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DIETARY RISK ASSESSMENT OF BISPHENOL A MIGRATION IN SOFT DRINKS

BY

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(BSc. Biochemistry)

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DECLARATION

I hereby declare that this submission is my own work and that, to the best of my knowledge, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree or diploma at Kwame Nkrumah University of Science and Technology, Kumasi or any other educational institution, except where due acknowledgment has been made in the text.

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DEDICATION

To God Almighty, my family, friends and all loved ones.

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ABSTRACT

In Ghana, there has been a steady increase in plastic products used in the food industry for packaging. This research investigated the effects of storage temperature, pH and storage time on migration of BPA from five brands of soft drinks packaged in polyethylene terephthalate (PET) bottles in Ghana. BPA extraction and clean-up was done on a total of 60 samples of soft drinks using a modified QuEChERS method. Palisade @Risk software was used to determine the hazard quotient (HQ), of consumption of the soft drinks to characterize the risk. BPA was detected in all the samples at all three temperature conditions. BPA concentrations ranged from 0.23 to 0.39 ng/mL, 0.23 to 1.3 ng/mL and 0.23 to 5.17 ng/mL for samples stored at refrigerated, room and elevated temperature respectively. pH remained relatively constant in the acidic range of 2.72 to 3.58 over the four-week period. Hazard quotients (HQ<1) of BPA at refrigerated and room temperature meant the study population were at no significant health risk. At elevated temperature, the 95^{th} percentile value of 1.11 implied that more than 5% of the study population were at a significant health risk (HQ>1), and therefore should not be neglected.

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ABBREVIATIONS

ANOVA	Analysis of Variance
APs	Alkylphenols
AT	Averaging Time
BPA	Bisphenol A
BW	Body Weight
CDC	Centers for Disease Control
CDI	Chronic Daily Intake
CEDI	Cumulative Estimated Daily Intake
CR	Contact Rate
DEHA	Di (2-Ethylhexyl) Adipate
DEHP	Di (2-Ethylhexyl) Phthalate
ED	Exposure Duration
EDs	Endocrine Disruptors
EF	Exposure Frequency
EFSA	European Food Safety Association
EPA	Environmental Protection Agency
EU	European Union
FCN	Food Contact Notification
FFQ	Food Frequency Questionnaire
FRF	Fat Reduction Factor
GRAS	Generally Regarded as Safe
HC	Hazard Concentration
HPLC	High Performance Liquid Chromatography

HQ	Hazard Quotient
LOD	Limit of Detection
LOQ	Limit of Quantification
LSGM	Least Squares Geometric Mean
NHANES	National Health and Nutrition Examination Survey
NIAS	Non-Intentionally Added Substances
PAEs	Phthalates Esters
PC	Polycarbonate
PET	Polyethylene Terephthalate
PVC	Polyvinyl Chloride
R_fD	Reference Dose
SML	Specific Migration Limit
TDI	Tolerable Daily Intake
TRI	Toxics Release Inventory
UK	United Kingdom
US	United States
US FDA	US Food and Drug Administration
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background

Protecting food from tampering or contamination from physical, chemical and biological sources is the main goal of food packaging (Prasad and Kochhar, 2014). Glass, metal, paper and paperboard, and plastics are the traditional materials used by this industry (Tang *et al*., 2012). Due to its functional properties, convenience, resistance, low weight and costs, plastic emerged as the main material used in primary food packaging in the past decades (Accorsi *et al.,* 2014; Shah *et al*., 2008).

There are different types of plastics, each with unique properties and application in the food industry, for example polycarbonate, high and low density polyethylene, styrene and polypropylene. These plastics are manufactured from various polymers and additives are used to improve elasticity, flexibility, color, resistance and durability.

According to Wagner and Oehlmann (2009), both plastic and additives can migrate from the packaging to the food or beverage over time as a result of an increase in temperature or mechanical stress. The presence of plastic components or additives in food, if not properly controlled, can affect the organoleptic properties of food and produce an endocrine disrupting effect if the levels exceed the legislated or toxicological values.

Many plasticizers and additives are considered as endocrine disruptors (EDs). Endocrine disruptors act by interfering with the biosynthesis, secretion, action, or

metabolism of naturally occurring hormones (Diamanti-Kandarakis *et al.*, 2009; Kavlock *et al.*, 1996). Given the importance of hormones in human physiology, there is concern in the scientific community over the potential for endocrine disruptors to adversely affect children's health, particularly in reproduction, early and adolescent development and behaviour (Diamanti-Kandarakis *et al.*, 2009). Some of these endocrine disruptors are phthalate esters (PAEs), alkylphenols (APs), 2, 2-bis (4-hydroxyphenyl) propane or Bisphenol A and di (2-ethylhexyl) adipate (DEHA) (Vom Saal and Hughes, 2005).

Bisphenol A (BPA) is an organic compound, first synthesized by a Russian scientist named A.P. Dianin in 1891 (Rubin, 2011). Among many other uses, BPA is utilized to produce polycarbonate (PC) plastics, epoxy resin for cans, toys, microwave containers and water pipes. Heat and contact with either acidic or basic foods, as the process of sterilization in cans or polycarbonate plastic, increase the hydrolysis of the ester bond linking BPA molecules in the polycarbonate and epoxy resins and BPA monomers are released into foods (Vom Saal and Hughes, 2005).

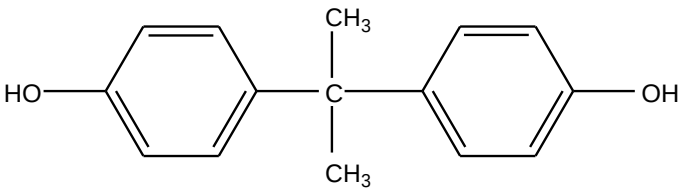

Figure 1.1: Molecular structure of BPA

Vandenberg *et al*. (2007) reviewed human exposure to BPA and highlighted that the potential BPA exposure is related to dietary sources, in particular foods stored in cans

with epoxy resin linings, drinking water, soft drinks, fruit juices and other carbonated beverages.

Soft drinks are non-alcoholic beverages that contain carbon dioxide, nutritive or non-nutritive sweeteners, natural or synthetic flavours, colours, acidification agents, chemical preservatives and emulsifiers in addition to other various functional agents (Ashurst, 2005). Soft drinks are formulated to provide an assimilated energy booster to the consumer and generally contain soluble sugars, which are easy to administer (Ashurst, 2005).

Ashurst (2005) also mentions that soft drinks are an essential source of hydration since they are more readily absorbed than water and hence can replace salt and energy quickly and are rapid thirst quenching. According to Heshmat (2011), the per capita soft drink consumption has increased almost 500% over the past 50 years. A third of teenagers drink at least three cans of soda a day and on the average adolescents get about 11% of their calories from soft drinks which corresponds to about 15 teaspoons of sugar (Grimm *et al*., 2004). A healthy diet during childhood and adolescence promotes optimal health, growth and cognitive development of the child, adolescent and may contribute to the prevention of chronic diseases in later life (Van Cauwenberghe, 2010).

Since BPA is widely used in food packaging, in recent years, there is an increasing concern regarding the level of BPA in the food system which could impact human health (Hammarling *et al*., 2000; Vandenberg *et al*., 2007). Thus, there is the need to

determine the level of BPA that migrate into food and how BPA is released into the food system from the packaging materials in order to ensure food safety.

1.2 Problem Statement and Justification

In Ghana, there has been a steady increase in plastic products used in the food industry for packaging certain foods like cereals, porridges, fruits juices, alcoholic and non-alcoholic beverages and carbonated soft drinks (Teye, 2012). Teye (2012) reported that it is very common to find mineral water, soft drinks and other beverages packaged in polyethylene terephthalate (PET) bottles on the Ghanaian market.

However, little work has been carried out on the extent to which BPA, a constituent of plastic bottles, can migrate into most carbonated soft drinks sold by vendors under different storage conditions. Therefore this study sought to evaluate the extent to which consumers are exposed to the effects of BPA migration in soft drinks packaged in plastic bottles.

1.3 Objectives

The general aim of the study was to evaluate the risk of BPA migration in soft drinks packaged in plastic bottles on the market. The specific objective was to determine the level of BPA in selected soft drinks under different storage temperatures and assess the health risk associated with consumption of these soft drinks in school-aged children.

CHAPTER TWO

LITERATURE REVIEW

2.1 BPA in the Food System

After Bisphenol A (BPA) was first documented to migrate from epoxy resin lining of canned food into its products in 1995 (Brotons *et al*., 1995), a significant body of work about the leaching of BPA into canned food has been performed by scientists worldwide. One of the early studies analyzing canned food contents was conducted in Goodson's laboratory in 2002. Sixty-two different canned foods were purchased from retail outlets in the United Kingdom. The can contents were homogenized before analysis by GC-MS. BPA was found at detectable levels in more than half of the foods (Goodson *et al*., 2002). Goodson's research team also studied the effects of damage to canned foods and storage time on the release of BPA into the food. In their analysis, they discovered that the foods and simulants acquired 80-100% of the total BPA present in the can coatings immediately after the sealing and sterilization processes. Denting of the cans did not increase the migration of BPA into food. After heating the food while it was still inside the can in boiling water, as one might do to prepare the food for consumption, no increase in BPA migration was seen. Allowing canned products to be stored for the entire shelf-life also does not increase BPA levels in the foodstuffs (Goodson *et al*., 2004).

Thomson and Grounds in 2005, conducted an exposure assessment of eighty different canned foods from retail outlets in New Zealand. They detected BPA in all of the foodstuffs except soft drinks. The highest concentrations of BPA were found in canned tuna, corned beef, and coconut cream. As part of their assessment, they constructed exposure doses by using the mean BPA concentration determined from

their food testing and 24-hour dietary recall information from over 4000 individuals. The mean exposure dose was determined to be 0.008 μg/kg bw/day and the maximum exposure was 0.29 μg/kg bw/day. Most of the individual scenarios that they modeled resulted in no BPA exposure. These exposure doses were based on adult consumption scenarios, and, therefore, cannot be used to make conclusions about exposures of other age groups (Thomson and Grounds, 2005).

Vivacqua's study conducted in Italy is one of the few studies that investigated the BPA content of fresh fruit and vegetables. Fourteen fresh foods were selected for analysis for the presence of BPA and nonylphenol. BPA concentrations were discovered in eight of the fourteen fresh foods in the range of 0.25 to 1.11 mg/kg. The study also explored the estrogenic activity of the contaminants. Estrogen-like activity was displayed in their tests with the estrogen dependent MCF7 breast cancer cells (MCF7wt), its hormone dependent but ER-positive variant MCF7SH, and the steroid-receptor- negative human cervical carcinoma HeLa cells (Vivacqua *et al*., 2003).

2.2 BPA from Non-Food Exposure Pathways

Although BPA exposures from dietary sources are the primary pathway, detectable levels of BPA can be found in soil, dust, air, water, and medical devices leading to exposure from inhalation and dermal absorption pathways (Kang *et al*., 2006; Von Goetz *et al*., 2010; Wilson *et al*., 2007). Occupational exposure to BPA mostly occurs through the inhalation and dermal pathways. Workers could be exposed during the manufacturing of BPA monomers, while BPA is being incorporated into commercial products such as epoxy resin powder paint, and throughout packaging and transport. It is possible to be exposed to BPA during the thermal processing used to recycle

plastics and make them into new commercial products (Tsai, 2006). Cashiers who frequently handle thermal receipts are at greater risk for BPA exposure. In models of exposure to BPA from daily intake and dermal absorption, occupationally exposed individuals were dosed at rates 100 times the general population (Liao and Kannan, 2011). In a study of the urinary BPA concentrations of pregnant women, cashiers were the occupational category most highly exposed. The study also revealed an additional source of BPA exposure, which is smoking. Women who actively smoked or were exposed to secondhand smoke had 20% higher urinary BPA concentrations than non-smokers. The BPA exposure is likely obtained from the tobacco smoke that becomes contaminated from the BPA-laden filter. Some cigarette filters are as much as 25% BPA by weight (Braun *et al*., 2011).

BPA levels in the air, water, and soil environments have been detected and should be closely monitored. It is known that BPA is released into the environment. According to the Toxics Release Inventory (TRI), 1.8 million pounds of BPA was released into the environment in 2003 in the United States. This waste was released from the industrial sector, municipal wastewater treatment plants, and landfills (Tsai, 2006). The EPA states that a million pounds of BPA are released into the environment (EPA, 2012a).

