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The Effect of Different Storage Conditions on The Migration of Chemicals from Polyethylene Terephthalate and Polycarbonate Bottles to Water

Gumrah Seyhan[1], Unsal Veli Ustundag[2], Ismail Unal[3], Perihan Seda Ates Kalkan[4], Derya Cansiz[2], Ebru Emekli Alturfan[5], Ahmet Ata Alturfan[6]

[1]Institute of Forensic Sciences, Istanbul University-Cerrahpaşa, Istanbul, Turkiye
[2]Department of Medical Biochemistry, Faculty of Medicine, Medipol University, Istanbul, Turkiye
[3]Institute of Health Sciences, Marmara University, Istanbul, Turkiye
[4]Department of Biochemistry, Istanbul Health and Technology University, Istanbul, Turkiye
[5]Department of Basic Medical Sciences, Faculty of Dentistry, Marmara University, Istanbul, Turkiye
[6]Department of Medical Biochemistry, Faculty of Medicine, Istanbul University-Cerrahpasa, Istanbul, Turkiye

ORCID ID: G.S. 0000-0002-2626-1757; U.V.U. 0000-0003-0804-1475; I.U. 0000-0002-8664-3298; P.S.A.K. 0000-0002-4905-1912; D.C. 0000-0002-6274-801X; E.E.A. 0000-0003-2419-8587; A.A.A. 0000-0003-0528-9002

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ABSTRACT

Objective: Polyethylene terephthalate (PET) and polycarbonate (PC) bottles have been used widely in the last years for the consumption of water and the increased use of these chemicals has raised many concerns regarding their adverse effects on health. Phthalates and bisphenol A (BPA) are the main endocrine disrupting chemicals (EDCs) that can migrate from these plastics into potable water.

Materials and Methods: The concentrations of phthalate and BPA were measured in water samples that were stored in PET and PC bottles at different storage conditions. The method of ELISA was used for the determination of phthalate and BPA levels. A standard curve is obtained from the standards prepared at known concentrations of phthalate, BPA, according to their absorbance at 450 nm. The BPA levels of the samples were obtained through the calculation of the absorbance values acquired using the standard curve.

Results: Different storage and heating processes applied on the samples significantly increased the levels of BPA and phthalate. One year of storage led to a statistically significant increase in phthalate levels when compared to the control group. Both BPA and phthalate levels detected in the water samples were higher than the control group depending on the storage conditions including exposure to high temperatures, sunlight and outdoor conditions.

Conclusion: Our results indicate the necessity to establish the environmental conditions that must be ensured during the production, transportation and storage processes of the bottles, on a legal basis with legal regulations.

Keywords: Polyethylene terephthalate, polycarbonate, water, phthalate, bisphenol A

INTRODUCTION

The endocrine system and the nervous system are the two major modulatory systems in physiology of the mammalians. The functions of cells, tissues and organs in the body are regulated by the interaction of various biological messenger systems called endocrine system. The endocrine system plays a major role in modulating functions such as metabolism, reproduction, development, electrolyte and water balance, and behavior (1).

Most of the man-made chemicals used in industry and agriculture are found in the environment as pollutants. These include pesticides, plasticizers, antimicrobial agents

Corresponding Author: Ahmet Ata Alturfan **E-mail:** ataalturfan@gmail.com
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and rust inhibitors. People are in contact with these chemicals, which are found in the air, water, food, and some personal care products. These chemicals, which are ubiquitous in the environment, act in the body by mimicking our body's complex and delicately regulated hormonal communication system or by antagonizing the effects of endogenous hormones. These chemicals are called endocrine disrupting chemicals (EDC) as a group (2).

Plastics are widely used for food packaging. However, the monomers in the structure of the packaging and various additives used in production can migrate into foods. One of the packaged consumer products is water. Polyethylene terephthalate (PET) and polycarbonate (PC) bottles are widely used in the packaging of potable water. The plasticizer di-(2-ethylhexyl) phthalate (DEHP) is among the chemicals that can migrate from PET bottles to water (3, 4). The building block of PC plastic is bisphenol A (BPA). These compounds have the potential for serious health problems due to their behavior to mimic hormonal activity. The use of DEHP and BPA in children's products under the age of three was banned in Sweden in 2000 and in the USA in 2009 (5). The European Union and the USA have imposed restrictions on the use of DEHP and BPA (5, 6).

