# Effects of Temperature and Storage Time on Bisphenol A Migration from Polycarbonate Bottles into Water: Analysis Using UV-Visible Spectrophotometric Method


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September, 2022

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## Abstract

Endocrine-disrupting chemicals (EDCs) have received widespread attention over the years due to their deleterious effects on human health. Bisphenol A (BPA) - a monomer used globally in producing polycarbonate plastics and epoxy resins, is a prototypic EDC that has received widespread attention due to its estrogenic activity. BPA has been detected in human serum, urine, amniotic fluid, placenta tissues, and umbilical cord blood. Its presence in the human population has been ascribed to consuming BPA-contaminated food due to its migration from polycarbonate plastics. However, little is known about the inimical health hazard of BPA migrating from polycarbonate bottles into food or drinks in Nigeria and how temperature and storage duration can influence its migration into any contact media. To address this problem, we scrutinized the effect of storage time and temperature on BPA migration from 3 selected polycarbonate water bottles and a brand of polycarbonate baby feeding bottles into a mixture of methanol and water in Makurdi, Nigeria, using the UV-Visible spectrophotometric method. We measured detectable levels of BPA right from day 1 at room temperature, suggesting a positive correlation between BPA release and residual BPA in the PC bottles. The amount of BPA migrated was in the range of (0.030 ± 0.012) ng/mL (day 1 at room temperature) to (5.620 ± 0.650) ng/mL (day 10 at 60 °C) for the 3 brands of polycarbonate feeding bottles and (0.080 ± 0.010) ng/mL (day 1 at room temperature) to (4.300 ± 0.100) ng/mL (day 10 at 60 °C) for a brand of polycarbonate water bottles studied. Both temperature and duration of storage generously influenced the migration of BPA exponentially. However, our study identified temperature as the dominant significant factor that enhanced the migration of BPA from PC bottles into the water. Therefore, it is safer for consumers to store foods in BPA-free bottles to avert health risks related to ingestion of BPA.

**Keywords**: BPA, migration, polycarbonate, plastics, spectrophotometric, EDC.

## Introduction

Packaging has become an indispensable element of manufacturing processes. Packing does not only adequately protect/preserve foods from microorganisms; biological, physical, and chemical changes, thus providing longer shelf life; it also makes transportation of food easier [1]. Several materials are utilized in food and beverage packaging including glass, metals, paper/paperboard, ceramics, wax, and wood. However, plastics remain desirable materials of choice, due to their light weight, durability, chemical inertness, thermosealabilty, microwavability, hydrophobicity, optical properties, malleability, and flexibility [2, 3]. They are inexpensive, versatile, hygienic, flexible, strong, highly durable, corrosion and chemical resistant materials with high thermal and electrical insulation properties. The diversity of plastics and the versatility of their properties are used to make a variety of products used in construction, packaging, automobiles, electronics, and household goods we use daily leading to advances in technology and medicine, energy savings, and several societal benefits [4]. Consequently, plastic production has augmented exponentially over the past years from approximately 0.5 million tons in 1950 to above 250 million tons today [5]. Hence, human exposure to plastic products is inevitable. Since plastics are extremely diverse in terms of possible applications, they are widely distributed in the environment. This expansion in polymer types and uses resulted in increasing complexity in the formulations of plastic polymers and hence the sophisticated nature of today's commercially available plastics [6].

Plastics themselves once created are described as non-toxic and feign no threats to human health and the environment because of their non-reactive disposition and generally are non-transferrable across biological membranes due to their large sizes [7]. Unfortunately, plastics are seldom used by themselves in their pure states [5], and typically, the polymer resins are aggregated with various additives to enhance the performance, functionality, and aging properties of the polymer [8]. These numerous additives together with non-polymeric substances like monomers or small oligomers, low molecular weight fragments, polymerization solvents, and catalyst remnants are hazardous to human health and the environment with persistent effects, e.g., styrene and vinyl chloride monomers are carcinogenic and mutagenic respectively while BPA is toxic to reproduction [7, 9]. Since non-polymeric compounds are usually of low molecular weight and are either weakly bound or non-bound at all to the polymer matrix, they and their degradative products can be emitted into the air, water, or other contact media [7,10] including food but no food contact material should release chemicals into food in quantities that can harm human health [2]. Once the concentrations of these migrants reached a specified limit, the safety and quality of food may be imperiled and consequently, health risks could potentially arisen when/if the concentration in food attains/exceeds a certain threshold that can be absorbed by the human body [11, 12]. Interaction between food and plastics is unpleasant and

a cause for concern if the concentration of the migrants is unknown or when their safety is uncertain [13]. Studies confirmed the contamination of about 90% of bottled water from world-leading brands with microplastics necessitating the review of plastics by the World Health Organization (WHO) and other regulatory bodies [14]. Perhaps, we are exposed to these chemicals concurrently from different sources, and as such, their measurements and impacts on human health become tremendously important [13].

Bisphenol A is an endocrine-disrupting chemical with pullulating toxicological health hazards on humans. It has been reportedly detectable in human serum, placental tissue, amniotic fluid, and umbilical cord blood [15], and its presence has been ascribed to oral ingestion of BPA-contaminated food due to its migration from polycarbonate bottles. Chronic exposure to even low-dose of BPA by humans is very detrimental. It can interfere with delicate hormonal coordination resulting in developmental, metabolic, and reproduction disorders and inflammatory pathways [16]. BPA is reportedly an obesogen that might intensify visceral adiposity, metabolic syndrome, and diabetes epidemics [16]. It is also purportedly associated with male and female infertility, breast and prostate cancer, polycystic ovarian syndrome (PCOS), recurrent miscarriages, karyotype aberrations, and endometrial hyperplasia [17, 18]. To mitigate this bad omen, the specific migration limit (SML) of BPA must be monitored to ensure that its daily intake is significantly lower than the tolerable daily intake.