Although BPA is known to be released into the air from TRI reports and has been measured in outdoor and indoor air, BPA's physical properties of a high boiling point and low vapour pressure do not allow BPA to easily evaporate. From this information, some scientists conclude that BPA inhaled from the air should not be of concern as an exposure route (Kang *et al*., 2006; Sajiki *et al*., 2007). Of the BPA releases reported

through TRI in 2003, 123,000 pounds were released to the air. This is a small amount compared to the overall releases of BPA to the environment (Tsai, 2006). In outdoor air, BPA's concentration ranges from <0.1 and 4.72 ng/m^3. In indoor air, BPA concentration is much higher, with a range of <0.1 to 29.0 ng/m^3. BPA levels in the air are from several sources. Indoor sources of BPA are hypothesized to be from household goods and furniture (Wilson *et al*., 2001; Wilson *et al*., 2003). Outdoor concentrations of BPA may be from combustion by-products such as from open-air barrel burns that total 79,000 kg per year (Sidhu *et al*., 2005).

Studies of BPA in the aquatic environment are numerous. BPA has been detected in leachates from industrial and municipal waste disposal sites at 8400 mg/L and 10,300 mg/L respectively. These levels are higher than aquatic toxicity value. BPA can also be detected in wastewater effluents from paper recycling plants (Fukazawa *et al*., 2001). BPA is considered to be readily biodegradable by bacteria in rivers under aerobic conditions with a half-life in freshwater of 3-5 days. In seawater, BPA persists much longer, about thirty days. In both aquatic environments, the aquatic organisms have been shown to have higher levels of BPA in their systems than is measurable in their aqueous environment. Caution should be taken in consuming seafood and freshwater fish from contaminated waters (Kang *et al*., 2006). Due to the way that drinking water is treated, through chlorination, ultraviolet light radiation, and ozonation, estrogenic compounds are destroyed. Even if BPA does leach from a PVC pipe, it will be destroyed by the chlorine in the water (Lee *et al*., 2004; Sajiki and Yonekubo, 2002).

Sediment can serve as a sink for BPA contamination due to BPA's organic carbon partition coefficient tested in the range of 2.5 to 4.64 (Staples *et al*., 1998). Soil and sediment samples from rivers in Taiwan and Germany show that BPA concentrations are higher in the soil than the water, showing evidence of its partitioning into the soil (Heemken *et al*., 2001; Lin, 2001; Stachel *et al*., 2005).

BPA is a component of dental composites and sealants. In a study of BPA residue after application of composite resins containing BPA, saliva was analyzed before treatment, after treatment, and after gargling. Saliva samples were collected after a patient chewed on a paraffin pellet for five minutes. BPA was detectable in the saliva directly after treatment, but was easily removed after gargling with tepid water for thirty seconds. BPA exposure from this route is therefore not chronic (Sasaki *et al*., 2005). Exposure assessment research was conducted on patients receiving dental sealants containing BPA. Only one of the two brands of sealants leached significant levels of BPA, on the same level where estrogen receptor –mediated effects have been seen in laboratory animal tests. Urinary BPA concentrations remain elevated for more than 24 hours (Joskow *et al*., 2006).

There is concern over possible BPA exposure from the plastic components of medical devices and equipment. Polyvinyl chloride (PVC) plastics are known components of medical tubing and bags. PVC is a concern because it can contain BPA and phthalate additives. In a recent study of premature infants that have received medical treatment in the neonatal intensive care unit, researchers classified babies into risk categories according to their degree of exposure to PVC plastic medical devices containing di (2-ethylhexyl) phthalate (DEHP). It was found that babies that received the most

intensive care had an order of magnitude higher urinary BPA concentration than the general population (Calafat *et al*., 2008)

2.3 BPA in the Human Population: Biomonitoring

BPA has been detected in the human body in blood, urine, saliva, breast milk, semen, amniotic fluid, and follicular fluid (Vandenberg *et al.*, 2007, 2010). Since BPA can be found in nearly the whole population at any given time even though is rapidly metabolized, it is believed that human exposure to BPA must be significant, continuous, and from multiple sources (Vom Saal and Hughes, 2005).

A valuable tool in researching associations of BPA body burdens with socioeconomic factors and adverse health outcomes in the United States population is the National Health and Nutrition Examination Survey, or NHANES. NHANES is a continuous, cross-sectional study that uses a complex, multi-stage sample design to achieve nationally representative samples. It entails dietary assessments, physical examinations, and laboratory testing. Data analysis and reporting of the laboratory testing performed in NHANES is completed by the Centers for Disease Control's Environmental Health Laboratory (CDC, 2012a). The CDC publishes the National Report on Human Exposure to Environmental chemicals with the latest being the Fourth National Report released in 2009. This report includes chemicals where there is concern of exposure and health effects (CDC, 2009).

Starting in 2003-2004, Bisphenol A has been tested in NHANES in a representative random, one-third subsample of NHANES participants 6 years and older. In this NHANES cycle, there were 10,122 subjects in the total sample and 2517 in the

subsample. Of the 2517 participants in this subset, urinary BPA concentrations were detected in 92.6% of this population. The unadjusted geometric mean urinary BPA concentration for 2003-2004 was 2.49 ng/mL (95% CI of 2.2 to 2.83) (Calafat *et al*., 2008).

Children have the highest urinary BPA concentrations of the ages tested in NHANES. An analysis of NHANES 2003-2004 data showed that the BPA concentration of 6-11 year olds, adjusted for sex, race/ethnicity, age group, creatinine concentration, and income, had a least squares geometric mean (LSGM) of 4.5 µg/L (95% CI of 3.9 to 5.1). The urinary BPA concentrations of this age group were statistically significantly higher than adolescents' ages 12-19 years old ($p < 0.001$). The adolescents, in turn, had higher BPA concentrations than adults ($p < 0.001$) (Calafat *et al*., 2008).

In the 2005-2006 NHANES cycle, the unadjusted geometric mean urinary BPA concentration declined to 1.79 ng/mL (95% CI of 1.64 to 1.96) (Melzer *et al*., 2010). In this same cycle, research was conducted to estimate BPA intakes derived from the NHANES individual urinary BPA concentrations and individual body weights. These calculations found the highest BPA median intakes in its youngest age groups, the 6-11 year olds, with an intake of 64.6 ng/kg-day, and 12-19 year olds, with an intake of 71.0 ng/kg-day. Each subsequent age category had decreasing median BPA intakes: 52.9 ng/kg-day for 20-39 year olds, 38.3 ng/kg-day for 40-59 year olds, and 33.5 ng/kg-day for subjects sixty years and older (Lakind and Naiman, 2011).

Analysis of the 2003-2004 NHANES data showed that urinary BPA concentrations varied by race/ethnicity. Race/ethnicity was stratified into three categories: non-

Hispanic Whites, non-Hispanic Blacks, and Mexican-Americans. In studies by Calafat, the analysis found that Mexican-American urinary BPA least squares geometric mean (LSGM) concentration of 2.3 µg/L was statistically significantly lower than non-Hispanic Blacks (3.0 µg/L) and non-Hispanic Whites (2.7 µg/L). There was no statistical difference between non-Hispanic Blacks and non-Hispanic Whites (Calafat *et al*., 2008). Lakind and Naiman's use of 2005-2006 NHANES data to compare BPA intakes according to ethnicity similarly found that Mexican-Americans and non-Hispanic Whites had statistically significantly lower urinary BPA than non-Hispanic Blacks (Lakind and Naiman, 2011).

Income analysis of the 2003-2004 NHANES showed that BPA exposures are highest for those with the lowest income bracket. In regression models of urinary BPA concentrations including the variables of sex, race/ethnicity, age group, creatinine concentration, and income, the adjusted LSGM was statistically significantly higher for the low household income category of <$20,000 (3.1 µg/L) as compared to high household income of >$45,000 (2.5 µg/L) (Calafat *et al*., 2008). In a recent study of NHANES cycles 2003-2008, an association was found showing families with lower income, lower food security, and that accessed emergency food assistance had higher urinary concentrations of BPA (Nelson *et al*., 2012).

The evidence for differences in BPA exposure levels by sex is not consistent among studies. In Lakind and Naiman's studies of NHANES 2003-2004 and 2005-2006, modeled median daily intake of BPA exposures for males is higher than for females (Lakind and Naiman, 2008; Lakind and Naiman, 2011). In Calafat's research, their regression model on BPA urinary biomarkers for NHANES 2003-2004 found

exposures for females higher than males (Calafat *et al*., 2008). In a small scale longitudinal study of Japanese school children, no statistically significant difference in BPA exposure levels was found between boys and girls (Yamano *et al*., 2008). NHANES dietary assessment tools were analyzed for indicators of dietary exposures that may lead to high urinary BPA concentrations. The only question from the food frequency questionnaire (FFQ) pertaining to food packaging inquires about the consumption of canned tuna. In the dietary recall data, information is recorded on intake of bottled or canned drinks. In analysis of these two pieces of dietary information, neither of these consumption areas was found to be associated with increased urinary BPA. Urinary BPA concentrations were found to be higher for study participants that drank more soda, although the packaging of the soda consumed is unknown. From FFQ data, urinary BPA concentrations were found to be statistically significantly higher for subjects that ate more school lunches and consumed more prepared food outside of the house (Lakind and Naiman, 2011).

To discover the body burden of BPA in the Asian population, Zhang's laboratory investigated urinary BPA concentrations in several Asian countries. The lab analyzed samples from 296 participants from Kuwait, Korea, India, China, Vietnam, Malaysia, and Japan. BPA was detected in 94.3% of the samples. Kuwait had the highest levels with an estimated median daily intake of BPA of 5.19 µg/day, and Japan had the lowest, with 1.61 µg/day. The age group with the highest intake were subjects less than or equal to 19 years of age. No significant gender difference was detected between subjects living in urban or rural area (Zhang *et al*., 2011).

2.4 BPA Metabolism in Humans

A handful of studies have researched the metabolism of BPA in humans after exposure. When BPA is ingested by humans, it is biotransformed in the liver on its first pass into bisphenol A-glucuronide, a highly water soluble metabolite. This metabolite is then rapidly excreted by the kidneys with urine (Volkel *et al*., 2002). By monitoring BPA doses in healthy adults from ingestion to excretion, evidence shows that BPA's half-life in the body is less than six hours and it is completely cleared from the body in 24 hours (Tsukioka *et al*., 2004; Volkel *et al*., 2002). This rapid clearance from the body through urine makes total urinary species, comprised of free plus conjugated BPA, the most appropriate BPA exposure assessment marker (Melzer *et al*., 2010). Dermal absorption and inhalation of BPA is of concern because exposures from these pathways are able to circumvent the first-pass metabolism of the liver and enter the circulatory system directly (Vandenberg *et al*., 2007).

BPA exposure in infants and children is a critical concern because their liver and kidneys are still developing. The kidneys do not reach full maturation until two years of age (Yamano *et al*., 2008). In their first year, the glomerular filtration volume of the kidneys develops. In the second year, the renal tubular function that excretes toxins increases to adult capacity (Yamano *et al*., 2008). Due to incomplete liver maturation, infant systems are unable to metabolize BPA through glucuronidation as adults do. It is theorized that infants metabolize BPA through a combination of glucuronidation and sulfation. Research on the biotransformation of acetaminophen shows neonates rely on sulfotransferases to metabolize this drug. This mechanism for metabolizing BPA is plausible because BPA is a substrate for sulfation; and

sulfotransferases, responsible for sulfation, develop earlier in neonates than UDP-glucuronosyltransferases, responsible for glucuronidation (Ginsberg and Rice, 2009).

BPA's metabolism in humans was also studied in a 36-hour dosing experiment where 10 men and 10 women ingested one of three specified meals made from grocery store food for breakfast, lunch, and dinner. Blood and urine samples were taken every hour to monitor BPA metabolism. It was discovered that serum levels of BPA were 42 times lower than in urine. Their findings closely matched the serum levels studied in Volkel's high dose studies (Volkel *et al*., 2002). The slight differences in timing of peak BPA in serum could be from the fact that in Volkel's study, the BPA was ingested from a hard gelatin capsule and in Teeguarden's study, the BPA dose was administered through a regular meal. The high comparability of serum pharmacokinetics between these two studies provides strong evidence that adsorption and elimination of BPA is linear in humans. This study learned that spot urine samples reflect exposures from the prior meal, in a 4-6 hour timeframe. The timing of spot urine sample in comparison to prior meals and taking into account the timing of the previous void which would eliminate accumulated BPA will also determine its ability to accurately measure exposures (Teeguarden *et al*., 2011).

