Identification of plastic material constituents in water and development of migration assays

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Tesi Doctoral

"IDENTIFICATION OF PLASTIC MATERIAL CONSTITUENTS IN WATER AND DEVELOPMENT OF MIGRATION ASSAYS"

Presentada per

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La Dra. Sílvia Lacorte Bruguera, amb NIF 37745208T, del Departament de Química Ambiental, Institut de Diagnosi Ambiental i Estudis de l'Aigua del Consell superior d'Investigacions Científiques, i el Dr. Antonio Borrell Azlor, amb NIF 39180001F, del Laboratori Dr. Oliver-Rodés, S.A.,

CERTIFIQUEN,

Que la present memòria titulada "*Identification of plastic material constituents in water and development of migration assays*", ha estat realitzada sota la nostra direcció pel Sr. Albert Guart Torres, amb NIF 46980723A, a l'Institut de Diagnosi Ambiental i Estudis de l'Aigua del Consell Superior d'Investigacions Científiques i al Laboratori Dr. Oliver-Rodés, S.A., i que tots els resultats presentats són fruits de les experiències realitzades pel citat doctorand.

I per a què així es faci constar, expedim i firmem el present certificat.

Barcelona, setembre 2013

Dra. Sílvia Lacorte Bruguera Dr. Antonio Borrell Azlor

"El azar afortunado suele ser casi siempre el premio del esfuerzo perseverante."

Santiago Ramón y Cajal

Agraïments

Són moltes les persones a qui vull agrair les seves aportacions, científiques i personals, i que han contribuït a la finalització d'aquesta tesi doctoral. En primer lloc als meus directors de tesi, la Sílvia i el Toni, que m'han acompanyat durant aquests anys i de qui he aprés moltíssim.

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Per poder expressar correctament el meu agraïment cal remuntar fins l'estiu de l'any 2008 en què vaig començar unes pràctiques amb el Laboratori Dr. Oliver Rodés, S.A. i el CSIC, i que em van permetre endinsar-me en el món de la química ambiental, l'anàlisi de contaminants en aigua i la migració de les ampolles de plàstic. Van ser moltes hores analitzant mostres amb el Jacob, la Sara i el Paco. Durant aquestes pràctiques al CSIC vaig tenir l'oportunitat de conèixer molts companys i amics d'arreu del món de qui he aprés moltíssim i que em van animar a endinsar-me en el món del doctorat.

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ÍNDEX

ABREVIATURES I ACRÒNIMS

ADI	Acceptable daily intake / Ingesta diària acceptable
AENOR	Asociación Española de Normalización y Certificación
ANSES	Agence Nationale de Sécurité Sanitaire
ATBC	Acetyl tributyl citrate / Acetil tributil citrat
ATRA	Acid retinoic all-trans / All-trans àcid retinoic
BADGE	Bisphenol A diglycidyl / Diglicil bisfenol A
BBP	Benzyl butyl phthalate / Benzil butil ftalat
BDW	Bottled drinking water / Aigua envasada per al consum humà
BHT	2,6-Di-tert-butyl-4-methylphenol / 2,6-Di-tert-butil-4-metilfenol
BP	Benzophenone / Benzofenona
BPA	Bisphenol A / Bisfenol A
bw	Body weight / Pes corporal
CAS	Chemical Abstracts Service Registry Numbers / Números de registre de substàncies químics
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids / Panel en Material en Contacte amb Aliments, Enzims, Aromatitzants i Coadjuvants d'Elaboració
DBP	Dibutyl phthalate / dibutil ftalat
DEHA	Di-(2-ethylhexyl) adipate / Dietilhexil adipat
DEHP	Di-(2-ethylhexyl) phthalate / Dietilhexil ftalat
DEP	Diethyl phthalate / Dietil ftalat
DMIP	Dimethyl isophthalate / Dimetil isoftalat
DMTP	Dimethyl terephthalate / Dimetil tereftalat
DMP	Dimethyl phthalate / Dimetil ftalat
DMSO	Dimethyl sulfoxide / Dimetilsulfòxid
2,4-DTBP	2,4-Di-tert-butylphenol / 2,4-Di-tert-butilfenol
DW	Drinking water / Aigua per al consum humà
E2	17β-estradiol / 17β-estradiol
EC	European Commission / Comissió Europea
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals / Centre Europeu d'Ecotoxicologia i Toxicologia de Substàncies Químics
ECHA	European Chemical Agency / Agència Europea de Substàncies i Mescles Químiques
ED	Endocrine disruptor / Disruptor endocrí

EDC	Endocrine disruptor compound / Compost amb activitat de disrupció endocrina
EE2	17α-ethinylestradiol / 17α-ethinilestradiol
EEC	European Communities / Comunitats Europees
EFSA	European Food Safety Authority / Autoritat Europea de Seguretat Alimentària
EN	European Standard / Norma Europea
EPA	Environmental Protection Agency / Agència de Protecció Ambiental
EU	European Union / Unió Europea
FDA	Food and Drug Administration / Administració d'Aliments i Medicaments
GC	Gas chromatography / Cromatografia de gasos
HDPE	High-density polyethylene / Polietilè d'alta densitat
hER	Human estrogen receptor / Receptor humà d'estrogen
HPLC	High-performance liquid chromatography / Cromatografia de líquids d'alta eficàcia
IPCS	International Programme on Chemical Safety / Programa Internacional per a la Seguretat de Substàncies Químiques
LC	Liquid chromatography / Cromatografia de líquids
LDPE	Low-density polyethylene / Polietilè de baixa densitat
LLE	Liquid-liquid extraction / Extracció líquid-líquid
LOD	Limit of detection / Límit de detecció
LOQ	Limit of quantification / Límit de quantificació
LVI	Large volume injection / Injecció de gran volum
MS	Mass spectrometry / Espectrometria de masses
NIAS	Non-intentionally added substances / Substàncies addicionades no intencionadament
NMW	Natural mineral water / Aigua mineral natural
NOAEL	No-Observed-Adverse-Effect Level / Nivell d'efectes adversos no observats
NP	Nonylphenol / Nonilfenol
4-NP	4- Nonylphenol / 4-Nonilfenol
OECD	Organisation for the Economic Co-operation and Development / Organització de Cooperació i Desenvolupament Econòmic
OP	Octylphenol / Octilfenol
4-OP	4- Octylphenol / 4-Octilfenol
PC	Polycarbonate / Policarbonat
PDMS	Polydimethylsiloxane / Polidimetilsiloxà

2-PE	2-Phenoxyethanol / 2-Fenoxiethanol
PET	Polyethylene terephthalate / Polietilè tereftalat
PoU	Point-of-use / Punt d'ús
PTFE	Polytetrafluoroethylene / Politetrafloroetilè
PS	Polystyrene / Poliestirè
PVC	Polyvinyl chloride / Policlorur de vinil
RARα	Retinoic acid receptor α / Receptor d'àcid retinoic α
RSD	Relative Standard Deviation / Desviació estàndard relativa
SBSE	Stir bar sorptive extraction / Extracció per barretes adsorbents
SCF	Scientific Committee on Food / Comitè Científic d'Aliments
SML	Specific migration limit / Límit de migració específica
SML(T)	Specific migration limit as a sum of substances / Límit de migració específica expressat com a suma de substàncies
SPE	Solid-phase extraction / Extracció en fase sòlida
SPI	Society of the Plastic Industry / Societat de la Indústria del Plàstic
SPME	Solid-phase microextraction / Microextracció en fase sòlida
SW	Spring water / Aigua de brollador
t	Time / Temps
T	Temperature / Temperatura
TDI	Tolerable daily intake / Ingesta diària tolerable
TM	Trademark / Marca registrada
TOC	Total organic carbon / Carboni orgànic total
UNE	Una Norma Española / Una Norma Espanyola
US	United States / Estats Units
USEPA	United States Environmental Protection Agency
UV	Ultraviolet / Ultraviolat
WHO	World Health Organization / Organització Mundial de la Salut
WWTP	Wastewater treatment plant / Planta de tractament d'aigües residuals
YAS	Yeast androgen screen / Assaig amb llevat modificat per a la detecció d'andròg ens
YAAS	Yeast antiandrogen screen / Assaig amb llevat modificat per a la detecció d'antiandròg ens
YAES	Yeast antiestrogen screen / Assaig amb llevat modificat per a la detecció d'antiestròg ens
YES	Yeast estrogen screen / Assaig amb llevat modificat per a la detecció d'estròg ens

JUSTIFICACIÓ DE LA TESI

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Objectius

L'objectiu principal d'aquesta tesi és identificar la presència de components del plàstic a l'aigua destinada al consum humà i avaluar el seu risc segons la seva capacitat de migració, toxicitat i ingesta diària. Per aconseguir aquest objectiu s'han desenvolupat i aplicat noves metodologies que permeten identificar compostos considerats com a possibles disruptors endocrins o que ja estan classificats com a tals. Els compostos que s'han estudiat han estat els ftalats, els alquilfenols i altres compostos com ara bisfenol A (BPA), dietilhexil adipat (DEHA), benzofenona (BP) i 2-fenoxietanol (2-PE). També s'han desenvolupat i aplicat proves de migració forçada que tenen com a objectiu identificar els compostos susceptibles a migrar del plàstic a l'aigua.

Els resultats d'aquests estudis pretenen contribuir a millorar els processos que rep l'aigua des de la seva captació a la deu fins arribar al consumidor final per tal de garantir la seva qualitat, en què es té en compte el procés d'envasat i l'emmagatzematge per a l'aigua envasada. També es pretén contribuir a millorar el tractament domèstic de l'aigua de xarxa pel què fa als materials en contacte amb l'aigua.

Els objectius específics són els següents:

1. Desenvolupar metodologies analítiques basades en l'extracció en fase sòlida (SPE) seguida de cromatografia de gasos acoblada a espectrometria de masses (GC-MS) i extracció per adsorció en barretes agitadores (SBSE) seguida de cromatografia de gasos acoblada a espectrometria de masses en tàndem (GC-MS/MS) per a la determinació de components del plàstic i altres contaminants ambientals en aigües de deus i aigües envasades.
2. Determinar la presència de plastificants i herbicides en els punts d'emergència o captació de deus i brolladors espanyols, en la corresponent aigua envasada i en l'aigua emmagatzemada durant un any per avaluar la migració de contaminants durant el procés d'envasat i emmagatzematge.
3. Determinar la presència de contaminants ambientals i els components del plàstic procedents de la migració dels envasos de polietilè tereftalat (PET) en aigües de vint-i-set països d'arreu del món.

4. Realitzar estudis de migració forçada en els principals tipus d'envasos comercials d'aigua per a avaluar el tipus i quantitat de compostos que es desprenen dels diferents materials polimèrics.

5. Avaluar l'activitat de disrupció endocrina del bisfenol A (BPA) i altres components del plàstic presents en els extractes de policarbonat (PC) i el seu possible substitut, el Tritan™, després d'haver estat sotmesos a un assaig de migració.

6. Realitzar assaigs de migració en aparells domèstics de tractament d'aigua i avaluar canvis en la composició d'aigua en referència a contaminants i altres paràmetres legislats.

Estructura

Així doncs, la tesi està estructurada en quatre parts:

- La introducció descriu els tipus d'aigua destinada al consum humà, ja sigui aigua de xarxa o aigua envasada (aigua mineral natural, aigua de brollador i aigua potable preparada). Pel què fa a l'aigua envasada, es descriuen els diferents materials polimèrics que s'utilitzen en l'envasat i la problemàtica de la migració de components del plàstic als aliments. Per avaluar el risc que comporta la presència de components del plàstic a l'aigua, es descriu la seva capacitat de disrupció endocrina i el càlcul de la ingesta diària. Es descriuen els diferents compostos que s'han analitzat al llarg de la tesi, així com les diferents tècniques analítiques utilitzades per a la determinació d'aquests compostos i tècniques toxicològiques per a la determinació de possibles efectes en els éssers vius.
- El capítol 2 i 3 estan dedicats a l'anàlisi d'aigües envasades. El capítol 2 descriu l'estudi realitzat en 131 deus o brolladors de tota Espanya i de com afecta l'envasat per a cadascun a les aigües de captació. D'aquesta forma s'ha realitzat una comparació entre tres possibles punts susceptibles de contaminació de l'aigua envasada abans d'arribar al consumidor final:

i. L'aigua abans de ser envasada.
ii. L'aigua després de l'envasat.
iii. L'aigua després d'estar emmagatzemada un any.

El capítol 3 inclou l'anàlisi de setanta-set aigües envasades en polietilè tereftalat (PET) de vint-i-set països d'arreu del món.

- En el capítol 4 i 5 es presenten els assaigs de migració. El capítol 4 descriu la realització de diferents assaigs de migració dels principals tipus de material polimèric utilitzats en l'envasat d'aigua. En el capítol 4 també s'avalua la capacitat de disrupció endocrina del monòmer que compon el policarbonat (PC) i del Tritan™ mitjançant assaigs toxicològics *in vivo* i *in vitro*. El capítol 5 descriu la realització d'assaigs de migració més complexos en aparells domèstics de tractament d'aigua.
- Finalment s'inclouen les conclusions generals obtingudes en el treball realitzat en aquesta tesi, així com la bibliografia corresponent.

1. INTRODUCTION

1. INTRODUCTION

1.1. Water resources

The Earth's appearance from space is like a blue planet due to the ocean reflection, which cover the 71 % of the Earth's area. In fact, ocean water corresponds to 96.5 % of total global water (Figure 1). From the remaining 3.5 % of world's water, only 2.5 % belongs to freshwater, where only a 31.4 % corresponds to groundwater, surface water and other freshwater directly available as a water resource for humans. Water resources are used for a widespread variety of human activities including agricultural (69% of the total used water), industrial (23%), household and human consumption (8%), energy production and recreational activities. Groundwater is the most used water for human activities or human consumption, which represents the 30.1 % of the fresh water and less than 1 % of the total global water.

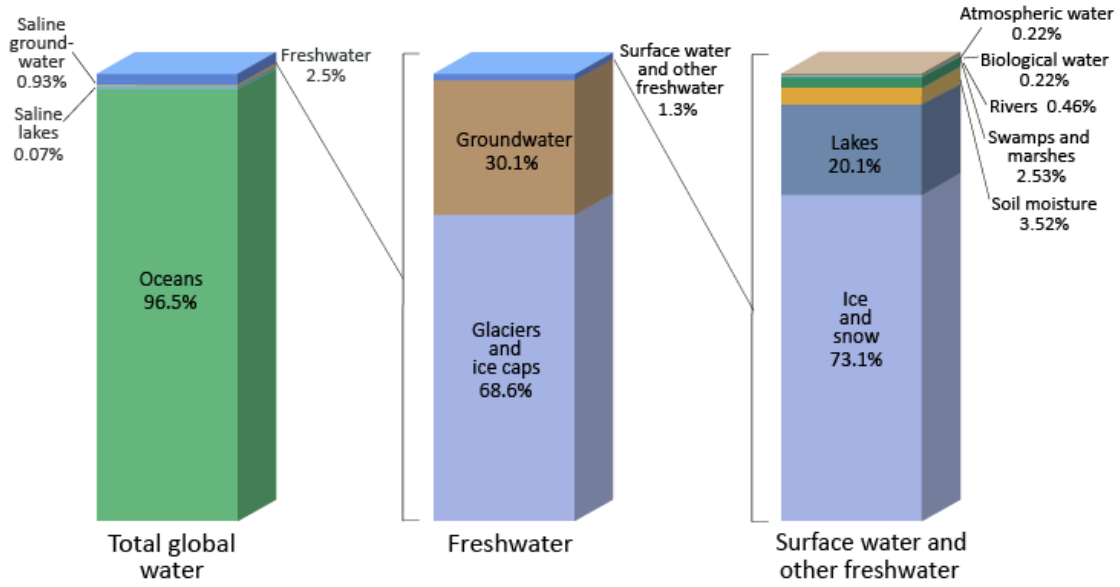


Figure 1. Distribution of the Earth's water (USGS Water Science School, 2013). Data from chapter "World fresh water resources" of "Water in Crisis: A guide to the World's Fresh Water Resources" book (Gleick, 1993).

Actually, from the point of view of the usable and non-usable water, only 1% of the total water in the Earth is usable by humans of which 99% belongs to groundwater (Figure 2). Groundwater is often used for human consumption due to the higher quality in comparison with surface water. Groundwater is also used for several activities such as domestic, recreation, industrial and agricultural activities.

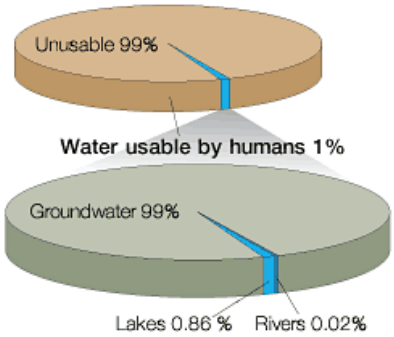


Figure 2. Usability of all the water in the Earth (USGS Water Science School, 2013).

When groundwater reaches an impermeable zone, it is accumulated and forms the so called aquifers. There are two types of aquifers:

- Unconfined aquifers: water reaches the aquifer directly from surface across empty spaces of the ground, hence these aquifers have a pressure similar to the atmospheric pressure.
- Confined aquifers: aquifer groundwater is separated from atmosphere across ground zones with low permeability (e.g. traditional boreholes), hence these aquifers have higher pressures than atmospheric pressure.

The extraction rhythm of water from an aquifer must be lower than its recharge to avoid overexploitation. In the case of an overexploitation (recharge is lower than exploitation), it may produce harmful effects in the environment and in the future use of the aquifer.

Groundwater contains organic and inorganic components. Within the inorganic constituents, there are cations (e.g. calcium, magnesium, sodium and potassium), anions (e.g. bicarbonate, chloride, sulphate, nitrate, fluoride) and non-ionic substances (e.g. silica). Moreover, these compounds can be classified as major ions, minor ions and trace elements. This classification is done according with the frequency of the constituents in groundwater, where major ions comprise at least 90 % of the total dissolved solids. On the other hand, there is the dissolved organic matter which is ubiquitous in natural groundwater and is defined by the total organic carbon (TOC) that is typically in the range of 0.1-3 mg/L. Groundwater contains dissolved gases such as nitrogen, oxygen that contribute to the properties of the water. Within the dissolved gases, carbon dioxide is originated from the equilibrium between carbonate and bicarbonate ions. Other gases as methane,

hydrogen sulphide and nitrous oxide are products of biologically related processes that can occur in confined aquifers (Senior and Dege, 2005).

The water source is defined as the point of water extraction, which may be a spring, a well, a borehole or another kind of source for groundwaters. Occasionally, a surface water source can be used as drinking water, but in most of the cases the source is located underground. A spring is a natural flowing source of groundwater (Figure 3). In contrast, a well is a man-made static source that can be subdivided into two groups according to their method of construction, as hand-dug wells and as machine-drilled wells or boreholes. Hand-dug wells are not usually constructed in developed countries because they only penetrate the superficial aquifers and the water extraction is slower than in boreholes. On the other hand, there are the boreholes which create a hydraulic gradient across the sides of the borehole and allow water to flow and to be recollected (Senior and Dege, 2005).

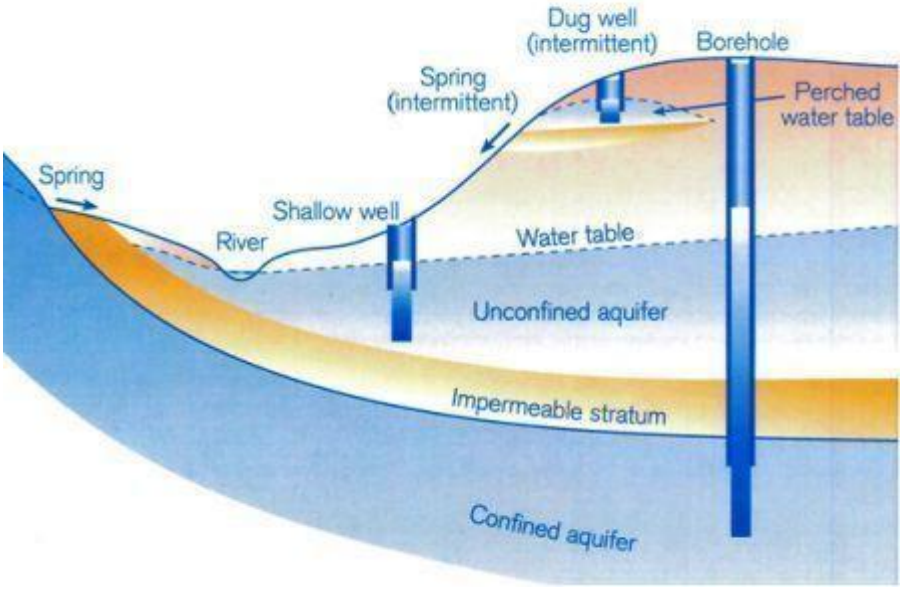


Figure 3. Diagram of the different exploitation sources of groundwater (Vale of Glamorgan Council, 2013).

1.2. Contamination of water intended for human consumption

In the last few decades, human activities have become the most important source of drinking water contamination. Several pollutants can contaminate the sources of water intended for human consumption. In large urban areas, the presence of industrial and agricultural activities near the source (e.g. the use of pesticides or the presence of farm animals) and other specific activities affect groundwater quality as many contaminants can leach to aquifers, increasing their vulnerability (Worrall et al., 2002).

Contamination of drinking water can be classified as microbiological, radiological and chemical contamination. The different contaminants of drinking water are described in Guidelines for drinking-water quality (WHO, 2011). Most microbial contamination refers to microbial pathogens such as viruses, bacteria and protozoa. Viruses (e.g. rotaviruses) are the smallest pathogens, hence are more difficult to remove by physical processes such as filtration. Bacteria (e.g. *Escherichia Coli* and *Legionella*) are generally the group of pathogens that are most sensitive to inactivation by disinfection. Protozoa (e.g. *Cryptosporidium*) is the group of pathogens that are less sensitive to inactivation by chemical disinfection.

Water sources can contain radionuclides of natural and human-made origin. Natural radionuclides (e.g. potassium-40, radium-226, radium-228, uranium-234, uranium-238 and lead-210) can be found in water as a result of either natural processes such as absorption from the soil or technological processes involving naturally occurring radioactive materials such as the mining and processing of mineral sands or phosphate fertilizer production. Human-made radionuclides may be present in water from discharges from nuclear fuel cycle facilities or from production and use in medicine or industry that entered into drinking-water supplies as a result of regular or incidental discharges.

Chemical contamination is produced by human activites. For example, intensive farming activities are the main cause of nitrate contamination, which at elevated concentrations can be used as an indicator of groundwater contamination. Nitrates can also leach from leakage of sewers, which can also contaminate groundwater with other organic compounds and bacteria. Other pollutants are heavy metals (e.g. mercury and lead) and petroleum oils, which can be the origin of low molecular weight hydrocarbons (e.g. benzene and toluene) which can cause widespread effects in humans as a consequence of exposure when they are present in excessive quantities.

Regarding organic microcontaminants, agriculture accounts for an important source of pesticides groundwater pollution, due basically to their use during long periods. Several studies report the leaching of insecticides and herbicides, as for example triazines, (Barbash et al. 2001; Gonçalves et al. 2007; Kolpin et al. 2002; Tappe et al. 2002) or pesticide coadjuvants (detergents, solvents, preservatives, etc.) (Latorre et al., 2003) to groundwater. In Spain, Hildebrandt et al. (2008) detected triazines in north Spain as the most ubiquitous herbicide with 72 % of the samples containing traces (38 % at levels above 0.1 mg/L European Union (EU) limit) for atrazine, 71 % (37 % above 0.1 mg/L EU limit) for atrazine-desethyl, and other triazines with results of 22 % (3 % above 0.1 mg/L EU limit) for terbuthylazine and 15 % (2 % above 0.1 mg/L EU limit) for terbuthylazine-desethyl.

As a result of groundwater contamination due to agricultural or other activities, source waters become vulnerable and may contain traces of pollutants, which affect the quality of water intended for human consumption. Triazines have already been detected in bottled water. Maggioni et al. (2013) detected the presence of triazines in 35 Italian cities and in bottled mineral water. Atrazine and atrazine-desethyl were detected in 18 out of the 35 waterworks in Italian cities at concentrations up to 0.013 µg/L and in 2 out of the 5 bottled water analysed showed maximum levels of 0.00012 and 0.00035 µg/L for atrazine and atrazine-desethyl, respectively. Terbuthylazine and its metabolite terbuthylazine-desethyl were also found (Maggioni et al. 2013) in 25 out of the 35 waterworks in Italian cities samples at concentrations up to 0.04991 µg/L and in bottled water samples at levels up to 0.00078 µg/L. To avoid groundwater pollution of waters intended for human consumption, it is established that the catchment area and the surrounding areas have to be free from any source of pollution. With the aim of preserving groundwater, in 2000 the European Water Framework (EU, 2000) was implemented to protect the European groundwater in general terms. There were three specific actions to achieve groundwater protection:

- The prohibition of direct discharges to groundwater.
- The requirement of monitoring groundwater bodies to detect pollution trends.
- The delimitation of protected areas of groundwater for human use.

1.3. Water intended for human consumption

In Europe, there are different classes of water intended for human consumption according to its origin: natural mineral water (NMW) and spring water (SW) which are bottled waters, and drinking water (treated water) which can be bottled or not (tap water). In the countries outside Europe, water can be classified different.

1.3.1. Non-bottled water

Tap water, so called drinking water, is taken from any type of source as long as it has no health risk for humans (Spanish Government, 2003). After treatment, it is distributed by the water supply network to the population. Normally, this kind of water requires several treatments before it is distributed and according with legislation. It is treated with the aim of ensuring that water intended for human consumption is wholesome and clean. Therefore, water must be free from any micro-organism and parasite and from any substance which may constitute a potential danger for human health, according to Spanish Royal Decree 140/2003 (Spanish Government, 2003), transposed from the European Directive 98/83/EC (EU, 1998). Parameters to be controlled after treatment are odour, taste, turbidity, colour, conductivity, pH, ammonium, *Escherichia Coli* and coliform bacteria. Moreover, at the exit of the general distribution water tanks after treatment, some parameters have to be monitored, such as iron and aluminium that are used for chemical coagulation, colony count at 22 ºC, *Clostridium perfringens* and nitrite, and free and combined chlorine when chlorination disinfection is used.

The other point of compliance for the legislation for treated water supplied from a distribution network is the point of supply, it means in the consumer tap or faucet. This control is done to check the distribution pipes and the other network elements in contact with the water. Materials used in the manufacture of pipes and tanks must not transfer undesirable components, nor micro-organisms and parasites. An early warning episode was produced by the undesirable transfer of lead to water and so lead pipes were changed to copper or plastic material.

Nowadays, there is a growing tendency to install point of use (PoU) water treatment devices to improve the organoleptic properties of tap water. PoU devices refer to several different types of devices that are usually installed under the kitchen sink (e.g. household reverse osmosis). They usually use the same

treatment technologies that have been used in treatment plants but they are only designed to treat domestic water. These devices also change tap water characteristics but they are not considered in legislation. The European legislation only refers to the water treatment of the device but it does not include the determination of micro-organisms or substances with harmful properties that can be transferred to human health. To fill this lack of information, the Spanish Standard UNE 149101 (AENOR, 2011) establishes the criteria to determine if a household water treatment device can be used without a transfer of undesirable or harmful substances, micro-organisms or properties. Therefore, it describes the sampling methodology for the subsequent microbiological and physicochemical analysis of PoU waters and the maximum permissible variations between the water supply and the water after the household treatment (EU, 1998; Spanish Government, 2003). In regard to plastic materials in contact with water/food, the standard refers to the European Commission (EU) No 10/2011 (EU, 2011).

1.3.2. Bottled water

Since 1980, bottled water has been divided in NMW and bottled drinking water (BDW) by two different European Directives (EU, 1980a; EU, 1980b). NMW means microbiologically wholesome water and it is a natural product which has its origin in an underground water deposit and emerges from a spring or a borehole. NMW can be clearly distinguished from ordinary drinking water by its nature, which is characterized by its mineral content, trace elements or other constituents and by its original purity. Original purity only refers to NMW and it means that water must be free of pollution. Both characteristics are preserved intact because of the underground origin of the water, which has been protected from all risk of pollution (EU, 1980a). Nowadays, tests for NMW are described in Directive 2009/54/EC (EU, 2009). This directive has been transposed to each Member State; in Spain this document has been transposed to Spanish Royal Decree 1798/2010 (Spanish Government, 2010a) for natural mineral water. It describes the required analyses in final bottled product which includes every day controls, every three months and every five years (Table 1).

SW was first incorporated in European legislation the year 1996 (EU, 1996) modifying the NMW directive (EU, 1980a). SW is a natural and non sterile product, as NMW, and recognition procedure is also required. Source recognition includes information on the catchment area, hydrogeology of the source, microbiological and

chemical analysis data and constant composition data taking into account flow rates, climatic and seasonal changes. The main difference between SW and NMW is that SW does not require a stable composition. Tests for SW are divided in two European directives. Microbiological tests are described in Directive 2009/54/EC (EU, 2009) and chemical analysis are indicated in Directive 98/83/EC (EU, 1998). These directives have also been transposed to each Member State; in Spain these documents have been transposed to the same NMW Spanish Royal Decree 1798/2010 (Spanish Government, 2010b).

Finally, BDW is a product which can be subjected to several treatment processes in accordance with current legislation and it can come from boreholes or from water supply network. Since these waters are intended for human consumption, controls of their properties and the absence of contaminants are done to ensure their quality. Tests for BDW are indicated in Directive 98/83/EC (EU, 1998), which is transposed to the Spanish Royal Decree 1799/2010 (Spanish Government, 2010b). The legislated parameters that must be controlled in each type of water are indicated in Table 1.

Preservation and maintenance of water quality are the main objectives when bottling. Member states have regulated the delimitation of wellhead protection areas for groundwater, specifically for NMW and SW, in different ways (García-García and Martínez-Navarrete, 2005). Sometimes water properties are not the desirable ones or water quality is not adequate for human consumption, hence there are several water treatments that can be used to improve water properties.

NMW and SW can be subject to specific treatments as long as they do not modify the essential composition and properties of the water (Spanish Government 2010a). These treatments include:

- Physical filtration.
- Separation of unstable natural elements (e.g. sulphur and iron) by filtration or decantation with a previous oxygenation.
- Separation of iron, manganese, sulphur and arsenic by air enriched with ozone. The use of ozone is not intended for disinfection.
- Separation of fluoride by activated alumina.
- Elimination of carbon dioxide by physical procedures.
- Addition of carbon dioxide.
- Addition of nitrogen as bottling gas to ensure the container stability.

BDW can undergo several treatments to improve its quality. It is habitual that BDW is treated before distribution. The most usual water treatments are the following (Spanish Government, 2010b; WHO, 2011):

- Physical filtration. Particulate matter can be removed from raw waters by rapid gravity, which is used to filter water that has been pretreated by coagulation and sedimentation, or slow sand filters.
- Addition of carbon dioxide.
- Addition of nitrogen as bottling gas to ensure the container stability.
- Chlorination. Chlorination is used for microbial disinfection. However, it also acts as an oxidant to remove or convert chemicals as, for example, decomposition of pesticides, oxidation of dissolved species to form insoluble products or oxidation of dissolved species to more easily removable forms. The use of chlorine has the disadvantage of reacting with natural organic matter to produce trihalomethanes and other halogenated disinfection by-products.
- Ozonation. Ozone can be used as a primary disinfectant. It reacts with natural organics to increase their biodegradability and it is effective for the degradation of a wide range of pesticides and other organic chemicals. Ozonation is normally used with subsequent treatment to avoid undesirable bacterial growth in distribution.
- UV radiation. UV radiation is emitted by a mercury arc lamp which has biocidal properties at wavelengths between 180 and 320 nm. It can be used to inactivate protozoa, bacteria, bacteriophage, yeast, viruses, fungi and algae and it can act as a catalyst in oxidation reactions when used in conjunction with ozone or hydrogen peroxide.
- Reverse osmosis. It is a purification system that uses a membrane together with pressure to remove non-desirable compounds from water (e.g. ions).

Table 1. Routine analyses for NMW, SW and BDW according with their periodicity.

Control periodicity	NMW	SW	BDW
Every day	*Escherichia coli* Fecal streptococcus *Pseudomonas aeruginosa* Colony count 22 ºC Colony count 37 ºC Sulphate-reducing bacteria Conductivity pH	*Escherichia coli* Fecal streptococcus *Pseudomonas aeruginosa* Colony count 22 ºC Colony count 37 ºC Sulphate-reducing bacteria Conductivity pH	*Escherichia coli* Enterococci *Pseudomonas aeruginosa* Colony count 22 ºC Colony count 37 ºC Conductivity pH
Every 3 months (include every day controls)	Anionic and cationic major components (e.g. bicarbonate, sulphate, chloride, calcium, magnesium and sodium) Characteristic components for each water (e.g. iron, manganese) Nitrite Nitrate	Anionic and cationic major components (e.g. bicarbonate, sulphate, chloride, calcium, magnesium and sodium) Characteristic components for each water (e.g. iron, manganese) Nitrite Nitrate	Aluminium Ammonium Chloride Colour Iron Manganese Odour Oxidability Sulphate Sodium Taste Turbidity Nitrite Nitrate *Clostridium Perfringens* Coliform bacteria

Table 1. Continuation.

Control periodicity	NMW	SW	BDW
Every 5 years (include every 3-month controls)	Antimony Total arsenic Barium Benzene Benzo(a)pyrene Cadmium Chromium Copper Cyanide Fluoride Lead Manganese Mercury Nickel Nitrate Nitrite Selenium Pesticides Total pesticides PAHs	Antimony Total arsenic Benzene Benzo(a)pyrene Boron Cadmium Chromium Copper Cyanide Fluoride Lead Mercury Nickel Nitrate Nitrite Selenium Pesticides Total pesticides PAHs Aluminium Ammonium Chloride Colour Iron Manganese Odour Sulphate Sodium Taste Turbidity Oxidability Coliform bacteria	Acrylamide Antimony Arsenic Benzene Benzo(a)pyrene Boron Bromate Cadmium Chromium Copper Cyanide 1,2-dichlormethane Epichlorohydrin Fluoride Lead Mercury Nickel Pesticides Total pesticides PAHs Selenium Tetrachloroethylene and trichloroethylene Total trihalomethanes Vinyl chloride Tritium Total indicative dose

1.4. Bottling process and packaging

Once a water source is ready to be bottled, it is necessary to maintain the water properties and the water quality during bottling and after bottling. Figure 4 shows the procedure of a bottling plant. Water is transported to a buffer tank before it is filtrated or treated. After that, water enters into a controlled environment where containers are rinsed and a machine fills the containers and bottles are sealed with caps, which are normally bought ready to use. There are two types of containers:

- Non-reusable container. It is bought as a preform, which is blow-moulded just before bottling and rinsed either with the same water that later will be used to fill it or with sterile air.
- Reusable container. It arrives directly from consumers and it is washed with detergent and rinsed with the same water that later will be used to fill it.

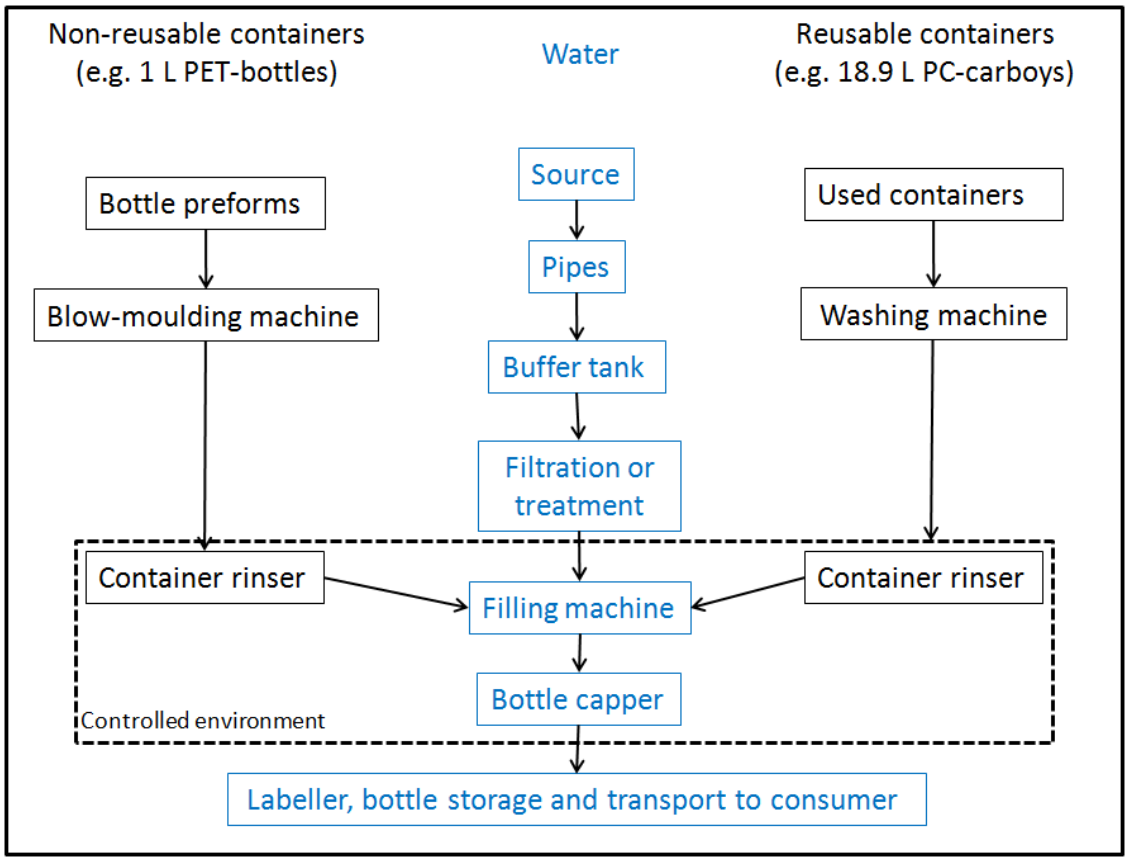


Figure 4. Bottling plant diagram.

In all these steps, all the materials in contact with water must be inert and cannot alter water composition. The most used material in bottling plants is stainless steel. Pipes have to be manufactured with materials approved for food contact. Moreover materials and devices must not react with food and their design has to allow a good hygienic maintenance. For example, pipes should be self-draining to avoid any residual water that could lead to contamination of product by the building-up of bacteria or microorganisms. Gaskets are controlled by using polytetrafluoroethylene (PTFE) which is a stable material with lubricious properties. Cleaning and disinfection of the complete filling system is done regularly with typical cleaning agents or disinfectants based in sodium hydroxide, phosphoric acid, peracetic acid, sodium hypochloride or with hot water sterilisation. Process air is used for the bottle cleaning equipment prior to filling and in cap vibratory bowls. This kind of air is of a very high microbiological and organoleptic quality.

Ideally, water should be bottled just after the collection, but it is often kept in stainless steel tanks before bottling. Bottle filling systems can fill bottles with still water (without gas) or carbonated water (with carbon dioxide gas that may be natural in groundwater or may be added when bottling). Also, there is the possibility of the addition of a small volume of flavouring to the bottled product. In this case, this final product is not considered as a bottled water, it is a flavoured water (Senior and Dege, 2005), so called soft drink.

Bottles are considered as food packaging whose manufacture is in continuous development. At the beginning of water packaging industry, glass was used as container for bottled water because it could be reused. However, glass is heavy and can be easily broken. Consequently, in the last years there has been an increase in the use of plastic materials in the bottled water market. There are several types of plastic with different characteristics according to their applications. The most common plastic materials used in water packaging are polyethylene terephthalate (PET), high-density polyethylene (HDPE), polycarbonate (PC), low-density polyethylene (LDPE) and polystyrene (PS) (WPO, 2008) (Table 2). Each polymeric material has been given a code, which is called resin identification coding system and it was developed for the Society of the Plastic Industry (SPI). There are other new plastics (resin code 7) that appeared in the last years, as Tritan™ copolyester. On the other hand, polyvinyl chloride (PVC) with resin code 3 is not used for water bottle manufacturing.

Table 2. Typical plastic packaging used for bottled water

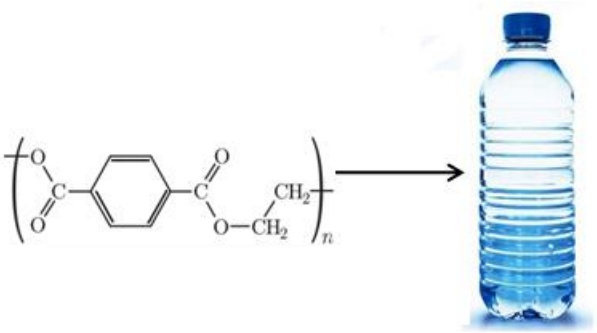
Polymer	Characteristics, use and structure
Polyethylene terephthalate (PET or PETE) 	Used in the manufacturing of bottles with volume between 0.25 and 8 L bottles and, nowadays, enterprises are studying to use PET for 20 L carboy. PET has got a density of 1.37 g/cm^3. PET is manufactured from terephthalic acid and etilenglycol. It has a good gas barrier and it is light and recyclable. 
High density polyethylene (HDPE) 	Used in the manufacturing of caps for PET bottles. Some brands used it for manufacturing 5 and 8 L bottles but, nowadays, they are being substituted by PET bottles. HDPE has got a density between 0.945-0.964 g/cm^3. It is manufactured from ethylene. It is harder than PET but with a worse gas barrier. 

Table 2. Continuation.

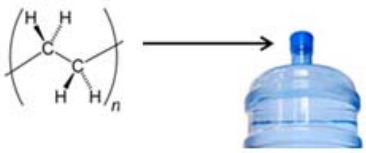
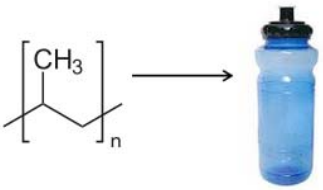
Low density polyethylene (LDPE) 	Used in the manufacturing of caps for PC carboys. It is also used in the manufacturing of water bags, although they are rarely used in Spain. LDPE has got a density between 0.915-0.940 g/cm^3. It has a good hardness and flexibility. 
Polypropylene (PP) 	Used in the manufacturing of 8L bottles, but it is not commonly used in not-reusable water bottles.  LDPE has got a density between 0.90-0.91 g/cm^3. It has a good water vapour barrier and fat resistance properties.

Table 2. Continuation.

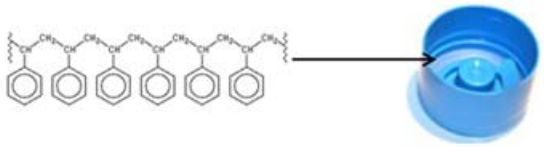
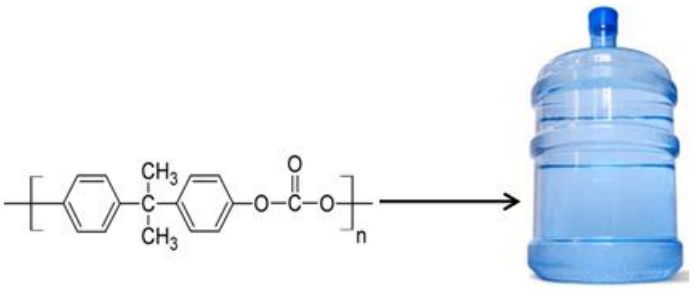
Polystyrene (PS) 	Used in the manufacturing of liner for LDPE caps, which are used in PC carboys. PS has got a density between 1.04 and 1.12 g/cm^3. PS may be copolymerisated with other monomers and it is often substituted for silicones in LDPE caps. 
Polycarbonate (PC) 	Used in the manufacturing of reusable bottles as 13, 18.9 and 20 L carboys. These carboys are coupled to the point-of-use (PoU) called "coolers", which are commonly placed in offices, hospitals, etc. PC has got a density between 1.20 and 1.24 g/cm^3. It is manufactured from BPA and it is reused after cleaning with detergents and water. 

Table 2. Continuation.

 Tritan™ (registered by Eastman Chemical Company)	Used in the manufacturing of reusable bottles as sport bottles. Nowadays, it is considered as a possible substitute for PC carboys. It is manufactured from dimethyl terephthalate (DMTP), 2,2,4,4-tetramethyl-1,3-cyclobutanediol and 1,4-cyclohexanedimethanol as principal monomers (Eastman, 2010). In spite of knowing the principal monomers, final structure is unknown. 

The different plastics used in the water bottling industry are manufactured of different polymeric materials, and within a type of plastic, different shapes, colours and strength are found. A polymeric material is composed by one or more polymers and additives. A polymer molecule is a repetition of small and simple chemical units which are connected with covalent bonds. These small units may be repeated, as BPA in PC, or may be different, as ethylenglycol and dimethyl terephthalate (DMTP) in PET. An additive is a substance which is incorporated to the polymer to give added value to the manufactured product. Several additives may be added in different manufacturing steps for a single polymeric material. This fact causes that sometimes all the additives used as processing aids are not declared in the final product (Piringer and Baner, 2008).

Plasticisers give the plastic flexibility, durability, stretchability and improve its processibility by decreasing their melt viscosity, temperature and elasticity of the final product without the alteration of the chemical character of the polymer. Normally, a combination of plasticisers is used in a single plastic to achieve the desired plastic characteristics. Due to their function, their characteristic requirements are: compatibility with the polymer, low extractability by water and solvents, stability to heat and light, good resistance to low-temperature properties, ease of processing and low odour, taste and toxicity. Since the monomers and

additives cannot react totally into the polymer, not reacted components can migrate to the surface of the finished plastic product and then evaporate or leach into the surrounding environment (Bolgar et al., 2008; Piringer and Baner, 2008). The most commonly plasticisers are phthalates.

The other main group is antioxidants, which give protection to the plastic against oxygen-triggered degradation and extend the plastic service life. They are used to protect the colour and molecular weight of the polymer during processing and have been shown to decompose peroxides, as well as chelate and react with metals. Due to their function, their characteristic requirements are: low concentration of use, compatibility with the substrate, stability, low toxicity, ease of use and cost (Bolgar et al., 2008; Piringer and Baner, 2008). In hydrocarbon polymers, the presence of tertiary hydrogen atoms makes the polymer prone to free radical formation, ultimately resulting in chain scission or crosslinking that degrades performance. So, antioxidants are used to terminate these chain reactions by removing radical intermediates (Bolgar et al., 2008; Piringer and Baner, 2008).

Plasticisers and antioxidants can easily migrate to foodstuff, especially when bottling and storage conditions are not properly controlled. Other compounds as precursors of monomers and additives (e.g. 2-phenoxyethanol (2-PE)) or additive or polymer production aid (e.g. benzophenone (BP)) can be found in food/simulant analysis.

1.5. Migration of plastic components

1.5.1. State of art

Nowadays, the safety in the use of polymeric materials is a subject of concern due to the transfer of plastic material constituents to water by a diffusion process called migration (Brocca et al., 2002; Bruchet and Janex-Habibi, 2004; Nerín and Asensio, 2007). Migration is a term used to describe the transfer of components from a certain material to the foodstuff in contact with this material. A compound placed in plastic material may migrate either because it did not react during manufacturing or it was released as a consequence of degradation by the contact with foodstuff or environment such as food acidity or ultraviolet (UV) light. These impurities are called as non-intentionally added substances (NIAS). Migration is divided in two classes:

- Overall migration, which is defined as the total quantity of non-volatile substances released from a material or object to the food or food simulant.
- Specific migration, which is defined as the quantity of a specific substance released from a material or article to the food or food simulant.

Determination of the migration is done by performing migration tests or assays, some of which are described in the European Legislation. These migration assays consist in the preparation of the plastic or object to favour the migration and they are important because they allow recreating a real long-period contact between material and food by performing short-period contact in laboratory controlled conditions (time, temperature and food simulant). That means the migration assays are standardized and may be recreated in different laboratories at the same conditions. A food simulant is a substitute of a food, which varies according to the kind of food that is in contact with plastic material. According to the Commission Regulation (UE) No 10/2011 (EU, 2011a), the food simulants used for specific migration are:

- Distilled water. It is used for migration assays of plastic intended to contain aqueous food with pH>4.5. According to current legislation (EU, 2011a) it can be used until 31th December 2015. After this date, only the Simulants A to E can be used.
- Simulant A - Ethanol 10 % (v/v). It is used for molasses, sugar syrups, honey, nuts paste or cream, fresh vegetables in form of purée, preserves,

and pastes or in its own juice, preserved vegetables and animal products in an oily medium, meat and in other products with fatty character.

- Simulant B - Acetic acid 3 % (w/v). It is used for migration assays of plastic intended to contain clear drinks such as water, ciders, clear fruit or vegetable juices, infusions, coffee, soft drinks and energy drinks, cloudy drinks such as juices with fruit pulp and liquid chocolates, fruit and vegetables in form of purée or preserves, animal products in aqueous medium, fermented milk such as yoghurt, cream and sour cream, processed cheese in aqueous medium, vinegar, sauces, mustard, concentrated extracts of an alcoholic strength ≥6 %.
- Simulant C - Ethanol 20 % (v/v). It is used for migration assays of plastic intended to contain clear drinks, alcoholic beverages of an alcoholic strength of between 6 % vol and 20 %, confectionary chocolate products in paste form as moist, fruit and vegetables in form such as purée or preserves, animal products in an aqueous medium and ice-creams.
- Simulant D1 - Ethanol 50 % (v/v). It is used for migration assays of plastic intended to contain cloudy drinks, alcoholic beverages of an alcoholic strength >20 % and all cream liquors, fruit and vegetables preserved in alcoholic medium, meat in aqueous medium, liquid and cooked eggs, milk, fermented milk, cream and sour cream, precessed milk and in aqueous medium, concentrated extracts of an alcoholic strength ≥6 %.
- Simulant D2 - Vegetable oil. It is used for migration assays of plastic intended to contain pastry, biscuits, cakes, bread, etc. with fatty substances, chocolate and its confectionary products in paste form with fatty substances on the surface, fruit and vegetables preserved in oily medium, nuts in paste or cream form, fats and oils, fish, crustaceans and molluscs preserved in oily medium, meat of all zoological species, preserved milk in a fatty or oily medium, natural cheese without rind or with edible rind and melting cheese, preserved cheese in oily medium, fried or roasted foods, cocoa paste, spices and seasoning in oily medium such as paste or curry paste and other products with fatty character.
- Simulant E – Poly(2,6-diphenyl-p-phenylene oxide). It is used for migration assays of plastic intended to contain all kind of cereals and chocolate not contemplated before, sugar and its products in crystal or powder form, dried or dehydrated fruits and vegetables, nuts without past or cream form, powdered, dried or frozen eggs, powdered milk, cheese (whole with not edible rind) and other foods not contemplated before such as cocoa powder, frozen foods, pepper and salt.

- Ethanol 95 % (v/v). It is used as substitute of simulant D2 for migration assays of plastic intended to contain undenaturated ethyl alcohol beverages.
- Isooctane. It is used as substitute of simulant D2 for migration assays when simulant D2 is considered as unstable.

According with current legislation, specific migration assays for plastic material in contact with water have to be done with the food simulant distilled water or simulant B and C. Tests with simulant B can be omitted if the water has a pH >4.5. As it was indicated before, other test conditions as contact time and temperature have to be set according with use (Table 3 and 4). The most used test conditions for bottled water are the incubation of water/simulant for 10 days at 40 ºC. Bottled water can be used in the migration assays as a simulant and represent the most real conditions.

Table 3. Test time for specific migration assays according to contact time in worst foreseeable use.

Contact time in worst foreseeable use	Test time
t ≤ 5 min	5 min
5 min < t ≤ 0.5 hour	0.5 hours
0.5 hours < t ≤ 1 hour	1 hour
1 hour < t ≤ 2 hours	2 hours
2 hours < t ≤ 6 hours	6 hours
6 hours < t ≤ 24 hours	24 hours
1 day < t ≤ 3 days	3 days
3 days < t ≤ 30 days	10 days
≥ 30 days	Test conditions which are recognised to be the most severe on the basis of scientific evidence (e.g. 10 days at 40 or 60 ºC)

Table 4. Temperature time for specific migration assays according to contact temperature in worst foreseeable use.

Contact temperature in worst foreseeable use	Test temperature
T ≤ 5 ºC	5 ºC
5 ºC < T ≤ 20 ºC	20 ºC
20 ºC < T ≤ 40 ºC	40 ºC
40 ºC < T ≤ 70 ºC	70 ºC
70 ºC < T ≤ 100 ºC	100 ºC or reflux temperature
100 ºC < T ≤ 121 ºC	121 ºC
121 ºC < T ≤ 130 ºC	130 ºC
130 ºC < T ≤ 150 ºC	150 ºC
150 ºC < T ≤ 175 ºC	175 ºC
T > 175 ºC	Adjust the temperature to a real temperature at the interface with the food

Several authors studied the specific migration in different polymeric materials using food simulants or the water inside the plastic bottles. Bentayeb et al. (2007) used PET to perform migration assays at 70 ºC using several food simulants (water, 3% acetic acid, 10% ethanol and 95% ethanol). This conditions recreated the normal use of PET bottles for soft drinks, where diethylenglycol and terephthalic acid were detected at the highest concentrations of 1.060 µg/kg and 0.841 µg/kg, respectively. Votavová et al. (2009) used the food simulants distilled water, 3% acid acetic and 95 % ethanol for 10 days at 40 ºC to determine the migration of nonylphenol (NP) in PVC films. They found that in 95 % ethanol, NP release was up to 0.449 mg/g polymer, for distilled water up to 0.091 mg/g polymer and for 3 % acetic acid up to 0.079 mg/g polymer. It was concluded that although NP is not used as a direct additive into polymers, it may be originated as a component of a more complex additive preparation (e.g. stabilizer). Li et al. (2010) determined the migration of BPA from baby bottles filled with Milli-Q grade water for 24h at different temperatures (24 °C, 40 °C and 100 °C), obtaining the highest value of 4.500 µg/L at 100 ºC. Amiridou and Voutsa (2011) analysed the migration from 5 brands of 1 L PET bottles and from 18.9 L PC reusable container. In this case, the own bottled water to perform the assays for each brand was used. The assay was performed analysing 3 samples first and then storing 2 more samples

outdoors and directly exposed to sunlight for 15 and 30 days. This assay showed a BPA increase with time from 0.112 to 0.170 µg/L in the PC container. In PET bottles, BPA, NP, di-(2-ethylhexyl) phthalate (DEHP), dibutyl phthalate (DBP) and diethyl phthalate (DEP) were detected at concentrations up to 0.350 µg DEHP/L. Casajuana and Lacorte (2003) determined the migration of phthalates, NP, BPA and bisphenol A diglycidyl (BADGE) using the own bottled water contained in PE, PET and glass containers which were analysed initially and after 10 weeks outdoor storage temperatures up to 30 ºC. In this study, there was also an increase of the detected compounds for all three kind of samples obtaining the highest value of 0.196 µg DEHP/L for PE samples after the 10 days storage. Le et al. (2008) performed assays for new and used PC and for HDPE water bottles with an incubation at room temperature up to 7 days to test the BPA migration. Along the 7 days, in all cases there was an increase of BPA in the migration. For new PC bottles the increase was of 0.36 to 1.33 µg/L, for used PC it was of 0.29 to 0.93 µg/L and for new HDPE it was of 0.08 to 0.19 µg/L. Furthermore, when PC migration was tested at 100 ºC, the BPA value was detected up to 7.67 µg/L.

On the other hand, other studies used a solvent such as dichloromethane to dissolve the plastic material and then identify their components. Monteiro et al. (1998) dissolved PET samples with dichloromethane, macerated for 6h and ultrasonic bath for 1h prior to an injection to GC-MS. Dichloromethane was used to dissolve polymer additives without dissolving PET polymer. Nerín et al. (2003) identified and quantified the compounds present in a commercially available PC container used for microwave applications. A total dissolution of the polymer was performed with dichloromethane and after reprecipitation of the polymer with methanol, compounds were analyzed by high-performance liquid chromatography (HPLC) with both ultraviolet (UV) and fluorescence detection. Gas chromatography coupled to mass spectrometry (GC-MS) was used for compound confirmation. This procedure showed BPA concentrations of 30 µg/g PC and 2,4-di-tert-butylphenol (2,4-DTBP) of 76 µg/g PC at room temperature in the PC container used for microwave. Votavová et al. (2009) studied the migration of NP in PVC films performing an extraction with methanol under reflux for 2h followed by GC-MS, after using several simulants, and found NP at a concentration up to 0.449 mg/g polymer. Biles et al. (1997) dissolved PC materials from baby bottles and cups with dichloromethane and also detected BPA ranging 7 to 58 µg/g PC.

