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Qualitative Determination of Bisphenol A and Phthalate Residues in Drinking Water Alternatives in Kayseri Province of Türkiye*

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Abstract: In this study, it was aimed to qualitatively determine Bisphenol A and dibutyl phthalate residues in carboy, pet bottled and tap waters used as drinking water in Kayseri-Türkiye. Being used as an additive in the production of many products and plastics frequently used in daily life, BPA and phthalates are associated with a variety of health issues and environmental problems. Within the scope of this study, a total of 20 drinking water samples (9 pet, 7 carboy and 4 tap water) were analyzed in terms of dibutyl phthalat (DBP), BPA and BPA derivatives (Bisphenol S and Bisphenol F). Among the pet bottled water samples; 8 (88.88%) were found to be contaminated with BPA, 5 (55.55%) with DBP and 7 (77.77%) with BPA derivatives, while 1(14.28%) of the carboy water samples were found contaminated with BPA and 7(100%) with DBP. Among the tap water samples, 2 (50%) were contaminated with BPA and 4 (100%) with DBP. No BPA derivatives were found in carboy and tap water samples while pet bottled waters were found positive for all three contaminants. On the other hand, all carboy and tap waters were found contaminated with phthalates. The findings of this study reveal that the most variety of contamination for these chemicals is determined in pet bottled waters. This study highlighted the situation of BPA and phthalate residues in drinking water sources. Measures should be taken to prevent the contamination of all types of drinking water and to systematically examine the BPA and phthalate-related safety of drinking waters. Further investigation is needed to determine and quantify the occurrence of the target compounds.
Keywords: Bisphenol-A, drinking water, endocrine disruptors, phthalate, public health

Türkiye'nin Kayseri İli İçme Suyu Alternatiflerinde Bisfenol A ve Ftalat Kalıntılarının Kalitatif Olarak Belirlenmesi

Öz: Bu çalışmada Kayseri/Türkiye'de içme suyu olarak kullanılan damacana, pet şişe ve çeşme sularında BPA ve ftalat kalıntılarının niteliksel olarak belirlenmesi amaçlanmıştır. Günlük hayatta sıklıkla kullanılan birçok ürünün ve plastiğin üretiminde katkı maddesi olarak kullanılan Bisfenol A ve dibütil fitalat , halk sağlığı ve çevre için risk oluşturmaktadır. Bu çalışma kapsamında toplam 20 adet içme suyu numunesi (9 pet şişe, 7 damacana ve 4 musluk suyu) dibütilftalat (DBP), BPA ve BPA türevleri açısından analiz edilmiştir. Pet şişe su örnekleri arasında; 8'inin (%88.88) BPA, 5'inin (% 55.55) DBP ve 7'sinin (%77.77) BPA türevleri ile kontamine olduğu, damacana su örneklerinin 1'inin (%14.28) BPA ve 7'sinin (100) DBP ile kontamine olduğu tespit edildi. DBP ile içme suyu numunelerinin 2'si (%50) BPA ile ve 4'ü (%100) DBP ile kontamine olmuştur. Damacana ve musluk suyu örneklerinde BPA türevi bulunmazken, pet şişe sularında her üç kirletici için de pozitif bulundu. Öte yandan, tüm damacana ve musluk sularının ftalatlarla kontamine olduğu bulundu. Bu çalışmanın bulguları, bu kimyasallar için en çeşitli kontaminasyonun pet şişe sularında belirlendiğini ortaya koymaktadır. Bu çalışma, içme suyu kaynaklarında BPA ve ftalat kalıntılarının durumunu ortaya koymuştur. Her türlü içme suyunun kirlenmesini önlemek ve içme sularının BPA ve ftalat ile ilgili güvenliğini sistematik olarak incelemek için önlemler alınmalıdır. Hedef bileşiklerin oluşumunu belirlemek ve ölçmek için daha fazla araştırmaya ihtiyaç vardır.
Anahtar kelimeler: Bisfenol A, endokrin bozucular, ftalat, halk sağlığı, içme suyu

Introduction

According to general perception, bottled water is more preferable than tap water in terms of safety and taste (Diduch et al., 2013; Saylor et al., 2011). However, some chemical residues had been frequently detected in bottled water, among which some endocrine disruptors have attracted special attention due to their serious effects on public health. (Al-Saleh et al., 2011; Amiridou and Voutsa, 2011; Casajuana and Lacorte, 2003; Diduch et al., 2013).

