

Contents lists available at [SciVerse ScienceDirect](http://SciVerse.Sciencedirect.com)

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Migration of phthalates, alkylphenols, bisphenol A and di(2-ethylhexyl)adipate from food packaging

Evelina Fasano^{a,*}, Francisco Bono-Blay^b, Teresa Cirillo^a, Paolo Montuori^c, Silvia Lacorte^b

^a Department of Food Science, University of Naples "Federico II", via Università n° 100, 80055 Portici, Naples, Italy

^b Department of Environmental Chemistry, IDAEA-CSIC, Jordi Girona 18-26, 08034 Barcelona, Catalonia, Spain

^c Department of Preventive Medical Sciences, University of Naples "Federico II", via c/Sergio Pansini 5, Naples, Italy

ARTICLE INFO

Article history:

Received 11 October 2011

Received in revised form

29 February 2012

Accepted 6 March 2012

Keywords:

Plasticizers

Migration tests

Food simulants

SPE

ABSTRACT

The migration of plastic components or additives from packaging to food can produce a risk for human health, in fact many of these plasticizers and additives are "Endocrine Disruptors", such as phthalates (PAEs), alkylphenols (APs), 2,2-bis(4-hydroxyphenyl)propane (bisphenol A or BPA) and di(2-ethylhexyl) adipate (DEHA). The evaluation of some PAEs, some APs, BPA and DEHA levels in common food packaging (oil and natural tuna cans, marmalade cap, yogurt packaging, polystyrene dish, teat, bread bag, film, baby's bottle, aseptic plastic laminate paperboard carton and plastic wine top) was carried out by migration tests. Furthermore to evaluate the potential migration of plasticizers and additives from plastic wine tops, two extraction methods were used, one through incubation at 40 °C for 10 days and one by ultrasounds extraction. The simulants employed were distilled water, acetic acid at 3%, ethanol at 15% for wine top. The food simulant was extracted by solid phase extraction (SPE) and analyzed by GC–MS. Comparing these results with EU restrictions all samples showed contaminant migration lower than SML and OML established. Finally, about the comparison of two extraction methods, the extraction carried out for 10 days at 40 °C may be better than the other one in order to detect all compounds.

© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

Food packaging is an important way to store food at different temperatures, to extend the shelf life of the product and to safeguard foods from natural agents, such as air, that can reduce or change their quality. Among other materials, plastics have emerged as a secure and comfortable practice for primary food packaging. There are different types of plastic, each with unique properties and application in the food sector, for example polycarbonate, high and low density polyethylene, styrene, polypropylene, etc. These plastics are manufactured from various polymers and additives are used to improve elasticity, flexibility, color, resistance, durability, etc. Both plastic and additives can migrate from the packaging to the food or beverage over time as a result of an increase in temperature or mechanical stress. The presence of plastic components or additives in food, if not properly controlled, can affect the organoleptic properties of food and produce an endocrine disrupting effects (Wagner & Oehlmann, 2009) if the levels exceed the legislated or toxicological values. Many plasticizers and

additives are considered as Endocrine Disruptors (EDs), and show activity on the reproductive system or act as carcinogens. Some of these chemicals are phthalate esters, alkylphenols (APs), 2,2-bis(4-hydroxyphenyl)propane, (bisphenol A or BPA) and di(2-ethylhexyl) adipate (DEHA).

Phthalates are synthetic organic chemicals introduced in the 1920s such as di(2-ethylhexyl)phthalate (DEHP), diethylphthalate (DEP), di-n-butylphthalate (DBP), butylbenzylphthalate (BBzP), di-iso-nonylphthalate (DiNP), di-n-octylphthalate (DnOP) and diiso-dodecylphthalate (DiDP). Since 1933, the enormous industrial success deriving from the addition of DEHP to polyvinyl chlorides (PVC) to increase flexibility, lead to an increasingly exponential applicability in solvent manufacture, adhesive, wax, ink, pharmaceuticals, cosmetic and insecticide and regenerated cellulose wrapped food production. They are not bound linked to plastics, therefore they can migrate to the food which are in contact. Food is a major source of exposure of several phthalates in humans (Fromme et al., 2007; Wormuth, Scheringer, Vollenweider, & Hungerbuhler, 2006). Packed foods, especially fatty foods, are the most affected, in particular by DEHP (Castle, Mercer, Startin, & Gilbert, 1988; Kavlock et al., 2002; Kueseng, Thavarungkul, & Kanatharana, 2007; Sheela, 2008; Spillmann, Siegrist, Keller, & Wormuth, 2009; Tsumura, Ishimitsu, Saito, Kobayashi, & Tonogai,

* Corresponding author. Tel.: +39 0812539408; fax: +39 0812539407.
E-mail address: evafas@libero.it (E. Fasano).

2001; Wormuth et al., 2006). Although in humans, phthalates are rapidly hydrolyzed to the monoesters and then further metabolized and excreted with urine and faeces (Itoh, Yoshida, & Masunaga, 2005), they have been detected in serum, amniotic fluids and breast milk (Ghisari & Bonefeld-Jorgensen, 2009). DBP, BBP and DEHP are introduced in the list of compounds suspected to have EDs properties (COM, 2001). To protect human health, the European Food Safety Authority (EFSA) established a Total Daily Intake (TDI) for some of these pollutants in particular 0.01 mg kg^{-1} body weight (bw) for DBP, 0.5 mg kg^{-1} bw for BBP, 0.15 mg kg^{-1} bw DiNP and 0.05 mg kg^{-1} bw for DEHP (EFSA, 2005a,b,c,d,e).

