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Monitoring the Leaching of Bisphenol-A From Feminine Hygiene Products Using Fluorescence Spectroscopy

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Monitoring the Leaching of Bisphenol-A From Feminine Hygiene Products Using Fluorescence Spectroscopy

written by

Madison Easley

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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Ouachita Baptist University

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ABSTRACT

Bisphenol-A (BPA) is a compound commonly used as a stabilizer in plastic products, including food storage containers and thermal paper receipts. Because BPA can bind to and activate estrogen receptors, it is linked to reduced fertility, altered development, and hormone-related cancers. A recent study at NYU Medical School confirmed the presence of BPA in pantyliners, pads, tampons, feminine washes and deodorants. This is concerning due to the high absorption capacity of the vulvar skin.

For the research performed in our lab this summer, the goal was to determine if fluorescence spectrophotometry could be used to determine the presence of BPA in feminine hygiene products by monitoring the release of BPA over time into a solution of 50% methanol/water. Fluorescence is a sensitive, selective and affordable method of analysis, and BPA is a fluorescent compound that absorbs energy at 278 nm and emits at 304 nm. First, a calibration curve was obtained and analytical figures of merit were determined: linear range, limit of detection and limit of quantitation. Due to the complex sample matrix and the small concentrations of BPA in these products, the standard addition method was employed for analysis. Pantyliners, tampons and tampon applicators were tested for the presence of BPA.

The top/outside layer of the feminine hygiene product which comes into direct contact with skin was removed and cut into small pieces. In the case of tampon applicators, the entire applicator was utilized. Samples were then placed into beakers containing 100 mL of 50% methanol/water solution, one per time point to be tested from 0 minutes to 6 hours. At each time point, aliquots of sample solution were removed and transferred to 25-mL volumetric flasks containing various concentrations of BPA stock solution and diluted to

25-mL with 1:1 methanol/water solution. Fluorescence emission intensities at 304 nm were obtained in quadruplicate, and standard addition graphs were utilized to determine the concentration of BPA that had leached from the sample at each time point. Graphs were prepared using these values to produce a visual representation of BPA leaching from the sample over time.

The results showed that both the generic and name-brand pantyliner and tampon samples leached BPA while the luxury brand's products did not. For both the generic and name-brand samples, the tampon applicator released more BPA into solution over time than the absorbent component. Of the generic brand's three products, the pantyliners leached the most BPA into solution. For the name-brand's three products, the tampon applicators leached the most BPA into solution.

INTRODUCTION

Health Consequences of BPA Exposure

Bisphenol-A (BPA) is a compound used in the synthesis of polycarbonate plastic products and epoxy resins. It is currently one of the highest volume chemicals produced worldwide (1). The compound is used in a wide array of commonly used products, with frequent exposure due to contact with BPA-containing food cans and containers, water bottles, and thermal paper receipts. BPA was first synthesized in the 1890's, and its artificial estrogen quality was identified in the 1930's (2). Around the 1950's, it gained traction as a compound used in the manufacturing of hard plastics and epoxy resins, especially in food-contact materials (3). Americans' exposure to BPA was not assessed by the FDA until 1996, and Canada was the first to deem it a "dangerous substance'' in 2008, who then took action to reduce BPA exposure (4).

The concern over the health effects of BPA stems from its estrogenic activity. BPA contains structural features, including a phenol group, that is able to mimic estrogen hormones and bind to both estrogen receptor subtypes, ERα and ERβ (5). The structure of BPA is shown below in Figure 1.

Both ERα and ERβ are ligand-activated transcription factors which undergo a conformational change in response to the binding of 17B-estradiol (E2), the most active estrogen (5). This conformational change allows for their migration into the nucleus (5). Nuclear-ligand bound estrogen receptors regulate E2-gene expression by interacting with corepressors, coactivators, and estrogen responsive elements in the promoters of target genes

(5). Estrogen, as well as BPA, also can rapidly induce change in extranuclear responses when the estrogen receptor palmitoylated and localized at the plasma membrane (5). With plasma membrane localization, estrogen receptors interact with signaling proteins and form multimolecular complexes that cause rapid signal transduction events (5).

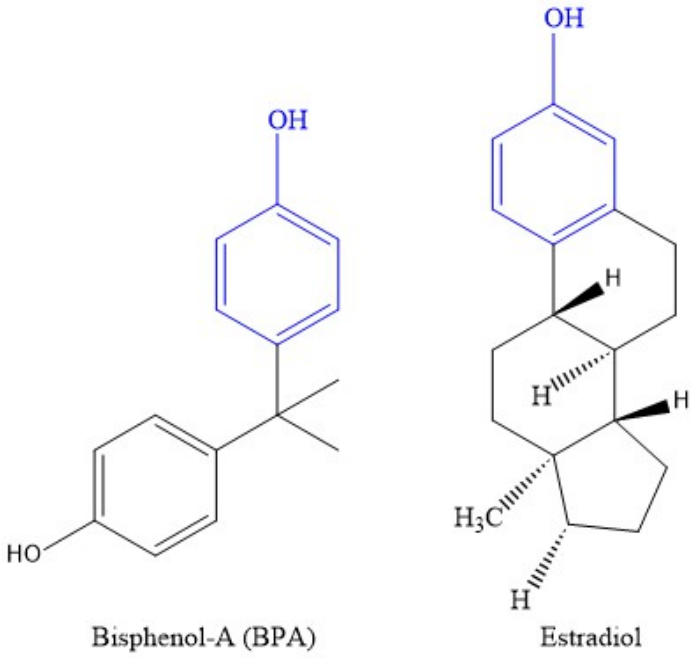

Figure 1: The chemical structures of BPA and estradiol, with structurally similar domains in blue

Estrogen-related receptors (ERR) are nuclear receptors considered a subfamily of ERα and ERβ (5). Three primary ERRs include ERRα, ERRβ, and ERRɣ (5). ERRs do not directly bind estrogens, but rather bind to estrogen response elements, thereby creating overlap between ER and ERR activity (5). BPA binds strongly to ERRs, which is another pathway that low-dose BPA could follow to stimulate estrogenic activity (5).

There are many negative health effects linked to BPA exposure due to BPA's interference with the function of the endocrine system. Such health consequences include hormone-related cancers, reduced fertility, and altered development (6). BPA is ubiquitous, as it has been shown to be present in 93% of adult urine samples (6). Furthermore, BPA has been detected in maternal breast milk, amniotic fluid, placental tissue, umbilical cord blood, and human fetal livers (5). With this high prevalence of BPA within human bodies, even in utero, the potential adverse health outcomes are of high concern.

