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Extraction and Quantification of Bisphenol-A Level in Infant Polycarbonate Feeding Bottles using High Performance Liquid Chromatography Technique

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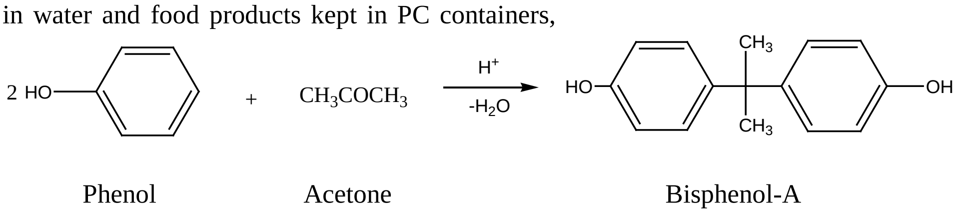
ABSTRACT

Polycarbonate plastics (PC) containing bisphenol A (BPA) are used for the production of infant feeding bottles, food storage containers, kitchen utensils and some components of medical devices. Trace amount of BPA have been reported in water and food products kept in PC containers. The leaching of bisphenol A from new and used polycarbonate feeding bottles into 10% ethanol (EU regulated simulant) and hot water was investigated using HPLC analysis. The bottles were filled to the corresponding nominal volumes with 10% ethanol, allowed to stand for 24hours and with boiled HPLC grade water for 2hours at 40°C, followed by extraction with ethyl acetate. The results indicated the release of high amount of BPA from new feeding bottles than the previously used ones. The BPA leachate in 10% ethanol was within the range of 20-61ng/mL and 14.13-27.30ng/mL in new and used bottle respectively, while the range in hot water was 12.53-92.65 ng/mL and 7.81-55.02ng/mL in new and used bottles respectively. The validation parameters were Limit Of Detection: 1.14 ng/mL, Limit Of Quantification: 3.80 ng/mL, while the percent recovery for spiked samples and efficiency of the extraction procedure were 98 –103 % and %RSD ranged between 2.00 – 5.61%.The estimated tolerable daily intake(TDI) using the average BPA leachate in boiled water has exceeded the new revised TDI of 4µg/kgbw/day established by European Food Safety Authority (EFSA).

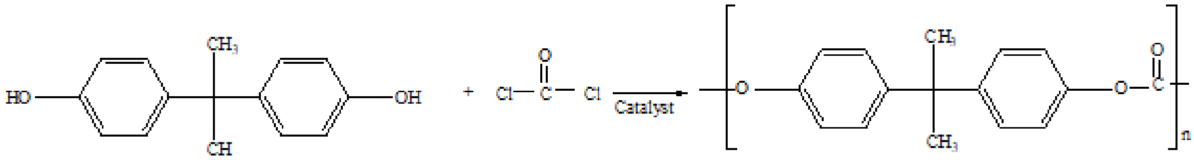
Keywords: Bisphenol A, Infant feeding bottles, Leachate, Leaching, Polycarbonate

INTRODUCTION

Bisphenol A, (2,2-bis(4-hydroxyphenyl) propane commonly known as BPA, is an industrial chemical produced in large quantities for use primarily in the production of polycarbonate plastics and epoxy resins. Polycarbonate plastics (PC) containing bisphenol A are used for production of infant feeding bottles, food storage containers, kitchen utensils and some components of medical devices. Epoxy resins are used to coat metal products such as food cans, drink containers and water supply pipes. BPA is a compound that is currently on the list of potential environmental hazardous chemicals, it is on the watch list of many countries, such as United States and Germany (Nicolas *et al.*, 2015). BPA can inevitably migrate into foodstuffs and beverages from packing of the product. Trace amount of BPA have been reported in water and food products kept in PC containers, which may lead to adverse effect (Cao *et al.*, 2011). BPA is synthesized from condensation of acetone (hence the suffix A) and two phenol molecules in an acidic or basic medium. Polycarbonates are polymers containing carbonate groups, produced from the reaction of bisphenol A and phosgene ($COCl_2$). Polycarbonate plastics are typically hard and clear and are marked with the resin identification code number '7'. The use of polycarbonate containers for the purpose of storage is controversial, because of their hydrolysis at high temperature to release BPA. This process is referred to as leaching or migration of BPA from the PC polymer to the contents stored in them (Hoekstra and Simoneau, 2013). Below are the scheme showing the production of Bisphenol-A from phenol and acetone and polycarbonate from bisphenol-A and carbonyl chloride.



