



# Evaluation of exposure to phthalate esters through the use of various brands of drinking water bottled in polyethylene terephthalate (PET) containers under different storage conditions

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## Abstract

**Background:** There are many concerns about the migration of phthalates into bottled water. These compounds are hazardous to consumers. In this study, the risk factors of the use of various brands of drinking water bottled in polyethylene terephthalate (PET) containers under different storage conditions were examined.

**Methods:** Phthalate esters were measured and analyzed using air-assisted liquid-liquid microextraction (AALLME) and gas chromatography-mass spectrometry (GC-MS). Subsequently, the risk of exposure to the desired compounds was evaluated by calculating the risk factor and excess cancer risk. Data were statistically analyzed using SPSS version 24 software.

**Results:** The average concentration of phthalate esters (DEHP, DBP, DIBP, DEP, and DMP) after 5 and 15 days of storage at incubator temperature of 42°C, and after 15, 45, and 75 days at incubator temperature of 25°C by increasing the initial level of the conditions was 7.28, 8.99, 1.78, 5.6, 6.45, and 8.55 ppb, respectively. The risk factor of non-carcinogenic effects of phthalates for consumers was low and can be ignored (HQ<1). Also, the risk of additional cancer due to the presence of diethylhexyl phthalate (DEHP) in bottled water is very low ( $4.8551 \times 10^{-6}$ ).

**Conclusion:** However, due to the increase in the concentration of phthalate esters in bottled water by increasing the storage time and temperature, as well as increasing the ethylene hexyl phthalate content from its established limit, the use of bottled water kept in unsuitable conditions for the society, and especially the sensitive groups, is not appropriate.

**Keywords:** Phthalate esters, Bottled water, Storage conditions, Risk assessment, Various brands

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## Introduction

Every year in the world more than 1.8 million people (mostly children) die from water-borne diseases, and these diseases have become the most common and important causes of mortality (1,2). Concerns about the quality of water plumbing, the lack of water in different areas, especially during warm years, the presence of adverse compounds due to disinfection of water, and changes in consumer habits, especially in recent years, are due to the increased use of bottled water in the world (3). One of the most important priorities in the countries is the optimal use of drinking water sources through the replacement of modern water supply methods, including

the use of packaging industries instead of urban water distribution networks, so that today, water is packaged as an essential commodity for the international community (4). The materials used to make water bottles vary from one country to another, but the most commonly used material is polyethylene terephthalate (PET). It contains some toxic and harmful compounds, such as phthalic acids and heavy metals, which in some cases, enter the water and threaten human health (5). As phthalates can accumulate in the human body, they have now considered as a serious risk to humans. The risk assessment of the use of products that human beings encounter when handling phthalates has been conducted by groups and experts



in the United States and Europe, such as the European Food Safety Authority (EFSA), the US Environmental Protection Agency (EPA), and the International Agency for Research on Cancer (IARC) (6). Phthalate acid esters (PAEs) are synthetic compounds, which are added to PET polymers as a lubricant for making plastic materials. Phthalates are used in many products, including food packaging, cosmetics, toys, medical equipment, etc (7,8). There are many concerns about the possibility of migration of chemical compounds from materials used in the manufacture of PET containers into the water and food stuffed therein. Inappropriate storage conditions (high temperatures, sunlight, freezing, etc) and storage time are proposed as the main causes of migration of these compounds into the content of these containers (3,9). In many studies, the presence of endocrine disruptor compounds, which produce estrogen hormone in humans, and disruption of the composition of the contents of PET bottles, have been reported (9,10). By binding to hormone receptors in the body, phthalates exhibit antiandrogenic activity, change the function and structure of testicular Sertoli cells, decrease the number and quality of sperm motility in the soft tissue, and change the levels of female sex steroid hormones, and lead to precocious puberty, preterm labor, and death of the fetus (8,11). In the United States and Europe, the use of PAEs in many products has been forbidden due to their harmful effects, and these compounds have been categorized in the list of priority chemicals by the EPA (12). There are different methods for the extraction of phthalates, including the solid-phase microextraction (13), dispersive liquid-liquid microextraction (14), and vortex-assisted liquid-liquid microextraction. However, with the introduction of an easy and fast micro-extracting method, air-assisted liquid-liquid microextraction (AALLME), the problems with other methods have been fixed. In this method, a very small amount of the organic solvent is used as a solvent extraction agent instead of a solvent (15). The AALLME is a fast, efficient, optimal, simple method, with low solvent consumption (16,17). When phthalates are used as plasticizers, they do not form an irreversible linkage with the polymer structure. As phthalate esters are not chemically bound (covalent bonds) to the polymer chain, but only physically and reversibly interact with the polymer structure, therefore, under physical conditions, they may isolate from the polymer structure and migrate to the contents of the packaging and present a risk to human health and the environment (18). The PAEs, commonly refer to phthalates, as industrial compounds with low molecular weight, including dimethyl phthalate (DMP), diethyl phthalate (DEP), di-n-butyl phthalate (DNBP), butyl benzyl phthalate (BBP), diethylhexyl phthalate (DEHP), diisobutyl phthalate (DIBP), and di-n-octyl phthalate (NOP) (19). Phthalate esters are commonly used in PET production, but human exposure to these materials, can lead to human health problems,

