2019

Determination of Bisphenol A (BPA) in Infant Oral Hygiene Devices using Fluorescence Spectrophotometry

Kaitlyn Thomason
Ouachita Baptist University

SENIOR THESIS APPROVAL

This Honors thesis entitled

Determination of bisphenol A (BPA) in infant oral hygiene devices using fluorescence spectrophotometry

written by

Kaitlyn Thomason

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.

Dr. Sara Hubbard, thesis director

Dr. Tiffany Eurich, second reader

Dr. Rachel Pool, third reader

Dr. Barbara Pemberton, Honors Program director

24 April 2019

Determination of bisphenol A (BPA) in infant oral hygiene devices using fluorescence spectrophotometry

OBU Honors Thesis

Kaitlyn Thomason

2019

Acknowledgments

- Ouachita Baptist University
- Dr. Sara Hubbard
- Dr. Tiffany Eurich
- Dr. Rachel Pool
- Dr. Barbara Pemberton

Abstract:

Bisphenol A (BPA) is an organic, solid substance that is structurally similar to estradiol, a naturally-occurring form of the female sex hormone estrogen. Since the 1957 discovery that BPA can function as an effective hardener, BPA-based plastics have been used to make a variety of consumer goods, such as water bottles, baby bottles, DVDs, eyeglass lenses, and medical devices, while BPA-based epoxy resins have been used to coat the insides of various food and drink containers, to line the insides of water pipes, and to create the thermal ink found on paper sales receipts. In recent years, however, numerous studies have suggested that BPA-exposure during fetal growth and childhood can lead to later problems like obesity, type 1 diabetes, anxiety, depression, hyperactivity, and aggression. While BPA has already been banned from baby bottles, no BPA regulations have been placed on children's oral hygiene devices as of yet. Some infant oral hygiene devices like toothbrushes claim to be BPA-free, while others do not make any statements regarding BPA content. In this research study, a technique was developed to determine the presence of BPA in toothbrushes made for infants and toddlers using fluorescence spectrophotometry. Using this technique, two different types of toothbrushes were analyzed: those labeled BPA-free and those not labeled. It was found that neither toothbrushes showed traces of BPA, although the toothbrush with no label showed an upward trend in regards to emission intensity that could possibly show the presence of BPA if more time was given in the solvent. This thesis will discuss literature detailing the effects of BPA on infant health, the results of the fluorescence spectrophotometry study done on the toothbrushes, and future research that can be done to both enhance the current study and further the exploration of BPA-exposure in infants.

Introduction:

BPA Background

BPA was first created in 1891 by Russian chemist Aleksandr Pavlovich Dianin as a side product of the catalyzed condensation reaction of a two-to-one mixture of phenol and acetone in concentrated hydrochloric acid (Figure 1).[1,2]

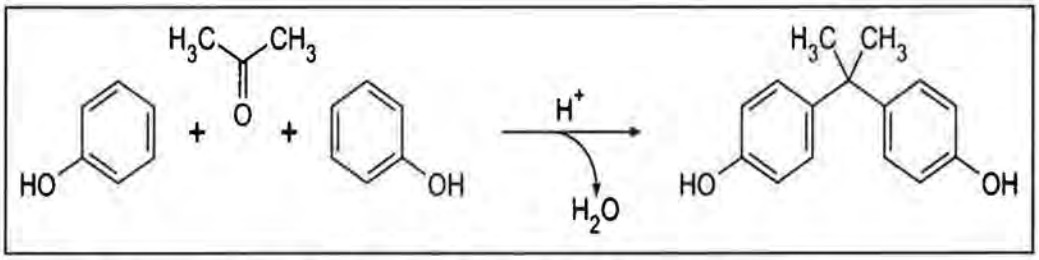


Figure 1. *The synthesis of BPA from two phenol molecules and one acetone molecule.*

In 1938, a British chemist by the name of Edward Charles Dodds discovered that BPA and estradiol, a naturally-occurring form of the estrogen hormone, shared similar chemical features—namely their phenol groups (Figure 2).[2,3] He soon began developing BPA to be a form of synthetic estrogen, although this endeavor was short-term, as another compound was found to produce better results. Interestingly, this compound would be later banned by the FDA in 1979 as it was found to be linked to rare vaginal cancers.[3]