## Literature review

### Polycarbonate as potential hazard in food contact applications

Effortless degradation of polycarbonate plastics via hydrolysis at high temperatures or by leaching has made the utilization of PC containers in storing food questionable, as seen in equation (1).

$$\frac{1}{n}[OC(OC_6H_4)_2CMe_2]_n + H_2O \rightarrow n\ (HOC_6H_4)_2CMe_2 + CO_2 \quad (1)$$

The BPA released into foods or drinks can be assimilated via different modes of chemical exposure by other tissues in our bodies which can result in dangerous health conditions. Inspecting the toxicokinetics of BPA, EFSA (2001) observed a threshold above which BPA intake can be harmful to the human immune system. This led to the establishment of 50 µg/kg bw/day as a tolerable daily intake (TDI) or a reference dose (RfD) for BPA corresponding to an SML of 0.6 mg/L [19]. To allow further comprehensive substance-specific extrapolation from animals to humans, taking into cognizant the surging magnitude of BPA toxicity, EFSA established a human equivalent dose (HED) of 609 g/kg bw/day which was used as a reference point to establish an accurate health-based guidance value for BPA. An uncertainty factor of 2.5 for inter-species differences and 10 for intra-species differences were used by the authority to establish this health-based guidance. An additional factor of 6 was

applied taking into cognizant the uncertainties surrounding the potential health effects of BPA on the mammary gland, metabolic, reproduction, neurobehavioral, and immune systems. As a result, an overall uncertainty factor of 150 was applied to establish a new TDI of 4 µg/kg bw/day which is 12.5 times lower than The TDI of 50 µg/kg bw/day [20]. Hence, based on the t-TDI, the allocation factor and the exposure assumption, a current SML of 0.05 mg of BPA per kg of food must be kept to ensure minimal exposure to BPA below t-TDI to mitigate it from endangering human health [20].

## Release of BPA from Polycarbonate

Several studies have been conducted to monitor and analyze the migration of BPA PC food contact materials under different parameters in the past years. Some of these studies reported the release of BPA from PC plastics either due to sensitivity or instability of PC plastics or both relative to different environmental impact factors like humidity, UV radiation, and temperature while others reported residual BPA migration from PC plastics but with no clear explanation. This release of BPA from PC occurs via a diffusion-controlled mechanism or PH-dependent decomposition of PC overtime at the polymer surface [21, 22]. Hydrolysis of PC at the surface has been supported by kinetic studies to be the major mechanism behind the release of BPA into an aqueous medium where the diffusion-controlled release of BPA plays a minor role in the overall release of BPA from PC [22]. This is accrued to the low diffusion rate of BPA and high decomposition rate of PC at elevated temperatures [21].

Hunt *et al* (2003) reported that sodium hypochlorite and other alkali cleaners catalyze the release of BPA from PC surfaces and recommended alcohol as an ideal solvent for cleaning grease and oil from polycarbonate containers [23]. Migration of BPA was also shown to be increasing with time and temperature [24, 25, 26, 27]. According to Cao and Corriveau (2008), the correlation between the migration of BPA and time which varied between 32 - 55 ng/mL (on day 1) to 228-516 ng/mL (on day 6) corresponds to a second-degree polynomial (Migration = $at^2 + bt + c$) when tested with water at 1-6 days at 70 °C [28]. Noted by the authors, the emergence of this variation in BPA migrated between samples may largely be due to different levels of residual BPA in the samples [28].

The residual level of BPA in PC was reported in the range of 1 - 70 mg/kg of the polymer [22, 29, 30]. One value of 140 mg/kg of residual BPA in PC was reported for a batch of baby bottles but was found to be lower than 26 mg/kg in a second batch of the same brand [30]. Migration of BPA was found to be weakly correlated with the residual level of BPA in PC resins [22] while Ehlert *et al* (2008) found no positive correlation between the residual content of BPA in the polymer material (1.4 - 35.3 mg/kg polymer) and the BPA release [29]. Nam *et al* (2010) observed that the BPA migration from PC bottles increased with prolonged usage. In this study, the authors reported concentrations of BPA released from brand-new PC

baby bottles in the range of 0.03 ppb to 0.13 ppb at 40 °C and 95 °C respectively while the concentrations increased to 0.18 ppb at 40 °C and 18.47 ppb at 95 °C respectively after been used for 6 months. They noted the spontaneous increase in BPA migration when the temperatures were above 80 °C [27]. The same conclusion was drawn by Neha and Himanshu, (2021) that reported the increased release of BPA from PC drinking water bottles into the water at the range of 0.38 - 44.48 ng/mL [31]. Release of BPA into hot water at an ambient temperature was reported by Maragou *et al* (2009) ranging from 2.4 –15.3 µg/kg for 45 mins [24] and Brede *et al* (2003) reported the migration of BPA within the same range of 2.4 - 14. µg/kg [32]. Migration of BPA was also observed to be increasing with time when incubated for 7 days with the amount of BPA migrated from new PC bottles in the range of 0.08 ng/mL (day 1) to 1.33 ng/mL (day 7) and 0.21 ng/mL (day 1) to 0.93 ng/mL (day 7) for old ones. According to the authors, the elevated temperature corresponding to the boiling point of water increased the migration of BPA by a factor of 15 - 55 as compared to room temperature [26]. Cao and Corriveau (2008) also reported the migration of BPA into boiling water between 1.7 - 4.1 ng/mL within 24 hrs [28]. pH also affects BPA migration from PC bottles [22, 32].

Several other authors also reported the BPA release into tap and deionized water with the highest concentration reported by Biles *et al* (2013) to be 1 mg/mL at 65 °C as cited by Simoneau and Hoekstra (2013) [33]. However, there is no clear explanation to the release of BPA from PC bottles into tap water at lower temperature. This study aimed to compare the effect of temperature and storage time on BPA release using UV-visible spectrophotometric method and to clarify the reason behind the release of BPA from PC bottles even at room temperature and short storage duration.

## Materials and Methods

### Materials/reagents

Spatula, Graduated cylinder, Beakers, Volumetric flasks, Graduated pipette, Test tubes, Sample bottles, Thermometer, Genlab oven; Model-OV/100/F, Lec laboratory fridge freezer; Model-IST56, ADAM's weighing balance; Model-AE437544, UV-visible spectrophotometer; Model-UV-2500PC Series, Differential scanning calorimeter, analytical graded Bisphenol A powder (LobaChemie Oct. Ltd., 107, Wodehouse Road, Jahangir villa, Mumbai - 400005, India. www.lobachemie.com), analytical graded Methanol (aq) (Hwatsi Chemical, Wadala East, Mumbai), Distilled water (Chemistry Department, Benue State University, Makurdi), analytical graded Nitric Acid (aq) Guangdang Guanghua Chemical Factory Co. Ltd., Suanhua, Guandang China, 51500. www.inhuada.com)

### Preparation of Working Standards

Equal volume of methanol and water was mixed to give 1:1 methanol:water

solution. Using this solution, 1 mg/mL of BPA stock solution was prepared in a dark amber volumetric flask by mixing 50 mg BPA standard with 50 mL 1:1 methanol:water solution and stored in a refrigerator calibrated at 4 °C away from sunlight. Using the stock solution, dilute calibration standard concentrations of 0.04, 0.20, 0.40, 0.80, 1.00, 2.00, 3.00, 4.00, 5.00 and 6.00 ng/mL were prepared using 1:1 methanol:water solution as the diluent via serial dilution.

