Bisphenol A migration study in baby feeding bottles of selected brands available in the Indian market

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The wide applications of polycarbonate (PC) and other polymers in the kitchen ware and food storage containers increase the risk of human exposure to bisphenol A (BPA), mainly through food and water. BPA results in endocrine disorders in humans; health impacts caused by the chemical vary with body weight and exposure dosage. The present study aims to test the safety of using PC bottles, for feeding infants with respect to BPA and the migration rate of BPA from the containers, while storing hot water at 70°C, for 1 h. Three different popular brands of PC baby feeding bottles were subjected to the tests. BPA residues were extracted with ethyl acetate and quantified using HPLC with PDA detector. The test reveals that BPA migrates from PC baby feeding bottles at 19 ng ml^{-1} of hot water (70°C), stored for 1 h.

Keywords: Bisphenol A, endocrine disruptor, feeding bottles, polycarbonate.

BISPHENOL A (BPA) is a solid organic compound, used globally in large amounts for the production of polycarbonate (PC) plastics and epoxy resins. Though the chemical is used in various processes, it has recently been revealed that it alters the human endocrine system; thus its name has been included in the list of endocrine disrupting chemicals (EDC; substances capable of mimicking/disrupting the innate hormonal mechanism of mammals, including human beings)[1–6]. The metabolic and reproductive abnormalities of BPA have been reported by several studies conducted on animal models.

Statement of problem

Epoxy resins and PC containers play a major role in food and package industries. Containers made of PC plastics are favoured for their sturdiness, light weight, shatter-resistance, durability, optical clarity, heat tolerance, electrical resistance, etc. For these reasons, most of the domestic kitchen ware, including baby feeding bottles are made with such plastics, using BPA as a plasticizer. The additive, BPA gets liberated from the food container, at particular environmental conditions and contaminates the food stuff stored and thereby the human body after intake. Due to the structural similarity of BPA with 17β-estradiol, the former compound binds with the estrogen receptor (ER) and alters its functions[7–9]. Hence continuous use of such plastic containers for food preparation and storage is likely to add up the body burden to a level that causes hormone-related problems; the impacts of BPA chemical residues on endocrine system vary with the dosage, body weight and synergistic actions of hormones. It has been reported that the acidity or alkalinity of food, mode of cooking/heating, temperature of food during storage, nature of cleaning detergents and age of the container are factors governing BPA migration[10–14].

From the literature review, it is evident that endocrine disorders are on the rise among people of all ages and sex. To emphasize, girls and women nowadays are seen to frequently encounter with thyroid disorders, early puberty, transgender, infertility, endometriosis, adenomyosis, etc. in comparison to their counterparts in the olden days. Many publications are blaming fast foods, enamel-coated kitchen ware, PC cooking ware, cosmetics and birth control pills for the occurrence of such endocrine disorders. Owing to the continuous exposure to the chemical via milk and solid foods, and lower body/mass index, children are highlighted as the prime risk group largely afflicted by endocrine disorders[15]. Due to the danger of BPA found in PC feeding bottles, Denmark, Canada and France have banned the use of BPA in feeding bottles since 2010 (ref. 11). However in India such awareness is less common; conversely plastics are put into multitude of uses. Feeding bottles are sold liberally in the Indian market without any label to mention the BPA concentration found in the container.

Tests were performed by employing three popular brands of PC baby feeding bottles in the market to find whether they are BPA-free and study the migration behaviour of BPA from the container at a particular pH,

