Environ Sci Pollut Res (2014) 21:1380–1390
DOI 10.1007/s11356-013-2020-3

RESEARCH ARTICLE

Preliminary toxicological assessment of phthalate esters from drinking water consumed in Portugal

José Santana · Clara Giraudi · Emílio Marengo · Elisa Robotti · Sara Pires · Inês Nunes · Elvira M. Gaspar

Received: 20 November 2012 / Accepted: 16 July 2013 / Published online: 31 July 2013

Abstract This paper reports, for the first time, the concentrations of selected phthalates in drinking water consumed in Portugal. The use of bottled water in Portugal has increased in recent years. The main material for bottles is polyethylene terephthalate (PET). Its plasticizer components can contaminate water by leaching, and several scientific studies have evidenced potential health risks of phthalates to humans of all ages. With water being one of the most essential elements to human health and because it is consumed by ingestion, the evaluation of drinking water quality, with respect to phthalate contents, is important. This study tested seven commercial brands of bottled water consumed in Portugal, six PET and one glass (the most consumed) bottled water. Furthermore, tap water from Lisbon and three small neighbor cities was analyzed. Phthalates (di-*n*-butyl phthalate ester (DnBP), bis(2-ethylhexyl) phthalate ester (DEHP), and di-*i*-butyl phthalate ester (DIBP)) in water samples were quantified (PET and glass) by means of direct immersion solid-phase microextraction and ionic liquid gas chromatography associated with flame ionization detection or mass spectrometry due to their high boiling points and water solubility. The method utilized in this study showed a linear range for target phthalates between 0.02 and 6.5 μg L^{-1}, good precision and low limits of detection that were between 0.01 and 0.06 μg L^{-1}, and quantitation between 0.04 and 0.19 μg L^{-1}. Only three phthalates were detected in Portuguese drinking waters: dibutyl (DnBP), diisobutyl (DIBP), and di(ethylhexyl) phthalate (DEHP). Concentrations ranged between 0.06 and 6.5 μg L^{-1} for DnBP, between 0.02 and 0.16 μg L^{-1} for DEHP, and between 0.1 and 1.89 μg L^{-1} for DIBP. The concentration of DEHP was found to be up to five times higher in PET than in glass bottled water. Surprisingly, all the three phthalates were detected in glass bottled water with the amount of DnBP being higher (6.5 μg L^{-1}) than in PET bottled water. These concentrations do not represent direct risk to human health. Regarding potable tap water, only DIBP and DEHP were detected. Two of the cities showed concentration of all three phthalates in their water below the limits of detection of the method. All the samples showed phthalate concentrations below 6 μg L^{-1}, the maximum admissible concentration in water established by the US Environmental Protection Agency. The concentrations measured in Portuguese bottled waters do not represent any risk for adult's health.

Keywords Phthalates (PAEs) · DnBP · DIBP · DEHP · Persistent organic pollutants · Profiles · Drinking water · Bottled water · Ionic liquid gas chromatography (IL-GC) · Portugal

Responsible editor: Markus Hecker

Part of this work was done under Erasmus students interchange program involving Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Portugal and University of Piemonte Orientale "Amadeo Avogadro, Italy."

Electronic supplementary material The online version of this article (doi:10.1007/s11356-013-2020-3) contains supplementary material, which is available to authorized users.

J. Santana · C. Giraudi · S. Pires · I. Nunes · E. M. Gaspar (✉)
Departamento de Química, CQFB-Requimte, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Quinta da Torre, Caparica, 2825-114 Lisbon, Portugal
e-mail: elvira.gaspar@fct.unl.pt

C. Giraudi · E. Marengo · E. Robotti
Faculty of Mathematical, Physical and Natural Sciences, University of Piemonte Orientale "Amadeo Avogadro", Allesandria, Piemonte, Italy

Introduction

There has been massive increase in bottled water use worldwide, driven in large measure by marketing, designed to convince the public of bottled water's purity and safety, and capitalizing on public concerns about tap water quality.

Bottled water has become a major industry. In 2004, the global consumption of bottled water was 154 billion liters, a 57 % increase from 1999 when 98 billion liters were consumed worldwide (Wood and Roberts 2011). Worldwide, consumers are steadily increasing with Portuguese and Italian citizens being good examples. In 2011, for example, each Italian consumed 179 L and each Portuguese consumed 122 L of bottled drinking water (APIAM 2011). Most of the time, consumers buy polyethylene terephthalate (PET) bottled water (Rodwan Jr 2011; EFBW 2010; Sax 2009), which is considered as a good alternative to glass as PET was judged safe and approved by the European Union (EU) as food packaging material (Borodinsky 2007). However, packaging may represent a source of contamination owing to the potential leaching of substances, such as residues from the polymerization process, degradation products, and plastic additives, into food and drinks (Staples et al. 2003; Sax 2009). Among the contaminants are the phthalic acid esters, also called phthalate esters or phthalates, a group of compounds of similar chemical structure with lipophilic properties. The most common phthalates ((dimethyl phthalate (DMP), diethyl phthalate (DEP), di-*n*-butyl phthalate (DnBP), butylbenzyl phthalate (BBP), bis(2-ethylhexyl) phthalate (DEHP), and di-*n*-octyl phthalate (DnOP) esters), as well as the plasticizer bis(2-ethylhexyl) adipate, have been included on the list of priority pollutants in several countries (Kamrin 2009). These compounds are continuously found to be present in products used during our everyday lives such as plastic tubing, gloves, bags, toys, and food packages including bottles. They are also found in building materials, home improvement products, personal care products, etc.

The US Environmental Protection Agency (EPA) has established a maximum admissible concentration (MAC) for DEHP and bis(2-ethylhexyl) adipate in water of 6 μg L^{-1} and 0.4 mg L^{-1}, respectively. DEHP is the most commonly used phthalate globally and it represents a quarter of the total production of plasticizers (Penãlver et al. 2000). In Europe, the EU Water Framework Directive 2000/06/CE announced in Annex X a list of 33 priority substances or groups of substances, which include metals, pesticides, polycyclic aromatic hydrocarbons, endocrine disruptors, and also phthalates. All these substances must be removed with the objective of obtaining good quality and preservation of good ecological status of water by 2015. Due to their potential risks for human health, authorities should pay particular attention to their industrial discharge into water because they have to ensure safety for the population in terms of both food chain consequences and environment. In addition, the Registration, Evaluation, Authorization, and Restriction of Chemicals regulation, which aims to identify dangerous chemicals and less dangerous replacements, was established in 2007 in Europe. The application of this regulation requires the removal of three phthalates (DEHP, DBP, and BBP) that were classified as carcinogenic and reproductive toxins or as being persistent in the environment (Deblonde et al. 2011; Wille et al. 2012). There are different and multiple routes of phthalate exposure, which must be considered in any exposure assessment: ingestion, inhalation, and skin contact. Regarding ingestion, drinking water plays a crucial role.

