Bringing BPA to Light: Determination of Bisphenol A in Thermal Receipt Paper Water Samples and Lake Water Samples Using Fluorescence Spectrophotometry

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SENIOR THESIS APPROVAL

This Honors thesis entitled

"Brands Crossing Borders: Cross-cultural Issues in Brand Localization in and from China"

written by

Molly Catherine Bowman

and submitted in partial fulfillment of
the requirements for completion of
the Carl Goodson Honors Program
meets the criteria for acceptance
and has been approved by the undersigned readers.

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May 5, 2015

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Abstract

Bisphenol A (BPA) is a chemical that is commonly used in harder plastic products, in the lining of food cans, and in thermal receipt paper. The usage of BPA in common products has become a concern for humans, especially for infants and young children, because of its effects as an endocrine disrupter. BPA has been linked to diabetes, cardiovascular disease, obesity, and certain developmental disorders. Because of the possible effects of BPA on the development of infants and young children, the use of BPA in baby bottles and other baby products has been outlawed. However, BPA is still used in the lining of canned goods and thermal receipt papers.

The purpose of this experiment was to use fluorescence spectrophotometry to test the amount of BPA that leached from thermal receipt papers into water samples that surrounded the paper. Also, lake water samples were tested for the presence of BPA. BPA was both detected and quantified in all of the receipt water samples and lake samples that were tested.

Introduction

Bisphenol A (BPA) is a monomer that is commonly used in making polycarbonate plastics, epoxy linings inside food cans, and thermal papers, as well as other commonly used items. In plastics, BPA helps provide durability, while in the lining of food cans, it helps to provide a barrier between the metal can and the food inside the can. [1-3] In thermal paper, BPA acts as a color developer that reacts with a thermochromic dye to produce colored writing when activated by heat. [4-8] However, BPA has been associated with several adverse health effects. The most widely investigated concern is the effect of BPA as an endocrine disrupter. BPA is similar in structure to estradiol and it binds to estrogen receptors, activating those receptors.

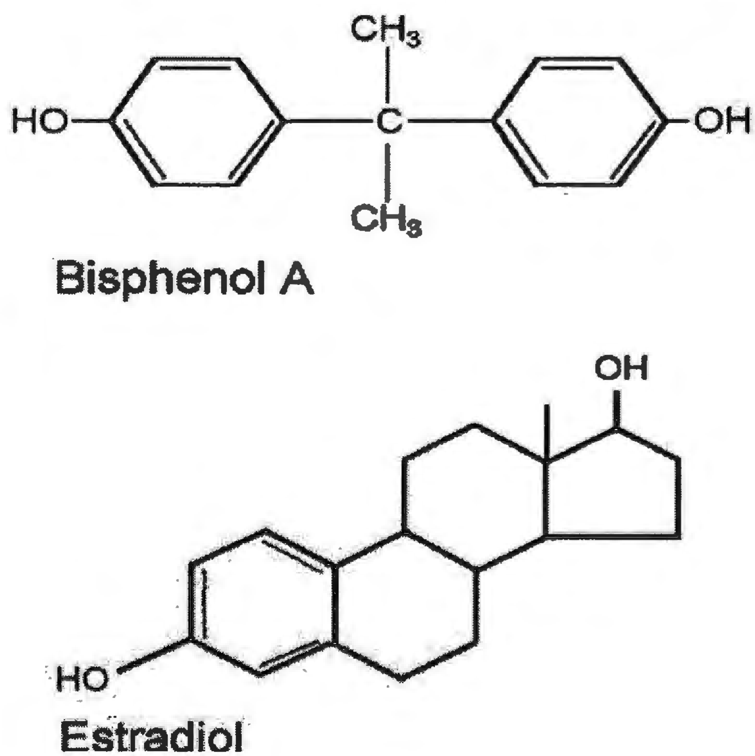

Figure 1. Comparison of BPA and estradiol[9]

Estrogen affects many different aspects of development, such as fetal development and reproductive and sexual development. Therefore, when abnormal amounts of estrogen receptors are activated, these developmental stages can be altered. Also, BPA exposure has been associated with diabetes, obesity, cardiovascular disease, and infertility. [1, 3-7, 10-16]

