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Study on the leaching of phthalates from polyethylene terephthalate bottles into mineral water

Szilvia Keresztes[1], Enikő Tatár[1], Zsuzsanna Czégény[2], Gyula Záray[1], Victor G. Mihucz[1,*]

[1]Laboratory of Environmental Chemistry and Bioanalytics, Department of Analytical Chemistry, Institute of Chemistry, Eötvös Loránd University, H-1117 Budapest, Pázmány Péter sétány 1/A

[2]Institute of Materials and Environmental Chemistry, Research Centre for Natural Sciences, Hungarian Academy of Sciences, H-1525 Budapest P.O. Box 17, Hungary

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*Corresponding author: Victor G. Mihucz, Laboratory of Environmental Chemistry and Bioanalytics, Department of Analytical Chemistry, Institute of Chemistry, Eötvös Loránd University, H-1117 Budapest, Pázmány Péter sétány 1/A, tel: +36-1-372-2555; 1227 ext.; fax: +36-1-372-2608; e-mail: vigami72@yahoo.es

Abstract

Carbonated and non-carbonated mineral water samples bottled in 0.5-L, 1.5-L and 2.0-L polyethylene terephthalate (PET) containers belonging to three different water brands commercialized in Hungary were studied in order to determine their phthalate content by gas chromatography - mass spectrometry. Among the six investigated phthalates, diisobutyl phthalate, di-n-butyl-phthalate, benzyl-butyl phthalate and di(2-ethyl-hexyl) phthalate (DEHP) were determined in non-carbonated samples as follows: <3.0 ng L^{-1} - 0.2 μg L^{-1}, <6.6 ng L^{-1} - 0.8 μg L^{-1}, <6.0 ng L^{-1} - 0.1 μg L^{-1} and <16.0 ng L^{-1} - 1.7 μg L^{-1}, respectively. Any of the above-mentioned phthalate esters could not be detected in carbonated mineral water samples. DEHP was the most abundant phthalate in the investigated samples. It could be detected after 44 days of storage at 22 ºC and its leaching was the most pronounced when samples were stored over 1200 days. Mineral water in PET bottles of 0.5 L had the highest phthalate concentrations compared to those obtained for waters of the identical brand bottled in 1.5-L or 2.0-L PET containers due to the higher surface/volume ratio. No clear trend could be established for phthalate leaching when water samples were kept at higher temperatures (max. 60 ºC) showing improper storage conditions. Phthalate determination by pyrolysis - gas chromatography/ mass spectroctrometric measurements in the plastic material as well as in the aqueous phase proved the importance of the quality of PET raw material used for the production of the pre-form (virgin vs. polymer containing recycled PET).

1. Introduction

Lately, due to the increasing popularity of mineral water consumption, several papers have drawn the attention to the occurrence of pollutants in bottled water that may pose a health risk to consumers. Among them, Sb and phthalates are considered to be being emerging pollutants in bottled mineral water. Phthalates are used as plasticizers to increase the flexibility of plastics such as PVC. As they are not chemically bound in plastics and such, they can be leached into the environment (Liu et al., 2008; Penalver et al., 2000; Pinto and Reali, 2009; Serôdio and Nogueira, 2006). Moreover, phthalates are lipophilic compounds, and have been found to bio-accumulate in fats (LaFleur and Schug, 2011). Exposure to phthalates can induce detrimental effects to human health. The larger molecular weight phthalates - di(2-ethyl-hexyl) phthalate (DEHP), di-n-butyl phthalate (DBP), diisononyl phthalate (DiNP) - are suspected carcinogens, as well as toxic to liver, kidneys (Gomez-Hens et al., 2003) and reproductive organs (Swan et al., 2005). Di-n-butyl phthalate, benzylbutyl phthalate (BBP), DEHP are weakly estrogenic. Some metabolites of phthalates such as mono-2-ethyl-hexyl phthalate (MEHP), mono-n-butyl phthalate (MBP) and monoethyl phthalate are also capable of disturbing the hormonal activity (2001/262/EC). Thus, it was reported that endocrine disruptor activity was found in most of the examined PET-bottled water samples (Plotan et al., 2012; Pinto and Reali, 2009; Wagner and Oehlmann, 2011). According to these reports, the oestrogenic, androgenic and progestogenic activities have not reached alarming levels. However, the safe level of glucocorticoid activity is still unknown. The tolerable daily intake (TDI) values established by the European Food Safety Authority (EFSA) panel (2013a; 2013b; 2013c) for BBP, DBP and DEHP are 500 μg/kg/bw/day, 10 μg/kg/bw/day and 50 μg/kg/bw/day, respectively. A TDI has not yet been defined for diisobutyl phthalate (DiBP)

as the necessary data from long-term toxicity studies with various DiBP doses are not available.

For bottled water, PET is the most widespread bottling material. Although the European Commission regulation No. 10/2011 as of 14 January 2011 does not authorize the use of phthalates for manufacturing food-contact materials, phthalates have been detected in PET material and in PET-bottled water. There are several possibilities for the occurrence of phthalates in bottled water such as: (i) quality of the raw material as well as the technology used in bottle production (Amiridou and Voutsa, 2011; Schmid et al., 2008) or perhaps chemicals used in the production process (Plotan et al., 2012, Wu et al., 2012)); (ii) use of recycled PET (Bach et al., 2012); (iii) contamination of the water sources with decomposed plastic wastes of dumps (Baram et al., 2000); (iv) cross-contamination in the bottling factory as phthalates are ubiquitous in the environment (Biscardi et al., 2003; Higuchi et al., 2004; Leivadara et al., 2008; Liu et al., 2008); (v) cap sealing resins (Hirayama et al., 2001) may present contamination.