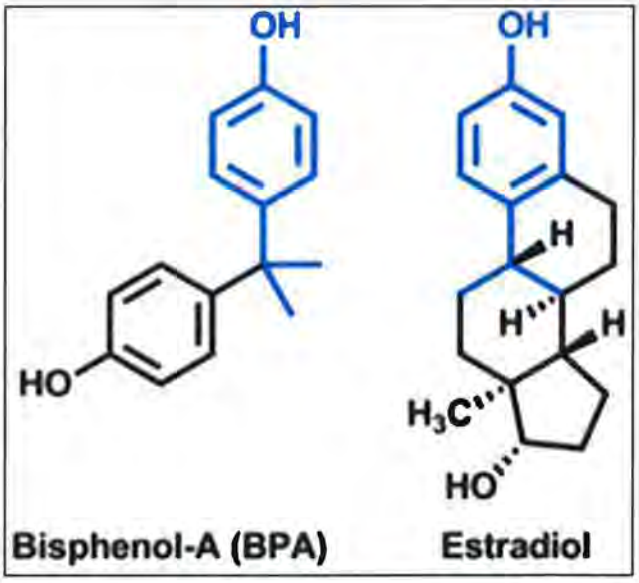


Figure 2. *The chemical structures of BPA and estradiol, with the blue regions showing the structural similarities.*

Less than twenty years later, however, one discovery would drastically change the applications of BPA forever. In 1953, chemists Dr. Hermann Schnell of Bayer and Dr. Daniel Fox of General Electric independently discovered how to use BPA to create a hard resin called polycarbonate by combining BPA with phosgene (Figure 3).[4] This hard resin was clear and rigid like glass, although much lighter and shatter-resistant. It was also more affordable than glass and thus easier to replace if broken. This polycarbonate product first appeared in the electrical industry in products like fuse boxes and plug connectors.[4] In the 1960s, the Food and Drug Administration (FDA) approved polycarbonate to be used in food packaging, and it was this that launched a world-wide boom in BPA-exposure.[4]

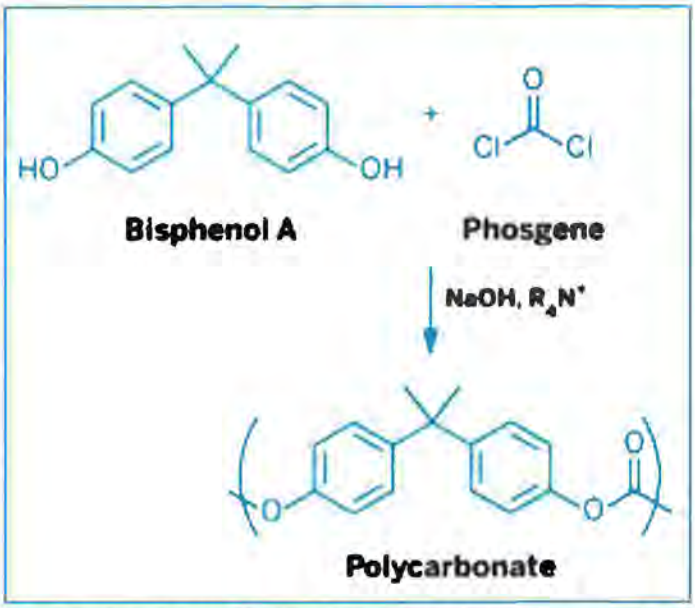


Figure 3. *The reaction of BPA and phosgene in basic solution results in the formation of polycarbonate, a strong plastic used in many consumer good.*

BPA became increasingly popular in consumer goods, and the estrogenic properties revealed by Dodds were ignored for nearly thirty years. This would soon change, however, in 1993, when Dr. David Feldman, a medical doctor and Stanford University professor discovered the leaching properties of BPA.[4] In a study to find estrogen-producing yeast, Feldman and his team were growing yeast in plastic flasks and noticed an estrogenic molecule in the growth medium.[5] After isolating the molecule and analyzing it with the use of nuclear magnetic resonance spectroscopy and mass spectroscopy, the team found that the molecule was BPA.[5] Successive testing done using flasks containing only distilled water showed that the presence of

BPA was not dependent on either the yeast or the growth medium.[5] This also disproved that the BPA was being synthesized by the yeast. Feldman and his team then repeated the studies using glass flasks in place of plastic and found no traces of BPA.[5] These findings led to the conclusion that BPA was being leached from the polycarbonate plastic and into the contents of the flask during the heating of the autoclaving process.[5] Also alarming was the revelation that BPA could bind to the estrogen receptors in rat uterus, as Feldman and his team discovered, which indicated that BPA was not just a form of synthetic estrogen but also an endocrine disruptor.[5]

BPA as an Endocrine Disruptor

Endocrine disruptors are chemicals that hinder the normal functioning of the endocrine system, usually by interfering with hormones. The most common way is through competitive inhibition (Figure 4). In this mechanism, the endocrine disrupter binds to a hormone receptor, thus inhibiting the hormone from binding and initiating its normal response.