The most common means of human exposure to BPA is through canned foods and foods that are contained in plastic packages. In 2013, Liao and Kannan tested the concentrations of BPA in 267 food samples collected from various stores in Albany, NY. They found that 199 out of the 267 food samples contained varying amounts of BPA. There were higher mean concentrations of BPA in the canned foods than in foods in plastic packages. [18] In an earlier study, Schecter, et. al. tested the concentrations of BPA in 105 canned foods bought from various stores in Dallas, TX. They found detectable concentrations of BPA in 63 of the 105 samples, with the highest concentration in cans of Del Monte Fresh Cut Green Beans. They also examined the relationship between the concentrations of BPA and the pH of the foods. It appeared that there were higher concentrations of BPA in foods that had a pH near 5. However, there was not a direct correlation signifying a relationship between pH and the concentration of BPA. [1]

Another source of concern for human exposure to BPA is through thermal receipt paper. In thermal receipt paper, BPA is in a more free state. Therefore, it is easier for the BPA to be transferred to other products or absorbed through the skin of the hands of consumers who touch the receipt paper. [3-5, 8, 19] Geens et. al. tested the concentration of BPA in several thermal paper receipts from various stores in Belgium. They found detectable amounts of BPA in each of the 44 thermal paper receipts. Because thermal receipt paper is something that most people handle, it is a concern that BPA is being absorbed through the skin of those who handle the paper. [5] In addition to consumers being exposed to BPA through handling thermal receipt paper, researchers are concerned that the recycling of receipt paper is causing concentrations of BPA to be in other recycled paper products. Liao et. al. tested several thermal paper receipt samples as well as other paper product samples made from both recycled and non-recycled paper. They found higher concentrations of BPA in the recycled papers than in the non-recycled papers. Thus, people may be even more exposed to BPA if thermal receipt papers are recycled along with other types of paper. In addition, they are concerned that receipt papers that end up in landfills could transfer BPA into the water that runs off of the landfills into the water supply. BPA leaching into the water supply could add to the concentration of BPA in the environment, thus exposing people to greater amounts of BPA. [8]

Objectives

The purpose of this experiment was to test the amount of BPA leaching into water samples surrounding thermal receipt papers both at room temperature and at elevated temperatures. Also, water samples from Degray Lake were sampled for the presence of BPA.

Methods

Instruments and Software

A Hitachi F-2000 fluorescence spectrophotometer was used to obtain fluorescence intensity readings for each receipt paper sample and each lake water sample. A Hewlett-Packard UV-Visible Instrument with UV-Visible ChemStation Software was used to determine the excitation and emission wavelengths, which were 278 nm and 304 nm, respectively. These wavelengths were in agreement with the wavelengths found during research in the previous summer.

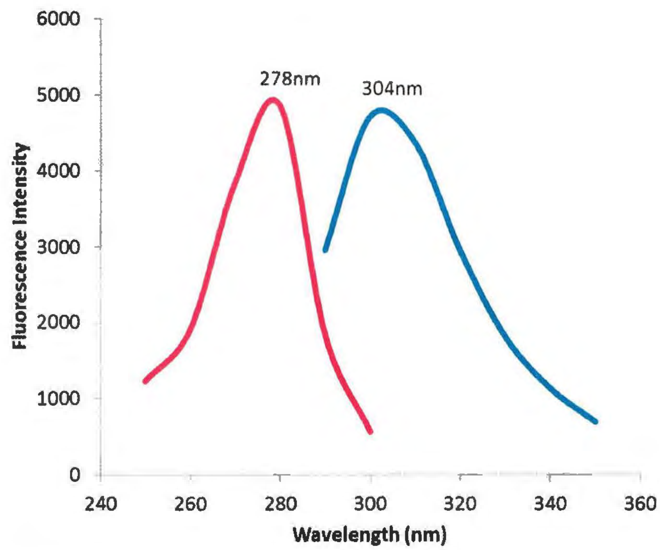

Figure 2. Excitation and emission wavelengths of BPA [20]

A Fischer Scientific Isotemp-205 water bath was used to heat the receipt water samples. A Fischer Scientific oven was used for drying all glassware. Microsoft Excel was used to organize and interpret data.

Reagents

HPLC-grade Methanol and Water were used to make a 1:1 methanol water solution, which was used as the solvent in making samples. Both of these compounds were purchased from Sigma-Aldrich. The HPLC-grade Water was also used as the liquid in which the receipt paper sat during the testing process. Bisphenol A purchased from Sigma-Aldrich was used to make the standard BPA solutions. Thermal receipt paper was purchased from Office Depot. Lake water was collected from Degray Lake. All glassware was cleaned with 50% HNO_3.

