Original Article

# Effect of Common Storage Condition on the Release of Phthalate Contaminants of Bottled Water in Polyethylene Terephthalate: A Chemical Analysis and Human Health Risk Assessment

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## Abstract

**Aims:** This survey aimed to investigate the impact of common storage conditions on the migration of phthalate esters (PEs) including di-2-(ethylhexyl) phthalate (DEHP), dibutyl phthalate (DBP), terephthalic acid (TPA), and phthalic anhydride from polyethylene terephthalate (PET) bottle into the water and to assess the potential human health risk using Monte Carlo simulation (MCS). **Materials and Methods:** Three different PET-bottled water brands were stored for 7 and 90 days at three temperatures: 5, 25, and >45°C. PEs were extracted from samples using the solid-phase extraction method with gas chromatography–mass spectrometry. **Results:** The highest concentrations were found for TPA in samples immediately after purchasing. DEHP and DBP were identified at 90 days in all of the samples. Based on the health risk assessment, the hazard quotient of four compounds in the MCS method was <1; therefore, it should not be considered as a matter of concern. However, excess lifetime cancer risk for DEHP ($3.09 \times 10^{-5}$) based on the maximum concentration was found to be more than $10^{-6}$. Furthermore, the adverse estrogenic effects of DEHP and DBP appeared to be significant. **Conclusion:** The probabilistic risk assessment revealed that high estrogen equivalence (DEHP and DBP) seemed to have adverse estrogenic effects on adults. Furthermore, adults were in carcinogenic risk of DEHP. The quality of water bottled in PET may change during the long period, and further research is recommended for the monitoring of phthalates in bottled water to ensure human health.

**Keyword:** Bottled water, estrogenic effects, Monte Carlo simulation, phthalates, risk assessment

## Introduction

Polyethylene terephthalate (PET), generally abbreviated PET, is the most famous packaging material worldwide for mineral water, which is constructed by condensation polymerization of terephthalic acid (TPA) or dimethyl terephthalate and ethylene glycol.[1] Phthalate esters (PEs) are industrial chemicals commonly used as a plasticizer in plastic products, personal care products, colors, and glaze to improve their flexibility and softness.[2] Due to their potential health risks for humans and the environment, many national and international organizations listed several PEs as priority substances. These compounds represent a wide range of chemicals, such as di-2-(ethylhexyl) phthalate (DEHP), dibutyl phthalate (DBP), benzyl butyl phthalate (BBP), diethyl phthalate (DEP), dimethyl phthalate (DMP). Among phthalates, DEHP and DBP were already found to be estrogenically active.[3] They are known as endocrine-disrupting compounds (EDCs) since

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they can affect fetal growth directly or indirectly in pregnant women by passing through the placental barrier and they have been associated with infertility, birth defects, as well as testicular cancer. Fetuses may have been exposed to these chemicals in the amniotic fluid.[4,5] Maximum contaminant levels (MCL) of DEHP and DBP by the US Environmental Protection Agency (EPA) have been set at 6 µg/L and 200 µg/L, respectively.[6] Some studies present that PET bottle can release phthalate additives used in the plastic molding process, particularly in critical conditions of use.[7,8] EDCs such as PEs disrupt some functional, structural, and epigenetic mechanisms that control lipid metabolism, energy homeostasis, appetite regulation, and adipogenesis. Furthermore, exposure to PEs may influence the steroid hormone receptors or nuclear receptor signaling pathways in preadipocytes or change serum levels of metabolic hormones.[9]

Risk assessment contains deterministic and probabilistic methodologies. The deterministic or "point estimation" method provides a single estimate of risk to describe a variable in the model and is most suitable for large populations. The probabilistic approach, using probability distribution functions to represent uncertainty and/or variability of model variables, is the most suitable risk assessment method for small communities with heterogeneous mixing models.[10] Monte Carlo simulation (MCS) method is used to include the uncertainties connected to several health risks. It has been identified as a means of quantifying variability and uncertainty in risk assessments.[11]

Due to the potential disrupting of the endocrine and estrogenic effects of DEHP and DBP, they were chosen as the target compounds in this study. Furthermore, we have chosen TPA and phthalic anhydride (PA) because they are the primary degradation product of diesters and precursor to phthalate esters, respectively. This study is aimed at describing the existence of PEs in bottled water. The effect of various storage conditions, including temperature and time on values of target compounds, were considered. Moreover, there are limited data on the health risk assessment of PEs in bottled water in Iran; therefore, the main purpose of the current study was to carry out a human health risk assessment of PE exposure for adults in Central Iran based on MCS approach in both human daily intake and estrogenic effect.