On the other hand, APs are primarily used as lubricants, detergents, resins, plasticizers, herbicides, cosmetics and as additives in plastics (Korkmaz, Ahabab, Kolankaya, & Barlas, 2010). In particular 4-tert-octylphenol (OP) and nonylphenol (NP) and its isomers are ubiquitous chemicals present in aquatic system (Shusheng et al., 2010) which exert endocrine disrupting effects both in vitro and in vivo experiments (Cespedes et al., 2004; Ferrara, Fabietti, Delisea, & Funari, 2005). In humans, the main sources of exposure to APs are inhalation, ingestion of contaminated food and dermal absorption. Various plastic food containers and wrappings have been found to produce the migration of alkylphenols residue into foodstuffs (Inoue et al., 2001; Loyo-Rosales, Rosales-Rivera, Lynch, Rice, & Torrents, 2004). In food packaging, nonylphenol originates from oxidation of the antioxidant additive trisnonylphenyl phosphite (TNPP) (McNeal et al., 2000), an antioxidant/antiozonant, added to polymeric materials such as polyvinyl chloride (PVC), polyolefins and acrylics (Kataoka, 2002). The intake may be significant via seafood where NP was detected in edible mussels at 696 ng g^{-1} and sea fish at $44\text{--}55 \text{ ng g}^{-1}$ fresh weight, respectively (Ferrara, Ademollo, Delise, Fabietti, & Funari, 2008; Ferrara, Fabietti, Delise, Piccoli Bocca, & Funari, 2001). Leaching from packaging can be an additional source of APs to humans, as demonstrated in milk where alkylphenols and phthalates ranged from 0.28 to $85.3 \text{ } \mu\text{g kg}^{-1}$ (Casajuana et al., 2004), in Taiwanese baby-food purees where OP and NP were detected between 0.6 and 21 ng g^{-1} wet weight (Chia-Tien et al., 2008) and bisphenol A diglycidyl ether (BADGE) and its derivatives were detected in soft drinks and canned food at $0.1\text{--}675 \text{ } \mu\text{g kg}^{-1}$ range (Gallart-Ayala, Moyano, & Galceran, 2011). At the low ng L^{-1} level, water supply can also be a source to APs as demonstrated by Casajuana et al. (2003) and Amirudou et al., (2011). Given its toxicological and ecotoxicological properties, NP has been included among the 11 pollutants for which a drastic policy of emissions reduction have been implemented in Europe (COM, 2001).

Among many other uses, BPA is utilized to produce polycarbonate plastics, epoxy resin for cans, toys, microwave containers and water pipes. Heat and contact with either acidic or basic foods, as the process of sterilization in cans or polycarbonate plastic, increase the hydrolysis of the ester bond linking BPA molecules in the polycarbonate and epoxy resins and compounds are released to foods (Vom Saal & Hughes, 2005). Vandenberg, Hauser, Marcus, Olea, and Welshons (2007) reviews human exposure to BPA and highlight that the potential BPA exposure is related to dietary sources, in particular foods stored in cans with epoxy resin linings or drinking water. The European Scientific Committee on Food estimated BPA exposure to be $0.48\text{--}1.6 \text{ g kg}^{-1}$ bw per day from food sources (SCF, 2002). As a result of the high daily intake of BPA, it has been detected in serum, urine, amniotic fluid and placental tissues (Ghisari & Bonefeld-Jorgensen, 2009). It shows moderate estrogenic activity and can influence reproduction. Some studies indicate that it has a potential to disrupt thyroid hormone action; proliferation of human prostate cancer cells and block of testosterone synthesis (Vom Saal & Hughes, 2005). EFSA TDI established for this compound is 0.05 mg kg^{-1} bw (EFSA, 2010). However, in

last few years many studies were carried out to define its use to preserve human health, in particular children, in fact this polymer was usually used to produce baby's bottle and it can migrate during reheating process.

Finally, di(2-ethylhexyl)adipate (DEHA) is utilized, at low levels, to produce polyvinyl chloride (PVC) films, so the main source for human exposure is the foodstuffs wrapped in PVC film. These films are $8\text{--}25 \text{ } \mu\text{m}$ thick and $10\text{--}30\%$ is constituted by plasticizers. DEHA main source for human exposure are wrapped foodstuffs. Goulas, Zygoura, Karatapanis, Georgantelis, and Kontominas (2007) review the migration of plasticizers from PVC films into various foods and food simulants. In particular DEHA was found in cheese wrapped in PVC films at levels ranging from 31 to 429 mg kg^{-1} while in fresh meat these levels ranged from 49 to 151 mg kg^{-1} . Considering DEHA toxicity, a study carried out in vivo in mice showed that this compound can produce cancer in liver, although it does not show effects on sexual maturation or on hormone levels (Ghisari & Bonefeld-Jorgensen, 2009) and it does not show antiandrogenic effects (Dalgaard et al., 2003). DEHA TDI is 0.3 mg kg^{-1} bw (EFSA, 2005f).

To guarantee human health, the European Union established limits for many compounds used in packaging and established regulations specifying migration tests using food simulants to determine their probable migration into food. The EU fixed Specific Migration Limits (SMLs) for single contaminants or group of contaminants in Regulation 10/2011. These values are in particular 0.3 mg kg^{-1} food simulant (fs) for DBP, 30 mg kg^{-1} fs for BBP, 1.5 mg kg^{-1} fs for DEHP and 18 mg kg^{-1} fs for DEHA. For compounds for which there are not SML, a restriction value of 60 mg kg^{-1} of food product is applied. For containers and other articles, for sheets and films in contact with less than 500 mL or g or more than 10 L and for materials and articles for which it is not possible to estimate the relationship between the surface area of such materials and the quantity of food in contact, the SML are expressed in mg kg^{-1} applying a surface to volume ratio of 6 dm^2 per kg of food. Overall, the plastic packaging must not be released to food simulants more than 10 mg of all compounds in one dm^2 of contact surface between food and packaging (Overall Migration Limit or OML) (Reg 10/11).

The objectives of this study were (i) to evaluate the migration of DMP, DBP, BBP, DEHP, OP, NP, BPA and DEHA from common food packaging, including cans, marmalade cap, yogurt packaging, polystyrene dish, teat, bread bag, film, baby's bottle, aseptic plastic laminate paperboard carton (tetrapack) and plastic wine top and to relate these levels with the limits established by the EU and, (ii) to compare the migration potential of plasticizers and additives from plastic wine tops using incubation at $40 \text{ }^\circ\text{C}$ (Extreme Conditions, EC) and ultrasonic extraction to validate the methodology.

