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Migration of phthalates, bisphenol A and alkylphenols from plastic containers and evaluation of risk

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Abstract

This study has investigated the potential migration of plasticizers, plastic components and additives from several plastic water bottles. Compounds studied were phthalates (dimethyl phthalate, di-n-butyl phthalate, benzylbutyl phthalate, bis(2-ethylhexyl) phthalate), bis(2-ethylhexyl) adipate, octylphenol, 4-nonylphenol and bisphenol A. Polycarbonate (PC), high density polyethylene (HDPE), low density polyethylene (LDPE), polyethylene terephthalate (PET) and polystyrene (PS) plastics used in the water bottling sector were tested using three kinds of total or specific migration tests: i) standard method UNE-EN ISO 177; ii) ultrasonic forced extraction and iii) standard method UNE-EN 13130-1. In addition, bottled waters contained in different plastic materials were analyzed to determine the potential migration of target compounds in real conditions. In all cases, samples were solid phase extracted using Oasis HLB 200 mg cartridges and analyzed using GC-MS in scan acquisition mode. Bisphenol A and 4-nonylphenol were detected in incubated samples, indicating that migration from food plastics can occur at the experimental conditions tested. The total daily intake was calculated according to the levels detected in bottled water and the assessment of the consumers' risk was evaluated taking into consideration toxicological and legislative values.

Keywords: migration; plasticizer; phthalate; alkylphenol; bisphenol A; nonylphenol; SPE; GC-MS.

Introduction

In the last years there has been an increase in the use of plastic materials in the food sector and consumer products, both for primary and secondary packaging. In the bottling industry, bottles are manufactured from specific polymers depending on the capacity of the container, each of them with unique characteristics as regards bottle strength, storage time, type of dispenser and disposal. Primary packaging is made with high density polyethylene (HDPE), polyethylene terephthalate (PET) and polycarbonate (PC) while caps are made of high density polyethylene (HDPE), low density polyethylene (LDPE) and polystyrene (PS) is used as septa in many caps (World Packaging Organization (WPO) 2008). These polymers contain additives such as antifogging, reinforcing and antistatic agents, blowing agents, colorants, fillers, lubricants, nucleating agents, optical brighteners, heat and light stabilizers, antiacids, antimicrobials, antioxidants, chain-breaking, photo- and hydroperoxide deactivating antioxidants, dehydrating agents, light screening pigments and UV absorbers (Bolgar 2008; Piringer 2008). Mixtures of plastic components and additives can be made to obtain improved plastic characteristics. In addition, several water bottle formats, shapes and colours are used in the different bottling industries.

The safety of some of polymeric materials is nowadays a subject of concern in the bottling sector due to the potential migration of plasticizers and additives to water by a diffusion process (Biscardi et al. 2003), as described for bisphenol A (BPA) (Biles et al. 1997; Casajuana et al. 2003; Le et al. 2008; Loyo-Rosales et al. 2004), phthalates (Casajuana et al. 2003; Peñalver et al. 2000) or nonylphenol (NP) (Casajuana et al. 2003). These substances are either plastic components, such as BPA used as monomer in the production of polycarbonate bottles (the so called “coolers”), or additives used to improve the plastic properties (such as NP), polymerization accelerators or agents to increase flexibility (phthalates) (Casajuana et al. 2003; Loyo-Rosales et al. 2004; Peñalver et al. 2000; Shen et al. 2007).

The presence of plastic components or additives in water can modify the organoleptic properties and if present at high concentrations, may trigger health problems due to the potential toxic properties of some chemicals. Epidemiological studies in test animals indicate an increase of some kinds of cancer, behaviour changes and anomalies in the reproductive and immunologic functions of some species (Casajuana et al. 2003; Rivas et al. 1997), reason that some of the target compounds are considered as endocrine disruptor compounds (EDC). Possible human health endpoints

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68 affected by these agents include breast cancer and endometriosis in women, testicular
69 and prostate cancers in men, abnormal sexual development, reduced male fertility,
70 alteration in pituitary and thyroid gland functions, immune suppression, and
71 neurobehavioral effects (Environmental Protection Agency (EPA) 1997). For safety
72 reasons, polymers used for packaging which are in contact with food must be analyzed
73 before use to prevent migration of any of its components to the food (Council of Europe
74 2002).

