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Sources of Bisphenol A Contamination in Drinking Water in Pakistan and Determination of Migration Rates

ABIDA TASKEEN, RABIA HAMEED and ISMAT NAEEM*

Department of Chemistry, Lahore College for Women University, Jail Road - 5400 Lahore.
*Department of Chemistry, Lahore College for Women University, Jail Road, Lahore, Pakistan.
*Corresponding author E-mail: ismat4_naeem@yahoo.co.in

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ABSTRACT

This is a first study of BPA contamination in stored drinking water in Pakistan. Commercial filters, installed at ten different locations at Lahore College for Women University, were selected for analyses of BPA in triplicate by reversed phase High Performance Liquid Chromatography (HPLC). Migration of BPA in 10% ethanol at different exposure timings (24, 48, 72, 96 and 120 hours) was also determined (5.78-31.2 ppb, M=11, SD=9.7). The BPA contents detected in commercial filter samples were 1.38-11.30 ppb (M=5.99, SD=2.96). BPA amounts detected in samples plastic bottled samples ranged from 11.89 ppb to 12.42 ppb (M=12.15, SD=0.2). In plastic material of bottle BPA contents ranged from 5.78 ppb to 6.34 ppb (M=6.0, SD=0.2) after accounting for free BPA present in plastic-ware. The daily intake of BPA calculated (60 kg body wt.) was 12.42 µg/L/ body weight. Which far exceeded the provisional TDI of BPA set by Health Canada (25 µg/kg/body weight).

Key words: Drinking water, Sources of BPA contamination, Migration rate.

INTRODUCTION

Bisphenol A is basically present in a number of chemical engendering and protraction of so many products including amber linings of most food cans, polycarbonate bottles, food containers and sealants used in dentistry [Timms, *et al.,* 2005]. In 2004, seemingly 2.3 billion pounds of plastic were produced and was used to manufacture polycarbonate bottles and also containers for food storage [Lee *et al.,* 2007]. The pervasiveness of BPA in such products is supposed to increase the level of risk to human health and noticeable fact is that it disrupts endocrine system. It is recently observed that the molecules of BPA can quite easily leach into food and beverages from plastics and surely these molecules can be hydrolyzed [Timms, *et al.,* 2005]. Center for Disease Control and Prevention presented a report in 2004 according to which 95% Americans were having this chemical in the urine [Zandonella, 2006]. BPA had been known to release from the lining present in canned foods [Environmental Working Group] and to a lesser extent, polycarbonate plastics that are washed with harsh detergents or soaps and are used to contain acidic or high temperature liquids. A Health Canada study recently found that the most of canned soft drinks had reasonable levels of BPA [Health Canada. 2009]. It has been found that most of exposure is through dietary sources. However, exposure could also occur through air and absorption through skin [Lang IA *et al.,* 2008]

MATERIALS AND METHODS

Reagents

Bisphenol A (minimum purity 97%) was obtained from Sigma Aldrich. HPLC grade dichloromethane, methanol, and acetonitrile were obtained from Merck. A standard solution containing

100 ppm of BPA and a sub stock containing 10 ppm of BPA were prepared in methanol and kept in the refrigerator. Solutions of the required concentrations were prepared daily by dilution.

Sampling plan

In this study 8 mineral water samples of commercial brands were purchased from the local market while 10 filtered water samples were collected from the Lahore College for Women University campus in April 2010. Most commonly used mineral water samples and filtered water were selected on the basis of availability. Samples used in study were tested as consumed. It should be noted that if any Pakistani brand is missing in the study this simply means that the brand was not included. No inference should be drawn.

All readings were taken in triplicate.

Sample preparation for HPLC analysis

All samples were extracted under similar conditions. To 1 ml of each sample dichloromethane (20 ml) was added with stirring and then 100 ml acetonitrile were mixed slowly while stirring constantly. A precipitate was formed; suspension was centrifuged at 3000 rpm for 10 minutes. Supernatant fluid was separated and concentrated under reduced pressure at 40°C to about 2ml. The concentrate and 8 ml acetonitrile were shifted to a volumetric flask and adjusted to 20 ml with distilled water. An appropriate amount was filtered and analyzed by HPLC.

Migration experiments

Migration experiments were carried out in 10% ethanol solution. One inch strip was placed in glass bottle in 10% ethanol and then dried in oven at 65°C. The migration test solution was taken out after 24,48,72,96 and 120 hours and analyzed.