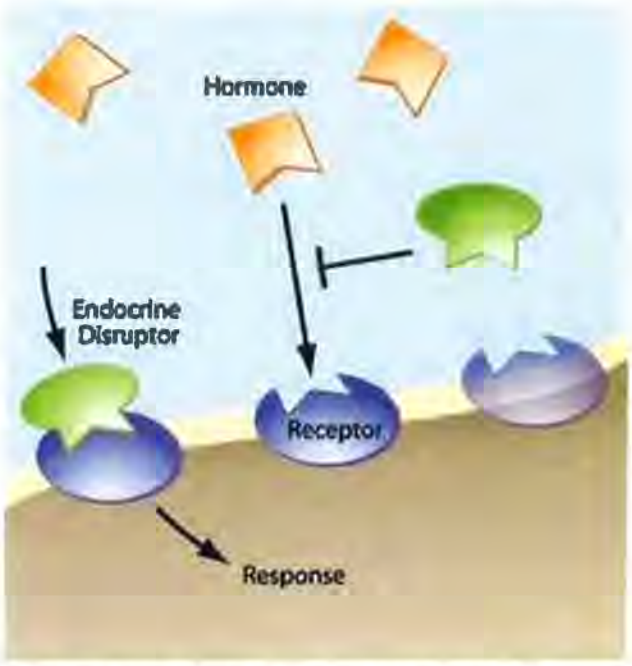


Figure 4. *An endocrine disrupter exhibiting competitive inhibition.*

BPA acts as an endocrine disrupter to the two types of estrogen receptors (ERs): ERα and ERβ.[6] Both of these receptors are ligand-activated, meaning that the binding of a ligand (i.e., estrogen) causes them to undergo a change in conformation.[6] After this shape change, the receptors are able to migrate into the nucleus of the

cell. It is here that the ERs act as transcription factors, interacting with activators and repressors associated with the target gene. If a transcription factor binds to an activator, the expression of the target gene increases, while the binding of a transcription factor to a repressor causes the expression to decrease.

Because the structural configuration of BPA is so similar to that of estrogen, the body is unable to distinguish between the two. As a result, BPA is able to successfully bind to both ERα and ERβ. However, because BPA and estrogen are not identical, the binding of BPA causes each type of ER to undergo a different conformation change.[6] When BPA binds to ERα, the receptor undergoes the same conformation change as would normally occur with estrogen as the ligand. Therefore, BPA acts agonistically to ERα, meaning that the binding of BPA to ERα initiates the same types of responses in the body as would occur if there were high levels of estrogen present, thus increasing estrogenic-related activities. On the other hand, the binding of BPA to ERβ causes an alteration in the receptor's conformation change, resulting in no response to occur. In this way, BPA acts antagonistically to ERβ, meaning that the binding of BPA to ERβ mimics that of having no estrogen present in the body, thus inhibiting estrogenic-related activities.

Effects of BPA on the Body

Although the discovery of the harmful nature of BPA is relatively recent, numerous studies have already been performed concerning the health risks of BPA exposure. In a study done by Kristin Junge and team, prenatal BPA exposure was found to be linked to obesity.[7] In this study, 408 infants were analyzed based on the levels of BPA they had been exposed to during prenatal development, as was determined by urine samples from the mothers. At birth, cord blood

samples were taken from these infants, and the DNA methylation of these samples was measured using MassARRAY. The results revealed that infants exposed to higher levels of BPA during prenatal development showed decreased methylation in the CpG islands associated with the promoter regions of MEST genes.[7] CpG islands are short segments of DNA that precede promoters, and the degree of methylation at these regions partly dictates whether a gene can be transcribed into RNA (Figure 5). When the CpG islands of a particular gene are highly methylated, transcription factors are unable to interact with the promoter and cannot turn it on, thereby inhibiting the transcription of the gene. When the CpG islands are unmethylated or hypomethylated, however, the promoter is more accessible to transcription factors, allowing transcription to freely occur. The MEST gene is said to control adipocyte size and thus the development of adipose tissue.[7] When the CpG islands associated with the MEST promoters are hypomethylated due to prenatal BPA-exposure, an increase in MEST RNA expression occurs, resulting in the accumulation of large amounts of fat cells and fat tissue. This increased MEST expression was found evident in the BMI studies conducted on the infants at age one, which found that the infants who had been exposed to greater levels of BPA during development showed higher BMI scores than their less-exposed cohorts.[7]