75 Several methods are used to evaluate the potential migration of plastic
76 components or additives. The migration capacity varies depending on conditions used in
77 migration tests and depending on the type of food simulant. Nerín et al. (2002) used
78 different migration tests, one consisted in two sorbents (Tenax and Porapak) in contact
79 with plastic, and another consisted in a total dissolution procedure with
80 dichloromethane and methanol (Nerín et al. 2003). These migration tests were set up for
81 several plastic containers used in microwave ovens. Schmidt et al. performed a
82 quantitative determination of the plasticizers bis(2-ethylhexyl) adipate (DEHA) and
83 bis(2-ethylhexyl) phthalate (DEHP) in PET bottles and revealed maximum
84 concentrations of 0.046 and 0.71 $\mu\text{g L}^{-1}$, respectively (Schmid et al. 2008). Casajuana et
85 al. (2003) detected BPA in HDPE and PET bottles at levels between 0.003 and 0.011 μg
86 L^{-1} after exposing water bottles at sunlight for 10 weeks. BPA was also detected in
87 waters of public distribution system at levels of 0.006 and 0.025 $\mu\text{g L}^{-1}$. The same
88 authors analyzed BPA in milk packed in Tetra Pack or HDPE bottles and concentrations
89 were between 0.28 and 2.64 $\mu\text{g kg}^{-1}$ of milk, depending on the brand (Casajuana et al.
90 2004). This indicates that fatty foods have a better ability to extract BPA from plastics
91 than water.

92 This study has investigated the potential migration of plasticizers and additives
93 from several plastic containers. Three migration tests were conducted: i) the standard
94 method UNE-EN ISO 177 to test overall migration from plastics (not necessarily food
95 contact materials) where plastics are in contact with an adsorbent and incubated at 40°C
96 during 10 days (European Communities 1988); ii) ultrasonic forced extraction of
97 plastics incubated in water at different times and iii) the standard method UNE-EN
98 13130 (2005, Part 1) in which plastics were incubated at 40°C in water for 10 days. This
99 last method is described and legislated in the Spanish Royal Decree 866/2008
100 (Ministerio de la Presidencia 2008) transposing Directives 82/711/EC (The Commission
101 of the European Communities 1982), 85/572/CEE (The Commission of the European

Communities 1985), UNE-EN 1186 of 2002 and 2007/19/CE (The Commission of the European Communities 2007). Analyses were performed using solid phase extraction (SPE) followed by gas chromatography coupled to mass spectrometry (GC-MS).

Materials and methods

Chemicals and reagents

Five phthalates, two alkylphenols and BPA were analyzed (Table 1). Analytical grade (98% purity) Phthalate Esters Mix including dimethyl phthalate (DMP), di-n-butyl phthalate (DPB), butyl benzyl phthalate (BBP), bis(2-ethyhexyl) phthalate (DEHP) and bis(2-ethyhexyl) adipate (DEHA) was purchased from Supelco (Bellefonte, PA, USA) at a concentration of 500 $\mu\text{g mL}^{-1}$ each in methanol. 4-nonylphenol (NP) was from Riedel-de Haën (Seelze, Germany) as a solid technical mixture of isomers; 4-tert-octylphenol (OP) was from Supelco (Bellefonte, PA, USA) as a solid and BPA was from Dr. Ehrenstorfer (Augsburg, Germany) as a solid. Stock standard solutions of each compound were prepared in ethyl acetate at a concentration of 5 $\mu\text{g mL}^{-1}$ and stored in the dark at -20°C until use. The surrogate standards used were NP-d₈ (100 $\text{ng } \mu\text{L}^{-1}$), diphenylphthalate-d₄ (DPP-d₄) and BPA-d₁₆ purchased as solids from Dr. Ehrenstorfer (Augsburg, Germany). The internal standard was anthracene-d₁₀, purchased from Supelco.

200 mg Oasis HLB cartridges were from Waters (Milford, MA, USA) and used with a Baker vacuum system (prod. No. 7018-94, J.T. Baker, The Netherlands). Chromatography grade methanol, acetone, dichloromethane, n-hexane, ethyl acetate and HPLC water were purchased from Merck (Darmstadt, Germany).

Samples

Polyethylene terephthalate (PET), polycarbonate (PC), two types of high density polyethylene (HDPE), low density polyethylene (LDPE) and polystyrene (PS) plastics used as cap septa were tested using three kinds of migrations test (see below). Prior to use, these plastics were rinsed with HPLC water and cut with scissors in circular chips of different diameter, according to the migration test used. In addition, bottled water samples in PET, PC and HDPE were analyzed to determine the presence of target compounds in real storing conditions.

Migration tests

Three methods were used to test the migration of phthalates, BPA and alkylphenols from water bottles, taps and septa. Except for UNE-EN ISO 177, specific migration tests were performed for all plastic types for comparability purposes. Although article filling is a conventional test to evaluate the migration of plastic components, total immersion was used because in our laboratory conditions, it was impossible to perform migration test of containers as big as 18 L (e.g. coolers). According to the specific migration test performed, values are given in $\mu\text{g dm}^{-2}$ because the samples were extracted from bottles as sheets and not as filled samples (Table 3). Also, the migration tests used in this study can be considered as total immersion tests, where the two plastic layers, inside (in contact with food) and outside (in contact with the environment) are in contact with the food stimulant. In European standards, it is considered the most severe test.