HPLC analysis parameters

The HPLC system (Waters 1500) consisted of a pump, a UV detector (2487) and a C18 column (250 x 4.6 mm, 5 mm particle sizes). The samples were qualitatively analyzed in isocratic mode, with acetonitrile /water (1:1) at a flow-rate of 1 ml min^{-1}. The injection volume was 10 µl and elute was monitored at 217 nm. The sonicated and filtered extracts (0.5 microns) of all water samples were injected under these conditions and compared with authentic standard of Bisphenol A, injected under similar conditions. All samples were analyzed by the Reversed phase HPLC.

Quality Control and Quality Assurance

Calibration of instrument

Instrument was calibrated using calibration standards. Calibration Standards were prepared from stock solution of Bisphenol A to obtain a calibration curve.

Precision and accuracy

Experiments were carried out in triplicate for maximum reliability of results and to assure precision and accuracy. When starting analysis, stability of baseline and response linearity of the detector was examined. Detector was able to detect Bisphenol A at a signal to noise ratio of 3:1. The same operating conditions of the HPLC system were maintained throughout the analysis of all water samples .Each solution was injected at least in duplicate. Samples were kept in refrigerator at +4°C in closed glass bottles with the exclusion of light.

Statistical analysis

Statistical analysis was carried out using SPSS v.16

RESULTS AND DISCUSSION

BPA contents in water samples from commercial filters

It has been reported that BPA poses serious health hazards. It is a part of almost all type of plastic ware. It has been reported by US FDA that BPA may leach into liquids from plastic bottles or containers. This is the first study in Pakistan based on analysis of BPA in drinking water purified through installed commercial filters. Water samples from different types of commercial water filters installed at Lahore College for Women University for student's consumption were collected and analyzed in triplicate to assess maximum reliability of results. All samples were found to contain detectable levels of BPA ranging from1.38 to 11.3(M=5.99, SD=2.96) ppb which is a cause of concern.

BPA contents in commercial brands of mineral water

Eight brands of commercial mineral water sold in Pakistan were selected randomly [Table: 2]. Water samples were analyzed for their BPA contents by HPLC. The BPA levels determined in the different samples ranged from 11.89 ppb to 12.42 ppb (M=12.15, SD=0.2) [Table: 2]. Free BPA in plastic samples of water bottles was also analyzed the concentrations ranged from 5.78 to 6.34 ppb (M=6.0, SD=0.2) [Table: 2]. There was no direct correlation between the level of BPA residues in the samples and their corresponding migration to the food and liquids, implying that high levels of residues remaining in the water bottle strip do not necessarily bring about high migration of BPA into the surrounding liquid, vice versa. [Figure: 2]. A possibility for this non-linear relationship could be due to the hydrolysis of BPA at the plastic surface, consequently migrating into the liquid.

Migration experiments in 10% ethanol were carried out to study the BPA elution at different times in microwave at time intervals of 24, 48, 72, 96 and 120 hours. Migration values ranged from 5.78 ppb to 31.2 ppb (M=11, SD=9.7) [Table; 3].

Migration studies

BPA migration in 10% ethanol ranged from 5.78 ppb to 31.2 ppb in for total of 5 days (24*5 hours) [Table: 3]. The results showed that BPA migration was greater when exposure time was increased. It was observed that migration of BPA was almost constant (only minor change) up to 96 hours (4 days), but it increased sharply after 120 hours.

Table 1: BPA contents in filtered water samples from commercial filters (at 25°C)

Filter no.	Brand names of commercial filters	Install ation date	BPA contents in ppb (*STD*)
1 filter	Water purifiers Aqua stars	2007	11.39±0.10
2 filter	Aqua save American Technology	2008	8.63±0.25
3 filter	Water at its best Aqua Gold	2007	4.83±0.10
4 filter	Breefa International (Pvt.) Ltd.	2009	1.38±0.20
5 filter	Aqua Safe water at its best	2009	2.78±0.17
6 filter	Aqua Cure water purification system	2009	6.17±0.24
7 filter	Aqua Stars	2007	8.59±0.10
8 filter	Life time safetyTriple water purification with sterilizer	2009	5.63±0.10
9 filter	Aqua Cure water purification system	2009	6.35±0.01
10 filter	Breefa International (Pvt.) Ltd.	2008	4.21±0.20
Unfiltered	tap water	2009	0.00±0.00

Table 2: Amount of Bisphenol A (ppb) in branded bottled water (500ml) (at 25C)

S. no	Brands	BPA contents in water (mean ± *STD* ppb)	Free BPA contents in plastic (mean ± *STD* ppb)
1	Nestle pure life	12.42 ±0.13	6.34±0.11
2	Aqua fina	12.22±0.10	5.78±0.10
3	Aquafresh	12.31±0.11	6.22±0.13
4	Sufi water	11.89±0.12	6.12±0.20
5	Kinley	12.34±0.13	5.81±0.14
6	Klinz	11.93±0.10	5.78±0.11
7	Avian	11.98±0.21	6.12±0.10
8	Aqua blue	12.16±0.22	5.92±0.13

Quality control in results

Triplicate analysis was carried out in order to ensure the accuracy and precision. Recognizing that calibration of the working standards was important for accurate analysis, care was taken to ensure that the calibration curve had a correlation as close to 1 as possible. The calibration curve was obtained by plotting the areas under the peaks of the standard versus the absolute amounts. BPA in the samples was identified by comparing its liquid chromatography retention time with that of the authentic standard of BPA with distilled water taken as blank [Fig. 1].