## Materials and Methods

### Standards and chemicals

In this study, analytical-grade DEHP, DBP, and TPA (Sigma–Aldrich Chemical Co.), PA (Merck Co.), were used. The solid-phase extraction (SPE) cartridges (CHROMABOND® C18ec – 3 mL/500 mg) and methanol (gas chromatography [GC] grade) were purchased from Macherey–Nagel Co., (Germany) and Merck Co., (Germany), respectively. Compound stock solutions were made in methanol and stored at 4°C. Calibration standards were then prepared by dilution of the individual stock solutions at five concentration levels (1–10,000 µg/l).

### Sample collection and extraction of phthalate esters

Three Iranian brands of PET-bottled water purchased from markets were analyzed.[12] Seven bottles from each brand were obtained. The first bottle was examined immediately upon purchase. The other samples were evaluated after 7 days and 3 months (90 days) at three different temperatures: refrigerator (5°C ± 1°C), room temperature (25°C ± 5°C), and high temperature (up to 45°C ± 5°C). Figure 1 shows the study design. PEs were extracted from samples using the SPE method, as reported by Zarean *et al*. [13] External calibration was done for quantification. Validation was performed in terms of $R^2$, the limit of detection, and limits of quantification (LOQ) for each target compound.

### Instrumental analysis

PEs were determined by a GC/MS. All the injection volumes were 3 µL in the splitless mode. The analysis was accomplished using an Agilent technology 7890A gas chromatograph equipped with a 5975C quadrupole mass selective detector. A Phenomenex HP5 column of 30 m length (0.25 µm film thickness and 0.32 mm i.d.) was used for GC separation. The operating conditions were made accordingly to our previously developed technique.[13]

### Statistical analysis

Quantitative variables in this study were reported as mean ± standard deviation. Concentration (µg/l) of PEs was compared using the bar plot by storage temperature. The differences in the mean values among the various brands, temperatures, and times were analyzed by one-way ANOVA. Differences between groups were considered significant at $P \leq 0.05$. Statistics were performed by SPSS software (version 22, SPSS Inc., Chicago, IL, USA).

### Monte Carlo Simulation Analysis

A MCS approach is a useful computer-based method based on statistical sampling techniques for estimating a probabilistic approximation to the solution of a mathematical model. For each variable in the model, the possible values are calculated according to a probability distribution, which is determined using goodness-of-fit tests. The goodness of fit was assessed using Kolmogorov–Smirnov, Cramer-von Mises, and Anderson–Darling statistics and also Q–Q plot. In this study, probability distributions that primarily fitted to PEs concentration, body weight, and Bottled Water Consumption (DI) were normal, lognormal, uniform, exponential, logistic, beta, gamma, and Weibull distributions. If a MCS is run for 10,000 trials, 10,000 possible outcomes are anticipated, and then, exposure and risk distributions of the population were estimated using these simulated values. In this study, the MCS analysis was done in 10,000 iterations by R free software (version 3.6.1). The sensitivity analysis was performed based on Spearman rank order correlation (ρ) on the MC: Concentration of PEs, DI, and body weight parameters.

### Risk assessment of phthalates in bottled water

The human health risk by the intake of PEs due to bottled water consumption was done using a probabilistic method

with variables defined using probability distributions, and risk of carcinogenic and noncarcinogenic was obtained by MSC analysis with 10,000 iterations by R software 3.4.3.

### Hazard identification

Given the severe concerns on public health, a lot of attention has been increasingly paid to PET in bottled water.[3] Two recent meta-analyses have documented the link between PE exposure (particularly DEHP and DBP) and reduced anogenital distance as the important clinical measure in the reproductive system and insulin resistance, respectively.[4,14]

### Exposure assessment

In this study, PE concentration and packaged water consumption data were used to obtain an assessment of the exposure level to PEs by the consumption of packaged water in Isfahan, a central province in Iran.

DI and body weight were fitted with the exponential and lognormal distributions, respectively. About PEs concentration, the DEHP, DBP, TPA, and PA concentrations were fitted with the logistic, logistic, gamma, and uniform distributions, respectively. In this study, DI and average body weights (kg) were surveyed based on questionnaires for adult consumers in Isfahan city. Exposure assessment based on the daily intake of PEs based on μg/kg person body weight/day equation (1):

$$EDI = \frac{MC \times DI}{BW} \quad (1)$$

where MC is a concentration of PEs in water (μg/L), BW is the body weight (kg), DI (bottled water consumption) is the volume of daily drinking water for the target group, and BW is the average body weight (kg).