Other authors detected contaminants in plastic material without using any food simulant or solvent. Dutra et al. (2011) placed pellets of recycled PET and recycled HDPE multilayer into a 20 mL vial and after 10 min the solid-phase

microextraction (SPME) fibre was exposed to the vapours. The results of this study showed that the presence of high levels of some contaminants such as 2,4-DTBP and 2,6-di-tert-butyl-4-methylphenol (BHT) could be attributed to the misuse of post-consumer PET material and a lack of control in the collection of this material, or due to recontamination in the recycling system or even by external contamination. Sanches-Silva et al. (2009) used HPLC-UV to perform mathematical models for the prediction of the migration of photoinitiators (e.g. benzophenone), which are used as catalysers for inks and lacquers that are cured with UV light and they can contaminate foodstuffs by mass transference.

1.5.2. Regulatory framework for plastic in contact with water

Nowadays, the current and general legislation for the safety of materials in contact with food is Regulation (EC) 1935/2004 (EU, 2004a), which repeals Directives 80/590/EEC (EU, 1980c) and 89/109/EEC (EU, 1989), and Regulation (EC) 2023/2006 (EU, 2006a), which describes the good manufacturing practice of food contact materials. These two regulations ensure that any molecule transferred to food does not raise changes on organoleptic properties or safety concerns.

From Regulation (EC) 1935/2004 (EU, 2004a), other regulations for specific materials were published. In the case of plastic materials, the current legislation is Commission Regulation (EU) No 10/2011 (EU, 2011a), which covers a list of substances that can migrate to food and a description of migration tests. Other regulations are explained and the reasons they are in force today together with Regulation (EU) No 10/2011 (EU, 2011a).

In 1982, Council Directive 82/711/EEC (EU, 1982), laying down the basic rules necessary for testing migration of the constituents of plastic materials and articles intended to come into contact with foodstuffs, was published to describe the simulants and test conditions (times and temperatures). These simulants and test conditions were carried out to select the conditions which correspond most closely to the normal or foreseeable conditions of contact for the plastic materials or articles being studied.

Since there was a lack of information about the use of simulants for the different foodstuffs, another Council Directive was published in 1985 (EU, 1985). It covers the specific properties of simulants and the use of each simulant for the different foodstuffs in the market (beverages, cereals, cereal products, pastry,

biscuits, cakes, bakers' wares, chocolate, sugar, confectionery products, fruits, vegetables, fats, oils, animal products, eggs, milk products and miscellaneous products).

Some years later, in 1993 and 1997, the Commission Directives 93/8/EEC (EU, 1993a) and 97/48/EC (EU, 1997) amending Council Directive 82/711/EEC (EU, 1982) were published to describe more in detail the migration test conditions and describing the basic rules for testing migration of constituents of plastic materials.

Next, Commission Directive 2002/72/EC (EU, 2002b) was published indicating a list of positive substances related to plastic materials intended to come into contact with foodstuffs. Moreover, other legislations were published in relation with plastic contact, as Regulation (EC) 1935/2004 (EU, 2004a), Council Directive 85/572/EEC (EU, 1985), and Commission Directives 2004/19/EC (EU, 2004b) and 2007/19/EC (EU, 2007). Furthermore, the Spanish government published the Royal Decree 866/2008 (Spanish Government, 2008) taking into account the European regulations.

In addition, in relation with these regulations, two series of European standards were published, EN 1186 (UNE-EN 1186:2002) and EN 13130 (UNE-EN 13130:2005) for overall and specific migration assays, respectively. Both European standards are interpretations of the regulations described before and indicate that the plastic material could be cut in pieces to perform the migration assays.

Taking into account all this set of regulations and directives, European countries decided to unify all of them into the Commission Regulation (EU) No 10/2011 (EU, 2011a). This regulation describes the overall and specific migration, food simulants and testing conditions for the different uses of plastics in contact with food. In fact, there are some changes concerning the use of different simulants in comparison with the previous regulations. For instance, this new Regulation indicates that distilled water cannot be used for specific migration tests. Implementation of Commission Regulation (EU) No 10/2011 (EU, 2011a) includes a transition period where: (i) until 31th December 2012, migration tests shall be based on Council Directive 82/711/EEC (EU, 1982); (ii) until 31th December 2015, migration tests may be based on Council Directive 82/711/EEC and Commission Regulation (EU) No 10/2011 (EU, 2011a); and (iii) from 1th January 2016, migration tests shall be based on Commission Regulation (EU) No 10/2011 (EU, 2011a).

Moreover, nowadays there are two regulations amending Commission Regulation (EU) No 10/2011 (EU, 2011a), Commission Regulations (EU) No 321/2011 (EU, 2011b), as regards of use of BPA in plastic infant feeding bottles, and No 1282/2011 (EU, 2011c), where some new substances are included.

Finally, Spanish government published Royal Decrees 846/2011 (Spanish Government, 2011a) and 847/2011 (Spanish Government, 2011b) about plastic materials intended to be in contact with foodstuffs. Royal Decree 847/2011 (Spanish Government, 2011b), establishes a positive list of permitted substances for the manufacture of polymeric materials. This list is based on a European list of 1987 for the Scientific Committee on Food (SCF) (SCF, 1987).

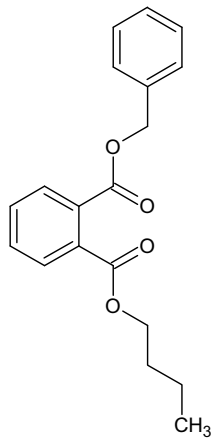
1.6. Tolerable daily intake

Substances intended to be hazardous for humans have been regulated according with the acceptable (ADI) or tolerable daily intake (TDI) defined for a single substance. The ADI or TDI means the estimate of amount of substance in food, expressed on a body weight basis, that can be ingested daily over a lifetime, without appreciable risk to any consumer, taking into account sensitive groups within the population (e.g. children and the unborn) (EU, 2005). TDI values are normally calculated from the No-Observed-Adverse-Effect Level (NOAEL) derived from toxicological studies (e.g. multigeneration study in rats) and applying an uncertainty factor of 500, which comprises 10 for interspecies differences and 10 for interindividual differences. Also, an additional uncertainty factor of 5 may be used for uncertainties in the database on reproductive and developmental toxicity. Additionally, TDI is calculated for a specific age range, for adults it is considered a weight of 60 kg and a consumption of 3 kg of commercial foods (1 kg solid foods and 2 kg or 2 L beverages) (EFSA, 2006). For bottled water, the consumption could be between 1.5 and 2 L water. These calculations result in a TDI number defined as mg of substance consumed in a day per body weight (mg/kg bw/day). Calculation of the amount of substance ingested allows knowing whether consumption of a single substance is above the TDI value.

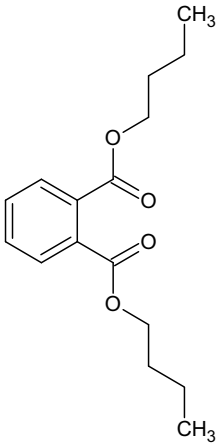
1.7. Description of target compounds

Contaminants studied in this thesis are substances which are present in plastic materials (monomers and additives as plasticisers, antioxidants and UV stabilizers). Compounds studied were benzyl butyl phthalate (BBP), dibutyl phthalate (DBP), di-(ethylhexyl) phthalate (DEHP), diethyl phthalate (DEP), dimethyl phthalate (DMP), dimethyl isophthalate (DMIP), di-(ethylhexyl) adipate (DEHA), 4-nonylphenol (4-NP), 4-tert-octylphenol (4-OP), 2,4-di-tert-butylphenol (2,4-DTBP), acetyl tributyl citrate (ATBC), benzophenone (BP), bisphenol A (BPA), 2,6-di-tert-butyl-4-methylphenol (BHT) and 2-phenoxyethanol (2-PE). Physicochemical properties of these compounds were searched in the Physical Properties Database (Physprop) (SRC, 2013).

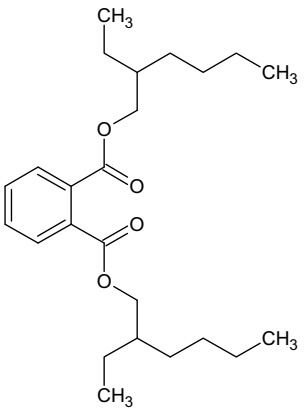
BBP. BBP is a plasticiser added to polymers to give flexibility and softness. It is used in flexographic inks for food packaging applications (Bolgar et al, 2008) and it is considered as a very toxic compound due to its mutagen, acute oral, reproductive and carcinogenicity toxicity (Bolgar et al., 2008; ECHA, 2013). Legislation (EU, 2011a) indicates that BBP is only used as a plasticiser in repeated use materials and articles; as a plasticiser in single-use materials and articles contacting non-fatty foods except for infant formulae o processed cereal-based foods and baby foods for infants and young children; and as a technical support agent in concentrations up to 0.1 % in the final product. Its specific migration limit (SML) is 30 mg/L and its specific migration limit as a sum of substances (SML(T)) is 60 mg/L (it is in the same group of ATBC).

<table>
<tr><td colspan="2">Name: Benzyl butyl phthalate</td></tr>
<tr><td>CAS Number: 85-68-7
Abbreviation: BBP
Molecular formula: $C_{19}H_{20}O_4$
Formula Weight = 312.37</td><td>  </td></tr>
<tr><td colspan="2">Synonyms: 1,2-benzenedicarboxylic acid, butyl phenylmethyl ester; butyl benzyl phthalate.
SMILES: O=C(OCc1ccccc1)c2ccccc2C(=O)OCCCC</td></tr>
<tr><td colspan="2">Physicochemical Properties
Water solubility: 2.69 mg/L — Log P/ Log Kow: 4.73
Melting Point: <25 ºC — Vapour pressure: $8.25E^{-6}$ mmHg
Boiling Point: 370 ºC — Henry's Law: $1.26E^{-6}$ atm.m^3/mol</td></tr>
</table>

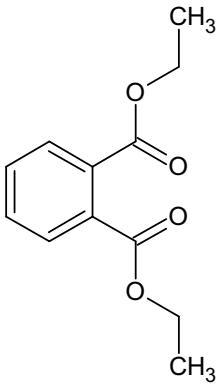
DBP. DBP can only be used as a plasticizer in repeated use materials and in articles in contact with non-fatty foods and as a technical support agent in polyolefins in concentrations up to 0.05 % in the final product (EU, 2011a). SML and SML(T) are 0.3 and 60 mg/kg, respectively. The European Chemical Agency (ECHA) (ECHA, 2013) established DMP use as a polymer and industrial plasticizer.

<table>
<tr><td colspan="2">Name: Dibutyl phthalate</td></tr>
<tr><td>CAS Number: 84-74-2
Abbreviation: DBP
Molecular formula: $C_{16}H_{22}O_4$
Formula Weight: 278.34</td><td>  </td></tr>
<tr><td colspan="2">Synonyms: 1,2-benzenedicarboxylic acid, dibutyl ester; n-butyl phthalate.
SMILES: O=C(OCCCC)c1ccccc1C(=O)OCCCC</td></tr>
<tr><td colspan="2">Physicochemical Properties
Water solubility: 11.2 mg/L | Log P/ Log Kow: 4.50
Melting Point: -35 ºC | Vapour pressure: $2.01E^{-5}$ mmHg
Boiling Point: 340 ºC | Henry's Law: $1.81E^{-6}$ atm.m^3/mol</td></tr>
</table>

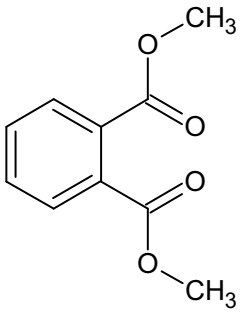
DEHP. DEHP can be used in repeated use materials and articles contacting with non-fatty foods and as a technical support agent in concentrations up to 0.1 % in the final product (EU, 2011a). SML and SML(T) are 1.5 and 60 mg/kg, respectively. It can also be used as plasticizer for resins and elastomers which are used to manufacture many products, including teething rings, pacifiers, soft squeeze toys, balls, vinyl upholstery, tablecloths, shower curtains, raincoats, adhesives, polymeric coatings, components of paper and paperboard, defoaming agents, enclosures for food containers and vinyl gloves used for medical examinations and surgery (Bolgar et al., 2008). The ECHA shows different uses for DEHP: for processing of formulations containing DEHP as plasticiser through compounding, calendering, spread coating, extrusion, injection moulding into articles and low energy manipulation of the resulting polymers; for manufacturing, distribution and use of DEHP as intermediate in Ziegler Natta catalyst (Ziegler Natta catalyst is a catalyst used in the synthesis of polymers of 1-alkenes); for formulation of DEHP in dry-blend and plastisol formulations; and formulation and use in polymers (ECHA, 2013).

<table>
<tr><td colspan="2">Name: Di-(2-ethylhexyl) phthalate</td></tr>
<tr><td>CAS Number: 117-81-7
Abbreviation: DEHP
Molecular formula: $C_{24}H_{38}O_4$
Formula Weight = 390.56</td><td>  </td></tr>
<tr><td colspan="2">Synonyms: dioctyl phthalate; bis(2-ethylhexyl)phthalate.
SMILES: CCC(CCCC)COC(=O)c1ccccc1C(=O)OCC(CC)CCCC</td></tr>
<tr><td colspan="2">Physicochemical Properties
Water solubility: 0.27 mg/L — Log P/ Log Kow: 7.60
Melting Point: -55 ºC — Vapour pressure: $1.42E^{-7}$ mmHg
Boiling Point: 384 ºC — Henry's Law: $2.7E^{-7}$ atm.m^3/mol</td></tr>
</table>

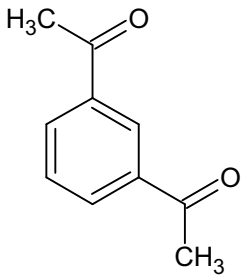
DEP. DEP is used as a plasticizer to improve plastic flexibility, commonly used in products such as toothbrushes, automobile parts, tools, toys, and food packaging. It is also used as a plasticizer in cellulose ester plastics such as photographic films and sheets, blister packaging, and tape applications (Bolgar et al., 2008). DEP is not legislated in Commission Regulation 10/2011 (EU, 2011). However it is legislated in Spanish Royal Decree 847/2011 (Spanish Government, 2011b) with a SML of 0.010 mg/kg.

<table>
<tr><td colspan="2">Name: Diethyl phthalate</td></tr>
<tr><td>CAS Number: 84-66-2
Abbreviation: DEP
Molecular formula: $C_{12}H_{14}O_4$
Formula Weight = 222.24</td><td>  </td></tr>
<tr><td colspan="2">Synonyms: 1,2-benzenedicarboxylic acid, diethyl ester; diethyl 1,2-benzenedicarboxylate; o-bis(ethoxycarbonyl)benzene; phthalic acid, diethyl ester.
SMILES: O=C(OCC)c1ccccc1C(=O)OCC</td></tr>
<tr><td colspan="2">Physicochemical Properties</td></tr>
<tr><td>Water solubility: 1080 mg/L
Melting Point: -40.5 ºC
Boiling Point: 295 ºC</td><td>Log P/ Log Kow: 2.42
Vapour pressure: 0.0021 mmHg
Henry’s Law: $3.1E^{-7}$ atm.m^3/mol</td></tr>
</table>

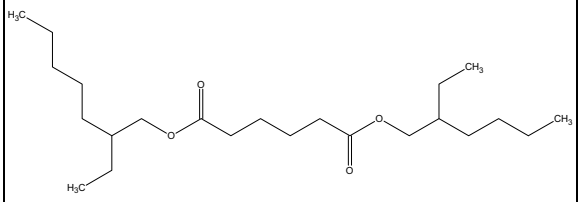
DMP. DMP can be used as a plasticizer and for the manufacturing of adhesives (ECHA, 2013). DMP isomers such as DMIP and DMTP can also be found in polymeric materials. The National Institute for Occupational Safety and Health (NIOSH) has statistically estimated that 57,908 workers (16,352 of these were female) were potentially exposed to DMP in the United States (NIOSH, 2008). Occupational exposure to DMP may occur through inhalation of aerosols and dermal contact with this compound at workplaces where DMP is produced or used. Monitoring data indicate that the general population may be exposed to DMP via inhalation of ambient air, ingestion of drinking water, and dermal contact with products containing DMP (U.S. National Library of Medicine, 2013).

<table>
<tr><td colspan="2">Name: Dimethyl phthalate</td></tr>
<tr><td>CAS Number: 131-11-3
Abbreviation: DMP
Molecular formula: $C_{10}H_{10}O_4$
Formula Weight = 194.19</td><td>  </td></tr>
<tr><td colspan="2">Synonyms: 1,2-benzendicarboxylicacid, dimethylester; 1,2-dimethyl phthalate; dimethyl 1,2-benzenedicarboxylate acid; dimethyl benzene-o-dicarboxylate; dimethyl benzeneorthodicarboxylate.
SMILES: COC(=O)c1ccccc1C(=O)OC</td></tr>
<tr><td colspan="2">Physicochemical Properties
Water solubility: 4000 mg/L — Log P/ Log Kow: 1.60
Melting Point: 5.5 ºC — Vapour pressure: 0.00308 mmHg
Boiling Point: 283.7 ºC — Henry's Law: $1.97E^{-7}$ atm.m^3/mol</td></tr>
</table>

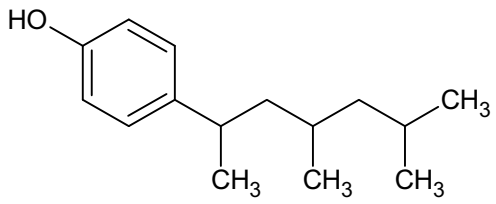
DMIP is authorised to be used as monomer or other starting substance or macromolecule obtained from microbial fermentation with a SML of 0.05 mg/kg (EU, 2011a). DMIP's production and use as a polyacrylate resin co-monomer and as a perfume fixative may result in its release to the environment through various waste streams. Occupational exposure to DMIP may occur through inhalation of aerosols and dermal contact with this compound at workplaces where DMIP is produced or used. Use data indicate that the general population may be exposed to DMIP via dermal contact with and inhalation of products containing this compound (U.S. National Library of Medicine, 2013).

<table>
<tr><td colspan="2">Name: Dimethyl isophthalate</td></tr>
<tr><td>CAS Number: 1459-93-4
Abbreviation: DMIP
Molecular formula: $C_{10}H_{10}O_4$
Formula Weight = 194.19</td><td>  </td></tr>
<tr><td colspan="2">Synonyms: 1,3-Benzenedicarboxylicacid, dimethyl ester; isophthalic acid, dimethyl ester; 1,3-di(methoxycarbonyl)benzene; dimethyl 1,3-benzenedicarboxylate; dimethyl m-phthalate; methyl 3-carbomethoxybenzoate.
SMILES: O=C(C)c1cccc(c1)C(C)=O</td></tr>
<tr><td colspan="2">Physicochemical Properties</td></tr>
<tr><td>Water solubility: 290 mg/L
Melting Point: 67.5 ºC
Boiling Point: 282 ºC</td><td>Log P/ Log Kow: 1.66
Vapour pressure: 0.00963 mmHg
Henry’s Law: $6.14E^{-8}$ atm.m^3/mol</td></tr>
</table>

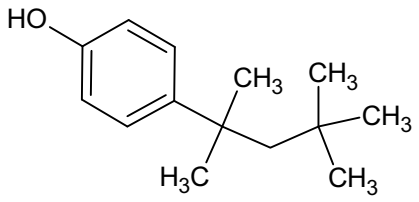
DEHA. DEHA is a plasticizer used primarily in food-contact wrapping, building materials and household furnishings (Bolgar et al., 2008). It is considered as an effective replacement for DEHP (Bolgar et al., 2008). Also, it can be used in formulation of plastics, as a plasticizer for PVC, acrylate, nitrocellulose and rubber products, to manufacture of rubbers and additives for coatings, inks and adhesives (ECHA, 2013). SML and SML(T) are 18 and 60 mg/kg, respectively (EU, 2011a).

<table>
<tr><td colspan="2">Name: Di-(2-ethylhexyl) adipate</td></tr>
<tr><td>CAS Number: 103-23-1
Abbreviation: DEHA
Molecular formula: $C_{22}H_{42}O_4$
Formula Weight = 384.59</td><td>  </td></tr>
<tr><td colspan="2">Synonyms: hexanedioic acid, bis(2-ethylhexyl) ester; dioctyl adipate; bis(2-ethylhexyl)hexanedioate.
SMILES: CCCCCC(COC(=O)CCCCC(=O)OCC(CC)CCCC)CC</td></tr>
<tr><td colspan="2">Physicochemical Properties
Water solubility: 0.78 mg/L | Log P/ Log Kow: 6.11
Melting Point: -67.8 ºC | Vapour pressure: $8.5E^{-7}$ mmHg
Boiling Point: 417 ºC | Henry's Law: $4.34E^{-7}$ atm.m^3/mol</td></tr>
</table>

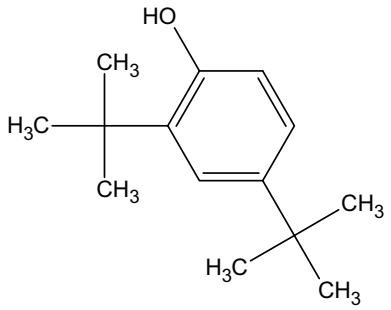
4-NP. NP is an alkylphenol whose name can apply to a large number of isomeric compounds of general formula C6H4(OH)C9H19. Nonylphenols may vary in two ways: the substitution position of the nonyl group on the phenol molecule, and the degree of branching of the nonyl group. Since the nonyl moiety is formed by polymerising propylene, the degree of branching may be considerable and varied. Many of the individual branched isomers have their own CAS numbers. It is understood that nonylphenol (CAS Number: 25154-52-3) as originally defined by CAS covered all nonylphenols. The main use of nonylphenol in the plastics industry is as a monomer in the production of phenol/formaldehyde resins. Other uses include as intermediate in the production of tri-(4-nonylphenyl) phosphite (TNPP) and as a catalyst in the curing of epoxy resins. Nonylphenol is not used as a free additive in resins, plastics or stabilisers and it may be present in detergents. There is a potential for consumer exposure due to the consumer use of epoxy resins. NP is legislated in Spanish Royal Decree 847/2011 with a SML of 0.010 mg/kg, which was assessed for risks to the environment and human health under the Existing Substances Regulation (ESR) 793/93/EEC (EU, 1993b) and is currently the subject of marketing and use restrictions under Council Directive 76/769/EEC (EU, 1976).

<table>
<tr><td colspan="2">Name: 4-Nonylphenol</td></tr>
<tr><td>CAS Number: 104-40-5
Abbreviation: 4-NP
Molecular formula: $C_{15}H_{24}O$
Formula Weight = 220.4</td><td>  </td></tr>
<tr><td colspan="2">Synonyms: p-nonylphenol; p-n-nonylphenol; 4-n-nonylphenol.
SMILES: Oc1ccc(cc1)C(C)CC(C)CC(C)C</td></tr>
<tr><td colspan="2">Physicochemical Properties
Water solubility: 6 mg/L — Log P/ Log Kow: 5.76
Melting Point: 43-44 ºC — Vapour pressure: $9.42E^{-5}$ mm Hg
Boiling Point: 293-297 ºC — Henry's Law: $4.3E^{-6}$ atm.m^3/mol</td></tr>
</table>

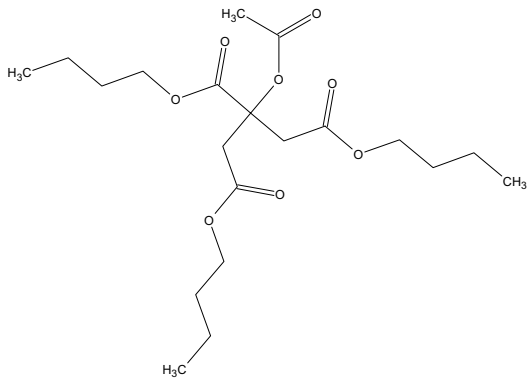
4-OP. OP is an alkylphenol that has been identified by industry as the only OP isomer currently commercially available in Europe. For the purposes of this study, therefore, unless otherwise specified, the term "octylphenol" or "OP" is assumed to refer to 4-tert-octylphenol. 4-tert-octylphenol is a high production volume chemical and is the most likely immediate replacement for NP. It is used in rubber industry, paints, printing inks, coatings industry, and adhesives formulation and in the production of polymers (ECHA, 2013).

<table>
<tr><td colspan="2">Name: 4-tert-Octylphenol</td></tr>
<tr><td>CAS Number: 140-66-9
Abbreviation: 4-OP
Molecular formula: $C_{14}H_{22}O$
Formula Weight = 206.33</td><td>  </td></tr>
<tr><td colspan="2">Synonyms: p-octylphenol; 4-t-octylphenol; p-t-octylphenol.
SMILES: Oc1ccc(cc1)C(C)(C)CCCCC</td></tr>
<tr><td colspan="2">Physicochemical Properties
Water solubility: 5 mg/L — Log P/ Log Kow: 5.28
Melting Point: 84.5 ºC — Vapour pressure: 0.000478 mmHg
Boiling Point: 158 ºC ($1.50E^{+1}$ mmHg) — Henry's Law: $6.89E^{-6}$ atm.m^3/mol</td></tr>
</table>

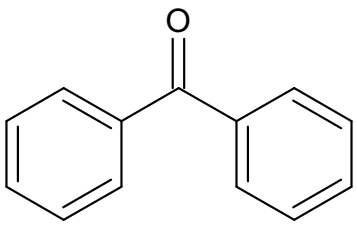
2,4-DTBP. 2,4-DTBP is an alkylphenol that is primarily used for the synthesis of triaryl phosphites and as antioxidant in plastics. It is also used to produce primary phenolic antioxidants and can be converted to benzotriazole derivates or to the ester 3,5-di-tert-butyl-4-hydroxybenzoic acid, both of which are used as UV stabilisers (EPA, 2013a).

<table>
<tr><td colspan="2">Name: 2,4-Di-tert-butylphenol</td></tr>
<tr><td>CAS Number: 96-76-4
Abbreviation: 2,4-DTBP
Molecular formula: $C_{14}H_{22}O$
Formula Weight = 206.33</td><td>  </td></tr>
<tr><td colspan="2">Synonyms: prodox146; prodox146A-85X; antioxidant 33
SMILES: CC(C)(C)c1cc(c1cc(ccc1O)C(C)(C)C</td></tr>
<tr><td colspan="2">Physicochemical Properties
Water solubility: 35 mg/L | Log P/ Log Kow: 5.19
Melting Point: 56.5 ºC | Vapour pressure: 0.00477 mmHg
Boiling Point: 263.5 ºC | Henry's Law: $3.74E^{-6}$ atm.m^3/mol</td></tr>
</table>

ATBC. ATBC can be used as a monomer or other starting substance (R. 10/2011). Furthermore, it is used as a plasticiser for flexible films made of vinylchloride-vinylidene chloride copolymer and cellulose and in food contact applications. ATBC provides adherence to metals, low volatility and resistance to yellowing. United States (US) Food and Drug Administration (FDA) approved it as a plasticiser in food-packaging materials in 1998, 21CFR178.3740; in adhesives as a component of articles intended for use in packaging, transporting, or holding food, 21CFR175.105; in the manufacture of resinous and polymeric coatings for safe use as a food-contact surface of articles, 21CFR175.300; in the manufacture of resinous or polymeric coatings in polyolefin films for the food-contact surface of articles intended for use in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding food, 21CFR175.320 (Bolgar et al., 2008). In terms of toxicology, ATBC has a low order of genetic toxicity, carcinogenicity and toxicity to reproduction (Bolgar et al, 2008; ECHA, 2013). It is legislated in Commission Regulation (UE) No 10/2011 (EU, 2011a) with a SML(T) of 60 mg/L.

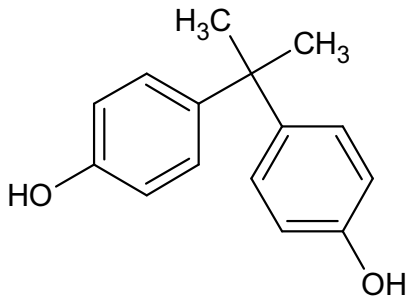
<table>
<tr><td colspan="2">Name: Acetyl tributyl citrate</td></tr>
<tr><td>CAS Number: 77-90-7
Abbreviation: ATBC
Molecular formula: $C_{20}H_{34}O_8$
Formula Weight = 402.54</td><td>  </td></tr>
<tr><td colspan="2">Synonyms: 2-Acetoxy-1,2,3-propanetricarboxylic acid, tributyl ester; acetyltri-n-butyl citrate; acetylcitric acid.
SMILES: CCCCOC(=O)CC(CC(=O)OCCCC)(OC(C)=O)C(=O)OCCCC</td></tr>
<tr><td colspan="2">Physicochemical Properties</td></tr>
<tr><td>Water solubility: 5 mg/L
Melting Point: -80 ºC
Boiling Point: 172 ºC at 1 mmHg</td><td>Log P/ Log Kow: 4.29
Vapour pressure: $4.55E^{-6}$ mm Hg
Henry's Law: $3.78E^{-10}$ atm.m^3/mol</td></tr>
</table>

BP. Benzophenones can be used as ultraviolet (UV) stabilizers, which prevent discoloration, cracking and loss of physical properties due to sunlight. BP can be used as additive or polymer production aid (EU, 2011a) and can be used as photoinitiator (PI) catalysers for inks and lacquers that are cured with ultraviolet light. This compound has been reported to migrate to foodstuffs by mass transference (Sanches-Silva et al., 2011; Rothenbacher et al., 2007), which can occur by set-off (as a result of the contact of the external printed face of the packaging with the inner non-printed face) or by a transfer through the substrate.

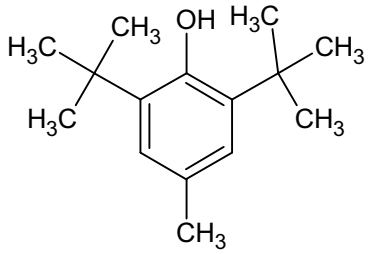
<table>
<tr><td colspan="2">Name: Benzophenone</td></tr>
<tr><td>CAS Number: 119-61-9
Abbreviation: BP
Molecular formula: $C_{13}H_{10}O$
Formula Weight = 182.22</td><td>  </td></tr>
<tr><td colspan="2">Synonyms: UV500; BLS 531; FEMA 2134; IHT-PI BP; Darocur BP; Kayacure bp; a-Oxoditane; Adjutan 6016
SMILES: O=C(c1ccccc1)c2ccccc2</td></tr>
<tr><td colspan="2">Physicochemical Properties
Water solubility: 137 mg/L — Log P/ Log Kow: 3.18
Melting Point: 47.8 ºC — Vapour pressure: 0.00193 mmHg
Boiling Point: 305.4 ºC — Henry’s Law: $1.94E^{6}$ atm.m^3/mol</td></tr>
</table>

BPA. BPA was synthesized in 1891 and it was investigated in 1930s as a possible synthetic estrogen. Nowadays, it is used in the manufacture of PC plastics and epoxy resins. PC is used in food contact plastics such as reusable beverage bottles, infant feeding bottles and storage containers, whereas epoxy resins are used in protective liners for food and beverage cans. From some years ago until now, there exists a concern related to the BPA ingestion from food and beverages. The ingestion can occur from the migration in plastic bottles. For this reason, the EFSA published several Scientific Opinions in 2006, 2008 and 2010 (EFSA, 2006; EFSA, 2008; EFSA, 2010). In 2006, the EFSA set a TDI for BPA of 0.05 mg BPA/kg bodyweight/day. The TDI was based on a NOAEL of 5 mg/kg bw/day, identified in two multi-generation reproductive toxicity studies in rodents, where the critical effects were changes in body and organ weights in adult and offspring rats and liver effects in adult mice, respectively. This TDI was set to protect human population for life-time exposure, including sensitive groups such as pregnant and lactating women, infants (01-12 months) and young children (12-36 months). In 2008, the EFSA reaffirmed this TDI, concluding that age-dependent toxicokinetics differences of BPA in animals and humans would have no implication for the default uncertainty factor of 100 for the TDI. In 2010, the EFSA's Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) identified some toxicological effects in developing animals which need further consideration. Furthermore, in 2011, the EFSA's CEF Panel published another Scientific Opinion related to a French Agency's Report (Food Environmental and Occupational Health Safety - "Agence Nationale de Sécurité Sanitaire (ANSES)"). It published two reports on BPA related to health effects and its uses. The ANSES report concluded that health effects have been proved in animals and suspected in humans, even at low levels of exposure that are below current regulatory threshold. It recommends no exposure to BPA of infants, young children, pregnant and breastfeeding women which they identified as most susceptible populations. The EFSA's CEF Panel concluded that the Panel would need more time to review more in depth the new studies. Also, BPA is considered as an endocrine-disrupting chemical (EDC) with estrogenic activity. Commission Directive 2002/72/EC of 6 August 2002 relating to plastic materials and articles intended to come into contact with foodstuffs authorised the use of BPA as a monomer for the manufacture of plastic materials and articles intended to come into contact with foodstuffs in accordance with the opinions of the SCF and the EFSA (EU, 2002b). In the Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food, BPA is indicated as an authorized substance to be used as monomer with a SML of 0.6 mg/kg (EU, 2011a). Commission Directive of 28 January 2011 amending Directive

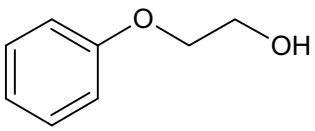
2002/72/EC restricted the use of BPA in plastic infant feeding bottles. Furthermore, the National Assembly of France introduced a bill seeking the suspension of the manufacture, import, export and placement on the market of all food packaging containing BPA on 12th October 2011. Later, on April 2013, the Agence Nationale se Sécurité Sanitaire Alimentation, environnement, travail (ANSES) published the press kit "Evaluation des risques sanitaires du bisphénol A", where it is indicated that it is necessary to reduce the exposure to BPA due to the potential risk for the health. This press kit also declared that more than 80 % of the population exposure to BPA is because of the food and the main BPA source (about 50 %) in food has the origin on cans. Also, it declared that PC water bottle is a BPA source.

<table>
<tr><td colspan="2">Name: Bisphenol A</td></tr>
<tr><td>CAS Number: 80-05-7
Abbreviation: BPA
Molecular formula: $C_{15}H_{16}O_2$
Formula Weight = 228.29</td><td>  </td></tr>
<tr><td colspan="2">Synonyms: 4,4'-dihydroxy-2,2-diphenylpropane; 4,4'-(propan-2-ylidene)diphenol; p,p'-isopropylidenebisphenol; 2,2-bis(4-hydroxyphenyl)propane.
SMILES: CC(C)(c1ccc(O)cc1)c2ccc(O)cc2</td></tr>
<tr><td colspan="2">Physicochemical Properties</td></tr>
<tr><td>Water solubility: 120 mg/L
Melting Point: 153 ºC
Boiling Point: 220 ºC (at 4 mmHg)</td><td>Log P/ Log Kow: 3.32
Vapour pressure: 3.91E^{-7} mm Hg
Henry's Law: 1E^{-11} atm.m^3/mol</td></tr>
</table>

BHT. BHT can be used as a monomer with a SML of 3 mg/kg (EU, 2011a). Also, it can be used as a phenolic antioxidant used for polyolefin applications such as petroleum, animal feed, and for food products and packaging. FDA approved it for food contact under 21CFR175.105 (adhesives (no limitations)), 175.125 (pressure sensitive adhesives (0.1% max.)) and 177.2600 (rubber articles intended for repeated use (5% max.)) (Bolgar et al., 2008). It is used in plastics such as non-rubber polymers, adhesives, coatings, dyes, inks, printing dyes, biodiesel, lubricant in machineries and rubber products (including tyres) (ECHA, 2013).

<table>
<tr><td colspan="2">Name: 2,6-di-tert-butyl-4-methylphenol</td></tr>
<tr><td>CAS Number: 128-37-0
Abbreviation: BHT
Molecular formula: $C_{15}H_{24}O$
Formula Weight = 220.35</td><td>  </td></tr>
<tr><td colspan="2">Synonyms: butylated hydroxytoluene; 2,6-di-tert-butyl-p-cresol.
SMILES: CC(C)(C)c1cc(C)cc(c1O)C(C)(C)C</td></tr>
<tr><td colspan="2">Physicochemical Properties
Water solubility: 0.6 mg/L — Log P/ Log Kow: 5.10
Melting Point: 71 ºC — Vapour pressure: 0.00516 mmHg
Boiling Point: 265 ºC — Henry's Law: $4.12E^{-6}$ $atm.m^3/mol$</td></tr>
</table>

2-PE. 2-PE is an aromatic ether used as a solvent for cellulose acetate, dyes, inks, resins, and in the organic synthesis of plasticizers and pharmaceuticals (Journal of the American College of Toxicology, 1990). It is used in manufacture of tyres and rubber products; emulsion polymerization; production of ethoxylates as an intermediate; formulation of adhesives; and production of polymers (ECHA, 2013). However, it is commonly used as part of cosmetics, where it is legislated (Journal of the American College of Toxicology, 1990).

<table>
<tr><td colspan="2">Name: 2-Phenoxyethanol</td></tr>
<tr><td>CAS Number: 122-99-6
Abbreviation: 2-PE
Molecular formula: $C_8H_{10}O_2$
Formula Weight = 138.17</td><td>  </td></tr>
<tr><td colspan="2">Synonyms: phenoxetol; ethylene glycol monophenyl ether; phenyl cellosolve.
SMILES: OCCOc1ccccc1</td></tr>
<tr><td colspan="2">Physicochemical Properties
Water solubility: 26700 mg/L — Log P/ Log Kow: 1.16
Melting Point: 14 ºC — Vapour pressure: 0.007 mmHg
Boiling Point: 245 ºC — Henry’s Law: $4.72E^{-8}$ atm.m^3/mol</td></tr>
</table>

1.8. Analytical techniques for the characterisation of plastic components in water intended for human consumption

Because plastic components in water are present at very low concentrations, it is necessary to develop and use analytical methods that provide very high sensitivity to obtain limits of detection (LODs) and quantification (LOQs) of parts per trillion. The most used analytical techniques for the detection of these compounds are gas chromatography (GC) and liquid chromatography (LC) coupled to mass spectrometry (MS) or tandem mass spectrometry (MS/MS).

GC has been one of the most popular techniques to its high separation capabilities and sensivility. According to the USEPA, GC technique is recommended for the analysis of phthalate and adipate esters in drinking water (USEPA, 1995) and phthalates in aqueous and solid matrices including groundwater, leachate, soil, sludge and sediment (USEPA, 1996). Within the wide range of volatile compounds that are normally analysed by GC-MS, phthalates have been largely studied in different water samples such as bottled waters (Farahani et al., 2007; Farahani et al., 2008; Zhang and Lee, 2013). Although GC is a good technique for the identification and quantification of plastic components, it may be necessary to derivatize phenolic compounds to improve GC-MS sensitivity (Gallart-Ayala et al., 2010; Stuart et al., 2005). Nerín et al. indicate that GC-MS is limited for some BPA derivates due to their low volatility (Nerín et al., 2002). Other plastic compounds have been characterized using GC. Monteiro et al. analysed UV stabilisers in PET samples performing ultrasonic bath extraction with dichloromethane followed by GC-MS (Monteiro et al., 1998).

On the other hand, LC is used for the analysis of non-volatile and more-polar compounds without the requirement of a derivatisation step. Avoiding this additional manipulation of the sample allows saving time and increases reproducibility (Gallart-Ayala et al., 2010). Bentayeb et al. (2007) used LC–MS to separate and analyze terephthalic acid and ethylenglycol that were extracted from recycled PET samples. This study indicated disadvantages of LC-MS for identifying unknown compounds due to the lack of mass spectra libraries and its relatively limited sensitivity, especially compared to GC–MS. However, authors also indicated the use of ultra performance liquid chromatography (UPLC) coupled to MS/MS systems as a solution to provide greater sensitivity and resolution of chromatographic peaks.

For the analysis of plasticizers and additives in water, several extraction methods have been developed. The most used extraction techniques for plastic components are liquid-liquid extraction (LLE), solid phase extraction (SPE), solid phase microextraction (SPME) and stir bar sorptive extraction (SBSE).

LLE was used in combination with automated large volume injection (LVI) and GC-MS analysis for the determination of phthalates in water samples (Tienpont et al., 2005) because it allowed extracting compounds from water to an organic solvent due to the relative solubility of the compounds. LLE is the extraction technique with more sample handling and use of solvents. LLE using dichloromethane as extraction solvent was applied to sea water and spring water for BPA analysis followed by GC-MS (Del Olmo et al., 1997) and a LOD of 0.6 µg/L was obtained. Therefore, this LOD may be too high for the trace determination of BPA in bottled water. Amiridou and Voutsa (2011) determined alkylphenols, phthalates and BPA in bottled water by LLE-GC/MS with derivatisation. Detection limits, were calculated from the standard deviation of seven replicates and ranged from 0.0022 µg/L for OP up to 0.030 µg/L for DMP. Mihovec-Grdič et al. (2002) also analysed several phthalates by LLE followed by GC-ECD in 77 groundwaters, 10 river waters and 9 drinking waters from Zagreb water supply, obtaining LODs of 0.005 and 0.040 µg/L for BBP and DEHP, respectively.

SPE technique is based on the specific interactions between a solid sorbent and an analyte from a sample matrix. These interactions can selectively retain and concentrate the target compounds. After preconcentration, analytes are eluted with one or more solvents and are thereafter evaporated to constitute the final extract. This technique is less time consuming than LLE and it can be easily automated. A study based in SPE and HPLC with UV detector without derivatisation was used for the analysis of BPA and alkylphenols in several kinds of water such as river and sea water, wastewater and drinking water in plastic bottles. This study did not detect BPA and NP above the LOQs (0.010 µg/L for BPA and 0.100 µg/L for NP) (Brossa et al., 2002). Using SPE and GC-MS, Casajuana and Lacorte (2003) analysed BPA, BADGE, alkylphenols and phthalates in bottled water obtaining recoveries between 72 and 80 %, except for DMP, DEP, DEHP and BADGE that were 56 %, 42 %, 21 % and 120 %, respectively. Due to the ubiquitous presence of phthalates in the environment and the complexity of their analysis, blanks were used to control the possible external contamination. Li et al. (2010) determined 4-NP, BPA and triclosan in tap water, bottled water and baby bottles by SPE-GC/MS with a prior derivatisation step. Three spiking levels of 5 ng, 100 ng, and 200 ng were added to 1 L of surface water, obtaining recoveries from 74 % to 118 %. In this case,

procedural blanks were also included in the analysis, and LODs and LOQs were calculated as 3 and 10 times the standard desviation of seven replicates of the spiked water at the concentration of 0.005 µg/L. The LOD values for 4-NP and BPA were of 0.002 and 0.0007 µg/L, while the LOQ values were 0.007 and 0.002 µg/L, respectively. Latorre et al. (2003) performed analysis of tert-octylphenol (t-OP), 4-NP and BPA in aquifer water by SPE-GC/MS and obtained recoveries between 89 and 94 %.

SPME is a technique developed in 1990 which uses a fibre coated with an extracting phase where compounds are attached for the posterior injection. SPME reduces the time necessary for sample preparation, decrease purchase and disposal costs of solvents and can improve detection limits (Coelho et al., 2008). In the last decade, SPME has been applied to the analysis of phthalates in water matrices due to its simplicity (Peñalver et al., 2000; Luks-Betlej et al., 2001; Polo et al., 2005). This technique requires a contact period to extract analytes which affects the extraction efficiency. For example, long periods and the use of salts, which changes the ionic strength, increase the amount of analyte extracted (Salafranca et al., 1999). Dévier et al., (2013) determined phthalates by SPME-GC/MS and detected phthalates in samples and in blanks at similar levels and demonstrated that the few detected compounds originated from the background laboratory contamination. Therefore, this study showed the complexity of reaching a reliable measure to qualify the contamination of a sample at ultra-trace level. SPME followed by GC-MS was also used for the determination of BPA and its derivate BADGE in different food simulants (distilled water, 3 % acid acetic and 10 % ethanol) (Salafranca et al., 1999). Nerín et al. (2002) used SPME for the analysis of BPA, bisphenol F (BPF) and derivates in aqueous foodstuffs with a previous derivatisation followed by HPLC. Luks-Betlej et al. (2011) analysed phthalates in drinking waters by SPME-GC/MS and obtained LODs between 0.005 and 0.04 µg/L. BP, naphthalene, BHT and 2,4-DTBP were analysed by SPME-GC/MS in recycled PET and recycled HDPE multilayer (Dutra et al., 2011) obtaining values of BHT and 2,4-DTBP above 320 ng/g HDPE.

SBSE technique was developed in 1999 by Baltussen et al. (Baltussen et al., 1999). It is based on the partitioning coefficient of substances diluted in an aqueous matrix. The principle is similar to SPME. However it has a greater surface of extraction than SPME and it allows having a higher sensibility. Stir bar, so called Twister® as the commercial name, is coated with polydimethylsiloxane (PDMS) which has a partitioning coefficient ($K_{PDMS/W}$) that is proportional to the octanol-water partitioning coefficient ($K_{o/w}$). PDMS used in SBSE showed high sample capacity, recovery and sensitivity improvement by a factor of 100–1000 compared

to SPME, decreasing the detection limits at the sub-ng/L level (Baltussen et al., 1999). SBSE technique is mainly used for the determination of semivolatile compounds in aqueous samples (Brossa et al., 2005). Additionally the use of organic solvents is drastically reduced in comparison to LLE and SPE (García-Falcón et al., 2004; Krüger et al., 2011). However, SBSE technique has the difficulty of extracting efficiently both hydrophobic and hydrophilic compounds (León et al., 2003; Sampedro et al., 2009). Both SPME and SBSE provide enhanced sensibility compared to traditional extraction procedures because the devices are introduced directly into the thermal desorption port without any losses (Sampedro et al., 2009). SBSE is also the technique with less handling and solvents are not necessary and its use is increasing for water analysis compared to other extraction procedures. The application to a variety of water samples makes this technique very useful. Some of samples applications are the analysis of river water and wastewater effluent (Chary et al., 2012), irrigation stream water (Peñalver et al., 2003), seawater and interstitial marine water (Pérez-Carrera et al., 2007), sea and estuarine waters (Prieto et al., 2007), groundwater (Tögyessy et al., 2011) and bottled water (Serôdio and Nogueira, 2004).

All these techniques allow the detection of micropollutants at very low concentrations. For this reason, it is necessary to ensure the correct analysis of target compounds in source water samples by calculating quality parameters such as limits of detection (LOD) and quantification (LOQ), recoveries and relative standard deviation (RSD). In addition, blank analysis is also used to avoid false positives. False positives in plastic components analysis is the result of phthalate contamination. Phthalates are widely used for the manufacturing of many items, hence they are present in air, water, organic solvents, adsorbed on glass and, of course, in plastic materials that could contaminate the samples (Capdeville and Budzinski, 2011; Munch et al., 1995). Therefore, it is important to minimise the risk of external contamination by using clean material, high quality water and organic solvents and a clean atmosphere. The use of HPLC or Milli-Q water blanks in parallel with the samples permit to know the possible contamination sources so they can be controlled or avoided. Nerín et al. (2003) observed contamination of DEHP in all of the analyses, which could be attributed to the contribution of plastic syringes, fittings, glass wool, and other common materials used in the laboratory. On the other hand, it is important to control the extraction efficiency. Using spiked water samples (e.g. HPLC or Milli-Q water) extracted and analysed together with blank and samples, losses and possible gains due to background contamination can be controlled.

1.9. Toxicological tests for endocrine disruptor compounds

Some monomers and additives are considered as potential endocrine disruptors (EDs) except for ATBC and BHT (EFSA, 2000). The World Health Organization (WHO)/ International Programme on Chemical Safety (IPCS) defined an ED as an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations (WHO/IPCS, 2002).

The endocrine system is a complex network of glands, hormones and receptors that are situated in various sites around the body. Its main function is to provide the key communication and control link between the nervous system and the functions of the body such as reproduction, immunity, metabolism and behaviour (EU, 2013). "Adverse health effects" is defined by WHO/IPCS as "adversity"; a change in morphology, physiology, growth, reproduction, development or lifespan of an organism which results in impairment of functional capacity or impairment of capacity to compensate for additional stress or increased susceptibility to the harmful effects of other environmental influences (IPCS, 2004).

Disruption of the endocrine system can occur in various ways: (i) some chemicals mimic a natural hormone, fooling the body into over-responding to the stimulus or responding at inappropriate times (agonistic effect); (ii) others block the effects of a hormone from certain receptors (antagonistic effect); (iii) some bind to transport proteins in the blood causing overproduction or underproduction of hormones; and (iv) still others interfere with the metabolic processes in the body, affecting the synthesis or breakdown rates of the natural hormones (EPA, 2011; EU, 2013).

Epidemiological studies in test animals indicate an increase of some kinds of cancer, behaviour changes and anomalies in the reproductive and immunologic functions in some species when exposed to EDs (Rivas et al., 1997). Possible human health endpoints affected by these agents include breast cancer and endometriosis in women, testicular and prostate cancers in men, abnormal sexual development, reduced male fertility, alteration in pituitary and thyroid gland functions, immune suppression, and neurobehavioral effects (EPA, 1997). For safety reasons, polymers used for packaging which are in contact with food must be analyzed before use to prevent migration of any of its components to the food at concentrations that may cause health effects (EU, 2002a).

Although there are many studies on the toxicity of EDs and their hormone response effects, it is difficult to establish a relationship between ingestion and adverse health effects as cancer (exposure-response relationship). Some EDs such as phthalates, BPA and alkylphenols are not persistent or they do not bioaccumulate in organisms, but they are constantly ingested along life and can produce effects long time after ingestion (lag time). Hence some authors consider them as pseudo-persistent. Furthermore, EDs can act at low doses. Traditionally, chemical testing focus doses ranging from 1 mg/kg bw (mg per kg of body weight) and upwards, but EDs could act at µg or ng/kg bw (EFSA, 2013a).

The 20th March 2013, EFSA published a Scientific Opinion on the hazard assessment of endocrine disruptors where a distinction between EDs and other groups of substances was done (EFSA, 2013b). EFSA Scientific Committee concluded that an ED is defined by three criteria: the presence of i) an adverse effect in an intact organism or a (sub)population; ii) an endocrine activity; and iii) a plausible causal relationship between the two.

There exists a large variety of tests that have been developed to detect endocrine disruptor activity of certain substances or samples. In general terms they are divided in *in vitro* and *in vitro* tests. Both kinds of tests separately have limitations so it is appropriate to use batteries of toxicological tests to ensure the endocrine disrupting activity of a substance or mixture. *In vitro* assays cannot reproduce the complex metabolic and kinetic interactions of a whole animal, but, opposite, in *in vivo* assays there may be a large variety of interactions without relation with ED activity (Baker, 2001).

1.9.1. *In vitro* assays

The main types of *in vitro* tests may be classified in (Baker, 2001):

- Receptor binding assays, which were developed to assess the ability of substances to bind directly to the hormone receptor. These assays have been widely used because they are easy to perform, rapid and relatively cheap, making them a good choice for large scale screening. However, they cannot distinguish between agonistic and antagonistic ED effects.
- Cell proliferation assays, which is one of the most widely used *in vitro* cell assays for the detection of oestrogenic compounds. In these *in vitro* systems, the ability of a test substance to stimulate the growth of oestrogen dependent cell lines

is measured (Soto et al., 1992; Soto et al., 1995). However, there are variations with interlaboratory due to variations in strains and culture conditions.

- Reporter gene assays analyse the ability of a substance to activate the transcription of a hormone sensitive promoter in eukaryotic cells (usually mammalian or yeast cells). They were developed in the Genetics Department at Glaxo for use in a test to identify compounds that can interact with the human estrogen receptor (hER). The principle is that the DNA sequence of a human receptor is integrated into the main chromosome of the yeast (Routledge and Sumpter, 1996). They may be used in several agonistic and antagonistic assays. Examples of the use of these reporter gene are the yeast estrogen screen (YES) for river water (Céspedes et al., 2005; Grover et al., 2011), for effluents from wastewater treatment plants (WWTP) (Li et al., 2010; Brix et al., 2010) and for bottled water (Wagner and Oehlmann, 2009; 2011); yeast androgen screen (YAS) for effluents from WWTP; yeast anti-androgen screen (YAAS) for river water (Grover et al., 2011); and retinoic acid receptor (RAR) for treated effluents from WWTP (Allinson et al., 2011).
- Other cell-based assays. There exist a large variety of other *in vitro* assays which been developed involving the use of diverse range of human and animal tissues/primary cultures and cell-free systems (ECETOC, 1996).

1.9.2. *In vivo* assays

There is a large range of *in vivo* bioassays (ECETOC, 1996). The most commonly mammalian species used are rats, mice, rabbits and dogs. The bioassays usually consist in the administration of the test substance for a determined period of time with an endocrine/reproductive endpoint (e.g. fertility and fecundity, abnormalities and malformations of foetuses, etc.). There also exist several environmental *in vivo* bioassays involving fishes, birds, reptiles and invertebrates (e.g. *Daphnia magna*).

The use of invertebrates as bioassays has been developed due to its sensitivity for EDCs (OECD, 2010). Molluscs are recommended as the most sensitive organisms for these substances. Among molluscs, there are the prosobranch snails which are important members of aquatic habitats and possess a high ecological relevance for marine and freshwater ecosystems. Their hormonal system is largely comparable to that of vertebrates (and humans), which makes them particularly qualified and promising test organisms for the identification of

endocrine disrupting chemicals (Duft et al., 2007). The principle of this *in vivo* bioassay is to expose adult female to a concentration range of a possible EDC for a specific period. After the exposure period, survival of the snails is determined and number of embryos, shelled and unshelled, is determined after shell removal and by opening the brood pounch (Department Aquatic Ecotoxicology, 2012; OECD, 2010).

2. ANÀLISI DE L'AIGUA ENVASADA AL MERCAT ESPANYOL

2. ANÀLISI DE L'AIGUA ENVASADA AL MERCAT ESPANYOL

2.1. Introducció

A Catalunya, Espanya i molts països europeus hi ha una tradició molt extesa de beure aigua envasada per les seves propietats minerals i organolèptiques. Per tant és necessari garantir la qualitat de les aigües subterrànies i el seu sistema de processat.

Actualment existeix una preocupació per part de la població pel progressiu augment en la quantitat d'espècies químiques que són incorporades a la dieta com additius alimentaris, fàrmacs o components de l'envàs. Degut al gran desenvolupament agrícola, industrial i urbà, les aigües subterrànies es converteixen en un medi especialment vulnerable. Els aqüífers poden contenir nivells elevats de compostos orgànics com a conseqüència de l'escassetat d'aigua i de la implantació progressiva de sistemes d'agricultura i ramaderia intensives, que a més a més d'utilitzar més aigua també la retornen més contaminada (mescla de fertilitzants, pesticides, additius industrials i fàrmacs). Malgrat el perímetre de seguretat implantat a tots els sistemes de captació d'aigües minerals, hi ha un risc de contaminació dels aqüífers que mai s'ha avaluat de forma rigorosa i sistemàtica.

Tanmateix, a part de la contaminació mediambiental, els processos d'envasat i emmagatzematge de les aigües poden ocasionar una disminució de llur qualitat com a conseqüència de la migració de components del plàstic utilitzats en les ampolles, taps i sèptums. La presència de components del plàstic a l'aigua a baixes concentracions pot provocar una modificació de les característiques organolèptiques de l'aigua i, degut a les seves propietats com a disruptors endocrins, poden produir efectes sobre la salut humana si els seus límits excedeixen el màxim permès o si no se'n controla llur presència.

Per aquesta raó, aquest estudi pretén investigar la presència de disruptors endocrins en aigües envasades des de la captació de l'aigua a l'aqüífer fins que aquesta és envasada, incloent els possibles tractaments fisicoquímics que pugui rebre l'aigua abans del seu envasat. Així mateix, es pretén avaluar la migració dels components del plàstic durant l'emmagatzemat de l'aigua. D'aquesta forma es vol contribuir a millorar les tècniques d'envasat i emmagatzematge que s'utilitzen en les plantes envasadores espanyoles per tal de garantir la qualitat de l'aigua i protegir la salut del consumidor.

Alguns dels compostos objecte d'aquest estudi estan inclosos en Directives Europees, mentre que d'altres es troben en procés de ser legislats. Com s'ha indicat a la introducció, els monòmers i additius són susceptibles de migrar a l'aigua envasada i, en el cas dels pesticides poden lixiviar a través del sòl. Per tant, cal avaluar la seva presència en les aigües subterrànies per a garantir les mesures de protecció dels aqüífers i així poder evitar possibles problemes de lixiviació d'aquests compostos cap als aqüífers, que podrien ser una font de contaminació de les aigües envasades.

Els compostos diana que s'han seleccionat ha estat els següents:

- Compostos alquilfenòlics: nonilfenol (NP) i octilfenol (OP).
- Compostos bisfenòlics: bisfenol A (BPA).
- Ftalats: dimetil, dietil, dibutil, butilbenzil, i di(2-etilhexil).
- Altres: di(3-etilhexil) adipat.
- Pesticides: triazines i cloroacetamides.