BPA levels

Different levels of BPA contents were found in drinking water samples which may be due to longer exposure times or increase in temperature during storage and transportation.

Provisional tolerable daily intake (TDI) values for BPA

The provisional tolerable daily intake (TDI) of 25 µg/kg body weight/day has been pre-established by Health Canada as a conservatively safe level for BPA presence in food [Health Canada.2009]. Average level of BPA calculated in all mineral water samples under this study was 12.15 µg /L. If an adult of 60 kg average body weight consumes one mineral water bottle (500 ml) per day, the intake of BPA would be equivalent to 6.075 µ*g* /day which is 0.405% of the allowed provisional TDI. For the canned drinks the highest BPA level estimated was 12.42*u*g/L and based on this finding, the daily intake was calculated was equivalent to 6.075 µg/day/body weight. Therefore, an adult (60 kg body weight) would have to consume approximately 2.55L (approximately 4 bottles of 500ml) of mineral water in one day to approach the provisional TDI set by Health Canada. This study clearly establishes the fact that the population of Pakistan consuming drinking water stored or bottled in plastics is exposed to dangerous levels of BPA.

Statistical analysis

In filtered water samples [Table: 1] the mean BPA concentration (M=5.99, SD =2.9, N= 10) was significantly greater than zero and 95 % CI was 3.89, 8.09 about the mean difference.

A paired t-test was performed to determine if the BPA in water and plastic [Table: 2] has any relation. In case of mineral water samples [Table: 2] the mean BPA concentration (M= 12.15, SD = 0.2, N= 8) for water and (M=6.01, SD=0.2, N=8) were significantly greater than zero, t (7) = 66.83, two-tail p = 4.41, providing evidence that the BPA leaching is effective in producing higher BPA contents in water sample than in plastic. A 95% C.I. about mean BPA difference is 5.842, 6.178.

It has been deduced from TWO-WAY ANOVA of migration experiments [Table: 3] that type of water samples does not interact with the time effect. It has been deduced because the residual (error) mean square (MS) is small compared with the mean squares for water sample (columns) or exposure time (rows). Because residual mean square is low so most variation in the data is accounted for by the separate effects of water

Table 3: Migration of Bisphenol A in Ethanol (10%) at 25^0 C at different time intervals

S. No.	Samples	Time duration(hours)				
		24	48	72	96	120
1	Nestle water	6.34±0.13 ppb	6.42±0.10ppb	6.68± 0.014ppb	6.72± 0.013ppb	29.44±0.20ppb
2	Aqua fina	5.78±0.11ppb	5.83±0.20ppb	5.92± 0.10ppb	5.97± 0.13ppb	30.21±0.20ppb
3	Aqua fresh	6.22±0.10ppb	6.24±0.11ppb	6.78± 0.20ppb	6.92± 0.12ppb	29.82±0.31ppb
4	Sufi water	6.12±0.13ppb	6.32±0.01ppb	6.56± 0.10ppb	6.87± 0.21ppb	31.2± 0.22ppb
5	Kinley	5.81±0.11ppb	5.93±0.11ppb	6.22± 0.13ppb	6.37± 0.23ppb	30.76±0.13ppb
6	Klinz	5.78±0.13 ppb	5.9± 0.22 ppb	5.99±0.11 ppb	6.23±0.10 ppb	31.43±0.01 ppb
7	Avian	6.12±0.12 ppb	6.33±0.21 ppb	6.54±0.10 ppb	6.78±0.11 ppb	30.92±0.21 ppb
8	Aqua blue	5.92±0.13 ppb	5.98±0.11 ppb	6.17± 0.32 ppb	6.35±0.21 ppb	29.37±0.10 ppb

