Existence of Bisphenol A (BPA) in Baby's Feeding Bottles in Ethiopia Evidence for Many Human Health Problems

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Abstract: ***Bisphenol A (BPA) is an industrial compound it is one of the highest volume chemicals produced worldwide, this compound is used to produce synthetic polymers, epoxy resins, which are used for beverage containers, metal cans, BPA also used to manufacture polycarbonates with applications in bottles of water and milk, studies have showed that BPA has been detected in majority of people. Which is an endocrine disrupter, and several reports have proposed on exposure to BPA will leads to adverse health effects, such as infertility, obesity, cancer. This study is proposed to evaluate the exposure levels to BPA in Ethiopian babies with by taking 2 to 24 months of age, and the bottles were made by China and Taiwan, and those are collected from Ethiopian market, Analyses were then made by taking consideration of the different food stuffs (water and milk) and different conditions.***

Keywords: Bisphenol A, Ethiopian babies, Milk bottles

1. Introduction

Bisphenol A (BPA; 4, 4′-(propane-2, 2-diyl) diphenol) is a phenolic compound, it is one ofthe highest volume chemicals produced worldwide [1], it is synthesized by the acid or alkaline catalysed condensation of acetone and phenol. BPA is used in the production of polycarbonate plastics, epoxy resins used to line metal cans, and in many plastic consumer products including toys, water pipes, drinking containers, eyeglass lenses, sports safety equipment, dental monomers, medical equipment and tubing, consumer electronics and baby's feeding bottles. polycarbonate plastics are the material of choice for high performance products in areas where safety, hygiene and durability are of key importance [2]. They are also used in the lining of metal food cans, in dental sealants and an additive to certain plastics used in children's toys [3].

Despite the ideal function it has been served in many industries, BPA is now identified to be extremely toxic chemical for human being. It is identified that exposure to BPA leads to potential health effects [4]. Like hormone disruptor, BPA strongly binds with estrogen-related receptors but does not replace the activity of estrogen. As a result, BPA may be adding a 'false' estrogen effect in the body, off-setting the hormonal balance required for healthy human development [5]. In general, the chemical is now believed as highly promoter for the health problem [6, 7]; neurological disruption, endocrine disruption, recurrent miscarriage, altered mammary gland development, cancer, altered brain development and insulin resistance.

Human mainly exposed to this chemical in to two ways; migration into food and beverages from packaging materials that directly contact food, specifically food and beverage containers with internal epoxy resin coatings and migration from polycarbonate repeat use containers such as baby bottles and drinking bottles [8].

Epoxy resins are used as an interior protective lining for food and beverage cans and for metal closures for some jars and bottles. As a result of these food contacts with it, very small quantities of the monomer Bisphenol A can migrate into the food and beverage contents of the containers. Migration of small amounts of bisphenol A from polycarbonate repeat-use containers such as baby bottles, drinking bottles, pitchers and carboys can also contribute to oral exposure. Studies have also determined that BPA can be measured in humans' serum, urine, amniotic fluid, follicular fluid, placental tissue and umbilical cord blood.

Children are especially vulnerable to bisphenol A because endocrine disruptors affect how their bodies grow and develop [9]. Young children still have immature organ systems, high metabolic rates, relatively low bodyweight and are going through rapid physical development; therefore, even low levels of repeated exposure may eventually lead to adverse health effects [10]. Baby's feeding bottles are products of this chemical helping us in order to feed children. Currently, some 90 percent of plastic baby bottles in the market are made with BPA InEthiopia [11].

The major objective of this study is to determine the level of BPA and assessing the level whether it is beyond the limit it causes an adverse effect in human health at different baby's feeding bottles which are imported and served in Ethiopia.

2. Materials and Methods

Chemicals and reagents:

Pure standard BPA (Sisco research laboratory) for quantification, HPLC grade methanol (which is obtained from Fischer) in order to prepare the standard and eluting the analyte from the sorbent and dichloromethane and ethanol (from Aldrich) in order to conditioning the cartridge were used. Volumetric flasks (50 mL, 100 mL and 500 mL) were used for the preparation of standards, Isolute SPE C18 columns which are obtained from International Sorbent Technology of UK and an instrument Flexar Quaternary pump HPLC instrument which is from Perkin Elmer of USA were used.