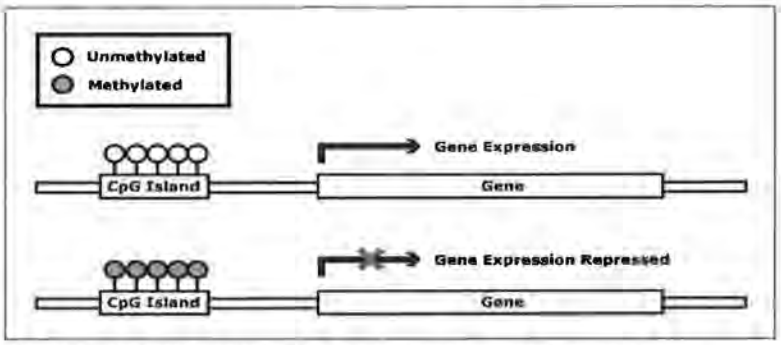


Figure 5. *The methylation of CpG islands greatly controls transcription.*

BPA has also been linked to the development of type 1 diabetes. In a study performed by Johanna Bodin and team, healthy weight, non-diabetic infant mice were administered doses of BPA via drinking water (1 mg/L) over the course of 35 weeks—doses considered to be under the

Fluorescence Spectroscopy

Fluorescence is the light energy given off when a certain type of compound absorbs energy, usually in the form of light or radiation. When the electrons in a fluorescent compound absorb a specific wavelength of energy, they become excited and begin to vibrate, moving from their ground energy state to a higher energy state. In fluorescence spectroscopy, the compound is excited by photons of light, causing the electrons to transition to a higher energy state. The electrons remain in this higher energy state for a short period of time, approximately 10^{-8}-10^{-4} seconds, before then losing energy and returning back to their ground state. The lost energy is released in the form of fluorescence (Figure 6). Because this emitted energy is less than the initial absorbed photon energy, the wavelength of the emitted fluorescent light is greater, due to the inverse relationship between energy and wavelength.

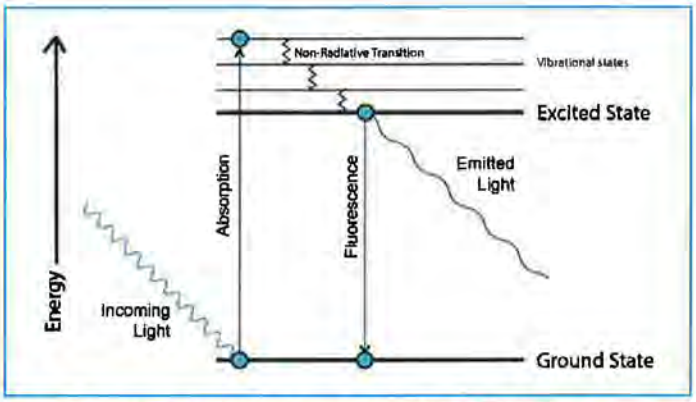


Figure 6. *Jablonski energy diagram illustrating fluorescence.*

The wavelength of the emitted fluorescence is unique for every compound. Previous research performed at Ouachita Baptist University found that BPA fluoresces at excitation and emission wavelengths equal to 278 nm and 310 nm, respectively (Figure 8). Therefore, it is possible to determine the

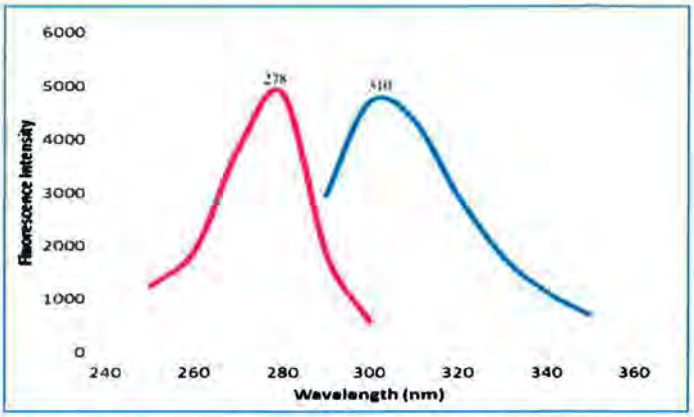


Figure 8. *Excitation and emission spectra of BPA in methanol water.*

presence of BPA in a sample by examining the wavelengths of the emitted fluorescence. If a sample contains BPA, the emission wavelengths will be 310 nm when excited by wavelengths of 278 nm. This can be tested using a fluorescence spectrophotometer (Figure 9). Within this instrument, a light source is emitted onto a prism and then a filter, which separates the light into a specified wavelength (278 nm, in this case). This specific wavelength is then directed to the sample, which is contained within a quartz cuvette. The electrons in the sample will become excited, travel to an increased energy state, and then release light energy on their way back to their ground state, as previously mentioned. This emitted light is passed through a detector, which records the wavelength. If the sample contains BPA, this wavelength will be approximately 310 nm.