### Dose Response Assessment and Risk characterization

The estimation of noncarcinogenic risk was based on the EPA hazard quotient (HQ) as the equation (2):

$$HQ = \frac{EDI}{RFD} \quad (2)$$

Here, RfD is the ingestion reference dose of PEs (μg/kg/day). The RfD values (μg/kg body weight/day) were obtained from the USEPA Integrated Risk Information System.[15] For HQ <1, there will be no evident risk; however, if HQ = 1, the contamination itself seems not to cause risk; and for HQ >1, we cannot exclude the possibility of harmful effects on human health.

In this study, the cancer risk for DEHP earned by equation (3):

$$ELCR = MC \times 4 \times 10^{-4} \quad (3)$$

Where, ELCR is excess lifetime cancer risks, and MC is a DEHP concentration in water (μg/L), and the reference carcinogenic unit risk from drinking water was $4 \times 10^{-4}$ μg/L. The EPA considers a cancer risk value ranging between $10^{-5}$ and $10^{-6}$ to be acceptable.[16,17]

In this study, a suitable concentration of DEHP in water (μg/L) determined based on equation (4) and equation (1):[18]

$$10^{-6} = EDI \times RFD \quad (4)$$

### Estrogenic activity assessment

Due to some PEs are estrogenic, for estimating the estrogenic activity, the estrogen equivalence (EEQ) was calculated using equation (5):

$$EEQ = \sum EPi \times ci \quad (5)$$

where EP represents the estrogenic potency of a specific estrogenic phthalate, and c denotes phthalate concentration in bottled water (μg/L). Selected as the standard compound, 17 β-estradiol (E2) has the strongest estrogenic activity. Therefore, the EP of this compound is established as 1. When the EP of one compound is above one means the estrogenic activity of it is stronger than E2.[3]

## Results

### Phthalate esters concentration in water bottled in polyethylene terephthalate directly after purchase

Method validation and quantification parameters are given in Table 1. In our study, the levels of DEHP, DBP, and PA in the preliminary analysis were below the recommended limit by US EPA and below LOQ in all initial samples.

The primary analysis results are shown in Table 2. The highest concentration of the investigated compounds was found for TPA in initial samples (ranging from 2000 to 15400 μg/L), while DEHP, DBP, and PA were not detected.

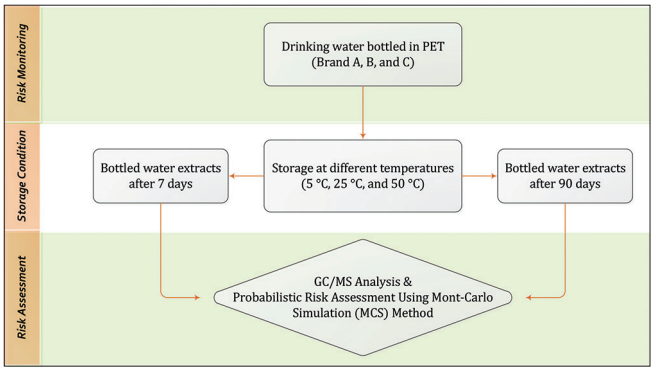


**Figure 1:** Scheme of the study design carried out on drinking water stored in polyethylene terephthalate bottles

**Table 1: Validation and quantification parameters for identification of phthalates via solid-phase extraction method and gas chromatography-mass spectrometry analysis**

| PEs | $R^2$ | LOD (μg/l) | LOQ (μg/l) |
|---|---|---|---|
| DEHP | 0.99 | 0.02 | 0.09 |
| DBP | 0.99 | 0.04 | 0.13 |
| TPA | 0.97 | 0.06 | 0.017 |
| PA | 0.98 | 0.5 | 0.16 |

PEs: Phthalates, LOD: Limit of detection, LOQ: Limits of quantification, DEHP: di-2-(ethylhexyl) phthalate, DBP: Dibutyl phthalate, TPA: Terephthalic acid, PA: Phthalic anhydride

Statistical analysis based on the ANOVA test showed that the comparison of PEs between three brands was no a significant difference ($P > 0.05$).

## Effect of storage condition on phthalate esters concentration

The concentrations of the target compounds in PET-bottled waters under various storage conditions are presented in Figure 2. After the experiment, a rise in the level of studied compounds was observed. The level of PEs in samples of three brands was ranging between 1 and 3100 µg/L. According to Figure 2, DEHP concentration is affected by storage time. Furthermore, based on the statistical analysis, there was a significant difference for DEHP concentration in 7 and 90 days ($P < 0.01$), but for other PEs was not similar to DEHP ($P > 0.05$). The results show that the maximum of TPA migration into the water was at a temperature of 5°C and >45°C. Furthermore, the results showed TPA concentration of water decreased within 7 days' storage time compared to samples that analyzed after the purchase. However, PA was not observed in almost every case, generally. The concentration of this compound only increased at the temperature of 5°C and >45°C in 90 days (bran A and C) and also at >45°C in 7 days (brand A).

