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Study of the possible migration risks of food contact materials for children under 3 years

Thesis submitted to obtain the degree of Doctor in Pharmaceutical Sciences at the University of Antwerp and the degree of Doctor at the University Jaume I

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NIETS VOOR NIETS…

List of abbreviations

ABS	Acrylonitrile-Butadiene-Styrene
ACC	American Chemistry Council
AcN	Acetonitrile
AhR	Aryl Hydrocarbon Receptor
APCI	Atmospheric Pressure Chemical Ionisation
AR	Androgen Receptor
BHT	Butylated Hydroxytoluene
BPA	Bisphenol-A
BPS	Bisphenol-S
bw	Body Weight
CAS	Chemical Abstracts Service
CCS	Collisional Cross Section
CE	Collision Energy
CHDM	1,4-Cyclohexanedimethanol
CMR	Carcinogenic, Mutagenic or Reprotoxic
CODA-CERVA	Veterinary and Agrochemical Research Centre
CoE	Council of Europe
DC	Direct Current
DCM	Dichloromethane
DCPS	4,4'-Dichlorodiphenyl Sulphone
DEHP	Bis(2-ethylhexyl)phthalate
DIPN	Diisopropyl Naphtalene
DMTP	Dimethyl Terephthalate
DNA	Deoxyribonucleic Acid
Doc	Declaration of Compliance
DPS	Diphenyl Sulphone
EA	Estrogen Activity
EC	European Commission
ECHA	European Chemicals Agency
EDC	Endocrine Disrupting Compound
EFSA	European Food Safety Authority
EI	Electron Impact
ER	Estrogen Receptor
ESI	Electrospray Ionisation
EtOAc	Ethyl Acetate
EtOH	Ethanol
EU	European Union
FCMs	Food Contact Materials

FDA	Food and Drug Administration
FWHM	Full Width at Half Maximum
GC	Gas Chromatography
GMP	Good Manufacturing Practice
GR	Glucocorticoid Receptor
HDPE	High-density Polyethylene
HE	High Energy
HPLC	High Performance Liquid Chromatography
HR	High Resolution
IAS	Intentionally Added Substances
IMS	Ion Mobility Spectrometry
IS	Internal Standard
ISO	International Organisation for Standardisation
LC	Liquid Chromatography
LDPE	Low-density Polyethylene
LE	Low Energy
LLE	Liquid-Liquid Extraction
LOAEL	Lowest Observed Adverse Effect Level
LOD	Limit of Detection
LOQ	Limit of Quantification
MeOH	Methanol
MRM	Multiple Reaction Monitoring
MS	Mass Spectrometry
MS/MS	Tandem Mass Spectrometry
MSD	Mass Selective Detector
MTBE	Methyl-*tert*-butyl Ether
MW	Molecular Weight
ND	Non-detectable
NIAS	Non-intentionally Added Substance
NIST	National Institute of Standards and Technology
NL	Not-listed
NMR	Nuclear Magnetic Resonance
NOAEL	No Observed Adverse Effect Level
OML	Overall Migration Limit
PA	Polyamide
PAA	Primary Aromatic Amines
PC	Polycarbonate
PDMS	Poly(dimethyl)siloxane
PES	Polyethersulphone
PET	Polyethylene Terephthalate
PETG	Polyethylene Terephthalateglycol

PIC	Polymer Identification Code
PP	Polypropylene
PPA	Polymer Production Aids
PPARγ	Peroxisome Proliferator Gamma Receptor
PR	Progesterone Receptor
PS	Polystyrene
PTV	Programmed Temperature Vaporising
PVC	Polyvinylchloride
QC	Quality Control
QqQ	Triple Quadrupole
QToF	Quadrupole Time of Flight
RASFF	Rapid Alert System for Food and Feed
RF	Radio Frequency
RP	Reversed Phase
RSD	Relative Standard Deviation
RSDr	Repeatability Relative Standard Deviation
RSDrw	Intra-laboratory Reproducibility Relative Standard Deviation
RT	Retention Time
SC	Scientific Committee
SCF	Scientific Committee on Food
SD	Standard Deviation
SI	Supplemental Information
SIM	Selected Ion Monitoring
SML	Specific Migration Limit
STER	Sterilisation
TDI	Tolerable Daily Intake
TMCB	2,2,4,4-Tetramethyl-1,3-cyclobutanediol
TOF	Time Of Flight
TPE	Thermoplastic Elastomer
TRβ	Thryoid Beta Receptor
TTC	Threshold of Toxicological Concern
TXIB	2,2,4-Trimethyl-1,3-pentanediol Diisobutyrate
UA	University of Antwerp
UHPLC	Ultra-high Performance Liquid Chromatrography
ULg	University of Liège
UV	Ultraviolet
VOCs	Volatile Organic Compounds
vPvB	very Persistent and very Bioaccumulative
VUB	Free University of Brussels
WIV-ISP	Scientific Institute of Public Health
XIC	Extracted Ion Chromatogram

Table of contents

Chapter 1:
Introduction

1.1 Food contact materials

Food can come into contact with a wide variety of articles and materials before its actual consumption during production, processing, storage, preparation and serving. These items are called food contact materials (FCMs) and include everything that is already into contact with food, is intended to be brought into contact with food, can be fairly brought into contact with food or transfer under normal or foreseeable use its constituents to the food. FCMs can include materials that are both into direct and indirect contact with the food, such as e.g. kitchen- and tableware, packaging materials, but also machinery to process food or containers for its transport and storage. Materials and articles in contact with water for human consumption fall also under this term (e.g. bottles), except for public or private water supply equipment (EFSA - European Food Safety Authority 2015a).

Since many types of materials, like plastics, paper, metal, woods, lacquers, adhesives, printing inks etc. are used for production, FCMs comprise a broad and complex area. These materials can be used as a single material or can be present as combinations, e.g., in complex multilayer materials. Moreover, especially for plastic FCMs, many different substances are used for the production of these materials. Plastic materials do not only consist of plastic polymers. In nearly all cases, the producer has made a formula (plastic compound) with different additives to improve the performance and ageing properties, as well as the processing properties of the plastic compound for the shaping process (injection moulding, extrusion, blow moulding, vacuum moulding, etc.). Therefore, next to monomers, a wide variety of other components, such as additives, plasticisers, stabilisers, solvents, pigments, etc. can be present in plastics.

As some of these (hazardous) chemicals can pass from the FCMs to the food, the safety of FCMs must be evaluated in order to safeguard consumer's health as stated in article 3 of EU Regulation No. 1935/2004. This holds that materials must be manufactured in compliance with European Union (EU) regulations, which includes good manufacturing practices in order to avoid that any potential transfer to the food does not raise safety concerns, changes the composition of the food in an unacceptable way or deteriorates its taste and odour (European Council 2004).

1.2 Migration

Migration is defined as the phenomenon that occurs when chemical substances present in a polymer migrate to the surface of the polymer item or to a medium in contact with the polymer (Figure 1.1). These polymer items can be anything, from FCMs over medical

devices to even car parts. Migration can occur from the surface of the polymer material towards the food, from the core to the surface of the materials and so towards the food or through the material into the food. Migration is relevant for compounds with molecular weights smaller than 1000 Dalton (Da) (EFSA - European Food Safety Authority 2008a).

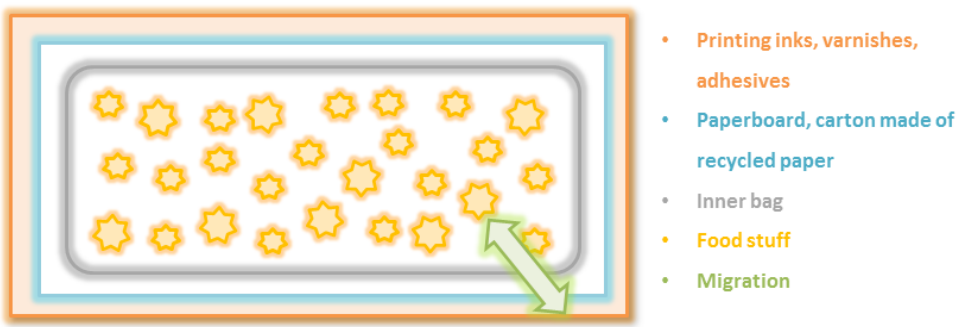


Figure 1.1: Migration phenomena in FCMs

In most cases, migration is not wanted, although in rare cases, it can be a desired property such as for the controlled release of drugs from a polymer matrix for precise dosage to patients. Unwanted migration can be for example the migration of plasticisers from medical devices into the patient's body. Migration of chemical substances from plastic packaging for food or medicine is generally undesired, since some of the migrating substances can be toxic, give an unpleasant taste or smell to the food or enhance the degradation of the active substances in a medicine.

Migration is mainly controlled by two processes; diffusion and sorption. The diffusion coefficients D_F and D_P represent the rate at which the migrant moves within the external matrix (foodstuff or simulant in this case) and within the polymer, respectively. The partition coefficient $K_{P/F}$ is an indication of the relative solubility of the migrant in the polymer and the food matrix. The extent to which migration occurs depends on various factors influencing these processes and their parameters.

The physico-chemical properties of the migrant, of the packaging material, and the food (e.g. fat and water content, acidity) are first important factors. Migration of organic substances from the polymer material depends for example on their size. Small molecules, such as monomers and residual solvents, will exhibit a fast migration since their small size facilitates an easier movement through the polymer matrix.

Moreover, the volatility of these compounds is generally high and monomers, such as formaldehyde or ethylene, are in the gas state at room temperature. Therefore, their

residuals have a high tendency to migrate quickly out of the polymer even at ambient temperature. Substances, such as additives, have generally molecular weights (MW) in the range of 200-2000 Da. The higher the molecular mass is, the larger the molecule and consequently the lower the migration rate will be, and vice versa. Most additives are designed deliberately with high MW structures in order to limit their migration rates (e.g., antioxidants). However, plasticisers and flame retardants (not-allowed in FCMs) based on this principle are mostly used to a minor extent because of the higher cost of these high molecular additives. Another requisite to avoid migration is that the solubility of the additive in the polymer should be high and not exhibit the tendency to migrate to the liquid (or food) in contact with the polymer. This is of course influenced by the nature of the food as well.

Furthermore, the initial concentration of the chemical substance in the polymer will naturally be the driving factor in the diffusion process. In addition to this, also the crystallinity of the polymer (e.g. amorphous or semi-crystalline) and the thickness and surface structure of the polymer item will influence the migration process.

The exposure temperature plays a critical role in the migration to a contact medium as well, since both the solubility and the mobility of the migrating substances depend hereon. Furthermore, the type of contact medium (gas, liquid, solid) is also an important parameter regarding migration. Finally, the storage and/or contact time will determine how much finally migrates to the contact medium.

To measure the migration of substances from plastics, practically, contact experiments under a worst case scenario are done. Some of these methods for FCMs (see section “Migration testing”) or pharmaceuticals have been standardised by EU Regulations and the Council of Europe (CoE). Health assessments are carried out and based on data from these contact migration studies.

Nowadays, infants and newborns are, next to breastfeed, mainly fed using a wide range of plastic FCMs, such as baby bottles, plastic cutlery, sippy cups etc. Baby bottles, the most used FCMs for infant feeding, were made mostly of polycarbonate (PC). However, the migration of the monomer bisphenol-A (BPA) which is used for PC production has recently raised concerns regarding its safety. As a precautionary measure, this has recently led to the ban of the production of BPA-containing PC baby bottles (European Union 2011a; Belgian statute book 2012).

1.3 Bisphenol-A

1.3.1 Production and use

Bisphenol-A (BPA) [2,2-bis-(4-hydroxyphenyl) propane, CAS No. 80-05-7] is an industrial chemical compound that is synthesised from the condensation of two phenol molecules, and one molecule of acetone (the A in bisphenol-A stands for acetone) in the presence of an acid catalyst (Pubchem Open Chemistry Database 2015) as shown in Figure 1.2.

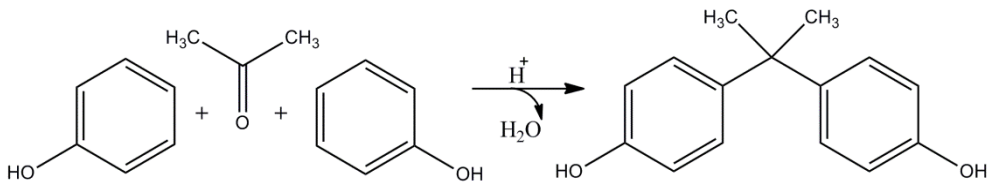


Figure 1.2: Synthesis and structure of bisphenol-A

BPA is mainly used as a monomer in the production of polymers, such as polycarbonate (PC) (Figure 1.3) (which has the largest end use (68.5%)) and epoxy resins (27%) (Hoekstra & Simoneau 2013). It has also applications as an additive such as an antioxidant, for the termination of the polymerisation in plastics (e.g. polyvinylchloride (PVC)) or in thermal paper. Furthermore BPA is also used in sunglasses, construction materials, CD-ROM, medical devices, dental materials, etc.

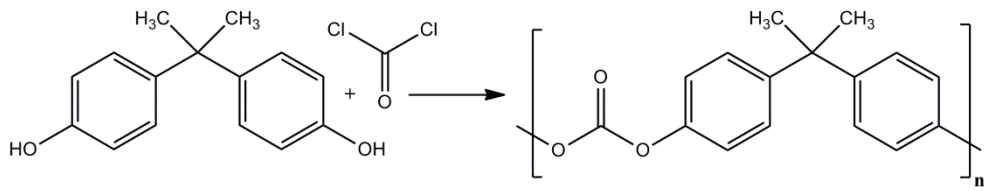


Figure 1.3: Synthesis of PC by the condensation of BPA and phosgene

PC is a versatile, durable, heat- and pressure-resistant and transparent thermoplast (European Information Centre on Bisphenol A 2015). Besides frequent use in all sorts of industries ,e.g., optical media, electrical products and electronics and construction materials it is also used in FCMs. Due to its high impact and temperature resistance, as well as its transparency, PC is a suitable material to use for reusable plastic bottles, (until recently) baby bottles, plates, mugs, cups, etc. (Plastics Europe 2011). Epoxy resins, the second largest application of BPA, can also be used in FCMs, namely as internal coatings for food and beverage cans. Yet, only 3% of the produced PC and only 10% of the epoxy resins is used in materials in contact with food (Plastics Europe 2011). The migration of residual BPA in the polymer, present because of incomplete polymerisation and

migration of BPA released by hydrolysis of the polymer from these PC materials into the foods and beverages with which they come into contact, has the potential to provide a source of dietary exposure to BPA (Nam et al. 2010).

1.3.2 Toxicity

The toxicity of BPA has been extensively studied compared to many other chemicals, yet for many years there was no consensus on what exposure levels of BPA pose a health risk (EFSA Scientific Committee (SC) 2013). For decades, BPA has been the subject of interest in scientific investigation regarding its properties as an endocrine disrupting compound (EDC). BPA was found to interact in particular with the human estrogen receptors, both in the nucleus and in the cell membrane, but with a capacity which is 1,000 to 5,000 times lower than 17β-estradiol, which is the natural ligand for this receptor (Rogers et al. 2013). Furthermore it also reduces the synthesis of some steroids at molecular level and results to be an androgen receptor antagonist (Wolstenholme et al. 2011; Kolsek et al. 2014; Feng et al. 2016). Low-dose effects of BPA on reproductive and mammary tissue, adipose, the immune and nervous system, the liver and in pancreatic and pituitary models were found in *in vitro* models (Wetherill et al. 2007). In rodent studies, changes in the brain physiology, brain structure, behaviour and sex differences in the brain were observed at exposure levels below 50 mg per kilogram body weight per day (mg/kg bw/day), which was previously defined as the lowest observed adverse effect level (LOAEL). BPA also affected the fertility and onset of puberty in females. Moreover, it caused changes in the mammary gland, uterus, vagina, ovary oocytes and affected the immune system and metabolism of test animals (Richter et al. 2007). Other effects such as carcinogenesis, adipogenesis and changes in male reproduction have been recently suggested as well (Gies & Soto 2013). BPA is however not likely to be genotoxic (EFSA - European Food Safety Authority 2015b).

Epidemiological research relates BPA exposure with diabetes, cardiovascular disease and altered liver enzyme levels. Furthermore, decreased semen quality, sperm DNA damage, altered thyroid function, metabolic syndrome, obesity, hypertension, peripheral arterial disease and coronary arterial stenosis could be associated with BPA exposure as well (Niederberger 2011; Lakind et al. 2014; Chrysant 2015; Goldstone et al. 2015).

About 90% of people in the western world have BPA and its metabolites in the urine. This indicates a general chronic exposure (Covaci et al. 2015). The most important sources of contamination are PC plastic FCMs and epoxy resins, but dust, consumer goods such as CDs, dental fillings and other materials can also have an important

contribution (Perez Lobato et al. 2016). Moreover, the migration can vary widely depending on the contents of the packaging or cans (Viñas et al. 2010).
Exposure to EDCs, such as BPA, is therefore usually not an acute event and results to be a more chronic and continuous process. Eating contaminated food, inhaling or ingesting contaminated house dust or working in an occupational setting form the major pathways of BPA exposure to humans. Since BPA is more dangerous during "critical periods" of life, such as intrauterine, perinatal, juvenile or puberty periods (Frye et al. 2012), this is one of the main reasons these specific groups should particularly be protected against EDCs exposure. Since infants have a lower body weight, a higher intake of leached plastic materials per kg of body weight is expected for this part of the population (Foster et al. 2010).

1.3.3 Regulations on BPA

1.3.3.1 European Union

Already in 1984, BPA was evaluated by the Scientific Committee on Food (SCF) for use in plastic materials and articles intended to come into contact with foodstuffs which set a tolerable daily intake (TDI) of 50 µg/kg bw/day (Scientific Committee on Food 1984). This TDI is an estimate of the amount of a contaminant in food or drinking-water, expressed on a body-weight basis that can be ingested daily over a lifetime without appreciable health risk to the consumer on the basis of all known facts at the time of the evaluation (International Food Safety Authorities Network (INFOSAN) 2009).

In the 1990s, Commission directive 90/128/EEC5 permitted the use of BPA as monomer with a specific migration limit (SML) of 3 mg/kg food (European Commission (EU) 1990). The SCF reduced the TDI in 2002 temporarily to 10 µg/kg bw/day (European Commission 2002), and subsequently the European Commission established a lower SML of 600 µg BPA/kg food from BPA-based FCMs (European Commission (EU) 2004). This amended the Commission Directive 2002/72/EC related to plastic materials and articles intended to come into contact with food, which also authorised the use of BPA as an additive (European Commission (EU) 2002).

The European Food Safety Authority (EFSA) established in 2006, a TDI of 50 µg BPA/kg bw/day, derived by applying a 100-fold uncertainty factor to the overall no observed adverse effect level (NOAEL) of 5 mg/kg bw/day, although the SML was kept at 600 µg/kg. EFSA updated its risk assessment on BPA in 2008 and 2010 and twice reconfirmed the TDI of 50 µg/kg bw/day (EFSA - European Food Safety Authority 2008b; EFSA - European Food Safety Authority 2010a; EFSA - European Food Safety Authority 2010b).

In January 2011, the European Commission placed a restriction on the use of BPA in the manufacture of PC baby bottles (from 1 March 2011) and the placing on the market and the import into the EU of such products (1 June 2011) based on the precautionary principle (European Union 2011a). The new Commission Regulation EU No. 10/2011 (European Union 2011b) on plastic materials and articles intended to come into contact with food, which was introduced as a replacement of the previous Commission Directive 2002/72EC, was subsequently amended in Commission Implementing Regulation No. 321/2011 (European Union 2011c). Yet, this new regulation on plastic FCMs still authorises the use of BPA as a monomer, however subjected to the earlier specified restrictions that BPA cannot be used for the manufacture of infant feeding bottles.

Previously, bans on the use of BPA in FCMs intended for children aged 0-3 years (infant feeding bottles, feeding cups, and packaging for baby food) have been already proposed by several European Union (EU) Member states. In May 2010, Denmark decided to invoke the precautionary principle and introduced a temporary national ban on BPA in all FCMs intended for children aged 0-3 (effective as of 1 July 2010) (Danish Veterinary and Food Administration 2011). Sweden chose to ban the use of BPA or BPA-containing compounds in varnishes or coatings intended for packaging food for children between 0-3 years old from 1 July 2013 (Svensk Författningssamling (SFS) 2013). France on the other hand introduced a more drastic ban and adapted a law that suspended the manufacturing, import, export and putting on the market of all FCMs that contain BPA. This law was gradually applied starting from 1 January 2013 for FCMs intended for children between 0-3 y passing to all FCMs from 1 January 2015 (OJ of the French Republic 2012). Austria prohibited the use of BPA in pacifiers and soothers from 6 October 2011 (Bundesministerium für Gesundheit 2011).

In Belgium, following the advice of the Superior Health Council (Superior Health Council 2010), a ban was also introduced from 1 January 2013 on the use of BPA in any FCMs intended for children younger than 3 years old (Belgian statute book 2012).

In January 2015, based on new data and methodologies, EFSA has reduced the TDI temporarily to 4 µg/kg bw/day (t-TDI) pending the outcome of an on-going long-term study in rats involving prenatal and postnatal exposure to BPA. Yet, EFSA experts concluded that BPA poses no health risk to consumers of any age group (including unborn children, infants, and adolescents) by comparing this t-TDI with current exposure levels. It was concluded that the highest estimates for dietary and non-dietary exposure to BPA are 3 to 5 times lower than the t-TDI depending on the age group (EFSA - European Food Safety Authority 2015b).

1.3.3.2 Canada

Health Canada has established a provisional TDI of 25 µg/kg bw/day as a conservatively safe level for BPA presence in food (Cao & Corriveau 2008). In March 2010, Canada was the first country in the world to prohibit BPA-containing baby bottles to be advertised, sold or imported. Furthermore, it also investigated ways to reduce BPA contamination of baby formula packed in metal cans as much as possible (Hengstler et al. 2011).

1.3.3.3 United States

The U.S. Food and Drug Administration (FDA) amended the food additive regulations in July 2012 to no longer provide for the use of PC resins in infant feeding bottles and spill-proof cups as a petition of the American Chemistry Council (ACC) showed that these uses had been abandoned by the industry (Food and Drug Administration 2012). In July 2013, a similar amendment was made concerning the use of BPA-based epoxy resins as coatings in packaging for infant formula, as these had been abandoned as well (Food and Drug Administration 2013). The FDA now continues to review the available information and studies on BPA and will update its assessment of BPA and take additional action if warranted. For the moment, the FDA still maintains a TDI of 50 µg/kg bw/day.

1.4 Alternative materials to PC for baby bottles

Until recently, most plastic baby bottles were made exclusively from PC. Due to the ban on the use of the PC monomer BPA for the production of baby bottles, PC was hastily replaced by other materials. These materials are also composed of a complex mixture of different monomers, together with a broad range of additives, such as plasticisers, antioxidants, etc. When these materials come into contact with food, one can expect migration of the monomers, additives and degradations products thereof into the foodstuff. However, this has been much less studied compared with the migration of PC components and specifically that of BPA (De Coensel et al. 2009;Munro et al. 2009; Namet al. 2010; Fasano et al. 2012; Bach et al. 2013; Cherif Lahimer et al. Forthcoming 2013; Mansilha et al. 2013; Ventrice et al. 2013). Preliminary research showed that different materials are used nowadays replacing PC for the production of baby bottles:

1.4.1 Polyethersulphone (PES)

Polyethersulphone (PES) [Poly(oxy-1,4-phenylenesulphonyl-1,4-phenyleen, CAS No. 25667-42-9] is a transparent, amorphous thermoplastic polymer that is amber in colour. It is distinguished by its high strength, rigidity and hardness and retains these capacities also at high (<150 °C) temperatures. Polysulphones are used in specialty applications and often are a superior replacement for polycarbonates (e.g. parts for medical equipment, aerospace, electrical and electronic components). Due to the ban on PC, PES has experienced a boom as a material for the production of baby bottles as well. PES is manufactured by the condensation reaction of its monomers 4,4'-dichlorodiphenyl sulphone (DCPS) and 4,4'-dihydroxydiphenyl sulphone, also known as bisphenol-S (BPS) (Figure 1.4) (KIK 2016; RTP Co. 2016).

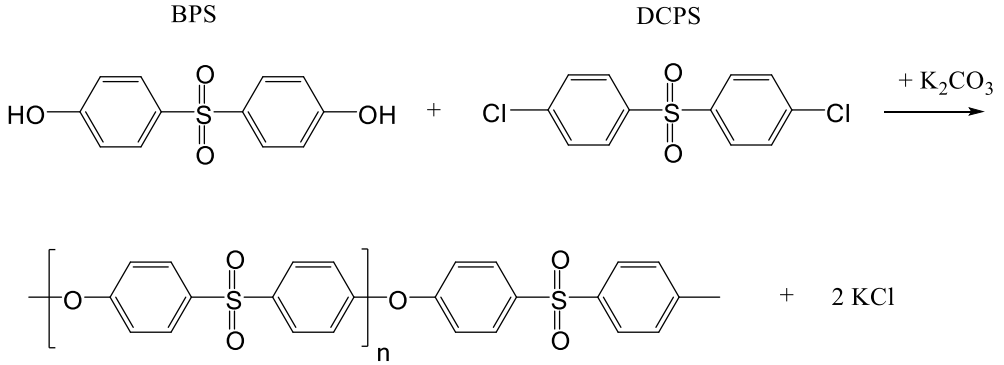


Figure 1.4: Synthesis of polyethersulphone

PES has the interesting characteristic that one of its monomers (BPS) has been recently shown in several studies to be similar to BPA in its ability to bind to the estrogen receptor, and is therefore also of potential concern (Kuruto-Niwa et al. 2005; Barret 2013; Viñas & Watson 2013; Kang et al. 2014). BPA-based epoxy resins in cans have been substituted with BPS as well (Viñas et al. 2010). Further, BPS is used in thermal papers replacing BPA (Liao, Liu, Guo, et al. 2012) and it has also been detected in recycled food carton and food packaging paper (Liao, Liu, Alomirah, et al. 2012).

Simoneau et al. (Simoneau et al. 2011) investigated the migration of the potential PES starting components diphenyl sulphone (DPS), DCPS and BPS by liquid chromatography coupled to mass spectrometry (LC-MS) and concluded that only DPS migrated, but far below the SML of 3000 μg kg^{-1}.

1.4.2 Polypropylene (PP)

Polypropylene (PP) is a thermoplastic polymer that has been one of the fastest growing plastics in recent years. The PP market is the second largest volume polymer business in the world today making up 25% of global polymer demand. Due to its low density (weight saving), high stiffness, heat resistance, chemical inertness, good transparency and recyclability it is the material of choice for a wide variety of applications such as packaging and labelling, textiles (e.g., ropes, thermal underwear and carpets), stationery, plastic parts of various types, laboratory equipment, loudspeakers and automotive components. The competitive costs of PP plastics combined with their versatile properties have made these plastics also the preferred type of packaging for a wide range of foodstuffs in all the common forms of food packaging: pots, containers, tubs, bottles, pouches and wrapping films. PP (Figure 1.5) is an addition homopolymer (all the same building blocks) made from the monomer propylene (Entec polymers 2016; Plastics Europe 2016).

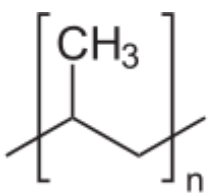


Figure 1.5: Structure of the repeating unit of PP

Most commercial PP is isotactic (regular relative orientation of the methyl groups; Figure 1.6) which is obtained by adding a Ziegler-Natta catalyst (e.g. $Al(C_2H_5)_3$) during the

polymerisation process and has an intermediate level of crystallinity (70-85%) between that of low-density polyethylene (LDPE) and high-density polyethylene (HDPE). When adding between 5 and 30% of ethylene in the polymerisation a copolymer is obtained which has a greater impact resistance than the homopolymer PP. Sometimes, a third monomer (1-butene) can also be added (Vasile 2000).

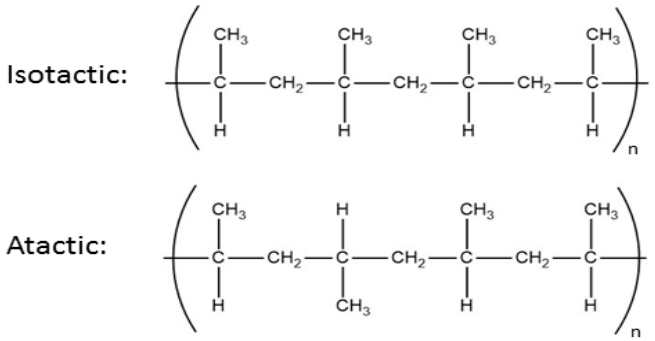


Figure 1.6: Isotactic and atactic PP

Although PP plastics are basically inert materials and usually do not present a health hazard to the consumer in either handling the plastics or consuming foodstuffs with which they have come into contact (King Plastic 2016), migration of some compounds can occur. Substances that may migrate from PP plastics to foodstuffs include residual monomers, low-molecular-weight polymer (oligomers) and any additives or other substances used in the formulations (Reingruber et al. 2010).

Since PP is for example subjected to chain degradation from exposure to heat and ultraviolet (UV) radiation, all commercial PPs are stabilised with antioxidants. The two main antioxidant types, the phenolic and phosphite types, are used at concentrations of 0.01–0.5 weight %. Alin et al. have found that upon heating in the microwave oven of PP, these antioxidants can migrate (Alin & Hakkarainen 2011). The three monomers, propylene, ethylene and 1-butene, used in the manufacture of PP plastics and the principal copolymers have not been assigned any SMLs or any other restrictions in the EU Regulation No. 10/2011 (see section “Legislative framework”). The decisions not to assign any restrictions to these monomers were based primarily on toxicological assessments by the European Commission’s Scientific SCF and the fact that these monomers are very volatile which makes their migration irrelevant.

McDonald et al. have shown that there may be leaching of bioactive compounds (e.g. quaternary ammonium products) and slip agents, such as oleamide from PP material as well (Mcdonald et al. 2008).

1.4.3 Polyamide (PA)

Polyamides (PAs) are linear polymers with regularly repeating amide (-CO-NH-) linkages along the backbone. The amide group can be considered as a condensation product of a carboxylic acid and an amine. The resulting bond is an amide bond, which is hydrolytically cleaved again during polymerisation. Proteins are examples of naturally occurring PAs whereas the best known manufactured PAs are often called nylons (the trade name given by the manufacturer, DuPont) which are aliphatic PAs. The nomenclature for describing these linear, aliphatic PA, such as PA 6 is based on the number of carbon atoms in the repeating unit. They are mainly used in textiles, automotive applications, carpets, sportswear and food packaging and utensils due to their high durability and strength (British Plastics Federation 2016). The transportation industry is the major consumer, accounting for 35% of polyamide (PA) consumption (Ceresana 2016). Figure 1.7 shows the polymerisation reaction of a typical PA (PA 6 or Nylon 6 from caprolactam).

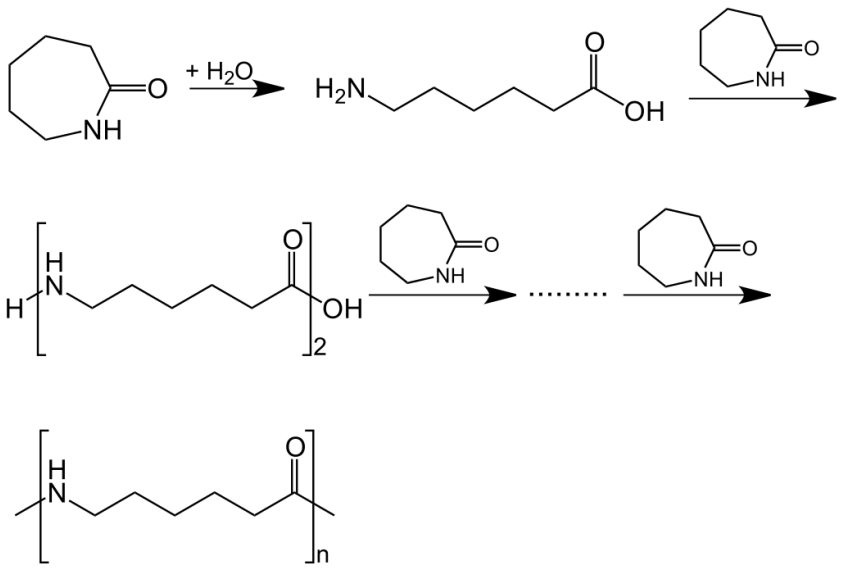


Figure 1.7: Polymerisation of caprolactam to Polyamide 6

Other manufactured PA can consist of a (semi-)aromatic repetitive unit and are known for their very high strength (respectively polyphthalamides (e.g. suitability for metal substitution applications) and aramides (e.g. Kevlar® used in bulletproof vests)).

It has been shown that high levels of primary aromatic amines (PAA) which are possibly carcinogenic to humans can migrate from polyamide kitchenware, such as spatulas (Trier et al. 2010; McCall et al. 2012; National Food Institute Norway 2014). However,

cyclic monomers and oligomers were shown to be the major migrating substances from PA FCMs (Heimrich et al. 2015).

1.4.4 Tritan™

Tritan™ is a new copolyester produced since 2009 by Eastman Chemical. Polyesters are combinations of diacids and diols and contain an ester function in their repeating unit. Copolyesters are formed when modifications are made to these polyesters by for example introducing other diols such as 1,4-cyclohexanedimethanol (CHDM). Tritan™ is synthesised from the monomers dimethyl terephthalate (DMTP), 2,2,4,4-tetramethyl-1,3-cyclobutanediol (TMCB) and CHDM (Guart et al. 2013)(Figure 1.8), yet the exact chemical composition is proprietary and therefore not known. Tritan™ is easily processed, has excellent transparency and major resistance to chemicals and heat. It is most often used for high-end reusable water bottles and also for baby bottles, next to applications in commercial houseware and small electro domestics.

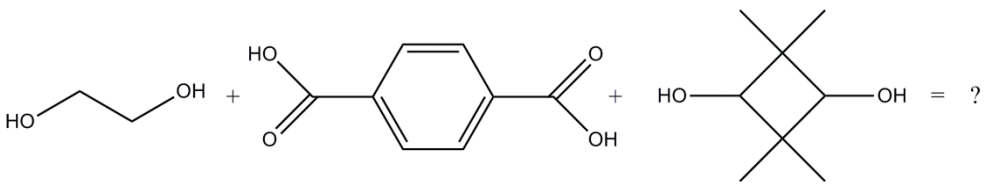


Figure 1.8: Monomers of Tritan™ co-polyester

One of its monomers, TMCB (an aliphatic diol), was recorded by EFSA in 2009 in EU Regulation No. 10/2011 and thus this monomer would be legally allowed to appear when performing migration tests. *In vitro* toxicological investigations revealed that none of these monomers had an effect on androgen and α-/β-oestrogen receptors (Osimitz et al. 2012). Yet, recent research has demonstrated that chemicals exhibiting estrogen activity (EA) can migrate from alternative materials to PC, amongst others also from Tritan™ (Guart et al. 2013; Bittner et al. 2014).

1.4.5 Silicone

Silicones (also known under the often used synonym siloxanes) are synthetic polymers that differ fundamentally from other polymer classes having a silicon-oxygen backbone (Figure 1.9), whereas the backbone of plastics mainly consists of C atoms. Each Si atom of such a backbone usually carries two organic groups, such as methyl or ethyl (e.g. poly(dimethyl)siloxane (PDMS)) (Forrest 2009).

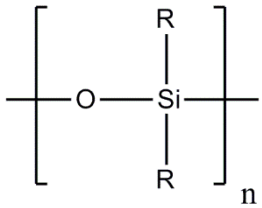


Figure 1.9: General repeating unit of a silicone polymer

By linking together the Si-O backbones by chemical reactions, a large variety of silicones can be formed, covering a broad range of properties and purposes. Silicones can be present as fluids, rubbers or resins depending on the length of the polymer chains and the degree and nature of crosslinking. By its chemical composition of silicon and oxygen it is flexible and soft, so it can adapt any form desired. Moreover, silicones do not stain, do not wear or age, have a low chemical reactivity and are resistant to oxygen, UV radiation and ozone.

Therefore, silicone products find a wide application as FCMs. In households, they are frequently used as baking moulds, spoons, coasters, spatulas, dough scrapers, brushes, containers, ice cube trays, stoppers for bottles, and many others (Helling et al. 2009). Silicone rubbers are next to natural rubber the material of choice to produce baby soothers, feeding teats and nipple shields for breast-feeding (Lund & Petersen 2002). Furthermore, silicones can amongst others also be added as additives to thermoplastic polymers, such as polyolefins (e.g. PP) to improve processing: enhance the flow during manufacture (since low percentages of silicone benefit the surface properties of thermoplastics), enhance fire resistance, etc.

A wide variety of substances can possibly migrate from silicone based FCMs into the food. Additives, catalysts, oligomers, breakdown and reaction products have been shown to be detectable in food that had been into contact with silicone FCMs (Meuwly et al. 2007; Helling et al. 2010; Helling et al. 2012; Zhang et al. 2012). Several analytical methods for the quantification of siloxane oligomers and also a predictive algorithm were described to assess overall and specific migration from silicones (Elskens et al. 2012). Some repeated-use articles showed that the migration of siloxane oligomers could exceed the legal limits especially during the first cycles of use, and a high fat content of the food led to an increase of this migration. A study on silicone baby bottles reported the migration of substances related to printing inks (e.g. benzophenone, diisopropyl naphthalene), but also of EDCs, such as phthalates (Simoneau et al. 2012). Yet, data on the food-related exposure to silicones, including cyclic and linear siloxane oligomers, are scarce.

These siloxane oligomers form the most typical and largest group of migrants from silicones FCMs, since they are commonly occurring as reaction by-products(Rücker & Kümmerer 2015). One of these compounds, hexadecamethylsiloxane, was added in 2013 to the Community Rolling Action Plan (CoRAP) of the European Chemicals Agency (ECHA) considering its potential carcinogenic, reprotoxic and mutagenic properties (European Chemicals Agency 2013). If a substance is on this list, it means that a member state has evaluated or will evaluate it over the coming years. Other linear siloxane oligomers, such as octamethyltri-, decamethyltetra- and dodecamethylpentasiloxane were recently added (March 2015) to the CoRAP regarding their potential bioaccumulative, persistent and toxic properties (European Chemicals Agency 2015). Evaluation of these four linear siloxanes is still ongoing, yet Health Canada already concluded in 2015 that, although limited empirical health effect data were available, effects on some organs, such as liver, kidney or lungs have been observed (Environment Canada-Health Canada 2015).

Cyclic siloxane oligomers that can migrate, e.g. octamethylcyclotetrasiloxane (D_4 – Figure 1.10) or decamethylcyclopentasiloxane (D_5), have been reported to exhibit effects on liver, lungs, kidney and thymus (Environment Canada-Health Canada 2008a; Environment Canada-Health Canada 2008b). Furthermore, D_4 was characterised as a weak estrogen (He et al. 2003), whereas D_5 exposure resulted in a statistically significant increase of uterine tumours in rats (Jeana et al. 2015). Currently, D_4 is labelled in the EU as being toxic to fertility (category III) and D_4 and D_5 were both judged to be very persistent and very bioaccumulative (vPvB) in the environment by the European Chemicals Agency (European Chemicals Agency 2014).

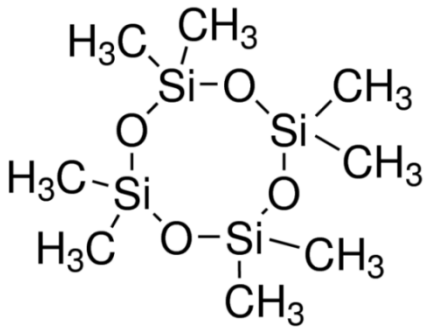


Figure 1.10: Structure of octamethylcyclotetrasiloxane (D_4)

1.5 Food contact materials regulations in Europe

1.5.1 General legislative framework

In Europe, national legislation and EU level legislation continue to coexist. At the EU level, various directives and regulations exist (EU legislative lists). While regulations are directly effective in member states, directives however need to be accepted by national parliaments in order to become operational. At the European Union level, the legislation on FCMs (including packaging, machinery, and kitchenware) is based on the Framework Regulation (EC) No. 1935/2004. This regulation establishes the general requirements for FCMs and the authorisation of new substances. It includes following basic requirements on FCMs:

- According to article 3 of the framework Regulation firstly FCMs must be safe. Secondly FCMs must not transfer their components into food in quantities that could endanger human health, change the composition of the food in an unacceptable way or deteriorate its taste and odour. Finally FCMs must be traceable throughout the production chain.
- Articles that are intended to come into contact with food must be labelled adequately or bear the typical glass and fork symbol (Figure 1.11). When food contact is obvious due to the nature of the article, e.g. a fork, a wine glass, etc. this labelling is not obligatory.
- Advertising, presentation and labelling of FCMs must not mislead the consumers.
- If necessary, information on the appropriate use of FCMs or articles must be provided.
- FCMs must be manufactured according to good manufacturing practice (GMP). GPM is described in the Commission Regulation (EC) No. 2023/2006 (European Union 2006). This Regulation sets the general principles for improving a GMP system applied to FCMs.

Figure 1.11: Glass and fork symbol indicating a FCM

Following Regulation (EC) No. 1935/2004, FCMs must be authorised by EFSA prior to their placement on the market. FCMs that have been authorised are listed in publicly

available online databases (Food Contact Materials Database) maintained by the EFSA and can be easily accessed at:
https://webgate.ec.europa.eu/sanco_foods/main/?event=display.

The Framework Regulation further allows for specific requirements on the seventeen individual FCMs covered which are active and intelligent materials and articles, adhesives, ceramics, cork, rubbers, glass, ion-exchange resins, metals and alloys, paper and board, plastics, printing inks, regenerated cellulose, silicones, textiles, varnishes and coatings, waxes and wood. Six such specific requirements have been adopted until now (see Table 1.1).

Table 1.1: Overview of EU legislations on FCMs

General Regulations on FCM	
Regulation EC 1935/2004 (on materials and articles intended to come into contact with food)	
Regulation EC 2023/2006 (on Good Manufacturing Practices)	
Specific Materials	
Plastics	Regulation EU 10/2011
Ceramics	Directive 84/500/EEC
Epoxy Resins	Regulation EC 1895/2005
Regenerated Cellulose Film	Directive 2007/42/EC
Recycled Plastics Material	Regulation EC 282/2008
Active and Intelligent Packaging	Regulation EC 450/2009
Specific Regulation on substances	
Regulation EU 321/2011 (restricting the use of bisphenol A in polycarbonate infant feeding bottles)	
Regulation EU 284/2011 (import procedures for polyamide and melamine plastic kitchenware from China and Hong Kong)	
Regulation EC 1895/2005 (restricting the use of certain epoxy resins)	
Directive 93/11/EEC (regulating the release of N-nitrosamines and N-nitrosable substances from rubber teats and soothers)	

Regulations must be automatically adopted by the member states, whereas directives still have to be transformed to a national legislation (e.g. royal resolution, decree, law). Other materials can be covered by specific measures at national level or at international recommendations such as Council of Europe (CoE) Resolutions may be taken into account (e.g. silicones) (Council of Europe 2004). These resolutions are the result of specific investigation in a particular field of FCMs with the aim to define an international

legislation for this type of FCM. However, the resolutions itself have no legislative character. Mostly, the specifically developed national legislations are based on these resolutions defined by the CoE. An overview of the resolutions and guidelines of the CoE on FCMs is given in Table 1.2.

Table 1.2: Resolutions and guidelines of the CoE on FCMs (source: Council of Europe 2016)

Resolutions
Resolution AP (89) 1 on the use of colourants in plastic materials coming into contact with food
Resolution AP (92) 2 on control of aids to polymerisation for plastic materials and articles
Resolution AP (96) 5 on surface coatings intended to come into contact with foodstuffs
Resolution AP (99) 3 on silicones used for food contact applications
Resolution AP(2002) 1 on paper and board materials and articles intended to come into contact with foodstuffs
Framework Resolution AP (2004) 1 on coatings intended to come into contact with foodstuffs
Resolution AP (2004) 2 on cork stoppers and other cork materials and articles intended to come into contact with foodstuffs
Resolution AP (2004) 3 on ion exchange and adsorbent resins used in the processing of foodstuffs (superseding Resolution AP (97) 1)
Resolution AP (2004) 4 on rubber products intended to come into contact with foodstuffs
Resolution AP (2004) 5 on silicones used for food contact applications
Resolution AP (2005) 2 on packaging inks applied to the non-food contact surface of food packaging materials and articles intended to come into contact with foodstuffs
Guidelines
Guidelines on metals and alloys
Guidelines on lead leaching from glass tableware into foodstuffs
Guidelines on tissue paper kitchen towels

There are at the moment for example no specific regulations for printing inks, waxes, paper and board and resins (other than those covered under regulation EC No. 1895/2005). EFSA recently published a report which inventories a list of substances that are commonly used in non-plastic FCMs. Since this report forms a first base for future legislations on these topics, more specific requirements are to be expected in the near future. Yet, although certain FCMs are only partially covered by EU regulation, they may already be specifically covered by nation legislation of some member states. Germany for example currently intends to regulate printed FCMs through an amendment to the German Ordinance on Materials and Articles, the so-called “Printing Ink Ordinance”. For Belgium, in first instance all EC directives on regulated materials were implemented.

Next, additional provisions were defined for some non-EU harmonised materials such as paper and board or glass. A summary thereof is given in Table 1.3.

Table 1.3: Summary of specific Belgian legislations for FCMs (source: European Commission 2015)

Implementation of EC Directives	
Regenerated Cellulose film	Royal Resolution of November 23 2004 relating to materials and articles made of regenerated cellulose film intended to come into contact with foodstuffs
Ceramic	Royal Resolution of May 1 2006 concerning the declaration of compliance and performance criteria of the analytical method for ceramic articles intended to come into contact with foodstuffs
Additional (non-EC) national regulations and recommendations	
Paper and board	Royal Resolution of May 11 1992 on materials and articles intended to come into contact with foodstuffs, modified by the Royal Resolution of June 2 2015
Glass	Royal Resolution of May 11 1992 on materials and articles intended to come into contact with foodstuffs, modified by the Royal Resolution of June 2 2015
Coating	Project of transposition in national law of the Resolution AP (2004) 1 of the Council of Europe on coatings intended to come into contact with foodstuffs
Metal and alloy	Project of transposition in national law of the resolution on metal and alloys from the council of Europe

1.5.2 Plastic materials

Since plastic is the most important packaging material and one of the most important FCMs on the market, the European Commission has focused its attention, risk assessment and legislation hereon. The requirements concerning plastic materials are defined in the specific European Commission (EU) Regulation No. 10/2011 (European Union 2011b). The Regulation consists of a consolidation of existing Directive 2002/72/EC (European Commission (EU) 2002) and also specifies directives related to migration tests conditions, food simulants and specific measures concerning vinyl chloride into a single Regulation.

Briefly, the Plastics Regulation specifies rules concerning the following aspects:

- It sets out a Union list of authorised substances (monomers, additives) that can be used in the manufacture of plastic layers of plastic materials and articles. It defines which types of substances are covered by the Union list and which are not and it sets restrictions and specifications for these substances. The Union list in annex I of the Regulation (EU) No. 10/2011 replaces annex II and annex III of Directive 2002/72/EC as amended.
- It sets out to which part of the plastic materials the Union list applies and to which not.
- Specific and overall migration limits for the plastic materials and articles are described, as well as specifications for the plastic materials and articles.
- A declaration of compliance (DoC) and the compliance testing requirements for plastic materials and articles are defined.