All sample manipulation was done using polytetrafluoroethylene (PTFE) tubing and glass material to avoid external contamination of the samples.

UNE-EN ISO 177 method was used as a screening tool for the determination of the overall migration of plasticizers, expressed as loss of weight in the incubated plastic and gain in the adsorbent. It applies to evaluate the tendency of plasticizers and additives to migrate from plastic materials to other materials placed in close contact with them. It cannot be compared with food legislation, although it shows the possible migration of plasticizers and represented the starting point for the next tests. First, C_{18} disks of 47 mm diameter and the plastic samples cut at the same diameter were weighed. The plastic sample was placed in a “sandwich” made of the plastic chip placed in between two C_{18} adsorbent disks, as it is shown in Figure 1. The “sandwich” was placed under a weight of 5 kg, and was introduced in an oven at 40°C during 10 days, as described in Directive 82/711/EC and their modifications (Directive 93/8/EC and Directive 97/48/EC). After that, the “sandwich” was removed and the adsorbent disks and samples were re-weighed to determine the weight difference. All polymers were tested except LDPE and PS sample which were adhered to the sorbent and could not be weighed after incubation.

Ultrasonic extraction method was developed as an accelerated method to force the migration of plastic components and additives to water. Although the results obtained in this method are not comparables with legislated values, it permits to identify the compounds that tend to migrate. 1 g of 0.5-1 cm^2 plastic chips was introduced in

100 mL of unbuffered HPLC water (used as food simulant at pH>4.5, as described in Directive 82/711/EC), and 5 ng of surrogates standards were added. Then samples were incubated in an ultrasonic bath during 5, 10 and 15 min (Figure 1). Afterwards, 100 mL of the incubated water was solid phase extracted (Céspedes et al. 2004) and analyzed by GC-MS to identify target compounds. All assays were performed in triplicate.

UNE-EN 13130 method was used for the identification of plastic components and to compare the concentrations obtained with legislated values (Table 1). 1 g of plastic chips was introduced in 100 mL of unbuffered HPLC water (used as food simulant at pH>4.5, as described in Directive 82/711/EC) and 5 ng of surrogates standards were added. Samples were incubated in a water bath at a temperature of 40°C during 10 days (Figure 1). After incubation, water was extracted by SPE and analyzed by GC-MS. GC-MS was used because it permitted to simultaneously analyze phthalates, alkylphenols and BPA, although UNE-EN 1313-13 describes the determination of BPA in food stimulants by high performance liquid chromatography (HPLC) with ultraviolet (UV) detection. All assays were performed in triplicate.

Solid Phase Extraction procedure

Resulting water from the migration tests was solid phase extracted using 200 mg Oasis HLB SPE cartridges using a Baker vacuum system with 12 cartridges capacity. All Baker vacuum system connections and tubing were of PTFE to avoid contamination of target compounds. Cartridges were conditioned prior to sample loading with 10 mL of hexane, followed by 10 mL of dichloromethane, 10 mL of methanol and 15 mL of HPLC water, all by gravity. This extensive cartridge cleaning was performed to eliminate any traces of target compounds. Then, 100 mL of water were preconcentrated at a flow of 8-13 mL min⁻¹ and afterwards the cartridge was dried under vacuum during 1 hour. Elution was performed with 10 mL of dichloromethane:hexane (1:1) and 10 mL dichloromethane:acetone (1:1). The extract was preconcentrated in a Turbovap nitrogen evaporator and extracts were reconstituted with 240 µL of ethyl acetate and 10 µL of anthracene d10 (10 ng µL⁻¹) was added as internal standard.

Instrumental analysis

Samples were analysed by gas chromatography coupled to a quadrupole mass spectrometer (Trace GC-2000 series from Thermo Electron, San José, CA, USA). The system was operated in electron ionization mode (EI 70 eV). The separation was

achieved with a 30 m x 0.25 mm I.D. DB-5MS column (J&W Scientific, Folsom, CA, USA) coated with 5% phenyl-95% dimethylpolysiloxane (film thickness 0.25 μm). The oven temperature was programmed from 70°C (holding time 2 min) to 135°C at 10°C min^{-1} , to 160°C at 3°C min^{-1} , to 175°C at 1°C min^{-1} , to 195 °C at 3°C min^{-1} and finally to 310°C at 10°C min^{-1} , keeping the final temperature for 5 min. 2 μL were injected in the splitless mode, keeping the split valve closed for 1 min. Helium was the carrier gas (1.2 mL min^{-1}). Injector, GC interface temperature and ion source temperatures were 280°C, 280°C and 200°C, respectively.