**Table 2: Concentration (µg/l) of phthalates identified in bottled drinking waters before experimental conditions**

| Brand ID | Type of PEs | Primary concentration (µg/l) |
|---|---|---|
| A | DEHP | ND |
| | DBP | ND |
| | TPA | 15,400 |
| | PA | ND |
| B | DEHP | ND |
| | DBP | ND |
| | TPA | 2000 |
| | PA | ND |
| C | DEHP | ND |
| | DBP | ND |
| | TPA | 2700 |
| | PA | ND |

DEHP: di-2-(ethylhexyl) phthalate, DBP: Dibutyl phthalate, TPA: Terephthalic acid, PA: Phthalic anhydride, ND: Not detected, PEs: Phthalates

## Risk assessment

In the current study, based on the goodness-of-fit statistics, probability distributions of exponential and log-normal were determined for DI and body weight, respectively. The probability distribution of logistics was determined for DBP and DEHP's MC. Finally, probability distributions for DBP and DEHP's MC were determined as uniform and gamma, respectively [Table 3].

The results for risk assessment are given in Table 4. The DI and average body weights are obtained at $0.1078 \pm 0.1074$ kg/L and $66 \pm 12.3$ kg, respectively. The results demonstrate that the 95% confidence interval for EDI (Exposure assessment based on the daily intake of PEs) with a probabilistic approach by MCS ranged from $7.93 \times 10^{-4} \pm 1.4 \times 10^{-6}$ to $1.405 \pm 0.00369$ l/person/day. The estimated HQ of DEHP, DBP, TPA, and PA for bottled water consumption were $0.654 \times 10^{-2}$, $0.670 \times 10^{-3}$, $3.96 \times 10^{-7}$, and $0.703 \times 10^{-3}$, respectively, that were far <1. Moreover, the ELCR value was estimated to be $3.09 \times 10^{-5}$ for DEHP [Figure 3].

The results of sensitivity analysis based on the Spearman's rho statistic showed that the MC had a significant main effect on these values for DBP, DEHP, and PA. For TPA, both MC and DI had the significant main impact on the HQ value [Figure 4]. Furthermore, the suitable MC value for limit the cancer risk from DEHP was estimated at $3.06 \times 10^{-5}$ µg/L using Equation (4).

In this study, we also refer to the guidelines for drinking water established by the EPA and WHO. According to Table 4, the MC of DEHP and PA were much more than MCL of EPA and WHO.

**Table 3: Distribution and goodness-of-fit statistics used in the Monte Carlo simulation**

| PEs | Parameter | Distribution | Goodness-of-fit statistics | | |
|---|---|---|---|---|---|
| | | | Kolmogorov-Smirnov | Cramer-von Mises | Anderson-Darling |
| DBP | MC | Logistic | 0.4178250 | 0.8343966 | 4.6988720 |
| | DI | Exponential | 0.2488795 | 1.3256670 | - |
| | BW | Log norm | 0.1133085 | 0.1507766 | 0.8003269 |
| DEHP | MC | Logistic | 0.3057270 | 0.3018247 | 1.7247742 |
| | DI | Exponential | 0.2488795 | 1.3256670 | - |
| | BW | Log norm | 0.1133085 | 0.1507766 | 0.8003269 |
| TPA | MC | Gamma | 0.12424752 | 0.06292291 | 0.41389785 |
| | DI | Exponential | 0.2488795 | 1.3256670 | - |
| | BW | Log norm | 0.1133085 | 0.1507766 | 0.8003269 |
| PA | MC | Uniform | 0.8333333 | 4.3166100 | - |
| | DI | Exponential | 0.2488795 | 1.3256670 | - |
| | BW | Log norm | 0.1133085 | 0.1507766 | 0.8003269 |

DEHP: di-2-(ethylhexyl) phthalate, DBP: Dibutyl phthalate, TPA: Terephthalic acid, PA: Phthalic anhydride, PEs: Phthalates, MC: Concentration of PEs, BW: Body weight, DI: Bottled Water Consumption

