T3 fibroblasts.

Undergraduate Research, August 2014
Working with James M. Harper, Ph.D
Evaluated the effect of BPA and DiPB on cytotoxin resistance in duck and mouse dermal fibroblasts and human pulmonary fibroblasts. The cytotoxins used were cadium, paraquat, and UV-C radiation.

Undergraduate Research, January 2013 – May 2014
Working with James M. Harper, Ph.D
Focused on deriving protocols for inducing stress-induced premature senescence in mouse cell lines.

Employment History

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Human Physiology, August 2015 – May 2016
Molecular Biology, January-May 2015
General Physiology, January-May 2015
Endocrinology, January-May 2015
Contemporary Biology, August-December 2014
Sam Houston State University, Huntsville, Texas.

Writing Center Tutor, August 2013 – May 2014
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Office Clerk, April 2012 – November 2012
Texas Surgery Center, Huntsville, Texas.

Conferences and Presentations

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Effect of Environmental Pollutants on Dermal Fibroblasts
Llano River Field Station, Texas Tech University
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Biological Sciences Graduate Research Symposium
Effect of Bisphenol A on Cellular Senescence
Texas Research Institute for Environmental Studies (TRIES)
Sam Houston State University
December 2014

Excellence in Basic and Translational Research
Effect of Environmental Pollutants on Dermal Fibroblasts
University of Texas at San Antonio
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Publications

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Community Outreach and Leadership

Undergraduate Research Symposium
Faculty Moderator
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Member
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Member
Sam Houston State University, Huntsville, Texas.
Lifetime Member as of 2013

Sam Houston Association of Medically Oriented Students (SHAMOS)
Historian
Sam Houston State University, Huntsville, Texas.
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Sam Houston Association of Medically Oriented Students (SHAMOS)
Member
Sam Houston State University, Huntsville, Texas.
2011-2014

Study Abroad Course
"Discoveries in Chemistry and Textiles"
Coordinator: Rick White, Ph.D
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Honors & Awards

College of Sciences Summer Stipend for Thesis Research
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Dean's List
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