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1 Migration of phthalates, bisphenol A and alkylphenols from plastic 2 containers and evaluation of risk

3
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9 10 11 **Abstract**

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

29
30 **Keywords:** migration; plasticizer; phthalate; alkylphenol; bisphenol A; nonylphenol;
31 SPE; GC-MS.

34 Introduction

35 In the last years there has been an increase in the use of plastic materials in the food
36 sector and consumer products, both for primary and secondary packaging. In the
37 bottling industry, bottles are manufactured from specific polymers depending on the
38 capacity of the container, each of them with unique characteristics as regards bottle
39 strength, storage time, type of dispenser and disposal. Primary packaging is made with
40 high density polyethylene (HDPE), polyethylene terephthalate (PET) and polycarbonate
41 (PC) while caps are made of high density polyethylene (HDPE), low density
42 polyethylene (LDPE) and polystyrene (PS) is used as septa in many caps (World
43 Packaging Organization (WPO) 2008). These polymers contain additives such as
44 antifogging, reinforcing and antistatic agents, blowing agents, colorants, fillers,
45 lubricants, nucleating agents, optical brighteners, heat and light stabilizers, antiacids,
46 antimicrobials, antioxidants, chain-breaking, photo- and hydroperoxide deactivating
47 antioxidants, dehydrating agents, light screening pigments and UV absorbers (Bolgar
48 2008; Piringer 2008). Mixtures of plastic components and additives can be made to
49 obtain improved plastic characteristics. In addition, several water bottle formats, shapes
50 and colours are used in the different bottling industries.

51 The safety of some of polymeric materials is nowadays a subject of concern in
52 the bottling sector due to the potential migration of plasticizers and additives to water
53 by a diffusion process (Biscardi et al. 2003), as described for bisphenol A (BPA) (Biles
54 et al. 1997; Casajuana et al. 2003; Le et al. 2008; Loyo-Rosales et al. 2004), phthalates
55 (Casajuana et al. 2003; Peñalver et al. 2000) or nonylphenol (NP) (Casajuana et al.
56 2003). These substances are either plastic components, such as BPA used as monomer
57 in the production of polycarbonate bottles (the so called “coolers”), or additives used to
58 improve the plastic properties (such as NP), polymerization accelerators or agents to
59 increase flexibility (phthalates) (Casajuana et al. 2003; Loyo-Rosales et al. 2004;
60 Peñalver et al. 2000; Shen et al. 2007).

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

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2
3 68 affected by these agents include breast cancer and endometriosis in women, testicular
4
5 69 and prostate cancers in men, abnormal sexual development, reduced male fertility,
6
7 70 alteration in pituitary and thyroid gland functions, immune suppression, and
8
9 71 neurobehavioral effects (Environmental Protection Agency (EPA) 1997). For safety
10
11 72 reasons, polymers used for packaging which are in contact with food must be analyzed
12
13 73 before use to prevent migration of any of its components to the food (Council of Europe
14
15 74 2002).

16 75 Several methods are used to evaluate the potential migration of plastic
17
18 76 components or additives. The migration capacity varies depending on conditions used in
19
20 77 migration tests and depending on the type of food simulant. Nerín et al. (2002) used
21
22 78 different migration tests, one consisted in two sorbents (Tenax and Porapak) in contact
23
24 79 with plastic, and another consisted in a total dissolution procedure with
25
26 80 dichloromethane and methanol (Nerín et al. 2003). These migration tests were set up for
27
28 81 several plastic containers used in microwave ovens. Schmidt et al. performed a
29
30 82 quantitative determination of the plasticizers bis(2-ethylhexyl) adipate (DEHA) and
31
32 83 bis(2-ethylhexyl) phthalate (DEHP) in PET bottles and revealed maximum
33
34 84 concentrations of 0.046 and 0.71 $\mu\text{g L}^{-1}$, respectively (Schmid et al. 2008). Casajuana et
35
36 85 al. (2003) detected BPA in HDPE and PET bottles at levels between 0.003 and 0.011 μg
37
38 86 L^{-1} after exposing water bottles at sunlight for 10 weeks. BPA was also detected in
39
40 87 waters of public distribution system at levels of 0.006 and 0.025 $\mu\text{g L}^{-1}$. The same
41
42 88 authors analyzed BPA in milk packed in Tetra Pack or HDPE bottles and concentrations
43
44 89 were between 0.28 and 2.64 $\mu\text{g kg}^{-1}$ of milk, depending on the brand (Casajuana et al.
45
46 90 2004). This indicates that fatty foods have a better ability to extract BPA from plastics
47
48 91 than water.