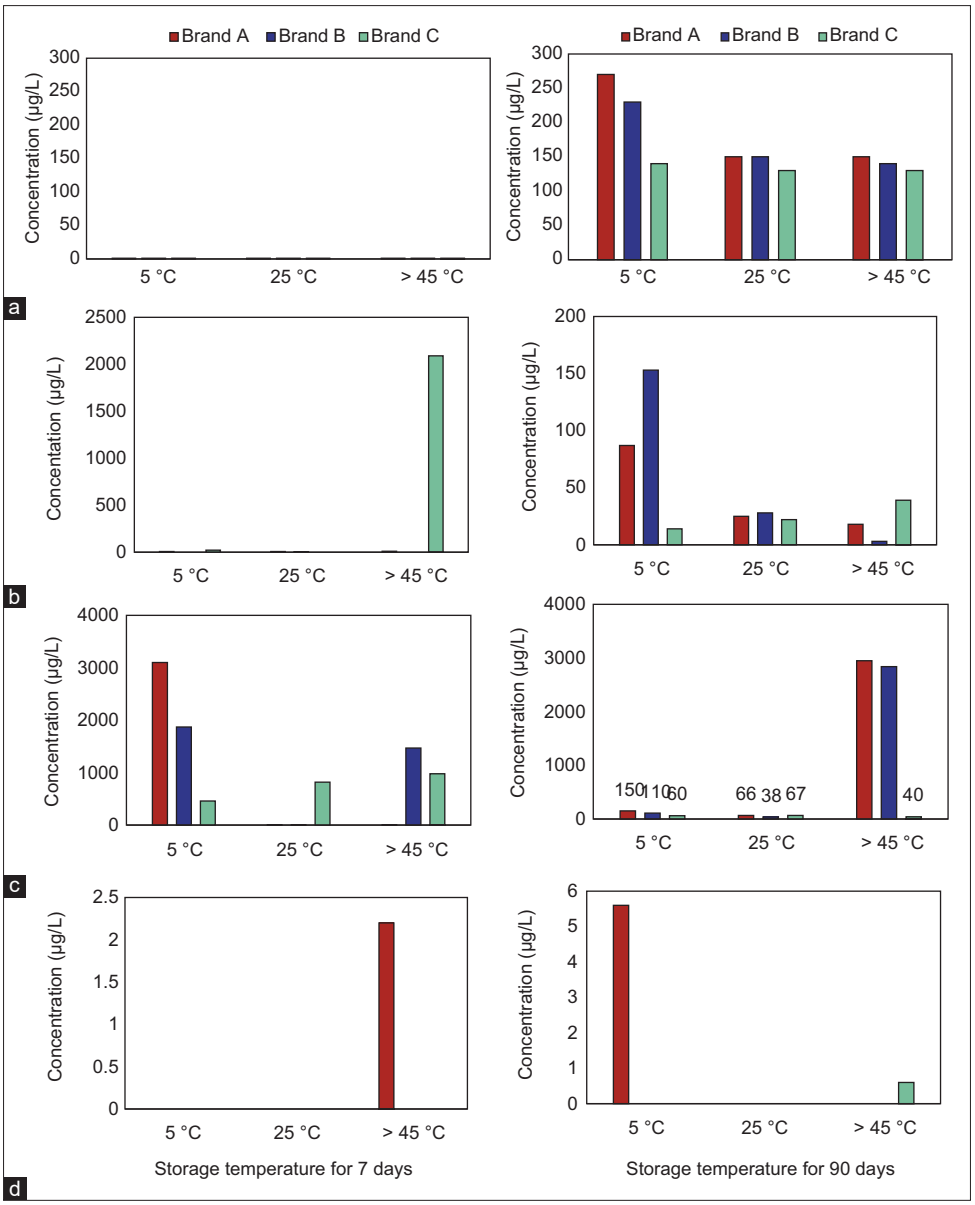


**Figure 2:** Concentration (µg/l) of phthalate esters identified in bottled drinking waters after experimental conditions. (a) di-2-(ethylhexyl) phthalate (b) dibutyl phthalate, (c) terephthalic acid, (d) Phthalic anhydrid

## Estrogenic activity assessment

To assess the potential estrogenic activities of DEHP and DBP, EEQ values were calculated and are summarized in Table 4. The EEQ of DEHP and DBP was 0.023 and 1.63 ng E2/L, respectively. The EEQ level for DBP was reasonably high. Besides, the calculated total EEQ was 1.653 ng E2/L.