As phthalates do not persist in the outdoor environment due to bio-, photo-, and anaerobic degradation, their quantitative determination is a challenging task. For this purpose, the most widespread used analytical technique is gas chromatography mass spectrometry (GC-MS) (Biscardi et al., 2003; Bošnir et al., 2007; Cao, 2008; Fierens et al., 2012) characterized by excellent detection limits in the low pg mL^{-1} concentration range. A sample preparation step allowing pre-concentration of the investigated phthalate esters is required (Psillakis and Kalogerakis, 2003) due to the low concentration of the target compounds (< 2 μg L^{-1} for diethyl phthalate –DEP-, DBP and DEHP). The problem for the quantitative determination of phthalates arises during sample preparation, because of their ubiquitous presence in laboratory wares made of plastic materials, reagents and sample preparation devices like solid phase extraction (SPE) cartridges. Thus, among several options (LaFleur

and Schug, 2011), liquid-liquid extraction (LLE), SPE, solid phase microextraction (SPME), cloud point extraction and stir bar sorptive extraction are used for this purpose. The more conventional LLE performed with dichloromethane (Amiridou and Voutsa, 2011; Ferretti et al., 2007 and Fierens et al., 2011), dichloromethane and pentane (Leivadara et al., 2008), hexane (Holadova and Hajslova, 1995; Schmid et al., 2008), ethyl acetate (Criado et al., 2005) or acetone (Biscardi et al., 2003) seems to be one the best choices as recovery values ranged between 70-100% for the most frequently occurring phthalate esters. After pre-concentration, the resulting extracts are dried with anhydrous sodium sulphate, evaporated to 1-2 mL volume and analyzed by GC-MS. Among phthalate esters occurring in soft drinks, DEHP is the most frequent and it can be determined in the highest concentration (Amiridou and Voutsa, 2011; Biscardi et al., 2003; Bošnir, et al., 2007; Schmid et al., 2008). DBP (Bošnir et al., 2007; Cao, 2008, Criado et al., 2005 ; Montuori et al., 2008), DiBP (Cao, 2008; Fierens et al., 2012; Montuori et al., 2008) and DEP (Bošnir et al., 2007; Cao, 2008; Montuori et al., 2008) also frequently occur at considerable concentrations, meanwhile BBP (Al-Saleh et al., 2011), dimethyl phthalate (DMP) and di-n-octyl phthalate (DnOP) are seldom detected. Phthalate ester concentrations reported in the literature do not exceed the specific migration limit values of 0.3 mg/kg, 30 mg/kg and 1.5 mg/kg for DnBP, BBP and DEHP, respectively, established by the EC directive No. 10/2011.

The phthalate concentration of PET bottled mineral water may vary with pH (Montuori et al., 2008); storage time (Biscardi et al., 2003; Criado et al., 2005), storage temperature (30 ºC - 60 ºC) (Casajuana and Lacorte, 2003; Schmid et al., 2008) and exposure to sunlight (Leivadara et al., 2008; Schmid et al., 2008). Photolysis may be a significant pathway for abiotic degradation of phthalates in waters (Peterson, 2003). As unequivocal conclusion from the above-mentioned studies could not be drawn, in this research, which is a complementary study of a previous report on Sb leaching by mineral water (Keresztes et al.,

2009), a systematic study was conducted on samples of three different mineral water brands bottled in PET material.

2. Experimental

2.1. Chemicals and reagents

Throughout the study, distilled water was used for sample/standard preparation. Dichloromethane and sodium sulphate anhydrous, both analytical reagent grade, were purchased from Molar Chemicals Ltd. (Budapest, Hungary) and LGC Standards GmbH (Wesel, Germany), respectively. Hydrochloric acid used for acidification of samples was of Suprapur® quality and purchased from Merck Ltd. (Budapest, Hungary). Phthalate standards (DEP, DMP, DiBP, DBP, BBP and DEHP) were purchased from Sigma Aldrich (St Louis, MO, US). Wool cotton fabricated according to British Pharmacopeia standards was used throughout the study.

2.2. Samples

Systematic investigation of three mineral water brands with similar chemical composition (labelled subsequently with A, B and C) was conducted. Carbonated and non-carbonated mineral water bottled in PET containers were purchased from supermarkets. All samples were stored in an air-conditioned laboratory in the dark at 22 °C, not longer than a week prior to processing. Therefore, for the determination of the temporal distribution of phthalates, C water samples bottled in 2-L PET containers were used. For the investigation of phthalate occurrence in carbonated and non-carbonated samples, mineral water bottled

in 1.5 L PET containers from all three water brands were purchased. For the contact surface study, 0.5-L, 1.5-L and 2.0-L water samples of brand C were used. For studying the effect of temperature on the leaching of phthalates, 0.5-L water samples were investigated for all three brands (A, B, and C). Water samples serving as blanks could be taken directly from the springs of the bottling companies for each brand into 1.5 L-glass bottles. Thermostation of samples at 40 °C, 50 °C and 60 °C for 24 h was achieved in a U-10 thermostat (VEB MLW, Germany) in an air-conditioned laboratory. Moreover, samples thermostated at 60 °C were further kept on standing at this temperature for additional 24 h and 48 h. The samples were analysed immediately after thermostation.

2.3. Sample preparation

First, water samples were manually homogenized and then aliquots of 480 mL were taken for subsequent liquid-liquid extraction. After setting pH=4 with cc. HCl, each sample was transferred into a 0.5-L glass separating funnel. Then 20 mL of dichloromethane was added. After this step, samples were mechanically shaken for 2 min. After the separation of the aqueous and the organic phases, the extraction was repeated twice for the same sample. Extracts were dispensed into a 100-mL beaker. These combined extracts were filtrated by passing through a layer of 6.42 g of anhydrous sodium sulphate placed over cotton wool in a small glass funnel. After filtration, this funnel was flushed with 2.5 mL dichloromethane and the resultant organic leachate was added to the filtrate. The extracts were concentrated by evaporation in the fume cupboard at 22 °C. The final volume of the extracts was 2 mL. They were stored at 4 °C in 4-mL tightly closed graded glass vials prior to analysis.

2.4. Preparation of stock solutions and standards

For the sensitivity check, a multi-component stock solution was prepared in dichloromethane from individual standards of DEP, DMP, DiBP, DBP, BBP and DEHP in concentration of 15 mg L^{-1}, 20 mg L^{-1}, 5 mg L^{-1}, 15 mg L^{-1}, 4 mg L^{-1} and 65 mg L^{-1}, respectively. Then, 20 μL were taken from this stock solution and placed in a 2-mL graded glass vial and filled up with dichloromethane. After homogenization, 1 μL from this solution was injected into the GC column. Quantification was done by using the external calibration method with 5 different standards prepared from the original multi-component stock solution showing linear regression correlation $R^2 > 0.98$ for all target analytes.