Scheme 1: Bisphenol A produced by the condensation of phenol and acetone in the presence of acid catalyst



Bisphenol-A Carbonylchloride(phosgene) Polycarbonate

Scheme 2: Polycarbonate produced from polymerization reaction of Bisphenol A and carbonyl chloride in basic solution and an amine catalyst

The majority of human exposures to BPA occur mainly via diet, through packaged food and beverages that are contaminated by BPA migrating from the packaging material (Almeida *et al.,* 2018). The highest estimated intakes of bisphenol A occur in infants and children, with highest estimated average of 0.375 ppb of body weight per day for infants till 3 years of age (EFSA, 2014).Their intake is greatest because BPA is suspected to leach from plastic baby bottles and the linings of cans of powdered and liquid formula. Many authors have studied migration of bisphenol A and its derivatives into food and simulants from the polymer packaging in which it is stored, especially at elevated temperatures e.g. microwave heating or other thermal process and as a consequence of incorrect use of packaging. (Hoesktra and Catherine (2013), Shrinithivihahshini *et al.,* (2014), Pedersen *et al.,* (2015), Torres *et al.,* (2015)) The preparation of aqueous food simulants and plastic samples of tests in the laboratory was laid under BS EN 1186-1: 2002 and BS EN 1186-3: 2002 framework and European Commission Directive EU 10/2011 to determine overall migration as: Food simulant (A) 10 % ethanol recommended for testing aqueous food pH > 4.5 and Food simulant (B) 3 % acetic acid recommended for testing aqueous food type pH < 4.5.

Bisphenol A has been detected using traditional analytical methods with low detection limits and good selectivity for routine analysis, these includes: high-performance liquid chromatography (HPLC) with different methods of detection (e.g. UV, MS, fluorescence), liquid chromatography (LC), gas chromatography coupled with mass spectrometry (GC-MS) and enzyme- linked immunosorbent assay. Since polycarbonate plastic are widely used in infant feeding bottles and children of < 3years are the most vulnerable to BPA-induced health hazard, the migration of BPA from the milk bottles into the milk that children drink is of concern to us. Hence the aim of this study was to investigate the migration of BPA from the new and used PC feeding bottles into EU regulated simulants, 10% ethanol and boiled water using HPLC analytical method.

Materials and Methods

HPLC 1260 infinity series from Agilent technologies USA equipped with ZORBAX SB- C 18 analytical column (Agilent, reverse phase 3.5µm 2.1 x 150mm) and UV detector were used. Methanol: water, 40:60 was used as the mobile phase at a flow rate of 0.7 mL/min. The injection volume was 15uL and the running time was 10 minutes with 30° C as the column temperature.

The target compound in the unknown sample was identified at 277nm using the retention time of the external standard (Average of 0.558 min) taken for elution. The chromatograms were processed by chemstation software also from Agilent technologies.

Preparation of Standards

BPA stock solution of 1mg/mL was prepared by dissolving 1 mg of analytical grade BPA D_{16} (>99%, Sigma-Aldrich, USA) in 1 ml of acetonitrile. The stock solution was diluted through serial dilution (3 folds) to get 1µg/mL standard, equivalent to1000 ng/mL. This was further diluted using dilution formula in which 2 – 10mL volumes were measured in 100 mL distilled water to prepare a calibration standards of 20 - 100 ng ml^{-1}. Both the external standards and the unknown samples were finally analyzed by HPLC.

