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Effects of Temperature & pH on BPA Leaching in Oral Hygiene Products Using Fluorescence Spectroscopy

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SENIOR THESIS APPROVAL

This Honors thesis entitled

"Effects of Temperature & pH on BPA leaching in Oral Hygiene Products using Fluorescence Spectroscopy"

written by

Mady Cate Rottinghaus

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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Abstract

Bisphenol-A (BPA) is a common chemical used in plastic production as well as in epoxy resin linings for metal food containers. Unfortunately, many are exposed to this chemical daily through plastic food and beverage containers as well as various other plastic products on the market.[1] Exposure to harmful BPA has been linked to negative effects on the body including cardiovascular diseases, reproductive issues, and effects on endocrine development. BPA binds to estrogen receptors and can activate them because of structural similarities to estradiol. These effects are of particular concern in infants and young children.

BPA is now regulated in many plastic products; however, it is not regulated in oral hygiene products, specifically children's toothbrushes. This research specifically tested for BPA in several infant oral hygeine products. Specific variables of temperature and pH levels were chosen to further mimic the conditions in the oral cavity and studied to observe the effect that changes in temperature and pH may have on the leaching of BPA from the toothbrushes. BPA is a fluorescent compound with an excitation wavelength of 278 nm and an emission wavelength of 304 nm. The presence of BPA was determined by measuring the fluorescnece intensity of BPA leached from toothbrush samples using the FS5 Spectrofluorometer by Edinburgh Instruments. A calibration curve and analytical figures of merit including linear range, limit of detection, and limit of quantitation were determined for BPA and further compared to previous work. These data were utilized to monitor the leaching of BPA from several brands of BPA-containing infant toothbrushes over time. Following the determinations at ambient conditions, toothbrush brands

[1] **"Bisphenol A (BPA) in U.S. Food"** Arnold Schecter, Noor Malik, Darrah Haffner, Sarah Smith, T. Robert Harris, Olaf Paepke, and Linda Birnbaum. *Environmental Science & Technology* **2010**

that were shown to contain BPA were then studied at varying temperatures and pH values to mimic warehouse conditions and/or the effects of brushing after eating.

Introduction

Bisphenol-A is a chemical that is used in the manufacturing of polycarbonate plastics, linings of canned goods, and many other products.[2] Variations in temperature and/or pH have been shown to increase migration of BPA from the polycarbonate to the outside environment due to polymer degradation.[3] BPA mimics and interferes with the action of estrogen, a hormone that helps in development and eventually in reproduction (Fig. 1).[4] Because of this, and due to its increasing amounts in many products, it has been found to have negative effects on the human body, which will be discussed in a later section.[5]

BPA was first used as a pharmaceutical hormone replacement for the therapeutic treatment of numerous female concerns.[6] The chemical industry first started using BPA to make

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[2] **"Bisphenol A"** William E. Luttrell and Bryce A. Baird. *Journal of Chemical Health & Safety* **2014**

[3] Pjanic, Milos. **"The role of polycarbonate monomer bisphenol-A in insulin resistance."** PeerJ vol. 5 e3809. 13 Sep. 2017, doi:10.7717/peerj.3809

[4] Schierow, L., Lister, S.A. (2008, May). **Bisphenol A (BPA) in Plastics and Possible Human Health Effects.** Congressional Research Service Report for Congress, The Library of Congress.

[5] Brent A. Bauer, M.D. **"What is BPA, and what are the concerns about BPA?"** Mayo Clinic, Mayo Foundation for Medical Education and Research, 18 Dec. 2019, www.mayoclinic.org/healthy-lifestyle/nutrition-and-healthy-eating/expert-answers/bpa/faq-20058331.

[6] Diamanti-Kandarakis, Evanthia et al. **"Endocrine-disrupting chemicals: an Endocrine Society scientific statement."** Endocrine reviews vol. 30,4 (2009): 293-342. doi:10.1210/er.2009-0002

hard plastics around the 1940's, which is how it is commonly used today. BPA is regulated in several plastics, particularly those that will come in contact with food and/or drink. The evidence of the regulation is evident in the commonly seen "BPA-free" labels on items like water bottles, plastic containers, and aluminum food and beverage cans. BPA-free items only account for about five percent of hard plastics, so BPA is still prevalent and should be highly considered in the health and safety realm.[7]

BPA in infant oral hygiene products still needs attention. The presence of BPA in the toothbrushes is not highly regulated and not widely researched. Previous work in our lab developed methods to monitor BPA leaching from plastics at ambient temperatures. For this thesis research project, the presence of BPA in different oral hygiene products was further investigated while altering the variables of temperature and pH. This information will help parents and guardians avoid exposing their children to BPA through the use of particular toothbrushes.