Preparation of Samples

For receipt paper samples:

A six-inch piece of thermal receipt paper was placed in 100 mL of HPLC-grade water inside a 150 mL beaker. Receipt water samples were tested in four different settings. The first group was allowed to sit in the dark at room temperature for seven hours. Aliquots were removed at 1-hour increments. The second group was allowed to sit in the dark at room temperature for a total of 96 hours, and aliquots were removed at 24-hour increments. The third group was heated at 37°C for 4 hours, with aliquots being removed at 1-hour increments. The last group was heated at 50°C for 4 hours, and aliquots were removed at 1-hour increments. The standard addition method was used to prepare the samples for testing.

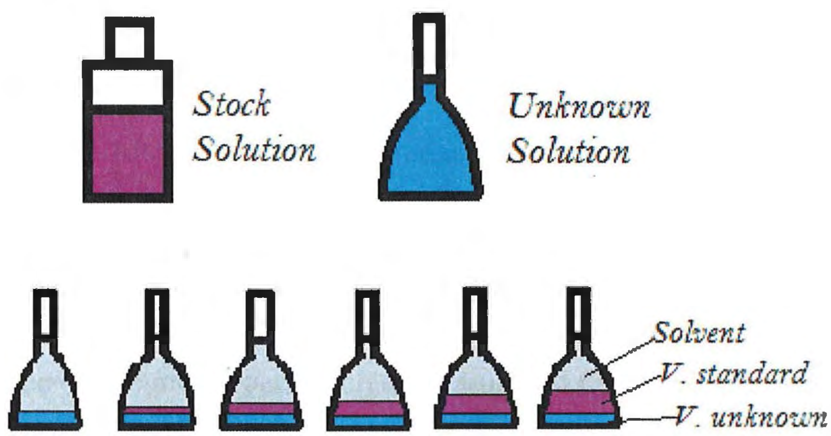

Figure 3. Standard addition method

The BPA standard solution was prepared by dissolving 0.0158 g of BPA in 50 mL of 1:1 methanol water solution for a concentration of 316 μg/mL. To prepare the samples, 1 mL of the receipt water sample was placed in each of 6 10-mL volumetric flasks. Next, 20-100 μL of BPA standard solution was placed in flasks 2-6, respectively. The first flask did not contain any BPA standard solution. Then, each flask was filled to the 10-mL mark with 1:1 methanol water solution and shaken to ensure homogeneity. Three aliquots of each sample were measured in the fluorescence spectrophotometer, with three intensity readings for each aliquot.

For lake water samples:

The lake water samples were prepared with the same method as the receipt water samples. Four different locations around Degray Lake (Lower Lake, Boat Ramp Near Iron Mountain Lower Mountain Trail Head, Hwy 7 Beach Area, and Lake Resort Beach) were chosen for testing. At each location, three samples were collected. For each sample, 1 mL of the sample was placed in each of 6 flasks. Then, 20-100 μL of standard were added to flasks 2-6, respectively. Flask 1 did not contain any standard. Three aliquots of each sample were tested using fluorescence spectrophotometry, with three intensity readings for each aliquot.

Calculations and Analysis

When preparing the BPA solutions, the dilution equation

$$C_1V_1=C_2V_2, \qquad \textbf{Equation 1}$$

where C is concentration and V is volume, was used to calculate the concentration of each new BPA solution. C_1 refers to the more concentrated solution and C_2 is the diluted solution. When making the standard addition curves, the equation

$$S_i*V_s/V_o \qquad \textbf{Equation 2}$$

was used to determine the x-axis values. S_i represents the concentration of the standard solution, V_s represents the amount of standard added to the flask, and V_o represents the total volume inside the flask. Then, the equation

$$I*V_s/V_o \qquad \textbf{Equation 3}$$

was used to determine the y-axis values. I represents the intensity readings for each flask, and V_s and V_0 are the same as in equation 2. The equation

$$y=mx+b \quad \textbf{Equation 4}$$

was used to calculate the concentration of BPA in the original solution for all samples. In this equation, y is a specific intensity, m is the slope of the curve for the samples, x is the concentration of BPA associated with the specific intensity, and b is the y-intercept. By solving for x, the concentration of BPA in the original solution can be found. The limit of detection was calculated using the equation

$$LOD=3s/m, \quad \textbf{Equation 5}$$

where s is the standard deviation of the y-axis and m is the slope of the calibration curve. The LOD was found to be 0.42 μg/mL. The limit of quantification was calculated using the equation

$$LOQ=10s/m, \quad \textbf{Equation 6}$$

where s and m are the same as in equation 5. The LOQ was found to be 1.28 μg/mL.