Selection of Polycarbonate Bottles

Twenty different brands of new polycarbonate baby feeding bottles of 60 ml and 120ml capacity were randomly purchased from stores and shops in the market, in Kano state north-west Nigeria. The bottles were selected based on physical properties of polycarbonate plastics, hard and clear feeding bottles with resin identification code '7' were selected during sampling. With the exception of one bottle coded NF2, the remaining PC bottles used in this study were made from China and India. The samples were further characterized by Fourier-transform Infrared Spectrometry (FTIR) and compared with polycarbonate reference material for confirmation. Home-used PC feeding bottles which had been used for two to twelve months were also obtained from nursing mothers, two of these bottles were excessively used probably for more than a year.

MIGRATION CONTACT EXPERIMENT

Extraction of BPA with 10% Ethanol

Washed and dried polycarbonate bottle was cut into small fragments of about 5×5 mm in size and weighed, and prepared as follows (Zhiqun *et al*., 2017). Exactly, 0.5 g fragment was soaked in 10% ethanol for 24 hours and ultrasonically extracted for 2 hours in a water bath at 95 ± 0.5°C. The leachate solution was cooled to room temperature, filtered with a 0.45 µm-filter membrane and transferred into a 2mL vial for HPLC analysis. BPA was then extracted from PC infant bottles as follows, the new PC bottles were rinsed with hot water before analysis, as according to manufacturer's instruction for the first time use and the water was discarded, the bottles were then dried. Migration contact experiments were carried out with 10% ethanol as food simulant according to European Commission Directive EU 10/2011. Ten new infant feeding bottles and ten used bottles each were filled corresponding to the nominal volume with 10% ethanol in water (v/v) and intermittently shaken for 10 minutes. The bottles were then kept in an oven at 40°C for 24 hours. The leachate solutions were concentrated in a water bath at 80°C and dissolved in 2 mL acetonitrile. It was then filtered, kept in a 2mL vial and stored at 4°C prior to HPLC analysis

Extraction of BPA with Hot water

Ten new and ten used feeding bottles coded NF1-NF10 and OF1-OF10 respectively, were filled with boiled HPLC grade water and allowed to stand for two hours. The leachate water was extracted according to (Shrinithvihahshini *et al.*, 2014).Where 60mL leachate water was transferred into a 250 ml seperatory funnel, 10 ml of 99% ethyl acetate added and the contents were swirled gently for 5 minutes and finally left undisturbed for separation into clear and distinct organic and aqueous layers. The organic phase was collected in a 50 ml glass test tube and the aqueous phase was drained out into a beaker for the second and third time extractions, each with 10 ml of ethyl acetate. The combined ethyl acetate extract was evaporated to dryness at 60°C in a water bath and the residue was dissolved in 2ml acetonitrile. This was filtered and transferred into 2 ml amber-colored glass vials and stored at 4°C for HPLC analysis.

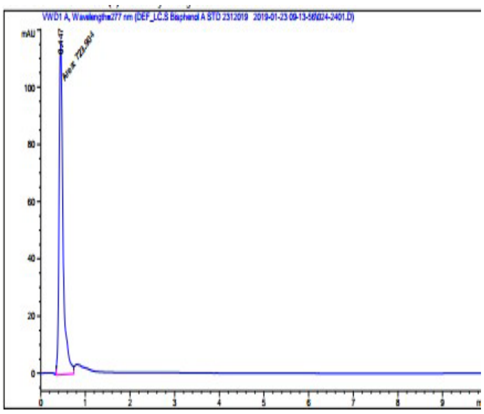
BPA Spike Samples (Recovery)

To check the recovery of BPA achieved in the extraction method employed in this study, BPA aqueous solution were prepared with HPLC grade water at 10, 50 and 100 ng/mL concentrations. The spiked solutions were extracted with ethyl acetate as done for the unknown samples in hot water analysis. The chromatograms of BPA spiked samples were compared to those of the external standards and the percentage efficiency of BPA extraction procedure as well as recovery of the method were evaluated.