49
50 92 This study has investigated the potential migration of plasticizers and additives
51
52 93 from several plastic containers. Three migration tests were conducted: i) the standard
53
54 94 method UNE-EN ISO 177 to test overall migration from plastics (not necessarily food
55
56 95 contact materials) where plastics are in contact with an adsorbent and incubated at 40°C
57
58 96 during 10 days (European Communities 1988); ii) ultrasonic forced extraction of
59
60 97 plastics incubated in water at different times and iii) the standard method UNE-EN
98
99 13130 (2005, Part 1) in which plastics were incubated at 40°C in water for 10 days. This
100
101 99 last method is described and legislated in the Spanish Royal Decree 866/2008
(Ministerio de la Presidencia 2008) transposing Directives 82/711/EC (The Commission
of the European Communities 1982), 85/572/CEE (The Commission of the European

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2
3 102 Communities 1985), UNE-EN 1186 of 2002 and 2007/19/CE (The Commission of the
4
5 103 European Communities 2007). Analyses were performed using solid phase extraction
6
7 104 (SPE) followed by gas chromatography coupled to mass spectrometry (GC-MS).
8
9 105
10 106

107 **Materials and methods**

108 *Chemicals and reagents*

109 Five phthalates, two alkylphenols and BPA were analyzed (Table 1). Analytical grade
110 (98% purity) Phthalate Esters Mix including dimethyl phthalate (DMP), di-n-butyl
111 phthalate (DPB), butyl benzyl phthalate (BBP), bis(2-ethylhexyl) phthalate (DEHP) and
112 bis(2-ethylhexyl) adipate (DEHA) was purchased from Supelco (Bellefonte, PA, USA)
113 at a concentration of 500 $\mu\text{g mL}^{-1}$ each in methanol. 4-nonylphenol (NP) was from
114 Riedel-de Haën (Seelze, Germany) as a solid technical mixture of isomers; 4-tert-
115 octylphenol (OP) was from Supelco (Bellefonte, PA, USA) as a solid and BPA was
116 from Dr. Ehrenstorfer (Augsburg, Germany) as a solid. Stock standard solutions of each
117 compound were prepared in ethyl acetate at a concentration of 5 $\mu\text{g mL}^{-1}$ and stored in
118 the dark at -20°C until use. The surrogate standards used were NP-d₈ (100 $\text{ng } \mu\text{L}^{-1}$),
119 diphenylphthalate-d₄ (DPP-d₄) and BPA-d₁₆ purchased as solids from Dr. Ehrenstorfer
120 (Augsburg, Germany). The internal standard was anthracene-d₁₀, purchased from
121 Supelco.

122 200 mg Oasis HLB cartridges were from Waters (Milford, MA, USA) and used
123 with a Baker vacuum system (prod. No. 7018-94, J.T. Baker, The Netherlands).
124 Chromatography grade methanol, acetone, dichloromethane, n-hexane, ethyl acetate and
125 HPLC water were purchased from Merck (Darmstadt, Germany).
126

127 *Samples*

128 Polyethylene terephthalate (PET), polycarbonate (PC), two types of high density
129 polyethylene (HDPE), low density polyethylene (LDPE) and polystyrene (PS) plastics
130 used as cap septa were tested using three kinds of migrations test (see below). Prior to
131 use, these plastics were rinsed with HPLC water and cut with scissors in circular chips
132 of different diameter, according to the migration test used. In addition, bottled water
133 samples in PET, PC and HDPE were analyzed to determine the presence of target
134 compounds in real storing conditions.
135