2.5. Instrumentation and operating conditions

2.5.1. Phthalate determination by GC-MS

Phthalates were determined by GC-MS without derivatisation. For the quantitative determination, a Varian 4000 tandem GC-MS system was used. The operating conditions were made accordingly to our previously developed method (Sebők et al., 2009). Briefly, on-column (a) injections were made at 100 ºC, and held at 100 ºC for 0.1 min, then heated to 300 ºC (200 ºC min^{-1}), with a 3 min hold at 300 ºC; (b) column temperature started at 100 ºC, for 1 min, then heated up to 300 ºC with a heating ramp of 20 ºC min^{-1}, and a 5.5 min hold at 300 ºC. Helium was used as a carrier gas. For the full scan spectra, the acquired m/z range varied from 76 to 400. The measurements were done in selected ion monitoring mode at m/z 149. For each sample, the phthalate concentrations calculated

corresponded to the average of three aliquots of 480 mL taken from the same batch purchased and subjected to the sample preparation protocol described in Section 2.3.

2.5.2. Pyrolysis–gas chromatography/mass spectrometry (Py–GC/MS)

In order to determine the phthalate content of PET bottles, Py-GC/MS experiments were performed. Pyrolysis experiments were carried out on a Pyroprobe 2000 pyrolyser (Chemical Data Systems). About 6 mg was cut from the neck of the bottle where contact of PET with water was minimal. Each sample was heated to 350°C (calibrated sample temperature) for 30 s in a quartz tube using helium as a carrier gas. Analysis of the volatile products was accomplished on line with a GC-MS (Agilent Techn. Inc. 6890 GC / 5973 MSD) using DB-1701 capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness). The pyrolysis interface was heated to 300°C. The GC injector was kept at 300°C. The GC oven was programmed to hold the temperature at 50°C for 1 min and then increase it to 280°C at a rate of 30°C min^{-1}. The mass spectrometer operated at 70 eV in the electron impact mode. Due to the low concentration of the analytes, the MS was operated in selected ion monitoring mode. The intensity of m/z 149 ion was selected as it is the most characteristic ion of the measured phthalate molecules. Peak area of m/z 149 ion chromatogram was integrated for qualitative comparison. The peak areas were related to the mass of the PET samples. For each brand, the phthalate peak areas calculated corresponded to the average of three polymer samples cut from three different bottles of the same batch purchased.

3. Results and discussion

3.1. Determination of phthalates in mineral water bottled in PET

The elution order of the phthalates separated by GC-MS was the following: DEP, DMP, DiBP, DBP, BBP and DEHP. Peaks of the phthalates were resolved in 8 min. However, the time for a complete chromatographic run was about 20 min. Among the above-mentioned phthalates, only DiBP, DBP, BBP and DEHP could be determined quantitatively independently of the mineral water brand (Figure 1 a). The limit of quantitation (LOQ) for DEP, DMP, DiBP, DBP, BBP and DEHP was: 22.2 ng L^{-1}, 6.8 ng L^{-1}, 3.0 ng L^{-1}, 6.6 ng L^{-1}, 6.0 ng L^{-1} and 16.0 ng L^{-1}, respectively. For blank subtraction, the phthalate concentrations obtained for the corresponding mineral water taken directly from the well were used. The water samples taken from the wells were subjected to the same analytical procedure thus, they can be considered as procedural blanks. Moreover, the RSD obtained for the peak area of DiBP, DBP, BBP and DEHP in the procedural blank was 5.6%, 4.9%, 2.7% and 8.0%, respectively. Generally, the obtained analyte concentrations were three times higher than the corresponding blank values.

A recovery study for the investigated phthalates was done at four different levels by spiking 1-, 2-, 5- and 10-times the concentration values corresponding to those obtained for DiBP, DBP, BBP and DEHP in mineral water C. The recovery rate ranged from 79% - 86% for DiBP, 80% - 92% for DBP, 70% - 89% for BBP and 78% - 87% for DEHP. The subsequently presented results were not corrected with the average recovery values obtained.

Mineral water A can be considered as the less affected by phthalate occurrence, which means that any phthalate could not be determined in the initial samples. The total concentration of phthalates in mineral water brand B and C bottled in 0.5-L PET containers originating from supermarkets did not exceed 1.8 μg L^{-1} and 1.6 μg L^{-1}, respectively. The concentration range of DiBP, DBP, BBP, DEHP in all investigated non-carbonated mineral

water samples was: <LOQ - 0.2 μg L^{-1}, <LOQ - 0.8 μg L^{-1}, <LOQ - 0.1 μg L^{-1} and <LOQ - 1.7 μg L^{-1}, respectively. With respect of the investigated phthalates, the highest concentrations were observed for DEHP in each water sample.

Compared to the literature data, generally, the same phthalates - DiBP, DBP, BBP and DEHP - were determined in the investigated water samples like in other reports dealing with determination of phthalate content in mineral water bottled in PET (Amiridou and Voutsa, 2011; Bošnir et al., 2007; Cao, 2008; Psillakis and Kalogerakis, 2003). However, Bošnir et al. (2007) reported higher DEHP and DBP concentrations in mineral water: 8.8 μg L^{-1} and 11.3 μg L^{-1}. Cao (2008) reported slightly lower concentration values for BBP and DEHP than in the present report: <0.085 μg L^{-1} and 0.102 μg L^{-1}, respectively. Our findings correlate better with the results of Psillakis and Kalogerakis (2003) as well as with those reported by Amiridou and Voutsa (2011). Thus, Psillakis and Kalogerakis (2003) reported a 0.1 μg L^{-1} and 0.4 μg L^{-1} concentration for DBP and DEHP in PET-bottled mineral water, respectively, by using an SPME method. According to Amiridou and Voutsa (2011), DEHP was the most abundant phthalate in bottled water, like in the present report, with a typical concentration of 0.35 μg L^{-1}. In the same report, the mean concentration of DBP was 0.044 μg L^{-1}. However, Bošnir et al. (2007), Psillakis and Kalogerakis (2003) as well as Amiridou and Voutsa (2011) also reported the occurrence of DEP: 0.11 μg L^{-1}, 0.05 – 0.13 μg L^{-1} and 0.033 μg L^{-1}, respectively.