There is some research challenging the commonly held belief that BPA exposures are almost exclusively from food sources. For NHANES, the urinary BPA concentration testing takes place after a period of overnight fasting for 9.5 hours for morning appointments and 6 hours of fasting for afternoon and evening appointments (CDC 2012b). As BPA levels remained higher than BPA half-life calculations predict, Stahlhut's study showed the possibility of people's exposure to non-food sources as

being more significant than previously estimated, in addition to the possibility that BPA is being stored in fat and slowly released (Stahlhut *et al*., 2009). In a study by Christensen, urinary BPA concentrations of 5 healthy individuals were monitored before, during, and after a 48 hour fasting period. The data showed that the BPA concentrations decreased significantly after 24 hours, and then remained at a constant low level for the remainder of the study. They concluded the remaining BPA concentrations are attributed to either non-dietary sources, mainly from dust, or that small reservoirs of BPA from past exposures are being released and excreted (Christensen *et al*., 2012).

Some research disagrees on regulatory frameworks based on the belief that rapid metabolism and excretion of BPA in humans also diminishes any concern about fetal and neonatal exposures. Regulations should consider, according to Ginsberg and Rice, the ability of fetal and neonatal deconjugation of BPA. The fetus and placenta have α-glucuronidase, which has the ability to deconjugate BPA. In rats, the placenta has high-levels of α-glucuronidase activity resulting in fetal exposure to deconjugated BPA. Also, although neonates conjugate BPA with sulfate using sulfotransferases, research on endogenous hormones has shown that biological activity does not end with sulfation. There is no reason to believe that BPA is completely de-activated by sulfation either. There are also local deconjugation reactions (Ginsberg and Rice, 2009). Ginsberg's theories of deconjugation are not commonly held and are disputed by Vandenberg saying that this theory does not have enough scientific support (Vandenberg *et al*., 2007).

2.5 Health Effects of BPA

Health concerns from BPA exposure to humans are numerous. Several studies reflect concern over BPA's endocrine disrupting properties' ability to adversely affect reproductive development in both sexes. BPA exposure could be contributing to an increase in rates of heart disease, diabetes, obesity, brain development issues, altered behaviors, and reproductive cancers (Melzer *et al*., 2010; Vandenberg *et al*., 2007). In the occupational setting, where exposures can be much higher than in the general population, there is concern that BPA exposure can affect reproductive hormones (NTP, 2008). BPA entered the mainstream spotlight in 2008 when Canada banned its use in baby bottles. The Canadian government was concerned with BPA leaching from infant formula cans, infant feeding bottles and drinking cups, and potentially having adverse health effects for infants (Environment Canada and Health Canada 2008a). BPA's possible estrogenic activity raised concerns since infants have a limited capacity to metabolize BPA and exposure to it is possibly associated with the early sexual development of children and some cancers. Only small changes in hormone activity during development can cause permanent effects (Welshons *et al*., 2003). There is also concern about prenatal maternal exposure possibly leading to reproductive and developmental issues for fetuses (Soto and Sonnenschein, 2010).

2.6 Effects of Temperature on Migration of BPA

Temperature is found to be one of the factors essential for the migration of BPA. Le *et al*. (2008) studied the effects of temperature on new and used bottles. The researchers concluded that the concentrations of BPA in heated samples are twice those in room temperature. A linear relationship is thus found between BPA and temperature. The higher the temperature the higher the concentration of BPA

migrated. The researchers justified their study by claiming that during cold weather hot water is stored in bottles. The work again found an elevated rate of BPA migration to have been achieved after hot bottles were cooled and incubated with room temperature water. This phenomenon is meant to explain that the leach of BPA is not only limited to heating but long term effects also take place (Le *et al.*, 2008).

The properties of polycarbonate plastic make it the ideal material to be used in the production of plastics. It is transparent, has good wear resistance and stable under sterilization. After washing bottles in dishwashers, boiling them and brushing, researchers found increases in the migration of BPA (Brede *et al*., 2003). The researchers tested and found BPA concentrations in 12 new bottles. After subjecting them to various treatments such as dishwashing, boiling and washing with brush significant increases were observed in the concentrations of BPA. Concentration of BPA decreased between 51 washings to 169 (Brede *et al*., 2003). These results are a confirmation of work by Le *et al.* (2008), who found no significant differences between used and new bottles.

Another team of researchers studied migration of BPA in baby bottles. They however used human participants (Maragou *et al.*, 2008). They examined migration of BPA using liquid chromatography. In all 21 baby bottles were used in the study. They were studied in water or an aqueous food substance. The bottles were cleaned with brush, dish washed and sterilized. At the end of the study, temperature was found to have significantly affected the results.

Howdeshell *et al*. (2003) conducted studies on migration of bisphenol A using animal cages. They studied the migration under different temperatures, using new and used cages. The concentrations were higher in elevated temperatures and lower at room temperature. Their work again measured the migration at room temperature and at neutral pH. The researchers incubated purified water at normal room temperature in new cages, used cages and glass containers (control). The results concluded that significant amounts of BPA were released from used cages, a little were released in the new cages and none released in the glass bottles. The results are clear indications that animals stored in such cages are at risk of being exposed to BPA.

The study by Le *et al*. (2008) acknowledges the difference in findings from their study (Howdeshell *et al*., 2003) about the increased migration of BPA after use. The main cause for such findings was because hot water with temperatures of 82 ºC was used in cleaning the cages. They thus practiced the laws of Association for Assessment and Accreditation of Laboratory Animal Care standards. In our homes however bottles are washed at temperatures within 50 ºC (Le *et al.,* 2008).

2.7 Effects of pH on Migration of BPA

The release of BPA into food matrix can be through leakage from plastic packaging. This leakage is caused by acidic or basic conditions which accelerate the hydrolysis of the ester bonds linking the plastic polymer therefore releasing monomers of BPA into the product in contact with the plastic material (Richter *et al.,* 2007). The concentrations of bisphenol A in packaged transparent plastic film increased with increase in storage time, pH, temperature and sugar levels. Sungur *et al.* (2013) conducted a study on the effects of glucose, sodium chloride and expiration date on

migration of bisphenol A into foods packaged in containers whose inner surface had been lined with plastic film, foods in glass jar and metal cans displayed for sale on the Turkish market. The study concluded that changes in the amount of bisphenol A in all foods are based on date of expiration, the amount of glucose and sodium chloride. They further concluded that the amount of bisphenol A increases with an increase in the amount of glucose, NaCl and expiration date.

2.8 BPA Risk Characterization

Minimal research has been conducted to characterize the risk of BPA exposure of children. From Japan, a longitudinal study was conducted tracking urinary BPA concentrations of elementary school children. It followed nearly a hundred school children from 1st grade to 6th grade. BPA was detected in 100% of these children's urine in the first grade sample, 97% in the third grade, and 86% in the sixth grade. The concentration of urinary BPA also decreased over time. A possible reason for the decrease in urinary BPA concentrations was the reduction in the use of BPA in canned food in Japan. Another possible explanation for the decrease in urinary BPA concentrations is that the consumption of canned foods decreased. Also, the polycarbonate plastic serving dishes used in school cafeterias were replaced with polyethylene terephthalate (PET) during the time of the study (Yamano *et al*., 2008).

In a United States study based in North Carolina, nine pre-school children aged 2-5 years were studied in two daycare centers for aggregate exposure to pesticides and persistent organic pollutants, including BPA. During the 48 hour sampling period, indoor and outdoor air, floor dust, play area soil, and duplicate diet samples were collected at the daycare center and at the homes of participating children.

Hand wipes and urine samples were also collected from each child. Time-activity diaries were completed to help correlate activities and exposures measured. This study concluded that the children's exposures were very low for most pollutants and that exposure levels were similar between the daycare center and home and between low- and middle-income households. As for BPA, the main environmental exposure was from indoor air. Overall, the primary route of exposure for BPA was from dietary ingestion (Wilson *et al*., 2003).

Wilson greatly expanded her exposure studies of BPA and other chemicals in young children to 257 preschoolers in 2006. Extensive environmental samples were taken in their homes and daycare for 48 hours. These included samples of food, beverages, indoor air, outdoor air, house dust, soil, swipes of participants' hands, and urine collection. BPA was measured in more than 50% of the samples of indoor air, hand wipes, solid food, and liquid food samples. It was estimated that highest potential aggregate dose, assuming 50% absorption, is 1.57 µg/kg-bw/day. This study concluded that BPA's main exposure is from dietary ingestion, accounting for 99% of exposure for children. The remaining 1% of BPA exposure is from inhalation (Wilson *et al*., 2007).

To study BPA metabolism and the effect of removing BPA from the diet, five families of four in the San Francisco Bay Area were closely monitored for eight days before, during, and after following a "BPA-free" diet. The basic structure of the study was that the families began urinary BPA monitoring while consuming their normal diets in the first two days of the study. Then, for the next three days, BPA exposures from the food supply were eliminated by supplying the families with catered meals

carefully prepared with fresh, organic, whole foods without any plastic cookware and stored in glass containers. The participants were only allowed to supplement their supplied food with fresh foods and foods from glass jars. The families then returned to their normal diets and monitoring continued for two more days. Monitoring results show that urinary BPA concentrations declined by 66% during the intervention period of the study. Post-intervention, the urinary BPA concentrations returned to the pre-intervention levels. The researchers acknowledge that not all sources of BPA can easily be eliminated from the food system. If this were possible, then they should have seen a 99% reduction since dietary BPA exposure is estimated to be 99%. One source of BPA exposure could be from milk that travels through PVC piping during processing. BPA has also been detected in whole eggs. The study also suggested that non-dietary exposures to BPA could be larger than previously estimated (Rudel *et al*., 2011).

In another BPA dietary study from the United States, study participants were assigned to eat canned soup or fresh soup using a randomized, single-blind, 2 x 2 crossover study design. They ate the type of soup they were assigned for lunch for five days. After a wash-out period of two days, the groups were switched and they ate the new type of soup for lunch for five days. Urine was collected on the fourth and fifth days of each study week. Consuming canned soup versus fresh soup increased the urinary BPA concentrations by 1221%. The urinary BPA concentrations of the participants after consuming canned soup were the highest measured in a non-occupational setting (Carwile *et al*., 2011).

Exposure assessment research from Japan investigated the relationship between source and exposure using two methods. The first method was to model aggregate exposure to BPA from inhalation and ingestion pathways using Monte Carlo simulation. The population was stratified into six age groups, including age appropriate BPA exposure sources such as toys, breast milk, formula, and baby bottles for infants. The dietary intake was based on information from the National Nutrition Survey in Japan that collects data for three consecutive days. The second method employed a backward calculation that used urinary BPA concentrations to estimate intake. This method also used Monte Carlo simulation techniques to account for uncertainty and variability of the model parameters. The average intake modeled in the aggregate exposure pathway model for male adults in 1995 was 0.43 µg/kg-bw/day and for 2002 was 0.16 µg/kg-bw/day. In comparison, in the backwards calculation from urinary BPA concentrations, the averagely exposed adult male was estimated to have a much lower exposure of 0.028 – 0.049 µg/kg-bw/day. This study considered the backward calculation as more reliable since the relationship between urinary BPA concentrations and BPA ingestion has been verified in human experimental studies. Its limitations are that it cannot provide information about sources of food exposure and the urinary BPA concentration data only included adults (Miyamoto and Kotake, 2006).

An aggregate risk assessment conducted in Switzerland studied the relative contributions of ingestion and inhalation exposure to BPA from a variety of sources. An aggregate exposure dose was calculated using ingestion and inhalation rates and known values of BPA contamination of food, house dust, air, and dental sealants from published literature. This research found that the main source of BPA exposure for

infants and children is from PC baby bottles and for adults is from canned food (Von Goetz *et al*., 2010).

2.9 Food Contact Materials Regulations

In the United States, food contact materials regulations originated with the Federal Food, Drug and Cosmetic Act of 1958, section on Food Additives (21USC348). All substances used as food additives not already approved for use before September 8, 1958 were subject to authorization under this act. There is no restriction on the quantity of an approved substance that can be present in a product, just a regulation on how much exposure could result from food contact with the substance. For a new substance to get approved, it must undergo testing and show that it will not exceed certain consumer exposure levels to food packaging contaminants. The consumer exposure levels are called the cumulative estimated daily intake, or CEDI. CEDI's are determined by leaching experiments into food simulants or through modeling (Muncke, 2009). The CEDI calculations are based on consumption factors, the percentage of a person's diet predicted to come in contact with a specific food-contact material, and food distribution factors, the percentage of all food contacting each material that is aqueous, acidic, alcoholic, or fatty (Duffy *et al*., 2006).