The temperature and pH values used in this study to monitor BPA leaching were selected in order to mimic those of the oral cavity. The oral cavity has a temperature of about 37°C and it was compared to room temperature at 20°C.[8] BPA leaching was also analyzed at pH values of 3, 6, and 10. Saliva has a pH normal range of 6.2-7.6 with 6.7 being the average pH.[9] The values of 3 and 10 were chosen to assess acidity or basicity based on consumption of food or a beverage right before brushing.

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[7] Vogel, Sarah A. **"The politics of plastics: the making and unmaking of bisphenol a "safety"."** American journal of public health vol. 99 Suppl 3,Suppl 3 (2009): S559-66. doi:10.2105/AJPH.2008.159228

[8] Green, Barry G. **"Oral Perception of the Temperature of Liquids."** *Perception & Psychophysics* , 1986, pp. 19–23., doi:BF03207579.

[9] Baliga, Sharmila et al. **"Salivary pH: A diagnostic biomarker."** Journal of Indian Society of Periodontology vol. 17,4 (2013): 461-5. doi:10.4103/0972-124X.118317

Health Effects of Bisphenol-A

Exposure to BPA has been linked to negative health effects such as cardiovascular diseases, reproductive abnormalities, neurobehavioral problems, and effects on endocrine development.[10] Researchers have also discovered possible links between BPA exposure and insulin resistance, increased formation and growth of fat cells, and reproductive health problems for both men and women.[11] Infants and young children are especially susceptible to the negative effects of BPA because their endocrine systems are still developing.

BPA acts as an endocrine disrupter, binding to estradiol's binding sites in the estrogen receptor.[12] Endocrine-disrupting chemicals (EDCs) are substances in our environment, food, and consumer products that interfere with hormone biosynthesis, metabolism, or action, resulting in a deviation from normal homeostatic control or reproduction. This leads to the initiation of pathways that are not supposed to be initiated, thus inhibiting the normal function of the hormone.[13] BPA is capable of causing this disruption because it has a phenol group that

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[10] Ellahi, Mujtaba, and Mamoon Ur Rashid. **"The Toxic Effects BPA on Fetuses, Infants, and Children** | IntechOpen."

[11] Green, Barry G. **"Oral Perception of the Temperature of Liquids."** *Perception & Psychophysics* , 1986, pp. 19–23., doi:BF03207579.

[12] Acconcia, Filippo et al. **"Molecular Mechanisms of Action of BPA."** *Dose-response : a publication of International Hormesis Society* vol. 13,4 1559325815610582. 7 Oct. 2015, doi:10.1177/1559325815610582

[13] Diamanti-Kandarakis, Evanthia et al. **"Endocrine-disrupting chemicals: an Endocrine Society scientific statement."** Endocrine reviews vol. 30,4 (2009): 293-342. doi:10.1210/er.2009-0002

resembles a phenol group on estradiol, making BPA able to bind to the same type of endocrine receptors as estradiol. (Fig. 1).