2. Materials and methods

2.1. Chemicals and reagents

All sample manipulation was done avoiding any contact with plastic material. Chromatography grade methanol, acetone, dichloromethane, n-hexane, ethyl acetate and HPLC water were from Merck (Darmstadt, Germany). Deuterated surrogate standards were NP D8 ($100 \text{ ng } \mu\text{L}^{-1}$), DPP D4 and BPA D16, purchased as solids from Dr. Ehrenstorfer (Augsburg, Germany) and anthracene D10, used as internal standard, was purchased at a concentration of $10 \text{ ng } \mu\text{L}^{-1}$ (Supelco Bellefonte, PA, USA). Phthalate Esters Mix was purchased at a concentration of $500 \text{ } \mu\text{g mL}^{-1}$ in methanol (Supelco, Bellefonte, PA, USA); NP was purchased as a solid technical mixture of isomers (Riedel-de Haën, Seelze, Germany); OP and BPA were purchased as solids from Supelco (Bellefonte, PA, USA) and Dr.

Ehrenstorfer (Augsburg, Germany), respectively. A working solution was prepared at concentration of $10 \mu\text{g mL}^{-1}$. A calibration solution was prepared at 0.01, 0.05, 0.1, 0.25, 0.5, 0.75 and $1 \mu\text{g mL}^{-1}$.

2.2. Migration tests

EU Directives 82/711/EEC and 85/572 EEC describe the migration test and specific the use of food simulants depending on the type of food. Relating to the food contact materials (FCM), four liquid simulants are described: distilled water for aqueous foods with a pH above 4.5; acetic acid at 3% in distilled water for acidic aqueous food with pH below 4.5; ethanol at 15% for alcoholic food and oil for fatty food. Considering that the packaging, the storage temperature and the contact time between food packaging and food are the most important parameters for the migration of contaminants into food, the best migration test conditions are 40°C for 10 days (Extreme Conditions or EC) concerning storage at room temperature for indefinite time (Grob, 2008). Testing migration conditions are currently described again in Regulation 10/2011 that replaced old directives. For plastic materials and articles not yet in contact with food the simulants listed are: ethanol 10% (v/v) (simulant A), acetic acid 3% (v/v) (simulant B), ethanol 20% (v/v) (simulant C), ethanol 50% (v/v) (simulant D1), vegetable oil (simulant D2) and poly(2,6-diphenyl-p-phenylene oxide), particle size 60–80 mesh, pore size 200 nm (simulant E). Food simulants A, B and C have to be used for foods that have a hydrophilic character, food simulants D1 and D2 are assigned for foods that have a lipophilic character and food simulant E is assigned for testing specific migration into dry foods. However the application of Plastics Implementing Measure (PIM) is characterized by a specific phased implementation period. In fact, until 1 January 2013, migration tests should be carried out according to the pre-PIM rules; while from 1 January 2013 to 31 December 2015, the pre-PIM rules or the rules in the PIM can be applied. Finally, from 1 January 2016, the rules from the PIM should be applied. For such reason, in this study, migration tests were set up considering the earlier directives (Directives 82/711/EEC and 85/572/EEC).

Samples were incubated in EC in an oven at 40°C during 10 days. The food simulant was then extracted by solid phase extraction (SPE) and analyzed by gas chromatography coupled to mass spectrometry (GC–MS) (UNI-EN 1186, 2002).

To test the performance of the EC method, an ultrasonic based migration method was used for plastic wine tops, similarly to Guart, Bono-Blay, Borell, and Lacorte (2011). Ten wine tops were incubated in extreme conditions at 40°C for 10 days (Extraction A) and ten were incubated in an ultrasonic bath for 15 min (Extraction B). In Guart study, three time ranges (5, 10 and 15 min) were tested, but as no differences in migration were observed, 15 min incubation period was used in the present study. In particular each top was put in a glass jar with 100 mL of food simulant (ethanol 15%) (Directive 82/711/EEC).

2.3. Food containers analyzed

Eleven packaging materials were studied. Epoxy resin coated oil and natural tuna cans and marmalade cap constituted by epoxy resins, low density polyethylene (LDPE) film and bread bag, aseptic plastic laminate paperboard carton commonly referred as tetrapack, high density polyethylene (HDPE) yogurt packaging, polystyrene dish, silicone teat, polycarbonate baby's bottle and 2 synthetic plastic wine tops from different brands were tested. They represent commonly used packaging materials used to wrap or to store foods. All packages were sampled in triplicate, they were weighted and the surface was calculated. As suggested by the

European Directive 85/572/EEC and considering the use of each packaging, the simulants employed were distilled water for film, natural tuna cans, marmalade cap, baby's bottle, teat, polystyrene, tetrapack, bread bag; acetic acid at 3% for yogurt packaging and oil tuna cans; and ethanol at 15% for plastic wine tops. Each packaging material or container was introduced in an adequate quantity of food simulant depending on the surface and volume of the item, as described in Directive 82/711/EEC. The packaging type, surface and quantity of simulant used are indicated in Table 1.

2.4. Solid phase extraction

The simulant used in the EC and ultrasonic migration tests were recovered and spiked with 50 ng of the surrogate standard. Waters were solid phase extracted using 200 mg Oasis HLB SPE cartridges (Waters Milford, MA, USA) using a Baker vacuum system (J.T. Baker, The Netherlands). To eliminate possible background contamination, cartridges were conditioned with 10 mL of hexane, followed by 10 mL of dichloromethane, 10 mL of methanol and 15 mL of HPLC water, all by gravity. Then, all quantity of simulant used was pre-concentrated at a flow of $8\text{--}13 \text{ mL min}^{-1}$ and afterward the cartridge was dried under vacuum during 1 h. The cartridges were eluted with 10 mL of dichloromethane:hexane (1:1) and 10 mL dichloromethane:acetone (1:1). The extracts were dried in a Turbovap nitrogen evaporator and then were reconstituted with 240 μL of ethyl acetate and 10 μL of anthracene D10 ($10 \text{ ng } \mu\text{L}^{-1}$).

