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RESEARCH ARTICLE

ANALYSIS OF BISPHENOL A MIGRATION IN REUSABLE POLYCARBONATE CONTAINERS AND CANNED PRODUCTS AVAILABLE IN INDIAN MARKETS.

Amanpreet Kaur, Ramesh Jhorar and Tapan Kumar Mukherjee.

Department of Biotechnology, Maharishi Markandeshwar University, Mullana (Ambala) India.

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*Corresponding Author

Amanpreet Kaur.

Abstract

The wide applications of Bisphenol A (BPA) in food and packaging industry have increased the exposure and risk of this chemical towards the human population with its endocrine disrupting effects. BPA is used among the various products with everyday use. The vulnerable population especially infants are at an increased risk of exposure as BPA is widely used in baby feeding bottles. The present study was carried out to determine the amount of BPA migrating in to the water samples from the polycarbonate material of baby feeding bottles, drinking water bottles, microwave safe plastic and canned food products. Different brands of polycarbonate feeding bottles, water bottles and microwave safe plastic were included in the study and BPA was determined in the water samples using HPLC with fluorescence detector. BPA leach out was also determined in the canned products which included canned fruits and canned vegetables. The test revealed 2.80 mg/L of BPA leaching for the feeding water bottles, 0.06 mg/L from the drinking water bottles, 0.008 mg/L from microwave safe plastic bowls and 0.067 mg/L in the canned products.

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Introduction:-

Bisphenol A, common name of 2,2-(4,4' dihydroxydiphenyl)propane, is a widely produced chemical which is used extensively as an intermediate in the production of polycarbonate plastics and epoxy resins. Polycarbonate plastics are being utilized for making various articles including baby feeding bottles, sippers, cups, water bottles, microwave ovenware, storage containers, returnable water and milk bottles, and refillable water containers. Numerous studies have shed light on the migration of BPA from various polycarbonate and canned products into the food and drinks including feeding bottles (Brede et al., 2003; Kubwabo et al., 2009), polycarbonate drinking bottles (Le et al., 2008), microwave polycarbonate containers (Nerin et al., 2003; Fasano et al., 2015), canned products (Sungur et al., 2014; Lorber et al., 2015) food packaging plastic film (Sungur et al., 2014) etc. Besides, the non-food sources have their equal contribution to the human exposure which could be from dust (Fu and Kawamura, 2010), thermal paper (Liao and Kannan, 2011), dental materials (Fleisch et al., 2010) and medical devices (Calafat et al., 2009; Haishima et al., 2001). Due to its high volume production and widespread use in plastics and cans, there is pervasive environmental contamination and well predictable and chronic exposure of BPA towards people of all ages. BPA is potential endocrine disruptor which exhibit low estrogenic and other endocrine disrupting activities (Krishnan et al., 1993; Laws et al., 2000; Chen et al., 2002). Moreover, many studies have come up exhibiting the low dose effects of BPA probably affecting the infants and babies which are more vulnerable towards the consequences of BPA. Besides, the presence of BPA in women of child bearing age is also worrisome as they provide the first environment for their developing babies (Padmanabhan et al., 2008). In addition, studies have also found higher levels of BPA in infants and young children as compared to adults (Calafat et al., 2009; Edginton and Ritter, 2009; Bushnik et al., 2010).

Materials and Methods:-

Materials and Reagents:-

Standard BPA of >97 % purity was purchased from HiMedia. The stock solution was prepared in methanol and used for further dilutions. The methanol of HPLC grade was also obtained from HiMedia. Solution of 500ppm was made as stock solution by adding BPA in methanol. Further external dilutions of concentrations 50, 100, 200 and 250 mg/L were made by adding BPA free-water treated through Milli-Q water system (Millipore Corporation, USA).