RESULTS AND DISCUSSION

Figures 1and 2 presents the HPLC chromatograms of some of the samples showing their retention times and peak areas. The concentration of BPA was derived from the peak area of external standards and BPA concentration by fitting into the linear curve equation $y = mx + c$; where y is the peak area, x the concentration of the analyte, m the slope of the curve and c the intercept. The mean retention time of the samples was found to be 0.535±0.03minute with 5.6% RSD. The limit of detection (LOD) and limit of quantification (LOQ) was determined from low calibration standard and slope of the calibration curve data and was found to be 1.14 and 3.80ng/mL respectively. The method recovery was determined at three different concentration and found to be within the range of 98-103% (Table 2) and %RSD ranged between 2.00 – 5.61%, this shows efficiency of the extraction procedure as well as good recovery of the method.

A



B

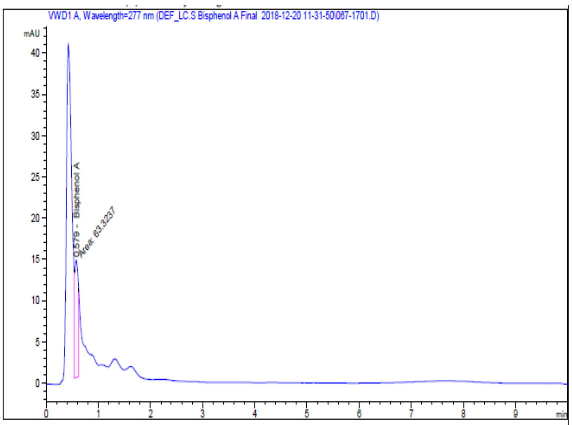


Figure 1: HPLC-UV Chromatograms for (A) Solvent Acetonitrile and (B) BPA Standard Peak Area 63.3237,(R=0.579)

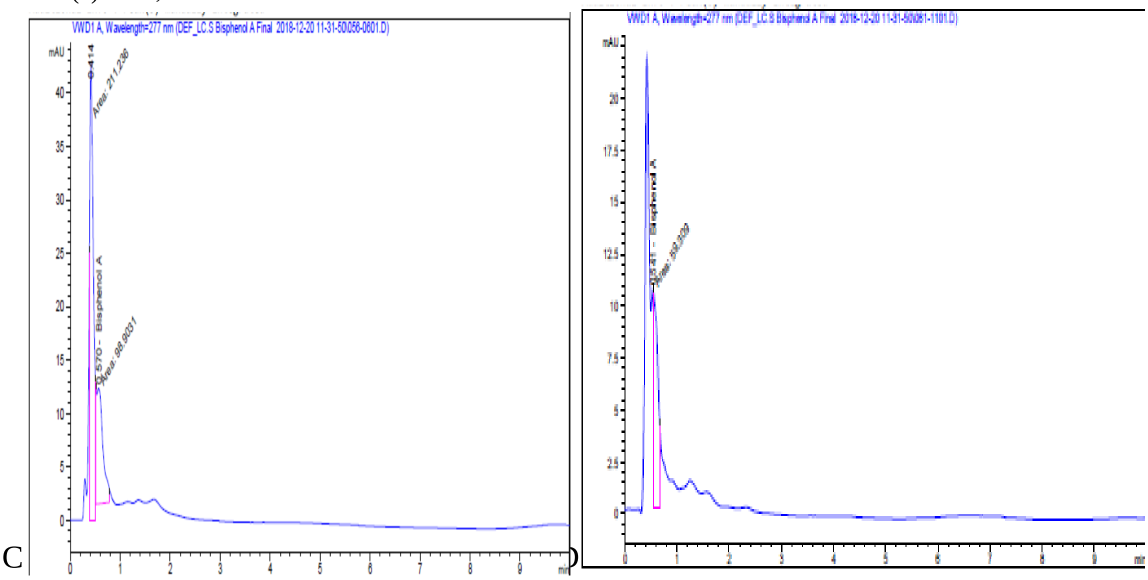


Figure 2: HPLC-UV chromatograms for (C) Hot water contained in new infant bottle (NF1) peak area= 98.9031, Ret. time=0.570min. and (D) in old infant bottle (OF6) peak area=59.309 Ret. time =0.541min.

