Food and Chemical Toxicology 128 (2019) 180–192

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

Estimates of dietary exposure of Spanish population to packaging contaminants from cereal based foods contained in plastic materials

Verónica García Ibarra[a,1], Raquel Sendón[a], Juana Bustos[b], Perfecto Paseiro Losada[a], Ana Rodríguez Bernaldo de Quirós[a,*]

[a] Faculty of Pharmacy, Department of Analytical Chemistry, Nutrition and Food Science, University of Santiago de Compostela, 15782, Santiago de Compostela, Spain
[b] National Food Centre, Spanish Agency for Consumer Affairs, Food Safety and Nutrition, 28220, Majadahonda, Spain

ARTICLE INFO

Keywords:
Food packaging materials
Migration
Exposure assessment
Composite foods
GC-MS
LC-MS/MS

ABSTRACT

Food packaging may be a potential source of contamination, through the migration of chemicals from the packaging into the food, thus food consumption is an important route of human exposure to packaging contaminants.

In the present study an approach to estimate the exposure to different chemicals transferred from food packaging was designed. As a first step a GC-MS screening was conducted to identify potential contaminants in the materials. Secondly, different chemicals previously identified in the packaging materials were selected for exposure assessment. The proposed methodology was applied to cereal based foods packed with plastic packaging. A variety of chemicals including e.g. acetyl tributyl citrate (ATBC), bis (2-ethylhexyl) adipate (DEHA) and diethyl phthalate (DEP) among others were identified and analyzed in the foodstuffs. For this purpose a LC-MS/MS method was developed.

The selected foodstuffs were pooled into three groups according to the population age (12–35 months, 3–9 years and 10–17 years) and based on the Spanish consumption data (Enalia).

In general, ATBC mean exposure was higher than that of phthalates and DEHA for the three groups considered, with mean dietary exposure values ranging from 1.01 μg/kg bw/day (pool 12–35 months) to 2.01 μg/kg bw/day (pool 3–9 years).

1. Introduction

Some chemicals present in food may pose a health risk to the consumers. People are exposed to contaminants, through several sources and it is recognized that food packaging may be a potential source of contamination, through the migration of substances from the packaging into the food. This is especially relevant when considering that most of the food is sold packaged in order to prevent the deterioration of the food and to prolong its shelf life. Unsafe levels of chemicals in food are currently known or suspected to be responsible of serious health problems of consumers including cancer, reproductive disorders and immune system suppression among others (Pocas and Hogg, 2007). The monitoring of this migration from food contact materials has become a priority issue in order to ensure the food safety. Total diet studies (TDSs) are an effective tool to estimate the levels of the population exposure to different chemicals, allowing the subsequent adoption of appropriate measures in order to protect the safety of consumers. In fact, The World Health Organization (WHO) considers TDSs as a cost-effective means for assuring that people are not exposed to unsafe levels of chemical compounds through food (EFSA, 2011). The TDSs are approaches designed specifically to establish through chemical analysis and the study of consumption habits, the intake of contaminants and other substances present in the foods of a typical diet. They are also appropriate for assessing macro and micronutrient intake.

Total diet studies (TDS) generally consist of the selection, collection and analysis of food products based on national food consumption data to represent a large portion of a typical diet. Foodstuffs are pooled into representative food groups, and analyzed for the determination of harmful and/or beneficial chemical substances. Pooled samples consist of creating a unique food sample by combining various individual food items. Finally, dietary exposure assessment is performed by combining the occurrence data with the available consumption data (EFSA, 2011). An interesting issue that should be mentioned here are the deviations from a typical TDS approach. This is the case, for example of studies not

* Corresponding author.
E-mail address: ana.rodriguez.bernaldo@usc.es (A. Rodríguez Bernaldo de Quirós).
[1] Present address: Faculty of Sciences and Food Engineering, Universidad Técnica de Ambato, Campus Huachi, Ambato, Ecuador.

https://doi.org/10.1016/j.fct.2019.04.003
Received 27 November 2018; Received in revised form 4 March 2019; Accepted 3 April 2019
Available online 08 April 2019
0278-6915/

including all food groups, namely those who are focused in a specific group, or studies not analysing foods as consumed, and so on. According the Joint guidance of EFSA, FAO and WHO related to harmonised Total Diet Study approach, these studies should be classified as TDS-like investigations (EFSA, 2011).

In the consumption surveys developed in several countries data on packaging type are rarely collected, however, for assessing the exposure to packaging chemicals is necessary to know what type of food is packaged in what type of material in order to evaluate which potential compounds may be present, as well as their concentration (Pocas and Hogg, 2007).

Several countries, including China, UK, Belgium, Swiss-German and USA, among others have assessed dietary exposure to chemical migrants from plastic food-contact materials; some of them focused primarily on the presence of phthalates in food (Bradley et al., 2013; Fierens et al., 2014; Schecter et al., 2013; Sui et al., 2014; Dickson-Spillmann et al., 2009). Bis (2-ethylhexyl) phthalate (DEHP), in particular, contributes to the overall exposure to these chemicals from all sources. Other diet studies have focused on the presence of Bisphenol A (BPA) (Howe and Borodinsky, 1998; Mariscal-Arcas et al., 2009; Rivas et al., 2016), phthalates and bis (2-ethylhexyl) adipate (DEHA) (Cao et al., 2015). The exposure in these studies was calculated combining the concentration of the contaminants in foods migrated from food packaging with the consumption data, and in some cases, probabilistic and semi-probabilistic modelling approaches were used.

In the present study the compounds selected for the exposure assessment comprise a wide range of additives used in food contact materials including plasticizers such as phthalates (DEHP, dibutyl phthalate (DBP), diisobutyl phthalate (DIBP), diethyl phthalate (DEP) and benzyl butyl phthalate (BBP)), citrates (acetyl tributyl citrate (ATBC)) and adipates (DEHA), UV stabilizers (octocrylene) and slip agents (erucamide). The chosen additives were previously identified in the packaging itself.

Phthalates are chemical compounds commonly used as additives in food packaging applications. Phthalates such as DEHP are mainly used as plasticizers for polyvinylchloride (PVC) resins as well as in other resins such as polyvinyl acetate, polyurethanes and cellulose. In fact, approximately 80% of annual phthalates production is destined for that purpose (Yang et al., 2015). Low molecular weight phthalates such as DEP are used as solvents in personal care products and plasticizers for cellulose acetate (Serrano et al., 2014). There are several routes of phthalates exposure including dermal, ingestion and inhalation. Besides plasticizers, phthalates have been used as solvents to hold color and also can be present in printing inks and in and adhesives. (Cao, 2010; Van Holderbeke et al., 2014; Kataoka et al., 2002). People are exposed to phthalates through ingestion, when phthalates are used as additives in food packaging. Thus food is expected to be a major source of phthalates exposure. Some phthalates have been associated with a variety of adverse effects including liver and kidney toxicity, carcinogenity, metabolic diseases and damage to reproductive system among others (Jeng, 2014). Since 2005 the European Food Safety Authority (EFSA) has specified tolerable daily intakes (TDIs) for some phthalates 0.01 mg/kg bw for DBP, 0.05 mg/kg bw for DEHP, 0.50 mg/kg bw for BBP, and 0.15 mg/kg bw for sum of DiDP and DiNP (EFSA, 2005a, 2005b; 2005c, 2005d; 2005e). For DEP a TDI of 0.50 mg/kg bw was also established (WHO, 2003).

DEHA is as plasticizer used in flexible PVC films as well as in coatings, printing inks and adhesives and also in a variety of home and office products. Just like phthalates people may be exposed to DEHA through its migration from food contact materials. A TDI of 0.3 mg/kg bw has been established for DEHA (Cao et al., 2013; SCF, 2000). Among citrates, ATBC is a plasticizer widely used for polyvinylidene, polyvinyl resins and is also used in rubber and cellulosic resins. This compound is commonly employed as phthalate substitute. People are exposed to ATBC through ingestion of food containing this compound, however dermal and oral contacts are other routes of exposition (Goulas et al., 2007).