Because a main source of BPA exposure for all individuals often occurs through food-contact materials, BPA levels through oral intake have been a primary focus of research. However, there are now a multitude of sources beyond food and drink that are further exposing the general population to BPA. Dermal absorption of the compound is another potential exposure route. A recent study published by Gao and Kannan at the New York School of Medicine confirmed the presence of BPA in various feminine hygiene products, which could be absorbed through the vulvar skin (7). For women of reproductive age using these products, this raises particular concern.

The presence of BPA in feminine hygiene products is concerning due to the extended duration of time such products are used, coupled with the acidic nature of the vagina and its increased absorption capacity (7). To further investigate the concentration of BPA which leaches from feminine hygiene products, pantyliner and tampon samples from a name brand, generic brand, and luxury brand- hereafter listed as Brand A, Brand B, and Brand C - were analyzed using fluorescence spectroscopy. Pantyliners were chosen for initial testing because Gao and Kannan's research in 2020 showed that of the tested feminine hygiene products, pantyliners contained the highest amount of BPA per gram of product (7). Tampons were also

chosen to be tested due to their popularity among consumers. Brands of different price ranges were chosen in order to assess for any discrepancies in BPA levels and affordability.

Fluorescence Spectroscopy

Fluorescence spectroscopy is a method which allows one to characterize the chemical of interest (analyte) within a solution. Upon excitation from a light beam, molecules absorb the light energy which takes them from the ground-state to a higher vibrational sub-level (8). As molecules collide, this excess energy is quickly lost and the molecule falls back to its ground-state. This lost energy can be released in the form of heat, or as in the case of fluorescent BPA, in the form of light (8). A diagram depicting this excitation pattern is shown below.

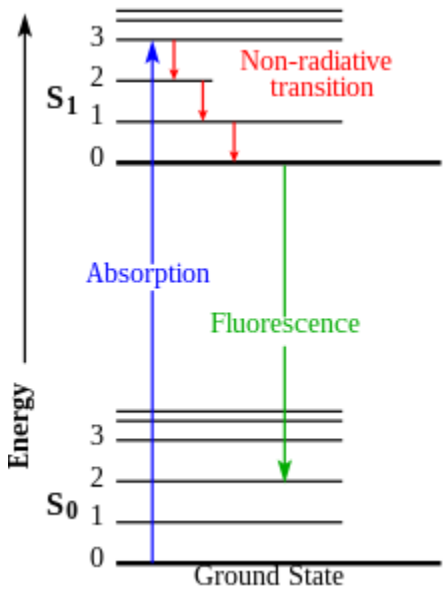

Figure 2: Jablonski diagram depiction of resulting electron fluorescence upon electronic state transition (10)

The wavelength at which light, in the form of photons, is absorbed is specific to the molecule. A calibration curve was run on samples of BPA stock solution, and it was shown

that BPA has an excitation wavelength of 278 nm and an emission intensity of 304 nm. The fluorescence excitation and emission spectra are shown in Figure 3 below, with peaks at the aforementioned wavelengths.

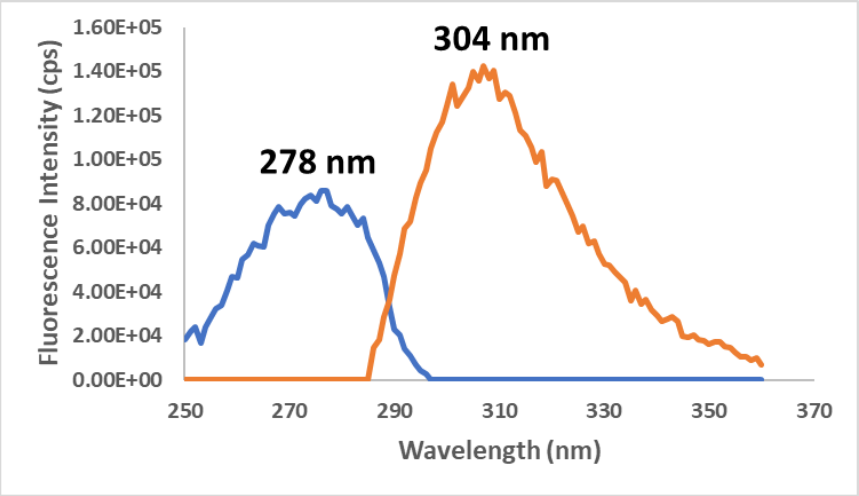

Figure 3: Fluorescence Spectroscopy Excitation and Emission Curves of BPA

The emission intensity, which measures the photons counted per second, correlates to the concentration of analyte (in this case, BPA) in the sample. The average emission intensity at 304 nm for each sample was graphed to determine the amount of BPA leaching into solution over time. It is at this wavelength that BPA fluoresces. Fluorescence is a more unique process than excitation, as all compounds absorb energy, but not all fluoresce. Thus, assessing fluorescence provides a more selective technique that will in turn produce less error when determining BPA presence.

MATERIALS/METHODS

Materials

The following chemicals were utilized during this experiment: Bisphenol-A (BPA), HPLC water, HPLC methanol, and HPLC nitric acid. Glassware used included 1-mL, 5-mL, and 10-mL pipets, nine 100-mL beakers, 50-mL beakers, 25-mL volumetric flasks, and 100-mL volumetric flasks. After each trial, all glassware was washed with a 10% nitric acid solution. This involved rinsing the glassware three times with the acid solution, then rinsing three times with distilled water. The instrument used for this experiment was the FS-5 spectrofluorometer from Edinburgh Instruments. Brand A, Brand B, and Brand C pantyliners and tampons were tested. Gloves, safety glasses, labels, and Parafilm were used as additional materials to ensure safety, maintain organization, and prevent evaporation. All materials were provided by Ouachita Baptist University.

Calibration Curve

To determine the analytical figures of merit, a calibration curve was obtained on the FS-5 Spectrofluorometer from Edinburgh Instruments. The calibration curve produced is shown below in Figure 4.

The limit of detection was 0.615 µg/mL and the limit of quantitation was 0.205 µg/mL. The linear range was 0-11.2 µg/mL. A 0.014-g sample of BPA was measured, then dissolved in a 100-mL volumetric flask of a 1:1 methanol/water solution. This 56 µg/mL BPA solution was serially diluted across eleven flasks, with increasing volumes of 0 to 25 mL, and each was run on the spectrofluorometer.