The individual members of the phthalate group have unique physical and chemical properties, and studies have suggested that they also affect organisms differently. In humans, phthalates are rapidly metabolized to their monoesters, and some of them can be further oxidized and conjugated with glucuronide before excretion in urine or feces (Preuss et al. 2005). Depending on the kind of ester, exposure over a reasonable period (around 1 year) could result in potential carcinogenic effects and in metabolic disorders on the hormonal and reproduction systems (Kluwe et al. 1982; Kamrin 2009). Several studies have been conducted with animals to investigate the toxicity of these compounds (Kamrin 2009; Sax 2009). The presence of elevated concentrations of phthalates (>23 ng L^{-1}) was shown to reduce male fertility and cause increased fetal mortality, low birth weights, and fetal malformation (Schuga et al. 2011). Over the course of this decade, concern regarding the exposure of infants and children to phthalates has been increasing.

Phthalates are one of the major contaminant groups found in bottled water, since they are incorporated into PET to generate a copolymer that enhances flexibility, and they can leach from the plastic (Sax 2009). However, phthalates are not covalently bound to plastics, so the residual monomers can pass into water with increasing time and use. Regulatory actions have been proposed by the European Union (Directive 2005/84/EC); European rules require a test on PET safety, which consists in storing the bottles at 40 °C for 10 days and then estimating the rate of contaminants leached from the plastic (Directive 82/711/EEC).

This paper reports, for the first time, the concentrations of phthalates in bottled drinking water consumed in Portugal. Using an improved, simple, one-step gas chromatographic (GC) method (direct immersion solid-phase microextraction (DI-SPME) coupled to ionic liquid gas chromatography with flame ionization detection, IL-GC-flame ionization detection (FID), and/or mass spectrometry, IL-GC-MS), phthalate concentrations in bottled water were characterized and discussed. This work will contribute to a better understanding of the quality of drinking water consumed in Portugal. Because the phthalates under investigation are persistent, bioaccumulative, and have the potential to cause adverse effects to humans, the study also contributes to the characterization of the exposure of Portuguese citizens to these compounds through drinking water.

Materials and methods

The analytical methodologies used in this study were based on previously published works (Penãlver et al. 2000; Penãlver et al. 2001; Prokůpková et al. 2002; Cortazar et al. 2002; Polo

et al. 2005). The study was divided in two different stages: (1) implementation and validation of the analytical methodology and (2) phthalate profile analysis and quantitation of phthalates in drinking water, including potable tap water and different bottled mineral waters commercially available in Portugal, making use of the tools developed in stage 1.

Chemicals and samples

Methanol, ethanol, propyl alcohol, isopropyl alcohol, isobutyl alcohol, *n*-butyl alcohol, isoamyl alcohol, 2-ethylhexyl alcohol, *n*-octyl alcohol, benzyl alcohol, phthalic acid, and hydrochloric acid were purchased from Sigma-Aldrich (Steinheim, Germany), analytical reagent grade. Phthalate esters were prepared from 10 mg of phthalic acid, adding five equivalents of each of the ten different alcohols and one drop of hydrochloric acid to each conic reacti-vials, sealed with polytetrafluoroethylene (PTFE)-faced silicone septum. The reacti-vials were homogenized using Vortex agitation (ZX3, VELP Scientifica, Usmate, Italy) and heated at 90 °C using the reacti-therm (LAB-LINE, Mumbai, India) for 1 h. After cooling at room temperature, the solutions were extracted three times with hexane, combined, and transferred to other vials. Two molecular sieves were added to remove vestigial water; then, solutions were concentrated under nitrogen stream and analyzed.

Stock standard solutions with a concentration of 1 g L^{-1} of each phthalate ester present in real samples were prepared in methanol, using vortex agitation and ultrasonication (5 min), and were stored at 0 °C. Using these stock solutions, standard solutions of each compound were prepared by dilution: di-*i*-butyl phthalate ester (DIBP) at concentrations of 0.2, 0.4, 2, and 4 μg L^{-1}; DnBP ester at concentrations of 0.04, 0.4, 2, and 4 μg L^{-1}; DEHP at concentrations of 0.04, 0.1, 0.2, and 0.4 μg L^{-1}. Only external calibration standards were used. Samples of Portuguese bottled waters were purchased in local supermarkets. Tap water samples were collected in Lisbon and in another three smaller cities.

SPME procedures

Based on the results from previous studies (Penãlver et al. 2000; Penãlver et al. 2001; Prokůpková et al. 2002; Cortazar et al. 2002; Polo et al. 2005; Cao 2008), two different types of SPME fibers were used in this work for the extraction of phthalates, 100 μm polydimethylsiloxane (PDMS) and 85 μm polyacrylate (PA), both purchased from Supelco (Bellefonte, PA, USA). Fibers were initially conditioned according to the manufacturer's instructions: each one was conditioned by insertion into the GC-FID injector during 1 h at 270 °C, in order to remove contaminants and to stabilize the polymeric phase before use. To avoid any background problem, this SPME preprocessing step was applied in all experiments.

Considering blank contamination problems that have been previously encountered during the application of SPME to phthalate analysis (Cortazar et al. 2002; Polo et al. 2005), a carefully selected spring water source (Serra da Estrela mountain) was adopted as the blank sample. Only DEHP was found at low concentrations (0.005±0.0009 μg L^{-1}) in blank samples. Standard addition calibrations were performed for the analysis of water samples. The limit of detection was estimated as the signal of the blank plus three times the standard deviation of this signal or as the offset plus three times the deviation of the offset when no peak was found for the compound in blank sample (Cortazar et al. 2002; Polo et al. 2005; Table 2).

Headspace-SPME (HS-SPME)

Samples (25 mL of all drinking waters) were placed into 50 mL EPA (chemically inert clear type I borosilicate glass) vials equipped with PTFE-coated magnetic bars and capped with a PTFE-faced silicone septum. NaCl was added to the samples to give a concentration of 180 g L^{-1} (Penãlver et al. 2000). The holder needle was inserted through the septum and both fibers—PA and PDMS—were exposed to the sample's headspace (HS-SPME). Each solution was extracted for 90 min at 45 °C with continuous magnetic stirring. After extraction, each fiber was withdrawn into the holder needle, removed from the vial, and introduced immediately into the GC-FID and/or GC-MS injector port for 15 min at 270 °C for thermal desorption of the analytes. Between sampling and sample extraction, a blank was run to avoid carry over processes. It was verified that the least volatile phthalates, DEHP, DnOP, and BBP, were not detectable by HS-SPME.