Quality controls were prepared with HPLC water, acetic acid (3%) and ethanol (15%) to determine the recoveries of target compounds in each migration condition. Recoveries consisted in adequate quantity of food simulants (30, 100 and 500 mL depending on the volume used to incubate food packaging) spiked with 50 μL of the standard solution at concentration of $2 \text{ ng } \mu\text{L}^{-1}$ and 10 μL of the surrogate standard solution at concentration of $5 \text{ ng } \mu\text{L}^{-1}$ and afterward were processed as a sample.

2.5. Instrumental analysis

Analysis was carried out by gas chromatography coupled to a quadrupole mass spectrometer (Agilent 6890 series GC System connected with a HP 5973 MS). The separation was achieved with a $30 \text{ m} \times 0.25 \text{ mm}$ 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 65°C (holding time 2 min) to 160°C at $15^\circ\text{C min}^{-1}$, to 170°C at

Table 1
Surface area, type and amount (in mL) of simulant used in the migration tests.

Packaging	Type of plastic	Surface area (cm^2)	Food simulant	Amount (mL)
Oil tuna can	Epoxy resin	128	Acetic acid 3%	65
Natural tuna can	Epoxy resin	134	Distilled water	60
Marmalade cap	Epoxy resin	126	Distilled water	35
Film	Low density polyethylene	1000	Distilled water	1000
Bread bag	Low density polyethylene	378	Distilled water	380
Tetrapack	Polyethylene laminate paperboard carton	624	Distilled water	1000
Yogurt packaging	High density polyethylene	171	Acetic acid 3%	100
Polystyrene dish	Styrene	337	Distilled water	380
Teat	Silicone	19	Distilled water	160
Baby's bottle	Polycarbonate	279	Distilled water	300
Plastic wine top 1	Mixed plastic foam	31	Ethanol 15%	50
Plastic wine top 2	Mixed plastic foam	31	Ethanol 15%	100

3 °C min⁻¹ and finally to 310 °C at 10 °C min⁻¹, keeping this condition for 10 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) and the inlet temperature was 290 °C.

Peak detection and integration were carried out using MSD ChemStation software. Acquisition was performed in Selected Ion Monitoring (SIM) using 3 ions per compound. Internal standard quantification was used and the method was linear over a concentration of 0.01–1 mg L⁻¹, with coefficient of regression (R^2) > 0.99.

2.6. Statistical analysis

To evaluate the differences between the two extraction methods (EC and UE) statistical analysis by SPSS 13.0 was carried out by log-transforming the values to approximate a normal distribution of the data. When the levels of contamination were not detected, the values considered were LOD estimated by blanks analyses. Analysis of variance was carried out by ANOVA. The level of significance was set at $p < 0.05$.

3. Results

3.1. Quality control analysis

The recoveries of target compounds varied according to the food simulant tested. All compounds were satisfactorily recovered in distilled water while OP, NP and BPA were not recovered in acetic acid and BPA was not detected in ethanol solution (Table 2). In relation to the two extraction methods using EC (incubation at 40 °C for 10 days) and by ultrasonic extraction (UE) (incubation for 15 min), all compounds were satisfactorily recovered except DMP, which was only recovered using EC (61 ± 9%) while it was not detected by ultrasound incubation and BPA which was not detected neither by EC or by UE (Table 2).

For each condition, blanks were prepared with HPLC water, acetic acid (3%) and ethanol (15%) and spiked with 10 µL of 5 ng µL⁻¹ surrogate standard solution and were processed together with each sample batch to determine the background contribution. Limits of Detection (LOD) for both EC and the ultrasound experiments, were calculated as mean blank value plus 3 times the standard deviation and Limit of Quantification (LOQ) as mean blank value plus 9 times the standard deviation (Table 3).

In particular LODs ranged from 17 (NP) to 451 ng mL⁻¹ fs (DEHP) for EC experiment; while LOD values in UE method varied from 13 (BBP) to 370 ng mL⁻¹ fs (NP and DEHA). Results on the contaminant levels in food packaging obtained using the migration experiments were reported only if the three replicates were >LOD.

3.2. Migration of plasticizers and additives from different packaging

From the migration experiments tested using the various simulants, at least one target compounds was identified in each packaging category and Table 4 shows the concentrations detected. Considering the 11 food packagings, the concentration of

Table 3

LODs in ng L⁻¹ calculated for each compound in extreme conditions (EC) and in ultrasonic extraction (UE) experiments.

Compound	LOD	
	EC (n = 4)	UE (n = 2)
DMP	18	21
DBP	114	86
BBP	42	13
DEHP	451	368
OP	85	228
NP	17	27
BPA	21	33
DEHA	155	370

OP > DEHP > NP > DEHA > DBP > BPA > BBP > DMP, even if a more variability of data was found in different packages. For all samples, the OP, that showed the highest mean value, the levels ranged from 6 to 27956 ng L⁻¹; on the contrary, DMP, a compound with lowest mean, the levels for all samples ranged from 6 to 421 ng L⁻¹. However, in terms of frequency of detection, DBP, BBP, DEHP, BPA and DEHA showed the lowest prevalence (33%, 36%, 55%, 55% and 64%, data not shown).

Epoxy coated containers are made of bisphenol polymers and this compound was expected to migrate. However, when acetic acid was used as food simulant, bisphenol could not be recovered and was not detected in oil tuna cans. However, in natural tuna cans, bisphenol was detected at 842 ± 148 ng L⁻¹. Other compounds detected in oil tuna cans were DMP and DEHP at 66 ± 20 ng L⁻¹ fs and 2668 ± 591 ng L⁻¹ fs, respectively. Marmalade cap contained the highest mean levels of DMP (338 ± 76 ng L⁻¹), BBP (275 ± 67 ng L⁻¹) and DEHP (7921 ± 738 ng L⁻¹). Finally, natural tuna can showed the highest NP levels, with a mean of 3912 ± 459 ng L⁻¹ along with traces of DMP and BBP. The presence of compounds other than BPA in epoxy resins can be attributed to the addition of phthalates and NP in the production of epoxy resins.