1
2
3 136 *Migration tests*
4

5 137 Three methods were used to test the migration of phthalates, BPA and
6 138 alkylphenols from water bottles, taps and septa. Except for UNE-EN ISO 177, specific
7 139 migration tests were performed for all plastic types for comparability purposes.
8 140 Although article filling is a conventional test to evaluate the migration of plastic
9 141 components, total immersion was used because in our laboratory conditions, it was
10 142 impossible to perform migration test of containers as big as 18 L (e.g. coolers).
11 143 According to the specific migration test performed, values are given in $\mu\text{g dm}^{-2}$ because
12 144 the samples were extracted from bottles as sheets and not as filled samples (Table 3).
13 145 Also, the migration tests used in this study can be considered as total immersion tests,
14 146 where the two plastic layers, inside (in contact with food) and outside (in contact with
15 147 the environment) are in contact with the food stimulant. In European standards, it is
16 148 considered the most severe test.

17 149 All sample manipulation was done using polytetrafluoroethylene (PTFE) tubing
18 150 and glass material to avoid external contamination of the samples.
19 151

20 152 UNE-EN ISO 177 method was used as a screening tool for the determination of
21 153 the overall migration of plasticizers, expressed as loss of weight in the incubated plastic
22 154 and gain in the adsorbent. It applies to evaluate the tendency of plasticizers and
23 155 additives to migrate from plastic materials to other materials placed in close contact
24 156 with them. It cannot be compared with food legislation, although it shows the possible
25 157 migration of plasticizers and represented the starting point for the next tests. First, C_{18}
26 158 disks of 47 mm diameter and the plastic samples cut at the same diameter were
27 159 weighed. The plastic sample was placed in a “sandwich” made of the plastic chip placed
28 160 in between two C_{18} adsorbent disks, as it is shown in Figure 1. The “sandwich” was
29 161 placed under a weight of 5 kg, and was introduced in an oven at 40°C during 10 days, as
30 162 described in Directive 82/711/EC and their modifications (Directive 93/8/EC and
31 163 Directive 97/48/EC). After that, the “sandwich” was removed and the adsorbent disks
32 164 and samples were re-weighed to determine the weight difference. All polymers were
33 165 tested except LDPE and PS sample which were adhered to the sorbent and could not be
34 166 weighed after incubation.

35 167 Ultrasonic extraction method was developed as an accelerated method to force
36 168 the migration of plastic components and additives to water. Although the results
37 169 obtained in this method are not comparables with legislated values, it permits to identify
38 the compounds that tend to migrate. 1 g of $0.5\text{-}1\text{ cm}^2$ plastic chips was introduced in

1
2
3 170 100 mL of unbuffered HPLC water (used as food simulant at pH>4.5, as described in
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5 171 Directive 82/711/EC), and 5 ng of surrogates standards were added. Then samples were
6
7 172 incubated in an ultrasonic bath during 5, 10 and 15 min (Figure 1). Afterwards, 100 mL
8
9 173 of the incubated water was solid phase extracted (Céspedes et al. 2004) and analyzed by
10
11 174 GC-MS to identify target compounds. All assays were performed in triplicate.

12 175 UNE-EN 13130 method was used for the identification of plastic components
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14 176 and to compare the concentrations obtained with legislated values (Table 1). 1 g of
15
16 177 plastic chips was introduced in 100 mL of unbuffered HPLC water (used as food
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18 178 simulant at pH>4.5, as described in Directive 82/711/EC) and 5 ng of surrogates
19
20 179 standards were added. Samples were incubated in a water bath at a temperature of 40°C
21
22 180 during 10 days (Figure 1). After incubation, water was extracted by SPE and analyzed
23
24 181 by GC-MS. GC-MS was used because it permitted to simultaneously analyze
25
26 182 phthalates, alkylphenols and BPA, although UNE-EN 1313-13 describes the
27
28 183 determination of BPA in food stimulants by high performance liquid chromatography
29
30 184 (HPLC) with ultraviolet (UV) detection. All assays were performed in triplicate.

