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Assessing human exposure to phthalic acid and phthalate esters from mineral water stored in polyethylene terephthalate and glass bottles

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Abstract
Phthalic acid and phthalate esters are of growing interest due to their significant usage and potential toxicity. Polyethylene terephthalate (PET) and glass are both widely used materials for bottled drinking water. In this study, phthalic acid (PhA), *bis*(2-ethylhexyl) phthalate (DEHP), dimethyl phthalate (DMP), diethyl phthalate (DEP), diisobutyl phthalate (DiisoBP) and dibutyl phthalate (DBP) were analysed in a large number of Italian bottled water samples. These samples showed different concentrations of phthalates are nearly 20 times higher in samples bottled in PET than those from glass bottles with total levels of phthalates of 3.52 and 0.19 $\mu g l^{-1}$, respectively. However, the observed levels do not represent a significant exposure pathway when considering the US Environmental Protection Agency (USEPA) reference dose (an estimate of a daily oral exposure to the human population, including sensitive subgroups, that is likely to be without an appreciable risk of deleterious effects during a lifetime). In addition, no significant correlation was found between the phthalate concentrations and the physicochemical properties of the different water samples, apart from the still/sparkling water parameter for the PET samples. In this instance, slightly higher concentrations were observed for the PET bottled still water samples than for the sparkling water samples, although no explanation has been found yet.

Keywords: *Bottled water, polyethylene terephthalate, phthalic acid, phthalate esters, solid-phase microextraction (SPME), electron-impact gas chromatography-mass spectrometry (EI GC-MS)*

Introduction

The bottled water industry in European countries and North America has expanded over the last 30 years, and it is also increasing rapidly in many developing countries (US Food and Drug Administration 2003). Consumption reached 155 L per capita per year in Italy, 136 L in Mexico, 112 L in France, 123 L in Belgium, 99 L in Germany, 98 L in Spain, 97 L in Switzerland, and 63 L in the USA in 2000/01 (Potera 2002; Thurman et al. 2002).

In Italy, as in many developed countries, polyethylene terephthalate (PET) is widely used as a container for commercial bottled water, and its use is also increasing rapidly due to its lower production costs in comparison with glass containers (Petrelli et al. 2006). PET is synthesized by reacting ethylene glycol ($C_2H_6O_2$) with either terephthalic acid or its methyl ester catalysed by antimony oxide. The reaction is carried out under vacuum at high temperatures to achieve high molecular weight polymers. Some studies have shown PET decomposition and phthalate migration in the absence of an accurate temperature and humidity control during PET synthesis (Castle et al. 1989; Calà et al. 2003). Other studies have shown that water, PET bottled, can release phthalate additives used in the plastic moulding process especially in critical conditions of use (e.g. long storage times) (Sauvant et al. 1995; Biscardi et al. 2003) as already shown for PVC (Hakkarainen 2003).

Phthalates display a variety of toxic effects in animal studies including decreased fertility in females (Biscardi et al. 2003), foetal defect (Saillenfait et al. 2001) and reduced survival of