including breast cancer, reproductive hormone disorders, obesity, and impaired function of enzymes involved in the maturation of sex cells (20). Chronic exposure to phthalate esters leads to the incidence of different types of cancers, such as breast cancer in women and prostate cancer in men (21), as well as disruption of the synthesis and activity of internal hormones (anti-androgenic), it affects thyroid gland function, teratogenic and mutagenic activity, sterilization (22), and results in hyperactivity in children, obesity, peroxisome enlargement, liver damage, and allergies (8,23,24). The degree of damages caused by these substances depends on the dose and contact with these substances (25). Considering toxicity profile of phthalate esters, many studies have been conducted on their effects on the human health. DEHP, DNBP, and BBP are classified according to the classification by the American Institute of Health in group 1 (as disturbing compounds which can disturb the endocrine and metabolism of the body) (26). The EPA's IARC and International Risk Information System (IRIS), have classified DEHP in a potentially carcinogenic group to humans (B2) (27). Registration Evaluation Authorization and Restriction of Chemical Substances (REACH) also classified phthalate esters (BBP, DNBP, and DEHP) as very hazardous substances for the reproductive system (B1) (28). The Canadian Environmental Protection Agency (CNEC) emphasized the need to monitor the levels of ethylhexyl phthalate in food contact products made of PET. Maximum contaminant level (MCL) and maximum contaminant level goals (MCLG) are standards that are set by the EPA for chemical water pollutants. The amount of MCL in drinking water has been determined only for ethylhexyl phthalate. According to the EPA standards, the MCLG for DEHP is zero. The MCL for DEHP in drinking water is 6 µg/L (29). The World Health Organization (WHO) and EU have set the MCL for DEHP in drinking water at 8 µg/L (30). The European Commission's regulations do not allow the use of phthalate esters in non-authorized food packages, but phthalates have been identified on various PET packets and PET-bottled water (31). According to Iran's National Standard 1053, the physical and chemical properties of drinking water, the maximum amount of DEHP is 0.008 mg/L (32). Given the increasing growth of the chemical industry in developed and developing countries, chemicals are an integral part of human life, and thus, the assessment of the risk of the exposure to these compounds in human life is one of the main priorities based on the principles of sustainable development (33). Many studies have been conducted in this field, but there are still many uncertainties about the risks of the use of these waters in different age groups. On the other hand, the safety of these products has already been studied. In this study, the effect of storage conditions on the release of phthalate esters into bottled water was investigated and analyzed for their migration using gas chromatography-mass spectrometry (GC-MS) due to its high accuracy. The

risk of exposure to these chemical compounds in various brands was investigated to ensure the safety of the use of this vulnerable group. The results of this research can be useful in increasing public awareness on the storage and use of bottled water, and designing plans for improving the status quo.

