Hindawi
Advances in Public Health
Volume 2023, Article ID 8278428, 6 pages
https://doi.org/10.1155/2023/8278428

Research Article

The Effect of Different Storage Conditions on the Levels of Bisphenol A in Bottled Drinking Water in Jeddah City, Saudi Arabia

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Received 30 January 2023; Revised 23 April 2023; Accepted 22 May 2023; Published 30 May 2023

Academic Editor: Dazhou Li

Bisphenol A (BPA) in drinking water sources is a significant concern in society because BPA is one of the endocrine disruption compounds (EDCs) that can cause hazards to human health even at extremely low concentration levels. This study investigated the leaching potential of BPA from drinking water bottles in five brands of bottled drinking water in Jeddah, Saudi Arabia, using high-performance liquid chromatography with a diode array detector (HPLC-DAD). To the best of our knowledge, this is the first study to evaluate the level of BPA in bottled water in the city of Jeddah. The separation was carried out under isocratic elution, and the detector was set to UV mode. Low levels of BPA were detected in all samples from polyethylene terephthalate (PET) containers. The mean concentration of BPA in water bottles stored at room temperature for 30 days was 9.46 $ng{\cdot}L^{-1}$, while the concentration of BPA in water bottles exposed to sunlight and boiling water bath was 16.13 $ng{\cdot}L^{-1}$ and 14.7 $ng{\cdot}L^{-1}$, respectively. Although the results show that the daily consumed concentration of BPA for an adult with 60 kg of body weight is 32.26 ng, which is lower than the total tolerable daily intake limit of BPA, health risks from the consumption of bottled water may increase after UV exposure for an extended time.

1. Introduction

Bottled water has increased in popularity as a thirst suppressant and a source of mineral constituents in the diet. Total bottled water volume sales increased in Saudi Arabia, particularly in the eastern region. Consumers believe that bottled water is necessary and that it is healthier and more pleasant than tap water [1].

Water bottles are made of a thin, translucent, and smooth plastic called polyethylene terephthalate (PET). It is anti-air plastic; it prevents oxygen from entering the container, thus preserving the water's taste. These plastic bottles must be stored away from high temperatures to avoid plastic breaking down and the leaching of harmful compounds such as acetaldehyde, antimony, phthalates, and bisphenol A (BPA) from PET [2].

BPA (2,2-bis[4-hydroxyphenyl] propane) is an important monomer used widely in the production of polycarbonate (PC) plastics and epoxy resins [3]. Since polycarbonates possess numerous physicochemical properties such as temperature, chemical resistance, impact resistance, optical properties, and moldability, they are widely used to produce commodity plastics (such as water bottles and food-contact plastics) [4, 5]. Additionally, epoxy resins are used as protective coatings on food and beverage containers [4]. Therefore, BPA's global demand is expected to increase. As an example, demand increased from 3.9 million tons in 2006 to approximately 5 million tons in 2010 [6]. In 2015, 7.69 million metric tons were estimated to be used for various applications of BPA, and this number was forecast to rise to 7.7 million in 2016. From 2016 to 2022, the compound annual growth rate (CAGR) was approximately 4.8%. Based on the broad applications of polycarbonate plastics and epoxy resins, production was expected to reach 10.7 million metric tons in 2020. BPA's global demand was expected to reach USD 22.49 billion in 2022 [7].

The USA Food and Drug Administration (USFDA) determined that the overall no-observed-adverse-effect level (NOAEL) of BPA is 5 mg/kg/day, derived from two multigenerational rodent studies [8]. However, the European Food Safety Authority (EFSA) established in 2015 a total tolerable daily intake (TDI) of 0.05 mg BPA/kg body weight [9]. The oral reference dose was determined as 100 μg/L as a total allowable concentration (TAC) [10]. The EFSA states that the temporary tolerable daily intake (t-TDI) of BPA is 4 μg/kg body weight/day [11].

Humans are exposed to BPA through polluted foods and drinks. Exposure to BPA in humans has extensive health effects and is therefore very concerning [12]. BPA has strong estrogenic activity, and it may increase macrophage activity, decrease antibodies, and lead to immune disorders [13]. Additionally, it is known to disrupt cell differentiation as well as distract cellular antioxidant mechanisms. Furthermore, BPA possesses lipophilic characteristics, which allow it to accumulate in fat. BPA is associated with pregnancy-related symptoms, such as miscarriages, preeclampsia, child anthropometric measures at delivery, and a reduced gestation size. In many countries, such as the European Union, Canada, and Denmark, BPA has been banned from baby feeding bottles because of its destructive effects [12].