Sample collection:-

A total of 15 samples were taken to check migration of BPA which include baby feeding bottles, drinking water bottle, microwave safe polycarbonate bowl and cans. Eight feeding bottles were taken under five different brand names (Brand A-E). Of them, five bottles (FB1-5) were newly purchased from the local market and the rest three used feeding bottles (FB6-8) were donated by anonymous people. Used polycarbonate bottles were of same brand as the new bottles. All the used bottles showed visible wear and opaque discoloration and used approximately for a period between 3-12 months, although the bottles were depicted as to be used under normal conditions. The capacity of the feeding bottles ranged from 125-250 ml. Four polycarbonate drinking water bottles were taken of a particular brand. Two of the drinking water bottles were newly purchased from the local market while two bottles were donated by anonymous people. A used microwave safe polycarbonate bowl of a particular brand (Brand F) was donated by an anonymous university student. The bowl was described to be used for microwaving for approximately six months. Two samples of canned products were collected from a local supermarket. One can comprised of canned fruit (pineapple) (CN 1) and second can comprised of canned green peas (CN 2). Both the canned products were made by different companies (Brand G and H).

Sample Preparation:-

A standard washing procedure was followed for each sample of feeding bottles, water bottles and microwave bowl. Each of the bottle and bowl were filled with distilled water treated through Milli-Q water system and rinsed well. Then detergent was added and bottles shaken for few times. The feeding bottles were washed by using a gentle brush. Again bottles were rinsed with distilled water and then washed with Isopropyl alcohol to remove any residue. A final wash of distilled water was given five times to remove any residue of alcohol. The bottles were then air dried until completely. They were then kept in boiling water in a beaker for about 5-10 minutes. Beakers were rinsed with distilled water and kept in hot air oven at 200°C to remove any water adsorbed on the surface of glass beaker. The beakers were then rinsed with methanol and air completely at 100°C for 1 hr. This was done to mimic the common sterilization technique followed at homes. The bottles were then taken out and filled with HPLC-grade water at 70°C upto a certain level and kept for 1 hr with gentle shaking in between. After 1 hr, the water was poured into an autoclaved beaker and kept for boiling to evaporate the water. Approximately 5ml of water was left behind and transferred into amber coloured 5 ml vials and kept in refrigerator until used for HPLC analysis. For water bottle sample, the bottles were washed following same procedure as described above. The air dried bottles were filled with HPLC-grade water at room temperature and kept for 24 hrs. After that, the water was transferred to a beaker and boiled for evaporation. 5ml water was reserved and stored in amber coloured glass vials in refrigerator until further use.

For sample preparation of microwave bowl, the washing procedure was same as followed for feeding bottles. After air drying the bowl in a laminar air flow hood, HPLC-grade water was poured in the bowl and kept in the microwave for about 2 minutes at medium-high intensity for boiling. After that, the bowl was kept at room temperature for about half an hour and 5 ml sample was taken with the help of autoclaved polypropylene micropipette tips into the glass vials.

For canned product samples, the products of the cans, both liquid and solid, were taken and put into the mixer for homogenization. 5ml of the semisolid product was taken out and poured into the polypropylene centrifuge tubes and 3 ml of dichloromethane was added and centrifuged at 3000 rpm for 10 minutes. The supernatant was collected and mixed with methanol and were stored in amber colored glass vials until further use.

HPLC analysis of Bisphenol A:-

The water samples of feeding bottles, water bottle and microwave bowl were analyzed by HPLC-fluorescence. For compound identification of BPA, the standard solution and the external dilutions prepared were analyzed using Shimadzu HPLC System equipped with 2475 Pump, C₁₈ analytical column (Perkin Elmer, USA; Reverse Phase; 250 mm x 4.6 mm i.d.; 5 µm) and Fluorescence detector. Injection volume of 20 µl was injected into the C₁₈ column.

Isocratic elution was carried out with a mixture of methanol:water (v/v) in the ratio of 65:35 at a flow rate of 1ml/min with equilibration time of 10 min. The column temperature was set at 30°C. The fluorescence detector was set at an excitation wavelength of 228nm and an emission wavelength of 313nm. The chromatograms were processed using Empower3 software.