### Spectroscopic Determination

The major instructive spectral property of BPA in a UV-visible spectrum is between the wavelength of 240-300 nm. The UV-visible spectrophotometer was used to analyze the stock solution and the wavelength of maximum absorbance was determined to be 277.2 nm. The instrument was set at this wavelength in order to generate a calibration curve. The absorbance of each working standard having different concentrations was then monitored at maximum wavelength of absorbance (277.2 nm). Using the absorbance data for working standards, calibration curve was prepared by plotting absorbance of working standards against their respective concentrations. Using the equation of straight line of the form $y = mx \pm c$, concentration equations were generated using a simple linear regression analysis in accordance with Beer Lambert law: $A = ECl$. An excellent correlation coefficient was obtained.

### Sample collection

Three different brands of polycarbonate baby feeding bottles (from three different manufacturers, A, B, and C) and a brand of polycarbonate drinking water bottles (from the same manufacturer) with RIC "7" were purchased in replicates from randomly selected supermarkets in Wurukum and Modern markets, Makurdi, Benue State of Nigeria. The bottles were confirmed to be polycarbonate using differential scanning calorimeter (DSC). The DSC shows thermal transition data such as glass transition temperature, melting point, crystallization, and the relief of residual stress from the manufacturing process. The DSC does this by comparing the heat flow between an empty pan and a pan that has plastic sample. The DSC rsmps the temperature of both pans at the same time and tries to keep the temperature of both pans equal. The DSC must provide additional heat to pans with sample that undergo melting (endothermic process) or a glass transition and less heat to samples undergoing crystallization (exothermic process).

### Sample preparation

For each of the items collected, control and treated samples were prepared in triplicates to ensure accurate results. 200 mL of blank 1:1 methanol:water solution without prior storage in PC containers or subjected to any of the set

experimental conditions was stored in a glass sample bottle. Control samples were prepared by filling each of the different PC containers with appropriate volume of 1:1 methanol:water solution in triplicate depending on the size, and stored at room temperature (~25 °C) for ten days without exposure to the various temperature conditions. Treated samples were prepared by filling three of each brand of the different PC plastic types with appropriate volume of 1:1 methanol:water solution. These three samples belonging to each of the two PC types were each stored in three different laboratory ovens calibrated at 40 °C, 50 °C, and 60 °C respectively for 4 hrs daily after which they were stored in the refrigerator until the next day. This process was repeated for ten consecutive days [36].

## Sample Analysis

UV spectrophotometer was used to analyze the samples. About 3 mL of blank sample was taken and analyzed using UV visible spectrophotometer and was confirmed to be BPA free. This means that if BPA ensue later in the analysis, it would be ascribed to migration of BPA from the PC bottles examined in this study. Also 3 mL of aliquots was taken from each control sample daily and analyzed using UV-visible spectrophotometer for ten consecutive days. For treated samples, 3 mL of the aliquots was taken from each sample and analyzed using UV visible spectrophotometer after been heated in the ovens for four hours daily and cooled in the refrigerator. The samples were kept in the refrigerator until the next day after analysis. This procedure was repeated for ten successive days and the concentrations of the analyte in the various samples were determined from the absorbance data by using the concentration equations generated from the calibration curves.

## Results

The absorbance of the working standards each has different concentrations (0.04, 0.20, 0.40, 0.60, 0.80, 1.00 2.00, 3.00, 4.00, 5.00, and 6.00) ng/mL was determined using a UV-Visible spectrophotometer and calibration curves were generated from the absorbance data by plotting the graph of absorbance against concentration (ng/mL) as shown in Figures 1a & b. Using linear regression analysis, concentration equations $y = 0.38x - 0.236$ (equation in figure 1a) and $y = 0.161x + 0.033$ (equation in figure 1b) were generated both with a very good correlation coefficient of $R^2 = 0.996$ from which the concentration of BPA in each sample was calculated as presented on table 1 and 2. A blank sample was analyzed and was confirmed to be BPA-free. Descriptive statistics of mean and standard deviation were used to obtain the concentration of BPA in each sample as readings were taken in triplicate.

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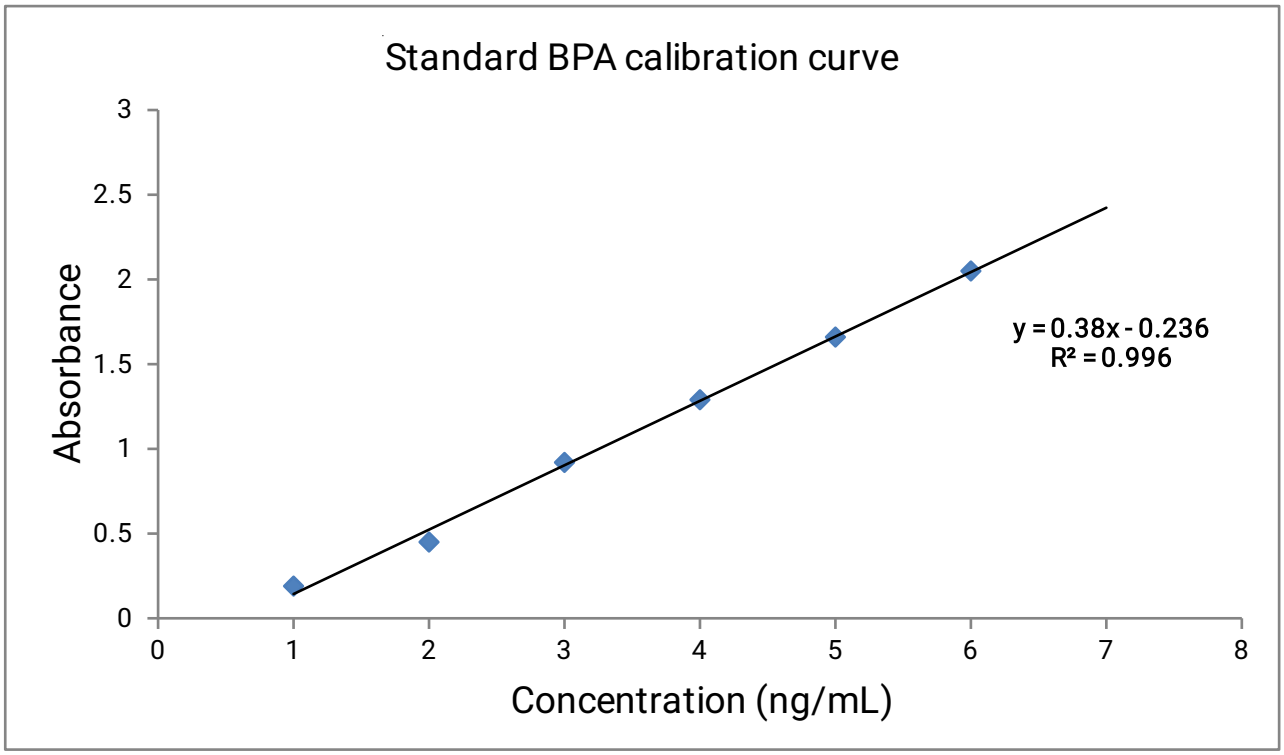