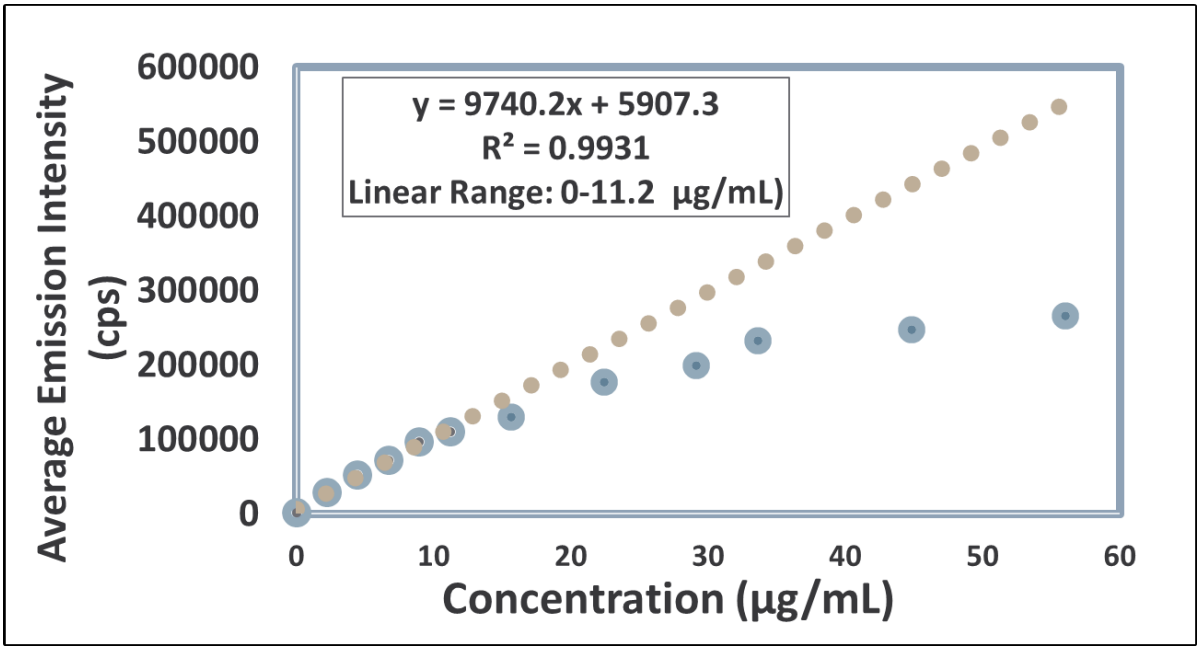

Figure 4: Fluorescence Calibration Curve of BPA stock in 1:1 methanol/water solution

Sample Preparation

Samples of pantyliners, tampons, and tampon applicators were prepared as described in the following sentences. For pantyliners, the top layer of netting material that comes into contact with the vulvar skin was removed and cut into ½ inch squares. Three pantyliners were used for each sample. For tampons, the outer netting layer, where available, which encompasses the cotton core of six tampons was removed and used for each sample. For tampon applicators, six applicators were cut into smaller pieces of ½ inch size and used for each sample.

Standard Addition Method

Due to the relatively low concentration of BPA within feminine hygiene products, the standard addition method was used to spike the samples with a known amount of BPA. By doing so, the fluorescent behavior of BPA is amplified, allowing more accuracy to be involved when concluding whether an emission intensity peak at 304 nm is due to BPA. For each experiment, samples of the feminine hygiene products were soaked in 1:1 methanol/water solutions for increasing intervals of time: 0 min, 20 min, 40 min, 1 hour, 1.5 hours, 2 hours, 3 hours, 4 hours, 5 hours, and 6 hours. Each specific time sample was then divided into five separate volumetric flasks, with 5-mL of sample in each flask. These samples were spiked with increasing volumes of BPA stock solution (also known as the standard): 0-mL, 2-mL, 5-mL, 7-mL, and 10-mL. Each sample was diluted to 25-mL with 1:1 methanol/water solution.

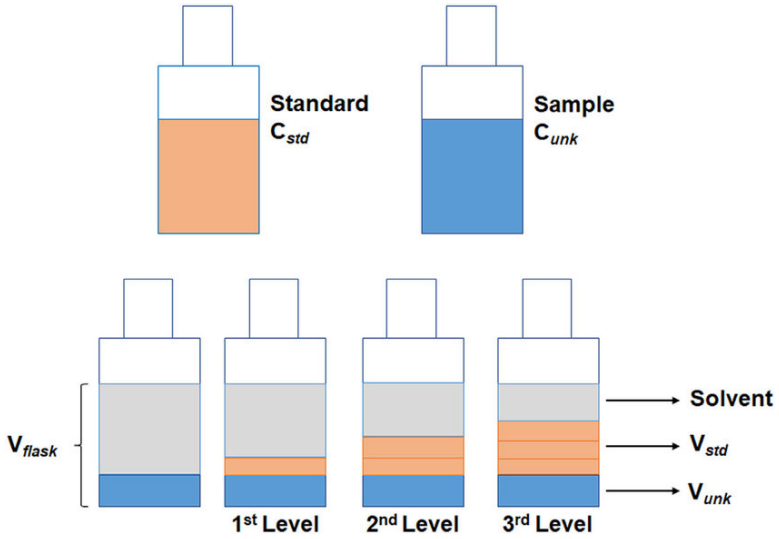

Figure 5: Standard Addition Method Visual Explanation (10)

Each sample was run on the spectrofluorometer and the emission intensity at 304 nm was recorded in quadruplicate. A diagram of this process is available in Figure 5. These

emission intensity values for each time point were averaged and plotted on a standard addition graph. The standard addition graphs allow for the creation of a curve that slopes up with increasing standard added. The line produced was then extrapolated to solve for the x-intercept, which translates to the unknown concentration of BPA in the sample. The standard addition equation (Eqn. 1) is provided below.

$$I_{s+u}\left(\frac{V_T}{V_u}\right) = I_u + \frac{I_u}{C_u}\left(c_s\right)\left(\frac{V_s}{V_u}\right) \quad (1)$$

Where I_{s+u} is the fluorescence intensity of the total solution which contains both standard BPA from the stock solution and unknown BPA from the sample. I_u is the fluorescence intensity of the unknown BPA sample only. C_u is the unknown concentration of BPA in the solution. C_s is the concentration of the BPA standard. V_s is the volume of the BPA standard used. V_u is the unknown volume of BPA leached into the solution from the sample. V_T is the total volume of the final solution, containing BPA standard, BPA unknown, and 1:1 methanol/water solution. An example standard addition graph for a specific time point is shown in Figure 6 on the next page.

A graph conveying the release of BPA into solution was obtained for each time point that sample was collected. For each time point graph, the line was extrapolated and the x-intercept was solved for. Each x-intercept value represents the unknown concentration of BPA leached from the product into solution at that particular time point. The x-intercept values were then compiled onto a single graph, showing the BPA concentration leaching from the product into solution over a period of six hours. An example of this is shown on the next page in Figure 7.