Study subjects:

Analysis has been performed for two groups of feeding bottles which were obtained from China and Taiwan based on temperature (at 25 °C and 80°C) and food simulants (water and milk). The amount of BPA leached from the bottles in to the contained food stimulant.

Extraction:

Each bottle was first filled with pure distilled water at room temperature. To test for leaching of BPA at high temperature, then other bottles were then filled with hot distilled water (approximately 80^{o}C). Again, to test the tendency of leaching BPA in milk, other new and used bottles were filled with milk simulant (50% of ethanol with water at room temperature) this ratio of mixture is believed to replace milk [12] and to test its leaching at high temperature, the same new and used bottles were filled with the same food simulant at 80°C. Finally, all the representative samples were left for 24 hours at room temperature. At the end of 24 hours the water and milk simulant from each bottle was subjected to solid phase-extraction process. The extraction process was performed using small columns with C18 sorbent [13].

Elution: Finally, the adsorbed components were eluted with 3 ml of methanol. The extract was then dried up and dissolved again in 0.5 ml of methanol [13] and the resulting sample was injected to the instrument (10 μL).

Validation Study:

Optimization was indispensable to provide complete separation of the compound studied and to eliminate possible matrix interferences in the shortest time. A good separation for analysis of the applied compound was obtained with a gradient elution. The peak was completely separated within 14.5 min. Attempt has also been made to find good choice of mixture of solvent. The different validation studies are summarized below in table 1, 2 and 3, 4.

Table 1: The gradient solvent flow with time

Step	Time (minute)	Solvent type	
		A (%)	B (%)
1	4	100	0
2	6	0	100
3	3	0	100
4	1	100	0

***Solvent A is1: 1 mixture of water and ethanol with 0.5% acetic acid and solvent B is pure methanol.

Table 2: The different parameters for the analysed compound

Parameters	
LOD (μg/L)	0.307
LOQ (μg/L)	0.980
IDL (μg/L)	0.077
Linear range (μg/L)	0.5-10
(r^2)	0.9993

*LOD is limit of detection, *LOQ, is limit of quantification, *IDL, is instrumental detection, * r^2 is the regression coefficient

Table 3: Three level recoveries of BPA from matrix spike

Concentration	7 (μg/L)	5 (μg/L)	2 (μg/L)
Recovery 1 (%)	85.4	84.0	81.3
Recovery 2 (%)	93.0	86.7	83.6
Recovery 3 (%)	88.6	82.4	79.7
Average recovery (%)	89	84.3	81.53
Standard deviation	3.816	2.218	1.96
Relative standard deviation	4.28	2.63	2.40

***** Results are average values of triplicate of reading

Table 4: Recovery of BPA from LCS spiked at 2 μg/L.

Sample	LCS1	LCS2	LCS3
Recovery 1 (%)	84.4	86.2	84.7
Recovery 2 (%)	81.6	83.9	82.6
Recovery 3 (%)	83	85.3	85.5
Average recovery (%)	83	85.13	84.27
Standard deviation	1.40	1.16	1.50
RSD	1.69	1.36	1.78

* RSD, relative standard deviation

Limit of Detection and Quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) of the analytical method were calculated as 3.14 and 10 times the standard deviation obtained from seven replicate blank samples spiked at 2μg/L. As table 3 shows the limit of detection of the method was as low as 0.307μg/L which indicates that that the method is good enough for the determination organic compounds at very low concentration.

Repeatability:

Repeatability of the method was examined by spiking three laboratory control samples and the resulting relative standard deviation was calculated. As table 4 and 5 shows all the relative standard deviation values lie between 1.36 to 4.28 which is indication of good repeatability or precision of analytical results.

Quality Control /Quality Assurance:

Analyses the quality of the validated analytical method and its results were controlled by analysing the results of matrix spike and matrix spike duplicates, laboratory control samples and reagent and method blanks. The percentage recoveries were in the range of 81.53-89% which is inacceptable range and the result of regent and method blanks are given at the appendices part. Accuracy of the analytical instrument was also controlled by analysing BPA standard solution of mid-point calibration concentration before each new bottle item was analysed and no significant variations were observed which indicates good calibration accuracy.4.3 Calibration and Linearity of Instrumental Response.