Peak detection and integration were carried out using Xcalibur software. Full scan data (from m/z 60 to 400) was used for the identification of target compounds in the migration tests.

Quantification and quality parameters

Internal standard quantification was performed using the deuterated surrogate standards corresponding to each chemical family (alkylphenols, phthalates and BPA) to correct any loss during sample manipulation. Calibration curves were constructed for all target compounds over a concentration of 0.01-1 mg L^{-1} . Limits of detection (LOD) were calculated using 3 times the standard deviation of n=5 blank samples, which is an adequate procedure when there is a contribution of target compounds in the blank samples due to the extraction and MS analysis. This procedure provides higher LOD than when calculated from a spiked sample at low concentration and using 3 times the noise signal. However, it is more realistic and provides better accuracy by reducing the risk of overestimation due to sample contamination by the ubiquitous presence of phthalates in laboratory air and even in the MS injection port. Quality controls were performed using HPLC water spiked at 1 $\mu\text{g L}^{-1}$ and were incubated in the absence of any plastic using the ultrasonic extraction and the UNE-EN 13130 method, in triplicate and using closed glass containers.

Results

Performance of the 3 migration tests

Migration tests permit to assess the potential leaching or migration of plastic components or additives. The UNE-EN ISO 177 method was used as a screening tool to determine the migration of plasticizers but was not adequate to identify specific

compounds. To identify those compounds that migrate from plastic and quantify their levels, ultrasonic and incubation of plastics at 40°C were used in combination of GC-MS whose capabilities permitted the determination of target compounds from test materials with good performance as regards to identification and precise quantification. The identification of plastic components was done in scan acquisition mode, which provided high selectivity and good identification capabilities and no response enhancement was observed due to the migration of other polymerized plastic to the extracts. The method allowed the determination of target analytes in water samples at levels of sub $\mu\text{g L}^{-1}$ (Table 1). The LOD of phthalates were high compared to other studies (Peñalver et al. 2000) because they were calculated from the 3 times the standard deviation of the blank samples which always contained traces of phthalates that originate both from the extraction and from the GC injection port. The use of surrogate standards to quantify each compound was necessary to achieve accurate results, taking into consideration the loss of analytes during incubation or extraction. The recoveries of the analytes were between 93 and 125% for the SPE-GC/MS method (Table 1).

Migration of plastic components and additives from bottled water and caps

UNE-EN ISO 177 provides an unspecific method capable to determine the total mass of compounds migrating from plastic containers. Table 2 proves that tested plastics incubated at 40°C lost weight, which was gained by the adsorbent, although there was some mismatch due to the fact that some volatile compounds were lost during incubation at 40°C, as considered in UNE-EN ISO 177. For example, for the PC plastic, there was a 0.1 mg increase in the plastic due to humidity increase in the weighing process but the gain of 2.3 mg in the sorbent after incubation should be taken into consideration. Overall, the lose in plastic weight provides first evidence on the overall migration of plasticizers from incubated plastics although this method failed to identify and quantify specific compounds prone to migrate.

Migration tests UNE-EN 13130 and ultrasonic incubation were able to identify target compounds. Ultrasonic method is a fast extraction method and was developed in an attempt to validate it by comparing the results with the incubation at 40°C. We found that qualitatively, both methods provided similar information, but quantitatively, compounds were extracted with different efficiency. Phthalates were not detected in any of the plastics tested and in any of the treatments used, and indicates that either these compounds were not added in the tested plastics or either that they do not migrate at the

conditions tested. The same holds for octylphenol. NP and BPA were the only compounds identified. The recoveries of NP and BPA using the ultrasonic incubation were of 45% and 76% for respectively, and using the UNE-EN 13130, recoveries were 92% and 97% for NP and BPA, respectively. The different recoveries using both migration tests reflect the losses that occur during the incubation period, which are higher when using ultrasonic extraction, especially for NP.

Ultrasonic incubation forces the migration of compounds by applying an ultrasonic wave which enhances the detachment of plastic components or additives which are released to water. Results showed that there was not any difference in the migration of plastic compounds using different extraction times (5, 10 or 15 min) and thus, an extraction time of 5 min was chosen. Using this technique, NP was identified in the incubated plastics at levels of 0.212-0.242 $\mu\text{g dm}^2$ while BPA was only detected at 1.870 $\mu\text{g dm}^2$ in the PC plastic (Table 3).