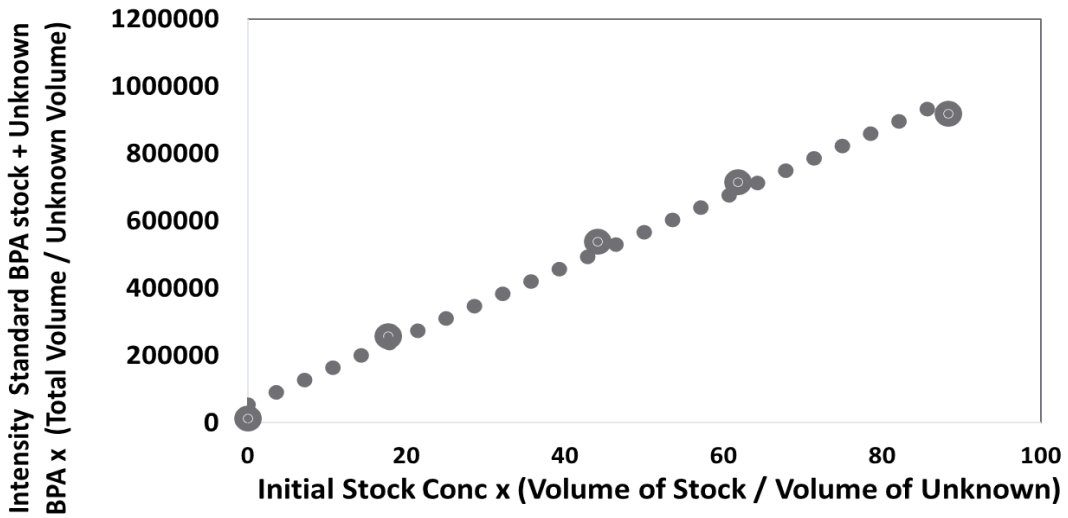

Figure 6: Standard Addition Graph of Tampon Applicator Sample

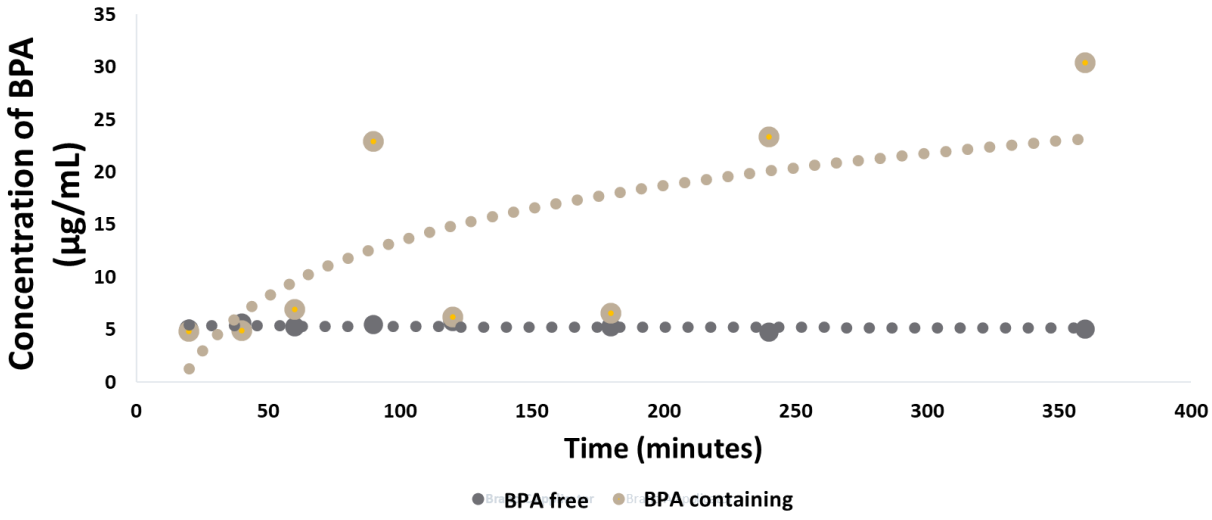

Figure 7: Curve of BPA-free product vs. curve of BPA-containing product

This graph contains two lines monitoring the release of BPA into solution from two different feminine hygiene products. The tan line shows a rise over time, indicating that the product was releasing BPA into solution as the six hours progressed. Contrastly, the dark gray line maintains a flat progression over time, indicating that little to no BPA was being released into solution over six hours and that this product likely does not contain BPA.

By compiling multiple lines onto a single graph, comparisons were made between the BPA-leaching behavior of different products over time. These results and comparisons will be discussed in the next section.

RESULTS AND DISCUSSION

The pantyliner and tampon products of three brands, A, B, and C, were utilized as samples for experimentation. Brands A, B, and C were a name-brand, generic brand, and luxury brand, respectively. The price for each product is shown in Table I.

Table I: Price Comparison of Brand A, Brand B, and Brand C Products

Brand	Brand A	Brand B	Brand C
Accessibility	Brand Name	Generic	Luxury
Price per Pantyliner	$0.08	$0.05	$0.19
Price per Tampon	$0.25	$0.14	$0.35

For the brand name, Brand A, pantyliners, tampons, and tampon applicators were tested. For the generic brand, Brand B, pantyliners, tampons, and tampon applicators were tested. For the luxury brand, Brand C, pantyliners and tampon applicators were tested.

The lines plotted on individual standard addition graphs showing the release of BPA for a given product over time were selectively compiled to create comparison graphs, as shown below.

Brand A, the brand name, shows a steepest rise over time in its tampon applicators (Fig. 8). The lines for its pantyliners and tampons remained relatively flat, with the tampons following a progression with a slightly higher concentration of BPA releasing over time. It is indicated that Brand A's tampon applicators contained the highest concentration of BPA, thereby releasing the most BPA into solution over time.