DI-SPME

Samples (25 mL of all drinking waters) were placed into 25 mL EPA glass vials equipped with PTFE-coated magnetic bars and capped with a PTFE-faced silicone septum. The holder needle was inserted through the septum and the fibers were immersed directly into the samples (DI-SPME). Waters were extracted for 20 min at 25 °C with continuous magnetic stirring. After extraction, each fiber (PA and PDMS) was withdrawn into the needle holder, removed from the vial, and introduced immediately into the GC-FID and/or GC-MS injector port for 15 min at 270 °C for thermal desorption of the analytes. Between sampling and sample extraction, a blank was run to avoid carry over processes. The same procedure was previously used with standard solutions (25 mL) to establish and to validate the method (Table 2).

Real samples (tap and bottled waters) were filtered through a 0.45-μm PTFE membrane filter (Whatman, Maidstone, UK) before analysis.

Instrumentation and chromatographic conditions

Three different columns, a fused silica capillary column Supelcowax (Supelco, Bellefonte, USA; 30 m×0.25 mm I.D., film thickness 0.25 μm), a fused silica capillary column, SLB™-IL76 (Supelco; 30 m×0.25 mm I.D., film thickness 0.2 μm), and a fused silica capillary column, SLB™-5 (Supelco; 60 m×0.25 mm I.D., with 0.25 μm of film thickness), were used. DI-SPME-IL-GC-FID analyses were performed simultaneously using the two chromatographic columns—Supelcowax and SLB IL-76, with an Agilent (Santa Clara, CA, USA), model 4890D chromatograph, equipped with a split/splitless injector and FID system. The separations were performed using oven temperatures programmed from 160 to 190 °C at 3 °C min^{-1} and 190 to 270 °C at 15 °C min^{-1}, with a 10-min hold. Hydrogen was used as carrier gas at a flow rate of 1.2 mL min^{-1}; column head pressure was 100 kPa. The injector was used in the splitless mode; the injector and detector temperatures were set both at 270 °C.

HS-SPME-GC-MS measurements were performed under identical chromatographic conditions, using the SLB™-5 MS column in an Agilent 6850 chromatograph coupled with 5975C MSD with triple-axis detector: mode EI^+ 70 eV, source temperature 250 °C, and interface temperature 280 °C. Helium was the carrier gas used at a flow rate of 1.2 mL min^{-1}. All compound identifications were confirmed using GC-MS. Full scan mode was used in the range of 45–400 amu, at a rate of 0.9 scan s^{-1}; *m/z* 149, the base peak of each compound, was selected for quantification under full scan acquisition mode.

Quantitation and quality control

In the analysis of phthalates in water samples, the level of phthalate esters found in the samples used as blanks is important. Spring water samples from Serra da Estrela mountain were used as blank signals (Table 3). These blank signals forced the performance of the method in terms of linear range and the limits of detection (LODs) and quantitation (LOQs) achieved (Table 2). Detection limits were estimated as the signal of the blank plus three times the standard deviation of this signal (LOD=blank signal+3SD) to DEHP and as the offset plus three times the deviation of the offset to DIBP and DnBP because no peaks were found for these compounds in blank (water) samples. Fiber blanks were run before each analysis to avoid carry over.

To evaluate the linearity of the SPME method, calibration studies were performed using multilevel standard solutions as follows: 0.2, 0.4, 2, and 4 μg L^{-1} for DIBP; 0.04, 0.4, 2, and 4 μg L^{-1} for DnBP; and 0.04, 0.1, 0.2, and 0.4 μg L^{-1} for DEHP. Background levels were subtracted from the results. Coefficients of determination (R^2) were equal or higher than 0.9613 (Table 2), demonstrating a proportional relationship between the extracted amount of phthalates and their initial concentration in the sample. Each sample was analyzed four times. The precision of the experimental data was evaluated by calculating the relative standard deviation (RSD) of replicates (n=4) (Table 3). RSD values were 11.33 % for DnBP, 11.71 % for DIBP, and 18.53 % for DEHP.

The analytical method was evaluated to prove its applicability to the analysis of all 10 target phthalates in 11 samples—five PET bottled mineral waters, one glass bottled water (the most consumed), four tap waters, and one 5-gal PET bottled water (IBWA 2013; Watercooler 2004). Four replicates (n=4) of each real sample were analyzed. RSD values are different for each phthalate are between 2.7 and 12.8 % for DIBP, 1.6 and 16.8 % for DnBP, and 1.6 and 19 % for DEHP (Table 3). Confirmation of phthalate identities was conducted by GC co-injection with standards and GC-MS mass spectra comparison.

Results and discussion

Separation of phthalates from water samples

Phthalate esters have boiling points varying between 230 and 486 °C (Table 1). These high boiling points contribute to their

Table 1 Physical–chemical properties of phthalates

No.	CAS no.	Compounds	Abbreviation	Boiling point (°C)	log Kow	H_2O solubility (mg L^{-1})
1	131-11-3	Dimethyl phthalate	DMP	283–284	1.61	4,200
2	605-45-8	Diisopropyl phthalate	DIPP	294	2.83	90.26
3	84-66-2	Diethyl phthalate	DEP	296–298	2.38	1,100
4	131-16-8	Dipropyl phthalate	DPP	305	3.63	108
5	84-69-5	Diisobutyl phthalate	DIBP	320	4.11	20
6	84-74-2	Di-*n*-butyl phthalate	DnBP	340	4.45	11.2
7	605-50-5	Diisoamyl phthalate	DIAP	347	5.45	0.252
8	117-81-7	Di-2-ethylhexyl phthalate	DEHP	361	7.5	0.003
9	117-84-0	Di-*n*-octyl phthalate	DnOP	380	8.06	0.0005
10	85-68-7	Butylbenzyl phthalate	BBP	370	4.59	2.7

usefulness as plasticizers, heat transfer fluids, and carders (Staples et al. 1997). Their water solubility (Table 1) is also an extremely important property because it influences their biodegradation and bio-accumulation potential, as well as aquatic toxicity and it is a determining factor controlling the environmental distribution of phthalates. Water solubility of phthalates is also important in context with the exposure of humans, as drinking water ingestion represents one of main