Aquest estudi tracta de realitzar un control de les aigües envasades d'Espanya més enllà dels paràmetres establerts a la legislació. S'avalua la presència de contaminants orgànics per poder determinar els orígens de la contaminació i proposar mesures per l'adequada conservació de la qualitat de l'aigua i, en el cas de les aigües minerals naturals, garantir la seva puresa original.

Per a poder realitzar aquesta avaluació s'ha seguit els següents passos:

(a) Presa de mostra de les aigües de captació d'un total de 131 brolladors arreu d'Espanya. El mostreig va ser realitzat a la sortida del pou o en el punt d'emergència de la deu, segons el tipus de punt de captació.

(b) Anàlisi de les aigües envasades corresponents a cadascuna de les aigües de captació. En el mateix dia del mostreig del punt anterior, es van agafar envasos comercials omplerts amb l'aigua de captació per a cada deu corresponent al punt anterior. En el cas de les aigües minerals naturals i les aigües de brollador no hi ha canvis respecte l'aigua de captació ja que no s'hi fan tractaments i per tant mantenen la seva composició. En aquest pas també es van afegir tres aigües envasades tractades de les quals no es disposa de la corresponent aigua de captació.

Els tipus d'envasos que es van estudiar van ser els que s'indiquen a continuació i que corresponen als envasos més comunament utilitzats a Espanya:

- Ampolla de PET amb tap de polietilè d'alta densitat (HDPE).
- Ampolla de HDPE amb tap de HDPE.
- Ampolla de vidre amb tap metàl·lic de tipus corona amb sèptum de plàstic.
- Ampolla de vidre amb tap metàl·lic de tipus roscat amb sèptum de plàstic.
- Ampolla de PC amb tap de polietilè de baixa densitat (LDPE) i sèptum que generalment és de poliestirè (PS) (tots els casos analitzats tenien aquests tipus de sèptum).
- Bossa de LDPE.

Pel què fa als envasos, cal destacar que tots els envasos de material polimèric, a excepció dels garrafons de PC, són fabricats en càmeres netes a partir de preformes just abans de l'envasat, mentre que els taps de plàstic els fabrica el propi proveïdor de taps. El cas dels garrafons de PC és molt similar al de les ampolles de vidre, per ambdós casos es tracta d'envasos reutilitzables i que un cop buits i retornats a l'envasadora són rentats diverses vegades amb aigua i detergent, generalment amb sosa addicionada, i finalment reomplerts amb l'aigua envasada.

Un cop l'aigua era envasada, es va realitzar l'anàlisi de tots els tipus de format d'envàs possibles per a cadascuna de les aigües de captació, des de 0.150 L fins a 20 L, i així poder determinar la contaminació associada als processos d'envasat. De la mateixa manera, també es va avaluar si l'addició de gas afavoria la presència de disruptors endocrins.

(c) Anàlisi de les mostres després d'un any d'emmagatzematge. Al moment d'agafar la mostra envasada per a l'anàlisi de l'aigua recent envasada també es va agafar una altra mostra idèntica per a guardar-la durant un any i realitzar l'anàlisi posterior. D'aquest forma es pot veure l'evolució de la migració de les concentracions dels components del plàstic després d'un llarg temps en contacte amb el plàstic.

L'anàlisi de les aigües en el punt de captació, de l'aigua envasada recent i de l'emmagatzemada un any permet determinar els punts on pot haver-hi una contaminació de l'aigua (captació, procés d'envasat i emmagatzematge) i per tal de fer una avaluació real del processos de migració dels components del plàstic dels diferents tipus d'envàs. Aquest estudi està descrit en dos articles científics; el

primer fa referència a l'aigua de captació i el segon fa una comparativa entre l'aigua envasada recent i l'emmagatzemada durant un any.

Per altra banda, el mètode utilitzat en els dos estudis també es va desenvolupar per a determinar herbicides que podrien provenir de la contaminació de l'aigua subterrània. Els herbicides analitzats pertanyen al grup de les cloroacetamides i les triazines. Ambdós grups de compostos poden lixiviar a través del sòl i arribar a les aigües subterrànies i per tant podrien trobar-se en aigua destinada al consum humà (WHO, 2011).

2.2. Treball experimental

El treball experimental d'aquest capítol consta de dos articles científics que s'ajunten a continuació. L'article científic I descriu l'optimització i validació d'un mètode d'extracció en fase sòlida (SPE) acoblat a cromatografia de gasos amb detector d'espectrometria de masses (GC-MS) i l'anàlisi de la majoria d'aigües de captació de l'Estat Espanyol pel què fa a la determinació de plastificants, additius i herbicides. L'article científic II descriu l'anàlisi de les aigües envasades en els formats d'envàs de cada casa comercial que hi ha al mercat per a cadascuna de les aigües descrites a l'article científic I. En ambdós treballs, el mètode d'anàlisi és el mateix.

La optimització del mètode SPE va consistir en determinar els cartutxos més adequats, i es van provar cartutxos de C18 i els polimèrics Oasis HLB. Es va obtenir millor recuperació amb els cartutxos Oasis, ja que amb els de C18 la recuperació dels ftalats era excessiva. També es va optimitzar el dissolvent d'elució, i mentre que el metanol donava recuperacions del 60-75%, l'elució d'una mescla de diclormetà i hexà (1:1; v/v) i una mescla de diclormetà i acetona (1:1; v/v) proporcionava recuperacions entre el 77 i el 117 %.

Malgrat que el mètode d'extracció va ser eficaç, el problema real de l'anàlisi de ftalats recau en la seva presència en els blancs. Els ftalats es troben a l'atmosfera, al material de vidre i al material de laboratori, fins i tot en el sistema d'injecció del GC. Per evitar o intentar controlar la contribució d'aquests compostos en els blancs, es van prendre mesures tant en la presa de mostra com en el procés analític. El material de presa de mostra va consistir en l'ús d'envasos nous, que es van rentar amb aigua Milli-Q, es van posar 10 minuts en ultrasons, es tornaven a rentar amb aigua Milli-Q seguit d'acetona i finalment es muflaven a 370 ºC per

eliminar qualsevol traça de contaminació orgànica. Un cop al lloc del mostreig, l'envàs de vidre es va rentar amb la pròpia aigua de captació i finalment es va omplir amb la mostra. Per evitar contaminacions pel contacte amb el tap, el sèptum utilitzat en els taps de les ampolles de mostreig era de tefló.

Durant el procés d'extracció, cal mantenir la zona de treball excepcionalment neta (campana i lleixa de treball netejada amb acetona), així com el laboratori en general, ja que durant l'extracció en fase sòlida les mostres es poden contaminar degut a la presència de ftalats a l'aire del laboratori. Finalment, s'intenta eluir amb un volum petit de dissolvents, ja que s'ha comprovat que aquests, en ser preconcentrats, contribueixen a la presència de ftalats en els blancs. D'aquesta manera, no s'obté mai un blanc "net" però si que es pot controlar la presència de ftalats en els blancs. La contribució dels ftalats en els blancs serveix per a calcular els límits de detecció, ja que instrumentalment els nivells són molt baixos però metodològicament, els límits de detecció són alts respecte altres compostos.

La determinació del conjunt de ftalats i plaquicides es va realitzar amb un mètode d'anàlisi basat en la cromatografia de gasos acoblada a l'espectrometria de masses (GC-MS). La tècnica de GC utilitza d'una rampa de temperatures (70ºC (2 min), 10ºC/min fins a 135ºC, 3ºC/min fins a 160ºC, 1ºC/min fins a 175ºC, 3ºC/min fins a 195ºC, 10ºC/min fins a 310ºC (5 min)) que permet una bona separació dels pics cromatogràfics sense obtenir cap coelució i, que conjuntament amb el detector de MS, permet assignar unes masses/càrrega (m/z) característiques per a cada compost estudiat (Taula 5).

Es van utilitzar patrons interns en cada mostra per a determinar l'eficàcia d'extrtacció i per a avaluar que no es perd sensibilitat al llarg de la seqüència cromatogràfica.

Taula 5. Massa molecular (M), temps de retenció (t_R), ions obtinguts per MS i la seva corresponent abundància i assginació molecular dels compostos estudiats.

Compost	t_R (min)	Ió	Assignació
Dimetil ftalat (DMP) M=194.2	12.98	163*(100%)	$[M\text{-}OCH_3]^+$
		135(7%)	$[M\text{-}CO_2CH_3]^+$
		77(21%)	$[C_6H_5]^+$
Dietil ftalat (DEP) M=222.1	16.54	149*(100%)	$[M\text{-}(OC_2H_5 + C_2H_4)]^+$
		177(21%)	$[M\text{-}OC_2H_5]^+$
		105(10%)	$[M\text{-}(C_2H_3O_2 + C_2H_2O_2)]^+$
Octilfenol (OP) M=206.3	16.79	135*(100%)	$[M\text{-}C_5H_{11})]^+$
		107(17%)	$[C_7H_7O]^+$
Atrazine desisopropyl-d_5 M=178.6	18.04	160*(100%)	$[M\text{-}CD_3]^+$
		178(98%)	$[M]^+$
		145(45%)	$[M\text{-}(C_2D_4 + H)]^{\cdot+}$
Desisopropil atrazina M=173.6	18.14	173*(100%)	$[M]^+$
		158(98%)	$[M\text{-}CH_3]^+$
		145(45%)	$[M\text{-}C_2H_4]^{\cdot+}$
Desetil atrazina-d_6 M=193.7	18.40	175*(100%)	$[M\text{-}CD_3]^+$
		177(42%)	$[M\text{-}CD_2]^+$
Desetil atrazina M=187.6	18.54	172*(100%)	$[M\text{-}CH_3]^+$
		174(34%)	$[M\text{-}CH]^+$
		145(26%)	$[M\text{-}C_3H_6]^+$
Nonilfenol (NP) M=220.4	19.97-21.74	135*(100%)	$[M\text{-}C_5H_{13}]^+$
		149(52%)	$[M\text{-}C_3H_8]^+$
		107(62%)	$[C_7H_7O]^+$
Simazina-d_5 M=206.7	21.15	206*(100%)	$[M]^+$
		178(46%)	$[M\text{-}C_2H_4]^{\cdot+}$
		188(46%)	$[M\text{-}CD_3]^+$
Simazina M=201.7	21.30	201*(100%)	$[M]^+$
		186(69%)	$[M\text{-}CH_3]^+$
		173(53%)	$[M\text{-}C_2H_4]^+$
Atrazina-d_5 M=220.1	21.62	205*(100%)	$[M\text{-}CH_3]^+$
		220(59%)	$[M]^+$
		178(43%)	$[M\text{-}C_3H_6]^{\cdot+}$

Taula 5. Continuació.

Compost	t_R (min)	Ió	Assignació
Atrazina M=215.1	21.77	200*(100%) 215(56%) 173(35%)	$[M-CH_3]^+$ $[M]^+$ $[M-C_3H_6]^{\cdot+}$
Propazina M229.7	22.15	214*(100%) 172(69%) 229(63%)	$[M-CH_3]^+$ $[M-C_4H_9]^+$ $[M]^+$
Di-n-propil ftalat-d_4 (DPP-d_4) M=254.3	22.62	153*(100%) 195(7%)	$[C_8HD_4O_3]^+$ $[M-C_3H_7O]^+$
Antracè-d_{10} M=188.3	22.79	188*(100%) 184(17%)	$[M]^+$
Terbutilazina M=229.7	22.90	214*(100%) 229(31%) 173(51%)	$[M-CH_3]$ $[M]^+$ $[M-C_4H_8]^{\cdot+}$
Secbutilazina M=229.7	25.62	200*(100%) 214(15%) 229(14%)	$[M-C_2H_5]^+$ $[M-CH_3]^+$ $[M]^+$
Nonilfenol-d_8 (NP-d_8) M=228.4	26.87	113*(100%) 112(93%) 228(48%)	$[C_7HD_6O]^+$ $[C_7H_2D_5O]^+$ $[M]^+$
Alaclor-d_{13} M=282.8	28.45	156*(100%) 170(46%)	$[C_{10}H_2D_{10}N]^+$ $[C_{11}H_4D_{10}N]^+$
Alaclor M=269.8	28.95	160*(100%) 188(77%) 146(61%)	$[C_{11}H_{14}N]^+$ $[C_{12}H_{14}NO]^+$ $[C_{10}H_{12}N]^+$
Prometrin M=241.4	29.80	184* (100%) 241(88%) 226(58%)	$[M-(C_3H_6 + CH_3O)]^+$ $[M]^+$ $[M-CH_3]^+$
Terbutrin M=241.4	31.02	226*(100%) 185(94%) 170(74%)	$[M-CH_3]^+$ $[M-C_4H_8]^+$ $[M-(CH_3 + C_4H_8)]^+$

Taula 5. Continuació.

Compost	t_R (min)	Ió	Assignació
Dibutil ftalat (DBP) M=278.3	32.10	149*(100%) 150(10%) 223(4%)	$[C_8H_5O_3]^+$ $[C_8H_6O_3]^+$ $[M-C_4H_7]^+$
Metolaclor M=283.8	32.84	162*(100%) 238(36%) 146(14%)	$[M-C_4H_6O_2Cl]^+$ ó $[M-C_5H_{10}OCl]^+$ $[M-C_2H_5O]^+$ $[M-C_5H_{10}O_2Cl]^+$
Cianazina M=240.7	33.95	225*(100%) 198(82%) 174(51%)	$[M-CH_3]^+$ $[M-C_2H_4N]^+$ $[C_5H_9ClN_5]^+$
Bisfenol A-d_{16} (BPA-d_{16}) M=244.4	40.62	224*(100%) 242(68%)	$[M-(CD_3 + D)]^+$ $[M-D]^+$
Bisfenol A (BPA) M=228.3	40.75	213*(100%) 228(53%) 119(70%)	$[M-CH_3]^+$ $[M]^+$ $[C_8H_7O]^+$
Butil benzil ftalat (BBP) M=312.4	43.75	149*(100%) 91(62%) 206(25%)	$[C_8H_5O_3]^+$ $[C_7H_7]^+$ $[M-C_7H_6O]^+$
Dietilhexil adipat (DEHA) M=370.6	44.44	129*(100%) 112(27%) 147(21%)	$[C_6H_9O_3]^+$ $[C_6H_8O_2]^+$ $[C_6H_{11}O]_4^+$
Dietilhexil ftalat (DEHP) M=390.6	46.01	149*(100%) 167(36%) 279(7%)	$[C_8H_5O_3]^+$ $[C_8H_7O_4]^+$ $[M-C_8H_{15}]^+$

M: pes molecular

* Ió de quantificació

Article científic I

Títol: Survey of phthalates, alkylphenols, bisphenol A and herbicides in Spanish source waters intended for bottling

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RESEARCH ARTICLE

Survey of phthalates, alkylphenols, bisphenol A and herbicides in Spanish source waters intended for bottling

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Abstract
Background, aim and scope Groundwaters and source waters are exposed to environmental pollution due to agricultural and industrial activities that can enhance the leaching of organic contaminants. Pesticides are among the most widely studied compounds in groundwater, but little information is available on the presence of phthalates, alkylphenols and bisphenol A. These compounds are used in pesticide formulations and represent an emerging family of contaminants due to their widespread environmental presence and endocrine-disrupting properties. Knowledge on the occurrence of contaminants in source waters intended for bottling is important for sanitary and regulatory purposes. So the aim of the present study was to evaluate the presence of phthalates, alkylphenols, triazines, chloroacetamides and bisphenol A throughout 131 Spanish water sources intended for bottling. Waters studied were spring waters and boreholes which have a protection diameter to minimize environmental contamination.
Materials and methods Waters were solid-phase extracted (SPE) and analysed by gas chromatography coupled to mass spectrometry (GC-MS). Quality control analysis comprising recovery studies, blank analysis and limits of detection were performed.
Results and discussion Using SPE and GC-MS, the 21 target compounds were satisfactorily recovered (77–124 %) and limits of quantification were between 0.0004 and 0.029 μg/L for pesticides, while for alkylphenols, bisphenol A and phthalates the limits of quantification were from 0.0018 μg/L for octylphenol to 0.970 μg/L for bis(2-ethylhexyl) phthalate. Among the 21 compounds analysed, only 9 were detected at levels between 0.002 and 1.115 μg/L. Compounds identified were triazine herbicides, alkylphenols, bisphenol A and two phthalates. Spring waters or shallow boreholes were the sites more vulnerable to contaminants. Eighty-five percent of the samples did not contain any of the target compounds.
Conclusions Target compounds were detected in a very low concentration and only in very few samples. This indicates the good quality of source waters intended for bottling and the effectiveness of the protection measures adopted in Spain. None of the samples analysed exceeded the maximum legislated levels for drinking water both in Spain and in the European Union.

Keywords Pesticides · Phthalates · Alkylphenols · Bisphenol A · Source waters · Gas chromatography

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1 Background, aim and scope

Groundwaters have a special interest because they often constitute a main drinking water source, either directly treated or for the bottling industry. Preserving groundwater quality is of vital importance to ensure the original water purity. In areas with high urban, industrial and agricultural

Survey of phthalates, alkylphenols, bisphenol A and herbicides in Spanish source waters intended for bottling

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Materials and methods Waters were solid phase extracted (SPE) and analyzed by gas chromatography coupled to mass spectrometry (GC-MS). Because of the complexity of analyzing phthalates, quality control analysis comprising recovery studies, blank analysis and limits of detection were performed.

Results and discussion Using SPE and GC-MS, the 21 target compounds were satisfactorily recovered (73-120%) and limits of quantification were between

0.0004 to 0.029 μg/L for pesticides, while for alkylphenols, bisphenol A and phthalates the limits of quantification were from 0.0018 μg/L for octylphenol to 0.970 μg/L for bis(2-ethylhexyl) phthalate. Among the 21 compounds analyzed, only 9 were detected at levels between 0.002 μg/L and 1.115 μg/L. Compounds identified were triazine herbicides, alkylphenols, bisphenol A, and two phthalates. Spring waters or shallow boreholes were the sites more vulnerable to contaminants. Eighty-five percent of the samples did no contain any of the target compounds.

Conclusions Target compounds were detected in a very low concentration and only in very few samples. This indicates the good quality of source waters intended for bottling and the effectiveness of the protection measures adopted in Spain. None of the samples analyzed exceeded the maximum legislated levels for drinking water both in Spain and in the European Union.

Keywords: pesticides; phthalates; alkylphenols; bisphenol A; source waters; gas chromatography.

1. Background, aim and scope

Groundwaters have a special interest because they often constitute a main drinking water source, either directly treated or for the bottling industry. Preserving groundwater quality is of vital importance to ensure the original water purity. In areas with high urban, industrial and agricultural pressure, groundwater is exposed to the presence of organic contaminants due to transport through soils (Flury 1996) and leaching to the aquifer, increasing its vulnerability (Worrall et al. 2002). Herbicides (Barbash et al. 2001; Gonçalves et al. 2007; Kolpin et al. 2002; Tappe et al. 2002) and pesticide coadjuvants [phthalates, bisphenol A (BPA) and alkylphenols] have been detected in groundwater as a result of their recurrent use in agriculture and to the application of sludge as organic fertilizer (Latorre et al. 2003; Hildebrandt et al. 2007). Phthalates, bisphenol A and alkylphenols have also been detected in groundwaters of industrial areas and in sew- age dumps (Fromme et al. 2002; Casajuana and Lacorte 2003; Ying et al. 2003). In addition, these compounds have been identified in bottled water (Leivadara et al. 2008; Li et al. 2010) but especially as a result of migra- tion from poor transport and storage conditions (Bach et al. 2012; Gallart-Ayala et al. 2011; Diduch et al. 2011). The

USA determined that 81 % of the 47 nation's water resources were contaminated, and bisphenol A (30 % of the samples) and 4-octylphenol (OP) monoethoxylate (19 % of the samples) were amongst the most frequent compounds detected (Barnes et al. 2008). In Europe, N,N- diethyl-meta-toluamide, caffeine, perfluorinated compounds, herbicides, carbamazepine , nonylphenoxy acetic acid (NPE1C) and bisphenol A were frequently detected in groundwaters at levels from few nanograms per litre to micrograms per litre (Loos et al. 2010). Because of the carcinogenic properties of triazines or the endocrine-disrupting properties of alkylphenols, phthalates and bisphenol A (Waring and Harris 2005), ground and drinking waters have to meet the legislated requirements for being consumed according to Directive 2009/54/EC (European Communities 2009) and Royal Decree 1798/2010 (Spanish Government 2010), and the organoleptic properties must be preserved to ensure their quality.

In the European bottle water market, there are three types of water: natural mineral water, spring water and treated water. Natural mineral water is packaged without any treatment as it springs out from the source. For this reason, precautions must be taken in the catchment to preserve the "original water purity" of the source of mineral water. In Spain, there are more than 150 water sources used in the water bottling indus- try, mostly located in remote sites far away from agricultural, industrial or urban areas. These areas have a protection diam- eter to minimize environmental contamination. However, aquifers are not static waters and may receive the pollution impact of nearby areas. While there is ample information on the presence of organic contaminants in groundwaters located in agricultural and industrial areas (Carabias-Martínez et al. 2000; Latorre et al. 2003; Hildebrand et al. 2007), there is a lack of information on the occurrence of contaminants in source waters intended for bottling.

The main objective of the present study was to deter- mine the presence of herbicides, phthalates, alkylphenols and bisphenol A in 131 source waters located throughout Spain. Because of the low levels expected in protected groundwaters, a gas chromatography coupled to mass spectrometry (GC-MS)-based method was optimized for the trace level determination of pesticides, phthalates, alkylphenols and bisphenol A in source waters intended for bottling. These compounds have been chosen due to their toxicity and ample environmental distribution, and because they are either legislated or represent emerging contaminants. Participating bottling companies are inter- ested in assessing that the protection limits adopted in each source are effective and water quality is not affected by environmental contamination.

2. Materials and Methods

2.1 Chemicals and Reagents

Phthalate Mix 525 (500 ng/µL each in methanol) con- taining dimethyl phthalate (DMP), diethyl phthalate (DEP), di-n-butyl phthalate (DBP), butyl benzyl phtha- late (BBP), bis(2-ethylhexyl) adipate (DEHA) and bis(2-eth- ylhexyl) phthalate (DEHP) was from Supelco (Bellefonte, PA, USA). Pesticide Mix 51 (10 ng/µL each in cyclohexane) containing atrazine, atrazine desethyl, atrazine desisopropyl, cyanazine, prometryn, propazine, sebuthylazine, simazine, terbuthylazine and terbutryn was purchased from Dr. Ehren- storfer (Augsbury, Germany). The chloroacetamides ala- chlor and metolachlor were from Riedel-de Häen (Seelzy, Germany) as solids. 4-Nonylphenol (NP) was from Riedel-de Häen (Seelzy, Germany) as a solid technical mixture of iso- mers. Bisphenol A was from Dr. Ehrenstorfer (Augsburg, Germany) as a solid and 4-tert-octylphenol from Supelco (Bellefonte, PA, USA) as a solid. The list of compounds analysed and their physico-chemical properties are indicated in Table 1.

Table 1. Properties of target compounds. Sources: SRC. Inc 201

Compound	CAS number	Molecular weight (g/mol)	Boiling point (°C)	Water solubility (mg/L)	Vapour pressure (mPa)	log K_{ow}	Henry's law (atm·m^3/mol)	Half-life from model river (avg, days)	Aerobic soil half-life (avg, days)	Adsorption coefficient log K_{oc}	GUS[a]
DMP	131-11-3	194.2	283.7	4,000	410.6	1.60	1.97E−07	173	30	1.68	3.43
DEP	84-66-2	222.2	295	1,080	280.0	2.42	3.94E−07	60	30	2.13	2.76
DBP	84-74-2	278.3	340	11.2	2.700	4.50	1.22E−06	23	17	3.28	0.88
BBP	85-68-7	312.4	370	2.69	1.100	4.73	4.22E−08	34	30	3.41	0.87
DEHA	103-23-1	370.6	417	0.78	0.1133	6.11[b]	5.16E−05	108	17	5.29	−1.58
DEHP	117-81-7	390.6	384	0.27	0.0189	7.60	1.18E−05	179	30	5.00	−1.47
OP	140-66-9	206.3	281.15[b]	4.821[b]	63.73	5.28[b]	4.50E−06	5	75	4.01	−0.02
NP	84852-15-3	220.4	295	5,000	12.56	5.77[b]	4.30E−06	8	75	4.28	−0.53
Bisphenol A	80-05-7	228.3	363.54[b]	120	0.0303	3.32	9.16E−12	75	75	3.10	1.70
Atrazine desisopropyl	1007-28-9	173.6	304.59[b]	670	28.13	1.15	1.16E−09	76	120	1.37	5.46
Atrazine desethyl	6190-65-4	187.6	310.23[b]	3,200	12.44	1.51	1.53E−09	60	120	1.57	5.05
Simazine	122-34-9	201.7	307.45[b]	6.2	0.0029	2.18	3.37E−09	28	110	2.53	3.00
Atrazine	1912-24-9	215.7	313.03[b]	34.7	0.0385	2.61	4.47E−09	30	146	1.99	4.40
Propazine	139-40-2	229.7	318.46[b]	8.60	0.0175	2.93	5.94E−09	22	120	2.34	3.46
Terbuthylazine	5915-41-3	229.7	321.23[b]	8.5	0.1493	3.21	3.94E−09	3	120	2.49	3.14
Sebuthylazine	7286-69-3	229.7	326.44[b]	45.6[b]	6.799	3.31[b]	5.94E−09	17	120	2.55	3.02
Prometryn	7287-19-6	241.4	346.68[b]	33	0.1653	3.73	9.09E−09	28	274	2.44	3.80
Terbutryn	886-50-0	241.4	349.08[b]	25	0.2253	3.74	9.09E−09	5	70	3.75	0.46
Cyanazine	21725-46-2	240.7	369.47[b]	170	0.2027	2.22	1.86E−12	3680	15	2.27	2.03
Alachlor	15972-60-8	269.8	378.17[b]	240	2.733	3.52	2.23E−08	30	20	2.12	2.45
Metolachlor	51218-45-2	283.8	382.78[b]	530	4.186	3.13	1.49E−09	200	26	2.28	2.44

Sources: SRC Inc. (2011)

[a] Groundwater ubiquity score—GUS= log $t_{1/2}$·(4−log K_{oc})

Phthalates surrogate standard was dipropylphthalate-3,4,5,6-d4 from Riedel-de Haën (Seelze, Germany), pur- chased as a solid. Surrogate standards for triazines were purchased from Dr. Ehrenstorfer (Augsbury, Germany) as a solution at 100 ng/µL. These surrogate standards were atrazine desisopropyl-d5 for atrazine desisopropyl quantifi- cation; desethyl atrazine-d6 for desethyl atrazine quantifica- tion; simazine-d5 for simazine quantification; atrazine-d5 for atrazine, propazine and terbuthylazine quantification and alachlor-d13 for prometryn, terbutryn, cyanazine, meto- lachlor and alachlor quantification. Alkylphenol surrogate standard was 4-n-nonylphenol-d8 from Dr. Ehrenstorfer (Augsburg, Germany) as a solution at 100 ng/µL in acetone, bisphenol A surrogate was bisphenol A-d16 from Sigma Aldrich (St. Louis, MO, USA) as a solid and anthracene d10 used as internal standard, from Dr. Ehrenstrofer as a solution of 10 ng/µL in cyclohexane.

Solid-phase extraction (SPE) cartridges Oasis HLB 200 mg sorbent in 6-mL syringe were from Waters, USA. GC-grade hexane, dichloromethane, methanol, acetone, ethyl acetate and HPLC-grade water were purchased from Merck (Darmstadt, Germany). Nitrogen for drying with 99.995 % of purity was from Air Liquid (Barcelona, Spain).

2.2. Sampling

Samples were collected directly from 131 water sources corresponding to 40 springs and 91 boreholes, distributed all over Spain. All these waters have the designation of natural mineral water or spring water. Table 2 lists the samples studied. Samples collected from the boreholes had depths between 24 and 400 m. At the moment of sampling, temperature values were between 4.1 and 57.2 °C. Temperature was more affected by the weather in springs than in boreholes (due to the depth). The warmest springs (>25 °C) correspond to very deep waters or areas of geothermic anomalies associated to fractures. When necessary and previous to bottling, these waters are kept in tanks until temperature has decreased. Conductivity was tested in order to demonstrate the stability of that aquifer, comparing with the historical data of the laboratory Dr. Oliver-Rodés. Conductivity values were between 29 and 4,800 µS/cm. In fact, one of the characteristics of natural mineral waters is their stable composition through time as regards to stable tempera- ture and conductivity. In those samples, total organic carbon values were always <1 mg/L.

Table 2. General characteristics of the 131 sources studied: regions of Spain, number and type of source water analyzed, temperature (T, ºC) and conductivity (µS/cm).

Region	Sources	Source no.	Source type	T (°C)	Conductivity (µS/cm)	Region	Sources	Source no.	Source type	T (°C)	Conductivity (µS/cm)
Andalucía	4	1	Spring	18.3	522	Castilla La Mancha	9	67	Borehole	20.5	428
		2	Borehole	14.4	338			68	Borehole	22.5	403
		3	Borehole	15.9	383			69	Borehole	21.5	379
		4	Spring	15.2	447			70	Borehole	22.2	362
Aragón	9	5	Borehole	29.4	796			71	Borehole	18.1	503
		6	Borehole	26.5	828			72	Borehole	22.8	348
		7	Borehole	8.4	199			73	Borehole	20.6	545
		8	Spring	29.4	103			74	Borehole	24.8	245
		9	Spring	7.4	205			75	Borehole	24.6	230
		10	Spring	9.3	305	Valencia	10	76	Borehole	14.3	503
		11	Borehole	10.3	327			77	Spring	13.2	355
		12	Spring	31.7	806			78	Borehole	19.2	404
		13	Borehole	10.5	48			79	Spring	11.0	414
Asturias—Cantabria	4	14	Spring	14.6	217			80	Spring	12.4	266
		15	Spring	29.8	841			81	Borehole	17.0	534
		16	Spring	13.5	29			82	Borehole	16.8	615
		17	Spring	11.0	338			83	Borehole	18.4	593
Castilla y León—Madrid	15	18	Borehole	14.0	403			84	Borehole	17.9	471
		19	Borehole	9.5	442			85	Borehole	19.4	751
		20	Spring	25.3	204	Extremadura	7	86	Borehole	23.1	61
		21	Spring	19.7	425			87	Borehole	20.3	116
		22	Borehole	13.4	354			88	Borehole	20.7	104
		23	Borehole	13.2	298			89	Borehole	17	577
		24	Borehole	11.1	381			90	Spring	NM	NM
		25	Borehole	11.9	399			91	Borehole	NM	NM
		26	Borehole	11.7	385			92	Borehole	NM	NM
		27	Spring	14.6	294	Galicia	13	93	Borehole	19.0	2,580
		28	Borehole	13.2	79			94	Borehole	16.8	141
		29	Borehole	14.8	308			95	Borehole	12.9	286
		30	Borehole	15.3	329			96	Borehole	18.6	2,150
		31	Spring	14.6	44			97	Borehole	13.8	57
		32	Borehole	NM	NM			98	Borehole	13.0	223
Catalunya	34	33	Borehole	17.6	2,580			99	Borehole	13.6	278
		34	Borehole	17.3	2,420			100	Borehole	15.7	129
		35	Spring	12.0	362			101	Borehole	13.8	306
		36	Spring	10.6	293			102	Borehole	14.2	125
		37	Borehole	14.0	505			103	Borehole	21.4	530
		38	Borehole	17.1	456			104	Borehole	20.8	451
		39	Spring	4.1	301			105	Borehole	14.3	432
		40	Spring	54.2	4,240	Balearic islands	7	106	Spring	14.8	805
		41	Spring	57.2	4,240			107	Spring	12.7	308
		42	Borehole	16.5	205			108	Spring	16.7	620
		43	Borehole	19.8	225			109	Spring	15.4	574
		44	Borehole	19.2	226			110	Spring	12.8	339
		45	Borehole	14.4	190			111	Spring	9.0	483
		46	Borehole	14.6	264			112	Spring	12.1	428
		47	Borehole	12.2	173	Canary Islands	13	113	Spring	14.7	84
		48	Borehole	13.0	253			114	Borehole	22.1	815
		49	Spring	33.4	270			115	Borehole	24.7	609

Table 2. Continued

Region	Sources	Source no.	Source type	T (°C)	Conductivity (µS/cm)	Region	Sources	Source no.	Source type	T (°C)	Conductivity (µS/cm)
		50	Borehole	15.1	268			116	Borehole	17.3	247
		51	Borehole	12.4	192			117	Borehole	16.6	237
		52	Borehole	15.2	201			118	Borehole	18.0	198
		53	Borehole	13.8	196			119	Borehole	19.6	279
		54	Borehole	14.5	297			120	Spring	20.1	467
		55	Borehole	14.5	236			121	Borehole	23.9	314
		56	Borehole	12.5	186			122	Spring	NM	189
		57	Borehole	19.7	440			123	Borehole	19.5	278
		58	Borehole	16.4	354			124	Borehole	15.0	243
		59	Spring	NM	NM			125	Spring	17.2	175
		60	Spring	9.3	1,507	Basque country—La Rioja	6	126	Spring	28.2	499
		61	Borehole	12.8	225			127	Borehole	15.6	656
		62	Borehole	11.8	236			128	Borehole	15.9	673
		63	Borehole	12.3	246			129	Spring	14.3	744
		64	Borehole	13.2	279			130	Spring	NM	NM
		65	Borehole	15.9	3,050			131	Borehole	NM	NM
		66	Spring	13.2	250						

NM not measured

In each Spanish water bottling industry, sampling was carried out by Laboratorio Dr. Oliver Rodés technical staff from 2007 to 2008. One litre of the sample was collected using a new amber glass bottle rinsed with acetone and baked at 100 °C, and capped with a Teflon septum cap. Before sampling, bottles were rinsed with the same water sample. Waters were stored at room temperature until anal- ysis, which was performed within 15 days.

2.3. Solid-phase extraction

Samples were analysed unfiltered. One litre of water was spiked with 10 µL of 5 ng/µL surrogate solution and afterwards was solid-phase extracted using Oasis HLB 200 mg sor- bent in 6-mL syringe cartridges set in a Baker SPE-12G apparatus, prod. no. 7018-94 (J.T. Baker, The Nether- lands). The cartridges were conditioned successively with 10 mL of hexane, 10 mL of dichloromethane, 10 mL of methanol and 15 mL of HPLC-grade water. This exten- sive conditioning of the cartridges was needed to ensure a complete elimination of any interfering compounds, especially phthalates. Water samples were loaded at 8–13 mL/min. After sample loading, cartridges were dried by vacuum system for approximately 60 min, and then eluted successively with 10 mL of a mixture of dichloro- methane and hexane (1:1, v/v) at 1 mL/min and 10 mL of a mixture of acetone and dichloromethane (1:1, v/v) at 1 mL/min, dropwise. The extracts were collected in a 40- mL vial and evaporated until almost dryness in a Turbo

Vap LV system (Caliper Life Sciences, UK). The extracts were transferred into a 1.5-mL vial using a small amount of ethyl acetate and evaporated to dryness under a nitrogen current in a Reacti-Vap III (Pierce, USA) system. At this point, the samples were reconstituted with 250 µL (240 µL of ethyl acetate and 10 µL of 10 ng/µL deuterated internal standard anthracene-d10).

2.4. Gas chromatography coupled to mass spectrometric analysis

Analysis was performed by gas chromatography coupled to a quadrupole mass spectrometer in a Thermo Electron Corporation (San José, CA, USA) Trace GC 2000 in- strument. Electron ionization was performed at 70 eV. The carrier gas used was helium with constant flow at 1.2 mL/min. A fused silica column DB-5MS (30 m × 0.25 mm × 0.25 µm) from J&W Scientific (Folsom, CA, USA) connected to a deactivated guard column was used with the following GC oven programme: 70 °C (2 min), 10 °C/min to 135 °C, 3 °C/min to 160 °C, 1 °C/min to 175 °C, 3 °C/min to 195 °C and 10 °C/min to 310 °C (5 min). The total analysis time was 55 min. The injection volume was 2 µL in splitless mode with splitless time of 1 min. Injector, GC interface temperature and ion source temperatures were 280, 280 and 200 °C, respectively. The detector voltage was set at 420 V. The samples were first analysed in scan mode using a mass range of 75–450 amu to optimize the acquisition conditions and then in time- scheduled select ion monitoring (SIM) mode for the iden- tification and quantification of target compounds. Target compounds were quantified with the internal standard quan- tification using the SIM chromatogram with the Xcalibur 1.4 software.

2.5. Quality Control and Quality Assurance

Calibration was performed over a concentration range be- tween 0.005 and 1 µg/mL. The precision of the method was evaluated by injecting one standard at 0.01 µg/mL (n 0 7) in consecutive days and expressed in terms of relative standard deviation (RSD).

Given the complexity of analysing phthalates due to background contamination, the analytical performance was continuously checked by analysing extraction solvents and by performing blank analysis and quality controls in each

extraction batch. The accuracy was evaluated by means of recovery experiments (n 0 7), analysing HPLC-grade water spiked at 0.01 and 0.1 μg/L concentration levels, although for phthalates, due to high blank contribution, recoveries were performed at 1 μg/L spiking level. Blank analysis was performed using HPLC water. Method limit of detection (LODs) and method limit of quantification (LOQ) for pesticides, alkylphenols and bisphenol A were determined through the signal-to-noise ratio of 3 and 10, respectively. Because of the blank contribution of phthalates, LODs were calculated using the arithmetical mean of the blank concentration plus three times the standard deviation (n 0 50) and for LOQ ten times the standard deviation was used. Surrogate standards were permitted to evaluate the extraction efficiency.

3. Results and discussion

3.1 Method performance

The multiresidue GC-MS permitted the resolution of all 21 compounds within 46 min with no coelutions (Fig. 1). Each compound was identified, when possible, at three mass fragments (Table 3) plus the retention time to ensure un- equivocal identification (Santos and Galceran 2002).

Since phthalates, alkylphenols, bisphenol A and herbicides are expected to be found at very low concentration in ground waters, the method was optimized to detect low microgram per litre levels. Table 3 shows the LOD which were in the low microgram per litre level for all compounds except for phtha- lates. For phthalates, LOD were much higher since they were calculated from the blank contribution and were in the range 0.010–0.46 μg/L. These are high values compared to other studies (Capdeville and Budzinki 2011) but ensure the quan- tification of phthalates in the sample with no need of blank subtraction. Table 3 also reports the LOQ of target compounds which ranged from 0.0018 to 0.970 μg/L for phthalates, OP, NP and BPA and from 0.0004 to 0.029 μg/L for herbicides. Target compounds were calculated at the concentration above the LOQ. The LOQ calculated herein are much lower than the environmental quality standards recently set by the EU (COM 2011 876 final) which range from 0.1 μg/L for OP to 1.3 μg/L for DEHP.

Recoveries are indicated in Table 3. For phthalates, values from 77 to 111 % were obtained at 1 μg/L spiking level. Pesticides, alkylphenols and bisphenol A were recovered from 84 to 124 % at 0.01 μg/L spiking level. At 0.1 μg/L, which is regarded

as the maximum level for individual pesticides in water intended for human consumption (Directive 98/83/CE), recoveries ranged from 88 to 106 % for alkylphenols and bisphenol A and 90 to 117 % for pesticides (Table 3). The RSD was in all cases <10 %, indicating that the method is highly repetitive and proves it robustness for the analysis of these series of compounds in source water intended for bottling.

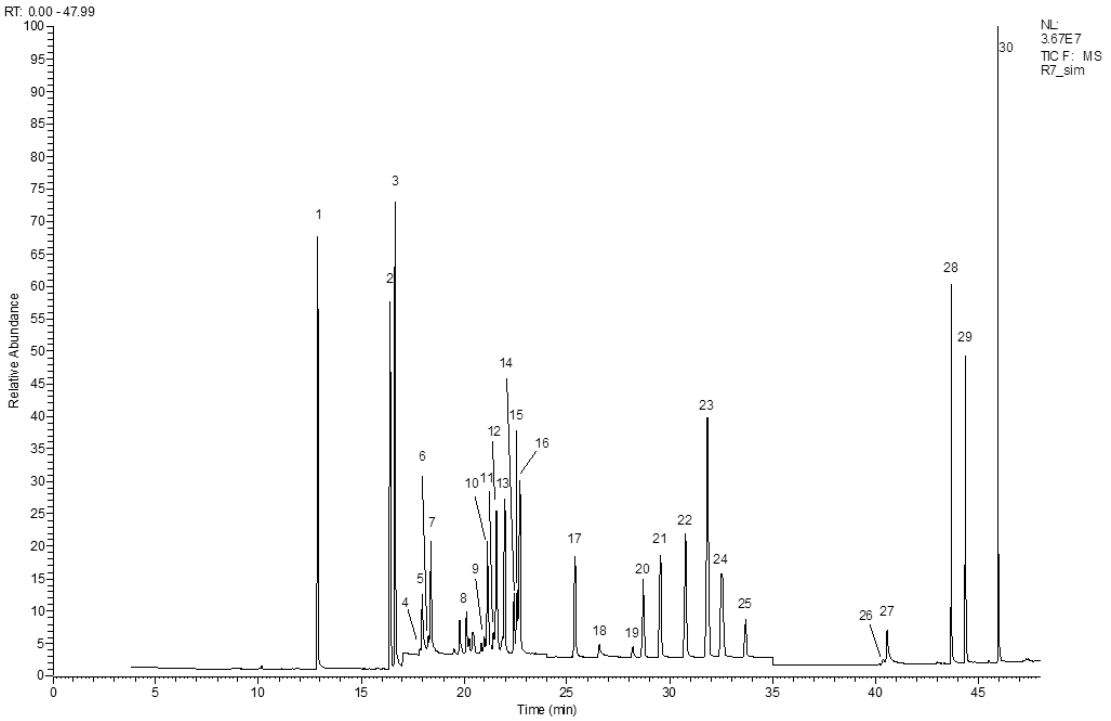

Figure 1. Chromatogram of 21 target compounds and their surrogates. 1. Dimethyl phthalate (12.88 min); 2. Diethyl phthalate (16.40 min); 3. Octylphenol (16.64 min); 4. Atrazine desisopropyl-d5 (17.85 min); 5. Atrazine desisopropyl (17.97 min); 6. Atrazine desethyl-d6 (18.25 min); 7. Atrazine desethyl (18.39 min); 8. Nonylphenol (19.79 – 21.54 min); 9. Simazine-d5 (20.99 min); 10. Simazine (21.12 min); 11. Atrazine-d5 (21.44 min); 12. Atrazine (21.57 min); 13. Propazine (21.97 min); 14. Di-n-propyl phthalate-d4 (22.44 min); 15. Anthracene-d10 (22.57 min); 16. Terbutylazine (22.70 min); 17. Secbutylazine (25.39 min); 18. Nonylphenol-d8 (26.57 min); 19. Alachlor-d13 (28.22 min); 20. Alachlor (28.71 min); 21. Prometryn (29.53 min); 22. Terbutryn (30.75 min); 23. Di-n-butyl phthalate (31.82 min); 24. Metolachlor (32.53 min); 25. Cyanazine (33.67 min); 26. Bisphenol A-d16 (40.35 min); 27. Bisphenol A (40.55 min); 28. Butylbenzyl phthalate (43.66 min); 29. Bis-(2-ethylhexyl) adipate (44.37 min); 30. Bis-(2-ethylhexyl) phthalate (45.95 min).

Table 3. Method quality parameters where the ions monitored are indicated, as well as the limits of detection (LOD), limits of quantification (LOQ), the recoveries at 2 spiking levels and the Relative Standard Deviation (RSD) of the SPE and GC-MS method.

Compound	Ions[a] (*m/z*)	LOD (µg/L)	LOQ (µg/L)	Recovery (%)		RSD (%)
				0.01 µg/L	0.1 µg/L (1 µg/L[b])	
DMP	*163*, 77, 194	0.01	0.018	<LOD	87[b]	5.9
DEP	*149*, 177, 105	0.33	0.837	<LOD	77[b]	1.6
DBP	*149*, 150, 223	0.23	0.687	<LOD	82[b]	4.4
BBP	*149*, 91, 206	0.19	0.525	<LOD	93[b]	4.3
DEHA	*129*, 112, 147	0.08	0.180	<LOD	111[b]	6.4
DEHP	*149*, 167, 279	0.46	0.970	<LOD	94[b]	7.1
OP	*135*, 107	0.001	0.0018	94	88	5.5
NP	*135*, 149, 107	0.017	0.057	112	106	4.1
Bisphenol A	*213*, 228, 119	0.009	0.029	89	93	5.4
Atrazine desisopropyl	*173*, 158, 145	0.0015	0.0051	103	99	5.1
Atrazine desethyl	*172*, 174, 145	0.0005	0.0016	107	104	5.2
Simazine	*201*, 186, 173	0.0023	0.0075	124	108	4.1
Atrazine	*200*, 215, 173	0.0007	0.0022	115	106	2.8
Propazine	*214*, 172, 229	0.0008	0.0025	109	93	1.9
Terbuthylazine	*214*, 229, 173	0.0004	0.0013	121	106	2.4
Sebuthylazine	*200*, 214, 229	0.0003	0.0010	119	117	5.9
Prometryn	*184*, 241, 226	0.0086	0.0290	110	99	1.0
Terbutryn	*226*, 185, 170	0.0003	0.0010	90	93	1.5
Cyanazine	*225*, 198, 174	0.0001	0.0004	84	93	0.6
Alachlor	*160*, 188, 146	0.0014	0.0045	113	95	2.8
Metolachlor	*162*, 238, 146	0.0006	0.0020	87	90	3.2

(1) In italics, quantification ion.

* For phthalates, the recovery was calculated at 1 µg/L spiking level.

3.2. Distribution of plasticizers and herbicides in source waters

Historically, actions to minimize the release of contaminants (nitrates, pesticides and biocides) to the environment and to prevent groundwater contamination have not always been effective. This situation is being changed in Europe, and according to the European Water Framework Directive (European Communities 2000), a precautionary approach has been launched for the general groundwater protection. It comprises (1) the prohibition of direct discharges to ground- water, (2) the requirement to monitor groundwater bodies so as to detect pollution trends and (3) to delimit protected areas of groundwater for human use. Taken together, these actions should ensure the protection of groundwater from contamina- tion events, according to the principle of minimum anthropo- genic

impact. According to these measures, member states have regulated the delimitation of wellhead protection areas in different ways (García-García and Martínez-Navarrete 2005). In Spain, wellhead protection areas for groundwater intended for human consumption can be divided into zones surround- ing the catchment, although no limit criteria are established by law (Martínez-Navarrete et al. 2008).

In this study, phthalates, alkylphenols, bisphenol A and herbicides were used as indicators of the original water purity of the source. The protection areas seem to be effec- tive in Spain since 111 sources out of 131 contained no traces of contamination, and in 20 of them (15 %), one to three target compounds were detected at a trace concentra- tion (Fig. 2). In 17 of these 20 sources, only one target compound was detected at concentrations between 0.002 and 1.115 µg/L. In two sources, two compounds were detected, and in one source, three compounds were detected (Fig. 2). These sources are either located near urban or industrial activity or correspond to less protected springs or superficial boreholes. These results can be considered in other terms. One hundred thirty-one sources were sampled, and 21 target compounds were studied in each source, generating a data matrix of 2,751 values. From this matrix, 24 data values were over the LOQ, representing less than 0.87 % of the total data values.

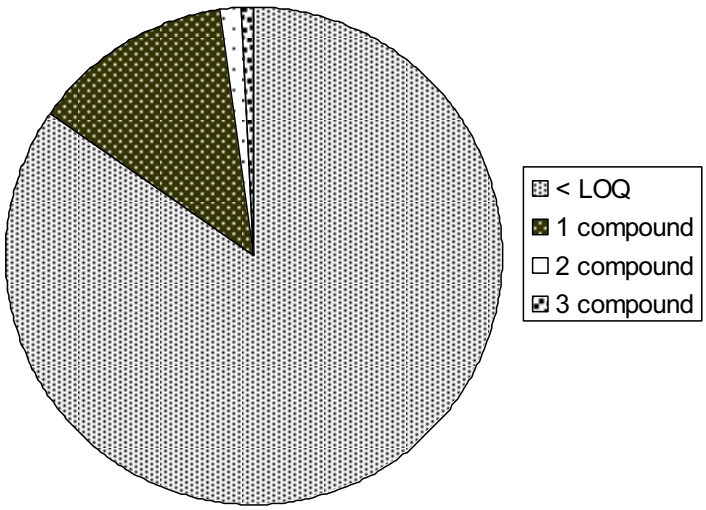

Figure 2. Frequency of detection of phthalates, alkylphenols, bisphenol A and herbicides in 131 source waters from Spain. In 84.7% of the samples, no traces were observed. In 13% of the samples, 1 compound was detected; in 1.5% of the samples, 2 compounds were detected; in 0.8% of the samples, 3 compounds were detected. In all cases, detected compounds were well below legislated values.

Bisphenol A was one of the most frequently detected compounds (in six sources) at concentrations between 0.031 and 0.203 µg/L. It was identified in shallow boreholes located in municipal areas or springs which could be affect- ed by surface waters. Bisphenol A has a water solubility of 120 mg/L, a relative low K_{OC} and soil half-lives of 75 days which enhance its lixiviation potential, as indicated by the GUS index (SRC Inc. 2011, Table 1). This compound has been previously detected in industrial groundwaters (Latorre et al. 2003). In another study, groundwater downgradient of an infiltration bed for secondary treated effluent contained nonyl/octylphenol and ethoxylates at 30 µg/L, while bisphe- nol A, nonylphenol monoethoxycarboxylate and nonyl/octyl- phenol tetraethoxylate were detected in some drinking water wells at concentrations up to 32.9 µg/L (Rudel et al. 1998). Ying reported that bisphenol A and OP remained almost unchanged in the aquifer material under aerobic conditions (Ying et al. 2003).

Among phthalates, DEP was detected once at 1.115 µg/L in a borehole near an industrial area and DEHA was detected at 0.192 µg/L in one spring water. DEHP and DEHA have a low lixiviation potential, given that they are strongly adsorbed to soil, and thus, mobilization is reduced. The exceptions are DMP and DEP, which have a high lixiviation potential due to their relative low K_{OC} and relative high half-life.

OP was detected four times in very small amounts, be- tween 0.002 and 0.003 µg/L, levels close to the LOQ. NP was only detected in one source at 0.058 µg/L. Both these compounds have a low GUS index, indicating that they will be preferably attached to soil and leaching can only occur when inputs are high. They are not regulated in Spanish legislation in R.D. 1798/2010, but in the European Directive 2008/105/EC, the maximum recommended concentration is 2 µg/L for NP and between 0.010 and 0.1 µg/L for OP in surface waters although there are no specific limits for water sources.

Triazine herbicides have been used in Spain for decades and can easily leach to groundwaters given their K_{OC} and soil half- lives (Table 1). Among herbicides, atrazine desisopropyl and atrazine desethyl were detected in two and seven sources, respectively, while atrazine and simazine were detected only once (Table 4). Concentrations found were from 0.002 to 0.059 µg/L. Other pesticides could not be observed even though they are nowadays used as replacements for atrazine and simazine. In all cases, the concentration of the individual pesticides was lower than the 0.1-µg/L limit set by Directive 98/83/CE. In Spain there have been several studies where herbicides were detected in surface and groundwaters (Brossa et al. 2005; Garrido et al. 2000; Hildebrandt et al. 2007). Carabias-Matínez et al. (2000)

detected alachlor and diflufeni- can, among other compounds, in agricultural groundwaters of Zamora and Salamanca (north-west Spain) at concentration higher than 0.1 µg/L. These authors indicated that while sur- face water pollution by triazine herbicides was related to the application and use of this herbicide, their presence in ground- water was associated to the frequency of application, soil permeability, rainfall and recharge rate of the aquifer (Carabias-Martínez et al. 2002, 2003). Another study identified atrazine and alachlor in surface water and groundwa- ter, with the highest concentration in surface water (Sánchez- Camazano et al. 2005). Quintana et al. (2001) detected sima- zine, terbuthylazine, atrazine and its metabolites desethylatra- zine and deisopropylatrazine in groundwater from the Llobregat river aquifer (north-east Spain) at concentrations lower than 0.1 µg/L. Hildebrandt et al. detected atrazine, sima- zine, terbuthylazine, their desethyl products, metalaxyl and metolachlor in agricultural groundwaters from the Duero, Ebro and Miño basins and concluded that agricultural groundwaters can be highly vulnerable because of the direct leaching from top soils where pesticides are directly applied (Hildebrandt et al. 2008). Issa and Wood (1999) suggested that the concentra- tion of some herbicides in groundwater is likely to take more than a decade to decrease significantly.

In terms of source water used in the bottling sector, con- tamination must be avoided for both sanitary and legislative reasons. The Royal Decree 1798/2010 (Spanish Government 2010) is the Spanish legislation relating to natural mineral waters and spring waters intended for bottling and specifies restrictions for several parameters. The target compounds analysed have no restrictions in Directive 2009/54/EC of the European Union (European Communities 2009) related to natural mineral waters, although annex I of this directive indicates that the original water purity must be preserved. On the other hand, Directive 2008/105/EC (European Com- munity 2008) and COM 2011 876 final (COM 2011) referring to environmental quality standards in the field of water policy limit certain substances, such as alachlor, atrazine, nonylphe- nol, octylphenol or simazine. However, these limits are not exceeded in any case in the studied source waters.

Table 4. Number wells studied (N), number of compounds detected above the LOQ, and minimum (min), maximum (max) and mean concentration (µg/L), and standard deviation (SD) of each compound.

Compound	*N*	*N* > LOQ	Min (µg/L)	Max (µg/L)	Mean (µg/L)	SD (µg/L)
DMP	131	0	–	–	–	–
DEP	131	1	1.115	1.115	–	–
DBP	131	0	–	–	–	–
BBP	131	0	–	–	–	–
DEHA	131	1	0.192	0.192	–	–
DEHP	131	0	–	–	–	–
OP	131	4	0.002	0.003	0.0024	0.004
NP	131	1	0.058	0.058	–	–
Bisphenol A	131	6	0.031	0.203	0.104	0.071
Atrazine desisopropyl	131	2	0.009	0.059	0.034	0.035
Atrazine desethyl	131	7	0.002	0.024	0.0069	0.0082
Simazine	131	1	0.018	0.018	–	–
Atrazine	131	1	0.007	0.007	–	–
Propazine	131	0	–	–	–	–
Terbutylazine	131	0	–	–	–	–
Sebuthylazine	131	0	–	–	–	–
Prometryn	131	0	–	–	–	–
Terbutryn	131	0	–	–	–	–
Cyanazine	131	0	–	–	–	–
Alachlor	131	0	–	–	–	–
Metolachlor	131	0	–	–	–	–
Total	131	24				

4. Conclusions

Source waters must be of high purity since this water is used untreated for human consumption, and their sanitary prop- erties have to be preserved. However, environmental pollu- tion can have an impact on this type of water. In this study it has been shown that the impact of herbicides, phthalates, alkylphenols and bisphenol A in 131 source waters intended for bottling from all over Spain is minimal and that protec- tion measures of wellhead waters from any direct anthropo- genic impact are effective. This means that water sources used in the bottling sector are not affected by the densely populated, agricultural or industrial activities that are carried out all over the Spanish geography. The few detected com- pounds were at the low microgram per litre level and mainly distributed in springs or superficial wells, which could be more easily contaminated by surface

waters. The identifica- tion of traces of phthalates, bisphenol A, alkylphenols and triazines in a few source water samples suggests their po- tential use as indicators of original purity for natural mineral waters. In all cases, detected compounds were well below the parametric values set by the European Union and the Spanish legislation and much lower than toxicological thresholds. According to this we can conclude that the Spanish sources are healthy and their water is appropriate for human consumption. Still, it is necessary to keep a careful and strict surveillance of sources to guarantee the original water purity.

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Article científic II

Títol: Effect of bottling and storage on the migration of plasticizers in Spanish bottled waters

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Effect of bottling and storage on the migration of plasticizers in Spanish bottled waters

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Abstract

Bottled water is packaged in glass or, in a greater extent, in plastic bottles with metallic or plastics caps of different material, shape and colour. Plastic materials are made of one or more monomers and several additives that can eventually migrate into water either during bottle manufacturing and water filling or during storage. The main objective of the present study was to carry out a comprehensive assessment of the quality of Spanish bottled water market in terms of (i) migration of plastic components or additives during bottling and during storage and (ii) evaluating the effect of the packaging material and bottle format on the migration potential. Studied compounds were 5 phthalates, diethylhexyl adipate, alkylphenols, and bisphenol A. A set of 362 of several commercial brands corresponding to 131 natural mineral waters and spring waters sources and 3 treated waters were analyzed immediately after bottling and after one-year stored (a total of 724 samples). Target compounds were detected in 5.6 % of the data values with median concentrations of 1.650, 0.061 and 0.139 µg/L for phthalates including diethylhexyl adipate, alkylphenols, and bisphenol A, respectively. Total daily intake was estimated and comparison with reference values is indicated.