On the other hand, octocrylene is used as ultraviolet stabilizer in food plastic packaging materials to prevent degradation of polymers. Erucamide is a slip agent used to reduce the coefficient of friction of the surface of a polymer. It is also used in polyolefin closures. The toxicological properties of this compound have not been thoroughly investigated (Wypych, 2005; Commission Regulation (EU) No 10/2011). All selected compounds are included in Regulation (EU) No 10/2011 on plastic materials and articles intended to come into contact with food, with exception of DEP and DIBP (Commission Regulation (EU) No 10/2011).

The first aim of this work was to establish a gas chromatography mass spectrometry (GC-MS) screening method for the identification of potential migrants from plastic packaging materials. Secondly, the purpose was to investigate the dietary exposure to nine food packaging chemicals in the Spanish food supply chain, specifically in cereal products using a suitable high-performance liquid chromatography-tandem mass spectrometry method (LC-MS/MS). The exposure was calculated by combining the concentration of compounds in the packaged foods with the consumption data.

2. Materials and methods

2.1. Chemicals and analytical standards

Analytical standards of acetyl tributyl citrate (ATBC) 99%, bis (2-ethylhexyl) phthalate (DEHP) 99% and bis (2-ethylhexyl) adipate (DEHA) were purchased by Fluka.

Diethyl Phthalate (DEP) 99.5%, diisobutyl phthalate (DIBP) 99%, dibutyl phthalate (DBP) 99%, Erucamide, Benzyl butyl phthalate 98% (BBP), octocrylene and internal standard diethyl phthalate-3,4,5,6-d4, 99,3% were provided by Sigma Aldrich. The physico-chemical properties and chemical structures of the mentioned compounds are detailed in Table 1.

Other standards were used only for identification purposes. These were: squalene, butylated hydroxytoluene (BHT) 99% and saturated alkane standard mixture C7-C30 (1000 μg/mL each component in hexane) were obtained from Sigma-Aldrich® (Schnelldorf, Germany).

Acetonitrile (ACN) HPLC grade and LC-MS grade, hexane HPLC grade, and methanol LC-MS grade were from Merck (Darmstadt, Germany). Formic acid LC-MS grade was supplied by Sigma-Aldrich®, (St.Louis, Mo, USA). Purified water (Type I) was obtained from an Autwomatic Plus purification system (Wasserlab, Navarra, Spain).

A primary stock solution of DEHA and ATBC, DEP, DEHP, DIBP, DBP, BBP, octocrylene, erucamide and internal standard diethyl phthalate-3, 4, 5, 6-d was prepared in methanol. All stock solutions were prepared at concentration of 1000 μg/mL. Working solutions of all compounds were prepared by subsequent dilution with methanol (see 2.7.2 LC-MS/MS analysis), and the internal standard solution, diethyl phthalate-3,4,5,6-d4, was added to have a final concentration of 0.5 μg/mL in all working solutions. The solutions were stored at 4 °C in the darkness and brought to ambient temperature prior to use.

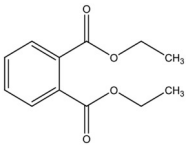
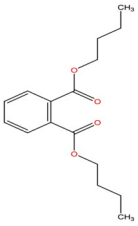
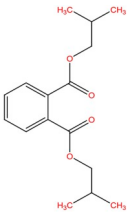
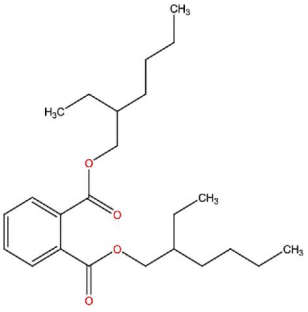
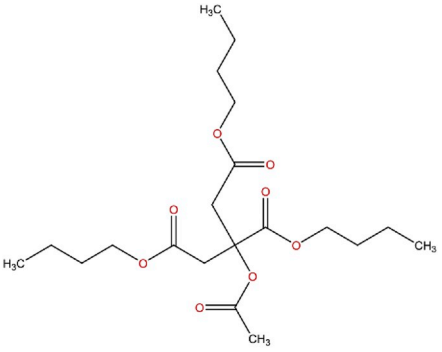
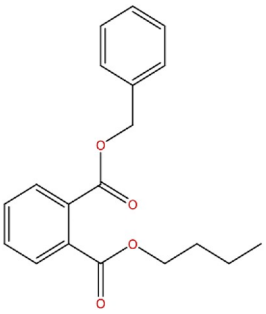
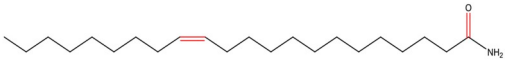
With the aim to minimize phthalate contamination a cleaning protocol was followed as described in section 2.9.

2.2. Samples

A total of seven plastic packaged foodstuffs containing cereal products were purchased from local supermarkets in Santiago de Compostela-Spain. Samples were of well-known brands consumed throughout Spain. All packaged foodstuffs were sampled in duplicate. An overview of all the analyzed samples is presented in Table 2.

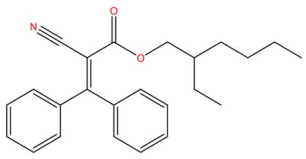
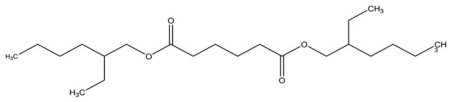
The selected cereal products including rice, pasta, breakfast cereal, bread and bread substitutes were pooled into three groups (according to the population age) based on the Spanish consumption data (ENALIA-AECOSAN) (ENALIA, 2014). Pools were prepared taken into account

Table 1
Chemical structures and specific migration limits of the selected compounds.

Structure	Compound	CAS N°	Molecular Weight g/ molssss	Melting point (°C)	Boiling Point (°C)	Vapor pressure (mm Hg)	SML mg/kg
	Diethyl phthalate (DEP)	84-66-2	222.23	4.05E + 01	295	0.0021	NL
	Dibutyl phthalate (DBP)	84-74-2	278.34	−35[a]	340[a]	2.01×10^{-5}	0.3 60*
	Diisobutyl phthalate (DIBP)	84-69-5	278.34	−64[a]	296[a]	6.65×10^{-3}	NL
	Bis(2-ethylhexyl) phthalate (DEHP)	117-81-7	390.56	−55[a]	384[a]	1.42×10^{-7}	1.5 60*
	Acetyltributyl citrate (ATBC)	77-90-7	402.48	8.00E + 01	172–174[a]	4.55E x 10^{-6}	60*
	Benzyl butyl phthalate (BBP)	85-68-7	312.36	−35[a]	370[a]	7.09×10^{-7}	30 60*
	Erucamide	112-84-5	337.58	75–80[a]	474.2[b]	3.69×10^{-9} [b]	**

(*continued on next page*)

Table 1 (*continued*)

Structure	Compound	CAS N°	Molecular Weight g/ molssss	Melting point (°C)	Boiling Point (°C)	Vapor pressure (mm Hg)	SML mg/kg
	Octocrylene	6197-30-4	361.48	–	478.5 [b]	2.56×10^{-9} [b]	0.05
	Di(2-ethylhexyl) adipate (DEHA)	103-23-1	370.57	−67.8[a]	214[a]	8.35×10^{-6} [b]	18 60*

NL: Not listed in Regulation 10/2011.
*SML (T) (group restriction).
** Specific migration limit of 60 mg/kg (Substances for which no specific migration limit or other restrictions are provided in Annex I Regulation 10/2011).

the consumption data (g/day). The pooled samples were stored in glass bottles with screw caps and frozen at −30 °C until analysis.