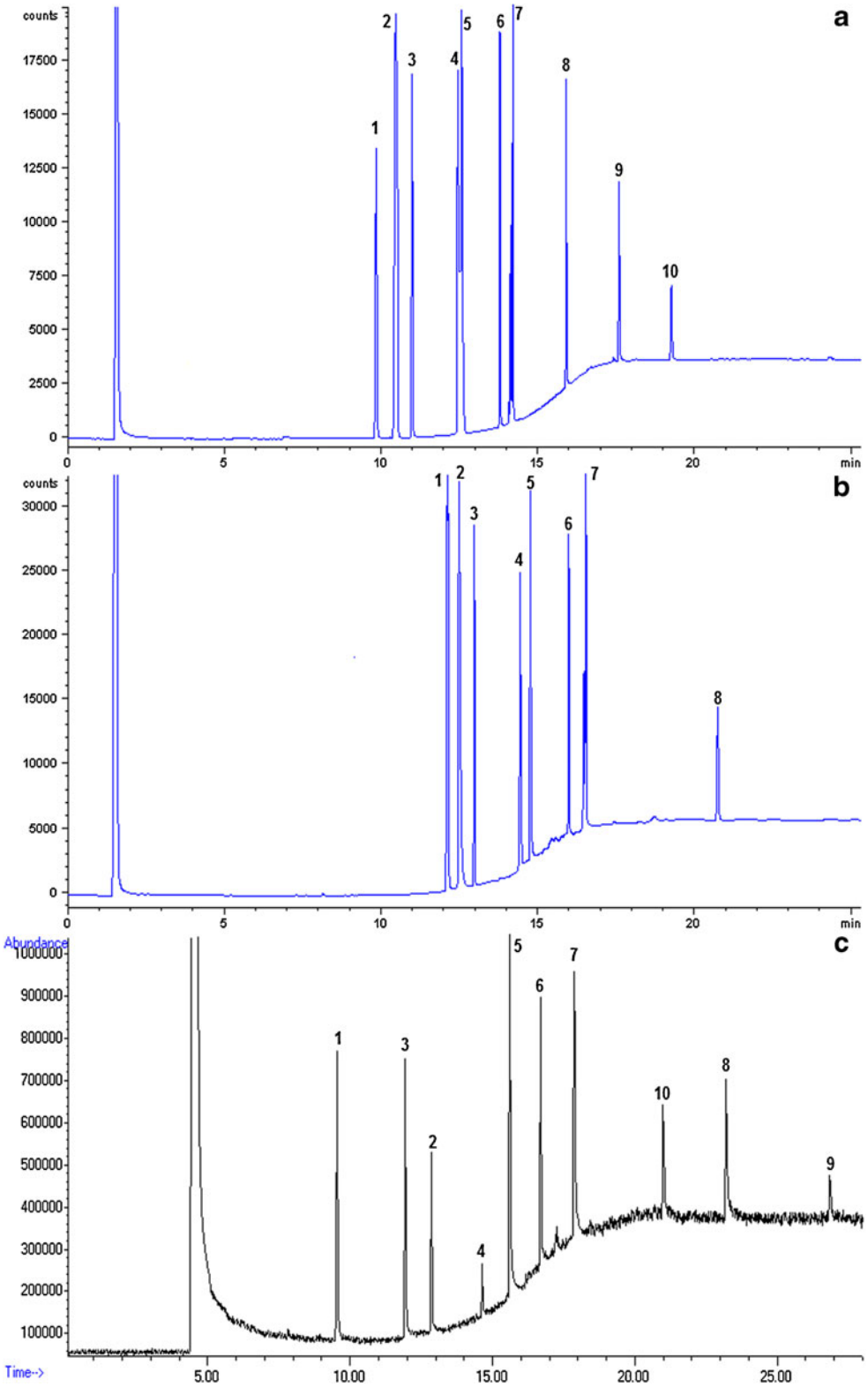

Fig. 1 Separation of liquid mixtures of phthalates; SLB IL-76 (**a**), polar Supelcowax (**b**), and unpolar SLB-5 (**c**). Number of compounds according to Table 1. See chromatographic conditions in "Material and methods" section

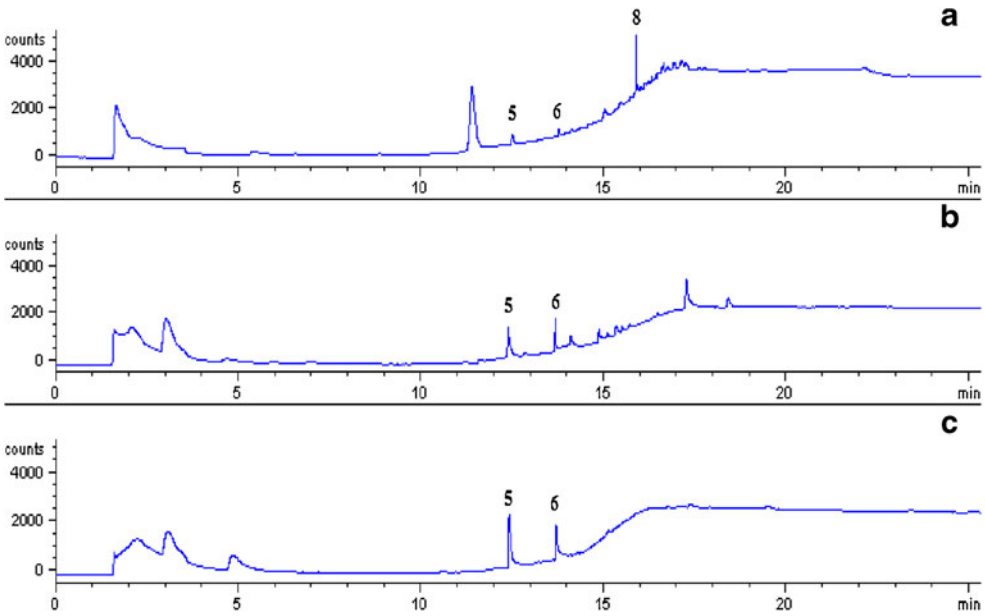

Fig. 2 Comparison of elution profiles using DI and HS-SPME using two fibers. **a** Chromatogram A DI-SPME(PDMS). **b** Chromatogram B HS-SPME(PDMS). **c** Chromatogram C HS-SPME(PA). See chromatographic conditions in "Material and methods" section

routes of uptake. The analytical approach of this work considered both of these chemical properties of phthalates. Gas chromatography of water samples was conducted using an ionic liquid column, IL-GC, due to ionic liquid (IL) properties such as low volatility, high thermal stability, and excellent selectivity for phthalates (Ragonesea et al. 2012). The column SLB-IL76 was chosen due to its excellent separation properties of low volatile phthalates.

Both IL-76 and SLB-5 columns gave good separation of the target compounds (Fig. 1). A literature search revealed that samples derived from water-rich matrices have been often analyzed using polyethylene glycol (Wax) GC columns; however, one limitation of Wax columns is their low usable maximum temperature of 280 °C, which is not sufficiently high for the analyses of all the target compounds. On the other hand, the intensive use of polymethylsiloxane columns (like SLB-5) with samples having water present caused irreversible column damage, performance loss, non-reproducible results, and column lifetime shortening; this may have been caused by some oxidation that may occur and the consequent thermal decomposition (Tooley et al. 2011).