If a compound is approved as an additive to food packaging by the Food and Drug Administration (FDA), it must comply with different requirements depending on its CEDI. The CEDI requirements fall into three categories. A Threshold of Regulation (TOR) applies to substances with CEDI's of 1.5 μg/person/day or below and if the substance does not demonstrate carcinogenicity or structural similarity to any compound with carcinogenicity. No experimental toxicological data is required if a

substance falls into this category. To qualify for a Food Contact Notification (FCN), a substance needs to have a CEDI below 3 mg/person/day. This method is the most expedient pathway for authorization, but this approval only allows usage to the applying company. For substances with an estimated CEDI greater than or equal to 3 mg/person/day, an Indirect Food Additive Petition is required. The final category of authorization for food contact materials are for substances Generally Regarded as Safe, or GRAS (Muncke, 2011).

In the United States, current food contact material regulations only require reproductive toxicity testing for intentionally added substances that might leach into food simulants at 1 ppm, or 1 mg/kg food or higher. This standard is 5 ppm or higher in the European Union (EU). Translated into an exposure dose, for an adult who is 60 kg and consumed 3 kg of food and liquids per day, an exposure up to 50 µg/kg bw/day could occur without requiring reproductive toxicity (Muncke, 2011).

Current food contact materials regulations focus on mutagenicity and genotoxicity testing. This narrow scope fails to examine endocrine disruption, the toxicity of mixtures, and developmental toxicity. If these approaches were incorporated into current toxicological regulatory frameworks, there would be greater protection to women of childbearing age and pregnant women who are more sensitive to these types of exposures. Another vulnerable population are overweight and obese persons whose bodies' ability to metabolize xenobiotics have been altered (Muncke, 2011).

In the European Union, the Framework Regulation 1935/2004 outlines the requirements for food packaging and its Article 3 pertains to food contact materials. The EU food contact materials regulations are based on substance migration from the

packaging to the food simulants. The general plastic packaging leaching limits are regulated by the Plastics Food Contact Material Directive. Individual substances authorized for use are governed by specific migration limits (SML). SML's are based on Tolerable Daily Intakes (TDI), so if a substance does not have a TDI, then there will not be an SML. BPA has an SML of 0.6 mg per kg of food in the EU. For starting substances such as monomers or other compounds that initially react to form a monomer are put on a "positive list". Substances that are considered non-intentionally added substances (NIAS) do not need specific authorization. NIAS are impurities added to the polymer chemistry in the manufacturing of plastic materials and side products of the polymerization process. There are tiers to the required types of toxicological testing based on potential migration. The EU regulations take special exception to packaging that have a functional barrier between it and the food. These compounds do not need to be authorized for use in food packaging. The Fat Reduction Factor (FRF) is applied to foods that contain more than 20% fat. Even though more lipophilic packaging compounds would migrate into the food, the government regulation assumption is that consumption is low. Essentially, there can be higher migration for these fatty substances because an FRF is applied to calculate this food's migration value (Muncke, 2009).

There is concern that current food contact materials regulations may underestimate risk because migration testing with food simulants instead of with actual food may not represent real world exposures. Since more than 50 endocrine disrupting chemicals are approved for use in US and EU, (Muncke, 2009) it is essential that the regulatory required toxicology testing reflects what is needed to accurately assess risk of food contact with endocrine disrupting chemicals.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

Five soft drink brands were purchased from wholesalers in Kumasi, Ashanti Region. Analytical chemical reagents; methanol, toluene, acetonitrile, magnesium sulphate, sodium chloride and potassium phosphate, used for the experiment were purchased from Merck (Darmstadt, Germany). Bisphenol A standard (>99%) was obtained from Sigma Aldrich Co. (St. Louis, MO, USA). The following equipment were used as part of the experiment; oven (Gen Lab, UK), orbital shaker (Eppendorf, USA), vortex (VortexGene, Holland), analytical balance (Ohaus, USA), centrifuge (Eppendoff, USA) and Cecil-Adept Binary Pump High Performance Liquid Chromatography (HPLC) with UV/Vis Detector (UK).

3.2 Methods

3.2.1 Source of Samples

Five commercially available brands of the most popularly consumed bottled soft drinks manufactured in Ghana were purchased from Kumasi Central Market. A total of five packs of each of the soft drink brands were purchased from wholesalers in the central business district. All the samples were purchased before their expiration dates. Only 350 mL samples were obtained for the brands selected for this study. The samples were coded T01 to T05 for the five brands for the purposes of this experiment.

3.2.2 Determination of Thickness

The thickness of the bottles from each brand was measured using a micro-meter screw gauge obtained from Singhla Scientific Industries (Mumbai, India). Five of the bottles from each brand were used for the determination and the averages recorded. This was done by closing the jaws of the gauge and checking for zero errors. The sample was placed between the anvil and the spindle end. The thimble was rotated until sample was firmly held between the anvil and the spindle. The measurement was then recorded.

Table 3.1: Thickness Characteristics of Sample Bottles

Brand	Material Type	Volume (mL)	Thickness (mm)
T01	PET	350	0.16
T02	PET	350	0.30
T03	PET	350	0.20
T04	PET	350	0.30
T05	PET	350	0.28

3.3 Sample Storage

The effect of storage temperature on potential BPA migration from the five soft drink brands was investigated by storing them at three different temperatures; Refrigerated (<8 ºC), Room (25±2 ºC) and Elevated temperature (50 ºC) for four consecutive weeks. Both pH and BPA content of the soft drinks was determined weekly using standard protocols.

3.4 Sample Preparation

The experiments were carried out in glassware. To minimize losses of BPA onto glass surfaces, all glassware was cleaned by briefly heating in an oven at 200 ºC to remove any water adsorbed on the glass surfaces, and soaked overnight in a 5%

trimethylchlorosilane solution in toluene. The glassware was rinsed with toluene and methanol and then dried in an oven at 100 ºC for one hour. Glassware, solvents and samples were carefully handled to avoid contamination. Analysis of the samples was carried out weekly on the same day each week for four weeks, when analysis of the final samples was carried out.

3.5 Determination of Bisphenol A

3.5.1 Extraction and Clean-up

Extraction and clean-up was based on QuEChERS as proposed by UCT (2013). A weight of 10 g of the sample was weighed into a 50 mL centrifuge tube and 10 mL of acetonitrile added. The tube was shaken vigorously at 400 rpm for 10 min. Subsequently, 4 g of $MgSO_4$ and 1 g of NaCl were added and the tube was vortexed for 10 sec to break up salt agglomerates. The tube was shaken at 400 rpm for 10 min and centrifuged at 4000 rpm for 5 min. The supernatant was dried at 40 ºC, reconstituted in 2 mL of mobile phase solution and 20 uL was injected into the HPLC.

3.5.2 HPLC Determination

Stock solutions of BPA (Fluka Analytical) were prepared in acetonitrile at final concentration of 1 µg/mL. Working standards were made by serial dilution from stock and calibration standards of 5 – 30 ng/g were prepared accordingly. High Performance Liquid Chromatography (HPLC) analysis using a Supelco Discovery C18, 5 µm, 4.6 x 15 µm, 5 µm maintained at 40 ºC. Mobile phase composition was water/methanol (45:65, v/v) at 0.9 ml/min and a wavelength of 225 nm. BPA peaks were identified using the retention time of the standard BPA chromatogram and quantification done using the calibration curve.

Recovery was determined to be 98±1.49% for samples spiked at 5 ng/g. Limit of detection (LOD) and limit of quantification (LOQ) was 0.05 ng/g and 0.1 ng/g respectively. Chromatographic data was processed using the PowerStream software (version 4) from Cecil-Adept, UK.

3.6 Data Analysis and Risk Assessment

Data was analyzed using StatGraphics Centurion software (version 18) for significant difference between factors at 95% confidence interval. The Palisade @Risk software (version 7.5, 2017) was used to fit the distribution of the hazard concentration (HC), contact rate (CR), exposure frequency (EF), exposure duration (ED), body weight (BW), averaging time (AT) and the risk estimated using (US EPA, 2002) recommended Equations 1 and 2.

$$\mathrm{CDI} = \frac{\mathrm{C} \times \mathrm{CR} \times \mathrm{EF} \times \mathrm{ED}}{\mathrm{BW} \times \mathrm{AT}} \qquad (1)$$

Where CDI is chronic daily intake, C is concentration of BPA (mg/g), CR is the contact rate (total mass of food consumed per day), EF is exposure frequency (days/year), ED is exposure duration (years), BW is body weight (kg), AT is averaging time of exposure (years).

$$\text{Hazard Quotient (HQ)} = \frac{\mathrm{CDI}}{\mathrm{R_fD}} \qquad (2)$$

Where R_fD is the reference dose of BPA.

The values of HC and CR (primary data) were obtained from HPLC analysis results and volume of the soft drinks respectively, whereas the values of EF, ED, BW, AT and R_fD were obtained from secondary data sources as presented in Table 3.2.

Table 3.2: Model Parameters, Primary and Secondary Data Sources Used for the Estimation of Risk

Risk Parameter	Data Source
Hazard concentration (HC)	Appendix B
Consumption rate (CR)	350 mL/day
Exposure frequency (EF)	180 days/year
Exposure duration (ED)	6 years (for children 6 – 12 years)
Body weight (BW)	WHO Child Growth Standards (2006)
Averaging time (AT) for carcinogens	365 days/year x 70 years (Gerba, 2006)
Averaging time (AT) for non-carcinogens	365 days/year x 30 years (Gerba, 2006)
Reference dose (R_fD)	0.00005 mg/g/day (US EPA, 2010)

The data was captured into Microsoft Excel (2010) and analyzed using Palisade @Risk software to simulate the potential health risk associated with consumption of the soft drink products packaged in plastic bottles. The corresponding values of the risk parameters from Table 3.2 were inserted into Equation 1 to obtain the dietary exposure Chronic Daily Intake (CDI). To characterize the hazard quotient (HQ) of BPA, reference dose (R_fD) of 0.00005 mg/g/day (US EPA, 2010) was used to run Equation 2, simulating at 100,000 iterations using Palisade @Risk software.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Effect of Storage Temperature on BPA Concentration (T01)

The leaching of BPA for product T01 is presented in Figure 4.1. There was no significant change in BPA levels when it was stored at refrigerated temperature (<8 ºC). There was no significant change in BPA for the first two weeks at room temperature (25±2 ºC), however BPA concentration doubled by week 3 from 0.25 ng/mL to 0.53 ng/mL and doubled from 0.53 ng/mL to 1.39 ng/mL from week 3 to week 4. BPA levels increased from 0.23 ng/mL to 2.32 ng/mL at elevated temperature (50 ºC) over the storage period.

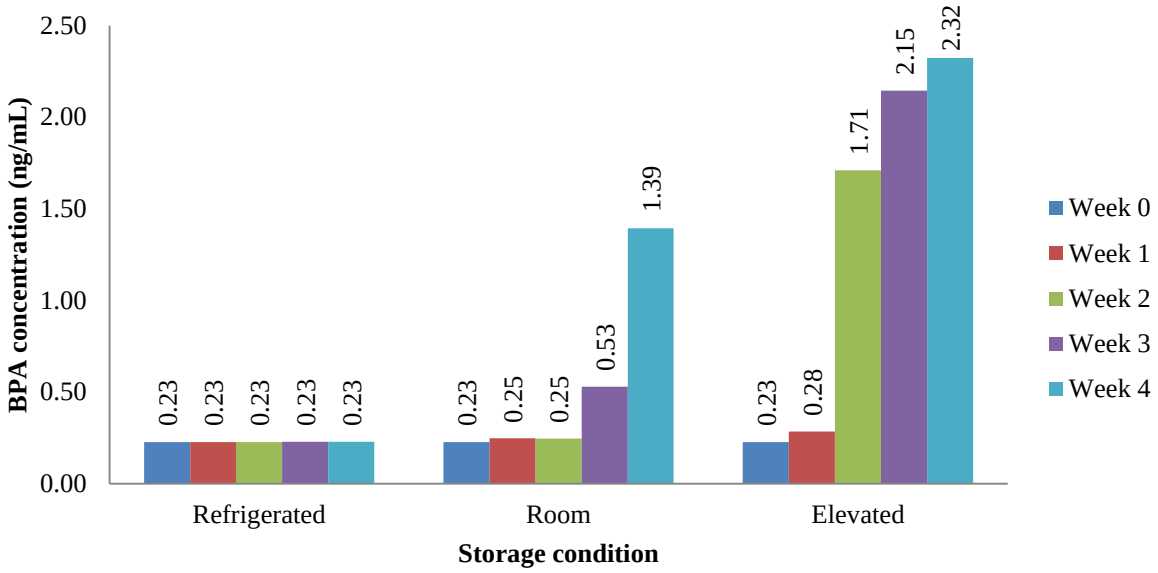

Figure 4.1: Effect of storage temperature on BPA migration over 4 weeks (T01)

4.2 Effect of Storage Temperature on BPA Concentration (T02)

Refrigerated samples had no significant change over the storage period for product T02. There was an increase from 0.38 ng/mL to 0.69 ng/mL for samples stored at room temperature while those stored at elevated temperature increased by 7 folds from 0.38 ng/mL to 2.90 ng/mL by the end of the fourth week.