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offspring (Gray et al. 2000), altered hormone levels (Thompson et al. 2004), uterine damage (Seidlova-Wuttke et al. 2004), and male reproduction abnormalities such as reduced sperm production and motility (Sharpe et al. 1995), Sertoli cell damage (Heindel and Powell 1992), Leyding cell tumours (Jones et al. 1993), cryptorchidism, and hypospadias, which may be manifestations of one condition termed as 'testicular dysgenesis syndrome' (Skakkebaek et al. 2001). The effects of human exposure to phthalates have not been fully studied (Colon et al. 2000; Health Care Without Harm 2002; Duty et al. 2003b). Long latency periods between relevant exposures and health impacts, unquantified exposures, and subtle effects that are difficult to detect are added difficulties to the few existing epidemiological studies of phthalate toxicity in humans (Health Care Without Harm 2002). In one of these few human studies, phthalates were investigated as a cause of precocious puberty in young Puerto Rican girls (Colon et al. 2000). In this study, the serum levels of phthalates obtained from 41 girls with premature appearance of breast tissue were compared with 35 controls. Phthalate esters were detected in 68% of the cases and in the 17% of the controls and they were found to lower levels of phthalates significantly than the cases. For the *bis*(2-ethylhexyl)phthalate (DEHP), the average concentration was 70 ppb in the controls compared with 450 ppb in the cases. Even if the study conclusions were limited due to small population size and the possibility of contaminated serum samples, the association between phthalates and premature thelarche is biologically plausible. Two studies present the first human data that demonstrate that phthalates are associated with increased DNA damage in sperm (Duty et al. 2003a, b). Finally, a recent study concludes that monobenzyl phthalate (MBzP) exposure was significantly associated with a 10% decrease in follicle stimulating hormones (FSH) concentration in adult men (Duty et al. 2005). Furthermore, phthalates are also chemicals of concern due to their large production volume and to a non-negligible human intake which has been estimated to range from, for example, 2 to 10 $\mu g\,kg^{-1}$ body weight day^{-1} of dibutyl phthalate (DBP) for the US population (Center for the Evaluation of Risks to Human Reproduction (CERHR 2007)) and from 3 to 30 $\mu g\,kg^{-1}$ body weight day^{-1} for DEHP (Latini 2005). In order to assess the safety issues of food containing phthalates their intake can be compared with the reference doses (RfD) defined by the US Environmental Protection Agency (USEPA). The RfD, being an estimate of a daily oral exposure to the human population (including sensitive subgroups), is likely to be without an appreciable risk of deleterious effects during a lifetime. The European Union has included several phthalates as priority substances for evaluation (European Union 2006) and dibutylphtalate has been proposed by the Committee on the Environment, Public Health and Food Safety to be included as a priority substance in the Water Framework Directive 2000/60/EC (Committee on the Environment, Public Health and Food Safety, European Union 2007).

Solid-phase microextraction (SPME) is a solvent free pre-concentration technique, which has recently been applied to the extraction of phthalates from aqueous matrices (Kelly and Larroque 1999). However, liquid–liquid extraction (LLE) (Jobling et al. 1995; Castillo and Barcelo 1997) with dichloromethane or hexane, and solid-phase extraction (SPE) (Castillo and Barcelo 1997; Holadova and Hajslova 1995; Jobling et al. 1995; Castillo et al. 1998) are usually applied. Nevertheless, a source of error of particular concern for phthalic acid and phthalate esters determination is their high levels in the procedural blanks originating from laboratory plastics, solvents and polymeric sorbents from the pre-concentration techniques (Durand and Barcelo 1993; Castillo et al. 1998). In this way, the USEPA reports that DEHP, along with other common phthalate esters, cannot be accurately or precisely measured at concentrations below 2 $\mu g\,l^{-1}$ due to high blank levels when the conventional methods LLE or SPE are employed (Lawrence 1995), and at the same time the USEPA has established a maximum concentration limit (MCL) in drinking water of 6 $\mu g\,l^{-1}$ in its National Primary Drinking Water Regulations (NPDWR) (US Environmental Protection Agency 1991). Nowadays, phthalates are not considered in the European Union drinking water regulations even if dibutylphtalate has been proposed by the Committee on the Environment, Public Health and Food Safety to be included as a priority substance in the Water Framework Directive 2000/60/EC (Committee on the Environment, Public Health and Food Safety, European Union 2007). Recent studies show that detection limits well below this level can be achieved when using SPME coupled to GC-MS (Peñalver et al. 2000; Alzaga et al. 2003). In this way, SPME diminishes the risk of contamination in the extraction of phthalates since it is a solvent-free technique and it minimizes the use of materials, which can be potentially polluted with phthalates (e.g. SPE cartridges, and solvents). At present there are different published papers in which the suitability of SPME phthalate esters extraction from water is shown (Moder et al. 1998) and even coupled to GC-MS (Peñalver et al. 2000, 2001; Suzuki et al. 2001; Alzaga et al. 2003) and to LC-UV (Kayali et al. 2006). However, in these studies only a small number of bottled water