UNE-EN 13130 describes the methods for determining the specific migration of plastic materials and UNE-EN 1186 describes the methods for determining the overall migration of materials, plastics and articles in contact with foodstuffs. This UNE-EN 13130 migration test was based on Part 1, guide to test methods for the specific migration of substances from plastics (see also UNE-EN 1186, Part 1 guide for the selection of conditions and test methods to calculate the overall migration). Using this method, NP and BPA were quantified at levels of 0.332-1.282 $\mu\text{g dm}^2$ and 0.128-3.423 $\mu\text{g dm}^2$, respectively (Table 3). This method was more efficient in extracting plasticizers and additives from plastics than ultrasonic extraction, and was able to determine NP in all types of plastic and BPA in PC, HDPE, LDPE and PS. Both NP and BPA can be used as plastic additives to improve the quality of plastic.

These two last methods are complementary and provide similar information on the migration potential, qualitatively. Ultrasonic bath provides a lower migration of BPA and NP compared to the UNE-EN 13130 (Table 3). Among both methods, ultrasonic extraction can be considered a fast extraction method but with lower efficiency than UNE-EN 13130 and so far, is not validated yet. Most important is that UNE-EN 13130 is based on a technical interpretation of Directive 2007/19/EC and provides maximum legislated migration levels for a large number of compounds. Thus, this technique was further used to determine the migration levels of target compounds in the 5 polymers studied and to compare the specific migration levels with legislated values.

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307 *Polymers tested and migration levels according to UNE-EN 13130*

308 NP and BPA were the only compounds identified in PC, HDPE, LDPE and PS plastics
309 and for PET and HDPE bottles NP was the only compound identified. The MS
310 chromatogram of BPA in the PC sample with UNE-EN 13130 method is shown in
311 Figure 2. PET was the plastic type for which NP migration was lower, followed by
312 HDPE bottles and PC. For caps material, NP levels increased from LDPE<PS<HDPE
313 with NP migration between $0.413 \mu\text{g dm}^{-2}$ and $1.282 \mu\text{g dm}^{-2}$. Contrarily, BPA was not
314 detected in PET or HDPE bottles while the highest migration was found in PC bottles at
315 $3.423 \mu\text{g dm}^{-2}$. BPA was also detected in caps and septa with levels ranging from 0.128
316 $\mu\text{g dm}^{-2}$ to $0.145 \mu\text{g dm}^{-2}$.

317 PET is one of the most common polymers used in bottled water container for its
318 lightness, its gas barrier and its possibility to be recycled, and it is generally used in
319 volumes from 0.33 up to 8 L bottles. Although in general it is for a single use, some
320 companies recycle up to 25% of this plastic in the manufacture of new bottles. NP was
321 detected at a mean concentration of $0.332 \mu\text{g dm}^{-2}$ (n=3) and BPA was not detected in
322 any replicate in this kind of plastic (Table 3). Casajuana et al. (Casajuana et al. 2003)
323 found the presence of DMP, DEP, 4-NP, DBP, BPA, BBP and DEHP in PET bottled
324 water samples at levels of 0.002 to $0.214 \mu\text{g L}^{-1}$ after 10 weeks storage at high
325 temperature and using SPE-GC-MS. Contrarily, Loyo-Rosales et al. (Loyo-Rosales et
326 al. 2004) describe that neither NP nor OP were found in extracts from water stored in
327 PET containers after 240 h. This discrepancy is attributed to the migration test and
328 conditions used. NP is not legislated by the European Community and thus, a
329 comparison with maximum permissible levels cannot be withdrawn.

330 HDPE is characterized by its strength and resistance to many solvents and is
331 used in bottles of 5-8 L. This material is also used in the manufacture of caps, since it is
332 resistant and it has a good sealing capacity. NP was released at concentrations $0.579 \mu\text{g}$
333 dm^{-2} and $1.282 \mu\text{g dm}^{-2}$ for HDPE bottles and caps, respectively (Table 3). For HDPE
334 caps, BPA was detected at $0.145 \mu\text{g dm}^{-2}$ and no traces were found in HDPE bottles
335 (Table 3). Other studies describe the presence of NP in water bottled in HDPE and PVC
336 containers at a concentration of $0.230 \mu\text{g L}^{-1}$ after 120 h at 40°C (Loyo-Rosales et al.
337 2004).

338 PC, a more resistant plastic, is used in containers over 10 L capacity and after
339 use, it is cleaned and reused. PC plastic is made of BPA monomers, which can migrate

from containers (Le et al. 2008). The BPA amount detected in PC plastic was of 3.423 $\mu\text{g dm}^{-2}$ and NP was detected at 0.694 $\mu\text{g dm}^{-2}$. Another study using bottled water in PC describes the migration of this compound from used and new bottles at 0.7 $\mu\text{g L}^{-1}$ and 1.0 $\mu\text{g L}^{-1}$, respectively, after 7 days at room temperature (Le et al. 2008). Nerín et al. (Nerín et al. 2003) detected that BPA migrated from PC plastic stored at room temperature. Biles et al. (Biles et al. 1997) detected BPA in PC baby bottles and cups at levels ranging from non detected to 5 $\mu\text{g L}^{-1}$.