### Materials and Methods

This research was conducted at Sari School of Public Health by the cooperation of the North Research Laboratory of the North of Iran at the Deputy for Research and Technology of Mazandaran University of Medical Sciences. DMP, DEP, DIBP, DBP, and DEHP standards were prepared with a purity of 99.9% from Sigma Aldrich (America) and Merck (Germany). First, by solving each of the phthalate esters in the chloroform solvent, a standard with a concentration of 100 mg/L was prepared. Then, by mixing all the standards prepared and diluting the obtained mixture with chloroform, a standard mixture with a concentration of 100 mg/L was prepared. Afterwards, by diluting the mixture with high-performance liquid chromatography (HPLC) purity, the standards were diluted to 1-20 µg/L (Figure 1). Bottled water used in this study were randomly selected from 5 common different brands in the market (city-level). Based on the design of the experiments and the number of levels of each factor, the sample size was estimated. Finally, 110 PET water bottles (5.0 L and 20 g transparent) were used in this study. Then, the standards were injected into the gas chromatography equipped with a mass detector. The linear regression analysis of these standards showed a correlation coefficient above 98%.

In the laboratory, various conditions of storage and distribution of bottled water were applied according to their actual conditions at the supply and storage centers in the market. In the study of each of the environmental factors, other factors and conditions were applied. The effects of each environmental factors, including incubator temperature (25-42°C) as ambient temperature, as well as freezer temperature (-18°C), refrigerator temperature (4°C), and sunlight (23 ± 2°C) on the release of phthalate esters from PET bottles into the bottled water were investigated. The standard 20 ppb was injected into the GC-MS device three times and the results of standard deviation are described in Figure 2. The lower limit



Figure 1. Preparation of standard solutions.

Std	Std1(20ppb)	Std2(20ppb)	Std3(20ppb)	Mean	SD
PHTHALAT					
DMP	19.69	20.33	20.14	20.05	0.32
DEP	20.05	20.11	19.5	19.88	0.33
DIBP	18.94	21.28	20.12	20.11	1.17
DBP	19.62	19.14	19.12	19.29	0.28
DEHP	20.29	19.64	20.31	20.08	0.38

Figure 2. Standard deviation.

of detection calculated in terms of computational and operational data for DMP, DEP, DIBP, DBP, and DEHP was 0.5, 0.2, 0.4, 0.3, and 0.2, respectively.

To perform the AALLME method, first, 5 mL of water was poured into a conical bottom falcon, then, using a HPLC syringe, 60 mL of chloroform as the solvent of phthalates, was added to the water sample for 10 times until all amounts of chloroform was mixed with water to extract the phthalate esters migrated into the water. Chloroform is heavier than water and it was centrifuged at 5000 rpm. Then, 1 µL of chloroform precipitated was removed with a special GC syringe and injected into the GC-MS to measure and analyze the compounds concentration. Chromatography was performed using the GC-MS device (Agilent Technologies 7890A, 5975c inert MSD detector). In this study, an injector set in split ratio mode of 2:1, was used. The best ratio of mass to load (m/z) was 163 for DMP, DBP (Figure 3A), and DEHP (Figure 3B), and 149 for other phthalates (Figures 3C and 3D), and used to determine their amount. Data were analyzed using SPSS version 24.

### Assessment of exposure to phthalate esters

To assess the risk of exposure to phthalate esters through the use of bottled drinking water, it is necessary to first determine the daily water intake of the subjects. This is usually based on the recommended daily intake of water based on age and body weight, which is expressed in liters per kilogram body weight per day (L/kg/d). Then, the maximum concentration of the desired substance (phthalate) in water, which is usually reported in micrograms per liter (µg/L), and the following formula, the exposure to phthalate esters is calculated by the water intake by body weight (EDI).