High density polyethylene has high tensile strength, harder and resist higher temperatures than low density polyethylene. Its use in a variety of packaging and wrapping containers has increased in the last years due to their unique properties of resistance, it is chemically inert, and has a cheap production. High and low polyethylene are thermo plastics derived from petroleum where additives and plasticizers are added to improve their properties. As other types of plastics, both plastic monomers or additives can migrate to food during storage. On the other hand, low density polyethylene is a flexible material used in many types of wrappings. Polyethylene bread back released higher amounts of plasticizers compared to polyethylene film. Similar data was obtained by Bonini, Errani, Zerbini, Ferri, and Girotti (2008) in relation to the migration of plasticizers from LDPE film in different test conditions, although no phthalate migration was found. In tetrapack containers, low levels of DMP, DBP, OP, NP and DEHA were found. Comparing polyethylene film, bread bag, yogurt packaging and Tetrapack, differences were observed on the detected compounds. It is possible that each plastic format uses several plasticizers and additives. It is also

Table 2

Recoveries (%; n = 2 except for acetic acid 3%) of phthalates, OP, NP, BPA and DEHA in the different food simulants used in this study.

FOOD SIMULANT	DMP mean ± sd	DBP mean ± sd	BBP mean ± sd	DEHP mean ± sd	OP mean ± sd	NP mean ± sd	BPA mean ± sd	DEHA mean ± sd
Acetic acid 3%	89	82	81	100	NR	NR	NR	74
Distilled water	79 ± 2	82 ± 18	92 ± 18	96 ± 5	81 ± 7	79 ± 7	80 ± 6	88 ± 12
Ethanol 15% EC	61 ± 9	85 ± 5	84 ± 9	143 ± 23	75 ± 7	100 ± 6	NR	66 ± 13
Ethanol 15% UE	NR	106 ± 8	97 ± 7	97 ± 12	72 ± 4	95 ± 6	NR	66 ± 10

NR = not recovered.

EC corresponds to Extreme Conditions and UE to Ultrasonic Extraction.

Table 4
Levels of phthalates, OP, NP, BPA and DEHA (mean \pm sd and range) in ng L⁻¹ fs in the different food packaging items considered in this study.

Packaging	DMP Mean \pm sd Range	DBP Mean \pm sd Range	BBP Mean \pm sd Range	DEHP Mean \pm sd Range	OP Mean \pm sd Range	NP Mean \pm sd Range	BPA Mean \pm sd Range	DEHA Mean \pm sd Range
Oil tuna can (n = 3)	66 \pm 20 50–90	<LOD	<LOD	2668 \pm 591 1986–3027	NR	NR	NR	<LOD
Natural tuna can (n = 3)	106 \pm 12 92–113	<LOD	39 \pm 4 35–43	<LOD	<LOD	3912 \pm 459 3382–4185	824 \pm 148 677–973	116 \pm 58 80–183
Marmalade cap (n = 3)	338 \pm 76 271–421	<LOD	275 \pm 67 210–344	7921 \pm 738 7141–8609	<LOD	<LOD	<LOD	340 \pm 71 279–418
Film (n = 3)	<LOD	30 \pm 8 25–39	<LOD	<LOD	17 \pm 5 11–21	57 \pm 28 27–81	<LOD	<LOD
Bread bag (n = 3)	19 \pm 3 15–21	65 \pm 15 49–80	<LOD	436 \pm 35 404–474	338 \pm 163 18–512	238 \pm 128 123–376	189 \pm 46 142–234	20 \pm 10 14–26
Tetrapack (n = 3)	12 \pm 5 6–15	40 \pm 15 42–68	<LOD	<LOD	12 \pm 6 6–19	24 \pm 6 18–30	<LOD	73 \pm 59 23–138
Yogurt packaging (n = 3)	58 \pm 37 19–94	<LOD	<LOD	<LOD	NR	NR	NR	1989 \pm 539 1876–2902
Polystyrene dish (n = 3)	19 \pm 6 15–26	<LOD	<LOD	701 \pm 479 323–1239	39 \pm 4 35–42	184 \pm 51 129–231	<LOD	40 \pm 10 24–48
Teat (n = 3)	<LOD	<LOD	18 \pm 4 14–22	<LOD	<LOD	378 \pm 130 364–476	<LOD	<LOD
Baby's bottle (n = 3)	<LOD	121 \pm 33 96–159	<LOD	<LOD	33 \pm 10 25–44	154 \pm 86 73–244	404 \pm 69 345–480	<LOD
Plastic wine top 1 (n = 3)	273 \pm 111 155–375	1684 \pm 281 1389–1948	274 \pm 108 151–355	14176 \pm 3360 10999–17694	26629 \pm 1185 25676–27956	958 \pm 490 415–1368	NR	1099 \pm 348 780–1470
All packaging (n = 33)	85 \pm 116 6–421	363 \pm 640 25–1948	116 \pm 135 14–355	5086 \pm 5348 323–17694	6619 \pm 20910 6–27956	720 \pm 1251 18–4185	315 \pm 300 142–973	651 \pm 875 14–2902

NR = not recovered (OP, NP and BPA not recovered in acetic acid 3%).

possible that the same plastic from different brands have a different composition that is translated to different migration potential of the components used.

Polystyrene is a polymer made of styrene and in its pure solid form, it is a colorless, hard plastic with limited flexibility. It can be cast into molds with fine detail and thus it is used in the packaging of yogurts and many lactic products. Yogurt packaging contained traces of DMP but the highest level of DEHA among packaging containers. This compound may leach from fatty food at high temperatures used during production. On the other hand, expanded polystyrene is used as trays for wrapping meats, fish, cheese, fruit, etc. Trays produced the release of some compounds such as DMP, DEHP, OP, NP and DEHA (Table 4). Again, the specific source of these compounds is difficult to establish since so far we do not know what additives are used in the production of polystyrene. Although it is known that they are added to plastics to improve their properties.