## Discussion

PEs are considered to be endocrine disruptors and estrogenic, which can migrate from the plastic container into water, and food and humans can be easily exposed them.[17] In our study, the concentrations of DEHP, DBP, and PA in the preliminary analysis were below the recommended limit by U. S EPA and below LOQ in all initial samples. Moreover, TPA was found in initial samples with the highest concentrations in brand A. As expected, a similar result was expectedly observed by Montuori *et al*. (2008) for TPA as the most abundant compound.[8] However, our findings oppose published studies that surveyed the contents of PEs in drinking bottled water directly after either production or purchase.[12,16,21] Cao (2008) found low levels of DEP, diisobutyl phthalate (DiBP), DBP, and DEHP in PET packaged water and stated that the concentration of DBP was higher (1.72 µg/L) than other phthalates in the water samples.[21] Keresztes *et al*. determined four phthalates (DEHP, BBP, DBP, and DiBP) in mineral water bottled in PET from three different brands as follows: <16 ng/L–1.7 µg/L, <6.0 ng/L–0.1 µg/L, <6.6 ng/L–0.8 µg/L,

**Table 4: Human exposure and risk assessment of phthalates in both human daily intake and estrogenic effect in water bottled in polyethylene terephthalate**

| Parameter | Mean±SD | | | | References |
|---|---|---|---|---|---|
| | DEHP | DBP | TPA | PA | |
| MC (µg/l) | 77.4±95.6 | 39.8±211 | 0.468±0.468 | 831±1392 | This study (MCS) |
| DI (L/day) | 0.1078±0.1074 | 0.1078±0.1074 | 0.1078±0.1074 | 0.1078±0.1074 | Questionnaire |
| BW (kg) | 66±12.3 | 66±12.3 | 66±12.3 | 66±12.3 | Questionnaire |
| Water quality guidelines (µg/l) | 6/8 | 200 | _ | 1 | [6] |
| TDI (µg/kg/bw/day) | 50 | 10 | _ | _ | [19] |
| EDI (µg/kg/day) | 1.31E-01±0.272 | 6.7E-02±0.522 | 7.93E-04±1.40E-03 | 1.405±3.69 | This study |
| RfD (µg/kg/day) | 20 | 100 | 2000* | 2000 | [20] |
| HQ | 0.00654±0.01360 | 0.000670±0.00522 | 3.96E-07±7.01E-07 | 0.000703±0.001843 | This study |
| ELCR | 3.09E-05±3.83E-05 | [a] | - | - | This study |
| EP | $3\times10^{-7}$ | $4.1\times10^{-5}$ | - | - | [3] |
| EEQ (ngE2/L) | 0.023 | 1.63 | - | - | This study |
| EEQ (ngE2/L) for total PEs | 1.653 | | | | This study |

[a]Not available, *For the TPA the RfD of the PA was considered. TDI: Tolerable daily intake, DEHP: di-2-(ethylhexyl) phthalate, DBP: Dibutyl phthalate, TPA: Terephthalic acid, PA: Phthalic anhydride, MC: Concentration of PEs, BW: Body weight, EDI: Estimated daily intake, HQ: Hazard quotient, ELCR: Excess lifetime cancer risk, EEQ: Estrogen equivalence, Bottled Water Consumption