A person weighing 70 kg and drinking daily 1500 mL of mineral water of 0.08 μg kg^{-1} BBP, 0.60 μg kg^{-1} of DBP, 2.98 μg kg^{-1} of DEHP and 0.20 μg kg^{-1} of DiBP corresponding to the maximum concentration values of these phthalates investigated in the mineral water samples in the present study, its estimated intake of BBP (0.12 μg/day), DBP (0.90 μg/day), DEHP (4.47 μg/day) and DIBP intake (0.30 μg/kg) would not exceed the TDI values

currently set for phthalates (EFSA 2013a; EFSA 2013b; EFSA 2013c) not even reaching 1% for DEHP.

3.2. Determination of phthalates in PET bottle by Py-GC/MS

A usual polymer sample mass for pyrolysis is around 0.5 mg. In our case, due to the low phthalate concentration of the polymer samples, a relatively high sample mass, about 6 mg was analysed by Py-GC/MS. Samples were heated to 350°C because at this temperature the polymer sample melts, but do not decompose and the phthalate content of PET sample evaporates. Higher pyrolysis temperature than 350 °C is not suitable, because the macromolecular chain of PET starts to decompose and the high amount of volatile decomposition products overload the column. In the PET material of mineral water brand A, only DEHP was detected. Among the other three phthalates determined in the water samples, BBP was not be detected in any PET sample, while DiBP and DBP was detected in PET bottle of mineral water brands B and C as traces (Figure 1 b). Although phthalate extraction from the PET bottle samples was not quantitative by melting, for example, similar leaching patterns could be established for DEHP in the PET material and all water samples. Moreover, the lowest occurrence of BBP in the different water samples explained why this phthalate was not expected to be detected by the mild Py-GC/MS. As calibration is not possible in the lack of a standard PET material with known phthalate content, the areas of the peaks after blank subtraction was divided by the mass of the sample in order to compare the different bottle materials. The standard deviation of these values for DEHP ranged between 9% and 30%. These peak area values related with the sample mass for DEHP in the PET material of the different brands decreased in the following order: B > C > A. The peak area ratio of DEHP for brands B and A was 4.2, while the same ratio for brands C and A was 3.2. These results

are in good agreement with the fact that virgin PET is used for bottling of mineral water brand A, while for the production of PET bottle of mineral water brands B and C, recycled PET flakes may be used in 20%-30% w/w as it was confirmed by the PET pre-form producer of each firm monitored in this study.

3.3. Leaching of phthalates as a function of storage time

For mineral water brand C, samples having different bottling time could be purchased. The time elapsed between bottling and chemical analysis ranged between 44 and 1283 days. The concentration of phthalates (DiBP, DBP, BBP and DEHP) in mineral water samples stored at 22 ºC was below LOQ in all samples stored for 44 days. After this period, a significant increase in the concentration of phthalates was observed (Figure 2). Especially, in the case of DEHP, a saturation curve was registered. The sharpest concentration increase over time was registered for DEHP, meanwhile for DiBP, DBP and BBP, a modest increase was observed (Figure 2). Thus, for DEHP, in about 25 and 40 months, the concentration increased by factor 1.5 and 1.7, respectively. However, the concentration of any detected phthalate ester did not reach values considered as harmful for human health. Our findings fit with literature data. Schmid et al. (2008) observed an increase in the DEHP concentration for deionized water bottled in PET containers stored in the dark at 17 °C. Under these conditions, the DEHP concentration varied between 0.14 and 0.24 μg L^{-1} in 17 hours. Thus Criado et al. (2005) reported an increase of 20% for DBP in Argentinian mineral water samples after a storage time of 5 months at room temperature (RT).

3.4. Occurrence of phthalates in carbonated and non-carbonated mineral water samples

Any of the phthalates investigated in the present study could not be detected in any carbonated mineral water sample. Therefore, all our further results refer to non-carbonated water samples. This finding is in good agreement with the report of Montuori et al. (2008) who observed slightly higher concentrations for the PET-bottled non-carbonated water samples compared to carbonated water samples. Moreover, Biscardi et al. (2003) demonstrated that the concentration of DEHP reached the detection limit by leaching in a non-carbonated mineral water sample bottled in PET after a 9-month-long storage at RT; while, the occurrence of DEHP in similarly stored carbonated mineral water could be detected only in the 10^{th} month of storage. Carbonated and non-carbonated water samples behave in a different way. The only difference in the carbonated and non-carbonated samples investigated was the pH, which plays an important role in the acid- or base-catalysed ester hydrolysis, a slow equilibrium process at RT. Lertsirisopon et al. (2009) investigated the abiotic degradability of BBP, DBP and DEHP in the aquatic phase over a wide pH range 5–9 at RT. The efficiency of abiotic degradation of the investigated phthalates *via* hydrolysis with relatively short alkyl chains, such as BBP and DBP, at neutral pH was significantly lower than that in the acidic or alkaline condition. However, the DEHP did not proceed significantly at any pH. Moreover, the polar character of the degradation products does not enhance their extraction by a non polar solvent like dichloromethane used in the present study. These findings may explain the incapability of phthalate ester detection in the carbonated samples of the present study.

3.5. The effect of the contact surface area on the phthalate concentration

As it was mentioned in our previous work (Keresztes et al., 2009), the contact surface area – expressed as the surface/volume ratio - is the highest in the 0.5-L PET containers. Thus, the phthalate concentration determined in water samples belonging to identical brand, but taken from PET containers having different volumes proved that the higher contact surface between water and PET material, the higher concentrations of DiBP, DBP, BBP and DEHP were observed. In Figure 3, the effect of contact surface area on the DBP, DiBP, BBP and DEHP concentration in mineral water C bottled in PET material is presented.