Direct immersion, DI-SPME, was selected rather than headspace, HS-SPME, due to the above-mentioned affinity for water and low volatility of phthalates. Lower molecular weight phthalates have moderate water solubility while higher molecular weight phthalates are less volatile and have lower water solubility (Table 1). Although higher molecular weight phthalates are less volatile and have a higher Henry law constant (*H*) (Polo et al. 2005) evaporating more rapidly from water, optimal results were obtained using DI-SPME.

Best results were obtained with PDMS fibers. It could be demonstrated that extraction of all target phthalates was not significantly influenced by temperature in the range between 15 and 25 °C. With regard to sorption time, often equilibrium could not be reached, and therefore, an alternative approach for extraction under non-equilibrium conditions which requires shorter extraction times had to be developed. Ai (1997a, b) proposed a dynamic model of SPME adsorption, in which the amount of analyte adsorbed from the sample onto the fiber is proportional to the initial analyte concentration in the sample matrix, if the agitation and the sampling time are held constant among samples. Therefore, according to Ai

Table 2 DI-SPME-IL-GC-FID method performance for the analysis of phthalates in drinking waters

Compound	Linearity			LOD (μg L^{-1})	LOQ (μg L^{-1})	Repeatability (RSD, %) (n=4)	Recovery (%)
	Range (μg L^{-1})	Calibration equation	R^2				
DIBP	0.20–4	y=702.86x+138.37	0.9931	0.06	0.19	11.71	100.7
DnBP	0.04–4	y=347.36x+147.4	0.9987	0.01	0.04	11.33	99.6
DEHP	0.04–0.4	y=16,741x+6.6696	0.9613	0.01	0.04	18.53	73.9

RSD relative standard deviation, *LOD* limit of detection, *LOQ* limit of quantification.

Table 3 Phthalates in Portuguese drinking waters

Sample		DIBP		DnBP		DEHP	
		Conc. (μg L^{-1})	RSD (%)[a]	Conc. (μg L^{-1})	RSD (%)[a]	Conc. (μg L^{-1})	RSD (%)[a]
	Blank sample	n.d.	–	n.d.	–	0.005	18.3
1	Potable tap water I (A)	0.17	5.4	n.d.	–	0.13	9.1
2	Potable tap water II	n.d.	–	n.d.	–	0.19	16.5
3	Potable tap water III	n.d.	–	n.d.	–	n.d.	–
4	Potable tap water IV	n.d.	–	n.d.	–	n.d.	–
5	Glass bottled water (B)	1.89	10.8	6.5	1.6	0.02	10.1
6	Plastic bottled water	0.22	12.7	0.06	8.9	0.16	1.6
7	Plastic bottled water (C)	1.42	11.9	1.68	13.9	0.18	18.7
8	Plastic bottled water[b]	0.1	7.5	0.12	12.4	0.09	16.1
9	Plastic bottled water	1.2	7.9	0.3	8.4	0.08	19.0
10	Plastic bottled water	0.76	12.8	0.42	6.5	0.1	14.6
11	5 gal bottled water (D)	1.12	2.7	2.94	16.8	0.07	9.5

n.d. not detected

[a] $n=4$

[b] Water pH 5.6

(1997a, b), SPME quantitation is feasible at non-equilibrium conditions. Hence, a time of 20 min was chosen as a compromise, since phthalates gave reproducible signals (precision and recovery RSDs less than 12 %, with exception of DEHP which was 18.5 %). Desorption time and injector temperature were limited by DnOP, the least volatile phthalate. A desorption time of 15 min was selected. This time was sufficient to ensure no significant memory effect when the same fiber was inserted for a second time. Assessment of elution profiles using direct immersion (DI) versus HS-SPME-IL-GC column revealed that by using headspace, the less volatile compound could not be detected in the samples, indicating that DI-SPME should be used for the analyses of phthalates from water (Fig. 2).

Method performance

Using optimized conditions for DI-SPME-IL-GC-FID, the quality parameters of the chromatographic methodology such as LODs and LOQs, linearity, repeatability, recovery, linearity range, precision, and sensitivity were calculated (Miller and Miller 1988; Table 2).

As described above, detection limits were estimated as the signal of the blank plus three times the standard deviation of this signal (LOD=blank signal+3SD) to DEHP and as the offset plus three times the deviation of the offset to DIBP and DnBP because no peaks were found for these compounds in blank (water) sample. Linearity was obtained by calibration studies performed using (4) multilevel spiked samples. Coefficients of determination (R^2) were higher than 0.9613, demonstrating the proportional relationship between the SPME-extracted amount and the initial concentration of phthalates in samples. The corresponding graphs are included in the Supplementary materials section. Precision was evaluated by calculating the RSD of four replicates of each point in the linearity range studies. Recovery results are also good (>73.9 %). LODs and LOQs were between 0.01 and 0.06 μg L^{-1} and between 0.04 and 0.19 μg L^{-1}, respectively. The method performance is in good agreement with previously published works (Penãlver et al. 2000; Penãlver et al. 2001; Prokůpková et al. 2002; Cortazar et al. 2002; Polo et al. 2005; Cao 2008).

Phthalates in Portuguese drinking water

Portuguese drinking waters (Table 3) revealed the presence of measurable concentrations of three phthalates—DEHP, DIBP, and DnBP. Each sample was analyzed four times; RDS values are different for each phthalate: between 1.6 and 19 % for DEHP, 2.7 and 12.8 % for DIBP, and 1.6 and 16.8 % for DnBP (Table 3 and Fig. 3). Identifications were confirmed by CG-FID co-elution with standards and GC-MS comparison of

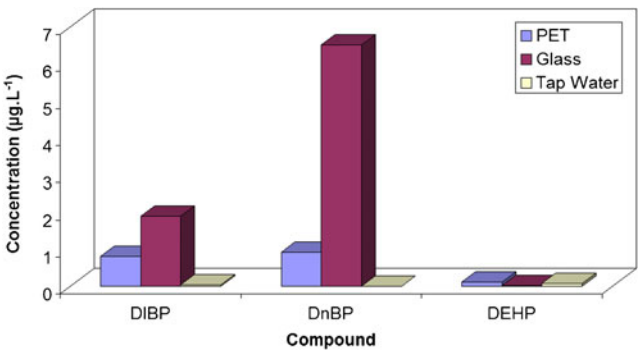

Fig. 3 Concentrations of DIBP, DnBP, and DEHP obtained in Portuguese PET, glass, and tap drinking waters; specifications according to Table 3. See chromatographic conditions in "Material and methods" section

