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Risk Assessment of Phthalate Compounds in Bottled Water Consumed in Isfahan, Iran

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HIGHLIGHTS

- Concentrations of phthalate compounds in some bottled water of Isfahan, Iran were higher than the permitted limits.
- Hazard Quito levels of some phthalate compounds were more than 1 in bottled water.
- Level of some phthalates in bottled water is alarming for public health in this region of Iran.

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Acronyms and abbreviations
DBP=di-buthyl phthalate
DEHP=di-2 ethylhexyl phthalate
DEP=di-ethyl phthalate
HQ=Hazard Quito
PA=Phthalic Anhydride
PET=polyethylene terephthalate
RFD=reference doses
TPA=terphthalic acid
USEPA=US Environmental Protection Agency
WHO=World Health Organization

ABSTRACT

Background: Phthalates are main ingredients of polyethylene terephthalate (PET) bottles used for storage of water. These compounds can cause adverse effects on human health. The purpose of this study was the quantification of the amounts of phthalates migrated in bottled water as well as the risk assessment of those compounds.

Methods: This cross sectional study was performed on 15 PET bottled water samples of popular brands distributed in Isfahan city, Iran. The samples were immediately sent to laboratory for analysis. Amounts of Phthalic Anhydride (PA), di-2 ethylhexyl phthalate (DEHP), di-buthyl phthalate (DBP), di-ethyl phthalate (DEP), and terphthalic acid (TPA) in bottled water samples were measured using Gas Chromatography-Mass Spectrometry (GC-MS) analysis. Risk assessment of migrated compounds were calculated and compared with Hazard Quito (HQ) standards.

Results: The ranges of PA, DEHP, DBP, and TPA were 2.3-26, 171-845, 30-2251, and 24-657 ppb, respectively. DEP was not found in none of the analyzed samples. HQ levels of DEHP, TPA, and DBP were more than 1 in bottled water.

Conclusion: In the current study, the concentration of DEHP, TPA, and DBP in some bottled water of Isfahan, Iran were higher than the permitted limits. Also, HQ levels of these three migrated compounds were more than 1 in bottled water that is alarming for public health in this region of Iran.

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Introduction

Safety assurance of water stored in plastic bottles is considered worldwide (Jin et al., 2010). Bottled water is principally produced from spring, ground, surface and tap water. This product is widely consumed based on easy

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use, suitable price, better taste, low impurity, and proper hygiene status (Ikem et al., 2002). Today, the most common polymer used for the bottling of drinking water is polyethylene terephthalate (PET). The usage of PET bottle for storage of water is increasing in last decades (Alonso et al., 2012; Ikem, 2010; Leivadara et al., 2008; Wagner and Oehlmann, 2011). The migration of ingredients of PET bottle to its content is always a controversy subjects globally. Bottled water can be contaminated in different phases of the production process, from supplying of the materials to handling, storing, and distribution (Bach et al., 2013; Salazar-Beltrán et al., 2017).

Despite PET being a material characterized as inactive chemical ingredients, various studies pointed out that different storage conditions such as exposure to sunlight and high temperatures has influences on the migration of PET ingredients to its contents (Amiridou and Voutsa, 2011; Pinto and Reali, 2009). These probable migrated compounds include phenols, phthalates, polycyclic aromatic hydrocarbons, and pesticides (Al-Mudhaf et al., 2009).

For increasing the flexibility of PET bottles, phthalate esters as plasticizer are applied in manufacturing of this product. These compounds are considered hazardous chemicals to environment and also human health since they have been associated to organ damage, infertility, birth defects as well as testicular cancer (Yan et al., 2010; Zarean et al., 2015; Zhang and Lee, 2013). For example, the results of an experimental study showed that di-2 ethylhexyl phthalate (DEHP) had diverse health hazards on different organs including reproductive, neurodevelopment, and respiratory systems. Also, in human studies, it is reported that exposure to this compound had carcinogenic effects and influenced neurodevelopment in early life (Zarean et al., 2016). Some phthalates such as DEHP, di-butyl phthalate (DBP), and butyl benzyl phthalate classified in Group 1 for the ability of disrupting of the endocrine and malfunction of the metabolism by the Institute of Health and the US Environmental Protection Agency (USEPA) (IEH, 2005). Furthermore, DEHP is introduced with probable carcinogenic effect in International Agency for Research on Cancer and Integrated Risk Information System. Phthalates cannot be connected by covalent bond to plastic materials and therefore it could be released into the water over the time (Yan et al., 2010; Zhang and Lee, 2013).

Reference doses (RFD) defined by the USEPA is used to evaluate the safety of foods transferring phthalate to human. For calculation of RFD, the estimation of daily oral exposure examines for human population in specific and sensitive subgroups (Montuori et al., 2008). So, it is important to evaluate the risk assessment of migrated phthalate compounds in PET bottles.