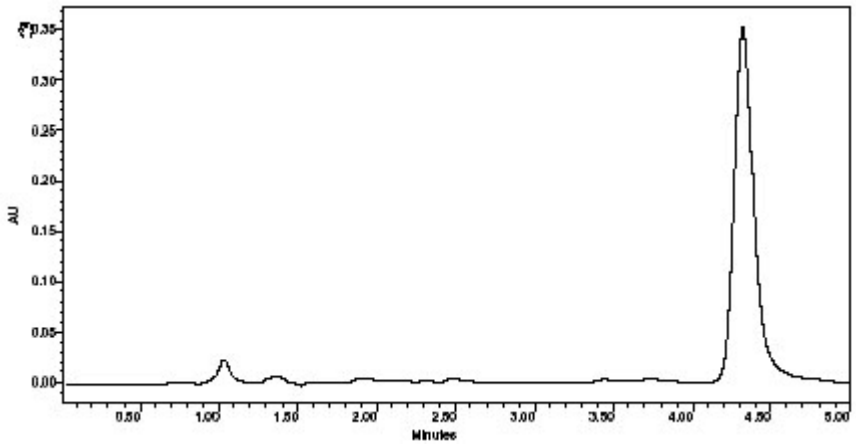

Fig. 1: HPLC chromatogram of Bisphenol A at room temp and pressure

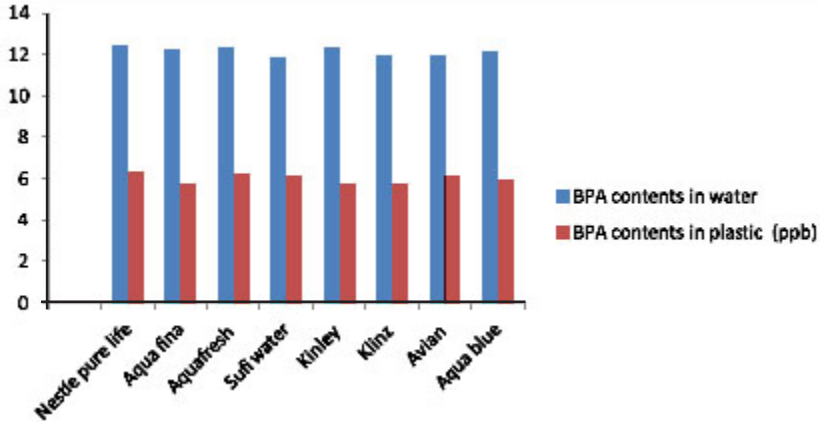

Fig. 2: Comparison of BPA contents in plastic and drinking water

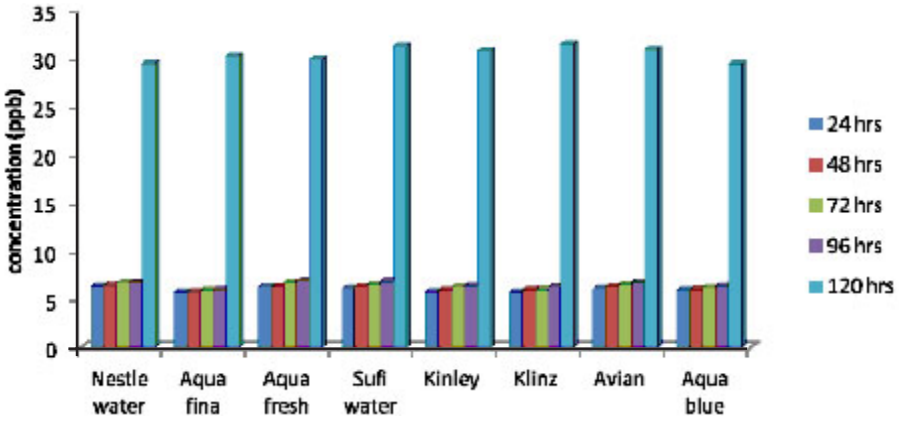

Fig. 3: Comparison of BPA migration rates in all water samples

samples and exposure time. In other words change of type of plastic bottle does not affect the migration rate of BPA.

CONCLUSION

By converting the values (in mg/inch^2) into the actual water bottle dimensions (0.5 L), the leachable amount of BPA in some of the samples is as high as 31.2 ppb, which is a cause for serious concern combined with the fact that all brands of mineral water and so called purified water samples coming out of commercial filters had detectable levels of BPA. Mitigation options such as frequent change of columns in branded water filters, avoiding longer periods of storage of branded mineral water in plastic bottles or containers and creating awareness about ill effects of BPA are highly recommended to ensure the safety of public health.

The detectable amount of BPA (though small) in tap water samples was a real cause of concern in relation to its excessive use in drinking and cooking. Effluents and leachates from landfills and paper mills were suspected to be the most important source that may results in BPA discharge and contaminate natural waters. This is a preliminary report on analysis of BPA in drinking water samples, the research is continuing and authors will publish new findings on analysis and bio-monitoring of BPA in near future.

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