LDPE is the polymer used in the cooler caps, its structure is the same as HDPE but the density is lower so LDPE is used in caps and not in bottles. NP and BPA migrated as shown in Table 3 at levels of 0.413 and 0.128 $\mu\text{g dm}^{-2}$, respectively.

Finally, PS was tested because it is used in the septum of several caps, such as the ones used in coolers. The amounts detected were 0.801 and 0.136 $\mu\text{g dm}^{-2}$ for NP and BPA, respectively. PS is one of the most produced plastics in food contact materials and can be copolymerized with many monomers. Products formed from PS are hard and transparent (Piringer 2008). However, cap septa are whitish due to additives that improve the brittleness and the sensitivity to stress cracking (Piringer 2008). These additives may migrate as it happens in other type of plastics.

The presence of phthalates and BPA in food in contact with plastic is legislated in the European Union. Directive 2002/72/EC, Directive 2004/19/EC and Directive 2007/19/EC (transposed to the Spanish Royal Decree 866/2008) establishes migration specific limits in food and food simulants of 600 $\mu\text{g kg}^{-1}$ or 100 $\mu\text{g dm}^{-2}$ for BPA, 1500 $\mu\text{g kg}^{-1}$ or 250 $\mu\text{g dm}^{-2}$ for DEHP, 300 $\mu\text{g kg}^{-1}$ or 50 $\mu\text{g dm}^{-2}$ for DBP and 30000 $\mu\text{g kg}^{-1}$ or 5000 $\mu\text{g dm}^{-2}$ for BBP. The levels for phthalates are explicit in Directive 2007/19/EC and are different according to i) whether the packing is a single or multiple use article, ii) whether it is in contact with aqueous or fatty foods and 3) whether it is for infants. So there are a variety of limits for different cases. From the different polymers tested, none of the samples exceeded the legislated value of 100 $\mu\text{g dm}^{-2}$ for BPA. These legislated levels are lower or equal with the former Directive 2002/72/EC, who established migration specific limits in food and food simulants of 3000 $\mu\text{g kg}^{-1}$ for BPA and 1500 $\mu\text{g kg}^{-1}$ for DEHP. Specifically, the levels of BPA decreased 5 times from 2002 to 2007 Directives because the increasing information and studies on BPA toxicity and the potential effects on humans if ingested daily (Bredhult et al. 2009; Ghisari et al. 2009; Huang et al. 2009; Le et al. 2008; Salian et al. 2009).

374 *Analysis of bottled water*

375 The presence of plasticizers in bottled water (PET, HDPE and PC) was determined
376 using the same SPE-GC/MS method extracting 1 L of water. In 1.5 L PET bottles, 2 out
377 of 10 samples contained one target compound; OP was detected in one sample at 0.003
378 $\mu\text{g L}^{-1}$ and NP in another at 0.019 $\mu\text{g L}^{-1}$. In HDPE bottles, OP was detected in two
379 samples at 0.003 $\mu\text{g L}^{-1}$ and 0.004 $\mu\text{g L}^{-1}$, (N=7, volume = 5-10 L). The trace presence
380 of these compounds is attributed to the use of OP and NP in the production of the
381 specific polymers which vary among brands.

382 Finally, all PC coolers (N=10, volume = 18.9-20 L) contained BPA at levels
383 ranging from 1.60 $\mu\text{g L}^{-1}$ to 4.44 $\mu\text{g L}^{-1}$, with an average concentration of 2.64 $\mu\text{g L}^{-1}$.
384 These levels are in agreement with other studies which indicate that BPA can migrate
385 from both PC and epoxy resins containers in contact with water at levels of few $\mu\text{g L}^{-1}$.
386 In a former study regarding the migration of BPA, the U.S. FDA (Food & Drug
387 Administration of the United States) analyzed BPA in water coolers stored for 39 weeks
388 and found BPA at levels between 0.1 and 4.7 $\mu\text{g L}^{-1}$ (Environmental Protection Agency
389 (EPA) 1993). In a 1997 study, it was found that BPA migrated when exposing
390 polymeric material to water, ethanol/water and Miglyol (an oil) to temperatures of 65°C
391 for 10 days. In the case of water in contact with polycarbonate bottles where the BPA is
392 the main monomer, the levels of BPA were from not detected to 5 $\mu\text{g L}^{-1}$ (Biles et al.
393 1997). In another study, the migration of BPA in PC bottles filled with water and
394 exposed to 100°C for 1 h showed BPA migration levels of $0.23 \pm 0.12 \mu\text{g L}^{-1}$, while
395 levels increased to $8.4 \pm 4 \mu\text{g L}^{-1}$ and $6.7 \pm 4 \mu\text{g L}^{-1}$ after using a domestic dishwasher
396 between 51 and 169 times (Unit on food contact materials, enzymes, flavourings and
397 processing aids (CEF) et al. 2008).