samples were analysed in order to evaluate the developed methodology. Recently, stir bar sorptive extraction (SBSE) technique has been used to analyse phthalates in water obtaining lower limits of detection than with SPME due to its higher volume of polymeric phase compared with SPME but as a drawback, it needs a dedicated analytical instrumentation (Serodio & Nogueira 2004, 2006).

The aim of the present study was to determine the phthalic acid and phthalate esters content in Italian mineral water both bottled in glass or in PET bottles. Moreover, the relevance of bottled water consumption in human exposure to phthalic acid and phthalate esters will be assessed. For this reason, phthalic acid (PhA) as the main degradation product of di-esters, *bis*(2-ethylhexyl) phthalate (DEHP), dimethyl phthalate (DMP), diethyl phthalate (DEP), diisobutyl phthalate (DiisoBP) and dibutyl phthalate (DBP) have been analysed in Italian samples of commercial mineral water stored in PET and in glass bottles using SPME in combination with GC-MS. To the best of our knowledge this study represents the first survey in which phthalic acid and phthalate esters, in an extensive sample pool, were investigated by using SPME and GC-MS.

Material and methods

Study area and sampling

The survey was conducted in Italy and a variety of commercial bottled water samples were collected. Commercial bottled water samples included different water type, such as spring water, mineral water, light water (low mineralization water) and sparkling water from miscellaneous commercial brands. The sampling of each commercial brand was carried out twice, packed in polyethylene and in glass bottles. A total of 71 commercial brands from 16 different Italian regions were collected. Therefore, 142 samples, 71 packed in PET and 71 in glass containers, were analysed.

Sample preparation and analysis

Water samples (5 ml) were placed in a 7-ml glass vial, the pH was adjusted to pH 2 with HCl and they were continuously stirred using a magnetic stirrer. Immersion SPME was carried out using the polydimethylsiloxane-divinylbenzene (PDMS/DVB) fibre from Supelco (Bellefonte, PA, USA) adapting an already reported methodology (Peñalver et al. 2001). After an extraction of 20 min at 25°C the fibre was thermally desorbed at 250°C (3 min splitless time) in the GC-MS standard split/splitless injector. The analysis was carried out using a quadrupole GC-MS QP5050A Shimadzu (Kyoto, Japan) with a GC-MS (version 1.1, data acquisition software), working in the electron-impact mode at 70 eV. A SPB 20 (20% diphenyl, 80% dimethylpolysiloxane) (60 m, 0.25 mm i.d.) coated with a 0.25 μm film thickness column was used. The gas chromatographic conditions were as follows. The initial oven temperature was 50°C for 2 min, then programmed from 50 to 250°C at 10°C min^{-1} with a final holding time of 30 min. The MS transfer line and ion source were kept at 250°C. Acquisition was carried out in the single-ion monitoring mode using two characteristic ions for each target analyte. Compound identification was carried out by comparing their retention times with standards, using two characteristic ions and their ratio for each target analyte. Furthermore, for the samples presenting higher concentrations, target analytes identity was confirmed in full-scan mode ($m/z = 60$–350). Quantification was done by using the external calibration method showing linear correlations with $R^2 > 0.98$ for all the target analytes from 0.01 to 1 $\mu g l^{-1}$.

Data analysis was performed with the statistical software SPSS (release 13.0, SPSS, Inc., Chicago, IL, USA). All data were presented as the mean ± standard deviation (SD). The level of significance was set at $p \leq 0.05$.