Keywords: bottled water; migration; phthalate; alkylphenol; bisphenol A; total daily intake.

1. Introduction

Bottled water has emerged as a drinking water which preserves the original water purity for natural mineral waters and in many cases as the only water available for human consumption in areas where there is a lack of potable distribution water. Bottle water demand grew during the period 2003 to 2008 by 6.7 % in the United States (as example of developed country) and by 15.6 % in China (as example of developing country) (Yasinsky et al., 2011). Depending on the source, there are different categories of bottled water according to its origin: natural mineral water, spring water and bottled drinking water (so-called treated water). Natural mineral water is directly drawn from its source, it is not sterile, it has a stable composition and it is characterized by a specific mineralization according to its chemical composition. Another important property is its original purity; it medians that natural mineral waters shall be free of anthropogenic pollution. Microbiological and physicochemical controls are undertaken in the source to ensure the original purity. Spring water is also a natural and not sterile product, but a stable mineral balance is not a requirement. Finally, bottled drinking water is a product which can be subjected to several treatment processes in accordance with current legislation and it may be obtained either from boreholes or from public distribution system. Besides, water can be carbonated water (with CO_2 gas) or still water (without gas). Since these waters are intended for bottling, analyses of their properties and the absence of contaminants are done to ensure their quality. Since 1980, natural mineral water and treated water have been separated in two different European Directives. Nowadays, chemical and microbiological tests for natural mineral water and microbiological tests for spring water are described in Directive 2009/54/EC (EU, 2009) and chemical analysis for spring water and both chemical and microbiological analysis for treated water are indicated in Directive 98/83/EC (EU, 1998).

The bottling industry pursues the production of high water quality. However, several factors can affect the quality of water: (i) leaching of pollutants from unprotected agricultural and industrial areas. To avoid this problem, a protection perimeter in the sources of natural mineral water and spring water has proved to be effective to avoid any contamination and preserve the original purity (Bono-Blay et al., 2012); (ii) bottling process where plastic components and additives can migrate into water from storage tanks and pipelines, and (iii) storage, where plastic components or additives can migrate into water depending on the packaging material and format (Diduch et al., 2011).

Bottle packaging is designed to act as a gas barrier to avoid interaction with the surrounding environment but they do not have a functional barrier, as for example aluminum layer (EU, 2011). Plastic material used to manufacture bottles intended to contain water consists of one or more monomers and several additives such as accelerators, catalyzers, stabilizers, antioxidants, coupling agents, flame retardants and plasticizers (Bolgar et al., 2008). Bach et al. (2012) reported that antioxidants, as alkylphenols, can be in contact with water during PET manufacturing or during the washing steps of containers. High temperatures and the presence of oxygen in PET melt process can promote thermo-mechanical and thermo-oxidative reactions (Romão et al., 2009; Zhang and Ward, 1995; Paci and La Mantia, 1998) which enhances migration of plastic material components. Phthalates may come from bottling lines (Higuchi et al., 2004), cap-sealing resins (Hirayama et al., 2001), water treatment facilities (Leivadara et al., 2008; Montuori et al., 2008) or migration during storage (Casajuana and Lacorte et al., 2003; Diduch et al., 2011, Bach et al., 2012). Several authors detected alkylphenols, as nonylphenol (NP) and octylphenol (OP) in bottled water (Li et al., 2010, Amiridou and Voutsa, 2011) and after migration assays (Guart et al., 2011). Other studies indicate that polycarbonate (PC) plastic and epoxy resins can be a source of bisphenol A (BPA) into water (Amiridou and Voutsa, 2011; Gallart-Ayala et al., 2011; Le et al., 2008; Nerín et al., 2003).

The main objective of the present study was to carry out a comprehensive assessment of the quality of bottled water and determine the effect of bottling, packaging type and format and storage time on water quality. To achieve this aim, 362 samples contained in different bottle types and formats corresponding to 131 sources (natural mineral waters and spring waters) and 3 treated waters were analyzed: (i) in fresh samples immediately after bottling and (ii) in one-year stored samples. The analyzed waters represent Spanish commercial brands. The studied compounds were phthalates, alkylphenols, and BPA.

2. Materials and methods

2.1. Chemical reagents

Phthalate Mix 525 (500 ng/µL each in methanol) containing dimethyl phthalate (DMP), diethyl phthalate (DEP), di-n-butyl phthalate (DBP), butyl benzyl phthalate (BBP), bis(2-ethylhexyl) adipate (DEHA) and bis(2-ethylhexyl) phthalate (DEHP) was from Supelco (Bellefonte, PA, USA). 4-nonylphenol (NP) was obtained

from Riedel-de Häen (Seelzy, Germany) as a solid technical mixture of isomers. BPA was purchased from Dr. Ehrenstorfer (Augsburg, Germany) as a solid and 4-tert-octylphenol from Supelco (Bellefonte, PA, USA) as a solid. Phthalates surrogate standard was dipropylphthalate-d_4 from Riedel-de Haën (Seelze, Germany), purchased as a solid. Alkylphenols surrogate standard was 4-n-nonylphenol-d_8 from Dr. Ehrenstorfer (Augsburg, Germany) as a solution at 100 ng/µL in acetone; BPA surrogate was BPA-d_{16} from Sigma Aldrich (St. Louis, MO, USA) as a solid; anthracene-d_{10} used as internal standard, was from Dr. Ehrenstrofer as a solution of 10 ng/µL in cyclohexane.

200 mg Oasis HLB cartridges were from Waters (Milford, MA, USA) and used with a Baker vacuum system (Product No. 7018-94; J.T. Baker, Deventer, the Netherlands). Chromatography-grade methanol, acetone, dichloromethane, n-hexane, ethyl acetate and HPLC water were purchased from Merck (Darmstadt, Germany). Nitrogen for drying with 99.995 % of purity was from Air Liquid (Barcelona, Spain).

2.2. Samples

Spanish bottled waters of 0.1, 0.15, 0.2, 0.25, 0.33, 0.5, 0.75, 0.92, 1, 1.25, 1.5, 2, 5, 6.5, 8, 10, 11, 13, 18.9 and 20 L of 94 brands were analyzed. Bottled water studied corresponded to (i) 85 glass bottles with metallic crown cap, 20 glass bottles with metallic screw-cap and 4 glass bottles with high density polyethylene (HDPE) cap; (ii) 1 polypropylene (PP) bottle with HDPE cap; (iii) 20 PC bottles with LDPE cap; (iv) 224 PET bottles with HDPE cap; (v) 7 HDPE bottles with HDPE cap and (vi) 1 low density polyethylene (LDPE) bag (Table 1). All these waters have the designation of natural mineral water, spring water or treated water. Samples were provided from main Spanish bottling industry. Two samples of each material and volume were collected after filling in the bottling lines of each company (a total of 724 samples). One sample was analyzed just after sampling (fresh sample) and the other was stored for 1 year and analyzed (one-year stored). Fresh waters were stored at room temperature until analysis, which was performed within 15 days from the sampling date. On the other hand, waters stored for 1 year in its original bottle were placed in an exterior warehouse in the dependencies of the enterprise Laboratorio Dr. Oliver Rodés (El Prat del Llobregat, Barcelona, Spain), protected from rain and sunlight. Average monthly minimum and maximum temperatures were 14.7 and 28.0 ºC, 12.4 and 28.9 ºC, and 12.5 and 30.1 ºC in

years 2007, 2008 and 2009, respectively (Weather Online, 2013, Airport of Barcelona, El Prat del Llobregat, at 4 km from the Laboratory).

2.3. Instrumental analysis

Samples were analyzed unfiltered and concentrated using solid phase extraction (SPE) followed by gas chromatography coupled to a quadrupole mass spectrometer (GC-MS) in a Thermo Electron Corporation (San José, CA, USA) Trace GC 2000 instrument. Electron ionization was performed at 70 eV. The carrier gas used was helium with constant flow at 1.2 mL/min. A fused silica column DB-5MS (30 m x 0.25 mm x 0.25 µm) from J&W Scientific (Folsom, CA, USA) was connected to a deactivated guard column. Target compounds were quantified with internal standard quantification using the selected ion monitoring (SIM) chromatogram with the Xcalibur 1.4 software. SPE-GC/MS method and conditions are described in a previous study (Bono-Blay et al., 2012) which provides quality control and quality assurance. In brief, because of the blank contribution of phthalates, LOQs were calculated using the arithmetical median of the blank concentration plus ten times the standard deviation (n=50). Surrogate standards permitted to evaluate the extraction efficiency in each sample (Bono-Blay et al., 2012).

Table 1. Description of the several types of packaging, volumes and the respective number of samples for each type analyzed in the study (Number inside brackets corresponds to the number of carbonated water).

Volume	Glass [a]	Glass [a]	Glass [a]	PP [a]	PC [a]	PET [a]	HDPE [a]	LDPE [c]
(L)	Metallic crown [b]	Metallic screw-cap [b]	HDPE [b]	HDPE [b]	LDPE [b]	HDPE [b]	HDPE [b]	-
0.1	1	-	-	-	-	-	-	-
0.15	-	-	-	-	-	1(1)	-	-
0.2	-	-	-	-	-	1	-	-
0.25	18(9)	2(1)	-	-	-	3	-	-
0.33	13(7)	1(1)	-	-	-	34	-	-
0.5	23(4)	3(2)	-	-	-	39(2)	-	-
0.75	4(4)	4(2)	-	-	-	-	-	-
0.92	3(1)	-	-	-	-	-	-	-
1	23(6)	10(6)	1(0)	-	-	-	-	1
1.25	-	-	-	-	-	3(2)	-	-
1.5	-	-	-	-	-	61(3)	-	-
2	-	-	-	-	-	5	-	-
5	-	-	-	-	-	56	3	-
6.5	-	-	-	-	-	1	-	-
8	-	-	2	1	-	18	3	-
10	-	-	1	-	-	1	1	-
11	-	-	-	-	2	-	-	-
13	-	-	-	-	1	-	-	-
18.9	-	-	-	-	12	-	-	-
20	-	-	-	-	5	1	-	-
Total	85 (31)	20 (12)	4 (0)	1 (0)	20 (0)	224 (8)	7 (0)	1 (0)

[a] Material of the bottle
[b] Material of the cap
[c] Bag format.

3. Results

3.1. Summary data

The effect of the water (still or carbonated), the bottling and the storage was evaluated for 8 packaging materials in terms of migration of phthalates, alkylphenols and BPA. Figure 1 shows the concentration range of total contaminants in water for each type of bottle, comparing fresh samples with one-year stored samples. Considering a data matrix of 3258 values (9 compounds x 362 samples) for each analysis period, fresh samples and one-year stored samples only showed a 4.0 % and 7.2 % values above LOQs, representing a low incidence of these compounds in bottled water. Considering all samples analyzed (724), 5.6 % were positive values for a total data matrix of 6516 values (9 compounds x 362 samples x 2 analysis periods). Median concentration levels were of 0.430 µg/L, and minimum and maximum levels were of 0.002 and 24.2 µg/L. As regards to the effect of storage on the migration of monomers and additives to water, similar total concentrations were observed in fresh samples and one-year stored, and in general, there was little variation between the concentration of target compounds in fresh waters and one-year stored water. Only glass with metallic crown cap, PET with HDPE cap and PC with LDPE cap showed an overall increase in the number of samples with levels >LOQ after storage. In addition, there was not a specific pattern on the migration of contaminants according to bottle format but rather, it depended on the packaging and type of water (still or carbonated). However, HDPE bottles with HDPE cap and LDPE (bag) were the only materials with a high increase in the concentration of target compounds, but since there were only a few bottles of these materials, conclusions cannot be drawn.

Considering all samples (n=724), BPA was detected in 10.9% of the samples, followed by DEHP (10.2%), NP (8.1%), DEP (7.7%), OP (7.3%), DEHA (3.6%), BBP (1.6%), DMP (0.9%) and DBP (0.1%). Table 2 summarizes the concentration of each target compound in fresh samples and one-year stored samples, classified according to the bottle material. BPA increased from 38 samples >LOQ in fresh samples to 41 in one-year stored. For DEHP, 28 samples with positive results increased to 46 in one-year stored. NP increased from 11 to 48 samples, DEP increased from 15 to 42. OP increased from 19 to 34 samples. DEHA increased from 10 samples to 16, and BBP from 4 to 8. However, DMP decreased from 6 to 1 and DBP from 1 to 0.

Considering the bottle material, DEHP was basically detected in glass bottles with metallic crown cap, whereas DEHA was basically found in PET bottles in one-

year stored samples. DEP, OP and NP were associated to glass bottle with metallic crown cap and to PET bottles, and in all cases there was an increase in the number of positive samples in one-year stored. BPA was mainly detected in PC bottles but also it was also detected in PET and in glass bottles with metallic crown cap.

Considering all the results obtained, two processes explain the scattered presence of target compounds. In fresh waters, the presence of contaminants is due to the bottle manufacturing or to the bottling process, where some of the tanks or pipes components could migrate from the different materials. Manufacturing bottle process could also be a source of plasticizers when the polymerization process has not been completed. In other cases, target compounds are detected only in one-year stored samples, indicating that compounds migrate from the plastic bottle, the cap or its liner. In very few cases, the same contaminants are detected in fresh water samples and in one-year stored, depending on the brand. Finally, the bottle volume or format did not essentially affect the migration of plasticizers. The migration of plasticizers from each type of bottle is indicated in the following sections.

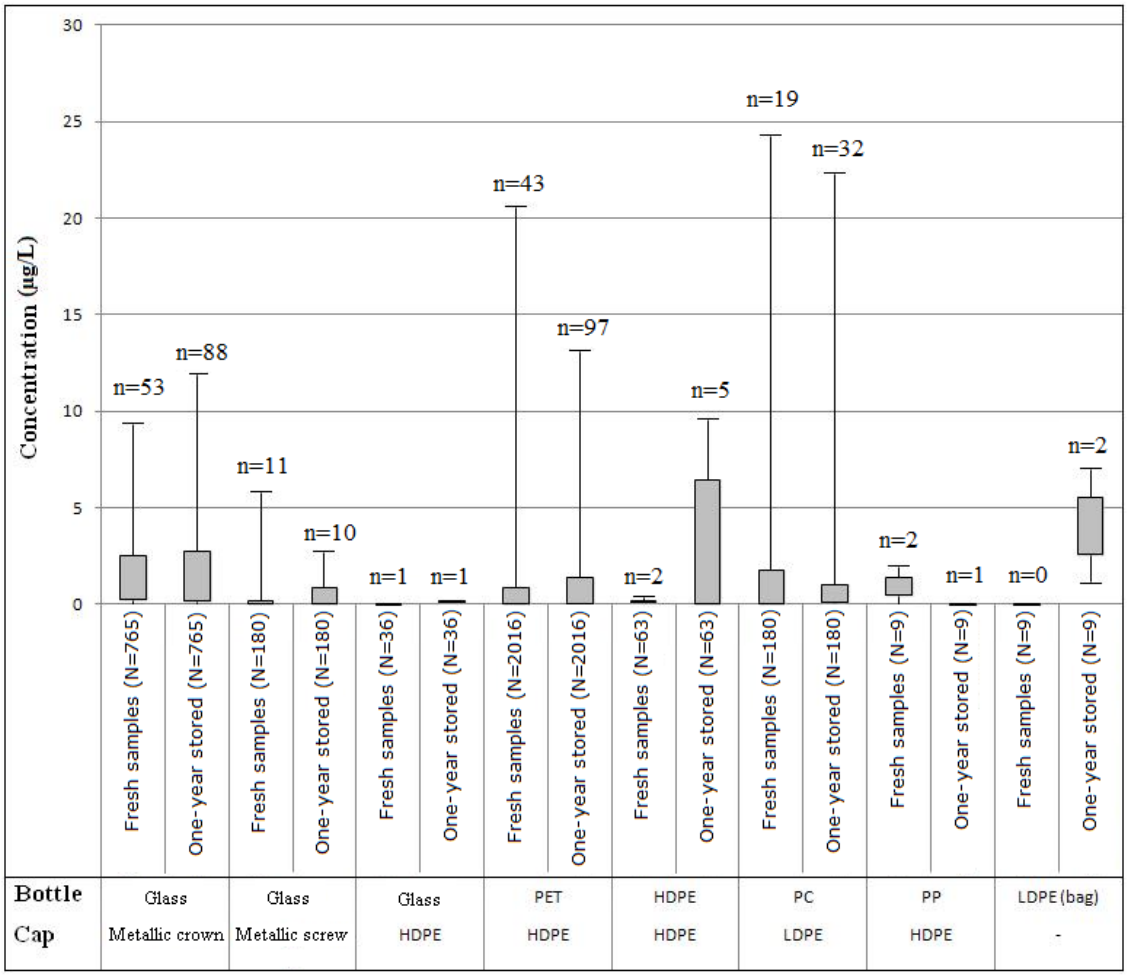

Figure 1. 25, 75% quartile, minimum and maximum concentration of target compounds for each type of container. "n" indicates the number of detected compounds for each container category, out of the total analyzed (N).

Table 2. Concentration of target compounds are shown as ranges, medians and number of positive samples in fresh waters and one-year stored waters for each compound and packaging format.

(µg/L)	Material	Bottle	Glass	Glass	Glass	PP	PC	PET	HDPE	LDPE (bag)
		Cap	Metallic crown	Metallic screw-cap	HDPE	HDPE	LDPE	HDPE	HDPE	-
DMP	Recent filled	Range	0.046	-	0.063	0.021	-	0.022	0.085-0.230	-
		Median	0.046	-	0.063	0.021	-	0.022	0.158	-
		n>LOQ (n total)	1(85)	-	1(4)	1(1)	-	1(224)	2(7)	-
	one-year stored	Range	0.174	-	-	-	-	-	-	-
		Median	0.174	-	-	-	-	-	-	-
		n>LOQ (n total)	1(85)	-	-	-	-	-	-	-
DEP	Recent filled	Range	1.170-9.340	-	-	-	0.920-2.460	1.020-20.50	-	-
		Median	6.550	-	-	-	1.690	1.310	-	-
		n>LOQ (n total)	5(85)	-	-	-	2(20)	8(224)	-	-
	one-year stored	Range	0.895-9.110	1.030-1.780	-	-	-	0.857-4.300	2.060-9.460	1.160
		Median	2.265	1.405	-	-	-	1.225	6.440	1.160
		n>LOQ (n total)	14(85)	2(20)	-	-	-	22(224)	3(7)	1(1)
DBP	Recent filled	Range	-	-	-	-	0.736	-	-	-
		Median	-	-	-	-	0.736	-	-	-
		n>LOQ (n total)	-	-	-	-	1(20)	-	-	-
	one-year stored	Range	-	-	-	-	-	-	-	-
		Median	-	-	-	-	-	-	-	-
		n>LOQ (n total)	-	-	-	-	-	-	-	-

Table 2. Continuation.

(µg/L)	Material	Bottle	Glass	Glass	Glass	PP	PC	PET	HDPE	LDPE (bag)
		Cap	Metallic crown	Metallic screw-cap	HDPE	HDPE	LDPE	HDPE	HDPE	-
BBP	Recent filled	Range	0.592-0.794	-	-	-	-	0.619-1.280	-	-
		Median	0.693	-	-	-	-	0.950	-	-
		n>LOQ (n total)	2(85)	-	-	-	-	2(224)	-	-
	one-year stored	Range	0.614-1.430	2.580	-	-	-	0.635-3.010	-	-
		Median	0.911	2.580	-	-	-	2.550	-	-
		n>LOQ (n total)	4(85)	1(20)	-	-	-	3(224)	-	-
DEHP	Recent filled	Range	0.985-5.510	1.640-5.780	-	1.840	2.810	1.520	-	-
		Median	2.260	3.710	-	1.840	2.810	1.520	-	-
		n>LOQ (n total)	23(85)	2(20)	-	1(1)	1(20)	1(224)	-	-
	one-year stored	Range	1.050-11.90	-	-	-	1.790	1.020-12.97	-	-
		Median	2.300	-	-	-	1.790	1.825	-	-
		n>LOQ (n total)	33(85)	-	-	-	1(20)	12(224)	-	-
DEHA	Recent filled	Range	0.232-2.400	0.182	-	-	-	0.283-1.470	-	-
		Median	0.457	0.182	-	-	-	0.507	-	-
		n>LOQ (n total)	5(85)	1(20)	-	-	-	4(224)	-	-
	one-year stored	Range	0.227	-	-	-	0.257	0.185-6.230	-	-
		Median	0.227	-	-	-	0.257	0.506	-	-
		n>LOQ (n total)	1(85)	-	-	-	1(20)	14(224)	-	-

Table 2. Continuation.

(µg/L)	Material	Bottle	Glass	Glass	Glass	PP	PC	PET	HDPE	LDPE (bag)
		Cap	Metallic crown	Metallic screw-cap	HDPE	HDPE	LDPE	HDPE	HDPE	-
OP	Recent filled	Range	0.004-3.160	0.003-0.005	-	-	0.004-0.037	0.002-0.011	-	-
		Median	0.005	0.004	-	-	0.010	0.005	-	-
		n>LOQ (n total)	3(85)	3(20)	-	-	3(20)	10(224)	-	-
	one-year stored	Range	0.003-0.040	0.007-0.051	-	0.002	0.002-0.128	0.002-0.023	0.007	-
		Median	0.005	0.029	-	0.002	0.009	0.005	0.007	-
		n>LOQ (n total)	7(85)	2(20)	-	1(1)	5(20)	18(224)	1(7)	-
NP	Recent filled	Range	0.061-0.430	0.064	-	-	0.537	0.062-0.538	-	-
		Median	0.114	0.064	-	-	0.537	0.129	-	-
		n>LOQ (n total)	4(85)	1(20)	-	-	1(20)	5(224)	-	-
	one-year stored	Range	0.068-0.933	0.057-0.430	0.219	-	0.061-2.420	0.058-2.030	0.059	6.980
		Median	0.131	0.120	0.219	-	0.138	0.125	0.059	6.980
		n>LOQ (n total)	18(85)	5(20)	1(4)	-	6(20)	16(224)	1(7)	1(1)
BPA	Recent filled	Range	0.037-4.040	0.045-0.135	-	-	0.032-24.20	0.042-0.191	-	-
		Median	0.104	0.087	-	-	0.365	0.159	-	-
		n>LOQ (n total)	10(85)	4(20)	-	-	12(20)	12(224)	-	-
	one-year stored	Range	0.035-1.620	-	-	-	0.074-22.17	0.037-0.819	-	-
		Median	0.101	-	-	-	0.550	0.058	-	-
		n>LOQ (n total)	10(85)	-	-	-	19(20)	12(224)	-	-

3.2. Glass bottle with metallic crown cap

Eighty-five samples bottled in glass were sampled in each period. Considering both periods, DEHP (56) was the most frequently detected compound, followed by NP (22), BPA (20), DEP (19), OP (10), BBP (6), DEHA (6) and DMP (2) while DBP was not detected in any sample.

More than 75% of positives for DEHP were detected in this specific material. DEHP was detected in 23 samples in fresh water and in 33 samples in one-year stored at concentrations of 0.985-5.510 µg/L (median 2.260 µg/L) and 1.050-11.90 µg/L (median 2.300 µg/L), respectively. Other phthalates identified were DEP, detected in 5 samples in fresh water and in 14 samples after one-year stored at concentrations of 1.170-9.340 µg/L (median 6.550 µg/L) and 0.895-9.110 µg/L (median 2.265 µg/L), respectively. BBP was detected in 2 fresh water samples and in 4 samples after one-year stored at concentrations of 0.592-0.794 µg/L (median 0.693 µg/L) and 0.614-1.430 µg/L (median 0.911 µg/L), respectively. DEHA was detected in 5 fresh water samples and in only 1 sample after one-year stored at concentrations of 0.232-2.400 µg/L (median 0.457 µg/L) and 0.227 µg/L, respectively. DMP was only detected at 0.046 µg/L in a one-liter carbonated fresh water sample and in the same sample after one-year stored the concentration increased to 0.174 µg/L. Given that this compound was only detected in 1 out of 85 samples analyzed, its presence is sporadic and relative to a specific brand.

BPA was detected in 10 samples in fresh water and in 10 different samples after one-year stored at concentrations of 0.037-4.040 µg/L (median 0.104 µg/L) and 0.035-1.620 µg/L (median 0.101 µg/L), respectively.

As for alkylphenols, OP was detected in 3 samples in fresh water and in 7 samples after one-year stored at concentrations of 0.004-3.160 µg/L (median 0.005 µg/L) and 0.003-0.040 µg/L (median 0.005 µg/L), respectively. In fresh waters, OP was always detected in carbonated waters and a 0.25 L sample (very small format) contained OP up to 3.160 µg/L. In those samples, OP was not detected in one-year stored samples, indicating that this compound might be degraded or volatilized during that period. In contrast to this, after one-year stored samples, OP was only detected in still water, and its presence is attributed to migration. NP was detected in 4 samples in fresh water and in 18 samples after one-year stored at concentrations of 0.061-0.430 µg/L (median 0.114 µg/L) and at 0.068-0.933 µg/L (median 0.131 µg/L), respectively. NP can either originate from cleaning of the glass bottle (Casajuana and Lacorte, 2003; Talmage, 1994; Votavová et al., 2009) or from the cap (Guart et al., 2011).

The presence of NP was not associated with carbonated waters. For only one still water brand contained in 0.33 L bottle, there was an increase in the concentration from 0.146 to 0.277 µg/L in one-year stored samples.

In many cases, the presence of monomers and additives was associated within a specific brand, where the bottling process or the quality of the materials used affect water quality. This is the case of Brand A, where target compounds were reiterated detected. In 1 L bottle filled with still water, there was a decrease of DEP from 6.550 to 1.670 µg/L after one-year stored; DEHP increased from 1.930 µg/L in fresh waters to 2.050 µg/L after one-year; and BPA decreased from 0.412 to 0.116 µg/L. In 0.5 L bottle filled with carbonated water of the same brand, BPA decreased from 0.860 to 0.175 µg/L. In 0.25 L bottle filled with carbonated water, DEHP increased from 2.390 to 4.370 µg/L after one-year stored. In three bottles of Brand B with volumes of 0.25 L still water, 0.25 L carbonated gas and 0.5 L still water, the concentrations of DEHP increased from 1.580 to 2.410 µg/L, from 1.280 to 3.660 µg/L and from 2.490 to 3.190 µg/L, respectively. In Brand C, two 0.25 L bottles filled with still and carbonated water showed increases from 4.650 to 5.580 µg/L and from 2.490 to 7.530 µg/L, respectively. In a 0.5 L still water of Brand D there was a decrease of DEHP from 2.050 to 1.570 µg/L in one-year stored sample. In still treated water of Brand D of volumes of 0.25, 0.5 and 1 L, DEHP decreased in one-year stored sample from 2.950 to 2.100 µg/L, from 2.720 to 2.300 µg/L and from 1.510 to 0.727 µg/L, respectively. In this case, the higher surface/volume area of small bottles produced a high migration of DEHP. In a 0.25 L bottle of still water of the same brand, BPA was found at the same concentration in fresh samples and in one-year stored at 0.090 and 0.085 µg/L, respectively. Considering fresh and one-year stored samples, NP concentration was of 0.082 and 0.089 µg/L in Brand E (1 L still water) and BPA increased from 0.068 to 0.145 µg/L. A 0.33 L bottle filled with still water showed an increase of DEHP from 1.180 to 3.310 µg/L while a carbonated water of 0.33 L bottle showed a decrease of DEHP from 4.040 to 1.620 µg/L.

Because of carbonated water is often bottled in glass containers, the presence of plasticizers or additives must be generated from the plastic liner placed inside the metallic crown cap. Carbon dioxide produces a decrease in water pH down to 6 hence it may enhance the migration of target compounds to water through plastic degradation and headspace contact. 31 of the 85 samples were carbonated waters (36 %), as it is indicated in Table 1. Positive carbonated waters represent the 49 % of total positive samples in fresh water and 27 % in one-year stored. From an overall point of view, carbonated waters showed higher concentrations of target compounds than still waters. From the total DEHP-positive

glass with crown cap samples, 43 % were carbonated for fresh waters and 36% were carbonated for one-year stored.

Summarizing, glass bottle with metallic crown cap is the type of container with the most positive results. All target compounds were found except DBP. The main migration compound was DEHP, followed by NP, BPA and DEP. The results indicate that migration of DEHP is highly correlated with the crown cap.

3.3. Glass bottle with metallic screw-cap

Total glass bottles with metallic screw-cap analyzed were 20 for each period. Phthalates were detected in 5 samples and DMP and DBP were never detected. DEP was detected in 2 carbonated samples after one-year stored at 1.030 µg/L and 1.780 µg/L; BBP was only detected in 1 carbonated sample after one-year stored at a concentration of 2.580 µg/L and DEHP was detected in 2 carbonated fresh water samples at 1.640 µg/L and 5.780 µg/L, respectively. On the other hand, DEHA was only detected in 1 fresh still water sample at 0.182 µg/L. OP was detected in 3 samples in fresh water and in 2 samples after one-year stored at concentrations of 0.003-0.005 µg/L (median 0.004 µg/L) and 0.007-0.051 µg/L (median 0.029 µg/L), respectively. NP was detected in 1 sample in fresh water and in 5 samples after one-year stored at concentrations of 0.064 µg/L and 0.057-0.430 µg/L (median 0.120 µg/L), respectively. The increase of NP with storage is explained by the migration of this compound through the time, as it was observed for crown caps. BPA was only detected in 4 samples in fresh water at concentrations from 0.045 to 0.135 µg/L (median 0.087 µg/L), traces were not observed after one-year stored. There was no relationship between fresh and one-year samples. Among fresh water and one-year stored water, the highest concentrations were detected after storage, indicating that the compounds migrate from plastic into water.

Among compounds, DEHP was only found in 2 out of 40 samples, which corresponds to the 5 % of the metallic screw-cap glass samples. Thus migration observed in this screw-on cap is less important than migration detected in crown caps. DEHP was found even at a higher concentration than in glass bottles with metallic crown cap, although the frequency of detection of DEHP was much lower than in glass bottles with crown caps. This difference may be due to the use of a different plastic material to manufacture crown cap and screw-cap liners.

Overall, the migration of most compounds was less significant than the migration observed for glass bottles with the crown cap, indicating that the cap has a strong influence on the presence of plasticizers in glass bottled water.

3.4. Glass bottle with HDPE cap

Total analyzed samples were 4 for each period. DEP, DBP, BBP, DEHP, DEHA, OP and BPA were not detected. In a 8 L bottle fresh water, DMP was only detected at a concentration of 0.063 µg/L and NP was detected at 0.219 µg/L after one-year stored. The results obtained on the plasticizers analyzed show a low potential of migration for this material. It is confirmed that the cap material produces the migration of contaminants in the other glass formats.

3.5. PP bottle with HDPE cap

Only one sample (8 L bottle) was analyzed for each period. DEP, DBP, BBP, DEHA, NP and BPA were not detected. DMP and DEHP were detected in fresh water at concentrations of 0.021 and 1.840 µg/L, respectively, and contained OP at 0.002 µg/L after one-year stored. So, there was not a relationship between fresh and one-year stored samples.

3.6. PC bottle with LDPE cap

Total analyzed samples were 20 for each period. PC bottles are used in watercoolers and they have a polystyrene (PS) or silicone liner inside the cap, which have been shown to contribute to the migration of plasticizers (Guart et al., 2011).

BPA was by far the most ubiquitous compound detected in 60% in fresh water (12 samples out of 20) and in 95% (19 samples) after one-year stored and at concentrations of 0.032-24.20 µg/L (median 0.365 µg/L) and 0.074-22.17 µg/L (median 0.550 µg/L), respectively. Therefore, there was a slight increase in the concentration of BPA with storage time due to migration, except for 3 samples that their amount decreased from 4.170 to 0.651 µg/L (20 L jug), from 24.20 to 0.490 µg/L (18.9 L jug) and from 4.140 to 0.975 µg/L (18.9 L jug). In 4 samples, BPA

exhibited concentrations of 24.20 µg/L (18.9 L jug, freshwater), 13.20 µg/L (18.9 L jug, freshwater), 15.64 µg/L (18.9 jug, freshwater) and 22.17 µg/L (18.9 L jug, one-year stored), although these high concentrations were outliers in comparison with median values. Overall, a high variability was observed (mean of 3.057 ± 6.844 µg/L and 3.533 ± 6.409 µg/L for fresh and one-year stored samples, respectively) and it suggests that the usage of PC bottles can affect the migration of BPA. This migration can occur from: (i) new bottles, by the transfer of non-polymerized BPA during PC container manufacturing; or (ii) reused bottles, by degradation of the PC. Nerín et al. (2003) detected BPA migration from PC plastic for microwave use when stored at room temperature at concentration of 30 µg/g of polycarbonate. Le et al. (2008) stored used and new PC bottles for 7 days at room temperature and obtained BPA concentrations of 0.7 and 1.0 µg/L for used and new PC bottles, respectively. Amiridou and Voutsa (2011) showed a small increase of BPA concentration from 0.112 to 0.170 µg/L after 30 days storage outdoor and direct sunlight exposition of a 18.9 L PC water reusable container. The U.S. FDA (Food & Drug Administration of the United States) analyzed BPA in 18.9 L PC bottles from water suppliers stored for 39 weeks and found BPA at levels between 0.1 and 4.7 µg/L (EPA, 1993). Biles et al. (1997) detected BPA in PC bottles at 5 µg/L. The Unit on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) (EFSA, 2006) exposed PC bottles to 100ºC for 1h showing BPA levels of 0.23 ± 0.12 µg/L, while levels increased to 8.4 ± 4 µg/L and 6.7 ± 4 µg/L after using a domestic dishwasher between 51 and 169 times. The results of this study corroborate BPA migration in reused containers after several washing cycles.

Other compounds were seldom detected. DMP and BBP were not detected in any sample. DEP was detected in 2 samples in fresh water at concentrations of 0.920 (18.9 L jug) and 2.460 µg/L (20 L jug). This last sample also contained DEHA, DEHP and BPA at concentrations of 0.257, 1.790 and 0.093 µg/L, respectively.

DBP was detected in 1 fresh water at a concentration of 0.736 µg/L (18.9 L jug) and DEHP was found in 2 samples, one in a fresh water sample and other one in a one-year stored sample at 2.810 µg/L and 1.790 µg/L, respectively. OP was detected in 3 fresh water samples of 18.9 L and in 5 samples after one-year stored at concentrations of 0.004-0.037 µg/L (median 0.010 µg/L) and 0.002-0.128 µg/L (median 0.009 µg/L), respectively. NP was detected in one fresh water at a concentration of 0.537 µg/L (13 L jug) and in 6 samples after one-year stored at concentrations of 0.061-2.420 µg/L (median 0.138 µg/L).

Overall, BPA was detected in 77% of the PC samples, indicating that BPA must be monitored in this kind of containers. It is important to indicate that the

high migration could be associated with bottles which have been washed several times (reused bottles). The ubiquitous presence of BPA is attributed to migration from PC containers, whereas the LDPE caps or the liner can be the source of APs and some phthalates, as demonstrated in a previous study with forced migration (Guart et al., 2011).

3.7. PET bottle with HDPE cap

PET bottles are the most commonly used water format in the Spanish market. Total analyzed samples were 224 for each period. Phthalates were detected in 12 fresh samples and in 37 one-year stored, at concentrations of 0.022-20.50 µg/L and 0.635-4.300 µg/L, respectively. DBP was not detected. DMP was only detected in one fresh water sample at a concentration of 0.022 µg/L (5 L bottle). DEP was detected in 8 samples in fresh water and in 22 samples after one-year stored at concentrations of 1.020-20.50 µg/L (median 1.310 µg/L) and 0.857-4.300 µg/L (median 1.225 µg/L), respectively. BBP was detected in 2 samples in fresh waters and in 3 samples after one-year stored at concentrations of 0.619-1.280 µg/L (median 0.950 µg/L) and 0.635-3.010 µg/L (median 2.550 µg/L), respectively. DEHP was detected in 1 sample in fresh water at 1.520 µg/L and in 12 samples after one-year stored at concentrations of 1.020-12.97 µg/L (median 1.825 µg/L). DEHA was detected in 4 samples in fresh water at 0.283-1.470 µg/L (median 0.507 µg/L) and in 14 samples after one-year stored at concentrations of 0.185-6.230 µg/L (median 0.506 µg/L), respectively. DEHA is considered as an effective replacement for DEHP (Bolgar et al., 2008).

OP was detected in 10 samples in fresh water and in 18 samples after one-year stored at concentrations of 0.002-0.011 µg/L (median 0.005 µg/L) and 0.002-0.023 µg/L (median 0.005 µg/L), respectively. NP was detected in 5 samples in fresh water and in 16 samples after one-year stored at concentrations of 0.062-0.538 µg/L (median 0.129 µg/L) and 0.058-2.030 µg/L (median 0.125 µg/L), respectively. BPA was detected in 12 samples in fresh water and also in 12 samples after one-year stored at concentrations of 0.042-0.191 µg/L (median 0.159 µg/L) and 0.037-0.819 µg/L (median 0.058 µg/L) µg/L, respectively. Overall, the number of samples with levels of detected compounds increased in one-year stored, although there was little variation in the concentrations detected.

These results corroborate other studies which investigates phthalates in PET. Cao (2008) identified the presence of phthalates in bottled water at 0.054-0.1 µg/L (DEP), 0.08-0.32 µg/L (DBP) and 0.05-0.093 µg/L (DEHP). Bošnir et al. (2007)

detected DEP, DBP and DEHP at ranges of <0.04-1 µg/L, <0.04-50 µg/L and <0.04-50 µg/L, respectively, in natural mineral water after 30 days exposure at a temperature of 22 ºC. Casajuana and Lacorte (2003) detected phthalates in PET bottled water stored for 10 weeks at 30ºC obtaining ranges of 0.082-0.355 (DEP), 0.020-0.070 (DBP), <0.004-0.010 (BBP) and <0.002-0.188 µg/L (DEHP). In addition, Leivadara et al. (2008) detected DEHP at <0.002-6.8 µg/L in natural mineral water after 3 months contact at temperatures up to 30 ºC. Schmid et al. (2008) detected DEHP at 0.10-0.71 µg/L after 17h contact in darkness/sunlight at room temperature and at 60 ºC. Regarding a previous study, forced plastic migration assays for PET bottles showed a migration of 0.332 µg/dm^2 for NP (Guart et al., 2011). Amiridou and Voutsa (2011) found 0.0046 µg/L of BPA in water bottled in PET and 0.112 µg/L in water bottled in PC. They repeated the test after 15 and 30 days, showing an increase in BPA up to 0.170 µg/L Keresztes et al. (2013) studied the leaching of phthalates from PET bottles into natural mineral water where DEHP was the most abundant phthalate up to 1.7 µg/L.

However, according to the PET report made by the Fraunhofer Institut for Process Engineering and Packaging (EFBW, 2013), PET does not contain plasticisers because they can make the plastic "softer". In opinion of the author, the aim for the PET bottle is to be stiff and rigid, so it would be a contradiction to use plasticizers in PET. The report also indicates that there is no BPA in PET. So, it remains unclear the occurrence and source of plasticisers and BPA detected in PET bottles. Guart el at. (2011) detected NP and BPA in HDPE caps at concentrations of 1.282 µg/dm^2 and 0.145 µg/dm^2; however BPA was not detected in the PET bottles cut in pieces. These results show the migration potential of plasticizers from the caps. Thus, as it was indicated in Bach et al. (2012), a source of BPA in PET-bottled water could be the containers' caps.

PET bottle with HDPE cap represents the most used container for bottled water and was the container with less incidence of the evaluated compounds which could leach from the cap.

3.8. HDPE bottle with HDPE cap

Total analyzed samples were 7 for each period. DBP, BBP, DEHP, DEHA and BPA were not detected. DMP was detected in 2 fresh water treated samples at concentrations of 0.085 and 0.230 µg/L. DEP was found in 3 samples in one-year stored at concentrations of 2.060 µg/L (5 L bottle), 6.440 µg/L (8 L bottle) and

9.460 µg/L (8 L bottle). OP and NP were detected in a 5 L bottle in one-year stored at concentrations of 0.007 and 0.059 µg/L, respectively.

Among all compounds analyzed, DEP was the only compound of concern which has been proved that migrates from this type of plastic, because it has been found in high amounts.

3.9. LDPE bag

Only one sample was analyzed for each period. DMP, DBP, BBP, DEHP, DEHA, OP and BPA were never detected whereas DEP and NP were detected at concentrations of 1.160 and 6.980 µg/L after one-year stored. Although there is only one sample, the amounts of these two compounds are high, especially for NP that was the highest of all the samples.

3.10. Daily intakes

The experts set up a safe level of exposure (acceptable daily intake (ADI) or tolerable daily intake (TDI)) and estimate the human exposure to chemicals from the diet and from specific foods. Such exposure assessments often are based on national or international data from the World Health Organisation (WHO) (WHO, 2009). As alkylphenols, phthalates and BPA are considered as endocrine disrupting compounds (EDCs) (WHO/IPCS, 2002), target compounds have an established TDI except for DMP and OP. Table 3 shows the calculated daily intake from bottled water for each compound and comparison with TDI. It was considered the highest concentration detected and 2 L water ingestion per day for a 60 kg person. In all cases, values are well below TDI. Furthermore, Table 3 shows the water consumption requirement to achieve the TDI by using the highest detected concentrations detected in bottled waters. DEP, DBP, BBP, DEHP, DEHA and BPA require more than 100 L water per day, and NP would require 43 L water consumption. So, taking into account the low number of positive samples and water daily intakes together, the possibility of getting health problems due to bottled water ingestion is non-existing.

Table 3. Comparison between the water daily intake of each compound vs. the stablished TDI.

Compound	Highest concentration [µg/L]	Water daily intake [mg/kg bw/day]	TDI [mg/kg bw/day]	TDI water consumption [L/day]
DMP	0.230	$0.077x10^{-4}$	-	-
DEP	20.50	$6.8x10^{-4}$	0.5[a]	1463
DBP	0.736	$0.24x10^{-4}$	0.01[b]	815
BBP	3.010	$1x10^{-4}$	0.5[c]	9967
DEHP	12.97	$4.3x10^{-4}$	0.05[d]	231
DEHA	6.230	$2.1x10^{-4}$	0.3[e]	2889
OP	3.160	$1x10^{-4}$	-	-
NP	6.980	$2.3x10^{-4}$	0.005[f]	43
BPA	24.20	$8.1x10^{-4}$	0.05[g]	124

[a] WHO (2003)
[b] EFSA (2005a)
[c] EFSA (2005b)
[d] EFSA (2005c)
[e] EFSA (2005d)
[f] Danish Environmental Protection Agency (2000)
[g] EFSA (2010)

4. Conclusions

Plastic components, as monomers or additives, migrate in very low amounts into water during bottling and storage with only 5.6 % of the 6516 data values being positive (9 compounds x 362 samples x 2 analysis periods). In all cases, all values were below TDI limits. Water characteristics and bottling process, which vary for each bottled water brand, can cause different migration rates from plastic into water. Bottles made of PET with HDPE cap represent the most part of Spanish bottled water market and they constitute the main part of the present study. Analyses of water in contact with PET showed that there was a low incidence of target compounds in contrast to glass bottles with metallic crown cap and PC bottles with LDPE cap showed the highest occurrence. The most remarkable case was BPA results in PC jugs; BPA was detected in the 60 % of samples in fresh samples and in the 95 % in one-year stored samples. In addition, DEHP was present in glass bottles with metallic crown-cap which increased from 27 % in fresh water to 39 % in one-year stored. The presence of specific contaminants depends on the plastic type rather than on the volume, where the cap material plays an important role in the migration of plasticizers. Another fact to take into account is the presence of gas (carbonated water) which may enhance the migration of some plastic constituents due to the lower pH of water. The obtained results can be

useful for bottling industries and for cap and resin distributors, which are constantly improving and developing their products to limit migration and maintain water source characteristics.

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2.3. Discussió dels resultats

2.3.1. Mètode analític

El mètode desenvolupat va permetre l'anàlisi dels 21 compostos (herbicides, ftalats, DEHA, alquilfenols i BPA) en aigua subterrània (article científic I) i en aigua envasada (article científic 2). Amb la rampa de temperatures utilitzades en la GC es va obtenir una bona resolució dels 21 compostos en 46 min de cromatograma i l'ús de la MS va permetre la correcta identificació i quantificació dels compostos.

Pel què fa als LODs i LOQs, es van calcular segons recomana la Comissió Europea (EU, 2002c) i segons la forma de calcular-los cal dividir els compostos en dos grups:

- No contribució en els blancs: els LODs i els LOQs es van calcular a partir d'una relació senyal/soroll (S/N) de 3 i de 10, respectivament, de la mostra fortificada de concentració més baixa de 0.01 µg/L per tots els compostos, excepte pels ftalats que era de 1 µg/L. Els compostos corresponents a aquest grup van ser el 4-OP, el 4-NP, el BPA i tots els herbicides, en què es van obtenir LODs entre 0.0001 i 0.0086 µg/L i LOQs entre 0.0004 i 0.0290 µg/L per als herbicides, mentre que els LODs i LOQs dels alquilfenols i BPA van ser d'entre 0.001 i 0.017 µg/L i d'entre 0.002 i 0.057 µg/L, respectivament.
- Contribució en els blancs: els LODs i els LOQs es van calcular a partir de l'equació $x = x_b + k.S_b$ (on x_b és la mitjana aritmètica de les concentracions dels blancs analitzats, k és 3 per calcular els LODs i 10 per calcular els LOQs, S_b és la desviació estàndard dels blancs, i x és la concentració corresponent al LOD o al LOQ). Els compostos corresponents a aquest grup van ser tots els ftalats i el DEHA, en què es van obtenir LODs entre 0.01 i 0.46 µg/L i LOQs entre 0.018 i 0.970 µg/L.

La utilització de blancs és molt important per a evitar els falsos positius. Pel què fa als components del plàstic, especialment pels ftalats, els punts més usuals de contaminació de la mostra són els dissolvents, els reactius, l'equipament del laboratori, els instruments analítics i l'atmosfera de la zona de treball (Capdeville and Budkinski, 2011). Així doncs, els blancs permeten conèixer les fonts de contaminació i poder eliminar-les o tenir-les controlades.

Pel què fa a les recuperacions, el mètode va permetre obtenir recuperacions entre el 77 i el 124 %, i amb desviacions estàndard relatives (RSD) d'entre 0.6 i 7.1 %, utilitzant mostres d'aigua Milli-Q fortificades amb els diferents compostos

diana a concentracions de 0.01 i 0.1 µg/L. Degut als elevats LOQs dels ftalats i DEHA es van utilitzar mostres fortificades a una concentració d'1 µg/L (article científic I).

2.3.2. Anàlisi de les mostres

L'anàlisi de les mostres va permetre determinar quins són els punts més susceptibles de contaminació, així com relacionar la migració dels anàlits segons el tipus d'envàs i tap (Taula 6).

En el punt de captació (article científic I), és a dir, en l'aigua abans d'envasar, es van detectar 9 dels 21 compostos analitzats. Tot i així, entre aquests 9 compostos, només es van detectar 24 vegades per sobre del LOQ d'un total de 2751 dades (131 mostres x 21 compostos), que significa un 0.87 %. Aquesta dada conjuntament amb el fet que en 111 mostres no es va detectar cap compost per sobre el LOQ, indica la bona qualitat de les aigües minerals naturals i de les aigües de brollador d'Espanya. Pel què fa als compostos detectats, el compost més freqüent va ser la desetil atrazina (n=7), seguit pel BPA (n=6) d'un total de 131 mostres, és a dir, aproximadament en un 5 % de les mostres analitzades. Les concentracions màximes trobades van ser de 1.115 µg/L pel DEP, 0.203 µg/L pel BPA i 0.192 µg/L pel DEHA, la resta de compostos es van detectar per sota de 0.059 µg/L. Les deus on es van detectar traces d'herbicides són zones poc protegides i aqüífers poc profunds, la qual cosa fa que la contaminació ambiental pugui afectar la qualitat de les aigües envasades. Això vol dir que en els casos en què el punt de captació estigui exposat a la contaminació ambiental, especialment d'origen agrícola, cal prendre mesures addicionals durant el procés d'envasat per evitar la presència de traces en l'aigua envasada. Per tant, el resultat d'aquest treball ha permès identificar punts problemàtics on es pot incidir per millorar la qualitat de l'aigua. Globalment, el més important és que a diferència dels aqüífers situats en zones agrícoles o industrialitzades, les aigües de captació estan lliures de contaminació, la qual cosa indica que les mesures de protecció dels aqüífers destinats a l'aigua envasada són adequades.

Un cop les aigües van ser envasades (article científic II), es va tornar a realitzar l'anàlisi dels diferents anàlits per a tots els formats d'envàs i per a cadascuna de les aigües de captació. El primer fet que cal indicar és que els herbicides es van trobar a les mostres d'aigua envasada corresponents a les

mostres d'aigua de captació en què s'hi van detectar, és a dir, les baixes concentracions d'herbicides trobades abans de l'envasat també són presents després de l'envasat. Aquest fet era d'esperar ja que l'aigua s'envasa directament i no pateix cap tractament per eliminar les baixes concentracions dels possibles compostos que es troben a les aigües subterrànies. En quant als components del plàstic, els ftalats, DEHA, alquilfenols i BPA van ser detectats, com a mínim, en una de les mostres. Es van detectar 132 compostos per sobre del LOQ d'un total de 3258 dades (9 compostos x 362 mostres), és a dir, només en un 4 %. Dins d'aquest 4 %, les concentracions més elevades corresponien al BPA amb 24.20 µg/L en un envàs de PC i al DEP amb 20.50 µg/L en un envàs de PET.

Després d'un any d'emmagatzematge dins el propi envàs (article científic II), els herbicides es continuaven detectant a concentracions semblants. Pel què fa als monòmers i additius del plàstic, es van detectar 8 dels 9 compostos estudiats. El DBP no va ser detectat en cap mostra. Es van detectar 236 compostos per sobre del LOQ d'un total de 3258 dades (9 compostos x 362 mostres), és a dir, en aproximadament un 7 %. Dins d'aquest 7 %, les concentracions més elevades corresponien al BPA amb 22.17 µg/L en un envàs de PC i al DEP amb 9.460 µg/L en un envàs de HDPE amb tap de HDPE.

Tenint en compte tots els resultats de forma global (articles científics I i II), es pot observar que la presència de contaminants a l'aigua de captació és molt minsa i, per tant, el perímetre de protecció establert a les deus es considera una mesura adequada de contenció de la contaminació de les aigües subterrànies. Per altra banda, tot i un augment de resultats per sobre el LOQ de 0.87 % de l'aigua de captació a 4 % en l'aigua envasada i a 7 % després d'un any d'emmagatzematge dins el propi envàs, el % de deteccions continua sent molt baix. Per tant, es pot afirmar que els mètodes d'envasat són adequats, així com la bona qualitat dels envasos utilitzats. A més a més, les concentracions trobades són molt baixes excepte per alguns casos excepcionals en què han estat més elevades, tal i com es mostra al segon article científic.

Per altra banda, aquest estudi ha permès avaluar la migració específica dels components del plàstic estudiats segons el tipus de material de l'envàs (article científic II). És possibles que degut a les diferents etapes de la producció d'un plàstic hi hagi una falta d'informació de tots els compostos que s'han utilitzat com a iniciadors de polimerització o dels additius que s'hi ha addicionat. Així doncs, a la Taula 6 s'han tingut en compte els compostos que estaven presents en l'aigua

continguda en un tipus d'envàs i que superaven la mitjana global en % (5.6 % tenint en compte les aigües envasades i les aigües emmagatzemades).

Entrant en detall en els diferents tipus d'envàs, en l'envàs de vidre amb tap corona o roscat i sèptum de plàstic s'han detectat alquilfenols que poden provenir d'una etapa inicial de la polimerització del plàstic i que hi resten com a residus, o de la neteja del envasos amb un detergent que contè NP. Per altra banda, els alquilfenols, el BPA i els ftalats poden migrar del sèptum de plàstic que està localitzat a l'interior de tap i en contacte amb l'aigua. En aquest estudi, el DEHP es va trobava majoritàriament en aigües carbonatades, és a dir, amb pH més baix. Aquest fet corrobora les conclusions de Bošnir et al. (2007) que indiquen que el pH per sota de 3 de les begudes refrescants, en contrast amb l'aigua mineral amb pH superior a 5, afavoriria la migració de ftalats de 5 a 40 vegades més.

En el PC s'han detectat el NP, l'OP i el BPA. Els alquilfenols podrien provenir de la migració del plàstic degut al seu ús com a additiu o en etapes inicials de la polimerització, tot i que no podem saber la seva procedència. El BPA, que és el monòmer utilitzat en la fabricació del PC, és el compost detectat de forma més ubiqua. Mercea (2009) explica que hi ha dos possibles mecanismes per a la migració de BPA des del PC als simulants alimentaris. Podria ser per un procés de difusió de BPA residual de la resina inicial durant l'etapa de fabricació del PC o degut a una hidròlisi a la superfície del plàstic. Aquest últim cas podria succeir sota condicions típiques d'ús quan el PC està en contacte amb l'aigua o durant l'etapa de rentat. També destaca la possibilitat que el PC es fabriqui de diferents formes i, per tant, això provocaria diferències en les propietats fisicoquímiques del PC. Una de les conclusions a les que arriba Mercea en el seu estudi és que rentant els contenidors de PC un major o menor nombre de cops, abans de ser omplerts amb aigua, no provoca canvis significatius en la migració de BPA. Per altra banda, conclou que el 98 % de la variabilitat observada en la migració de PC depèn de la temperatura, el pH de l'aigua, la concentració d'ozó a l'aigua, la interacció entre pH i temperatura i la interacció entre ozó i temperatura.

Per altra banda, l'envàs de PET i tap de HDPE, que és el més utilitzat per a l'envasat d'aigua, ha demostrat ser l'envàs en què menys quantitat de compostos s'hi ha detectat. Només s'ha detectat el DEP (7 %) i OP (6 %) per sobre de la mitjana trobada a l'estudi (5.6 %) i a concentracions molt baixes, tal i com es pot observar en el segon article científic.

Pel què fa a l'envàs de vidre amb tap de HDPE, l'envàs de HDPE, l'envàs de PP i la bossa de LDPE, els compostos trobats han migrat del plàstic ja que poden haver estat utilitzats com a additius dels polímers. Cal tenir en compte el reduït nombre de mostres que s'han analitzat per aquests tipus d'envàs en comparació amb els altres envasos, ja que el seu ús en el mercat espanyol és més reduït.

Taula 6. Migració dels diferents compostos en relació amb el material de l'envàs i del tap (s'indiquen el compostos que s'han detectat per sobre la mitjana de les aigües envasades i emmagatzemades, 5.6 %).

Material de l'envàs	Material del tap	Compostos identificats (% >LOQ)
Vidre (n=170)	Metàl·lic de tipus corona amb sèptum de plàstic	DEHP (33%) NP (13 %) BPA (12 %) DEP (11 %)
Vidre (n=40)	Metàl·lic de tipus roscat amb sèptum de plàstic	OP (12 %) BPA (10 %)
Vidre (n=8)	HDPE	DMP (12 %) NP (12 %)
PP (n=2)	HDPE	DMP (50 %) DEHP (50 %) OP (50 %)
PC (n=40)	LDPE	BPA (77 %) OP (20 %) NP (17 %)
PET (n=448)	HDPE	DEP (7 %) OP (6 %)
HDPE (n=14)	HDPE	DEP (21 %) DMP (14 %) OP (7 %) NP (7 %)
LDPE (bossa) (n=2)	-	DEP (50 %) NP (50 %)

A partir dels valors obtinguts es va determinar la ingesta diària de cada compost a través del consum d'aigua i es va comparar amb la ingesta diària tolerable (TDI). Tenint en compte les concentracions màximes de cada compost, un pes d'una persona de 60 kg, que es begui 2 L d'aigua al dia i considerant que no hi ha cap altra contribució per part dels compostos en la ingesta d'altres aliments, s'haurien de beure entre 43 i 9967 L per a arribar a la TDI establerta pels diferents compostos estudiats.

2.4. Conclusions

- El mètode de SPE-GC/MS és un mètode eficaç per al control de plastificants i herbicides en aigua subterrània i aigua envasada. S'han detectat ftalats en els blancs analitzats i per aquesta raó els LODs i LOQs són més elevats que pels altres compostos analitzats.
- El perímetre de protecció de les aigües de captació és efectiu.
- L'aigua emmagatzemada presenta un petit augment en el nombre de deteccions per sobre del LOQ respecte a l'aigua envasada recent i, per tant, es pot concloure que hi ha migració de monòmers i additius del plàstic a l'aigua envasada.
- El BPA es troba present en gairebé totes les mostres d'aigua envasades en garrafons de PC amb tap de LDPE i sèptum de PS
- El DEHP es troba present en un elevat % de les mostres d'aigua envasades en ampolles de vidre amb tap corona i sèptum de plàstic.
- Els valors estimats pels diferents compostos mostren concentracions molt per sota dels indicats a les TDI.