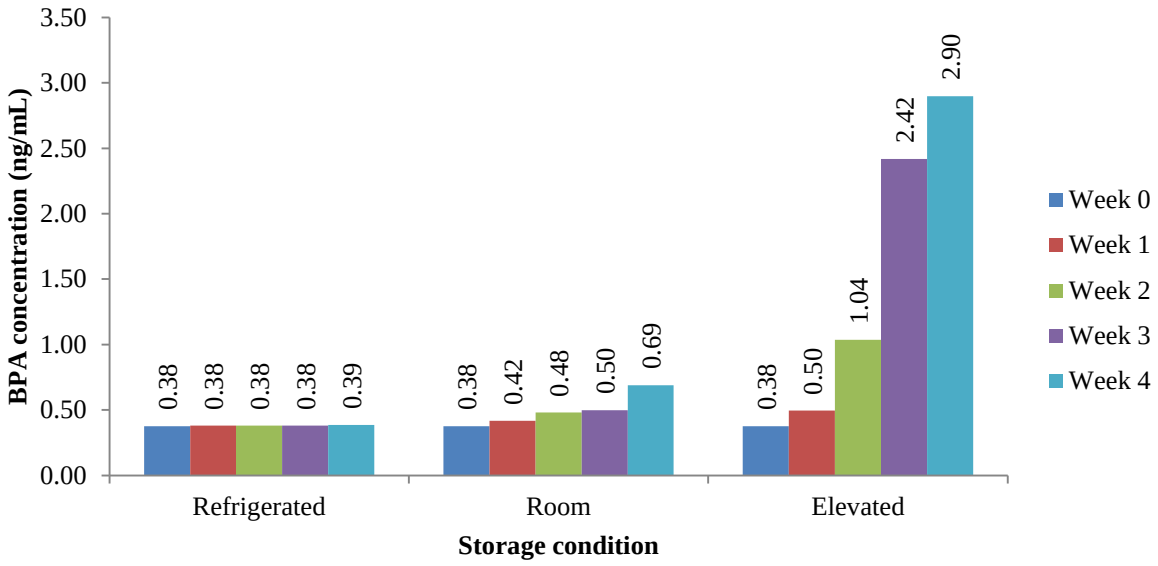

Figure 4.2: Effect of storage temperature on BPA migration over 4 weeks (T02)

4.3 Effect of Storage Temperature on BPA Concentration (T03)

A similar trend like product T02 was observed for product T03 when samples were stored at refrigerated temperature (<8 ºC). Samples stored at room temperature increased from 0.38 ng/mL to 0.45 ng/mL with an increase from 0.38 ng/mL to 2.85 ng/mL for samples stored at elevated temperature.

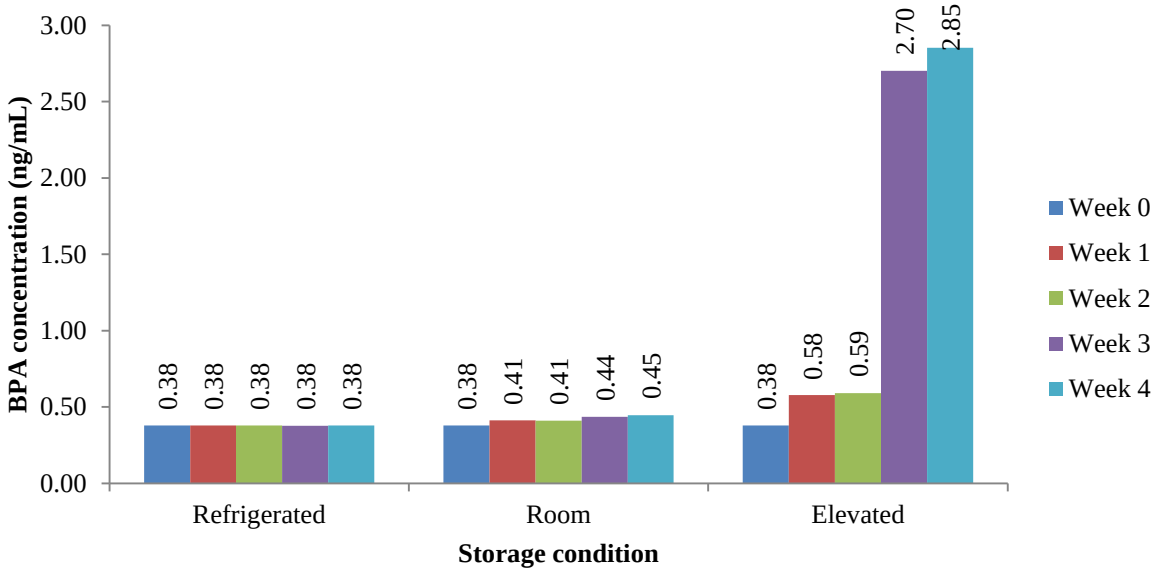

Figure 4.3: Effect of storage temperature on BPA migration over 4 weeks (T03)

4.4 Effect of Storage Temperature on BPA Concentration (T04)

There was a sharp increase in the first week from 0.41 ng/mL to 0.85 ng/mL with a steady increase from 0.85 ng/mL to 0.98 ng/mL over the storage period. BPA concentration doubled by week 1 for samples stored at elevated temperatures with a 5 fold increase in BPA concentration from week 1 (1.16 ng/mL) to week 4 (5.17 ng/mL).

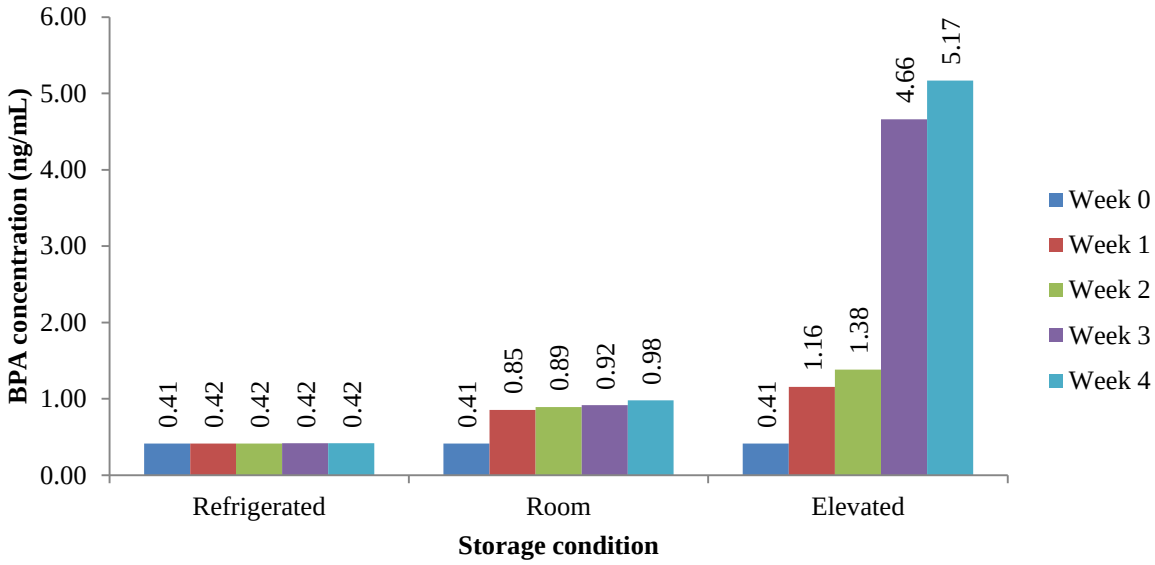

Figure 4.4: Effect of storage temperature on BPA migration over 4 weeks (T04)

4.5 Effect of Storage Temperature on BPA Concentration (T05)

Product T05 had a steady increase from 0.52 ng/mL to 0.94 ng/mL by the end of the storage period at week 4 for samples stored at room temperature. There was fast rise from 0.52 ng/mL to 0.96 ng/mL by week 1 at elevated temperature. BPA concentration increased by 3 fold from 0.96 ng/mL to 3.24 ng/mL at elevated temperature. Refrigerated samples had no significant increase in BPA.

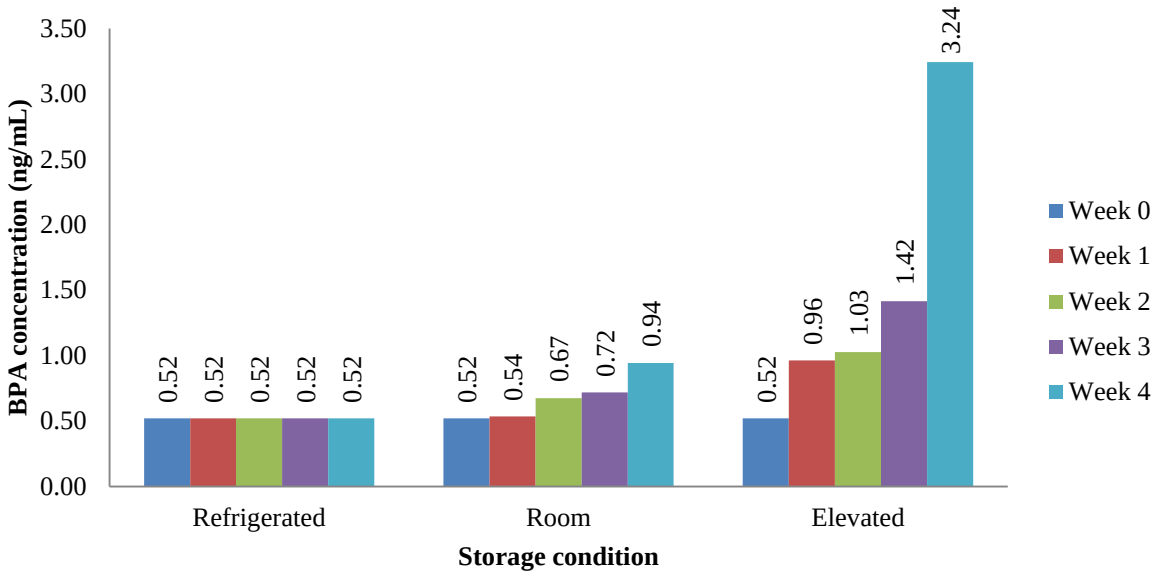

Figure 4.5: Effect of storage temperature on BPA migration over 4 weeks (T05)

Le *et al*. (2008) studied the effects of temperature on new and used bottles. The researchers concluded that the concentrations of BPA in heated samples were twice those in room temperature. A linear relationship is thus found between BPA and temperature. The higher the temperature the higher the concentration of BPA migrated. The work again found an elevated rate of BPA migration to have been achieved after hot bottles were cooled and incubated with room temperature water. This phenomenon is meant to explain that the leaching of BPA is not only limited to heating but long term effects also take place (Le *et al.*, 2008). This supports the current study's findings of BPA concentration increasing with increase in storage temperature for all the samples over the study duration.

4.6 Effect of Storage Time on BPA Concentration

BPA concentration in the soft drink products was evaluated with storage time from 0 – 4 weeks. With the exception of the refrigerated samples, which recorded very little to no change in the concentration of BPA over the duration of the experiment, both

samples stored at room and elevated temperatures had a steady increase in BPA concentration from week 1 through to week 4. Figures 4.1 to 4.5 show the trend of BPA concentration increasing with increase in storage time, which is the period between the time of purchase of the products and that of the analysis of the samples.

In a similar study carried out by Howdeshell *et al*. (2003) involving polycarbonate animal cages, the team of researchers made comparable observations about the migration of BPA into water at room temperature with increase in storage time. These results also support the findings of Le *et al*. (2008), who found that the concentration of BPA released from polycarbonate bottles increased over time into water at room and elevated temperature.

4.7 Effect of pH on BPA Concentration

The pH of the samples used for the study were all in the acidic range of 2.72 to 3.58. Sample T03 recorded the lowest pH of 2.71 followed by T05 with 2.96, T04 with 3.15, T02 with 3.13 and T01 with 3.58. Slight increases in pH were observed in all the samples after the storage period irrespective of the storage conditions. However, the increase was not statistically significant at $p<0.05$.