Recent studies have demonstrated that high exposure to BPA may be harmful to humans and considered an endocrine disruptor because it can bind to estrogen receptors (ERα and ERβ) and contributes to estrogen-linked diseases, such as female infertility. Furthermore, it may interfere with the body's own hormone signals, contributing to the development of hormone-dependent diseases such as prostate and breast cancer [14, 15]. It can also cause tumors, oxidative stress, and liver damage due to mitochondria dysfunction, lipid peroxidation reaction, inflammation, apoptosis, and cell death [16].

The primary reason for the presence of BPA in bottled water is still unknown. For example, the detected BPA could be either from water resources before packaging, from recycled PET plastic, or from the caps of the bottles [17].

BPA has been found in food and drinks in recent years, which is a direct result of its usage as a starting material for such polymers. In general, this migration is caused by the hydrolysis of the ester bond in polycarbonate or epoxy resins. By heating, such as during sterilization or cooking, and by acidic or basic conditions, ester bond hydrolysis can be sped up [4].

Many factors assist the migration of BPA from the food containers to their content, for instance, the long-time manufacture of resins, the temperature of the canning process, outdoor ultraviolet (UV) radiation, the presence of NaCl, acids, vegetable oils, and storage time of canned foods (usually for 12 days in markets and months at home) [18].

For the detection of BPA, several chromatographic and spectroscopic analytical techniques have been developed; however, they are time-consuming, complex, and expensive [19]. As a result, it is imperative to select a sensitive, specific, and executive detecting method. High-performance liquid chromatography with a diode array detector (HPLC-DAD) is an effective technique that provides fast and good separation for the qualitative and quantitative determination of phenolic compounds [20, 21]. A study was conducted using the HPLC-DAD system for the determination of bisphenols migration from disposable plastic lunch boxes to contacting water [22]. The DAD gathers chromatograms over a wide range of wavelengths simultaneously during a single run, providing more information on sample composition than a single wavelength detector [23].

Bottled water consumption is significant in Saudi Arabia because it is the primary source of drinking water. This study was conducted to investigate the effects of storage conditions on the release of BPA from bottled drinking water samples purchased at local supermarkets in Jeddah city, Saudi Arabia by using the HPLC method. To the best of our knowledge, this is the first study to evaluate the level of BPA in bottled water in the city of Jeddah. The aim of measuring the BPA concentration in bottled water after exposure to room temperature, outdoor temperature, and boiling water bath was to assess the effect of heat on the release of BPA into drinking water.

2. Experimental Methods

2.1. Sample Collection and Storage. Samples were bottled drinking water from five different well-known factories in Saudi Arabia. These brands were almost identical in production date, and for each bottle, there was one year of shelf life left before the expiration date. Water bottles were similar in shape and had the same volume (200 mL). The plastic material of the samples was PET according to the global identification number in the recycling triangle on the surface of each bottle. The store-bought bottled water samples were divided into three groups according to the storage conditions: Group I, water samples were stored for thirty days at room temperature (23 to 25°C); Group II, water samples were stored for thirty days exposed to sunlight (40 to 43°C); and Group III, water samples were placed in a boiling water bath (100°C) for 30 min after storage for thirty days at room temperature. All water samples were stored for thirty days after purchasing from local markets. The BPA in the samples was identified using standard BPA (BPA, purity of ≥99%, Sigma-Aldrich, Taiwan). Glass-bottled samples were used as a negative control. Five repetitions were conducted for the quantitative measurement of BPA in each sample.

To investigate the migration of BPA from the disposable plastic bottles to the contacting water, all water samples were subjected to solid phase extraction (SPE), followed by HPLC analysis of the eluted fractions.