Results

For receipt water samples:

Most samples were tested at 1-hour increments using the standard addition method. The fluorescence spectrophotometer was used to quantify the concentration of BPA in each sample. After gaining intensity readings from the fluorescence spectrophotometer, a standard addition curve (as seen in Figure 3) was made to calculate the concentration of BPA in the original receipt water sample using the slope and y-intercept. Figure 3 is an example of a standard addition for one of the hourly testing increments. The standard addition curves provided a means to calculate the concentration of BPA in the original receipt paper solution at that specific time point. After testing the samples for several hours, the concentrations of BPA found from each standard addition curve were used to make a graph showing the change in concentration over time (as seen in Figure 4).

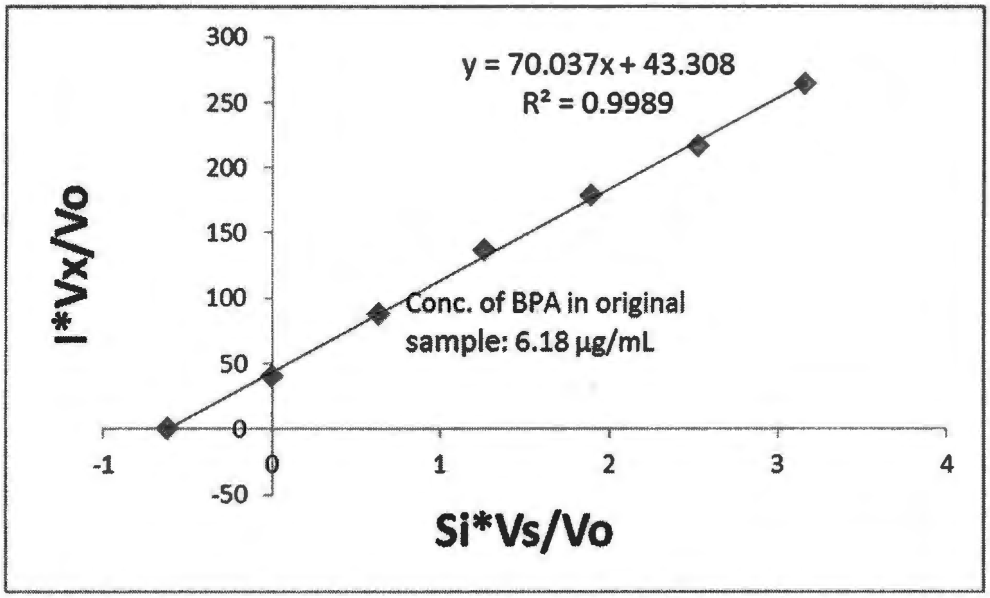

Figure 4. Standard addition curve with calculated concentration of BPA

Figure 4 shows the average change in concentration over a period of 7 hours between three different samples. The concentration ranged between 4.50 ± 0.28 µg/mL to 6.81 ± 2.38 µg/mL between all hourly data points. There was not a significant difference in concentration between the first and seventh hours, for the concentrations found at the first and seventh hours were 4.94 ± 1.11 µg/mL and 5.25 ± 0.93 µg/mL, respectively.