2.3. Food consumption data

Food consumption data were obtained from a Spanish National dietary survey on children and adolescents (ENALIA) developed between November 2012 and July 2014 using food consumption data and other information about eating habits and physical activity on children and adolescents (6 months- 17 years old).

The ENALIA survey was carried out on a representative sample at national level including 1862 children and adolescents. Food consumption and related data were collected and four sub groups were established: Infants (from 6 to 11 months inclusive), toddlers (from 12 months to 35 months inclusive), children (from 3 to 9 years inclusive) and adolescents (from 10 years to 17 years inclusive) (ENALIA, 2014). Average daily consumption for selected cereal products of Spanish population is shown in Table 3.

In the ENALIA survey the dietary information was collected, firstly on the daily diet, that is, specifying the type of food and quantities consumed (g/day) during two no consecutive days and secondly on the frequency of consumption of foods and food supplements. In addition, information is collected on the general sociodemographic characteristics, the physical activity performed and the anthropometric measures of weight and height of the participants (Marcos et al., 2016). In the present work, we have focused on the consumption (g/day) of cereal products, in order to estimate the dietary exposure.

Considering the types of foods selected in this work, were considered three sub groups of population between 12 months and 17 years.

Table 3
Consumption data for cereal based foods of the Spanish population.

Consumption g/ day			
Food category	12–35 months	3–9 years	10–17 years
Cereals			
Rice	6.939	12.41	13.30
Toasted bread	0.178	0.337	0.269
Bread	4.856	16.914	18.70
Alternative to bread	1.015	0.754	0.865
Pasta	9.651	17.502	20.84
Breakfast cereals	9.231	1.937	1.777

2.4. Calculation of dietary intake

The exposure was calculated by combining the concentration of selected compounds in the packaged foods with the national consumption data. In the same way the exposure was derived for each group of population, namely toddlers (12 months to 35 months), children (3–9 years inclusive) and adolescents (10 years to 17 years).

2.5. Packaging materials (screening)

Extraction of packaging samples were performed in two solvents: ACN and hexane. A sample of the material of known surface area 0.8 dm^2 was immersed in 25 mL of ACN and maintained in an oven at 70 °C for 24 h. An aliquot of the extract (10 mL) was then removed and

Table 2
Details of the food packaging materials and the packaged food.

Code	Type of sample	Type of material		Packaging container	Thickness (μm)	Fat content
		Internal side	External side			
CR_CD_01_A	Breakfast cereal	PP	PP	Pouch	61	5.2 g/100g (Satur. 1.7g)
CR_AR_01_A	Rice	PP	PP	Pouch	58.5	0.8 g/100g (Satur. 0.2g)
CR_PM_01_A	Bread	PE	PET	Pouch	30	3 g/100g (Satur. 0.5g)
CR_PT_01_A	Toasted bread	PP	PP	Pouch	52.5	12 g/100g (Satur.5.6g; Monoinsatur.4.8g; Poliinsatur. 1.6g)
CR_PP_01_A	Alternative to bread (picos)	PP	PP	Pouch	41.5	1.3 g/100g (Satur. 0.2g)
CR_PA_01_A	Pasta based on durum wheat semolina	PP	PP	Pouch	52.5	1.5 g/100g(Satur. 0.3g)
CR_PA_02_A	Pasta with vegetables	PP	PP	Pouch	50.5	2 g/100g (Satur. 0.5g)

PP: Polypropylene.
PET: Polyethylene terephthalate.
PE:Polyethylene.

concentrated under nitrogen stream (RapidVap Vertex Evaporator, Labconco) to 1 mL. After that, the extract was filtered through a 0.45 μm PTFE membrane filter (Advanted; Toyo Roshi Kaisha, Japan) and analyzed by GC-MS. Duplicate extractions were done for each sample, and the two concentrated extracts obtained were analyzed independently.

The extraction in hexane was carried out following the same procedure as with ACN, but extraction was performed at 60 °C for 4 h. In all cases a reagent blank was analyzed prior to each sample, and background subtraction was employed.

Cramer rules were used to estimate the toxicity of identified compounds. An open source application called Toxtree v2.6.13 (Ideaconsult Ltd.) was used for this purpose (Toxtree v2.6.13, 2015). The software applies a decision tree in order to estimate toxic hazard of chemical compounds based mainly on the chemical structure of the molecule. Chemical compounds are divided into three structural classes according to the concern for their potential toxicity: class I (low toxicity) for substances of simple chemical structure that suggests a low order of oral toxicity, class II (intermediate toxicity) for substances that may suggest significant toxicity and class III (high toxicity) for substances with chemical structures that may suppose a significant toxicity or have reactive functional groups (Patlewicz et al., 2008).

2.6. Sample preparation-foodstuffs

Some compounds previously identified in packaging materials were selected for their analysis in the foodstuffs in order to assess their exposure. Selected compounds were phthalates (DEP, DEHP, DIBP and DBP), ATBC, DEHA, erucamide and octocrylene.

Before analysis, foodstuffs were first homogenized with a grinder and pooled into the three groups (12–35 months, 3–9 years and 10–17 years). The different composite samples (pools) were prepared by combining the corresponding amounts of each foodstuff, according to the national consumption data for each group (see Table 3). Pools were homogenized and 1g of pooled sample was weighed into a glass centrifuge tube. Each pooled sample was extracted with ACN (10 mL). After vortex agitation for 2 min the samples were extracted in ultrasonic bath for 15 min. The mixture was centrifuged (10 min, −5 °C at 3500 rpm) and the supernatant were freeze for 30 min at 4 °C in the refrigerator. The procedure was repeated twice more, and the three sequential extracts together were concentrated in a rotary evaporator at 50 °C until aprox. 0.5 mL, 50 μL of internal standard (10 μg/mL) was added and finally it was made up with methanol to a volume of 1 mL in a volumetric flask. Following the addition of internal standard, samples were filtered through a 0.45 μm PTFE membrane filter and 0.22 μm PTFE membrane filter (Advanted; Toyo Roshi Kaisha, Japan) and analyzed by LC-MS/MS. Duplicate samples were prepared in this way.

To perform recovery tests, the pooled sample assigned to the 12–35 months group was selected, since this contained a more representative quantity of each of the cereal products included in this study. The recovery of the method was evaluated by spiking the pooled food samples, at three different concentrations (0.25; 0.5 and 1 μg/g). The spiked samples were extracted in the same way as mentioned before.

Procedure blanks samples (in the absence of the foodstuff) were processed, along with the samples, following the same procedure.

2.7. Instrumental analysis

2.7.1. GC-MS analysis -packaging materials (screening)

A Thermo Scientific Trace 1300 Series Gas Chromatograph (Thermo Fisher Scientific, San José, CA, USA) with a Trace ISQ LT mass detector and an AI 1310 automatic injector was used to perform GC analysis. GC column ZB-5MS (30 m × 0.25 mm x 0.25 μm) from Phenomenex® (Torrance, CA, USA) was used. Helium was used as carrier gas, at a flow rate of 1 mL/min. The injector temperature was 300 °C. Samples were injected in splitless mode. The injection volume was 1.0 μL. The oven temperature profile used was 40 °C for 2 min, then 300 °C at 9 °C/min, with a holding time of 3 min for samples extracted with ACN, and 40 °C for 2 min, then 300 °C at 9 °C/min, with a holding time of 10 min for samples extracted with hexane.

The mass spectra were obtained with a mass selective detector under electron impact ionization at a voltage of 70 eV. The MS was operated in full scan mode over m/z range of 35–500. Xcalibur 3.0.63 software (Thermo Fisher Scientific Inc) was used to process chromatograms. The identification was carried out by using the NIST/EPA/NIH 11 Mass spectral library (version 2.0) and Wiley RegistryTM 8th edition.

2.7.2. LC-MS/MS analysis

The HPLC-MS/MS system consisted of an Accela autosampler, an Accela 1250 pump with a degasser, a column thermostatting system and a PDA detector, coupled to a triple quadrupole mass spectrometer TSQ Quantum Access max, controlled by Xcalibur software (Thermo Fisher Scientific, San José, CA, USA).