Procedural blanks were carried out and used for the calculation of the limits of detection (LOD). They ranged from 0.01 $\mu g l^{-1}$ for the DBP to 0.08 $\mu g l^{-1}$ for phthalic acid. The limits of quantification (LOQ) ranged from 0.02 $\mu g l^{-1}$ for DEHP to 0.1 $\mu g l^{-1}$ for phthalic acid.

Results and discussion

Despite few data being available, the results reported here are compared in Table I with bibliographic phthalate concentrations for tap and bottled water. It is important to note that the scarcity of data on this topic is mainly due to the analytical difficulties in the determination of phthalate. In this way, the large number of non-detected samples in the USA National Resources Defence Council study (NRDC 2006) (DEHP was not detected in 98.5% of samples) is explained by the high detection limits of the applied methodologies (e.g. USEPA 2 $\mu g l^{-1}$). However, for the most studied compounds such as DEP, DnBP and DEHP the results found in this study are in agreement with those in the literature.

The use of SPME GC-MS as an analytical technique achieved lower detection limits due to its improved blank level. In this way, lower target analytes concentrations were detected avoiding the production of a large number of non-detected samples as occurred in the NRDC study (2006).

Table I. Comparison of bibliographic data on the content of phthalates in tap and bottled water. The results are the mean of the different analysed samples expressed in $\mu g l^{-1}$.

Sample	National Resources Defence Council (NRDC) (2006) Bottled water	Casajuana and Lacorte (2003) Bottled water	Peñalver et al. (2001) Bottled water	Kayali et al. (2006) Bottled water	Serodio and Nogueira (2006) Bottled water	Serodio and Nogueira (2006) Tap water	Luks-Betlej et al. (2001) Tap water, Poland	Luks-Betlej et al. (2001) Tap water, Germany	Present study Bottled water
Samples number	132	20	3	4	1	1	1	1	142
Phtalic acid (PhA)	–	–	–	–	–	–	–	–	1.28
Dimethylphthalate (DMP)	–	0.002	n.d.	–	n.d.	0.04	–	–	0.07
Diethylphthalate (DEP)	–	0.254	0.24	–	0.04	0.19	0.16	0.20	0.17
Diisobutylphthalate (DiisoBP)	–	–	–	–	–	–	–	–	0.20
Di-*n*-butyl-phthalate (DBP)	–	0.047	0.10	–	0.35	0.52	0.64	0.38	0.21
Bis(2-ethylhexyl)phthalate (DEHP)	8.5[1]	0.164	1.0	n.d.	0.17	0.06	0.06	0.05	0.02

[1]DEHP was only detected in two samples.
n.d., Not detected; –, not analysed.

Table II shows the quartiles of the target analytes concentrations for the glass and PET bottled water. PET bottled water shows, as expected, a higher content for all phthalates, being PhA, degradation product of the phthalates esters, the most abundant individual compound. Box plots of the different target analytes concentrations depending on bottle material are shown in Figure 1. It is evident here that higher concentrations are found for all the analysed phthalates in the PET bottled water. However, these differences were confirmed using statistical tools and significant differences (Mann–Whitney *U*-test, $p < 0.05$) were found between glass and PET for all the compounds. It should be pointed out that different phthalate patterns were also obtained in the two cases (Figure 2). For the PET bottles PhA (69%) and DnBP (10%) are the most abundant compounds and for the glass bottles the most abundant are DiisoBP (25%) and the DnBP (15%). In both cases the lowest concentrations were found for DEHP, which was always far below the USEPA regulation limit of $6\,\mu g\,l^{-1}$ with a maximum of $0.17\,\mu g\,l^{-1}$ for PET bottles and of $0.02\,\mu g\,l^{-1}$ for glass bottles. These results confirm that the use of PET containers is the main cause of higher concentrations of phthalates in bottled water, as the concentration of the sum of the studied compounds is more than 12 times higher in PET than in glass bottled water. The presence of phthalates in glass bottled water could come from the other water processing steps (PVC tubing, storage tanks, filtration steps, cap-sealing). In the case of PET, four different bottle volumes have been sampled (0.75, 1, 1.5 and 2 L) but no correlation was found between the bottle volume and the phthalate content. Moreover, no correlation was found between the phthalates content and several water physicochemical parameters such as

Table II. Target analytes quartiles in ($\mu g\,l^{-1}$) for glass and PET bottled water.