3. ANÀLISI CONTINENTAL DE L'AIGUA ENVASADA

3. ANÀLISI CONTINENTAL DE L'AIGUA ENVASADA

3.1. Introducció

El llibre "The World's Water" (Gleick et al., 2011) descriu l'augment del consum d'aigua als països on hi ha un major consum. Espanya es troba al setè lloc amb un augment en el consum d'aigua envasada de 102 a 124 L/persona a l'any des de 1999 a 2010; darrera de Mèxic (243 L/persona), Itàlia (187 L/persona), Emirats Àrabs Units (153 L/persona), Bèlgica-Luxemburg (148 L/persona), Alemanya (134 L/persona) i França (132 L/persona) a l'any 2010. Altres països a destacar són Els Estats Units d'Amèrica amb 107 L/persona i Xina/Hong Kong amb 95 L/persona.

Aquest estudi es va centrar en la determinació de components del plàstic que poden estar presents en l'aigua envasada i en la determinació d'altres compostos semivolàtils descrits a la legislació d'aigües envasades en mostres d'aigua envasada de diferents continents arreu del món. Aquests altres compostos pertanyen als grups de triazines, pesticides organofosforats (OPPs) i organoclorats (OCPs), piretroids, hidrocarburs aromàtics policíclics (PAHs) i bifenils policlorats (PCBs).

Els OPPs són insecticides que s'usen en l'agricultura contra diverses plagues. Són compostos que poden ser hidrolitzats per l'aigua, adsorbits en el sediments o degradats en el sòl. Es poden trobar en l'aigua per al consum humà i s'utilitza per al control dels mosquits, mosques o diverses plagues en cultius, plagues domèstiques i larves aquàtiques. Segons la WHO, no es recomana la seva addició en l'aigua de xarxa com a larvicida amb fins sanitaris, però és possible que en alguns països s'utilitzi com a larvicida (WHO, 2011).

Els piretroids són insecticides que s'utilitzen domèsticament i a l'agricultura per al control dels mosquits. El seu ús ha augmentat com a substitut dels OPPs degut a què són menys tòxics per a la salut humana, per a les aus i per als mamífers (EPA, 2013b).

Els OCPs són pesticides utilitzats en l'agricultura de països en vies de desenvolupament i són coneguts com a contaminants orgànics persistents o "persistent organic pollutants" (POPs). Són molt persistents i bioacumulables. Alguns d'ells, com ara, el dieldrin, el clordè, el DDT, l'heptaclor i l'hexaclorbenzè, es poden arribar a trobar en aigües per al consum humà, representant fins a 1 % de la

TDI (WHO, 2011). També cal destacar que el DDT es continua utilitzant però només per al control de mosquits que puguin ser transmissors de la malària (EPA, 2012).

Els PAHs es troben de forma natural al medi ambient però també són productes derivats de l'activitat humana. Els PAHs es creen a partir de la combustió incompleta de productes com el carbó, el petroli i el gas. Alguns PAHs, com el naftalè, s'utilitzen per a fabricar tints, plàstics, pesticides i alguns medicaments. Degut a tots aquests possibles orígens, les fonts d'exposició més comunes per l'ésser humà són el fum dels vehicles, el carbó, l'asfalt, els incendis forestals, focs agrícoles i fum del tabac. Cal destacar que els PAHs són persistents i poden romandre en el medi ambient durant períodes llargs de temps i no es descomponen fàcilment a l'aigua (USEPA, 2008).

Els PCBs són substàncies químiques fabricades per l'ésser humà i són coneguts com a POPs. Des de 1930 fins el 1980 es van fabricar a gran escala en tot el món. Els PCBs són químicament estables, són resistents a la calor i s'utilitzen en diversos sectors (European Environmental Agency, 2011). Segons les seves aplicacions, es poden classificar per a sistemes tancats, com ara en fluids dielèctrics de transformadors, condensadors i sistemes hidràulics, i per a sistemes oberts, com ara en pesticides, plàstics, pintures i adhesius (OSPAR, 2004). Els PCBs poden ser transportats a grans distàncies a través del medi ambient i s'han arribat a trobar en punts remots del món que es troben molt lluny del lloc on són fabricats i utilitzats (European Environmental Agency, 2011).

Per a realitzar l'anàlisi conjunt de tots aquests compostos (n=69) es va desenvolupar un mètode basat en l'extracció per barretes adsorbents (SBSE) acoblada a la cromatografia de gasos i espectrometria de masses en tàndem (GC-MS/MS). Aquest mètode s'ha aplicat a l'anàlisi de setanta-set mostres que s'han recollit en vint-i-set països d'arreu del món. Totes les aigües estan envasades en PET, que és el plàstic més utilitzat per a la fabricació d'envasos no reutilitzables destinats a l'envasat d'aigua. A més a més, el fet de tenir totes les mostres envasades en PET permet eliminar la variable del tipus d'envàs i així permetre una comparació més objectiva entre les diferents mostres (article científic III).

3.2. Treball experimental

El treball experimental descrit a l'article científic III descriu el desenvolupament d'un mètode d'anàlisi multiresidual, basat en la GC-MS/MS, en què es van utilitzar tres modes d'operació diferent:

1. Scan d'ions precursor (en anglès "precursor" or "parent ion scan").

El Q1 es disposa en mode scan per un rang de 50 a 450 m/z (massa/càrrega), Q2 actua com a cel·la de col·lisió i Q3 transmet els ions producte fins al detector. L'espectre de masses resultant permet seleccionar els ions precursors adequats per cada compost.

2. Scan d'ions producte o fills (en anglès "product" o "daughter ion scan").

El Q1 transmet els ions precursor seleccionats, Q2 els fragmenta per dissociació induïda per col·lisió i Q3 opera en mode scan (50-450 m/z). El resultat és un espectre de masses obtingut a partir dels ions precursors a partir del qual es seleccionen els ions producte. En el cas dels PAHs no hi ha fragmentació ja que són compostos amb una estructura molt estable.

3. Monitoratge d'una reacció seleccionada (en anglès "selected reaction monitoring (SRM)" o "multiple reaction monitoring (MRM)".

El Q1 es disposa per a seleccionar un nombre limitat d'ions precursor (a la Figura 5), Q2 continua com a cel·la de col·lisió i Q3 transmet un nombre limitat d'ions producte. L'espectre de masses resultant mostra una proporció entre l'abundància dels ions precursor i els ions producte (transicions), com es pot apreciar a la Figura 6. Aquest mode d'operació permet obtenir uns resultats més específics i sensibles que en un MS simple S'utilitza una transició com a quantificadora i una com a qualificadora.

Un cop desenvolupat el mètode d'anàlisi es van determinar els 69 compostos en aigües envasades de tot el món tal i com s'indica a l'article científic III.

Acquisition | Instrument | Chromatogram

Scan segments

	Compound name	ISTD?	Precursor ion	MS1 resolution	Product ion	MS2 resolution	Dwell	Collision energy
▶	BHT	☐	205	Wide	177	Wide	10	15
	BHT	☐	205	Wide	145	Wide	10	25
	DTBP	☐	191	Wide	175	Wide	10	20
	DTBP	☐	191	Wide	57	Wide	10	25
	DMIP	☐	163	Wide	135	Wide	10	20
	DMP	☐	163	Wide	135	Wide	10	15
	DMTP	☐	163	Wide	135	Wide	10	10
	DMIP	☐	163	Wide	120	Wide	10	20
	DMTP	☐	163	Wide	103	Wide	10	20
	DMP	☐	163	Wide	77	Wide	10	25

Figura 5. Pantalla extreta del programa Masshunter d'Agilent on s'hi seleccionaven les diferents transicions per cadascuna de les finestres del cromatograma.

En la Figura 6, es pot veure el cromatograma amb la transició 163>135 característica del DMTP (8.512 min) i DMIP (8.688 min). Degut a què tenen el mateix pes molecular i una estructura molt semblant, es diferencien per l'abundància de les transicions i pel temps de retenció. Per tant, va ser necessari injectar-los prèviament per a saber quin temps de retenció corresponia a cadascun. Aquesta mateixa transició també es pot apreciar en 2,4-DTBP (8.608 min).

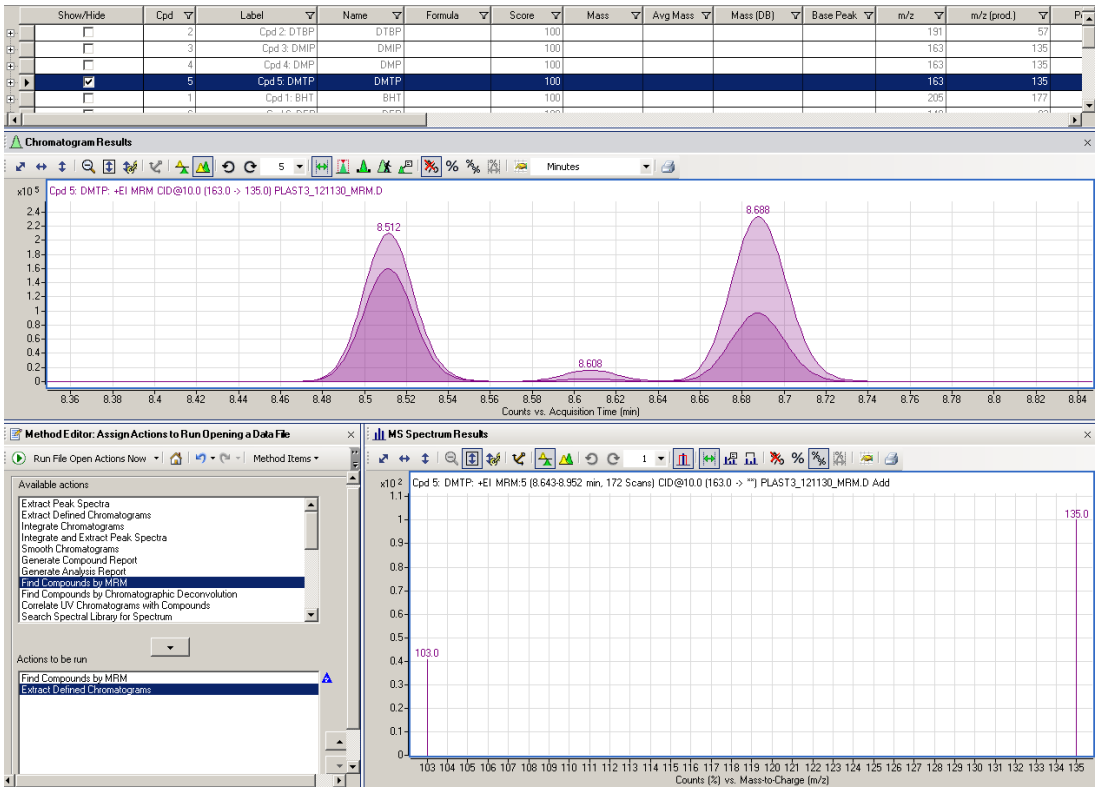

Figura 6. Cromatograma i espectre de masses de la transició 163>135 corresponent al DMTP i DMIP (Agilent Masshunter per a l'anàlisi qualitatiu).

Article científic III

Títol: Continental bottled water assessment by stir bar sorptive extraction followed by gas chromatography-tandem mass spectrometry (SBSE-GC-MS/MS)

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Continental bottled water assessment by stir bar sorptive extraction followed by gas chromatography-tandem mass spectrometry (SBSE-GC-MS/MS)

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Abstract

Background, aim and scope This study was aimed to determine the presence of 69 organic contaminants in 77 representative bottled water collected from 27 countries all over the world. All waters samples were contained in polyethylene terephthalate (PET) bottles. Target compounds were (i) environmental contaminants including 13 polycyclic aromatic hydrocarbons (PAHs), 31 pesticides including organochlorine (OCPs) and organophosphorus (OPPs) and pyrethroids, 7 polychlorinated biphenyls (PCBs) and 7 triazines, and (ii) plasticizers including 6 phthalates and 5 other compounds.

Materials and methods Samples were analyzed by stir bar sorptive extraction followed by gas chromatography-tandem mass spectrometry (SBSE-GC-MS/MS).

Results and discussion PAHs, OCPs, PCBs and triazines, which are indicators of groundwater pollution, were not detected in most of the samples, except for naphthalene (0.005-0.202 µg/L, n=16). On the other hand, plastic components were detected in 77 % of the samples. Most frequently detected compounds were dimethyl phthalate (DMP) and benzophenone (BP) at concentrations of 0.005-0.125 (n=41) and 0.014-0.921 (n=32), respectively.

Conclusions Levels detected are discussed in terms of contamination origin and geographical distribution. Target compounds were detected at low concentrations. Results obtained showed the high quality of bottled water in the different countries around the world.

Keywords: bottled water; migration; polyethylene terephthalate; pesticides; phthalate.

1. Introduction

The worldwide bottled water consumption is constantly increasing. Between 1994 and 2002, the bottled water market grew from 58 to 144 billion litres (Senior and Dege 2005). As some recent examples, the top country in per-capita bottled water consumption is Mexico with a 243 L per person in year 2010. Other countries as the United Arab Emirates, Germany, Spain, United States of America and China/Hong Kong consume 153, 134, 124, 107 and 95 L per person in year 2010, respectively, in accordance with Beverage Marketing Corporation (BMC) (Gleick et al. 2011). Most times, bottled water is extracted from groundwater and thus, its quality must be guaranteed. However, some concern has arouse due to the leaching of organic contaminants to groundwater (Flury 1996) and the potential contamination of aquifers, In Spain, triazines and organophosphorus pesticides (OPPs) have been detected in groundwater from the Ebro river basin as a result of their recurrent use in agriculture (Hildebrandt et al., 2007). In industrial sites from Spain, nonylphenol and octylphenol were detected in groundwater in 13 out of 14 samples indicating that groundwater can act as a reservoir for organic pollutants (Latorre et al. 2003). Triazines such as atrazine, desethyl atrazine and simazine, polycyclic aromatic hydrocarbons (PAHs), bisphenol A (BPA) and benzophenone (BP) were detected in groundwater from the United Kingdom (UK) (Stuart et al. 2012). Therefore, contaminants generated by human activities increase the vulnerability of groundwater (Worrall et al. 2002) and can affect groundwater quality and its further use for drinking purposes. However, when considering waters intended for bottling, a recent study demonstrated the high quality of source waters from Spain, suggesting that the protection of wellhead areas is effective to prevent groundwater contamination (Bono et al. 2012). Other studies report the presence of triazines in 29 out of 35 drinking waters from the waterworks of Italian cities, as well as in 2 out of 5 bottled waters packaged in polyethylene terephthalate (PET) (Maggioni et al. 2013). In Mexico, organochlorine pesticides (OCPs) where detected in 1.5 L bottled drinking water at concentrations up to 0.152 µg/L (Díaz et al. 2009).

Once bottled, an additional concern is related to the potential migration of plastic components as monomers or additives into the bottled water. One of the

most common materials used for plastic bottles is PET which is usually manufactured from terephthalic acid (TPA) or isopthalic acid (IPA) and ethylene glycol. Additives are incorporated to the polymer to allow better processing, to increase stability and to give specific material properties (Piringer and Baner 2008). After bottling, transport and storage of bottles are considered as other points of release of plastic constituents by migration (EU 2011). Casajuana and Lacorte (2003) detected phthalates, alkylphenols and BPA in PET bottles after 10 weeks storage. Similarly, Amiridou and Voutsa (2011) detected phthalates, alkylphenols and BPA in PET samples of five brands when samples were taken and after 14 and 30 days storage.

Stir bar sorptive extraction (SBSE) coupled to gas chromatography coupled to tandem mass spectrometry (GC-MS/MS) has emerged as a promising alternative to other analytical methods for the trace level determination of organic compounds in water. SBSE has been applied to analyze semivolatile compounds in water, where factors affecting SBSE were studied (León et al., 2003; León et al. 2006). Multiresidue methods have been developed to analyze PAHs, OCPs, OPPs, polychlorinated biphenyls (PCBs), triazines and phthalate esters in river water and wastewater effluent (Chary et al. 2012), in irrigation stream water (Peñalver et al. 2003), in seawater and interstitial marine water (Pérez-Carrera et al. 2007), in sea and estuarine waters (Prieto et al. 2007; Sánchez-Avila et al., 2010), in groundwater (Tögyessy et al. 2011) and in bottled water (Serôdio et al. 2004).

This study is aimed to analyze 77 bottled water samples taken from 27 different countries all over the world. Compounds analyzed included (i) 58 environmental contaminants, including 13 PAHs, 31 pesticides belonging to organochlorine (OCP), organophosphorus (OPP) and pyrethroids, 7 PCBs and 7 triazines and (ii) 11 plasticizers that can migrate during bottling and storage including 6 phthalates and 5 other plastic components. The expected low concentrations of these compounds in bottled water and the complexity of phthalates analysis due to their extensive presence in environment were the reasons of optimizing a multiresidue method based in SBSE-GC-MS/MS.

2. Materials and methods

2.1 Chemicals and reagents

13 PAHs including naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, indeno(1,2,3,cd)pyrene, dibenzo(a,h)anthracene and benzo(g,h,i)perylene were purchased as Mix M-8270-13-ASL from Accustandard (New Haven, Connecticut, USA) at a concentration of 2000 mg/L. Twenty-three OCPs and pyrethroids including α-HCH, β-HCH, γ-HCH (Lindane), δ-HCH, heptachlor, aldrin, chlorpyrifos, heptachlor epoxide, endosulfan I, dieldrin, p,p'-DDE, endrin, endosulfan II, chlorobenzilate, p,p'-DDD, endosulfan sulphate, p,p'-DDT, methoxychlor, were purchased as Mix M-508P-A and trifluralin, hexachlorobenzene, DCPA, cis-permethrin (500 mg/L) and trans-permethrin (1500 mg/L) were purchased as Mix M-508P-B-R from Accustandard (New Haven, Connecticut, USA), both mixes at a concentration of 1000 mg/L each. Chlorpyrifos methyl was purchased as a solid from Dr. Ehrenstorfer (Augsburg, Germany) Seven OPP including diazinon, fenthion, parathion methyl, phorate, fenchlorfos (ronnel), trichloronate and tokuthion were purchased as Mix M-8140M-5X from Accustandard (New Haven, Connecticut, USA) at a concentration of 200 mg/L. Seven PCBs including 2,4,4'-trichlorobiphenyl (PCB 28), 2,2',5,5'-tetrachlorobiphenyl (PCB 52), 2,2',4,5,5'-pentachlorobiphenyl (PCB 101), 2,3',4,4',5-pentachlorobiphenyl (PCB 118), 2,2',3,4,4',5'-hexachlorobiphenyl (PCB 138), 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153), 2,2',3,4,4',5,5'-heptachlorobiphenyl (PCB 180) were purchased as PCB-Mix 3 from Dr. Ehrenstorfer (Augsburg, Germany) at a concentration of 10 mg/L each. Atrazine, atrazine-desethyl, prometryn, propazine, simazine, terbuthylazine and terbutryn were purchased as Pesticide-Mix 51 from Dr. Ehrenstorfer (Augsburg, Germany) at a concentration of 10 mg/L each. Phthalate Mix 525 (500 mg/L each in methanol) containing dimethyl phthalate (DMP), diethyl phthalate (DEP), di-n-butyl phthalate (DBP), butyl benzyl phthalate (BBP), bis(2-ethylhexyl) adipate (DEHA), bis(2-ethylhexyl) phthalate (DEHP) was purchased from Supelco (Bellefonte, PA, USA). Dimethyl terephthalate (DMTP, ≥99%) and dimethyl isophthalate (DMIP, ≥99%) were purchased from Sigma-Aldrich (St. Louis, MO). 4 other plastic components including benzophenone (BP) (≥99%), butylated hydroxytoluene (BHT) (≥99.0%) and 2,4-di-tert-butylphenol (2,4-DTBP) (≥99%) were purchased from Sigma-Aldrich (St. Louis, MO) as solids and 4-nonylphenol (4-NP) from Riedel-de Haën (Seelze, Germany) as a solid technical mixture of isomers. Acenaphthene-d_{10} (100 %), chrysene-d_{12} (100 %), naphthalene-d_8 (98.9 %),

perylene-d_{12} (99.4 %) and phenanthrene-d_{10} (97.6 %) were purchased as individual surrogate standards from Accustandard (New Haven, Connecticut, USA) at a concentration of 4000 mg/L each. All standard and surrogate mixes were solved with methanol (HPLC grade) and stored in the dark at -20 ºC at concentrations >10 mg/L. Spiking solutions were prepared the same day of analysis. Milli-Q water was produced with a Milli-Q Integral Water Purification System (Millipore, Billerica, Massachusetts, USA).

2.2. Sampling

A total of 77 bottled water samples were collected between years 2010 and 2012 in several countries around the world (Table 1). Samples included 66 natural mineral waters, 10 treated waters and 1 natural spring water. World distribution of the sampling is showed in Figure 1. One bottled water was from United Arab Emirates (AE), 1 from Argentina Republic (AR), 3 from Austria (AT), 1 from Bolivia (BO), 2 from Canada (CA), 5 from Brazil (BR), 3 from People's Republic of China (CN), 1 from Costa Rica (CR), 5 from Czech Republic (CZ), 8 from Germany (DE), 1 from Egypt (EG), 1 from Finland (FI), 4 from France (FR), 5 from Croatia (HR), 2 from Indonesia (ID), 1 from Israel (IL), 9 from India (IN), 3 from Italy (IT), 4 from Morocco (MA), 3 from Mexico (MX), 2 from Malaysia (MY), 1 from Norway (NO), 1 from Poland (PL), 4 from Portugal (PT), 4 from Serbia (RS), 1 from Turkey (TR) and 1 from Taiwan (TW) (Table 1). All samples were packaged in polyethylene terephthalate (PET) bottles with high-density polyethylene (HDPE) caps. Volumes of bottles were between 0.2 and 1.2 L. Storage was done in a laboratory closet at room temperature as they are in the market. Samples were analyzed before expiration date.

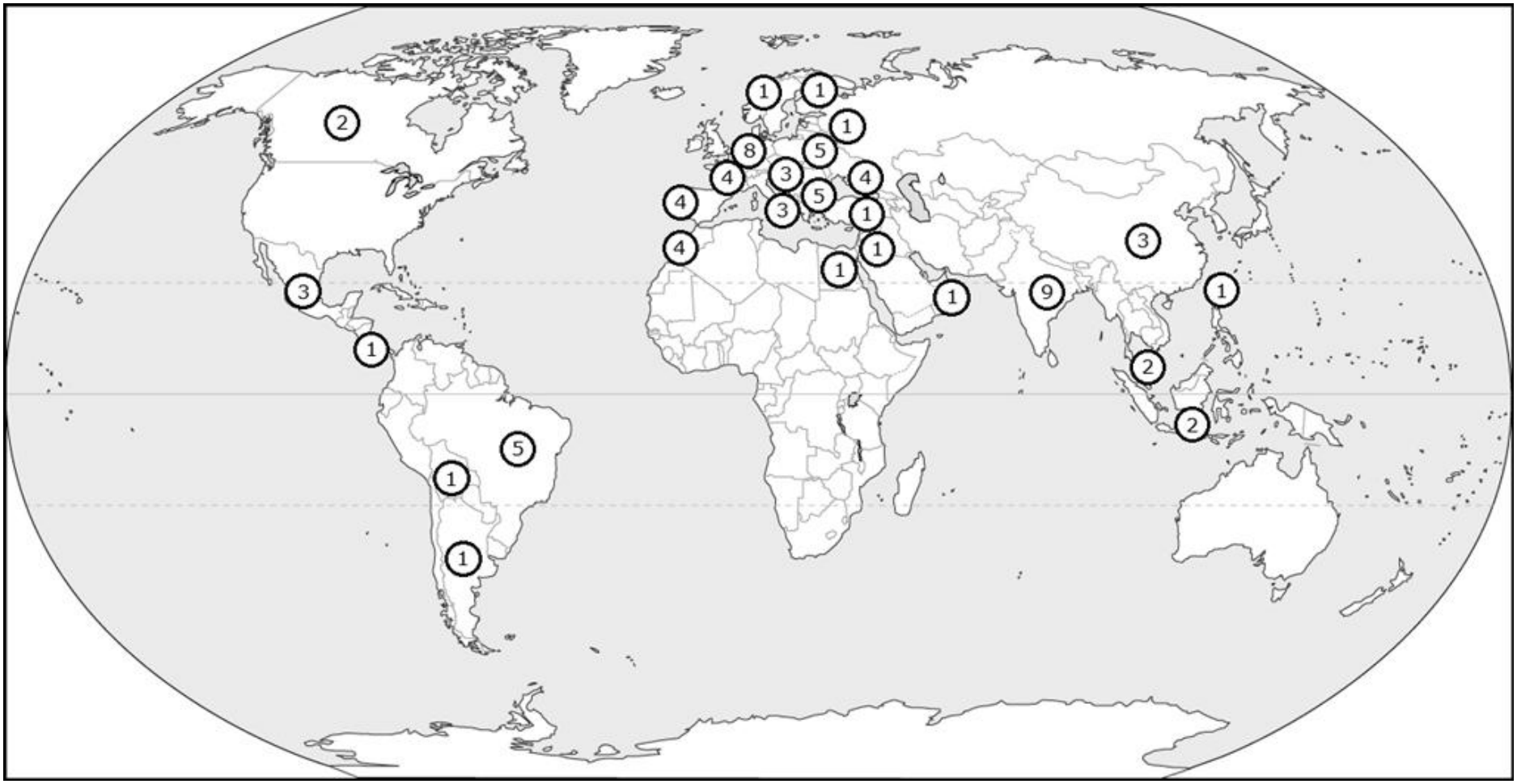


Figure 1. World map of the sampling sites. The number of samples per country is indicated in circles.

Table 1. Sample code and principal characteristics of each analyzed sample. All water samples were packaged in PET bottles with HDPE caps.

Sample code	Country	Type of water	Volume (L)	Sample code	Country	Type of water	Volume (L)	Sample code	Country	Type of water	Volume (L)
AE1	United Arab Emirates	NSW	0.6	DE5	Germany	NMW	0.5	IN9	India	NMW	1.0
AR1	Argentina Republic	NMW	0.5	DE6	Germany	NMW	0.5	IT1	Italy	NMW	1.5
AT1	Austria	NMW	0.75	DE7	Germany	NMW	0.5	IT2	Italy	NMW	0.5
AT2	Austria	NMW	0.2	DE8	Germany	NMW	0.5	IT3	Italy	NMW	0.75
AT3	Austria	NMW	0.5	EG1	Egypt	NMW	0.6	MA1	Morocco	NMW	1.5
BO1	Bolivia	NMW	2	FI1	Finland	NMW	0.5	MA2	Morocco	NMW	0.5
BR1	Brazil	NMW	0.5	FR1	France	NMW	0.5	MA3	Morocco	NMW	1.5
BR2	Brazil	NMW	0.5	FR2	France	NMW	0.5	MA4	Morocco	NMW	1.5
BR3	Brazil	NMW	0.51	FR3	France	NMW	0.5	MX1	Mexico	DW	1
BR4	Brazil	NMW	0.51	FR4	France	NMW	0.33	MX2	Mexico	DW	1
BR5	Brazil	NMW	0.51	HR1	Croatia	NMW	1.5	MX3	Mexico	DW	1
CA1	Canada	NMW	0.5	HR2	Croatia	DW	0.5	MY1	Malaysia	NMW	0.6
CA2	Canada	NMW	0.5	HR3	Croatia	NMW	0.5	MY2	Malaysia	NMW	0.5
CN1	People's Republic of China	NMW	0.55	HR4	Croatia	NMW	0.5	NO1	Norway	NMW	0.5
CN2	People's Republic of China	DW	1.5	HR5	Croatia	NMW	1.5	PL1	Poland	NMW	1.5
CN3	People's Republic of China	NMW	0.55	ID1	Indonesia	NMW	1.5	PT1	Portugal	NMW	0.33
CR1	Costa Rica	NMW	0.6	ID2	Indonesia	NMW	0.6	PT2	Portugal	NMW	0.33
CZ1	Czech Republic	NMW	0.5	IL1	Israel	NMW	1.5	PT3	Portugal	NMW	0.33
CZ2	Czech Republic	NMW	0.5	IN1	India	NMW	0.5	PT4	Portugal	NMW	0.5
CZ3	Czech Republic	NMW	0.5	IN2	India	DW	1.0	RS1	Serbia	NMW	0.5
CZ4	Czech Republic	NMW	0.5	IN3	India	DW	1.0	RS2	Serbia	NMW	0.5
CZ5	Czech Republic	NMW	0.5	IN4	India	DW	1.0	RS3	Serbia	NMW	0.5
DE1	Germany	NMW	0.5	IN5	India	DW	1.0	RS4	Serbia	NMW	1.5
DE2	Germany	NMW	0.5	IN6	India	DW	0.5	TR1	Turkey	NMW	0.5
DE3	Germany	NMW	0.5	IN7	India	NMW	0.5	TW1	Taiwan	NMW	0.6
DE4	Germany	NMW	0.5	IN8	India	NMW	0.5				

NSW: natural spring water
NMW: natural mineral water
DR: drinking water

2.3 Experimental setup

100 mL of water were introduced into a glass erlenmeyer, which was previously rinsed three times with water and once with methanol and dried at 100 ºC. Sample was spiked with 10 µL of 100 µg/L surrogate standard mix (DPP-d_4, naphthalene-d_8, acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{10}, perylene-d_{12}) to give a concentration of 0.01 µg/L. After that, a stir bar (2 cm length and 0.5 thickness coated with PDMS) (Twister® - Gerstel GmbH & Co. KG, Mulheim a/d Ruhr, Germany) was immersed into the erlenmeyer and closed with a glass cap. Extraction was performed without any addition of substances as sodium chloride (NaCl) or methanol (MeOH) because they affect the recoveries of some compounds (Tögyessy et al. 2011). Sample was stirred for 14 h at an agitation speed of 900 rpm. After the stirring period, the stir bar was removed with a clean magnetic bar, rinsed with Milli-Q water, dried with a lint-free tissue and placed into the liner of the GC-MS/MS rack.

A blank sample and Milli-Q water samples spiked with target compounds were analyzed. Blank sample consisted in 100 mL Milli-Q water spiked with 10 µL of 100 µg/L surrogate standard mix. Recoveries were performed using Milli-Q water samples spiked at a level of 0.020 µg/L (20 µL of 100 µg/L native mix to 100 mL Milli-Q water) for PAHs, pesticides, PCBs and triazines and 0.100 µg/L (100 µL of 100 µg/L native mix to 100 mL Milli-Q water) for phthalates and the other plastic components since LOQs are above 0.020 µg/L due to blank contribution. Calibration curve was performed at 0.005-0.450 µg/L (eight calibration points). LOQs (Table 2) were calculated using 10 times the signal to noise (S/N) ratio of the 0.005 µg/L calibration point or, in the case of blank contribution, using the arithmetical mean of the blank concentration plus 10 times the standard deviation (n=9), respectively.

Table 2. GC-MS/MS parameters and curve parameters for each target compound.

Compound	RT (min)	ISTD	Quantifier CE (eV)	Quantifier Transition	Qualifier CE (eV)	Qualifier transition	Qualifier relative response	Lineal range	r^2	LOQ (µg/L)	0.020 µg/L recovery ± RSD (%)
PAHs											
Naphthalene	5.345	Naphthalene-d_8	0	128 > 128	0	127 > 127	11	0.005-0.450	0.9962	0.005	88 ± 14
Acenaphthylene	7.961	Acenaphthene-d_{10}	0	152 > 151	0	152 > 150	5	0.005-0.450	0.9934	0.005	76 ± 15
Acenaphthene	8.420	Acenaphthene-d_{10}	0	153 > 153	0	154 > 154	94	0.005-0.450	0.9953	0.005	90 ± 10
Fluorene	9.910	Acenaphthene-d_{10}	0	166 > 166	0	165 > 165	93	0.005-0.450	0.9961	0.005	92 ± 6
Phenanthrene	13.822	Phenanthrene-d_{10}	0	178 > 178	0	179 > 179	15	0.005-0.450	0.9968	0.005	96 ± 7
Anthracene	14.044	Phenanthrene-d_{10}	0	178 > 178	0	179 > 179	15	0.005-0.450	0.9971	0.005	94 ± 5
Fluoranthene	20.839	Phenanthrene-d_{10}	0	202 > 202	0	201 > 201	13	0.010-0.100	0.9988	0.010	110 ± 5
Pyrene	22.202	Phenanthrene-d_{10}	0	202 > 202	0	201 > 201	15	0.010-0.100	0.9982	0.011	118 ± 7
Benzo(a)anthracene	28.323	Chrysene-d_{10}	0	228 > 228	0	229 > 229	20	0.005-0.450	0.9966	0.005	97 ± 6
Chrysene	28.472	Chrysene-d_{10}	0	228 > 228	0	227 > 227	5	0.005-0.450	0.9986	0.005	96 ± 8
Indeno[1,2,3-cd]pyrene	37.861	Perylene-d_{12}	0	276 > 276	0	137 > 137	17	0.005-0.450	0.9980	0.010	75 ± 7
Dibenzo[a,h]anthracene	38.105	Perylene-d_{12}	0	278 > 278	0	139 > 139	40	0.005-0.100	0.9638	0.010	91 ± 4
Benzo[g,h,i]perylene	39.133	Perylene-d_{12}	0	276 > 276	0	138 > 138	26	0.005-0.250	0.9964	0.010	93 ± 8
OCPs											
Trifluralin	11.637	Phenanthrene-d_{10}	15	306 > 264	5	264 > 160	33	0.005-0.450	0.9993	0.005	95 ± 8
α-HCH	12.084	Phenanthrene-d_{10}	15	181 > 145	30	181 > 109	113	0.005-0.450	0.9960	0.005	95 ± 10
Hexachlorobenzene	12.377	Phenanthrene-d_{10}	35	284 > 214	25	284 > 249	99	0.005-0.450	0.9985	0.005	97 ± 10
β-BHC	13.200	Phenanthrene-d_{10}	15	181 > 145	20	181 > 109	21	0.005-0.100	0.9816	0.005	101 ± 4
γ-HCH (Lindane)	13.461	Phenanthrene-d_{10}	12	181 > 145	30	181 > 109	123	0.005-0.050	0.9950	0.005	103 ± 9
δ-HCH	14.544	Phenanthrene-d_{10}	15	181 > 145	20	181 > 109	123	0.005-0.450	0.9990	0.005	128 ± 9

Table 2. Continuation.

Compound	RT (min)	ISTD	Quantifier CE (eV)	Quantifier Transition	Qualifier CE (eV)	Qualifier transition	Qualifier relative response	Lineal range	r^2	LOQ (µg/L)	0.020 µg/L recovery ± RSD (%)
OCPs											
Chlorpyrifos methyl	16.593	Phenanthrene-d_{10}	16	286 > 271	26	288 > 93	32	0.005-0.450	0.9996	0.005	100 ± 5
Heptachlor	16.796	Phenanthrene-d_{10}	25	272 > 237	40	272 > 117	10	0.005-0.100	0.9899	0.005	89 ± 6
Aldrin	18.528	Phenanthrene-d_{10}	30	263 > 193	30	263 > 191	66	0.005-0.450	0.9993	0.005	101 ± 8
Chlorpyrifos	19.234	Phenanthrene-d_{10}	15	197 > 169	40	197 > 107	36	0.005-0.450	0.9998	0.005	96 ± 13
DCPA	19.433	Phenanthrene-d_{10}	24	301 > 223	14	301 > 273	55	0.005-0.100	0.9977	0.005	99 ± 9
Heptachlor epoxide	20.722	Phenanthrene-d_{10}	25	237 > 143	25	237 > 141	68	0.005-0.450	0.9978	0.005	96 ± 8
Endosulfan I	22.637	Phenanthrene-d_{10}	10	241 > 206	20	241 > 170	30	0.005-0.100	0.9932	0.007	108 ± 8
Dieldrin	23.870	Phenanthrene-d_{10}	30	263 > 193	30	263 > 191	66	0.005-0.100	0.9891	0.008	110 ± 7
p,p'-DDE	24.021	Phenanthrene-d_{10}	30	246 > 176	20	246 > 211	14	0.010-0.100	0.9999	0.010	112 ± 8
Endrin	24.745	Phenanthrene-d_{10}	30	263 > 193	30	263 > 191	67	0.005-0.100	0.9972	0.005	104 ± 7
Endosulfan II	25.158	Phenanthrene-d_{10}	5	195 > 159	15	241 > 206	83	0.005-0.100	0.9909	0.010	99 ± 6
Chlorobenzilate	25.395	Phenanthrene-d_{10}	12	251 > 139	15	139 > 111	93	0.005-0.100	0.9931	0.005	107 ± 5
p,p'-DDD	25.686	Phenanthrene-d_{10}	20	235 > 165	20	237 > 165	63	0.005-0.100	0.9903	0.005	98 ± 6
Endosulfan sulfate	26.760	Phenanthrene-d_{10}	20	272 > 237	40	272 > 117	9	0.005-0.250	0.9938	0.057	83 ± 11
p,p'-DDT	26.982	Phenanthrene-d_{10}	20	235 > 165	20	237 > 199	32	0.005-0.250	0.9846	0.009	66 ± 13
Methoxychlor	28.862	Chrysene-d_{10}	30	227 > 169	35	227 > 141	80	0.005-0.100	0.9988	0.010	79 ± 6
Pyrethroids											
cis-Permethrin	31.369	Chrysene-d_{10}	15	183 > 168	38	183 >77	63	0.010-0.100	0.9948	0.010	117 ± 4
trans-Permethrin	31.550	Chrysene-d_{10}	15	183 > 168	38	183 >77	63	0.010-0.100	0.9915	0.010	115 ± 4

Table 2. Continuation.

Compound	RT (min)	ISTD	Quantifier CE (eV)	Quantifier Transition	Qualifier CE (eV)	Qualifier transition	Qualifier relative response	Lineal range	r^2	LOQ (µg/L)	0.020 µg/L recovery ± RSD (%)
OPPs											
Phorate	11.962	Phenanthrene-d_{10}	10	75 > 47	10	75 > 41	37	0.005-0.450	0.9969	0.005	81 ± 15
Diazinon	14.466	Phenanthrene-d_{10}	20	179 > 137	20	179 > 121	20	0.005-0.100	0.9966	0.005	97 ± 18
Parathion methyl	16.594	Phenanthrene-d_{10}	15	263 > 109	30	263 > 79	16	0.005-0.450	0.9985	0.015	85 ± 4
Fenchlorphos (Ronnel)	17.330	Phenanthrene-d_{10}	15	285 > 270	30	285 > 240	62	0.005-0.450	0.9997	0.005	96 ± 14
Fenthion	19.120	Phenanthrene-d_{10}	20	278 > 109	40	278 > 125	12	0.005-0.100	0.9940	0.005	100 ± 5
Trichloronate	19.840	Phenanthrene-d_{10}	15	297 > 269	30	297 > 223	27	0.005-0.450	0.9989	0.005	100 ± 14
Tokuthion (Prothiofos)	23.752	Phenanthrene-d_{10}	5	267 > 239	40	162 > 63	70	0.005-0.450	0.9974	0.010	109 ± 6
PCBs											
PCB 28	16.100	Phenanthrene-d_{10}	30	256 > 186	50	256 > 151	23	0.010-0.100	0.9849	0.014	96 ± 9
PCB 52	17.800	Phenanthrene-d_{10}	34	292 > 220	34	292 > 222	99	0.005-0.450	0.9984	0.005	82 ± 10
PCB 101	22.550	Phenanthrene-d_{10}	34	326 > 256	34	326 > 254	67	0.005-0.100	0.9910	0.010	102 ± 9
PCB 118	25.335	Phenanthrene-d_{10}	27	326 > 256	27	326 > 254	66	0.005-0.100	0.9949	0.005	101 ± 6
PCB 153	26.132	Phenanthrene-d_{10}	35	360 > 290	16	360 > 325	55	0.005-0.100	0.9951	0.005	104 ± 5
PCB 138	27.068	Phenanthrene-d_{10}	30	360 > 290	15	360 > 325	38	0.005-0.100	0.9943	0.005	89 ± 4
PCB 180	29.153	Chrysene-d_{10}	34	394 > 324	15	394 > 359	55	0.005-0.050	0.9930	0.005	93 ± 10

Table 2. Continuation.

Compound	RT (min)	ISTD	Quantifier CE (eV)	Quantifier Transition	Qualifier CE (eV)	Qualifier transition	Qualifier relative response	Lineal range	r^2	LOQ (µg/L)	0.020 µg/L recovery ± RSD (%)
Triazines											
Atrazine-desethyl	11.254	Phenanthrene-d_{10}	34	172 > 43	22	172 > 69	72	0.005-0.050	0.9925	0.005	121 ± 18
Simazine	12.909	Phenanthrene-d_{10}	2	201 > 173	2	201 > 186	42	0.005-0.050	0.9828	0.015	103 ± 12
Atrazine	13.159	Phenanthrene-d_{10}	20	200 > 94	20	200 > 104	93	0.005-0.050	0.9810	0.005	104 ± 7
Propazine	13.363	Phenanthrene-d_{10}	5	214 > 172	20	214 > 104	30	0.005-0.050	0.9772	0.005	103 ± 6
Terbuthylazine	13.810	Phenanthrene-d_{10}	18	214 > 104	8	214 > 132	88	0.005-0.050	0.9764	0.005	99 ± 11
Prometryn	17.340	Phenanthrene-d_{10}	10	241 > 184	10	241 > 226	49	0.005-0.050	0.9933	0.005	105 ± 5
Terbutryne	17.973	Phenanthrene-d_{10}	20	226 -> 96	20	226 > 83	94	0.005-0.100	0.9905	0.005	104 ± 7
Phthalates											
DMP	7.914	DDP-d_4	15	163 > 135	25	163 > 77	158	0.005-0.450	0.9994	0.005	103 ± 1*
DMTP	8.509	DDP-d_4	10	163 > 135	20	163 > 103	34	0.005-0.450	0.9946	0.005	110 ± 1*
DMIP	8.683	DDP-d_4	20	163 > 135	20	163 > 120	18	0.005-0.450	0.9991	0.005	101 ± 1*
DBP	15.952	DDP-d_4	15	149 > 121	15	149 > 93	98	0.010-0.250	0.9974	0.046	88 ± 15*
BBP	27.007	DDP-d_4	15	149 > 93	15	149 > 121	90	0.010-0.100	0.9933	0.046	57 ± 7*
DEHP	29.655	DDP-d_4	20	149 > 93	20	149 > 121	70	0.020-0.450	0.9753	0.066	145 ± 24*
Other plasticizers											
2,4-DTBP	8.604	DDP-d_4	25	191 > 57	20	191 > 175	20	0.030-0.450	0.9980	0.030	61 ± 12*
BHT	8.700	DDP-d_4	25	205 > 177	15	205 > 145	83	0.030-0.450	0.9976	0.040	47 ± 4*
BP	10.685	DDP-d_4	20	105 > 77	15	105 > 51	19	0.005-0.450	0.9997	0.014	88 ± 2*
4-NP	12.110	DDP-d_4	25	135 > 107	25	135 > 77	65	0.030-0.450	0.9949	0.039	68 ± 1*
DEHA	27.742	DDP-d_4	5	129 > 101	5	129 > 111	64	0.030-0.450	0.9951	0.045	55 ± 6*

* Recoveries of quality controls spiked at 0.100 µg/L.

2.4. Instrumental settings

The coated stir bars were thermally desorbed with a commercial thermal desorption TDS-2 (Gerstel GmbH & Co. KG, Mulheim a/d Ruhr, Germany) connected to a programmed temperature vaporization (PTV) injector CIS-4 (Gerstel GmbH & Co. KG, Mulheim a/d Ruhr, Germany) by a heated transfer line set at an initial and final temperatures of 30 ºC and 300 ºC, respectively, increasing at 10 ºC/seg. The PTV was installed in an Agilent 6890A GC System (Agilent Technologies, Palo Alto, CA, USA) interfaced to a 7000A triple quadrupole mass spectrometer system (Agilent, USA) in electronic ionization (EI+) at +70 eV. A Masshunter Workstation (ver. B.06) was used for data acquisition, instrument control and retention time locking, which was performed with methyl chlorpyrifos. Compound separation was performed with a Agilent HP-MS5 capillary column (5 % phenylmethylsiloxane-95 % dimethylsiloxane) 30 m x 0.25 mm i.d. x 0.25 µm film thickness. The oven temperature was set at 70 ºC for 2 min, increased to 150 ºC at 25 ºC/min (held for 0 min), to 200 ºC at 3 ºC/min (held for 0 min), and finally to 280 ºC at 8 ºC/min (held for 10 min).

3. Results and discussion

3.1 Performance of the SBSE-GC-MS/MS method

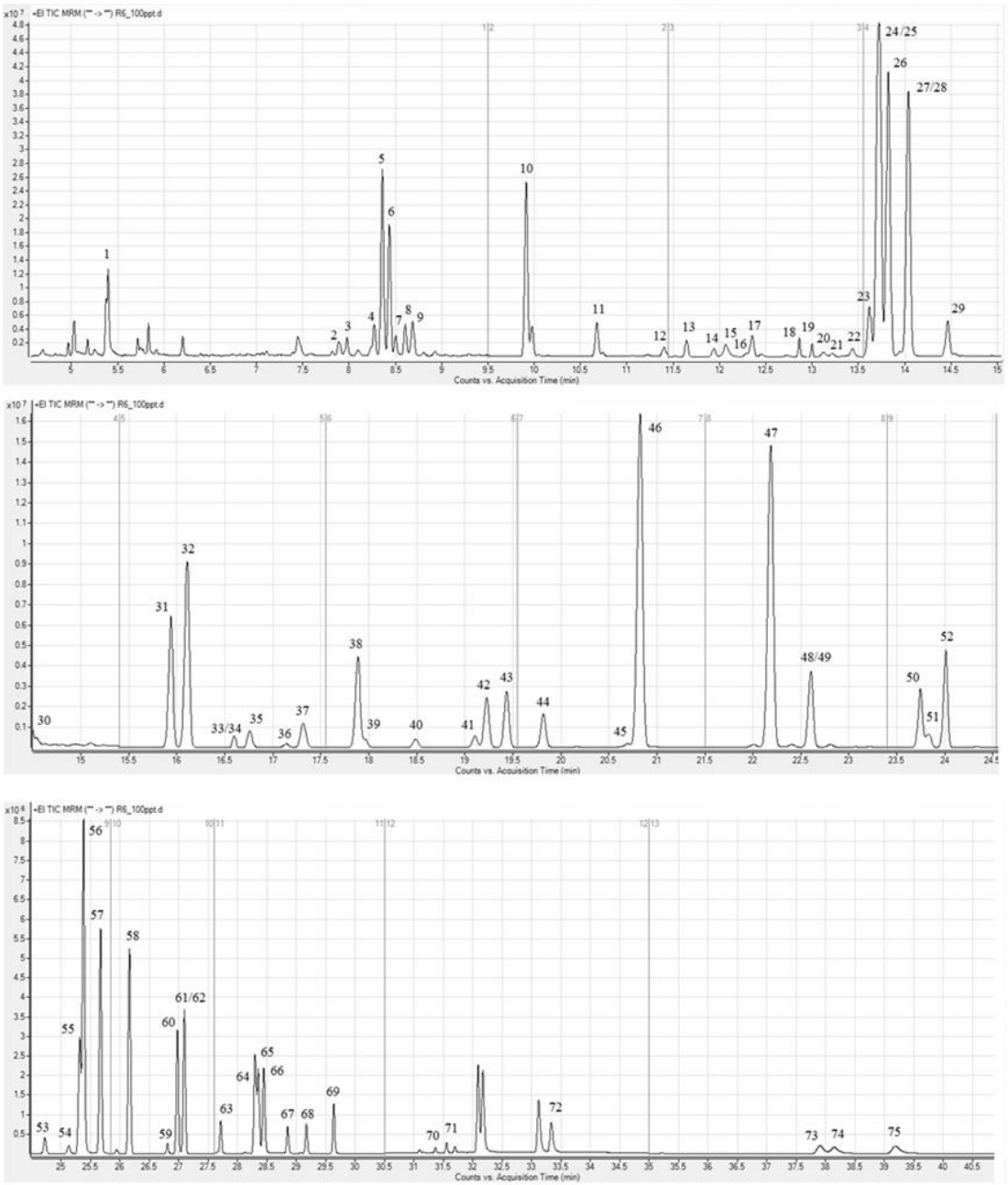
GC-MS/MS method was performed according with following steps: (i) precursor ion scan was performed to obtain the retention time (RT) and to isolate a single precursor ion for each target compound (Table 2); (ii) product ion scan was performed at several collision energy (CE) voltages from 0 to 40 eV and 2 ions were selected for each target compound; and (iii) selected reaction monitoring (SRM) was performed using a precursor ion and two product ions, where GC-MS/MS automatically selected one quantifier and one qualifier transition. RT, internal standard (ISTD), quantifier and qualifier transitions with corresponding CE voltages are indicated in Table 2 for each target compound. PAH CEs were set at 0 eV because their fragmentation was low even at high CE. The use of qualifier relative response for peak confirmation was limited at a 20 % relative error.

Calibration curves were performed from 0.005 to 0.450 µg/L for each target compound. Most PAHs were lineal at a range from 0.005 to 0.450 µg/L. OCPs were lineal from 0.005 to 0.100 or 0.450 µg/L. Most OPPs were lineal from 0.005 to 0.100 µg/L. Most PCBs were lineal from 0.005 to 0.100 µg/L. Triazines were lineal

from 0.005 to 0.050 µg/L. Phthalates and the other compounds were lineal from 0.005-0.030 to 0.450 µg/L. The Total Ion Chromatogram (TIC) of a spiked samples at a concentration of 0.100 µg/L is shown in Figure 2.

All recoveries were between 70 and 130 %, except for p,p'-DDT which was of 66 at 0.020 µg/L spiking level and for 2,4-DTBP, BBP, DEHP, BHT, 4-NP and DEHA which were of 61 %, 57 %, 145 %, 47 %, 68 % and 55 %, respectively for 0.100 µg/L spiking level (Table 2). BHT is degraded in contact with water forming different products (OECD 2002) and it may explain its low recovery.

Figure 2. Total ion chromatogram (TIC) of a spiked sample at a concentration of 0.100 µg/L.



(1) Naphthalene (5.345 min); (2) DMP (7.914 min); (3) acenaphtylene (7.961 min); (4) acenaphthene-d_{10} (8.336 min); (5) acenaphthene (8.420 min); (6) DMTP (8.509 min); (7) 2,4-DTBP (8.604 min); (8) DMIP (8.683 min); (9) BHT (8.700 min); (10) fluorene (9.910 min); (11) BP (10.685 min); (12) desethyl atrazine (11.254 min); (13) trifluralin (11.637 min); (14) phorate (11.962 min); (15) α-HCH (12.084 min); (16) 4-NP (12.110 min); (17) hexachlorobenzene (12.377 min); (18) simazine (12.909 min); (19) atrazine (13.159 min); (20) β-HCH (13.200 min); (21) propazine (13.363 min); (22) γ-HCH (13.461 min); (23) di-n-propyl phthalate (13.670 min); (24) phenanthrene-d_{10} (13.687 min); (25) terbuthylazine (13.810 min); (26) phenanthrene (13.822 min); (27) anthracene-d_{10} (13.900 min); (28) anthracene (14.044 min); (29) diazinon (14.466 min); (30) δ-HCH (14.544 min); (31) DBP (15.952 min); (32) PCB 28 (16.100 min); (33) chlorpyrifos methyl (16.593 min); (34) Parathion methyl (16.594 min); (35) heptachlor (16.796 min); (36) ronnel (17.330 min); (37) prometryn (17.340 min); (38) PCB 52 (17.800 min); (39) terbutryne (17.973 min); (40) aldrin (18.528 min); (41) fenthion (19.120 min); (42) chlorpyrifos (19.234 min); (43) DCPA (19.433 min); (44) trichloronate (19.840 min); (45) heptachlor epoxide (20.722 min); (46) fluoranthene (20.839 min); (47) pyrene (22.202 min); (48) PCB 101 (22.550 min); (49) endosulfan I (22.637 min); (50) tokuthion (23.752 min); (51) dieldrin (23.870 min); (52) p,p'-DDE (24.021 min); (53) endrin (24.745 min); (54) endosulfan II (25.158 min); (55) PCB 118 (26.132 min); (56) chlorobenzilate (25.395 min); (57) p,p'-DDD (25.686 min); (58) PCB 153 (26.132 min); (59) endosulfan sulfate (26.760 min); (60) p,p'-DDT (26.982 min); (61) BBP (27.007 min); (62) PCB 138 (17.068 min); (63) DEHA (27.742 min); (64) benzo(a)anthracene (28.323 min); (65) chrysene-d_{10} (28.400 min); (66) chrysene (18.472 min); (67) methoxychlor (28.862 min); (68) PCB 180 (29.153 min); (69) DEHP (29.655 min); (70) cis-permethrin (31.369 min); (71) trans-permethrin (31.550 min); (72) perylene-d_{12} (33.305 min); (73) indeno[1,2,3-cd]pyrene (37.861 min); (74) dibenzo[a,h]anthracene (38.105 min); (75) benzo[g,h,i]perylene (39.133 min).

3.2. Analysis of water samples

Among the 69 target compounds, only 13 compounds were detected (Table 3). Two origins of the pollutants can be defined: (i) from source water (PAHs, OPPs, OCPs, PCBs and triazines) and (ii) from packaging migration (phthalates and other plastic components). Among the 77 water samples analyzed, twenty-seven samples had 1 compound >LOQ, twenty samples had 2 compounds, twelve samples had 3 compounds, one sample had 4 compounds, one sample had 5 compounds and one sample had 6 compounds. The other 15 samples did not have any traces of

pollution and they were AE1 from United Arab Emirates, AT2 from Austria, BR1 from Brazil, CA1 from Canada, CZ5 from Czech Republic, DE1 and DE2 from Germany, HR3 and HR4 from Croatia, ID1 and ID2 from Indonesia, IN7 from India, MA1 from Morocco, MY2 from Malaysia and RS4 from Serbia (Table 1).

3.2.1. Contaminants from source water

Within the 77 bottled water samples, the target contaminants detected in source water were naphthalene (n=16 samples), indeno[1,2,3-cd]pyrene (n=4), benzo[g,h,i]perylene (n=3), p,p'-DDE (n=2), simazine (n=2), atrazine-desethyl (n=1) and atrazine (n=1) (Table 3). In the case of the natural spring water (NSW in Table 1) no compounds was detected. Among drinking waters (DW in Table 1; n=10), 4 of them had not any compound, 4 of them had only naphthalene and 2 of them had >1 positive compound. All the other compounds were detected in natural mineral waters (NMW in Table 1; n=66).

p-p'-DDE was detected in 2 samples DE8 (Germany) and BO1 (Bolivia) at 0.012 and 0.014 µg/L, respectively. Other OCPs, OPPs and PCBs were not detected in any sample. In the case of OCP and PCBs, these compounds have a Koc ranging from 871 to 851138 (SRC 2013) and therefore their leaching potential is very low because rather they would be adsorbed to soil. On the other hand, the use of OPP is limited in many world regions to be substituted by pyrethroids. When compounds are not reiteratelly used, the leaching potential is very low and become compounds with no risk for groundwater. PAHs were detected in 18 out of 77 samples at levels between 0.005 and 0.202 µg/L. The most detected compound was naphthalene, which was detected in 16 samples at concentrations of 0.005-0.202 µg/L (median 0.012 µg/L) (Table 3). The 3 samples from China (CN) had naphthalene at 0.007-0.033 µg/L and 6 out of 9 samples from India (IN) had naphthalene at 0.005-0.202 µg/L. The extensive industrial activities of some areas in both countries increase the leaching of this compound to groundwater. In sample MX3 (Mexico), naphthalene, indeno[1,2,3-cd]pyrene and dibenz[a,h]anthracene were detected at concentrations of 0.020, 0.017 and 0.015 µg/L, respectively. Díaz et al. (2009) studied the presence of OCPs in bottled drinking waters from Mexico City with 6 positive samples out of 36 (1.5 L and 19 L bottles), where concentrations were ranged from 0.001 to 0.152 µg/L. Triazines were detected in 3 samples: 0.005 µg/L simazine in MA4 (Morocco), 0.005 µg/L atrazine desethyl in DE8 (Germany), and 0.023 and 0.022 µg/L of simazine and atrazine, respectively, in IL1 (Israel).

Maggioni et al. (2013) detected triazines in 2 bottled water brands out of 5 contained in 1.5 L PET bottles from Italy, where 1 brand had atrazine, atrazine-desethyl, terbuthylazine and terbuthylazine-desethyl at levels of 0.00012, 0.00027, 0.00050 and 0.00480 µg/L, respectively, and the other brand had atrazine, atrazine-desethyl and terbuthylazine at 0.00011, 0.00035 and 0.00078 µg/L, respectively.