The chromatographic separation was performed using a reversed-phase column Kinetex biphenyl (100 × 3 mm x 2.6 μm) (Phenomenex®, Torrance, CA, USA), thermostatted at 30 °C, with a mobile phase composed by methanol and water, both containing 0.1% (v/v) formic acid. A gradient elution method was applied, during the first minute the mobile phase was consisted of 30% of water and 70% of methanol, then the concentration of methanol gradually increased reaching 100% at minute 10, and this composition was held constant until minute 15 (in order to allow the elution of the most retained compounds). The flow rate was 0.4 mL/min, and the injection volume was 10 μL. The total run time was 15 min.

The mass spectrometer was operated in positive ESI mode. Nitrogen was used as the sheath gas at a pressure of 35 psi, and as auxiliary gas (pressure 10 arbitrary units) and argon was used as the collision gas at a pressure of 1.0 mTorr. The spray voltage was 3000 V. The vaporizer and capillary temperatures were 340 and 350 °C, respectively. Other conditions are given in Table 4.

Quantification was performed using calibration curves based on different calibration levels and the response areas. The response area was calculated as the ratio of each compound area and the area of the internal standard. Xcalibur 2.1.0 software (Thermo Fisher Scientific Inc) was used to process chromatograms.

Table 4
MS/MS conditions for the selected compounds, retention times and typical ion ratio.

Compound	Retention time (min)	Precursor ion	Product ions *	Collision energy (V)
DEP	3.31	223.1	149.1	17
			177.1	5
DIBP	6.15	279.2	149.0	20
			121.1	32
DBP	6.59	279.2	149.0	19
			205.1	5
BBP	7.37	313.1	91.1	26
			149.0	20
ATBC	7.71	403.2	129.0	24
			139.0	24
Erucamide	9.33	338.4	321.4	10
			97.3	19
Octocrylene	9.66	362.2	232	20
			250	10
DEHA	9.90	371.3	129.0	11
			111.1	20
DEHP	10.22	391.3	149.0	24
			167.0	10
DEPd	3.25	227.2	153.1	17
			181.2	5

*The first listed product ion is used for quantification and the second for qualification purposes.

Calibration curves were prepared by diluting the stocks solutions with methanol, to have concentrations ranging from 0.0025 to 2 μg/mL for BBP, 0.005–2 μg/mL for ATBC, 0.025–2 μg/mL for DEP, 0.01–2 μg/mL for DEHA and 0.1–2 μg/mL for DIBP, DBP, DEHP, erucamide and octocrylene, in all cases using a constant concentration of 0.5 μg/mL of the internal standard. Each point of the calibration curve is the average of two peak-area measurements.

2.7.3. FT-IR

Infrared spectra were acquired using an ATR-FTIR spectrometer (FT-IR 4700, Jasco, Japan) equipped with a diamond optical element, in the range from 4000–650 cm^{-1}. ATR-FTIR spectrometer was controlled by the software Spectra Manager™ Suite. The identification was carried out by using the Sigma Aldrich libraries.

2.8. Thickness

The film thickness for each packaging material was measured with a manual digital micrometer (Mitutoyo-Japan) and mean values were estimated from three measurements.

2.9. Control of blank concentrations for phthalates analysis

Several precautions were taken in order to minimize the blank problems associated with the omnipresence of phthalates in the environment. All plastic material was avoided during the procedure including handling samples and making dilutions. Glass material were used instead of plastic materials, which was carefully cleaned and thermally treated at 400 °C for 4 h, then was covered with aluminium foil and stored in a clean environment until its use. In the same way all solvents used in the procedure were tested for background levels of phthalates.

3. Results and discussion

3.1. Screening of packaging materials

In this study, for screening purposes a simple extraction method was used to leach out chemicals from the plastic packaging materials. Two solvents, ACN and hexane, were used to assure the screening of a wide polarity range of compounds. Despite the different polarity range among the additives, significant differences between both solvents were not found.

A variety of compounds including plasticizers (phthalates, adipates, citrates, sebacates), slip additives, phenolic compounds, acids, carboxylic acids, aldehydes, alkanes among others were detected in the plastic materials extracts. More than 50 different extractable compounds were detected by GC-MS. Identification of compounds was performed comparing the mass spectra of the peak detected in the samples with that of the NIST/EPA/NIH 11 Mass spectral library (version 2.0) and Wiley RegistryTM 8th edition. Only the compounds with the best coincidences found during the library search are listed in Table 5. Compounds with matching factors SI (direct matching factor for the unknown and the library spectrum) and RSI (reverse search matching factor ignoring any peaks in the unknown that are not in the library spectrum) in a value from 800 to 1000 were selected (900 or greater is considered an excellent match; 800–900, a good match; and 700–800, a fair match). In order to confirm the identity of some compounds, the injection of the respective standards was performed. The remaining detected peaks that could not be finally confirmed were considered as tentatively identified.

Several types of plasticizers were found in this screening, including phthalates, citrates and adipates. The main groups of plasticizers found were phthalates and citrates. Five different phthalates were detected, including DEP, DIBP, DEHP, BBP and DBP. DEP, DEHP and DIBP were found in all samples while BBP and DBP in 4 samples. Phthalates are mainly used for PVC applications. Exposure to phthalates is related with several diseases and due to their endocrine disrupting properties, some phthalates are recognized as substances of very high concern (Heudorf et al., 2007).

ATBC was found in all studied samples. It was extracted with both solvents. It is the most widely used phthalate substitute plasticizer for vinyl applications and other resins. It is also employed as a flavor ingredient in non-alcoholic beverages (Ash, 2004). Other citrates that were found are triethyl citrate and tributyl citrate. Both compounds were found in one sample (CR-PA-01) and these substances are classified as high toxicity (class III), according Cramer rules. Triethyl citrate is used as plasticizer in natural resins, in food contact coatings and for printings inks. Tributyl citrate as well as triethyl citrate is used as plasticizer in inks, adhesives and coatings (Ash, 2004).

DEHA was found in samples CR-AR-01, CR-CD-01 and CR-PA-01. A better extraction of this compound was observed in ACN. DEHA is used mainly as plasticizer for synthetic resins such as PVC used to wrap foods. A TDI (tolerable daily intake) of 0.3 mg/kg bw has been established for this compound (EFSA, 2005f). Other adipates that were found are mono (2-ethylhexyl) adipate and bis (2-butoxyethyl) adipate. Additionally, other plasticizers detected were tributyl aconitate and dibutyl sebacate, the first was identified in six samples (CR-AR-01, CR-CD-01, CR-PA-01, CR-PM-01, CR-PP-01, CR-PT-01) and the second one in one sample (CR-PM-01), respectively.

The phenolic antioxidant butylated hydroxytoluene (BHT) was detected in only one sample. This compound is used as antioxidant to prevent the aging process of plastic materials (Ash, 2004). Other compounds such as 2,4-di-tert-butylphenol and 7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione are degradation products of commercial antioxidants used in plastic polymers. The latter compound, 7,9 di-tert-butyl-1-oxaspiro(4,5)deca-6-9-diene-2,8-dione, is a byproduct of the antioxidant Irganox 1010®, and it was one of the most predominant compounds found in all samples (CR-AR-01, CR-CD-01, CR-PA-01, CR-PA-02, CR-PM-01, CR-PP-01, CR-PT-01). This compound is classified in class III according to Cramer rules (Lago and Ackerman, 2016; Félix et al., 2012). The alkyl phenol 2,4-di-tert-butylphenol was found in three samples (CR-AR-01, CR-CD-01, CR-PM-01). This compound is a degradation product of the antioxidant Irgafos 168® (Dupáková et al., 2010). Irgafos 168® may degrade and generate non intentionally added substances (NIAS) during polymer processing, one of these NIAS is 2,4-di-tert-butylphenol (Yang et al., 2016).