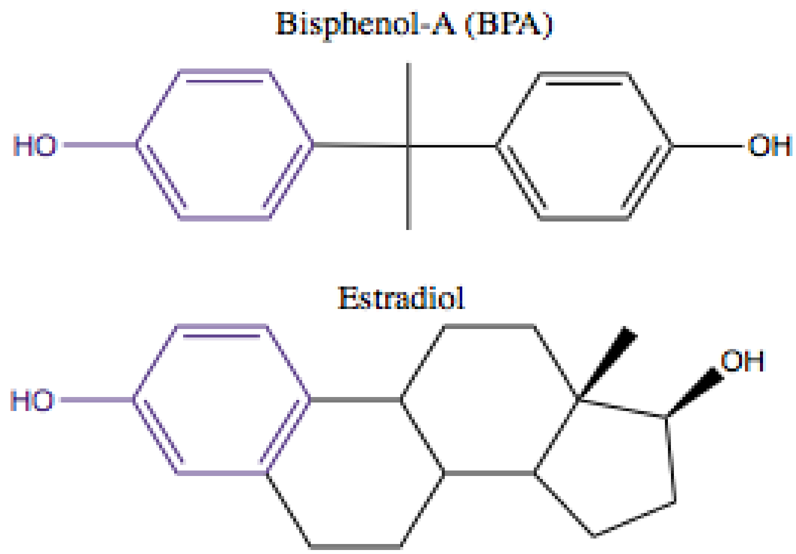

Figure 1. Chemical Structures of BPA (top) and Estradiol (bottom). Both compounds contain a phenol group (purple), which can react with estrogen receptors.

The use of BPA-containing products in daily life makes exposure ubiquitous, and the potential human health risks of this chemical are a major public health concern.[14] The increased susceptibility of infants and young children to the effects of BPA makes it especially important to study the oral hygiene products that they are using. It is imperative to analyze this data and lower their potential exposure to BPA in these everyday products.

Observation of Oral Health

Oral Health is a large component of overall health, well-being, and quality of life which has been included to highlight this as an aspect of my directed study. Oral health is important to

[14] **Are Bisphenol A(BPA) Plastic Products Safe for Infants and Children?** *National Center for Health Research*, 10 Aug. 2017.

me as I am pursuing a career in Dentistry and want to be knowledgeable about the products I am using in my practice. Comparing oral health and potential BPA leaching to see if there is a correlation would be a great analysis in future experimentation. According to the World Health Organization, oral health encompasses a range of diseases and conditions that include dental caries, periodontal disease, tooth loss, oral cancer, oral manifestations of HIV infection, and birth defects such as cleft lip and palate.[15] The Global Burden of Disease Study in 2017 estimated that oral diseases affect 3.5 billion people worldwide.[16] This alarming statistic encourages people to look at the impact and further respond in prevention. By brushing two times a day, cleaning in between the teeth, eating a healthy diet, and consulting with dental professionals, this statistic can be lowered ensuring that oral health will be protected and healthy smiles will be the outcome.[17]

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[15] Inadera H. (2015). **Neurological Effects of Bisphenol A and its Analogues**. International journal of medical sciences, 12(12), 926–936. https://doi.org/10.7150/ijms.13267

[16] **"Oral Health**." *World Health Organization*, World Health Organization, www.who.int/health-topics/oral-health/#tab=tab_1.

[17] "Brushing and Beyond: Key Oral Health Tips for Anyone with a Smile." *Mouth Healthy TM*, www.mouthhealthy.org/en/oral-health-recommendations.

After an electron absorbs light and excites into higher energy states, vibrational relaxation occurs and heat is lost in a non-radiative transition as shown in the Jablonski Diagram (Fig. 2).[18] The electron then returns to the ground state and fluorescent light is emitted. The instrumentation used in this research is an FS5 Spectrofluorometer by Edinburgh Instruments (Fig. 3). It was used to monitor levels of BPA throughout experimentation and a key component in measuring the intensities of light in emission and excitation spectra of BPA in methanol/water solutions.

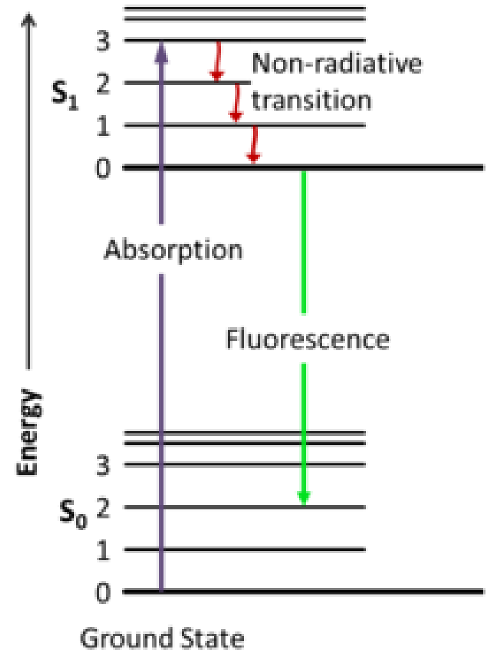


Figure 2. The Jablonski Diagram. Used as a model in Florescence Spectroscopy to illustrate the excited states of a molecule and the transitions that occur between them as well.