The first analysis on fragments carried out to detect the presence of BPA in PC bottle, indicate average BPA leaching of 60.20±7.12 ng/mL. HPLC result of 10% ethanol simulant is presented in figure 3, BPA obtained in new feeding bottles ranged from 20-61 ng/mL, while the used ones leached less BPA ranging from 14.13-27.30 ng/mL. The hot water leaching experiment indicated the migration of BPA ranged from 12.53 – 92.65 $ngml^{-1}$ and 7.81 – 55.02 $ngml^{-1}$ in new and used bottles respectively as shown in Fig. 4. The results showed that both new and old infant feeding bottles released varied amount of BPA. The lowest BPA leachate was obtained from a branded “Avent” feeding bottle made in England (NF2) and the highest value was obtained from “Linco baby love”(NF4) made from China, all used for hot water analysis. The variation in BPA leachate among the feeding bottles could be due to different sources of raw materials used and the different processing conditions by the manufacturers. Overall the released of BPA from the bottles support the hypothesis that suggests,” The hydrolysis of the PC as the source of BPA migration from the polymer to the aqueous medium” (Torres *et al.* 2015).

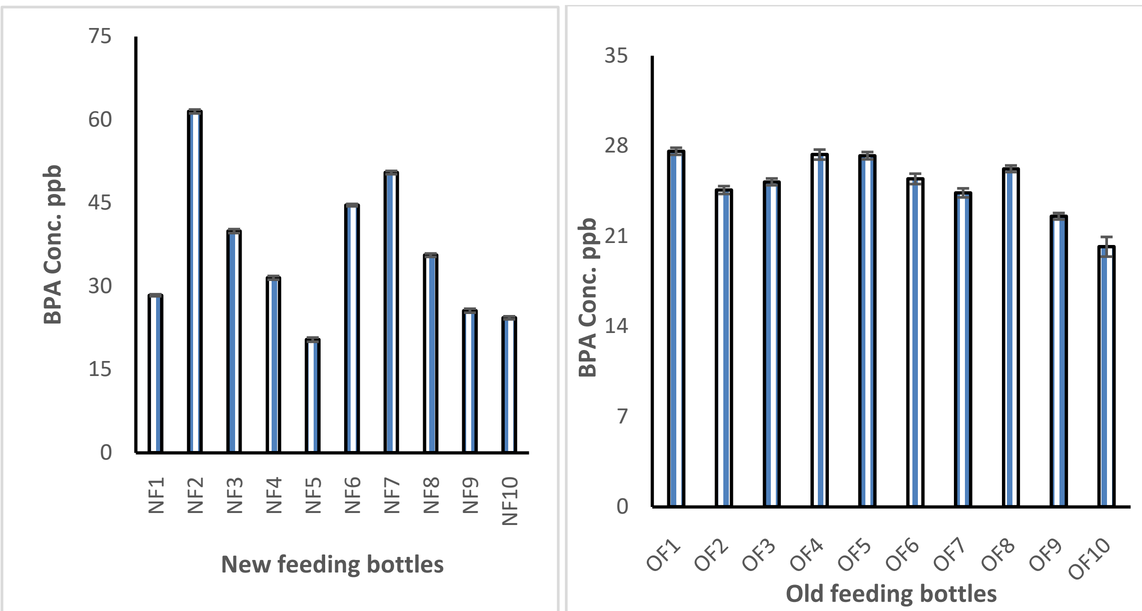


Figure 3: BPA leachate from new and used feeding bottles by storing in 10% Ethanol simulant