Slip agents such as erucamide and hexadecanamide were found in several samples, and were extracted with both solvents. Erucamide was found in all samples (CR-AR-01, CR-CD-01, CR-PA-01, CR-PA-02, CR-PM-01, CR-PP-01, CR-PT-01) while hexadecanamide was only detected in two samples (CR-AR-01, CR-PM-01). Slip additives are internal lubricants that reduce the surface friction of polymers and create a better processability of polymers (Wypych, 2005).

Moreover, fatty acids such as octadecanoic acid, palmitic acid and 2-monostearin were also found in some samples. Specifically, octadecanoic acid was identified in five samples (CR-CD-01, CR-PA-01, CR-PA-02, CR-PM-01, CR-PP-01) whereas palmitic acid was detected in all samples analyzed (CR-AR-01, CR-CD-01, CR-PA-01, CR-PA-02, CR-PM-01, CR-PP-01, CR-PT-01) and 2-monostearin was only found in two samples (CR-PP-01, CR-PT-01). These compounds can serve as lubricants in plastic materials. In the case of octadecanoic acid, it is used in plastic applications in combination with other slip and antiblocking additives (Wypych, 2005).

Octocrylene, used as an ultraviolet light absorber for plastics and paints, was found in four samples (CR-AR-01, CR-CD-01, CR-PM-01, CR-PP-01).

Other compounds detected were alkanes and aldehydes (nonanal, octadecanal, hexadecanal). Alkanes, besides their use as solvents, can also be oligomers originating from polyolefines. Information on the compounds found in the screening of the packaging samples are listed in Table 5. It is interesting to note that only 14 out of the 45 compounds

Table 5
Compounds identified in plastic packaging materials with the level of toxicity (TC) according to Cramer rules.

Compound	IUPAC Name	CAS	RT (min)	Uses	TC	SML	CR-AR-01		CR-CD-01		CR-PA-01		CR-PA-02		CR-PM-01		CR-PP-01		CR-PT-01	
							ACN	HEX	ACN	HEX	ACN	HEX	ACN	HEX	ACN	HEX	ACN	HEX	ACN	HEX
Nonanal	Nonanal	124-19-6	9.97	Odorous compound from adhesives	I	NL									X	X				
Nonanoic acid	Nonanoic acid	112-05-0	12.66	Used in lacquers, plastics and as vinyl plasticizer	I	NL									X	X				
*Butylated Hydroxytoluene	2,6-ditert-butyl-4-methylphenol	128-37-0	16.20	Antioxidant for plastics in contact with food; stabilizer in hot-melt adhesives and coatings for food packaging	II	3	X	X												
2,4-di-tert-butylphenol	2,4-ditert-butylphenol)-	96-76-4	16.23	Degradation product.	I	NL	X	X	X	X					X	X				
Mono(2-ethylhexyl) adipate	6-(2-ethylhexoxy)-6-oxohexanoic acid	4337-65-9	16.79	Hydrolysed product of DEHA	I	NL	X	X	X	X										
*Diethyl Phthalate	Diethyl benzene-1,2-dicarboxylate	84-66-2	17.33	Solvent to hold color	I	NL	X	X	X	X	X	X	X	X	X	X	X	X	X	X
*Hexadecane	Hexadecane	544-76-3	17.46	Compound identified in adhesive	I	NL									X	X				
Decyl decanoate	decyl decanoate	1654-86-0	18.00	Ink component	I	NL			X	X							X	X		
Bis(2-butoxyethyl) adipate	Bis(2-butoxyethyl) hexanedioate	141-18-4	18.00	Plasticizer	I	NL					X	X	X	X						
*Octadecane	Octadecane	493-45-3	19.95	Compound identified in adhesive		NL									X	X				
Hexadecanal	Hexadecanal	629-80-1	20.17	Aldehyde	I	NL													X	X
Isopropyl myristate	Propan-2-yl tetradecanoate	110-27-0	20.22	Plasticizer for cellulosic and Pigment dispersant.	I	NL													X	X
Triethyl citrate	Triethyl 2-hydroxypropane-1,2,3-tricarboxylate	77-93-0	20.37	Plasticizer for cellulose acetate, cellulose acetate butyrate, cellulose nitrate, chlorinated rubber, ethyl cellulose etc. Solvent for inks, adhesives, coatings.	III	60					X	X								
*Diisobutyl phthalate	Bis(2-methylpropyl) benzene-1,2-dicarboxylate	84-69-5	20.66	Plasticizer for cellulose nitrate.	I	NL	X	X	X	X	X	X	X	X	X	X	X	X	X	X
7,9 Di-Tert-Butyl-1-Oxaspiro (4,5)Deca-6-9-Diene-2,8-Dione	7,9-ditert-butyl-1-oxaspiro [4.5]deca-6,9-diene-2,8-dione	82304-66-3	21.19	Antioxidant degradant	III	NL	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Methyl palmitate	Methyl hexadecanoate	112-39-0	21.38	Intermediate for detergents, emulsifiers stabilizers, resins, plasticizers	I	NL		X												
*Dibutyl phthalate	Dibutyl benzene-1,2-dicarboxylate	84-74-2	21.74	Plasticizer for rubber and vinil materials, solvent for nitrocellulose lacquers	I	0.3	X	X							X	X	X	X	X	X
Palmitic acid	Hexadecanoic acid	57-10-3	21.79	Slip agent degradant. Food grade additive.	I	**	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Ethyl palmitate	Ethyl hexadecanoate	628-97-7	22.12	Lubricant	I	NL			X	X										
*Eicosane	Eicosane	112-95-8	22.20	Alkane	I	NL									X	X	X	X		
Octadecanal	Octadecanal	638-66-4	22.40	Aldehyde	I	NL	X	X			X	X	X	X					X	X
Isopropyl palmitate	Propan-2-yl hexadecanoate	142-91-6	22.43	–	I	NL	X	X							X	X	X	X		
*Heneicosane	Heneicosane	629-94-7	23.30	Alkane	I	NL	X	X											X	X
Tributyl citrate	Tributyl 2-hydroxypropane-1,2,3-tricarboxylate	77-94-1	23.22	Plasticizer for food contact materials. Used in adhesives, coatings, inks.	III	NL					X	X								
Tributyl aconitate	Tributyl (1E)-1-propene-1,2,3-tricarboxylate	7568-58-3	23.81	Plasticizer	I	NL	X	X	X	X	X	X			X	X	X	X	X	X
Octadecanoic acid	Octadecanoic acid	57-11-4	23.89	Internal lubricant for use in plastics, lubricant, stabilizer.	I	**			X	X	X	X	X	X	X	X	X	X		
Ethyl oleate	Ethyl (Z)-octadec-9-enoate	111-62-6	23.92	Solvent, plasticizer, lubricant, water-resisting agent, flavoring.	I	NL			X	X										
Dibutyl Sebacate	Dibutyl decanedioate	109-43-3	23.94	Plasticizer for food contact materials.	I	60									X	X				

(*continued on next page*)

Table 5 (*continued*)