	Quartiles (%)	PhA	DMP	DEP	DiisoBP	DBP	DEHP	Total
Glass	25	<LOD	<LOQ	<LOQ	0.02	0.02	<LOQ	0.13
	50	<LOD	0.02	0.02	0.03	0.04	<LOQ	0.19
	75	<LOD	0.04	0.06	0.06	0.09	<LOQ	0.36
PET	25	1.24	<LOQ	0.14	0.221	0.17	<LOQ	2.11
	50	2.20	0.06	0.22	0.32	0.23	<LOQ	3.52
	75	3.50	0.10	0.35	0.45	0.52	0.02	4.81

LOD, limit of detection; LOQ, limit of quantitation.

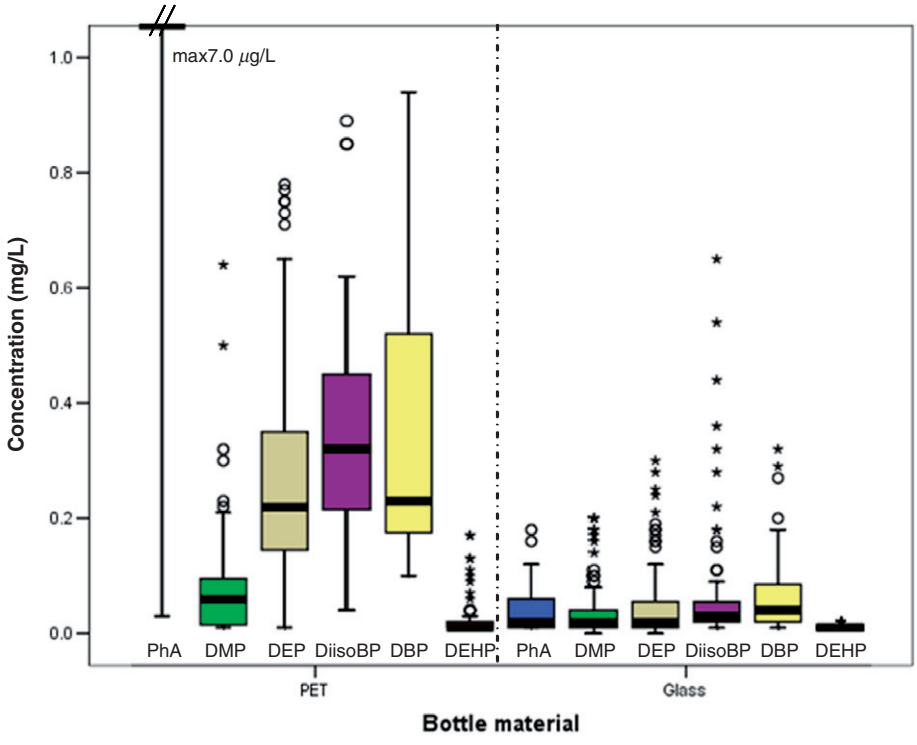

Figure 1. Box plots showing the phthalates concentrations depending on the bottle material.

conductivity, pH, solid residue, sodium, calcium, potassium, magnesium, chlorine, fluorine, sulphates, carbonates, nitrates and silicates (data taken from the bottle label). Therefore, it could be considered that these parameters are not relevant in controlling the leaching of the phthalates from the bottle to the water.