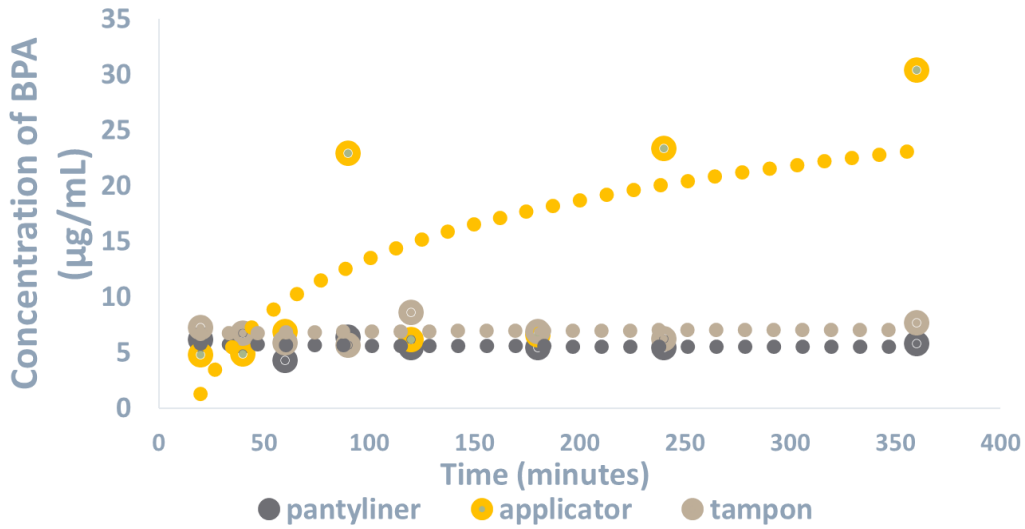

Figure 8: Concentration of BPA Leaching from

Brand Name Brand A pantyliner, applicator, and tampon

Generic Brand B's pantyliner curve has a steeper increase in BPA concentration over time than its applicators and tampons (Fig. 9). The tampon curve maintains the flattest progression, indicating it has the least BPA present and the pantyliner has the most BPA present.

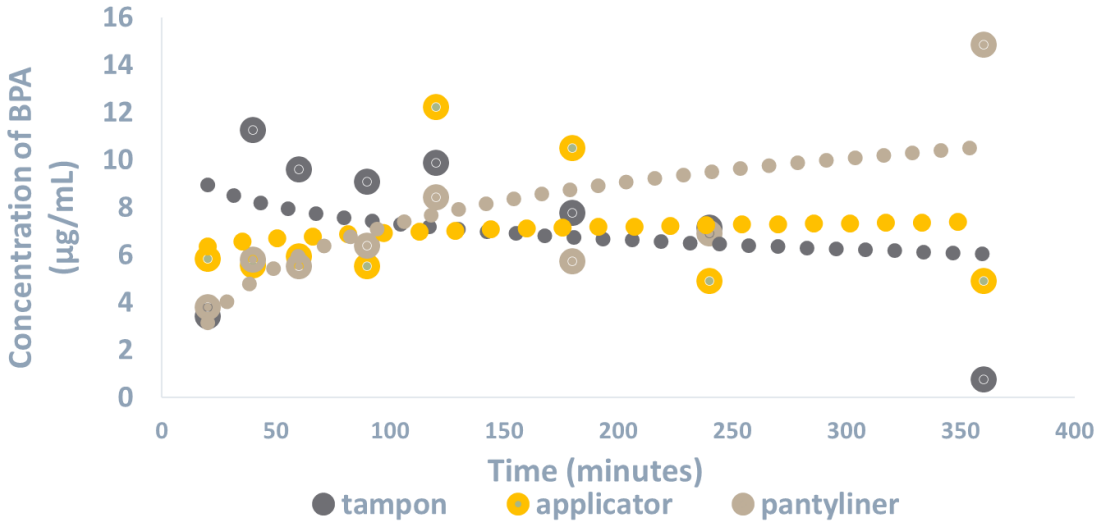

Figure 9: Concentration of BPA Leaching from generic Brand B pantyliner vs. applicator vs. tampon

Both the pantyliners and tampon applicators of Brand C, a luxury brand which includes a BPA-free claim on its packaging, maintained a steady concentration of BPA in their solutions over 6 hours (Fig. 10). This indicates that BPA concentrations in the brand's pantyliners and applicators were either very low or zero. The absorbent component of this brand's tampons were not tested, due to the lack of an outer netting layer to take for sample collection. Rather, the tampons consisted of only cotton, and could not be used for sampling.

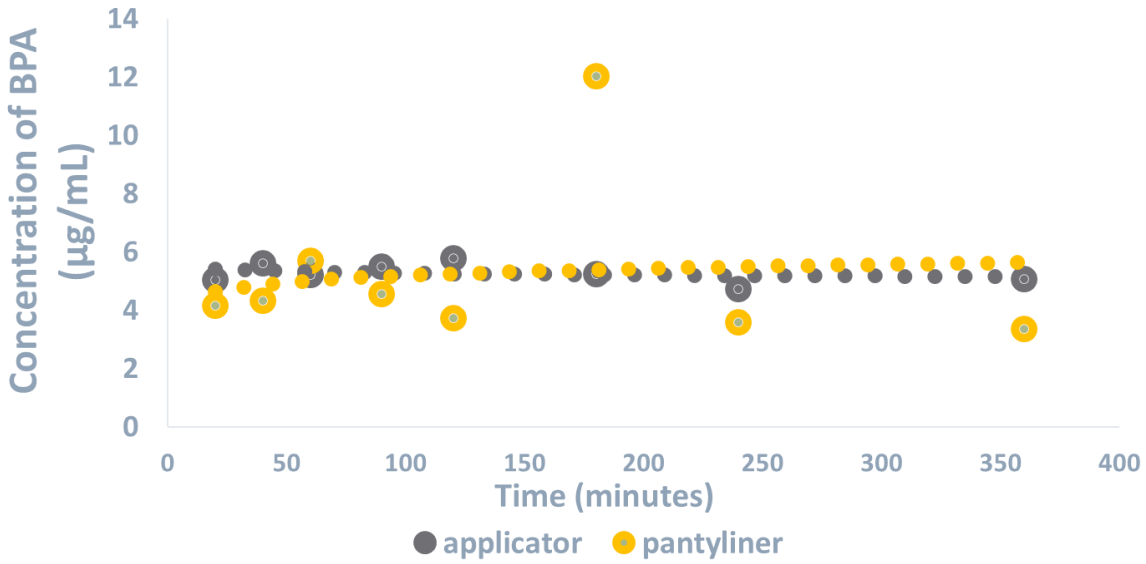

Figure 10: Concentration of BPA Leaching from Luxury Brand C Applicators vs Pantyliners

Previous graphs focused on variations between product types for a particular brand. Analysis was continued by comparing BPA leaching between brands for each product type. The following figure compares the BPA leaching over time of Brand A, Brand B, and Brand C tampon applicators (Fig. 11). Brand A showed a steep rise in BPA concentration of its applicators over time. Brand B's applicators showed a slight increase over time. It had more of an increase in concentration over time than Brand C, but less than Brand A. This indicates the presence of BPA in Brand A and Brand B applicators, but none in Brand C. The amount in Brand B may be slight.

Due to the steeper rise in both Brand A and Brand B's applicators' BPA concentration level compared to the tampon, the presence of BPA is higher in the tampon applicators compared to the absorbent component of the tampons.