The incubation of silicone teat in water produced the migration of BBP and NP, both probably usually used as plasticizers as they confer great ductility and an anatomical shape.

Baby's bottle made of polycarbonate released DBP, OP, NP and BPA. This latter compound was expected since it is the monomer used in the synthesis of PC plastic. De Coensel et al. (2009) carried out a study to detect this compound in baby's bottle but in different conditions, such as heating of the bottles in a microwave or in a baby's bottle heater for 100 times at a maximum of temperature of 86 °C. In this study a relation between heating temperature and BPA migration was found and BPA diffused only during heating as suggested by Vom Saal and Hughes (2005). As a result of this, PC has been avoided in the manufacture of baby's bottles (Directive 8/2011).

Finally, wine tops were the material which produced the highest release of phthalates, alkylphenols and DEHA. Mean concentrations detected are indicated in Table 4. Probably these levels depended on the plastic wine top composition, in fact these plastic wine tops are made with elastomers and foams (ethylene, propylene, urethane, silicones or their copolymers with different additives), to

obtain top with uniform cellular structure or variable geometry. However, during our experimental conditions, the wine top samples were totally in contact with the food simulant, differing from real conditions where the wine top is not in direct contact with the wine product. Therefore, the amount of compounds migrating from wine tops do not reflect the real conditions where the top is only in contact with the headspace of the wine bottle.

According to the aims of this study, phthalates, NP, OP, BPA and DEHA levels were compared with EU maximum levels, all samples showed contaminant migration lower than SML and OML established (Reg 10/2011).

3.3. Migration from plastic wine tops

Recoveries and levels of contamination obtained from the two incubation experiments, i.e. in EC and by UE, were compared. In particular the percentages of recovery were quite similar for all chemicals considered, even though DMP was not detected using UE. The results obtained by the evaluation of the two extraction methods are shown in Table 5. Comparing these values with data reported in Table 4 on synthetic wine tops 1, it was found a different level of contamination for BBP, DEHP, OP, NP and DEHA. It was probably due at different chemical composition of these tops because the tops considered for migration tests were different from tops used in this step in terms of color, porosity and texture. The incubation at 40 °C (EC) was more efficient for extracting food contaminants than UE. Specifically, 100% of EC samples contained all compounds except for DEHA, while in UE samples, 100% of tops were contaminated by DBP, DEHP and OP, only 60% by BBP and none contained NP and DEHA. Only for DBP and DEHP, mean EC results were lower than UE ones, with levels of 1855 \pm 372 ng L⁻¹ and 4878 \pm 2295 ng L⁻¹ for DBP and of 3157 \pm 934 ng L⁻¹ and 6907 \pm 2171 ng L⁻¹ for DEHP. Anyway significant differences between EC and UE method were found only for DBP ($p = 0.015$), BBP and OP ($p = 0.001$ for BBP and $p = 0.000$ for OP). Since EC method showed better recoveries for each chemical and the prevalence of contamination was similar or higher than UE, EC method

Table 5

Levels of phthalates, OP, NP, BPA and DEHA (percentage of positive samples, mean \pm sd and range) in ng L⁻¹ fs in plastic wine tops 2 ($n = 10$) processed in extreme conditions (EC) and in ultrasonic incubation (UE).

Compounds	Extraction EC ($n = 10$)		Extraction UE ($n = 10$)	
	% POS	Mean \pm sd Range	% POS	Mean \pm sd Range
DMP	100	407 \pm 168 235–792	NR	
DBP	100	1855 \pm 372 1426–2492	100	4878 \pm 2295 1819–7385
BBP	100	1004 \pm 235 596–1280	60	57 \pm 33 28–110
DEHP	100	3157 \pm 934 1728–4482	100	6907 \pm 2171 3352–9436
OP	100	2760 \pm 788 1833–3625	100	580 \pm 187 357–935
NP	100	181 \pm 42 101–233	<LOD	
BPA	NR		NR	
DEHA	40	301 \pm 97 163–371	<LOD	

NR = not recovered.

is recommended for the detection of plasticizers and plastic additives in migration experiments using simulants.

4. Conclusions

Phthalates, alkylphenols, BPA and DEHA were analyzed in 11 common packaging containers using specific simulants depending on the food product. The use of simulants is useful to identify compounds that migrate from containers and the data can be compared to legislated maximum concentrations. However, they do not reflect real conditions and in subsequent steps, migration experiments should be performed directly in food. More than one target compound was detected in tested packaging containers at levels from the low ng L⁻¹ to μ g L⁻¹. In particular, plastic wine tops showed the highest level of migration although wine tops are not in contact with the wine but in the headspace of the bottle. In other containers, the compounds detected may reflect the composition of the material, although in many instances it is difficult to determine the origin of phthalates, alkylphenols and DEHA since knowledge on the type and amounts of plasticizers and additives added during the manufacturing of plastics and containers is unspecified. This study provides information on the release of compounds which have endocrine disrupting properties. These compounds, if found in high concentrations in food, may produce health effects in exposed population. Therefore, their presence needs to be monitored in packaged foods which are daily ingested to minimize human exposure.

Acknowledgments

This study was financed by the Ministerio de Ciencia e Innovación of Spain, project INNPACTO (IPT-2011-0709-060000). Dr. Roser Chaler, Dori Fanjul and Maria Comesaña are acknowledged for MS support.

References

Amiridou, D., & Voutsas, D. (2011). Alkylphenols and phthalates in bottled waters. *Journal of Hazardous Materials*, 185, 281–286.

Bonini, M., Errani, E., Zerbinati, G., Ferri, E., & Girotti, S. (2008). Extraction and gas chromatographic evaluation of plasticizers content in food packaging films. *Microchemical Journal*, 90(1), 31–36.