The first discovery of BPA, which is used in the construction material of polycarbonate plastics, which are used quite frequently in household products, in the production of polyvinyl chloride (PVC) plastics, and in

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the content of many products such as the inner coating of food and beverage containers, carboys and pet bottle waters, was by Dianin in 1891. CD, DVD BPA, which has additional uses in the coating of various electronic materials, sports equipment, paper coatings and automobiles, is one of the chemicals whose use has been increasing rapidly from past to present. Plastics, which are frequently used in living spaces, have a polymeric structure. Polymers are composed of monomers containing carbon, hydrogen, oxygen atoms and silicon in their structure. Many monomers are heated and polymerized to form these polymers (Brunelle, 2005).

Large amounts of BPA produced worldwide each year can cause BPA pollution in rivers and lakes, negatively affecting wildlife and people who use these sources for drinking water in many ways (Prokop et al., 2004).

Phthalates are also widely used plasticizer chemicals to provide the durability and flexibility of polyvinyl chloride containing plastic products, including packaged food products, toys, textiles, feeding bottles, pacifiers, medical equipment, carboys and pet bottles. Phthalates are oil-soluble, as are organic pollutants. Phthalates are transported by water, air and organic matter. Since they are not covalently bonded to plastics, migration from products to the environment can be easier. Due to the risk of health, the use of plastic in materials that come into contact with food has been limited by legal regulations. Phthalate esters, especially DEHF (diethylhexyl phthalate) and DBP (dibutyl phthalate), have been detected in studies carried out in surface waters, underground waters, drinking water, carboys, pet bottles and tap waters, and it has been observed that their levels are affected by seasonal changes and water storage conditions (Johnson et al., 2002).

Phthalates are plasticizing chemicals that are used to ensure the durability and flexibility of plastic products; such as packaged food products, toys, textiles, baby bottles, carboy and pet bottled waters. The public health risks of phthalate and BPA contaminated water and food constitute the most intense agenda of the scientific community indicating the associations between endocrine disrupting chemicals (EDCs) and obesity, diabetes (Diamanti-Kandarakis et al., 2010; Legler et al., 2015) and insulin resistance (James-Todd et al., 2012; Lind et al., 2012; Stahlhut et al., 2007).

Neurodevelopmental, cardiovascular and reproductive disorders and endocrine disruption are reported among the most critical adverse effects of BPA and phthalates (Harris et al., 1997; Engel et al., 2009; Heudorf et al., 2007; Martino-Andrade and Chahoud, 2010; Swan, 2008) as well as reproductive abnormalities in case of perinatal exposure (Gray et al., 2000; Swan et al., 2005; Foster, 2006).

In order to prevent the risks of these chemicals; exposure routes and quantities should be determined which is an important step in terms of tracking risks, taking necessary precautions and protecting the environment and public health. In this context, this study aimed to determine the presence and public health risks of BPA DBP and BPA derivates in bottled drinking water.

Materials and Methods

1. Solvents, reagents and materials

N-hexane-ethyl acetate, were used in column chromatography and separations in laboratories. Ethyl acetate can be separated by spraying compressed air in a hot water bath due to its volatility and low boiling point in organic chemistry and especially in experiments. N-Hexane was used as a degreaser. The main purpose of using hexane is to perform the separation process by evaporating the solvent. NaCl, separating funnel was used in liquid-liquid extraction. This method was applied by making use of the density difference of two liquids. When the mixture was placed in the separatory funnel, the liquid with the lower density was collected at the top and the other one at the bottom.

2. Sample collection

A total of 20 water samples collected from the market (9 pet bottled (P1-P9), 7 carboy (C1-C7) and 4 tap water (T1-T4) from various districts, were collected in 3 days and BPA DBP and BPA derivate analyses were performed. The study was carried out with commercially sold pet bottled and carboy waters and tap water collected from four different districts of Kayseri. Most frequently used and most common brands are preferred to represent commercial pet bottled and carboy water samples.