Figure 1a. Standard BPA calibration curve (Concentration range 1.00-6.00 ng/mL)

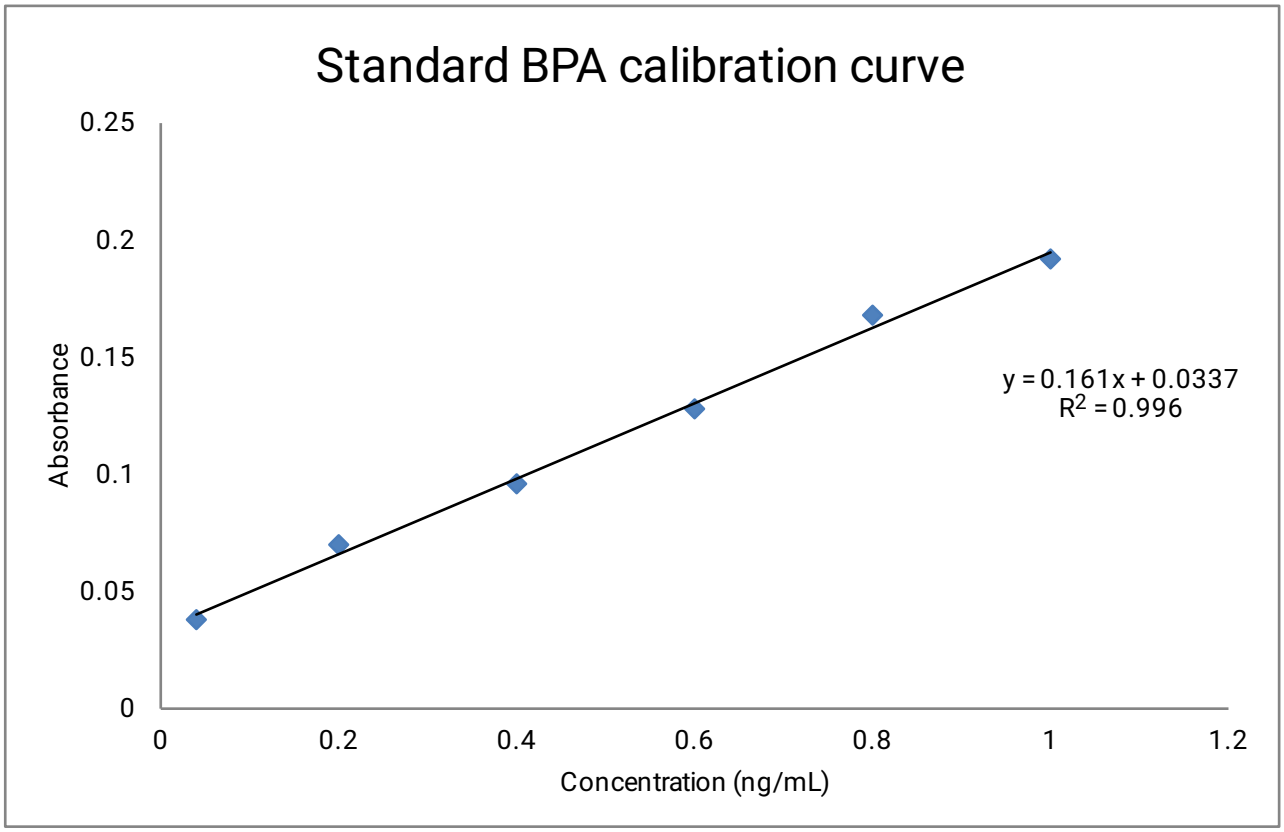



**Figure 1b. Standard BPA calibration curve (Concentration range 0.04-1.00 ng/mL)**

**Table 1.** BPA Concentrations in Three Brands of PC Baby Feeding Bottles (ng/mL)

| Storage time | Samples | Temperatures | | | |
|---|---|---|---|---|---|
| | | Room Temperature (~25 °C) | 40 °C | 50 °C | 60 °C |
| Day 1 | A | 0.040 ± 0.016 | 0.420 ± 0.015 | 1.690 ± 0.130 | 2.800 ± 0.130 |
| | B | 0.039 ± 0.012 | 0.430 ± 0.015 | 1.800 ± 0.120 | 2.360 ± 0.170 |
| | C | 0.036 ± 0.010 | 0.510 ± 0.020 | 1.700 ± 0.130 | 2.600 ± 0.170 |
| Day 2 | A | 0.041 ± 0.015 | 0.450 ± 0.017 | 1.800 ± 0.360 | 2.860 ± 0.150 |
| | B | 0.042 ± 0.012 | 0.482 ± 0.015 | 1.900 ± 0.120 | 2.510 ± 0.200 |
| | C | 0.034 ± 0.010 | 0.513 ± 0.031 | 1.720 ± 0.150 | 2.830 ± 0.140 |
| Day 3 | A | 0.052 ± 0.013 | 0.49 1± 0.012 | 1.830 ± 0.200 | 2.890 ± 0.180 |
| | B | 0.046 ± 0.009 | 0.505 ± 0.060 | 1.940 ± 0.360 | 2.530 ± 0.150 |
| | C | 0.045 ± 0.011 | 0.520 ± 0.082 | 1.760 ± 0.230 | 2.880 ± 0.170 |
| Day 4 | A | 0.060 ± 0.011 | 0.530 ± 0.030 | 1.840 ± 0.190 | 2.920 ± 0.150 |
| | B | 0.053 ± 0.013 | 0.590 ± 0.015 | 1.950 ± 0.320 | 2.570 ± 0.160 |
| | C | 0.056 ± 0.012 | 0.590 ± 0.010 | 1.790 ± 0.160 | 2.920 ± 0.170 |
| Day 5 | A | 0.063 ± 0.015 | 0.538 ± 0.042 | 1.960 ± 0.150 | 2.970 ± 0.160 |
| | B | 0.062 ± 0.007 | 0.660 ± 0.036 | 2.160 ± 0.320 | 2.680 ± 0.170 |
| | C | 0.059 ± 0.006 | 0.596 ± 0.016 | 1.890 ± 0.210 | 3.150 ± 0.190 |
| Day 6 | A | 0.065 ± 0.015 | 0.632 ± 0.015 | 2.150 ± 0.340 | 3.190 ± 0.130 |
| | B | 0.064 ± 0.018 | 0.670 ± 0.020 | 2.290 ± 0.520 | 2.790 ± 0.150 |
| | C | 0.063 ± 0.011 | 0.630 ± 0.008 | 2.030 ± 0.350 | 3.300 ± 0.250 |
| Day 7 | A | 0.069 ± 0.014 | 0.649 ± 0.030 | 2.170 ± 0.260 | 3.250 ± 0.160 |
| | B | 0.068 ± 0.012 | 0.670 ± 0.007 | 2.320 ± 0.360 | 3.000 ± 0.230 |
| | C | 0.066 ± 0.015 | 0.700 ± 0.015 | 2.170 ± 0.270 | 3.360 ± 0.170 |
| Day 8 | A | 0.161 ± 0.021 | 0.700 ± 0.015 | 2.190 ± 0.280 | 3.310 ± 0.220 |
| | B | 0.122 ± 0.008 | 0.726 ± 0.020 | 2.420 ± 0.260 | 3.260 ± 0.150 |
| | C | 0.096 ± 0.005 | 0.820 ± 0.009 | 2.260 ± 0.160 | 3.820 ± 0.120 |
| Day 9 | A | 0.169 ± 0.012 | 0.820 ± 0.038 | 2.200 ± 0.160 | 4.130 ± 0.160 |
| | B | 0.130 ± 0.018 | 0.930 ± 0.012 | 2.500 ± 0.250 | 3.980 ± 0.120 |
| | C | 0.120 ± 0.012 | 0.960 ± 0.050 | 2.280 ± 0.160 | 4.250 ± 0.160 |
| Day 10 | A | 0.180 ± 0.015 | 0.872 ± 0.018 | 2.560 ± 0.360 | 5.130 ± 0.360 |
| | B | 0.142 ± 0.009 | 0.938 ± 0.032 | 2.800 ± 0.160 | 5.021 ± 0.250 |
| | C | 0.168 ± 0.011 | 1.060 ± 0.026 | 2.620 ± 0.210 | 5.620 ± 0.650 |

Key: values are mean ± standard deviations of three determinations