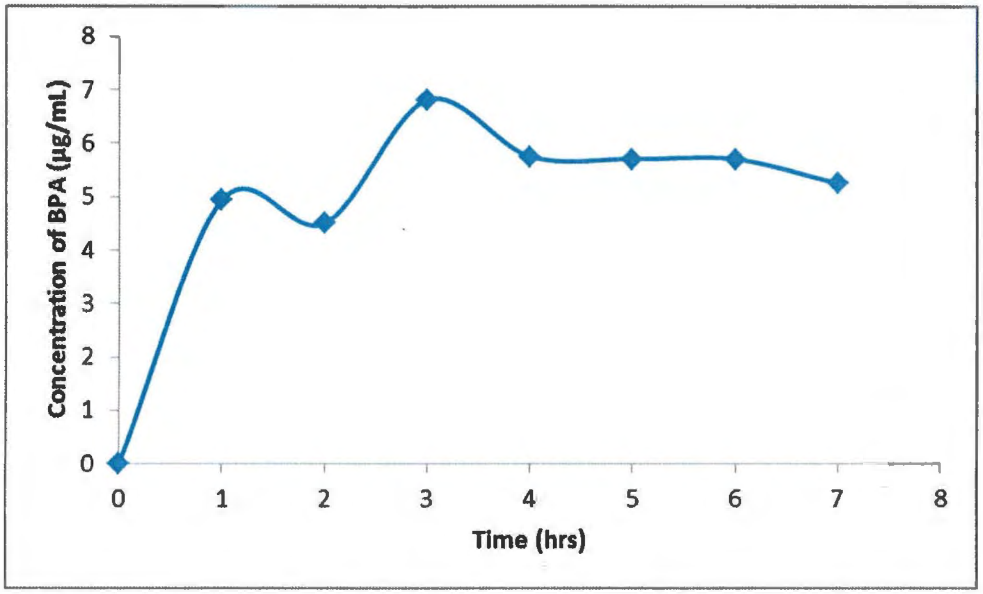

Figure 5. Change in concentration of BPA in receipt water sample over a period of 7 hours

Also, two different samples were tested over longer times. One sample was tested at 24, 48, and 72 hours, while the other sample was tested at 24 and 96 hours. By compiling the fluorescence intensities from these samples, the change in concentration over longer periods of time was more evident (Figure 5). The range in concentration among all data points was 7.50-8.25 µg/mL. Again, there was not a significant change in concentration of the original sample between the first and last testing times.

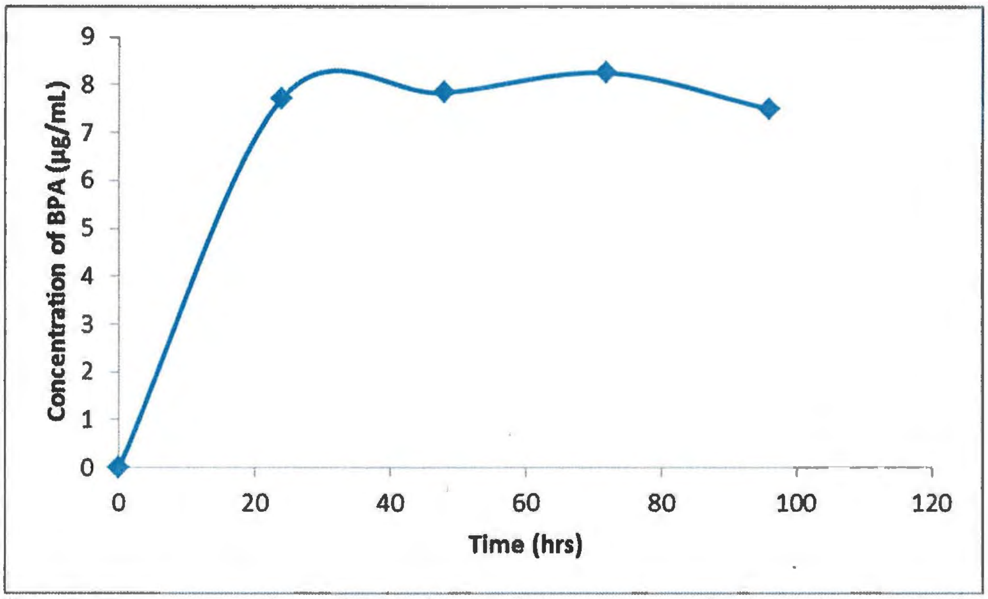

Figure 6. Change in concentration of BPA in receipt water samples tested every 24 hours for 96 hours

Then, the receipt water samples were tested at elevated temperatures. The results from the receipt water samples tested at 37 and 50°C are shown in Figures 6 and 7, respectively. The data found if Figure 6 represents the average concentrations found from three trials. The range of concentrations found at the time intervals was 7.01 ± 1.93 to 8.30 ± 0.76 µg/mL. Figure 7 represents concentrations found for only one trial. The experiment represented in Figure 7 should be repeated to verify results. The range of concentrations found at the 1-hour increments when heating the sample at 50°C was 5.17 to 6.59 µg/mL. Both of these figures seem to exemplify the same trends that have been shown in Figures 4 and 5.