Nutrition, inhalation, intravenous injection, and skin absorption are potential routes of EDC exposure. The most likely route of exposure to DEHP is through food. DEHP can migrate from plastics to food during manufacture and storage (7). The DEHP exposure of the population in the USA is thought to be 3–30 µg/kg/day. Since a significant part of this is through nutrition, there is a concern that continuous exposure may exceed the acceptable level, and precautions are being taken to prevent this (8).

BPA, a synthetic estrogen, is one of the highest-produced chemicals worldwide. In the United States alone, BPA has a production volume of 800,000 tons per year, and the five companies that produce it generate revenues of US$6 billion per year (9).

BPA is an EDC and can affect different endocrine pathways. In animal experiments, BPA was shown to mimic the role of estrogen and can inhibit sex differentiation. It may affect reproduction and behavior in some rodents even at doses below the safe exposure limit for humans set at 50µg/kg body weight per day as prescribed by the EPA in 1988 (10, 11).

The amount of EDCs leaking from bottles to drinking water varies according to the environmental conditions they are located in. This reveals the importance of storage conditions. In our study, which we started by considering the health risks of PET and PC bottles stored in adverse conditions, we exposed PET and PC bottle samples of different brands to natural climatic conditions, high temperatures and different levels of microwave applications for various periods of time, and investigated the effects of these processes to the migration of BPA and DEHP from PC and PET bottles to drinking water.

MATERIALS AND METHODS

A total of 85 bottled water samples were used in the study. For the determination of BPA migration, the PC group consisted of 8 groups (7 experimental and 1 control group) each including 5 PC bottles, and a total of 40 bottles. For the determination of DEHP migration, the PET group consisted of 9 groups (8 experimental and 1 control group) each including 5 PET bottles, and a total of 45 bottles. The PC groups are given in Table 1. The PET groups and the processes applied are given in Table 2.

BPA Measurement

BPA analysis was performed by BPA recognition through specific monoclonal antibodies using the Ecologiena Supersensitive BPA ELISA kit. Samples containing BPA and a mixture of BPA-enzyme conjugate (Coloring enzyme labeled BPA) were added to each well of the microplate, and compete for the binding sites of specific antibodies fixed to the surface of the wells. Unbound BPA and excess BPA-enzyme conjugates were washed away. The presence of BPA was evaluated through the addition of a chromogenic substrate. Then the enzyme-labeled BPA, which binds to the BPA antibody, catalyzed substrate con-

Table 1. PC groups and the processes applied.

Groups (n=5)	Processes applied
PC Control	PC bottles of different brands supplied on the day of the experiment
PC1 Group	PC bottles of brand A kept outdoor for 1 year
PC2 Group	PC bottles of brand B kept outdoor for 1 year
PC3 Group	PC bottles of brand A kept outdoor for 2 years
PC4 Group	100^{0}C boiling water poured into plastic containers and cooled
PC5 Group	Water heated in microwave in low-power for 10 min in plastic containers
PC6 Group	Water heated in microwave in medium-power for 10 min in plastic containers
PC7 Group	Water heated in microwave in high-power for 10 min in plastic containers

Table 2. PET groups and the processes applied.

Groups (n=5)	Processes applied
PET Control	PET bottles of different brands supplied on the day of the experiment
PET1 Group	100^{0}C boiling water poured into PET bottles of brand A and cooled
PET2 Group	PET bottles of brand A kept outdoor for 1 month in July
PET3 Group	PET bottles of brand A kept in car for 1 month in July
PET4 Group	PET bottles of brand A kept outdoor for 1 year
PET5 Group	PET bottles of mineral water brand B kept outdoor for 1 year
PET6 Group	PET carboys of brand A kept outdoor for 1 year
PET7 Group	PET carboys of brand B kept outdoor for 1 year
PET8 Group	PET carboys of brand C kept outdoor for 1 year

version to colored product. After the incubation, the reaction was inhibited by adding diluted acid. The intensity of the color formed, hence the measured absorbance, varied inversely with the amount of BPA in the samples. A standard curve was obtained according to the absorbance at 450 nm from BPA standards with known concentrations. The BPA concentration in each sample was calculated using the absorbance values obtained from the standard curve.