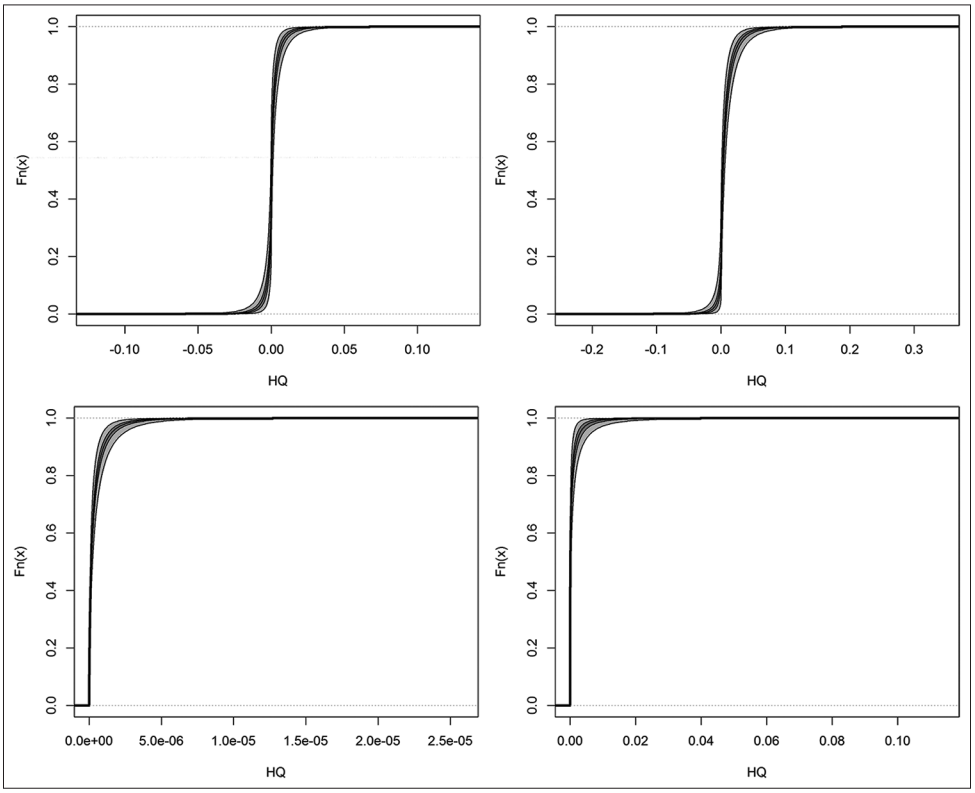


**Figure 3:** Variability cumulative distribution plots of the phthalate esters hazard quotient. For each percentile of variability (y value), the corresponding x value is the point estimate of hazard quotient. The x value of the corresponding points on the light gray lines corresponds to the 95% credible interval

and <3 ng/L–0.2 µg/L, respectively.[12] Recently, a systematic review that assesses a concentration summary of PEs in bottled waters in several countries stated Thailand and Mexico with concentrations of 61.1 and 45.1 µg/L, respectively, as having the highest concentration of DEHP and DBP. The variation in the obtained types and values of PEs between our survey and others might be due to regional differences as well as different production methods and uses of PEs. Furthermore, it might originate from numerous sources such as source water, the raw material of container and packaging, production and bottling processes, and the use of recycled PET as well as municipal and industrial activities. It seems that the major cause of PEs contamination in bottled water originated from plastic bottles.[3,22]

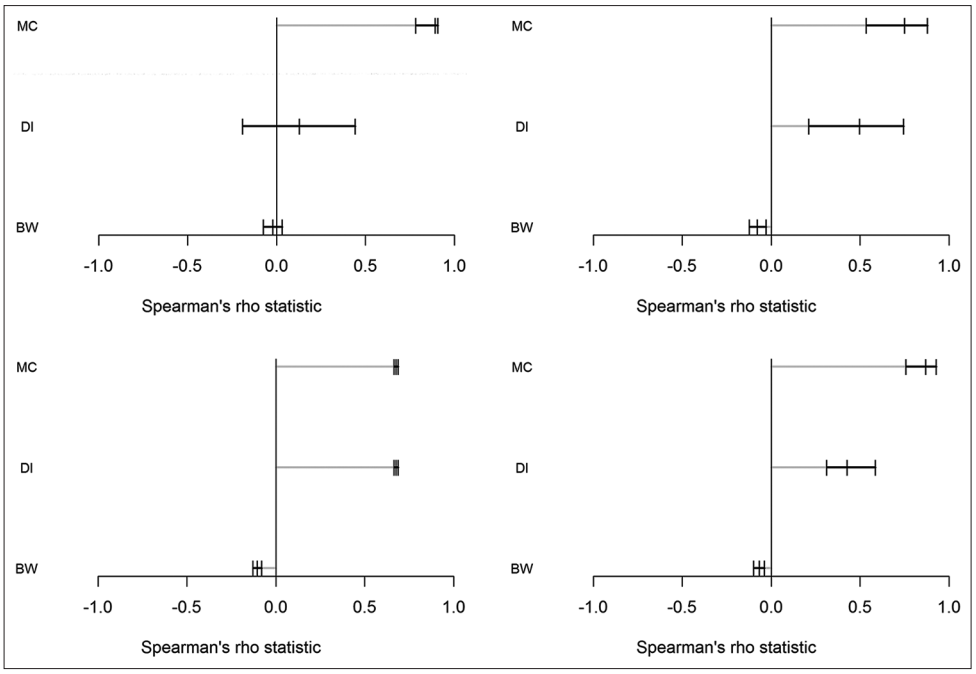


**Figure 4:** Tornado charts showing the spearman's rank correlation between the input variables (MC (Concentration of PEs), DI and body weight) and the output (hazard quotient)