31 185

32 186 *Solid Phase Extraction procedure*

33 187 Resulting water from the migration tests was solid phase extracted using 200 mg Oasis
34
35 188 HLB SPE cartridges using a Baker vacuum system with 12 cartridges capacity. All
36
37 189 Baker vacuum system connections and tubing were of PTFE to avoid contamination of
38
39 190 target compounds. Cartridges were conditioned prior to sample loading with 10 mL of
40
41 191 hexane, followed by 10 mL of dichloromethane, 10 mL of methanol and 15 mL of
42
43 192 HPLC water, all by gravity. This extensive cartridge cleaning was performed to
44
45 193 eliminate any traces of target compounds. Then, 100 mL of water were preconcentrated
46
47 194 at a flow of 8-13 mL min⁻¹ and afterwards the cartridge was dried under vacuum during
48
49 195 1 hour. Elution was performed with 10 mL of dichloromethane:hexane (1:1) and 10 mL
50
51 196 dichloromethane:acetone (1:1). The extract was preconcentrated in a Turbovap nitrogen
52
53 197 evaporator and extracts were reconstituted with 240 µL of ethyl acetate and 10 µL of
54
55 198 anthracene d10 (10 ng µL⁻¹) was added as internal standard.

56 199

57 200 *Instrumental analysis*

58 201 Samples were analysed by gas chromatography coupled to a quadrupole mass
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60 202 spectrometer (Trace GC-2000 series from Thermo Electron, San José, CA, USA). The
203 system was operated in electron ionization mode (EI 70 eV). The separation was

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3 204 achieved with a 30 m x 0.25 mm I.D. DB-5MS column (J&W Scientific, Folsom, CA,
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5 205 USA) coated with 5% phenyl-95% dimethylpolysiloxane (film thickness 0.25 μm). The
6
7 206 oven temperature was programmed from 70°C (holding time 2 min) to 135°C at 10°C
8
9 207 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
10
11 208 310°C at 10°C min^{-1} , keeping the final temperature for 5 min. 2 μL were injected in the
12
13 209 splitless mode, keeping the split valve closed for 1 min. Helium was the carrier gas (1.2
14
15 210 mL min^{-1}). Injector, GC interface temperature and ion source temperatures were 280°C,
16
17 211 280°C and 200°C, respectively.

18 212 Peak detection and integration were carried out using Xcalibur software. Full
19
20 213 scan data (from m/z 60 to 400) was used for the identification of target compounds in
21
22 214 the migration tests.

23 215

24 216 *Quantification and quality parameters*

25
26 217 Internal standard quantification was performed using the deuterated surrogate standards
27
28 218 corresponding to each chemical family (alkylphenols, phthalates and BPA) to correct
29
30 219 any lose during sample manipulation. Calibration curves were constructed for all target
31
32 220 compounds over a concentration of 0.01-1 mg L^{-1} . Limits of detection (LOD) were
33
34 221 calculated using 3 times the standard deviation of $n=5$ blank samples, which is an
35
36 222 adequate procedure when there is a contribution of target compounds in the blank
37
38 223 samples due to the extraction and MS analysis. This procedure provides higher LOD
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40 224 than when calculated from a spiked sample at low concentration and using 3 times the
41
42 225 noise signal. However, it is more realistic and provides better accuracy by reducing the
43
44 226 risk of overestimation due to sample contamination by the ubiquitous presence of
45
46 227 phthalates in laboratory air and even in the MS injection port. Quality controls were
47
48 228 performed using HPLC water spiked at 1 $\mu\text{g L}^{-1}$ and were incubated in the absence of
49
50 229 any plastic using the ultrasonic extraction and the UNE-EN 13130 method, in triplicate
51
52 230 and using closed glass containers.