$$EDI = MC \times \text{Water Consumption}$$

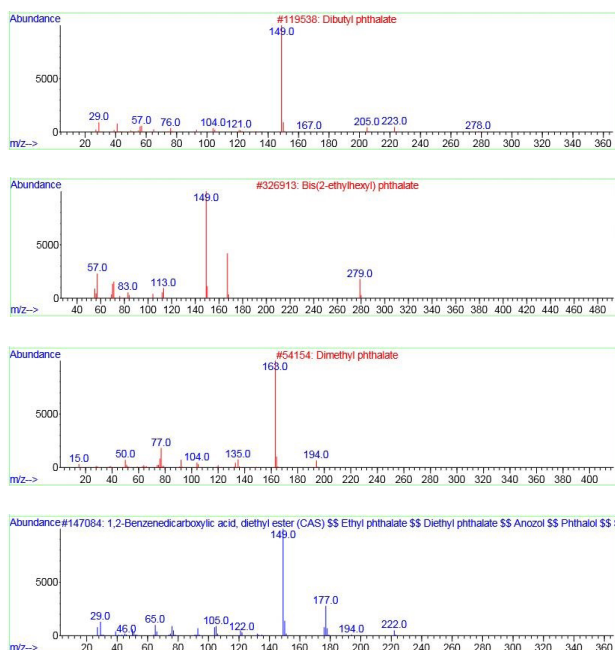
where *MC* is the maximum concentration of the compounds in the bottled water (µg/L) and *Water Consumption* is the recommended daily intake of water based on body weight (L/kg/d).

To calculate the risk of exposure to phthalate esters through the use of bottled water to produce non-carcinogenic complications, the hazard quotient (HQ) method was used.

The ratio between the exposure to the mixture due to the use of bottled water and the permissible limits for phthalate esters obtained from all strains, was calculated as follows:

$$HQ = \frac{EDI}{RFD}$$





**Figure 3.** (A) DBP, (B) DEHP, (C) DMP, and (D) DEP mass spectrum.

Where EDI is the exposure to phthalate esters (mg/kg/d) and RfD is oral reference dose ( $\mu\text{g}/\text{kg}/\text{d}$ ).

RfD is daily exposure to phthalate esters ( $\mu\text{g}/\text{kg}/\text{d}$ ) without causing non-carcinogenic effects in humans due to all the resources and contexts that human is exposed to. According to the US EPA, the maximum oral reference dose (RfD) is 0.02 mg/kg/d (20  $\mu\text{g}/\text{kg}/\text{d}$ ) for DEHP, 0.2 mg/kg/d (200  $\mu\text{g}/\text{kg}/\text{d}$ ) for BBP, and 0.1 mg/kg/d (100  $\mu\text{g}/\text{kg}/\text{d}$ ) for DBP (34).

Considering that only DEHP in the category of compounds is likely to be carcinogenic to humans, therefore, the risk of excess lifetime cancer resulting from the presence of a maximum amount of DEHP in the bottled water is calculated using following equation.

$$\text{ELCR} = \text{Drinking Water Unit Risk} \times \text{MC}$$

ELCR is the Excess Lifetime Cancer Risks due to exposure to chemicals through the use of bottled water (without unit).

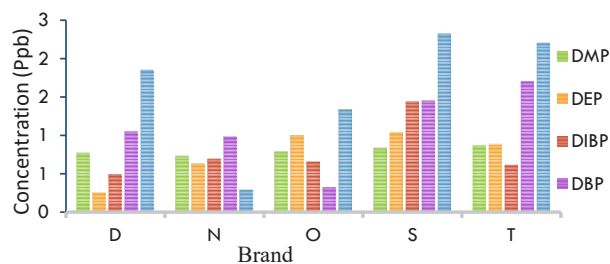
where *Drinking-Water Unit Risk* is the unit risk defined per  $\mu\text{g}$  of ethylenehexyl phthalate in water, which is equal to  $4.7 \times 10^{-7} \mu\text{g}/\text{L}$ . And *MC* is the highest concentration of ethylenehexyl phthalate in bottled water in micrograms per liter.