Taking into consideration the PET container sizes (0.5 L, 1.5 L and 2.0 L), the increase of DEHP concentration in 0.5-L PET containers was about 1.2 and 1.5 times when dividing the corresponding DEHP concentration with that obtained in 1.5-L and 2.0-L PET containers, respectively. This finding can be explained with the fact that DEHP is not chemically bound in plastics. The outcome of this part of the present study is important as, generally, the 0.5-L PET bottles are the most preferred by consumers. Moreover, up to our knowledge, other reports do not stress the importance of the contact surface area.

3.6. The effect of storage temperature on the phthalate concentration

Experiments carried out at 22 ºC, 40ºC, 50 ºC and 60 ºC with non-carbonated mineral water bottled in 0.5-L PET containers revealed that, generally, a pronounced increase in the concentration of DiBP, DBP, BBP and DEHP was observed at 60 ºC after 24 hours in the case of mineral water C (Figure 4). For this mineral water brand, the concentration of DiBP, DBP, BBP and DEHP increased by factor of 1.6, 1.4, 2.6 and 2.5, respectively, at 60 °C after 24 hours compared to the initial concentration values determined prior to heating. Moreover, phthalate concentration detected in mineral water B was less affected by temperature increase. In this case, there was no significant

difference for the concentration of DiBP, BBP and DBP at any of the 4 different temperature values applied after 24 hours. In mineral water A, characterized by not detectable phthalate levels at RT, DEHP was the only phthalate that could be determined in significant concentration after 24-h thermostation at 60 ºC (Figure 4).

Interestingly, a prolonged thermostation at 60 ºC for 72 h resulted in a drastic reduction in the phthalate concentrations (Figure 5). However, in the case of mineral water C, the concentration of DiBP, DBP and DEHP decreased by 90%, 77% and 45%, respectively, after thermostation at 60 ºC for 72 h compared to the initial values determined prior heating, meanwhile the concentration of BBP in this water brand fell below the LOQ. Even for the most persistent phthalate, DEHP, its concentration fell under the LOQ in all almost every case.

According to our results obtained at 40 °C, it seems that two antagonistic effects were acting by increasing the temperature: increase of the dissolution rate of phthalates and increase of the decomposition rate. The dissolution rate is related to diffusion, which in return is also related to the polymer structure of the bottle.

Among the phthalate leaching studies conducted at temperatures higher than RT, the work of Al-Saleh et al. (2011) investigated the phthalate concentration in mineral waters from Arabian Saudi supermarkets bottled in PET containers and stored in three different ways: (i) at 4 °C for 1 month, (ii) at RT for 2 months; (iii) outdoors (> 45 °C) for three months. The levels of DMP, DEP, BBP and DEHP in bottled waters stored at 4 °C were significantly higher than those for the other two storage modalities; whereas, the opposite trend was observed for DBP, especially when water was stored at RT. Casajuana and Lacorte (2003) stated that poor storage conditions (e.g., outdoors for 10 weeks, at temperatures higher than 30 ºC) increased the DMP, DEP, DnBP and DEHP concentrations in bottled (PET and PE) water. They reported the following mean

concentrations after a storage in PET at 30 °C for 10 weeks: DEHP 0.134 μg L^{-1}, DBP 0.046 μg L^{-1}, DEP 0.214 μg L^{-1}, DMP 0.002 μg L^{-1} and BBP 0.01 μg L^{-1}, while the above-mentioned phthalates could not be quantified in the initial samples. Leivadara et al. (2008) observed the concentration of DEHP increased from the initial less than 0.5 μg L^{-1} to 2 μg L^{-1} if bottled water was stored at 24 ºC in the dark for 3 months. At the same time, DEHP was not detected in the samples when bottled waters were stored at 30 °C under outdoor conditions for 3 months. Thus, photolysis may be an important pathway for abiotic degradation of phthalates in waters (Peterson, 2003). At increased temperatures, decomposition due to biodegradation is even more pronounced.

By investigating the phthalate concentration of water treated by solar disinfection, Schmid et al. (2008) stated that the occurrence of phthalates in deionised water stored in PET bottles at a maximum of 34 °C for 17 hours under direct sunlight, depended mainly on the country of origin of the bottle. For example, the DEHP concentration in these water samples ranged between 0.1 and 0.38 μg L^{-1}, which meant an increase compared to the DEHP concentration obtained in the dark at RT. In the case of exposure of bottles to sunlight at 60 °C, the DEHP concentration ranged between 0.15 μg L^{-1} and 0.71 μg L^{-1}. Like in the present study, this tendency was not unequivocally proven; the results depended on the type of bottle.

4. Conclusions

Determination of four phthalate esters as emerging pollutants in mineral water bottled in PET containers was performed from three different mineral water brands. The applied method requires a careful selection of the procedural blanks as these contaminants are ubiquitous in the nature. According to our results, their occurrence depends strongly on

the PET bottle material (virgin vs. polymer containing recycled PET), pH (carbonated vs. non-carbonated samples), packaging volume and temperature. Therefore, the selection of the appropriate material and storage conditions play a decisive role. However, taking into consideration the maximum phthalate ester concentration of mineral water determined in the present study, the calculated TDI values proved to be not yet a thread for human health.

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Figure captions

Figure 1. (a) Typical GC-MS chromatogram of a standard (up) and water sample (down) at m/z 149. Elution order from left to right: DiBP, DBP, BBP and DEHP; (b) Typical Py-GC/MS chromatogram of PET bottle at m/z 149.

Figure 2. Evolution of phthalate concentration in non-carbonated mineral water brand C bottled in 2.0-L PET containers over time.

Figure 3. The effect of contact surface area of 0.5-L, 1.5-L and 2.0-L PET bottles on the phthalate concentration of non-carbonated mineral water brand C.

Figure 4. The effect of storage temperature (22 °C, 40 °C, 50 °C and 60 °C) on phthalate (a: DiBP, b: DBP, c: BBP and d: DEHP) concentration for three different non-carbonated mineral water samples bottled in 0.5-L PET containers.

Figure 5. The effect of prolonged exposure time (24 h, 48h, 72h) at 60 °C on the phthalate concentration (a: DiBP, b: DBP, c: BBP and d: DEHP) for three different non-carbonated mineral water samples bottled in 0.5-L PET containers.