A = sample bottle brand A

B = sample bottle brand B

C = sample bottle brand B

SML of BPA set by EFSA in 2018 is 0.05 mg/L corresponding toTDI of 4 µg/kg

| Storage time/Temperature | Room temperature | 40 °C | 50 °C | 60 °C |
|---|---|---|---|---|
| Day 1 | 0.08 ± 0.01 | 0.18 ± 0.05 | 1.07 ± 0.08 | 3.20 ± 0.10 |
| Day 2 | 0.28 ± 0.01 | 0.32 ± 0.01 | 1.28 ± 0.03 | 3.44 ± 0.20 |
| Day 3 | 0.36 ± 0.05 | 0.42 ± 0.17 | 1.38 ± 0.03 | 3.48 ± 0.15 |
| Day 4 | 0.45 ± 0.07 | 0.53 ± 0.07 | 1.43 ± 0.07 | 3.52 ± 0.08 |
| Day 5 | 0.50 ± 0.06 | 0.67 ± 0.05 | 1.59 ± 0.15 | 3.56 ± 0.12 |
| Day 6 | 0.61 ± 0.20 | 0.79 ± 0.02 | 1.65 ± 0.50 | 3.59 ± 0.31 |
| Day 7 | 0.69 ± 0.10 | 0.82 ± 0.08 | 1.82 ±0.20 | 3.60 ± 0.07 |
| Day 8 | 0.78 ± 0.12 | 0.96 ± 0.15 | 1.89 ± 0.09 | 3.63 ± 0.08 |
| Day 9 | 0.98 ± 0.15 | 1.07 ± 0.18 | 1.96 ± 0.25 | 3.69 ± 0.15 |
| Day 10 | 1.02 ± 0.03 | 1.28 ± 0.80 | 2.31 ± 0.08 | 4.03 ± 0.10 |

bw/day.

**Table 2.** BPA concentrations in one brand of PC drinking water bottles (ng/mL).

Key: values are mean ± standard deviations of three determinations

SML of BPA set by EFSA in 2018 is 0.05 mg/L corresponding to TDI of 4 µg/kg bw/day

## Discussion

BPA is a prototypic EDC that its toxicological impacts on human health have been the subject of debate over the last few decades as a result of its deleterious effect on human health. Studies confirm the presence of BPA in human serum, amniotic fluid, placental tissue and umbilical cord blood, and its presence in human tissues have been linked to the oral ingestion of foods contaminated with BPA due to its migration from PC bottles into foods.

The results obtained in this work agree with results previously reported as detectable levels of bisphenol A (BPA) were observed right from day 1 at room temperature for all the samples of water in the PC bottles that were studied suggesting the presence of residual BPA in the studied PC bottles. BPA levels migrated from the three different brands of PC baby feeding bottles ranging from 0.036 ± 0.012 ng/mL (day 1 at room temperature) to 5.620 ± 0.650 ng/mL (day 10 at 60 °C). The levels of BPA detected in PC drinking water bottles were in the range of 0.08 ± 0.01 ng/mL (day 1 at room temperature) to 4.03 ± 0.10 ng/mL

(day 10 at 60 °C). The mean levels of BPA migrated rapidly increased when the temperatures were above 40 °C and then exponentially above 40 °C. The highest concentration of 5.620 ng/mL or 5.620 µg/L reported in this study is below the acceptable SML of 0.05 mg/L corresponding to TDI of 4 µg/kg bw/day. These values are similar to and in line with results reported previously by many authors in literature as reviewed in this work.