Casajuana, N., & Lacorte, S. (2003). Presence and release of phthalic esters and other endocrine disrupting compounds in drinking water. *Chromatographia*, 57, 649–655.

Casajuana, N., & Lacorte, S. (2004). New methodology for the determination of phthalate esters, bisphenol A, bisphenol A diglycidyl ether, and nonylphenol in commercial whole milk samples. *Journal of Agricultural and Food Chemistry*, 52, 3702–3707.

Castle, L., Mercer, A. J., Startin, J. R., & Gilbert, J. (1988). Migration from plasticized films into foods. 3. Migration of phthalate, sebacate, citrate and phosphate esters from films used for retail food packaging. *Food Additives & Contaminants*, 5, 9–20.

Céspedes, R., Petrovic, M., Raldúa, D., Ursula, S., Pina, B., Lacorte, S., et al. (2004). Integrated procedure for determination of endocrine-disrupting activity in surface waters and sediments by use of the biological technique recombinant yeast assay and chemical analysis by LC–ESI–MS. *Analytical and Bioanalytical Chemistry*, 378, 697–708.

Chia-Tien, L., Chin-Yuan, C., & Wang-Hsien, D. (2008). Determination of alkylphenol residues in baby-food purees by steam distillation extraction and gas chromatography–mass spectrometry. *Food and Chemical Toxicology*, 46, 803–807.

Commission Directive 2011/8/EU of 28 January 2011 amending Directive 2002/72/EC as regards the restriction of use of bisphenol A in plastic infant feeding bottles. *Official Journal of the European Union L Series*, 26, 11–14.

Commission regulation 10/ 2011/EU of 14 January 2011 on plastic materials and articles intended to come into contact with food. *Official Journal of the European Union L Series*, 12, 1–89.

Communication from the Commission to the Council and the European Parliament COM (2001) on the implementation of the community strategy for endocrine disruptors – a range of substances suspected of interfering with the hormone systems of humans and wildlife COM (1999) 706, 262 final, Brussels.

Council Directive 82/711/EEC of 18 October 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. *Official Journal of the European Union L Series*, 297, 26–30.

Council Directive 85/572/EEC of 19 December 1985 on laying down the list of simulants to be used for testing migration of constituents of plastic materials and articles intended to come into contact with foodstuffs. *Official Journal of the European Union L Series*, 372, 14–21.

Dalgaard, M., Hass, U., Vinggaard, A. M., Jarfelt, K., Lam Henrik, R., Sørensen, I. K., et al. (2003). Di(2-ethylhexyl) adipate (DEHA) induced developmental toxicity but not antiandrogenic effects in pre- and postnatally exposed Wistar rats. *Reproductive Toxicology*, 17, 163–170.

De Coenselet, N., David, F., & Sandra, P. (2009). Study on the migration of bisphenol-A from baby bottles by stir bar sorptive extraction–thermal desorption–capillary GC–MS. *Journal of Separation Science*, 32, 3829–3836.

European Food Safety Authority (EFSA). (2005a). Opinion of the scientific panel on food additives, flavourings, processing aids and material in contact with food (AFC) on a request from the commission related to di-butylphthalate (DBP) for use in food contact materials. Question N° EFSA-Q-2003-192. *EFSA Journal*, 242, 1–17.

European Food Safety Authority (EFSA). (2005b). Opinion of the scientific panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on a request from the commission related to butylbenzylphthalate (BBP) for use in food contact materials. Question N° EFSA-Q-2003-190. *EFSA Journal*, 241, 1–14.

European Food Safety Authority (EFSA). (2005c). Opinion of the scientific panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on a request from the commission related to di-isononylphthalate (DINP) for use in food contact materials. Question N° EFSA-Q-2003-194. *EFSA Journal*, 244, 1–18.

European Food Safety Authority (EFSA). (2005d). Opinion of the scientific panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on a request from the commission related to di-isodecylphthalate (DIDP) for use in food contact materials. *EFSA Journal*, 245, 1–14.

European Food Safety Authority (EFSA). (2005e). Opinion of the scientific panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on a request from the commission related to bis(2-ethylhexyl)phthalate (DEHP) for use in food contact materials. Question N° EFSA-Q-2003-191. *EFSA Journal*, 243, 1–20.

European Food Safety Authority (EFSA). (2005f). Opinion of the scientific panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on a request from the commission on the application of a total reduction factor of 5 for di(2-ethylhexyl)adipate used as plasticizer in flexible PVC food packaging films. Question No EFSA-Q-2003-071. *EFSA Journal*, 217, 1–5.

European Food Safety Authority (EFSA). (2010). Scientific opinion on bisphenol A: evaluation of a study investigating its neurodevelopmental toxicity, review of recent scientific literature on its toxicity and advice on the Danish risk assessment of bisphenol A. *EFSA Journal*, 8(9), 1–116.

Ferrara, F., Fabietti, F., Delise, M., Piccioli Bocca, A., & Funari, E. (2001). Alkylphenolic compounds in edible molluscs of the Adriatic sea (Italy). *Environmental Science & Technology*, 35, 3109–3112.

Ferrara, F., Fabietti, F., Delise, M., & Funari, E. (2005). Alkylphenols and alkylphenol ethoxylates contamination of crustaceans and fishes from the Adriatic Sea (Italy). *Chemosphere*, 59, 1145–1150.

Ferrara, F., Ademollo, N., Delise, M., Fabietti, F., & Funari, E. (2008). Alkylphenols and their ethoxylates in seafood from the Tyrrhenian Sea. *Chemosphere*, 72, 1279–1285.