Results:-

Detectable levels of BPA were observed in the present study in all the samples of plastic ware and cans as well. Table 1 depicts the results of BPA concentration obtained in all the samples irrespective of new and old. The BPA concentration was derived using the peak area of external standard versus the peak area of sample solutions and employing the linear curve equation, $y = mx + c$, where, y is the peak area, x is the concentration of analyte and m is the slope of the curve and c is the intercept. These results are a well illustration of BPA leaching from the plastic water bottles, feeding bottles and cans. Significant levels of BPA were detected in the present work which was slightly higher than the levels found in other studies (Nam et al., 2010; Shrinithiviahshini et al., 2014). The highest BPA leachout was obtained in sample FB 3 with concentration of 52.54 mg/L in a used feeding bottle. The value is corresponding to the average value of BPA observed in feeding bottle by Shrinithiviahshini et al. (2014). BPA was also detected in drinking water bottles at minimum concentration of 0.06 mg/L. Thermicrowave safe polycarbonate cans also leached out BPA at concentration of 0.008 mg/L. Similarly, the BPA was also detected in cans, with minimum concentration of 0.067 mg/L. Studies conducted by Yoshida et al. (2001) also revealed the BPA migration from the internal lining of epoxy resin into the food product. The changing lifestyle is captivating the urban population mainly to depend on canned food and drinks, thus exposing the body with more of these chemicals.

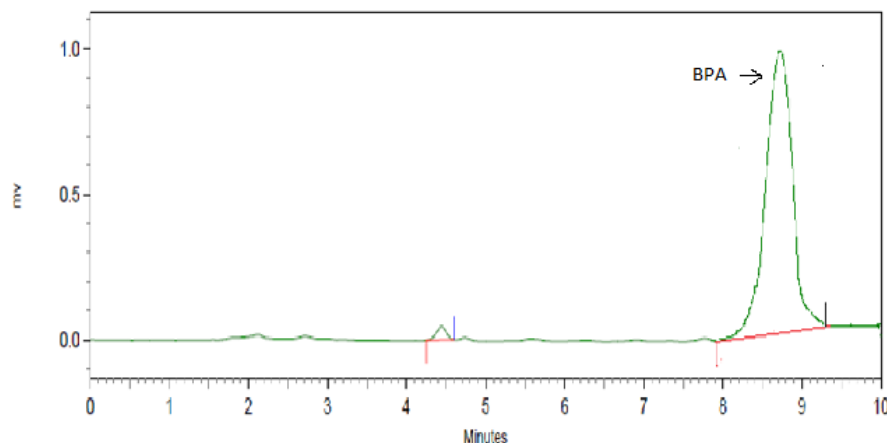
Discussion:-

Although, the BPA concentration observed in samples of the present study is below the maximum acceptable or reference dose of "0.05 mg/kg of the body weight/day" (50µg/kg body weight/day), established by the United States Environmental Protection Agency (USEPA) and European Union (EU), however, the observed values could not be considered as safe, as the daily intake of liquid is far more than the samples taken for this study. It has been observed that the daily intake of milk by an infant of about 4.5 kg of body weight is approximately 700 ml, whereas the samples taken for this were about of 100 ml. Moreover, many studies have shown that exposure to even low doses of this chemical which probably may be through food packaging, can increase the risk of many hormone related disorders.