Several authors reported detectable levels of BPA right from day 1 even at a short duration of time, about 30 min. However, this depends on the way samples were prepared before the analysis and the assigned temperature conditions corresponding to the ways that consumers normally treat their PC containers at home before beverages are being stored in them. Cao and Corriveau 2008 reported migrated concentrations of bisphenol A at the range of 32-55 ng/mL (day 1) to 228-516 ng/mL (day 6) at 70 °C [28]. In another work conducted by a group of researchers, a concentration range between 5.5-7 ng/mL was reported at 80 °C after 1 h [34]. Biedermann-Brem and Grob in 2008 also reported BPA concentration range of 0.5-18 ng/mL [35]. In all of these studies, there is a positive correlation between the release of BPA from PC plastics and temperature & time. In another study by Maragou *et al* (2008), the concentrations of BPA migrated from PC baby bottles in the range of 2.4-14.3 ng/mL [24]. According to a study by Nam *et al* (2008), the concentrations of BPA migrated from brand-new PC baby bottles were reported to be 0.03 ng/mL to 0.13 ng/mL at 40 °C and 95 °C respectively whereas the concentrations range of 0.18 ng/mL to 18.47 ng/mL were reported for the bottles that were used for 6 months at 40 °C and 95 °C respectively. It was observed that the levels of BPA migrated rapidly increased when the water temperatures were above 80 °C for 6-months used PC baby bottles. The authors concluded that temperature is the significant factor that favors BPA migration from PC bottles to water [27]. Results reported in this work compared favorably with the work by Brede *et al* (2003) that reported the concentration of BPA from PC baby bottles at the range of 0.11-0.43 ng/mL (1 time of testing corresponding to new PC bottles at 70 °C) [32] and a work by Le *et al* (2008) that reported concentrations of BPA migrated from PC water bottles at the range of 0.08 ± 0.06 ng/mL (day 1 at room temperature) to 1.33 ± 0.09 ng/mL (day 7 at room temperature) for 3 brands of PC bottles studied [26]. They stated succinctly that exposure to elevated temperatures above those typically used for washing by consumers but not outside normal household practice (e.g., boiling to sterilize infant feeding bottles) or outdoor applications (addition of very hot or boiling water or beverages to drinking bottles) greatly elevated the rate of migration [26].

Results reported here clearly show that migration occurs daily as can be seen in tables 1 and 2 above but the difference between the levels of BPA migrated each day is not statistically high enough for us to conclude that storage duration has a very high impact on the BPA migration. This may be because the time scale

chosen for this experiment (1-10 days) is not sufficient enough compared to some works that extended up to six (6) months. After all, prolonged usage may weaken the polymer surface. This would further increase the surface area of the polymer and increase the contact ratio between the food/beverages including water and the polymer which would subsequently enhance the rate of BPA migration into food/beverages in the future. This would increase the migration of BPA from PC plastics at a rate > 0.049 ng/mL per feeding due to the enhancement of average interspacing (d-spacing) of PC bottles, by repeated use leading to an increase in the diffusion of BPA unto the surface of the PC bottles for contact with food and beverage [27].

Again there is a significant difference between the levels of BPA migration at different temperatures pointing to the fact that temperature greatly influences BPA migration from PC bottles into the water as compared to storage time. According to our observation, the rate of increment in BPA surged at temperatures greater than 40 °C which is lower than but agrees with Nam *et al* (2010) and Le *et al* (2008) that the levels of BPA migration from PC bottles would rapidly increase at elevated temperature (above 65 °C) more rapidly than the range observed here. This observation succinctly insinuates temperature as the major crucial factor that favors the migration of BPA from PC bottles into the water as it increases the diffusion of BPA to the surface for contact with food. The release of BPA at a room temperature at day 1 is largely due to the residual amount of BPA in the PC bottles.

## Conclusion

Results reported in this study showed that BPA migrated from PC bottles even at room temperature. The release of BPA at room temperature at day 1 is due to the non-polymerized BPA in the PC bottles. Both duration of storage and temperature influenced the migration of BPA from PC bottles into the water. However, this study indicates succinctly that the impact of temperature on BPA release from PC bottles into water is significantly greater than that of storage time. The concentrations of BPA migrated were in the range of 0.036 ± 0.012 ng/mL (day one at room temperature) to 5.620 ± 0.650 ng/mL (day 10 at 60 °C) for the three brands of PC baby feeding bottles that were studied whereas the concentrations in the PC water bottles were in the range of 0.08 ± 0.01 ng/mL (day 1 at room temperature) to 4.03 ± 0.10 ng/mL (day 10 at 60 °C). The highest concentration detected (5.620 ng/mL or 5.620 µg/L is below the SML of 0.05 mg/L corresponding to TDI of 4 µg/kg bw/day set by EFSA 2018 [20]. However, we cannot say with certainty that this level is safe for consumers because the temperature and time conditions used in this study are lower than what most people practice at home, and the presence of other factors can further trigger its release from PC bottles. Moreover, chronic accumulation of a small dose of BPA can have lifetime implications for humans. Other factors such as pH of water [35] and residual level of alkaline detergent remaining on the surface of PC bottles

after washing may further increase the release of BPA. Manual washing for cleaning the PC bottles does not raise the release of BPA [24]. BPA migration also depends on the heating mode (heating in a microwave may result in more BPA release than heating in an oven).

Summarily, our study confirmed the migration of bioactive BPA from the PC bottles with a positive correlation to temperature and storage time. However, a critical investigation of the results obtained succinctly revealed that temperatures augmented the migration of BPA compared to storage duration. The release of BPA at a very low temperature during a short period of time is due to the residual level of BPA on the PC bottles during the manufacturing processes. The level of BPA migrated as reported in this work may not be safe because of the influence of other parameters that may further increase the release of BPA from PC bottles.

## Competing interests

The authors do not have any conflict of interest to declare. All authors have seen and approved the final contents of the manuscript being submitted and therefore, acknowledge that the article is a product of our original work and has not received prior publication nor is it under consideration for publication elsewhere. We also admit that no financial/personal interest, external funding or research grant stimulates the design of this research work.