399 *Daily Intake and toxicological values*

400 The Tolerable Daily Intake (TDI) is a set of reference values for the acceptable intake of
401 a variety of nutrients, as well as energy. A way to express the toxicity of a chemical
402 specie is to calculate the TDI, which represents a lifetime exposure level that is
403 considered to be safe. In this study, BPA is the only compound considered for
404 evaluating the TDI since it was the only one detected in all samples analyzed. Based on
405 the migration data from bottled water, the total exposure to BPA from PC plastic was
406 estimated to be of $4.00 \cdot 10^{-5} \text{ mg BPA kg}^{-1} \text{ bw day}^{-1}$ (1.5 L water day^{-1} , 60 kg person and
407 $1.60 \mu\text{g BPA L}^{-1}$) and an upper range exposure of $1.48 \cdot 10^{-4} \text{ mg BPA kg}^{-1} \text{ bw day}^{-1}$ (2 L

water day⁻¹, 60 kg person and 4.44 µg BPA L⁻¹). The Scientific Committee on Food (SCF) in 2002 considered that the overall oral No-Observed-Adverse-Effect Level (NOAEL) for BPA was 5 mg kg⁻¹ bw day⁻¹ and set a temporary TDI of 0.01 mg kg⁻¹ bw day⁻¹ (European Food Safety Authority (EFSA), 2006), which was changed in 2008 by a TDI of 0.05 mg kg⁻¹ bw day⁻¹ (Unit on food contact materials, enzymes, flavourings and processing aids (CEF) et al. 2008). In a former study realized in 1993, the U.S. EPA had already suggested a reference value of 0.05 mg kg⁻¹ of body weight (Environmental Protection Agency (EPA) 1993). These organizations state that PC products are safe for being used in products in contact with food and beverage and pose no known risk to human health. The levels of BPA from PC coolers were below the NOAEL and TDI, although it should be considered that BPA consumption from PC bottled water is only a part of the total oral Daily Intake.

Many studies have been carried out on the toxicity and potential estrogenic effects of BPA using various animal models, cellular lines and at molecular and biochemical level. These studies set the initial basis to carry out risk assessment studies of BPA on humans. However, discrepancies have been observed in these on going studies regarding both the effects of BPA and the levels that cause these effects. From a toxicological point of view, if the aquatic toxicity is considered using *Daphnia magna*, EC₅₀ values range between 1 and 20 mg L⁻¹ (Table 1), and although these concentrations cannot be extrapolated to a human model toxicity, they are an indicator that effects caused to a very sensitive specie are initiated at the mg level. Cespedes et al. developed a more specific method to assess the estrogenicity of BPA using recombinant yeast using the human estrogen receptor (Céspedes et al. 2004). It was demonstrated that the estrogenicity of BPA is 1644 ± 380 µg L⁻¹, much lower than the natural hormone estradiol (0.04 µg L⁻¹), used as positive control (Table 1). That means that at concentrations below 1644 µg L⁻¹, BPA does not activate the human estrogen receptor and therefore does not trigger any estrogenic effect. Concluding, the concentration of BPA from PC containers in the samples analyzed were below the aquatic toxicity, the EC₅₀ obtained with the recombinant yeast assay and the legislated levels (Table 1). However, epidemiological studies carried out by Vandenberg et al. indicate that BPA is present in human fluids at concentration levels higher than those that induce effects *in vitro* and one order of magnitude higher than the levels that induce effects in animal models (Vandenberg et al. 2007). Another study detected that 92.6% of the examined

US population had BPA in urine due to the routine ingestion of this compound from plastic food containers (Calafat et al. 2008).

Conclusions

Given the increased use of plastic in the bottling packaging sector, migration tests permit the identification of plasticizers and additives with endocrine disrupting properties that can cause human health effect when ingested continuously. Out of the 3 migration tests used, the UNE-EN 13130 method permitted identification of compounds leaching from plastics and the ability to compare the migration levels with legislative values. The ultrasonic extraction was an alternative to evaluate compounds migrating from different polymers and qualitatively had a comparative performance although with less efficiency and therefore, is not yet a validated and accepted method. From 5 polymers tested, phthalates were not present in the plastics while NP and BPA were identified at concentrations from 0.128 up to 3.423 $\mu\text{g dm}^{-2}$. PET and HDPE bottles were the bottles with the lowest amount of EDCs. The biggest problem compound was BPA which was detected in all polycarbonate bottled waters analyzed and although the total daily intake was below the legislative values, it may contribute to the total daily intake of this compound considering all types of food. Numerous studies indicate that BPA causes adverse effects in experimental animals and the constant exposure, metabolism and long term risk in humans is a matter of concern. The European Food Safety Authority (EFSA), the US Environmental Protection Agency and other Agencies are currently undertaking studies to integrate data from migration tests, exposure levels and toxicity endpoints to assess the possible effects of BPA on humans and initiate actions to protect human health. At this point, BPA has recently been banned in Europe (by summer 2011).