Figure 1a

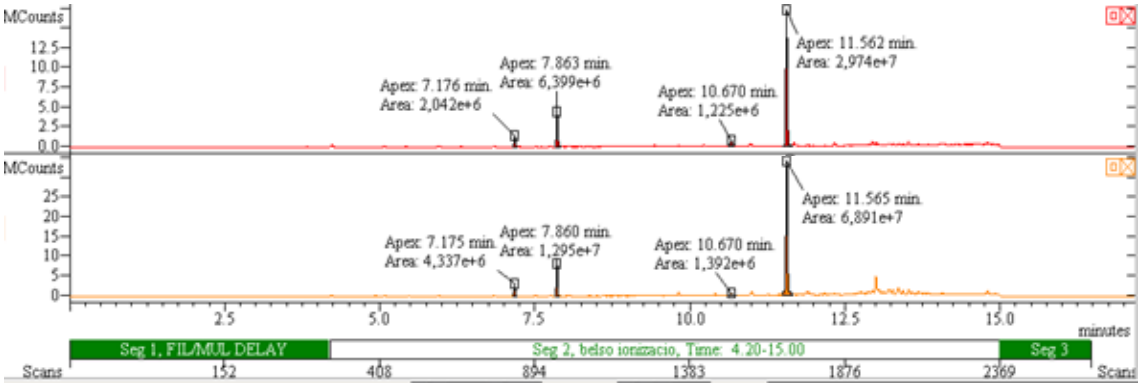


Figure 1b

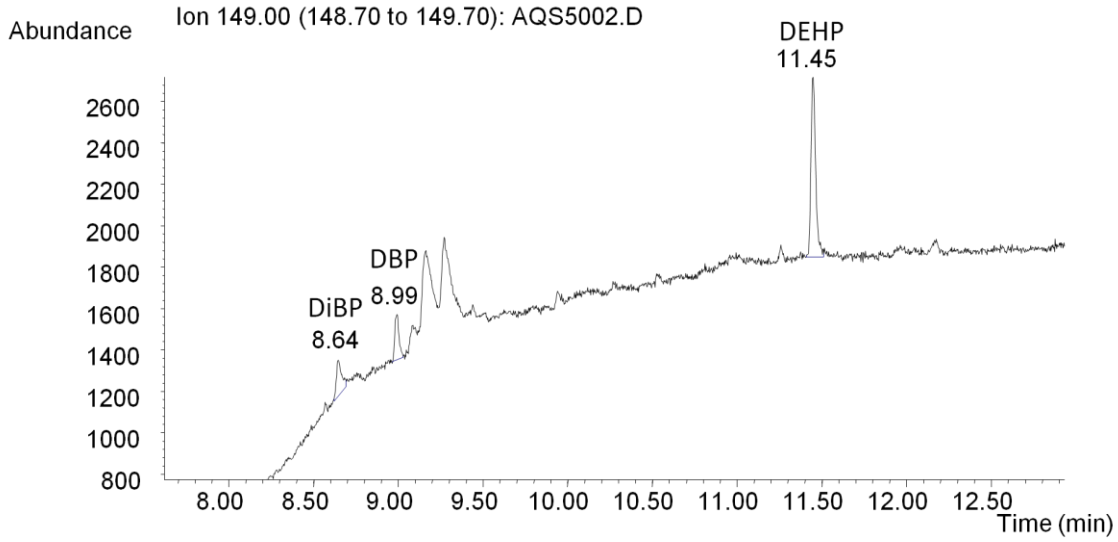


Figure 2

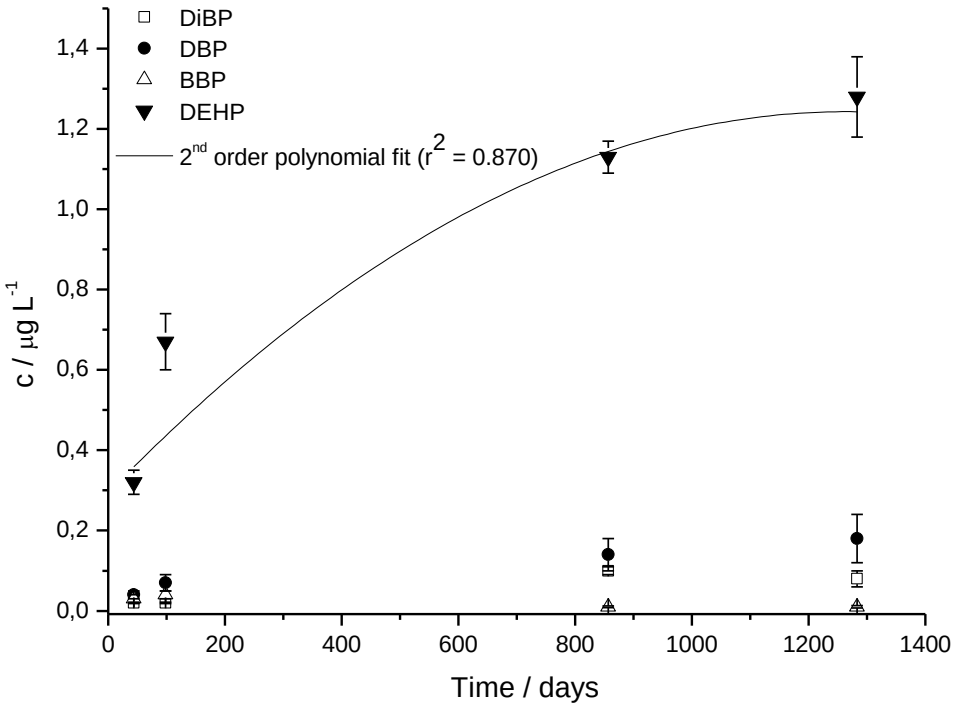


Figure 3

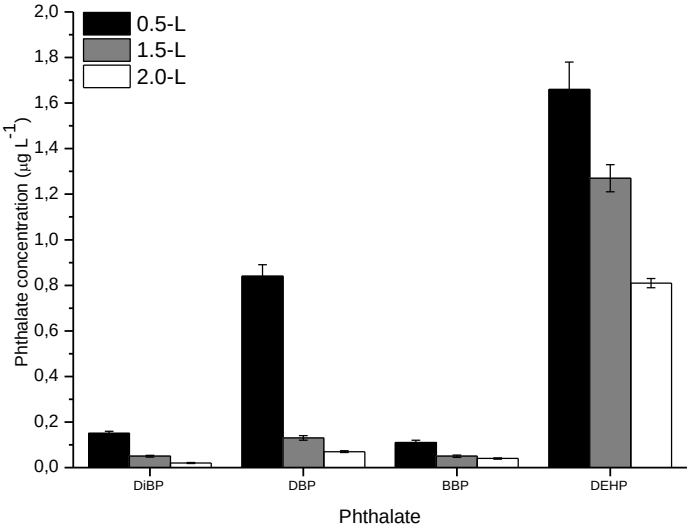
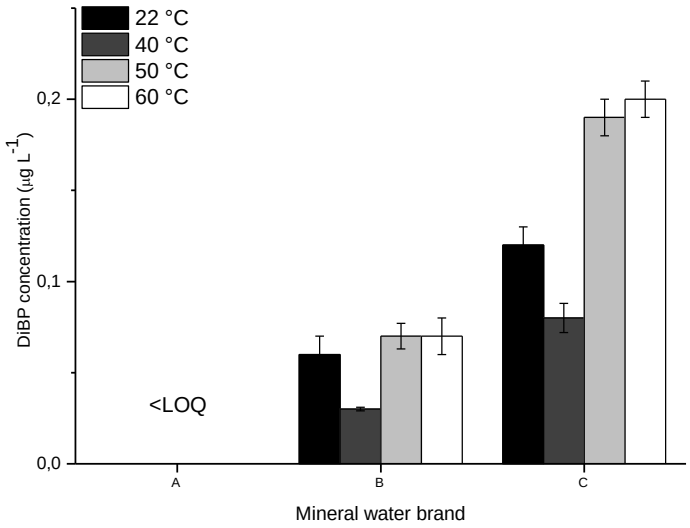
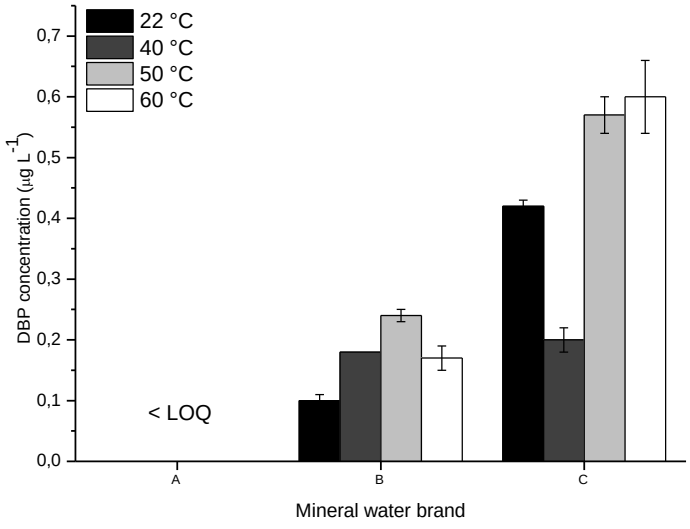


Figure 4

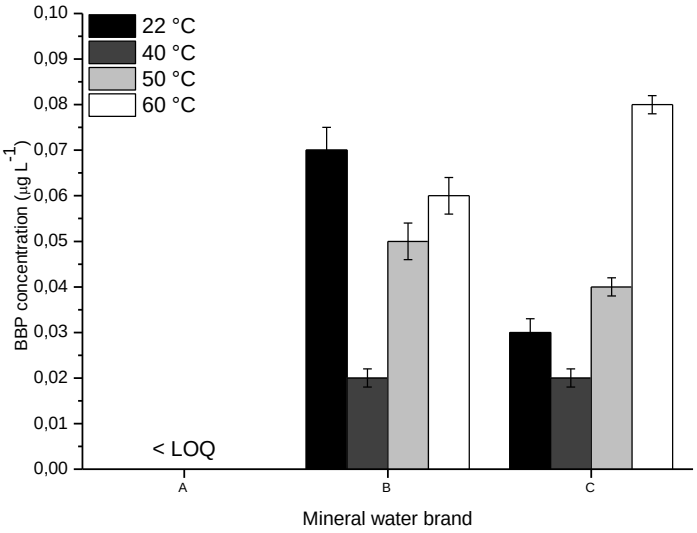
a)


22 °C
40 °C
50 °C
60 °C
DiBP concentration (µg L-1)
0,0
0,1
0,2
<LOQ
A
B
C
Mineral water brand

b)


22 °C
40 °C
50 °C
60 °C
DBP concentration (µg L-1)
0,0
0,1
0,2
0,3
0,4
0,5
0,6
0,7
< LOQ
A
B
C
Mineral water brand

c)


22 °C
40 °C
50 °C
60 °C
BBP concentration (µg L-1)
0,10
0,09
0,08
0,07
0,06
0,05
0,04
0,03
0,02
0,01
0,00
< LOQ
A
B
C
Mineral water brand

d)