The concentrations of the target compounds under different storage conditions are presented in Figure 2. According to Figure 2, storage time enhanced the migration or formation of DEHP from PET bottles. These results agree with previous studies showing an increasing pattern in the concentrations of PEs.[12,17] Several reports emphasized that migrations of PEs from PET bottles to water have a positive correlation with temperature. For instance, the finding of Al-Saleh *et al*. study that analyzed 150 bottled waters in three different storage conditions verified that the concentration of DEHP, BBP, DMP, and DEP in samples (stored at 4°C for 1 month) were significantly higher than those two groups; however, the opposite trend was observed for DBP.[23] On the contrary, some studies report no essential changes in PEs concentration after storage at various times and temperatures.[12,24,25] For instance, in 2009, Ceretti *et al*. (2010) analyzed six commercial brands PET packaged and glass water stored at 40°C for 10 days based on standard European Commission Directive total migration test.[24] The results of our study indicated that TPA (a monomer of PET) concentration significantly decreased after 7 days in all storage temperatures. Kim and Lee examined migration values of TPA from PET bottle under conditions of Asian legislation (60°C/0.5 h) and EU legislation (40°C/10 d) and found 0.07–0.39 and 0.14–0.39 mg/dm$^2$, respectively.[1] However, increasing of PA concentration was not observed in all almost every case, generally. In fact, the PA concentration was less affected by time and temperature increase. In our study, we also refer to the guidelines for drinking water established by the EPA and WHO. According to Table 4, the MC of DEHP, DBP, and PA was much more than MCL of EPA and WHO. The EPA and WHO set a maximum permitted value for DEHP, DBP, and PA.[6,20] This is in contrast the Jeddi *et al*. (2015). Jeddi *et al*. (2015). They reported that the DEHP concentration in all examined conditions was far below the MCL.[16]

Many PE compounds can produce carcinogenic effects at very low levels, and risk assessment is only a quantitative approach, which can present an estimation of the risk.[26] The HQ for target compounds in water (based on the maximum concentration of PEs under the different storage conditions) was low, and the health-related risk to the adults was negligible. Therefore, there were no adverse health impacts through the consumption of PET bottled water even at the maximum values determined in this study (HQ <1). Previous research documented bottled water as being safe for human consumption.[8,16,17] The ELCR value indicated carcinogenic risks based on the DEHP compound, with $3.09 \times 10^{-5}$, which was higher than the acceptable risk level of $10^{-6}$. Then, probably, consuming drinking water in PET bottles has a carcinogenic hazard. Jeddi *et al*. (2016) demonstrated that ELCR was below the accepted risk level and was negligible.[17] As mentioned above, the suitable concentration for limit the cancer risk from DEHP was calculated by $3.06 \times 10^{-5}$ µg/L. Therefore, the quality of water storage in the PET bottle is the result of numerous factors such as its initial composition, treatment processes, the bottling process, recycling, and storage condition.[27]

Estrogenic compounds at significant concentrations can adversely affect animals, disrupting infertility and mortality.[28] Concerning potential endocrine disruption activities, estrogenic activity determined in PET bottled water. The EEQ level for DBP was reasonably high (1.63 ng E2/L) and could not be ignored. Because, at the low EEQ value (0.27 ng E2/L), the

egg mortality was seen in zebrafish. According to Table 4, the calculated total EEQ levels were at the level of 1.653 ng E2/L, which was 6.1 times more than that causing estrogenic effects on zebrafish as reported by Soares *et al*.[29] A recent systematic review estimated EEQ levels in bottled waters for DEHP, DBP, BBP, and DEP from four different countries (Saudi Arabia, Pakistan, Mexico, and Thailand). Luo *et al*. (2018) reported that the highest and lowest average EEQ of 6.289 and 1.328 ng E2/L were found in drinking bottled water from Saudi Arabia and Thailand, respectively. Our findings are following previous studies; thus, bottled water consumption would likely pose adverse estrogenic impacts on human health.[3,25]

## Conclusion

The migration of four compounds (DEHP, DBP, TPA, and PA) in PET-bottled waters stored for 7 and 90 days under three temperature conditions was investigated in three different brands. In parallel, this study assesses potential risks based on human daily intakes and estrogenic effects via the consumption of bottled water. Among target compounds, only TPA was identified in the water samples that evaluated directly after purchasing. We also demonstrated that the long time (90 days) period increased the migration of DEHP and DBP in PET-bottled water used in this study. Generally speaking, the probabilistic risk assessment by MCS method revealed that studied compounds in PET bottled water are safe (HQ <1); however, the high EEQ values (DEHP and DBP) seemed to have adverse estrogenic effects to adults and serious concern on public health. Furthermore, adults were in carcinogenic risk of DEHP (ELCR $>10^{-6}$). Therefore, the quality of water bottled in PET may change during the long period, and because of widespread use, the long-term monitoring of PEs compounds in PET bottled water is entirely crucial.

### Acknowledgment

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### Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.,) have been completely observed by the authors.

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### Conflicts of interest

The authors declared that there is no conflicts of interest.

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