Before sampling, the bottles that will be used to sample were rinsed twice with the water samples. All samples were transported to the laboratory and were stored at 4^0C before analysis. Samples were extracted within 6-7 days of collection.

3. BPA and DBP determination

Water samples were taken into a 2000 ml separatory funnel and n-hexane-ethyl acetate solution was prepared in 500 ml glass flasks. 1000 ml of water sample was extracted with 300 ml of n-hexane-ethyl acetate (8:2) and shaken for 60-80 minutes. 25 g of NaCl was added to the water sample and shaken again for 60-70 minutes. The resulting phase is then transferred into the glass beaker and evaporation was carried out at 200 pressure at 90 rpm and at 37^0C. The oily residue in the flask was dissolved in a small amount of n-hexane and taken into 1 ml glass vials. These vials were transferred and analyzed in the Technology Research and Application Center (TAUM) by Gas Chromatography-Mass Spectrometry (GC-MS) method.

BPA and DBP analyzes were done using GC-MS method and SHIMADZU QP2010 device was used. It consists of high capacity turbo molecular pump, high performance ion source and ion optics, configurations and optional units that can be selected in accordance with analytical needs. Values GC-MS conditions are shown in Table 1.

Results

An example of BPA and DBP values representing the pet bottled, carboy and tap water samples examined within the scope of the study is shown in Figure 1, Figure 2 and Figure 3 respectively.

In this study, 88.88% (8/9) of pet bottled, 14.28% (1/7) of carboy water and 50% (2/4) of tap water samples were found positive in terms of BPA, while 55.55% (5/9), 100% (7/7) of carboy and 100% (4/4) of tap water samples were found to be contaminated with DBP. The contamination rates of DBP, BPA and BPA derivatives in the analyzed drinking water samples are summarized in Table 2.

Table 1. GC-MS conditions

GC-MS Conditions	Values
Column oven temperature	50ºC
İnjection temperature	280ºC
Sampling time	4 minute
Pressure	208.3 kPa
Total flow	11 mL/min
Column flow	4 mL/min
Linear velocity	72.6 cm/sec
Purge flow	3 mL/min
Split ratio	1

Table 2. Contamination rates of DBP, BPA and BPA derivates in drinking water samples

	BPA (%)	DBP (%)	BPA DERİVATİVES (%)
Pet Bottled (n=9) (P1-P9)	8 (88.88)	5 (55.55)	7 (77.77)
Carboy (n=7) (C1-C7)	1 (14.28)	7 (100)	-
Tap water(n=4) (T1-T4)	2 (50)	4 (100)	-
Total: 20	**11 (55)**	**16 (80)**	**7 (35)**

BPA: Bisphenol A, DBP: Dibutyl phthalate, BPA Derivates: Bisphenol F and Bisphenol S.

4. Quality assurance and quality control

In the study, the samples were collected in glass bottles, to avoid the possibility of bottle to be contaminated with bpa and phthalate. The sterilization process was carried for the glass bottles before the sampling. The bottles were washed with the sampled water in order to prevent other contaminations from the laboratory environment. After being rinsed twice with the sampled water, the glass bottles were filled with the water samples. While performing the analysis, attention was paid to ensure that the equipment used were not plastic for bpa and phthalate contamination; Care was taken to ensure that the beaker used in the extraction process, the balloon used in the evaporation process, and the vials in which the reading process took place in the GC/MS device were glass. The samples, with protection from heat and light, were transported to the laboratory and analyzed. They were stored at room temperature, in an area out of direct sunlight and were subjected to the analyses as soon as possible.

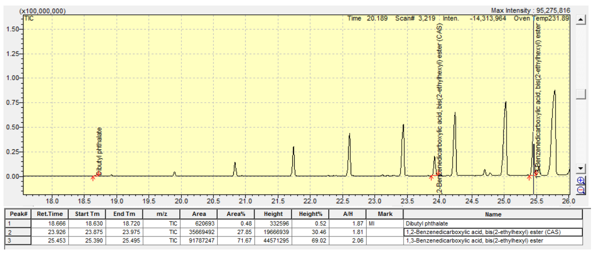


Figure 1. DBP peak values in pet bottle water samples.