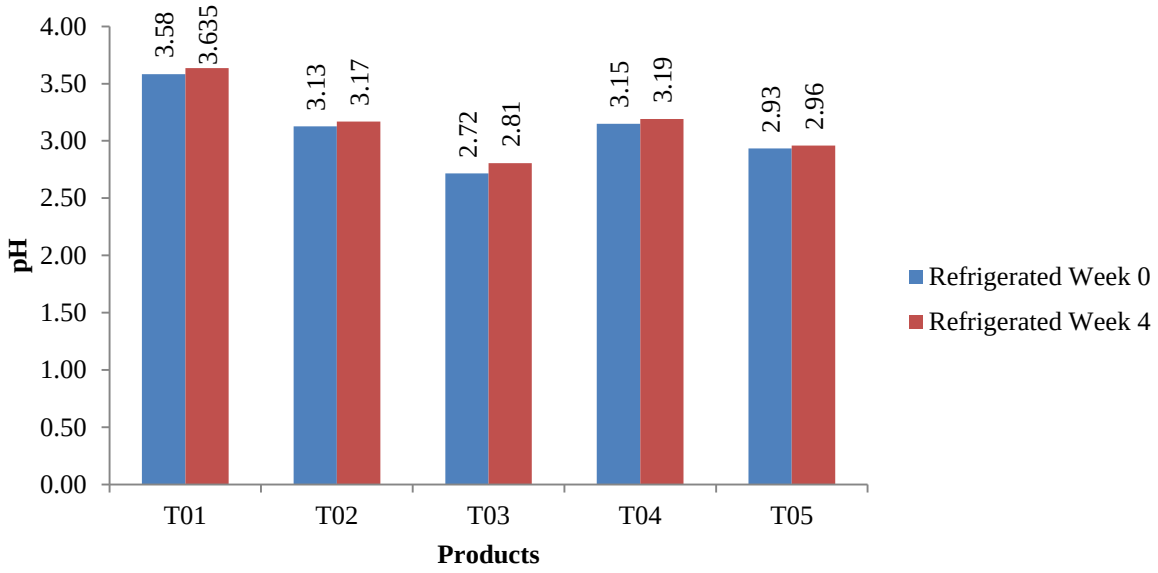

Figure 4.6: Change in pH of samples from week 0 – 4 at Refrigerated temperature

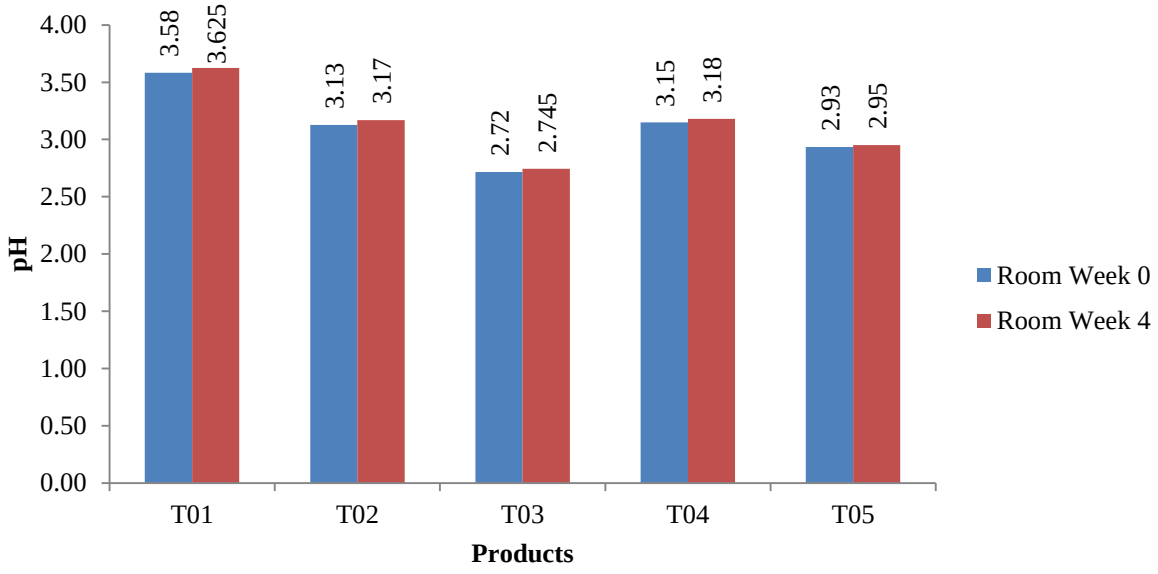

Figure 4.7: Change in pH of samples from week 0 – 4 at Room temperature

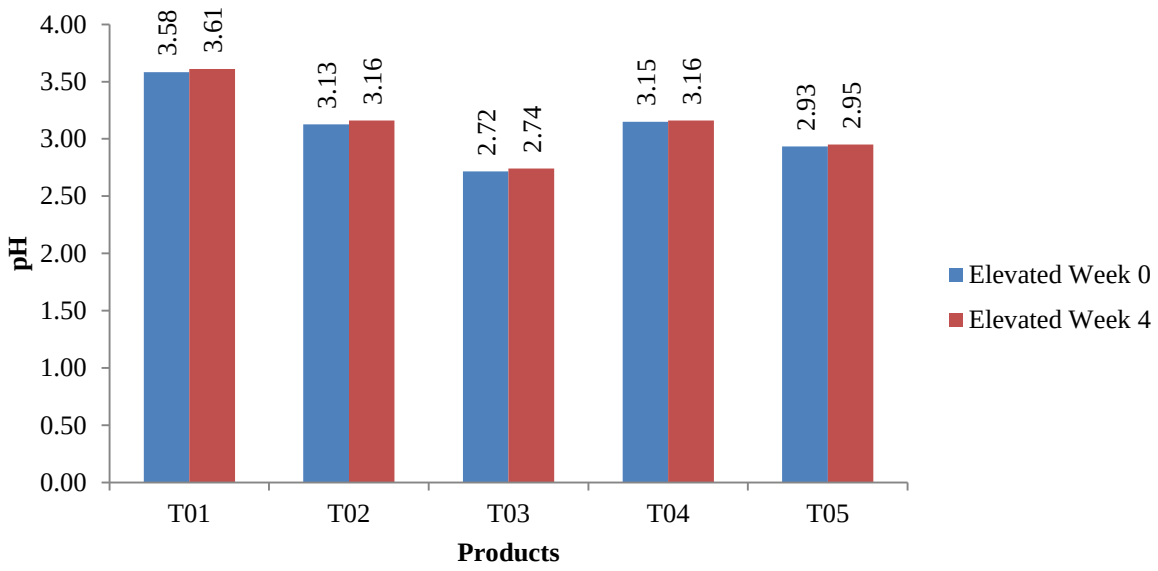

Figure 4.8: Change in pH of samples from week 0 – 4 at Elevated temperature

Under acidic or basic conditions, the ester bonds linking the plastic polymer are susceptible to hydrolysis therefore releasing monomers of BPA into the product in contact with the plastic material (Richter *et al.*, 2007). This could account for the presence of some traces of BPA even at the start of the study (week 0).

4.8 Effect of Material Thickness on BPA Levels (Refrigerated)

The percentage increase in BPA migration after 4 weeks at refrigerated temperature ranged from 0.39% to 0.47% for plastics with 0.16 mm and 0.3 mm thickness respectively. The rate of BPA migration was 0.28 mm <0. 20 mm <0.16 mm <0.30 mm in increasing order.

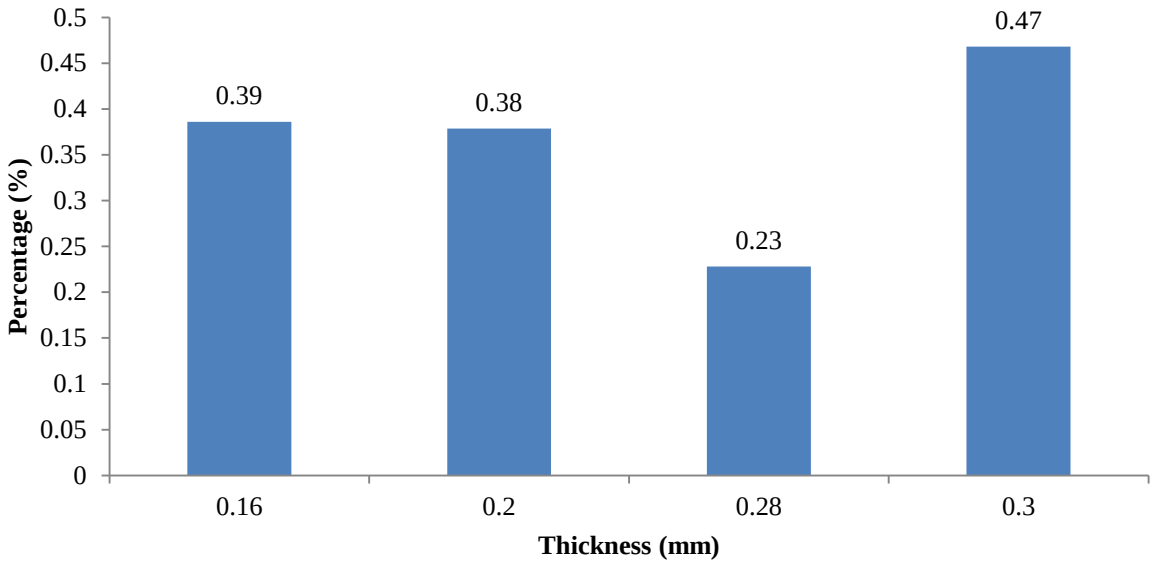

Figure 4.9: Effect of thickness on BPA levels at Refrigerated temperature after 4 weeks

4.9 Effect of Material Thickness on BPA Levels (Room)

Plastics with thickness size of 0.28 mm recorded the highest increase in BPA migration at 83.70% with 0.20 mm recording the least of 15.38% after four (4) weeks of storage. The rate of BPA migration was 0.20 mm <0.16 mm <0.30 mm <0.28 mm in increasing order.

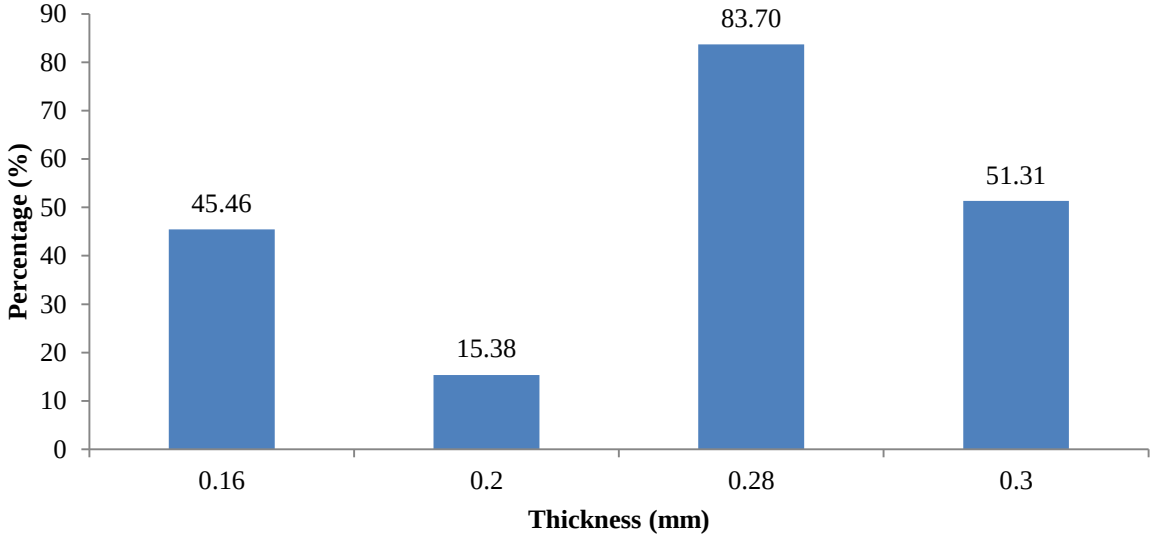

Figure 4.10: Effect of thickness on BPA levels at Room temperature after 4 weeks

4.10 Effect of Material Thickness on BPA Levels (Elevated)

The least increase (86.75%) in BPA migration was observed in plastics with thickness size of 0.20 mm with 0.28 mm having the highest increase of 90.22%. The rate of BPA migration was 0.20 mm <0.16 mm <0.30 mm <0.28 mm in increasing order.