No significant differences were found between sparkling and still waters when there was no segregation of the data between PET and glass containers. However, when considering PET bottles alone, DEP was found to be significantly higher in still water ($n = 55$) than in sparkling water ($n = 16$) ($p < 0.05$). Until now no reason has been found to explain this result.

From a toxicological point of view, and considering that a MCL is only available for DEHP (6 $\mu g\,l^{-1}$ USEPA limit for drinking water), the concentrations found are lower. Another way to assess the health impact of phthalates is to study the importance of drinking PET bottled water referred to the USEPA available phthalates reference doses (RfD) (US Environmental Protection Agency 2006) (0.1 $mg\,kg^{-1}\,day^{-1}$ for the DnBP, 0.8 $mg\,kg^{-1}\,day^{-1}$ for the DEP, 2 $mg\,kg^{-1}\,day^{-1}$ for PhA, and 0.02 $mg\,kg^{-1}\,day^{-1}$ for DEHP). In this way we will consider a body weight of 70 kg and a daily water consumption of 2 L. Table III shows the proportion of the different RfDs, which can be achieved by drinking PET bottled water. The results show that, due to the low phthalate concentrations, PET bottles do not represent any health risk related to the phthalate intake as they do not contribute significantly to the estimated RfD. The maximum expected contribution will be 0.051% of the RfD for the DnBP and only for the most contaminated sample. However, it is important to point out the lack of data on this subject and the need of further studies involving both food analysis and epidemiological research to estimate the phthalates uptake in a comprehensive way.

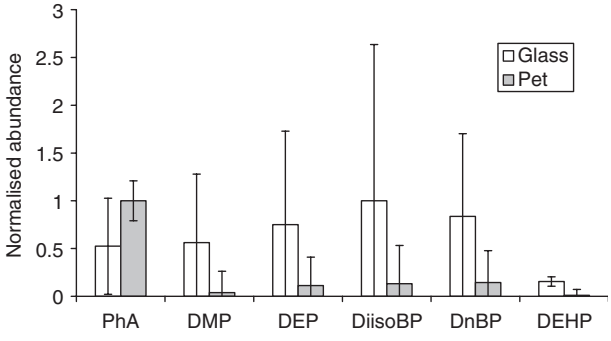

Figure 2. Normalized phthalates patterns for the PET and the glass bottled water.

Conclusions

Due to analytical difficulties in achieving low detection limits, there is a lack of data on the occurrence of phthalate in drinking water. The use of SPME and GC-MS as an analytical technique has proven to be suitable for the analysis of phthalates at low concentrations and for a large number of samples. The use of PET bottles has been clearly correlated with the concentration of phthalates in the bottled water. In this way, phthalates concentrations are significantly higher (nearly 20 times) in this water than in glass bottled water. However, the concentrations found do not represent any risk for human health as can be seen by comparing the concentrations found to the existing USEPA regulation (only available for DEHP) or by referring to the USEPA RfDs. The contribution from drinking water is always below 0.1% of the RfD for all the target analytes. Therefore, the main conclusion of this study is that Italian bottled water does not represent a relevant ingestion source of phthalates esters and phthalic acid for the population who consume bottled water. Due to similarities in the materials being used and in the technologies in water processing industries all around Europe, similar

Table III. Minimum, maximum and quartiles values of the water consumption contribution to the RfDs for the PET bottled water.

		RfD PhA*	RfD DEP	RfD DnBP	RfD DEHP
Reference value ($mg\,kg^{-1}\,day^{-1}$) (Environmental Protection Agency 2006)		2	0.8	0.1	0.02
Samples RfD contribution (%)	Minimum	<0.001	<0.001	0.003	<0.001
	25	0.002	0.001	0.005	<0.001
	50	0.003	0.001	0.007	<0.001
	75	0.005	0.001	0.015	0.001
	Maximum	0.010	0.003	0.051	0.008

*For the PhA the RfD of the phthalic anhydre was considered.

conclusions to the ones presented here should be expected for the whole European Union.

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