Compound	IUPAC Name	CAS	RT (min)	Uses	TC	SML	CR-AR-01		CR-CD-01		CR-PA-01		CR-PA-02		CR-PM-01		CR-PP-01		CR-PT-01	
							ACN	HEX	ACN	HEX	ACN	HEX	ACN	HEX	ACN	HEX	ACN	HEX	ACN	HEX
Hexadecanamide	Hexadecanamide	629-54-9	24.08	Slip agent	III	NL	X	X							X	X				
*Acetyl tributyl citrate	Tributyl 2-acetyloxypropane-1,2,3-tricarboxylate	77-90-7	24.75	Plasticizer for food contact materials and Plasticizer for inks, adhesives and coatings	I	60	X	X	X	X	X	X	X	X	X	X	X	X	X	X
*Tricosane	Tricosane	638-67-5	25.23	Compound identified in adhesive	I	NL			X	X	X	X					X	X		
*Benzyl butyl phthalate	2-O-benzyl 1-O-butyl benzene-1,2-dicarboxylate	85-68-7	25.67	Component in printing inks and adhesives	I	30 60				X		X						X		X
*Bis(2-ethylhexyl) adipate	Bis(2-ethylhexyl) hexanedioate	103-23-1	26.04	Plasticizer in food contact polymers mainly for PVC, rubber	I	18 60	X	X	X	X		X								
Octadecanamide	Octadecanamide	124-26-5	26.04	Slip agent	III	**	X	X							X	X				
*Tetracosane	Tetracosane	646-31-1	26.15	Compound identified in adhesive	I	NL			X	X					X	X				
cis-13-Eicosenoic acid		17735-94-3	26.99	Migrant from printing inks	I	NL	X	X	X	X										
Pentacosane	Pentacosane	629-99-2	27.09	Compound identified in adhesive		NL					X	X	X							
2-Palmitoylglycerol	1,3-dihydroxypropan-2-yl hexadecanoate	23470-00-0	27.14	.	I	NL					X	X					X	X	X	X
*Bis(2-ethylhexyl) phthalate	Bis(2-ethylhexyl) benzene-1,2-dicarboxylate	117-81-7	27.32	Plasticizer used in the production of polyvinyl chloride (PVC)	I	1.5	X	X	X	X	X	X	X	X	X	X	X	X	X	X
cis-11-Eicosenamide	(Z)-icos-11-enamide	10436-08-5	27.65	Used in adhesives and components of coatings	III	**	X	X	X	X	X	X	X	X	X	X				
*Octocrylene	2-ethylhexyl 2-cyano-3,3-diphenylprop-2-enoate	6197-30-4	28.34	UV-B absorver	III	0.05	X	X	X	X					X	X	X	X		
2-Monostearin	1,3-dihydroxypropan-2-yl octadecanoate	621-61-4	28.90	Lubricant	I	NL											X	X	X	X
*13-Docosenamide	(Z)-docos-13-enamide	112-84-5	29.40	Slip release agent	III	**	X	X	X	X	X	X	X	X	X	X	X	X	X	X
*Squalene	2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene	111-02-4	29.63	Used as oxygen-scavenging	I	NL			X	X			X	X	X	X	X	X		
Glycerol tricaprylate	2,3-di(octanoyloxy)propyl octanoate	538-23-8	30.57	Lubricant	I	NL			X	X										

*Confirmed with standards.

TC: Cramer Toxicity.

NL: Not listed in Regulation 10/2011.

** Specific migration limit of 60 mg/kg (Substances for which no specific migration limit or other restrictions are provided in Annex I Regulation 10/2011).

Table 6
Linear regression of selected compounds and their limits of detection and quantification.

Compound	Equation	R^2	LOD μg/g	LOQ μg/g	Range μg/mL
DEP	y = 1.448x + 0.0279	0.9997	0.01	0.025	0.025–2
DIBP	y = 3.0639x + 0.0473	0.9995	0.025	0.1	0.1–2
DBP	y = 3.831x + 0.0648	0.9999	0.05	0.1	0.1–2
BBP	y = 4.1714x + 0.0551	0.9995	0.001	0.0025	0.0025–2
ATBC	y = 9.3931x-0.0094	0.9995	0.0025	0.005	0.005–2
Erucamide	y = 6.7967x + 0.268	0.9990	0.05	0.1	0.1–2
Octocrylene	y = 3.2159x + 0.3581	0.9993	0.05	0.1	0.1–2
DEHA	y = 12.335x + 0.209	0.9994	0.005	0.01	0.01–2
DEHP	y = 8.8964x + 0.4014	0.9995	0.05	0.1	0.1–2

listed in Table 5 are authorized substances for the manufacture of plastic materials and articles that are intended to come into contact with foodstuffs (Commission Regulation (EU) No 10/2011).

3.2. Optimization of extraction procedure and development of the LC-MS/MS method

In order to evaluate the extraction efficiency of the target compounds, recovery assays were performed by spiking samples. Two different extraction solvents, ACN and hexane, were tested and better recoveries for all compounds were obtained with ACN; therefore, it was chosen as the extraction solvent for the following studies.

Different extraction times (15, 30 and 60 min) in an ultrasonic bath were tested. The results revealed similar extraction efficiencies at the three tested times. Taking into account that larger extraction times involve the heating of the organic solvent as well as losses by evaporation, 15 min extraction time was selected. This extraction method combined with a vortex agitation for 2 min improved the extraction efficiency.

It was observed that during the solvent concentration step, after the extraction, the evaporation of the solvent to dryness was associated with irreproducible results and loss of some analytes. Hence, evaporation was stopped when the solvent volume reached approximately 0.5 mL. No further clean-up was made in order to prevent losses of compounds.

During the development of the LC chromatographic method four columns were tested: Luna C18 (150 × 3 mm x 5 μm), Phenosphere ODS (2) (150 mm × 3.20 mm x 3 μm), Kinetex C18 (100 × 3 mm x 2.6 μm) and Kinetex biphenyl (100 × 3 mm x 2.6 μm). With C18 columns an acceptable separation of all compounds was obtained except in the case of phthalates DBP and DIBP, which were not completely separated. The Kinetex biphenyl (100 × 3 mm x 2.6 μm) column was finally chosen because of a better separation efficiency for all compounds, including DBP and DIBP, as well as for its great sensitivity. The peak shape and peak height, were also considered for the selection of this column.

Two mobile phases consisting of acetonitrile or methanol containing 0.1% (v/v) formic acid, and 0.1% (v/v) formic acid in water were assayed. Methanol showed more efficiency in separating all compounds, also more symmetrical peaks were obtained. Thus the mobile phase containing methanol as the organic phase was selected. Then, different gradient profiles were tested and finally a gradient starting from water–methanol (30:70 v/v) to 100% methanol in 10 min was selected as the best compromise between analysis time, suitable separation and resolution of the target compounds. Several flow rates 0.4, 0.5 and 0.6 mL/min were also assayed, and the best separation was achieved at 0.4 mL/min.

Regarding MS operating conditions, the ESI interface in the positive SIM mode was chosen for the identification and quantification, because of its better suitability for the selected compounds.

To evaluate the MS-generated precursor ion and product ions for each compound, individual methanolic standard solutions of each compound was analyzed by direct infusion by use of a built-in syringe pump. Full scan data acquisition was performed to 100–700 m/z. In all cases, the more intensive precursor ion corresponded to the protonated molecular ion $[M+H]+$. For each compound, one product ion was selected for quantification purposes and other for confirmation. The ratio between the two selected transitions selected was used for the verification of the identity of the analytes in the samples.

Selected multiple reaction monitoring transitions (MRM), retention times and adjusted voltage settings are shown in Table 4.

3.3. Method validation

Nine compounds previously identified in the packaging materials were selected for the dietary exposure assessment, including phthalates (DEP, DEHP, BBP, DIBP, and DBP), ATBC, DEHA, erucamide and octocrylene.

Criteria used for the selection of these compounds included toxicological properties and human health hazards such as is the case of phthalates and DEHA. Other compounds were selected because they are among the most abundant compounds found in almost all packaging samples. The availability an appropriate analytical method for their analysis was also taken into account in planning the study.

The method was validated in terms of linearity, recovery, reproducibility, limits of quantification (LOQ) and detection (LOD). The linearity of the method was tested by using a series of standards solutions of known concentration. The compounds were quantified based on the ratio of the analyte response and that of the internal standard. Calibration curves were obtained for each compound by linear regression plotting the peak area ratio against the injected concentration. All studied compounds showed good linearity in the studied concentration range with coefficients of determination (R2) equal or greater than 0.9990 in all cases. Table 6 shows the linear equation, the R2 values and the concentration ranges for all compounds.