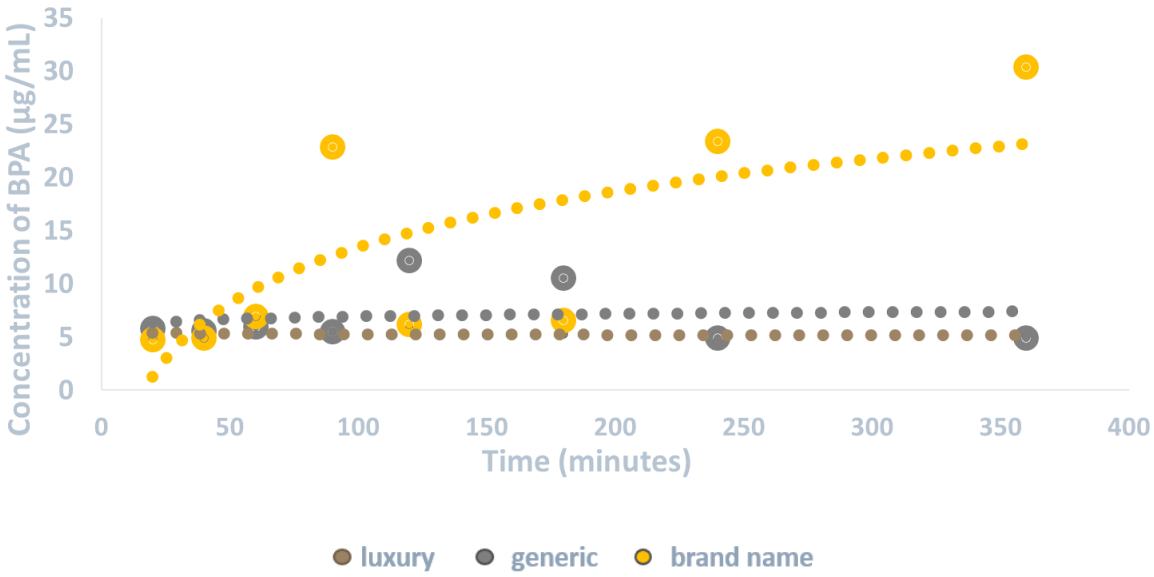

Figure 11: Concentration of BPA leaching from luxury Brand C vs. Brand B Applicators vs. Brand A Applicators

The BPA concentration values for both Brand A and Brand C pantyliners maintain a relatively flat progression over the six hours (Fig. 12). Brand B's line exhibits a steep rise, indicating that much more BPA was present in the generic pantyliners than in either the brand name or luxury brand.

Brand B pantyliners showed a steeper increase in the concentration of BPA leached into solution over time compared to Brand A pantyliners, indicating a higher level of BPA is in Brand B.

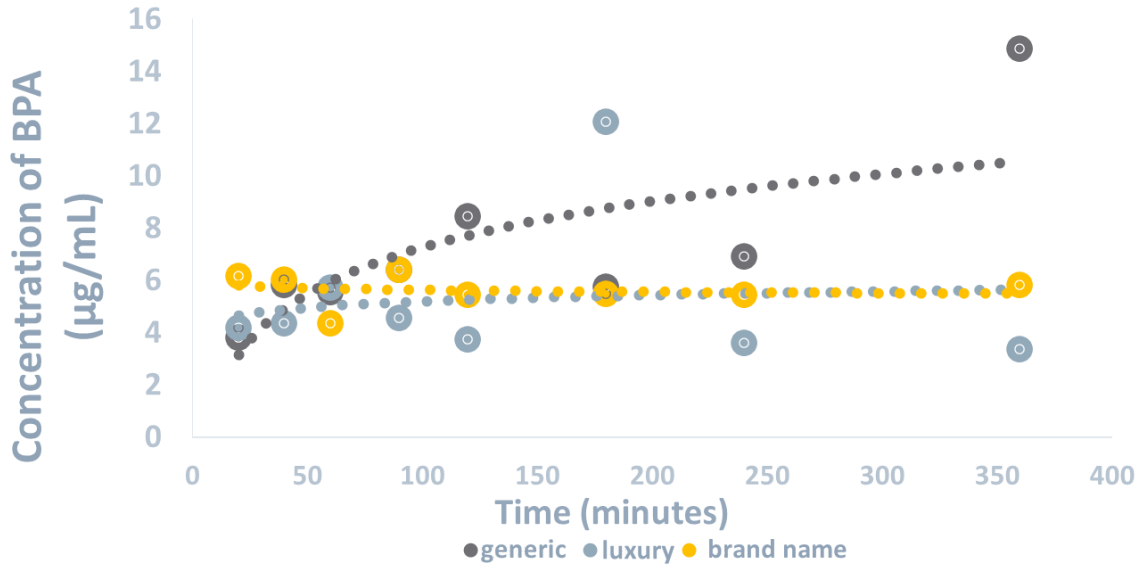

Figure 12: Concentration of BPA Leaching from Brand A vs. Brand B vs. Brand C pantyliner

CONCLUSIONS

Based on the results of this experiment, it is shown that BPA is present within the tested feminine hygiene products, including pantyliners, tampons, and tampon applicators. Among the three products of the name brand product, Brand A, its applicators appeared to leach the highest concentration of BPA into solution over time. Among the three products of the generic brand, Brand B, its pantyliners appeared to leach the highest concentration of BPA into solution over time. For both Brand A and Brand B, the tampon applicators leached more BPA into solution than the absorbent component of the tampon. Both the tampon

applicator and pantyliner samples of the luxury brand, Brand C, produced results that were consistent with the brand's BPA-free claim. These results are summarized in the table below.

Table II: Level of BPA Leaching from Each Product

Brand	Pantyliner	Tampon	Tampon Applicator
A	low	low	high
B	high	low	moderate
C	low	x	low

Thus, there appears to be a correlation between exposure to BPA and price point. From the sample of products tested in this experiment, it is conveyed that a higher price must be paid for one to purchase feminine hygiene products that do not contain BPA. These products must be bought regularly for the average woman, and the significantly higher price point of Brand C, which is a price comparable to other BPA-free claiming brands, is unaffordable for many women.

FUTURE WORK

Future work will involve conducting statistical analysis to further validate all results, including the comparisons made between brands. To further investigate the trend of potential price disparity, more brands will be tested. Future research will involve retesting pantyliners and tampon components in a solution that more closely mimics the physical and chemical

properties of vaginal fluid. Once BPA presence within the products has been confirmed, retesting in a vaginal fluid simulant will provide a better indication of whether BPA is likely to be leaching from the hygiene product to the vulvar skin.