## Results

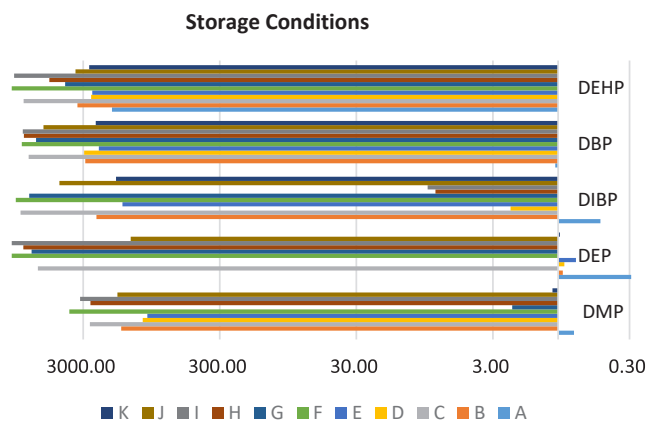
The mean initial concentration of phthalate esters in drinking water bottles measured in the first week of the production for each of the phthalate esters, including DIBP, DMP, DBP, DEHP, and DEP was 1.6, 1.1, 0.8, 0.77, and 0.75 ppb, respectively (Figure 4).

The MCL and MCLG are a set of rules that the EPA has set for chemical agents. Except for DEHP, for other phthalates, MCL levels in drinking water are not specified. According

to the EPA rules, the MCLG for DEHP is zero. The MCL level for DEHP in drinking water is 6  $\mu\text{g}/\text{L}$  (35). The WHO and the EU have set an authorized MCL limit of 8  $\mu\text{g}/\text{L}$  for DEHP in drinking water (33). The drinking water bottles that were exposed to a temperature of 25°C for 75 days were kept at an average concentration of phthalate esters (DEP > DEHP > DIBP > DBP > DMP) of 8.55 ppb. DEHP with a concentration of 12.67 ppb had the highest mean concentration among the released phthalate esters at this temperature and storage time. The mean concentration of DEHP in the samples tested under these conditions was 9.62 ppb. This amount was greater than that declared by the regulators. The mean concentration of phthalate esters in the samples that was refrigerated at refrigerator temperature (4°C), freezer temperature (-18°C), sunlight ( $23 \pm 2^\circ\text{C}$ ), incubator temperature (25-42°C) were 1.63, 2.22, 3.35, 1.76, and 7.28 ppb, respectively, and increased to 63, 121, 233, 78, and 625%, respectively. Figure 5 shows the effect of each of the environmental factors applied on the rate of migration of phthalate esters into the bottled water, and 5 days of incubation at incubator temperature of 42°C had the greatest effect. According to Figure 5, DIBP had the highest concentration among other phthalates (8.62 ppb). The average concentration of DEHP at 42°C was 8.18 ppb. This amount exceeded the limit established by the regulatory authorities. Figure 5 show that in drinking water bottles exposed to sunlight for 5 days, the concentration of DBP was higher than other phthalate esters with an average concentration of ppb 5.86. In drinking water bottles that were exposed to the incubator at 42°C for 15 days, the mean concentration of phthalate esters (DEP > DEHP > DIBP > DBP > DMP) was 8.99 ppb. DEP with concentration of 13.03 ppb had the highest mean concentration among other phthalate esters released at this temperature and storage time. The average DEHP concentration in the samples tested under these conditions was 10.33 ppb. This amount was greater than that announced by the WHO and EU. The effect of each of the above-mentioned conditions on the migration of phthalate esters into the bottled water was significant ( $P < 0.05$ ). In general, the concentrations of each of phthalate esters, including DEHP, DBP, DIBP, DEP, and DMP were found to be 6.93, 6.53, 6.43, 5.9, and 2.23 ppb, respectively, in which the S, O, N, N, and S



**Figure 4.** The initial concentration of phthalate esters (in the production) in five different brands of water bottles.



**Figure 5.** Concentration of phthalate esters under different storage conditions. A= Initial concentration, B= Freezing point (-18°C), E= Refrigerator temperature (4°C), K= Incubator temperature (25°C) for 5 days, G= Incubator temperature (25°C) for 15 days, H= Incubator temperature (25°C) for 45 days, I= Incubator temperature (25°C) for 75 days, C= Incubator temperature (42°C) for 5 days, D= Fluorescent light, F= Incubator temperature (42°C) for 15 days, J= Sunlight temperature (23±2°C).