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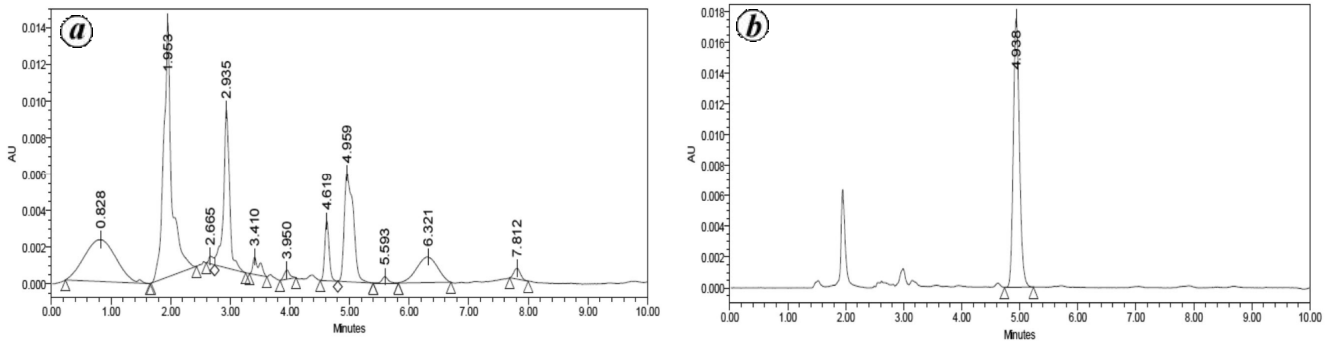

Figure 1. HPLC-UV chromatograms showing the peak (***a***) BPA present in a sample (R_t: 4.9) and (***b***) 15 ng ml^{-1} standard BPA (R_t: 4.9).

temperature and contact period using water as a food simulator, and finally to quantify the residue in the test medium, BPA-free water. The work was done preliminarily to confirm its presence and migration; further work on BPA migration using other variables is in progress and is not discussed here, considering that they are not relevant to the present topic.

Materials and methods

Three different brands of PC baby feeding bottles of 75 ml capacity with resin identification code '7' were purchased in replicates from randomly selected super markets of Tiruchirappalli, Tamil Nadu, India. Heat-resistant capacity of the bottles as found in the label was 120–140°C.

Initially the PC bottles were rinsed with hot water to imitate the real use conditions, where bottles are normally rinsed with water at its boiling point to kill the germs, before pouring milk into them. BPA-free water, treated through Milli-Q water system (Millipore Corporation, USA) was taken in a 1 litre borosilicate beaker and heated to 70°C using thermostatically controlled mantle. Next 60 ml of hot water was transferred separately into the three different brands of baby bottles, in triplicate and was made to stand for 1 h. After 1 h, the water was transferred into a 250 ml separatory funnel, to which 10 ml of 99% ethyl acetate (Merck Specialties Pvt Ltd, India) was added; the contents were swirled gently for 30 sec and finally left undisturbed for the formation of 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 collective 30 ml of the organic phase (ethyl acetate extract) was evaporated to dryness at 60°C using a water bath. The dried residue was redissolved in 1 ml of acetonitrile, and then filtered through 0.2 µm PTFE (polytetrafluoroethylene, Nupore Filtration System Pvt Ltd, India) syringe filter using a glass syringe. The filtered extracts were transferred into 5 ml amber-coloured glass vials and stored at 4°C for further analyses.

For compound identification through HPLC, BPA stock solution was prepared by dissolving 1 mg of analytical grade BPA (>99%, Sigma-Aldrich, USA) in 1 ml of acetonitrile (Fisher Scientific, India). From this, 5, 10, 15, 25 and 50 ng ml^{-1} of external standards were prepared. Both the external standards and the unknown samples (A, B and C) in triplicate were finally analysed using Waters™ HPLC System, equipped with 2475 Pump, C_{18} analytical column (Perkin Elmer, USA; Reverse Phase; 250 mm × 4.6 mm i.d.; 5 µm) and UV photo diode array (PDA) detector. Methanol (Qualigens Fine Chemicals Pvt Ltd, India) – water was used as mobile phase at a flow rate of 1 ml min^{-1}. The elution was achieved through isocratic mode at 70 : 30 (methanol/water; v/v). The injected sample volume was 20 µl. The target compound found in the unknown test samples was identified at 277 nm using the retention time of the external standard (4.9 min) taken for elution (Figure 1). The chromatograms were processed by using Empower2 (Waters Corporation, USA) software.

Recovery

To check the recovery rate of BPA achieved in the extraction method employed in this study, BPA aqueous solution in the concentration of 10, 25 and 50 ng ml^{-1} was made and the solutions extracted with ethyl acetate, similar to that of the samples. Finally, chromatograms of the BPA extracts prepared from the known concentrated BPA aqueous solutions were compared to those of the external standards (5, 10, 15, 25 and 50 ng ml^{-1}) and percentage efficiency of BPA extraction procedure was calculated.