Edor Uche Godwin:…….

Ogbene Gillian Igbum:…….

## Authorship Contributions

- Edor Uche Godwin: Conceptualization and design of the study, material sourcing, data acquisition, analysis, and interpretation; crafting of the manuscript, review for essential intellectual property, and approval for publication.
- Ogbene Gillian Igbum: Crafting of the manuscript, review for essential intellectual property, approval for publication, supervision, and funding.

## Acknowledgment

Words are not ineffable for us to use in appreciating the laboratory technicians Mr. Sa'id Hassan Musa of National Research Institute for Chemical Technology (NARICT), Zaria, and Mr. Utange of Benue State University, Makurdi for the assistance rendered in the laboratory at the cause of carrying out the analysis.

## Abbreviations

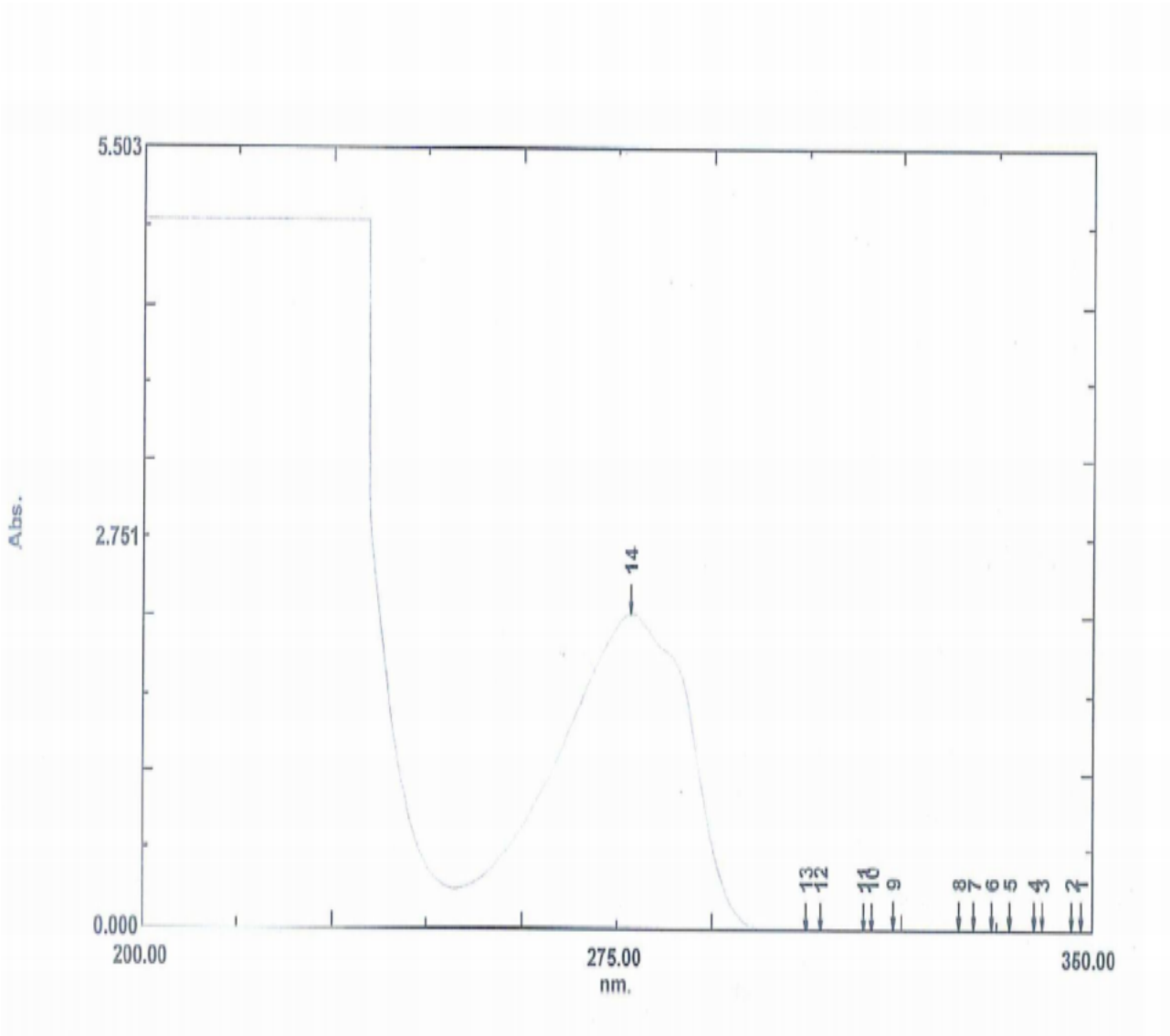
BPA – Bisphenol A
DSC – Differential Scanning Calorimeter
EFSA – European Food and Safety Administration
HED – Human Equivalent Dose
PC – Polycarbonate
PCOS – Polycystic Ovarian Syndrome
$R_fD$ – Reference Dose
SML – Specific Migration Limit
TDI – Total Daily Intake
UV – Ultraviolet WHO – World Health Organization