their retention times and mass spectra (Fig. 4; mass spectra of all other materials are provided in the Supplementary materials section). Phthalates were found at concentrations ranging between 0.06 and 6.5 μg L^{-1} for DnBP), between 0.005 and 0.19 μg L^{-1} for DEHP and between 0.1 and 1.89 μg L^{-1} for DIBP. The mean values of concentrations of these phthalates in bottled (PET and glass) and tap waters showed to be different (Fig. 3). Regarding DIBP, a literature search revealed that only three other studies involving drinking waters from Italy, Iran, and Canada also revealed the presence of DIBP (Montuori et al. 2008, Cao 2008; Asadollahzadeh et al. 2010). DIBP was analyzed by DI-SPME-GC-MS using a PDMS-DVB fiber (Montuori et al. 2008), by HS-SPME-GC-MS (Cao 2008) using a PDMS-DVB fiber, and by DI-SPME-GC-FID using a carbon nanotube/polypyrrole composite homemade fiber (Asadollahzadeh et al. 2010), emphasizing the role of the SPME pre-concentration technique in the extraction of phthalates from aqueous matrices. Portuguese waters seem to have higher DIBP concentrations (Table 3) than Canadian (0.133 to 0.481 μg L^{-1}), Italian (mean 0.2 μg L^{-1}), and Iranian (not detected to1.2 μg L^{-1}) bottled waters. DnBP and DEHP seem to be common in worldwide drinking waters (Montuori et al. 2008, Asadollahzadeh et al. 2010; Cao 2008; Prokůpková et al. 2002; Schmid et al. 2008).

All three phthalates were detected in the most consumed Portuguese glass bottled water with the amount of DnBP being surprisingly high (6.5 μg L^{-1}). Previous studies (Prokůpková et al. 2002; Montuori et al. 2008) attributed elevated phthalate concentrations in glass bottles to metal caps that were sealed with PVC inserts, which represent a potential origin of this phthalate. However, the 5-gal PET bottled water consumed in Portugal also revealed a high content of DnBP (Table 3); perhaps in this case, it was derived from plastic pipe and taps belonging to the apparatus associated to this container. The lowest concentrations of phthalates were measured in sample 5—a PET bottled water with a pH of 5.6, the most acidic water commercialized in Portugal (Table 3 and Fig. 5). These results, obtained by the analyses of Portuguese drinking waters with an optimized direct immersion solid-phase microextraction and ionic liquid gas chromatography

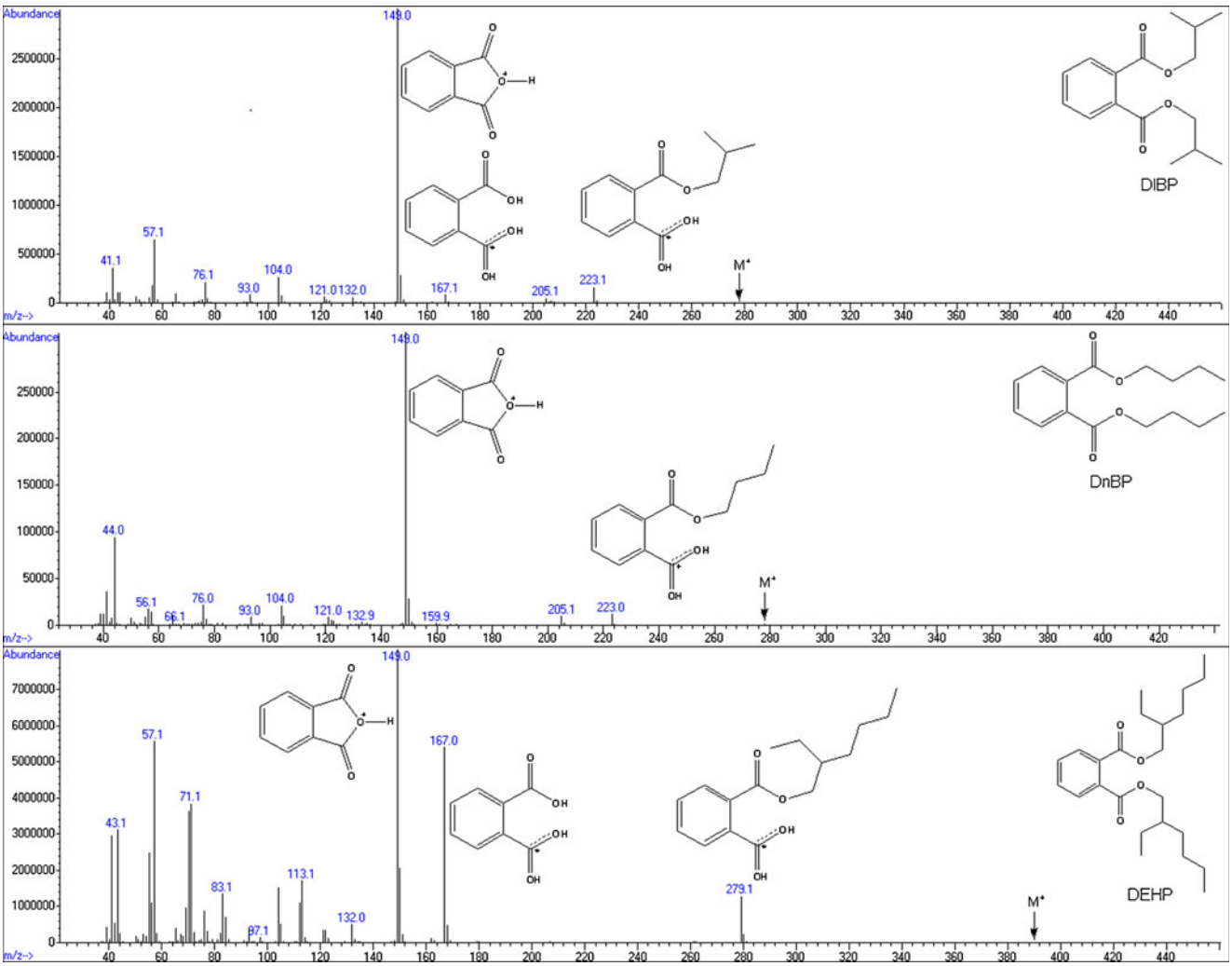

Fig. 4 DIBP (5), DnBP (6), and DEHP (8) mass spectra. See chromatographic conditions in "Material and methods" section