Table 3. Compounds detected, number of times detected (n), maximum, minimum and median concentration (µg/L) of each detected compound.

Compound	n	Minimum	Maximum	Median
DMP	41	0.005	0.125	0.016
BP	32	0.014	0.921	0.073
Naphthalene	16	0.005	0.202	0.012
2,4-DTBP	8	0.041	0.290	0.118
Indeno[1,2,3-cd]pyrene	4	0.014	0.040	0.020
DBP	4	0.058	0.220	0.100
Benzo[g,h,i]perylene	3	0.015	0.068	0.031
BBP	3	0.077	0.131	0.086
p,p'-DDE,	2	0.012	0.014	0.013
Simazine	2	0.005	0.023	0.014
Atrazine-desethyl	1	0.005	0.005	0.005
Atrazine	1	0.022	0.022	0.022
4-NP	1	0.054	0.054	0.054

3.2.2. Plastic component contaminants from packaging migration

The type of polymer and the bottling process or storage conditions are the factors that affect the presence of phthalates and other plastic components by the migration from plastic bottles (Guart et al. 2011). All DW, NMW and NSW analyzed in this study were packaged in PET bottles and HDPE caps, although bulk polymeric materials can differ among countries and hence, the migration of plastic components can vary in rate and in composition.

DMP was the most ubiquitous compound detected 41 times at concentrations of 0.005-0.125 µg/L (median 0.016 µg/L). This fact was in accordance with the study of Bošnir et al. (2007) that showed a DMP migration from PET containers into soft drinks. Regarding the geographical distribution of DMP, it was detected in all the water samples from France (n=5/5) and People's Republic of China (n=3/3). It was also detected in almost all the water samples from India (n=5/7) and Turkey (n=4/5).

BP was detected 32 times at concentrations of 0.014-0.921 µg/L (median 0.073 µg/L). BP can be used as additive or polymer production aid (EU, 2011) and can be used as photoinitiator (PI) catalysers for inks and lacquers that are cured with ultraviolet light. This compound has been reported to migrate to foodstuffs by mass transference (Rothenbacher et al. 2007; Sanches-Silva et al. 2011), by set-off (as a result of the contact of the external printed face of the packaging with the inner non-printed face) or by a transfer through the substrate. Regarding the geographical distribution of BP, it was detected in all the water samples from France (n=5), Mexico (n=3) and Serbia (n=4). Shinohara et al. (1981) determined BP in tap water in Japan at a concentration of 8.8 µg/L and Loraine and Pettigrove (2006) detected BP in drinking water after being treated in a water filtration plant in the San Diego County (USA) at a concentration of 0.26 µg/L. To our knowledge, it has not been reported previously in bottled water.

Other plastic components detected were 2,4-DTBP, DBP, BBP and 4-NP. 2,4-DTBP was detected 8 times at the highest median (0.118 µg/L) among all detected compounds, followed by DBP with a median of 0.100 µg/L (n=4). DE8 (Germany) contained DMP, 2,4-DTBP and BP at concentrations of 0.016, 0.288 and 0.143 µg/L, respectively; AT1 (Austria) contained DMP, 2,4-DTBP and BP at concentrations of 0.008, 0.109 and 0.036 µg/L, respectively; AT3 (Austria) contained DMP, 2,4-DTBP and BP at concentrations of 0.025, 0.290 and 0.248 µg/L, respectively; MX3 (Mexico) contained DBP, 2,4-DTBP and BP at concentrations of 0.220, 0.050 and 0.021 µg/L, respectively; and CN2 from People's Republic of China contained DMP, DBP and BP at concentrations of 0.010, 0.058 and 0.213 µg/L, respectively. Other studies report 2,4-DTBP in bottled water and its concentration increased from 0.4 to 0.7 µg/L in water after an exposure of 10 days at 60ºC into PET bottles (Bach et al., 2013). The presence of phthtalates and alkylphenols in PET bottle has been previously reported. Amiridou and Voutsa (2011) determined the presence of NP, DEHP, DBP and diethyl phthalate (DEP) at concentrations of 0.0079, 0.350, 0.044 and 0.033 µg/L, respectively, in 1 L PET bottles. Casajuana and Lacorte (2003) detected DBP in PET bottled water at a concentration of 0.059 µg/L, but after 10 weeks outdoor storage, DMP, DEP DBP, BBP, DEHP and 4-NP were detected at 0.002-0.214 µg/L. Although phthalates are commonly associated with packaging, Mihovec-Grdič et al. (2002) found them in 76 out of 77 underground water of Zagreb (Croatia) at maximum levels of 3.603, 14.886, 18.157, 7.001, 5.344 and 2.817 µg/L for DMP, DEP, DBP, BBP, DEHP and dioctyl phthalate (DOP), respectively. This means that although phthalates are often associated with the

migration from the plastic material, in some areas they have become emerging groundwater contaminants.

4. Conclusions

The multiresidue SBSE-GC-MS/MS method developed proved to be effective for the low level detection of organic contaminants in bottled water. This technique allowed to analyze target compounds at concentrations of ng/L with a high sensitivity and selectivity. Additionally, the low level of manipulation of the sample and therefore the low contamination of the sample allowed obtaining low limits of quantification. DMP was the most ubiquitous compound with 53 % of positive samples (41 out of 77) followed by BP and naphthalene with 42 % (32 out of 77) and 21 % (16 out of 77), respectively. In all cases, detected compounds were in very low concentrations indicating the good quality of bottled water worldwide.

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3.3. Discussió dels resultats

3.3.1. Mètode analític

El mètode d'extracció per SBSE va demostrar ser un mètode eficaç i altament reproduïble (article científic III). Les avantatges respecte la SPE són que la SBSE permet minimitzar la manipulació de la mostra ja que la barreta agitadora s'introdueix directament dins de la mostra d'aigua, a diferència de la SPE en què la mostra passa a través d'un cartutx. En la SBSE tampoc s'utilitzen dissolvents per a eluir els anàlits. Les barretes adsorbents es poden reutilitzar fins a 100 vegades, segons la casa comercial, sense que hi hagi pèrdua d'adsorció, ja que per mostres "netes" la vida útil és més llarga. Al Laboratori Dr. Oliver Rodés s'ha realitzat un control de les barretes per a saber el nombre de vegades que es poden utilitzar en aigües envasades i s'ha determinat que és d'unes 30 vegades. Per altra banda, en les condicions definides no es pot analitzar el BPA conjuntament amb la resta de compostos ja que es necessari realitzar una derivatització per augmentar l'adsorció a les barretes agitadores. Un inconvenient de la SBSE es que les mostres extretes no es poden tornar a analitzar perquè tots els compostos adsorbits a la barreta agitadora són desorbits al cromatògraf. Per això, sovint cal agafar més mostra de la necessària per a possibles problemes durant l'anàlisi.

El fet d'utilitzar l'espectrometria de masses en tàndem permet identificar un gran nombre de compostos, degut a la utilització dels dos ions producte, que conjuntament amb el temps de retenció, permet evitar errors en la identificació. El desavantatge que presenta aquest mètode recau en què el rang lineal de les rectes de calibrat és molt reduït per cert compostos, com ara les triazines que és de 0.005-0.050 µg/L. El DEP i el 4-OP tampoc no es van poder analitzar degut a què les rectes de calibrat no eren lineals.

Degut a la molt bona sensibilitat del GC-MS/MS, ja que tota la mostra extreta es desorbeix. D'aquesta manera, els LODs i LOQs dels plastificants són inferiors respecte a l'anàlisi per SPE-GC/MS (Taula 7).

Taula 7. Comparació dels LOQs per SPE-GC/MS (article científic I i II) i per SBSE-GC-MS/MS (article científic III) en aigües envasades

Compostos	LOQs de SPE-GC/MS (µg/L)	LOQs de SBSE-GC-MS/MS (µg/L)	Tendència dels LOQs
DMP	0.018	0.005	Baixa
DMTP	NA	0.005	-
DMIP	NA	0.005	-
DEP	0.837	NA	-
DBP	0.687	0.046	Baixa
BBP	0.525	0.046	Baixa
DEHP	0.970	0.066	Baixa
DEHA	0.180	0.045	Baixa
4-OP	0.0018	NA	-
4-NP	0.057	0.039	Baixa
BPA	0.029	NA	-
2,4-DTBP	NA	0.030	-
BHT	NA	0.040	-
BP	NA	0.014	-

NA: no analitzat
- : sense comparació

3.3.2. Aigua envasada

L'extens mostreig d'aigua envasada recol·lectada arreu del món va permetre avaluar la seva qualitat en relació a la contaminació medioambiental (aigua de captació) i per la migració del plàstic de l'envàs de PET i del tap de HDPE (article científic III). Tot i que els compostos com ara els ftalats, alquilfenols i altres components del plàstic també poden tenir com origen l'aigua de captació, a l'anterior estudi es va demostrar que el principal origen d'aquests compostos era la migració des del plàstic.

Tenint en compte les 77 mostres analitzades, en 15 d'elles no s'hi va detectar cap compost, en 27 mostres es va detectar un sol compost, en 20 mostres només dos compostos, en 12 mostres es van detectar tres compostos, en 1 mostra quatre compostos, en 1 altra cinc compostos i en una última 6 compostos (Figura

7). D'aquesta forma s'ha pogut comprovar que les aigües de captació estan ben protegides i que l'envàs de PET i el tap de HDPE és un tipus de contenidor apte per a envasar aigua ja que hi ha una baixa migració de components del plàstic.

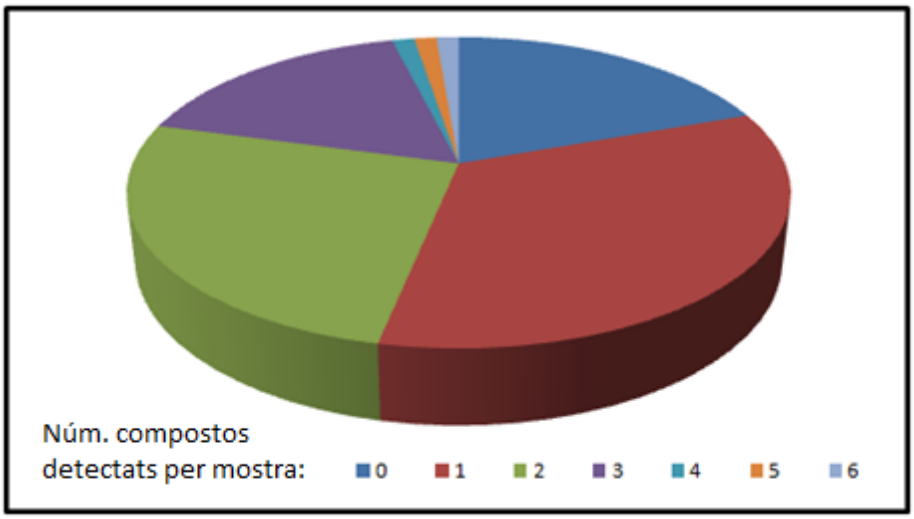

Figura 7. Distribució de les 62 mostres positives de les 77 mostres analitzades segons el número de compostos detectats.

Pel què fa als 69 compostos estudiats només es van detectar 13, és a dir, aproximadament un 19 %. Els compostos més detectats van ser amb diferència el DMP (n=41), la BP (n=32) i el naftalè (n=16). La concentració mínima, màxima i els quartils al 25 i 75 % dels compostos detectats igual o més de 3 cops es poden apreciar a la Figura 8. La resta de compostos, desetil atrazina, atrazina i 4-NP que només es van detectar 1 cop a concentracions entre 0.005 i 0.054 µg/L, i p,p'-DDE i simazina es van detectar 2 cops a concentracions entre 0.005 i 0.023 µg/L.

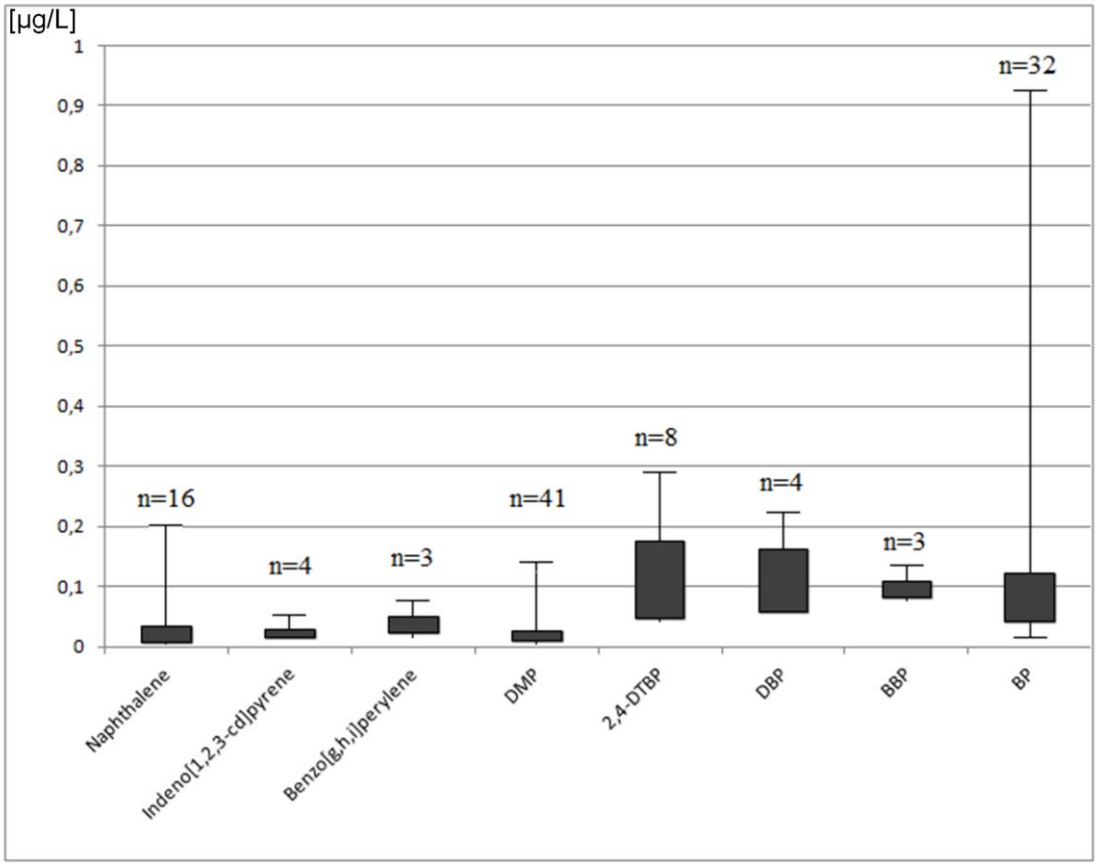

Figura 8. Màxims, mínims i quartils 25 i 75 % per als compostos detectats n≥3.

Tenint en compte tots els valors globalment, es van detectar 118 resultats per sobre del LOQ d'un total de 5313 valors (69 compostos x 77 mostres), és a dir, aproximadament el 2 %. També es pot apreciar que els components del plàstic i alguns PAH, especialment el naftalè, són els compostos més detectats i que caldria controlar-los. Aquests resultats demostren la bona qualitat de l'aigua envasada arreu del món des del punt de vista dels compostos orgànics analitzats.

3.4. Conclusions

- La SBSE ha demostrat ser un mètode d'extracció molt eficaç per a la determinació d'un elevat nombre de contaminants ja que la manipulació de la mostra és mínima.
- Els LOQs del mètode SBSE-GC-MS/MS són més baixos que els del mètode SPE-GC/MS.
- Les aigües envasades d'arreu del món gaudeixen de bona qualitat ja que la presència de contaminants és molt baixa.
- Tal i com s'ha demostrat a l'anterior estudi, el plàstic PET resulta ser un molt bon material per a envasar aigua des del punt de vista de la migració específica.

4. ASSAIGS DE MIGRACIÓ D'AMPOLLES DE PLÀSTIC I IMPLICACIONS TOXICOLÒGIQUES

4. ASSAIGS DE MIGRACIÓ D'AMPOLLES DE PLÀSTIC I IMPLICACIONS TOXICOLÒGIQUES

4.1. Introducció

Històricament, els envasos per aliments s'han utilitzat com a medi de transport fins arribar al consumidor. Actualment, els envasos també preserven els aliments, donen informació al consumidor i protegeixen el producte envasat de contaminacions externes. Per a dur a terme aquestes accions, és necessària la utilització de materials d'envasat adequats que protegeixin l'aliment durant la conservació, transport i distribució. Tanmateix, el contacte entre l'aliment i l'envàs introdueix la possibilitat de transferir components de l'envàs a l'aliment. Per a què aquest fet no esdevingui un problema per a la salut humana, els materials són sotmesos a investigació i a legislacions específiques, com és el cas dels envasos de plàstic.

Per a què els envasos siguin adequats cal complir amb la legislació vigent a diferents nivells de la seva fabricació i distribució (fabricant del polímer, transformador del polímer fabricat, envasador i comerciant). El fabricant del polímer ha d'assegurar que els monòmers i additius utilitzats estan a la llista de substàncies permeses que descriu la legislació. El transformador ha de disposar de les especificacions del fabricant i ha de transferir-les a l'envasador. L'envasador ha de tenir totes les especificacions del producte final; a vegades, com és el cas de les ampolles d'aigua de polietilè tereftalat (PET), l'envasador també és el transformador ja que es fabriquen a la mateixa planta envasadora. Finalment, el comerciant és l'última baula de la cadena d'envasat d'aliment i ha de poder disposar de tota la certificació dels seus subministradors.

Un dels requisits que contempla la legislació de materials polimèrics en contacte amb els aliments és la realització d'assaigs de migració global i específica que garanteixin que, en cas d'haver migració de components del plàstic, la migració estigui per sota dels nivells legislats i, per tant, no esdevinguin un risc per a l'ésser humà. Per altra banda, degut a què poden haver diverses transformacions dels polímers i diverses addicions d'additius, és possible que el comerciant final no tingui tota la informació sobre tots els components de l'envàs. Aquest fet també succeeix en el cas d'importacions i exportacions entre països que, en haver diferents legislacions, desemboca en una falta de transferència d'informació al llarg de la cadena d'envasat.

Per aquesta raó, en el següent estudi, es descriuen assaigs de migració dels materials polimèrics més utilitzats per a l'aigua envasada (PET; HDPE, PC; LDPE i PS), en què, a part dels anàlits determinats anteriorment, també es realitza una identificació d'altres compostos amb la finalitat de determinar tots els compostos susceptibles de migrar (article científic IV). D'acord amb els resultats exposats en els estudis anteriors, també es vol demostrar la relació entre la migració de BPA i la utilització del plàstic PC. Però aquesta afirmació desemboca en una altra pregunta: les concentracions trobades poden afectar a l'ésser humà? Per a contestar aquesta pregunta cal retrocedir fins l'any 1993, en què Krishnan et al. (1993) va suggerir per primera vegada l'estrogenicitat del BPA que imita l'acció dels estròGens, i, per tant, és capaç d'alterar el sistema endocrí en organismes que hi estan exposats. Tot i això, era difícil preveure les concentracions en què es produeixen efectes. Degut a la polèmica que ha generat la presència de BPA en aliments i en aigües envasades en PC, s'està intentant trobar nous substituts polimèrics lliures de BPA. Entre altres, el copolièster Tritan™.

Per a avaluar si el copolièster Tritan™ podria arribar a ser un bon substitut del PC en quant a migració de compostos potencialment tòxics, es van realitzar assaigs de migració en garrafons reutilitzables de PC i en envasos esportius i prototips de garrafons de Tritan™, també reutilitzables. Un cop identificats els compostos que migraven s'han pogut aplicar diferents mètodes d'assaigs *in vivo* i *in vitro* per a la caracterització de l'activitat de disrupció endocrina de les mostres d'aigua dels envasos sotmesos als assaigs de migració i dels compostos identificats a les concentracions detectades (article científic V).

4.2. Treball experimental

4.2.1. Tècniques analítiques

En el treball experimental descrit en els articles IV i V, es van realitzar tres tipus d'assaigs de migració diferents:

1. Mètode basat en la legislació aplicable en el moment (el Reial Decret 866/2008 i les respectives normes europees, així com en la norma UNE-EN 13130 per a assaigs de migració específica) en què cada plàstic analitzat es va incubar en un bany d'aigua a 40 ºC durant 10 dies (articles científics IV i V).
2. Mètode basat en la norma UNE-EN ISO 177 de l'any 1988 que s'usa en plàstics en general i utilitza discs adsorbents per a afavorir la migració, per tant

no és aplicable específicament per a plàstics en contacte amb aliments; aquest mètode està establert per a avaluar la migració global. Es va adaptar a les condicions legislades de 10 dies a 40 ºC, tot i que no es va utilitzar cap simulant alimentari (article científic IV).

3. Mètode basat en l'extracció per ultrasons utilitzant aigua destil·lada en contacte amb el plàstic; aquest mètode es va utilitzar per a avaluar si utilitzant unes condicions més forçades en menys temps es poden obtenir resultats comparables als de la legislació aplicable a materials plàstics en contacte amb aliments (article científic IV).

4.2.2. Tècniques toxicològiques

4.2.2.1. Assaigs *in vitro*

Els assaigs basats en llevats recombinants (article científic V) van ser desenvolupats per a identificar compostos que podien interaccionar amb els receptors humans d'estròg ens (hER). Normalment les cèl·lules dels llevats no contenen receptors d'estròg ens, de tal manera que s'hi ha d'introduir seqüències de DNA dels receptors hER dins el cromosoma del llevat. Les cèl·lules dels llevats també contenen plàsmids d'expressió que contenen el gen lac-Z que codifica l'enzim β-galactosidasa i que és utilitzat per a mesurar l'activitat dels receptors. El procés que succeeix durant aquest assaig es pot descriure en 6 passos (Figura 9):

1. El receptor hER s'expressa des del genoma situat al nucli del llevat.
2. El receptor hER és capaç d'unir-se als elements de resposta a estròg ens que s'ubiquin en el promotor fort del plasmidi d'expressió.
3. Quan el llevat modificat s'exposa a un contaminant estrogènic, aquest s'uneix al receptor hormonal i es transforma en un promotor actiu.
4. El receptor actiu interacciona amb factors de transcripció que modulen la transcripció del gen. Això provoca l'expressió del gen resposta lac-Z i la producció de l'enzim β-galactosidasa.
5. L'enzim β-galactosidasa es secreta dins el medi del llevat, on metabolitza el substrat cromogènic clorfenol vermell- β-gatactopiranosida (CPRG).
6. El CPRG canvia de color (de groc a vermell) i permet mesurar la seva absorbància a una longitud d'ona de 540 nm i així determinar l'activitat estrogènica (Routledge and Sumpter, 1996).

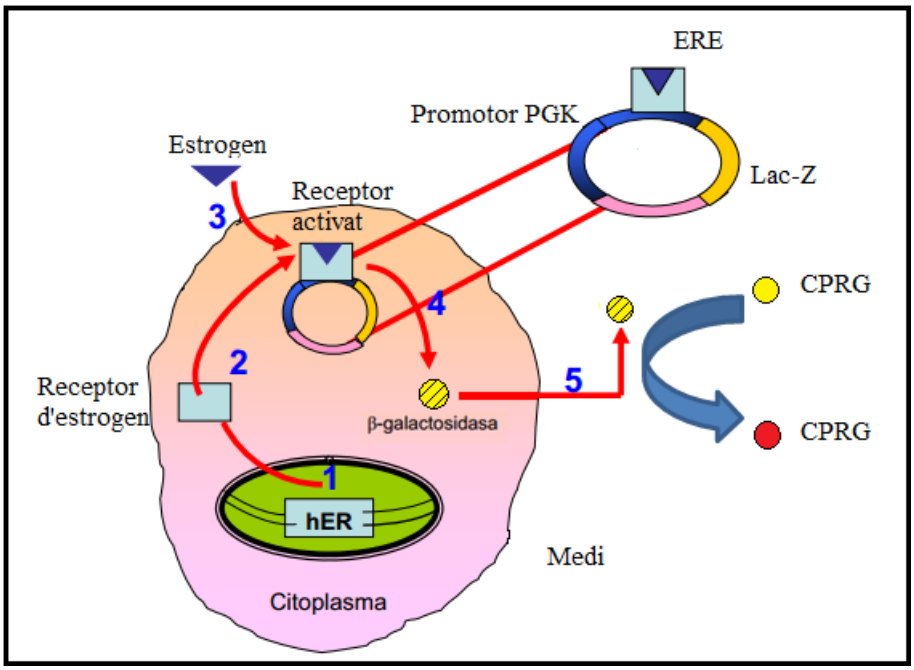

Figura 9. Esquema del funcionament d'un assaig *in vitro* per a detectar compostos amb activitat estrogènica. Figura adaptada de l'estudi realitzat per Routledge i Sumpter (1996).

Es va utilitzar aquest mateix mecanisme per a receptors d'estròg ens en assaigs *in vitro* per a realitzar cinc tipus d'assaigs més amb diferents receptors, els quals estan indicats en la següent taula:

Taula 8. Assaigs *in vitro* utilitzats en aquest estudi (article científic V).

Nom de l'assaig	Tipus d'activitat	Funció en l'ésser humà	Control positiu a l'assaig	Color final si l'assaig és positiu (λ=540mm)
Yeast Estrogen Screen (YES)	Estrogènica	Hormona sexual femenina	17β-estradiol (E2)	Vermell
Yeast Androgen Screen (YAS)	Androgènica	Hormona sexual masculina	Testosterona (T)	Vermell
Yeast Antiestrogen Screen (YAES)	Antagonista al receptor d'estrogen	Inhibeix l'hormona sexual femenina	Antiestrogen 4-hidroxitamoxifen	Groc
Yeast Antiandrogen Screen (YAAS)	Antagonista al receptor d'androgen	Inhibeix l'hormona sexual masculina	Flutamida	Groc
Retinoic Acid Receptor α (RARα)	Anàleg a la Vitamina A[1]	Utilitzats en malalties de la pell i tumors[2]	Àcid retinoic *All-trans* (ATRA)	Vermell
Vitamin D_3 Receptor (VDR)	Anàleg a la Vitamina D_3	Manté l'homeòstasi del calci[3], inhibeix la proliferació cel·lular i la modulació de la funció immunocel·lular[4]	1α,25-Dihidroxivitamina-D3 (Calcitriol)	Vermell

[1] (Zouboulis and Orfanos, 2000)
[2] (Elewa and Zouboulis, 2011)
[3] (Norman, et al., 1982)
[4] (Gombart et al., 2006)

4.2.2.2. Assaig *in vivo*

Un cop identificats els compostos procedents de la migració es va realitzar un assaig de toxicitat (article científic V) amb el cargol d'aigua dolça *Potamopyrgus antipodarum* (GRAY 1843). Aquest cargol pertany als mol·luscs, classe Gasteròpode, subclasse Prosobranchia, ordre Mesogastropoda i família Hydrobiidae; és originari de Nova Zelanda però ha estat introduït en altres parts del món. El seu habitat típic és l'aigua corrent de petits rierols, estanys o estuaris, on la seva reproducció acostuma a ser molt intensa (Department Aquatic Ecotoxicology, 2012; OECD, 2010). Per a aquest estudi s'han utilitzat cargols d'un cultiu del laboratori del Department of Aquatic Ecotoxicology de la Goethe Universität de Frankfurt am Main, que prèviament eren originaris d'una població natural de cargols d'un rierol de Dörente, a prop de Ibbenbüren (North Rhine-Westphalia, Germany).

Per a aquest estudi es van utilitzar cargols adults femella. Cal destacar que les poblacions europees són gairebé totes femelles (Ponder, 1988; Wallace, 1979) essent molt estrany trobar cargols mascle i al laboratori no se n'han trobat mai. El principi de l'assaig és exposar un nombre determinat de cargols a diferents concentracions del ED per un temps específic (en aquest cas 28 dies) i, un cop passat el període d'assaig, determinar el número d'embrions. Aquests cargols són sensibles als estròg ens, els quals faran augmentar la seva reproducció i, per tant, augmentar el nombre d'embrions.

Per a poder avaluar si hi ha hagut un augment del nombre d'embrions, cal realitzar un blanc que consisteix en no addicionar cap tipus de substància, un control de dissolvent que consisteix en addicionar 8 µL de dissolvent dimetilsulfòxid (DMSO), el qual s'utilitzava per a dissoldre els compostos a estudiar i així avaluar l'efecte del dissolvent, i un control positiu de 17α-ethinylestradiol (EE2) a una concentració de 0.025 µg/L que produïa un augment en el nombre d'embrions. Per a poder fer l'assaig més robust i poder fer un tractament estadístic de les dades obtingudes es van realitzar replicats per a cadascun dels controls, el blanc i les diferents concentracions dels compostos estudiats (Department Aquatic Ecotoxicology, 2012; OECD, 2010). A la Figura 10 es poden apreciar els cultius i l'assaig per a cada concentració de compost estudiat i els corresponents replicats. Els tubs de color verd són tubs homologats per a poder fer arribar oxigen als cargols on, al final de cada tub, s'hi va addicionar una pipeta Pasteur de vidre. D'aquesta manera, l'única part en contacte amb l'aigua dels cargols era de vidre i així s'evitaven possibles contaminacions per part del plàstic.

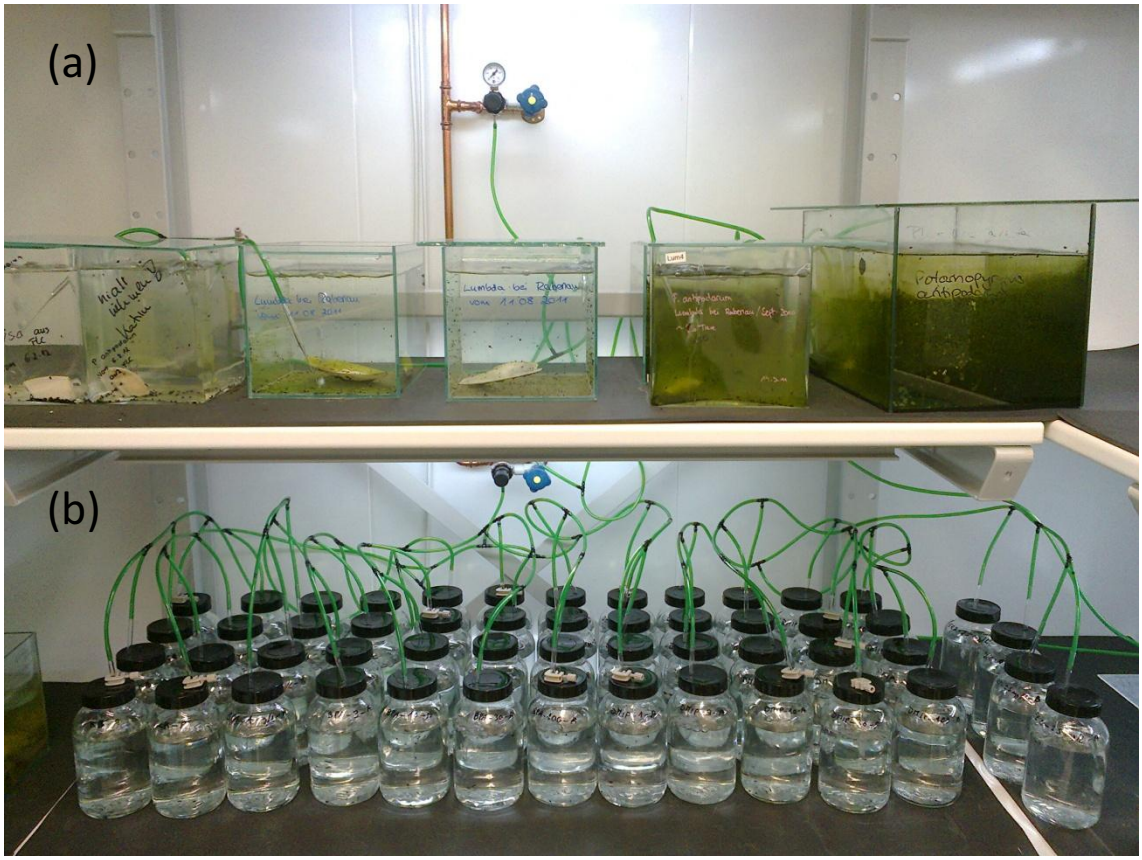

Figura 10. (a) Cultius i (b) assaig *in vivo* amb el cargol *Potamopyrgus antipodarum.*

Article científic IV

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

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

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This study investigates the potential migration of plasticisers, 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: (1) standard method UNE-EN ISO 177; (2) ultrasonic forced extraction; and (3) standard method UNE-EN 13130-1. In addition, bottled waters contained in different plastic materials were analysed 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 analysed 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; plasticiser; 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 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 stabilisers, anti-acids, antimicrobials, antioxidants, chain-breaking, photo- and hydroperoxide deactivating antioxidants, dehydrating agents, light screening pigments and UV absorbers (Bolgar et al. 2008; Piringer and Baner 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 plasticisers and additives to water by a diffusion process (Biscardi et al. 2003), as described for bisphenol A (BPA) (Biles et al. 1997; Casajuana and Lacorte 2003; Loyo-Rosales et al. 2004; Le et al. 2008; Gallart-Ayala et al. 2011), phthalates (Peñalver et al. 2000; Casajuana and Lacorte 2003) or nonylphenol (NP) (Casajuana and Lacorte 2003). These substances are either plastic components, such as BPA used as a monomer in the production of polycarbonate bottles (the so-called 'coolers'), or additives used to improve the plastic properties (such as NP), polymerisation accelerators or agents to increase flexibility (phthalates) (Peñalver et al. 2000; Casajuana and Lacorte 2003; Loyo-Rosales et al. 2004; 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

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

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Abstract

This study is aimed to investigate the potential migration of plasticizers and alkylphenols from several water plastic bottles. Compounds under study 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 migration tests: i) standard method UNE-EN ISO 177; ii) ultrasonic extraction and iii) standard method UNE-EN 13130-1. In addition, bottled waters of different plastic material were analyzed to determine the potential migration of plasticizers and alkylphenols in real conditions. In all cases, samples were solid phase extracted using Oasis HLB 200 mg cartridges and analyzed using gas chromatography coupled to mass spectrometry (GC-MS) in a 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. Total Daily Intake is calculated according to the levels detected in bottled water and the assessment of the consumers' risk was evaluated taking into consideration toxicological and legislated values.

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

1. 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 to 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 antioxidants, photoantioxidants, 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.

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

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

This study is aimed to investigate the potential migration of plasticizers and additives from several plastic containers. Three migration tests were tested: i) the standard method UNE-EN ISO 177 (UNE-EN ISO 177 1988) where plastics are in contact with an adsorbent and incubated at 40ºC during 10 days (European Communities 1982); ii) ultrasonic extraction of plastics incubated in water at different times and iii) the standard method UNE-EN 13130 (Part 1) (EN 13130 2005), in which plastics were incubated at 40ºC in water for 10 days. This 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 Communities 1985) and 2007/19/CE (The Commission of the European Communities *2007*), as well as UNE-EN 1186 (UNE-EN 1186 2002).

Analyses were performed using solid phase extraction method followed by gas chromatography coupled to mass spectrometry.

2. Materials and methods

2.1. Chemicals and reagents

Five phthalates, two alkylphenols and bisphenol A were analyzed (Table 1). 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 μg 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 bisphenol A (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 μg mL^{-1} and stored in the dark at -20ºC until use. The surrogate standards used were NP-d_8 (100 ng μL^{-1}), diphenylphthalate-d_4 (DPP-d_4) and BPA-d_{16} 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 (J.T. Baker, The Netherlands). Chromatography grade methanol, acetone, dichloromethane, n-hexane, ethyl acetate and HPLC water were purchased from Merck (Darmstadt, Germany).

2.2. 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 Milli-Q 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.

2.3. Migration tests

Three methods were used to test the migration of plasticizers and alkylphenols from water bottles, taps and septa. All the sample manipulation was done to avoid contact with plastic, always using polytetrafluoroethylene (PTFE) tubing and glass material.

UNE-EN ISO 177 method: this method is used for the determination of migration of plasticizers as a screening tool, expressed as loss of weight in the incubated plastic and gain in the adsorbent. It applies to the evaluation of the tendency of plasticizers to migrate from plastic materials to other materials or plastics placed in close contact with them. It can't be compared with food legislation, although it shows the possible migration of plasticizers and it is the starting point of 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 40ºC 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.

Figure 2. Illustration of the three tests.

Ultrasonic extraction method: this 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 legislation values, it permits knowing what compounds tend to migrate. 1 g of 0.5-1 cm^2 plastic chips was introduced in 100 mL of 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). Total immersion was done to take a regular piece of plastic and to avoid migration from other parts of the container tested, as printed letters or glue used to attach labels. Afterwards, 100 mL of the incubated water was solid phase extracted (Céspedes et al. 2004) and analyzed by gas chromatography coupled to mass spectrometry (GC-MS) to identify target compounds. All assays were performed in triplicate.

UNE-EN 13130: this 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 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 40ºC during 10 days (Figure 1). Total immersion was done to take a regular piece of plastic to avoid migration from other parts of the container tested, as printed letters or glue used to attach labels. As before, water was then extracted by solid phase extraction (SPE) and analyzed by GC-MS. GC-MS was used to analyze phthalates, alkylpohenols and BPA, although UNE-EN 1313-13 describes the determination of BPA in food stimulants by high performance liquid chromatography (HPLC) with ultra violet (UV) detection. All assays were performed in triplicate.

2.4. 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.

2.5. 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 impact 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 70ºC (holding time 2 min) to 135ºC at 10ºC min^{-1}, to 160ºC at 3ºC min^{-1}, to 175ºC at 1ºC min^{-1}, to 195 ºC at 3ºC min^{-1} and finally to 310ºC at 10ºC 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 and ion source temperatures were 280ºC, 280ºC and 200ºC, respectively.

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

2.6. 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 lose during sample manipulation. Calibration curves were constructed for all target compounds over a concentration of 0.01-1 mg L^{-1}. Limits of detection were calculated using 3 times the standard deviation of n=5 blank samples, injection of standards at amounts of 1 and 0.1 µg L^{-1} were injected to avoid overestimated values. Quality controls were performed using HPLC water spiked at 1 µg L^{-1} and were incubated in the absence of any plastic using the ultrasonic extraction and the UNE-EN 13130 method.

3. Results

3.1. 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 able 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 40ºC 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 polymerized plastic to the extracts. The method allowed the determination of target analytes in water samples at levels of ng L^{-1} (Table 1). The LOD of phthalates were high compared to other studies (Peñalver et al. 2000) because they were calculated from the blank samples which always contain 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).

Table 1. Plasticisers and additives studied, their molecular formula, water–octanol coefficient (log Kow), toxicity using Daphnia magna and recombinant yeast assay (RYA), limits of detection calculated from three times the standard deviation of the blank samples (n¼5), recoveries calculated at 1 mg l_1 and legislated values.

Compound	Acronym	Molecular formula	Log K_{ow}	Aquatic toxicity ($\mu g\,l^{-1}$)[a]	RYA ($\mu g\,l^{-1}$)[b]	LOD ($\mu g\,l^{-1}$)	Recovery (%)	Legislative level ($\mu g\,dm^{-2}$)	Legislative level ($\mu g\,kg^{-1}$)[c]
Dimethyl phthalate	DMP	$C_{10}H_{10}O_4$	1.60	45900	4080 ± 1200	0.010	112	n.l.	n.l.
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]	30 000[cd]
Bis(2-ethylhexyl) adipate	DEHA	$C_{22}H_{42}O_4$	6.11	n.f.	n.f.	0.080	97	n.l.	n.l.
Bis(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	1000–20 000	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	n.l.	n.l.
Nonylphenol	NP	$C_{15}H_{24}O$	5.92	90–470	78 ± 20	0.017	98	n.l.	n.l.

Notes: [a]EC50 – 48 h, *Daphnia magna*.
[b]EC50 – recombinant yeast assay (EPA/630/R-96/012).
[c]Spanish Royal Decree 866/2008.
[d]According to Directive 2007/19/EC.
[e]According to Directive 2004/19/EC.
n.f., Not found; n.l., not legislated.

3.2. 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 as a screening tool. Table 2 proves that tested plastics incubated at 40ºC 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 40ºC, as considered in UNE-EN ISO 177. This lose in 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. Moreover the PC plastic had a mass increase for plastic and disk, plastic increase could be explained for the gain of environment water vapour when "sandwich" system was retired from oven, next tests shows specific migration from PC plastic.

Table 2. UNE-EN ISO 177 weights for four 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

Migration tests UNE-EN 13130 and ultrasonic incubation were able to identify target compounds. With these methods, 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 the analytes using the ultrasonic incubation were of 45% and 76% for NP and BPA respectively, and the extraction was performed using closed glass containers. The recoveries of the analytes using the UNE-EN 13130 were 92% and 97% for NP and BPA respectively, and the extraction was also performed using closed glass containers. The different recoveries are explained because for ultrasonic incubation the surrogates were added before incubation to take in account the loss of analytes. In our study, values are given in

μg dm^{-2} because the samples were extracted from bottles as sheets and not as filled samples (Table 3). In total immersion the two phases: inside (in contact with food) and outside (in contact with environment) are in contact with simulant, in European standards it is considered the most severe test.

Table 3. Compounds identified using ultrasonic extraction and the conditions specified in UNE-EN 13130 expressed in mg dm^{-2} of plastic_standard deviation and percentage recoveries of target compounds in the 1 mg L^{-1} spiked HPLC water quality control.

		Bottles			Caps			
Method	Compound	PET (μg dm^{-2})	HDPE (μg dm^{-2})	PC (μg dm^{-2})	HDPE (μg dm^{-2})	LDPE (μg dm^{-2})	Septum PS (μg dm^{-2})	Recovery (%)
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

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 and BPA were the only compounds identified in the incubated plastics at levels of 0.212-0.242 μg dm^2 and 1.752-2.176 μg dm^2, respectively (Table 3), using the tested area samples as described in Directive 2007/19/EC. It seems ultrasonic extraction is more efficient than temperature of 40 ºC for BPA migration, for this reason these results are not compared with legislated values. This method permitted to know what compounds can migrate faster than other methods as UNE-EN 13130 and UNE-EN 1186 describes.

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 μg dm^2 and 0.128-3.423 μg dm^2, respectively (Table 3).

These two last methods are complementary and provide similar information on the migration potential qualitatively. Ultrasonic bath provides a higher migration of BPA compared to the UNE-EN 13130, except for PC plastic. NP also migrates after 10 days of incubation, however the migration in the ultrasonic bath is lower (Table 3). Because UNE-EN 13130 is based on a technical interpretation of Directive 2007/19/EC, provides maximum legislated migration levels for a large number of compounds, this technique was further used to determine the migration levels of target compounds in the 5 polymers studied.

3.3. Polymers tested and migration levels

NP and BPA were the only compounds identified in PC, HDPE, LDPE and PS plastics and for PET and HDPE bottles NP was the only compound identified. The MS chromatograms of the two incubation methods are shown in Figure 2 for BPA in a PC plastic. PET was the plastic type which NP migration was lower, followed by HDPE bottles and PC. For caps material, NP levels increased from LDPE<HDPE<PS with NP migration between 0.413 μg dm^{-2} and 1.282 μg dm^{-2}. Contrarily, BPA was no detected in PET or HDPE bottles while the highest migration was found in PC bottles at 3.423 μg dm^{-2}. BPA was also detected in caps and septums with levels ranging from 0.128 μg dm^{-2} to 2.176 μg dm^{-2}.

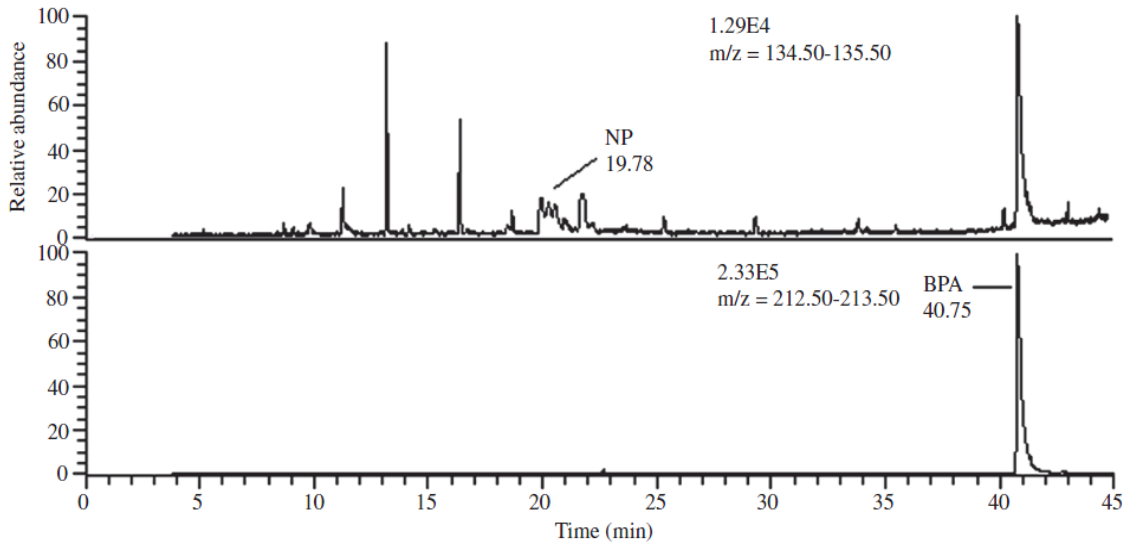

Figure 2. GC-MS TIC chromatogram of a PC sample with the UNE-EN 13130 method.

PET is one of the most common polymers used in bottled water container for its lightness, its gas barrier and its possibility to be recycled, and it is generally

used in volumes from 0.33 up to 8 L bottles. Although in general it is for a single use, some companies recycle up to 25% of this plastic in the manufacture of new bottles. NP was detected at a mean concentration of 0.332 μg dm^{-2} (n=3) and BPA was not detected in any replicate in this kind of plastic (Table 3). Casajuana et al. (Casajuana et al. 2003) found the presence of DMP, DEP, 4-NP, DBP, BPA, BBP and DEHP in PET bottled water samples at levels of 0.002 to 0.214 μg L^{-1} after 10 weeks storage at high temperature and using SPE-GC/MS. Contrarily, Loyo-Rosales et al. (Loyo-Rosales et al. 2004) describe that neither NP nor OP were found in extracts from water stored in PET containers after 240 h. This discrepancy is attributed to the migration test and conditions used. NP is not legislated by the European Community and thus, a comparison with maximum permissible levels cannot be withdrawn.

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

PC, a more resistant plastic, is used in containers over 10 L capacity and after 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 μg dm^{-2} and NP was detected at 0.694 μg dm^{-2}. Another study using bottled water in PC describes the migration of this compound from used and new bottles at 0.7 μg L^{-1} and 1.0 μg L^{-1}, respectively, after 7 days at room temperature (Le et al. 2008). Nerín et al. (Nerín et al. 2003) detected 30 mg of BPA for kg of PC plastic stored at room temperature used for microwaves oven. Biles et al. (Biles et al. 1997) detected BPA in PC baby bottles and cups at levels ranging from 7 to 58 mg kg^{-1} plastic.

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 μg 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 μg 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 septums 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 bisphenol A in food in contact with plastic was first legislated in Directive 2002/72/EC and modified in Directive 2007/19/EC which is transposed to the Spanish Royal Decree 866/2008. This directive establishes migration specific limits in food and simulators of 600 μg kg^{-1} or 100 μg dm^{-2} for BPA, 1500 μg kg^{-1} or 250 μg dm^{-2} for DEHP, 300 μg kg^{-1} or 50 μg dm^{-2} for DBP and 30000 μg kg^{-1} or 5000 μg dm^{-2} for BBP. From the different polymers tested, none of the samples exceeded the legislated value of 100 μg 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 simulators of 3000 μg kg^{-1} for BPA and 1500 μg kg^{-1} for DEHP. Specifically, the levels of BPA decreased 5 times from 2002 to 2004 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).

3.4. Analysis of bottled water

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

Finally, all PC coolers (N=10, volume = 18.9-20 L) contained BPA at levels ranging from 1.60 μg L^{-1} to 4.44 μg L^{-1}, with an average concentration of 2.64 μg L^{-1}. These levels are in agreement with other studies which indicate that BPA can migrate from both PC and epoxy resins containers in contact with water at levels of

few μg L^{-1}. In a former study regarding the migration of BPA, the U.S. FDA (Food & Drug Administration of the United States) analyzed BPA in water coolers stored for 39 weeks and found BPA at very low levels, between 0.1 and 4.7 μg L^{-1} (Environmental Protection Agency (*EPA) 1993*). In a 1997 study, it was found that BPA migrated when exposing polymeric material to water, ethanol/water and Miglyol (an oil) to temperatures of 65ºC for 10 days. In the case of water in contact with polycarbonate bottles where the BPA is the main monomer, the levels of BPA were from not detected to 5 μg L^{-1} (Biles et al. 1997). In another study, the migration of BPA in PC bottles filled with water and exposed to 100ºC for 1h showed BPA migration levels of 0.23 ± 0.12 μg L^{-1}, while levels increased to 8.4 ± 4 μg L^{-1} and 6.7 ± 4 μg L^{-1} after using a domestic dishwasher between 51 and 169 times (Unit on food contact materials, enzymes, flavourings and processing aids (CEF) et al. 2008).

3.5. Daily Intake and toxicological values

The Tolerable Daily Intake (TDI) is a set of reference values for the acceptable intake of a variety of nutrients, as well as energy. A way to express the toxicity of a chemical specie is to calculate the TDI, which represents a lifetime exposure level that is considered to be safe. In this way BPA is the only compound considered for evaluating the TDI since it was detected in all samples analyzed. Based on the migration data from bottled water, the total exposure to BPA from PC plastic was estimated to be of 4.00 10^{-5} mg BPA kg^{-1} bw day^{-1} (1.5 L water day^{-1}, 60 kg person and 1.60 μg BPA L^{-1}) and an upper range exposure of 1.48 10^{-4} mg BPA kg^{-1} bw day^{-1} (2 L water day^{-1}, 60 kg person and 4.44 μg BPA L^{-1}). In infants (6-12 months), these values would be of 2.05 10^{-5} and 5.69 10^{-5} mg BPA kg^{-1} bw day^{-1} for the lower and upper range, respectively (0.1 L water day^{-1}, 7.8 kg person and range 1.60-4.44 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 already suggested a reference value of 0.05 mg kg^{-1} of body weight (Environmental Protection Agency (*EPA) 1993*). These organizations state that polycarbonate 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 are 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 *D. magna*, EC_{50} values range between 1 and 20 mg L^{-1} (Table 1), and although these concentrations cannot be extrapolated to 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 1.644 ± 0.388 mg 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 1.644 mg 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 EC_{50} 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 US population examined had BPA in urine samples due to the routine ingestion of this compound from plastic food containers (Calafat et al. 2008).

4. Conclusions

Given the increase use of plastic in the bottling packaging sector, migration tests permit to identify 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 1186 permitted to identify the compounds leaching from plastics and to compare the migration levels with legislated values while the ultrasonic extraction was an alternative to evaluate compounds migrating from different polymers and had a comparative performance although is not yet a

validated and accepted method. From 5 polymers tested, phthalates were not present in plastic while NP and BPA were identified at concentrations from 0.128 up to 3.423 μg dm^{-2}. PET and HDPE bottles were the bottles with the lowest amount of EDCs. The most problem compound was BPA which was detected in all polycarbonate bottled waters analyzed and although the total daily intake was below the legislated values, it may contribute to the total daily intake of this compound considering all types of food. BPA could be present in caps and septums as an additive to improve plastic properties, although in principle these plastics are BPA-free. 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 Environmental Protection Agency of the United States 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.

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Migration of plasticisers from Tritan™ and polycarbonate bottles and toxicological evaluation

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ABSTRACT

This study is aimed to compare Tritan™ and polycarbonate (PC) from a point of view of migration of monomers and additives and toxicological evaluation. Migration assays were performed according with Commission Regulation (UE) No. 10/2011. Samples were incubated at 40 °C for three consecutive periods of 10 days. Identification and quantification of the compounds intended to migrate was done using solid phase extraction (SPE) followed by gas chromatography coupled to mass spectrometry (GC–MS) in scan mode. Compounds identified in Tritan™ were 2-phenoxyethanol (2-PE), 4-nonylphenol (4-NP), bisphenol A (BPA), benzylbuthyl phthalate (BBP) and dimethyl isophthalate (DMIP) at levels from 0.027 ± 0.002 to 0.961 ± 0.092 μg/kg, although in the 3rd migration period, BBP and DMIP were the only compounds detected well below the specific migration limit. On the other hand, BPA was the only compound detected in PC polymers at a mean concentration of 0.748 μg/kg. *In vitro* bioassays for (anti)estrogenic, (anti)androgenic as well as retinoic acid- and vitamin D-like activity were negative for Tritan™ and PC migrates. BPA and DMIP were estrogenic in high concentrations. Exposure of the estrogen-sensitive molluskan sentinel *Potamopyrgus antipodarum* confirmed the estrogenic activity of BPA *in vivo* at 30 μg/L.

1. Introduction

Plastic is a processable material based on polymers and is widely used around the world for primary packaging. In the water bottling sector, polyethylene terephthalate (PET) for single use bottles (World Packaging Organisation (WPO), 2008) and polycarbonate (PC) for repeated use are employed in different volumes and shapes. In inappropriate storage conditions, both plastic monomers and additives can migrate to the water, can change its organoleptic properties (Park et al., 2004; Song, Al-Taher, & Sadler, 2003) and, if the migrating compound is toxic or has endocrine disrupting properties, can cause acute or long term health effects (McLachlan, Simpson, & Martin, 2006; Safe, 2004; Waring & Harris, 2005). The most prominent and severe case of migration of plastic components to water is 4,4′-dioxy-diphenyl-2,2-propane (bisphenol A, BPA), a well-known endocrine disruptor interfering with hormone signalling (Kang, Kondo, & Katayama, 2006; Vandenberg, Maffini, Sonnenschein, Rubin, & Soto, 2009; Welshons, Nagel, & Saal, 2006). BPA is used as monomer in the fabrication of PC, in epoxy resins (lining metal cans) and in blends with other types of plastic products (Piringer & Baner, 2008).

Because the PC bond created by BPA is unstable, this chemical can eventually leach into food or beverages in contact with the plastic (Piringer & Baner, 2008). Biles et al. showed that BPA migrated from PC containers to food simulants under controlled time/temperature conditions at concentrations from 7 to 58,000 μg/kg (Biles, McNeal, Begley, & Hollifield, 1997). Casajuana and Lacorte (2003) detected BPA and phthalates in distribution water and natural mineral water bottled in polyethylene, PET and glass containers exposed at temperatures up to 30 °C (Casajuana & Lacorte, 2003). Le et al. determined that after 7 days of exposure at room temperature, BPA was released from new PC bottles at 1.0 μg/L, and at 0.7 μg/L from used PC bottles, using enzyme-linked immunosorbent assay (ELISA). BPA was also detected at 3.84 and 7.67 μg/L after incubation of new PC bottles at 100 °C for 24 h (Le, Carlson, Chua, & Belcher, 2008). Gallart-Ayala et al. detected BPA in eleven canned soft drinks including soda, beer, cola beverages, tea and energy drinks at concentrations ranging from 0.044 to 0.607 μg/L (Gallart-Ayala, Moyano, & Galceran, 2011).

From a legal point of view, the use of BPA in plastics intended to be in contact with foodstuffs has been restricted due to the migration of this compound to food at levels which can cause health hazards. In Canada, BPA is considered as a substance that may constitute a danger to human life or health (Government of Canada, 1999, 2008). In Europe, BPA polycarbonate infant feeding bottles have been banned (The European Commission, 2011a)

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ABSTRACT

This study is aimed to compare Tritan™ and polycarbonate (PC) from a point of view of migration of monomers and additives and toxicological evaluation. Migration assays were performed according with Commission Regulation (UE) No 10/2011. Samples were incubated at 40ºC for 3 consecutive periods of 10 days. Identification and quantification of the compounds intended to migrate was done using solid phase extraction (SPE) followed by gas chromatography coupled to mass spectrometry (GC-MS) in scan mode. Compounds identified in Tritan™ were 2-phenoxyethanol (2-PE), 4-nonylphenol (4-NP), bisphenol A (BPA), benzylbuthyl phthalate (BBP) and dimethyl isophthalate (DMIP) at levels from 0.027±0.002 to 0.961±0.092 µg/kg, although in the 3rd migration period, BBP and DMIP were the only compounds detected well below the specific migration limit. On the other hand, BPA was the only compound detected in PC polymers at a mean concentration of 0.748 µg/kg. *In vitro* bioassays for (anti)estrogenic, (anti)androgenic as well as retinoic acid- and vitamin D-like activity were negative for Tritan™ and PC migrates. BPA and DMIP were estrogenic in high concentrations. Exposure of the estrogen-sensitive molluskan sentinel *Potamopyrgus antipodarum* confirmed the estrogenic activity of BPA in vivo at 30 µg/L.

Keywords: Tritan™; polycarbonate; bisphenol A; dimethyl isophthalate; toxicology.