Fluorescence Spectroscopy

[18] **Fluorescence Spectroscopy.** Eleftherios P. Diamandis**.** *Analytical Chemistry* **1993** *65* (12), 454-459 DOI: 10.1021/ac00060a616

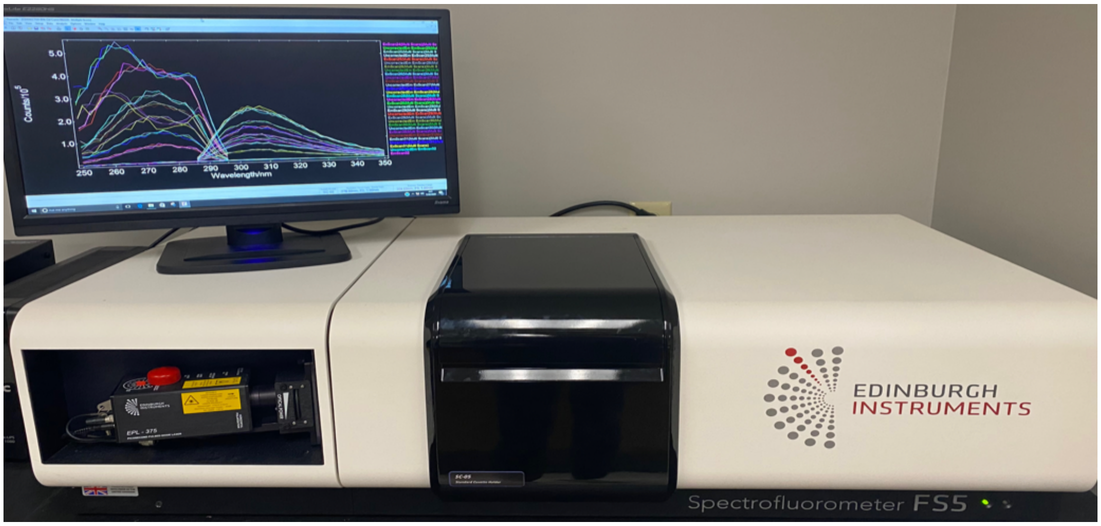


Figure 3. Edinburgh Instruments Spectrofluorometer. Instrument used to measure levels of BPA.

Based on the calibration curve with the various amounts of BPA sampled, the excitation wavelength is around 278 nm whereas the emission wavelength is around 304nm (Fig. 4). This is crucial information because we can see observe higher levels of BPA as the wavelengths are increased.