2.2. Solid Phase Extraction (SPE). SPE was carried out to reduce the possibility of background contamination and improve the binding between BPA and the sorbent, following the optimized protocol of [24, 25] using an SPE C18 cartridge (3 mL; 200 mg, ikeme, China). The cartridge was first conditioned with 10 mL of methanol, followed by 10 mL of water. After loading the sample (500 mL) to ensure that sufficient organic phase could be collected for the chromatographic analysis, the cartridge was washed with 3 mL of water and then 3 mL of methanol to remove interferences. A vacuum was applied through the cartridge for 15 min to ensure the removal of residual moisture. Finally, the BPA entrapped in the C18 cartridge was eluted with 1 mL of methanol. The elution step was repeated three times, and the eluents were pooled and evaporated using a vacuum centrifuge Concentrator plus Eppendorf SE, Germany, in preparation for HPLC analysis.

2.3. HPLC Analysis. The chromatographic analyses were performed by a SHIMADZU LC-2030C 3D Plus HPLC system equipped with a diode array detector on an Agilent ZORBAX Extend-C18 column (4.6 × 150 mm × 5 Micron). Bottled water samples were analyzed under isocratic elution mode for ten minutes at 30°C with a binary solvent of acetonitrile-water (35 : 65, v/v) as the mobile phase at a constant flow rate of 1.5 mL/min. All solvents used were of HPLC grade purchased from Honeywell, Germany. The detection wavelength and sample injection volumes were 228 nm and 25 μL, respectively. The HPLC method was optimized and modified after the method of [22]. Chromatographic data were processed using LabSolutions software version 5.97 SP1.

2.4. Statistical Analysis. MegaStat version 10.1 was used in two-way ANOVA, confidence interval tests, and the regression statistics test. $P \leq 0.05$ was set as statistical significance.

3. Results

BPA was detected in the five examined bottled water brands, and chromatograms are illustrated in the supplementary figure (S1). The analyzed glass bottled water samples were free of BPA. Figure 1 shows the different concentrations of BPA in water samples. The mean concentration of BPA in water bottles stored indoors at room temperature for 30 days was 9.46 ng·L^{-1}, while the mean concentration of BPA in water bottles exposed to sunlight and boiling water bath was 16.13 ng·L^{-1} and 14.7 ng·L^{-1}, respectively. The concentration of BPA in bottled water stored outdoors under sunlight and the concentration of BPA in bottled water that was boiled for 30 minutes were significantly higher than those stored indoors at room temperature ($P \leq 0.05$). Among the water brands stored indoors, the difference between their BPA concentrations was not significant. The same observation applies to those stored outdoors under sunlight and in boiling water baths. Among water bottles stored at room temperature, brand 1 showed a significant increase in the concentration of BPA (12.4 ng·L^{-1}, $P = 0.02$). On the other hand, for the water bottles exposed to a boiling water bath, brand 5 showed a nonsignificant increase in the concentration of BPA (20.1 ng·L^{-1}, $P = 0.2$), while the water bottles stored outdoor under sunlight showed a significant increase in BPA concentration in brand 1 and 2 ($P = 0.0003$). This led to considering the effect of sunlight UV on the materials used in these brands.

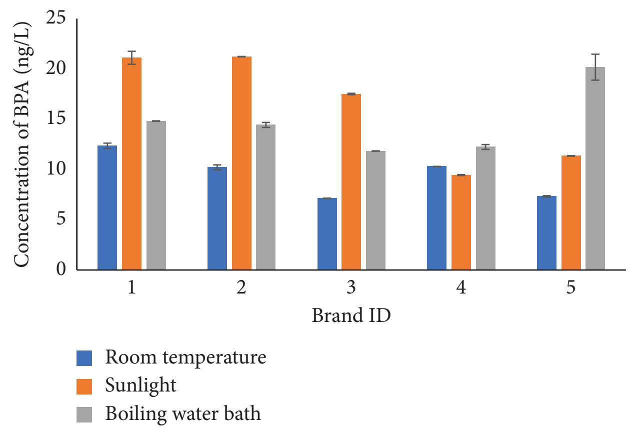

Figure 1: Comparison of BPA concentrations (ng·L^{-1}) in different storage conditions. Values are means ± SD. BPA was detected in the five examined bottled water brands. The mean concentration of BPA in water bottles stored at room temperature for 30 days was 9.46 ± 2.21, while the concentration of BPA in water bottles exposed to sunlight and boiling water bath was 16.13 ± 5.48 and 14.7 ± 3.32, respectively. The concentration of BPA in bottled water stored outdoors under sunlight was significantly higher ($P \leq 0.05$) than that stored indoors at room temperature.