Calibration was performed for a standard solution of the analysed compound. To determine the linear range of the response to direct injection, the different level of standard solutions was injected. The calibration curve was then constructed using the five concentration levels. A simple solution at each concentration level was injected in triplicate. The calibration plots were approximated by the equation y = ax + b, where y is the peak area, and x is the concentration of determination compounds in µg/L. Good correlation coefficients, higher than 0.99, were obtained. Shown in (Fig.1)

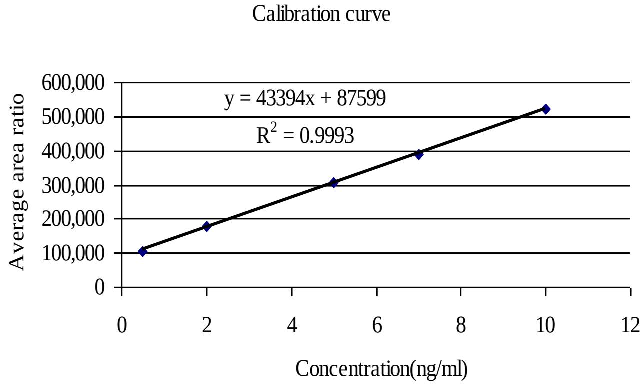


Figure 1: Calibration curve for standard of BPA

Statistical Analysis:

In order to test whether there was a significance difference in the detected concentration of BPA between the new and old, water and milk simulant, a two tailed t-test was performed on bottles obtained from China and Taiwan, at 25°C and 80°C. Accordingly, for the mean concentrations of BPA between old vs new, in all cases i.e. under water and milk stimulant at room temperature as well as at 80°C, a significant difference was not observed at 99 % confidence interval (p=0.01) for new and old bottles processed in China while a significant difference was observed for bottles processed in Taiwan. Comparison has also been made at 99 % confidence interval (p=0.01) on average concentration of BPA detected on the new bottles as well as on the old bottles obtained from China and Taiwan at 25°C vs 80°C. As a result, a significant difference was observed between the mean concentrations of BPA detected at this temperature difference in any of the bottles obtained from these two countries. Again, the test was made to compare average concentration of BPA detected in water vs milk stimulant, still the test did notify that there was a significant difference observed in mean concentration of BPA detected in the two food simulants. Finally, attempt has also been made to compare the average concentration of BPA detected on the new and old bottles of China vs Taiwan. And the same test did show that there was a significant difference on the average concentration of BPA detected between the two countries.

3. Results

BPA Detected in Different Babies' Feeding Bottles

Analysis has been performed for two groups of feeding bottles which were obtained from China and Taiwan based on temperature (at 25 °C and 80°C) and food simulants (water and milk). The amount of BPA leached from the bottles in to the contained food stimulant is summarized below in table 5.

Table 5: Amount of BPA detected in water and milk simulant in the bottles

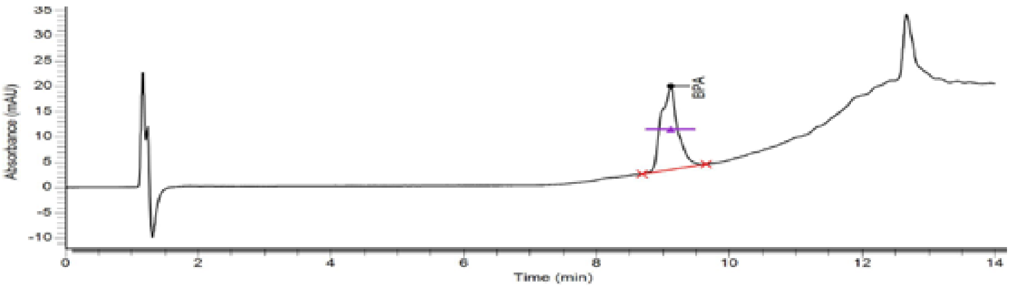
Made of	Bottle type	Amount of BPA detected in water (µg/L) ± sd		Amount of BPA detected in milk simulant (µg/L) ± sd	
		At 25°C	At 80°C	At 25°C	At 80°C
China	New	ND	5.26 ± 0.203	2.46 ± 0.088	6.80 ± 0.425
	Old	ND	5.77 ± 0.274	2.58 ± 0.093	7.78 ± 0.671
Taiwan	New	2.09 ± 0.098	6.87 ± 0.520	5.65 ± 0.261	8.98 ± 0.692
	Old	1.6 ± 0.093	4.31 ± 0.332	3.04 ± 0.184	5.64 ± 0.527