51 231

52 232

53 233 **Results**

54 234 *Performance of the 3 migration tests*

55
56 235 Migration tests permit to assess the potential leaching or migration of plastic
57
58 236 components or additives. The UNE-EN ISO 177 method was used as a screening tool to
59
60 237 determine the migration of plasticizers but was not adequate to identify specific

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2
3 238 compounds. To identify those compounds that migrate from plastic and quantify their
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5 239 levels, ultrasonic and incubation of plastics at 40°C were used in combination of GC-
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7 240 MS whose capabilities permitted the determination of target compounds from test
8
9 241 materials with good performance as regards to identification and precise quantification.
10
11 242 The identification of plastic components was done in scan acquisition mode, which
12
13 243 provided high selectivity and good identification capabilities and no response
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15 244 enhancement was observed due to the migration of other polymerized plastic to the
16
17 245 extracts. The method allowed the determination of target analytes in water samples at
18
19 246 levels of sub $\mu\text{g L}^{-1}$ (Table 1). The LOD of phthalates were high compared to other
20
21 247 studies (Peñalver et al. 2000) because they were calculated from the 3 times the
22
23 248 standard deviation of the blank samples which always contained traces of phthalates that
24
25 249 originate both from the extraction and from the GC injection port. The use of surrogate
26
27 250 standards to quantify each compound was necessary to achieve accurate results, taking
28
29 251 into consideration the loss of analytes during incubation or extraction. The recoveries of
30
31 252 the analytes were between 93 and 125% for the SPE-GC/MS method (Table 1).

32 253

33 254 *Migration of plastic components and additives from bottled water and caps*

34 255 UNE-EN ISO 177 provides an unspecific method capable to determine the total mass of
35
36 256 compounds migrating from plastic containers. Table 2 proves that tested plastics
37
38 257 incubated at 40°C lost weight, which was gained by the adsorbent, although there was
39
40 258 some mismatch due to the fact that some volatile compounds were lost during
41
42 259 incubation at 40°C, as considered in UNE-EN ISO 177. For example, for the PC plastic,
43
44 260 there was a 0.1 mg increase in the plastic due to humidity increase in the weighing
45
46 261 process but the gain of 2.3 mg in the sorbent after incubation should be taken into
47
48 262 consideration. Overall, the lose in plastic weight provides first evidence on the overall
49
50 263 migration of plasticizers from incubated plastics although this method failed to identify
51
52 264 and quantify specific compounds prone to migrate.

53 265 Migration tests UNE-EN 13130 and ultrasonic incubation were able to identify
54
55 266 target compounds. Ultrasonic method is a fast extraction method and was developed in
56
57 267 an attempt to validate it by comparing the results with the incubation at 40°C. We found
58
59 268 that qualitatively, both methods provided similar information, but quantitatively,
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270 269 compounds were extracted with different efficiency. Phthalates were not detected in any
271
270 271 of the plastics tested and in any of the treatments used, and indicates that either these
271
270 271 compounds were not added in the tested plastics or either that they do not migrate at the

1
2
3 272 conditions tested. The same holds for octylphenol. NP and BPA were the only
4
5 273 compounds identified. The recoveries of NP and BPA using the ultrasonic incubation
6
7 274 were of 45% and 76% for respectively, and using the UNE-EN 13130, recoveries were
8
9 275 92% and 97% for NP and BPA, respectively. The different recoveries using both
10
11 276 migration tests reflect the losses that occur during the incubation period, which are
12
13 277 higher when using ultrasonic extraction, especially for NP.