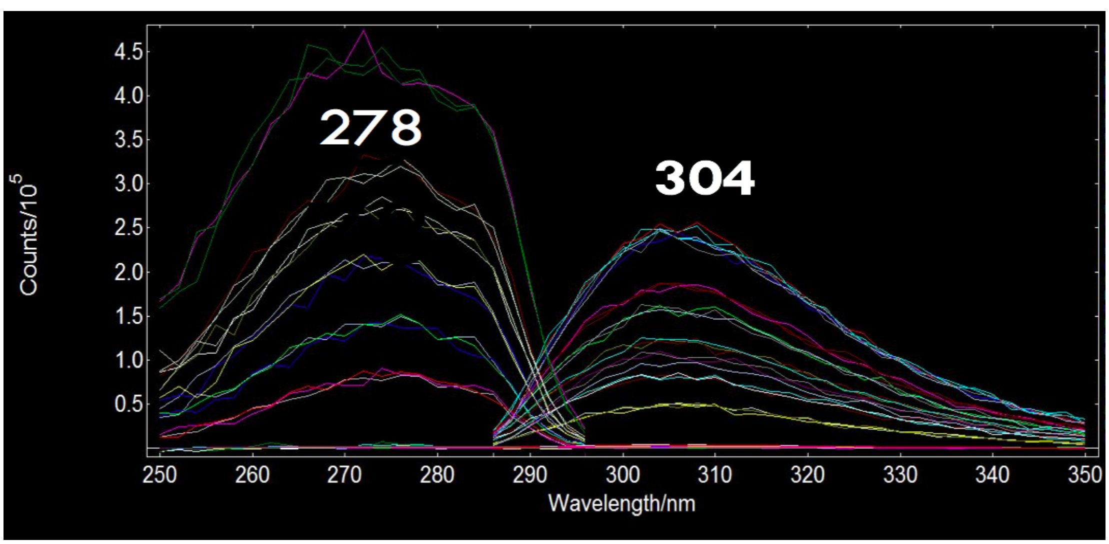
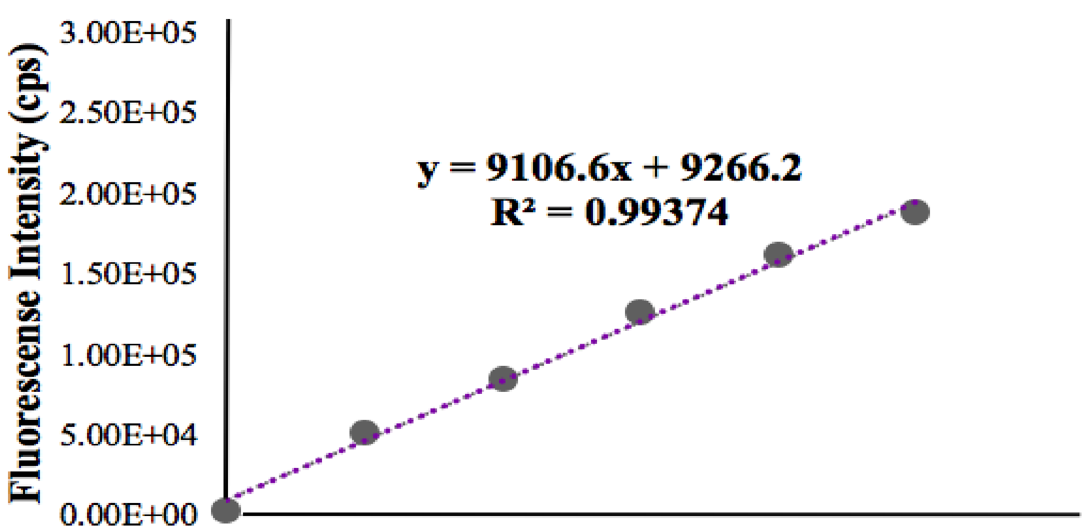


Figure 4. Fluorescence Excitation and Emission spectra obtained using the FS5 Spectrofluorometer from Edinburgh Instruments. The excitation wavelength is on the left (around 278nm) and the emission wavelength is on the right (around 304nm).

Materials and Methods

The following chemicals were utilized in these experiments: Bisphenol-A (BPA), HPLC water & HPLC methanol were used to prepare the solutions. The glassware used in this research included 100-mL volumetric flasks, 25-mL volumetric flasks, 5-mL volumetric flasks, and 100-mL beakers. The instrument used was the FS5 Spectrofluorometer from Edinburgh Instruments. Four brands of BPA-containing toothbrushes were used in comparison to a previously studied BPA-free toothbrush. Labeling materials were used with the toothbrushes to minimize error. Micropipettes were used for accurate extraction of the samples. Safety measures were taken into place with the use of goggles, gloves, and a lab coat. Cleaning of all glassware was performed with 10% nitric acid. Hydrochloric acid and sodium hydroxide were used in pH experimentation to prepare acidic and basic solutions, respectively.

A calibration curve was prepared to determine the correlation between BPA concentration and fluorescence intensity (Fig. 5). To prepare standard slutions for the calibration curve, 0.203g of BPA was weighed and transferred to a 100-mL volumetric flask and filled to the line with 50% methanol/water solution (M/W). This stock solution was then used to prepare standard solutions for the fluorescence calibration curve with concentrations from 0-20 µg/mL, which were measured in the FS-5 Spectrofluorometer.