1.5.3 Migration testing

Regulation No. 10/2011 sets that, to determine the extent of chemical transfer from FCMs into food, migrants are not to be measured in actual foodstuffs, but in food simulants. Only for materials and articles that are already in contact with the food the compliance testing shall be carried out in the food itself.

When the materials or article is not into contact with the food, verification of compliance is to be carried out in food simulants. These food simulants are used as substitutes for food due to simplification of the chemical analysis. They vary in terms of their chemical properties, thus representing several particular food types: hydrophilic (water-based), lipophilic (fatty foods) or amphiphilic (foods with both watery and fatty properties). Butter and other amphiphilic foods are for example simulated by a 50% ethanol in water solution. For oily foods, vegetable oil is the prescribed food simulant, whereas simulants 10% ethanol or 3% acetic acid in water have to be applied for water-based foods and drinks. For dry foods, a synthetic polymer with a defined pore size, namely poly(2,6-diphenyl-phenylene oxide), also commercially known as Tenax, is to be used as a simulant (Table 1.4).

Testing is performed by putting the material into contact with the appropriate simulant. Testing conditions, such as time and temperature, are selected taking into consideration the final use of the item, respecting the worst case principle and consequently selecting the highest possible temperature and the longest period of time, considering the worst reasonably foreseeable final use (Table 1.5).

Table 1.4: List of food stimulants as defined in EU Regulation No. 10/2011

Reference	Simulant	Food type
A	10% Ethanol	Aqueous Food
B	3% Acetic acid	Foods that have a hydrophilic character and are able to extract hydrophilic substances and which have a pH below 4.5
C	20% Ethanol	Foods that have hydrophilic character and are able to extract hydrophilic substances, alcoholic foods with alcohol content of up to 20 % and foods containing a relevant amount of organic ingredients that render the food more lipophilic
D1	50% Ethanol	Alcoholic foods with an alcohol content of above 20 % and dairy products
D2	Vegetable oil	Fatty food and foods which contain free fats at the surface
E	poly(2,6-diphenyl-phenylene oxide), particle size 60-80 mesh, pore size 200 nm	Dry foods

Table 1.5: Selection criteria for contact time (left) and temperature (right) for specific migration testing. Time and temperature are selected independently of each other.

Contact time in worst foreseeable use	Test time	Contact temperature in worst foreseeable use	Test temperature
t ≤ 5 min	5 min	T ≤ 5 °C	5 °C
5 min < t ≤ 0.5 hour	0.5 hour	5 °C < T ≤ 20 °C	20 °C
0.5 hours < t ≤ 1 hour	1 hour	20 °C < T ≤ 40 °C	40 °C
1 hour < t ≤ 2 hours	2 hours	40 °C < T ≤ 70 °C	70 °C
2 hours < t ≤ 6 hours	6 hours	70 °C < T ≤ 100 °C	100 °C or reflux temp
6 hours < t ≤ 24 hours	24 hours	100 °C < T ≤ 121 °C	121 °C (*)
1 day < t ≤ 3 days	3 days	121 °C < T ≤ 130 °C	130 °C (*)
3 days < t ≤ 30 days	10 days	130 °C < T ≤ 150 °C	150 °C (*)
Above 30 days	Specific conditions specified	150 °C < T < 175 °C	175 °C (*)
		T > 175 °C	Adjust temperature to real temperature at interface with food (*)

(*) means that this temperature shall be used only for food simulants D2 and E. For applications heated under pressure migration testing under pressure at the relevant temperature may be performed. For food simulants A, B, C or D1 the test may be replaced by a test at 100 °C or at reflux temperature for duration of four times the time selected according to the time conditions in the left side of the table.

1.5.3.1 Specific migration

Within the regulations, table 1 of Annex I in the Regulation provides the European Union's list of the authorised chemicals which can be used in the production of articles intended to be in contact with foodstuffs. It also lists the way the chemical is authorised to be used, for example as an additive or a monomer unit. However, for many of these chemicals, there are limits for the amounts which can be released into the food (SML). SMLs are fixed on the basis of a toxicological evaluation and are set according to the acceptable daily intake (for authorised substances) or the tolerable daily intake (for contaminants) established by the Scientific Committee on Food. The limit is set on the assumption that every day throughout an individual's lifetime, a person weighing 60 kg eats 1 kg of food packed in plastics containing the substance in the maximum permitted quantity. For substances for which no SML or other restrictions are provided in Annex I of the Regulation, a generic SML limit of 60 mg kg^{-1} applies. Specific migration testing is performed according to the previously described conditions to assess migration for the individual authorised (and unauthorised) substances. If the material or article is intended to come into repeated contact with foods (such as baby bottles), the migration test(s) shall be carried out three times on a single sample using another portion of food simulant on each occasion. Its compliance shall be checked on the basis of the level of the migration found in the third test. However, when the specific migration limit is set as non-detectable (in practice detection < 10 µg kg^{-1}) and for non-listed substances behind a plastic functional barrier these limits already have to be respected in the first migration test (European Union 2011b).

1.5.3.2 Overall migration

The overall migration limit (OML) is the maximum permitted amount of non-volatile substances that can be released into the food. This is determined by exposing a product to an (aqueous) simulant for a specified length of time, after which the extracted residue is dried (105-110 °C) and weighed. When vegetable oil is to be used as a simulant, the overall migration in the oil is determined as the loss in the mass of specimens after the contact with the simulant.

There are two requirements for the OML:

- No more than 10 mg of total constituents can be released per dm^2 of food contact surface (mg/dm^2)
- In cases where the item is intended to be brought into contact with food for infants and young children, the overall migration cannot exceed more than 60 mg of total of constituents released per kilogram of food simulant (mg/kg).

1.6 Analytical techniques

This section gives a detailed overview of the mass spectrometry techniques that were used during the framework of this PhD study.

1.6.1 Gas chromatography electron ionisation (tandem) mass spectrometry

In gas chromatography (GC) the analytes are separated on the GC column due to differences in volatility and interactions with the stationary phase. Once the analytes are eluted from the column they reach the ionisation source. For electron ionisation (EI), ionisation occurs as follows: electrons are released from a heated filament after which they migrate towards a cathode due to a 70 eV potential difference. Along their way they can encounter and collide with analyte molecules that are in the gas phase. These 70 eV electrons possess sufficient energy to provoke that due to this impact, an electron is removed from the analyte molecule forming a radical cation. This reaction is shown by the following equation:

$$M + e^- \rightarrow M^{+\bullet} + 2\ e^-$$

A positively charged plate located on one side of the ionisation chamber then drives the radical cations formed by a system of lenses toward the mass spectrometer (MS). There, the radical cations and the derived fragments are separated according to their respective *m/z* values.

To this end, the GC is coupled further in this work to a quadrupole mass spectrometer. A quadrupole mass analyser is built up by four parallel hyperbolic rods to which certain direct current (DC) and radio frequency (RF) voltages are applied. An equal but opposite DC voltage superimposed with a RF voltage is applied to the diagonally placed pair of rods. Ions can then be selected based on their path stability in the applied oscillating electric field between the four rods. By applying certain DC and RF voltages only ions with a specific *m/z* value will be able to pass through the quadrupole and reach the detector. The quadrupole can also be operated in the scan mode, implying that all *m/z* values within a specified range are able to reach the detector (Bart 2005). Since EI has the ability to produce highly reproducible fragmentation spectra, mass spectra databases are available to which experimentally obtained spectra can be compared.

A triple quadruple MS consists of three quadrupoles, where in fact of the two addition quadrupoles the second one is not *m/z* selective but serves as a collision cell (therefore generally abbreviated as QqQ). Here the selected ions from the first quadrupole are fragmented by impact with an inert collision gas (mostly nitrogen). This additional characteristic fragmentation increases the specificity since the third quadrupole can then isolate specific ion fragments (product ions) to let them pass to the electron multiplier detector (Ho et al. 2003)(Figure 1.12).

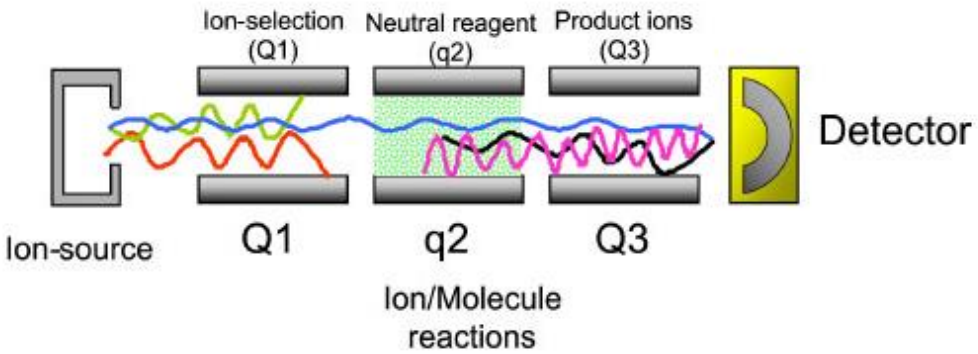


Figure 1. 12: Schematic overview of a triple quadrupole mass spectrometer

1.6.2 GC time-of-flight EI-MS

Using high resolution time-of-flight mass spectrometry (TOF-MS), the identification process improves as accurate masses of the ions are obtained. In TOF-MS, ions are accelerated by an electric field in a flight tube (~ 1m long) in which they travel to the detector. The time necessary to reach the detector is measured since this depends on the accurate mass and charge of the accelerated ions (Figure 1.13). Characterised by its high scanning speed and broad measuring range the sensitivity of TOF-MS is notably higher than of the quadrupole MS when working in full spectrum acquisition (Stachniuk & Fornal 2016). The compounds tentatively identified by library matching can be confirmed by checking the accurate masses of the product ions, and the molecular ion (if present in the EI spectrum) and ambiguous results in the library search can be partly resolved (Hernández, Portolés, et al. 2011).

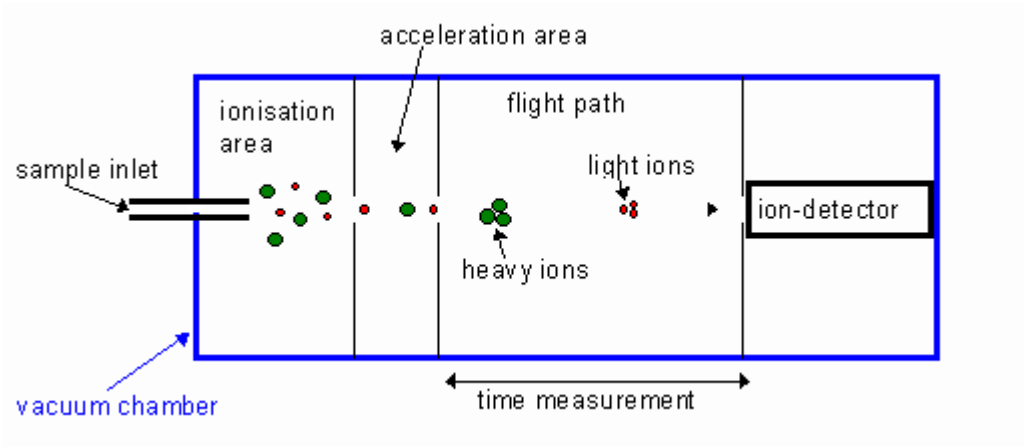


Figure 1.13: Schematic overview of Time of Flight Mass Spectrometer

1.6.3 GC-Atmospheric Pressure Chemical Ionisation-MS

One of the major drawbacks faced during the identification of unknown compounds when EI is used as an ionisation technique is often the absence/low abundance of the molecular ion ($M^{+\bullet}$). Due to the "hard" ionisation a high degree of fragmentation occurs and very often the molecular ion cannot be seen in the EI spectrum anymore. This is an important deficit when facing structural elucidation, as the presence of the molecular ion in a mass spectrum, especially if measured at accurate mass, provides crucial information about the character of the unknown compound. 'Soft' ionisation techniques that produce spectra with less fragmentation and keep the molecule intact are required here. Recently, a new ionisation source called atmospheric pressure chemical ionisation (APCI) has been made commercially available for coupling to GC. The APCI technique produces a soft and universal ionisation, benefiting the presence of the (quasi)molecular ion. This implies a higher sensitivity and specificity especially for compounds that show significant fragmentation in EI. Using a reagent gas (nitrogen) and a lower energy than EI, by means of charge transfer from the generated plasma ($N2^{+\bullet}$ and $N4^{+\bullet}$) to the analyte molecules the $M^{+\bullet}$ is formed. Simultaneously, another reaction mechanism is possible with the traces of water vapour present in the source, generating the protonated molecule $[M+H]^+$ by means of a protonation process starting from the $[H_3O]^+$ ion. The formation of this protonated molecule can be enhanced by the addition of modifiers in the interior of the source such as water or methanol (Figures 1.14 and 1.15).

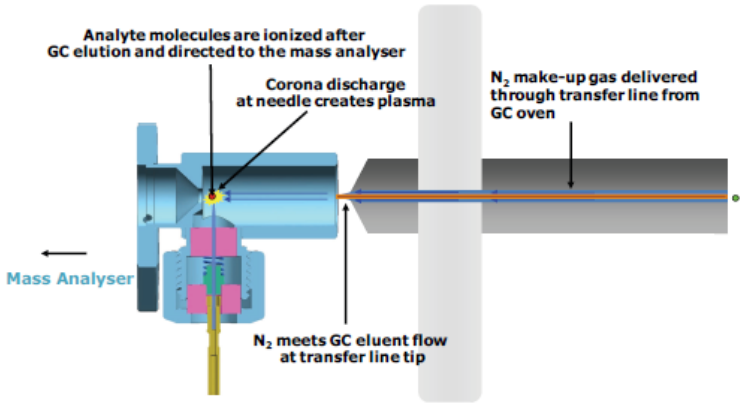


Figure 1.14: APCI source coupled to GC (Adapted from Waters Corporation)

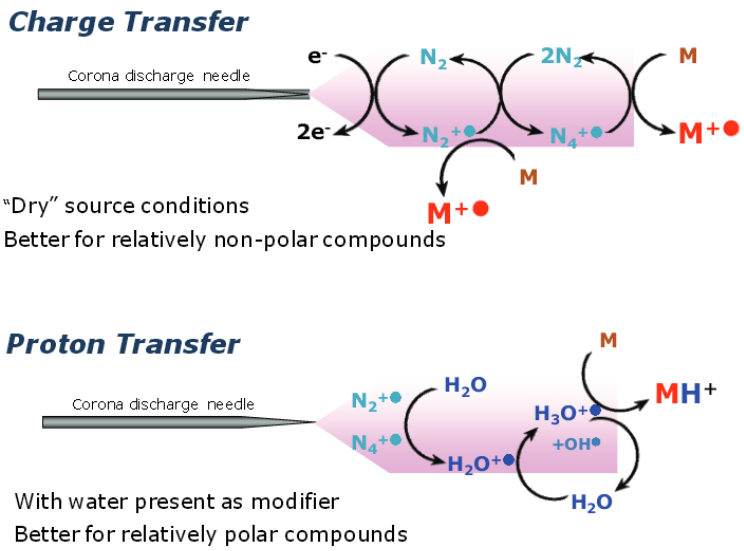


Figure 1.15: APCI ionisation process (Adapted from Waters Corporation)

This highly facilitates a rapid and sensitive large-scope screening based on the investigation of the molecular ion/protonated molecule which in turn eases the derivation of possible molecular formulae. The APCI interface is very promising as it is more universal than Chemical Ionisation (CI) which is more restricted to specific chemical classes. Furthermore, it permits coupling of GC with a wide range of high resolution mass spectrometers (HRMS: MS/MS, TOF, QTOF) initially developed for liquid chromatography (LC)-MS. The potential of GC-(APCI)TOF-MS has recently been demonstrated in other fields, such as pesticide residue or water analysis (Portolés et al. 2012; Portolés et al. 2014; Pintado-Herrera et al. 2014). To our knowledge, its application to the analysis of migrants from plastic FCMs has been rather limited. This technique has been explored for the analysis of adhesives and non-intentionally added

substances (Canellas et al. 2012; Domeño et al. 2012; Canellas et al. 2014), although no work applying the APCI source was yet conducted on plastic baby bottles.

1.6.4 Liquid chromatography mass spectroscopy

For liquid chromatography (LC), generally the stationary phase used is a column filled with silica particles, in which functional groups are implanted. In the case of reversed-phase (RP) chromatography, the stationary phase is relatively non-polar. The mobile phase is a solvent or solvent mixture and is relatively polar in case of RP. Isocratic elution (same mobile phase composition during the run) or gradient elution (in which the composition of the mobile phase will change in function of time) can be used. The separation of the analytes that are placed on the column is carried out by establishing an equilibrium for the analytes between the stationary and the mobile phase. For each analyte, this equilibrium is different, since it depends on the polarity, charge, etc. of the molecule. The mobile phase then takes the analytes through the column in the direction of the mass spectrometer.

To study the migration of non-volatile compounds from FCMs, LC-MS with electrospray ionisation (ESI) is the most suitable approach to be applied (Gallart-Ayala et al. 2013). As shown in Figure 1.16, next to the aforementioned GC-MS techniques the application of LC-ESI-MS is necessary to cover also the medium and highly polar migrants. Other ionisation techniques for LC such as Atmospheric Pressure Photo Ionisation (APPI) or Atmospheric Pressure Chemical Ionisation are also available. However, their applicability is rather limited to non-polar compounds, which are here supposedly covered by the GC-MS techniques.

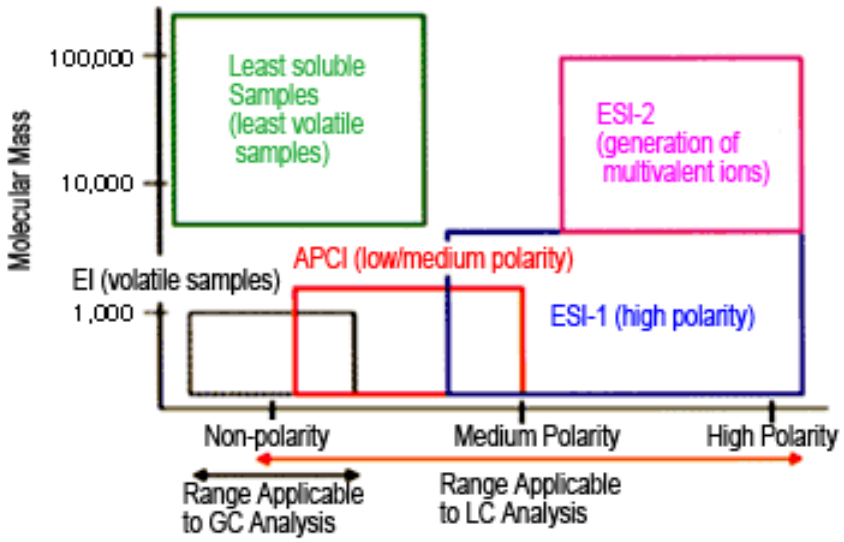


Figure 1.16: Ionisation methods and applicable compounds (source:(Shimadzu 2016)

In order to enable detection of the analyte molecules they have to be ionised, in our case by the application of ESI. With this method, the mobile phase that leaves the chromatographic column is introduced into the ionisation source through a stainless steel capillary. At the outlet of the capillary, the sample dissolved in the solvent is exposed at atmospheric pressure to an elevated voltage (3-6 kV) and a strong nebulising gas (mostly nitrogen). This results in the atomisation of the sample into charged microdroplets, which exhibit the same polarity as the capillary voltage. Then, the solvent is vaporised from the droplet surface with a stream of (mostly) nitrogen drying gas until the ions are desorbed and charged ions in the gas phase are formed which finally enter the mass analyser through focusing lenses (Ho et al. 2003; Williams & Fleming 2007; Stachniuk & Fornal 2016). Figure 1.17 shows how positive ions are formed. For the negative ionisation mode, the capillary voltage is reversed (turned into negative) as well as the sampling cone voltage (+ here). Ions of analytes are generated in ESI either by charge separation or by adduct formation. Examples of ion formation in ESI of a common compound in the positive mode are given below:

$R\text{-}NH_2 + H^+ \rightarrow R\text{-}NH_3^+$ (protonation)
$C_6H_{12}O_6 + Na^+ \rightarrow C_6H_{12}O_6Na^+$ (adduct formation)

Whilst for the negative mode ion formation is as follows:

$R\text{-}COOH \rightarrow R\text{-}COO^- + H^+$ (deprotonation)
$C_6H_{12}O_6 \rightarrow C_6H_{11}O_6^- + H^+$ (deprotonation)
$C_6H_{12}O_6 + A^- \rightarrow C_6H_{12}O_6A^-$ (adduct formation, A= Cl^-, $CH3COO^-$, $HCOO^-$ etc.)

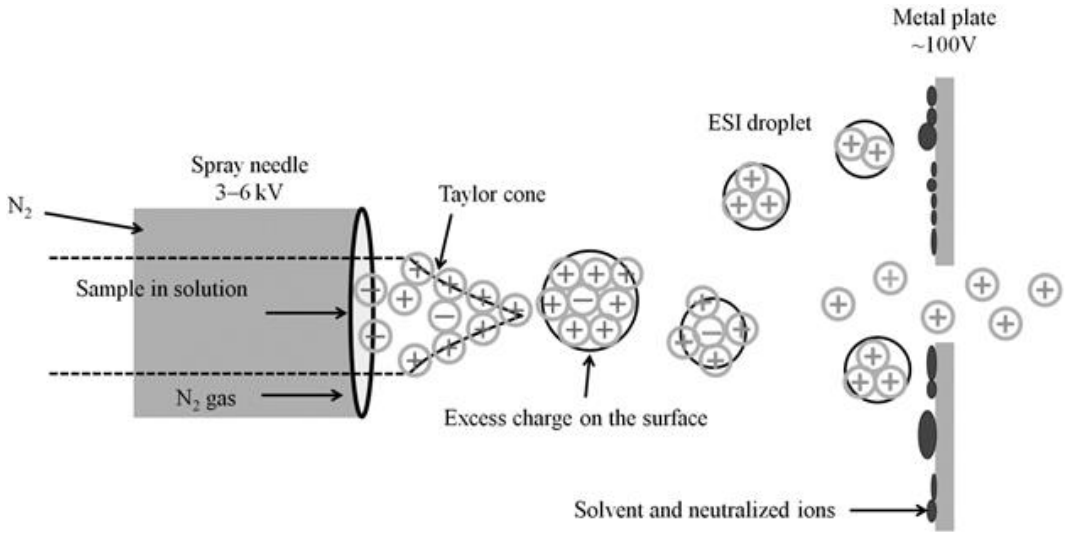


Figure 1.17: Schematic overview of an ESI source operating in the positive ionisation mode (NPTEL 2016)

Only for few classes of compounds, such as pharmaceuticals or pesticides, LC mass spectral libraries are available due to the prominent spectral differences induced by the use of different ionisation sources. Therefore, until now, most of the analysis of non-volatile plastic migrants has been limited to targeted approaches by monitoring pre-selected families of compounds, such as phthalates, ultraviolet (UV)-ink photoinitiators or antioxidants (Gallart-Ayala et al. 2013). For these target approaches, LC is coupled to QqQ mass analysers based on the same principles as explained in section 1.6.1. On the other hand, the use of HRMS is mandatory for screening purposes. LC-TOF-MS has already shown its efficiency for screening and confirmation in the analysis of forensic (illicit drugs) and environmental samples (pesticides, flame retardants, etc.) (Hernández et al. 2008; Hernández, Bijlsma, et al. 2011; Masia et al. 2013; Vandeneede et al. 2013; Ibáñez et al. 2013; Hernández et al. 2015). Furthermore, few non-targeted studies have been published on possible contaminants migrating from FCMs (Félix et al. 2012; Aznar et al. 2012; Biedermann & Grob 2013; Isella et al. 2013; Vera et al. 2013; Cherta et al. 2015)

References

Alin J, Hakkarainen M. 2011. Microwave heating causes rapid degradation of antioxidants in polypropylene packaging, leading to greatly increased specific migration to food simulants as shown by ESI-MS and GC-MS. J Agric Food Chem. 59:5418–5427.

Aznar M, Rodriguez-Lafuente A, Alfaro P, Nerín C. 2012. UPLC-Q-TOF-MS analysis of non-volatile migrants from new active packaging materials. Anal Bioanal Chem. 404:1945–1957.

Barret J. 2013. Assessing the Safety of a Replacement Chemical: Nongenomic activity of bisphenol-S. Environ Health Perspect. 121:97.

Bart JC. 2005. Additives in polymers-Industrial Analysis and Applications. John Wiley & Sons, editor. Hoboken, NJ, USA.

Belgian statute book. 2012. Law of 4 September 2012 amending the Law of 24 January 1977 on the protection of the health of the users in terms of food and other products, to ban Bisphenol A in food packaging. Brussels.

Biedermann M, Grob K. 2013. Is comprehensive analysis of potentially relevant migrants from recycled paperboard into foods feasible? J Chromatogr A. 1272:106–115.

Bittner GD, Yang CZ, Stoner MA. 2014. Estrogenic chemicals often leach from BPA-free plastic products that are replacements for BPA-containing polycarbonate products. Environ Health. 13:41.

British Plastics Federation. 2016. Nylons (Polyamide) [Internet]. [cited 2016 Jan 25]. Available from: http://www.bpf.co.uk/plastipedia/polymers/polyamides.aspx

Bundesministerium für Gesundheit. 2011. Verbot der Verwendung von Bisphenol A in Beruhigungssaugern und Beissringen. Bundesgesetzblatt für die Republik Österreich. II:2011.

Canellas E, Vera P, Domeño C, Alfaro Tena P, Nerín C. 2012. Atmospheric pressure gas chromatography coupled to quadrupole-time of flight mass spectrometry as a powerful tool for identification of nias. J Chromatogr A. 1235:141–148.

Canellas E, Vera P, Nerín C. 2014. Atmospheric pressure gas chromatography coupled to quadrupole-time of flight mass spectrometry as a tool for identification of volatile migrants from autoadhesive labels used for direct food contact. J Mass Spectrom. 49:1181–1190.

Cao X-L, Corriveau J. 2008. Survey of bisphenol A in bottled water products in Canada. Food Addit Contam Part B. 1:161–164.

Ceresana. 2016. Market study: engineering plastics [Internet]. [cited 2016 Jan 3]. Available from: http://www.ceresana.com/en/market-studies/plastics/engineering-plastics/

Cherta L, Portolés T, Pitarch E, Beltran J, López FJ, Calatayud C, Company B, Hernández F. 2015. Analytical strategy based on the combination of gas chromatography coupled to time-of-flight and hybrid quadrupole time-of-flight mass analyzers for non-target analysis in food packaging. Food Chem. 188:301–308.

Chrysant SG. 2015. Association of Exposure to Bisphenol A and Incidence of Cardiovascular Disease and Hypertension. J Clin Hypertens. 17:737–739.

Council of Europe. 2004. Resolution ResAP(2004)5 on silicones used for food contact applications. Off J Eur Union [Internet]. Available from:

https://wcd.coe.int/ViewDoc.jsp?id=797547&Site=CM
Council of Europe. 2016. Food Contact Materials [Internet]. [cited 2016 Mar 20]. Available from: http://www.coe.int/t/e/social_cohesion/soc-sp/public_health/food_contact/presentation.asp#TopOfPage
Covaci A, Hond E Den, Geens T, Govarts E, Koppen G, Frederiksen H, Knudsen LE, Morck TA, Gutleb AC, Guignard C, et al. 2015. Urinary BPA measurements in children and mothers from six European member states: Overall results and determinants of exposure. Environ Res. 141:77–85.
Danish Veterinary and Food Administration. 2011. Bekendtgørelse om fødevarekontaktmaterialer [Internet]. [cited 2016 Feb 12]. Available from: https://www.retsinformation.dk/forms/r0710.aspx?id=152320
Domeño C, Canellas E, Alfaro P, Rodriguez-Lafuente A, Nerín C. 2012. Atmospheric pressure gas chromatography with quadrupole time of flight mass spectrometry for simultaneous detection and quantification of polycyclic aromatic hydrocarbons and nitro-polycyclic aromatic hydrocarbons in mosses. J Chromatogr A. 1252:146–54.
EFSA - European Food Safety Authority. 2008a. Note for guidance. :1–5.
EFSA - European Food Safety Authority. 2008b. Toxicokinetics of Bisphenol A. EFSA J. 759:1–10.
EFSA - European Food Safety Authority. 2010a. Statistical re-analysis of the Biel maze data of the Stump et al (2010) study : “Developmental neurotoxicity study of dietary bisphenol A in Sprague-Dawley rats.” EFSA J. 8:1–67.
EFSA - European Food Safety Authority. 2010b. 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 J. 8:18–29.
EFSA - European Food Safety Authority. 2015a. Food Contact Materials [Internet]. [cited 2015 Dec 7]. Available from: http://www.efsa.europa.eu/en/topics/topic/foodcontactmaterials
EFSA - European Food Safety Authority. 2015b. Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs. EFSA J. 13:1–23.
EFSA Scientific Committee (SC). 2013. Scientific Opinion on the hazard assessment of endocrine disruptors: Scientific criteria for identification of endocrine disruptors and appropriateness of existing test methods for assessing effects mediated by these substances on human health and the env. EFSA J. 11:31–32.
Elskens M, Vloeberghs D, Van Elsen L, Baeyens W, Goeyens L. 2012. Multiple testing of food contact materials: A predictive algorithm for assessing the global migration from silicone moulds. Talanta. 99:161–166.
Entec polymers. 2016. Polypropylene homopolymer [Internet]. [cited 2016 Jan 26]. Available from: http://www.entecpolymers.com/products/polypropylene-homopolymer/
Environment Canada-Health Canada. 2008a. Screening Assessment for the Challenge Octamethylcyclotetrasiloxane (D4).
Environment Canada-Health Canada. 2008b. Screening Assessment for the Challenge Decamethylcyclopentasiloxane (D5).
Environment Canada-Health Canada. 2015. Screening Assessment for the Challenge Trisiloxane, octamethyl-(MDM).

European Chemicals Agency. 2013. Justification for the selection of a candidate CoRAP substance: Formaldehyde. :1–7.
European Chemicals Agency. 2014. Identification of PBT and VpVB substance, results of evaluation of PBT/vPvB properties. :1–171.
European Chemicals Agency. 2015. Community rolling action plan (CoRAP) update covering years 2014 , 2015 and 2016. :1–31.
European Commission (EU). 1990. Commission Directive 90/128/EE: of 23 February 1990 relating to plastic materials and article intended to come into contact with foodstuffs. Off J Eur Union. L75:19–40.
European Commission (EU). 2002. Commission Directive 2002/72/EC of 6 August 2002 relating to plastic materials and articles intended to come into contact with foodstuffs. Off J Eur Union. L220:18–58.
European Commission (EU). 2004. Commission directive 2004/19/EC of 1 March 2004 amending Directive 2002/72/EC relating to plastic materials and articles intended to come into contact with foodstuffs. Off J Eur Union. L71:8–21.
European Commission. 2002. Opinion of the Scientific Committee on Food on Bisphenol A [Internet]. [cited 2016 Mar 15]. Available from: http://ec.europa.eu/food/fs/sc/scf/out128_en.pdf
European Commission. 2015. References of the European and national legislations-working document [Internet]. [cited 2016 Mar 7]:1–71. Available from: http://ec.europa.eu/food/safety/docs/cs_fcm_non-harmonised.pdf
European Council. 2004. Regulation (EC) No 1935/2004 of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC. Eur Off J.:1–20.
European Information Centre on Bisphenol A. 2015. European Information Centre on Bisphenol A [Internet]. [cited 2016 Feb 24]. Available from: http://www.bisphenol-a-europe.org/en_GB/what-is-bisphenol-a
European Union. 2006. Commission Regulation (EC) No 2023/2006 of 22 December 2006 on good manufacturing practice for materials and articles intended to come into contact with food. Off J Eur Union. L384:75–78.
European Union. 2011a. 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. Off J Eur Union. L26:11–14.
European Union. 2011b. Commission Regulation (EU) No 10/2011 of 14 Januari 2011 on plastic materials and articles intended to come into contact with food. Off J Eur Union. L12:1–89.
European Union. 2011c. Commission implementing regulation (EU) No. 321/2011 of 1 April 2011 amending Regulation (EU) No. 10/2011 as regards the restriction of use of Bisphenol A in plastic infant feeding bottles. Off J Eur Union. L87:1–2.
Félix JS, Isella F, Bosetti O, Nerín C. 2012. Analytical tools for identification of non-intentionally added substances (NIAS) coming from polyurethane adhesives in multilayer packaging materials and their migration into food simulants. Anal Bioanal Chem. 403:2869–2882.
Feng Y, Jiao Z, Shi J, Li M, Guo Q, Shao B. 2016. Effects of bisphenol analogues on steroidogenic gene expression and hormone synthesis in H295R cells.

Chemosphere. 147:9–19.
Food and Drug Administration. 2012. Indirect Food Additives: Polymers. Fed Regist. 77:41899.
Food and Drug Administration. 2013. Indirect Food Additives: Adhesives and Components of Coatings. Fed Regist. 78:41840.
Forrest M. 2009. Food contact materials- Rubbers, silicones, coatings and inks. Shawbury, Shrewsbury, Shropshire, United Kingdom: Smithers Rapra.
Foster E, Mathers JC, Adamson AJ. 2010. Packaged food intake by British children aged 0-6 years. Food Addit Contam Part A. 27:380–388.
Frye C, Bo E, Calamandrei G, Calzàe L, Dessì-Fulgheri F, Fernández M, Fusani L, Kah O, Kajta M, Page Y Le, et al. 2012. Endocrine disrupting Compounds: a review of some sources, effects and mechanisms of actions on behaviour and neuroendocreine systems. J Neuroendocrinol. 24:144–159.
Gallart-Ayala H, Nuñez O, Lucci P. 2013. Recent advances in LC-MS analysis of food-packaging contaminants. TrAC - Trends Anal Chem. 42:186–204.
Gies A, Soto AM. 2013. Bisphenol A: contested science, divergent safety evaluations. Late lessons from early Warn Sci precaution, Innov. II:215–239.
Goldstone AE, Chen Z, Perry MJ, Kannan K, Louis GMB. 2015. Urinary bisphenol A and semen quality, the LIFE study. Reprod Toxicol. 51:7–13.
Guart A, Wagner M, Mezquida A, Lacorte S, Oehlmann J, Borrell A. 2013. Migration of plasticisers from Tritan and polycarbonate bottles and toxicological evaluation. Food Chem. 141:373–380.
He B, Rhodes-Brower S, Miller MR, Munson AE, Germolec DR, Walker VR, Korach KS, Meade BJ. 2003. Octamethylcyclotetrasiloxane exhibits estrogenic activity in mice via ER α. Toxicol Appl Pharmacol.:254–261.
Heimrich M, Nickl H, Bönsch M, Simat TJ. 2015. Migration of Cyclic Monomer and Oligomers from Polyamide 6 and 66 Food Contact Materials into Food and Food Simulants: Direct Food Contact. Packag Technol Sci. 28:123–139.
Helling R, Kutschbach K, Simat TJ. 2010. Migration behaviour of silicone moulds in contact with different foodstuff. Food Addit Contam Part A. 27:396–405.
Helling R, Mieth A, Altmann S, Simat TJ. 2009. Determination of the overall migration from silicone baking moulds into simulants and food using 1H-NMR techniques. Food Addit Contam Part A. 26:395–407.
Helling R, Seifried P, Fritzsche D, Simat TJ. 2012. Characterisation and migration properties of silicone materials during typical long-term commercial and household use applications: a combined case study. Food Addit Contam Part A. 29:1489–1500.
Hengstler JG, Foth H, Gebel T, Kramer P-JP-J, Lilienblum W, Schweinfurth H, Völkel W, Wollin K-MK-M, Gundert-Remy U. 2011. Critical evaluation of key evidence on the human health hazards of exposure to bisphenol A. Crit Rev Toxicol. 41:263–91.
Hernández F, Bijlsma L, Sancho J V, Díaz R, Ibáñez M. 2011. Rapid wide-scope screening of drugs of abuse, prescription drugs with potential for abuse and their metabolites in influent and effluent urban wastewater by ultrahigh pressure liquid chromatography-quadrupole-time-of-flight-mass spectrometry. Anal Chim Acta. 684:87–97.
Hernández F, Ibáñez M, Portolés T, Cervera MI, Sancho J V, López FJ. 2015. Advancing towards universal screening for organic pollutants in waters. J Hazard Mater.

282:86–95.
Hernández F, Portolés T, Pitarch E, López FJ. 2011. Gas chromatography coupled to high-resolution time-of-flight mass spectrometry to analyze trace-level organic compounds in the environment, food safety and toxicology. Trends Anal Chem. 30:388–400.
Hernández F, Sancho JV, Ibáñez M, Grimalt S. 2008. Investigation of pesticide metabolites in food and water by LC-TOF-MS. Trends Anal Chem. 27:862–872.
Ho CS, Lam CWK, Chan MHM, Cheung RCK, Law LK, Lit LCW, Ng KF, Suen MWM, Tai HL. 2003. Electrospray ionisation mass spectrometry: principles and clinical applications. Clin Biochem. 24:3–12.
Hoekstra EJ, Simoneau C. 2013. Release of bisphenol a from polycarbonate-a review. Crit Rev Food Sci Nutr. 53:386–402.
Ibáñez M, Bijlsma L, van Nuijs ALN, Sancho J V, Haro G, Covaci A, Hernández F. 2013. Quadrupole-time-of-flight mass spectrometry screening for synthetic cannabinoids in herbal blends. J Mass Spectrom. 48:685–94.
International Food Safety Authorities Network (INFOSAN). 2009. International Food Safety Authorities Network (INFOSAN) BISPHENOL A (BPA) - Current state of knowledge and future actions by WHO and FAO. :1–6.
Isella F, Canellas E, Bosetti O, Nerín C. 2013. Migration of non intentionally added substances from adhesives by UPLC-Q-TOF/MS and the role of EVOH to avoid migration in multilayer packaging materials. J Mass Spectrom. 48:430–7.
Jeana PA, Plotzkea KP, Sciallib AR. 2015. Chronic toxicity and oncogenicity of decamethylcyclopentasiloxane in the Fischer 344 Rat. Regul Toxicol Pharmacol. 74:S57–S66.
Kang JS, Choi J-S, Kim W-K, Lee Y-J, Park J-W. 2014. Estrogenic potency of bisphenol S, polyethersulfone and their metabolites generated by the rat liver S9 fractions on a MVLN cell using a luciferase reporter gene assay. Reprod Biol Endocrinol. 12:102.
KIK. 2016. Polyethersulfon [Internet]. [cited 2016 Jan 10]. Available from: http://www.kik.nl/polyethersulfon-pes
King Plastic. 2016. Polypropylene safety data sheet [Internet]. [cited 2016 Jan 23]. Available from: http://www.kingplastic.com/wp-content/uploads/2014/05/King_KPC_Polypropylene_SDS.pdf
Kolsek K, Gobec M, Mlinaric Rascan I, Sollner Dolenc M. 2014. Screening of bisphenol A, triclosan and paraben analogues as modulators of the glucocorticoid and androgen receptor activities. Toxicol Vitr. 29:8–15.
Kuruto-Niwa R, Nozawa R, Miyakoshi T, Shiozawa T, Terao Y. 2005. Estrogenic activity of alkylphenols, bisphenol S, and their chlorinated derivatives using a GFP expression system. Environ Toxicol Pharmacol. 19:121–30.
Lakind JS, Goodman M, Mattison DR. 2014. Bisphenol A and indicators of obesity, glucose metabolism/type 2 diabetes and cardiovascular disease: a systematic review of epidemiologic research. Crit Rev Toxicol. 44:121–50.
Liao C, Liu F, Alomirah H, Loi VD, Mohd MA, Moon H-B, Nakata H, Kannan K. 2012. Bisphenol S in urine from the United States and seven Asian countries: occurrence and human exposures. Environ Sci Technol. 46:6860–6866.
Liao C, Liu F, Guo Y, Moon H-B, Nakata H, Wu Q, Kannan K. 2012. Occurrence of eight bisphenol analogues in indoor dust from the United States and several Asian

countries: implications for human exposure. Environ Sci Technol. 46:9138–45.
Lund KH, Petersen JH. 2002. Safety of food contact silicone rubber: Liberation of volatile compounds from soothers and teats. Eur Food Res Technol. 214:429–434.
Masia A, Ibáñez M, Blasco C, Sancho JV, Picó Y, Hernández F. 2013. Combined use of liquid chromatography triple quadrupole mass spectrometry and liquid chromatography quadrupole time-of-flight mass spectrometry in systematic screening of pesticides and other contaminants in water samples. Anal Chim Acta. 761:117–127.
McCall E, Keegan J, Foley B. 2012. Primary aromatic amine migration from polyamide kitchen utensils: method development and product testing. Food Addit Contam Part A. 29:149–160.
Mcdonald GR, Hudson AL, Dunn SMJ, You H, Baker GB, Whittal RM, Martin JW, Jha A, Edmondson DE, Holt A. 2008. Bioactive Contaminants Leach from disposable laboratory plasticware. Science. 322:917.
Meuwly R, Sager F, Brunner K, Dudler V. 2007. Migration of Siloxane Oligomers in Foodstuffs from Silicone Baking Moulds. Dtsch Leb Rundschau. 103:561–568.
Nam S-H, Seo Y-M, Kim M-G. 2010. Bisphenol A migration from polycarbonate baby bottle with repeated use. Chemosphere. 79:949–52.
National Food Institute Norway. 2014. Analysis of primary aromatic amines (PAA) in black nylon kitchenware 2014 [Internet]. [cited 2016 Feb 15]:1–24. Available from: www.food.dtu.dk/~/media/Institutter/Foedevareinstituttet/Publikationer/Pub-2015/Rapport_Analysis of PAA in black nylon kitchenware 2014.ashx?la=da+&cd=1&hl=es&ct=clnk&gl=be
Niederberger C. 2011. Re: Urine bisphenol-A (BPA) level in relation to semen quality. J Urol. 186:625–631.
NPTEL. 2016. Schematic overview of an ESI source [Internet]. [cited 2016 Feb 17]. Available from: http://nptel.ac.in/courses/102103044/module2/lec11/7.html
OJ of the French Republic. 2012. Regulation No 1442/2012 of 24 December 2012 aiming at banning the manufacture, import, export and commercialisation of all forms of food packaging containing bisphenol A. :Text 2 of 154.
Osimitz TG, Eldridge ML, Sloter E, Welsh W, Ai N, Sayler GS, Menn F, Toole C. 2012. Lack of androgenicity and estrogenicity of the three monomers used in Eastman's Tritan copolyesters. Food Chem Toxicol. 50:2196–2205.
Perez Lobato R, Mustieles V, Calvente I, Diaz Jimenez I, Ramos R, Caballera Casero N, Jiminez Lopez JF, Rubio S, Olea N, Fernandez MF. 2016. Exposure to bisphenol A and behavior in school-age children. Neurotoxicology. 53:12–19.
Pintado-Herrera MG, González-Mazo E, Lara-Martín P a. 2014. Atmospheric pressure gas chromatography-time-of-flight-mass spectrometry (APGC-ToF-MS) for the determination of regulated and emerging contaminants in aqueous samples after stir bar sorptive extraction (SBSE). Anal Chim Acta. 851:1–13.
Plastics Europe. 2011. Applications of Bisphenol A [Internet]. Available from: http://www.bisphenol-a-europe.org/uploads/EN_BPA applications 2.pdf
Plastics Europe. 2016. Polypropylene (PP) [Internet]. [cited 2016 Jan 21]. Available from: http://www.plasticseurope.org/what-is-plastic/types-of-plastics-11148/polyolefins/polypropylene.aspx
Portolés T, Mol JGJ, Sancho J V, Hernández F. 2012. Advantages of atmospheric pressure

chemical ionization in gas chromatography tandem mass spectrometry: pyrethroid insecticides as a case study. Anal Chem. 84:9802–10.
Portolés T, Mol JGJ, Sancho J V, Hernández F. 2014. Use of electron ionization and atmospheric pressure chemical ionization in gas chromatography coupled to time-of-flight mass spectrometry for screening and identification of organic pollutants in waters. J Chromatogr A. 1339:145–53.
Pubchem Open Chemistry Database. 2015. Bisphenol-A [Internet]. [cited 2015 Dec 2]. Available from: http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=6623
Reingruber E, Himmelsbach M, Sauer C, Buchberger W. 2010. Identification of degradation products of antioxidants in polyolefins by liquid chromatography combined with atmospheric pressure photoionisation mass spectrometry. Polym Degrad Stab. 95:740–745.
Richter CA, Birnbaum LS, Farabollini F, Newbold RR, Rubin BS, Talsness CE, Vandenbergh JG, Walser-Kuntz DR, Saal FS vom. 2007. In Vivo Effects of Bisphenol A in Laboratory Rodent Studies. Reprod Toxicol. 24:199–224.
Rogers JA, Metz L, Yong VW. 2013. Review: Endocrine disrupting chemicals and immune responses: A focus on bisphenol-A and its potential mechanisms. Mol Immunol. 53:421–430.
RTP Co. 2016. Polyethersulfone (PES) [Internet]. [cited 2016 Jan 12]. Available from: http://www.rtpcompany.com/products/product-guide/polyethersulfone-pes/
Rücker C, Kümmerer K. 2015. Environmental Chemistry of Organosiloxanes. Chem Rev. 115:466–524.
Scientific Committee on Food. 1984. Reports of the Scientific Committee for Food (seventeenth series) [Internet]. Available from: http://ec.europa.eu/food/fs/sc/scf/reports/scf_reports_17.pdf
Shimadzu. 2016. Introduction to LC-MS [Internet]. Available from: http://www.shimadzu.com/an/hplc/support/lib/lctalk/47/47intro.html
Simoneau C, Van Den Eede L, Valzacchi S. 2012. Identification and quantification of the migration of chemicals from plastic baby bottles used as substitutes for polycarbonate. Food Addit Contam Part A. 29:469–480.
Simoneau C, Valzacchi S, Morkunas V, Van Den Eede L. 2011. Comparison of migration from polyethersulphone and polycarbonate baby bottles. Food Addit Contam Part A. 28:1763–1768.
Stachniuk A, Fornal E. 2016. Liquid Chromatography-Mass Spectrometry in the Analysis of Pesticide Residues in Food. Food Anal Methods. 9:1654–1665.
Superior Health Council. 2010. Publication of the Superior Health Council number 8697: Bisphenol A. Brussels.
Svensk Författningssamling (SFS). 2013. Regulation No 991/2012 of 20 December 2012 amending the Food Regulation No 813/2006. :p. 1.
Trier X, Okholm B, Foverskov A, Binderup M-L, Petersen JH. 2010. Primary aromatic amines (PAAs) in black nylon and other food-contact materials, 2004-2009. Food Addit Contam Part A. 27:1325–1335.
Vandeneede N, Maho W, Erratico C, Neels H, Covaci A. 2013. First insights in the metabolism of phosphate flame retardants and plasticizers using human liver fractions. Toxicol Lett.:9–15.
Vasile C. 2000. Handbook of polyolefins. Second edi. New York-Basel: Marcel Dekker.

Vera P, Canellas E, Nerín C. 2013. Identification of non-volatile compounds and their migration from hot melt adhesives used in food packaging materials characterized by ultra-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry. Anal Bioanal Chem. 405:4747–4754.

Viñas P, Campillo N, Martínez-Castillo N, Hernández-Córdoba M. 2010. Comparison of two derivatization-based methods for solid-phase microextraction-gas chromatography-mass spectrometric determination of bisphenol A, bisphenol S and biphenol migrated from food cans. Anal Bioanal Chem. 397:115–125.