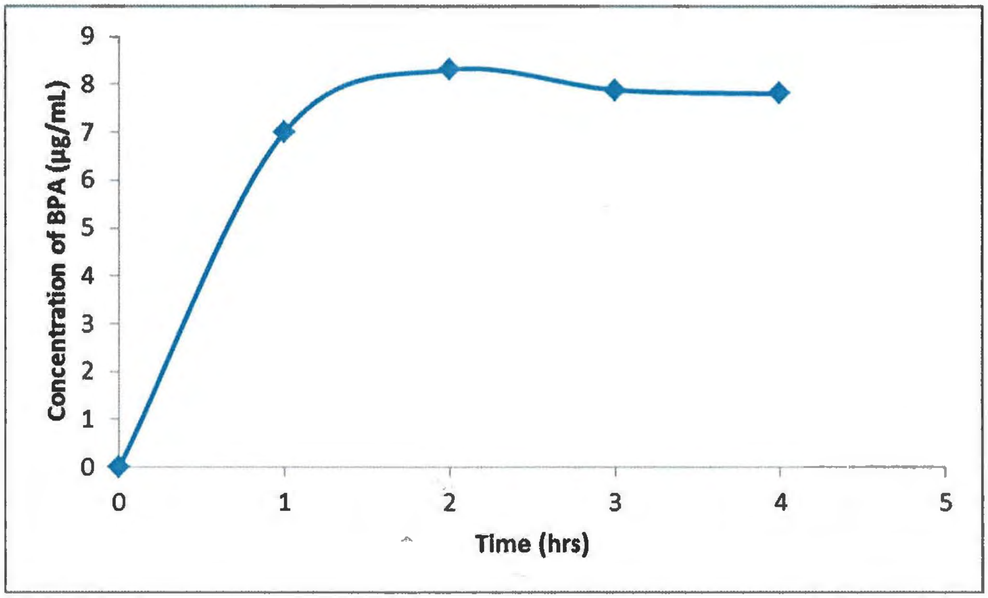

Figure 7. Change in concentration of BPA in receipt water sample heated at 37°C

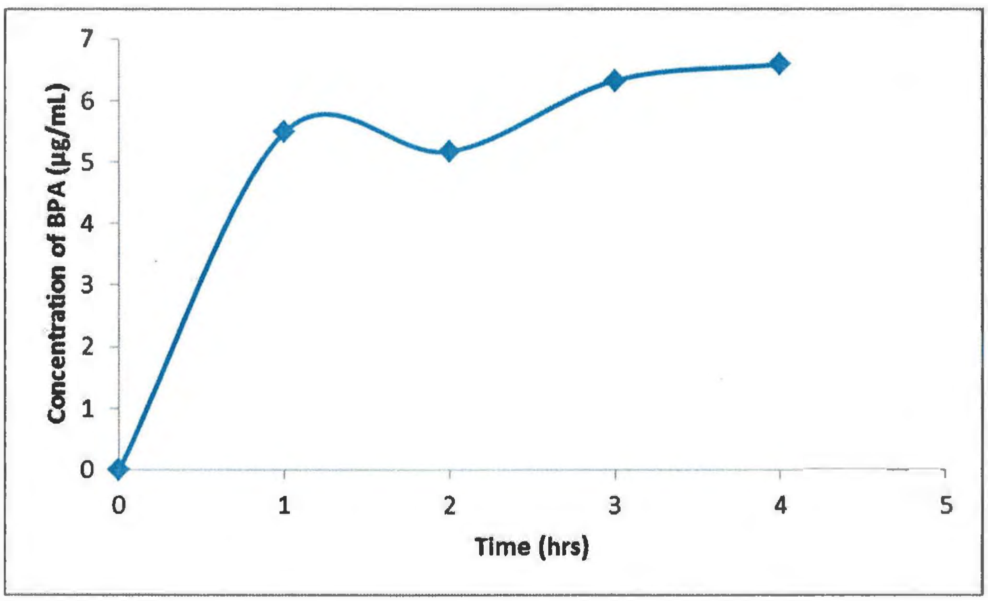

Figure 8. Change in concentration of BPA in receipt water sample heated at 50°C

Figure 9 shows the comparison of the effects of heating the samples. There did not appear to be a significant difference in BPA leaching between heating trials based on the comparison in this graph.