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Table 1. Plasticizers and additives studied, their molecular formula, water octanol coefficient (log K_{ow}), toxicity using *Daphnia magna* and Recombinant Yeast Assay (RYA), limits of detection calculated from 3 times the standard deviation of the blank samples (n=5), recoveries calculated at 1 µg L⁻¹ and legislated values.

Compound	Acronym	Molecular formula	Log K _{ow}	Aquatic toxicity ^a µg L ⁻¹	RYA ^b µg L ⁻¹	LOD µg L ⁻¹	Recoveries %	Legislative level µg dm ⁻²	Legislative level ^c µg kg ⁻¹
Dimethyl phthalate	DMP	C ₁₀ H ₁₀ O ₄	1.60	45900	4080 ± 1200	0.010	112	nl	nl
Di-n-butyl phthalate	DBP	C ₁₆ H ₂₂ O ₄	4.50	2990	3960 ± 1500	0.230	104	50 ^c	300 ^{cd}
Benzylbutyl phthalate	BBP	C ₁₉ H ₂₀ O ₄	4.73	960	5160 ± 2160	0.190	125	5000 ^c	30000 ^{cd}
Di(2-ethylhexyl) adipate	DEHA	C ₂₂ H ₄₂ O ₄	6.11	nf	nf	0.080	97	nl	nl
Di(2-ethylhexyl) phthalate	DEHP	C ₂₄ H ₃₈ O ₄	7.60	160	2310 ± 1460	0.460	107	250 ^c	1500 ^{cd}
Bisphenol A	BPA	C ₁₆ H ₁₅ O ₂	3.32	1000-20000	1640 ± 380	0.009	97	100 ^c	600 ^{ce}
Octylphenol	OP	C ₁₄ H ₂₂ O	5.28	90-1140	180 ± 80	0.001	93	nl	nl
Nonylphenol	NP	C ₁₅ H ₂₄ O	5.92	90-470	78 ± 20	0.017	98	nl	nl
^a EC50 – 48 hr, <i>Daphnia magna</i> . ^b EC50 - Recombinant Yeast Assay (EPA/630/R-96/012). ^c Spanish Royal Decree 866/2008 ^d According Directive 2007/19/EC ^e According Directive 2004/19/EC nf: not found, nl: not legislated									

Table 2. UNE-EN ISO 177 weights for 4 test plastics where it is observed that plastics lose weight while the adsorbent gains weight.

	Δm plastic mg	Δm disks mg
PET bottle	-0.3	8.1
HDPE bottle	-2.7	5.0
PC bottle	0.1	2.4
HDPE cap	-5.1	5.9
Blank	–	0.3

Table 3. Compounds identified using ultrasonic extraction and the conditions specified in UNE-EN 13130 expressed in mg dm^{-2} of plastic $\pm$ standard deviation and % recoveries of target compounds in the $1 \mu\text{g L}^{-1}$ spiked HPLC water quality control.

Method	Compound	Bottles			Caps		Septum	Recov. (%)
		PET $\mu\text{g dm}^{-2}$	HDPE $\mu\text{g dm}^{-2}$	PC $\mu\text{g dm}^{-2}$	HDPE $\mu\text{g dm}^{-2}$	LDPE $\mu\text{g dm}^{-2}$	PS $\mu\text{g dm}^{-2}$	
Ultrasonic extraction	NP	<LOD	0.242 ± 0.005	<LOD	<LOD	<LOD	0.212 ± 0.034	45
	BPA	<LOD	<LOD	1.870 ± 0.088	<LOD	<LOD	<LOD	76
UNE-EN 13130	NP	0.332 ± 0.022	0.579 ± 0.008	0.694 ± 0.091	1.282 ± 0.178	0.413 ± 0.004	0.801 ± 0.176	92
	BPA	<LOD	<LOD	3.423 ± 0.217	0.145 ± 0.026	0.128 ± 0.019	0.136 ± 0.028	97

Figure 1. Illustration of the three tests.

Figure 2. GC-MS TIC chromatogram of PC sample with UNE-EN 13130 method.