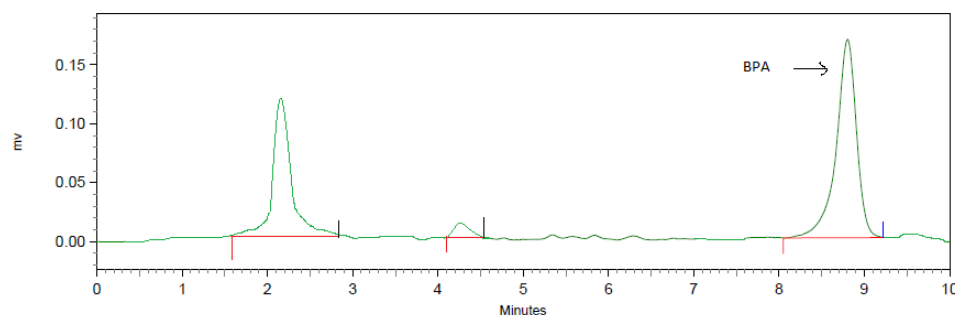
There also exists a slight difference between the BPA concentrations in new and old PC bottles with old PC bottles leaching higher concentration of BPA. This might be due to the fact that with due course of time and repeated washing of the bottle result in weakening of the polymer, with enhanced leaching of the chemical in to the food and drinks. Nam et al. (2010) have stated in their studies that the BPA leaching is possibly to increase with the age of the container at the rate of 0.049 ngml⁻¹ per feeding from the PC baby feeding bottles. Moreover, high temperature put an additional effect in leaching of the BPA, as the BPA leach out was observed to be more in feeding bottles as hot water was put in to them, while the BPA concentration came out to be less in PC water bottles. The same was also observed by Le et al. (2008) in their studies in which they established a clear linking of BPA concentration at elevated temperature. It is well clear from the studies that BPA is leaching at detectable levels at various concentrations depending upon the factors comprising age, temperature and nature of food material. Though, the results obtained in the present study are less than the minimum TDI values, however, further work needs to be done taking different variables including temperature, pH, solvents, age of material etc.

Graph 1: BPA Standard graph

- BPA standard- 200mg/L
- Retention time- 8.883 min



Graph 1: HPLC graph showing BPA peak of sample FB 4

**Table 1:-** BPA concentration in different samples of polycarbonate products and cans.

| Sample No. | Type of Sample | BPA conc. (in ppm) | Condition of the sample |
|------------|-----------------------------|--------------------|-------------------------|
| FB1 | Feeding Bottle | 3.58 | New |
| FB2 | Feeding Bottle | 19.63 | New |
| FB3 | Feeding Bottle | 52.54 | New |
| FB4 | Feeding Bottle | 2.80 | New |
| FB5 | Feeding Bottle | 5.27 | New |
| FB6 | Feeding Bottle | 25.02 | Used |
| FB7 | Feeding Bottle | 35.32 | Used |
| FB8 | Feeding Bottle | 26.91 | Used |
| WB1 | Drinking water bottle | 0.06 | New |
| WB2 | Drinking water bottle | 0.083 | New |
| WB3 | Drinking water bottle | 0.71 | Used |
| WB4 | Drinking water bottle | 0.28 | Used |
| MP1 | Microwave safe plastic bowl | 0.008 | Used |
| CN1 | Canned fruits | 0.067 | New |
| CN2 | Canned vegetables | 0.09 | New |

Table 1 depicts the results of BPA migration into the samples which were further detected by high performance liquid chromatography with fluorescence detector. The highest concentration among the polycarbonate product was found in used feeding bottle of sample FB3 with BPA concentration of 52.54 mg/L.

Conclusion:-

The present study was carried out to determine the BPA leaching in the feeding bottles of different brands, water bottles and cans available in Indian markets, hence revealing leaching of a minimum of 2.80 mg/L of BPA from feeding bottles, 0.06 mg/L from water bottles at room temperature and 0.067 mg/L in cans. Moreover, the leaching is also enhanced with various factors of age, type of container and temperature. Besides, the human body gets exposed to BPA through various other sources including dental sealants, thermal paper receipts, food packaging plastic films etc. adding more of load of this chemical into the body. Further research is a prerequisite to be carried out on the effect of this chemical on the Indian populations as there no stringent laws against the chemical in the country to protect especially the vulnerable population including infants, children and adolescents.

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