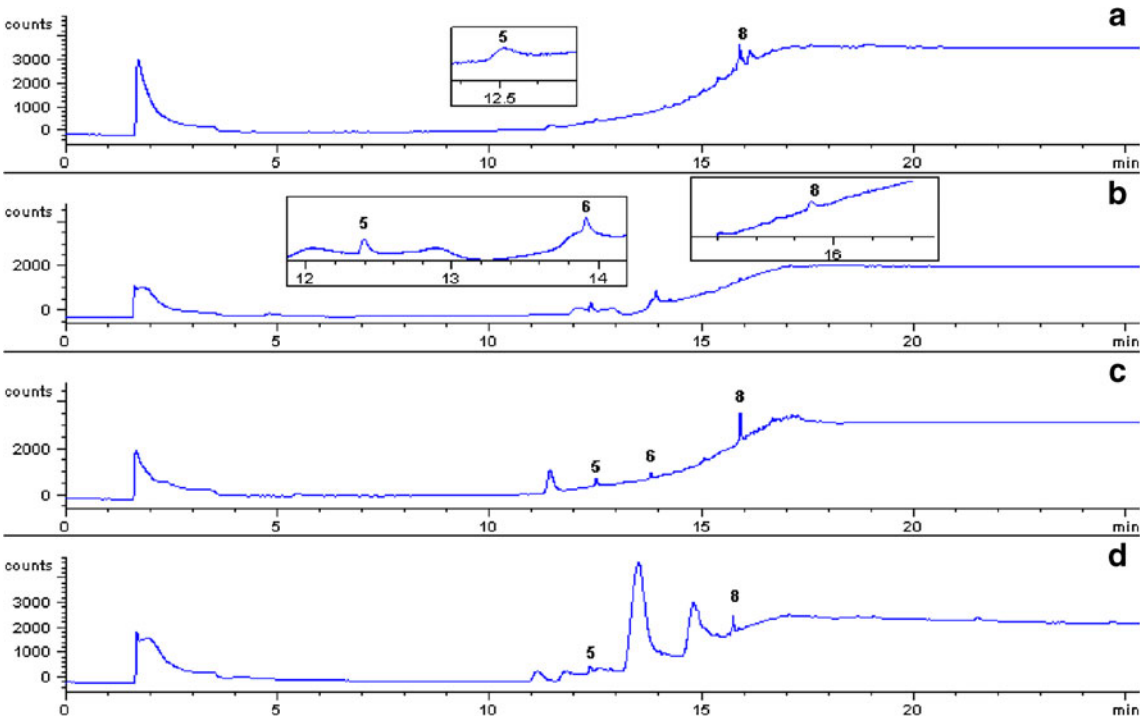

Fig. 5 DI-SPME-IL-GC-FID analyses of Portuguese drinking waters. **a** Chromatogram A, potable tap water; **b** chromatogram B, glass bottled water; **c** chromatogram C, plastic bottled water, sample 5; **d** chromatogram D, 5-gal bottled water. See chromatographic conditions in "Material and methods" section

associated with flame ionization detection or mass spectrometry (DI-SPME-IL-GC-FID(MS)) method, are comparable to those previously reported in the Czech Republic, Italy, and Canada by other authors (Prokůpková et al. 2002, Montuori et al. 2008, Cao 2008). Regarding potable tap waters from Lisbon and three small neighbor cities, the results (Table 3) were very different and only DIBP and DEHP were detected. Two of the cities showed concentrations of all three phthalates in their water below the limits of detection of the method. The results seem to indicate that the type of packaging material used affects phthalate concentrations (Table 3). Concentrations of DEHP were up to five times higher in PET than in glass bottled water. All the samples showed phthalate concentrations below 6 μg L^{-1}, the MAC in water established by the US EPA. Samples 5 and 11 (Fig. 5, chromatograms b and d) also showed the presence of other non-identified compounds, demonstrating the usefulness of the DI-SPME-IL-GC-FID(MS) methodology to efficiently concentrate substances present at ultra-trace levels and to identify a range of organic substances contained in aqueous samples.

Contribution to the evaluation of Portuguese citizen's exposure

An adult Portuguese person will consume around 122 L of bottled water plus 243 L of tap water per year (APIAM 2011). Based on the results obtained in this work (Table 3), each year, the ingestion of phthalates by drinking water by a Portuguese adult is estimated to be between 53.5 and 271.9 μg of DIBP ((0.17 μg L^{-1}×243 L)+(0.1 μg L^{-1}×122 L) and (1.89 μg L^{-1}×122 L)), between 34 and 53.6 μg of DEHP ((0.13 μg L^{-1}×243 L)+(0.02 μg L^{-1}×122 L) and (0.18 μg L^{-1}×122 L)), and between 7.3 and 793 μg of DnBP ((0.06 μg L^{-1}×122 L) and (6.5 μg L^{-1}×122 L)). Adding the content of phthalates that were shown to be present in Portuguese drinking waters, this means that each Portuguese adult may ingest between 94.8 (53.5+34+7.3) and 1,118.5 (271.9+53.6+793) μg of phthalates per year. According to EPA data, the no observed adverse effect level (NOAEL)[1] of BBP is 159 μg/kg/day and the lowest observed adverse effect level (LOAEL[2]) of DEHP is 19 mg/kg/day (EPA Risk Information for DEHP 2012). So, this first study indicates that the water ingested could be a significant source of phthalates in Portugal. However, PET bottles do not represent a health risk related to phthalate intake.

Due to the potential adverse effects of phthalates with respect to their endocrine properties and associated potential decline in fertility (Shen et al. 2012) as well as their potential carcinogenic properties (Deblonde et al. 2011), this first evaluation of Portuguese adult exposure indicates the necessity of further studies in order to identify other important sources of phthalates in Portugal, in particular those obtained by ingestion (others than water). With regard to drinking water, a close

[1] NOAEL denotes the level of exposure of an organism, found by experiment or observation, at which there is no biologically or statistically significant.

[2] LOAEL is the lowest concentration or amount of a substance, found by experiment or observation, which causes an adverse alteration of morphology, functional capacity, growth, development, or life span.

monitoring is recommended due to the fact that it contributes to a daily continuous ingestion of phthalates. This study also indicates that there is a need for epidemiologic studies to evaluate the potential effects of chronic phthalate exposure in Portuguese people.

Conclusions

The use of DI-SPME/IL-GC-MS(FID), due to the high boiling points and water solubility of phthalates, has proven to be a suitable analytical technique for the analysis of phthalates at low concentrations and for a large number of samples. The concentrations found in Portuguese drinking waters do not represent any risk for adult's health.

This study constitutes a first evaluation of the exposure of adult Portuguese citizens to phthalates. Due to toxicity, persistence, and bioaccumulative properties of phthalates, studies are needed to identify other important sources of these compounds in Portugal, in particular those obtained by ingestion (other than water).

Acknowledgments The authors gratefully thank Sigma-Aldrich/Supelco (Silep program). The authors also acknowledge Dr. Pedro Gutiérrez Rivas for his support. Inês Nunes and José Santana acknowledge Portuguese Science and Technology Foundation (FCT) for their PhD grants. Clara Giraudi acknowledges her grant from Erasmus interchanging program.