4. Discussion

Bottled water is preferred by many people over tap water. When bottled water is stored in polycarbonate plastic for an extended period of time, some harmful substances may leak into the water, which may cause health problems [26]. Among the chemicals used to make polycarbonate plastic used in making water bottles is BPA [2]. The purpose of this study was to determine whether storage conditions affect BPA release from PET water bottles. BPA was detected in the five examined bottled water brands, while the analyzed glass bottled water samples were free of BPA. In this study, the mean concentration of BPA in water bottles exposed to sunlight and boiling water bath was 16.13 ng·L^{-1} and 14.7 ng·L^{-1}, respectively, indicating a correlation between the increase in BPA content in bottled water and the increase in storage temperature. Based on the analysis, it was found that BPA was high in samples exposed to UV sunlight in brands 1, 2, 3, and 5. BPA leached from these bottles might be due to photolytic formation or degradation of the organic compounds in the plastic that causes BPA migration to contact water. In contrast, brand 4 was unaffected by UV sunlight at an outside temperature range between 40 and 43°C, indicating that the bottle material was of high quality. On the other hand, when brand 5 was exposed to a boiling water bath, it produced the highest concentration of BPA (20.2 ng·L^{-1}) when compared to the other brands under the same condition and a conformational change was observed in the bottle during boiling, indicating a low-quality material plastic. Similarly, BPA was detected in all water bottles stored at room temperature for 30 days and the mean concentration of BPA was 9.46 ng·L^{-1}, but brand 1 showed a significantly higher concentration of BPA than the rest, which suggested that the bottle material was not of high quality. Based on the worldwide identifying number visible inside the recycling triangle on the bottom of each bottle, the plastic substance in brand 1 was determined to be PET, which allows BPA to migrate into the water without applying any heat stress. Wang et al. [27] found that the amount of BPA that migrated from PET bottles was lower than that migrated from PC bottles, which indicates that PET is a better material than PC.

This study's results show that significant amounts of BPA leached from bottled containers into the water. Based on this study analysis, brand 4 showed the lowest level of BPA increase after prolonged exposure to sunlight and boiling water. In order to reduce the risk of human exposure to BPA, bottled water should not be stored under direct sunlight for long periods of time.

A number of factors may contribute to the differences in BPA content among the tested samples, e.g., the material used in the bottle, materials used in the cap, the method of storage during production and sale, and the material used in the installation to draw water and fill the bottles.

Similar studies have reported BPA levels in plastic water bottles in multiple countries worldwide [26]. The results of this study are consistent with other studies that found BPA in water stored in bottles. Elobeid et al. [28] conducted the first study in Saudi Arabia using gas chromatography to assess the leaching of BPA from seven local brands of water bottles under the same storage conditions (stored at 25°C and outdoors at 40°C). They found that the mean concentration of BPA leached from water bottles stored at room temperature was 4.03 ng·L^{-1}, while the mean concentration of BPA leached from water bottles stored under sunlight was 7.5 ng·L^{-1}. Our results showed higher levels of BPA than their findings, and the reason may be due to the use of manufacturing materials worse than those used in 2012 and the use of different technique of separation and detection.

In 2014, a study investigated the effects of storage temperature and duration on the release of BPA from 16 brands of drinking water bottles in China. After one week of storage, BPA release increased from 0.26–18.7 ng/L at 4°C to 0.62–22.6 ng/L at 25°C and to 2.89–38.9 ng/L at 70°C. BPA release increased with a storage duration of up to four weeks [29]. Our results showed similarly increased levels of BPA in water bottles stored at room temperature for one month.

Likewise, a study in Iran assessed the migration of bisphenol A from disposable plastic containers and mineral water into the water at different temperatures. In order to identify and quantify BPA, gas chromatography with a mass detector was used, and in order to quantify it, high-performance liquid chromatography with a fluorescence detector was used. The presence of BPA in the water of mineral water bottles was detected, and the increase in temperature also increases the rate of migration of BPA into water. As a result of improper storage of mineral water bottles as well as the use of disposable plastic containers for hot drinks, consumers are exposed to more BPA [30]. According to this study, similar results were obtained.