Viñas R, Watson CS. 2013. Bisphenol S disrupts estradiol-induced nongenomic signaling in a rat pituitary cell line: effects on cell functions. Environ Health Perspect. 121:352–8.

Wetherill YB, Akingbemi BT, Kanno J, McLachlan JA, Nadal A, Sonnenschein C, Watson CS, Zoeller RT, Belcher SM. 2007. In vitro molecular mechanisms of bisphenol A action. Reprod Toxicol. 24:178–198.

Williams DH, Fleming I. 2007. Spectroscopic Methods in Organic Chemistry. 5th editio. McGraww-Hill Education Europe, editor. London.

Wolstenholme JT, Rissman EF, Connelly JJ. 2011. The role of Bisphenol A in shaping the brain, epigenome and behavior. Horm Behav. 59:296–305.

Zhang K, Wong JW, Begley TH, Hayward DG, Limm W. 2012. Determination of siloxanes in silicone products and potential migration to milk, formula and liquid simulants. Food Addit Contam Part A. 29:1311–1321.

Chapter 2:
Outline and Objectives

This PhD study was part of ALTPOLYCARB, a project founded by the Belgian Ministry of Public Health, whose main focus was to determine the possible migration risks of chemicals present in FCMs for children below 3 years. In this framework, the PhD aimed to answer the following research questions:

1. Which alternatives to PC are currently used as FCMs for baby bottles in Belgium?
2. Which materials are used as FCMs or are intended to come into contact with food for children under 3 years?
3. Which substances can migrate from the materials (from question 1 and 2) into the food (or food simulant)?
4. Under which circumstances and in what quantities do these components migrate from the material?

In parallel with this PhD, a consortium of different research institutes (University of Antwerp (UA), Scientific Institute of Public Health (WIV-ISP), University of Liège (ULg), Free University of Brussels (VUB) and the Veterinary and Agrochemical Research Centre (CODA-CERVA)), each skilled with their own expertise in the domain of FCMs, further collaborated to answer questions related to toxicity and biological activity of the migrating substances. In parallel with the chemical identification, the toxicity of the total migration solution was assessed in terms of genotoxicity, mutagenicity and endocrine disrupting effects. Since the transition of migration solutions to *in vitro* cell lines is not evident, due to the incompatibility of the cell lines with the selected simulant (H_2O-EtOH), in first instance the toxicity of the pure components was evaluated. This work was done by the other partners and it was not discussed in this thesis. Moreover, they also assessed the risks for children younger than 1 year and children between 1 and 3 years exposed to substances migrating from packaging materials.

To complete the first objective of this PhD, a thorough literature study was conducted on which alternatives were present to PC baby bottles. Afterwards, a field study documented the presence of these alternative materials on the Belgian market. In addition, the plastics which children under 3 years get in touch with via the diet were mapped to accomplish objective 2. **Chapter 3** discusses these data that were obtained from the market study in collaboration with the ULg.

Although intentionally all FCMs for children < 3 y were documented, the main focus of this PhD study was made on the chemical identification and quantification of substances that could migrate from the alternatives to PC baby bottles. The extensiveness of different materials encountered for FCMs other than baby bottles made it impossible to perform migration testing for all these materials as well. Furthermore, baby bottles are by far the most used FCM to feed infants, and were therefore the main FCM of interest.

However, the results of this market study on FCMs other than baby bottles could be a viable initial platform for future research projects.

Subsequently, we selected the plastics that had to be evaluated for their migration potential. In addition, the migration conditions were also determined based on the current European regulations (EU No. 10/2011) **(Chapter 4)**.

Prior to this PhD study, no expertise was available at the Toxicological Centre for the determination of migrants from baby bottles. Therefore, new analytical techniques had to be developed and optimised within the framework of this project. This was done by an intensive collaboration with the Scientific Institute of Public Health. Since these contaminants are present in trace levels (ng ml^{-1}), subsequent detection techniques with sufficient selectivity and sensitivity had to be used. Therefore, to answer objective 3, a generic liquid-liquid extraction (LLE) method was developed in order to extract an as broad as possible spectrum of chemicals from the migration solutions. These extracts were in first instance analysed by gas chromatography coupled with mass spectrometry (GC-MS) (**Chapter 4.1**) and liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (LC-QTOF-MS). For the identification, the largest peaks from the different chromatograms were identified, because these are probably the products that are migrating in the largest amounts from the alternative materials. Peaks that could not be identified by GC-MS or the initial LC-QTOF-MS screening were studied more into detail by a combination of GC coupled to accurate mass techniques and extended LC-QTOF-MS analysis. This work was done in collaboration with the University Jaume I in Castellon (Spain). The results of this chemical identification are described in **Chapter 4.2**.

Since it was of the utmost importance that the detected compounds comply with the specific migration limits specified in legislation to guarantee a safe use of these baby bottles, migrants had to be accurately quantified as well. To this end, based on the previous experiments, a selection of components for quantification was made. Considering their migrating abundance and evaluated toxicity, a prioritisation of the compounds to be monitored in Objective 4 was made. Therefore, the previously developed LLE was further optimised and the performance of the method was evaluated by the determination of several validation parameters. Accurate quantification of the selected compounds from the selection of baby bottles present on the Belgian market was then done by means of validated GC-QqQ-MS and LC-QqQ-MS. These data were discussed in **Chapter 5.1**.

Furthermore, with these validated methods, the effect of real-life use conditions on the baby bottles such as warming in the microwave, use of the dishwasher and sterilisation was quantitatively determined to assess more into the detail the degree of the

consumer's exposure to these substances. By performing duration tests, the influence of each parameter on the migrating concentrations of the selected compounds was evaluated. In **chapter 5.2,** the results are described of microwave, sterilisation and dishwasher duration tests that had the goal to mimic "real-life use" of these bottles. Again, the influence of these treatments on the monitored compounds was measured with GC- and LC-QqQ MS. Finally, in **Chapter 5.3,** a comparison by means of specific software kits was made between the chromatograms obtained before and after a number of each specific treatment. Consequently, not only the presence of the target compounds, but also any possible formation of other degradation products from the polymers after a specific treatment was checked. To this end, GC-TOF-MS was applied. Future work can focus also on the LC-QTOF-MS analysis hereof.

The obtained qualitative and quantitative results were critically discussed in **Chapter 6**. The major outcomes of each chapter were placed in a wider perspective, and the relevance of these findings was assessed. Furthermore, based on the outcome of this PhD, some suggestions for future work were defined.

Chapter 3:
Market study

3.1 Introduction

A study of the Belgian market was realised with the intention to document the different polymers used as FCMs for infants under 3 years.

3.2 Methods

Initially, a worldwide study was conducted on the Internet to document the globally available polymer alternatives to PC baby bottles and cups. Therefore, research was done in all independent and brand-related online stores. Next the Belgian market was investigated. Samples were taken in October and November 2012. The name of the different products, the brand, the country of manufacture, the type of polymer (if this information was available it was mostly mentioned on the item itself or found on the packaging or the website) and the manufacturer's recommendations were collected from the items. This was done during a period of one month in 14 pharmacies, 13 specific stores for baby articles (e.g. Dreambaby, Fun, Baby 2000, etc.) and five food retailers (e.g. Carrefour, Cora, etc.) near Antwerp and Liège, this in order to give a representative image of the Belgian market. Based on these data, a database was made by type of FCM. In addition, there was also a photo of each item added to this database. Based on the Belgian market study, the relative frequency of occurrence of different polymer materials in the different categories of FCMs (baby bottles, cups, tableware, accessories, storage materials, etc.) was calculated. Each item was counted once every time it was seen and in this way an idea was formed of the relative market share of each type of polymer. All materials made (partly) of polymer material(s) were included in this study. Since migration of organic substances was not expected for FCMs made of glass, they were not investigated here. The identity of the polymers encountered was verified based on the PIC (Polymer Identification Code) and information specified by the producer. However, for several items this information was lacking. Although it was visually clear that different unidentified materials were used, considering the absence of information, they were all categorised under the term “unspecified”.

3.3 Results & Discussion

3.3.1 Belgian market study of FCMs used for infants

The results of the Belgian market study showed that 24 different bottles were encountered. The most widely present material for baby bottles in Belgian shops was PP with a relative frequency of more than half of the sold bottles (61.8%). Other materials offered were among others PES (13.0%), PA (8.6%), silicones (5.3%) and stainless steel (1.3%). The market study revealed that a number of samples were made of unspecified materials. After inquiring the producers, most of these unspecified bottles resulted to be made of the new co-polymer Tritan™ (7.3%). It was noticeable that some PC baby bottles (2.7%) were still present on the shelves, though these were found in some specific local shops and were still sold due to a lack of knowledge of the owners of the new EU and Belgian regulations. The results are summarised in Figure 3.1.

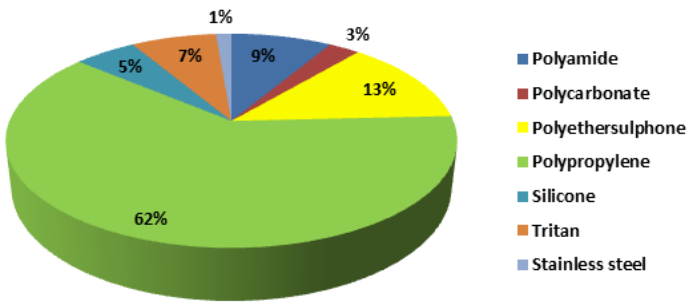


Figure 3.1: Belgian market study of the polymer baby bottle alternatives to PC baby bottles

For the baby cups, 85 different products were found. Hereof, about 70% of the products were made of an unspecified material. The best-known material was PP exhibiting around 20% of the market share. Cups made of melamine, PA, and Low Density Polyethylene (LDPE) were identified. In addition, materials from the polymer category PIC 7 were also found, such as ABS (acrylonitrile-butadiene-styrene) (or possibly PC). The results are shown in figure 3.2:

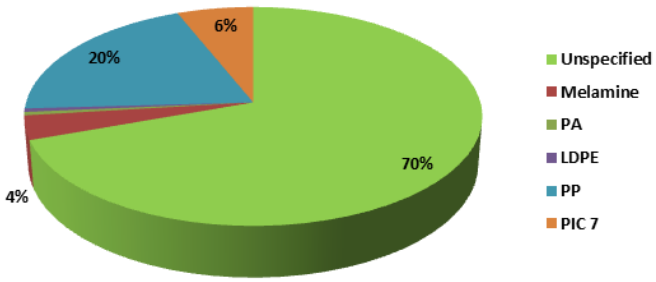


Figure 3.2: Overview of the materials used of baby cups on the Belgian market (LDPE and PA share < 1%)

For the teats present on the Belgian market, more than 80% of the 88 found products were made from silicone. The majority of the remainder was made of latex (15%) (Figure 3.3). The same pattern was observed for pacifiers where 76% was made of silicone and 24% of latex.

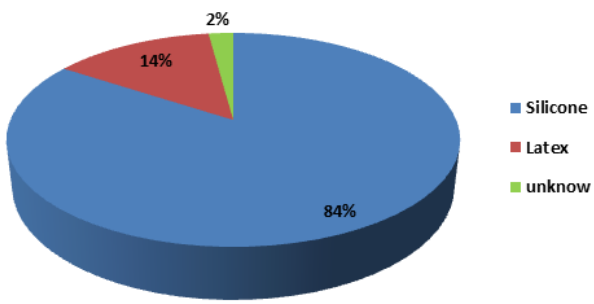


Figure 3.3: Overview of the materials used for teats on the Belgian market

A number of 27 different teethers were found on the Belgian market, more than 60% of which was made of an unspecified material. Identified materials were among others ethylene vinyl acetate, polyethylene terephthalate (PET), PVC, silicone, ABS and a copolymer of acrylamide and sodium acrylate. The results are shown in Figure 3.4.

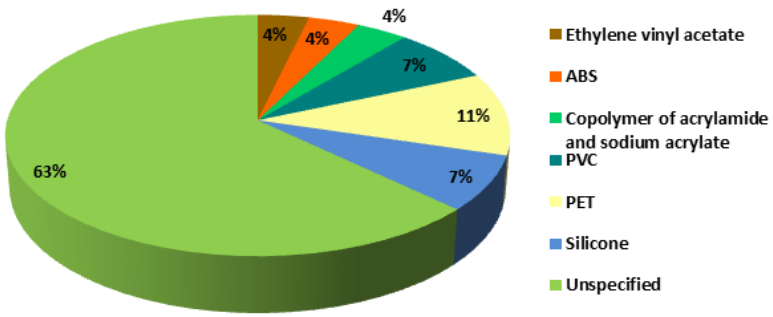


Figure 3.4: Teether materials on the Belgian market

Also for the examined cutlery and dinnerware for babies, more than 60% of the products consisted of an unspecified material. The most common materials were PP (18%) and melamine (13%). Other observed materials were silicone, PC, and PP in a combination with a thermoplastic elastomer (TPE). Figure 3.5 gives a summary of the encountered materials.

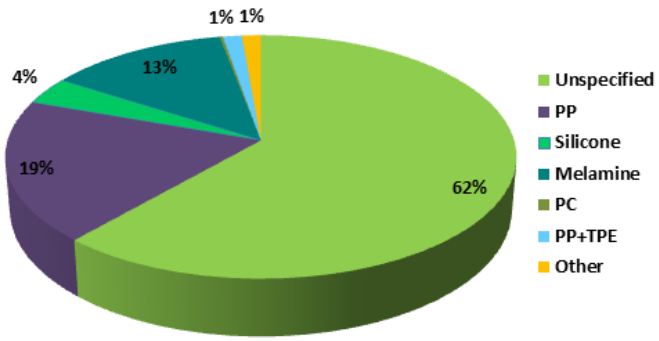


Figure 3.5: Overview of the baby dinnerware materials on the Belgian market

The accessories found on the market for infants and young children, such as storage jars for food, breast pumps, etc. were made for almost 80% of an unidentified polymer. PP was also here the most sold known material (17%). Other identified materials were TPE, silicone and polyethylene terephthalate glycol (PETG). Figure 3.6 gives an overview of the results.

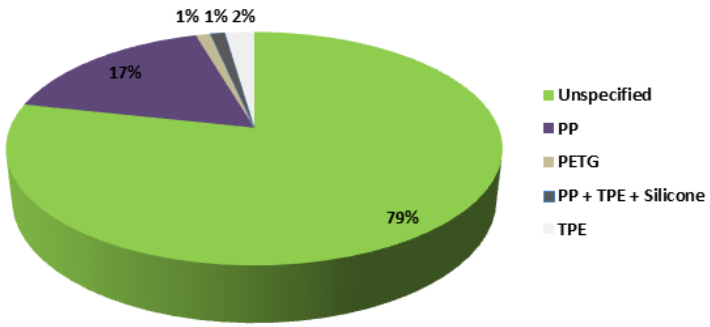


Figure 3.6: Materials used for infants FCMs accessories on the Belgian market

For the packaging of food for children younger than 3 years old, the same trend as previously observed was seen. The majority of the products in this category were made available from an unspecified polymer (44%). PP is again the most commonly found material that could be identified with about 14% market representation. Other materials encountered consisted of among others HDPE, polystyrene (PS), PET, etc. The results are shown in Figure 3.7.

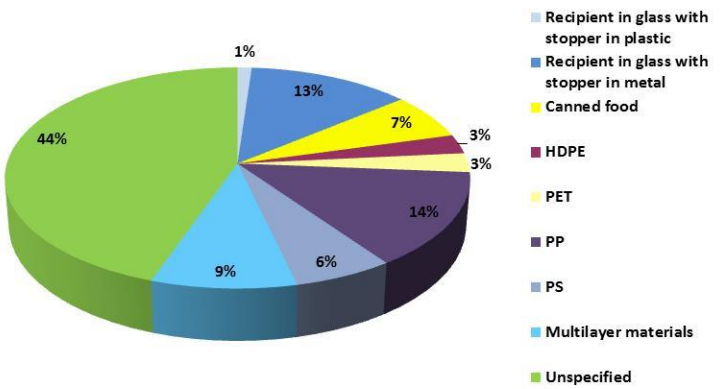


Figure 3.7: Baby food packaging materials on the Belgian market

3.3.2 Internet market study

A number of 126 different polymer baby bottles were found during the worldwide Internet market survey. PP is the most offered material, which represents about 43.7% of the items present. Other materials that were found are silicones (7.1%), PES (4.8%), PA (3.2%), stainless steel (5.6%), Tritan™ (3.2%), and Kostrate (0.8%). 26.2% of the

bottles seen were made of an undisclosed, but declared "BPA-free", material. Another 5.6% was made of an unidentified material. Of the 110 different baby cups encountered, the BPA-free label made more than half of the market share (54%), followed by PP and completely unspecified materials (14%). Results are summarised in Figures 3.8 and 3.9.

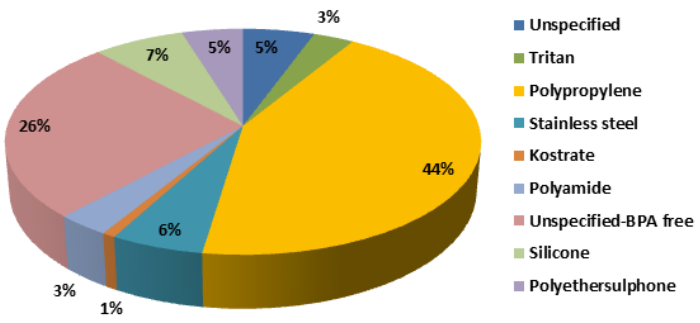


Figure 3.8: Overview of the materials used for baby bottles sold on the internet

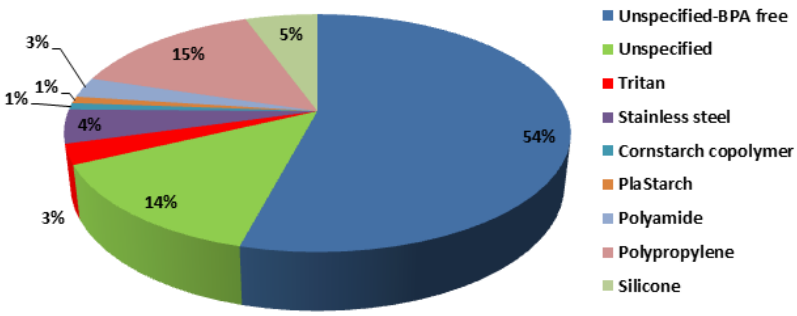


Figure 3.9: Overview of the materials used for baby cups sold on the internet

The Belgian market seemed to exhibit a dominant presence of PP to other alternative materials compared to the worldwide image, though one must take into account that on the internet several bottles and cups were made of an unspecified material which could still influence this tendency significantly. However, although nowadays internet shopping becomes slowly more and more habitual amongst the general public in Belgium (Comeos 2013a), the share of internet sales in Belgium in 2013 still only amounted to 3 percent of total retail sales (Trends 2014). Apparently, consumers generally still perceive more risk in electronic commerce since they cannot visit a physical store and feel and touch products prior to purchase online (Li et al. 2014). Moreover, baby bottles and other FCMs for infants only form a small percentage of these online purchases (Comeos 2013b). Therefore, it does not seem to be expected that many people in Belgium would purchase these unspecified materials that are sold

on the internet. Furthermore, due to the increased media attention on the possible toxic effects of BPA and the consequent ban of this material for the use in baby bottles, awareness concerning the possible issues that could occur from using (poor/unknown) quality FCMs for infants is rising among the general public (Gezinsbond België 2015). This is certainly to be considered as an extra factor that nowadays could influence the behaviour of Belgian consumers regarding the purchase of FCMs for infants.

3.4 Conclusions

The variety of FCMs for infants encountered on both the Belgian and international market resulted to be broad. Moreover, the materials of which these FCMs were made exhibited a wide variety as well. Of the materials that could be identified, PP was generally predominant. Yet, for almost all FCMs (except baby bottles), the polymer(s) of which they were made could not be identified. Considering this lack of information and the major importance of baby bottles for infant feeding, it was decided to limit migration experiments to baby bottles present on the Belgian market.

References

Comeos. 2013a. 6 Belgen op 10 kochten al online - E-commerce blijft groeien [Internet]. [cited 2016 Feb 6]. Available from: http://www.comeos.be/menu.asp?id=10542&lng=nl

Comeos. 2013b. E-commerce in België [Internet]. [cited 2016 Feb 6]. Available from: http://www.comeos.be/files/docs/10542_1_nl.pdf

Gezinsbond België. 2015. " Er zitten 300 verschillende chemische stoffen in een baby " [Internet]. Available from: https://www.gezinsbond.be/persberichten/Documents/Persbericht 5 oktober 2015.pdf

Li H, Jiang J, Wu M. 2014. The effects of trust assurances on consumers' initial online trust: A two-stage decision-making process perspective. Int J Inf Manage. 34:395–405.

Trends. 2014. Belgische e-commerce moet tandje bijzetten [Internet]. [cited 2016 Feb 7]. Available from: http://trends.knack.be/economie/e-business/belgische-e-commerce-moet-tandje-bijzetten/article-normal-262647.html

Chapter 4 :
Qualitative evaluation of migrants from polymeric baby bottles by screening and elucidation methods

4.1 Evaluation of migrants by a GC-MS screening method

Based on the following publication:

Onghena M, Van Hoeck E, Vervliet P, Scippo ML, Simon C, Van Loco J, Covaci A. Development and application of a non-targeted extraction method for the analysis of migrating compounds from plastic baby bottles by GC-MS., *Food Additives and Contaminants*. Part A 31: 2090–102 (2014)

4.1.1 Introduction

Nowadays, there is an increasing concern over the presence of hazardous chemicals in FCMs (Grob 2014; Geueke et al. 2014). Many of these FCMs are made of plastics, which, next to the polymer, contain complex mixtures of compounds, such as monomers, additives, catalysts or degradation products. Consequently, migration of these chemicals from the plastic FCMs into the food could arise, resulting in off-flavours and taints in the food or even harmful effects to human health. For plastic FCMs, all authorised starting substances have been assembled in a Union List in EU Regulation No. 10/2011 together with their migration limit and/or restricted use (European Union 2011a). Furthermore, the use of BPA was banned for the manufacture of PC infant feeding bottles and their placement on the European market (European Union 2011b). As a consequence, baby bottles made of other polymer types, such as PP, PES, PA, Tritan™ or silicone, are now present on the market.

Recently, Simoneau et al. (Simoneau et al. 2011; Simoneau et al. 2012) have studied to some extent these alternative polymers to PC baby bottles. An extraction method with iso-octane was applied to the simulant for rapid GC-MS analysis. This resulted in the identification of several migrating compounds, though many could not be identified. Except for PA bottles which unexpectedly released BPA (abnormal for this polymer), generally no issues for PP bottles were found because of the low quantities of released compounds. PP released some substances not present on the European Union positive list and for silicone bottles, migration of phthalates could be evidenced. However, further detailed research is still needed on the compounds that migrate from plastic baby bottles into the foodstuff and their possible effects of exposure on the consumer's health.

While the migration phenomenon in plastics has already been studied extensively (Simoneau et al. 2011; Yang et al. 2011; Simoneau et al. 2012; Li et al. 2013; Castillo et al. 2013; Kirchnawy et al. 2014; Bittner et al. 2014; Maiolini et al. 2014), the present study complements previous findings by using a broader scope for the identification of chemicals originating from plastics. Therefore, the principal aim of this study was to detect migrants from the alternative polymer materials currently used for baby bottles. To this end, an extraction method was developed in order to encompass as much as possible chemicals migrating from the polymers. Afterwards, the method was applied to the migration solutions obtained during the testing of different polymer baby bottles representative for the Belgian market. After GC-MS analysis, the identification of the migrating chemicals was done using the Wiley and NIST library. The link between the obtained analytical results and the impact of these findings on the consumer's health will be assessed in future work.

4.1.2 Materials

4.1.2.1 Market survey and sampling

Based on the previously conducted market survey a total of 24 different bottles were selected considering all the different materials produced by various manufacturers and available in the shops. The selected bottles included PP (n =17), PES (n =2), PA (n =2), Tritan™ (n = 1), silicone (n = 1) and also stainless steel materials (n = 1). All bottles were tested in duplicate.

4.1.2.2 Chemicals

Methanol (gradient grade for LC LiChrosolv), ethyl acetate (for LC LiChrosolv), dichloromethane (for analysis EMSURE®), methyl *tert*-butyl ether (for LC) and iso-octane (ECD for GC and FID SupraSolv) were purchased from Merck (Darmstadt, Germany). N-hexane (for residue analysis and pesticides, 95%) was purchased from Acros Organics (Geel, Belgium). Ultrapure water was prepared by means of an Elga Purelab Prima (Tienen, Belgium). Butylated hydroxytoluene (≥ 99%), 2,4-di-*tert*-butylphenol (99%), cyclohexylamine (≥ 99%), 4-(methylthio)-benzaldehyde (95%), cyclododecene (96%), 9-octadecenamide (≥ 99%), 2,2,4-trimethyl-1,3-pentanediol diisobutyrate (≥ 98.5%), eucalyptol (99%), camphor (96%), benzophenone (≥ 99%), hexadecanoic acid (≥ 99%), methyldodecanoate (≥ 98%), 2,6-diisopropylnaphthalene (purity not specified by the manufacturer), *tris*(2,4-di-*tert*-butylphenyl) phosphite (Irgafos 168, 98%), Cyclohexanone, 3,3,5-trimethyl (98%), acetophenone (≥ 99.0%), 4-methylbenzaldehyde (≥ 97.0%), 2-phenyl-2-propanol (97%), fenchone (≥ 98%), ethanol, 2-(2-ethoxyethoxy)-, acetate (99%), α-terpineol (≥ 90.0%), 4-propylbenzaldehyde (97%), 2-undecanone (99%), p-propenylanisole (≥ 99.5%), butoxyethoxyethyl acetate (≥ 99.2%), oxacyclotridecan-2-one (98%), *p*-*tert*-octylphenol (purity not specified by the manufacturer), cedrol (purity not specified by the manufacturer), tetradecanoic acid (≥ 99.5%), azacyclotridecan-2-one (98%), tetradecanoic acid, ethyl ester (≥ 99.0%), hexadecanoic acid, ethyl ester (≥ 98.5%), methyl oleate (≥ 99.0%), octadecanoic acid (≥ 98.5%), octadecanoic acid, methyl ester (≥ 99.5%), cyclohexanone (≥ 99.0%), 2-cyclohexen-1-one, 3,5,5-trimethyl (97%), acetic acid, 2-ethylhexyl ester (99%), naphthalene (99%), 2,4-dimethylbenzaldehyde (≥ 90%), 2-ethylhexyl acrylate (≥ 99.5%), 2,4,6-trimethylbenzaldehyde (98%), 2,6-di-*tert*-butylbenzoquinone (98%), diisobutyl phthalate (99%), hexadecanoic acid, methyl ester (≥ 99%), dibutyl phthalate (99%), octadecanoic acid, ethyl ester (≥ 99%) and 4-phenylbenzophenone (99%) were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). *p*-Cresol (≥ 99.7%) was purchased from Fluka Analytical (Buchs, Switzerland). 2,6-Di-*tert*-butyl-4-methylphenol-D_{24} was purchased from Campro ScientificGmbH (Berlin, Germany). Helium (99.999%) and nitrogen (99.99%) were purchased from Air Liquide (Liège, Belgium).

4.1.3 Methods

4.1.3.1 Migration testing

Before performing the migration tests, baby bottles were sterilised according to the recommendations of the manufacturer. Therefore, the bottles were filled over ten min with boiling water; this is the only way of sterilising the bottles that allows collection of the sterilisation solution for further analysis. Afterwards, the conventional migration test for 'hot fill conditions', i.e. two h at 70 °C, was carried out on the baby bottles. These conditions at which the migration tests should be carried out are prescribed in European Union Regulation No. 10/2011 (European Union 2011a). During the preparation of infant formula, heating will take place and afterwards the residues of the food will not be stored, as specified in the method of preparation of one of the manufacturers of infant formula (Nutricia baby - Danone Group 2014). The conditions that accorded the best with the preparation of infant formula were those mentioned in chapter 2 of annex V of the 10/2011 Regulation (European Union 2011a). The choice of the simulant was also in accordance with the guidelines of the Regulation No. 10/2011 (European Union 2011a). Since milk is the predominant nutritional product for infants during the first months of life, H_2O-EtOH (50:50, v/v), determined by the same European Union regulation to mimic the use of milk, was chosen as simulant for the migration tests (European Union 2011a). After preheating the simulant on a heating plate to 70 °C, baby bottles were filled to the specified volume of each bottle. The bottles were then sealed with a plastic cap delivered with the bottle to avoid losses of the simulant by evaporation. Filled bottles were placed in a convection oven for two h at 70 °C. European Union legislation prescribes that when materials, such as baby bottles, are intended to come into repeated contact with foods, the migration test has to be carried out three times using a fresh aliquot of the food simulant for each occasion. After each migration, the bottles were rinsed with ultrapure water and the simulant was transferred to a glass recipient and stored at four °C. Both the sterilisation and the migration solutions were analysed.

4.1.3.2 Liquid-Liquid extraction (LLE) method development

The main goal of LLE in this case, that is the use of an organic solvent or mixtures with a low boiling point to avoid losses of the most volatile migrating compounds, led to a limited choice of solvents. These included *n*-hexane, iso-octane, ethyl acetate (EtOAc)/*n*-hexane (1:1), EtOAc/*n*-hexane (1:3), methyl-*tert*-butyl ether (MTBE), *n*-hexane/dichloromethane (DCM) (1:1) and *n*-hexane/ DCM (1:3). For each solvent (mixture), the extraction was carried out in triplicate for spiked simulant samples and in duplicate for blank samples. Spiked simulant samples were samples prepared by adding the selected standard mixture in the H_2O-EtOH (50:50, v/v) simulant solution. The 14 substances shown in Table 4.1 were used for the optimisation of the method. Blank

samples contained the simulant without any addition of components of the standard mixture.

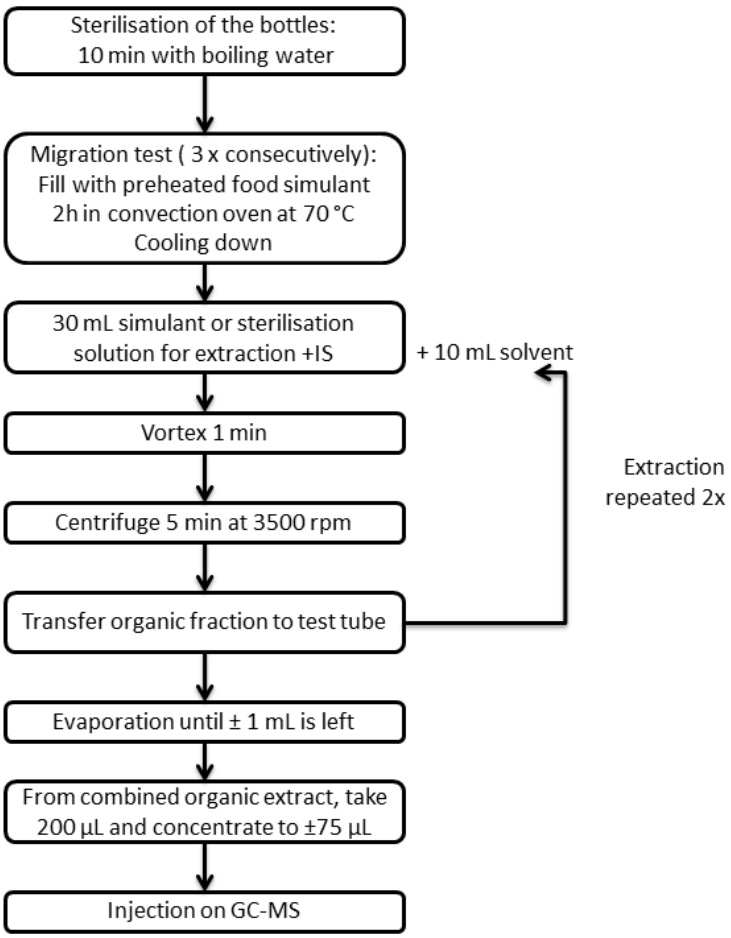


Figure 4.1: Schematic overview of the liquid–liquid extraction (LLE) procedure.

To prepare a spiked sample, a solution of five µg ml^{-1} of the standard mixture was prepared in the selected simulant. For each simulant sample, 30 ml containing five µg ml^{-1} of the standard mixture were extracted twice with ten ml of the solvent (mixture) to be tested. Deuterated 2,6-di-*tert*-butyl-4-methylphenol-D_{24} used as internal standard was added to the simulant prior to LLE and the mixture was vortexed for one min in a 50 ml glass pear. The sample was then transferred into a 50 ml glass centrifuge tube and centrifuged for five min at 2205 g using an Eppendorf 5804 centrifuge (Eppendorf AG, Hamburg, Germany). Subsequently, the organic phase was transferred to a test tube and the entire procedure was repeated. The combined organic extracts were evaporated under a gentle nitrogen stream at 30 °C until about one ml remained. From this, 200 µl

were taken and further concentrated by evaporation in a vial to about 75 µl for GC-MS analysis. A schematic overview is shown in Figure 4.1.

One of the major problems in the analysis of migrants from plastic FCMs is the possible contamination from the environment. Plastic materials are also frequently used in laboratories for sample preparation steps, water storage or in the chromatographic systems (tubings, sealings, etc.). Therefore, the analysis of blank samples is very important for this research. A blank sample is an aliquot of the food simulant (e.g. H_2O-EtOH (50:50, v/v) for milk) which has passed through the whole sample treatment procedure. This was done in order to evaluate possible sources of contamination with plastic leachables. Compounds that were detected in both the samples and the blanks were considered only as tentative candidates for further research when the signal observed in the samples was ten times higher than that in the blanks.

4.1.3.3 GC-MS analysis

For the determination of the recoveries of the standard mixture, a specific GC-MS method was developed. The LLE extracts were analysed by GC-MS by monitoring fragmentation ions specific for each analyte and the internal standard (2,6-di-*tert*-butyl-4-methylphenol-D_{24}), as shown in Table 4.1. The performance of the method was tested by determining precision, accuracy and recovery. These analyses were performed with an Agilent 6890 gas chromatograph (Agilent JW Scientific, Diegem, Belgium) coupled to an Agilent 5973 mass selective detector (MSD) equipped with an electron impact (EI) ionisation source and operated in selected ion monitoring (SIM) mode, enabling the recording of only the selected ions, characteristic of the selected compounds.

The quadrupole and ion source temperatures were set at 150 and 230 °C, respectively. The multiplier voltage was 2200 V. In order to improve the number of cycles per second, three acquisition segments were created with different dwell times (20, 15 and 20 ms, respectively). A one µl extract was injected into a PTV injector in pulsed splitless mode with an injection temperature of 280 °C. The GC column was a 30 m × 0.25 mm × 0.25 µm DB-5ms column (Agilent JW Scientific). The temperature of the oven was set at 60 °C for three min, and was then increased to 300 °C at a rate of ten °C min^{-1} where it was held for 15 min. The total run time was 42 min. Helium was used as a carrier gas; this with a constant flow rate of one ml min^{-1}.

Table 4.1: SIM parameters for the determination of the selected standard compounds by GC-MS. Compounds are ordered according to their retention times. Ion 1 (Q) indicates the ion used as quantifier, ions 2 and 3 are used as qualifiers.

SIM segment	Component	RT (min)	Ion 1 (*m/z*)	Ion 2 (*m/z*)	Ion 3 (*m/z*)
1	cyclohexylamine	4.2	56.0 (Q)	99.0	70.0
1	Eucalyptol	7.6	108.0 (Q)	154.0	139.0
1	*p*-cresol	8.6	107.0 (Q)	90.0	77.0
1	Camphor	9.7	95.0 (Q)	152.0	108.0
1	Cyclododecene	12.5	82.0 (Q)	166.0	96.0
2	4-(methylthio)-benzaldehyde	14.1	151.9 (Q)	122.8	107.8
2	Butylated hydroxytoluene (BHT)	14.7	205.0 (Q)	220.0	145.0
2	2,4-di-*tert*-butylphenol	14.7	191.2(Q)	206.2	163.3
2	Dodecanoic acid, methyl ester	14.9	74.0 (Q)	214.0	87.0
2	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate (TXIB)	15.7	71.0 (Q)	111.0	43.0
2	Benzophenone	16.4	105.0 (Q)	182.0	77.0
3	Hexadecanoic acid	20.0	213.0 (Q)	256.0	129.0
3	9-Octadecenamide (oleamide)	23.4	59.0 (Q)	280.7	72.0
3	*Tris*(2,4-di-*tert*-butylphenyl) phosphite (Irgafos 168)	32.8	57.0 (Q)	646.6	441.0

For the analysis of real baby bottle simulant samples, the same GC-MS equipment as before was used. However, since we performed an untargeted search, the MS was operated in full-scan mode from *m/z* 40 to 700. The same column and oven programme were used as for the previously developed SIM method. A volume of two µl extract was injected so that a sufficiently detectable amount of analyte was brought on the column. Since EI has the ability to produce highly reproducible fragmentation spectra, the MS spectra obtained for the migrating chemicals extracted by the simulant were compared with commercially available WILEY and NIST mass spectra libraries by use of the Agilent MSD Chemstation® for peak identification. Deuterated 2,6-di-*tert*-butyl-4-methylphenol-D_{24} was added as internal standard to the simulant prior to LLE to correct for potential variations in the extraction method or instrumental response. A cut-off value of 10% of the area of the internal standard peak was set for the identification of the unknown peaks. Only library matches above 90% were accepted as tentative candidates; standards were bought, when commercially available, to confirm the presence of the suggested compounds. When the returned match was below 90%, peaks were defined as 'unidentified'. Because of their large similarity between MS spectra of the

homologues, compounds such as alkanes, alcohols and aromatics were identified only as classes.

4.1.4 Results and discussion

4.1.4.1 LLE development

The official simulant for milk is H_2O-EtOH (50:50, v/v) (European Union 2011a). Several methods, such as overnight evaporation at 110 °C, rotary evaporation, were tested to evaporate the simulant (H_2O-EtOH (50:50, v/v)). Since large volumes of water had to be evaporated, any chemicals with a lower boiling temperature were evaporated as well, therefore massive losses (almost 100%) of the migrating compounds occurred. Considering our previous experiments, we developed a new method for the chemical identification based on a LLE using a solvent mixture with a low boiling point. To develop a robust and universal method, a mixture of 14 chemicals previously identified as potential migrants (Simoneau et al. 2012) and covering a wide variety in polarity and chemical functionality was chosen. In this way, the wide variety of potentially migrating chemicals was taken into account.

To find a suitable extraction solvent, the extraction efficiency was compared between different solvents (mixtures). The mixtures ethyl acetate–*n*-hexane (1:1) and dichloromethane–*n*-hexane (1:1) were the most efficient extraction solvents for the milk simulant. For MTBE, no phase separation was observed. Results were normalised to the best solvent and are summarised in Table 4.2.

Table 4.2: Extraction efficiencies (%) of the different solvents tested normalised to the recoveries of EtOAc–*n*-hexane (1:1).

Compound	*n*-hexane	Iso-octane	EtOAc – *n*-hexane (1:1)	EtOAc–*n*-hexane (1:3)	DCM – *n*-hexane (1:1)	DCM – *n*-hexane (1:3)
Cyclohexylamine	/	/	/	/	/	/
Eucalyptol	90	89	100	87	98	82
p-cresol	43	35	100	65	163	88
Camphor	92	84	100	87	108	90
Cyclododecene	83	82	100	84	86	73
4-(Methylthio)-benzaldehyde	74	59	100	80	121	100
BHT	82	76	100	85	85	72
2,4-Di-*tert*-butylphenol	79	68	100	83	91	72
Dodecanoic Acid, Methylester	79	73	100	85	84	71
TXIB	83	78	100	84	91	76
Benzophenone	83	72	100	88	94	82
Hexadecanoic acid	50	66	100	68	148	63
Oleamide	67	46	100	87	96	86
Irgafos 168	102	89	100	70	71	73

For further experiments, it was decided to continue the method development with the non-chlorinated solvent regarding environmental and health concerns. The recovery results of the complete LLE procedure using the EtOAc–Hex (1:1) mixture, including the evaporation step, are shown in Table 4.3 and were calculated by applying a correction for the presence of the internal standard. Cyclohexylamine could not be detected as this compound was eluted before the solvent delay. Good recoveries were obtained for the majority of the rest of the selected compounds varying generally between ±80% and 120%. Hexadecanoic acid and oleamide sometimes gave problematic recoveries because a derivatisation step would be required for GC analysis, and therefore results were not applicable. Since our goal was to develop a universal and not specific extraction method that would cover a wide range of possible target analytes, a derivatisation step was not included in the procedure. Although recoveries of some compounds, such as *p*-cresol or 4-(methylthio)-benzaldehyde, were rather low (about 30%), the developed method was still a better alternative for the concentration of migrating compounds from the simulant solution then the tested conventional evaporation methods. The repeatability (as % RSD) and linearity (as R^2, correlation coefficient) of this developed semi-

quantitative screening method were satisfying for most compounds, with RSD values generally < 7% and R^2 values generally > 0.995.

Table 4.3: Analytical parameters (recovery, precision, R^2, LOD and LOQ) of the standard compounds used for LLE development with EtOAc-Hex (1:1). n.a. - not applicable.

Compound	Recovery (%)	RSD (%)	R^2	LOQ (ng ml^{-1} simulant)	LOD (ng ml^{-1} simulant)
Cyclohexylamine	n.a.	n.a.	n.a.	n.a.	n.a.
Eucalyptol	105.3	3.7	0.9981	1.8	0.6
p-cresol	34.4	6.8	0.9812	5.4	1.6
Camphor	87	4.6	0.9978	1.3	0.4
Cyclododecene	120.6	3.4	0.9995	1.4	0.4
4-(methylthio)-benzaldehyde	30.9	0.1	0.9477	56.2	16.9
BHT	103	0.8	0.9972	1.6	0.5
2,4-Di-*tert*-butylphenol	111.1	3	0.9957	0.1	0.02
Dodecanoic Acid, Methylester	134.5	1.5	0.9986	1.2	0.4
TXIB	135.4	1.8	0.9979	1.2	0.4
Benzophenone	76.3	4.2	0.9989	2.3	0.7
Hexadecanoic acid	n.a.	n.a.	n.a.	n.a.	n.a.
Oleamide	58.5	27.3	0.9826	62.1	18.7
Irgafos 168	95.2	21.5	0.9953	4.9	1.47

4.1.4.2 Migration testing of real baby bottles

Substances allowed to be used for production can consequently migrate from plastic food contact materials and are defined with their specific migration limit (SML) in European Union Regulation No. 10/2011. This defines a 'positive' list for plastic food contact materials authorised by European Union legislation and for which the migration conditions are described (European Union 2011a). For substances for which no SML or other restrictions are provided in Annex I of this legislation, a generic SML of 60 000 µg kg^{-1} of food is applied. In European Union Regulation No. 10/2011, a migration limit of 10 µg kg^{-1} is used for substances that are not classified as carcinogenic, mutagenic or reprotoxic (CMR) behind a functional barrier. This limit has been further used as a threshold value to prioritise the substances detected above this value for their possible effects towards public health. For substances migrating in concentrations below 10 µg kg^{-1}, the CMR characteristics still need to be evaluated, but this is out of the scope of this publication.

4.1.4.2.1 Migration patterns

The possible migration of unknown chemicals from PP, PES, PA, Tritan™, silicone and stainless steel baby bottles was determined by applying the developed LLE method to the baby bottle simulant samples. The extracts were consequently analysed by GC-MS. Significant differences in the migrating patterns (= compounds and their intensities) were observed among the different types of polymers and also among the same polymers from different producers. Differences in the migration patterns were perceived between the sterilisation liquid and the simulants, as well within the different simulants of the consecutive migrations. Silicone, Tritan™ and PP exhibited a wide variety of migrating compounds, whereas PES and PA showed a lower amount of migrants, though sometimes in relatively large amounts. Figure 4.2 shows the differences in the migration patterns between PP and PA bottles.

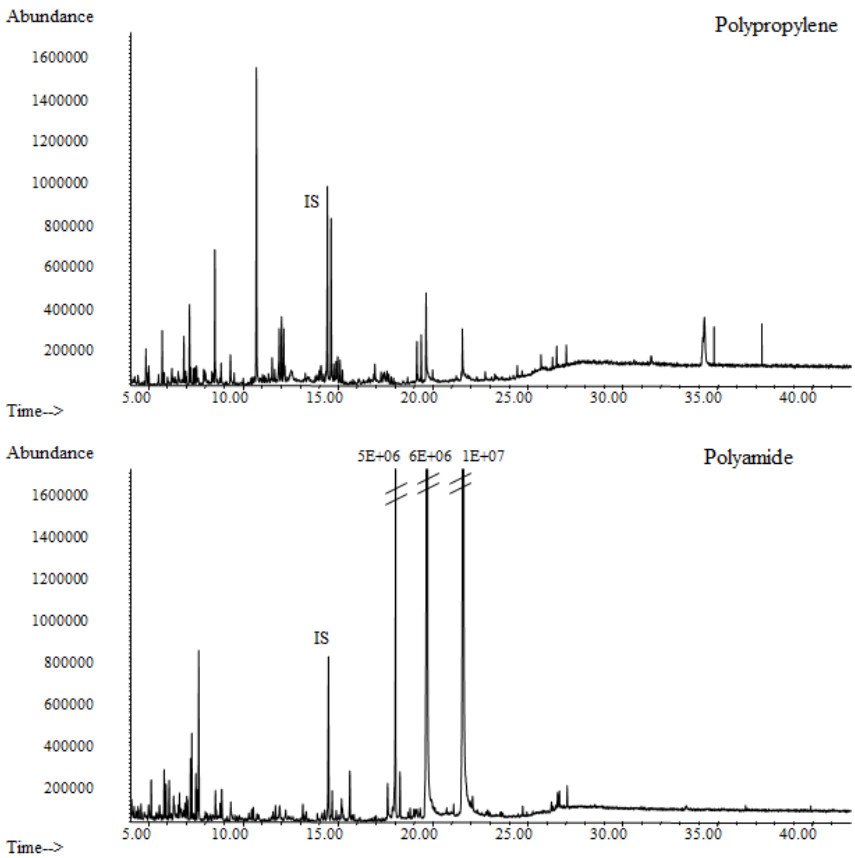


Figure 4.2: Differences in the migration pattern between the first migration of a polypropylene (PP) and a polyamide (PA) bottle (the internal standard level is the same in both bottles).

Since the main aim of this initial study was to perform a screening and identification of compounds migrating from the alternatives to PC baby bottles, a detailed quantification fell therefore outside the scope of this project. A semi-quantitative estimation of the concentration ranges of the detected compounds was made based by comparison of their intensities with that of the internal standard (supposing the ionisation capacity is comparable) or when available with the standard of the detected compound itself. Once the elucidation of all migrating compounds is performed, a quantitative method will be developed for the compounds of interest to determine if the concentrations in which they are migrating present a public health concern for infants when using a particular type of baby bottle.

When comparing the chromatograms of the sterilisation liquids and those of the simulants, distinctive differences in the amount and intensity of migrating compounds were observed. In general, hardly any compounds were perceived in the sterilisation solutions, whilst starting with the first migration, many organic compounds migrated from the tested polymers. It could therefore be concluded that almost no superficial contamination was present in the baby bottles. The detection of some compounds in the migration solutions such as, for example, benzophenone (around ± 90 µg kg^{-1}) or diisopropyl naphtalene (DIPN, estimated <10 µg kg^{-1}) was suggested to originate from printing inks used for paper or cardboard (Simoneau et al. 2012), such as the instruction leaflets sometimes added inside the baby bottles. If these compounds could migrate from these leaflets, then the contamination would only be rather superficial. Sterilising the baby bottles would wash away these components and they would be detected in the sterilisation solution. As this was not always the case, it was most likely that these compounds originate from the polymer itself, though one should take into account that compounds such as benzophenone and DIPN are much more soluble in a H_2O-EtOH (50:50, v/v) solution than in water only.