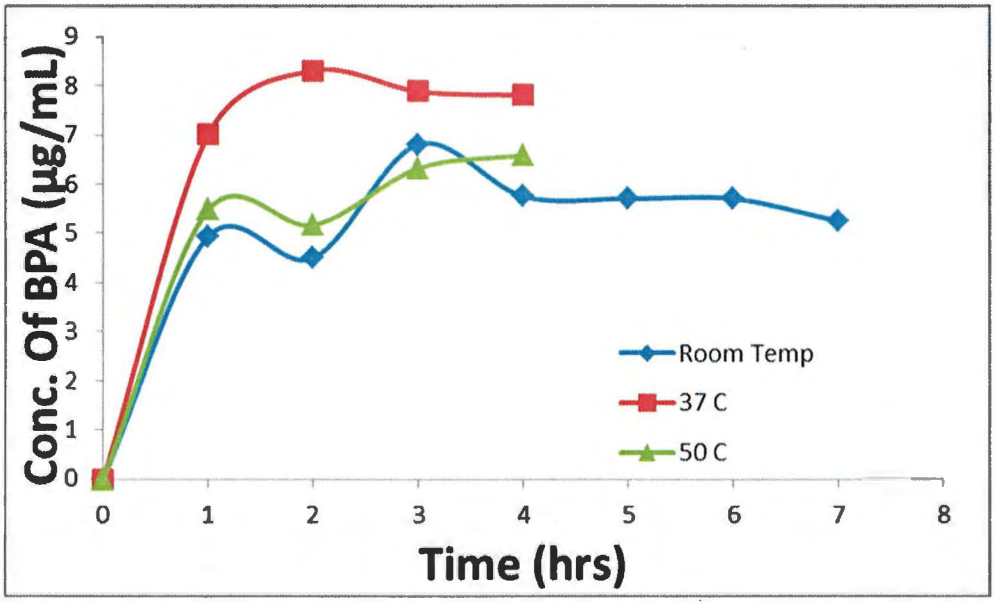

Figure 9. Comparing change in concentration of BPA between samples kept at room temperature and samples heated at 37 and 50°C

For lake water samples:

Measurable amounts of BPA were found in all lake water samples that were tested. A total of 12 samples were tested, with three samples from each location. The lowest concentration found from any of the samples was 1.06 µg/mL and the highest concentration was 1.99 µg/mL. The average concentrations of BPA at each location are shown in Table 1.

Table 1. Average concentrations of BPA found at each location around lake

Location	Average Concentration (µg/mL)
Lower Lake	1.42±0.14
Boat Ramp	1.56±0.37
Hwy 7 Area	1.4±0.26
Lake Resort	1.3±0.38

Discussion

Based on the data in Figures 4-7, we saw that the concentrations of BPA found in the receipt water samples plateaued after the first time point. The largest increase in concentration of BPA in the water sample surrounding the receipt paper occurred within the time between the placing the paper in the water and removal of the first sample. The plateau in BPA concentration could have occurred either because the water sample had become saturated with BPA at that point and, therefore, could not absorb any more BPA, or all of the BPA had leached from the receipt paper into the water sample. Therefore, allowing the receipt paper to sit in water for longer periods of time did not affect the amount of BPA that leached into the water sample.

We could not make definitive conclusions about the effects of heating the receipt paper samples based on Figure 8. When comparing the difference in concentration that leached into the water between the room temperature samples and the 37°C samples, it appears that a greater amount of BPA would leach out of the receipt paper when the receipt paper samples were heated. However, the results from heating the samples at 50°C contradicted the thought that heating the samples would increase the concentration of BPA in the water sample. Although the results from the samples heated at 50°C were contradictory of our hypothesis, we cannot rule out the idea that heating the samples would increase leaching of BPA, since we were only able to complete one trial of heating the samples at 50°C. We would need to perform more trials in order to verify our results.

We found that there were detectable concentrations of BPA in the water samples from Lake Degray. We also found that these concentrations were fairly consistent among all locations. Possible errors in determining the actual concentration of BPA in each sample could have resulted because the calculated concentrations were near the LOQ.