Detection and quantification limits, (defined as three and ten times the height signal of the noise level, respectively) were calculated in accordance with American Chemical Society (ACS, 1980) and are shown in Table 6. The proposed method exhibited an excellent sensitivity with LODs equal or lower than 0.05 μg/g.

Precision and recovery of the method was determined by running spiking experiments on food samples. For that purpose, six replicate analyses of pooled food samples spiked at levels of 0.25, 0.5 and 1 μg/g were performed. In the case of ATBC recovery experiments were performed at two levels 0.5 and 1 μg/g. Each sample used for the recovery experiments was also analyzed without addition of the selected compounds in order to correct the recoveries for the sample concentration. Recoveries varied between 78% and 109% (Table 7). The repeatability values for the different compounds, expressed as the relative standard deviation (RSD), are listed in Table 7 and ranged between 4 and 18%, except in the case of octocyrylene, where higher values up to 25% were obtained.

The precision values obtained in this study are in concordance with other studies with RSD values lower than 20% for phthalates and DEHA

Table 7
Recovery and Repeatability of the method in food samples.

Pool 12–35 months						
Compound	Recovery (%)			Repeatability (RSD %)		
	0.25 μg/g	0.5 μg/g	1 μg/g	0.25 μg/g	0.5 μg/g	1 μg/g
DEP	94.7	104	104	13.5	13.5	8.91
DIBP	91.3	109	98.5	13.6	7.80	17.8
DBP	90.0	93.5	90.3	7.97	9.43	14.2
BBP	81.1	106	103	4.18	7.09	10.8
ATBC	–	107	108	–	10.7	12.1
Erucamide	78.6	85.4	78.7	7.78	15.6	6.07
Octocrylene	77.5	95.4	80.6	5.75	21.8	24.7
DEHA	80.7	100	105	10.5	14.4	13.6
DEHP	97.8	91.1	87.3	16.3	13.6	8.38

(Bradley et al., 2013; Cao et al., 2015; Fierens et al., 2012; Xu et al., 2010); or in the case of ATBC, lower than 15% (Lambertini et al., 2016).

3.4. Food concentration of selected packaging chemicals

The contaminants considered for the dietary exposure assessment were identified previously in several packaging samples (Table 5). Among the 9 target chemicals measured, only five of them were detected in the pooled food samples, DEP, DIBP, DEHP, ATBC and DEHA while DBP, BBP, erucamide and octocrylene were not detected. The results obtained are shown in Table 8.

Each analytical sequence was composed of at least 2 procedural blanks at the beginning and the final of the sequence, several solvent blanks, calibration standards and the extracted food samples. The purpose of the blank analysis was to verify the absence of any procedural contamination that could lead to quantification errors.

DEP was detected at concentrations between 0.0608 and 0.118 μg/g, being the highest concentration in the pooled sample assigned to the 12–35 months group. DIBP was detected but at levels below the LOQ (0.1 μg/g). DEHP concentrations ranged from 0.102 to 0.265 μg/g. The highest concentration was found in the pooled sample corresponding to the 10–17 years group.

Bradley et al. (2013) developed a GC-MS method for the analysis of phthalates in 20 food groups, including cereal products. In this last group they identified DEP, DIBP, DBP and DEHP at concentrations of 13 μg/kg, 81 μg/kg, 14 μg/kg and 104 μg/kg, respectively, with DEHP being the compound found at higher concentrations. Our findings for DEP in cereal based products were higher than those reported in that study; in the case of DEHP, depending on the food group category, these are similar (12–35 months) or either higher (3–17 years). In another published survey the concentration of phthalates found in cereal and cereal products varied between ND to 558 μg/kg for DEP, ND to 1054.0 μg/kg for DIBP and ND to 1073.0 μg/kg for DEHP. Other phthalates such as dimethyl phthalate (DMP), DBP, BBP, dicyclohexyl phthalate (DCHP) and di-n-octyl-phthalate (DnOP) were also found in concentrations up to 61μg/kg (Fierens et al., 2012).

Table 8
Concentration of selected compounds in the cereal based pooled samples assigned to the different consumer groups.

Compound	μg/g		
	12–35 months	3–9 years	10–17 years
DEP	0.118	0.0839	0.0608
DIBP	< LOQ	< LOQ	< LOQ
DBP	nd	nd	nd
BBP	nd	nd	nd
ATBC	0.262	0.508	0.432
Erucamide	nd	nd	nd
Octocrylene	nd	nd	nd
DEHA	0.0270	< LOQ	< LOQ
DEHP	0.102	0.196	0.265

Regarding other plasticizers, ATBC was found at levels ranging from 0.262 μg/g (12–35 months group) to 0.508 μg/g (3–9 years group). DEHA was only measured at a concentration of 0.0270 μg/g, which corresponds to the group of 12–35 months. In general, ATBC was the compound found at higher concentrations in the three pooled samples. These migration values, in cereals with low fat content, are considerably lower than those reported in other studies in fatty food, with DEHA migration values varying from 133.9 to 345.5 mg/kg in three different types of cheese and ATBC values of 2–8 mg/kg in cheese too (Castle et al., 1988; Goulas et al., 2000).

3.5. Dietary exposure to selected packaging chemicals

Estimated dietary exposure values to the selected migrants, in the cereal-based foods analyzed are presented in Table 9. Mean dietary exposure to DEP varied from 0.179 μg/kg bw/day (pool 10–17 years) to 0.458 μg/kg bw/day (pool 12–35 months), DIBP from 0.0864 μg/kg bw/day (pool 12–35 months) to 0.262 μg/kg bw/day (pool 3–9 years) and DEHP from 0.395 μg/kg bw/day (pool 12–35 months) to 0.782 μg/kg bw/day (pool 10–17 years) (Table 9).

The mean exposure value for DEP in the pooled samples for the 12–35 months and 3–9 years were more than twice the exposure of the 10–17 years group.

95th percentile exposure to DEP varied from 0.588 μg/kg bw/day (pool 10–17 years) to 2.38 μg/kg bw/day (pool 12–35 months), while the 50th percentile varied from 0.0996 μg/kg bw/day (pool 12–35 months) to 0.184 μg/kg bw/day (pool 3–9 years).

The mean exposure to DIBP was similar for the 12–35 months group and 10–17 years group, and it was higher for the 3–9 year group.

95th percentile exposure to DIBP varied from 0.296 μg/kg bw/day (pool 10–17 years) to 0.943 μg/kg bw/day (pool 3–9 years), while the 50th percentile varied from 0.0187 μg/kg bw/day (pool 12–35 months) to 0.262 μg/kg bw/day (pool 3–9 years).

In the case of DEHP mean dietary exposure for the group 3–17 years was more than twice that of children from 12 to 35 months. 95th percentil exposure to DEHP varied from 2.06 μg/kg bw/day (pool 12–35 months) to 2.79 μg/kg bw/day (pool 3–9 years). The percentile 50th varied from 0.0860 μg/kg bw/day (pool 12–35 months) to 0.489 μg/kg bw/day (pool 10–17 years). In all cases, the values estimated were lower than the TDI of 0.05 mg/kg bw established by EFSA for this chemical.

Regarding ATBC, mean dietary exposure varied from 1.01 μg/kg bw/day (pool 12–35 months) to 2.01 μg/kg bw/day (pool 3–9 years) and DEHA from 0.0100 μg/kg bw/day (pool 10–17 years) to 0.104 μg/kg bw/day (pool 12–35 months). Mean dietary exposure values to ATBC were higher for the infants and the 3–9 years group, in comparison with the other two consumer groups. In general, exposure to ATBC was higher than that to phthalates and DEHA for the three groups considered. 95th percentil exposure to ATBC varied from 4.18 μg/kg bw/day (pool 10–17 years) to 7.23μg/kg bw/day (pool 3–9 years), while the percentile 50th varied from 0.220 μg/kg bw/day (pool 12–35 months) to 1.11 μg/kg bw/day (pool 3–9 years).