BPA POLICY ACROSS THE GLOBE

The modern world is highly industrialized and scientifically advanced. Mass production and international trade fuels many first- and second-world economies. Reasonably, the manufacturers of the goods sold to the public want to create products with cheap ingredients that have extended shelf lifes and chemical stability of the product. However, at what point does manufacturing convenience outweigh the wellbeing of the public?

Bisphenol-A was synthesized by scientists in 1891, and was determined to behave as an artificial estrogen in the 1930's (2). In the 1950's, BPA began to be used as a stabilizer in polycarbonate and epoxy resin products in America (3). Eventually, BPA became an incredibly prevalent compound in plastic products across the globe. In the 1990's, increasing scientific research provided evidence of the toxicity of BPA exposure even at very low levels. In 1997, FDA tests revealed infant formula was contaminated with BPA from the BPA-lined cans it was packaged in (12). Likewise, infant bottles were made with a BPA-containing polycarbonate plastic. In 1999, it was determined that repeated washing and heating exacerbated the leaching of BPA from such products (13). It was not until 2008, eleven years after the finding, that Canada became the first country to ban BPA in infant bottles and formula cans (14).

It is alarming that it took eleven years for a country to begin making any stride toward reducing BPA exposure, particularly among infants and young children. The FDA even publicly asserted the safety of infants utilizing these products in 1999, assuring that the level of BPA exposure was too low to cause harm (15). Despite this claim, evidence of BPA toxicity at levels lower than the safety standard set by the EPA in 1988 (50 micrograms of BPA per kilogram of body weight per day) grew (3). In 1997, a few months before BPA leaching from formula cans was confirmed, Fred vom Saal at the University of Missouri-Columbia found that low level BPA exposure of 2ug/kg/day was linked to prostate cancer in male offspring (16). Over 100 epidemiological studies followed that concluded BPA was associated with disease/dysfunction in animal models (16). In 1999, scientists from the University of Missouri reported that female mouse fetuses exposed to 2.4 ug/kg/day of BPA altered the postnatal growth rate and induced early puberty (17). In 2002, Italian scientists found that female mice administered 10 ug/kg of bodyweight of BPA daily during gestation spent less time nursing their pups and more time out of the nest than the control group, indicating an impact of BPA on neuroendocrine functioning (18).

Beyond the United States of America, plastics are an abundantly used material. From toothbrushes, to food packaging, to athleticwear, to electronics, plastics are used. Plastic products are cheap, versatile, and durable to manufacture. Their stability is famously enhanced by bisphenol-A, by the increased rigidity it provides (19). Thus, BPA-containing plastic is circulating around the globe for its convenience and effectiveness. The United States was the place of initial synthesis of the compound, and subsequent industrial use, but BPA has since become prevalent in a multitude of countries. As discussed, the United States began using the compound heavily in industry in the 1950's, and no government assessments

concluded that BPA was harmful until 2007, when the National Institute of Environmental Health Sciences Chapel Hill expert panel stated that the chemical had "potential to impact human health at current levels of exposure" (3).

Still, policy change was not enforced at the national level. The current concern at this time was for infants, specifically regarding exposure from formula cans and bottles, and beginning in 2010, individual states began to ban BPA in these products, including California, Maryland, Missouri, New Jersey, New Mexico, New York, Pennsylvania, Vermont, Washington State, Wisconsin, Maryland, and Vermont (20). This banning of BPA in infant feeding products was an appropriate decision, as in December of 2009, an EWG study confirmed the presence of BPA in 9 out of 10 umbilical cord samples collected from newborns (21). Before even entering this world, infants are exposed to BPA through their mother. This reinforces that BPA consumption and exposure remains an issue through adulthood.

Just across the pond from America, the European Food Safety Authority did not complete its first full risk assessment of BPA until 2006 (22). In 2011, BPA-containing polycarbonate infant bottles were banned (23). In 2015, a re-evaluation of BPA exposure was published, and the tolerable daily intake was largely reduced from 50 to 4 μg/kg bodyweight (24). In 2018, the European Union employed stricter limits on the levels of BPA within food-contact materials. The standard migration limit of 0.05 mg of BPA per kg of food was set for all food-contact materials to assist in keeping the people's exposure to below the updated daily tolerable intake (25).

In 2017, the Member State Committee of the European Chemicals Agency supported a proposal from France to identify BPA as a substance of very high concern based on

evidence that it causes endocrine dysfunction (26). France was the first country to suspend BPA in all food packaging in 2012 (27). In addition to food-contact materials, Article 16(1) of Regulation (EC) No 1935/2004 communicates that business operators are responsible for providing written declarations of compliance with the aforementioned limits with their varnishes and coatings of materials and articles (25). While BPA is not banned in the EU, efforts to decrease the public's exposure to it are relatively respectable.

Moreover, in April 2022, the European Commission released a working document entitled "Restrictions Roadmap under the Chemicals Strategy for Sustainability" (28). A long list of common-use chemicals which is suspected to cause human and/or environmental harm is included. BPA is included on the proposed restriction list, "for all uses" (28). BPS is specifically listed as a potential substitute to BPA in thermal receipt paper (28). A "to-be-determined" risk assessment of BPA analogues is proposed in the document, however (28). While many think that "BPA-free" labeling indicates safety, such products typically contain BPA-alternatives, such as BPF and BPS, which are similarly linked to human health concerns due to comparable mechanisms of action (29). Hopefully these necessary risk assessments of BPA and BPA analogues will be conducted and policy change will occur correspondingly. This initiative to investigate and potentially restrict BPA, as well as its analogues, is a step in the right direction.

As of 2021, Canada also has claimed to have begun investigative research into the safety of 188 BPA analogs and alternatives (30). As previously discussed, Canada was the first country to complete a comprehensive assessment of human exposure to BPA and to in turn implement BPA restriction measures nationally (13). However, after the infant formula scare, Canada - like nearly all other countries -has become less concerned with any further

restriction or monitoring. The current tolerable daily intake in Canada is 25 μg/kg bodyweight/day, which was established in 1996 (31). In 2018, a BPA risk evaluation was performed in Canada, but addressed only infants (30). Thus, the only results were that there was a 96% decrease in exposure of infants since banning BPA in formula cans and infant feeding bottles (32). This is certainly significant, however, BPA continues to circulate throughout the population. Exposure to BPA is still ubiquitous. Infant exposure to BPA is even possible through maternal breast milk (33). Even with BPA eliminated from infant bottles and formula cans, infants – who are highly susceptible to the health consequences of BPA – still endure exposure to BPA as soon as their first feeding.

In other countries, BPA regulation is even less prevalent than in the United States, European Union, and Canada. Australia announced it would be phasing BPA out of use in baby bottles in 2010 (34). However, no updated information concerning BPA regulation or assessment has been reported since this statement. In 2018, a report was posted to the Australian Food Standards government website which stated that the phase-outs of BPA in products seen in Canada, the European Union, and the United States were "not supported by the risk assessment conclusions on the safety of BPA," and reassures the safety of Australians at current exposure levels (35). This seems quite misleading. It also makes clear that the Australian government is not concerned with regulating BPA in its country (35).