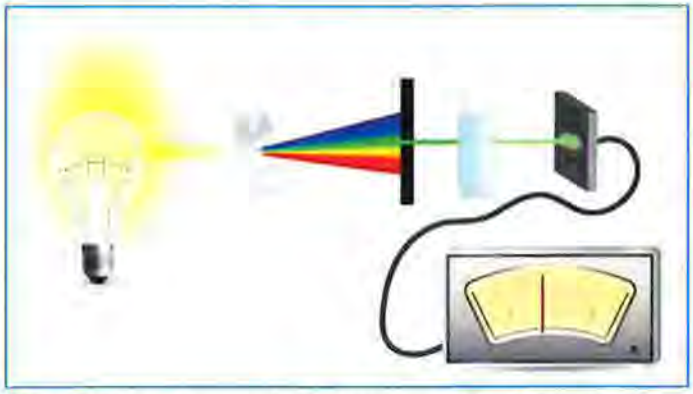


Figure 9. *The mechanism of a fluorescence spectrophotometer.*

Materials and Methods:

In order to become familiarized with the instrument and to set a standard for the rest of the fluorescence intensity values, a calibration curve was created using known concentrations of BPA: 0 M, 1.5 M, 3.04 M, and 4.56 M (Figure 10). These concentrations were

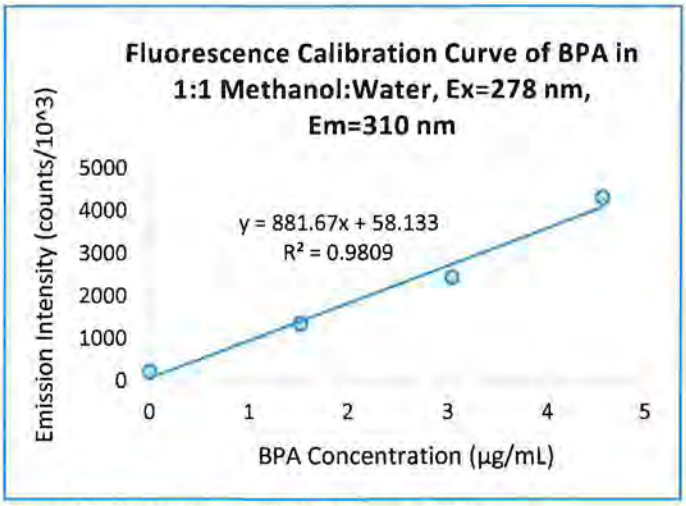


Figure 10. *Fluorescence calibration curve of BPA.*

made using 0 mL, 2 mL, 4 mL, and 6 mL of BPA, respectively. All concentrations were run in triplicates.

To begin the study, two toothbrushes designed for infants and toddlers were obtained: one labeled BPA-free (Toothbrush A) and one with no label (Toothbrush B). Each toothbrush was placed in separate 100-mL beakers containing 100 mL of Milli-Q water. Over the course of one week, 10-mL aliquots of the Milli-Q water were taken from each beaker at various times and placed in 10-mL volumetric flasks. On the starting day of the experiment, samples were taken at the onset (time = 0 hours) and then every twenty minutes for two hours (time = 0.33, 0.66, 1.00, 1.33, 1.66 hours). Samples were then taken every twenty-four hours for three days (time = 24, 48, 72 hours) and then once more on the seventh day (time = 168 hours). For control, two 10-mL samples of Milli-Q water were taken at the start of the experiment (time = 0 hours). Samples were analyzed using the FS-5 Spectrofluorometer from Edinburgh Instruments. A portion of each sample was pipetted into the quartz cuvette, which was then placed into the instrument. The parameters of the instrument were set to include excitation wavelengths of 278 nm and emission wavelengths of 310 nm. Samples were done in triplicates, with thorough washings between each.

Results and Discussion:

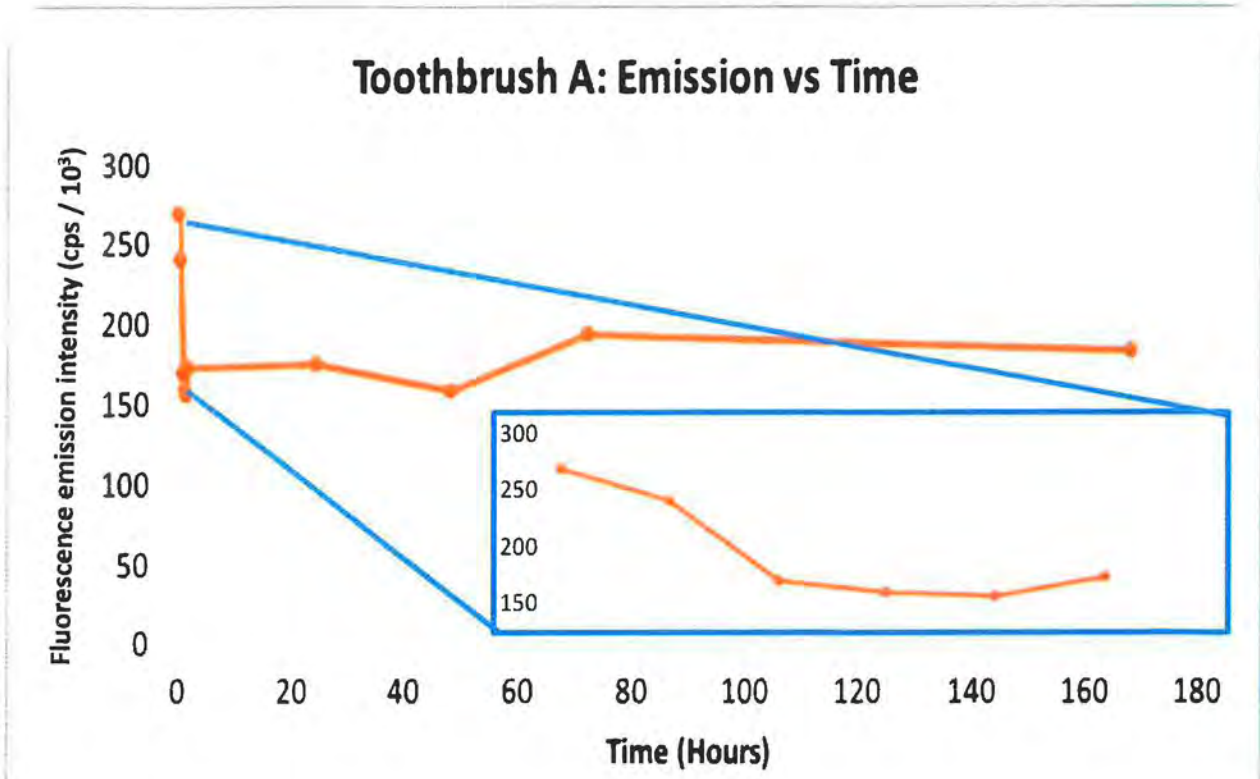
For all samples, the following values were recorded: emission wavelength, emission intensity, excitation wavelength, and excitation intensity (Tables 1 & 2).

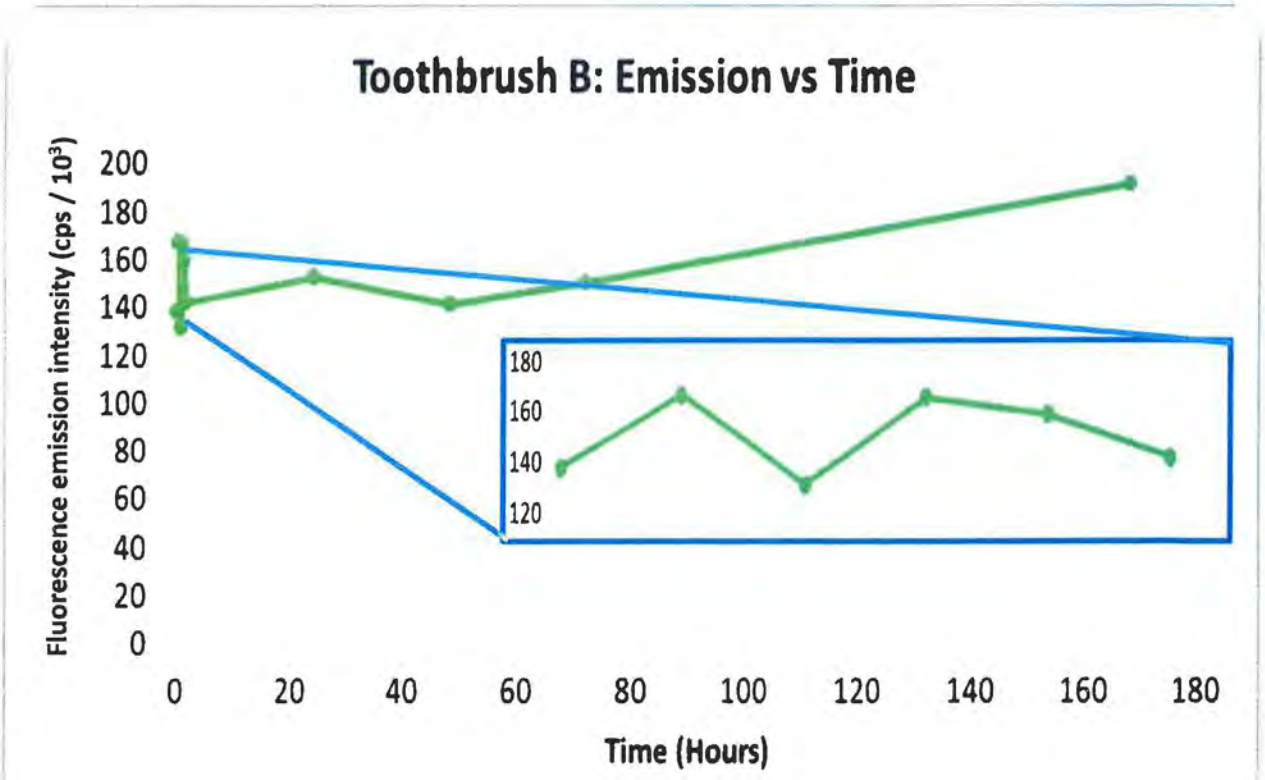
Time of Absorbance (Hours)	Toothbrush Labeled BPA-Free			
	Emission Wavelength	Emission Intensity (counts/10^3)	Excitation Wavelength	Excitation Intensity (counts/10^3)
0	308	267	281	126.3
0.33	308	239	280	142.7
0.67	307	168	280	89.96
1	307	158	281	90.42
1.33	308	154	280	81.56
1.67	307	171	280	93.55
24	307	173	280	86.36
48	307	156	280	85.76
72	307	192	280	98.35
168	308	181	280	97.75