Another important observation was the relative decrease in the intensity of the migrating compounds through the three consecutive migrations, as shown in Figure 4.3. The first migration exhibited the highest abundances of migrating components, after which these decreased in intensity during the following migrations. Some compounds that were detected in the first/second migration disappeared in the third migration.

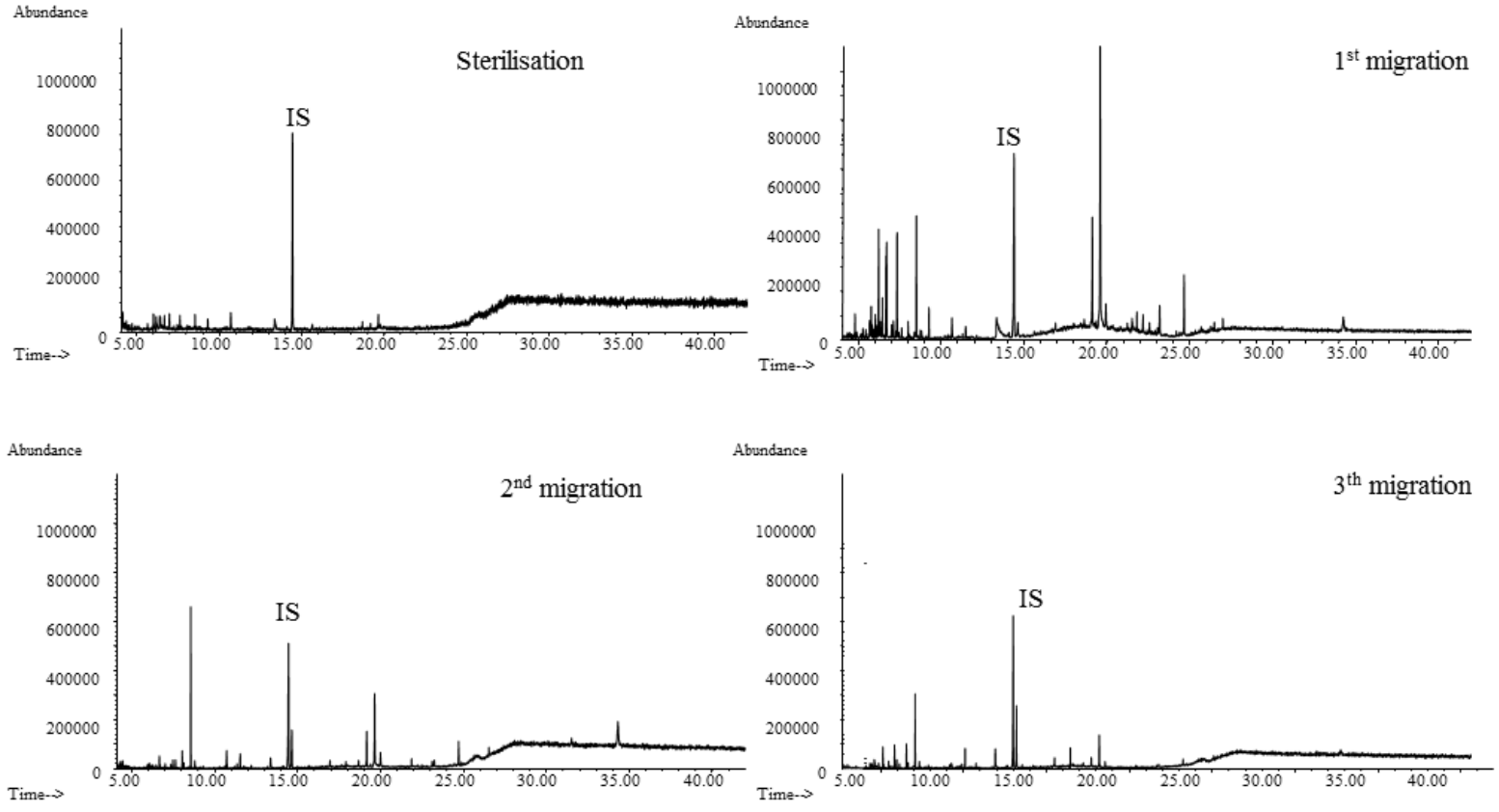


Figure 4.3: Differences in the migration pattern between the sterilisation and the consecutive migrations of a polypropylene (PP) bottle.

4.1.4.2.2 Commonly migrating compounds

The variety of migrating compounds detected from the different polymer alternatives was very high, though some compounds were detected on a regular basis. An overview of the migrating components is given in Table 4.4. A full inventory of the migrating compounds from the different polymers is given in Tables SI-1 and SI-2 in the Supplementary Information included at the end of this chapter. 3,4-dimethyl benzaldehyde was shown to migrate from all tested polymer types. This compound sometimes was estimated migrating up to 20 μg kg^{-1}, whereas none of the dimethyl-benzaldehyde isomers was mentioned in European Union Regulation No. 10/2011 and therefore its origin should be investigated. Naphthalene and acetophenone (except for the PA) were also found, though in small amounts (estimated < 10 μg kg^{-1}). 2-Butoxyethyl acetate, not mentioned in European Union Regulation No. 10/2011 either, migrated from all the bottles (except for silicone) at low concentrations (expected around or just above the 10 μg kg^{-1} threshold). For one specific brand, this compound was detected even at concentrations above 300 μg kg^{-1}. Also here, the origin of this substance should be investigated.

4.1.4.2.3 Polypropylene (PP) baby bottles

PP was the most used alternative polymer material to PC baby bottles on the Belgian market, with about 62% of the market share. It was already demonstrated (Mcdonald et al. 2008; Alin & Hakkarainen 2010) that chemicals present in PP could migrate into food. The migration tests performed on PP baby bottles revealed a wide variety of compounds migrating to the simulant. Of the 17 different PP bottles tested, more than 94% exhibited the presence of alkanes and in 41%, benzene derivatives were seen. In two bottles, migration of several siloxanes was also perceived. The antioxidant Irgafos 168 (*tris*-(2,4-di-*tert*-butylphenyl) phosphite) was found in 76% of the PP samples and its oxidised form *tris*-(2,4-di-*tert*-butylphenyl) phosphate was seen in every PP bottle, though the amounts migrating were much lower (about ppb range) than the SML (60 000 μg kg^{-1}) prescribed for this compound. 2,4-Di-*tert*-butylphenol, a possible degradation product of Irgafos 168, was detected in > 90% of the PP bottles. Generally, the detected range was rather low (estimated around or just above the 10 μg kg^{-1} specified threshold), though further research with a quantitative method is needed to draw adequate conclusions about its exact concentration.

It was noticeable, though, that for some bottles of lower quality brand the specific PP variant used released up to 10-fold higher amounts of this specific compound than for most other bottles. 2,6-Di-*tert*-butylbenzoquinone, previously detected only in silicone bottles (Simoneau et al. 2012) and a possible degradation product of another antioxidant, namely Irganox 1010, was also seen in five out of 17 samples (low concentrations, estimated < 10 μg kg^{-1}). The presence of these possible degradation

products can be explained by the fact that hindered phenolic primary antioxidants such as Irganox 1010 can undergo oxidation by reacting with peroxide radicals when preventing polymer degradation (Chanda & Roy 2006). The use of Irganox 1010 in plastic food contact materials is compliant with the European legislation (SML = 60 000 µg kg^{-1} food), but nothing is mentioned on its degradation products and therefore the origin of these compounds should be investigated as well.

Other degradation products that were previously not identified in PP baby bottles, such as 7,9-di-*tert*-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione, were found in all 17 PP bottles at levels as high as that of the internal standard. This compound was seen in all three migrations and sometimes already even in the sterilisation step. Since it is a degradation product formed by oxidation of Irganox 1076 (octadecyl 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl) propionate; SML = 6000 µg kg^{-1} food), this suggests the initial presence of Irganox 1076 in the PP samples as well. Methyl-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl) propionate was detected in all PP samples in accordance with the previous findings that it can be a degradation product of Irganox 1010 and/or Irganox 1076. Five PP bottles also showed the presence of 4-propylbenzaldehyde in all migration solutions (estimated up to ±25 µg kg^{-1}). Dibutyl phthalate was detected in one specific bottle (around 40 µg kg^{-1}) just as its isomer diisobutyl phthalate which was seen in one other PP bottle (±10 µg kg^{-1}).

4.1.4.2.4 Polyethersulphone (PES) baby bottles

PES is the most closely related alternative material to PC as one of its building blocks is 4,4′-dihydroxydiphenyl sulphone (or bisphenol S), which may have similar endocrine disrupting properties as BPA (Kuruto-Niwa et al. 2005; Barret 2013; Viñas & Watson 2013). The GC-MS analysis of the various migration solutions showed that hardly any migration took place and that the amounts migrated were small (expected < 10 µg kg^{-1}). One PES bottle exhibited the presence of 4-methylbenzaldehyde in all three migrations as well as in the sterilisation, while this compound was not detected in the PES bottle from another brand. The same was found for a dimethyl-benzaldehyde isomer, present only in the second PES bottle. This suggests that different polymer producers use different polymerisation agents in their production process and that different chemicals can therefore migrate from PES polymers of different origin (and generally from the different polymer types).

It would be though very premature to state that considering the low amount of migration from this material, this would be the safest polymer alternative to PC baby bottles, especially taking into account the similar properties of BPS that could not be detected by GC-MS. Simoneau et al. (2011) investigated the migration of the potential

PES starting components diphenyl sulphone (DPS), 4,4´-dichlorodiphenyl sulphone (DCPS) and BPS by LC-MS and concluded that only DPS migrated, but far below the SML of 3000 µg kg^{-1}. Further LC analysis of PES bottles is necessary before any claims about the safety of this material can be done.

4.1.4.2.5 Polyamide (PA) baby bottles

Two PA bottles of different brands available on the Belgian market were tested. Similar to the PES bottles, the number of migrating compounds from PA was rather low, but the few compounds that migrated were detected in relatively large quantities. Azacyclotridecan-2-one was detected in both PA samples at relatively high concentrations compared with other migrants (estimated up to 250 µg kg^{-1}), but still with a migration far below the SML of 5000 µg kg^{-1}. As certain types of PA are formed by a ring opening polycondensation of azacyclotridecan-2-one, the presence of this compound is logical (Troughton 2008). In one PA bottle, a component with a similar mass spectrum and retention time was seen, but no adequate library match was obtained. As a negative mass difference of 2 atomic mass units (amu) was present through the mass spectrum, most probably the structure of this compound included a double-bond in the ring structure (e.g. azacyclotridec-3-en-2-one). Hexa- and octadecanoic acid were also found in both PA samples in large concentrations. Taking into account the low response factor observed for hexadecanoic acid (similar response is expected for octadecanoic acid), the concentrations of these compounds had to be rather high as their intensities seen were up to six times higher than the internal standard for hexadecanoic acid and up to 12 times for octadecanoic acid. Their use as lubricants in the polymer production process can explain their omnipresence (also seen in PP and Tritan™) (Faghihnejad & Zeng 2013).

4.1.4.2.6 Tritan™ baby bottles

Only one baby bottle type made of Tritan™ co-polymer was found on the Belgian market, most probably due to the novelty of this polymer. In contrast with earlier findings on Tritan™ baby bottles (Simoneau et al. 2012), the number of migrants from this material detected by us was high. Similar to the PA bottles, hexa- and octadecanoic acids were also here the most abundant peaks and migrated in similar concentrations. 2,6-Di-*tert*-butylbenzoquinone and 2,4-di-*tert*-butylphenol, which indicate the presence of antioxidants, were seen at low levels (up to ±5 µg kg^{-1}). 2,2,4-Trimethyl-1,3-pentanediol diisobutyrate (TXIB), a substance authorised by European Union legislation to migrate at a level of 5000 µg kg^{-1} food, but only from single-use gloves, was found migrating in trace amounts as well. As this restriction does not apply to baby bottles, this compound should not be present here under any circumstances. Diisobutyl phthalate, a possible endocrine disruptor not authorised for the use in FCMs, was

detected at concentration levels estimated around 30 µg kg^{-1}. On the other hand, none of the monomer building blocks could be identified in the migration solutions.

4.1.4.2.7 Silicone baby bottles

Even though silicones are not classified by the European Union as plastic materials (Council of Europe 2004), these materials had to be investigated because silicone baby bottles are also being sold and were already indicated as a possible source of interest for the migration of endocrine disrupting compounds (Simoneau et al. 2012). On the Belgian market only one brand of silicone bottle was found. This bottle tended to release a high amount of migrating components. Besides from a variety of siloxanes (e.g. octamethyl-cyclotetrasiloxane, decamethyltetrasiloxane, etc.), previously identified compounds, such as TXIB, DIPN, 2,6-di-*tert*-butylbenzoquinone, 2,4-di-*tert*-butylphenol etc., were also evidenced in the migration solutions. Diisobutyl phthalate was detected here at relatively high levels (estimated up to 80 µg kg^{-1}) and also minor amounts of dibutyl phthalate were seen (±25 µg kg^{-1}). As silicones are not regulated under European Union legislation for FCMs, no limitations concerning the migration of these compounds are specified. Consequently, the toxicity of the compounds should be known to assess the risk for the consumer.

4.1.4.2.8 Stainless steel baby bottles

Only one brand of stainless steel baby bottle was found on the Belgian market. The only compounds evidenced in the migrations of this bottle were siloxanes (differing from those detected in the silicone bottle, such as, for example, hexadecamethyl-cyclooctasiloxane), most probably released from the interior coating.

Table 4.4: Schematic overview of the confirmed and tentatively identified (italic) migrating compounds per polymer type.

Compound	PES	PA	Tritan	Silicone	PP
Cyclohexanone	X			X	X
Eucalyptol		X	X		X
Cyclohexanone, 3,3,5-trimethyl			X		
Cyclohexanol, 3,3,5-trimethyl			X		
Acetophenone	X		X	X	X
4-Methylbenzaldehyde	X				X
2-Butoxyethyl acetate	X	X	X		X
2-Phenyl-2-propanol				X	
Fenchone			X		
2-Cyclohexen-1-one, 3,5,5-trimethyl				X	
Acetic acid, 2-ethylhexyl ester	X		X		X
Camphor			X	X	X
Ethanol, 2-(2-ethoxyethoxy)-, acetate					X
Diisopropyl xanthate					X
Naphthalene	X	X	X	X	X
alpha-terpineol				X	
3,4-Dimethylbenzaldehyde	X	X	X	X	X
2-Ethylhexyl acrylate				X	X
4-Propylbenzaldehyde					X
2-Undecanone				X	
2,4,6-Trimethylbenzaldehyde				X	X
2-Methylnaphthalene				X	
Butoxyethoxyethyl acetate			X		X
Naphthalene, 2,6-dimethyl				X	
2,6-di(t-butyl)-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one				X	
2,6-Di-*tert*-butylbenzoquinone			X	X	X
2,4-Di-*tert*-butylphenol			X	X	X
4-tert-Octyl-o-Cresol					X
Benzoic acid, 4-ethoxy-, ethyl ester					X
Oxacyclotridecan-2-one		X			
Dodecanoic acid		X			
TXIB			X	X	X
Hexanoic acid, 2-ethyl-, 2-ethylhexyl ester					X
p-tert-octylphenol					X
Cedrol				X	
Benzophenone			X	X	X
Phenol, 2-methyl-4-(1,1,3,3-tetramethylbutyl)					X
1,1'-Biphenyl, 2,2',5,5'-tetramethyl					
2,6-Diisopropylnaphthalene			X	X	X
Octanoic acid, 2-ethylhexyl ester					X
Tetradecanoic acid		X			
3,5-di-tert-Butyl-4-hydroxybenzaldehyde					X
Azacyclotridecan-2-one		X			
Tetradecanoic acid, ethyl ester				X	
3,5-di-tert-Butyl-4-hydroxyacetophenone					X

Compound	PES	PA	Tritan	Silicone	PP
Azacyclotridec-3-en-2-one		X			
Diisobutyl phthalate			X	X	X
Decanoic acid, 2-ethylhexyl ester					X
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione					X
Hexadecanoic acid, methyl ester					X
Methyl-3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate					X
Dibutyl phthalate				X	X
Hexadecanoic acid		X	X		X
Hexadecanoic acid, ethyl ester				X	X
Methyl oleate					X
Octadecanoic acid		X	X		X
Octadecanoic acid, methyl ester					X
Octadecanoic acid, ethyl ester					X
4-Phenylbenzophenone					X
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester					X
Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester					X
Irgafos 168					X
Oxidized Irgafos 168					X

4.1.5 Conclusions

A market survey showed that five polymer alternatives to PC baby bottles, of which PP was clearly the most dominant (±62%), are currently being sold on the Belgian market.
An LLE method was developed for the universal extraction of migrating compounds from the milk simulant (H_2O-EtOH (50:50, v/v)). It showed that the mixture ethyl acetate–*n*-hexane (1:1) was the best universal extraction solvent for a mixture of 14 chemicals previously identified as potential migrants with a wide variety in polarity and chemical functionality (Simoneau et al. 2012).
Migration tests with the milk simulant were performed on the different polymer alternatives for PC baby bottles present on the Belgian market. Prior to the migration tests, the baby bottles were sterilised.
The analysis of the simulants led to the following conclusions. PA and PES showed a low release of substances migrating, though for PA the concentrations were relatively high. PP, Tritan™ and silicone bottles demonstrated a high variety of migrating compounds, some not being approved by European Union legislation for FCMs, which makes further investigation on their origin needed. Compounds authorised by European Union Regulation No. 10/2011 did not indicate to exceed the defined SMLs. On the other hand, the concentrations of these migrants were generally rather low. Further investigation is needed to elucidate all unknown compounds that migrate and accurately determine the

concentrations of migrants with a dedicated quantitative method, also under real-life use conditions of the baby bottles.

4.1.6 Supplemental Information

Table SI-4.1: Compounds detected in PES, PA, Tritan™, and silicone bottles. Compounds in italic are only tentatively identified (match > 90%), other compounds were confirmed by an analytical standard. S indicates the sterilisation, I, II & III the consecutive migrations. * indicates that the specific compound was still present but with an intensity below the cut-off value of 10 % of the area of the IS peak. NL means not listed in the EU Regulation No. 10/2011. [a]Only to be used in single-use gloves

Polymer -->> Bottle number -->> Compound	SML (mg kg $^{-1}$)	PES 1	PES 4	PA 3	PA 23	Tritan 6	Silicone 10
Cyclohexanone	NL	I, II, III					S, I, II, *
Eucalyptol	NL			I, II, III		I, II, III	
Cyclohexanone, 3,3,5-trimethyl	NL					I, II, III	
Cyclohexanol, 3,3,5-trimethyl	NL					I, II, III	
Acetophenone	NL	S, I, *	*			*, I, II, III	S, I, *
4-methylbenzaldehyde	NL		S, I, II, III				
2-Butoxyethyl acetate	NL	I, II, III		I, II, *	*	I	
2-phenyl-2-propanol	NL						S, *
Fenchone	NL					I, II, III	
2-Cyclohexen-1-one, 3,5,5-trimethyl	NL						I, II
Acetic acid, 2-ethylhexyl ester	NL	I				I, II, III	
Camphor	60					I, *	I, *
Ethanol, 2-(2-ethoxyethoxy)-, acetate	NL						
Diisopropyl xanthate	NL						
Naphthalene	NL	I	I		*	I, *	I, II, *
alpha-terpineol	NL						I, II, *
3,4-Dimethylbenzaldehyde	NL	S, I, II, III			I	I, *	*
2-Ethylhexyl acrylate	0.05						I, II, *
4-propylbenzaldehyde	NL						
2-Undecanone	NL						I, II, *
p-Propenylanisole	NL					I, II, III	
2,4,6-Trimethylbenzaldehyde	NL						
2-Methylnaphthalene	NL						I, II, *
Butoxyethoxyethyl acetate	NL					I, II, III	
Naphthalene, 2,6-dimethyl	NL						*

Polymer -->> Bottle number -->> Compound	SML (mg kg $^{-1}$)	PES 1	PES 4	PA 3	PA 23	Tritan 6	Silicone 10
2,6-di(t-butyl)-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one	NL						I, II, *
2,6-Di-*tert*-butylbenzoquinone	NL					I, II, III	I, II, *
2,4-Di-*tert*-butylphenol	NL					I, II, III	I, II, *
4-tert-Octyl-o-Cresol	NL						
Benzoic acid, 4-ethoxy-, ethyl ester	NL						
Oxacyclotridecan-2-one	NL			I, *			
Dodecanoic acid	NL				I		
TXIB	5[a]					I, II, III	I, II, *
Hexanoic acid, 2-ethyl-, 2-ethylhexyl ester	NL						
p-*tert*-octylphenol	NL						
Cedrol	NL						I, II, *
Benzophenone	0.6					*	I, II, *
Phenol, 2-methyl-4-(1,1,3,3-tetramethylbutyl)	NL						
1,1'-Biphenyl, 2,2',5,5'-tetramethyl	NL						
2,6-Diisopropylnaphthalene	NL					*	I, II, *
Octanoic acid, 2-ethylhexyl ester	NL						
Tetradecanoic acid	60			I, II, III	I		
3,5-di-tert-Butyl-4-hydroxybenzaldehyde	NL						
Azacyclotridecan-2-one	5			I, II, III	I, II, III		
Tetradecanoic acid, ethyl ester	NL						II
3,5-di-tert-Butyl-4-hydroxyacetophenone	NL						
Azacyclotridec-3-en-2-one	NL			I, II, III	I, II, III		
Diisobutyl phthalate	NL					I, II, III	I, II,*
Decanoic acid, 2-ethylhexyl ester	NL						
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	NL						
Hexadecanoic acid, methyl ester	NL						
Methyl-3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate	NL						
Dibutyl phthalate	0.3						I, II,*
Hexadecanoic acid	60			I, II, III	S, I, II, III	S, I, II, III	
Hexadecanoic acid, ethyl ester	NL						II, *
Methyl oleate	NL						
Octadecanoic acid	60			I, II, III	S, I, II, III	S, I, II, III	
Octadecanoic acid, methyl ester	NL						
Octadecanoic acid, ethyl ester	NL						
4-phenyl-benzophenone	NL						
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	NL						
Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	NL						

Polymer -->> Bottle number -->> Compound	**SML (mg kg $^{-1}$)**	**PES 1**	**PES 4**	**PA 3**	**PA 23**	**Tritan 6**	**Silicone 10**
Irgafos 168	60						
Oxidized Irgafos 168	NL						
Alkanes							X
Benzenes							
Siloxanes							X
Aldehydes							

Table SI-4.2. Compounds detected in PP bottles. Compounds in italic are only tentatively identified (match > 90%), other compounds were confirmed by an analytical standard. S indicates the sterilisation, I, II & III the consecutive migrations. *indicates that the specific compound was still present but with an intensity below the cut-off value of 10 % of the area of the IS peak. NL means not listed in the EU Regulation No. 10/2011.

[a]Only to be used in single-use gloves

Polymer -->>	**SML**	**PP**															
Bottle number -->>		**2**	**5**	**7**	**8**	**9**	**11**	**12**	**17**	**18**	**19**	**20**	**21**	**22**	**24**	**25**	**26**	**27**
Compound	**(mg kg $^{-1}$)**																	
Cyclohexanone	NL	S, I, *	I, II, III			*	*		I, *, III							S, II	II, III	*, II, III
Eucalyptol	NL			*, I, II, III										I, II				
Cyclohexanone, 3,3,5-trimethyl	NL																	
Cyclohexanol, 3,3,5-trimethyl	NL																	
Acetophenone	NL	S		*										*				
4-Methylbenzaldehyde	NL		I, II, III															
2-Butoxyethyl acetate	NL	S, I, II, III	*								S, I, II, III			I, *		S, I, II, III		
2-phenyl-2-propanol	NL																	
Fenchone	NL																	
2-Cyclohexen-1-one, 3,5,5-trimethyl	NL																	
Acetic acid, 2-ethylhexyl ester	NL	*																
Camphor	60			I, II, *														
Ethanol, 2-(2-ethoxyethoxy)-, acetate	NL															S, I, II, III		
Diisopropyl xanthate	NL												I, II, III					
Naphthalene	NL				I, *	*	*	*		I, II, *					I, II, III	I, II, *		
alpha-terpineol	NL																	
3,4-Dimethylbenzaldehyde	NL	S, I, II, III				I, II, *			S, I, II, III		I, II, III	S, I, II, III	I, II, III	I, II, *	I, II, III	S, I, II, III	S, I, II, III	S, I, II, III
2-Ethylhexyl acrylate	0.05					*												
4-Propylbenzaldehyde	NL		I, II, III	I, II, III	*, I, II, *		I, II, III	I, II, III		*								
2-Undecanone	NL																	
p-Propenylanisole	NL																	
2,4,6-Trimethylbenzaldehyde	NL		I, II, III															
2-Methylnaphthalene	NL																	
Butoxyethoxyethyl acetate	NL								I, II, III									S, I, II, III

Polymer -->> Bottle number -->> Compound	**SML (mg kg $^{-1}$)**	**PP 2**	**5**	**7**	**8**	**9**	**11**	**12**	**17**	**18**	**19**	**20**	**21**	**22**	**24**	**25**	**26**	**27**
Naphthalene, 2,6-dimethyl	NL																	
2,6-di(t-butyl)-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one	NL																	
2,6-Di-*tert*-butylbenzoquinone	NL					*	I, II, *					I, II		I, II		I, *		
2,4-Di-*tert*-butylphenol	NL	I, II, III		S, I, II, III		I, II, III	S, I, II, III	I, II, III	*, II	II	I, II, III	I, II, III	I, II,*	I, II	I, II, III	I, II, *	*	I,*
4-tert-Octyl-o-Cresol	NL			I, II, III														
Benzoic acid, 4-ethoxy-, ethyl ester	NL														I, II, *			
Oxacyclotridecan-2-one	NL																	
Dodecanoic acid	NL																	
TXIB	5[a]								I, II, *						I, *			
Hexanoic acid, 2-ethyl-, 2-ethylhexyl ester	NL																	I, II, III
p-*tert*-octylphenol	NL			I, II, III														
Cedrol	NL																	
Benzophenone	0.6		*					S, I, II, III		S, I, II, III						I		
Phenol, 2-methyl-4-(1,1,3,3-tetramethylbutyl)	NL			I, II, III														
1,1'-Biphenyl, 2,2',5,5'-tetramethyl	NL																	
2,6-Diisopropylnaphthalene	NL					*	I, II, *											
Octanoic acid, 2-ethylhexyl ester	NL																	I, II, III
Tetradecanoic acid	60																	
3,5-di-tert-Butyl-4-hydroxybenzaldehyde	NL					*						I, II, *	*			I, *		
Azacyclotridecan-2-one	5																	
Tetradecanoic acid, ethyl ester	NL																	
3,5-di-tert-Butyl-4-hydroxyacetophenone	NL											*				I, *		
Azacyclotridec-3-en-2-one	NL																	
Diisobutyl phthalate	NL					I, II, III												
Decanoic acid, 2-ethylhexyl ester	NL																	I, II, III

Polymer -->>	**SML**	**PP**															
Bottle number -->>	**(mg**	**2**	**5**	**7**	**8**	**9**	**11**	**12**	**17**	**18**	**19**	**20**	**21**	**22**	**24**	**25**	**26**	**27**
Compound	**kg $^{-1}$)**																	
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	NL	I, II, III	I, II, III	I, II, III	I, II, III	I, II, *	I, II, *	I, II, III	I, II, III	I, II, III	I, II, III	I, II,*	I, II, III	I, II, *	S, I, II, III	S, I, II, III	I, II, III	I, II, III
Hexadecanoic acid, methyl ester	NL								I, II, III			II						
Methyl-3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate	NL	I, II, III	*	I, II, III	I, II, III	I, II, III	I, II, *	I, II, III	I, II, III	I, II, *	*	I, II,*	I, II, III	*	I, II, III	I, II, III	I,*	I, II, III
Dibutyl phthalate	0.3										I, II, III							
Hexadecanoic acid	60	S, I, II, III	S, I, II, III	S, I, II, III	S, I, II, III	S, I, II	I, II	S, I, II, III	I, II	I, II	I, II, III	I, II	S, I, II		S, I, II, III	S, I	I	
Hexadecanoic acid, ethyl ester	NL	I, II, III	*		I, II, III			I, II, III		I, II, III	I, II, III	I, II	I, II, III	I,*	I, II, III	*		
Methyl oleate	NL								I, II, III							I, II		
Octadecanoic acid	60	I, II, III	I, II, III	S, I, II, III	S, I	I, II		S, I, II, III			I	I			I			
Octadecanoic acid, methyl ester	NL								I, II, *									
Octadecanoic acid, ethyl ester	NL	I, II, III			I, II, III			I		I, II, *	I, II		I, II, III	I, *	I, II	*		
4-Phenylbenzophenone	NL												*					
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	NL	I, II, III		III														
Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	NL	I, II, III	III	III														
Irgafos 168	60	I, II, III	I, II, III	I, II, III	I, II, III	I, II, III	I, II, *	I, II, III	II	I, II, III	I, II			I, II	I, *		I	
Oxidized Irgafos 168	NL	I, II, III	I, II, III	I, II, III	I, II, III	I, II, III	I, II, *	I, II, III	II	I, II, III	I, II,*	I, II, III	I, II, III	I, II	I, II, III	I, II, *	I	I,*
Alkanes		X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Benzenes		X						X	X				X			X	X	X
Siloxanes								X							X			
Aldehydes		X																

4.2 Elucidation of unknown migrants

Based on the following publication:

Onghena M, Van Hoeck E, Van Loco J, Ibáñez M, Cherta L, Portolés T, Pitarch E, Hernandéz F, Covaci A. Identification of substances migrating from plastic baby bottles using a combination of low and high resolution mass spectrometric analyzers coupled to gas and liquid chromatography. *Journal of Mass Spectrometry* 50(11): 1234-1244 (2015)

4.2.1 Introduction

The migration phenomenon in the alternative materials for baby bottles has been understudied up to now, and little is known about the possible migrants from these polymer alternatives. GC-quadrupole-MS with electron impact (EI) ionisation source has been used before to investigate the presence of unknown compounds in food simulant that has been in contact with the alternative baby bottle polymers (Simoneau et al. 2012; Onghena et al. 2014). The drawback of this approach is that a conclusive library match cannot always be obtained when comparing experimental and library EI spectra, as many migrating compounds can be new, unregulated or even non-intentionally added substances (NIAS), e.g. degradation products of polymerisation reaction, and are thus not included in commercially available libraries.

The aim of this work was to develop and apply a methodology for the identification of unknowns observed during non-targeted screening of migrants from baby bottles, based on the use of low resolution and high resolution MS. GC and LC hyphenated to a variety of mass analysers were used for this purpose. To our knowledge, this is the first time that a combination of these techniques has been applied in a non-targeted approach to elucidate unknown migrants from polymer baby bottles. Since it was not the goal of this work to give a complete overview of all detected compounds in the tested baby bottles (Onghena et al. 2014), some particular examples have been selected to demonstrate the potential of the applied methodology for the elucidation of unknown migrants.

4.2.2 Materials and methods

4.2.2.1 Materials

4.2.2.1.1 Samples and sample treatment

Ten PP baby bottles and one PA baby bottle from the Belgian market (Onghena et al. 2014), consisting the majority of the market share, were selected for the application of the developed methodology. The use of simulants is prescribed in the EU Regulation No. 10/2011 to mimic the migration testing towards real foods, leading to the selection of simulant D1 H_2O-EtOH (50:50, v/v) as a simulant for milk (European Union 2011a). After sterilisation of the bottles during ten min with boiling water, three consecutive migrations for two h at 70 °C were performed with the water–EtOH simulant. Afterwards, a non-targeted LLE with EtOAc:*n*-hex (1:1) was performed on the simulant samples as previously described (Onghena et al. 2014). The obtained organic extracts were then further concentrated to ±75 µl under a gentle N_2 stream for analysis by GC or evaporated until dryness and dissolved in 75 µl of MeOH for LC injection. All bottles

were tested in duplicate. Deuterated 2,6-di-*tert*-butyl-4-methylphenol-D_{24} (Campro Scientific GmbH, Berlin, Germany) was added as an internal standard (IS) for GC analysis to the simulant prior to LLE to correct for potential variations in the extraction method or instrumental response. For LC, $^{13}C_{12}$-bisphenol-A was selected (Cambridge Isotope Laboratories, Inc. Andover, Massachusetts, USA).

4.2.2.1.2 Chemicals

Methanol (gradient grade for liquid chromatography LiChrosolv) and ethyl acetate (for liquid chromatography LiChrosolv) were purchased from Merck (Darmstadt, Germany). N-hexane (for residue analysis and pesticides, 95%) was purchased from Acros Organics (Geel, Belgium). Ultrapure water was prepared by means of an Elga Purelab Prima (Tienen, Belgium). Helium (99.999%) and nitrogen (99.99%) were purchased from Air Liquide (Liège, Belgium). For GC-(Q)TOF-MS analysis hexane for ultra-trace analysis grade was purchased from Scharlab (Barcelona, Spain). For UHPLC-quadrupole-TOF (QTOF)-MS analysis HPLC-grade methanol (MeOH), acetonitrile and sodium hydroxide (>99%) were purchased from ScharLab (Barcelona, Spain). Formic acid (HCOOH) (>98% w/w) was obtained from Fluka. HPLC-grade water was obtained from deionised water passed through a Milli-Q water purification system (Millipore, Bedford, MA, United States). Dicyclopentyl(dimethoxy)silane (>98%) was purchased from TCI chemicals (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan). Pentaerythritol tetrakis(3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propionate) (98%)was purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany).

4.2.2.2 Methods

4.2.2.2.1 GC-(EI)MS

Initial non-target analyses of simulant extracts were performed with an Agilent 6890 gas chromatograph coupled to an Agilent 5973 mass selective detector (MSD) equipped with an EI ionisation source and operated in full scan mode from *m/z* 40 to 700. The GC column was a 30 m × 0.25 mm × 0.25 µm DB-5ms column (Agilent JW Scientific, Diegem, Belgium). The temperature of the oven was set at 60 °C for three min and was then increased to 300 °C at a rate of 10 °C min^{-1} where it was held for 15 min. The total run time was 42 min. Helium was used as a carrier gas, with a constant flowrate of 1.0 ml min^{-1}. A volume of two µl of extract was injected so that a sufficiently detectable amount of analyte was brought on the column. The MS spectra obtained for the migrating chemicals extracted by the simulant were compared with commercially available WILEY and NIST mass spectra libraries by use of the Agilent MSD Chemstation® for peak identification. These analyses were carried out at the University of Antwerp. All

the following analyses in this chapter were performed at the University Jaume I of Castellon (Spain).

4.2.2.2.2 GC-(EI)TOF-MS

An Agilent 6890N GC system (Palo Alto, CA, United States) equipped with an Agilent 7683 autosampler, was coupled to a GC TOF mass spectrometer (Waters Corporation, Manchester, UK), operating in EI mode (70 eV). The GC separation was performed using the same column type and oven programme as for the GC-(EI)MS. The interface and source temperatures were both set to 250 °C, and a solvent delay of 3 min was selected. The TOF-MS was operated at one spectrum/s acquisition rate over the mass range *m/z* 50-700, using a multichannel plate voltage of 2800 V. TOF-MS resolution was approximately 8500 at full width at half maximum (FWHM) at *m/z* 614. Heptacosafluorotributylamine (Sigma Aldrich, Madrid, Spain), used for the daily mass calibration and as lock mass, was injected via syringe in the reference reservoir at 30 °C to monitor the *m/z* ion 218.9856. The application manager ChromaLynx, also a module of MassLynx software, was used to investigate the presence of unknown compounds in samples. Library search was performed using the commercial NIST library.

4.2.2.2.3 GC-(APCI)QTOF-MS

An Agilent 7890A GC system(Palo Alto, CA, United States) coupled to a quadrupole TOF mass spectrometer XevoG2QTOF (Waters Corporation, Manchester, UK) with an APCI source was used. The quadrupole–time-of-flight (Q-TOF) tandem mass spectrometer has similarly to the QqQ a series of quadrupoles connected, where the third one is replaced by a TOF analyser (Stachniuk & Fornal 2016). The instrument was operated under MassLynx version 4.1 (Waters Corporation). Sample injections were made using an Agilent 7693 autosampler. The GC separation was performed using the same conditions as described in the previous two GC techniques. About one µl was injected at 280 °C under splitless mode. Helium was used as a carrier gas at 1.2 ml min^{-1}. The interface temperature was set to 310 °C using N2 as auxiliary gas at 150 l h^{-1}, makeup gas at 300 ml min^{-1} and cone gas at 16 l h^{-1}. The APCI corona pin was operated at 1.6 µA with a cone voltage of 20V. The ionisation process occurred within an enclosed ion volume, which enabled control over the protonation/charge transfer processes. Xevo QTOF-MS was operated at 2.5 spectra/s, acquiring a mass range *m/z* 50– 1200. TOF-MS resolution was approximately 18 000 (FWHM) at *m/z* 614. For MS^E measurements, two alternating acquisition functions were used applying different collision energies: a low-energy (LE) function, selecting four eV, and a high-energy (HE) function. In the latter case, a collision energy ramp (25–40 eV) rather than a fixed higher collision energy was used. Heptacosafluorotributylamine (Sigma Aldrich, Madrid, Spain) was used for the daily mass calibration. Internal calibration was performed using a background ion coming from the GC-column bleed as lock mass (protonated molecule of octamethyl-

cyclotetrasiloxane, *m/z* 297.0830). MassFragment software (Waters) was used to explain the fragmentation behaviour of the detected compounds. This software applies a bond disconnection approach to suggest possible structures for the product ions from a given molecule.

4.2.2.2.4 LC-QTOF-MS

A Waters Acquity UHPLC system (Waters, Milford, MA, United States) was interfaced to a hybrid quadrupole-orthogonal acceleration-TOF mass spectrometer (XEVO G2 QTOF, Waters Micromass, Manchester, UK), using an orthogonal Z-spray-ESI interface operating in positive and negative ionisation modes. The UHPLC separation was performed using an Acquity UHPLC BEH C_{18} 1.7 µm particle size analytical column 100 mm l × 2.1 mm internal diameter (I.D.) (Waters) at a flow rate of 300 µl min^{-1}. The mobile phases used were A=H_2O with 0.01% HCOOH and B=MeOH with 0.01% HCOOH. The percentage of organic modifier (B) was changed linearly as follows: 0 min, 10%; 14 min, 90%; 16 min, 90%; 16.01 min, 10%; and 18 min, 10%. Nitrogen (from a nitrogen generator) was used as the drying and nebulising gas. The gas flow was set at 1000 l h^{-1}. The injection volume was 20 µl. The resolution of the TOF mass spectrometer was approximately 20 000 at FWHM at *m/z* 556. MS data were acquired over an *m/z* range of 50–1200. A capillary voltage of 0.7 and 2.5 kV was used in positive and negative ion modes, respectively. A cone voltage of 20V was used. Collision gas was argon 99.995% (Praxair, Valencia, Spain). The interface temperature was set to 600 °C and the source temperature to 130 °C. The column temperature was set to 40 °C.

For MS^E experiments, two acquisition functions with different collision energies were created. The first one is the low-energy (LE) function, selecting a collision energy of 4 eV, and the second one is the high-energy (HE) function, with a collision energy ramp ranging from 25 to 40 eV in order to obtain a greater range of product ions. The LE and HE functions settings were for both a scan time of 0.4 s.

Calibrations were conducted from *m/z* 50 to 1200 with a 1:1 mixture of 0.05 M NaOH: 5% HCOOH diluted (1:25) with acetonitrile:water (80:20). For automated accurate mass measurement, the lock-spray probe was used; using as lockmass, a solution of leucine enkephalin (10 µg ml^{-1}) in acetonitrile:water (50:50) at 0.1% HCOOH was pumped at 20 µl min^{-1} through the lock-spray needle. The leucine enkephalin $[M+H]^+$ ion (*m/z* 556.2771) for positive ionisation mode and $[M-H]^-$ ion (*m/z* 554.2615) for negative ionisation were used for recalibrating the mass axis and to ensure a robust accurate mass measurement over time. It should be noted that all the exact masses shown in this work have a deviation of 0.55 mDa from the 'true' value, as the calculation performed by the MassLynx software uses the mass of hydrogen instead of a proton when

calculating $[M+H]^+$ exact mass. However, because this deviation is also applied during mass axis calibration, there is no negative impact on the mass errors presented in this article. MS data were acquired in centroid mode and processed by the ChromaLynx XS application manager (within MassLynx v 4.1; Waters Corporation).

4.2.2.3 Data processing

4.2.2.3.1 GC data processing

A schematic overview of the GC approach is given in Figure 4.4. The analytical strategy to perform a non-target analysis with GC-MS techniques started from the results obtained in our previous work (Onghena et al. 2014). In a first screening based on GC-(EI)MS data using commercially available WILEY and NIST libraries with Agilent MSD Chemstation software, peaks with an area of at least 10% of the area of the IS were selected for identification. Only compounds with library matches above 90% were accepted as tentative candidates. When the returned match was below 90%, peaks were defined as 'unidentified' as they were most probably not included in the commercial libraries, and further research was conducted with GC-(EI) TOF-MS based on accurate mass data.

By means of the ChromaLynx Application Manager, a module of MassLynx software, the remaining unidentified peaks were deconvoluted and searched again in the commercial nominal mass NIST02 library. A hit list with five positive matches>700 was generated. Next, an elemental composition calculator (maximum deviation of 5 mDa) was applied to determine the five most likely formulae of the five most intense ions acquired in the accurate mass spectrum. The proposed formulae of these five fragments were then compared with the proposed molecular formulae of the top five library hits using criteria like mass error and isotopic fit. When a possible molecular formula could be derived in this way, candidates with this particular empirical formula were searched in the Chemspider database. By using the ChromaLynx MassFragment, which is a tool for fragmentation prediction, the obtained accurate mass EI spectrum could be compared with the predicted fragments of a selected possible structure, and scorings were given. In this way, a differentiation could also be made between different structures with the same empirical formula and those that generate fragments, which are not in accordance with the obtained experimental spectrum, could be rejected.

When no conclusive match could be obtained (e.g. more than one identity fit of possible molecular formulae with the experimental GC-(EI)TOF spectrum), the samples could be re-injected into the GC-(APCI)QTOF system to confirm or exclude preceding tentative GC-(EI)TOF identifications. Because of the reduced fragmentation generally occurring in the APCI source, a search was conducted for the accurate mass molecular ion and the

protonated molecule of the suggested molecular formulae candidates from the (EI)TOF. If one of the two was present, a narrow window-extracted ion chromatogram (nw-Extracted Ion Chromatogram (XIC), ±0.02 Da) resulted in a chromatographic peak eluting approximately two min earlier than the values obtained in the GC-(EI)TOF-MS. If no chromatographic peak appeared performing the nw-XIC for the selected masses, the obtained spectrum at the expected retention time was manually examined for other possible ions that could be the $M^{+\bullet}$ or $[M+H]^+$. Often the (EI)TOF spectrum still contains minor amounts of $M^{+\bullet}$ (or $[M+H]^+$), which are more abundant in the (APCI)QTOF. Consequently, by comparing the (EI)TOF and the (APCI)QTOF spectra, generally, $M^{+\bullet}$ or $[M+H]^+$ could be retrieved. Again, the elemental composition software (±5 mDa) was used to determine the molecular formula of the unknown compound. Then, the fragmentation pattern in the (APCI)QTOF of the unknown compound was studied by examining the MS^E data, which provide useful further information about the fragmentation. Normally, the HE mode offers most information about how the compound fragments as the presence of $M^{+\bullet}$ or $[M+H]^+$ diminishes and fragmentation increases. For some compounds, quite severe fragmentation occurs already in the LE mode. Experimentally recorded fragmentation patterns can also here be compared with software generated ones for possible candidates by the use of MassFragment. When commercially available, standards were bought to confirm the actual presence of the suggested compounds.

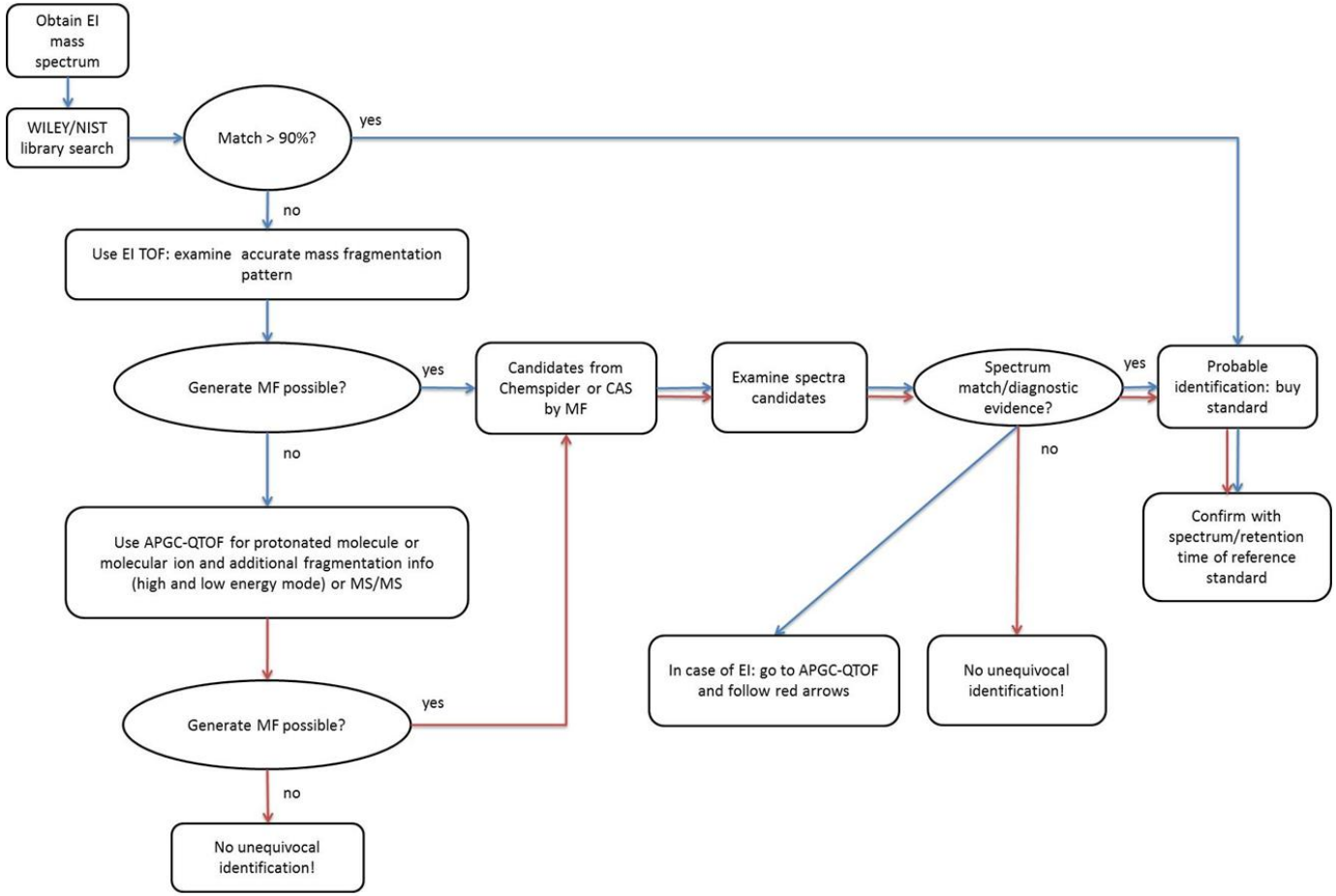


Figure 4.4: Schematic overview of GC methodology for the non-target screening and elucidation of unknown plastic migrants. MF = molecular formula

4.2.2.3.2 LC data processing

A graphical overview of the LC workflow was given in Figure 4.5. No commercial MS libraries of common plastic migrants are available for LC-MS, and a genuine non-target approach of the raw data would result in a far too laborious data processing. Therefore, we constructed a home-made database to facilitate a wide-scope suspect screening. By including the empirical formula of a compound in the database, the ChromaLynx software processes this against the obtained accurate mass spectra, and positive matches are returned if the mass error (±0.002Da) is appropriate. First, approximately 50 migrants that were previously detected in the alternative plastics to PC baby bottles were included in this list (Simoneau et al. 2012; Onghena et al. 2014). Because all analytical standards of these compounds were available to us, their experimental data (retention time and product ions) were also included in the database. Second, the empirical formulae of around 190 common plastic additives were added, because these compounds could also migrate from the alternative polymers. Last, more than 800 compounds authorised for plastic FCMs by the European Union Regulation no. 10/2011 (European Union 2011a) were included in the database.