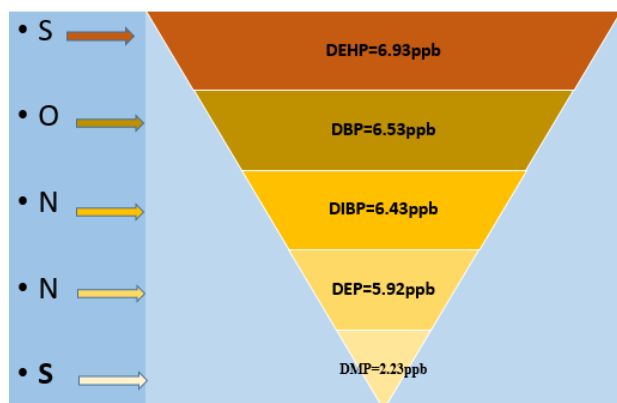
had respectively the highest concentration of phthalates. The mean concentrations of phthalates measured in each brands D, O, S, N, T were 3.71, 4.45, 4.83, 4.96, and 5.53 ppb, respectively (Figure 6).

However, the aim of the study was to evaluate the risk of exposure to phthalate esters through the use of bottled water in PET containers under various conditions of care for community, especially sensitive groups. Since children and lactating women are among the vulnerable groups in the face of chemical compounds and this vulnerability is higher than that of other groups, therefore, the risk assessment and excess cancer risk, specifically for children group, lactating women, and adolescents is of great importance. The assessment is based on the daily water intake of children and other target groups, and is only intended for the use of bottled water in PET containers. For this purpose, the HQ for two ester phthalates, including DEHP and DBP, in preschool-age children (1-6 years) was determined. According to Table 1, the HQ for DEHP and DBP was calculated to be 0.04 and 0.0064, respectively. According to  $HQ < 1$ , the risk of the use of bottled water

in children is very low and can be ignored. On the other hand, the EPA's IARC and IRIS have classified disclose DEHP in the potentially carcinogenic group of humans (B2). The risk of cancer due to the presence of DEHP in bottled water was determined and intended for the use of water by children and other two target groups and only for DEHP. It was revealed that the risk of cancer, which is based on the highest level of DEHP migration, was very low ( $4.8551 \times 10^{-6}$ ) and can be ignored.

**Discussion**

The mean level of phthalate esters in bottled water (control samples) was 1 µg/L. MCL levels for ethylhexyl phthalate in drinking water has been defined as 6 to 8 µg/L by the WHO and the EPA. The concentration of DEHP in all control samples was less than the limit value. The levels of DEHP increased compared to other phthalate esters at higher temperatures and storage times, and its concentration was less than that after 5-day storage period and at lower temperatures compared to other phthalates. The maximum amount of DEHP was 10.33 ppb at above-mentioned conditions, and this was attributed to samples



**Figure 6.** The highest concentration of each of the phthalate esters in the total applied conditions.

**Table 1.** Assessment of the risk of exposure to phthalate esters in bottled water in PET containers

	Diethyl Hexyl Phthalate	Di-butyl Phthalate
Maximum concentration (µg/L)	10.33	8.45
Daily receipts (EDI)		
Preschool-age children (1-6 years)	0.78	0.64
Adolescents	0.44	0.36
Lactating women	0.98	0.8
Risk factor (HQ)		
Preschool-age children (1-6 years)	0.04	0.0064
Adolescents	0.02	0.0036
Lactating women	0.05	0.008
Excess lifetime cancer risk (ELCR)	$4.8551 \times 10^{-6}$	-