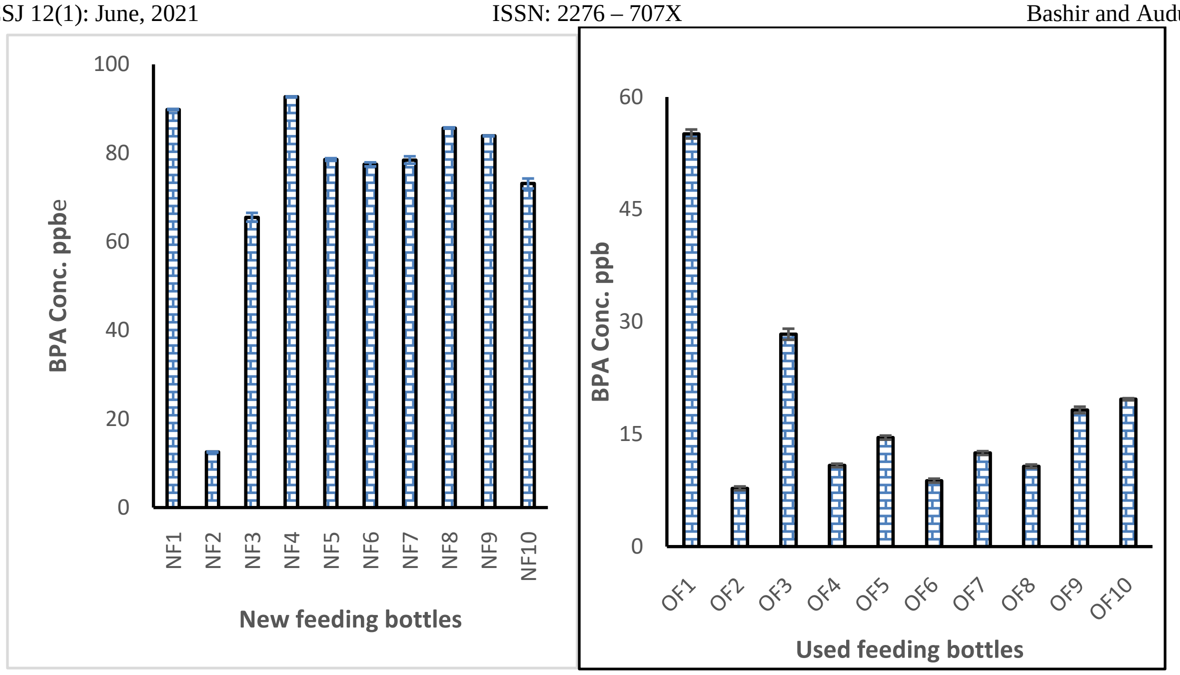


Figure 4: BPA leachate from new and used feeding bottles by storing in boiled water

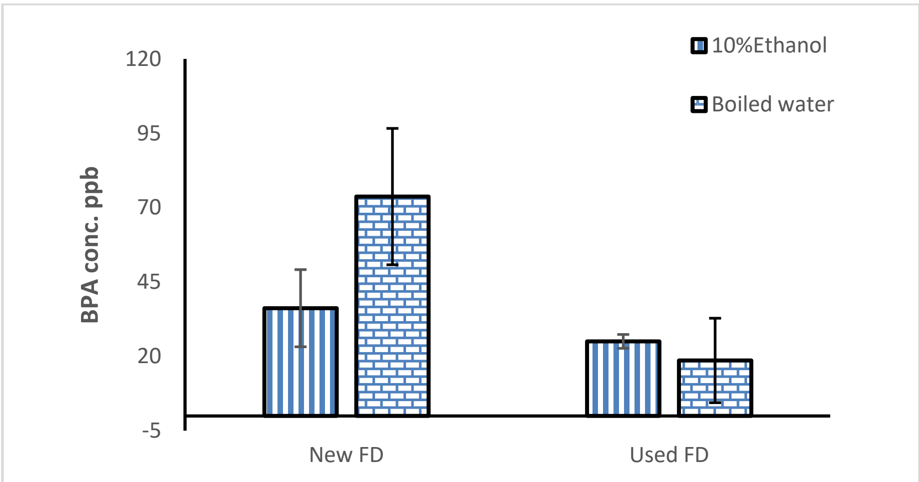


Figure 5: Comparison of BPA migration from new and used bottles in two stimulants

Significant different at 5% probability level was indicated between the average concentration of BPA leachate from new and used feeding bottles in both the two simulants. The higher value in new feeding bottles (Figure 5) could be attributed to diffusion of residual BPA that remained after the manufacturing process. It can also be attributed to free or non-polymerized molecules on the inner layer of PC bottles that are gradually released by repeated use of these bottles (Moghadem *et al* 2015). With regard to the two simulants, BPA migration was greater in hot water than in 10% ethanol, especially in new feeding bottles. This could be attributed to elevated temperature used in hot water simulant, as indicated from the results of earlier studies that the concentration of BPA migration from PC containers increased with temperature of the food (Shrinithivihahshini, 2014 and Torres *et al.*, 2015). According to European Scientific committee on Food an infant of 4.5 kg could consume about 700mL of milk per day (SCF, 2012), with the average BPA leachate in boiled water in the present studies that infant is likely to consume 10.26µg of BPA per kg of its body weight per day. The amount has exceeded the recently reviewed Tolerably Daily Intake (TDI) of 4µg/kgbw/day (EU, 2018).