In 2022, Ginter-Kramarczyk et al. conducted research in Poland about the influence of temperature on the amount of bisphenol A in bottled drinking water. The analytical method was based on liquid chromatography coupled with the tandem mass spectrum (LC-MS/MS). The concentrations of BPA were detected at low levels (at the ng/L range) in water bottles that were analyzed immediately after their purchase. The obtained values correspond well with the values found in the literature, ranging from 4.04 ng·L^{-1} to 4.6 ng·L^{-1}. Afterwards, the content of this compound was determined in the water samples after incubation of the bottles at various temperatures for a period of 24 h. Similarly, results indicated that high temperatures and exposure time increased BPA leaching. The findings from the analyses of various water bottles showed that the chemical has varying propensities to release at various temperatures. Several factors can contribute to the variance between the study's results and those reported in the literature, including the quality and composition of the bottle material, the testing conditions, and the storage conditions of the samples before purchase [26].

In 2015, EFSA issued a scientific opinion assessing the risks associated with BPA exposure. Various groups of the human population were exposed in three different ways: (1) externally (by drinking water, diet, inhalation, and dermal contact with cosmetics and thermal paper); (2) internally (absorbed dose of BPA and the sum of conjugated and unconjugated BPA); and (3) aggregated (by dust, diet, cosmetics, and thermal paper). A total uncertainty factor of 150 was applied by the EFSA's expert Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF Panel) (for interspecies and intraspecies differences and reproductive, neurobehavioural, immune, and metabolic system effects) to establish a temporary tolerable daily intake (t-TDI) of 4 micrograms per kilogram of body weight per day. Comparing this t-TDI with exposure estimates led the CEF Panel to determine that dietary exposure poses no health concerns for any age group, while aggregate exposure poses only low health risks. The CEF Panel noted considerable uncertainty in nondietary exposure estimates, while relatively low uncertainty was noted in dietary exposure estimates [31].

In 2021, EFSA re-evaluated the risks of BPA in food and proposed to considerably lower the TDI compared to its previous assessment in 2015. It set a temporary TDI of 4 micrograms per kilogram of body weight per day in its 2015 risk assessment of BPA. CEF has established a daily TDI of 0.04 nanograms per kilogram based on its draft re-evaluation of BPA published in 2021. BPA's TDI was lowered based on studies that have appeared from 2013 to 2018, particularly those indicating adverse effects on the immune system. The EFSA reported that consumers with both average and high levels of exposure to BPA in their diets had exceeded the new TDI, indicating potential health risks [32].

Based on WHO guidelines, the recommended daily water intake for an adult with 60 kg of body weight is 2 L per day [33]. The TDI of BPA is 0.05 mg/kg body weight, which equals 3×10^6 ng/60 kg. Based on our results, the average concentration of BPA in bottled water stored under poor storage conditions was 16.13 ng·L^{-1}. Hence, the daily consumed concentration of BPA for an adult with 60 kg of body weight is 16.13 ng·L^{-1} × 2 L = 32.26 ng, which is lower than the TDI limit of BPA, and it does not imply a serious threat to human health.

However, according to the new EFSA TDI, consumers appear to exceed the new TDI, indicating health concerns.

5. Conclusion

The consumption of bottled water is one of the major routes through which humans are exposed to BPA. Until now, there has been no study to evaluate the level of BPA in Jeddah city. Although BPA was detected in this study, it is still unclear if it came from the bottle, the water itself, or both. In the future, we need further studies to investigate this. It is concluded from the results of this study that bottled water stored under poor storage conditions contains less BPA than the WHO TDI limit. Therefore, it does not imply a serious health risk. Despite the low level of BPA present in bottled water (ng·L^{-1}), the cumulative dose in the body may be much higher than the quoted level due to the multitude of everyday products containing BPA, and the new EFSA TDI indicates that consumers appear to exceed the new TDI, which indicates health concerns. Considering the harmful properties of BPA as well as the potential risk to human health, the concentrations of this compound should be monitored regularly.

Data Availability

The data supporting the findings of this study are available within the article. More detailed data used to support the findings of the current study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This work was funded by Mawakeb Alajer Association, Jeddah, Saudi Arabia, through the Science Research and Innovation Unit at the Faculty of Science and King Abdulaziz University, Jeddah, Saudi Arabia, under research number (scigriu41/02).

Supplementary Materials

Chromatograms from HPLC analysis. (*Supplementary Materials*)

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