kept for incubation at 42°C for 15 days. This amount is higher than the regulatory limit set by the regulatory agencies. The APA's IARC, uses only phthalate esters to detect dehydroepiandrosterone (DHEA) as a potentially carcinogenic to humans (B2). Considering the effects of the environmental factors mentioned separately on the release of phthalate esters in bottled water and the mean concentration of released phthalate esters that migrated into bottled water, and according to the results of Figure 5, it can be concluded that incubator temperature of 42°C for 15 days, storage time at incubator temperature of 25°C for 75 days, sun exposure for 5 days, freezing for 5 days, and refrigerator temperature of 4°C, respectively, had the greatest effect on the increase of the specific migration rate of phthalate esters in bottled water in PET containers. The refrigerator temperature of 4°C provided better conditions for the storage of bottled water, with the lowest level of release of phthalates at this temperature. The analysis results of two-way ANOVA by SPSS software, it was revealed that under different conditions, there was a significant effect on the migration of phthalate esters ( $P < 0.05$ ). There was also a significant difference between different temperatures and periods used to store bottled water. Therefore, it can be concluded that by increasing time and temperature, the migration of phthalate esters increases. Also, the interaction of temperature with time showed that there is a significant difference between the mean of migration, and the factors of temperature and time have a direct effect on the migration.

In a homogeneity study, the conditions applied to investigate the effect the release of phthalates were similar to all conditions in the release of the DEHP, DEP, and DIBP plasticizers in the present study. And by being in a subset, the same conditions were obtained in the release of these three analytes. DEP, DIBP, and DEHP were the most abundant phthalate esters found in the samples. Casajuana et al reported that unsuitable storage conditions for water bottles (at 30°C for 10 weeks) increased the concentration of phthalates, such as DBP, DEHP, and BBP in water. Also, the number of phthalates in PET water bottle was 20 times higher than that in glass water bottle (36). In 2014, Zare Jeddi et al (34) conducted a study at Tehran University of Medical Sciences on the use of mineral water packaged in PET bottles. The results indicated that over time, the concentration of all three compounds, ethylenehexyl phthalate, dibutyl phthalate, and BBP increased in water, and after 12 months storage, the concentration of the compounds compared to that in the first week of production became 811.8, 2545.2, and 832% higher. However, over the entire storage period, the amount of ethylhexyl phthalate did not exceed the limit specified by the EPA (6 µg/L). They stated that given children's daily use of mineral water packaged in PET bottles and low migration of phthalate, the consumption of mineral water packaged in PET bottles does not pose a risk to children, and these waters are healthy and safe. In the present

study, the risk factors for non-carcinogenic complications caused by this exposure (in all target groups) in the range of 0.005 to 0.02 µg/kg bw alone was low and can be omitted through the use of bottled water in all groups (risk factor < 1). Also, the excess lifetime cancer risk due to the presence of DHEP in bottled water is not significant ( $9.9 \times 10^{-7}$ ). Comparison of the results of the present study with those of the above-mentioned study shows that the risk factor for non-carcinogenic complications calculated in this study was slightly higher in the case of drinking water, ranging from 0.04 to 0.008 g/kg bw (Table 1) (34). The results also showed that the total non-carcinogenic risk of DEP and DEHP was lower than 1, indicating that there would be unlikely incremental non-carcinogenic risk to humans. Both carcinogenic and non-carcinogenic risks of PAEs in drinking water to female were slightly higher than those to male (37). Based on the results of the present study, the risk of fungal infections in lactating women due to phthalates of DHEP and di-butyl phthalate in water with values of 0.05 and 0.008 µg/kg/d, respectively, was higher than that in other groups. It was also revealed that the risk assessment of phthalate esters by using drinking water in PET containers was not significant in different brands. However, considering the effect of each of the above-mentioned conditions on the migration of phthalate esters into bottled water was significant ( $P < 0.05$ ). At high temperatures, the concentration of phthalate esters in bottled water increased, so assessment of the risk of exposure to phthalate esters indicated that the risk of use of bottled water was higher at high temperatures. In a study conducted in Portugal, concentrations of the compounds measured in Portuguese water did not present any risk to adults health (38).