*****Values are reported as a mean of three replicate samples**.**
***** ND, is not detected.

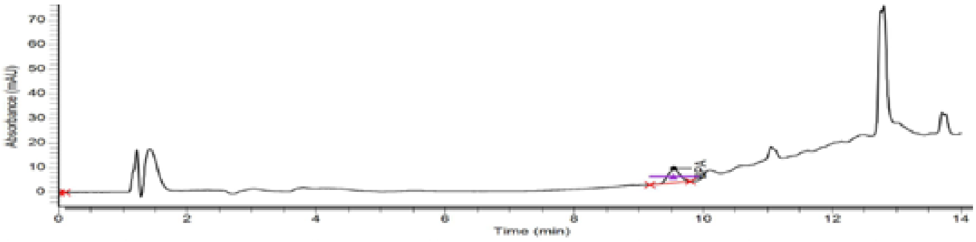
As the above table shows, in bottles filled with water at room temperature the bottles obtained from Taiwan showed migration of BPA while the compound wasn't detected in Chinese bottles. Both of them showed significantly high level of BPA under the water which is filled at 80oC. On the analysis made for milk stimulant at room temperature as well as 80°C, all the bottles obtained from these two countries showed high level of leaching. The old Chinese bottles did show a slightly higher level of BPA than the new ones while a lower level was detected in bottles obtained from Taiwan. Comparing the food simulants, water and milk, all bottles did show notable amount of BPA on milk sample. In general, the bottles did not show notable levels of leaching at room temperature but all bottles showed significant levels of leaching in the range of 2.4-8.98 in milk stimulant and under high temperature.

Comparison of Concentration of BPA Detected with Other Reported Values:

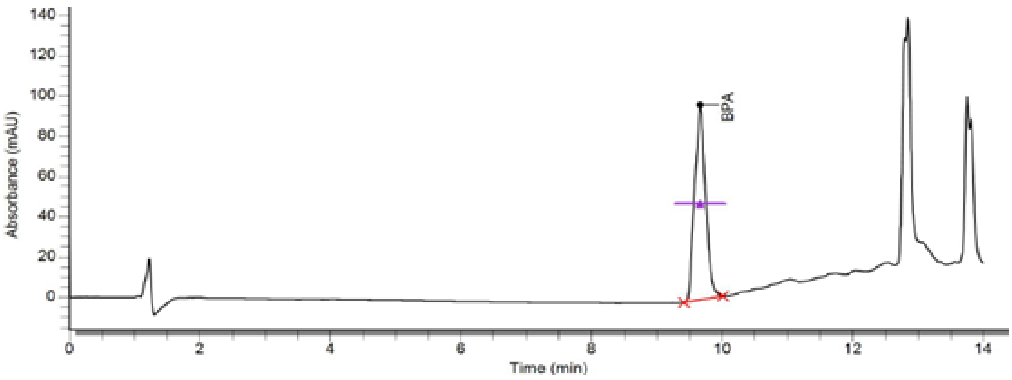
For the last 10 years a subsequent study, in USA, Canada and in some countries of Europe, have examined leaching of BPA from polycarbonate baby bottles using a variety of methods including HPLC-UV, LC-ED, and GC-MS. BPA leaching has been observed from polycarbonate baby bottles manufactured in many different countries [16]. Different results have been obtained from various groups studying the effects of washing, boiling and brushing on BPA leaching. The result of the study made by Maragou and his co-worker [14], which was made on bottles obtained from different brands under different realistic use conditions, showed a detected of BPA concentration from 2.4 to 14.3 µg/L Researchers from Sweden [15] also detected BPA from 20 to 50 μg/L under harsh condition. In relation to this, the Norwegian Food Safety Authority [17] investigated the effect of migration of BPA from polycarbonate feeding bottle and they concluded that under aggressive conditions BPA contents could exceed 10 μg/L. Generally, considering differences in methods of sampling, sample preparation and analytical techniques, the result obtained in this study is in a good agreement with the result of the study made by environmental defence of Canada [18] and the work group for safe markets which was made in USA [19]



a)



b)



c)