Fromme, H., Gruber, L., Schlummer, M., Wolz, G., Boehmer, S., Angerer, J., et al. (2007). Intake of phthalates and di(2-ethylhexyl)adipate: results of the

- integrated exposure assessment survey based on duplicate diet samples and biomonitoring data. *Environment International*, 33, 1012–1020.
- Gallart-Ayala, H., Moyano, E., & Galceran, M. T. (2011). Fast liquid chromatography-tandem mass spectrometry for the analysis of bisphenol A-diglycidyl ether, bisphenol F-diglycidyl ether and their derivatives in canned food and beverages. *Journal of Chromatography A*, 1218(12), 1603–1610.
- Guart, A., Bono-Blay, F., Borell, A., & Lacorte, S. (2011). Migration of plasticizers phthalates, bisphenol A and alkylphenols from plastic containers and evaluation of risk. *Food Additives & Contaminants: Part A*, 28, 676–685.
- Ghisari, M., & Bonefeld-Jorgensen, E. C. (2009). Effects of plasticizers and their mixtures on estrogen receptor and thyroid hormone functions. *Toxicology Letters*, 189, 67–77.
- Goulas, A. E., Zygoura, P., Karatapanis, A., Georgantelis, D., & Kontominas, M. G. (2007). Migration of di(2-ethylhexyl) adipate and acetyltributyl citrate plasticizers from food-grade PVC film into sweetened sesame paste (halawa tehneh): kinetic and penetration study. *Food and Chemical Toxicology*, 45, 585–591.
- Grob, K. (2008). The future of simulants in compliance testing regarding the migration from food contact materials into food. *Food Control*, 19, 263–268.
- Inoue, K., Kondo, S., Yoshie, Y., Kato, K., Yoshimura, Y., Horie, M., et al. (2001). Migration of 4-nonylphenol from polyvinyl chloride food packaging films into food simulants and food. *Food Additives & Contaminants*, 18(2), 157–164.
- Itoh, H., Yoshida, K., & Masunaga, S. (2005). Evaluation of the effect of governmental control of human exposure to two phthalates in Japan using a urinary biomarker approach. *International Journal of Hygiene and Environmental Health*, 208, 237–245.
- Kataoka, H., Ise, M., & Narimatsu, S. (2002). Automated on-line in-tube solid-phase microextraction coupled with high performance liquid chromatography for the analysis of bisphenol A, alkylphenols, and phthalate esters in foods contacted with plastics. *Journal of Separation Science*, 25, 77–85.
- Kavlock, R., Boeckelheide, K., Chapin, R., Cunningham, M., Faustman, E., Foster, P., et al. (2002). NTP center for the evaluation of risks to human reproduction: phthalates expert panel report on the reproductive and developmental toxicity of di(2-ethylhexyl)phthalate. *Reproductive Toxicology*, 16, 529–653.
- Korkmaz, A., Ahbab, M. A., Kolankaya, D., & Barlas, N. (2010). Influence of vitamin C on bisphenol A, nonylphenol and octylphenol induced oxidative damages in liver of male rats. *Food and Chemical Toxicology*, 48, 2865–2871.
- Kueseng, P., Thavarungkul, P., & Kanatharana, P. (2007). Trace phthalate and adipate esters contaminated in packaged food. *Journal of Environmental Science and Health, Part B*, 42, 569–576.
- Loyo-Rosales, J. E., Rosales-Rivera, G. C., Lynch, A. M., Rice, C. P., & Torrents, A. (2004). Migration of nonylphenol from plastic containers to water and a milk surrogate. *Journal of Agricultural and Food Chemistry*, 52, 2016–2020.
- McNeal, T. P., Biles, J. E., Begley, T. H., Craun, J. C., Hopper, M. L., & Sack, C. A. (2000). Determination of suspected endocrine disruptors in foods and food packaging. In McNeal, T. P., Biles, J. E., Begley, T. H., Craun, J. C., Hopper, M. L., & Sack, C. A. (Eds.). (2000). *Analysis of environmental endocrine disruptors*, Vol. 747 (pp. 33–52). Washington, DC: American Chemical Society.
- Scientific Committee on Food (SCF) Opinion of the Scientific Committee on Food on Bisphenol A of 3 May 2002. *SCF/CS/PM/3936 final*.
- Sheela, S. (2008). Phthalates and children's health. *Current Problems in Pediatric and Adolescent Health Care*, 38, 34–49.
- Shusheng, L., Ling, F., Xiaowei, W., Hongtao, L., Gangfeng, O., Chongyu, L., et al. (2010). Determination of octylphenol and nonylphenol in aqueous sample using simultaneous derivatization and dispersive liquid-liquid microextraction followed by gas chromatography-mass spectrometry. *Journal of Chromatography A*, 1217, 6762–6768.
- Spillmann, M. D., Siegrist, M., Keller, C., & Wormuth, M. (2009). Phthalate exposure through food and consumers' risk perception of chemicals in food. *Risk Analysis*, 29, 1170–1180.
- Tsumura, Y., Ishimitsu, S., Saito, H., Kobayashi, Y., & Tonogai, Y. (2001). Eleven phthalate esters and di(2-ethylhexyl) adipate in one-week duplicate diet samples obtained from hospitals and their estimated daily intake. *Food Additives & Contaminants*, 18, 449–460.
- UNI-EN 1186. (2002). *Materials and articles in contact with foodstuffs-plastics*. BS EN 1186-6.
- Vandenberg, L. N., Hauser, R., Marcus, M., Olea, N., & Welshons, W. V. (2007). Human exposure to bisphenol A (BPA). *Reproductive Toxicology*, 24, 139–177.
- Vom Saal, F. S., & Hughes, C. (2005). An extensive new literature concerning low-dose effects of bisphenol A shows the need for a new risk assessment. *Environmental Health Perspectives*, 113(8), 926–933.
- Wagner, M., & Oehlmann, J. (2009). Endocrine disruptors in bottled mineral water: total estrogenic burden and migration from plastic bottles. *Environmental Science and Pollution Research*, 16, 278–286.
- Wormuth, M., Scheringer, M., Vollenweider, M., & Hungerbuehler, K. (2006). What are the sources of exposure to eight frequently used phthalic acid esters in Europeans? *Risk Analysis*, 26, 803–824.