For most compounds in this database, the only criterion to obtain a positive match was to search by the exact mass of the empirical formula. This commonly led to several false positive hits. Therefore, every positive hit (a peak detected, commonly corresponding to the exact mass of the (de)protonated molecule) was checked manually evaluating the product ions and characteristic isotopic ions, leading to the tentative identification of the candidate, based on structure compatibility and comparison with the available literature data. Adducts, such as $[M+Na]^+$ or $[M+K]^+$, were also included to facilitate the detection of some compounds in those cases where information existed on their possible formation. Also here, the analytical standards were purchased for confirmation when commercially available.

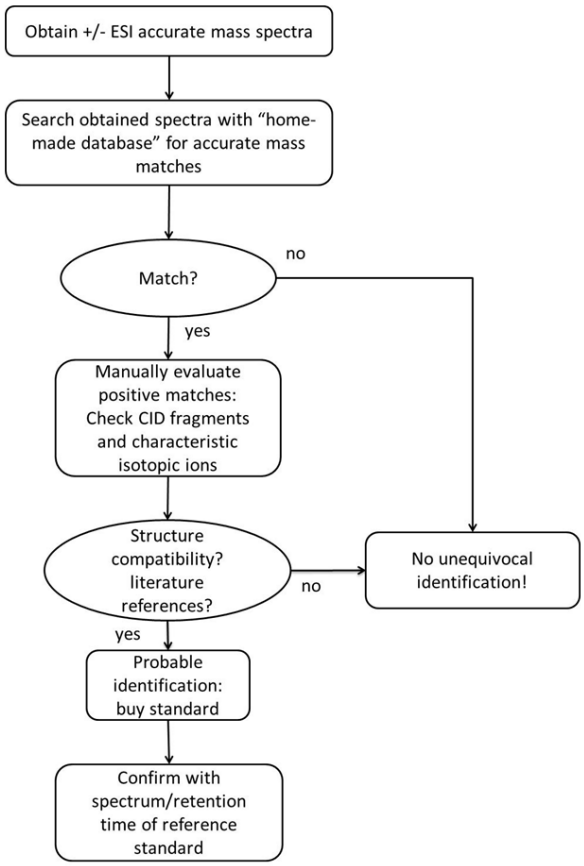


Figure 4.5: Schematic overview of LC methodology for the non-target screening and elucidation of unknown plastic migrants.

4.2.3 Results and discussion

4.2.3.1 Selection of techniques

Until now, most analytical methods employed for the determination of plastic migrants have been focused on the targeted analysis of a restricted number of a priori selected compounds (Gärtner et al. 2009; Mezcua et al. 2012; Reinas et al. 2012). However, potential migrating compounds other than the target analytes cannot be detected using this approach. EI ionisation used in GC produces highly reproducible fragmentation spectra, which make the identification of unknown compounds possible by comparison with commercially available mass spectral libraries (e.g. Wiley and NIST). Because of its ability to obtain sensitive full scan data and accurate mass measurements (Krauss et al. 2010; Hernández, Portolés, et al. 2011; Hernández et al. 2012), GC-TOF-MS and hybrid QTOF-MS are powerful mass analysers for a wide variety of non-target applications for semi-volatiles (Hernández, Bijlsma, et al. 2011; Cajka 2013). Because of a high degree of

fragmentation in EI ionisation, the molecular ion often has a low abundance. This is an important limitation for structural elucidation, as the presence of the molecular ion in a mass spectrum, especially if measured at accurate mass, provides crucial information. In APCI ionisation, a stable (quasi)molecular ion is formed by means of charge transfer ($M^{+\bullet}$) and/or by protonation ($[M+H]^+$). The APCI interface used in GC can be coupled with a wide range of high resolution mass analysers (TOF, QTOF).

For LC analysis, the accurate mass product ion spectra obtained in the MS/MS mode on the QTOF-MS provide relevant structural information. However, because the pre-selection of analyte precursor ions has to be carried out in the quadrupole, this results in the usual loss of isotopic pattern information. This drawback can be overcome by MS^E data acquisition, in which both accurate mass (de)protonated molecule (LE function) and product ions (HE function) are obtained in the same injection without the need of selecting any precursor ion. The sequential collection of LE and HE data during sample analysis is a significant advantage towards the structural elucidation of unknown compounds in a non-targeted screening approach (Bijlsma et al. 2011).

In this manuscript, we have included a selection of examples to demonstrate the developed strategy for the elucidation of unknown migrants from polymer baby bottles. The selection of the cases was based on their ability to illustrate the contribution of each ionisation technique and mass analyser towards the final identification. A detailed overview of all identified compounds and the used techniques can be found in Table 4.5. Because most migrating compounds are small molecules (molecular weight of <1200Da), the parameters to calculate the possible molecular formulae with the elemental composition software were generally set as follows: C: 0–50, H: 0–100, O: 0–10, N: 0–10 and P: 0–5. Other atoms were included in the search if after manual inspection of the spectrum the isotope pattern indicated the presence of other elements. A maximum deviation of 2 mDa from the measured mass was applied. When searching for the $M^{+\bullet}$ (if existing), the option 'odd electron ions only' was added. For $[M+H]^+$, this option was 'even electron ions only'. For fragments, both odd and even options were selected.

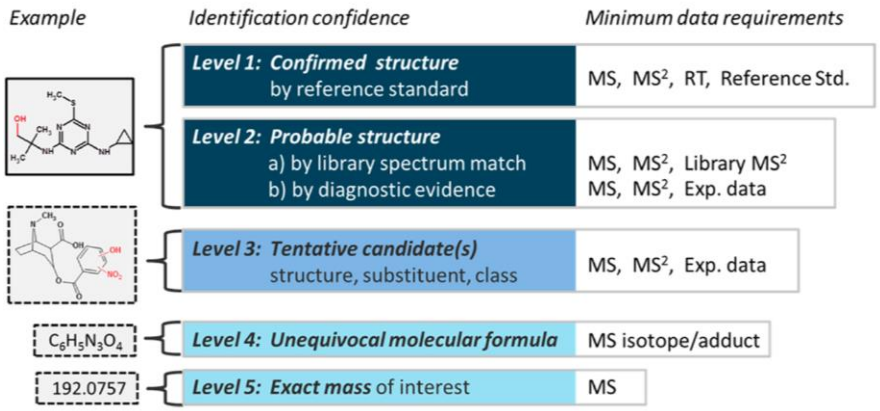


Figure 4.6: Proposed identification confidence levels in high resolution mass spectrometric analysis (Schymanski et al. 2014)

Within the workflows proposed in Figure 4.4 and 4.5, the criteria introduced by Schymanski et al. (Schymanski et al. 2014) were used towards the acceptance of an unambiguous identification of a compound. Here, five different levels of identification were defined, each with their corresponding requirements varying from a level 5 mass of interest identification to an unequivocal molecular formula (level 4), tentative candidate (level 3), probable structure (level 2) and confirmed structure (level 1) (Figure 4.6). Because of the lack of commercial availability or sometimes relatively high prices of some products (sometimes up to 300-400€ per standard), not all analytical standards of tentatively identified migrants were obtained. Here, identification was only carried out until level 2 of these criteria.

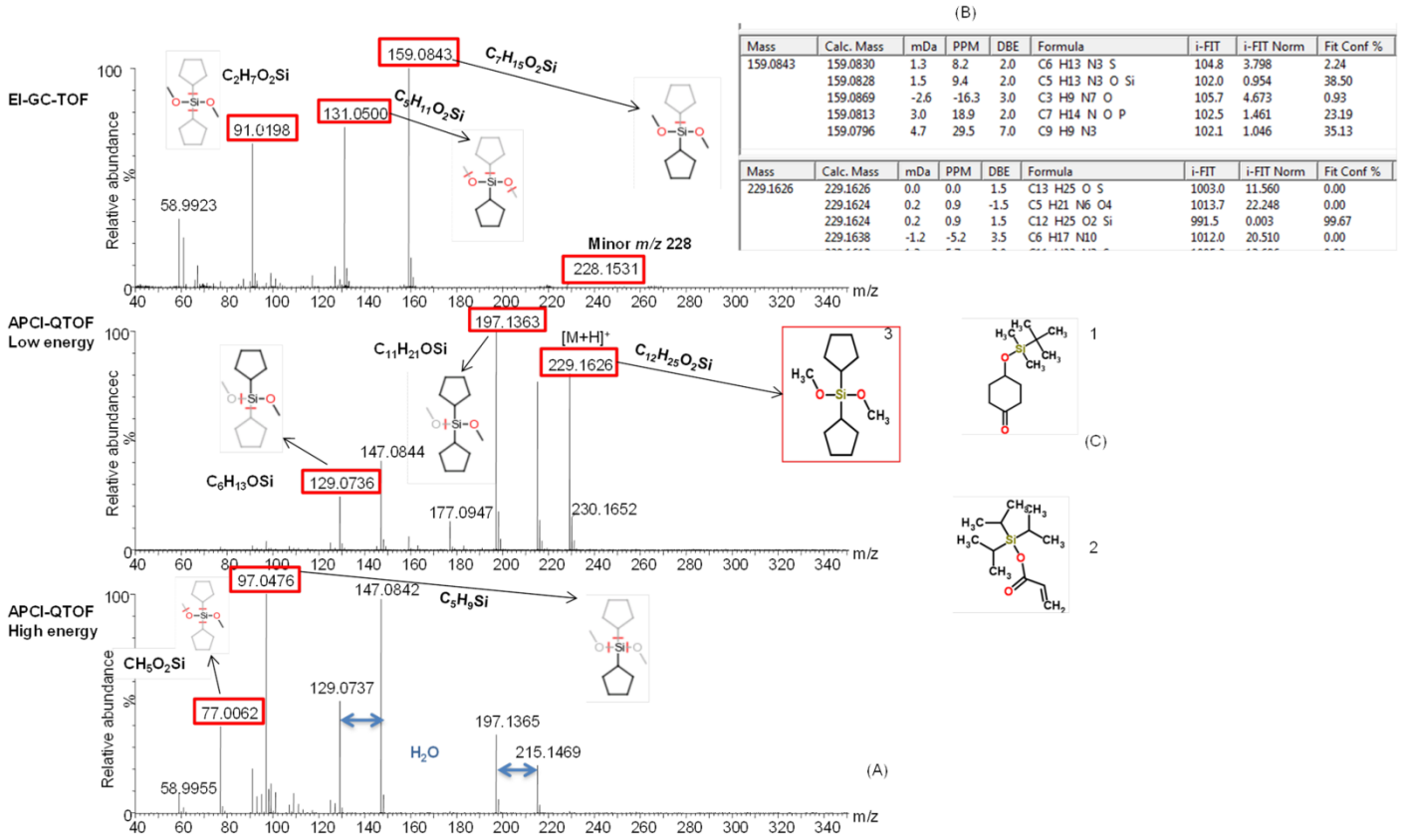
Table 4.5: Summary of detected compounds, techniques used and related errors.

Compound name	Identification level	Techniques used for identification	GC-(EI)TOF-MS error (ppm)	GC-(APCI)QTOF-MS error (ppm)	LC-QTOF-MS error (ppm)
Dicyclopentyl(dimethoxy)silane	1	GC-(EI)MS / GC-(EI)TOF-MS / GC-(APCI)QTOF-MS	-6.6	0.9	/
(2)-hydroxypropylstearate / (3)-hydroxypropylstearate / 1-hydroxypropan-2-yl-stearate	2	GC-(EI)MS / GC-(EI)TOF-MS / GC-(APCI)QTOF-MS	-7.6	-0.9	/
Laurolactam monomer / dimer / trimer	2	GC-(EI)MS / GC-(EI)TOF-MS / GC-(APCI)QTOF-MS / LC-QTOF-MS	-13.4	0.0	-3.0
bis(3,4-dimethylbenzylidene)sorbitol	2	LC-QTOF-MS	/	/	-0.7
2,5-bis(5'-*tert*-butyl-2-benzoxaolyl)thiophene	2	LC-QTOF-MS	/	/	-0.9
Irganox 1010	1	LC-QTOF-MS	/	/	4.9
p-*tert*-octylphenol	1	GC-(EI)-MS /GC-(EI)TOF-MS	3.4	/	/
Diisopropylxanthate	2	GC-(EI)MS / GC-(EI)TOF-MS / GC-(APCI)QTOF-MS	6.7	0.6	/
Dibutyl phthalate	1	GC-(EI)-MS / LC-QTOF-MS	/	/	1.4
Diisobutyl phthalate	1	GC-(EI)-MS / LC-QTOF-MS	/	/	-0.4
Benzoic acid, 4-ethoxy-, ethyl ester	2	LC-QTOF-MS	/	/	1.5

4.2.3.2 Case study 1

In the GC-(EI)MS, an unknown chromatographic peak with a retention time of 14.30 min was detected in most PP samples tested. No firm library match was obtained, and scores were very poor (<70%). Because of its detection frequency and because the intensity was comparable with that of the IS (± 10 µg kg^{-1} assuming an equal response factor), this compound was of major interest. Therefore, the compound was analysed further with GC-(EI)TOF-MS (Figure 4.7). When performing a database search using the accurate mass fragmentation data obtained, no improvement in the match factors was perceived. Regarding the (EI)TOF spectrum (Figure 4.7), the ion *m/z* 159.0843 would be assumed to be the possible $M^{+\bullet}$. A clear isotope pattern at M+1 and M+2 was seen, and therefore both S and Si were included for the elemental composition search. This resulted in five possible molecular formulae, although only two of them ($C_6H_{13}N_3S$ and $C_5H_{13}N_3OSi$) could possibly explain the isotope pattern seen.

Looking at the LE APCI spectrum (Figure 4.7), *m/z* 229.1626 is the highest mass acquired, suggesting that this would be the $M^{+\bullet}$ or $[M+H]^+$ of the unknown compound and that 159.0843 is a major fragment ion. Indeed, a very small and hardly visible peak was perceived at *m/z* 228.1531 in the (EI)TOF spectrum, suggesting that *m/z* 229.1626 was $[M+H]^+$. A large number of molecular formulae (>20) were calculated, but after considering the mass errors, only three formulae remained. Of these three, already one could be discarded, as $C_5H_{21}N_6O_4$ is not an existing chemical structure. This reduced the possible empirical formulae to $C_{13}H_{24}OS$ or $C_{12}H_{24}O_2Si$. Investigating the isotope ratios and the elemental compositions of the fragments starting from these two formulae, the option implying a Si atom clearly fitted best to the obtained spectra. A number of 116 positive hits were returned when searched in the Chemspider database. At this point, a literature search using the term '$C_{12}H_{24}O_2Si$+PP' quickly returned the suggestion of dicyclopentyl(dimethoxy)silane (structure 3, Figure 4.7). This alkyl silane is used in combination with Ziegler–Natta catalysts to increase the isotactic index of PP (Xu et al. 2006). This structure was also suggested by Chemspider as the third most cited one. The first two structures (Figure 4.7) were considered as well, but already when checking the APCI spectrum with the MassFragment prediction software, the ions *m/z* 197.1363 (loss of CH_4O), 159.0844 (loss of C_5H_{10})or 129.0736 (loss of $C_6H_{12}O$) could only be explained by structure 3. The respective masses *m/z* 215.1469, 177.0947 and 147.0844 could be explained as the addition of a water molecule to these fragments. The inclusion of a small amount of water in the APCI source to promote the formation of the $[M+H]^+$ could explain this phenomenon as already described by Wachsmuth et al. (Wachsmuth et al. 2014) Therefore, dicyclopentyl(dimethoxy)silane was retained as the probably identified migrant. The presence of this compound (level 1 identification) was afterwards unambiguously confirmed by injection of the purchased commercial standard.



Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %
159.0843	159.0830	1.3	8.2	2.0	C6 H13 N3 S	104.8	3.798	2.24
	159.0828	1.5	9.4	2.0	C5 H13 N3 O Si	102.0	0.954	38.50
	159.0869	-2.6	-16.3	3.0	C3 H9 N7 O	105.7	4.673	0.93
	159.0813	3.0	18.9	2.0	C7 H14 N O P	102.5	1.461	23.19
	159.0796	4.7	29.5	7.0	C9 H9 N3	102.1	1.046	35.13

Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %
229.1626	229.1626	0.0	0.0	1.5	C13 H25 O S	1003.0	11.560	0.00
	229.1624	0.2	0.9	-1.5	C5 H21 N6 O4	1013.7	22.248	0.00
	229.1624	0.2	0.9	1.5	C12 H25 O2 Si	991.5	0.003	99.67
	229.1638	-1.2	-5.2	3.5	C6 H17 N10	1012.0	20.510	0.00

Figure 4.7: (A) (EI)TOF (top), (APCI)QTOF low-energy (middle) and high-energy (bottom) spectra of unknown 1 with indicated fragments originating from structure number 3. (B) Possible elemental compositions for *m/z* 159.0843 and 229.1626. (C) Top three Chemspider possible structures for $C_{12}H_{24}O_2Si$.

4.2.3.3 Case study 2

Two peaks with an EI spectrum that exhibited similarities to those of the previously identified, respectively, hexa- (22.54 min) and octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (24.22 min), were found in a PP sample at high intensities (more than six times the area of the IS). Library matching gave poor results (<70%) and did not suggest any structures with realistic possibilities either. The abundant presence of ion *m/z* 343.3209 in the LE function of the (APCI)QTOF suggested that for the compound related to the octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester this *m/z* had to be the $[M+H]^+$. The low abundant presence of ion *m/z* 342.3108 in the (EI)TOF spectrum indeed confirmed that ion *m/z* 343.3209 was the protonated molecule, resulting in a molecular formula of $C_{21}H_{42}O_3$. Chemspider returned 59 possible structures for this empirical formula. The presence of ions *m/z* 284.2723 and 285.2791 in the (EI)TOF and LE (APCI)QTOF spectrum, respectively, indicated the presence of an integral stearic acid moiety ($C_{18}H_{36}O_2$) in the structure, which made us discard all other possible molecular structures; and thus, only five possibilities remained (Figure 4.8(B)). The detection of this *m/z* also revealed that, for the remaining C_3H_6O moiety, the position of the third O-atom of this molecule had to be at the ultimate or the penultimate C-atom, whether or not incorporated as an ether (structures 1 and 2) or as an alcohol group (structures 3–5) (Figure 4.8(B)). Indeed, to explain the presence of fragment *m/z* 284.2723, the rules of the McLafferty rearrangement had to be applied, stating that the sixth atom starting from the carbonyl-O has to be a hydrogen atom. In this way, structure 2 (Figure 4.8(B)) could already be rejected as a possibility. The presence of *m/z* 325.3109 in the LE (APCI)QTOF spectrum, explained by the loss of a water molecule, suggests, on the other hand, the presence of a free alcohol group instead of an ether, because the loss of water is easier and more probable in this case, which eliminates structure 1 as well. Within the available MS spectra, it was not possible though to differentiate between the remaining structural isomers of structures 3–5 to determine which the actual unknown migrant was and only a probable identification could be reached (level 2). Injection of the different analytical standards is the only way to bring a decisive answer here. These standards were however only obtainable in large quantities (kilograms) and at corresponding prices. For the hexadecanoic acid-based unknown migrant, the same conclusions could be drawn.

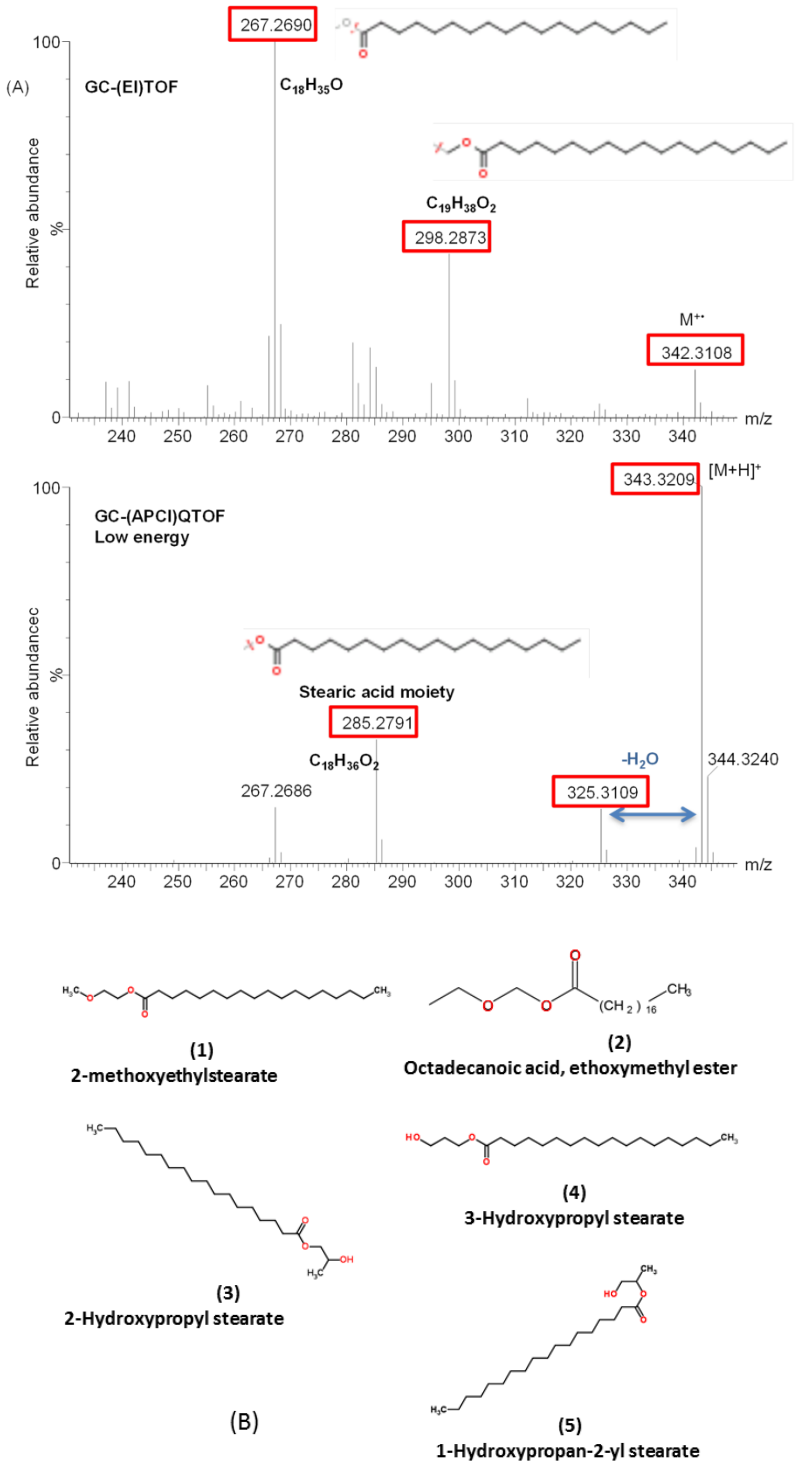


Figure 4.8: (A) (EI)TOF (top) and (APCI)QTOF low-energy spectra of unknown 2 with structures of the most abundant fragments and (B) possible molecular structures for unknown 2 with molecular formula $C_{21}H_{42}O_3$.

4.2.3.4 Case study 3

In this case, an unknown compound with a double intensity of the IS peak was seen in the first migration step of the PA bottle, although it completely disappeared in the next migration steps. Both GC-(EI)MS and GC-(EI)TOF-MS database searches gave poor matches (<40%), indicating that the structure of the unknown migrant was very different from the structures present in the database. The abundant ion *m/z* 394.3612 in the GC-(EI)TOF-MS (RT 31.79 min) seemed to be the $M^{+\bullet}$, which was indeed confirmed by the highly abundant presence of *m/z* 395.3638 (protonated molecule) in the LE GC-(APCI)QTOF-MS spectrum. Because no significant isotope patterns were noticed, an elemental composition search including only elements C, O, H and N resulted in a molecular formula of $C_{24}H_{46}N_2O_2$ (mass error of -0.2 mDa) for which Chemspider returned 32 hits. For this molecular formula, all fragment ions of both GC-(EI)TOF-MS and the HE of the GC-(APCI)QTOF-MS could be explained with very low mass errors (generally <2 mDa for the TOF and <0.2 mDa for the QTOF), differentiating clearly the realistic possible fragments. It was noticeable that the most abundant (EI)TOF-MS ion (*m/z* 198.1868, $C_{12}H_{24}NO$) and the second most abundant (APCI)QTOF-MS fragment ion (*m/z* 197.2014, $C_{12}H_{25}N_2$) exhibited a mass difference of only 1 amu with different though very similar empirical formulae, suggesting a common origin (Figure 4.9).

This observation, together with the presence in this sample of a large amount of laurolactam, a PA monomer with *m/z* 197.1780 and a molecular formula of $C_{12}H_{23}NO$, (GC-(EI)TOF-MS RT 17.08 min) suggested that this unknown might be a dimer of laurolactam, because its molecular formula is exactly the double of this compound and the ion *m/z* 395.3638 is two times the mass of the protonated form of laurolactam. Another evidence is the disappearance of this unknown compound after the first migration step. Because this dimer is a side product of the polymerisation reaction, it is probably unbound in the polymer skeleton. Therefore, it can easily be transferred to the migration solution and disappear in the second migration step. Although data were rather conclusive, LC-QTOF-MS was also used to confirm the presence of this dimer, because no commercial standard was available. Indeed, the protonated monomer (*m/z* 198.1861, $C_{12}H_{23}NO$, RT: 7.41 min), the dimer (*m/z* 395.3626, $C_{24}H_{46}N_2O_2$, RT: 7.74 min) and even the trimer (*m/z* 592.5419, $C_{36}H_{70}N_3O_3$, RT: 8.39 min, most probably not eluted on GC) were seen in the LC-QTOF-MS (Figure 4.10). The MS spectra of these oligomers were undeniably confirmed by Stoffers et al. (Stoffers et al. 2003). Regarding the identification criteria proposed by Schymanski et al. (Schymanski et al. 2014), this leads us only to a level 2a identification: probable structure, unambiguous literature spectrum-structure match, but not confirmed by a reference standard. It has to be noticed although that, in this particular case, the degree of confirmation could already be considered as high, because three different ionisation techniques (EI, APCI and ESI)

have been applied. Yet, this is not always possible, because some compounds are not suited for both GC and LC.

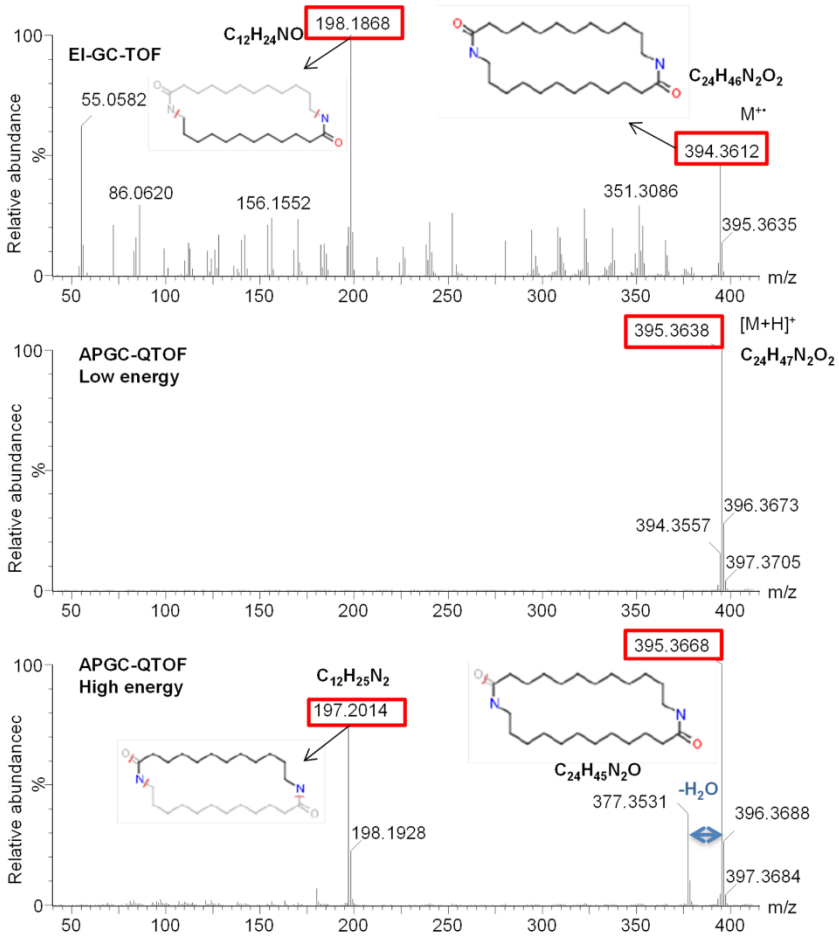


Figure 4.9: GC-(EI)TOF (top), GC-(APCI)QTOF low-energy (middle) and high-energy (bottom) spectra of unknown 3 with empirical formulae and fragments of the most abundant peaks.

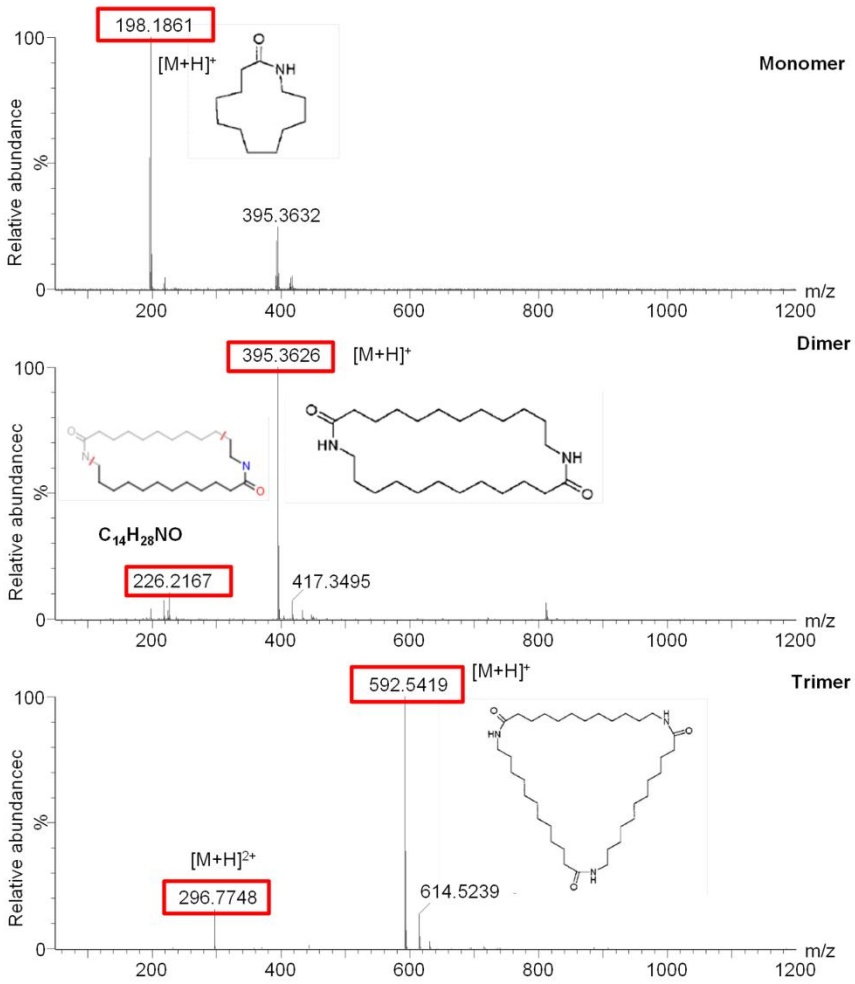


Figure 4.10: LC-QTOF spectra of laurolactam monomer (top), dimer (middle) and trimer (bottom). (Source structures (Stoffers et al. 2003)).

4.2.3.5 Case study 4

The following case study was based on a positive accurate mass match of a peak eluted in the LC with RT of 7.85 min having the accurate mass of bis(3,4-dimethylbenzylidene)sorbitol ($C_{24}H_{30}O_6$, Millad 3988, a nuclear clarifying agent for PP) (McDonald et al. 2008), with the processed LC data in ESI(+) mode. For nine out of ten PP bottles, the protonated mass of *m/z* 415.2118 was matched with an error of <2 mDa and with good isotope fittings. To confirm its presence, a literature search was conducted to compare the obtained MS spectra with the available literature. McDonald et al. (McDonald et al. 2008), provided characteristic MS data for this compound, which indeed matched with our data (Figure 4.11). The protonated molecule *m/z* 415.2121 was in the LE mode and also the most abundant ion. Furthermore, the $[M+Na]^+$ and

$[M+K]^+$ adducts were also identified with masses *m/z* 437.1941 and 453.1682, respectively. The *m/z* 119.0862 (C_9H_{11}), which originates from the loss of one of the two dimethylbenzene moieties, was already seen in the LE function, and this ion was the most significant in the HE spectrum. Ions *m/z* 397.2010 (loss of H_2O), 295.1187 ($C_{15}H_{19}O_6$) and 277.1802 ($C_{15}H_{17}O_5$) were also retrieved in the HE function, although in relatively small abundances. The elemental composition calculator confirmed that all these fragments were indeed present, calculating their empirical formulae with low mass errors (less than ±0.8 mDa). It was noteworthy that 3,4-dimethylbenzaldehyde, a degradation product of Millad 3988, was retrieved in the GC-MS injections of all PP samples that contained this compound, confirming indirectly its presence. Therefore, we conclude the identification of Millad 3988 with a high confidence (level 2) as migrant from most PP baby bottles. An analytical standard was difficult to purchase (available only from China).

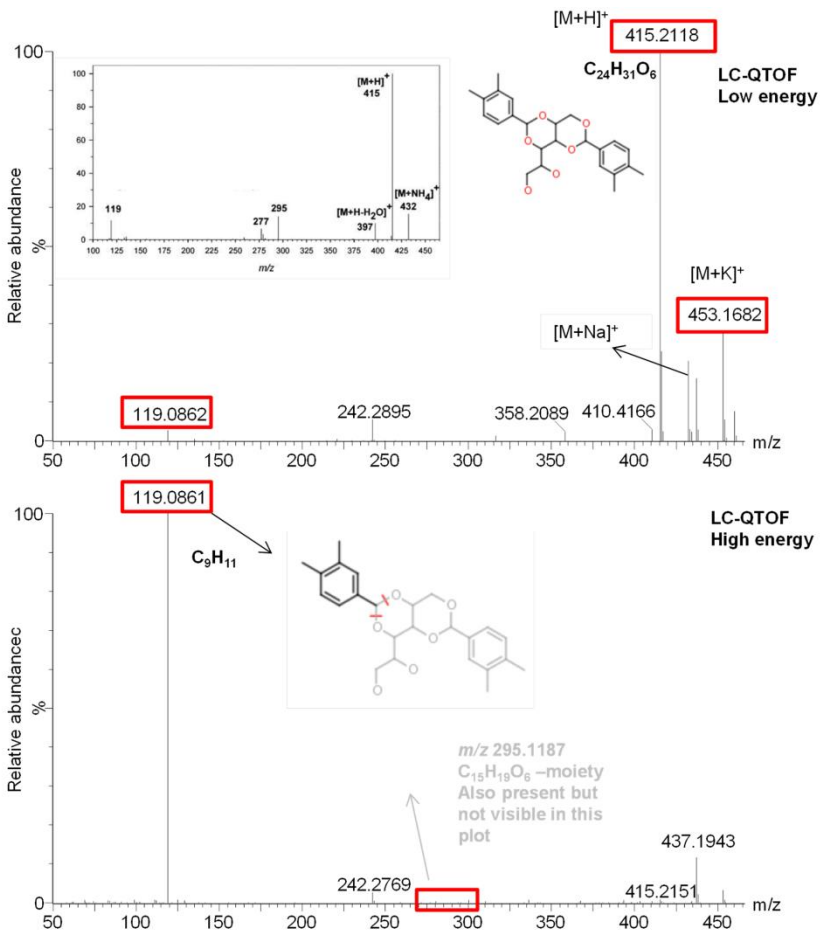


Figure 4.11: Literature (McDonald et al. 2008) +LC-MS spectrum (upper left corner) compared with the spectra obtained by us on ESI(+) LC-QTOF MS (upper right LE mode, lower right HE mode) for suggested compound bis(3,4-dimethylbenzylidene)sorbitol.

4.2.3.6 Case study 5

The accurate mass of the protonated molecule $C_{26}H_{27}N_2O_2S$, *m/z* 431.1789 (LC RT 11.9 min), corresponding to 2,5-bis(5'-*tert*-butyl-2-benzoxaolyl)thiophene, an optical brightening agent for polymers, was returned as a possible positive hit when comparing a PP sample acquired in ESI(+) mode to the LC database part containing plastic additives (mass error 0.4 mDa) (Figure 4.12). Literature search (Guo et al. 2013) supported this finding, as besides the protonated molecule, it also explained the fragments *m/z* 415.1467 and 401.1303, which were seen in the HE mode and matched by the elemental composition calculator as $C_{25}H_{23}N_2O_2S$ (1 mDa error) and $C_{24}H_{21}N_2O_2S$ (2.6 mDa error), respectively. No further fragments could be seen because of the complexity of this structure. To obtain a higher confidence degree in the identification of the compound, more fragments are necessary to be obtained by applying higher collision energies. At the time of elucidation, the analytical standard was not commercially available.

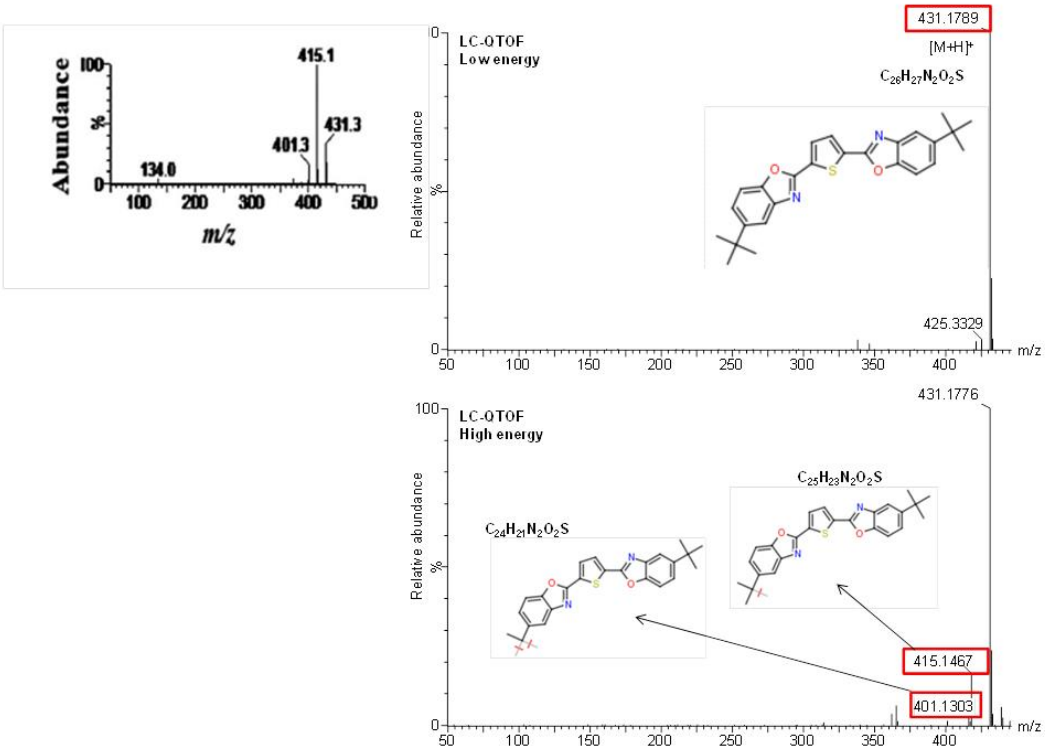


Figure 4.12: Literature (Guo et al. 2013) (+) LC-MS spectrum (left) compared to the spectra obtained by us on ESI(+) LC-QTOF MS (upper right LE mode, lower right HE mode) for suggested compound 2,5-bis(5'-*tert*-butyl-2-benzoxaolyl)thiophene.

4.2.3.7 Case study 6

The last example involves the compound pentaerythritol tetrakis(3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propionate), an antioxidant better known under its commercial name Irganox 1010. An accurate mass matching for mass *m/z* 1175.7821 ($C_{73}H_{107}O_{12}$) was obtained for this compound in all PP samples injected under ESI(-) mode in LC-QTOF-MS. Although the protonated molecule was not present in the positive mode, its deprotonated molecule was seen in the ESI(-) mode. Comparison of our experimental spectra with literature data only could confirm the deprotonated molecule (Woodman 2003). However, the injection of an available reference standard of Irganox 1010 matched perfectly in retention time and fragmentation pattern confirming in this way the unequivocal identification of this compound.

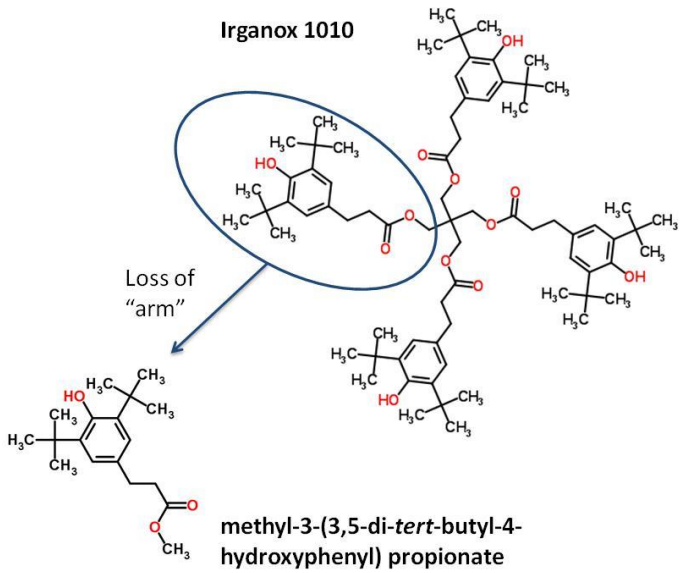


Figure 4.13: Indication of the loss of methyl-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl) propionate (found in GC-MS analysis) from the original structure of Irganox 1010 (found by LC-MS).

The presence of Irganox 1010 was already suggested in our previous work because several potential degradation products of this compound were found by GC-(EI)MS analysis (Onghena et al. 2014). The compound methyl-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl) propionate ($C_{18}H_{28}O_3$), originating from a loss of one of the four 'arms' of the original antioxidant (Figure 4.13), was detected in all PP samples tested before, although until now, no concrete link with its origin from Irganox 1010 could be established. This example demonstrates again the power of the simultaneous use of these complementary techniques for the analysis of unknown migrants from polymer products.

4.2.4 Conclusions

An efficient analytical strategy based on the combination of several mass analysers coupled to both gas and liquid chromatography has been applied for non-target analysis of migrating components from polymer baby bottles. The complementary use of GC-(EI)MS, GC-(EI) TOF-MS, GC-(APCI)QTOF-MS and UHPLC-QTOF-MS allowed an efficient and wide-scope target and non-target screening on samples based on a food simulant, in this case H_2O-EtOH (50:50, v/v), that had been previously into contact with baby bottles. The methodology was applied to six case studies to illustrate the analytical challenges when the mass spectra of the unknown compounds did not match with commercially available GC-(EI)MS libraries. Furthermore, the use of a home-made database including a large number of compounds of interest for detection of compounds via LC-QTOF was discussed into detail. The strategy applied in this work has been proven to be successful for the elucidation of several unknown migrants, from non-polar volatile compounds to semi-polar non-volatiles. Despite the success of the (tentative) identification of some relevant compounds, the successful elucidation of unknowns is not only a matter of easily following a standardised procedure. It is a process that also requires experience and creative insight of the analyst, next to the use of several analytical techniques, which still makes it a challenging and quite tedious labour.

References

Alin J, Hakkarainen M. 2010. Type of Polypropylene Material Significantly Influences the Migration of Antioxidants from Polymer Packaging to Food Simulants During Microwave Heating. J Appl Polym Sci. 118:1084–1093.

Barret J. 2013. Assessing the Safety of a Replacement Chemical: Nongenomic activity of bisphenol-S. Environ Health Perspect. 121:97.

Bijlsma L, Sancho J V, Hernández F, Niessen WM. 2011. Fragmentation pathways of drugs of abuse and their metabolites based on QTOF MS/MS and MS(E) accurate-mass spectra. J Mass Spectrom. 46:865–75.

Bittner GD, Yang CZ, Stoner MA. 2014. Estrogenic chemicals often leach from BPA-free plastic products that are replacements for BPA-containing polycarbonate products. Environ Health. 13:41.

Cajka T. 2013. Gas chromatography-time-of-flight mass spectrometry in food and environmental analysis. In: Compr Anal Chem. Vol. 61. 1st ed. Amsterdam: Elsevier B.V.; p. 271–302.

Castillo R, Biedermann M, Riquet A-M, Grob K. 2013. Comprehensive on-line HPLC-GC for screening potential migrants from polypropylene into food: The effect of pulsed light decontamination as an example. Polym Degrad Stab. 98:1679–1687.

Chanda M, Roy SK. 2006. Plastics technology handbook. New York: Taylor and Francis group.

Council of Europe. 2004. Resolution ResAP(2004)5 on silicones used for food contact applications. Off J Eur Union [Internet]. Available from: https://wcd.coe.int/ViewDoc.jsp?id=797547&Site=CM

European Union. 2011a. Commission Regulation (EU) No 10/2011 of 14 Januari 2011 on plastic materials and articles intended to come into contact with food. Off J Eur Union. L12:1–89.

European Union. 2011b. 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. Off J Eur Union. L26:11–14.

Faghihnejad A, Zeng H. 2013. Fundamentals of Surface Adhesion, Friction, and Lubrication. In: Polym Adhes Frict Lubr (ed H Zeng). Hoboken, NJ, USA: John Wiley & Sons; p. 1–57.

Gärtner S, Balski M, Koch M, Nehls I. 2009. Analysis and migration of phthalates in infant food packed in recycled paperboard. J Agric Food Chem. 57:10675–10681.

Geueke B, Wagner CC, Muncke J. 2014. Food contact substances and chemicals of concern: a comparison of inventories. Food Addit Contam Part A. 31:1438–50.