Phthalate Measurement

The test is a direct competitive ELISA based on phthalates recognition by specific antibodies by using the Abraxis Phthalates ELISA kit. Phthalate-containing samples and a phthalate-enzyme conjugate were added to the wells of the microplate. The phthalates and phthalate-enzyme conjugate present in the samples competed for the binding sites of the anti-phthalate antibodies in solution. The phthalate antibodies were then bound by a second antibody (goat anti-rabbit), which was fixed to the surface of the wells. After the washing step and substrate solution addition, a color developed. The color reaction was stopped after 20 minutes through addition of a diluted acid. The intensity of the blue color was inversely proportional to the phthalate concentration in the sample. The standard curve was obtained according to the absorbance at 450 nm from phthalate standards with known concentrations. The phthalate concentration in each sample was calculated using the absorbance values obtained from the standard curve.

Statistical Analyses

For all statistical analysis, GraphPad Prism 5.0 (GraphPad Software, San Diego, USA) was used. All data were expressed as mean±standard deviation (SD). Analysis of variance ANOVA test was used for comparison of multiple groups; Tukey test was used for binary comparisons between groups. A value of $p<0.05$ was considered significant.

RESULTS

Results of BPA Analysis of Water Samples Stored in PC Bottles

One way ANOVA test showed that the difference between the means was significant ($p<0.0001$). When the control group and the experimental groups were compared, the mean values of PC1, PC2 and PC5 were found to be 9.35%, 9.43%, and 5.86% lower than the mean of the control group. However, the most dramatic increase was found in the PC3 group. In addition, the mean values of PC4, PC6 and PC7 were found to be 15.69%, 7.13%, and 54.99% higher than the mean of the control group (Figure 1).

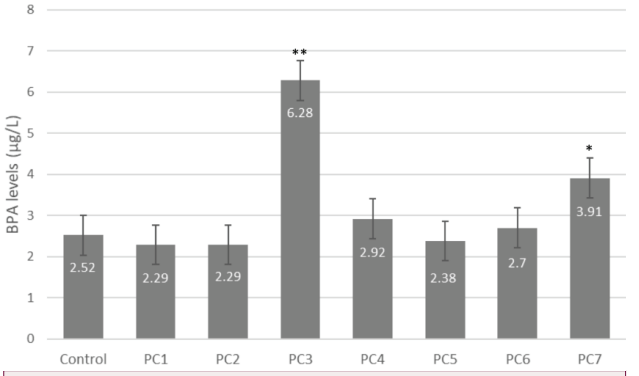

Figure 1. Average BPA levels of the groups. *$p<0.001$; **$p<0.0001$, Significantly different when compared with the control group.

Results of Phthalate Analysis of Water Samples Stored in PET Bottles

One-way ANOVA test showed that the difference between the means of phthalate levels were significant ($p<0.0001$). When the control and experimental groups were compared, it was observed that the phthalate ratio increased in all experimen-

tal groups as 31.39 %, 37.14 %, 181.18%, 187.00% and 163.94% in the PET1, PET3, PET4, PET6, and PET7 groups, respectively. It was observed that the mean phthalate levels of PET8 was 208.07% higher than the mean of the control group. It was determined that the mean phthalate levels of the PET5 group represented the highest increase as 381.77% when compared with the control group. The sample with the least increase was the PET2 sample with an increase of 1.37% (Figure 2).