## Appendices

**Appendix 1: Absorbance data of BPA working standards at different concentrations.**

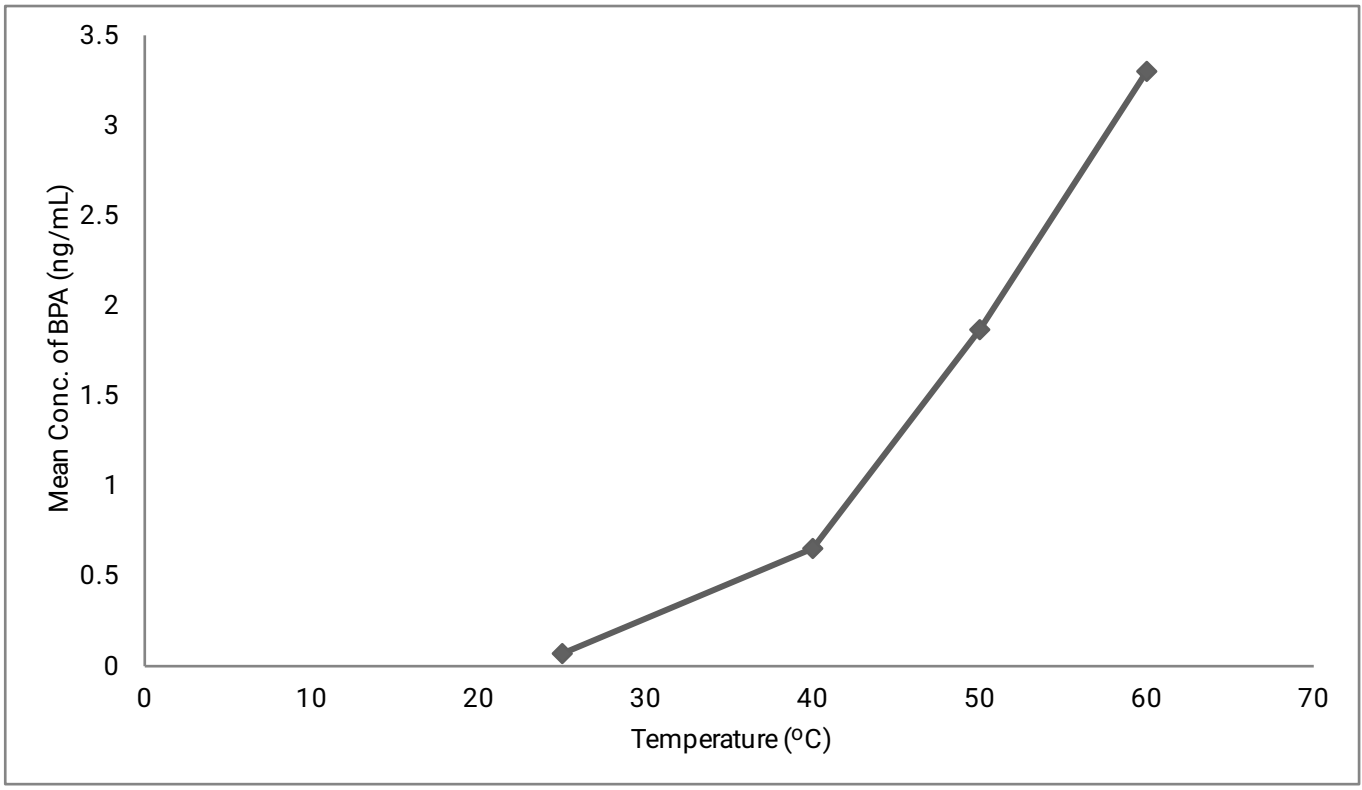
| Concentration (ng/mL) | Wavelength (nm) | Absorbance |
|---|---|---|
| 0.040 | 277.200 | 0.038 |
| 0.200 | 277.200 | 0.070 |
| 0.400 | 277.200 | 0.096 |
| 0.600 | 277.200 | 0.128 |
| 0.800 | 277.200 | 0.168 |
| 1.000 | 277.200 | 0.190 |
| 2.000 | 277.200 | 0.450 |
| 3.000 | 277.200 | 0.920 |
| 4.000 | 277.200 | 1.290 |
| 5.000 | 277.200 | 1.660 |
| 6.000 | 277.200 | 2.050 |

Appendix 3: Absorption spectrum of 1 mg/mL BPA standard solution with maximum wavelength of absorbance at 277.2 nm



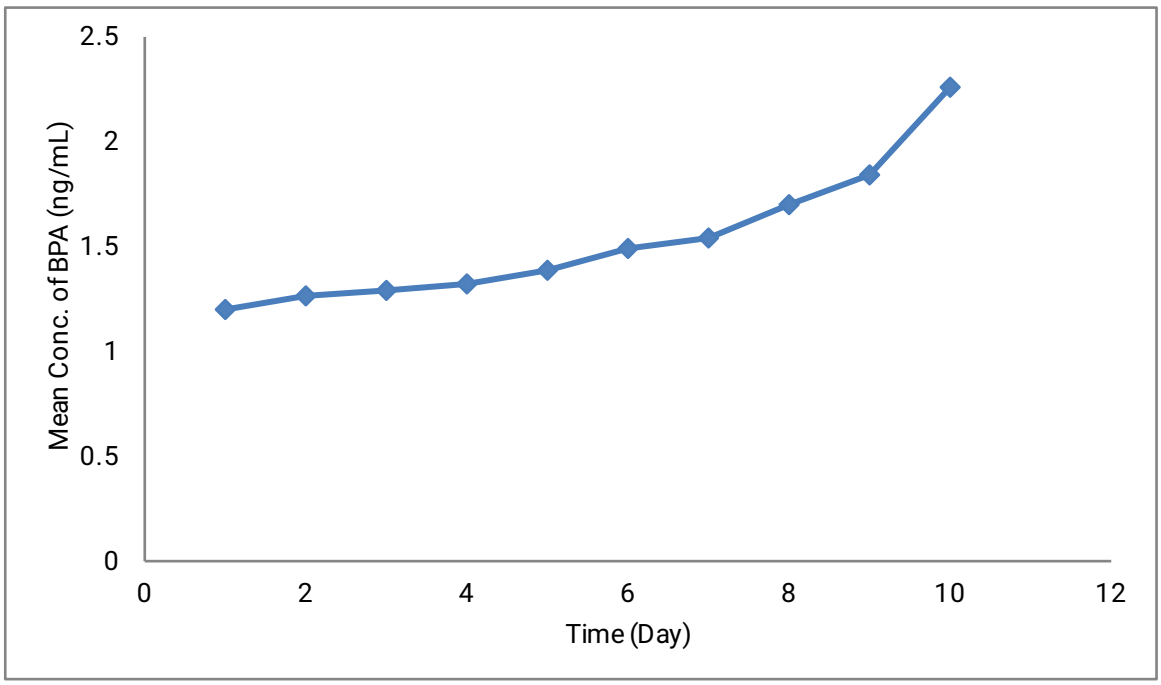
## Appendix 4: Effect of temperature on the migration of BPA from PC bottles

To further clarify the effect of temperature on BPA migration from PC bottles, mean concentrations of BPA migrated at each temperature for ten days were calculated (i.e., summation of all the concentrations of BPA migrated at each temperature divided by the total number of readings). The table below shows the calculated mean concentration of BPA migrated at various temperatures. Then, a plot of mean concentration against temperature was made as shown 3.



## Appendix 5: Effect of storage time on the migration of BPA from PC bottles

To further elucidate the effect of storage time on BPA migration from PC bottles, mean concentration of BPA migrated at various temperatures for ten days were calculated for each day and the calculated mean concentrations were tabulated as shown below. Then a plot of mean concentrations against time (day) was made as shown on figure 4.



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