In the case of DEHA, significantly higher exposure was detected for the population from 12 up to 35 months. In all cases mean exposure values were below the TDI of 0.3 mg/kg bw established for this compound. The 95th percentil exposure to DEHA varied from 0.0330 μg/kg bw/day (pool 10–17 years) to 0.543 μg/kg bw/day (pool 12–35 months). In the case of percentile 50th varied from 0.00628 μg/kg bw/day (pool 10–17 years) to 0.0227 μg/kg bw/day (pool 12–35 months).

In this work several contaminants were detected in the same composite samples (pools); therefore, a simultaneous exposure to different substances cannot be excluded. When an exposure to a wide variety of chemicals is present, the known “cocktail effect” has to be considered.

Table 9
Estimated dietary exposure (through cereal based foods comsumption) to the selected packaging chemicals of Spanish population (μg/kg bw per day).

	Dietary exposure μg/kg bw per day								
	12–35 months			3–9 years			10–17 years		
	Mean	P50	P95	Mean	P50	P95	Mean	P50	P95
DEP	0.458	0.0996	2.38	0.332	0.184	1.19	0.179	0.112	0.588
DIBP	0.0864	0.0187	0.449	0.262	0.145	0.943	0.0902	0.0564	0.296
ATBC	1.01	0.220	5.27	2.01	1.11	7.23	1.27	0.795	4.18
DEHA	0.104	0.0227	0.543	0.0204	0.0113	0.0733	0.0100	0.00628	0.0330
DEHP	0.395	0.0860	2.06	0.779	0.431	2.79	0.782	0.489	2.57

The combination of various chemicals can produce adverse reactions in humans, even at low levels of the individual substances. Potential effects of multiple exposure, is another important factor that should be considered in total diet studies.

Several studies in the literature have reported on the assessment of exposure to migrants from food contact materials. Thus, in a survey run in 2012, mean dietary exposure to DEHP for the Chinese population was estimated at 2.34 μg/kg bw/day for the general population, 4.51 μg/kg bw/day for children aged 2–6 years, 3.41 μg/kg bw/day for children aged 7–12 years, 2.46 μg/kg bw/day for adolescents aged 13–17 years and 2.03 μg/kg bw/day for adults. Results of this study showed that in children and adults cereals were the most important food category contributing to DEHP dietary intake (Sui et al., 2014). Exposure values found in this study are higher than those found for DEHP in the present work.

In another study, phthalate exposure through food in the adult Swiss-German population was estimated with exposure values of 1.90 μg/kg bw/day for DEHP, 0.39 μg/kg bw/day for DBP, 0.14 μg/kg bw/day for BBP and 0.02 μg/kg bw/day for DEP. The food categories contributing most to the dietary exposure of DEHP in this study were fatty, sweet, ready meal and health supplements. Regarding DEP, all clusters showed low exposure. According to their findings, daily exposure of consumers to phthalates through food did not exceed the TDIs (Dickson-Spillmann et al., 2009). The mean exposure values in our study are below the range reported for DEHP, and higher than those reported for DEP. However, in the previously mentioned study, other food groups were considered and only the adult population was taken into account.

Schecter et al. (2013) investigated dietary exposure to phthalates from food purchased in New York. The total estimated intake for children and adults based on mean concentrations was 0.673 μg/kg/day for DEHP, 0.033 μg/kg/day for DEP and 0.020 μg/kg/day for DIBP. Our findings for DEHP mean dietary exposure were comparable with their values, especially for the 3–17 years group. However, the estimated exposure in our work for DEP and DIBP is higher than those reported in the study by Schecter et al. (2013).

Fierens et al. (2014) estimated the dietary exposure to four phthalates including DEP: 0.039 μg/kg/day and DEHP: 1.45 μg/kg/day of the Belgian adult population, using a semi-probabilistic modeling approach. For DEP, grains and grain-based products (39.5%) contributed the most to the total dietary DEP intake, and for DEHP meat and meat products (18.7%) were the main contributors to the exposure. Predicted exposure values in this study were higher for DEHP and lower for DEP than our estimations for the Spanish population through cereal based foods.

Cao et al. (2013) developed a GC-MS method for the simultaneous analysis of DEHA and 20 phthalates in food samples selected from the 2013 Canadian Total Diet Study. DEHA and other five phthalates including DEHP and DIBP were detected in the cereal products. Reported levels of DEHA were from 5.04 ng/g to 1300 ng/g, for DEHP ranged from 18.8 to 153 ng/g and for DIBP from 2.89 to 15.0 ng/g. Reported DEHP values are within the range of our estimations; for DEHA the higher exposure value we found was below the level found by Cao et al. (2013).

In the case of ATBC our results were comparable with those obtained by using the FACET exposure tool (Facet 3.0.2, 2008–2012 Creme Software Ltd.). This software was developed within the European Commission 7th Framework project (Flavourings, Additives and Food Contact Materials Exposure Task) and allows to assess the exposure to flavours, additives and chemicals from food contact materials by using a probabilistic approach. For consumption data, surveys from different European countries (UK, Hungary, France, Poland, Finland, Ireland, Italy, and Portugal) are available. In our case, we used the UK survey data, and similar mean exposure values were found, slightly higher for the 1–3 years group (1.5 μg/kg bw day) and a somehow lower for the other groups 1.52 μg/kg bw/day (3–9 years) and 0.9362 μg/kg bw/day (10–17 years), respectively.

When differences were found in our food exposure estimations compared to other studies, these might be due to, among other factors, the type of foodstuffs considered for the exposure estimation, since our study is limited to cereal-based foods. Also, the variability of nutrition habits, as well as the group of population selected for the exposure studies can contribute to some variations.

4. Conclusions

In the present work an approach to address the dietary exposure to chemicals transferred from food packaging materials is reported. The proposed methodology involves the following steps: first, a GC-MS method was applied to identify potential contaminants in the plastic packaging of cereal based foodstuffs. Secondly, a LC-MS/MS method was developed and validated to quantify the target compounds in composite food samples. The proposed LC-MS/MS method is satisfactory in terms of sensitivity, precision and accuracy for the detection and quantification of the selected compounds. The results of this study confirm that Spanish consumers are exposed to packaging related chemicals through food consumption. In this study the identified compounds included phthalates (DEP, DIBP and DEHP), ATBC and DEHA. Exposure was assessed by combining the concentration of compounds in the composite food samples (pools) targeted to different groups of population, with the national food consumption data reported in the ENALIA Spanish survey.

Although the levels found in foods were low, it is important to consider that migration of chemical compounds from food packaging materials is one source of exposure and can contribute to a risk for public health and safety. For the selected compounds in this study the estimated exposures were below the available TDI values.

Results of the current exposure study are consistent with those observed in other studies showing the presence of phthalates in a range of foods commonly consumed in the diet. In our case only cereal products in plastic packaging were considered so far for the study. Thus, for a better estimation of packaging chemical exposure, other food categories should be taken into consideration. Additionally, in the case of phthalates other sources of exposure would also need to be considered for a

complete risk assessment.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

The study was financially supported by the “Ministerio de Economía y Competitividad”, "Fondo Europeo de Desarrollo Regional (FEDER) and by "Agencia Estatal de Investigación" Ref.No. AGL2015-69609-P “MIGRAEXPO”. (MINECO/FEDER,UE). V. García Ibarra is grateful for her grant from SENESCYT-Ecuador. We gratefully acknowledge Mrs. Victoria Marcos Suarez, Spanish Agency for Consumer Affairs, Food Safety and Nutrition for critically reading the manuscript before submission.

Transparency document

Transparency document related to this article can be found online at https://doi.org/10.1016/j.fct.2019.04.003

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