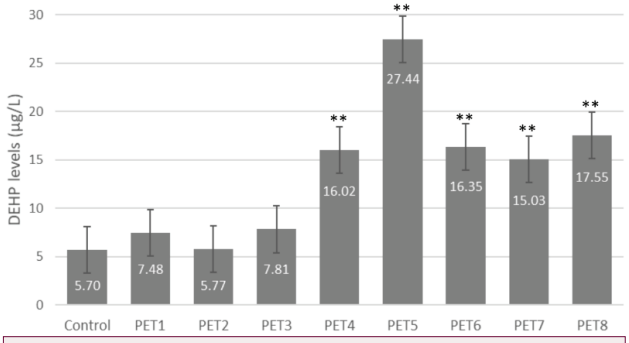

Figure 2. Average DEHP levels of the groups. **p<0.0001, Significantly different when compared with the control group.

DISCUSSION

With the developing technology, the contents of plastic containers are developed by adding various chemicals to improve elasticity, durability and transparency. These plastics contain various chemicals, and are relatively cheaper and less breakable than glass. Especially in the last 10 years, some toxic properties of these chemicals have been discovered, and with the development of determination methods, it has been reported that some of the final product plastics are not toxic, but some monomers used may show toxic properties. The bonds of these monomers may weaken and break due to light, heat, storage time and the contents of the stored liquids, and the monomers separated from the plastic may migrate into the container and pose health risks (12).

Migration from plastic materials that come into contact with water and food poses a danger and risk to public health, primarily in chronic exposures. The chemicals contained in these plastics should not migrate into water and food and should not contain toxic substances. However, it has been shown in the literature that the chemicals in plastic materials can migrate to water and food depending on storage conditions (3, 4, 7).

Today, especially in big cities, bottled water consumption is preferred more than tap water consumption, considering it is safer, pure and of high quality, despite its high price. The preferred water for drinking purposes at homes and workplaces is PET carboys and PET water. This intense demand also focused attention on the effects of phthalates on health. According to the drinking water directives of the World Health Organization (WHO) and the Food and Drug Administration (FDA), the amount of phthalates in bottled water should not exceed 8 µg/L (13, 14).

In our study, when the total phthalate levels of the control group and the PET4 group (water in a 5L PET bottle kept outside for 1 year) were compared, a statistically significant difference was found between them. Interestingly, we also detected phthalates in newly bottled PET bottled water, which we accepted as control. It has been suggested that the reason for this is due to contamination during bottling and water treatment (15) and natural water source including EDC (16). In addition, the EDC values detected in the water samples were found to be higher than the control differed depending on the storage conditions (exposure to high temperatures, sunlight, outdoor conditions, etc.).

Evandri et al. (17) investigated the toxic effects of EDCs transferred from PET bottles to water in different storage conditions and at different times with the *Allium cepa* test. They stated that exposure to high temperatures and sunlight can cause chromosomal changes due to the migration of toxic chemicals that pass from PET bottles to water. Linssen et al. (18) stated that acetaldehyde, which is produced during the condensation and melting of PET plastic bottles at high temperatures, is a volatile and heat degradation product, and showed that this EDC migrates from PET bottles to mineral waters.

In our study, when the phthalate levels of the control group were compared with the phthalate levels of the PET3 group (water in a PET bottle kept in the car in July), no statistically significant difference was found. We associate this insignificance with the fact that PET bottles were kept in the trunk of the car, not in direct sunlight, and the storage period was relatively short. No statistically significant difference was observed between the phthalate levels of the control group and the PET2 group (water in a PET bottle kept outside in July). However, the phthalate contents of the PET3 group were found to be higher than the PET2 group.

Boiling water at 100°C was poured into a PET bottle and stored to cool to investigate whether phthalates could be migrated from the PET bottle to the water. It was observed that boiling water caused increased phthalate levels in the PET bottle, although statistically not significant. In our study, when the amounts of phthalates in the water in carboys of different brands were compared with the control group, a high statistically significant difference was found between each group and the control group. We encountered high phthalate levels due to temperature increase, long-term exposure to ultraviolet rays and the length of the storage period in water stored in PET carboys for 1 year outdoors. However, we observed that this increase was below the levels determined as toxic by the EPA and the WHO. Our findings revealed the necessity of paying attention to the storage conditions of PET bottles, especially during distribution and use. During our research, we determined that phthalates were migrated from PET bottles to mineral waters after 1 year. According to the data obtained, when the mineral waters in the PET bottles were compared with the control group, a statisti-

cally significant difference was found between the two groups. Biscardi et al. (19) investigated DEHP concentrations in mineral waters in PET bottles stored at room temperature for 9 months, and they did not find phthalates in their samples in the first 8 months. DEHP was detected in the ninth month.