In some studies performed previously in Iran, some chemical compounds such as nitrate, nitrite, and organic chemical compound were quantified in bottled water (Ebrahimi et al., 2016; Moazeni et al., 2014). Therefore, it is purposed a research to quantify the amounts of phthalate compounds such as Phthalic Anhydride (PA), DEHP, DBP, di-ethyl phthalate (DEP), and terphthalic acid (TPA) in water bottles consumed in Isfahan, Iran. Afterwards, the comparison of results was performed with standards defined in international organization such as World Health Organization (WHO) and USEPA. Then, the risk assessment of phthalates was determined through the consumer's exposure with consumption of bottled water in Isfahan, Iran.

Materials and methods

Materials

In this study, the standards of phthalate compounds were provided from Sigma Aldrich Company, America. Methanol and methyl tert-butyl ether (MTBE) with 99.9% purity were purchased from Merck Company (Germany).

Sampling

This cross sectional study was performed on 15 PET bottled water samples of popular brands distributed in Isfahan city, Iran from January to June 2016. The samples were immediately sent to laboratory for analysis.

Extraction and measurement of phthalates

Each water sample was analyzed for quantification of 5 types of phthalates including PA, DEHP, DBP, DEP, and TPA by Gas Chromatography-Mass Spectrometry (GC-MS) analysis (Agilent technology 7890A model and mass detector spectrometry 5915C model Equipped by a HP5 column). Derivation of inorganic (rigid) phase accomplished with vacuum system by the usage of cartridge C_{18} (CHROMABOND® C_{18}ec-3 ml/500 mg, Germany).

First, cartridge C_{18} was washed with methanol and deionized water. Around 300 ml of the sample passed through the vacuum. The cartridge was washed with 2 ml methanol and kept in a small vial. Afterwards, derivational solution was dried with nitrogen gas; and phthalates were diluted by 100 µl of MTBE for injection to GC-MS. Helium with 99.99% purity was used as a carrier gas in flow of 1 ml/min. The temperature program was 100 °C for 1 min, increased to 210 and 250 °C in 10 and 5 °C/min. Finally, temperature increased to 280 °C in 10 °C/min (Ebrahimi et al., 2016).

Comparison of measured phthalates with standards

The maximum amount of allowable emissions for some phthalate compounds is described in drinking water by USEPA and WHO. For DEP and PA, no limit is defined through both organizations. It is mentioned 6 and 8 ppb for DEHP by USEPA and WHO, respectively. The standard of TPA and DBP is expressed in 1 and 200 ppb by USEPA. However, no limit for these compounds is outlined by WHO.

Risk assessment of phthalate compounds

Evaluation of risk assessment of phthalate compounds migrated to PET bottles was determined by equation 1:

$$EDI=(MC\times DI)/BW \quad (1)$$

In equation 1, EDI, MC, DI, and BW are Estimated Daily Intake via drinking water (mg/kg body weight/day), Maximum Concentration of phthalate in bottle water (mg/l), the recommended Daily Intake of water consumption each day in Iran (0.1 l/d) and the humans body weight (60 Kg), respectively. Equation 2 was used for the estimation of the risk of phthalates exposure through using the bottled water for creation of non-carcinogenic effects in humans.

$$\text{Hazard Quito (HQ)}=EDI/RFD \quad (2)$$

In equation 2, HQ is defined as hazard quotient as well as RFD is the amount of permissive facing with phthalate esters without creation non-carcinogenic effects in human exposure from all sources and routs (mg/Kg BW/day). RFD of PA, DEHP, DBP, DEP, and TPA are defined 0.5, 0.02, 2, 0.8, and 0.01, respectively (USEPA, 2000).

Results

Table 1 shows the results of phthalates concentrations in PET bottled water. PA was detected in 4 out of 15 water samples in the range of 2.3 to 26 ppb as well as DEHP in 10 out of 15 samples in the range of 171 to 845 ppb. DBP was found in all samples from 30 to 2251 ppb. A different amount of TPA was observed in 14 samples of water from 24 to 657 ppb. DEP was not found in none of analyzed samples.

The concentration of DEHP in all samples was more than standard limit defined by the USEPA (6 ppb) and WHO (8 ppb). Permissive extent of DBP in accordance with USEPA is 200 ppb that the amount of these compounds in one sample was much more than standards. TPA in bottled waters permitted in 1 ppb according to USEPA standards. The results of this study showed that the amounts of this compound were more than permitted limit in all tested samples.

The results of risk assessments of phthalate compounds in bottled water are shown in Table 2. HQ levels of DEHP, TPA, and DBP were more than 1 in bottled water, showing health risks for the consumers.

Table 1: Concentration of phthalate compounds in bottled water of Isfahan, Iran

Sample Code	Concentration of phthalate compounds (ppb)				
	di-ethyl phthalate	terphthalic acid	di-buthyl phthalate	di-2 ethylhexyl phthalate	phthalic anhydride
1	ND	24	30	171	ND
2	ND	36	57	845	ND
3	ND	91	45	701	26
4	ND	172	33	ND	ND
5	ND	39	71	ND	ND
6	ND	200	46	845	ND
7	ND	657	49	813	ND
8	ND	392	34	ND	ND
9	ND	115	50	341	ND
10	ND	459	2251	ND	19.4
11	ND	68	63	226	2.3
12	ND	110	66	282	ND
13	ND	57	68	ND	ND
14	ND	ND	52	314	ND
15	ND	94	38	235	4

ND: Non Detectable

Table 2: Phthalates risk assessment resulting from the consumption of bottled water in Isfahan, Iran

Phthalates*	Maximum concentration (ppb)	Daily intake (l/d)	Estimated daily intake (mg/Kg BW/day)	Hazard Quito
phthalic anhydride	26	0.1	0.04	0.08
di-2 ethylhexyl phthalate	845	0.1	1.4	70
di-buthyl phthalate	2251	0.1	3.7	1.85
terphthalic acid	657	0.1	1.09	109

* di-ethyl phthalate was not detected in samples.