14 278 Ultrasonic incubation forces the migration of compounds by applying an
15
16 279 ultrasonic wave which enhances the detachment of plastic components or additives
17
18 280 which are released to water. Results showed that there was not any difference in the
19
20 281 migration of plastic compounds using different extraction times (5, 10 or 15 min) and
21
22 282 thus, an extraction time of 5 min was chosen. Using this technique, NP was identified in
23
24 283 the incubated plastics at levels of 0.212-0.242 $\mu\text{g dm}^2$ while BPA was only detected at
25
26 284 1.870 $\mu\text{g dm}^2$ in the PC plastic (Table 3).

27 285 UNE-EN 13130 describes the methods for determining the specific migration of
28
29 286 plastic materials and UNE-EN 1186 describes the methods for determining the overall
30
31 287 migration of materials, plastics and articles in contact with foodstuffs. This UNE-EN
32
33 288 13130 migration test was based on Part 1, guide to test methods for the specific
34
35 289 migration of substances from plastics (see also UNE-EN 1186, Part 1 guide for the
36
37 290 selection of conditions and test methods to calculate the overall migration). Using this
38
39 291 method, NP and BPA were quantified at levels of 0.332-1.282 $\mu\text{g dm}^2$ and 0.128-3.423
40
41 292 $\mu\text{g dm}^2$, respectively (Table 3). This method was more efficient in extracting
42
43 293 plasticizers and additives from plastics than ultrasonic extraction, and was able to
44
45 294 determine NP in all types of plastic and BPA in PC, HDPE, LDPE and PS. Both NP and
46
47 295 BPA can be used as plastic additives to improve the quality of plastic.

48 296 These two last methods are complementary and provide similar information on
49
50 297 the migration potential, qualitatively. Ultrasonic bath provides a lower migration of
51
52 298 BPA and NP compared to the UNE-EN 13130 (Table 3). Among both methods,
53
54 299 ultrasonic extraction can be considered a fast extraction method but with lower
55
56 300 efficiency than UNE-EN 13130 and so far, is not validated yet. Most important is that
57
58 301 UNE-EN 13130 is based on a technical interpretation of Directive 2007/19/EC and
59
60 302 provides maximum legislated migration levels for a large number of compounds. Thus,
303 this technique was further used to determine the migration levels of target compounds in
304 the 5 polymers studied and to compare the specific migration levels with legislated
305 values.

306

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

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3 340 from containers (Le et al. 2008). The BPA amount detected in PC plastic was of 3.423
4
5 341 $\mu\text{g dm}^{-2}$ and NP was detected at 0.694 $\mu\text{g dm}^{-2}$. Another study using bottled water in PC
6
7 342 describes the migration of this compound from used and new bottles at 0.7 $\mu\text{g L}^{-1}$ and
8
9 343 1.0 $\mu\text{g L}^{-1}$, respectively, after 7 days at room temperature (Le et al. 2008). Nerín et al.
10
11 344 (Nerín et al. 2003) detected that BPA migrated from PC plastic stored at room
12
13 345 temperature. Biles et al. (Biles et al. 1997) detected BPA in PC baby bottles and cups at
14
15 346 levels ranging from non detected to 5 $\mu\text{g L}^{-1}$.

16 347 LDPE is the polymer used in the cooler caps, its structure is the same as HDPE
17
18 348 but the density is lower so LDPE is used in caps and not in bottles. NP and BPA
19
20 349 migrated as shown in Table 3 at levels of 0.413 and 0.128 $\mu\text{g dm}^{-2}$, respectively.

21 350 Finally, PS was tested because it is used in the septum of several caps, such as
22
23 351 the ones used in coolers. The amounts detected were 0.801 and 0.136 $\mu\text{g dm}^{-2}$ for NP
24
25 352 and BPA, respectively. PS is one of the most produced plastics in food contact materials
26
27 353 and can be copolymerized with many monomers. Products formed from PS are hard and
28
29 354 transparent (Piringer 2008). However, cap septa are whitish due to additives that
30
31 355 improve the brittleness and the sensitivity to stress cracking (Piringer 2008). These
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33 356 additives may migrate as it happens in other type of plastics.