The Limit of Detection (LOD) was calculated to be 4.676 µg/mL and it is the lowest concentration that one is certain has BPA. The Limit of Quantification (LOQ) was 15.59 µg/mL and its value is the lowest concentration at which one can quantify the amount of BPA. The linear range for the calibration curve was from 0-20.0 µg/mL. The importance for finding these values is to accurately determine at what concentrations BPA can be successfully measured.

A variety of children's toothbrush brands were selected and tested for the presence of BPA (Tab. I and Fig. 6). Four toothbrushes were labeled: A, B, C, and D. These toothbrushes had been previously shown to contain BPA. They were further tested for this research and compared to a known BPA-free toothbrush as a negative control. Each toothbrush was then placed in a beaker with 100 mL of 50% methanol/water solution. Five-mL aliquots were taken from the beaker at 0 minutes, 5 minutes, 10 minutes, 15 minutes, 20 minutes, 40 minutes, 60 minutes, 80 minutes, 100 minutes, 120 minutes, 4 hours, 5 hours, 6 hours, and 24 hours after the toothbrush was placed in the solution. These samples were then analyzed in the FS5 Spectrofluorometer to record and collect the emission intensity, emission wavelength, excitation intensity, and the excitation wavelength for each sample.

Table I. List of Children's Toothbrushes tested & their labels.

Toothbrushes Tested
Baby's First Toothbrush
Colgate- ANIMALS
Oral-B - DORY

Colgate - MINIONS	A
SmileCare- BUBBLE	B

All of this information was graphed using Microsoft Excel, and the BPA-containing toothbrushes were compared using statistical analysis at the 95% confidence level to the known BPA-free toothbrush that was utilized as a negative control. Temperature and pH were further studied in these samples to align with the oral cavity in order to predict if there is more BPA leaching going on in the mouth under varying conditions.

Results and Discussion

For the temperature experiment, each toothbrush was measured in triplicate at temperatures of 20°C (room temp) and 37°C (body temp). The fluorescence emission intensities of each sample were plotted versus time to monitor changes in BPA concentration as it is leached from the toothbrushes overtime. A representative graph of toothbrush A is shown in Figure 7. The BPA intensity increased over time for all toothbrushes at each temperature, but toothbrush A showed the most dramatic difference between behavior at 20°C and 37°C. A t-test comparing average intensities at 20°C and 37°C was performed for each toothbrush (Tab II). Each average value at 37°C was statistically significantly different than the 37°C values.

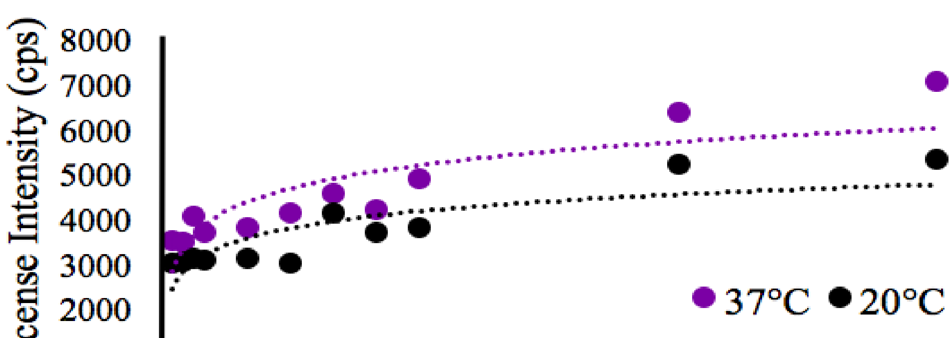


Figure 7. Representative data for Toothbrush A Temperature Analysis at room temperature (20°C) & temperature of oral cavity (37°C)

Table II. Temperature statistical analysis of BPA-containing toothbrushes compared to BPA-free at the 95% confidence level.