In South America, the trade bloc Mercosur, passed three resolutions to ban BPA in infant bottles and other food packaging for children which were not effective until 2022 (36). Also in 2022, the BPA migration limit was lowered to 0.05 mg/kg for other food contact materials (36). This is noticeably greater than the concentrations of BPA shown to cause human harm in scientific studies. With minor exceptions, BPA regulation is virtually absent

in South America (37). A similar statement can be made for the Caribbean, where biomonitoring of maternal urine samples indicates a high level of BPA exposure, and thus, a need for regulation (38).

BPA restriction is also virtually absent in Southeast Asia and Africa (37). Since 2000, China's BPA consumption has increased 10-fold, to a startling 3 million tons per year (39). The largest source of its exposure is electronics– which is alarming for a growing technological world (39). Another major source of exposure in China, Japan, and South Korea, was shown to be household dust, which conveys just how ubiquitous exposure is (40). Testing of urinary BPA concentration in China, India, Japan, Korea, Kuwait, Malaysia, and Vietnam all indicate concerning levels of exposure (41). In another region of the world, Africa, an increase in industrial production has been seen. In turn, Africa has experienced an increase in BPA exposure through BPA-containing products (42). BPA has been found at a significant level in Saudi Arabia's wastewater (43). In Egyptian markets, BPA is found in the food packaging products (44).

Due to the length of time that negative health effects of BPA exposure have been evidenced by research, it is disheartening that humans all over the globe are routinely consuming it, unawares. When formula-fed infants were identified as a highly exposed group due to the BPA's leaching ability, it took over a decade for any country to implement any restrictions. Once restrictions concerning infant exposure through food contact materials were in place in several countries, any government concern surrounding BPA seemed to dissipate. With the increasing empirical research indicating that BPA exposure, even at very low levels, is harmful to human health, an alarm should be sounding all around the world that

restriction is necessary. This is especially so due to the near-constant BPA exposure experienced each day, as it is in a multitude of daily-use products, foods, and drinks.

Traditional toxicology studies utilize a protocol which assumes the tested compound follows a monotonic dose-response curve (46). However, increasing empirical evidence that hormones and hormone-mimicking compounds exhibit a more unpredictable, non-monotonic dose-response curve have changed how compounds such as BPA must be analyzed for determining the lowest observed adverse effect level (LOAEL).

When assessing a compound for its LOAEL, the traditional approach in toxicology involves beginning testing the response level at a dose far higher than the daily exposure to determine the maximal tolerable dose (MTD) (46). From there, the dose is sequentially lowered until the LOAEL is determined (46). Although a linear, monotonic dose-response relationship may be expected, in which as dose increases or decreases, the response to the compound steadily rises or falls accordingly, this is not the pattern seen for many hormones. Examples of monotonic and non-monotonic dose-response curves are shown in Figure 13.

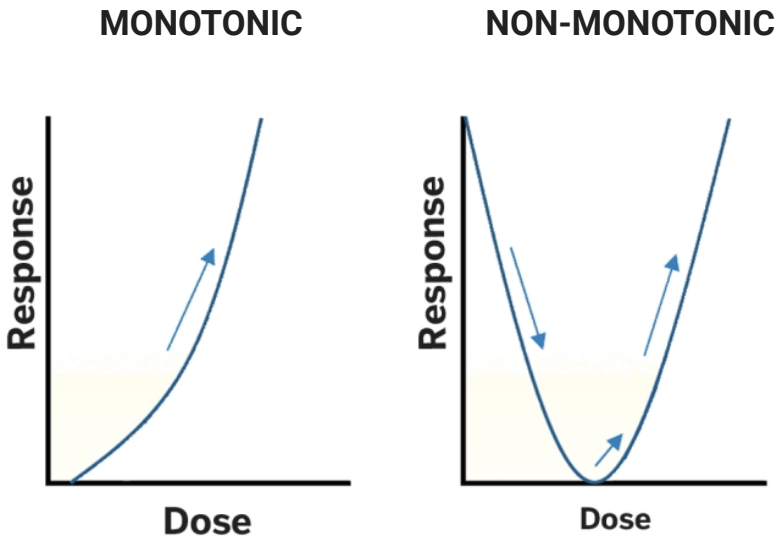

Figure 13: Representative Monotonic vs. Non-monotonic Curve (45)

Non-monotonic dose-response curves often follow a "U" or "inverted U" shape due to mechanisms such as binding kinetics and the specific actions of different tissues; these cause a response at a low dose that is not seen at a higher dose (6). Due to this non-linearity, there is potential for doses below the LOAEL to indeed induce a response, thus falsifying the previously presumed LOAEL (6). A visual representation of this is shown in Figure 14. Such a non-monotonic dose-response relationship is exhibited by BPA. Numerous studies have shown BPA to have significant effects at doses below the currently set tolerable daily intake (16). While historically a common argument has been that the dose of exposure to BPA is too low for public concern, evidence of the health risk of daily low-dose BPA exposure is increasingly abundant.

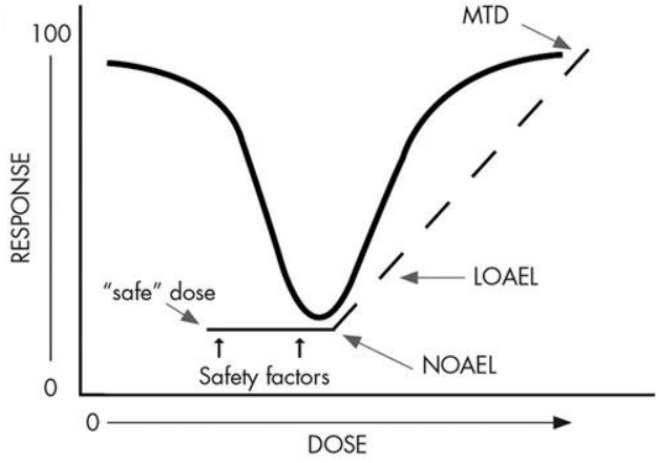

Figure 14: Traditional Toxicology Lowest Observed Adverse Effect Level Measurement Representation (46)

Fortunately, assessments of BPA and its analogs are being conducted in the European Union, and of BPA analogs in Canada (28, 30). However, it has been seen before that despite scientific evidence, more concern seems to be placed on maintaining the ease and affordability of BPA use as a structural component in plastic products. Hopefully, continued research further exposing the harm inflicted by BPA will drive change.

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