Grob K. 2014. Work plans to get out of the deadlock for the safety assurance of migration from food contact materials? A proposal. Food Control. 46:312–318.

Guo X, Xian Y, Luo H, Wu Y, Luo D, Chen Y, Lu Y, Xu D. 2013. Quantitative determinations of seven fluorescent whitening agents in polystyrene and polyvinyl chloride plastics by ultrahigh performance liquid chromatography–tandem mass spectrometry. Anal Methods. 5:6086.

Hernández F, Bijlsma L, Sancho J V, Díaz R, Ibáñez M. 2011. Rapid wide-scope screening of drugs of abuse, prescription drugs with potential for abuse and their metabolites

in influent and effluent urban wastewater by ultrahigh pressure liquid chromatography-quadrupole-time-of-flight-mass spectrometry. Anal Chim Acta. 684:87–97.
Hernández F, Portolés T, Pitarch E, López FJ. 2011. Gas chromatography coupled to high-resolution time-of-flight mass spectrometry to analyze trace-level organic compounds in the environment, food safety and toxicology. Trends Anal Chem. 30:388–400.
Hernández F, Sancho J V, Ibáñez M, Abad E, Portolés T, Mattioli L. 2012. Current use of high-resolution mass spectrometry in the environmental sciences. Anal Bioanal Chem. 403:1251–64.
Kirchnawy BC, Mertl J, Osorio V, Hausensteiner H, Washüttl M, Bergmair J, Pyerin M, Tacker M. 2014. Detection and Identification of Oestrogen-Active Substances in Plastic Food Packaging Migrates. Packag Technol Sci. 27:467–478.
Krauss M, Singer H, Hollender J. 2010. LC-high resolution MS in environmental analysis: from target screening to the identification of unknowns. Anal Bioanal Chem. 397:943–51.
Kuruto-Niwa R, Nozawa R, Miyakoshi T, Shiozawa T, Terao Y. 2005. Estrogenic activity of alkylphenols, bisphenol S, and their chlorinated derivatives using a GFP expression system. Environ Toxicol Pharmacol. 19:121–30.
Li X, Xiong W, Lin H, Zhuo L, Lv S, Tang X, Chen M, Zou Z, Lin Z, Qiu B, Chen G. 2013. Analysis of 16 phthalic acid esters in food simulants from plastic food contact materials by LC-ESI-MS/MS. J Sep Sci. 36:477–84.
Maiolini E, Ferri E, Pitasi AL, Montoya A, Di Giovanni M, Errani E, Girotti S. 2014. Bisphenol A determination in baby bottles by chemiluminescence enzyme-linked immunosorbent assay, lateral flow immunoassay and liquid chromatography tandem mass spectrometry. Analyst. 139:318–24.
Mcdonald GR, Hudson AL, Dunn SMJ, You H, Baker GB, Whittal RM, Martin JW, Jha A, Edmondson DE, Holt A. 2008. Bioactive Contaminants Leach from disposable laboratory plasticware. Science. 322:917.
McDonald J, Cummins CL, Barkley RM, Thompson BM, Lincoln HA. 2008. Identification and Quantitation of Sorbitol-Based Nuclear clarifying agents extracted from common laboratory and consumer plasticware made of PP. Anal Chem. 80:5532–5541.
Mezcua M, Martinez-Uroz MA, Gomez-Ramos MM, Gomez MJ, Navas JM, Fernandez-Alba AR. 2012. Analysis of synthetic endocrine-disrupting chemicals in food: A review. Talanta. 100:90–106.
Nutricia baby - Danone Group. 2014. Flesvoeding maken [Internet]. Available from: http://www.nutriciababy.be/NL/babymaanden/Flesvoeding/Paginas/Flesvoeding-maken.aspx
Onghena M, van Hoeck E, Vervliet P, Scippo ML, Simon C, van Loco J, Covaci A. 2014. Development and application of a non-targeted extraction method for the analysis of migrating compounds from plastic baby bottles by GC-MS. Food Addit Contam Part A. 31:2090–102.
Reinas I, Oliveira J, Pereira J, Machado F, Poças MF. 2012. Migration of two antioxidants from packaging into a solid food and into Tenax. Food Control. 28:333–337.
Schymanski EL, Jeon J, Gulde R, Fenner K, Ru M, Singer HP, Hollender J. 2014. Identifying

Small Molecules via High Resolution Mass Spectrometry: Communicating Confidence. Environ Sci Technol. 48:2097–2098.

Simoneau C, Van Den Eede L, Valzacchi S. 2012. Identification and quantification of the migration of chemicals from plastic baby bottles used as substitutes for polycarbonate. Food Addit Contam Part A. 29:469–480.

Simoneau C, Valzacchi S, Morkunas V, Van Den Eede L. 2011. Comparison of migration from polyethersulphone and polycarbonate baby bottles. Food Addit Contam Part A. 28:1763–1768.

Stachniuk A, Fornal E. 2016. Liquid Chromatography-Mass Spectrometry in the Analysis of Pesticide Residues in Food. Food Anal Methods. 9:1654–1665.

Stoffers NH, Brandl F, Linssen JPH, Franz R. 2003. Development and validation of analytical methods for monomeric and oligomeric migrants from nylon 12 packaging materials. Food Addit Contam Part A. 20:410–6.

Troughton MJ. 2008. Handbook of plastics joining: A practical guide. 2nd ed. Norwich: William Andrew.

Viñas R, Watson CS. 2013. Bisphenol S disrupts estradiol-induced nongenomic signaling in a rat pituitary cell line: effects on cell functions. Environ Health Perspect. 121:352–8.

Wachsmuth CJ, Dettmer K, Lang SA, Mycielska ME, Oefner PJ. 2014. Continuous water infusion enhances atmospheric pressure chemical ionization of methyl chloroformate derivatives in gas chromatography coupled to time-of-flight mass spectrometry-based metabolomics. Anal Chem. 86:9186–95.

Woodman M. 2003. Screening and Qualitative Identification of Antioxidant Polymer Additives by HPLC with UV / VIS and APCI-MS Detection Application [Internet]. [cited 2016 Jan 17]. Available from: https://www.agilent.com/cs/library/applications/5988-8610EN.pdf

Xu Z, Liao S, Wang W. 2006. Synthesis of Highly Pure Ex—Donor(DCPMS) and Its Application in Propylene Polymerization. Chem Ind times. 1:23–32.

Yang CZ, Yaniger SI, Jordan VC, Klein DJ, Bittner GD. 2011. Most plastic products release estrogenic chemicals: a potential health problem that can be solved. Environ Health Perspect. 119:989–996.

Chapter 5 :
Quantitative evaluation of important migrants from polymeric baby bottles

5.1 Optimisation and application of a LLE method for the target analysis of important migrants from baby bottles

Based on the following publication:

Onghena M, Negreira N, Van Hoeck E, Quirynen L, Van Loco J, Covaci A. Quantitative determination of migrating compounds from plastic baby bottles by validated GC-QqQ-MS and LC-QqQ-MS methods., *Food Analytical Methods (In press)* DOI: 10.1007/s12161-016-0451-4

5.1.1 Introduction

The migration patterns from the alternative materials that are nowadays present have been studied only very briefly (Simoneau et al. 2011; Simoneau et al. 2012). Only recently a more thorough screening based on a non-target liquid-liquid extraction method (LLE) has been conducted (Onghena et al. 2014; Onghena et al. 2015). This non-target screening approach employed low and high resolution mass spectrometric analysers coupled to GC and LC to identify and semi-quantify the most abundant migrating compounds. A wide variety of chemical compounds were determined (e.g. 2,4-di-*tert*-butylphenol, butyl phthalates, 2-butoxyethylacetate, ...), some of which are not authorised by the European Union Regulation No. 10/2011 (European Union 2011) which defines a Union list of substances that can be used for plastic FCMs together with their SMLs. Compounds which are not listed in the legislation may only be used if the migration levels are confirmed to be non-detectable by an agreed sensitive method. In practice, migrated concentrations in food should remain below 10 µg kg^{-1} (Barlow 2009; Baughan 2015).

To assess the prioritisation of these detected migrating compounds several criteria were taken into account. Firstly, information on the genotoxic hazard of the identified substances was collected using the ECHA database, the in silico prediction tools ToxTree and Derek NexusTM and the Vitotox® test for detecting DNA damage and a decision tree combining the collected genotoxic information was applied to classify the substances into different priority groups (Mertens et al. 2016) (e.g. high priority: 2,6-di-*tert*-butylbenzoquinone). Furthermore, receptor gene assays were applied to determine the estrogen (ER), androgen (AR), progesterone (PR), glucocorticoid (GR), thyroid beta (TRβ), peroxisome proliferator gamma (PPARγ) and aryl hydrocarbon receptor (AhR) mediated transactivational activity, since these nuclear receptors are involved in several regulating processes in the human body (Zoeller et al. 2012; Osimitz et al. 2012; Pereira-Fernandes et al. 2013). Based on this series of bioassays, an overall cumulative toxicity scoring was assigned to each identified compound.

The assessment of the activity of the chemical was based on the relative response of the cell line, expressed as a percentage of the maximal response induced by the reference agonistic ligand. A summary of the activations/inhibitions exhibited by the prioritised chemicals on the different receptors was shown in Table 5.1 (Simon et al. 2016). Finally, the semi-quantitatively estimated concentrations of the migrants were also considered for the prioritisation of the substances (e.g. 2-butoxyethyl acetate, 2,4-di-*tert*-butylphenol). Considering these scorings and the detected abundance, a selection was made of the migrating compounds with the highest priority. In order to safeguard any possible risks for public health, it was of the utmost importance to investigate if the

specified migration limits of these priority compounds were exceeded in the baby bottle migration samples.

For this purpose, the use of validated analytical methods with a sufficiently low limit of quantification ((LOQ) - 10 µg kg^{-1} in simulant) was needed to accurately quantify both the volatile (GC) and less volatile (LC) compounds of concern. Triple quadrupole tandem mass spectrometry (QqQ) is generally the technique of choice for this type of target analysis. To validate the GC- and LC-QqQ methods, the precision (generally accepted as repeatability and intra-laboratory reproducibility), the accuracy (often evaluated by repetitively spiking the matrix), and the limit of detection (LOD) were determined as a minimum requirement (European Union 2002; Weitzel et al. 2007).

This work presents the further optimisation of a generic extraction method, as well as the validation of both GC- and LC-QqQ quantitative methods to accurately determine the concentrations of 26 previously selected compounds migrating from baby bottles on the Belgian market. Earlier research conducted on the quantification of toxic compounds migrating from baby bottles mainly focused on only one (e.g. BPA) or a modest number (Simoneau et al. 2011) of target compounds. In other cases, quantification was performed by a semi-quantitative estimation of concentrations when applying a screening approach (Simoneau et al. 2012; Onghena et al. 2014). Therefore, to our knowledge, this is the first time that an accurate quantification by combined GC-MS/MS and LC-MS/MS techniques of a group of migrants selected on the basis of their toxicological profile, from baby bottles was performed.

Table 5.1: Overview of the activation or inhibition of steroid and non-steroid hormone receptors by the prioritised chemicals. "+" means that an agonistic or antagonistic effect (respectively: increase of the relative response of more than 10 % or decrease of the relative response for two consecutive points of more than 10 %) was observed. "++": for agonistic effect: relative response > 50 %, for antagonistic effect: complete inhibition of the cell response. Empty space = no effect. (Source: Simon et al. 2016)

Tested compound	Agonistic effect								Antagonistic effect							
	ER1	ER2	AR	GR	PR	TRβ	PPARγ	AhR	ER1	ER2	AR	GR	PR	TRβ	PPARγ	AhR
Acetophenone										+					+	
4-Methylbenzaldehyde										+	+		+		+	+
2-Butoxyethyl acetate																
3,4-Dimethylbenzaldehyde												++		+	+	
4-Propylbenzaldehyde								+	+	++	++		++			
2-Undecanone										++	++	++				
2,4,6-Trimethylbenzaldehyde										++		++	++		+	
2,6-Di-*tert*-butylbenzoquinone																
Dicyclopentyl(dimethoxy)silane	+															
2,4-Di-*tert*-butylphenol																
Oxacyclotridecan-2-one	+						+									
2,2,4-Trimethyl-1,3-pentanediol diisobutyrate (TXIB)	++	+														
Cedrol	++	++														
Benzophenone	++	++														
2,6-Diisopropylnaphthalene	+	+				++	+									
3,5-Di-*tert*-butyl-4-hydroxybenzaldehyde									+		++	++	++	+	+	
Diisobutyl phthalate	++	+					+							+		
Dibutyl phthalate	++						+									
Methyl oleate	+						+	++								

Tested compound	Agonistic effect								Antagonistic effect							
	ER1	ER2	AR	GR	PR	TRβ	PPARγ	AhR	ER1	ER2	AR	GR	PR	TRβ	PPARγ	AhR
Bis(2-ethylhexyl)phthalate (DEHP)	++	+							++	++				+	++	
Azacyclotridecan-2-one									++		++	++	++			
4-Phenylbenzophenone	++	+				+										+
Bisphenol-S	++	++					+								+	
Bisphenol-A	++	++					+									
p-*tert*-Octylphenol	++	++														
4-n-Nonylphenol	++	+														

5.1.2 Materials

5.1.2.1 Market survey and sampling

Samples were selected based on the market study conducted in our previous research (Onghena et al. 2014). Briefly, the 24 selected bottles included polypropylene (n = 17), polyethersulphone (n = 2), polyamide (n = 2), Tritan™ (n = 1), silicone (n = 1) and stainless steel materials (n = 1).

5.1.2.2 Chemicals

Ethanol (absolute for analysis EMSURE®, Reag. Ph Eur.), ethyl acetate (for LC LiChrosolv®), *n*- hexane (EDC for GC and FID SupraSolv®), sodium chloride (ACS, ISO, Reag. Ph Eur), sodium sulphate (ACS , ISO, Reag. Ph Eur.), formic acid (for analysis EMSURE® ACS, Reag. Ph Eur), acetonitrile (for LC LiChrosolv®), ammonia (for analysis EMSURE® ACS,Reag. Ph Eur) and ammonium sulphate (extra pure) were purchased from Merck KGaA (Darmstadt, Germany). Ultrapure water was prepared with Elga Purelab flex system of Veolia Water Solutions & Technologies (Tienen, Belgium). Acetophenone (≥ 99.0%), 4-methylbenzaldehyde (≥ 97.0%), 2-butoxyethoxyethyl acetate (≥ 99.2%), 3,4-dimethylbenzaldehyde (98%), 4-propylbenzaldehyde (97%), 2-undecanone (99%), 2,4,6-trimethylbenzaldehyde (98%), 2,6-di-*tert*-butylbenzoquinone (98%), dicyclopentyl-(dimethoxy)silane (98%), 2,4-di-*tert*-butylphenol (99%), oxacyclotridecan-2-one (98%), 2,2,4-trimethyl-1,3-pentanediol diisobutyrate (TXIB, 98.5%), p-*tert*-octylphenol (98.5%), cedrol (purity not specified by the manufacturer), benzophenone (≥ 99%), 2,6-diisopropylnaphthalene (purity not specified by the manufacturer), 2,6-di-*tert*-butyl-4-hydroxybenzaldehyde (98%), azacyclotridecan-2-one (98%), diisobutyl phthalate (99%), dibutyl phthalate (99%), 4-phenylbenzophenone (99%) and methyl oleate (≥ (99%) were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). The deuterated internal standard (IS) 2,6-di-*tert*-butyl-4-methylphenol-D24 was purchased from Campro Scientific GmbH (Berlin, Germany). Helium (ALPHAGAZTM, 99.999%) and nitrogen (99.99%) were purchased from Air Liquide (Liege, Belgium).

5.1.3 Methods

5.1.3.1 Migration testing

Migration testing was performed according to the conditions described in Chapter 4.1.3.1.

5.1.4.2 Liquid-liquid extraction (LLE) optimisation

Further optimisation of the previously developed LLE method (Onghena et al. 2014) was necessary for the accurate quantification by GC analysis of the simulant samples

considering the relatively low detection levels required (< 10 µg kg^{-1} in simulant). Due to the rather low absolute recoveries for the majority of the selected target compounds and the lack of availability of internal standards for all selected compounds, an increase in these absolute recoveries was aimed. Therefore, several modifications were performed to determine the conditions which produced the best absolute extraction efficiencies for the priority migrants. Originally, the LLE was based on an extraction of 30 ml of H_2O-EtOH (50:50, v/v) simulant with a low boiling point organic solvent mixture (ethyl acetate (EtOAc): *n*-hexane (50:50, v/v)) in order to minimise the loss of volatile compounds during concentration of the sample. This extraction was repeated with 10 ml of EtOAc-*n*-hexane by vortexing this entire mixture one minute. Then, after centrifugation during five min the organic extracts were combined in a test tube and evaporated until ± one ml was left (method A).

Optimisation of this method was performed with a H_2O-EtOH (50:50, v/v) simulant spiked with the target species at 25 µg kg^{-1}. In method B, three extractions with ten ml of EtOAc-*n*-hexane were carried out. Method C differed from method A by the way of transferring the organic phase, as in this method by pipetting more from the interphase a large amount of the lower aqueous phase was transferred to the test tube as well. In methods D to G, salts were added to increase the ionic strength of the aqueous phase and so to decrease the water solubility of the analytes. For methods D and E, respectively 1 g of NaCl and 0.3 g of $(NH_4)_2SO_4$ were added to the H_2O-EtOH before addition of the organic solvent mixture. For the modified methods, a standard vortex time of two min was applied in order to enhance the transfer of the migrants to the organic phase. The sample was evaporated until ± five ml to avoid losses of volatile compounds. In this way, the concentration factor compared to the simulant was six. After extraction, approximately 100 mg of anhydrous Na_2SO_4 was added and vortexed for 30 sec to dry the organic phase. The method with the best results (Method B, final procedure shown in Figure 5.1) was afterwards validated and employed for the sample preparation of the migration solutions.

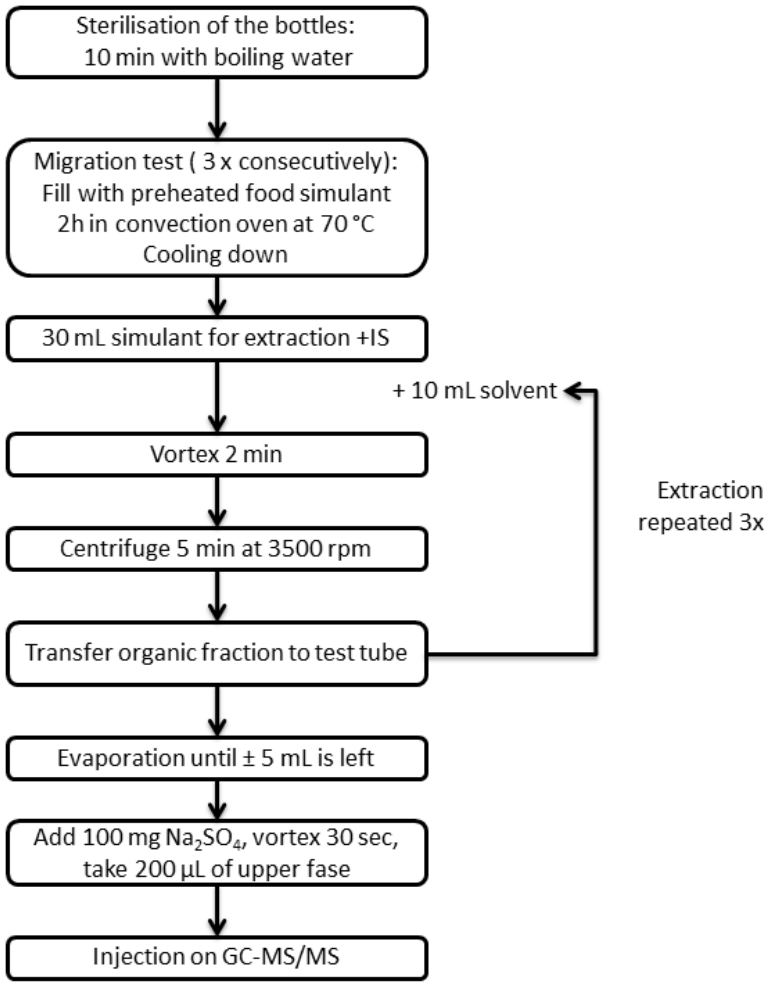


Figure 5.1: Overview of the validated and applied LLE method B

5.1.3.3 Instrumentation

5.1.3.3.1 GC-MS analysis

The EtOAc-*n*-hexane (1:1) extracts of the 3rd migration solutions were analysed with an Agilent 7890A gas chromatograph (Agilent JW Scientific, Diegem, Belgium). One µl of extract was injected into a PTV injector in pulsed splitless mode with an injection temperature of 300 °C. Separations were carried out in a DB-5ms column (95 % polydimethylsiloxane, 30 m × 0.25 mm × 0.25 µm) (Agilent JW Scientific). The temperature of the oven was initially set at 60 °C for 3 min, and was then increased to 115 °C at a rate of 7 °C min^{-1}. Next an increase in temperature of 10 °C min^{-1} until 240 °C followed by another augmentation of 15 °C min^{-1} until 300 °C was applied. Finally, this temperature was maintained during 15 min. The total run time was 42.36 min. Helium was used as a carrier gas; this with a constant flow rate of 1.0 ml min^{-1} during 22 min. Then, the flow was increased to 1.5 ml min^{-1} for 5 min after which it was set back to 1 ml

min^{-1} for the remaining analysis time. The GC system was coupled to an Agilent 7000 GC-MS triple quadrupole (QqQ) equipped with an electron impact (EI) ionisation source.

The QqQ was operated in multiple reaction monitoring (MRM) mode for the detection and quantification of compounds. The quadrupole and ion source temperatures were set at 150 and 230 °C, respectively. N_2 was used as collision gas at a flow of 1.5 ml min^{-1}. The multiplier voltage was 1537 V and 1630 V, respectively for a gain of 10 and 20. In order to improve the number of cycles per second, several acquisition segments were created with different dwell times (15 and 20 ms, respectively). The specific mass spectrometric parameters were tailored for each compound individually in order to monitor the fragmentation ions for each analyte and the internal standard (2,6-di-*tert*-butyl-4-methylphenol-D_{24}) (Table 5.2).

Table 5.2: Optimised MRM transitions and retention times for selected compounds analysed by GC/MS and the deuterated internal standard.

Compound name	Retention time (min)	Precursor ion (*m/z*)	Quantifier		Qualifier	
			Product ion (*m/z*)	Collision energy (V)	Product ion (*m/z*)	Collision energy (V)
Acetophenone	7.8	105	51	40	77	15
4-Methylbenzaldehyde	8.2	119	91	15	65; 39	30; 50
2-Butoxyethyl acetate	8.2	87	43	17	/	/
3,4-Dimethylbenzaldehyde	10.3	133	105	10	79; 77	20; 32
4-Propylbenzaldehyde	11.3	148	91	25	119; 65	7; 45
2-Undecanone	11.5	71	43	10	41	17
2,4,6-Trimethylbenzaldehyde	11.7	147	119	10	91; 41	22; 25
2,6-Di-*tert*-butylbenzoquinone	13.9	177	91	30	77; 43	50; 20
Dicyclopentyl(dimethoxy) silane	14.1	159	91	12	131; 61	5; 30
2,6-Di-*tert*-butyl-4-methylphenol-D_{24} (IS)	14.2	225	66	10	46	35
2,4-Di-*tert*-butylphenol	14.4	191	57	25	163; 41	10; 35
Oxacyclotridecan-2-one	14.9	98	83	10	70; 55	10; 20
2,2,4-Trimethyl-1,3-pentanediol diisobutyrate (TXIB)	15.4	71	43	5	41; 27	17; 22
Cedrol	15.8	119	91	15	77; 51	30; 45
Benzophenone	16.0	182	105	17	152; 77	35; 35
2,6-Diisopropylnaphthalene	17.1	197	155	15	167; 43	30; 22
3,5-Di-*tert*-butyl-4-hydroxybenzaldehyde	17.4	219	191	10	175; 57	20; 20
Diisobutyl phthalate	18.3	149	65	30	93; 39	20; 55
Dibutyl phthalate	19.3	149	65	30	93; 39	20; 55
Methyl oleate	20.7	96	67	12	81; 41	12; 30
Bis (2-ethylhexyl) phthalate (DEHP)	24.3	149	65	30	93; 39	20; 55

5.1.3.3.2 LC-MS analysis

The migration solutions were directly analysed by LC-MS (without LLE extraction). These analyses were performed at the Scientific Institute of Public Health (WIV-ISP) in Brussels. The liquid chromatographic system was a Waters Acquity Ultra High Pressure LC

(UHPLC) fitted with a degasser, a binary high- pressure gradient pump, a thermostated column compartment and an autosampler module. Chromatographic separation was achieved using a Waters Acquity UHPLC C_{18} BEH column (100 mm x 2.1 mm x 1.7 µm) at a flow rate of 0.4 ml min^{-1}. An injection volume of 10 µl was applied and during analysis the column was maintained at a constant temperature of 30 °C. The mobile phases used for the LC were for the positive ionisation mode: H_2O with 0.1 % HCOOH (mobile phase A) and AcN with 0.1% HCOOH (mobile phase B), while for the negative mode H_2O with 0.1 % NH_3 (mobile phase A) and AcN with 0.1% NH_3 (mobile phase B) were used. The following gradient was used for both ionisation modes: 0 min: 5% B; 0-6 min: 5-95% B; 6-8 min: 95% B; 8-10 min: 5% B. The LC system was coupled to a Waters XevoTQ-S triple quadrupole mass spectrometer with an electrospray interface (ESI) which was operated in both positive and negative modes for the detection and quantification of the compounds. Source parameters were as follows: a capillary voltage of 3 kV was applied for the ESI (+) mode and 2.5 kV for the negative mode. Cone Voltage (30 V), cone gas flow (50 l h^{-1}), source temperature (150 °C), desolvation gas flow (800 l h^{-1}) and collision gas flow (0.15 l h^{-1}) were the same for both polarities. Two compounds were measured in ESI (+) mode and four in ESI (-) mode. Mass spectrometer parameters were optimised for each compound individually (Table 5.3).

Table 5.3: Optimised MRM transitions and retention times for selected compounds analysed by LC-MS.

Compound name	Retention time (min)	Precursor ion (*m/z*)	Quantifier Product ion (*m/z*)	Quantifier Collision energy (V)	Qualifier Product ion (*m/z*)	Qualifier Collision energy (V)
ESI positive mode						
Azacyclotridecan-2-one	2.0	235	57	25	179	20
4-Phenylbenzophenone	4.1	259	105	25	181	15
ESI negative mode						
Bisphenol-S	0.4	249	108	25	249	20
Bisphenol-A	3.8	227	133	25	212	20
p-*tert*-Octylphenol	6.3	205	133	25	/	/
4-n-Nonylphenol	7.2	219	106	25	/	/

5.1.3.4 Method optimisation and validation

5.1.3.4.1 Mass spectrometer parameters

In order to select the specific MRM conditions, individual standard solutions of the selected compounds were injected using the MS spectrometer operating in the scan mode to identify the precursor ion, and in the product ion mode to select the transitions and collision energies (CE). Table 5.2 (GC-MS/MS parameters) and Table 5.3 (LC-MS/MS parameters) summarise the retention times, the most intense MRM transitions, and the CE selected for monitoring of the various target analytes. The collision energies were optimised to acquire if possible two (or three) MRM transitions (at least one quantifier and if possible one or two qualifiers) for each compound and for the internal standard. The most abundant transition in terms of signal to noise ratio (S/N) was chosen as quantifier (Q) and the second most abundant transition as qualifier (q). The MRM1/MRM2 ratio was monitored for variation (relative standard deviation (RSD <30%) to provide an additional identification criterion besides the retention time (RSD <5%).

5.1.3.4.2 Method validation

The performance of the method was evaluated by an in-house validation of the method. The following characteristics were assessed: precision, accuracy, selectivity-specificity, linearity, calibration range, recovery, matrix effects, lower limit of quantification (LOQ) and sensitivity.

For the GC-method, multi-component calibration curves (n=3) based on an internal standard (IS) with ten calibration points were made. Carryover was evaluated by injecting a blank sample fortified with internal standard after the highest concentrated (1000 µg kg^{-1}) calibration standard injection in the instrumental sequence.

For each compound, a calibration curve was established with R^2 according to the linear model, which was examined by the Mandel's fitting test (Mandel 1964). The precision in the form of repeatability and intermediate precision (reproducibility) were examined based on the Horwitz equation for a low (10 µg kg^{-1}), intermediate (50 µg kg^{-1}) and high (150 µg kg^{-1}) control concentration. For repeatability, five replicates of the control sample were analysed by the same person on the same day. For intra-laboratory reproducibility, the control sample was analysed by the same person in the same conditions, with five replicates per day for three different days. The recoveries were calculated following the method B. LOQ and limit of detection (LOD) were calculated for signal to noise ratio (S/N) 10 and 3, respectively, based on five replicates. In addition, a zero extract sample (processed matrix sample without analyte with IS) and a quality control (QC) sample at an intermediate concentration in the expected sample concentration range (50 µg kg^{-1}) were included. Matrix effects were evaluated and

quantified during method optimisation based on a blank simulant sample for the proposed method B. The responses of extracts of blank simulant samples spiked at 125 µg kg^{-1} (n=5) after LLE were compared to the responses of the target analytes (after subtracting the peak areas corresponding to the native analytes present in the sample) in pure EtOAc-*n*-hexane. Specificity and selectivity were checked based on retention times and MRM ratios (MRM1/MRM2).

The LC-method was validated based on the same principles, though some minor practical changes were adapted here, as simulant samples were injected directly into the LC-MS/MS without any pre-treatment. Calibration curves were prepared in the same medium as the samples (H_2O-EtOH (50:50, v/v)) with seven calibration points ranging from 1 to 100 µg kg^{-1} and thus no matrix effects were studied. A blank sample consisted in blank simulant without any treatment, while simulant spiked at 50 µg kg^{-1} served as a QC sample. Linearity was also evaluated with the Mandel's fitting test, and precision and accuracy were determined again and compared to the Horwitz equation. The concentrations used here (low (5 µg kg^{-1}), intermediate (10 µg kg^{-1}) and high (75 µg kg^{-1})) were different considering that the expected concentrations were lower than for the GC method since no concentration step was included.

5.1.4 Results and discussion

5.1.4.1 LLE method development

5.1.4.1.1 Optimisation of the extraction conditions

The results of the absolute recoveries of the previously developed LLE method (method A) showed low values for the most of the selected compounds within the first and second extraction step (generally between 20 and 50%) (Table 5.4). A third extraction step was therefore added in method B in order to obtain quantitative recoveries. Although the labour intensity of the method was significantly higher adding this extra extraction step, higher recoveries were obtained (generally between 70 and 110%), yet azacyclotridecan-2-one remained at low values even after 3 extraction steps (Table 5.4). For method C, the transferred extract still contained an important amount of water and precaution had to be taken when pipetting 200 µL of concentrated extract to a vial for GC injection, since water should not be present to avoid damage of the GC column. For this adaptation, a slight increase in the absolute recoveries was observed, although they were still lower than for method B (Table 5.4).

Table 5.4: Optimisation of extraction conditions: Absolute recoveries (%) and RSDs for the different methods.

Compound name	Abs. rec. meth. A(%)	RSD (%)	Abs. rec. meth. B(%)	RSD (%)	Abs. rec. meth. C(%)	RSD (%)	Abs. rec. meth. D(%)	RSD (%)	Abs. rec. meth. E(%)	RSD (%)
2,2,4-trimethyl-1,3-pentanediol diisobutyrate (TXIB)	54	18	92	17	84	18	57	6	59	36
2,4,6-Trimethylbenzaldehyde	47	13	86	11	69	12	83	5	60	19
2,4-Di-*tert*-butylphenol	54	16	93	20	91	14	28	46	78	24
2,6-Diisopropylnaphthalene	64	12	107	16	98	13	81	10	84	25
2,6-Di-*tert*-butylbenzoquinone	57	15	109	12	105	15	59	3	82	23
2-Butoxyethyl acetate	33	14	67	16	47	11	67	5	45	21
Methyl oleate	21	32	96	63	61	55	87	61	44	100
Benzophenone	31	22	80	23	55	19	48	9	46	35
Diisobutyl phthalate	31	25	89	36	68	28	63	31	49	51
Dibutyl phthalate	23	29	85	43	59	36	60	46	43	65
Acetophenone	26	13	57	14	36	9	56	5	38	19
4-Methylbenzaldehyde	31	14	68	15	45	11	64	6	44	21
4-Propylbenzaldehyde	43	18	90	14	70	14	88	7	60	23
2-Undecanone	56	20	96	14	86	15	96	7	67	25
Oxacyclotridecan-2-one	62	12	97	11	89	12	50	14	75	19
p-*tert*-Octylphenol	22	25	20	68	32	25	22	45	29	40
Cedrol	44	14	52	34	58	18	36	38	51	25
3,5-Di-*tert*-butyl-4-hydroxybenzaldehyde	24	41	99	40	61	35	66	32	47	58
Azacyclotridecan-2-one	2	102	9	68	0	107	31	27	1	138
4-Phenylbenzophenone	9	14	93	71	38	82	90	77	38	113
Dicyclopentyl(dimethoxy) silane	70	9	100	10	99	11	83	37	85	15
3,4-dimethylbenzaldehyde	35	14	78	12	55	11	75	6	52	20
Bis(2-ethylhexyl)phthalate (DEHP)	9	20	83	22	26	82	39	69	26	121

The addition of NaCl to the H_2O-EtOH mixture before extraction showed an improvement of the extraction response for some analytes (e.g. 2,4,6-trimethylbenzaldehyde; 2-butoxyethyl acetate). For some compounds, the addition of salt played an opposite effect as recoveries even became lower than before (e.g. 2,4-di-*tert*-butylphenol) or splitting of the chromatographic peak occurred (not accurately quantifiable anymore, e.g. benzophenone) which made this addition not appropriate. The influence of $(NH_4)_2SO_4$ was first tested for the addition of 0.3 g, demonstrating only a slight increase in the recoveries of most compounds. When adding 1.5 and 5 g of $(NH_4)_2SO_4$, a 3-phase system was formed. Both methods were therefore discarded.

When comparing the tendency of the recovery values and repeatability of the target compounds between the different methods, it was clear that method B exhibited the most significant performance enhancement. This method was therefore validated and afterwards applied to real baby bottle samples. An overview of the results for each of the validation parameters was shown in Table 5.5. Figure 5.2 gives a graphical overview of the extracted ion chromatograms for the target compounds measured by the GC-MS.

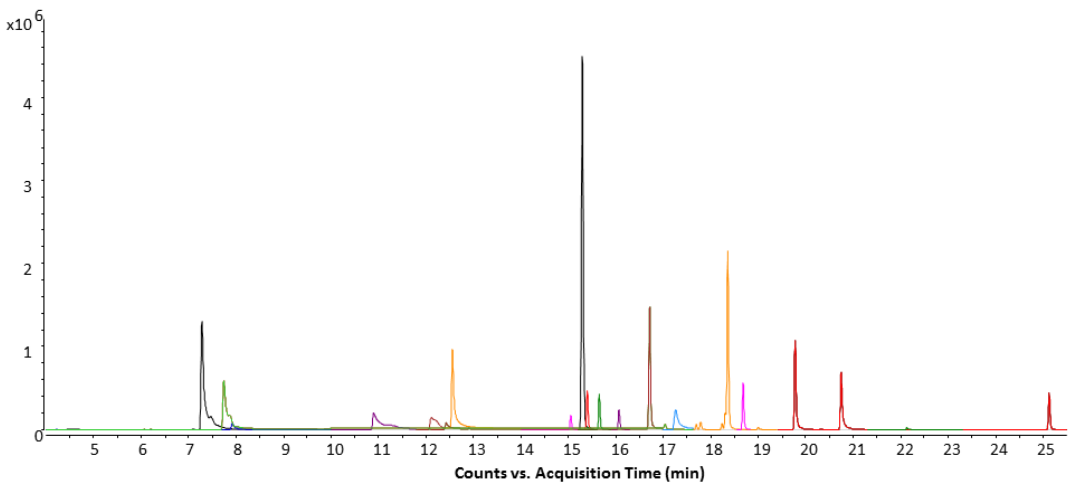


Figure 5.2: GC-MS extracted ion chromatograms of the quantifier transitions of the monitored compounds for a blank sample spiked at 100 µg kg^{-1}

Table 5.5: Extraction method and GC-MS validation results for method B (* indicates that the LOQ was higher than the spiking level). RSDr = Repeatability Relative Standard Deviation, RSDRw = Intra-laboratory reproducibility Relative Standard Deviation.

Compound name	R^2 cal. curve	RSDr (%)			RSDRw (%)			Recoveries			LOQ (µg kg^{-1} in simulant)	Instrumental LOQ (pg $µL^{-1}$)
		10 µg kg^{-1}	50 µg kg^{-1}	150 µg kg^{-1}	10 µg kg^{-1}	50 µg kg^{-1}	150 µg kg^{-1}	10 µg kg^{-1}	50 µg kg^{-1}	150 µg kg^{-1}		
2,2,4-trimethyl-1,3-pentanediol diisobutyrate (TXIB)	0.9972	3	8	10	6	8	12	92	93	98	6.4	6.7
2,4,6-Trimethylbenzaldehyde	0.9984	11	8	7	12	25	7	86	95	110	1.7	2.2
2,4-Di-*tert*-butylphenol	0.9989	13	10	12	14	11	15	71	102	108	6.2	2.1
2,6-Diisopropylnaphthalene	0.9976	*	15	14	*	25	23	*	84	101	13.4	2.4
2,6-Di-*tert*-butylbenzoquinone	0.9961	5	9	9	31	26	9	96	93	88	5.6	2.2
2-Butoxyethyl acetate	0.9989	7	8	8	13	14	9	73	75	82	5.6	11.3
Methyl oleate	0.9939	*	11	19	*	29	19	*	81	108	25.0	11.1
Benzophenone	0.9916	7	11	12	11	13	12	88	82	106	3.6	2.0
Diisobutyl phthalate	0.9949	5	12	13	5	12	13	65	74	94	8.0	17.1
Dibutyl phthalate	0.9939	10	13	12	12	13	15	87	93	97	4.4	16.6
Acetophenone	0.9994	9	10	8	9	19	24	73	72	70	1.7	5.1
4-Methylbenzaldehyde	0.999	10	8	8	10	13	10	66	79	76	3.4	6.8
4-Propylbenzaldehyde	0.9961	3	8	8	3	12	10	74	90	90	0.6	2.3
2-Undecanone	0.9978	*	12	10	*	19	19	*	94	87	6.7	34.2
Oxacyclotridecan-2-one	0.9948	12	10	7	20	18	19	86	96	100	2.5	0.7
p-*tert*-Octylphenol	0.9822	13	18	19	22	23	29	88	85	76	5.7	3.4
Cedrol	0.9955	*	11	13	*	15	17	*	102	102	9.7	7.7
3,5-Di-tert-butyl-4-hydroxybenzaldehyde	0.9838	9	8	14	16	11	16	77	91	90	3.1	2.2
Azacyclotridecan-2-one	0.9765	*	14	13	*	14	22	*	34	28	9.6	2.3
4-Phenylbenzophenone	0.9797	13	16	17	17	23	17	102	94	113	5.7	2.2
Dicyclopentyl(dimethoxy) silane	0.9988	12	6	7	13	9	8	101	114	117	0.8	4.7
3,4-dimethylbenzaldehyde	0.9989	8	9	8	10	11	9	98	86	95	5.6	3.4
Bis(2-ethylhexyl)phthalate (DEHP)	0.9931	16	13	16	16	20	16	46	87	75	5.1	1.4

5.1.4.2 Method validation

Since no matrix effects were present, an external calibration against standards prepared in EtOAc:*n*-hexane (1:1) was used to measure the levels of those compounds in simulant samples.

The linearity of the method was investigated with standards prepared in EtOAc:*n*-hexane (1:1) at ten different concentrations, from 1 µg kg^{-1} (or the limit of quantification if higher) to 1000 µg kg^{-1} (0.7; 3.5; 7; 17.5; 35; 70; 175; 350; 700 and 1000 µg kg^{-1}). The concentration of the IS was in all cases 300 µg kg^{-1}. The calibration curves were obtained by plotting the peak areas of the analyte and the internal standard versus the spiked concentrations and were not weighted. An "F value" was calculated for each substance. The obtained "F values" were compared with a tabulated F value, corresponding to the F-distribution with 1 and n−3 degrees of freedom and a probability of 99%. The F=0.99 was 16.26 for all compounds. According to the Mandel's fitting test, the straight line regression model is preferred when the calculated "F value" is below F 0.99. All compounds gave a linear response in the above described calibration range (TV < F), with determination coefficients (R^2) higher than 0.990, except for 2,6-di-*tert*-butyl-benzoquinone, p-*tert*-octylphenol, 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde, azacyclo-tridecan-2-one and 4-phenylbenzophenone with R^2 values between 0.977 and 0.984 (Table 5.5). The instrumental limits of detection (LODs) and quantification (LOQs) were experimentally estimated from the lowest level included in the calibration curve as the concentration of analyte giving S/N of 3 and 10, respectively. Instrumental LODs varied between 0.2 and 10.3 µg kg^{-1}, whereas the LOQs (S/N = 10) ranged between 0.7 and 34.2 µg kg^{-1}.

The precision of the method was evaluated with simulant spiked at three different concentrations: 10, 50 and 150 µg kg^{-1} extracted within the same day (repeatability) and on different days (intra-laboratory reproducibility) (n=5). The repeatability (r) and the intra-laboratory reproducibility (Rw) were evaluated by calculating the relative standard deviations (RSD) for these two parameters (RSDr and RSDRw) according to the ISO-5725-2 guidelines (Interscience publications 1994). These RSD's are compared to the RSD's obtained from the Horwitz equation (Horwitz et al. 1980; Horwitz & Wood 2000) and modified by Thompson (Thompson 2000). The calculations are carried out for each concentration level of the validation and the results are given in Table 5.5. When the obtained RSDs are significantly below the RSDs derived from the Horwitz equation, the method showed good precision at the levels of interest for the compound. However, some compounds exhibited already a lower repeatability when applying the previously optimised extraction (e.g. p-*tert*-octylphenol; 4-phenylbenzophenone; azacyclotridecan-2-one) and were therefore included in the LC-MS analysis method.

Intra-laboratory reproducibility was evaluated for n=5 extractions processed for 3 consecutive days (Table 5.5). Compounds which previously exhibited a poor repeatability showed the same pattern here and generally did not attain the tolerance level calculated from the Horwitz equation for reproducibility (22, 22, and 21% for the different concentrations). 2,6-Di-*tert*-butyl-benzoquinone also presented higher RSDs values than allowed with 31 and 26% for the 10 and 50 µg kg^{-1} concentration respectively, together with benzaldehyde, 2,4,6-trimethyl (25% for 50 µg kg^{-1}) and acetophenone (24% for 50 µg kg^{-1}). Since this is a multi-residue method, it was therefore not possible to optimise all parameters equally well for different compounds and sometimes compromises had to be made. 2,6-diisopropylnaphthalene (DIPN) gave poor results for both repeatability and reproducibility due to blank contamination. Procedural blanks, performed with simulant without fortification, showed the absence of significant contamination problems for most compounds. However, both DIPN and bis(2-ethylhexyl)phthalate (DEHP) were systematically detected in blanks extracts at varying concentrations and were therefore quantified only semi-quantitatively.

The achieved method LOQs ranged from 0.6 to 8.0 µg kg^{-1} simulant, with the exceptions of cedrol (9.7 µg kg^{-1}) and azacyclotridecan-2-one (9.6 µg kg^{-1}) which were just around the proposed "non-detection limit" of 10 µg kg^{-1}. Methyl oleate exhibited a high LOQ (25 µg kg^{-1}) due to its low sensitivity. For DIPN (13.4 µg kg^{-1}), the high LOQ was of course observed due to its presence in the procedural blanks. The same issue was also faced for DEHP, a common contaminant present in almost any plastic material used for laboratory work (Nguyen et al. 2008). For both compounds, rather high RSDs (>25%) were obtained. Since DIPN and DEHP were not detected in the previous screening method (Onghena et al. 2014), we still decided to include them in our method considering their endocrine activity in different bioassays (Simon et al. 2016) and public concern. When analytes were present in the procedural blanks, the mean of the concentrations in those blanks was used. If the latter value was lower than 3 x SD, this was adopted as LOQ.

Absolute recoveries were calculated by comparing the analyte peak area obtained in spiked samples (after subtracting the amount of the analyte in the blank (n = 5), if present) and in standard solutions with equivalent concentrations. Relative recoveries were calculated by comparing the absolute recoveries obtained for each analyte and the internal standard. Relative recoveries calculated with respect to the IS were between 65 ± 2% and 117 ± 5% for the different concentration levels.

Finally, for positive confirmation of the presence of a compound in a sample, the GC retention time of the compound in the sample had to match that of the standard with a margin of ± 5%, and its precursor-product ion ratio could not deviate more than 30%

(depending on the MRM1/MRM2 value) from the ratio in the standard (European Union 2002).

For the LC-MS validation, linearity was investigated with standards prepared in H_2O-EtOH at eight different concentrations (1, 2, 5, 10, 25, 50, 75 and 100 µg kg^{-1}). The Mandel's fitting test demonstrated also here a linear response in the proposed calibration range with R^2 for all compounds between 0.995 and 0.999. For p-*tert*-octylphenol and 4-n-nonylphenol only one MRM transition was selected (quantifier). The LOQ was determined at 1 µg kg^{-1} for all components. Repeatability and reproducibility were tested at three levels (5, 10 and 75 µg kg^{-1}) and all RSDr and RSDRw resulted bellow the required Horwitz values (Table 5.6) consequently demonstrating the adequate measurement of those compounds that resulted to be troublesome with GC-MS analysis. The ratio of the concentrations obtained and the theoretical concentration for the 10 µg kg^{-1} calibration level resulted in recoveries between 98 and 110%.

Table 5.6: LC-MS validation parameters.

Compound name		RSDr (%)			RSDRw (%)			10 µg kg^{-1} Rec.	LOQ (µg kg^{-1} in simulant)
	R^2 cal. curve	5 µg kg^{-1}	10 µg kg^{-1}	75 µg kg^{-1}	5 µg kg^{-1}	10 µg kg^{-1}	75 µg kg^{-1}		
ESI positive mode									
Azacyclotridecan-2-one	0.9992	3	8	3	8	8	5	100	1
4-phenylbenzophenone	0.9999	3	7	2	6	9	7	107	1
ESI negative mode									
Bisphenol-S	0.9997	2	9	2	4	9	2	98	1
Bisphenol-A	0.9960	5	8	3	8	8	5	99	1
p-*tert*-Octylphenol	0.9996	5	7	3	10	7	4	105	1
4-n-Nonylphenol	0.9955	10	8	6	12	10	11	109	1

5.1.4.3 Migration from baby bottles

The experiments to test the migration from the selected baby bottles were all carried out following the EU legislation which defines a Union list of substances that can be used in plastic food contact materials together with their SMLs (European Union 2011). The substances not included in the Union list can be used if they are not classified as carcinogenic, mutagenic or reprotoxic (CMR) and if they are not detectable in the food

with an appropriate sensitive method. In practice, the concentrations of these substances in food should remain below 10 µg kg^{-1} (Barlow 2009).