34 357 The presence of phthalates and BPA in food in contact with plastic is legislated
35
36 358 in the European Union. Directive 2002/72/EC, Directive 2004/19/EC and Directive
37
38 359 2007/19/EC (transposed to the Spanish Royal Decree 866/2008) establishes migration
39
40 360 specific limits in food and food simulants of 600 $\mu\text{g kg}^{-1}$ or 100 $\mu\text{g dm}^{-2}$ for BPA, 1500
41
42 361 $\mu\text{g kg}^{-1}$ 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 $\mu\text{g kg}^{-1}$
43
44 362 or 5000 $\mu\text{g dm}^{-2}$ for BBP. The levels for phthalates are explicit in Directive
45
46 363 2007/19/EC and are different according to i) whether the packing is a single or multiple
47
48 364 use article, ii) whether it is in contact with aqueous or fatty foods and 3) whether it is for
49
50 365 infants. So there are a variety of limits for different cases. From the different polymers
51
52 366 tested, none of the samples exceeded the legislated value of 100 $\mu\text{g dm}^{-2}$ for BPA.
53
54 367 These legislated levels are lower or equal with the former Directive 2002/72/EC, who
55
56 368 established migration specific limits in food and food simulants of 3000 $\mu\text{g kg}^{-1}$ for
57
58 369 BPA and 1500 $\mu\text{g kg}^{-1}$ for DEHP. Specifically, the levels of BPA decreased 5 times
59
60 370 from 2002 to 2007 Directives because the increasing information and studies on BPA
371
372 toxicity and the potential effects on humans if ingested daily (Bredhult et al. 2009;
373 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

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3 408 water day⁻¹, 60 kg person and 4.44 µg BPA L⁻¹). The Scientific Committee on Food
4
5 409 (SCF) in 2002 considered that the overall oral No-Observed-Adverse-Effect Level
6
7 410 (NOAEL) for BPA was 5 mg kg⁻¹ bw day⁻¹ and set a temporary TDI of 0.01 mg kg⁻¹
8
9 411 bw day⁻¹ (European Food Safety Authority (EFSA), 2006), which was changed in 2008
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11 412 by a TDI of 0.05 mg kg⁻¹ bw day⁻¹ (Unit on food contact materials, enzymes,
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13 413 flavourings and processing aids (CEF) et al. 2008). In a former study realized in 1993,
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15 414 the U.S. EPA had already suggested a reference value of 0.05 mg kg⁻¹ of body weight
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17 415 (Environmental Protection Agency (EPA) 1993). These organizations state that PC
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19 416 products are safe for being used in products in contact with food and beverage and pose
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21 417 no known risk to human health. The levels of BPA from PC coolers were below the
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23 418 NOAEL and TDI, although it should be considered that BPA consumption from PC
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25 419 bottled water is only a part of the total oral Daily Intake.

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27 420 Many studies have been carried out on the toxicity and potential estrogenic
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29 421 effects of BPA using various animal models, cellular lines and at molecular and
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31 422 biochemical level. These studies set the initial basis to carry out risk assessment studies
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33 423 of BPA on humans. However, discrepancies have been observed in these on going
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35 424 studies regarding both the effects of BPA and the levels that cause these effects. From a
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37 425 toxicological point of view, if the aquatic toxicity is considered using *Daphnia magna*,
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39 426 EC₅₀ values range between 1 and 20 mg L⁻¹ (Table 1), and although these
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41 427 concentrations cannot be extrapolated to a human model toxicity, they are an indicator
42
43 428 that effects caused to a very sensitive specie are initiated at the mg level. Cespedes et al.
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45 429 developed a more specific method to assess the estrogenicity of BPA using recombinant
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47 430 yeast using the human estrogen receptor (Céspedes et al. 2004). It was demonstrated
48
49 431 that the estrogenicity of BPA is 1644 ± 380 µg L⁻¹, much lower than the natural
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51 432 hormone estradiol (0.04 µg L⁻¹), used as positive control (Table 1). That means that at
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53 433 concentrations below 1644 µg L⁻¹, BPA does not activate the human estrogen receptor
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55 434 and therefore does not trigger any estrogenic effect. Concluding, the concentration of
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57 435 BPA from PC containers in the samples analyzed were below the aquatic toxicity, the
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59 436 EC₅₀ obtained with the recombinant yeast assay and the legislated levels (Table 1).
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437 However, epidemiological studies carried out by Vandenberg et al. indicate that BPA is
438 present in human fluids at concentration levels higher than those that induce effects *in*
439 *vitro* and one order of magnitude higher than the levels that induce effects in animal
440 models (Vandenberg et al. 2007). Another study detected that 92.6% of the examined