Comparing the results of the present studies with what was reported from the literature: A similar results reported by Moghadam *et al*, (2015), in which new feeding bottles leached more BPA than the old ones, ranging from 0.49-8.58µg/L and 0.63-2.47µg/L respectively. Also Cao and Corriveau (2008) reported the concentration of BPA released from new bottles as 228 - 521µg/ L and from used bottles was only 0.63-2.47µg/mL. The results of BPA leachate from new feeding bottles in the present studies is found to be higher than the value reported by some authors, Maragou *et al*. (2008), reported 14 $ngml^{-1}$ by storing hot water at 70°C for 1 hour. Bledermann-Brem, and Grob, (2008) reported a leaching of $18ngml^{-1}$. Similarly Shrinithvihahshini *et al.,* (2014) reported a value of $19ngml^{-1}$ in some brands of baby feeding bottles available in the Indian market. The high value obtained in this study could probably be due to the use of hotter water at 100°C in the migration experiment and kept for longer contact time of two hours than the earlier studies mentioned above. Likewise other results were significantly higher than the present study as well, ShagunKapil , (2019) reported BPA migration between 0.1 and 98.4 ppm in baby feeding bottles and from ND to 14.9 ppm in Sippy cups in twenty samples for both branded and local samples from India, in a study "Bottles can be toxic". Mostafa *et al.* (2011) also reported BPA leaching by HPLC, using MTBE as simulant ranged from $10.7ngml^{-1}$ - $5620ngml^{-1}$ for all the twelve baby milk bottle tested in Egypt, and reported a migration test study in both oil and 10% ethanol simulant, from $4.01\text{-}141ngml^{-1}$ for 34 infant feeding bottles samples. The results were equally lower than what was reported by Niki *et al.* (2008), the release of BPA for all the bottle samples studied in the concentration range from 2.4 - 14.3 μgkg^{-1} when filled with boiled water and left at ambient temperature for 45min.

CONCLUSION

The present study revealed a significant amount of BPA leachate from both new and used PC feeding bottles into 10% ethanol simulant and boiled water. Average BPA concentration from new PC bottles was found to be 36.14±6.07 ng/mL in 10% ethanol and 66.68±7.30ng/mL in boiled water. While the used bottles leached lower amount of BPA, average of 22.18±3.50ng/mL in 10% ethanol and 18.64±4.20ng/mL in boiled water.

It is disturbing that the average concentration of BPA leachate in boiled water experiment was higher than in 10% ethanol simulant. Because boiled water at that particular temperature is normally used by nursing mothers for feeding babies, after keeping in the PC feeding bottle for long hours. The tolerable daily intake (TDI) using the average BPA leachate in boiled water was found to be 10.26 µg/kgbw/day, which exceeded the newly established TDI of 4µg/kgbw/day (EU, 2018). And there are still evidences of continuous and enhanced level of BPA migration due to the effect of temperature, pH of food and contact time. The use of hot water in infant feeding bottles, Sippy cups and bowls made of polycarbonate enhanced the leaching of BPA into the food, which is the main source of exposure in infant and children. From the findings of this research the use of polycarbonate feeding bottles cannot be regarded as safe for the level of BPA consumed at every feeding of the infant.Exclusive breastfeeding is a great way of reducing potential BPA exposure to infants.

RECOMMENDATION

It is recommended, for future work on Bisphenol-A to study the effect of different temperatures for the hot water simulant and the effect of contact time in polycarbonate feeding bottles in order to obtain the conditions for optimum release of BPA, so that they can be avoided.

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