Furthermore, the Regulation also defines the use of simulants and the testing conditions to be implied. For baby bottles, a H_2O-EtOH (50:50, v/v) solution was therefore used as simulant for milk and three consecutive migrations were performed during 2 h at 70 °C. These consecutive migrations are prescribed by the Regulation to mimic the repetitive use of a FCM. To assess its final compliance, the concentrations of migrating compounds measured in the third migration step have to be compliant with those specified in the Regulation. On the one hand, the previously optimised and validated extraction method was applied to detect and quantify the migration from baby bottles made of polymer alternatives to PC for the more volatile compounds. On the other hand, for the more polar migrants that needed to be analysed with LC-MS, direct injection of the H_2O-EtOH (50:50, v/v) simulant samples was possible.

5.1.4.3.1 Polypropylene (PP) baby bottles

PP resulted to be the most used polymer as an alternative to PC baby bottles on the Belgian market, representing more than 60% of the market share. Previous studies already demonstrated that a wide variety of chemicals present in PP polymers could migrate towards the food (Mcdonald et al. 2008; Alin & Hakkarainen 2010; Chang et al. 2016). For baby bottles, severe differences in the identity and the concentration of the compounds migrating from the different PP bottles were observed (Simoneau et al. 2012; Onghena et al. 2014) which agreed with the data obtained in this study. Indeed, when quantifying those compounds that were previously selected to be of priority interest, some bottles exhibited relatively large concentrations of migrants that in other bottles were not even seen. An overview of the results is given in Table 5.7. Bottles number 5, 7 and 25 in particular exhibited a wide variety of migrants at relatively high concentrations (e.g. 2-butoxyethylacetate in n° 25: 946 µg kg^{-1}) whereas from other bottles such as 9, 26 or 27, only 1 or 2 compounds migrated at concentrations just above the LOQ.

Generally only 2 compounds with a specified SML were detected, benzophenone and dibutyl phthalate. Benzophenone was measured above the LOQ in only 3 out of 17 PP bottles, though in one case it was found up to 97 µg kg^{-1} (bottle 12). Yet, for this specific compound the detected migrating concentrations remained far below the SML of 600 µg kg^{-1}. Dibutyl phthalate (SML= 300 µg kg^{-1}) was identified in one bottle at very low concentration (5 µg kg^{-1}). None of the other targeted compounds detected in PP bottles were listed in the EU Regulation as authorised substances. Some of them migrated at concentrations significantly higher than 10 µg kg^{-1} (e.g. dicyclopentyl(dimethoxy)silane: 117 µg kg^{-1} in bottle 8) and therefore their origin and corresponding toxicological profile

should be further investigated. 2,4-di-*tert*-butylphenol was detected in >90% of PP bottles though only for 4 specific bottles the concentrations exceeded the LOQ varying from 12 to 118 µg kg^{-1}. LC-QqQ analyses only revealed the presence of p-*tert*-octylphenol (7 µg kg^{-1} in bottle 7) and 4-phenyl-benzophenone (7 µg kg^{-1} in bottle 21). It has to be stated that all these concentrations are of course not fixed values, but they should be considered as a "concentration range" taking into account the measurement uncertainties mentioned in Table 5.6.

Table 5.7: Concentrations (in µg kg^{-1}) of migrating compounds in PP bottles on the Belgian market measured after the 3rd migration experiment ± the measurement uncertainties. Bottle numbers are given in the first row. [a] not in EU No. 10/2011. *The method was not proven to be quantitative at these concentrations.

		Baby bottle number																
Compound name	**SML (µg kg^{-1})**	**2**	**5**	**7**	**8**	**9**	**11**	**12**	**17**	**18**	**19**	**20**	**21**	**22**	**24**	**25**	**26**	**27**
2,4,6-Trimethylbenzaldehyde	[a]	-	5±0.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2,4-Di-*tert*-butylphenol	[a]	12±3	-	72±19	-	-	12±3	-	-	-	-	118±31	-	-	-	-	-	-
2-Butoxyethyl acetate	[a]	15±4	22±5	-	-	-	-	-	-	-	45±11	-	-	-	-	946 *	-	-
Methyl oleate	[a]	-	-	-	-	-	-	-	34±12	-	-	-	-	-	-	-	-	-
Benzophenone	**600**	-	14±3	-	-	-	-	97±23	-	35±8	-	-	-	-	-	-	-	-
Dibutyl phthalate	**300**	-	-	-	-	-	-	-	-	-	5±1.3	-	-	-	-	-	-	-
Acetophenone	[a]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3±0.5	-	-
4-Methylbenzaldehyde	[a]	-	32±7	-	-	-	-	-	4±0.8	-	-	-	-	-	-	26±5	-	-
4-Propylbenzaldehyde	[a]	-	20±3	12±2	11±2	-	17±3	11±2	-	3±0.5	-	-	-	-	-	-	-	-
Dicyclopentyl(dimethoxy)silane	[a]	-	-	31±6	117±23	-	-	-	1±0.2	-	-	9±2	-	-	-	13±3	5±1	1±0.2
3,4-dimethylbenzaldehyde	[a]	59±12	-	-	-	11±2	-	-	13±3	-	-	13±3	-	6±1	-	53±11	6±1	6±1
4-Phenylbenzophenone	[a]	-	-	-	-	-	-	-	-	-	-	-	7±1	-	-	-	-	-
p-*tert*-Octylphenol	[a]	-	-	7±1	-	-	-	-	-	-	-	-	-	-	-	-	-	-

5.1.4.3.2 Baby bottles made of other materials

Other baby bottle materials present on the Belgian market were PES, PA, Tritan™, silicones and stainless steel. In the PES bottles, none of the selected compounds except for acetophenone (at very low concentration: 3 µg kg^{-1}) were encountered. It was interesting to take into account though that BPS, a building block for PES with similar endocrine disrupting properties (EDC) to BPA (Kuruto-Niwa et al. 2005), was not detected in the samples. These results were consistent with an earlier study (Simoneau et al. 2011).

PA bottles exhibited just like PES a very low variety of migrating compounds, though the PA monomer azacyclotridecan-2-one was detected at relatively high concentrations (924 and 1091 µg kg^{-1}). Yet, this was still far below the SML of 5000 µg kg^{-1} of this compound.

The Tritan™ bottle showed, in contrast to both PES and PA, a rather wide variety of migrants. Nevertheless the concentrations detected in the third migration step were low (e.g. dicyclodipentyl(dimethoxy)silane: 10 µg kg^{-1}; 2,4-di-*tert*-butylphenol: 8 µg kg^{-1}) and only for 4-propylbenzaldehyde (27 µg kg^{-1}) the concentration was higher than 10 µg kg^{-1}.

Table 5.8: Concentrations (µg kg^{-1}) of migrating compounds in PES, PA, Tritan™, Silicone, and stainless steel bottles on the Belgian market measured after the 3rd migration experiment ± the measurement uncertainties. [a] not in EU No. 10/2011. [b] For single-use gloves only. *The method was not proven to be quantitative at these concentrations.

Compound name	SML (µg kg^{-1})	PES 1	PES 2	Tri	Sil	Steel	PA 1	PA 2
2,2,4-trimethyl-1,3-pentanediol diisobutyrate (TXIB)	**5000**[b]	-	-	-	348*	-	-	-
2,4-Di-*tert*-butylphenol	[a]	-	-	8±2	-	-	-	-
2,6-Di-*tert*-butylbenzoquinone	[a]	-	-	-	8±3	-	-	-
Benzophenone	**600**	-	-	-	9±2	-	-	-
Diisobutyl phthalate	[a]	-	-	-	15±3	-	-	-
Dibutyl phthalate	**300**	-	-	-	11±3	-	-	-
Acetophenone	[a]	3±0.5	-	-	-	-	-	-
4-Propylbenzaldehyde	[a]	-	-	27±4	1±0.2	-	-	-
Dicyclopentyl(dimethoxy)silane	[a]	-	-	10±2	-	-	-	-
3,4-dimethylbenzaldehyde	[a]	-	-	-	15±3	-	-	-
Azacyclotridecan-2-one	**5000**	-	-	-	-	-	924±93	1091±109

The migration profile of the silicone baby bottle found on the Belgian market was also tested. Since EU Regulation 10/2011 is only applicable for plastics, it cannot be used for

silicones. However, a resolution of the Council of Europe that contains inventory lists of substances is available and can be used for the interpretation of the data observed in this study (Council of Europe 2004). Previous research (Simoneau et al. 2012; Onghena et al. 2014) indicated already the migration of possible EDCs. Indeed, several components with a potential endocrine activity such as phthalates were identified (diisobutyl phthalate: 15 µg kg^{-1}; dibutyl phthalate: 11 µg kg^{-1}). Furthermore 3,4-dimethylbenzaldehyde was encountered at 15 µg kg^{-1} and 2,2,4-trimethyl-1,3-pentanediol diisobutyrate (TXIB) at a concentration of more than 348 µg kg^{-1}. This substance was authorised by European Union legislation, but may only migrate at a level of 5000 µg kg^{-1} from single-use gloves according to EU Regulation 10/2011. 2,6-Di-*tert*-butylbenzoquinone (8 µg kg^{-1}) and benzophenone (9 µg kg^{-1}) were both detected below 10 µg kg^{-1}. For the stainless steel bottle none of the targeted compounds could be detected. Results are summarised in Table 5.8. Also here the measurement uncertainties are mentioned to indicate the actual measured concentration range.

5.1.5 Conclusions

A LLE method was optimised for the extraction of chemicals migrating from baby bottles and which were identified as of toxicological interest. GC- and LC-MS/MS analysis methods were successfully validated to accurately determine the concentrations of these compounds in the selected food simulant. The analysis of the migration solutions confirmed the presence of previously detected compounds and they were adequately quantified. Compounds with an SML which were regulated by the EU Regulation No. 10/2011 did not exceed these specified values. Yet, other migrants which were not specified in the EU No. 10/2011 positive list with authorised materials were detected sometimes above 10 µg kg^{-1}. Therefore, further research is needed to understand the potential health risks associated with the migration of these quantified compounds. Finally, Bisphenol-A and Bisphenol-S were not detected in any of the migration solutions, indicating that the new polymers used as the replacement of polycarbonate are not leaching these bisphenols.

5.2 Quantitative evaluation of the migration under standardised and real-life use conditions

Based on the following publication:

Onghena M, Van Hoeck E, Negreira N, Quirynen L, Van Loco J, Covaci A. Evaluation of the migration of chemicals from baby bottles under standardised and duration testing conditions. *Food Additives and Contaminants Part A (In press)* DOI: 10.1080/19440049.2016.1171914

5.2.1 Introduction

In Chapter 5.1, the migration of the selected priority substances was determined under conventional EU repetitive use conditions (3 migrations during 2 h at 70 °C with H_2O-EtOH (50:50, v/v, milk simulant))(European Union 2011) and quantification was done by GC- and LC-QqQ (Onghena et al. 2016).

However, the results of the migration using the conventional EU repetitive use conditions can be compared to the migration when daily use conditions are applied. These conditions can be mimicked by duration tests, which consist in stressing the polymer by applying one specific parameter (e.g. sterilisation) for several repeated cycles to determine its resistance and possible degradation. Several studies already investigated the migration of BPA from PC baby bottles under different circumstances. Not only standardised EU migrating conditions (Wong et al. 2005), but also the mimicking of real-life use conditions of baby bottles such as microwave heating (Ehlert et al. 2008; Biedermann-Brem & Grob 2009), sterilisation (Mountfort et al. 1997) or use of a dishwasher (Brede et al. 2003; Maragou et al. 2008) have been studied thoroughly. No information regarding the migration under such real-life circumstances is yet available for baby bottles made of materials other than PC.

However, for FCMs other than PC, this field has only been explored for the analysis of the influence of microwave heating on PP food containers (Alin & Hakkarainen 2010; Alin & Hakkarainen 2011), which reported an increased release of antioxidants compared to conventional heating. This clearly indicates the need for more research in this field, especially for FCMs that are intended for young children such as baby bottles.

Therefore, the aim of this work was to determine the migration of the priority substances from baby bottles undergoing duration tests (e.g. microwave, sterilisation and dishwasher). Finally, these results were compared to the previously collected data of the 3rd migration of the EU repetitive use experiment in order to evaluate the appropriateness of the conventionally used migration conditions.

5.2.2 Materials

5.2.2.1 Market survey and sampling

Samples were selected based on the market study conducted in our previous research (Onghena et al. 2014). Due to the labour intensity of these duration tests, one bottle of each different polymer type (PP, PES, PA, Tritan™, and silicone) was selected. Due to large market share of PP-bottles, an additional PP bottle exhibiting another variety of

migrating compounds was selected for the duration tests. The selected baby bottles correspond to baby bottles 2 and 9 for PP, PES 1, PA 1, Tritan™, and silicone from our previous research (Onghena et al. 2016).

5.2.2.2 Chemicals

All chemicals used were the same as described in section 5.1.2.2.

5.2.3 Methods

5.2.3.1 Migration testing: EU repetitive use conditions

The migration from the selected baby bottles under EU repetitive use conditions was evaluated for selected substances according to the procedure described by Onghena et al. (Onghena et al., 2016). Briefly, baby bottles were sterilised by filling them during 10 min with boiling water and afterwards three consecutive migrations were executed using H_2O-EtOH (50:50, v/v) (milk simulant) for 2 h at 70 °C following EU Regulation No. 10/2011 on plastic materials and articles intended to come into contact with food (European Union 2011). The third migration solution was analysed.

5.2.3.2 Migration testing: Duration tests

In order to evaluate the impact of the duration test on the migration, a set of baby bottles was also filled with simulant at 40 °C and kept at room temperature for 30 min. Afterwards, the simulant was transferred into glass containers and stored at +4 °C prior to analysis; this was repeated five times. These experiments will serve as reference to evaluate whether migration of compounds occurs when the baby bottles have not been subjected to any pre-treatment.

Four different types of duration treatments were applied to determine the migration of targeted compounds when mimicking real-life conditions: microwave heating, dishwasher cleaning, steam and cooking sterilisation.

Firstly, the influence of microwave heating was simulated with the following procedure: bottles were filled with H_2O-EtOH (50:50, v/v) at room temperature (23 °C) up to the indicated volume and sealed. They were then placed individually in the centre of the microwave oven (Whirlpool Gusto GT288WH) and evenly heated to a temperature of 40 °C with their respective heating time by the three-dimensional release of microwave radiation in combination with a rotating baking dish. The power was set at 500 W and the heating time was adjusted depending on the size and volume of each bottle to ensure that a temperature of 40 °C was reached. After heating, bottles were placed at

room temperature for 30 min to simulate the real drinking process by infants. Finally, the simulant was transferred into glass containers and stored at +4 °C. After each migration, baby bottles were rinsed with 50 ml of Milli Q water and refilled with new simulant for a new migration test. To simulate aging under the influence of microwave radiation, the bottles were subjected to a total of 100 cycles in the microwave oven and the solutions obtained after cycles 1 to 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90 and 100 were analysed. However, it should be noted that the bottles were not sterilised prior to the experiment.

Secondly, the impact of the repetitive use of the dishwasher was examined. Six new bottles were subjected to a dishwasher treatment. In each cycle, bottles were washed in a dishwasher operated at "eco-mode" (2 h 55 min at 55-60 °C) using a common detergent. The inclination of the bottles in the dishwasher was adjusted such that the whole internal surface was in contact with the water spray. After each cycle, bottles were rinsed with 50 ml of Milli Q water, filled with preheated H_2O-EtOH (50:50, v/v) simulant (40 °C) and left for 30 min. Finally, the simulant was transferred into glass containers and stored at +4 °C. To simulate aging under the influence of the dishwasher, the bottles were subjected to a total of 10 cycles and the migration solutions obtained after 1, 2, 4, 6, 8 and 10 cycles were analysed.

The objective of the third and fourth treatment was to investigate the influence of different sterilisation techniques on the migration. A typical electric steam steriliser (available in specialised baby shops (Philips Avent 3-in-1 electric steam steriliser)) was used. Bottles were placed together with 100 ml of tap water and steamed for approximately 10 min (according to the conditions mentioned in the user manual). Also a cooking sterilisation was applied for which bottles were boiled in tap water for 10 min. After sterilisation, bottles were also rinsed with 50 ml of Milli Q water, filled with simulant (40 °C) and kept for 30 min at room temperature before storage. Again, 10 cycles were performed using new samples for both types of treatment and the migration solutions obtained after 1, 2, 4, 6, 8 and 10 cycles were analysed.

For all types of duration tests, a glass bottle was taken through the entire procedures and afterwards filled with simulant as a blank control sample.

5.2.3.3 Analysis of the migration solutions

The obtained migration solutions were processed with a previously optimised and validated liquid-liquid extraction (LLE) method with ethyl acetate (EtOAc)-*n*-hexane (50:50, v/v) (Onghena et al. 2016) for GC-QqQ-MS analysis or directly analysed by LC-QqQ-MS. Briefly, the LLE consisted in extracting 30 ml of simulant 3 times with 10 ml of EtOAc-*n*-hexane (1:1) and evaporating the organic extract to ± 5 ml of which 200 µL was

taken for injection. In addition to the zero extract sample (processed matrix sample from the glass bottle without analyte, but with IS), a quality control (QC) sample spiked at an intermediate concentration in the expected sample concentration range (50 µg kg^{-1}) was included as well. Next, the obtained extracts were analysed by GC-QqQ-MS or LC-QqQ-MS.

5.2.3.4 Instrumentation

GC-QqQ-MS and LC-QqQ-MS analysis were performed according to the same conditions as described in sections 5.1.4.3.1 and 5.1.4.3.2.

5.2.4 Results and discussion

5.2.4.1 EU repetitive use experiment

A harmonised European Regulation is available for plastic FCMs (European Union 2011), specifying both migration conditions (three migrations for repetitive use materials such as baby bottles) and simulants (H_2O-EtOH (50:50, v/v) to simulate milk).

The Regulation sets out an EU list of authorised substances that can be intentionally used in the manufacture of plastics, together with restrictions, specifications and SMLs. For substances for which no SML or other restrictions are established, Article 11(2) of this Regulation describes that the specific migration of these substances shall not exceed a generic SML of 60 mg kg^{-1}. To assess a materials final compliance, the concentrations of migrating compounds measured in the 3rd migration step should not exceed those specified in the Regulation. Derogation from the latter can be applicable when the substance is separated from the food by a functional barrier. In this case, substances not present on the Union list can be used if they do not migrate into the food at detectable concentrations (with a detection limit of 10 µg kg^{-1}) (Barlow 2009) and if they do not exhibit carcinogenic, mutagenic or reprotoxic (CMR) properties. If the SML of a substance is specified as non-detectable (ND), a detection limit of 10 µg substance per kg food is applicable unless specified differently for the individual substance. In both these cases, the material or article must already respect the SML in the first migration.

The prescribed migration conditions were applied on the polymer baby bottles as well as on the stainless steel and silicone bottles (Onghena et al., 2016). Table 5.9 summarises the results of the 3rd migration step of the EU repetitive use experiment for the six bottles that were selected to be subjected to duration tests as well. None of the authorised compounds exceeded the SML, yet some none listed compounds were detected above the proposed threshold of 10 µg kg^{-1} (Onghena et al. 2016).

Table 5.9: Concentrations (in µg kg^{-1}) of migrating compounds (with their respective SMLs) in the 3rd migration when applying the conventional EU repetitive use migration conditions. - means non-detectable. Compounds which were not detected at all are not shown. [a] not in EU No. 10/2011. [b] For single-use gloves only.

Compound name	SML (µg kg^{-1})	PP Brand A	PP Brand B	PES	PA	Tri	Sil
2,2,4-trimethyl-1,3-pentanediol diisobutyrate (TXIB)	**5000**[b]	-	-	-	-	-	348
2,4-Di-*tert*-butylphenol	[a]	12	-	-	-	8	-
2,6-Di-*tert*-butylbenzoquinone	[a]	-	-	-	-	-	8
2-Butoxyethyl acetate	[a]	15	-	-	-	-	-
Benzophenone	**600**	-	-	-	-	-	9
Diisobutyl phthalate	[a]	-	-	-	-	-	15
Dibutyl phthalate	**300**	-	-	-	-	-	11
Acetophenone	[a]	-	-	3	-	-	-
4-Propylbenzaldehyde	[a]	-	-	-	-	27	1
Dicyclopentyl(dimethoxy)silane	[a]	-	-	-	-	10	-
3,4-dimethylbenzaldehyde	[a]	59	11	-	-	-	15
Azacyclotridecan-2-one	**5000**	-	-	-	924	-	-

5.2.4.2 Reference migration testing

To be able to adequately compare the influence of the duration tests on the selected baby bottles of different polymer types available on the Belgian market, a set of bottles was first subjected to a reference treatment. This consisted in filling the bottles 5 times with pre-heated simulant (40 °C) and leaving them at room temperature for 30 min to simulate the direct use of the bottles without any pre-treatment by the consumer. The results of this experiment are given in Table 5.10 and are used further through this manuscript as a reference for comparison with the other treatments. The encountered concentrations reached a maximum in the first migration step and showed a decreasing tendency towards the consecutive migration steps. Azacyclotridecan-2-one (PA), dicyclodipentyl(dimethoxy)silane (Tritan™) and acetophenone (PES and silicone) were some of the compounds detected in the reference treatment. Furthermore, for the silicone bottle also 2,2,4-trimethyl-1,3-pentanediol diisobutyrate (TXIB), benzophenone, di(iso)butylphthalate and 3,4-dimethylbenzaldehyde were identified. Yet, the detected concentrations were relatively low (mostly non-detectable or <LOQ (Limit of Quantification).

Table 5.10: Concentrations of targeted migrating compounds (µg kg^{-1}) from six selected baby bottles of the Belgian market when filled five times with preheated simulant (40 °C) during the reference treatment. * means < LOQ, - means non-detectable. Compounds which were not detected at all are not shown.

	Filling	2,2,4-trimethyl-1,3-pentanediol diisobutyrate (TXIB)	2,4-Di-*tert*-butylphenol	2,6-Di-*tert*-butylbenzoquinone	Benzophenone	Diisobutyl phthalate	Dibutyl phthalate	Acetophenone	Cedrol	3,5-Di-*tert*-butyl-4-hydroxybenzaldehyde	Dicyclopentyl(dimethoxy)silane	3,4-dimethylbenzaldehyde	Azacyclotridecan-2-one
PP brand-A	1	-	*	*	-	-	-	*	-	-	-	-	-
	2	-	*	*	-	-	-	-	-	-	-	-	-
	3	-	*	*	-	-	-	-	-	-	-	-	-
	4	-	*	*	-	-	-	-	-	-	-	-	-
	5	-	*	*	-	-	-	-	-	-	-	-	-
PA	1	-	*	*	-	-	-	*	-	-	-	-	70
	2	-	*	*	-	-	-	-	-	-	-	-	32
	3	-	*	*	-	-	-	-	-	-	-	-	10
	4	-	*	*	-	-	-	-	-	-	-	-	31
	5	-	*	*	-	-	-	-	-	-	-	-	15
PES	1	-	*	*	-	-	-	2	-	-	-	-	-
	2	-	*	*	-	-	-	*	-	-	-	-	-
	3	-	*	*	-	-	-	2	-	-	-	-	-
	4	-	*	*	-	-	-	-	-	-	-	-	-
	5	-	*	*	-	-	-	-	-	-	-	-	-
PP brand-B	1	-	*	*	-	-	-	*	-	-	-	-	-
	2	-	*	*	-	-	-	*	-	-	-	-	-
	3	-	*	*	-	-	-	*	-	-	-	-	-
	4	-	*	*	-	-	-	-	-	-	-	-	-
	5	-	*	*	-	-	-	-	-	-	-	-	-
Silicone	1	118	*	*	12	15	9	27	*	*	-	6	-
	2	102	*	*	4	12	7	11	-	*	-	-	-
	3	64	*	*	*	*	*	5	-	*	-	-	-
	4	71	*	*	*	*	*	4	-	*	-	-	-
	5	67	*	*	-	*	*	4	-	*	-	-	-
Tritan	1	11	*	*	-	-	-	*	-	-	2	-	-
	2	10	*	*	-	-	-	-	-	-	1	-	-
	3	7	*	*	-	-	-	-	-	-	1	-	-
	4	8	*	*	-	-	-	-	-	-	1	-	-
	5	-	*	*	-	-	-	-	-	-	1	-	-

5.2.4.3 Duration tests: Microwave heating

Microwaves are often used to warm infant feeding formula in a short time. The processes involved in heat transfer during microwave heating are different from those during conduction or convection heating, and consequently unpredictable migration behaviour could be expected (Letellier & Budzinski 1999; Alin & Hakkarainen 2012).

Microwave heating of the selected bottles during 100 cycles showed that only a few compounds were released and in low concentrations (µg kg^{-1} level). The detected concentrations were continuously decreasing cycle after cycle and were below LOQ or LOD after 25 cycles.

Some of the targeted substances previously seen in the 3rd migration of the EU repetitive use and reference experiment were released also here. Generally, the concentrations detected were significantly lower (Table 5.10) than those of the 3rd migration step, most probably due to the higher temperature (70 °C instead of 40 °C) applied in the repetitive experiment. Concentrations of migrating compounds were more in the range of those observed in the reference experiment.

Maximum concentrations of migrating substances were measured in the first cycles and showed a downwards tendency afterwards. For both PP bottles, none of the targeted compounds were detected or they were present at levels < LOQ (data not shown), whereas the concentration of some compounds (e.g. 3,4-dimethylbenzaldehyde: 59 µg kg^{-1}) were significantly higher in the EU repetitive use experiment indicating their actual presence in the polymer and the possibility to be released. The PA bottle only exhibited the presence of azacyclotridecan-2-one at 124 µg kg^{-1} after the 1st microwave treatment, though in the subsequent steps this concentration rapidly decreased to 31 after the 2nd microwave treatment (Table 5.11). Yet, it was noteworthy that this was the only bottle which demonstrated a continuous release (concentrations > 5 µg kg^{-1}) of one of its migrants (azacyclotridecan-2-one) throughout the entire 100 cycles. For the Tritan™ bottle only few compounds were detected at measurable concentrations (e.g. 4-propylbenzaldehyde: 8 µg kg^{-1} and disappearing after 8 cycles; dicyclopentyl-(dimethoxy)silane: 1 µg kg^{-1}). TXIB was released continuously at levels between the LOQ and 13 µg kg^{-1} during the first 25 cycles after which its concentration dropped below the LOQ (Table 5.11). For the silicone bottle, TXIB was found between LOQ and 11 µg kg^{-1} during the first 25 cycles, although for the reference experiment the concentration reached more than 118 µg kg^{-1}. Dibutyl phthalate was even not detected in the silicone bottle in the microwave experiments whereas in the reference treatment it was detected starting at 9 µg kg^{-1}. Yet generally, the other components detected in the reference experiment of the silicone bottle displayed higher concentrations in the microwave extracts (e.g. acetophenone 43 µg kg^{-1} (1st cycle microwave-experiment) vs.

27 µg kg^{-1} (1st reference-experiment)). Although detected in the microwave and reference treatment, acetophenone was not seen in the 3rd migration of the EU repetitive experiment of the silicone bottle. This is most probably due to the fact that it already disappeared entirely after the first two EU repetitive use migrations.

For the PES material, acetophenone was the only detected compound at a concentration of 6 µg kg^{-1} and disappeared after 8 cycles.

When comparing the values obtained in these microwave duration tests to those of the reference experiment (Table 5.10), two conclusions could be drawn. Firstly, the concentrations detected during the microwave treatments were higher for almost all compounds compared to the reference experiment. For example, 4-propyl-benzaldehyde, which was not detected in the reference treatment of the Tritan™ bottle, was measured after the first microwave cycle at 8 µg kg^{-1}. Although the increase induced by the microwave treatment was rather modest, the same phenomenon was generally perceived also for the other bottle types, e.g. azacyclotridecan-2-one from the PA bottle: 124 µg kg^{-1} after the first microwave heating vs. 70 µg kg^{-1} in the first reference-experiment. Secondly, the few compounds that were detected in the reference treatment at higher or similar concentrations dropped below the LOQ within a few cycles, whereas for the microwave treatment, their release was longer, such as for benzophenone (silicone, 8 cycles before <LOQ) or TXIB (Tritan™, up to 25 cycles). The latter compound was rather particular, as it was the only one that was present at higher concentrations in the reference treatment compared to the microwave duration test (10 vs. 118 µg kg^{-1}). However, this behaviour was only noticed for the silicone bottle, while the concentration of TXIB after the first microwave heating and the first reference experiment was very similar for the Tritan™ material (9 vs. 11 µg kg^{-1}).

In conclusion, the microwave treatment not only systematically increased the release of substances in general, but it also substantially prolonged the number of cycles in which the target compounds were detected.

Table 5.11: Summary of the concentrations (in µg kg^{-1}) of the detected migrating compounds in different polymer types during migration after microwave heating. * means < LOQ, - means non-detectable.

Polymer type	Compound	Microwave heating cycle																					LOQ (µg kg^{-1} simulant)
		1	2	3	4	5	6	7	8	9	10	15	20	25	30	40	50	60	70	80	90	100	
PES	Acetophenone	6	2	2	2	2	3	2	2	*	*	*	*	*	-	-	-	-	-	-	-	-	1.7
PA	Azacyclotridecan-2-one	124	31	19	22	26	25	14	19	34	17	15	7	22	5	5	8	7	6	11	14	7	9.6
Tritan	2,2,4-trimethyl-1,3-pentanediol diisobutyrate (TXIB)	9	9	7	9	8	*	*	10	9	10	8	9	13	*	*	*	*	*	*	*	*	6.4
	4-propylbenzaldehyde	8	4	3	3	2	2	1	*	-	-	-	-	-	-	-	-	-	-	-	-	-	0.6
	Dicyclopentyl(dimethoxy)silane	1	1	1	1	1	1	*	1	1	1	1	*	1	*	*	*	*	*	*	*	*	0.8
Silicone	TXIB	10	8	*	*	*	*	7	9	8	*	*	8	11	*	*	*	*	*	*	*	*	6.4
	Benzophenone	14	10	6	6	6	4	5	6	*	*	*	*	*	*	-	-	-	-	-	-	-	3.6
	Diisobutyl phthalate	24	19	14	15	15	10	14	19	12	15	*	17	17	*	*	*	*	*	*	*	*	8
	Acetophenone	43	24	14	14	13	7	7	8	5	6	2	*	*	*	*	*	*	*	*	*	*	1.7
	3,4-dimethylbenzaldehyde	10	6	*	*	*	*	*	*	*	*	-	-	-	-	-	-	-	-	-	-	-	5.6
PP Brand A & B	No compounds detected																						

5.2.4.4 Duration tests: Dishwasher cleaning

After treatment of the baby bottles with a dishwasher programme between 55-60 °C during almost 3 h, hardly any of the target compounds could be detected. For the PP and PES bottles, no compounds were detected and for PA, only the monomer azacyclotridecan-2-one was seen at decreasing concentrations (from 98 to 39 µg kg^{-1} in 10 cycles). Silicone and Tritan™ exhibited the presence of some of the targeted compounds (TXIB, benzophenone, di(iso)butyl phthalate,…), though only at low concentrations. When comparing the observed levels to the reference treatment, they were generally slightly higher after using the dishwasher (Table 5.12), indicating that the washing programme could cause a slight increase in the release of some compounds (e.g azacyclotridecan-2-one: 98 vs. 70 µg kg^{-1}). For compounds such as benzophenone or di(iso)butyl phthalate, the detected levels remained also higher during more cycles than compared to the reference treatment. Moreover, dibutyl phthalate even exhibited a small increase in concentrations after several dishwasher treatments (from 7 to 13 µg kg^{-1}). Nevertheless, other target compounds were already partially removed and therefore migrated in lower concentrations in the subsequent migration experiment (e.g. in the silicone bottle: TXIB 36 vs. 118 µg kg^{-1} in reference; acetophenone ND vs. 27 µg kg^{-1}). Most probably the elevated temperature (55-60 °C) and long washing time (almost 3h) were the main causes of this phenomenon. Anyhow, the detected concentrations for all compounds remained at low levels and far below the SMLs.

Table 5.12: Summary of the concentrations (in µg kg^{-1}) of the detected migrating compounds in different polymer types during migration after dishwasher treatment. * means < LOQ, - means experimental failure.

Polymer type	Compound	Dishwasher cycle						LOQ (µg kg^{-1} simulant)
		1	2	4	6	8	10	
PA	Azacyclotridecan-2-one	98	18	13	55	22	39	9.6
Tritan	2,2,4-trimethyl-1,3-pentanediol diisobutyrate (TXIB)	-	13	8	11	8	8	6.4
	Dicyclopentyl(dimethoxy)silane	1	3	3	3	3	3	0.8
Silicone	TXIB	-	36	34	27	23	22	6.4
	Benzophenone	-	20	13	12	7	7	3.6
	Diisobutyl phthalate	-	13	16	17	14	16	8.0
	Dibutyl phthalate	-	7	10	11	11	13	4.4
	2,4-Di-*tert*-butylphenol	*	*	*	7	*	*	6.2
PP Brand A & B	No compounds detected							
PES	No compounds detected							

It should be considered though that only the levels of previously selected target compounds were monitored here, and that no information was available on any possible polymer degradation products formed by this treatment. Therefore, this should be further studied into detail.

5.2.4.5 Duration test: Steam and cooking sterilisation

Steam sterilisation of the selected bottles clearly resulted in a quick elimination of the monitored compounds, since both PP and PES showed no migration. For PA, the detected concentrations were considerably lower than compared to the reference treatment (starting at 59 instead of 70 µg kg^{-1} and rapidly decreasing), as well as for Tritan™ for which only dicyclopentyl(dimethoxy)silane was released at low concentrations. The same was observed for acetophenone which migrated at very low concentrations from Tritan™ before it disappeared (2 µg kg^{-1} after the first cycle). For the silicone bottle, all detected compounds were seen in decreasing concentrations and most of them also disappeared after a few sterilisation cycles (e.g. 3,4-dimethylbenzaldehyde; di(iso)butyl phthalate; acetophenone; 2,4-di-*tert*-butylphenol) (Table 5.13). Generally, the detected concentrations were lower (e.g. TXIB 28 vs. 118 µg kg^{-1}) or similar (di(iso)butyl phthalate) to those seen after the reference treatment. Benzophenone was initially released at higher concentrations after steam sterilisation, and it was detected during the entire 10 treatments, whereas for the reference treatment, it disappeared after 2 cycles. Moreover, other compounds were not released (2,4-di-*tert*-butylphenol) or at much lower concentrations (3,4-dimethylbenzaldehyde) during the reference treatment. This might indicate that the high temperatures applied during sterilisation could influence (and even increase) the release of some particular compounds. Yet, concentrations remained relatively low and still exhibited a decreasing tendency. Therefore, when overlooking the general tendency for the majority of the targeted compounds and considering also the other tested baby bottles, it could clearly be concluded that sterilisation is generally recommended to be performed before using a baby bottle in order to remove residual chemicals after production, but also to suppress the microbial contamination.

The cooking sterilisation generally showed the same pattern as seen in the steam sterilisation. The PES and both PP bottles did not display any migration of the selected compounds after they were subjected to a sterilisation in boiling water during 10 min whereas for PA the tendency was similar to the steam sterilisation as well. The Tritan™ bottle showed the release of 4-propylbenzaldehyde (previously seen here in the EU experiments as well) at 12 µg kg^{-1} in the 1st migration step, but it was not detected anymore after the 2nd sterilisation. Cooking sterilisation of the Tritan™ polymer resulted also in the migration of 4-n-nonylphenol, which was not detected after any of the other

treatments, starting at 6 µg kg^{-1} after the first cycle and decreasing afterwards. The results of this treatment on the latter polymers therefore suggested mainly the same conclusions as drawn from the steam sterilisation, namely that the application of harsh conditions such as high temperatures, and in this case even physical movement of the bottles during the cooking, results in temporary release of compounds. Therefore, it might be advised to perform some sterilisation cycles before the first use of new baby bottles. Yet again, only the previously selected compounds were monitored and no data were available on the possible degradation products formed during this cooking sterilisation.

Whereas none of the aforementioned polymers released hardly any substances after the cooking treatment, the silicone bottle showed a totally different migration pattern. Here, an increase in concentrations of the previously observed compounds was seen, and even some substances that were not detected in the reference treatment were released (e.g. cedrol: 13 µg kg^{-1}; 3,5-di-*tert*-Butyl-4-hydroxybenzaldehyde: 4 µg kg^{-1}) although they disappeared after 2 cycles. Benzophenone, acetophenone, and dibutyl phthalate exhibited a similar migration pattern compared to the steam sterilisation, though for diisobutyl phthalate (starting at 29 µg kg^{-1} and maintaining higher concentrations during more cycles) and TXIB (247 µg kg^{-1} after the first treatment), the detected concentrations were significantly higher than for the steam sterilisation and the reference treatment (Table 5.13).

Cooking sterilisation might be less suitable than steam sterilisation for silicone bottles due to the physical contact with boiling water which seems to enhance the release of some compounds. Therefore, the rather aggressive conditions of the cooking sterilisation are not recommendable for the silicone bottle. Generally, considering the lower concentrations and number of compounds released during steam sterilisation, this type of sterilisation would be preferable. Further non-target analyses of migrants of the other polymers still needs to be done to draw appropriate conclusions.

Table 5.13: Summary of the concentrations (in µg kg^{-1}) of the detected migrating compounds in the different polymer types during migration after steam or cook sterilisation treatment. * means < LOQ, - means non-detectable. X means sample lost during experiment.

Polymer type	Compound	Steam sterilisation cycle						Cook sterilisation cycle						LOQ (µg kg^{-1} simulant)
		1	2	4	6	8	10	1	2	4	6	8	10	
PA	Azacyclotridecan-2-one	59	33	10	4	24	6	51	13	9	19	7	21	9.6
Tritan	2,2,4-trimethyl-1,3-pentanediol diisobutyrate (TXIB)	-	13	8	11	8	8	2	*	*	*	*	*	6.4
	4-n-Nonylphenol	-	-	-	-	-	-	6	X	5	2	2	1	1.0
	Dicyclopentyl(dimethoxy)silane	1	3	3	3	3	3	2	X	1	1	1	1	0.8
	Acetophenone	2	-	-	-	-	-	-	-	-	-	-	-	1.7
	4-propylbenzaldehyde	-	-	-	-	-	-	12	-	-	-	-	-	0.6
Silicone	TXIB	28	27	23	15	16	9	247	201	178	145	127	128	6.4
	Benzophenone	53	66	26	14	9	12	58	36	25	12	7	7	3.6
	Diisobutyl phthalate	10	9	*	*	*	*	29	29	24	19	17	15	8.0
	Dibutyl phthalate	11	14	7	7	-	-	10	12	11	9	8	8	4.4
	2,4-Di-*tert*-butylphenol	7	*	*	*	*	*	12	7	*	*	*	*	6.2
	2-Butoxyethyl acetate	-	-	-	-	-	-	6	-	-	-	-	-	5.6
	Acetophenone	14	8	*	*	-	-	14	4	*	*	*	-	1.7
	Cedrol	*	*	*	*	*	*	13	11	*	*	*	*	9.7
	3,5-di-*tert*-Butyl-4-hydroxybenzaldehyde	-	-	-	-	-	-	4	4	*	*	3	*	3.1
	3,4-dimethylbenzaldehyde	18	15	-	-	-	-	6	-	-	-	-	-	5.6
PP Brand A & B	No compounds detected													
PES	No compounds detected													

5.2.4.6 Comparison of treatments

When comparing the different duration tests to each other, the lowest release of substances was generally exhibited after the microwave process, showing generally slightly higher concentrations than those of the reference treatment. Figure 5.3A & B shows the examples of benzophenone from the silicone bottle and azacyclotridecan-2-one from the PA. Furthermore, the concentrations observed after the dishwasher treatment were slightly higher than the microwave whereas (for some of the compounds some of) the sterilisation treatments displayed the highest concentrations released.

All targeted compounds detected after application of the different duration tests were in accordance with the EU repetitive use experiment. Yet, this did not necessarily mean that these target compounds were still present in the 3rd step of the repetitive use experiment (Table 5.9), as they could have migrated already entirely from the polymer material during the first two migrations (Onghena et al. 2014). When comparing the concentrations observed in the duration tests (e.g. after 10 cycles) to those of the EU repetitive use experiment, most compounds detected in the latter experiments clearly displayed higher levels than any of the individual experiments (e.g. TXIB, 2-10 times higher compared to the 10th cycle of the duration tests). Therefore, the EU repetitive use experiment seemed to overestimate the migration originating from the normal daily use of baby bottles for some compounds, sometimes with even more than a factor 10. Yet, for others such as benzophenone (Figure 5.3A) or butyl phthalate the concentrations after 10 duration cycles were in the same range as those of the EU repetitive use experiment.

Most importantly, the EU repetitive experiment did not make an underestimation of the concentrations released in the duration tests for any of the target compounds. However, it has to be taken into account that the conditions applied for the EU repetitive experiment represented a worst-case scenario for direct migration from the polymer, whereas during the duration tests (dishwashing and sterilisation), potential migrants could already been washed away, resulting in lower actual migration to the simulant solution afterwards. Furthermore, all duration tests were conducted individually and therefore the influence of a combination of these treatments on the migration still has to be investigated.

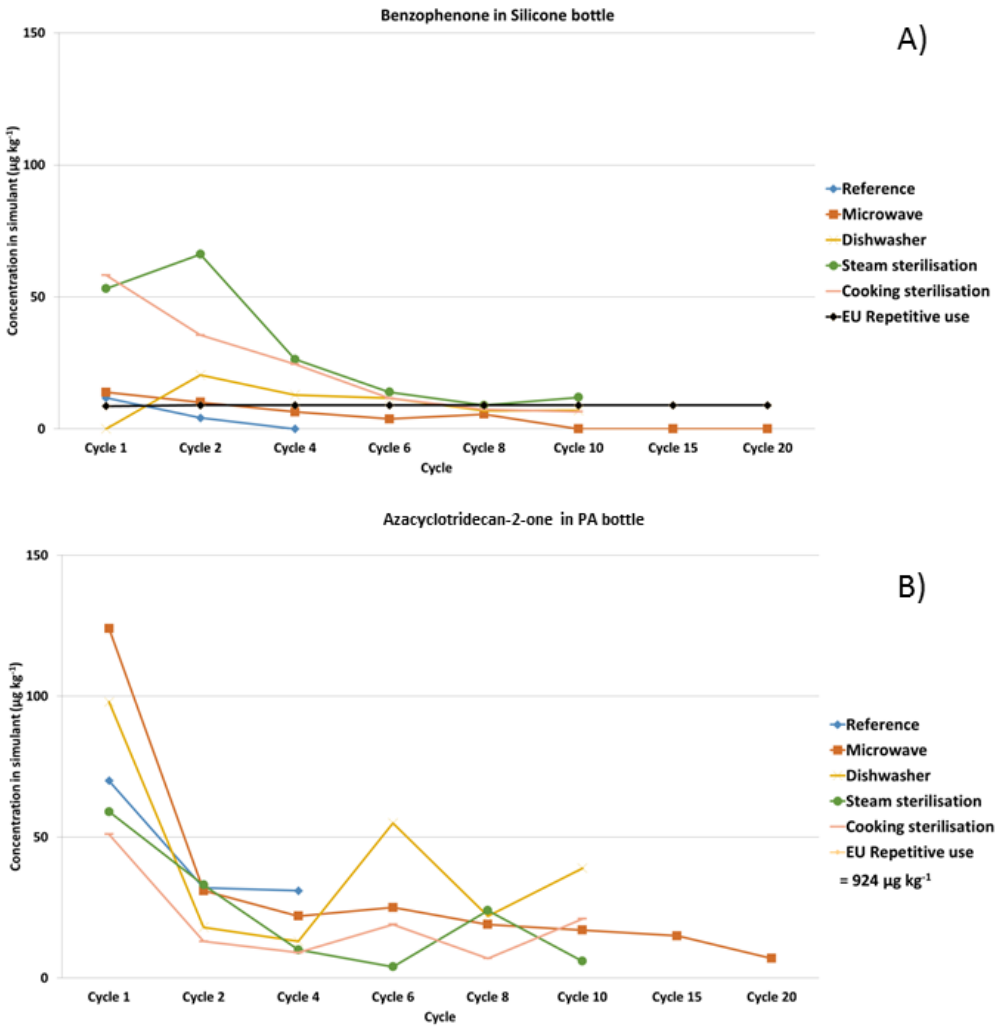


Figure 5.3: Summary of the detected concentrations (µg kg^{-1}) after the different treatments for A) benzophenone in the silicone bottle and B) azacyclotridecan-2-one in the polyamide bottle.

5.2.5 Conclusions

Migration experiments on the polymer alternatives to PC baby bottles were done following the conditions specified in EU Regulation No. 10/2011 and by performing duration tests to simulate "real-life use" conditions. The experiments following the EU conditions showed that for compounds authorised by EU Regulation, none of the specified SMLs were exceeded.

The duration tests showed the release of the same substances as detected in the EU repetitive use experiment, though the detected concentrations were lower (mostly

<LOQ or ND) and a downwards tendency of migrant concentrations towards the subsequent treatment cycles was seen. Yet, the use of the microwave led to a slightly increased and/or prolonged release of migrating substances compared to a “regular-use reference treatment”. However, migration was below detection limits after 30 microwave cycles. The concentrations detected after dishwasher and sterilisation treatments remained considerably low.

These experiments give more insight in the migration behaviour of baby bottles and show that repeated use of baby bottles under “real-life” conditions will not increase the migration of relevant compounds. On the contrary, the migration of these compounds became insignificant after a number of cycles. The results are indeed somewhat contra intuitive, but the current work monitors only a selection of compounds, and further research on other possible degradation products formed during these treatments needs to be conducted. To further reduce the exposure of young children, the consumer could be advised to perform some cycles of steam sterilisation before the first use of baby bottles.

5.3 Screening for possible degradation products after application of duration tests by a fingerprinting approach

5.3.1 Introduction

In chapter 5.2, we have studied the influence of duration tests on the migration of those compounds that were previously identified to be of major priority. However, due to the targeted approach applied there, no information was obtained on the evolution of other possible migrants. It was shown in that chapter that the migrating concentrations of the monitored compounds decreased throughout the subsequent cycles of the different treatments. Yet, no information is available if any other compounds (more specifically degradation products, e.g. release of monomers, antioxidant breakdown products) might be newly formed and exhibit therefore an increasing concentration profile throughout the subsequent treatment cycles.

For PC based bottles, previous studies (Mercea 2009) showed that under the influence of high temperatures, BPA and low molecular weight species accumulated at the surface, resulting in increased BPA levels during the migration testing. Despite this, no similar studies are available at the moment considering the polymer alternatives to PC for baby bottles. Nevertheless, to fully safeguard consumers' safety, not only the monitored target compounds, but also other possible migrants that might appear due to the influence of the different type of duration tests have to be studied.

To this end, a fingerprinting approach was applied to document the entire migration profile after one treatment step of each duration test. Subsequently, this was compared to the migration profile after a notable number of cycles. In this way, the detection of peaks that initially were not present, and whose appearance consequently origins from the influence of the duration test treatment, was enabled.

5.3.2 Materials

5.3.2.1 Samples

The baby bottles that were selected in section 5.2.2.1 for the application of the duration tests were used for the fingerprinting approach. For each different duration test, the first and the tenth sample were analysed. Metolachlor (a pesticide) was added to the sample extracts at a concentration of one ng μL^{-1} to ensure a constant response of the GC instrument. This was of the utmost importance since the comparisons and consequent conclusions on the (dis)appearance of peaks are based on the detected peak areas.