Figure 2: Typical chromatograms; a) a standard at 7μg/L, b) unspike sample, c) a sample spiked at 7 μg/L)

Exposure Estimates

The risk to humans of BPA exposure has been assessed during the last 10 years by several regulatory authorities, in all cases potential adverse health effects of BPA were identified and evaluated, and human exposure levels were estimated in order to draw conclusions about health risks at current exposure levels [20]. The updated 2008 European Union Risk Assessment Report on bisphenol A, published in June 2008, by the European Commission and European Food Safety Authority (EFSA), concluded that Tolerable Daily Intake (TDI) level for BPA of0.0015 mg/kg bodyweight. In September 2010, the European Food Safety Authority (EFSA) published its latest scientific opinion, based on a comprehensive evaluation of recent toxicity data, concluded that; exposure of infants in the age of 0-6 month is divided into three groups. Breast-fed infants re-estimated to be exposed to 0.0002 mg/kg bw/day (body weight per a day), infants fed with a non-polycarbonate containing bottle to 0.0023 g/kg bw/day and infants fed with polycarbonate bottles to 0.011 mg/kg bw/day [21]. On the other hand, according to the United States National Health and Nutrition Examination Survey (NHANES) and other studies, exposure to a very low level (up to 0.1ng/mL) of biologically active compound BPA could be a reason for many different physiological problems [22]. The scientific committee of food (SCF) established realistic worst-case exposure to BPA based on migration data into food. The SCF noted that their conclusions were based on a draft version of the EU Risk Assessment Report. This worst-case exposure was set to be 0.0016 mg/kg bw/day for new-borns and infants of 0-4 month, and 0.0008 mg/kg bw/day for infants of 6-12 month, respectively. The exposure data also showed that infants and young children may be more exposed to BPA than adults [23]. After all these, on 25 November 2010, the European Union Executive Commission decided to ban the manufacturers by 1 March 2011 and ban the marketing and market placement of polycarbonate baby bottles containing the organic compound bisphenol A (BPA) by 1 June 2011, though it is opposed by a majority of EU governments [24]. In general, no matter how the tolerable daily intake (TDI) values differ from one to another, all of the results detected in this study (2.64-11.34 µg/kg) were above the values given by any of these organizations.

Table 6: Average BPA detected expressed in mg/kg

Bottle processed	Temperature	Food simulant	Concentration of BPA detected in	
			µg/L	In mg/kg
In Chinese	At 25°C	In milk	2.520	0.00318
		Water	ND	-
	At 80°C	In milk	7.290	0.00920
		Water	5.515	0.00696
In Taiwan	At 25°C	In milk	4.345	0.00548
		water	1.845	0.00233
	At 80°C	In milk	7.31	0.00923
		water	5.59	0.00706

* ND non detected**.**

Most risk assessments take an exposure value from an animal study (dose in mg/kgbw/day) and divide it by several uncertainty factors to arrive an acceptable dose in humans [22]. And the tolerable daily intake values, which are given above (Table 6), are recommended based on the test of less acute human health problems, but according to European Union (EU) and US Environmental Protection Agency, the TDI could reach up to 0.05 mg/kg bw/day for highly acute human health problems like Cancer [24].

4. Conclusions

This study has attempted to assess the level of BPA in babies' feeding bottles obtained from two countries, China and Taiwan. The assessment was made based on two food simulants (water and milk) and temperature conditions (25°C and 80°C). Based on the result of this study the following conclusions are drawn.

Except the test made at room temperature on food simulant of water, all bottles showed notable level of BPA in any other parameter conditions. The detection level ranges from 1.6 ng/mL to 8.98 ng/mL. All bottles did show significant level of leaching at 80°C than the test made at 25°C and the same pattern was observed in milk simulant when compare with water. Bottles obtained from Taiwan showed higher average level of leaching than other bottles processed in China.

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