Plastic Toothbrush	T Cal	T Table	Statistically Significantly Different?
Temperature of Oral Cavity (37°C)			
A	6.7	4.3	YES
B	28.6	4.3	YES
C	28.8	4.3	YES
D	5.52	4.3	YES

For the pH experiment, each toothbrush type was measured in triplicate at pH values of 3, 6, and 10. The pH of 6 mimicked normal oral pH, while the pH of 3 represented acidic food consumption (like soda) and the pH of 10 represented alkaline food consumption (like most vegetables). The fluorescence emission intensities of each sample were plotted versus time to monitor changes in BPA concentration as it is leached from the toothbrushes overtime. A representable graph of toothbrush A is shown in Figure 8. BPA leaching increased slightly at pH 3 compared to pH 6, but not by a large amount. At pH 10, fluorescence was difficult to obtain, so little to no change occurred over time at alkaline conditions. A t-test was performed on pH 10 sample data comparing intensity values of each toothbrush to those of the BPA-free toothbrush also measured at pH 10 (Tab. III). Only toothbrush A was statistically significantly different at pH 10 from the BPA-free toothbrush at the same pH. Further analysis is needed.

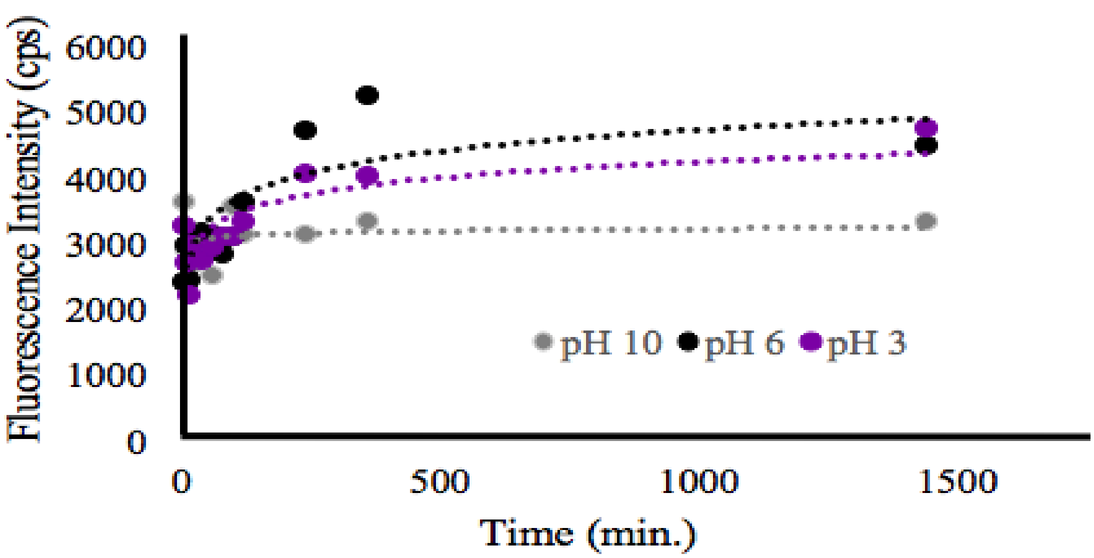


Figure 8. Representative data for Toothbrush A pH Analysis at pH values of 3, 6, 10.

Table III. pH Statistical analysis of BPA-containing toothbrushes compared to BPA-Free at 95% confidence level.

Plastic Toothbrush	T Cal	T Table	Statistically Significantly Different?
pH 10			
A	10.26	4.3	YES
B	2.08	4.3	NO
C	3.31	4.3	NO

Conclusions

In analyzing the results of this study, it was concluded that increasing the temperature from 20°C and 37°C led to an increase in BPA leaching from the BPA-containing toothbrushes.

With the samples measured at a pH of 3, 6 and 10, there was a slight increase observed on BPA leaching at pH 3 and 6, while at a pH of 10 the signal was too low to obtain results. Statistical analysis was performed on the temperature and pH data at a 95% confidence level. High temperature 37°C results were compared to room temperature results 20°C for each toothbrush, and found to be statistically significantly different at the 95% confidence level. This indicates that the increase in intensity is authentic. Additional testing is needed in the future to replicate this data and to quantify the amount of BPA present in the toothbrushes.

Future Experimentation

It would be useful to monitor the presence of BPA in toothbrushes at even higher temperatures to mimic the conditions of warehouses and/or delivery trucks in which the toothbrushes are stored. It is also imperative to replicate the work that has been done and verify that the data is correct by performing further statistical analysis. An accurate measure of the presence of BPA can be obtained by performing additional experimentation. With further research on BPA findings in children's oral hygiene products, the risks will potentially be better known.

References