Table 1. *Fluorescence spectrophotometry results for Toothbrush A.*

Time of Absorbance (Hours)	Toothbrush Not Labeled			
	Emission Wavelength	Emission Intensity (counts/10^3)	Excitation Wavelength	Excitation Intensity (counts/10^3)
0	308	137	280	65.97
0.33	307	166	280	83.36
0.67	306	130	280	83.36
1	308	165	280	86.36
1.33	307	158	280	92.95
1.67	307	141	280	84.56
24	308	151	279	88.15
48	308	140	280	86.96
72	307	149	280	103.7
168	307	190	281	78.44

Table 2. *Fluorescence spectrophotometry results for Toothbrush B.*


Toothbrush A: Emission vs Time
Fluorescence emission intensity (cps / 10³)
300
250
200
150
100
50
0
0
20
40
60
80
100
120
140
160
180
Time (Hours)
300
250
200
150


Toothbrush B: Emission vs Time
Fluorescence emission intensity (cps / 10³)
200
180
160
140
120
100
80
60
40
20
0
0
20
40
60
80
100
120
140
160
180
Time (Hours)
180
160
140
120

The toothbrush labeled BPA-free (Toothbrush A) did not show traces of BPA. The toothbrush with no label also did not show traces of BPA (Toothbrush B), although the line did exhibit an upward progression that could possibly reveal BPA if given more time.

Summary and Conclusions:

Bisphenol A is a very prevalent molecule, as it has been used in the widespread manufacturing of consumer goods since the 1960s. However, recent research suggests that BPA exposure is harmful, as the molecule binds to estrogen receptors in the body and elicits a number of estrogenic responses. This is particularly true for infants and children, as they do not have a well-developed purification system like adults. Studies show that BPA exposure during development leads to increased MEST gene expression, which can result in higher BMI scores and thus an increased risk of obesity. Exposure to BPA has also been linked to type 1 diabetes, as BPA depletes phagocytic macrophages that serve to lessen hyperglycemia and insulitis. Furthermore, infants are being exposed to BPA in many ways, including through the intake of both liquid and formula formulas, and by contact with medical devices like those found in the neonatal intensive care unit. In 2012, BPA-based plastics were banned from being used in the manufacturing of baby bottles. However, no BPA regulations have been places on oral hygiene devices made for children.

In this study, I wanted to determine if BPA was present in toothbrushes designed for children. I also wished to see if the toothbrushes labeled BPA-free were truly void of BPA. This was an unexplored field, so I first had to design a way to test the toothbrushes for BPA. I then used fluorescence spectrophotometry to determine the presence of BPA.

Future Work:

In this study, only a single BPA-free labeled toothbrush and a single non-labeled toothbrush were tested. Therefore, to get a better understanding of BPA leaching from infant toothbrushes, it would be ideal to perform this experiment again with a variety of different brands and styles. Furthermore, many infants and toddlers drink a wide variety of fruit juices (i.e., apple juice, white grape juice, orange juice). For this reason, it would be interesting to determine to what effect the acidity of these different juices would have on BPA leaching, if any at all. In addition, a gentleman at the research conference where I presented inquired about the Milli-Q water that I used. He noted that the purity of this water could possibly be inhibiting the leaching of BPA from the toothbrush, as there are no impurities for the BPA molecules to interact with.