1. Introduction

Plastic is a processable material based on polymers and is widely used around the world for primary packaging. In the water bottling sector, polyethylene terephthalate (PET) for single use bottles (World Packaging Organisation (WPO), 2008) and polycarbonate (PC) for repeated use are employed in different volumes and shapes. In inappropriate storage conditions, both plastic monomers and additives can migrate to the water, can change its organoleptic properties (Park, Kim, Gee, Watanabe, Ahn & Hammock, 2004; Song, Al-Taher & Sadler, 2003) and, if the migrating compound is toxic or has endocrine disrupting properties, can cause acute or long term health effects (McLachlan, Simpson & Martin, 2006; Safe, 2004; Waring & Harris, 2005). The most prominent and severe case of migration of plastic components to water is 4,4'-dioxy-diphenyl-2,2-propane (bisphenol A, BPA), a well-known endocrine disruptor interfering with hormone signalling (Kang, Kondo & Katayama, 2006; Welshons, Nagel & Saal, 2006; Vandenberg et al.j 2009). BPA is used as monomer in the fabrication of PC, in epoxy resins (lining metal cans) and in blends with other types of plastic products (Piringer & Baner, 2008).

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From a legal point of view, the use of BPA in plastics intended to be in contact with foodstuffs has been restricted due to the migration of this compound to food at levels which can cause health hazards. In Canada, BPA is considered as a substance that may constitute a danger to human life or health (Government of Canada, 1999; Government of Canada, 2008). In Europe, BPA polycarbonate infant feeding bottles have been banned (The European Commission, 2011a) and for food products, a specific migration limit of 600 µg/kg has been established. The United States Environmental Protection Agency (US EPA) (1993) and European Food Safety Authority (EFSA) (2010) set a maximum reference dose or tolerable daily intake (TDI) of 50 µg/kg bodyweight/day.

Due to the worldwide concern about the human exposure to BPA from food and water and its potential endocrine disrupting effects (Kang, Kondo & Katayama, 2006; Welshons, Nagel & Saal, 2006) the packaging industry is searching for new plastics. Tritan™ copolyester, a BPA free plastic, is a potential substitute of PC. There are some brands commercializing Tritan™ as a reusable package in 0.5 and 1 L bottles. Furthermore it could be a substitute for 18.9 L reusable carboys used in bottled water coolers. Tritan™ copolyester was introduced in 2007 as a polymer produced from dimethyl terephthalate (DMTP), 1,4-cyclohexanedimethanol and 2,2,4,4-tetramethyl-1,3-cyclobutanediol (Eastman, 2010).

The present study investigates the migration of organic compounds, either monomers or additives, from Tritan™ copolyester using the migrations conditions described in the Commission Regulation (EU) No 10/2011 (The European Commission, 2011b). A comparison between PC and Tritan™ was done from the point of view of migration of plasticizers and additives, comparison with legislated values and evaluation of their toxicological effects. Water used in migration assays was extracted using solid phase extraction (SPE) and analyzed by gas chromatography coupled to mass spectrometry (GC-MS). To evaluate the toxicological effects, the samples from migration assay and the identified compounds were analyzed *in vitro* for their activity at several hormone receptors (e.g., estrogen and androgen receptor) and *in vivo* in the molluskan model *Potamopyrgus antipodarum*.

2. Materials and methods

2.1. Samples and assays

Samples analyzed were: (i) one 18.9 L reusable PC-carboy (PC-carboy); (ii) three 1 L reusable Tritan™-bottles (Tritan™-bottles) used in sport activities purchased in a shop in Barcelona (Spain) in March 2011 and (iii) two types of 18.9 L reusable Tritan™-carboy prototype (Tritan™-carboy-1 and Tritan™-carboy-2) used in bottles intended for use in water coolers. Samples were totally filled with distilled water; except Tritan™-carboy-1 which was cut in three 1 dm^2 pieces to perform 3 replicates. Furthermore, 45 water samples were directly extracted from 18.9 L reusable PC carboys and analyzed to determine the minimum, media and maximum concentrations of BPA in water. These results were also used in *in vitro* tests.

After migration assays, PC-carboy and Tritan™-carboy-2 samples were used to analyze their endocrine disruptor activity. The *in vitro* tests Yeast Estrogenic Screen (YES), Yeast Antiestrogenic Screen (YAES), Yeast Androgenic Screen (YAS), Yeast Antiandrogenic Screen (YAAS), Retinoic acid receptor (RAR) and Vitamin D receptor (VDR) activity using yeast-based recombinant receptor-reporter gene bioassays were used to test the water samples from migration experiments and the specific individual compound. *In vivo* tests were performed for the compounds found in PC-carboy and Tritan™-carboy-2 samples.

2.2. Migration assays

Migration assays were performed at 40ºC during 10 days, according to Commission Regulation 10/2011 (The European Commission, 2011b), which updates Directive 82/711/EC (Commission of the European Communities, 1982) and Directive 85/752/ECC (Commission of the European Communities, 1985). According with this legislation, plastics used in the migration tests must be new (The European Commission, 2011b). Directive 82/711/CE indicates that tests have to be carried out taking a new sample of the plastic material and using distilled water or water of equivalent quality (= simulant A). In this study, distilled water was used as simulant for the migration assay and was produced in the laboratory in a Milli-Q Integral Purification System.

Migration assays were performed in 3 consecutively periods as it is indicated for reusable containers. The 1st incubation period was used to identify compounds intended to migrate; after identification, the 1st, 2nd and 3rd incubation periods were used to quantify the migration compounds. The 3rd incubation period was compared with specific migration limits indicated in the current legislation. All results are shown in µg/kg taking into account the water density of 1 kg/L. In the case of PC-carboy sample the compounds analyzed were NP and BPA due to the prior experience with this material (Guart et al., 2011).

2.2.1. Chemicals and reagents

A preliminary list of compounds susceptible to migrate was done. These compounds included analytical grade (98% purity) Phthalate Esters Mix including dimethyl phthalate (DMP), diethyl phthalate (DEP) di-n-butyl phthalate (DBP), 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 mg/mL each in methanol. 4-nonylphenol (4-NP) as a technical mixture was from Riedel-de Haën (Seelze, Germany) as a solid technical mixture of isomers; BPA was from Dr. Ehrenstorfer (Augsburg, Germany) as a solid; benzophenone (BP, ≥99%), dimethyl terephthalate (DMTP, ≥99%) and dimethyl isophthalate (DMIP, ≥99%) were from Sigma-Aldrich (St. Louis, MO) as a solid purified by sublimation; and 2-phenoxyethanol (2-PE, ≥99,5%) was from Fluka (Neu-Ulm, Germany) at analytical grade (≥99.5%). Stock standard solutions of each compound were prepared in ethyl acetate at a concentration of 5 mg/mL and stored in the dark at -20ºC until use. Surrogate standards dipropylphthalate-d_4 (DPP-d_4) and BPA-d_{16} were purchased as solids from Dr. Ehrenstorfer. The internal standard was anthracene-d_{10} (A-d_{10}), purchased from Supelco (Bellefonte, PA, USA). 200 mg Oasis HLB cartridges were from Waters (Milford, MA, USA) and used with a Baker vacuum system (J.T. Baker, The Netherlands). Chromatography grade methanol, acetone, dichloromethane, n-hexane and ethyl acetate were purchased from Merck (Darmstadt, Germany). Distilled water was produced from an Integral Water Purification System from Millipore (Billerica, MA, USA). It is controlled every week by analysing total organic carbon (TOC) (4-5 µg/L) and conductivity (<0.066 mS/cm) that provides a pH of 6.2.

2.2.2. Solid Phase Extraction procedure

SPE procedure has been described previously for some of the target compounds (Bono-Blay et al., 2012; Guart et al., 2011). Although, sample volumes and standard concentration were different in this study because of the new target compounds. 0.5 L of resulting water from migration tests were spiked with 50 µL of 5 ng/µL surrogate standards DPP-d_4 and BPA-d_{16} and afterwards 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 Teflon to avoid contamination of phthalates. 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 phthalates. Then, 0.5 L of sample were preconcentrated at a flow of 8-13 mL/min and afterwards were dried under vacuum during 1 hour. If 1 L of sample was extracted, breakthrough occurred for 2-PE. 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-d_{10} (5 ng/µL) was added as internal standard.

2.2.3. Instrumental analysis

Samples were analyzed by gas chromatography coupled to a quadrupole mass spectrometer (Thermoquest GC 8000 Top/Finnigan Voyager MS, ThermoFinnigan, Bremen, Germany). The system was operated in electron impact mode (EI 70 eV). The separation was achieved with a 30 m x 0.25 mm BPX5 capillary column (SGE Analytical Science) coated with 5% phenyl polysilphenylene-siloxane (film thickness 0.25 µm). The oven temperature was programmed from 60ºC (holding time 3 min) to 310ºC at 10ºC min^{-1}, keeping the final temperature for 15 min. Two µL were injected in the splitless mode, keeping the split valve closed for 1 min. Helium was used as carrier gas (1.0 mL/min). Injector, GC interface temperature and ion source temperatures were 275ºC, 250ºC and 200ºC, respectively.

Peak detection and integration were carried out using Xcalibur 1.4 software of Thermo Fisher Scientific (San Jose, CA, USA). Full scan data (50-450 m/z) was

used for the identification of plasticizers and additives in the 1st incubation period. Then Select Ion Monitoring (SIM) was used to quantify target compounds at each migration period.

2.2.4. Quantification and quality parameters

Internal standard quantification was performed using the deuterated surrogate standards (BPA-d_{16} and DPP-d_4) to correct any lose during sample manipulation. Calibration curves were constructed for all target compounds over a concentration of 0.04-1 μg/mL. Instrumental limits of detection (iLOD) were calculated from the less concentrated point of the curve (0.04 μg/mL). Method limits of detection (LOD) was determined through the signal-to-noise ratio of 3, from the 0.2 μg/L spiked samples when there was no contribution in blank samples. In the case there was contribution in blank samples, LODs were calculated using the arithmetical mean of the blank concentration plus 3 times the standard deviation (n=10), respectively. Recoveries were performed using distilled water spiked at 0.2 and 1 μg/kg (Table 1). Example of a spiked water sample is shown in Figure 1. All analyses were done in triplicate. Distilled water used in blanks, quality controls and migration assays is produced in the same Milli-Q Integral Purification System.

Table 1. Quality parameters for the identified compounds, indicating retention time (RT), monitored ions, instrumental detection limits (iLOD), method detection limits (LOD) and recoveries (rec) at a spiking level of 0.2 and 1 lg/kg.

Compound	RT [min]	Ions [m/z]	iLOD [ng]	LOD [μg/kg]	Rec. 0.2 μg/kg [%]	Rec. 1 μg/kg [%]
2-PE	10.87	94[a], 138, 77	0.040	0.385	<LOD	75 ± 4.2
DMIP	14.32	163[a], 77, 194	0.001	0.014	81 ± 0.5	NA
4-NP	17.08-17.62	135[a], 149, 107	0.014	0.092	89 ± 1.8	77 ± 3.1
BPA	22.09	213[a], 228, 119	0.003	0.016	83 ± 1.4	87 ± 1.2
BBP	23.44	149[a], 91, 206	0.006	0.003	108 ± 0.2	118 ± 4.0

NA= not analyzed
[a] Quantification ion

2.3. *In vitro* bioassays

Yeast-based reporter gene assays were used to investigate the migration of chemicals with endocrine activity from the PC-carboy and Tritan™-carboy-2. A blank sample from the migration experiments was analyzed as a control for potential contaminations. Water samples coming from the migration assay, as well as BPA and DMIP at concentrations found in samples, were analyzed for estrogenic and androgenic activity in the Yeast Estrogen and Androgen Screen (YES and YAS, respectively) (Routledge & Sumpter, 1996; Sohoni & Sumpter, 1998). Antagonistic activity on estrogen and androgen receptor were investigated in the corresponding Yeast Antiestrogen and Antiandrogen Screen (YAES and YAAS, respectively). In addition, the samples' potential to activate Retinoic acid receptor α (RARα) and Vitamin D3 receptor (VDR) was investigated in yeast two hybrid assays (Nishikawa et al., 1999; Inoue et al., 2009).

Assay procedures and data analysis have been described previously (Behr et al., 2011; Stalter et al., 2011; Wagner & Oehlmann, 2009). In brief, for testing samples, 75 µL of water sample was diluted with 25 µL 5-fold medium and 20 µL yeast suspension. On the other hand, for testing BPA and DMIP, 25 µL substance dissolution was diluted with 75 µL ultrapure water and 20 µL yeast suspension. BPA and DMIP were dissolved in dimethylsulfoxid (DMSO) and tested at concentrations from 0.5 to 1000 µg/L. Each sample was tested in eight replicates. After 18 h (YES, YAES, RAR, VDR) and 20 h (YAS, YAAS) incubation at 30 °C cell growth (and potential cytotoxicity) was monitored by determining the optical density at 595 nm. Activation or inhibition of the respective receptor induces or represses the expression of the reporter gene β-galactosidase. β-galactosidase activity (corresponding to receptor activity) was quantified by adding the substrate chlorophenolred-β-D-galactopyranoside (CPRG) and determining the absorbance at 540 nm wavelength. 250 U/mL lyticase (Sigma-Aldrich, St. Louis, MO) was used to lyse the yeast cells.

Negative (ultrapure water) and solvent controls of ethanol and DMSO were included in each experiment. Positive controls were as follows: 0.133 to 13315 µg/L 17β-estradiol (E2; CAS 50-28-2; >98%; Sigma-Aldrich) for the YES, 138.4 to 6922 µg/L testosterone (T, CAS 58-22-0; > 99%; Sigma-Aldrich) for YAS. 0.81 µg/L E2 or 0.0007 µg/L T dissolved in ethanol was used in the YAES and YAAS to submaximally activate the respective receptor. Hence, the inhibition of estrogen and androgen receptor results in a decreased reporter gene signal. 0.186 to 18600 µg/L of the antiestrogen 4-hydroxytamoxifen (CAS 68392-35-8; >70% Z isomer;

Sigma-Aldrich) and 132.6 to 6629 µg/L of the antiandrogen flutamide (CAS 13311-84-7, Sigma-Aldrich) served as positive control in the YAES and YAAS, respectively. 144 to 432633 µg/L µg/L of *all-trans* retinoic acid (ATRA; CAS 302-79-4; Molekula, Shaftesbury, UK) and 20.0 to 59996 µg/L of 1α,25-Dihydroxyvitamin-D3 (Calcitriol, CAS 32222-06-3; >99%; Molekula) were used for the yeast assays with RAR and VDR, respectively.

Data analysis was performed as described previously (Wagner & Oehmann 2009). The corrected absorbance is presented here as measure of the reporter gene activity. It is calculated from the optical density at 540 nm (CPRG cleavage) relative to the cell number (optical density at 595 nm) corrected by the according blank values (Wagner & Oehlmann 2009, Stalter et al. 2011). Statistical analysis was performed using GraphPad Prism (5.03, GraphPad Software, Inc., San Diego, USA). Nonparametric Kruskal–Wallis tests (with Dunn's multiple comparison test) were applied to compare bioassay data. A p value of <0.05 was regarded as significant.

2.4. *In vivo* bioassay with *Potamopyrgus antipodarum*

To investigate the effects of BPA and DMIP identified in migration experiments an *in vivo* test with the molluskan sentinel *Potamopyrgus antipodarum* (GRAY 1843) was performed. The test is described in detail by the Organisation for Economic Co-operation and Development OECD (2010) and in Sieratowicz et al., (2011). In brief, *P. antipodarum* (Gastropoda, Prosobranchia) is an invasive freshwater species introduced from New Zealand more than 100 years ago. The European populations consist almost exclusively of females with a parthenogenic mode of reproduction. Numerous studies (reviewed in OECD 2010) have shown that the snail's reproduction is sensitive to exposure to endocrine disrupting chemicals: Estrogen-like compounds for example will increase the number of embryos produced by *P. antipodarum*.

The experiment was conducted with the following specifications: 800 mL culturing medium was spiked with 10, 30 and 100 µg/kg BPA and DMIP (dissolved in DMSO), respectively. The solvent concentration was 0.001%. Negative (without DMSO) and solvent controls (0.001% DMSO) were included. 25 ng/L 17α-ethinylestradiol (EE2, CAS 57-63-6) was used as positive control. Three replicates

with 10 snails each (from a laboratory stock, average shell height 3.7±0.3 mm) were used for all control/treatment groups.

The test duration was 28 days (16±1 ºC, constant aeration and 16/8 h light/dark rhythm). During the experiment the medium was renewed twice a week. Feeding (2 µg TetraPhyll per individual) and spiking with the test chemicals was done after each water renewal. Ammonium, nitrite and nitrate concentrations were checked weekly to ensure the quality of test medium. Furthermore, SPE extracts of the water were obtained to check the presence of the compounds in the medium used in the *in vivo* test.

After 28 days of exposure, reproduction of *P. antipodarum* was investigated as described by OECD (2010) and the number of unshelled (young) as well as the total number of embryos was recorded. GraphPad Prism was used for data analysis. One-way ANOVA (with Dunnett's post test) was used to compare the controls/treatments. A p value of <0.05 was regarded as significant.

3. Results

3.1. First incubation period

In order to evaluate the migration in PC polymer and Tritan™ copolyester, the 3 incubation periods described in Regulation 10/2011 (The European Commission, 2011b) for reusable containers were used for different purposes. For Tritan™, the 1st incubation period was used to identify the migration compound, considering both monomers and all the additives incorporated in every manufacturing step. This period generally corresponds to the highest migration values, as the plastic container has not been used before testing. The 2nd incubation period was used to evaluate successive migration of compounds considering that containers are to be refilled. Finally, the 3rd incubation period was used to compare with legislation values.

Table 2 shows the physico-chemical properties of identified compounds in Tritan™ in the 1st incubation assay. Identified compounds were 2-PE, 4-NP, BPA, BBP and DMIP. Concentrations are shown in Table 3. BP, DMP, DEP, DBP, DEHP, DEHA and DMTP were not detected. Their LODs were 0.091, 0.043, 0.387, 0.146, 0.257, 0.055, 0.186 µg/L, respectively.

Table 2. Compounds identified in the 1st incubation period.

Compound	CAS Number	Molecular formula	Molecular Weight [g/mol]	Boiling Point [ºC]	Water solubility [mg/L]	log Kow
2-PE	122-99-6	$C_8H_{10}O_2$	138.2	245	26700	1.16
DMIP	1459-93-4	$C_{10}H_{10}O_4$	194.2	282	290	1.66
4-NP	104-40-5	$C_{15}H_{24}O$	220.4	293-297*	6	5.76
BPA	80-05-7	$C_{15}H_{16}O_2$	228.3	220 (at 4 mmHg)	120	3.32
BBP	85-68-7	$C_{19}H_{20}O_4$	312.4	370	2.69	4.73

SRC Inc. 2012. Interactive Physiprop Database.
* Chemical book webpage

In Tritan™-bottles, BBP and BPA were the only compounds detected. The presence of 0.032 µg/kg of BBP can be attributed to its use as additive or polymer production aid, as a plasticiser in reusable materials and articles and as a technical support agent in concentrations up to 0.1 % in the final product (The European Commission, 2011b). Casajuana and Lacorte found BBP in a PET sample after 10 weeks storage and in distribution water collected from public fountains at a concentration of 0.010 µg/L and up to 0.017 µg/L, respectively (Casajuana & Lacorte, 2003),. Petersen and Breindahl detected BBP in baby diet samples at a range of 17-19 µg/kg (Petersen & Breindahl, 2000). On the other hand, BPA was detected at 0.027 µg/kg. Opposite to our study, Cooper et al. indicated that BPA did not migrate from Tritan™ plastic water bottles (Cooper, Kendig & Belcher, 2011). In Tritan™-carboy-1, 2-PE was detected at 0.961 µg/kg. 2-PE is an aromatic ether used as a solvent for cellulose acetate, dyes, inks, and resins, and in the organic synthesis of plasticizers and pharmaceuticals (American College of Toxicology, 1990). Its presence in Tritan™ can be associated to the organic synthesis of plasticizers involved in the manufacturing process. NP was detected at 0.162 µg/kg. NP is a starting substance for the manufacture of plastic materials established in the provisional lists of Scientific Committee on Food (SCF) list 8 (Commission of the European Communities, 1987) and Spanish Royal Decree 847/2011(Ministerio de la Presidencia, 2011). It is limited at a migration value of 10 µg/kg, but it is not legislated in Commission Regulation 10/2011 (The European Commission, 2011b). It is present in many different plastic polymers as shown in previous studies (Loyo-Rosales et al., 2004, Guart et al., 2011). Similar to Tritan™-bottles, BBP was the only phthalate detected at 0.087 µg/kg. Finally, BPA was detected at 0.039 µg/kg. The presence of BPA could be attributed to the

manufacturing process in the casts, where PC containers were manufactured beforehand. In the successive incubation periods, BPA was not detected.

In Tritan^{TM}-carboy-2, BBP and DMIP were detected at concentrations of 0.046 and 0.902 µg/kg, respectively. DMIP can be used as monomer or other starting substance, although it cannot be used as additive or polymer production aid (The European Commission, 2011b). Stareczek et al. studied the migration of this compound and dimethyl terephthalate (DMTP) in distilled water, aqueous 10% (v/v) ethanol and aqueous 3% (m/v) acetic acid as food simulants and neither DMTP nor DMIP were found in PET bottles samples (Stareczek et al., 1999). Differences between containers were attributed to the different shapes, manufacture process and monomers used that can produce a different compound migration.

Table 3. Migration assays results for the three periods and comparison with specific migration limits.

Compound	1st period [μg/kg]	2nd period [μg/kg]	3rd period [μg/kg]	Specific migration limit [μg/kg]
Tritan™-bottles (n = 3)				
2-PE	<LOD	<LOD	<LOD	–
DMIP	<LOD	<LOD	<LOD	50[a]
4-NP	<LOD	<LOD	<LOD	10[b]
BPA	0.027 ± 0.002	<LOD	<LOD	600[a]
BBP	0.032 ± 0.003	0.043 ± 0.001	0.042 ± 0.002	30,000[a]
Tritan™-carboy-1 (n = 3, pieces)				
2-PE	0.961 ± 0.092	<LOD	<LOD	–
DMIP	<LOD	<LOD	<LOD	50[a]
4-NP	0.162 ± 0.002	<LOD	<LOD	10[b]
BPA	0.039 ± 0.002	<LOD	<LOD	600[a]
BBP	0.087 ± 0.001	0.050 ± 0.002	0.046 ± 0.002	30,000[a]
Tritan™-carboy-2 (n = 1)				
2-PE	<LOD	<LOD	<LOD	–
DMIP	0.902	0.145	0.085	50[a]
4-NP	<LOD	<LOD	<LOD	10[b]
BPA	<LOD	<LOD	<LOD	600[a]
BBP	0.046	0.042	0.040	30,000[a]

[a] Commission Regulation (EU) No. 10/2011.
[b] Spanish Royal Decree 847/2011.

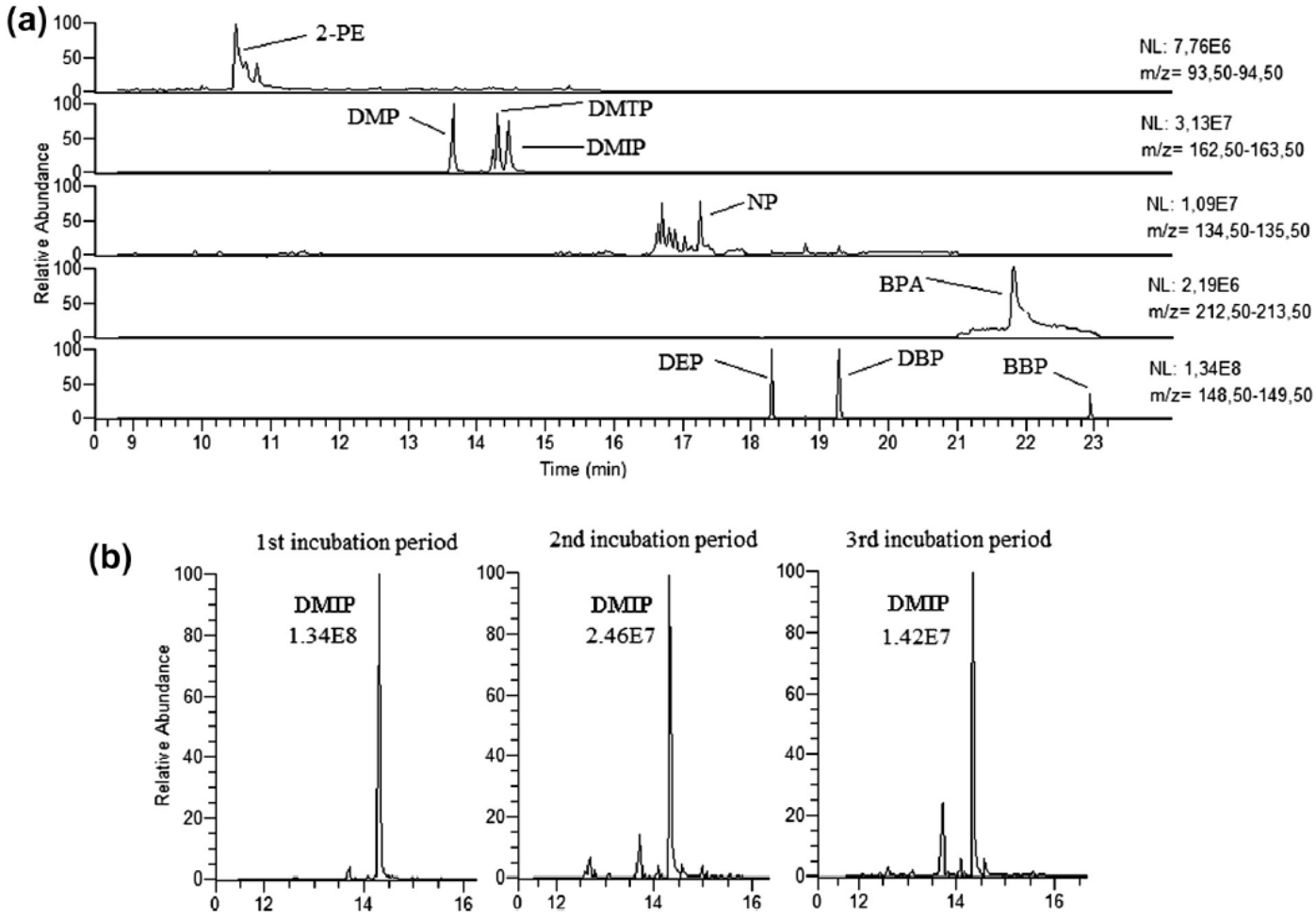


Figure 1. GC–MS chromatograms of (a) distilled water spiked at a concentration of 0.2 lg/kg; (b) DMIP in the Tritan™-carboy-2 sample (monitored at m/z = 163).

3.2. 2nd and 3rd incubation period

Concentration levels in the 2nd and 3rd incubation period are shown in Table 3. In Tritan™-bottles, BBP was slightly higher in the 2nd period than in the 1st, and this concentration was maintained in the 3rd. BPA was not detected in the 2nd or 3rd period. In Tritan™-carboy-1, only BBP was detected in the 2nd and 3rd period, in decreasing concentrations. Similar to Tritan™-bottles, BPA was not further detected, neither 2-PE nor 4-NP. Finally, in Tritan™-carboy-2, the concentration of BBP was maintained throughout the 3 migration periods. DMIP decreased from 0.902 µg/kg in the 1st period to 0.085 µg/kg in the 3rd, as shown in Figure 1.

According with the current legislation (The European Commission, 2011b) for reusable articles intended to be in contact with food, concentrations from the 3rd incubation period must be compared with the specific migration limits. The comparison with legislated limits is shown in Table 3 and in all cases the results are well below legislated values.

In summary, 2-PE, 4-NP and BPA were only identified in the 1st migration period probably as remains of the manufacturing process. On the other hand, BBP and DMIP were detected in the 3 migration periods. These two compounds are limited in EU Regulation 10/2011 (2011b) on plastic materials and articles intended to come into contact with food. It mentions that only compounds detected in the 3rd migration period have to be compared with the limit values of legislation.

On the other hand, in PC-carboy sample, NP was not detected and BPA was detected at concentrations of 0.681, 1.714 and 2.416 µg/kg in the first, second and third incubation periods, respectively. In contradiction to the other detected compounds as DMIP, BPA increased along the incubation periods.

3.3. Direct analyses of BPA in PC water carboys

The analysis of 45 water samples from 18.9 L reusable PC-carboys provided the minimum, median and maximum BPA values of 0.045, 0.748 and 4.445 µg/kg, respectively. The differences between the minimum and the maximum concentrations are explained because of the differences in the PC-carboys, which were not new and had different usage times and conditions. However, it is important to note that PC-carboy migration results were inside the range found in

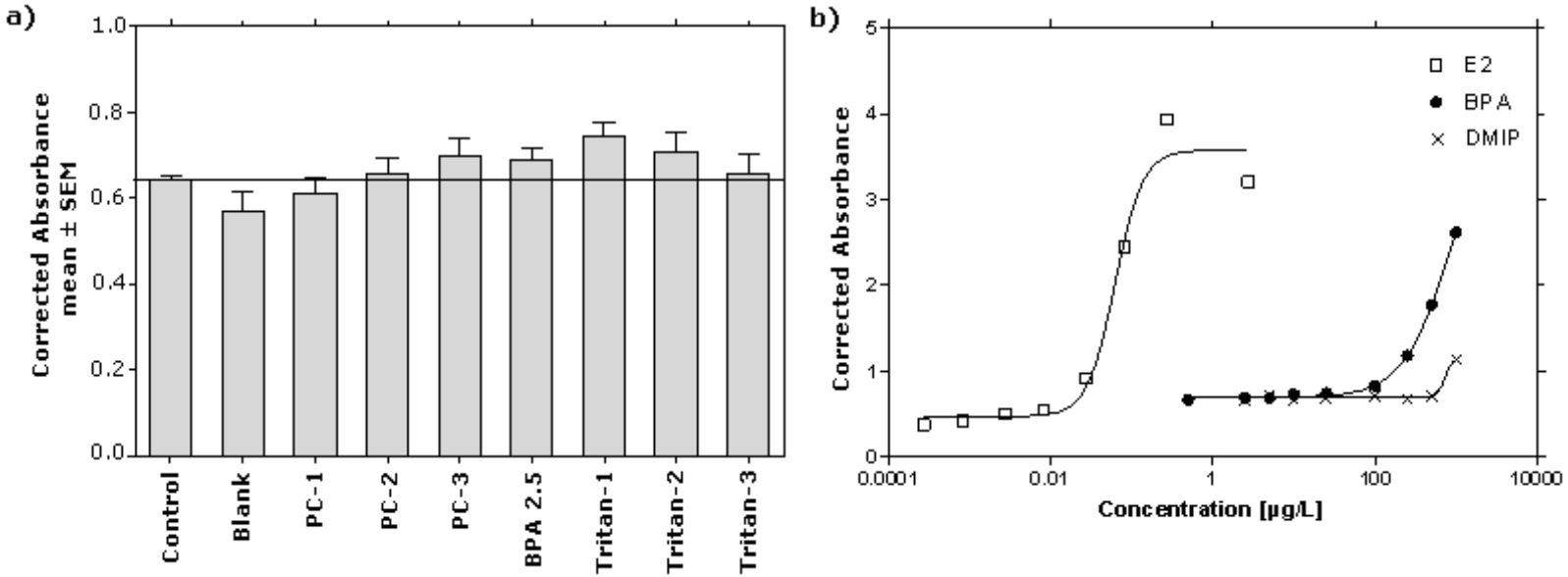
direct analyses of bottled water; it indicates that the migration method and simulant assay worked properly.

3.4. *In vitro* bioassays

PC-carboy and Tritan™-carboy-2 samples were used for *in vitro* tests. Tritan™-carboy-2 sample was chosen because DMIP was identified in the three incubation periods. On the other hand, PC-carboy sample was used to achieve a comparison between Tritan™ and PC materials. In addition, DMIP and BPA, the major 2 compounds identified in the Tritan™ and PC migration assays, respectively, were tested using the different *in vitro* tests to compare the endocrine activity of individual compounds with the samples. YES, YAES, YAS, YAAS, RAR and VDR were negative for DMIP at concentrations of 0.902, 0.145 and 0.085 µg/L (considering 1 L water =1 kg water as mentioned before) and for BPA at concentrations of 0.681, 1.714 and 2.416 µg/L corresponding to the three consecutive migrations incubations. Also, for 0.045, 0.748 and 4.445 µg/L of BPA detected in PC carboys, the results were negative. Only in the YES test it was possible to appreciate a little estrogenic activity (Figure 2). "Control" refers to negative and solvent samples; they were considered together because they had the same corrected absorbance. PC-1 to PC-3 and Tritan™-1 to Tritan™-3 samples refer to the consecutive incubation periods of PC-carboy and Tritan™-carboy-2 in the migration experiments, respectively. In PC samples, an increase in estrogenic activity was observed along the migration assay obtaining the highest absorbance in the 3rd incubation period (PC-3; 2.416 µg/L BPA) with a similar value comparing with the 2.5 µg/L BPA concentration (Figure 2). In Tritan™ samples, a decrease in absorbance was observed along the migration assay obtaining the lowest absorbance in the 3rd incubation period (Tritan™-3; 0.085 µg/L DMIP). However, no significant differences were observed in these samples in comparison with Control because activity is too low in the water samples.

As it is known, BPA has estrogenic activity. For this reason, a curve between 0.05 and 1000 µg/L was done and tested for YES. Also, the same concentration curve and test were used for DMIP. The results are shown in Figure 2 in comparison with E2 which serves as positive control. E2 absorbance started increasing with significant difference at 0.013 µg/L; whereas BPA started at 25 µg/L and DMIP at 500 µg/L.

Figure 3. In vitro YES test: a) Absorbance of Control, Blank (distilled water) and samples from migration assays; b) Estrogenic activity of E2, BPA and DMIP.



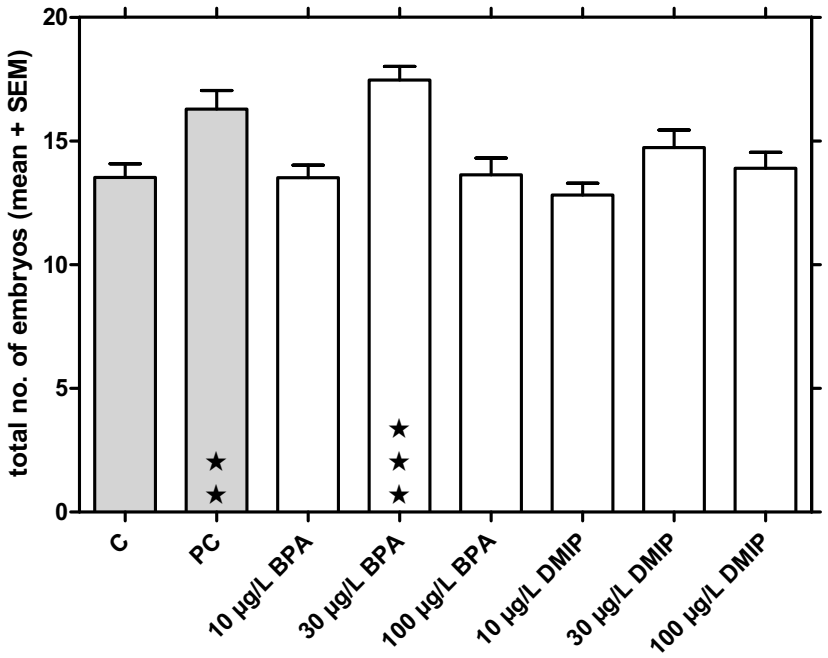
3.5. *In vivo* bioassay with *Potamopyrgus antipodarum*

To investigate potential endocrine effects *in vivo P. antipodarum* was exposed to BPA and DMIP for 28 days. At the end of the test, all the snails had an average shell length of 3.7±0.1 mm without any significant differences between treatment and controls (one-way ANOVA with Dunnett's post test). That showed that the test compounds did not reduce the growth of the animals. Reproduction of the animals was assessed counting the total number of embryos produced by each female. Reproduction of animals from the negative and solvent control groups did not differ significantly (data not shown). This demonstrates that the solvent DMSO did not have an effect by its own. Exposure to 25 ng/L EE2 used as positive control significantly enhanced the reproduction of *P. antipodarum* (Figure 3). This is in accordance to previous studies (Wagner and Oehlmann, 2009; Statler, Magdeburg & Oehlmann, 2010; Magdeburg et al. 2012) and demonstrates that the snail's reproduction is sensitive to estrogens.

Likewise, exposure to 30 µg/L BPA resulted in a statistically significant increase of reproduction. These estrogenic effects of BPA on *P. antipodarum* have been observed previously (Magdeburg et al. 2012, Sieratowizc et al. 2012). In the treatment group with the highest BPA concentration (100 µg/L), reproduction was reduced to control level. These nonmonotonic dose-response curves are typically observed for endocrine disruptors and have been described for *P. antipodarum* before (Jobling et al. 2004). DMIP effects were less pronounced. In snails exposed to 30 µg/L DMIP a slight but not significant increased embryo production was observed. This fact is in accordance with our in vitro data: In the YES, DMIP was less estrogenic than BPA.

Taken together, our *in vivo* data demonstrate the estrogenic effect of the well-known endocrine disruptor BPA on a molluskan model organism. Compared to that, DMIP did not enhance the snail's reproduction significantly. Since *P. antipodarum* serves as a sentinel for estrogen-like compounds, this demonstrates that DMIP is – if at all – only very weakly estrogenic in this model.

Figure 3. *In vivo* test with *Potamopyrgus antipodarum*. Total number of embryos produced after 28 days of exposure to 25 ng/L EE2 (used as positive control, PC), BPA and DMIP (n = 27–30). Significant differences to pooled negative and solvent controls (C, n = 55) are determined by one-way ANOVA and Dunnett's post test. ★★ p<0.01; ★★★ p<0.001.



4. Conclusions

Tritan™ has emerged as a possible substitute of polycarbonate polymer, to be used for water bottling. This study has demonstrated that Tritan™ copolyester has a low migration potential of both monomers and additives. Using distilled water as simulant, BBP and DMIP were detected in the 3 migration periods tested according to Regulation 10/2011 (The European Commission, 2011b) at concentrations 10^5 and 600 times, respectively, below legislated limits. 2-PE, NP and BPA were only detected after the 1st incubation period. BPA was detected at concentrations close to the LOD in 2 of the 3 containers. We have used GC-MS in scan mode to identify compounds with potential endocrine disrupting properties which can migrate from Tritan™ containers, but we do not rule out that other more polar and soluble components can migrate. From the point of view of toxicology, Tritan™ and PC samples were analyzed, as well as the main compounds found in the migration tests, using 6 different *in vitro* tests and an *in vivo* test with *Potamopyrgus antipodarum*. *In vitro* results did not show positive endocrine disruptor activity at the concentrations found in migration assays. Furthermore, it

was probed that for YES test, higher concentrations (25 µg/L BPA and 500 µg/L DMIP) were necessary to observe significant differences comparing with E2-estrogenic activity. In the in vivo assay, 30 µg/L BPA produced an increase in the number of total embryos, while 30 µg/L DMIP did not affect significantly the total embryo production. As a final conclusion, both in Tritan™ and PC materials incubated at 40ºC for 10 days showed the migration of DMIP and BPA, respectively, but at levels that produced negligible endocrine disrupting effects, according to *in vitro* and *in vivo* tests.

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4.3. Discussió dels resultats

4.3.1. Assaigs de migració

El primer pas per a realitzar els assaigs de migració va consistir en fer una comparació entre l'assaig legislat per aliments (Directiva 82/711/EU, Reial Decret 866/2008 i UNE-EN 13130), un altre mètode establert per a plàstics en general (UNE-EN ISO 177) i un mètode basat en ultrasons (desenvolupat al laboratori). Aquesta comparació està descrita a l'article científic IV.

Un cop es van obtenir els resultats dels tres mètodes es va comprovar que el mètode UNE-EN ISO 177 només era adequat per a la determinació de la migració global, és a dir, la quantitat total de plàstic que migra, tal i com s'indica a la norma. Aquest mètode no era adequat per a la determinació de la migració específica, és a dir, per a la identificació de compostos i la seva posterior quantificació. Tot i així es van intentar extreure els discos adsorbents, però no es va poder avaluar la migració específica degut a què els discos adsorbents es quedaven enganxats a la mostra de plàstic i dificultaven la seva extracció, i els cromatogrames d'aquests extractes no permetien la identificació de compostos ja que tenien una línia de fons irregular i molt soroll de fons.

Els altres dos mètodes permetien la identificació i quantificació de compostos corresponents a la migració del plàstic al simulant alimentari (aigua). El mètode d'ultrasons va resultar ser molt més ràpid que el mètode UNE-EN 13130 ja que no requeria de 10 dies d'incubació. Una altra diferència entre els dos mètodes va ser en les recuperacions del NP i el BPA, en què en el mètode d'ultrasons s'obtenien recuperacions de 45 i 76 %, respectivament, i en el mètode UNE-EN 13130 de 92 i 97 %, respectivament. Pel què fa als resultats, ambos casos proporcionaven resultats qualitativament similars, però no quantitativament ja que les concentracions eren diferents degut a la diferent eficiència en l'extracció. Això es va veure reflectit en què el mètode d'ultrasons va mostrar nivells de migració més baixos respecte el mètode UNE-EN 13130.

Tenint en compte l'estudi que es va realitzar per aquests tres tipus d'assaigs de migració, a partir de llavors es va decidir seguir amb el mètode de la legislació i de la norma UNE-EN 13130 que després esdevindria descrit en el Reglament (UE) No 10/2011.

4.3.2. Resultats dels assaigs de migració

En aquests estudis es van realitzar assaigs de migració per als materials polimèrics més utilitzats en l'envasat d'aigua (PET, HDPE, PC, LDPE i PS a l'article científic IV) i el Tritan™ (article científic V). En tots els casos els assaigs de migració es van realitzar per immersió completa després d'haver tallat el material polimèric en porcions (expressat en µg de compost per dm^2 de plàstic) i, en el cas del PC i el Tritan™, també es van realitzar assaigs de migració en què s'omplien les ampolles (expressat en µg de compost per litre de simulant). En aquest últim cas es van realitzar tres períodes d'incubació de 10 dies a 40 ºC en què es canviava el simulant però el contenidor de plàstic era el mateix.

La Taula 9 mostra un resum dels diferents compostos detectats en els assaigs de migració segons el material.

Taula 9. Compostos trobats segons el material.

Tipus de material	Compostos identificats
PET	4-NP
HDPE ampolla	4-NP
HDPE tap	4-NP BPA
LDPE tap	4-NP BPA
PS sèptum	4-NP BPA
PC	4-NP BPA
Tritan™	2-PE 4-NP DMIP BBP BPA

El PET va resultar ser el material amb menys migració específica en què només s'hi va detectar 4-NP a una concentració de 0.332 ± 0.022 µg/dm^2 de plàstic (article científic IV).

Es va analitzar el HDPE utilitzat per a la fabricació d'ampolles i de taps. El HDPE de l'ampolla va mostrar resultats similars als del PET, en què només hi havia migració de 4-NP a nivells de 0.579 ± 0.008 μg/dm^2 de plàstic. El HDPE dels taps contenien 4-NP i BPA a nivells de 1.282 ± 0.178 μg/dm^2 de plàstic i 0.145 ± 0.026 μg/dm^2 de plàstic, respectivament. La diferència de migració entre ampolla i tap va ser deguda a què el seu ús és diferent i, per tant, és d'esperar que els additius en ambdós casos siguin diferents (article científic IV).

El LDPE també va mostrar traces de 4-NP i BPA a nivells de 0.413 ± 0.004 μg/dm^2 de plàstic i 0.158 ± 0.019 μg/dm^2 de plàstic, respectivament (article científic IV).

El PS, que es caracteritza per la seva gran flexibilitat, també presentava concentracions de 4-NP i BPA a nivells de 0.801 ± 0.176 μg/dm^2 de plàstic i 0.136 ± 0.028 μg/dm^2 de plàstic , respectivament (article científic IV).

El PC va ser analitzat de dues formes diferents:

- Porcions tallades d'un garrafó per immersió total (article científic IV): es van detectar traces de 4-NP a nivells de 0.694 ± 0.091 μg/dm^2 de plàstic, i el BPA es va detectar a elevades concentracions en comparació amb els altres materials polimèrics. La concentració de BPA que es va trobar després de la incubació del plàstic en aigua va ser de 3.423 ± 0.217 μg/dm^2 de plàstic.
- Garrafó de 18.9 L reutilitzable per ompliment (article científic V): degut a què el garrafó analitzat era reutilitzable, es van realitzar 3 períodes d'incubació consecutius de l'aigua dins el garrafó, en què es canviava l'aigua en cada període d'incubació, obtenint tres concentracions de BPA corresponents al tres períodes de migració de 0.681, 1.714 i 2.416 μg/L de BPA en el simulant. Hi va haver un augment de la concentració en relació al temps de migració, és a dir, el tercer període d'incubació mostrava més migració que el primer període.

Detectar BPA en aquest tipus de material és habitual ja que es tracta del monòmer a partir del qual està fabricat el PC. En els assaigs de migració realitzats per Mercea (2009) es demostra que un augment en la temperatura de l'assaig de 40 ºC a 60 i 80 ºC produeix un augment de la concentració de l'ordre de 2 i 4 vegades, respectivament, en 10 dies d'assaig. També conclou que per a assaigs de 10 dies, després d'un cert temps es produeix una disminució de la migració del BPA, que atribueix a la inhibició de la hidròlisi a la superfície del PC al llarg del

temps, i a l'absorció del BPA a la mateixa mostra de PC quan l'aigua excedeix una certa concentració. Els resultats obtinguts a l'estudi realitzat en aquest capitol (als tres períodes de migració amb concentracions de 0.681, 1.714 i 2.416 µg/L de BPA, respectivament) no es pot comprovar aquest fet ja que als 10 dies d'assaig es procedia a canviar el simulant i, per tant, el BPA podia no arribar a excedir la concentració de la que indica Mercea (2009). Per altra banda, Mercea (2009) també inclou assaigs amb diferents simulants (diferents mescles d'etanol/aigua, oli d'oliva i àcid acètic al 3 %) concluint que hi ha un augment de la migració de BPA en augmentar el % d'etanol i una disminució de la migració amb l'oli d'oliva. Aquest fet és rellevant ja que, a partir de 2016, els assaigs de migració no es faran amb el simulant aigua, sinó amb etanol al 20 % i, segons el cas, amb àcid àcetic al 3 %.

Finalment, es va analitzar el plàstic Tritan™. L'anàlisi per GC-MS en mode scan (50-450 m/z) va permetre identificar quins compostos havien migrat (article científic V). Es van analitzar tres tipus de mostres:

- Ampolles d'1 L reutilitzables (n=3): es va detectar BPA en el primer període d'incubació a una concentració de 0.027 µg/L de simulant i BBP a valors que es mantenien en els tres períodes d'incubació a concentracions de 0.032, 0.043 i 0.042 µg/L de simulant.
- Porcions tallades d'un garrafó per immersió total (n=3): en el primer període d'incubació es van detectar 2-PE, 4-NP i BPA a concentracions de 0.961, 0.162 i 0.039 µg/L, mentre que el BBP es va tornar a trobar al primer, segon i tercer períodes d'incubació a concentracions de 0.098, 0.050 i 0.046 µg/L de simulant, respectivament.
- Garrafó de 18.9 L reutilitzable per ompliment: en el primer, segon i tercer períodes d'incubació, el BBP es va trobar a concentracions de 0.046, 0.042 i 0.040 µg/L de simulant, respectivament, i el DMIP es va detectar a concentracions de 0.902, 0.145 i 0.085 µg/L.

El fet més destacable en l'anàlisi del Tritan™ és que en dos tipus de mostra es va detectar BPA quan aquest material es comercialitza com a lliure de BPA. L'única explicació plausible és que aquestes ampolles s'hagin fabricat en els mateixos motlles on es fabricava PC i hi hagin quedat restes adherides. En aquest cas també s'explicaria perquè només s'ha detectat BPA en el 1er període d'incubació. Per altra banda el BBP sempre està present a concentracions molt baixes i en un dels garrafons s'ha detectat DMIP en els tres períodes d'incubació. El fet de detectar DMIP en els tres períodes d'incubació podria denotar que és un

residu de la polimerització, potser degut a una impuresa del dimetil tereftalat (DMTP), compost a partir del qual es fabrica el Tritan™.

4.3.3. Ingesta diària de bisfenol A en aigua

Degut a la migració del monòmer BPA del PC a l'aigua, es va decidir analitzar 55 mostres d'aigua agafades de garrafons de 18.9-20 L de PC per a determinar el BPA present en aigua i així avaluar quina és la seva ingesta diària per mitjà de l'aigua (article científic IV).

Les mostres d'aigua analitzades contenien BPA a concentracions entre 0.045 i 4.445 µg/L d'aigua, d'aquesta manera es va demostrar la presència de BPA en totes les mostres de garrafons. Sabent la concentració mínima i màxima trobades a l'aigua envasada (0.045 i 4.445 µg/L), el pes d'una persona adulta mitja (60 kg) i que de mitjana una persona veu entre 1.5 i 2 L d'aigua al dia, es pot determinar que la ingesta diària de BPA d'un adult per mitjà de l'aigua és entre $1.50x10^{-6}$ i $1.48x10^{-4}$ mg BPA per kg de pes corporal i per dia, molt per sota del valor de TDI fixada per l'EFSA i l'EPA que és de 0.05 mg BPA per kg de pes corporal i per dia.

4.3.4. Efectes dels components del plàstic

Els assaigs *in vitro* (article científic V) que es van realitzar a partir de les mostres d'aigua procedents de la migració dels garrafons de PC i Tritan™ van permetre determinar que no hi havia activitat de disrupció endocrina pels sis tipus de receptors. El mateix va succeir en analitzar mostres d'aigua Milli-Q fortificades amb BPA a 0.681, 1.714 i 2.416 µg/L i amb DMIP a 0.902, 0.145 i 0.085 µg/L, concentracions obtingudes en els diferents períodes d'incubació en els assaigs de migració. Tot i no haver-hi una diferència significativa, en el cas de l'assaig YES es va poder apreciar un augment en l'absorbància relativa en concordança amb l'augment de BPA al llarg dels tres períodes d'incubació del PC. De fet, l'absorbància relativa de la mostra d'aigua de l'assaig del garrafó de PC va ser molt similar a l'obtinguda en analitzar l'aigua Milli-Q fortificada a una concentració de 2.5 µg/L de BPA. També es va apreciar una disminució de l'activitat estrogènica en disminuir la concentració de DMIP com passava al llarg dels tres períodes d'incubació del plàstic Tritan™.

Per altra banda, els assaigs *in vitro* també permeten fer gràfics dosi-resposta per a determinar quin és el rang de concentració en què un compost presenta activitat de disrupció endocrina. A la Figura 11 es pot veure una placa de 96 cel·les en què el segment D correspon a diferents concentracions de l'estrogen 17β-estradiol (E2), a partir d'aquesta placa es va realitzar el gràfic dosi-resposta de l'E2. El canvi de color cap a un vermell més intens denota l'augment de concentració de E2 (d'esquerra a dreta) i, per tant, un augment de l'activitat estrogènica.

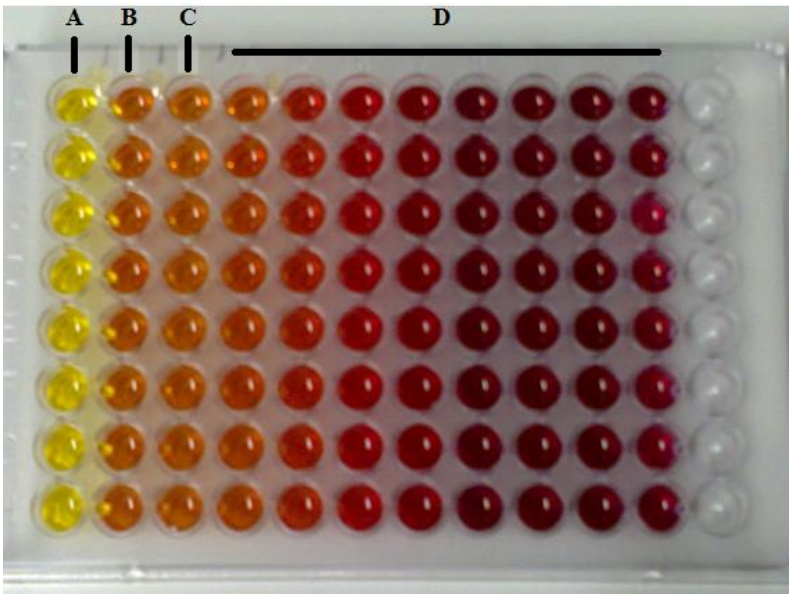

Figura 11. Test de YES. (A) Cel·les sense llevat. (B) Cel·les del blanc amb aigua Milli-Q. (C) Cel·les del blanc amb la mateixa quantitat d'etanol que les mostres (dissolvent usat per a dissoldre l'E2). (D) Cel·les amb E2, d'esquerra a dreta augmenta la concentració.

Pel què fa a altres assaigs *in vitro* realitzats en aigua envasada o en simulants en contacte amb ampolles de PET, cal destacar els estudis realitzats per Wagner i Oehlmann (2009; 2011) i per Bach et al. (2013). Wagner i Oehlmann (2009; 2011) van concloure que hi havia activitat estrogènica en analitzar aigua envasada en ampolles de PET i es va atribuir al contacte amb l'ampolla de plàstic. Bach et al. (2013) va estudiar la migració de diferents compostos d'ampolles de PET a aigua a diferents temperatures, trobant migració de 2,4-DTBP (com en algunes mostres de PET descrites al Capítol 3 d'aquesta tesi) entre d'altres compostos. En aquest estudi, els assaigs *in vitro* van concloure que no hi havia cap tipus de toxicitat (citotoxicitat, genotoxicitat o activitat de disrupció endocrina), la qual cosa concorda amb els resultats obtinguts en aquesta tesi.

Finalment, es van realitzar assaigs *in vivo* (article científic V) per a determinar la capacitat d'actuar com a disruptors endocrins i afectar als éssers vius. Tenint en compte la bibliografia del BPA, es van triar les concentracions de 10, 30 i 100 µg/L per al BPA i les mateixes concentracions per al DMIP per a poder comparar els dos compostos, utilitzant l'estrogen 17α-etinilestradiol (EE2) com a control positiu. A la Figura 12 es pot veure la fotografia d'un *Potamopyrgus antipodarum* amb els seus embrions feta amb un microscopi electrònic.

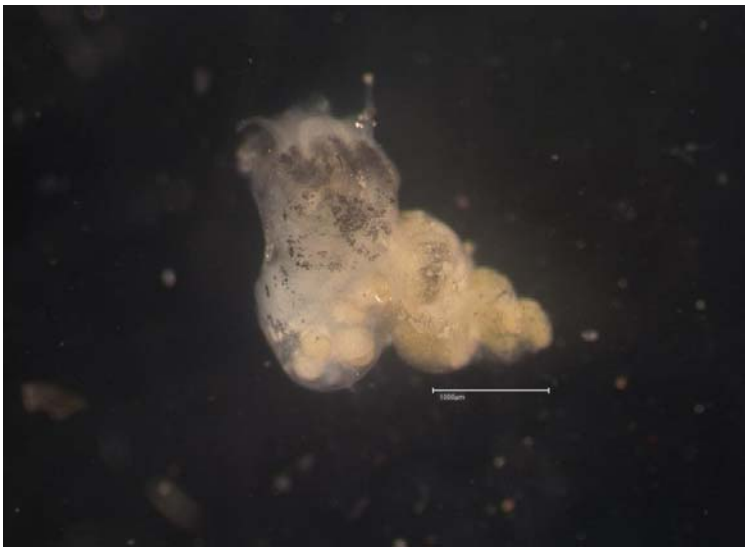

Figura 12. *Potamopyrgus antipodarum* adult sense closca.

Els resultats obtinguts es poden veure a la Figura 13, en què es mostren quatre gràfics corresponents a:

- (A): Els cargols de l'assaig havien de tenir una llargada de la closca compresa entre 3.7 i 4.3 mm per a què tots els cargols de l'estudi fossin adults i es reproduïssin de forma similar. La llargada es mesura abans i després de l'assaig.
- (B): Es van obtenir resultats molt semblants en el nombre d'embrions amb closca per al control positiu (EE2) i pel BPA a 30 µg/L. Tot i així, només en el cas del BPA hi havia una diferència significativa ja que, en el cas del control positiu, la desviació estàndard era una mica més gran i no hi havia diferència significativa. La diferència entre embrions amb closca i sense closca roman en què els embrions amb closca estan més desenvolupats.
- (C): Es va obtenir una diferència significativa en quant a l'augment en el nombre d'embrions sense closca per a la concentració de 30 µg/L de BPA i de DMIP. En el cas del BPA, l'augment és més significatiu.