Results and discussion

The concentration of BPA was derived using the peak area of external standards versus 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 results show that the containers of all three brands tested leach BPA at a significant level, which is slightly higher than the value reported by Maragou *et al.*[16] (14 ng ml^{-1}) using hot water. The result also provides evidence for BPA leaching from the containers to the water stored at minimum temperature (70°C for 1 h) and pH (pH of the BPA-free water, 7). More importantly, unlike the earlier experiments, the present leaching study was done using hot water (70°C) and not boiling water.

The concentrations of BPA reported by various workers are: 5.5–7 ng ml^{-1} (ref. 17), 2.4–14.3 ng ml^{-1} (ref. 16) and 0.5–18 ng ml^{-1} (ref. 10). The BPA residues observed in the present study (Table 1) are highly significant to those previously reported values. The findings of the present study are in line with the concentration of BPA reported by Biles *et al.*[18] in 1 g of PC polymer.

Although the average leaching value of BPA recorded in the present study is less than the tolerable daily intake (TDI) of 50 μg kg^{-1} bw^{-1} (ref. 19), it cannot be considered as safe, because the present work was carried out using water at 70°C with neutral pH. The volume of water used for migration study was also only 60 ml, whereas an infant of 4.5 kg consumes minimum of at least 700 ml of milk per day[20]. Residual content of BPA in PC containers and hydrolysis of PC are two prime reasons for BPA and origin[21]. Prolonged usage may weaken the polymer surface. This would further increase the surface area of the polymer to food for contact; which could subsequently enhance the rate of BPA migration into foods in future. Similar inference was also given by Nam *et al.*[22], who examined the BPA migration in PC baby bottles using hot water (40–100°C) stored for 30 min. They have reported that BPA diffusion into food was enhanced due to the enhancement of average interspacing (*d*-spacing) of PC bottles, by repeated usage. Futher they stated that migration of BPA from the PC baby feeding bottles likely to increase with the age of the container at a rate of 0.049 ng ml^{-1} per feeding. It is clear from the results of the earlier studies that the concentration of BPA that migrated from PC containers increases with age, nature and temperature of food in addition to the polymer composition of the container. Therefore, even though the present level of BPA found from the migration study is lesser than the TDI value, it cannot be considered safe and requires further work by taking variables like pH, quantity of solvent, wide range of temeperature, etc.

Table 1. Average of bisphenol A (BPA) migrated into water from the sample container

Sample	Migrated BPA (ng/ml)	Average concentration (ng/ml)
A1	22	19
A2	19	
A3	17	
B1	19	17
B2	17	
B3	17	
C1	18	17
C2	17	
C3	18	

Conclusion

The present study reveals that the PC baby feeding bottles available in the Indian market are likely to leach around 19 ng of BPA ml^{-1} of milk. According to the European commission's Scientific Committee on Food, an infant of 4.5 kg could consume about 700 ml of milk per day[20]; if the present BPA migration level of 19 ng ml^{-1} exists, an infant by feeding through such PC bottle is likely to get 2.9 μg of BPA d^{-1} kg^{-1} of its bodyweight. It can be argued that the container may not leach the same amount of BPA every time. But there are evidences for continuous and enhanced level of BPA migration due to the effect of temperature, pH of food and age of the container.

In addition, the recommended TDI of BPA kg^{-1} of body weight has been recently revised to 5 μg (ref. 23) from the previous value of 50 μg (ref. 19). The recent change made by the EFSA on the TDI value of BPA itself, outlines the increased risk of BPA on human health. Therefore, detailed studies are needed, involving a wide range of temperature, pH of the food, age of the container, contact time, action of cleaning detergents to completely understand the migration behaviour of BPA and its rate of migration. Further, the milk formulations available for infants also require thorough study for the presence of BPA as plastic linings are used in such containers. Such studies would also reveal the exact amount of BPA likely to enter into the body system of children and the possible health risks posed by such plastic ware. The impact study of BPA on children in both the young and adolescent stages is also needed in India, where only limited information is available on such matters.

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