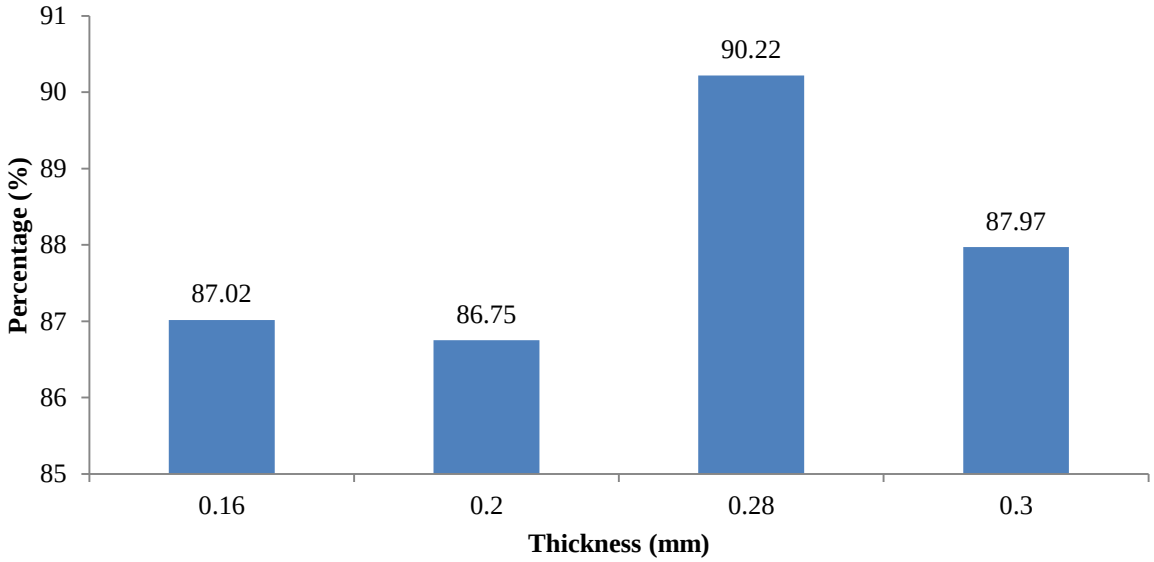

Figure 4.11: Effect of thickness on BPA levels at Elevated temperature after 4 weeks

4.11 Health Risk Hazard Quotient

Table 4.1 presents the estimated health risk indices for BPA in the soft drinks through oral route by the study population.

Table 4.1: Statistical distribution metrics of health risk hazard quotient of BPA

Sample	Mean	Median	Mode	5^{th} Percentile	95^{th} Percentile
Refrigerated	0.08	0.08	0.08	0.04	0.15
Room	0.13	0.12	0.04	0.03	0.27
Elevated	0.38	0.26	0.05	0.05	1.11

The hazard quotient (HQ) must be greater than 1 (HQ>1) for a study population to be at a significant health risk or exposed to an adverse health effect (Gerba, 2006). However, if the value of HQ is less than 1 (HQ<1), it can be concluded that there is no significant health risk or no adverse health effect is expected.

For samples stored at refrigerated temperature, the mean, modal, median, 5^{th} and 95^{th} percentile values of the hazard quotient posed no significant health risk. Also for samples stored at room temperature, the mean, modal, median, 5^{th} and 95^{th} percentile values from Table 4.1 reinforced the observation that school-aged children between the ages of 6 – 12 years consuming 1 bottle (350 mL) of any of the sampled brands of soft drinks per day were exposed to no significant health risk, since HQ<1.

However, at elevated temperature, the 95^{th} percentile of BPA recorded a HQ greater than 1 (HQ>1). Even though at the same elevated temperature, the mean, modal, median and 5^{th} percentile values of the hazard quotient posed no significant health risk, the 95^{th} percentile value of 1.11 implied that more than 5% of the population of school-aged children assumed to be consuming the soft drinks were at a potential risk of suffering the adverse health effects of BPA in the soft drinks.

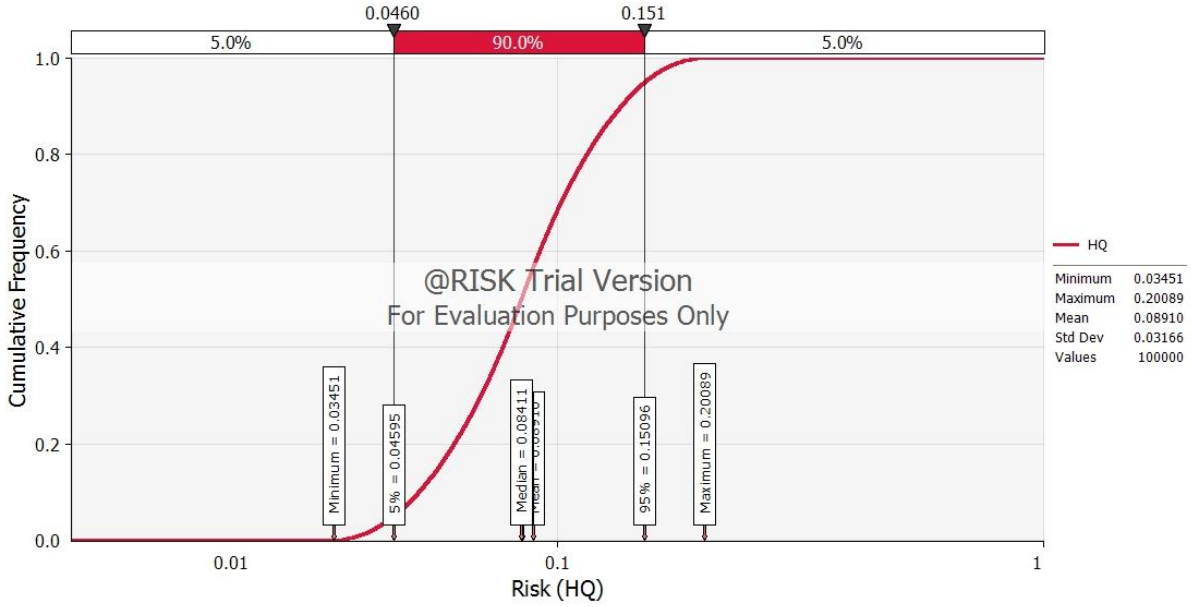

Figure 4.12: Risk (hazard quotient) for BPA at Refrigerated temperature

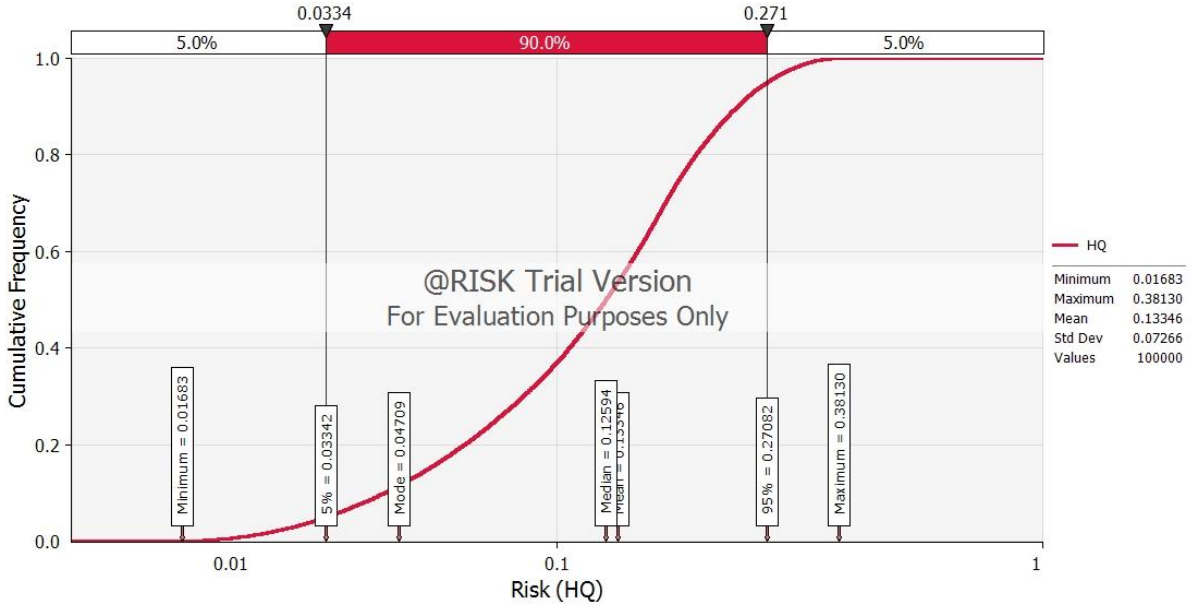

Figure 4.13: Risk (hazard quotient) for BPA at Room temperature

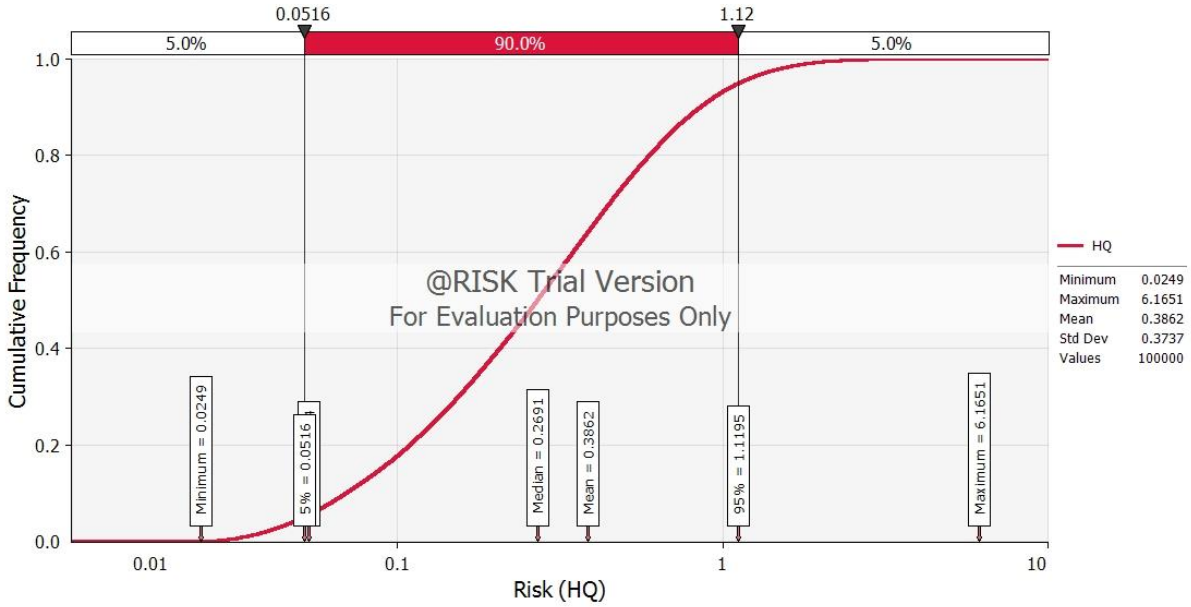

Figure 4.14: Risk (hazard quotient) for BPA at Elevated temperature

4.12 Sensitivity Analysis

Correlation coefficient is a statistical measure of the strength of relationship between two variables. Variables which exhibit this feature are considered to depend on one another in some way. Values between +1 and -1 are used to connote correlation coefficient. A coefficient of +1 indicates a perfect positive correlation. That is, an increase or decrease in the value of one variable will result in an increase or decrease in the value of the other variable. A coefficient of -1 indicates a perfect negative correlation. That is an increase in the value of one variable will result in a decrease in the value of the other variable and vice versa.

Since the values for volume consumed, exposure frequency, exposure duration and averaging time remained relatively constant in the estimation of the risk, concentration of BPA and body weight were the two parameters that had an effect on the risk.

From Figure 4.15, Figure 4.16 and Figure 4.17, of the two risk parameters, only concentration of BPA had a strong to extremely strong positive correlation to the level of hazard quotient. Thus 0.72 mg/g, 0.90 mg/g and 0.97 mg/g for refrigerated, room and elevated temperature respectively. Conversely, body weight had a weak to very weak negative correlation to the hazard quotient. Thus -0.67 kg, -0.41 kg and -0.25 kg for refrigerated, room and elevated temperature respectively. This can be attributed to the fact that concentration of BPA is directly proportional to the Chronic Daily Intake (CDI), whereas body weight is inversely proportional to the CDI.