References

Ai J (1997a) Solid phase microextraction for quantitative analysis in nonequilibrium situations. Anal Chem 69:1230–1236

Ai J (1997b) Headspace solid phase microextraction. Dynamics and quantitative analysis before reaching a partition equilibrium. Anal Chem 69:3260–3266

APIAM (2011) http://www.apiam.pt/conteudo/Um-mercado-sustentado/-/10. Accessed 19 Nov 2012

Asadollahzadeh H, Noroozian E, Maghsoudi S (2010) Solid-phase microextraction of phthalate esters from aqueous media by electrochemically deposited carbon nanotube/polypyrrole composite on a stainless steel fiber. Anal Chim Acta 669:32–38

Borodinsky L (2007) Technical Advisor's Report to the Food, Drug, and Cosmetic Packaging Materials Committee. http://www.plasticsindustry.org/files/about/fdcpmc/techpdfs/FDCPMCTechAdvisorReportJune07.pdf. Accessed 10 June 2013

Cao X-L (2008) Determination of phthalates and adipate in bottled water y headspace solid-phase microextration and gas chromatography/mass spectrometry. J Chromatogr A 1178:231–238

Cortazar E, Zuloaga O, Sanz J, Raposo JC, Etxebarria N, Fernández LA (2002) Multiplex optimisation of the solid-phase microextration-gas chromatographic-mass spectrometric determination of polycyclic aromatic hydrocarbons, polychlorinated biphenyls and phthalates from water samples. J Chromatogr A 978:165–175

Deblonde T, Cossu-Leguille C, Hartemann P (2011) Emerging pollutants in wastewater: a review of the literature. Int J Hyg Environ Health 214:442–448

Directive 2005/84/EC. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2005:344:0040:0043:en:PDF. Accessed 19 Nov 2012

Directive 82/711/EEC. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:1982L0711:19970901:EN:PDF. Accessed 19 Nov 2012

EFBW—European Federation of Bottled Waters (2010) Bottled water facts. http://www.efbw.eu/images/file/Bottled%20Water_Facts%20.pdf. Accessed 19 Apr 2013)

EPA Risk information for DEHP (2012) http://www.epa.gov/iris/subst/0014.htm. Accessed 20 Nov 2012

IBWA—International Bottled Water Association (2013) http://www.bottledwater.org/buyersguide/plastipak-packaging. Accessed 19 Apr 2013

Kamrin MA (2009) Phthalate risks, phthalate regulation, and public health: a review. J Toxicol Environ Health B 12:157–174

Kluwe WM, McConnell EE, Huff JE, Haseman JK, Douglast JF, HartwelIt WV (1982) Carcinogenicity testing of phthalate esters and related compounds by the National Toxicology Program and the National Cancer Institute. Environ Health Perspect 45:129–133

Miller JC, Miller JN (1988) Statistics for analytical chemistry. Ellis Horwood, Chichester

Montuori P, Jover E, Morgantini M, Bayona JM, Triassi M (2008) Assessing human exposure to phthalic acid and phthalate esters from mineral water stored in polyethylene terephthalate and glass bottles. Food Addit Contam 25:511–518

Penãlver A, Pocurull E, Borrull F, Marce RM (2000) Determination of phthalate esters in water samples by solid-phase microextraction and gas chromatography with mass spectrometry detection. J Chromatogr A 872:191–201

Penãlver A, Pocurull E, Borrull F, Marcé RM (2001) Comparison of different fibers for the solid-phase microextration of phthalate esters from water. J Chromatogr A 922:377–384

Polo M, Llompart M, Garcia-Jares C, Cela R (2005) Multivariate optimization of a solid-phase microextraction method for the analysis of phthalate esters in environmental waters. J Chromatogr A 1072:63–72

Preuss R, Koch HM, Angerer J (2005) Biological monitoring of the five major metabolites of di-(2-ethylhexyl)phthalate (DEHP) in human urine using column-switching liquid chromatography–tandem mass spectrometry. J Chromatogr B 816:269–280

Prokůpková G, Holadová K, Poustka J, Hajšlová J (2002) Development of a solid-phase microextraction method for the determination of phthalic acid esters in water. Anal Chim Acta 457:211–223

Ragonesea C, Sciarrone D, Tranchida PQ, Dugo P, Mondello L (2012) Use of ionic liquids as stationary phases in hyphenated gas chromatography techniques. J Chromatogr A 1255:130–144

Rodwan Jr JG (2011) Bottled water 2011: the recovery continues, U.S. and International Developments and Statistics. International Bottled Water Association, IBWA. http://www.bottledwater.org/files/2011BWstats.pdf. Accessed 18 Apr 2013

Sax L (2009) Polyethylene terephthalate may yield endocrine disruptors. Environ Health Perspect 118:445–448

Schmid P, Kohler M, Meierhofer R, Luzi S, Wegelin M (2008) Does the reuse of PET bottles during solar water disinfection pose a health risk due to the migration of plasticisers and other chemicals into the water? Water Res 42:5054–5060

Schuga TT, Janesickb A, Blumbergb B, Heinde JJ (2011) Endocrine disrupting chemicals and disease susceptibility. J Steroid Biochem Mol Biol 127:204–215

Shen H, Liu L, Bao H, Liu F, Zhang J (2012) Phthalates exposure of Chinese reproductive age couples and its effect on male semen quality, a primary study. Environ Int 42:78–83

Staples CA, Peterson DR, Parkerton TF, Adams WJ (1997) The environmental fate of phthalate esters: a literature review. Chemosphere 35:667–749

Staples CA, Stanley MK, Robillard KA (2003) Part Q. Phthalate esters. In: Staples CA (ed) The handbook of environmental chemistry, vol 3. Springer, Berlin

Tooley W, Feghhi S, Han S, Wang J, Sniadecki N (2011) Thermal fracture of oxidized polydimethylsiloxane during soft lithography of nanopost arrays. J Micromech Microeng 21:960–1317

Watercooler (2004) http://en.wikipedia.org/wiki/File:Watercooler_Wikinews.jpg. Accessed 19 Apr 2013

Wille K, De Brabander HF, De Wulf E, Van Caeter P, Janssen CR, Vanhaecke L (2012) Coupled chromatographic and mass-spectrometric techniques for the analysis of emerging pollutants in the aquatic environment. Trends Anal Chem 35:87–108

Wood A, Roberts S (2011) Economic geography: places, networks and flows. Routledge, New York. http://books.google.pt/books?id=w_b0i1sBFw0C&pg=PT165&lpg=PT165&dq=worldwide+consumption+of+bottled+water&source=bl&ots=E1hzx3PIbZ&sig=U1l8FlPwxp84b2D1yQf7JaelTzU&hl=pt-PT&sa=X&ei=JiNrULi4HsiXhQfN0YGgCw&ved=0CEgQ6AEwBA. Accessed 10 Nov 2012