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3 441 US population had BPA in urine due to the routine ingestion of this compound from
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5 442 plastic food containers (Calafat et al. 2008).
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11 446 **Conclusions**

12 447 Given the increased use of plastic in the bottling packaging sector, migration tests
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14 448 permit the identification of plasticizers and additives with endocrine disrupting
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16 449 properties that can cause human health effect when ingested continuously. Out of the 3
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18 450 migration tests used, the UNE-EN 13130 method permitted identification of
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20 451 compounds leaching from plastics and the ability to compare the migration levels with
21
22 452 legislative values. The ultrasonic extraction was an alternative to evaluate compounds
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24 453 migrating from different polymers and qualitatively had a comparative performance
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26 454 although with less efficiency and therefore, is not yet a validated and accepted method.
27
28 455 From 5 polymers tested, phthalates were not present in the plastics while NP and BPA
29
30 456 were identified at concentrations from 0.128 up to 3.423 $\mu\text{g dm}^{-2}$. PET and HDPE
31
32 457 bottles were the bottles with the lowest amount of EDCs. The biggest problem
33
34 458 compound was BPA which was detected in all polycarbonate bottled waters analyzed
35
36 459 and although the total daily intake was below the legislative values, it may contribute to
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38 460 the total daily intake of this compound considering all types of food. Numerous studies
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40 461 indicate that BPA causes adverse effects in experimental animals and the constant
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42 462 exposure, metabolism and long term risk in humans is a matter of concern. The
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44 463 European Food Safety Authority (EFSA), the US Environmental Protection Agency and
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46 464 other Agencies are currently undertaking studies to integrate data from migration tests,
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48 465 exposure levels and toxicity endpoints to assess the possible effects of BPA on humans
49
50 466 and initiate actions to protect human health. At this point, BPA has recently been
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52 467 banned in Europe (by summer 2011).
53

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13 480
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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, *Daphnia magna*.
^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

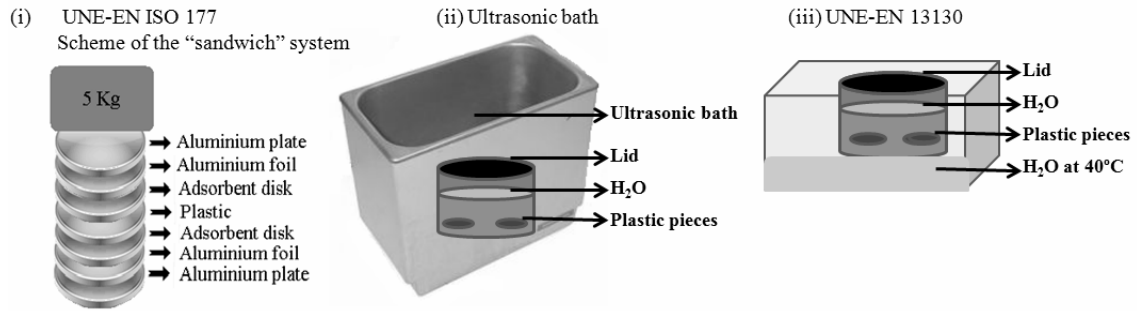
Table 2. UNE-EN ISO 177 weights for 4 test plastics where it is observed that plastics lose weight while the adsorbent gains weight.

	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.



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