Alternatives for BPA

Since BPA has been noted as an endocrine disruptor, potentially causing many adverse health effects, researchers have worked to find alternatives for BPA that will be more stable in extreme conditions, such as high temperatures, and less likely to interact with receptors in the body that control vital functions. However, the alternatives that are being used are still bisphenols, but they include different molecules attached to the phenol group. So, researchers have hypothesized that these alternatives will still cause adverse health effects even at small concentrations.[22-25] Many researchers have completed experiments testing for the concentrations of these other bisphenols and testing the effect of certain bisphenols on certain receptors in the body.

Liao and Kannan measured the concentrations of BPA, BPB, BPF, BPS, and other forms in certain food items bought from Albany, NY.

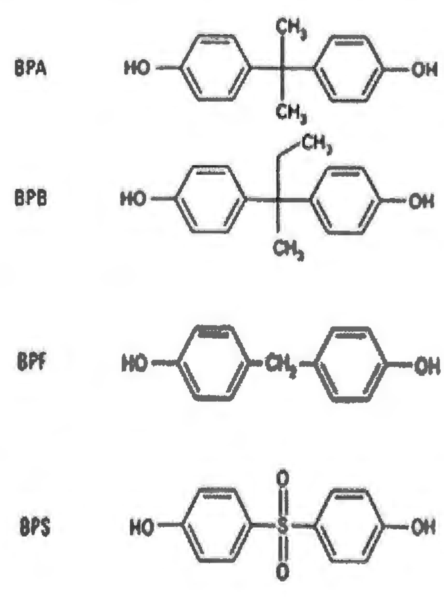

Figure 10. Comparison of BPA, BPB, BPF, and BPS[26]

They measured the amount of these bisphenols that leached from packaging into water surrounding the foods and into the foods themselves. They found that many of the different forms of bisphenols were present in most of the food items. The most common bisphenol found among the food items was BPA, and BPF was the second most common. However, BPF was only found in about 10% of the food items, and the other forms, including BPS, BPAF, and BPAP, each were found in less than 10% of the samples. Overall, this experiment showed that other forms of bisphenols were being used in the making of many polycarbonate plastics and that these bisphenols were still migrating from the plastic packaging. However, the migration was much less than that of BPA.[21]

The effects of these bisphenol analogues have also been investigated. One experiment tested the effects of BPS on the activation of certain kinases. They compared the activity in the presence of only physiologic estrogen (E_2), only BPS, and a combination of E_2 and BPS. They found that there was greater activation of the kinases in the environment with both E_2 and BPS, leading them to believe that when a person is exposed to BPS while the body is already producing E_2, many adverse effects could occur, including desensitization of the receptor kinases or activation of other pathways that were not intended to be activated. Even though BPS does not leach as much into samples because it is more stable, it is still able to leach in small concentrations, and it was found that BPS can have an effect even at very small concentrations.[22]

Also, many researchers have studied the ability of bisphenol analogues to bind to estrogen receptors and activate those receptors. They have found that BPA is able to bind to the estrogen receptor ERRγ better than BPAF, but BPAF can bind better to estrogen receptors ERα and ERβ. As BPA and BPAF bind to the estrogen receptors, they activate abnormal production of estrogen in the body.[23] In another experiment, Riu et. al. determined that bisphenols that were chlorinated or brominated could also bind to the ERα and ERβ receptors.[24] Furthermore, Delfosse et. al. confirmed that bisphenol analogues act as endocrine disruptors, but they act differently in different tissues. The bisphenol analogues bind to the endocrine receptors similarly to E_2 in some tissues, but would not bind to the receptors in other tissues.[25] All in all, many researchers have determined that even though bisphenol analogues that are more resistant to heat have been manufactured, the analogues still pose a health risk. The analogues are still able to activate many receptors, mainly the endocrine receptors, causing issues in many bodily functions. Therefore, more research needs to be completed to find safe alternatives for BPA.

Future Research

In further research, the experiment heating the receipt water samples at 50°C needs to be repeated to verify the results. Also, testing the samples in 24-hour increments needs to be repeated to confirm that the results are reproducible. Then, testing the effects of heating the receipt water samples on the rate of BPA leaching would be important. Finally, it would be informative to test the leachate from various landfills for the presence of BPA.

Acknowledgments

Dr. J. D. Patterson Summer Research Program

Ouachita Baptist University Department of Chemistry

Dr. Sara E. Hubbard, thesis director

Dr. Timothy E. Hayes, second reader

Dr. Joe Nix, third reader

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