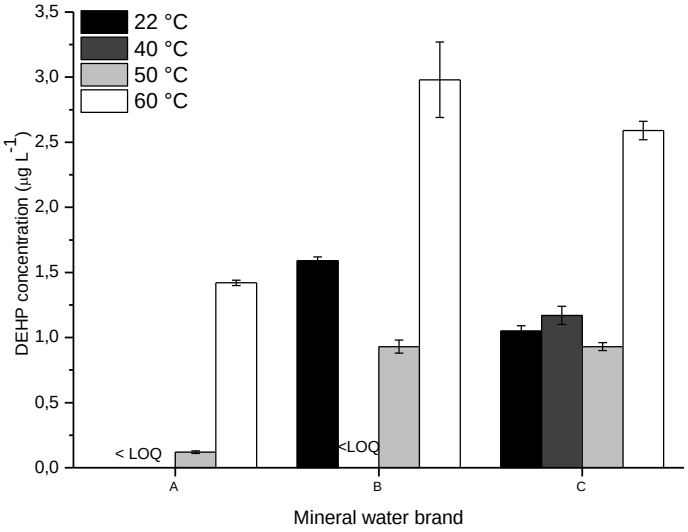
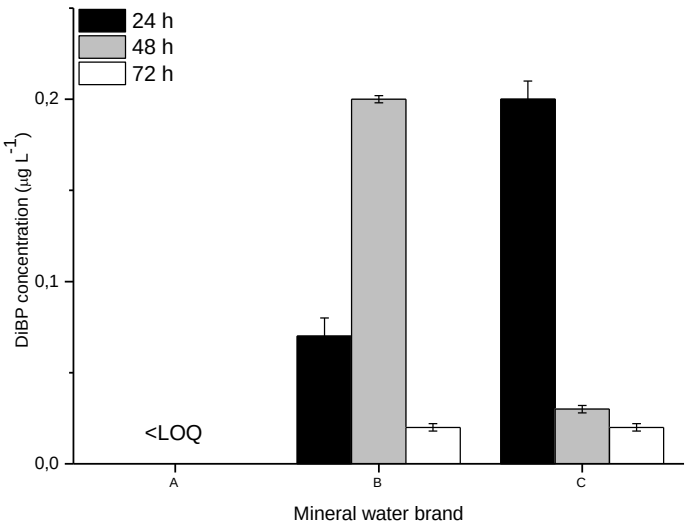
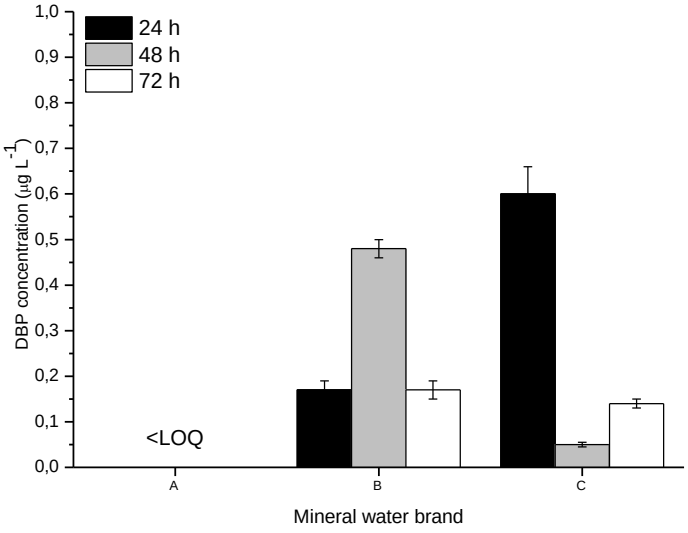

22 °C
40 °C
50 °C
60 °C
DEHP concentration (µg L-1)
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
< LOQ
<LOQ
A
B
C
Mineral water brand

Figure 5

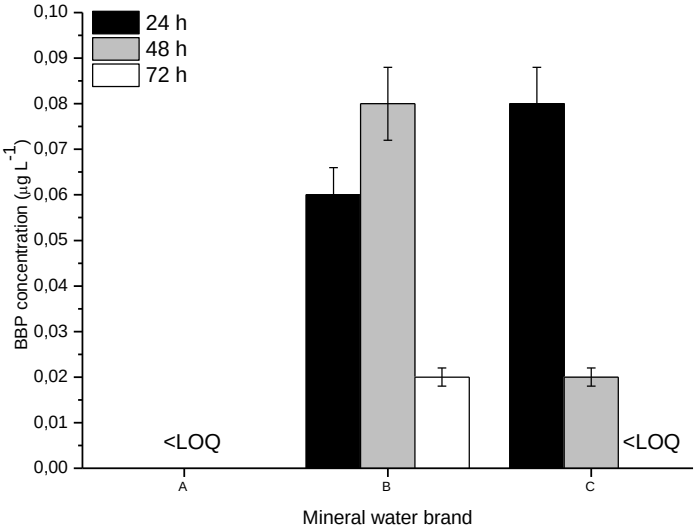
a)


24 h
48 h
72 h
DiBP concentration (µg L-1)
0,0
0,1
0,2
<LOQ
A
B
C
Mineral water brand

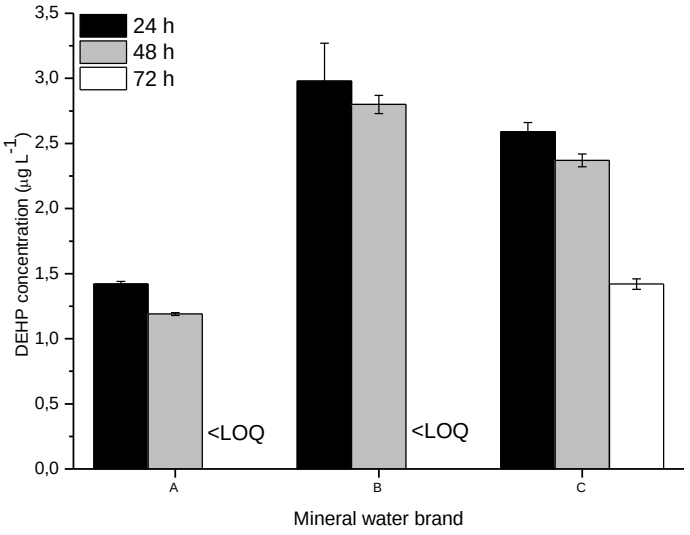
b)


24 h
48 h
72 h
DBP concentration (µg L-1)
0,0
0,1
0,2
0,3
0,4
0,5
0,6
0,7
0,8
0,9
1,0
<LOQ
A
B
C
Mineral water brand

c)


24 h
48 h
72 h
BBP concentration (µg L-1)
0,10
0,09
0,08
0,07
0,06
0,05
0,04
0,03
0,02
0,01
0,00
<LOQ
<LOQ
A
B
C
Mineral water brand

d)


24 h
48 h
72 h
DEHP concentration (µg L-1)
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
<LOQ
<LOQ
A
B
C
Mineral water brand