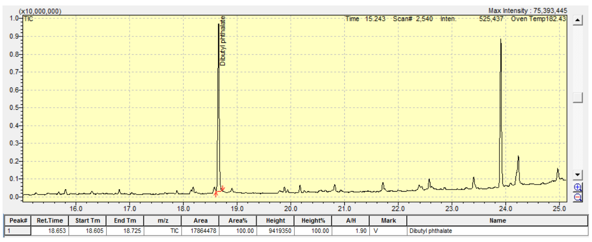
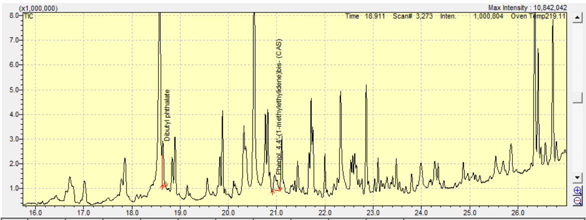


Figure 2. DBP peak values in carboy water samples.



Peak#	Ret.Time	Start Tm	End Tm	m/z	Area	Area%	Height	Height%	A/H	Mark	Name
1	18.642	18.620	18.700	TIC	3891418	57.80	1797709	75.47	2.16	V	Dibutyl phthalate
2	20.950	20.920	21.070	TIC	2841377	42.20	584383	24.53	4.86		Phenol, 4,4'-(1-methylethylidene)bis- (CAS)

Figure 3. BPA and DBP peak values in tap water water samples.

Drinking water samples collected in this study were analyzed only for DBP, BPA and BPA derivates, and no quantification were made in the water matrix. However, when the findings of the study are evaluated in terms of peak values; DBP, BPA and BPA derivate levels in pet bottled, carboy and tap water samples were highly variable.

Discussion and Conclusion

According to the drinking water criteria determined by WHO and USA; phenol compounds up to 0.001-0.002 mg / L are permitted (WHO 2010). On the other hand, EFSA reports the limit values for BPA and DBP in drinking water as 50 ppb and 0.005 mg / L respectively (EFSA 2005). Similarly, in Turkish Food Codex (TGK), the maximum BPA value allowed in food products is reported to be 600 ppb (TGK 2005) and up to 0.002 mg / L of phenolic substances was allowed only in the group of substances that affect the drinkable feature (TS 2019).

Different levels of DBP, BPA and BPA derivatives contamination rates were found for carboy, tap and pet waters in this study. These differences determined in pet bottled and carboy water may be related to the DBP, BPA and BPA derivative amounts used in the production of plastics of different brands, as well as the storage time and conditions of the packaged waters. Likewise, the lowest contamination levels were obtained from the water brands of nearest source of origin in this study which means shorter transport and storage time before being consumed.

In this study, 88.88% (8/9) of pet bottled samples, 14.28% (1/7) of carboy water samples and 50% (2/4) of tap water samples were found positive in terms of BPA, while Shao et al. (2005) reported that no detectable BPA contamination was found in water and other beverages sold in plastics or cans. On the other hand, in a study conducted by Maggioni et al. (2012), 15 (28.57%) of 35 Italian city waters were found contaminated with BPA. Similarly, Colin et al. (2014) determined the incidence of BPA contamination in pet water as 14.4%.

Much higher contamination rates have been reported in a study conducted by Santhi et al. (2012) in Malaysia, in which the BPA contamination in drinking waters were reported to be 93%. In a study conducted by Li et al. (2010) in China, BPA contamination in tap waters was determined to be 80.95%. Kuch (2001) and Notardonato et al. (2018); reported the prevalence of BPA in drinking water as 100% and 98.1%, respectively. Also in studies related to BPA contamination of tap waters in various countries; contamination rates in the USA, Lebanon, India and Europe, were reported as 94%, 94%, 82% and 72% respectively (Kosuth et al., 2017).