Although the DEHP level in the study of Zare Jeddi et al was less than the observed values in this study, the findings of this study showed that the average concentration of DHEP was higher than the permitted values reported by the regulatory authorities (34). However, the risk assessment and the concern about the excess lifetime cancer risk due to the exposure to DHEP in similar studies based on the maximum concentration of these phthalate esters was low and irreversible. In a study by Hosseini et al, the migration of these compounds into mineral water packaged in PET containers was investigated (39), which confirm the inappropriateness of PET bottles. Applying different storage conditions on bottled drinking water showed that, in all cases, the environmental factors affected by the concentration of phthalate esters were higher than the measured initial level. The effect of each of the environmental factors, including enhancing temperature, prolongation of storage time, light and freezing, was significant on the migration level of phthalate esters ( $P < 0.05$ ). The bottled water with high storage time had a longer shelf-life, and in the case of refrigeration (4°C), the release of phthalates into the water was less. In a study by Heudorf et al, phthalates had low acute toxicity with LD<sub>50</sub>

values of about 1-30 g/kg. They also found that due to the carcinogenic effects of phthalates, their mutagenicity and genotoxicity were negative (40). The EFSA has set the maximum daily limit of human intake for some phthalates (DEHP 0.05, DNP 0.15, DDP 0.15 DBP 0.01, and BBP 0.5 mg/kg/d) (41). The mean migration of phthalate esters was significant in only brands S and T for DEHP and DMP, respectively ( $P=0.001$ ). In other cases, there was no significant difference in the level of migration between the brands ( $P>0.05$ ) (Figure 7).

Biscardi et al evaluated the migration of mutagenic and carcinogenic substances in the water packaged in PET, by biological and chemical tests. Mineral water was stored for 1-12 months, and several samples were chemically and biologically analyzed each month. DEHP with a maximum value of 3.22 mg/L, was detected in mineral water by GC/MS in the 9th month of storage, which did not have a mutagenic effect on the level of DEHP, but its effect on the extent of liver cancers was determined (42).

## Conclusion

Although the carcinogenic risk of phthalate esters in bottled water and the risk factors of non-carcinogenic complications caused by exposure to these compounds only by the use of bottled water, is low and negligible, however, due to the increase in the concentration of phthalate esters in bottled water by increasing the storage time and temperature, as well as increasing the ethylene hexyl phthalate content from its established limit, the

use of bottled water kept in unsuitable conditions for the society, and especially the sensitive groups, is not appropriate. Certainly, further studies are needed.

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## Ethical issues

The authors certify that all data collected during the study are as stated in the manuscript, and no data from the study has been or will be published separately elsewhere.

## Competing interests

The authors declare that they have no conflicts of interests.

## Authors' contributions

All authors contributed equally in the data collection, analysis, and interpretation. All authors critically reviewed, refined, and approved the manuscript.

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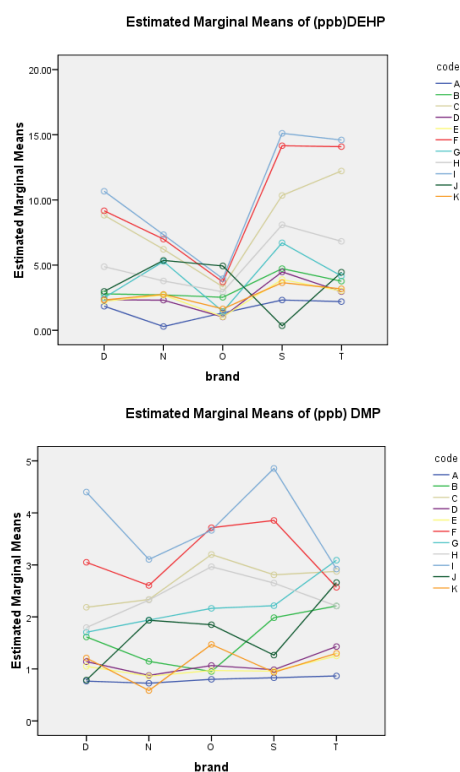


Figure 7. Profile plots for DEHP and DMP.



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