Discussion

In the present study, concentration of PA, DEHP, DBP, and TPA varied in the water samples. Nearly, DEHP content in the tested samples of bottled water was higher than other phthalate esters. For risk assessment, HQ levels of DEHP, DBP, and TPA in bottled water were more than 1 representing adverse effect on health. According to the previous studies in other countries, concentrations of phthalate esters depend on to the time, temperature, pH, and different types of bottled waters. For instance, in a study in Croatia, the level of phthalate in samples of plastic containers, soft drinks, and mineral water packed in PET ranged from 20.22 to 819.40 ppb. However, risk assessment showed that there was no risk for human health for the local consumers (Bošnir et al., 2007).

Based on survey of Leivadara et al. (2008) on bottled drinking waters in Greek, no volatile and carbonyl constituents as well as other carcinogen and hormone disrupter phthalates were recognized except for the plasticiser phthalate and DEHP. These authors concluded that the existence of organic compounds was influenced by parameters such as conditions of storage and type of water. In a research performed on a large number of Italian bottled water samples, different concentration of compounds such as DEHP, dimethyl phthalate, DEP, diisobutyl phthalate, and DBP were found with a range of 2.11 to 4.81 ppb. In glass bottle, the amounts of aforementioned migrated compounds were from 0.13 to 0.36 ppb. Nearly, the concentration of phthalates was 20 times higher in samples bottled in PET than glass container. However, the observed levels do not represent a significant exposure pathway when comparing with the standards (Montuori et al., 2008). In 2009, a total of 623 samples of household drinking water and 568 samples bottled water available in Kuwaiti markets were examined for the existence of different semi volatile compounds. Eight of these compounds were detected in the household water, including four phthalate and one adipate esters, one polyaromatic hydrocarbon (anthracene), 2,6-dinitrotoluene, and hexachlorobenzene. But all bottled waters were found to be totally lack of the aforementioned compounds. All detected migrated semi volatile compounds in household and bottled waters, except styrene, were measured at amount much lower than those established as safe by WHO and USEPA (Al-Mudhaf et al., 2009). According to another survey, analytical results of phthalates showed the amount of 9.1, 10.3, and 11.3 ppb for dioctyl phthalate, di isooctyl phthalate, and DBP in bottled water samples in Korea (Yan et al., 2010). In Greece, most of alkylphenols and phthalates were detected in different brands of bottled water purchased from local market. The maximum concentration of DEHP, DEP, bisphenol A, as well as nonylphenol were 0.580, 0.070, 0.170, and 0.150 ppb, respectively (Amiridou and Voutsa, 2011). Differences in the results of the present study with other surveys can be related to differences in the number of collected samples, method of detection, and the kind of proceudre for PET production and water purifecion in processing plants.

In a research conducted in France, the impact of variable temperatures on the release of PET bottle constituents into water were investigated as well as the potential health hazard was assessed using *in vitro* bioassays with bacteria and human cell lines. DEHP as an intermediary monomer was found in PET bottled waters. However, the PET-bottled water immigrants did not induce toxic activity in the bioassays after exposure (Bach et al., 2013). Unlike our findings in Isfahan (Iran), the total daily phthalate intake via consumption of PET bottled waters in France was below the recommended limits (Martine et al., 2013).

Most of the organic chemical compounds including phthalate, alkyl phenol, alkene, and organic acid were previously detected in bottled water collected from Isfahan, Iran (Ebrahimi et al., 2016). In addition, Zaki and Shoeib (2018) showed the presence of DEHP and DBP in Egyptian bottled waters with a mean concentration of 0.104 and 0.082 ppb, respectively. These researchers stated that there were significant positive correlations between the storage time and the concentration of phthalate compounds. The estimated daily intake of phthalate compounds from Egyptian PET bottled water was below the tolerable level, showing no adverse health effects. Conversely, we found considerable phthalates in bottled water of Isfahan, Iran which were higher than permitted levels. These differences may be due to variations in storage time and temperature of bottled waters.

Conclusion

In the current study, the concentration of DEHP, TPA, and DBP in some bottled water of Isfahan, Iran were higher than the permitted limits. Also, HQ levels of these three migrated compounds were more than 1 in bottled water that is alarming for public health in this region.

Author contributions

F.E., Z.E., and H.P. designed the study; F.E., M.M., and H.P. did the experiments; F.E., H.K., M.M., Z.E., M.Z., and H.P. analyzed data and wrote the manuscript. All authors read and approved the final manuscript.

Conflicts of interest

There is no conflict of interest to declare.

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