Works Cited:

1. Rogers, Kara. "Bisphenol A." *Encyclopædia Britannica*, Encyclopædia Britannica, Inc., 1 Sept. 2014, www.britannica.com/science/bisphenol-A#ref1178413.
2. Monti, Mie. "Bisphenol A." *University of Bristol - School of Chemistry*, School of Ramiro De Maeztu, Madrid, Spain, Aug. 2013,www.chm.bris.ac.uk/motm/bisphenolA/Bisphenol A.pdf.
3. Vogel, Sarah A. "The politics of plastics: the making and unmaking of bisphenol a "safety." *American Journal of Public Health,* vol. 99, no. 3, Nov. 2009: S559-66. doi:10.2105/AJPH.2008.159228
4. Caliendo, Heather. "BPA in Packaging: A Lucrative Past, a Controversial Present, and a Tentative Future." *PlasticsToday*, Informa PLC., 2 July 2013, www.plasticstoday.com/content/bpa-packaging-lucrative-past-controversial-present-and-tentative-future/52470083117654.
5. Feldman, David, et al. "Bisphenol-A: an estrogenic substance is released from polycarbonate flasks during autoclaving." *Endocrinology*, vol. 132, no. 6, 1 June 1993, pp. 2279-2286. *Oxford Academic*, doi:10.1210/en.132.6.2279.
6. Junge, Kristin M., et al. "MEST Mediates the Impact of Prenatal Bisphenol A Exposure on Long-Term Body Weight Development." *Clinical Epigenetics*, vol. 10, no. 58, 20 April 2018, doi:10.1186/s13148-018-0478-z.
7. Acconcia, Filippo et al. "Molecular Mechanisms of Action of BPA." *Dose-Response,* vol. 13, no. 4, 7 Oct. 2015, doi:10.1177/1559325815610582.

8. Bodin, Johanna, et al. "Exposure to Bisphenol A, but Not Phthalates, Increases Spontaneous Diabetes Type 1 Development in NOD Mice." *Toxicology Reports*, vol. 2, 22 Feb. 2015, pp. 99–110., doi:10.1016/j.toxrep.2015.02.010.
9. Espinoza-Jiménez, Arlett, et al. "Alternatively Activated Macrophages in Types 1 and 2 Diabetes." *Mediators of Inflammation*, vol. 2012, 3 Dec. 2012. *Hindawi*, doi:10.1155/2012/815953.
10. Gao, Hui, et al. "Bisphenol A and Hormone-Associated Cancers." *Medicine*, vol. 94, no. 1, Jan. 2015, doi:10.1097/md.0000000000000211.
11. Goetz, Natalie Von, et al. "Bisphenol A: How the Most Relevant Exposure Sources Contribute to Total Consumer Exposure." *Risk Analysis*, vol. 30, no. 3, 8 Mar. 2010, pp. 473–487., doi:10.1111/j.1539-6924.2009.01345.x.
12. Corrales, Jone, et al. "Global Assessment of Bisphenol A in the Environment: Review and Analysis of Its Occurrence and Bioaccumulation." *Dose Response*, vol. 13, no. 3, 29 July 2015, doi:10.1177/1559325815598308.
13. FDA. "Questions & Answers on Bisphenol A." *Questions & Answers on Bisphenol A (BPA) Use in Food Contact Applications*, FDA, www.fda.gov/food/food-additives-petitions/questions-answers-bisphenol-bpa-use-food-contact-applications.
14. Mendonca, K., and Sdf Dsf. "Bisphenol A Concentrations in Maternal Breast Milk and Infant Urine." *International Archives of Occupational and Environmental Health*, vol. 87, no. 1, Jan. 2014, doi:10.1007/s00420-012-0834-9.
15. Balakrishnan, Biju. "Transfer of Bisphenol A across the Human Placenta." *American Journal of Obstetrics and Gynecology*, vol. 202, no. 4, 27 Mar. 2010, doi:10.1016/j.ajog.2010.01.025.

16. Cirillo, Teresa, et al. "Exposure to Di-2-Ethylhexyl Phthalate, Di-N-Butyl Phthalate and Bisphenol A through Infant Formulas." *Journal of Agricultural and Food Chemistry*, vol. 63, no. 12, 23 Mar. 2015, pp. 3303–3310., doi:10.1021/jf505563k.

17. Calafat, Antonia M., et al. "Exposure to Bisphenol A and Other Phenols in Neonatal Intensive Care Unit Premature Infants." *Environmental Health Perspectives*, vol. 117, no. 4, Apr. 2009, pp. 639–644., doi:10.1289/ehp.0800265.