There are many studies reporting the presence of phthalates in drinking waters (Benotti et al. 2008; Loraine and Pettigrove, 2006; Santana et al., 2014). In this study, 55.55% (5/9) of pet bottled water samples, 100% (7/7) of carboy samples and 100% (4/4) of tap water samples were contaminated with DBP. This similarity in tap and carboy waters could be due to the network of distribution pipes and carboy material to be made of hard plastic for which DBP is used for hardening the plastic. Also, it is the fact that water consumption from carboy is much slower than the pets and that could be associated with increased contact time and longer DBP release.

Consistent to our study results; Serrano et al. (2014) also reported higher DBP contamination rates in tap and carboy waters than other beverage samples; and they pointed out high-fat foods, fatty dairy products, and poultry for human phthalate exposure. Likewise, Notardonato et al. (2018); reported 97.5% of tap water samples to be contaminated with DBP.

Serôdio and Nogueira (2006) stated that DBP was the most common phthalate derivate found in mineral waters. Similarly, in a study by Li et al. (2010) evaluating drinking water samples from various regions in China for 6 different phthalate derivatives; the most common phthalate contaminant in drinking water was found to be DBP (99.6%). The researchers stated that no water sample exceeded “Chinese drinking water quality standards”, and they concluded that phthalates in drinking water could not be considered as a cause of cancer in Northern China.

The data obtained as a result of this study revealed that drinking water consumed in Kayseri/Türkiye is contaminated with DBP, BPA and BPA derivatives. Drinking water in pet bottles has been demonstrated to be contaminated with all contaminants of concern, therefore pet waters preserved in thin plastic material could constitute the most risky alternative as drinking water for public health. Although there are limit values for BPA and DBP in the national legislation, content of these chemicals in drinking water are not routinely tested and monitored in Türkiye. Since the level of contaminations could not be determined, the risk assessments could not be evaluated in this study.

These values indicate certain levels of BPA and DBF contamination in both mains and commercial waters. It is of great importance to carry out studies to prevent BPA and DBP from mixing with water resources. In our country, studies on the use of alternative materials for all kinds of materials that contain BPA and DBP and come into contact with food and water should be implemented. Comprehensive studies are needed to determine the sources of exposure for the risk group, which has a higher risk of exposure, especially for children and other sensitive individuals in the society, and to take precautions. Rather than ignoring the public health risks of these chemicals, it is critical to develop a more realistic approach for the invisible dangers faced by modern lives. Therefore it is necessary i) to establish reference laboratories to implement fast, reliable and quantity-measuring methods for the routine control of phthalate and bisphenol contaminations in drinking water ii) to identify sources of contamination and iii) to take necessary legal measures and protect public health.

Risk analysis; especially for the sensitive population group (women, infants and children during pregnancy and lactation), should be performed and announced to the population. In this context, it is essential to raise public awareness with the comprehensive and well-planned educational activities, especially for pregnant women and those who plan to become pregnant, about the importance of avoiding exposure in the critical pregnancy periods. The laws that prevent the use of BPA in food containers should effectively be implemented since they may lead to high amount of exposure for the most vulnerable group (the children up to 3 years).

Global industrial BPA production is increasing and is expected it to grow by 4.8% in 2020 (Almeida et al., 2018). In areas where the use of BPA is legally restricted or prohibited, studies on the alternative use of chemicals such as bisphenol F and S, for which the health effects have not been fully revealed, should be completed and relevant legal regulations should be put into force. Some studies, however, have already concluded the "BPA-like" endocrine disrupting effects of other bisphenol derivatives. Therefore, it seems to be the best option to prefer materials such as glass rather than bisphenol-containing plastics for contact surfaces and containers to reduce exposure and adverse health effects.

As a result, taking into account the proven health effects of these chemicals; this study aims to develop a BPA and DBP targeted perspective in daily life, and reveals that efforts to obtain more environment friendly and healthier products should be increased to protect human and environmental health.

It is necessary to raise public awareness about the potential health consequences of these chemicals and to limit the exposure by continuous and well-coordinated training activities.

This study, which we have done, was conducted with a limited sample and is a preliminary study, and new studies are needed by conducting more quantitative studies.

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