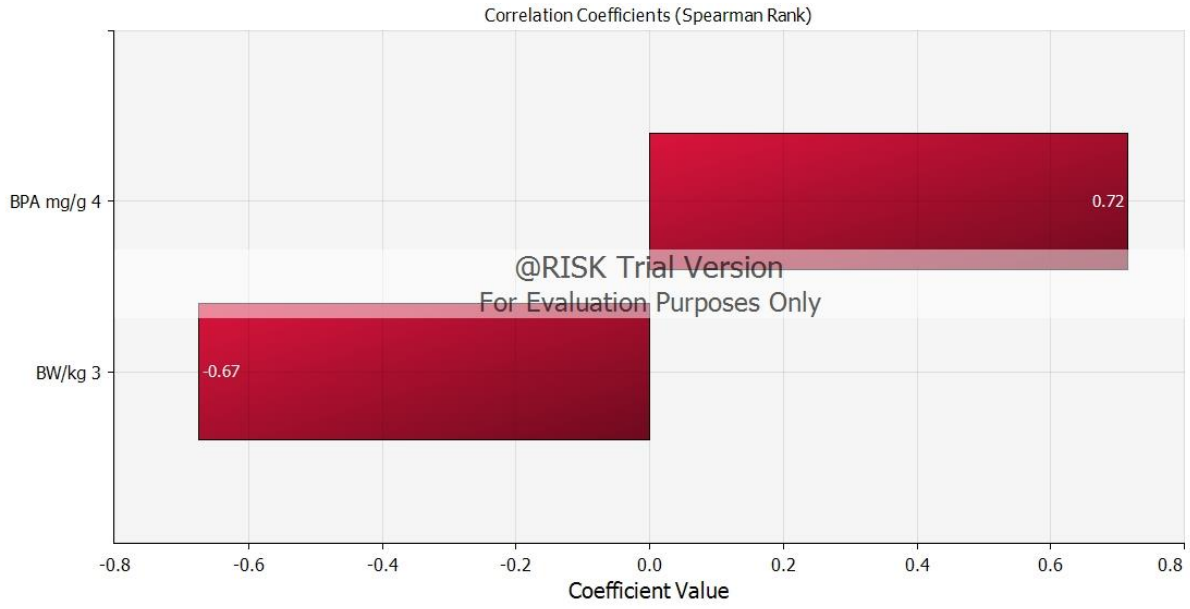

Figure 4.15: Correlation coefficient for BPA at Refrigerated temperature

Figure 4.16: Correlation coefficient for BPA at Room temperature

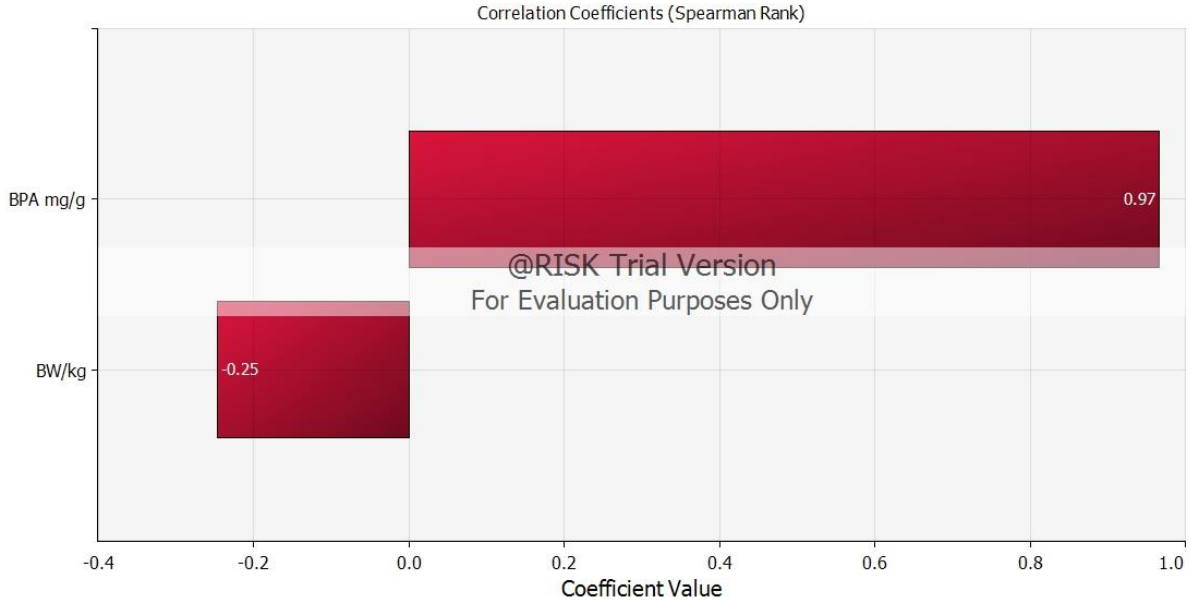

Figure 4.17: Correlation coefficient for BPA at Elevated temperature

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Bisphenol A migration from five brands of soft drinks packaged in plastic bottles was investigated, as influenced by storage temperature, pH and bottle thickness for four weeks. BPA was detected in all the samples at all three storage temperatures over the study duration. Though BPA levels remained fairly constant for refrigerated samples, there was a general trend of BPA concentration increasing with increase in temperature and storage time for samples stored at room and elevated temperature.

pH remained relatively constant in the acidic range of 2.72 to 3.58. Slight increases in pH were observed in all the samples, even though the increase was not statistically significant at $p<0.05$. The overall rate of BPA migration was 0.20 mm <0.16 mm <0.30 mm <0.28 mm in increasing order of bottle thickness.

The study further showed that samples stored at refrigerated and room temperature posed no significant health risk. However, at elevated temperature, the 95^{th} percentile recorded a HQ greater than 1 (HQ>1), implying that more than 5% of the study population were at a potential risk of suffering the adverse health effects of BPA in the soft drinks, and therefore should not be neglected.

5.2 Recommendations

It is recommended that prolonged storage of soft drinks packaged in plastic bottles under elevated temperature conditions (above 50 ºC), or under direct sunlight should be avoided to reduce the risk of human exposure to BPA. Regulatory agencies must monitor and educate the general public, especially soft drink vendors on the acceptable storage conditions to minimize BPA migration into these products.

Further studies on migration of other plasticizers and polyethylene packaging materials, such as phthalates, used in the Ghanaian food industry must be carried out, to help regulatory agencies sanction new legislations to regulate the type and quantity of plasticizers used during the manufacturing of food packaging materials.

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APPENDICES

Appendix A

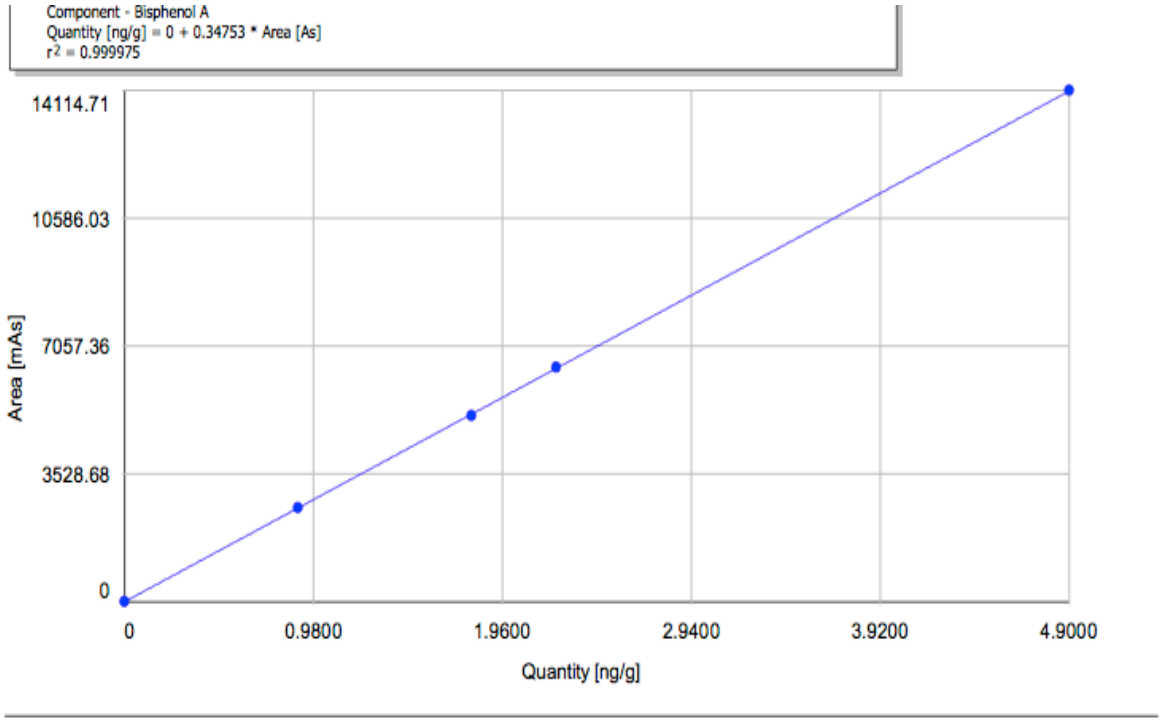

Figure A1: Bisphenol A Concentration Calibration Curve

Appendix B

Table B1: Concentration of BPA migration under different storage conditions for sample T01 over 4 weeks

Storage Condition	Weeks				
	0	**1**	**2**	**3**	**4**
Refrigerated	0.227	0.227	0.227	0.229	0.228
Room	0.227	0.247	0.246	0.529	1.394
Elevated	0.227	0.284	1.710	2.145	2.324

Table B2: Concentration of BPA migration under different storage conditions for sample T02 over 4 weeks

Storage Condition	Weeks				
	0	**1**	**2**	**3**	**4**
Refrigerated	0.376	0.380	0.380	0.380	0.386
Room	0.376	0.416	0.482	0.499	0.689
Elevated	0.376	0.495	1.036	2.418	2.897

Table B3: Concentration of BPA migration under different storage conditions for sample T03 over 4 weeks

Storage Condition	Weeks				
	0	**1**	**2**	**3**	**4**
Refrigerated	0.378	0.379	0.378	0.377	0.378
Room	0.378	0.412	0.411	0.435	0.446
Elevated	0.378	0.577	0.591	2.702	2.853

Table B4: Concentration of BPA migration under different storage conditions for sample T04 over 4 weeks

Storage Condition	Weeks				
	0	**1**	**2**	**3**	**4**
Refrigerated	0.415	0.415	0.415	0.417	0.416
Room	0.415	0.852	0.893	0.915	0.980
Elevated	0.415	1.156	1.384	4.661	5.168

Table B5: Concentration of BPA migration under different storage conditions for sample T05 over 4 weeks

Storage Condition	Weeks				
	0	**1**	**2**	**3**	**4**
Refrigerated	0.519	0.520	0.521	0.520	0.52
Room	0.519	0.535	0.674	0.718	0.944
Elevated	0.519	0.962	1.026	1.415	3.243

Appendix C

Multifactor ANOVA - BPA
Dependent variable: BPA
Factors:
Thickness
Storage

Table C1: Analysis of Variance for BPA - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Main Effects					
A:Thickness	1.2719	3	0.423966	0.68	0.6187
B:Storage	18.8943	2	9.44713	15.24	0.0268
Interactions					
AB	2.22544	6	0.370907	0.60	0.7290
Residual	1.85943	3	0.619811		
Total (Corrected)	29.5586	14			

All F-ratios are based on the residual mean square error

The StatAdvisor

The ANOVA table decomposes the variability of BPA into contributions due to various factors. Since Type III sums of squares (the default) have been chosen, the contribution of each factor is measured having removed the effects of all other factors. The P-values test the statistical significance of each of the factors. Since one P-value is less than 0.05, this factor has a statistically significant effect on BPA at the 95.0% confidence level.

Table C2: Multiple Range Tests for BPA by Storage

Method: 95.0 percent Tukey HSD

Storage	*Count*	*LS Mean*	*LS Sigma*	*Homogeneous Groups*
Refrigerated	5	0.36528	0.368217	X
Room	5	0.873255	0.368217	X
Elevated	5	3.07001	0.368217	X

Table C3: Significance Tests between Storage Conditions

Contrast	*Sig.*	*Difference*	*+/- Limits*
Elevated - Refrigerated	*	2.70473	2.17431
Elevated - Room	*	2.19676	2.17431
Refrigerated - Room		-0.507975	2.17431

* denotes a statistically significant difference

Table C4: Correlation between BPA and Storage

Correlation	BPA
Storage	0.8467
Sample Size	(15)
P-Value	0.0001

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This table shows Pearson product moment correlations between each pair of variables.

These correlation coefficients range between -1 and +1 and measure the strength of

the linear relationship between the variables. Also shown in parentheses is the number of pairs of data values used to compute each coefficient.

The third number in each location of the table is a P-value which tests the statistical significance of the estimated correlations. P-values below 0.05 indicate statistically significant non-zero correlations at the 95.0% confidence level. The following pairs of variables have P-values below 0.05: Storage and BPA.