Guart et al. (20) found DEP levels to be 20.5 µg/L, and their results are close to the values measured at the end of the 1-year storage period in our study. However, researchers reported that they could not detect DBP and DMP (20). Zaki and Shoeib found the average DEHP concentrations as 0.274 and 0.396 µg/L, respectively, in their measurements after 2 months and 6 months of storage at an average room temperature of 25 degrees, and stated that the increases were 2.6 and 4 times, respectively, compared to the control group. In this study, the amount of phthalate showed a statistically significant increase compared to the control group depending on time (21).

The WHO and the EPA have determined the maximum contaminant level for phthalates in drinking water to be 6-8 µg/L (22, 23). In our study, the control group, PET1, PET2 and PET3 groups were within these limits. However, we observed that phthalate levels rise above this range as the storage conditions deteriorate, that is, the waters are kept outside for a long time such as 1 year and are exposed to seasonal temperature changes. The data we obtained overlap with the results of other researchers (16, 24, 25).

Polycarbonates are formed when the BPA monomer reacts with carbonyl chloride in the presence of sodium hydroxide to form the carbonate bond in the polymer. As a result of the incomplete reactions in the polymer, the unreacted BPA monomers remain in the PC plastic. These monomers migrate to their environment under the influence of heat, light, friction, pH and some minerals. According to the European Community (EC) directives, the specific migration limit of BPA is 3 mg/kg (18, 26).

Maragou et al. (27) investigated whether BPA migrated to the water by putting boiled water in baby bottles made of PC plastic, and showed that the values obtained were in the range of 2.4 to 14.3 µg/kg. Guart et al. (28) reported that BPA levels were between 1.60 and 4.44 µg/L, and the mean concentration was 2.64 µg/L in water samples stored in PC plastic containers. Nerín et al. (29) detected 30 µg/g BPA in their samples in PC plastic storage containers with the HPLC technique. They reported that 6.5 µg/g of this amount migrated to water and nutrients as a result of heating in a microwave oven.

In our experiments, BPA migration was observed, albeit slightly, into water samples exposed to low and medium microwave levels in PC plastic storage containers, although not statistically significant. However, statistically significant BPA migration was detected from PC plastic storage containers into the water at high level microwaves. But, this level is well below the toxic level specified by the WHO.

In our study, we investigated the effects of different storage and usage conditions on the chemical migration from PET and PC bottles and storage containers to water, which are frequently used by the public. While designing and performing our study, we tried to consider the habits of the producer and consumer of using plastic containers. As a result of our study, the highest BPA level found in the analysis of water samples in PC bottles was 6.28 µg/L. When we evaluate our study according to the report of European Food Safety Authority (EFSA), the level of BPA that people living in our country are exposed to in terms of drinking water is at an acceptable level. In our water samples analysis in PET bottles, the highest phthalate level was found to be 17.55 µg/L in drinking water and 27.44 µg/L in mineral waters. We found that most of the data we obtained confirms and supports previous studies showing the migration of BPA from PC plastics and phthalates from PET plastics. The results are well below the toxic limits for both endocrine disruptors. On the other hand, food packaging, medical supplies, kitchen utensils, toys, cosmetic products, detergents, in short, tools and consumer products found in all areas of our daily life also contribute to BPA and phthalates exposure. Considering all these potential sources of EDC, the amount of daily EDC taken into the body increases. When our study is evaluated in this direction, it has been concluded that the levels of EDC that can be exposed from drinking water should be kept as low as possible. For this purpose, it is necessary to establish the environmental conditions that must be ensured during the production, transportation and storage processes of the bottles, and legal regulations should be applied.

Ethics Committee Approval: Ethics committee approval is not required because of no material or experimental animal that would require permission.

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Conflict of Interest: The authors have no conflict of interest to declare.

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