- (D) Pel què fa al total d'embrions, es pot observar un augment significatiu per al control positiu (EE2) i pel BPA a una concentració de 30 µg/L.

Aquesta espècie de cargol es reprodueix durant tot l'any, però té oscil·lacions en què hi ha mesos de l'any que es reprodueixen més. Durant el desenvolupament d'aquest estudi segurament va coincidir en una època de més reproducció ja que els cargols dels controls negatius i de dissolvent van tenir una mitja d'uns 13 embrions totals que es considera elevat, segons l'experiència del Departament of Ecotoxicology de la Goethe Universität.

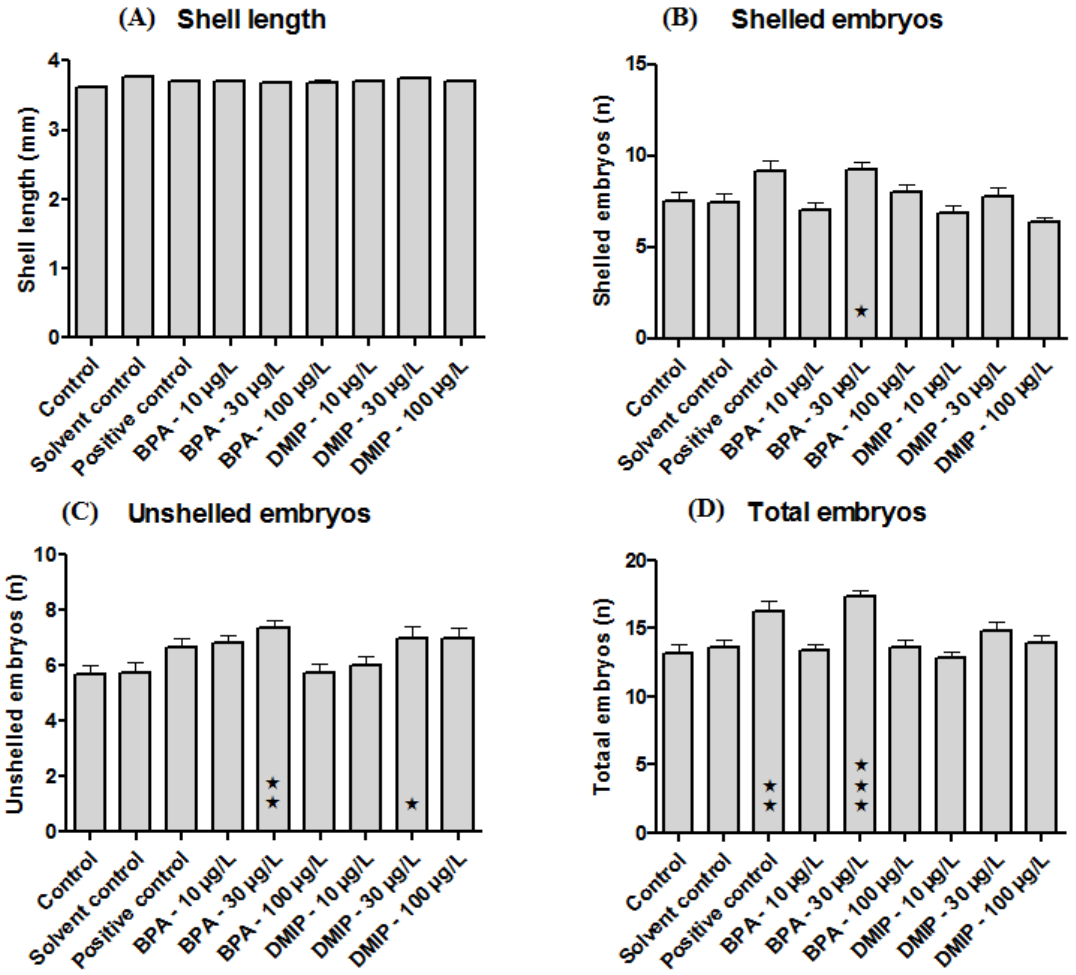

Figura 13. Assaig *in vivo*. (A) Llargada de la closca dels cargols. (B) Número d'embrions amb closca. (C) Número d'embrions sense closca. (D) Número total d'embrions. Les diferències significatives respecte al control (n=55) es van determinar segons els tests ANOVA d'un factor i Dunnett on *p<0.1, **p<0.01 i ***p<0.001.

4.3.5. Efectes toxicològics del bisfenol A detectat als garrafons de policarbonat

A l'aigua envasada dels garrafons de PC analitzats en aquest capítol (article científic V) es van trobar concentracions de BPA amb una mediana de 0.748 µg/L, les quals són molt semblants als valors trobats en el segon capítol d'aquesta tesi (article científic II) amb una mediana de 0.457 µg/L, malgrat les concentracions en algunes mostres puntuals fossin molt més elevades i fora de la normalitat, és el cas de les concentracions de 24.20 µg/L per a l'aigua envasada i de 22.17, 15.64 i 13.20 µg/L per a l'aigua emmagatzemada un any. Tenint en compte els resultats obtinguts en els assaigs *in vitro* i *in vivo* (article científic V), les mostres de 24.20 i 22.17 µg/L podrien causar efectes estrogènics en el test del llevat recombinant i causar un augment dels embrions del *P. antipodarum*. Cal destacar que aquestes concentracions van ser detectades en casos puntuals (article científic II) i la major migració de BPA es podria atribuir a què els garrafons eren nous (major migració de BPA degut a la presència de residual després de la polimerització) o a què els garrafons estaven molt reutilitzats (major migració de BPA degut a la degradació del PC). En la major part de les ampolles, les concentracions de BPA detectades eren molt inferiors a les concentracions necessàries per a poder causar efectes estrogènics.

4.4. Conclusions

- L'assaig de migració basat en el mètode descrit a la legislació i a la norma UNE-EN 13130 (incubació amb aigua durant 10 dies a 40 ºC) va demostrar ser el més adequat per a la identificació i quantificació de compostos susceptibles a migrar del plàstic.
- La concentració de BPA en els garrafons de PC sotmesos als assaigs de migració, així com les aigües analitzades procedents d'aquests envasos estava molt per sota de la ingesta diària tolerable que aplica la Unió Europea.
- Els assaigs de migració de prototips de garrafons reutilitzables de Tritan™ van indicar que hi ha migració de DMIP i BBP. El BPA només va ser detectat al primer període d'incubació, fet que indica que el BPA no és un monòmer o additiu del Tritan™.
- Pel què fa als efectes toxicològics de les mostres d'aigua obtingudes en els assaigs de migració pel PC i pel Tritan™, els assaigs *in vitro* van ser negatius per als 6 tipus de receptors humans. Aquest fet demostra que els compostos que van migrar no es troben a concentracions que puguin afectar la salut humana.
- Pel què fa als efectes toxicològics del BPA i el DMIP, els assaigs *in vitro* van indicar que són necessàries concentracions més elevades de les trobades habitualment (medianes de 0.457 µg BPA/L al segon capítol i 0.748 µg BPA/L en aquest capítol pel PC) per a causar efectes estrogènics en el YES (25 µg/L pel BPA i 500 µg/L pel DMIP).
- L'augment significatiu d'embrions en l'assaig *in vivo* del BPA a 30 µg/L permet afirmar que hi ha una correlació amb els assaigs *in vitro*, els quals van mostrar activitat estrogènica a 25 µg/L.

5. ASSAIGS DE MIGRACIÓ D'EQUIPS DE TRACTAMENT D'AIGUA

5. ASSAIGS DE MIGRACIÓ D'EQUIPS DE TRACTAMENT D'AIGUA

5.1. Introducció

L'aigua de la xarxa és aigua potable que ha estat tractada abans de procedir a la seva distribució. Cada cop amb més freqüència s'instal·len petits equips domèstics de tractament d'aigua a les aixetes per millorar les característiques de l'aigua. Aquest equips poden estar fabricats amb plàstic i metall en els que s'hi pot produir una migració de components dels plàstics.

La legislació europea (EU, 1998) i l'espanyola (Spanish Government, 2003) estableixen els criteris necessaris sobre la qualitat de l'aigua de consum humà, en què es declara que els processos de potabilització no transmetran substàncies o propietats que contaminin o degradin la qualitat de l'aigua i suposin un incompliment dels requisits especificats en aquestes legislacions, incloent els aparells de tractament d'aigua en els edificis. Pel què fa a l'aigua de subministrament de la xarxa hi ha un gran control durant el tractament, transport a través de les canonades d'abastament i pels diferents tractaments dels interiors de les cases, així com els possibles dipòsits d'emmagatzematge, de manera que no es transfereixin substàncies no desitjades a l'aigua. Són segurament les propietats organolèptiques de l'aigua, requisits d'una zona i els requeriments dels consumidors que indueixen a la instal.lació de PoU, que proporciona una aigua en què hi ha un tractament addicional basat generalment en la filtració o osmosi inversa.

La Norma Espanyola UNE 149101 (AENOR, 2011) estableix els criteris per a determinar si un equip de tractament d'aigua en edificis pot ser utilitzat sense transmetre a l'aigua substàncies, microorganismes o propietats indesitjables o perjudicials per la salut. És a dir, estableix la metodologia de mostreig dels diferents paràmetres fisicoquímics i microbiològics segons el tipus d'aparell que es vulgui controlar i estableix quines són les variacions màximes dels paràmetres sense mai sobrepassar els límits legislats a la Directiva 98/83/CE (EU; 1998) i al Reial Decret 140/2003 (Spanish Government, 2003). Pel què fa a la part de materials plàstics, la Norma declara que els materials plàstics han de complir amb la legislació vigent aplicable, amb el Reglament (UE) 10/2011 (EU, 2011).

Tenint en compte el Reglament (EU, 2011) i la Norma (AENOR, 2011), sorgeixen una sèrie de contradiccions entre els dos documents que es discuteixen en aquest capítol.

En primer lloc, cal detallar que l'àmbit d'aplicació del citat Reglament és per a materials i objectes i les seves parts que constin exclusivament de matèries plàstiques (Article 2 del reglament). Per tant, no està contemplat que es facin assaigs de migració d'objectes que puguin tenir part metàl·lica o d'algun altre material no plàstic (en aquest cas l'objecte no és tan obvi com una ampolla, sinó que és tot l'equip de tractament, tot i estar compost per diferents materials i objectes). Aquest problema suposa que per als objectes o equips de tractament amb altres tipus de materials caldrà prendre aquest Reglament com a legislació de referència i no com a legislació aplicable.

En el Reglament també es fa distinció en la realització dels assaigs de migració segons si el material o objecte ha estat o no en contacte amb l'aliment (Annex V del reglament). Pel què fa a un objecte en contacte amb l'aliment (cas 1) (per exemple aliments envasats que necessitin ser introduïts en un microones abans de la seva ingesta) s'ha d'emmagatzemar tal i com indica l'etiqueta d'embalatge o en condicions adequades i retirar l'aliment abans de la data de caducitat per a la seva anàlisi. Per altra banda, pel què fa a un objecte que encara no hagi estat en contacte amb l'aliment (cas 2), s'ha de comprovar la conformitat de la migració utilitzant els simulants adequats en condicions de temps i temperatura el més extrems previsibles per a l'ús real de l'objecte (en els casos vistos en els altres capítols es tractava de 10 dies a 40 ºC).

També es declara que el material o objecte es tractarà tal i com s'indiqui a les instruccions adjuntes o que disposi la declaració de conformitat (la declaració de conformitat inclou tota la documentació referent a les característiques del material o objecte contemplat en el Reglament 10/2011).

Paral·lelament, la Norma UNE 149101 (AENOR, 2011) estableix uns períodes de rentat necessaris per a poder realitzar correctament el mostreig per a l'anàlisi fisicoquímic i microbiològic. Això implica que els assaigs de migració cal realitzar-los abans del rentat, quan l'equip de tractament d'aigua és nou.

En els casos descrits en aquest estudi, els equips de tractament que s'instal·len en els edificis cal posar-los a punt segons es descriu a les instruccions de cada equip, que en molts casos significa fer passar aigua de la xarxa d'una determinada forma i un cert nombre de cops per l'equip i que, per tant, s'hauria de seguir el cas 1 descrit prèviament, però no existeix data de caducitat per saber quant temps pot residir l'aigua dins els conductes o dipòsit d'un equip.

En conclusió, es va seguir el Reglament com a legislació aplicable o de referència i es va procedir a fer un rentat previ segons les instruccions de l'equip de tractament d'aigua i es van seguir les condicions d'un assaig de migració segons el cas 2 descrit prèviament.

En aquest capítol (article científic VI) es descriuen els assaigs de migració de tres equips que s'utilitzen per al tractament domèstic de l'aigua de xarxa:

- Una osmosi inversa amb dipòsit que consta d'un filtre de carbó actiu, un filtre de sediments, una membrana d'osmosi, un dipòsit de pressió recobert de material polimèric i finament un altre filtre de carbó actiu.
- Una font que consta d'un filtre de carbó actiu, uns tubs interns de material polimèric i d'un serpentí metàl·lic per si es desitja refredar l'aigua.
- Una gerra amb un filtre que consta d'un filtre de carbó actiu, un filtre d'intercanvi iònic i partícules de plata per a evitar la proliferació de microorganismes.

5.2. Treball experimental

La Figura 14 fa referència al circuit d'aigua instal·lat al Laboratori Dr. Oliver Rodés per a realitzar el mostreig dels tres equips de tractament d'aigua en el punt d'ús (en anglès "point of use (PoU)") (osmosi (PoU-1), font (PoU-2) i gerra (PoU-3)). Aquesta instal·lació és necessària per a mostrejar les mostres per a l'anàlisi microbiològic perquè cal tenir un bon control del tram de xarxa previ als equips per tal que l'augment de les colònies sempre sigui semblant. Els diferents conductes de la Figura 14 fan referència a dues línies d'aigua amb connexió d'aigua freda i calenta per a fer altres tipus d'assaigs. El tanc de drenatge s'utilitza per enviar l'aigua al desaigua. L'ordre de la presa de mostra realitzat a l'estudi és:

1. Instal·lació i posada a punt de l'equip de tractament d'aigua; en tots els casos era necessari passar aigua de xarxa.
2. Assaig de migració (72h a 20 ºC) per triplicat, és a dir, es van realitzar tres períodes consecutius de migració ja que els equips són reutilitzables.
3. Període de rentat de l'equip, previ a la presa de mostra microbiològica i química, per a simular el funcionament normal de l'equip (té una durada de 3 dies)
4. Presa de mostra microbiològica i química de la xarxa i de cada equip de tractament d'aigua.

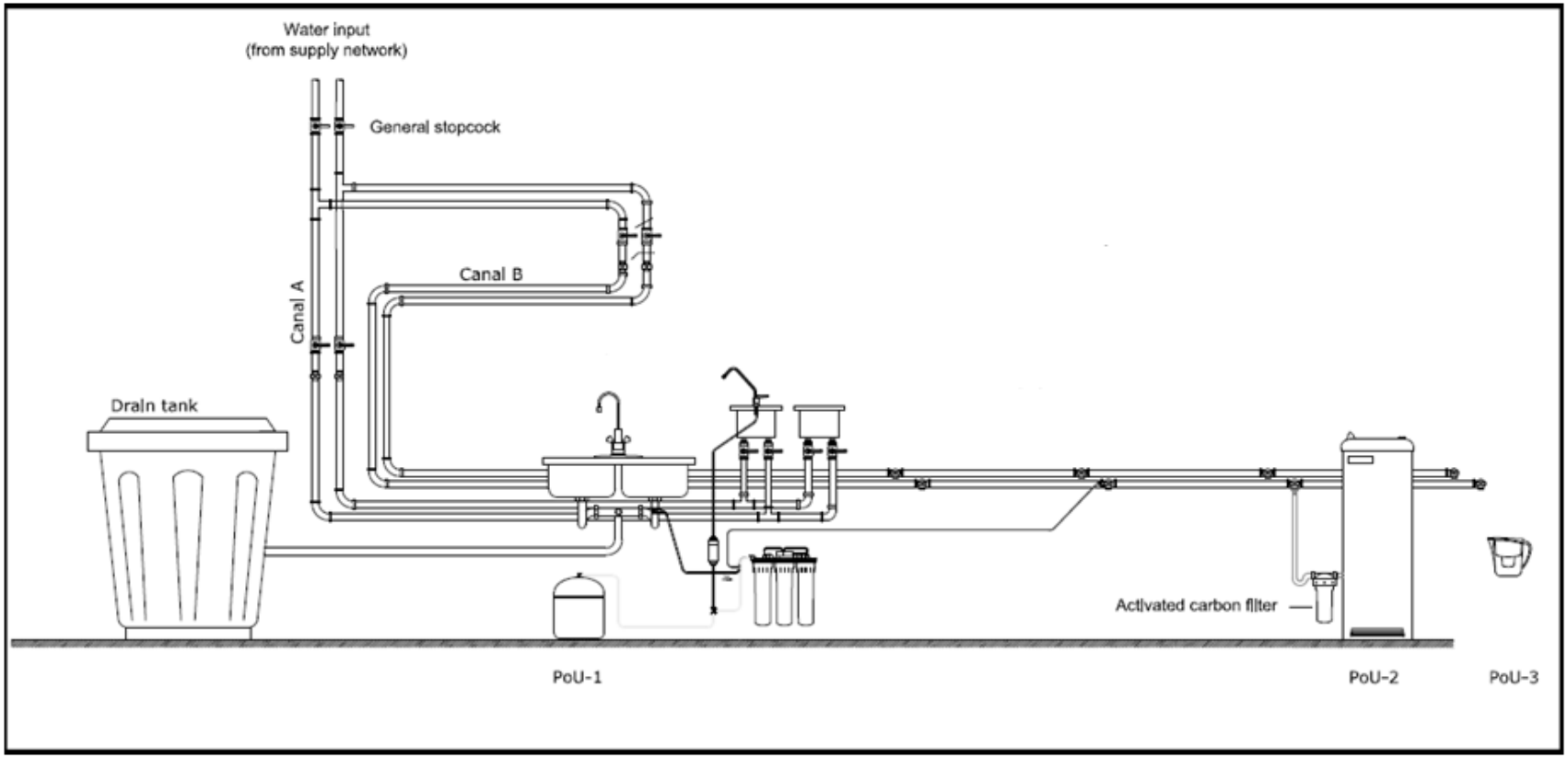

Figura 14. Instal·lació del circuit d'aigua per a realitzar el mostreig de les diferents mostres per a l'anàlisi de migració, fisicoquímic i microbiològic.

Article científic VI

Títol: Migration of plastic components from point of use domestic water treatments devices

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Migration of plastic components from point of use domestic water treatments devices

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Abstract

Three domestic point of use (PoU) water treatment devices, a fountain with a carbon filter, a reverse osmosis system and a jug, were studied to evaluate the migration of plasticizers and additives from the polymeric materials and to determine microbiological and physico-chemical water characteristics that can affect water quality. Migration assays were performed filling the PoU devices following the temperature and time conditions established in Commission Regulation (UE) No 10/2011, which applies to plastic in contact with food. 4-nonylphenol (4-NP), bisphenol A (BPA), acetyl tributyl citrate (ATBC) and 2,6-di-t-butyl-4-methylphenol (BHT) were identified in migrations assays. Chemical and microbiological analyses were done before and after the PoU domestic water treatments with water taken from El Prat de Llobregat (Catalonia, Spain). After the domestic water treatment, pH in the jug and nickel in the fountain did not comply the legislation limits (Directive 98/83/EC and Spanish Royal Decree 140/2003). Also, the osmosis system and the jug analyzed had a Langelier Index below the recommended value.

Keywords: point of use; osmosis; carbon filter; treated water; migration.

1. Introduction

Point of Use (PoU) refers to a treatment device applied to tap water or faucet to improve the quality of water intended for human consumption. There are several treatment devices composed by different materials (e.g. polymeric and metallic materials). The most common PoU devices use filters as carbon, reverse osmosis membranes and ion exchange resins which are connected to the water supply network. Other PoU devices as jugs are not connected to water supply network. Water refrigeration is also considered as a physical treatment because the chlorine taste disappears with lower temperatures.

PoU devices are used to remove undesirable compounds present in tap water and to improve organoleptic properties. Substances that may affect taste and odour to water are chlorination by-products as trihalomethanes (THMs) and haloacetic acids (HAAs) whose presence in drinking water depends on the area studied and the season (Legay et al. 2011). Karavoltsos et al. (2008) analysed several parameters indicated in Directive 98/83/EU (EU 1998) for drinking water from several regions of Greece and found that 2.7 % of the samples analysed exceeded the legislated value for lead, 2.4 % for chloride, 2.1 % for nickel and around 2 % for ammonium, sodium, fluoride, sulphates, nitrates and conductivity. Substances in tap water quality depend on the natural variability of source water and on the deterioration of pipes due to corrosion along the water supply network (Karavoltsos et al. 2008; Khadse et al. 2011; Lehtola et al. 2004). This deterioration is the consequence of water corrosion, which is influenced by many factors such as minerals from the source water, microorganisms that grow in the biofilm of pipes or the chlorine added to the treated water to decrease the growth of microorganisms can affect water quality (Bloetscher et al. 2010; Srinivasan et al. 2008).

However, the installation of PoU devices adds another treatment to treated water that changes its characteristics. Furthermore, the use of plastic components may produce a migration of monomers or additives to the drinking water. Within the European migration framework, Commission Regulation (UE) No 10/2011 (EU, 2011a) describes the parametric limits for plastics intended to be in contact with food. Directive 98/83/EC (EU, 1998) describes the chemical and microbiological that must be controlled in consumer faucet. Maximum legislated values are provided for metals, ionic substances, pesticides and microorganisms.

This study is aimed to evaluate the water quality after PoU water treatments in terms of migration, chemical and microbiological parameters. Devices studied were: (i) a fountain with a carbon filter; (ii) a reverse osmosis and (iii) a jug with an active carbon treatment cartridge. The migration assay used followed the European Directive (EU 2011a). On the other hand, chemical and microbiological analyses were done in the drinking water before and after the domestic treatments. Results are discussed regarding maximum legislated values and benefits/disadvantages of PoU treatments in relation to parametric values.

2. Materials and methods

2.1. Chemicals and reagents

4-nonylphenol (4-NP) was purchased from Riedel-de Haën (Seelze, Germany) as a solid technical mixture of isomers. Bisphenol A (BPA) was purchased from Dr. Ehrenstorfer (Augsburg, Germany) as a solid. Acetyl tributyl citrate (ATBC) (≥98 %) and 2,6-di-t-butyl-4-methylphenol (BHT) (≥99.0 %) were purchased from Sigma Aldrich (St. Louis, MO, USA). Stock standard solutions of each compound were prepared in ethyl acetate at a concentration of 5 mg ml^{-1} and stored in the dark at -20ºC until use. Dipropylphthalate-d_4 (DPP-d_4) and BPA-d_{16} were purchased as solids from Dr. Ehrenstorfer (Augsburg, Germany). The internal standard was anthracene-d_{10}, purchased from Supelco (Bellefonte, PA, USA). 200 mg Oasis HLB cartridges were from Waters (Milford, MA, USA) and used with a Baker vacuum system (J.T. Baker, The Netherlands). Chromatography grade methanol, acetone, dichloromethane, n-hexane, ethyl acetate were purchased from Merck (Darmstadt, Germany). Distilled water was produced with Milli-Q Integral Water Purification System and osmotized water was produced with an Elix 5 UV Purification System, both from Millipore (Billerica, Massachusetts, USA). The chemicals and reagents used for the chemical and microbiological analyses are described in the supplementary data.

2.2. Migration assays for PoU water treatment devices

PoU devices tested were: (i) fountain (PoU-1-Fountain) without accumulation tank composed by a carbon filter, a metallic coil to refrigerate water and metallic tubes. Consumers can choose to drink refrigerated water or not; (ii) reverse osmosis (PoU-2-Osmosis) connected to the water supply network was composed by a carbon and a 5 µm sediment filter, an osmosis membrane filter of aromatic polyamide, a 5 L plastic pressure tank and another carbon filter; and (iii) a 2.5 L jug (PoU-3-Jug) which is filled with distribution water. The jug was composed by a cartridge that acts as an ion exchange and as a carbon filter. It also had silver to inhibit microbiological growth.

Migration assays were performed in new and not used devices following the usual starting/cleaning procedure which is described in each PoU handbook. Assays were done at 20ºC and water was enclosed in the PoU system during 3 days according with Commission Regulation (UE) No 10/2011 (EU 2011a). The migration assay was done in 3 consecutive periods of 3 days each because samples were from reusable devices. The 1^{st} incubation period was used to identify compounds intended to migrate and quantify their contribution. The 2^{nd} and 3^{rd} incubation periods were used to quantify the migrating compounds and finally the 3^{rd} incubation period was compared with parametric values. To compare the results with legislated values, the water density of 1 kg/dm^3 was considered and not the total plastic surface in contact with drinking water. It was done this way because it was very difficult to determine the total surface in contact with water.

After migration assay, PoU devices were exposed to a cleaning period of 3 days simulating the normal working order in terms of time. After that, the tap water sample was taken from water supply network for a posterior comparison with PoU device samples. Then, each PoU device was filled with tap water and water was reposed for 24h simulating the normal use of the device after one night of inactivity. After the 24h contact period, PoU device samples were taken from the water exit for each PoU device.

The tap water sample was taken from the El Prat de Llobregat (Barcelona, Spain) water supply network which is originated in a deep groundwater aquifer. This water undergoes a treatment process that consists in an air stripping of organic compounds removal, a filtration step and a reverse osmosis (Sanz et al. 2013).

2.3. Parameters tested and procedures

4-NP, BPA, ATBC and BHT were analysed by solid phase extraction (SPE) using Oasis 200 mg SPE cartridges as described in Guart et al. (2011) followed by gas chromatography coupled to mass spectrometry (GC-MS) (Thermoquest GC 8000 Top/Finnigan Voyager MS, ThermoFinnigan, Bremen, Germany). The MS system was operated in electron ionization mode (EI 70 eV). The GC separation was achieved with a 30 m x 0.25 mm BPX5 capillary column (SGE Analytical Science) coated with 5% phenyl polysilphenylene-siloxane (film thickness 0.25 μm). The oven temperature was programmed from 60ºC (holding time 3 min) to 310ºC at 10ºC min^{-1}, keeping the final temperature for 15 min. 2 μL were injected in the splitless mode, keeping the split valve closed for 1 min. Helium was the carrier gas (1.0 mL/min). Injector, GC interface and ion source temperatures were 275ºC, 250ºC and 200ºC, respectively. Peak detection and integration were carried out using Xcalibur software. First, MS was set as scan mode (50-450 m/z) for identification of compounds prone to migrate in 1^{st} incubation period and posterior injections were set as SIM mode for quantification in 1^{st}, 2^{nd} and 3^{rd} incubation periods (Table 1). Internal standard quantification was performed using the deuterated surrogate standards (BPA-d_{16} and DPP-d_4) to correct any lose during sample manipulation. Calibration curves were constructed for all target compounds over a concentration of 40-1000 μg/L. Limits of quantification (LOQ) were determined through the signal-to-noise ratio of 10 from the 0.2 μg/L spiked samples when there was not any contribution in blank samples. In the case there was contribution in blank samples, LOQs were calculated using the arithmetical mean of the blank concentration plus 10 times the standard deviation (n=10) (Table 1). Recoveries were between 83 and 99 %, except for BHT that was always 25 %. This is explained because it is degraded in contact with water forming different products (OECD 2002; Schwope et al. 1987).

The analysis of water properties according to European (EU, 1998) and Spanish (Spanish Government 2003) legislation included chemical and microbiological methods which are described in supplementary data. In brief, boron and nitrite were analysed by spectrophotometry UV-Vis. Trihalomethanes were analysed by head-space followed by gas chromatography coupled to mass spectrometry (HS-GC/MS). Chloride, nitrate and sulphate were analysed by ion-exchange chromatography. Calcium and magnesium were analysed by flame atomic absorption spectrometry (FAAS). Chlorine was analysed by colorimetry. Conductivity and pH were analysed by electrometry. Oxidizability was analysed by redox volumetry. Bicarbonate and total alkalinity were analysed by volumetric

titration. Turbidity was analysed by nephelometry. Colony count at 22 and 37 ºC were analysed by recounting cfu. In microbiological and chemical analyses (Table 2) blanks, spiked samples and interlaboratories tests with their respective quality parameters (linearity, precision, selectivity, limits of detection and quantification and uncertainty) were done according to ISO 17025:2005 (ISO 2005). Water characteristics are defined by Langelier index, hardness and alkalinity. Langelier index describes the potential corrosion of the water and it is the difference between the actual pH of the water and its "saturation pH", this being the pH at which a water of the same alkalinity and calcium hardness would be at equilibrium with solid calcium carbonate. Hardness in water is caused by a variety of dissolved metallic ions, predominantly calcium and magnesium. Alkalinity refers to the concentration of carbonate and bicarbonate (WHO, 2011).

Table 1. Parameters of the migration assay analysis and results obtained in the three periods for each PoU device.

		BHT	4-NP	BPA	ATBC
RT [min]		14.47	17.08-17.62	22.09	22.13
Ions [m/z]		205*, 220, 57	135*, 149, 107	213*, 228, 119	185*, 129, 259
LOQ [µg/L]		0.078	0.154	0.053	0.027
Recovery 0.2 µg/L [%]		25 ± 0.5	89 ± 1.8	83 ± 1.4	99 ± 1.4
Tap water		<LOQ	<LOQ	<LOQ	<LOQ
PoU-1 –Fountain [µg/L]	**1st period**	<LOQ	0.316	0.077	<LOQ
	2nd period	<LOQ	<LOQ	<LOQ	<LOQ
	3rd period	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**
PoU-2-Reverse Osmosis [µg/L]	**1st period**	1.320	1.092	0.619	<LOQ
	2nd period	1.569	0.370	0.354	<LOQ
	3rd period	**1.889**	**0.333**	**0.334**	**<LOQ**
PoU-3-Jug [µg/L]	**1st period**	<LOQ	<LOQ	<LOQ	0.658
	2nd period	<LOQ	<LOQ	<LOQ	0.613
	3rd period	**<LOQ**	**<LOQ**	**<LOQ**	**0.128**
Parametric value [µg/kg]	**[µg/kg]**	3000[a]	10[c]	600[a]	60000[ab]

<LOQ: below limit of quantification

[a] Commission Regulation (EU) No 10/2011 (EU, 2011a)

[b] Considered as a sum of substances in legislation

[c] Spanish Royal Decree 847/2011 (Spanish Government, 2011)

3. Results and discussion

3.1. PoU-1-Fountain

Compounds identified in the 1st incubation period were BPA and 4-NP at concentrations of 0.077 and 0.316 µg/L, respectively (Table 1), attributed to migration from the internal tubes of the fountain. After the 2nd and 3rd migration periods, these compounds were not detected. BPA concentration in 1st incubation period was very low in comparison with parametric value of 600 µg/L set by Commission Regulation (EU) No 10/2011 (EU 2011a) for BPA. On the other hand, 4-NP is a starting substance for the manufacture of plastic materials established in the provisional lists of Scientific Committee on Food (SCF) list 8 (EU 1987) and Spanish Royal Decree 847/2011 (Spanish Government 2011), and its use in the plastic materials manufacture is restricted to the migration value of 10 µg/kg. The NP concentration is well below in comparison with legislated value. Its presence has been described in several plastic polymers such as polyethylene terephthalate (PET), high density (HDPE) and low density polyethylene (LDPE), polycarbonate (PC) and polystyrene (PS) which were tested by Guart et al. (2011). Tests were performed by cutting samples of these polymers in pieces and introducing the pieces in contact with water at 40 ºC for 10 days, obtaining the highest NP value of 1.282 µg/dm^2 for HDPE used in bottle caps for bottled water (Guart et al. 2011).

After 24 h contact time in fountain treatment, chemical and microbiological analyses showed an increase of bicarbonate, nickel, calcium, potassium and TAC; a decrease of aluminium, chloride, magnesium, pH, THMs, sodium and sulphate; and similar values of conductivity, hardness, Langelier index and nitrate parameters (Table 2). Aluminium was quantified in tap water at 16 µg/L but was not detected in the fountain; it is used as coagulant in drinking-water treatment to reduce turbidity and as a consequence to maintain a good quality of water in terms of microbiology because microorganisms are attached to the particles in suspension. WHO describe a good operating conditions of treatment at concentration under 100 µg/L (WHO 2011). In comparison with tap water analysed previously, nitrate concentration slightly decreased from 6.6 mg/L to 4.1 mg/L. Nitrates can sometimes reach both surface water and groundwater as a consequence of agricultural activity (WHO 2011). Nickel increased due to the contact of water with the metallic part covering the carbon filter. This hypothesis was corroborated by introducing water inside the metallic cover of the filter and analysing nickel after 1 day contact. THMs are formed from chlorine or others chlorine disinfectants and in the presence of a small quantity of organic matter in water. THMs decreased from

45 µg/L (tap water) to <LOQ. The levels of THMs in El Prat de Llobregat tap water range from 1.3 to 19 µg/L (Sanz et al. 2013). THMs formation is affected by standing water, temperature, quantity of residual chlorine, quantity of organic matter and quantity of bromide in water. Bromide is characteristic from Llobregat basin and after chlorination treatment it is habitual a bromoform formation. Boron, which was detected at 0.3 mg/L in tap water, may come from seawater where boron is present at 4-6 mg/L (WHO 2011). On the other hand, colony count in water in contact with the fountain at 22 and 37 ºC increased up to 5000 and 1300 cfu/mL in comparison with tap water due to the chlorine degradation. This increase in colony counts was checked as a usual variation for tap water stored for 24h in a section of the laboratory water network, obtaining 2470 and 55 cfu/mL for colony count at 22 and 37 ºC, respectively.

3.2. PoU-2-Reverse osmosis

In Reverse osmosis, the compounds identified in the 1st incubation period were BHT, BPA and 4-NP at concentrations of 1.320, 0.619 and 1.092 µg/L, respectively (Table 1). In the 2nd incubation BPA and 4-NP concentrations decreased to 0.354 and 0.370 µg/L, while BHT increased to 1.569 µg/L. In the 3rd incubation period, BPA and 4-NP remained at the same levels, at 0.334 and 0.333 µg/L, respectively. BHT further increased to 1.889 µg/L. BHT is used as a phenolic antioxidant used in food products and packaging (Bolgar et al. 2008) and it is legislated in Commission Regulation (EU) No 10/2011 (EU, 2011a). Unlike the other identified compounds, BHT concentration increased along incubation periods. It can be explained by the hypothesis of Schwope et al. (1987) that describes a continuous BHT migration from a LDPE polymer counterbalanced by a decomposition reaction.

Chemical analyses showed a decrease in chlorine due to its removal in the carbon filter, which was placed before osmosis membrane because chlorine breaks the reverse osmosis membranes. A decrease of bicarbonate, calcium, potassium, sodium and sulphate due to the osmosis action caused a decrease in conductivity, hardness, Langelier index, pH, THMs and TAC parameters. In accordance with the results obtained for THMs, Mazloomi et al. (2009) indicated that domestic reverse osmosis can be used for the removal of THMs. The presence of nitrite may be due to reduction of nitrate by the effect of carbon. Langelier Index shows the water tendency to precipitate or to dissolve calcium carbonate and in equilibrium

conditions it has a theoretical value of zero; the reference value considered in legislation is ±0.5 (Spanish Government 2003). Water turned aggressive according with the Langelier Index value of -3.5. An easy solution to correct the Langelier Index was to combine the treated water with network water. From a microbiological point of view, colony count at 22ºC and 37ºC increased because water is stored in a tank before consumption. Along time, an increase in colony count can produce bad odour and taste and biofilm growth, so it is necessary to clean the tank periodically as it is described in osmosis instructions. A hygienic solution could be the combination of osmosis water with fresh tap water.

3.3. PoU-3-Jug

The compound identified in the 1st incubation period was ATBC at 0.658 µg/L (Table 1). In the posterior migration periods this compound decreased to 0.128 µg/L. ATBC is used as a plasticizer in food contact applications and it provides adherence to metals, low volatility, and resistance to yellowing (Bolgar et al. 2008). ATBC is legislated in Commission Regulation (EU) No 10/2011 (EU 2011a) with a parametric value of 60000 µg/L as sum of ATBC and other substances. According to this parametric value, the levels detected should not pose a health risk.

Chemical and microbiological analyses showed out two processes. The first process is an ion exchange produced by bicarbonate and calcium which were exchanged by potassium whose concentration increased. This ion exchange made a decrease in hardness, Langelier index and alkalinity. As a consequence of these changes, pH decreased from 7.82 to 6.13 obtaining a value below the legislated limit (Table 2). The second process is the preservation or rejection of microorganisms. Table 2 showed that there were not changes in colony count because jug cartridges contained silver that acts as a bacteriostatic and bactericide agent, hence microorganism colonies cannot grow obtaining the same range of colony count at 22ºC than in tap water.

Table 2. Comparison of drinking water before (Tap water) and after Chemical and microbiological results.

Parameter	LOQ	Tap water	PoU-1-Fountain	PoU-2-Reverse Osmosis	PoU-3-Jug	Royal Decree 140/2003	Directive 98/83/EC	Units
pH*	Range 2-10	7.82	7.50	6.73	6.13	6.5-9.5	-	pH units
Conductivity*	1.1	1073	1016	73	905	2500	2500	µS/cm
Langelier Index*	-	0.3	0.1	-3.5	-2.6	-	-	-
Turbidity*	0.2	<LOQ	<LOQ	0.2	0.3	5	Acceptable to consumers without anomaly changes	UNF
Bicarbonate	10.0	153.0	201.0	15.1	19.4	-	-	mg/L
Chloride*	0.2	215	185	16.2	216	250	250	mg/L
Nitrate*	0.5	6.6	4.1	2.1	3.4	50	50	mg/L
Nitrite*	0.02	<LOQ	<LOQ	0.07	<LOQ	0.5	0.5	mg/L
Sulphate*	1.0	106.7	96.4	<LOQ	104.0	250	250	mg/L
Calcium*	0.2	74.6	85.7	1.1	33.7	-	-	mg/L
Magnesium*	0.1	25.8	21.0	0.3	21.2	-	-	mg/L
Potassium*	0.5	5.2	9.6	0.8	6.9	-	-	mg/L
Sodium*	0.5	113.8	101.0	14.4	112.0	200	200	mg/L
Hardness total*	1.0	292.3	300.1	4	171.2	-	-	mg/L
Hardness total (ºF)*	1.0	29.2	30.0	0.4	17.1	-	-	ºF
Alkalinity*	10.0	126.0	165.0	12.4	15.9	-	-	mg/L

Table 2. Continuation.

Parameter	LOQ	Tap water	PoU-1-Fountain	PoU-2-Reverse Osmosis	PoU-3-Jug	Royal Decree 140/2003	Directive 98/83/EC	Units
Chlorine combined*	0.10	<LOQ	<LOQ	<LOQ	<LOQ	2	-	mg/L
Chlorine free*	0.10	0.26	<LOQ	<LOQ	<LOQ	1	-	mg/L
Chlorine total*	0.10	0.33	<LOQ	<LOQ	<LOQ	-	-	mg/L
Oxidizability*	0.5	0.7	0.6	<LOQ	0.6	-	-	mg O_2/l
Aluminium*	10	16	<LOQ	<LOQ	<LOQ	200	200	µg/L
Boron*	0.2	0.3	<LOQ	<LOQ	<LOQ	1	1	mg/L
Copper*	0.20	0.73	<LOQ	<LOQ	<LOQ	2	2	mg/L
Nickel*	10	<LOQ	173	<LOQ	<LOQ	20	20	µg/L
Trihalomethanes Total	11	45	<LOQ	<LOQ	<LOQ	100	100	µg/L
Bromoform	2	37	<LOQ	<LOQ	3	-	-	µg/L
Dibromochloromethane	2	8	<LOQ	<LOQ	<LOQ	-	-	µg/L
c-1,2-Dichloroethylene	2	8	<LOQ	<LOQ	<LOQ	-	-	µg/L
Colony count 22 ºC*	3	11	5000	265000	22	-	Without anomaly changes	cfu/mL
Colony count 37 ºC*	3	<LOQ	1300	50000	-	-	-	cfu/mL

* Accredited by ISO 17025:2005 - General requirements for the competence of testing and calibration laboratories (ISO, 2005).

4. Conclusions

Three PoU were evaluated in terms of migration of plastic components and chemical and microbiological characterization. PoU migration assays permitted to identify compounds from the polymeric material that migrate into drinking water, although values were well below the concentrations indicated in the reference legislation (EU 2011). In incubation periods, concentrations of BPA, 4-NP and ATBC decreased but BHT concentration increased up to 1.889 µg/L in PoU-2-Reverse Osmosis. BPA and 4-NP were also detected in PoU-2-Reverse Osmosis as a result of migration from plastic components, although the levels detected in the 3rd incubation period were below the parametric value. Regarding the other parameters analysed, PoU-1-Fountain showed a nickel concentration above legislated limit due to the metallic cover of the carbon filter. PoU-3-Jug showed an acidic pH, which was below the legislated value. Also it is necessary to take into account that in PoU-2-Osmosis and PoU-3-Jug, the Langelier Index was negative and very low. It is recommended to mix non-treated tap water with the treated tap water to correct the Langelier Index.

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Supplementary data

Reagents and chemicals

In pH analysis: pH buffer solutions of 2.00, 4.00, 7.00 and 10.00 were purchased from Merck (Darmstadt, Germany). In conductivity analysis: calibration standards of 1.41 mS/cm and 0.147 mS/cm with NIST traceability were purchased from Merck (Darmstadt, Germany). Potassium chloride was purchased from Merck (Darmstadt, Germany) as a solid. Control standards CKSC20 of 18.06 µS/cm and CKSC5 of 4.48 µS/cm at 20ºC were from Reagecon (County Clare, Ireland). In turbidity analysis: formazin and Gelex secondary turbidity standards were purchased from Hach (Loveland, Colorado, USA). In chloride, nitrate and sulphate analyses: sodium carbonate and sodium bicarbonate were purchased from Merck (Darmstadt, Germany) as solids. As standards of calibration, chlorine, nitrate and sulphate were purchased from Merck (Darmstadt, Germany) at a concentration of 1000 mg/L each. As standards of control, chlorine, nitrate and sulphate were purchased from Merck (Darmstadt, Germany) at a concentration of 1000 mg/L each with NIST traceability. In nitrite analysis: NED-sulfanilamide as a solid, phosphoric acid at 85% and nitrite at 1000 mg/L were purchased from Merck (Darmstadt, Germany). In calcium and magnesium analyses: EDTA solution was purchased from Panreac (Barcelona, Spain) at a concentration of 0.1 mol/L. Potassium hydroxide was from Merck (Darmstadt, Germany) at a concentration of 0.05 mol/L. For buffer and complexing agent, tris(hydroxymethyl)aminomethane as a solid and acetylcetone were purchased from Merck (Darmstadt, Germany). For the standard solution of calcium carbonate, carbonate calcium and concentred chlorhydric acid were from Merck (Darmstadt, Germany); and ammonia at 30% and methyl red indicator were purchased from Panreac (Barcelona, Spain). Magnesium control standard was purchased from Merck (Darmstadt, Germany) at a concentration of 1000 mg/L. In potassium and sodium analyses: potassium and sodium were purchased from Merck (Darmstadt, Germany) at a concencentration of 1000 mg/L each. In total alkalinity (T.A.) and complete alkalinity titration (T.A.C) analyses: sodium carbonate as a solid was from Merck (Darmstadt, Germany) and chlorhydric acid at 1 N was from Panreac (Barcelona, Spain). In oxidizability analysis: chlorhydric acid at 37% and potassium permanganate at 0.1 N were purchased from Panreac (Barcelona, Spain). Sulphuric acid at 25%, resorcinol and sodium oxalate as a solid were purchased from Merck (Darmstadt, Germany). In aluminium, cooper, and nickel analyses: nitric acid at 65% was from Merck (Darmstadt, Germany). Aluminium, copper and nickel were purchased from Merck (Darmstadt, Germany). In boron analysis: chlorhydric acid concentrated was

purchased from Panreac (Barcelona, Spain). Ethanol at 96% was purchased from Prolabo. Oxalic acid was purchased from Merck (Darmstadt, Germany). Boron was purchased from Merck (Darmstadt, Germany) at a concentration of 1000 mg/L. In total trihalomethanes analysis: benzene, bromoform (tribromomethane), dibromochloroethylene, chlorobenzene, chloroform (trichloromethane), 1,2-dichlorobenzene, 1,3-dichlorobenzene, 1,4-dichlorobenzene, 1,1-dichloroethane, 1,2-dichloroethane, 1,1-dichloroethylene, c-1,2-dichloroethylene, t-1,2-dichloroethylene, dichloromethane, 1,2-dichloropropane, c-1,3-dichloropropylene, t-1,3-dichloropropylene, ethylbenzene, hexachlorobutadyene, tetrachloroethylene, tetrachloromethane, 1,2,3-trichlorobenzene, 1,2,4-trichlorobenzene, 1,3,5-trichlorobenzene, 1,1,1-trichloroethane, 1,1,2-trichloroethane, trichloroethylene, trichlorofluoromethane and toluene. Sodium chlorine, sodium carbonate and methanol were purchased from Merck (Darmstadt, Germany). Toluene-d_8 was purchased from Accustandard (New Haven, Connecticut, USA) at a concentration of 2500 mg/l. No halogenated volatile solvent VOC-Mix 236 was purchased from Dr. Ehrenstorfer (Augsburg, Germany) at concentrations of 1000 mg/L for benzene, toluene and ethylbenzene. VOC-Mix 237 was purchased from Dr. Ehrenstorfer (Augsburg, Germany) at concentration of 200 mg/L each. Mix M-624 was purchased from Accustandard (New Haven, Connecticut, USA) at a concentration of 200 mg/L each. In colony count at 22 and 37 ºC analyses: saline solution at 0.85% and sterile phosphate buffer were purchased from Panreac (Barcelona, Spain). Plate count agar (PCA) was from VWR International LLC (Radnor, PE, USA).

Instrumental analysis

In aluminium, cooper, nickel, potassium and sodium analyses: atomic absorption spectrometer Thermo M6AA System and graphite furnace Thermo GF95Z were purchased from Thermo Fisher Scientific (San Jose, CA, USA). In trihalomethanes analysis: gas spectrometer Focus DSQ and Automatic Triplus Sampler by head-space injection were from Thermo Fisher Scientific (San Jose, CA, USA). In boron analysis: atomic absorption spectrophotometer Thermo M6AA System was purchased from Thermo Fisher Scientific (San Jose, CA, USA) and spectrophotometer filter Unicam W392 was from Unicam (Cambridge, UK). In chloride, nitrate and sulphate analyses: Dionex Ionpac AS14A, Dionex ICS-1000 ion.exchange chromatograph, Dionex ASRS-ULTRA 4 mm suppressor column and AS40 Automated Sampler were purchased from Dionex (Sunnyvale, CA, USA). Ultrasonic bath was from Selecta (Barcelona, Spain). In calcium and magnesium

analyses: 809 automatic titrator, control computer with TIAMO 1.0 software and ion-selective electrode were purchased from Metrohom AG (Ionenstrasse, Switzerland). In conductivity analysis: 4 Star Bechtop and Orion cell conductivity model 990101 were purchased from Thermo Fischer Scientific (San José, CA, USA). In colony count at 22 and 37ºC analyses: 22ºC and 37ºC incubation ovens/refrigerators were from Radiber SA (Barcelona, Spain), thermostatic bath from Selecta (Barcelona, Spain), 90 mm diameter Petri plates from Afora (Barcelona, Spain) and Reax3 shaker plate from Heidolph Instruments Gmbh &. Co. KG (Schwabach, Germany). In oxidizability analysis: VMS-C7-2 heating block was from VWR International LLC (Radnor, PE, USA) and digital buret from Metrohm AG (Ionenstrasse, Switzerland). In the pH analysis: pHmeter with Ross sure flow 8172 BNWP was purchased from Thermo Fischer Scientific (San José, CA, USA). In total alkalinity (T.A.) and complete alkalinity titration (T.A.C) analyses: 809 automatic tritator, 814 automatic sampler and glass ion-selective electrode were purchased Metrohm AG (Ionestrasse, Switzerland). In turbidity analysis: 2100A turbidimeter and Pocket Colorimeter II were purchased from Hach Company (Loveland, CO, USA).

Table Supplementary data. Description of the methods used for the analysis of the chemical and microbiological parameters.

Parameter	Method	Method	LOQ	Units
Aluminium*	GFAAS	Standard Methods 3113 B	10	µg/L
Bicarbonate*	Volumetry	Standard Method 2320 B UNE-EN ISO 9963-1:1996	10000	µg/L
Boron*	Spectrophotometry UV-Vis	Standard Methods 4500-B B	200	µg/L
Bromoform	HS-GC/MS	Internal procedure	2	µg/L
Calcium*	FAAS	Standard Methods 3111 B	200	µg/L
Chloride*	Ion-exchange Chromatography	UNE-EN ISO 10304-1	200	µg/L
Chlorine combined*	Estimation	Estimation from colorimetry analyses	100	µg/L
Chlorine free*	Colorimetry	Method from Pocket colorymetry II Hach manual	100	µg/L
Chlorine total*	Colorimetry	Method from Pocket colorymetry II Hach manual	100	µg/L
Colony count 22ºC*	Count	UNE-EN ISO 6222:1999 Heterotrophic plate count: Pour plate method	3	cfu/plate
Colony count 37ºC*	Count	UNE-EN ISO 6222:1999 Heterotrophic plate count: Pour plate method	3	cfu/plate
Conductivity*	Electrometry	UNE-EN 27888:1994	1,1	µS/cm
Copper*	GFAAS	Standard Method 3113 B	10	µg/L
Dibromochloromethane	HS-GC/MS	Internal procedure	2	µg/L
c-1,2-Dichloroethylene	HS-GC/MS	Internal procedure	2	µg/L

Table Supplementary data. Continuation.

Parameter	Method	Method	LOQ	Units
Hardness total*	Estimation	UNE 77-040:2002 Spanish "Orden de 1 de Julio 1987"	1000	µg/L
Hardness total (ºF)*	Estimation	UNE 77-040:2002 Spanish "Orden de 1 de Julio 1987"	1	ºF
Langelier Index*	Estimation	Standard Method 2330	-	-
Magnesium*	FAAS	Standard Methods 3111 B	100	µg/L
Nickel*	GFAAS	Standard Methods 3113 B	10	µg/L
Nitrate*	Ion-exchange chromatography	UNE-EN ISO 10304-1	500	µg/L
Nitrite*	Spectrometry UV-Vis	Standard Method 4500 NO2 B	20	µg/L
Oxidizability*	Volumetry	Spanish document "Orden de 1 de Julio 1987"	500	µg O_2/l
pH*	Electrometry	Spanish document "Orden del 1 de julio de 1987" Standard Method 4500 H+ I	Range 2-10	pH units
Potassium*	GFAAS	Standard Methods 3113 B	500	µg/L
Sodium*	Atomic emission spectroscopy	NF T 90-019	500	µg/L
Sulphate*	Ion-exchange chromatography	UNE-EN ISO 10304-1	1000	µg/L
Alkalinity*	Volumetry	Standard Method 2320 B UNE-EN ISO 9963-1:1996	10000	µg/L
Trihalomethanes Total	Estimation	Estimation from single THMs analysed by LLE-GC/MS (Standard Method 6410 B)	11	µg/L
Turbidity*	Nephelometry	UNE-EN ISO 7027:2001	0,1	UNF

* Accredited by ISO 17025:2005 - General requirements for the competence of testing and calibration laboratories.

5.3. Discussió dels resultats

L'article científic VI es va realitzar en base a la UNE 149101 (AENOR, 2011), Norma que regula els increments acceptables per a una sèrie de paràmetres descrits a la legislació d'aigües (EU, 1998; Spanish Government, 2003). Però, pel què fa al contacte amb els materials dels aparells de tractament d'aigua, fa referència a la legislació actual (EU, 2011a), que considera l'aigua com a un aliment. Tenint en compte aquest fet es van realitzar assajos de migració per a tres aparells diferents.

Els assaigs de migració descrits a l'article científic VI van permetre identificar quatre compostos (BHT, BPA, 4-NP i ATBC), el BPA i el 4-NP s'han trobat anteriorment en estudis descrits en aquesta tesi (articles científics I, II, IV i V) degut als materials polimèrics utilitzats per a l'envasat d'aigua. L'ATBC es va detectar en la gerra, fet que es pot explicar perquè aquesta substància s'utilitza en la fabricació de resines o revestiments polimèrics destinats al contacte amb aliments. El BHT es va trobar en l'osmosi inversa, fet que es pot explicar perquè aquesta substància s'utilitza com a antioxidant en poliolefines en productes en contacte amb aliments. EL BHT va ser l'únic dels quatre compostos que va augmentar la seva concentració en l'aigua al llarg dels tres períodes d'incubació. Tenint en compte tots els compostos estudiats en aquesta tesi, el BHT en aquest tipus d'osmosi inversa es comporta de forma similar al BPA del garrafó de PC estudiat al capítol 4.

Existeixen molts tipus d'equips domèstics de tractament d'aigua i poden estar fabricats a partir de diversos materials polimèrics i utiltizar una gran varietat d'additius per a millorar les seves propietats. Per aquesta raó, el mètode de mostreig i anàlisi descrits en aquest estudi permeten fe run pas endavant per a realitzar assajos de migració, analísis químiques i microbiològiques en aparells de tractament d'aigua domèstics. Més endavant, aquests assajos es podrien estandarditzar per a determinar la migració del material en contacte amb l'aigua quan l'aparell està en funcionament i així no només avaluar el material sense tenir en compte el procés de tractament d'aigua tal i com es fa actualment.

5.4. Conclusions

- Els quatre compostos detectats (BHT, BPA, 4-NP i ATBC) es van detectar a concentracions molt per sota del nivells legislats en el Reglament (UE) No 10/2011 (EU, 2011) i, per tant, els materials utilitzats en aquests equips de tractament d'aigua són de bona qualitat per estar en contacte amb l'aigua.
- Aquest estudi va permetre desenvolupar un mètode per a avaluar la migració de components del plàstic en diferents tipus d'equips de tractament d'aigua tenint en compte el tractament d'aigua.

CONCLUSIONS GENERALS

CONCLUSIONS GENERALS

- Els dos mètodes utilitzats en els diferents estudis, SPE-GC/MS i SBSE-GC-MS/MS, han estat eficaços per a la determinació de components del plàstic, així com de pesticides i altres compostos semivolàtils.

    - El mètode basat en SPE-GC/MS ha permès l'anàlisi de 9 components del plàstic (ftalats, DEHA, BPA, 4-OP i 4-NP) i herbicides (triazines i cloroacetamides) en aigües subterrànies, aigua envasada, aigua de xarxa i aigua provinent d'assaigs de migració. S'han obtingut LOQs entre 0.004 i 0.970 µg/L i amb bona repetitivitat.

    - El mètode basat en SBSE-GC-MS/MS ha permès l'anàlisi de 69 compostos (OCPs, OPPs, PCBs, PAHs, piretroids, triazines, ftalats i altres components del plàstic) en aigua envasada. És un mètode més sensible i selectiu que la SPE-GC/MS i permet obtenir LOQs de 0.005 a 0.066 µg/L.

- Els brolladors d'Espanya estan lliures de contaminants i, per tant, es pot garantir la bona qualitat de l'aigua destinada a l'envasat, així com el manteniment de la puresa original en aigües minerals naturals. Per tant, cal concloure que el perímetre de protecció dels brolladors i els controls previs a l'envasat són efectius.

- L'avaluació dels diferents punts susceptibles de contaminació durant l'envasat i l'emmagatzematge de les aigües envasades (aigua mineral natural, aigua de brollador i aigua per al consum humà) ha permès determinar que, tot i un lleu augment en el nombre de deteccions de components del plàstic durant l'envasat i emmagatzematge, les tècniques d'envasat i els propis envasos són adequats per a garantir la bona qualitat del producte envasat.

- Els assaigs de migració han permès determinar quins compostos migren en els diferents materials polimèrics més utilitzats en l'envasat d'aigua. El fet que els assaigs de migració estiguin estandarditzats ha permès comparar els resultats obtinguts amb la legislació vigent. Els nivells de migració obtinguts sempre han estat molt per sota dels valors legislats.

- L'activitat de disrupció endocrina de mostres del polímer PC i de mostres del copolièster Tritan™ mitjançant assaigs *in vitro* i *in vivo* han permès determinar que són necessàries concentracions molt més elevades de BPA i DMIP per a provocar efectes adversos en l'ésser humà.

- Els assaigs de migració d'equips domèstics de tractament d'aigua han mostrat que hi ha migració de components del plàstic a concentracions per sota dels nivells legislats.

- Els estudis de TDI indiquen que en cap cas es superen els màxims legislats pel BPA, DEP, DBP, BBP, DEHP, DEHA i NP a través del consum d'aigua envasada.

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