5.3.3 Methods

5.3.3.1 Instrumentation

5.3.3.1.1 GC-(EI)TOF-MS

An Agilent 6890N GC system (Palo Alto, CA, United States) equipped with an Agilent 7683 autosampler, was coupled to a TOF mass spectrometer (Waters Corporation, Manchester, UK), operating in EI mode (70eV). The GC separation was performed using a 30 m × 0.25 mm × 0.25 µm DB-5MS column type and the oven programme was as follows: initial oven temperature was 60 °C and was increased at a rate of 12 °C min^{-1} until 240 °C. Then, a rate of 30 °C min^{-1} was applied until a temperature of 300 °C was reached which was held for two min. This resulted in a total run time of 20 min. Helium was used as a carrier gas at constant flow of one ml min^{-1}. The injection volume was 1 µl.

The interface and source temperatures were both set to 250 °C, and a solvent delay of three min was selected. The TOF-MS was operated at one spectrum/s acquisition rate over the mass range *m/z* 40–750, using a multichannel plate voltage of 2800 V. TOF-MS resolution was approximately 8500 at full width at half maximum (FWHM) at *m/z* 614. Heptacosafluorotributylamine (Sigma Aldrich, Madrid, Spain), used for the daily mass calibration and as lock mass, was injected via syringe in the reference reservoir at 30 °C to monitor the *m/z* ion 218.9856.

5.3.3.2 Data analysis

For each different duration test, the first and the tenth sample were analysed (for the microwave treatment the first and the hundredth sample) and compared. To this end, samples were first individually investigated by the “identify samples” feature of the Chromalynx application manager, a module of MassLynx software. Then, samples were compared one by one to each other to detect any possible differences by the “compare samples” feature. Since the identification of the EU repetitive use samples in Chapter 4 already accounted for those peaks that could be detected in the first sample, in the comparison focus was only made on those peaks that could newly appear after a number of treatments. To investigate the identity of newly formed compounds, library search was performed using the commercial NIST library.

5.3.4 Results and discussion

Comparison of the fingerprints of the initial samples of each duration test to those of samples that underwent a significant number of cycles displayed that for the PES, PA, Tritan™, and silicone bottles no new peaks appeared after a number of treatments. Moreover, for the compounds that were detected in these bottles after the first treatment, only a decrease in concentrations was seen. This was in accordance with the conclusions of Chapter 5.2, since the same was already described for the target compounds. The non-target screening approach that was applied here confirmed that also for the other migrants that were previously not monitored this was the case (Figure 5.4).

These findings were in accordance with those found in literature for the testing of PC baby bottles, where for both steam sterilisation (Mountfort et al. 1997), as for cooking sterilisation (Maragou et al. 2008), no increased release of BPA after the treatments nor a consequent degradation of the polymer could be observed. Also for the dishwasher (Mountfort et al. 1997) and microwave (Biedermann-Brem & Grob 2009) treatment similar conclusions were made for PC baby bottles.

However for one of the PP bottles, in the fingerprint of the migration solution after ten cooking sterilisations, a peak was detected at 11.16 min that exhibited a tenfold higher intensity than after the first sterilisation. Library search identified this peak as butylated hydroxytoluene (BHT), a phenolic antioxidant commonly used in polymer materials (Dopico-García et al. 2007). Since this compound was not detected before (EU repetitive use experiments) or was detected only at levels similar to those seen in the blanks, its appearance here was rather remarkable. This indicated again, as seen for the silicone bottle in Chapter 5.2, that the cooking sterilisation might increase the release of certain substances that are already present in the polymer, but normally not released. For the other PP that was tested, an increase in BHT concentration was also perceived, yet to a much lower extent. For the other treatments applied to the PP bottles, no new or increasing peaks were seen. Nevertheless, this observation for the cooking sterilisation of the PP bottles, none of the applied treatments seemed to cause degradation of any of the tested polymer types.

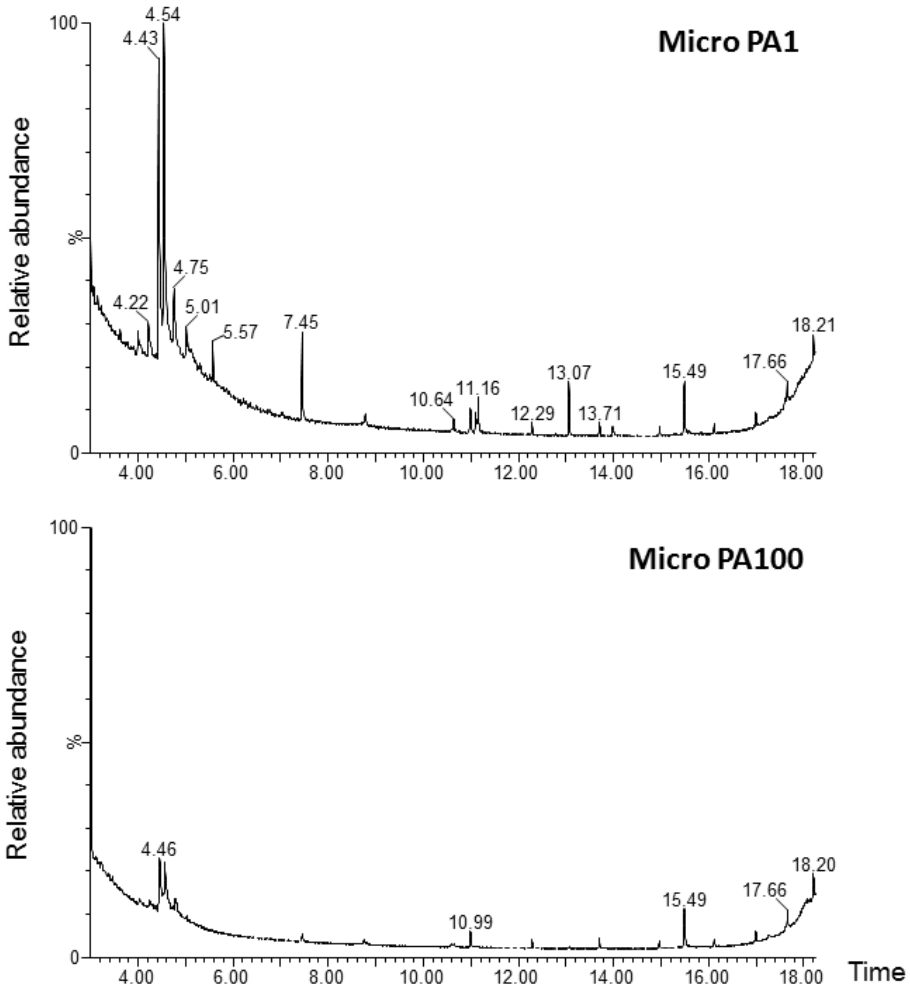


Figure 5.4: Comparison of chromatograms of the migration solution of the PA baby bottle after 1 and 100 microwave treatments

5.3.5 Conclusions

A comparison of the fingerprints obtained after one and several treatment(s) of each type of duration test demonstrated that generally no new peaks were detected as a result of these treatments. Moreover, the concentrations of the observed compounds decreased towards the subsequent cycles. Only the cooking sterilisation of the PP bottles demonstrated an increased release of BHT when comparing the tenth cycle to the first. However, LC-MS analysis still needs to be conducted to complete these data with information on the possible less volatile, more polar degradation products.

References

Alin J, Hakkarainen M. 2010. Type of Polypropylene Material Significantly Influences the Migration of Antioxidants from Polymer Packaging to Food Simulants During Microwave Heating. J Appl Polym Sci. 118:1084–1093.

Alin J, Hakkarainen M. 2011. Microwave heating causes rapid degradation of antioxidants in polypropylene packaging, leading to greatly increased specific migration to food simulants as shown by ESI-MS and GC-MS. J Agric Food Chem. 59:5418–5427.

Alin J, Hakkarainen M. 2012. Migration from polycarbonate packaging to food simulants during microwave heating. Polym Degrad Stab. 97:1387–1395.

Barlow SM. 2009. Risk assessment of food-contact materials: past experience and future challenges. Food Addit Contam Part A. 26:1526–1533.

Baughan JS. 2015. Global Legislation for Food Contact Materials. 1 edition. Sawston, Cambridge: Woodhead Publishing.

Biedermann-Brem S, Grob K. 2009. Release of bisphenol A from polycarbonate baby bottles: Water hardness as the most relevant factor. Eur Food Res Technol. 228:679–684.

Brede C, Fjeldal P, Skjevrak I, Herikstad H. 2003. Increased migration levels of bisphenol A from polycarbonate baby bottles after dishwashing, boiling and brushing. Food Addit Contam. 20:684–689.

Chang N, Zhang C, Zheng F, Huang Y, Zhu J, Zhou Q, Zhou X, Ji S. 2016. Migration of toluene through different plastic laminated films into food simulants. Food Control. 59:164–171.

Council of Europe. 2004. Resolution ResAP(2004)5 on silicones used for food contact applications. Off J Eur Union [Internet]. Available from: https://wcd.coe.int/ViewDoc.jsp?id=797547&Site=CM

Dopico-García MS, López-Vilariñó JM, González-Rodríguez M V. 2007. Antioxidant content of and migration from commercial polyethylene, polypropylene, and polyvinyl chloride packages. J Agric Food Chem. 55:3225–3231.

Ehlert K a, Beumer CWE, Groot MCE. 2008. Migration of bisphenol A into water from polycarbonate baby bottles during microwave heating. Food Addit Contam Part A. 25:904–10.

European Union. 2002. Commission Directive 2002/657/EC. Off J Eur Union. L221:8.

European Union. 2011. Commission Regulation (EU) No 10/2011 of 14 Januari 2011 on plastic materials and articles intended to come into contact with food. Off J Eur Union. L12:1–89.

Horwitz W, Kamps LR, Boyer KW. 1980. Quality assurance in the analysis of food for trace constituents. J Assoc Off Anal Chem. 63.

Horwitz W, Wood R. 2000. Relationship of (known) control values to (unknown) test values in proficiency studies of pesticide residues. J AOAC Int. 83:399–406.

Interscience publications. 1994. ISO 5725-2:1994, Accuracy (trueness and precision) of measurement methods and results - Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method. Hoboken, NJ, USA: John Wiley & Sons.

Kuruto-Niwa R, Nozawa R, Miyakoshi T, Shiozawa T, Terao Y. 2005. Estrogenic activity of alkylphenols, bisphenol S, and their chlorinated derivatives using a GFP expression system. Environ Toxicol Pharmacol. 19:121–30.

Letellier M, Budzinski H. 1999. Microwave assisted extraction of organic compounds. Analysis. 27:259–271.

Mandel. 1964. The Statistical Analysis of Experimental Data. New York: Interscience.

Maragou NC, Makri A, Lampi EN, Thomaidis NS, Koupparis MA. 2008. Migration of bisphenol A from polycarbonate baby bottles under real use conditions. Food Addit Contam Part A. 25:373–383.

Mcdonald GR, Hudson AL, Dunn SMJ, You H, Baker GB, Whittal RM, Martin JW, Jha A, Edmondson DE, Holt A. 2008. Bioactive Contaminants Leach from disposable laboratory plasticware. Science. 322:917.

Mercea P. 2009. Physicochemical Processes Involved in Migration of Bisphenol A from Polycarbonate. Appl Polym Sci. 112:579–593.

Mertens B, Simon C, Van Bossuyt M, Onghena M, Vandermarken T, Van Langenhove K, Demaegdt H, Van Hoeck E, Van Loco J, Vandermeiren K, et al. 2016. Investigation of the genotoxicity of substances migrating from polycarbonate replacement baby bottles to identify chemicals of high concern. Food Chem Toxicol. 89:126–137.

Mountfort KA, Kelly J, Jickells SM, Castle L. 1997. investigations into the potential degradation of polycarbonate baby bottles during sterilization with consequent release of bisphenol A. Food Addit Contam Part A. vol.14:4–7.

Nguyen DH, Nguyen DTM, Kim E. 2008. Effects of di-(2-ethylhexyl)phthalate (DEHP) released from laboratory equipments. Time. 25:1136–1139.

Onghena M, Hoeck E Van, Loco J Van, Ibáñez M, Cherta L, Portolés T, Pitarch E, Hernandéz F, Lemière F, Covaci A. 2015. Identification of substances migrating from plastic baby bottles using a combination of low-resolution and high-resolution mass spectrometric analysers coupled to gas and liquid chromatography. J Mass Spectrom. 50:1234–1244.

Onghena M, Van Hoeck E, Negreira N, Quirynen L, Van Loco J, Covaci A. 2016. Quantitative determination of migrating compounds from plastic baby bottles by validated GC-QqQ-MS and LC-QqQ-MS methods. Food Anal Methods. :DOI 10.1007/s12161–016–0451–4.

Onghena M, van Hoeck E, Vervliet P, Scippo ML, Simon C, van Loco J, Covaci A. 2014. Development and application of a non-targeted extraction method for the analysis of migrating compounds from plastic baby bottles by GC-MS. Food Addit Contam Part A. 31:2090–102.

Osimitz TG, Eldridge ML, Sloter E, Welsh W, Ai N, Sayler GS, Menn F, Toole C. 2012. Lack of androgenicity and estrogenicity of the three monomers used in Eastman's Tritan copolyesters. Food Chem Toxicol. 50:2196–2205.

Pereira-Fernandes A, Demaegdt H, Vandermeiren K, Hectors TLM, Jorens PG, Blust R, Vanparys C. 2013. Evaluation of a screening system for obesogenic compounds: screening of endocrine disrupting compounds and evaluation of the PPAR dependency of the effect. PLoS One. 8:774–781.

Simon C, Onghena M, Covaci A, Van Hoeck E, Van Loco J, Vandermarken T, Van Langenhove K, Demaegdt H, Mertens, Birgit Vandermeiren, Karin Scippo, Marie-

Louise Elskens M. 2016. Screening of endocrine disrupting activity of compounds migrating from plastic baby bottles using a multi-receptor panel of in vitro bioassays. Submitt to Toxicol in Vitr.

Simoneau C, Van Den Eede L, Valzacchi S. 2012. Identification and quantification of the migration of chemicals from plastic baby bottles used as substitutes for polycarbonate. Food Addit Contam Part A. 29:469–480.

Simoneau C, Valzacchi S, Morkunas V, Van Den Eede L. 2011. Comparison of migration from polyethersulphone and polycarbonate baby bottles. Food Addit Contam Part A. 28:1763–1768.

Thompson M. 2000. Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing. Analyst. 125:385–386.

Weitzel M, Lee SM, Smoot M, Viafara N, Brodsky M. 2007. How to Meet ISO 17025 Requirements for Method Verification. ALACC Guid [Internet]. [cited 2016 Jan 12]:18. Available from: http://www.aoac.org/imis15_prod/AOAC_Docs/LPTP/alacc_guide_2008.pdf

Wong KO, Leo LW, Seah HL. 2005. Dietary exposure assessment of infants to bisphenol A from the use of polycarbonate baby milk bottles. Food Addit Contam Part A. 22:280–288.

Zoeller RT, Brown TR, Doan LL, Gore a. C, Skakkebaek NE, Soto a. M, Woodruff TJ, Vom Saal FS. 2012. Endocrine-Disrupting Chemicals and Public Health Protection: A Statement of Principles from The Endocrine Society. Endocrinology. 153:4097–4110.

Chapter 6 :

General discussion

6.1 Discussion

The main goals of this thesis were on one hand to investigate the identity of compounds released from the polymer alternatives to PC FCMs used for children under 3 y old (e.g., baby bottles), and on the other hand to quantitatively assess the migration of these compounds under real-life use conditions tested by means of duration tests. Consequently, their compliance of the observed migration with the current legislation was determined. Within this chapter, the achievement of these predefined goals of this thesis will be discussed and some critical considerations will be made.

As defined in the first goal, we initially focused on the identification of compounds that could possibly be released by the current alternative materials for PC baby bottles, as up to date, only few studies have addressed this issue (Simoneau et al. 2011; Simoneau et al. 2012). Since the use of simulants is mandatory by the EU Regulation No. 10/2011 (European Union 2011) to investigate the migration from plastic FCMs towards food, we had to develop a fast and generic analytical method that enabled a wide scope screening of the possible migrants via both GC-MS and LC-MS. Since the non-target analytical determination of these migrants requires a thorough sample preparation owing to the low concentrations (µg kg^{-1} range) that have to be measured, **chapter 4.1** describes the development and consequently the application of this generic screening method by GC-MS analysis. The advantage of developing such a general method is that a broad spectrum of chemically different compounds can be covered which enables the aimed wide scope screening to detect as much compounds as possible. However, a consequent limitation is that the method exhibited a rather poor performance for certain classes of compounds.

The results of this initial screening demonstrated that a wide variety of compounds migrated from the polymer alternatives for PC present on the Belgian market and we observed significant differences in the migrating patterns between the different polymer types. Although silicones are not covered by EU Regulation 10/2011 on plastics, they were included in this study considering their relevant release of organic compounds. Furthermore, a first semi-quantitative estimation of the concentrations of the identified migrants during the third repetitive use experiment was already made to obtain an initial idea regarding the compliance of these PC baby bottle alternatives, and to assess which compounds were of major importance to be specifically monitored afterwards.

Next, some more volatile compounds separated by GC that could not yet be confirmed with a conclusive library match were further elucidated by means of accurate mass techniques, in a similar way as the less volatile migrants separated by LC, as described in **chapter 4.2**. Although several previously unidentified compounds were elucidated by means of these accurate mass techniques, this identification process remains a time-consuming task and the successful outcome still highly depends on the availability of spectral libraries.

About 75% of the migrating compounds could be identified in this way, indicating that, even with powerful analytical techniques, a full identification of complex polymer migration samples still remains a daunting task. For the migrants for which the identity could be elucidated, the results of the identification demonstrated that only a minor percentage of the migrants was present in the EU Regulation No. 10/2011 positive list. Normally, for monomers, other starting substances and additives, only substances authorised by the EU on this positive list may be intentionally used in plastic FCMs. These substances are called intentionally added substances (IAS). Yet, certain substances are not subject to authorisation and listing in the Union list and may be present such as polymerisation and polymer production aids (PPA), colorants, and substances used behind a functional barrier. Also the non-intentionally added substances (NIAS) are exempted from the authorisation and inclusion in the Union list. However, in certain cases, Annex I and Annex II (restrictions on materials and articles) to the Plastics Regulation may include restrictions on some NIAS. Since NIAS can originate from different sources, including impurities present in authorised substances, degradation products or undesired side products and contaminants from polymer recycling processes (Figure 6.1), consequently their variety and extend in which they can migrate can be very broad. However, for the not-listed substances that are not used behind a functional barrier, no official limit has been specified in the Regulation, though to ensure compliance with general safety requirements and avoid any potential health risk, a “threshold” migration limit of 10 µg kg^{-1}, based on the limit specified for not-listed substances used behind a functional barrier (European Union 2011), is recommended and generally assumed for those substances that are not yet evaluated and therefore they should be assessed in accordance with internationally recognised scientific principles on risk assessment (Art. 19 of European Regulation No. 10/2011 (European Union 2011).

It is the responsibility of the manufacturers to ensure compliance with the general safety requirements (art. 3) of the Framework Regulation No. 1935/2004 (European Council 2004) and to asses any potential health risk in the final material arising from their use or presence. One of the major drawbacks that arises here, is that most often, the identity, presence or origin of many of these compounds is not even known (Bradley & Coulier 2007) (**Chapter 4**). However, several studies regarding the toxicity of packaging materials have shown that it is often not possible to explain the toxicity of materials only based on the toxicity of the identified substances, which emphasises the importance of the identification of NIAS (Nerin et al. 2013). The approach applied in this thesis has shown to deliver complementary results for the characterisation of NIAS from polymer baby bottles (and by extension polymer materials in general), and the various experiments described in **Chapters 4.1 and 4.2** emphasise the innovativeness and usefulness of this combination of analytical techniques for the elucidation of unknown migrants.

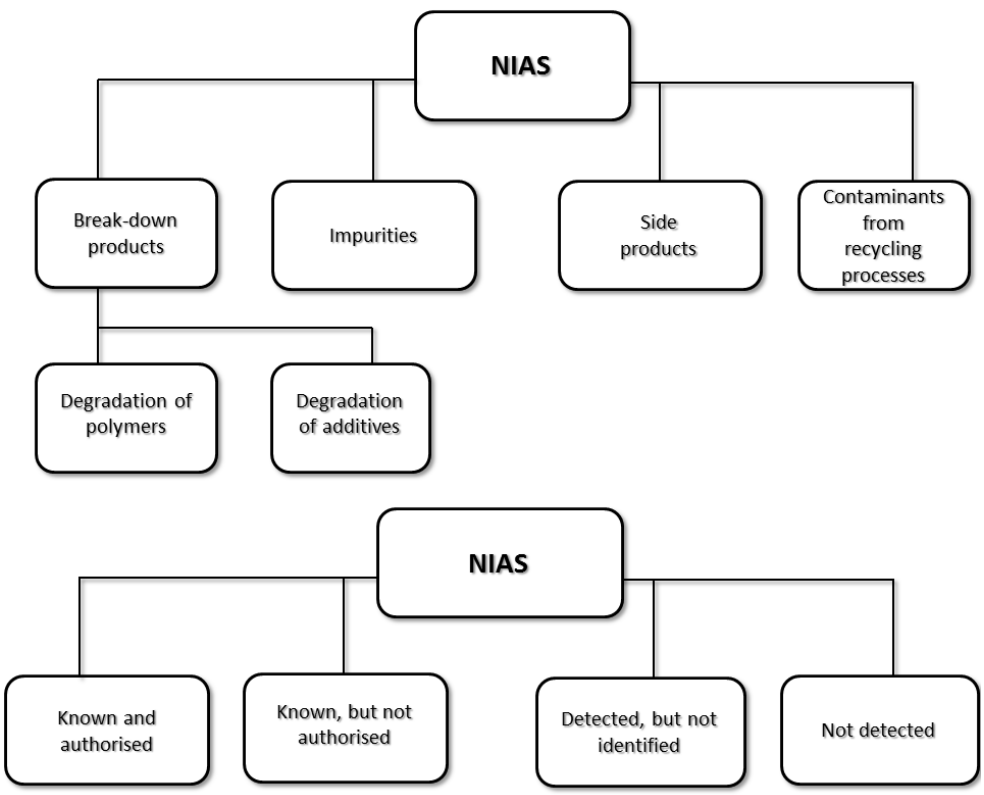


Figure 6.1: Schematic overview of NIAS (Geueke, 2013)

When considering the EU Regulation No. 10/2011 to assess the compliance for the prioritised compounds (IAS and NIAS) of the baby bottles tested following the prescribed EU repetitive use conditions, it was clearly seen in the results of **Chapter 5.1** that none of the compounds with a SML defined in the Regulation was detected at values exceeding the specified values (e.g., benzophenone SML= 600 µg kg^{-1}, found up to 97 µg kg^{-1}). Yet, on the other hand, several substances that were not present in the EU list were identified throughout this thesis, and, based on their toxicity studied by our project partners, some of them were prioritised and adequately quantified. However, often it was unclear if these compounds fell under the category of not-listed substances that are still authorised (such as e.g., PPA, etc.) or were (un)intentionally used without authorisation (e.g., NIAS for which risks should be consequently assessed). Since several compounds used in the production process of polymers can cover a wide range of possible applications, this question does not provide an easy answer.

The complexity of the issue concerning NIAS was illustrated with 2,4-di-*tert*-butylphenol. This compound was detected in PP bottles at levels clearly exceeding 10 µg kg^{-1} (up to 118 µg kg^{-1}). Therefore, awareness was raised since this substance was not present in the EU list. Its origin could be explained as a possible degradation product (NIAS) of the authorised compound *tris*(2,4-di-*tert*-butylphenyl)phosphite, commercially known as the antioxidant Irgafos 168. The detection of this compound was therefore theoretically authorised, provided that any possible health risk occurring due to its presence was assessed by the manufacturer. This remains a doubtful interpretation that is open for discussion, since it is not always possible for the (especially small-scale) plastic manufacturers to assign the nature and toxicity of possible NIASs formed (or other non-authorised substances) in order to conduct a proper risk assessment. Moreover, with this approach, still no proof was provided that 2,4-di-*tert*-butylphenol was not directly added to the polymer as an additive. In this case, more information should be asked to the manufacturer about its origin.

The same issues were faced for other non-EU regulated substances. Other antioxidants degradation products, such as 3,4-dimethylbenzaldehyde (from Millad 3988, up to 59 µg kg^{-1}) were frequently encountered. Dicyclopentyl(dimethoxy)silane was seen in one particular PP bottle up to 117 µg kg^{-1}, though its presence could be justified by its application as a Ziegler-Natta catalyst. The detection of methyl oleate (34 µg kg^{-1} in one PP bottle) was explained by its use as a lubricant in the polymer production process. The origin of

4-methylbenzaldehyde remained rather unclear and considering that it was detected up to 34 µg kg^{-1} could make it relevant for further investigation as well. 2-butoxyethyl acetate, most probably originating from its use as solvent/production aid, showed concentrations above 946 µg kg^{-1} in one bottle. Regarding the possible ambiguous interpretation of the Regulation, more transparency and clarity on this matter is urgently needed here.

Table 6.1 summarises the most important data that were obtained for the different polymer types that were tested.

Table 6.1: Summary of the properties of the investigated baby bottles

Polymer type	Estimated market share (%)	N° compounds identified	Most frequently detected migrants	Included in quantitative method	Concentration range (µg kg^{-1}) (EU repetitive use 3rd step)
PP	62	± 40	2,4-Di-*tert*-butylphenol	Yes	12-118
			3,4-Dimethylbenzaldehyde	Yes	6-59
			4-Propylbenzaldehyde	Yes	3-20
			Dicyclopentyl(dimethoxy) silane	Yes	1-117
			Other antioxidant degradation products (e.g. methyl-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl) propionate)	No (concentration estimated)	± 5-15
			Fatty acids	No (concentration estimated)	±5-100
PES	13	6	Acetophenone	Yes	3
PA	9	8	Azacyclotridecan-2-one	Yes	924-1091
			Fatty acids	No (concentration estimated)	±100-200
Tritan™	7	19	TXIB	Yes	8
			Dicyclopentyl(dimethoxy) silane	Yes	10
			2,4-Di-*tert*-butylphenol	Yes	8
			4-Propylbenzaldehyde	Yes	27
Silicone	5	25	TXIB	Yes	348
			Di(iso)butyl phthalate	Yes	11-15
			3,4-Dimethylbenzaldehyde	Yes	15
			Siloxanes	No	5-15

It was clearly shown that PP, Tritan™ and silicone exhibited the widest variety of possible migrants, whereas for PA, the variety was rather low and for PES, almost no migration was detected. For PA, the two bottles analysed showed that, next to the intense migration of fatty acids, the migrants consisted mainly of the PA monomer azacyclotridecan-2-one, which was consequently included in the quantitative method. The decreasing concentrations of this compound in the conducted experiments showed that its presence could be explained due to its high residual content in the polymer matrix after an incomplete polymerisation reaction, Although a SML of 5000 µg kg^{-1} is specified for azacyclotridecan-2-one, the findings of these high concentrations of migrating monomer indicate that the PA polymer may be not very suitable as a FCM, or that at least the polymerisation reaction should be optimised for its use as a FCM.

For PP, the most sold material in Belgium (62%; 17 bottles tested), NIAS such as antioxidant degradation products formed the major part of the migrants, and most of these compounds were accurately quantified in **Chapter 5.1**. This demonstrated that not the polymer was the problem, but substances such as additives, catalysts etc. that were added by the producers were the major issue considering migration from PP FCMs. Therefore, the safety of PP as a FCM seems to be highly determined by its specific production process, fact confirmed since also significant differences in the migration of certain compounds were observed among manufacturers. For Tritan™, although only bottle was tested, similar observations as for PP were made.

For silicone however, next to the expected presence of siloxane oligomers, several other compounds (±25) such as plasticisers were seen, some of them at relatively high concentrations (e.g. TXIB). Yet, according to the silicone industry, these plasticisers are not intentionally added and should therefore not be detected. Yet, no plausible explanation for their presence could be given. Nevertheless, silicone baby bottles exhibited a relatively low market share (5%; 1 bottle tested), this issue will be further discussed later in the future perspectives.

Although the European Commission commented the existence of NIAS in both the EC No. 1935/2004 and EU No. 10/2011 Regulation, until now no guidance is determined on how NIAS should be analysed and assessed, nor on which approach a manufacturer could follow to demonstrate the safety and consequent compliance of its final articles with the EU Regulations, nor on how inspectorates should control the final validity of articles. Considering these ambiguities, more

focus is needed also on the finished materials and articles, and not only on the starting substances. To this end, EFSA has published in January 2016 a scientific opinion concerning this matter (EFSA - European Food Safety Authority 2016). The document entitled “Recent developments in the risk assessment of chemicals in food and their potential impact on the safety assessment of substances used in food contact materials” discussed the newest opinions related to the safety assessment of chemicals in food. This opinion provides the European Commission a new scientific basis for the implementation of possible risk assessments for plastic FCMs, such as the baby bottles investigated here. In accordance with the results of this thesis and what was previously stated in this discussion, the EFSA opinion suggests an update of the EU guidelines in such a manner that they account for a more comprehensive approach that not only evaluates the safety of the starting substances, but of all migrating substances, whether they are intentionally present or not, since the NIAS often constitute the main part of the migrants.

To this end, in first instance extensive databases of possible migrating compounds should be made available at EU community level to facilitate the identification of possible migrants. Recently, the Scientific Institute of Public Health of Belgium started such an initiative: https://fcm.wiv-isp.be. As shown in this thesis, the assessment of the identity of migrants (IAS and NIAS) often still results in an initial time-consuming step and a first serious bottleneck in the safety evaluation process of polymer FCMs. However, this could be partially encompassed by the general availability of such databases. Moreover, a clearer transfer of information during the production chain of a polymer FCM by means of e.g. obligatory listing of all ingredients would largely facilitate the identification process. Secondly, more toxicity data on possible migrants is still needed. At the moment, most often laborious testing protocols are still required to properly determine the toxicity of migrants, as it was faced by the partner universities in the ALTPOLYCARB project. Also here, the availability of more extensive toxicity databases specifically for both IAS and NIAS could significantly reduce the time needed for the safety evaluation of polymer FCMs. Consequently, this commands a change of the present system of listing substances to provide transparency on what has been already evaluated. Although it is acknowledged that ensuring the safety of all possible NIAS from a polymer FCM can be a difficult and daunting task, a material will not be legally suitable for food contact if its safety cannot be demonstrated.

Although the estimation of the concentrations (**Chapter 4.1**) was initially done only by a semi-quantitative screening purpose, it is important to notice that for some compounds, such as 2-butoxyethyl acetate (initially estimated around 300 µg kg^{-1}) or azacyclotridecan-2-one (estimated around 250 µg kg^{-1}), a serious underestimation of the actually present concentrations was observed when these compounds were accurately quantified with an optimised target method (**Chapter 5.1**). Instead, 2-butoxyethyl acetate was quantified at a level of 946 µg kg^{-1} and azacyclotridecan-2-one even up to 1091 µg kg^{-1}, indicating that both the assumption of a broadly equal response factor in the detector and presuming rather similar recoveries could provoke an important under- (or over)estimation of the migrating concentrations.

However, it is important to emphasise that rather low concentrations were measured for most of the polymer types under the tested real-life conditions (**Chapter 5.2**). Microwave heating of the bottles showed an increase in the concentrations of the monitored compounds. This was in accordance with the phenomenon that was previously described by Alin et al. (Alin & Hakkarainen 2011) for migrating compounds from PP. Yet, the increase in the concentrations of migrating compounds was moderate and after few cycles most of the compounds were detected in negligible concentrations or were below LOQ. The same pattern was observed for the dishwasher treatment, yet here, up to date no other studies have addressed this issue other than those focusing on the release of BPA after dishwasher treatment (Maragou et al. 2008). Anyhow, also here the detected concentrations remained low. Furthermore, it was remarkable that for the sterilisation applied during the screening approach (filling 10' with boiling water at 100 °C - **Chapter 4.1**) afterwards in the first migration step a wide variety of components was detected, also at significant concentrations, whereas after both sterilisation techniques applied during the real-life use experiments (**Chapter 5.2**), the concentrations of the target compounds were generally low. Most probably, both steam sterilisation and cooking sterilisation already give rise to a partial removal of the possible migrants as indicated by the data obtained **Chapter 5.2**. Yet, since migration experiments after sterilisation were performed during 30 min at 40 °C, it was logical that less migration will occur. Compared to the screening approach, where more favourable conditions for migration were applied (2 h at 70 °C), this was a logical consequence.

Although we concluded that the EU repetitive experiment seems to overestimate the migration originating from real-life use conditions, it is crucial to note here

that all duration tests were performed individually and then compared to the EU repetitive use experiment. This is an important footnote to be made, since McCombie et al. recently demonstrated that underestimation of the actual migration due to simulation may still be an issue (McCombie et al. 2015). Moreover, although defined as real-life use conditions, these tests were still carried out with simulant, and no data for the migration under these conditions with real foodstuff, such as milk, are yet available. Therefore, future research should focus also hereon in order to assess even more accurately the possible risks of using such baby bottles. Also the use of 3% acetic acid, a simulant for orange juice, would be interesting to be tested as well.

A major outcome of these real-life experiments was the recommendation to perform some cycles (5-10) of steam sterilisation. We proved in this thesis that performing sterilisation before the first use of baby bottles significantly diminishes the amount of contaminants migrating from plastic FCMs for infants.

Chapter 5.3 compared the initial fingerprints of the real-life experiments with profiles of samples that underwent a number of cycles. This showed that no degradation of the polymers occurred under these treatments. Only the cooking sterilisation demonstrated to increase the release of one particular compound (BHT) from PP bottles, confirming its ability to enhance the release of some compounds present in the polymers, as was seen before in **Chapter 5.2**.

Although migration from FCMs, and particularly from the alternatives to PC plastic baby bottles is a rather “new” and hot research field (also due to the increased media attention focusing on this matter), this thesis has shown that such research can provide interesting and useful information regarding the identity and amounts of migrants to which infants are exposed nowadays. Moreover, this work has the potential that it can be directly linked in a future risk assessment study to the toxicological data that were obtained in parallel with this work. In this way, this research can be applied to provide answers to questions that cannot be solved with the existing data regarding the safety and possible health concerns that might occur due to the use of the alternative materials to PC baby bottles.

6.2 Future perspectives

To assess the possible migration risks related to NIAS, several approaches have been proposed so far. The classical way, as applied in the project of which this PhD forms part, is based on a screening of the migration solutions and consequent identification of the migrating compounds by means of (a combination of) analytical techniques. Then, toxicological testing of the identified compounds can be performed. However, the variety of toxicological tests to be applied on each individual compound is very costly and time-consuming. Yet, the main drawback of this approach is, as faced in this study as well (and many examples in literature (Nerin et al. 2013)), that not all compounds (and especially NIAS) can be easily identified, and consequently toxicological testing cannot be performed for these compounds. Here, the question still arises how risk assessment of these particular compounds will be done. Nevertheless, this approach exhibits clear advantages concerning sensitivity and specificity .

A second approach consists of performing bioassays on the whole migrate. This is a much quicker and cheaper way of testing than performing bioassays for all the individual compounds, however no information is obtained here on the toxicity of specific compounds and relevant toxicological endpoints may not even be covered. Moreover, often an incompatibility of the bioassay cell lines with the simulant solution can be encountered, such as faced in the work that was done in parallel with this PhD thesis. Finally, bioassays are not easily performed in a routine laboratory environment (Geueke 2013).

The third approach, which has recently gained increasing attention, is the application of the TTC concept to NIAS (Koster et al. 2011; Koster et al. 2014). The TTC approach is a screening and prioritisation tool for the safety assessment of chemicals when hazard data are incomplete and human exposure can be estimated. Here, a generic human exposure threshold value for unidentified substances that allows the determination of safe levels of exposure was established, and supposedly below this threshold (90 µg kg^{-1}, corresponding to the threshold for Cramer class III substances) a very low probability of risk to human health exists. When the identity of the NIAS is known, this threshold can be adapted accordingly to the available toxicity data. In a first step, a screening by means of different analytical techniques is done and a semi-quantitative estimation is made to determine if some particular NIAS exceed the 90 µg kg^{-1} threshold. Next, the presence of certain groups of substances of relatively high

toxicity (e.g. aflatoxin-like substances) that have to be excluded from the TTC approach is investigated by target analytical techniques and information on the starting materials. Following this initial exclusion step, substances exhibiting a (possible) genotoxic potency, identified by means of bioassays or analytical techniques, are assessed using a threshold of 0.15 µg/person/day. Finally, migrating substances that were semi-quantitatively estimated above the 90 µg kg^{-1} threshold have to be identified and submitted to a substance-specific risk assessment, including a search for toxicological information (Koster et al. 2014; International Life Sciences Institute Europe 2015).

Some future developments could be made that would fundamentally support this TTC approach. An analytical methodology that detects genotoxicity structural alerts (e.g. aromatic amines functional group) at low concentrations would be of considerable added value in order to decide if an unidentified peak is a possible genotoxic compound. Many assays will give an indication of the genotoxic potential of substances, however most of these were developed to test pure known substances at high concentrations (Koster et al. 2011). Furthermore, the TTC concept does not cover the cumulative toxicity effects of mixtures. This could be overcome by the application of sufficiently reliable and suitable bioassays, yet these are currently lacking. Finally, the TTC does not take into account possible endocrine disrupting effects of the migrating substances. Since compounds such as BPA are banned on the basis of possible estrogenic activity, this is an important footnote to make. Again bioassays, as performed by the partner universities, combined with existing lists of EDCs can offer additional valuable information here.

Concerning the growing complexity of polymer FCMs, NIAS and their consequent detection, identification and risk assessment will remain a hot topic in the near future. However, each of the three proposed approaches to assess the risks related to the migration of NIAS still contain some gaps that inhibit a 100% safety assurance as summarised in Table 6.2. Although EU authorities have recognised the importance of risk assessment for NIAS, at the moment, guidance at this level is lacking. Therefore, appropriate guidelines should be developed by law-enforcement authorities to warrant the safety of these FCMs including their NIAS.

Firstly, an official threshold value for not-listed substances is clearly urged. To this end, the generally assumed (but not officially accepted) 10 µg kg^{-1} threshold, originating from the “non-detection limit” for not-listed substances used behind a

functional barrier should be evaluated. A firm value, if necessary adapted according to e.g. the nature of specific compounds, should be established to bring more clearance here. Therefore, more additional toxicity data, also considering criteria on the endocrine disrupting properties of compounds, are demanded from the scientific community for many already "known" NIAS and IAS.

Table 6.2: (Dis)advantages of the different risk assessment approaches for NIAS

Risk Assessment Approach	Advantages	Disadvantages
Individual identification and toxicological characterisation	Good sensitivity and specificity	No easy identification of all migrants possible Individual toxicological evaluation is complex and expensive Often no risk assessment possible for all compounds
Bioassays of the whole migrate	Quick and cost efficient Cumulative effect of mixes is covered	No information about specific substances Possible incompatibility of cell lines with simulant Possibly hazardous substances present at low concentrations might not be detected
TTC-concept	Good pragmatic approach when little data available Relatively rapid risk assessment method Provides risk assessment possibility when peak number is so high that not all peaks can be completely evaluated with available resources	No cumulative effect of mixtures is covered Endocrine disrupting effects are not taken into account Compounds might not be assigned to the proper risk class or not detected at all Genotoxicity tests for trace level substances in complex matrices are lacking Limited to unexpected substances that have been detected before already

Secondly, clear guidelines should be defined for producers on how to perform a proper risk assessment. Now, producers can chose between the more practical approaches where they try to identify all migrants and evaluate their individual toxicity by bioassays or perform bioassays on the entire migrate, whereas the TTC concept is a more theoretical approach that is mostly valuable when little data are available. Although EFSA stated in its opinion on TCC (EFSA Scientific Committee

(SC) 2012) that this concept is a useful screening tool, it is certainly not an optimal approach yet. Therefore, a harmonised approach must be defined by EU law-enforcement bodies, preferably into the direction of a combined method which uses the TTC concept completed with more detailed identification (such as those obtained in Chapter 4 and 5) and toxicity data that should be made readily available as stated before. Future developments in analytical chemistry and genotoxicity assays should also reduce the present limitations of the TTC concept. Moreover, realistic exposure models are wanted as well (WHO & EFSA 2016).

Finally, based on the two previous perspectives, also guidelines for inspectorates should be described how to control the compliance of polymer FCMs regarding their safety and NIAS related risks. Checklists and decision trees to be followed, such as the one developed by Mertens et al. (Mertens et al. 2016) for genotoxicity, could be a useful and hands-on stepwise approach to be used by these control agencies when assessing the (non-)compliance of a material.

Although analytical techniques are constantly evolving nowadays, certain compounds might still remain undetected or unidentifiable. The application of new, cutting-edge techniques could offer a possible solving for this. The use of equipment that is capable of accurate mass measurements is almost mandatory for the adequate elucidation of unknown migrants. Although QTOF-MS is the most widespread platform used for this elucidation due to its relative lower cost, significantly higher resolution and subsequent elucidation power can be obtained by means of Orbitrap-MS. Moreover, Orbitrap-MS interfaced to GC has been made available only very recently, implementing a significant advance in the analysis of unknown volatile compounds (Thermo Scientific 2015).

Another technique that has gained renewed attention for elucidation purposes is that of ion mobility spectrometry (IMS), and several commercial brands have marketed new equipment focusing on this feature. By separating unknown compounds not only chromatographically and by their *m/z* values, but also according to their drift time and corresponding collisional cross section (CCS) values, IMS adds an additional dimension and subsequent powerful increase in confidence level to the identification process. CCS provides chromatographic retention time independent data points, enabling confident identification of analytes and reducing the risk of false positives/negatives. Even during initial sample analysis, without any retention time knowledge, theoretical exact mass

and CCS library values can be used to identify unknowns (Waters Corporation 2015).

Another issue which must be looked at in the future is that of silicone as a FCM. Since the results of this work indicated a large variety of migrants occurring from this material, silicones form one of the non EU-regulated groups of FCMs that is particularly considered of special concern. A resolution of the Council of Europe (CoE) outlines some specific requirements and an inventory list of substances that can be used for the manufacture of silicone products, though this latter document is only intended to provide guidance and is not legally binding unless the CoE countries transpose them partially or totally into national law (Council of Europe 2004). Some countries (Germany, France (Matériaux au contact des denrées alimentaires, Produits de nettoyage de ces matériaux: Arrêté du 25 novembre 1992; Franck et al. 2004)) have regulated silicone FCMs under the scope of national legislation, yet for Belgium, no particular regulation on silicones is existing at the moment.

However, silicone products have found a wide area of applications as FCMs in the last decade (Helling et al. 2009). In households, they are frequently used as baking moulds, spoons, coasters, spatulas, dough scrapers, brushes, containers, ice cube trays, stoppers for bottles, and many others. Furthermore, silicone rubbers are next to natural rubber the material of choice to produce baby soothers, feeding teats, and nipple shields for breast-feeding (Lund & Petersen 2002). Yet, there are only few publications documenting the migration behaviour of silicone polymers in contact with foodstuffs or food simulants, although notifications to the EU Rapid Alert System for Food and Feed (RASFF) mentioned high levels of overall migration (OM), volatile organic compounds (VOCs) and colour migration (Castro et al. 2012).

Previous analyses by GC and LC coupled to mass spectrometry and analysis with nuclear magnetic resonance (NMR) (Meuwly et al. 2005; Meuwly et al. 2007; Helling et al. 2009; Helling et al. 2010; Helling et al. 2012; Zhang et al. 2012) have shown that a wide variety of substances can possibly migrate from silicone-based FCMs into the food. Some repeated-use articles, especially those used at elevated temperatures (e.g. baking moulds), showed that the migration of VOCs and linear and cyclic siloxane oligomers could exceed the limits specified by the CoE, especially during the first cycles of use, and that a high fat content of the food

(e.g. cake) led to an increase of this migration (Meuwly et al. 2005; Meuwly et al. 2007; Castro et al. 2012).

Research has been mainly focused on the latter compounds, however, only little data exist about the(ir) migration into real food and realistic conditions of use. Yet, it was shown that for silicone FCMs, such as baking moulds, that the results of migration experiments in simulants could strongly differ from those conducted in real foodstuffs (Helling et al. 2010). Furthermore, only very limited data exist on the presence of NIAS other than siloxane oligomers that can migrate from silicones. Yet, a recent study on silicone baby bottles, which was also confirmed in this thesis, reported the migration of substances related to printing inks (e.g. benzophenone, diisopropyl naphthalene), but also of other EDCs, such as phthalate plasticisers (Simoneau, Van Den Eede, & Valzacchi, 2012).

Therefore, comprehensive and stepwise research is necessary regarding the elucidation of migrants from silicone FCMs, their pathways of exposure to different food matrices, the extent to which the general population is currently exposed to these migrants and the possible toxic properties (e.g. estrogenicity) of these migrants. Moreover, it is also needed that regulatory limits for migrating compounds from silicones are laid down to protect the vulnerable population of babies and young children who frequently use this material.

References

Alin J, Hakkarainen M. 2011. Microwave heating causes rapid degradation of antioxidants in polypropylene packaging, leading to greatly increased specific migration to food simulants as shown by ESI-MS and GC-MS. J Agric Food Chem. 59:5418–5427.

Bradley E, Coulier L. 2007. Report FD 07/01: An investigation into the reaction and breakdown products from starting substances used to produce food contact plastics [Internet]. [cited 2016 Feb 12]:629. Available from: http://www.foodpackagingforum.org/wp-content/uploads/2014/06/Bradley-and-Coulier-2007.pdf

Castro A, Bettencourt A, Pereira J, Selbourne M, Poças M. 2012. Overall migration from silicone kitchen utensils. Effect of time , temperature , contacting surface area and repeated use. 5th Int Symp food Packag.

Council of Europe. 2004. Resolution ResAP(2004)5 on silicones used for food contact applications. Off J Eur Union [Internet]. Available from: https://wcd.coe.int/ViewDoc.jsp?id=797547&Site=CM

EFSA - European Food Safety Authority. 2016. Scientific Opinion on Recent developments in the risk assessment of chemicals in food and their potential impact on the safety assessment of substances used in food contact materials. EFSA J. 14:1–28.

EFSA Scientific Committee (SC). 2012. Scientific Opinion on Exploring options for providing advice about possible human health risks based on the concept of Threshold of Toxicological Concern (TTC). EFSA J. 10:1–103.

European Council. 2004. Regulation (EC) No 1935/2004 of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC. Eur Off J.:1–20.

European Union. 2011. Commission Regulation (EU) No 10/2011 of 14 Januari 2011 on plastic materials and articles intended to come into contact with food. Off J Eur Union. L12:1–89.

Franck R, Wieczorek H, (Eds). 2004.): Kunststoffe im Lebensmittelverkehr, Empfehlungen des Bundesinstitutes für Risikobewertung, XV. Silicone, 53. Lieferung, Carl Heymanns Verlag. Germany: BfR.

Geueke B. 2013. Dossier – Non-intentionally added substances (NIAS) [Internet]. [cited 2016 Jan 15]. Available from: http://www.foodpackagingforum.org/wp-content/uploads/2015/11/FPF_Dossier03_NIAS.pdf

Helling R, Kutschbach K, Simat TJ. 2010. Migration behaviour of silicone moulds in contact with different foodstuff. Food Addit Contam Part A. 27:396–405.

Helling R, Mieth A, Altmann S, Simat TJ. 2009. Determination of the overall migration from silicone baking moulds into simulants and food using 1H-NMR techniques. Food Addit Contam Part A. 26:395–407.

Helling R, Seifried P, Fritzsche D, Simat TJ. 2012. Characterisation and migration properties of silicone materials during typical long-term commercial and household use applications: a combined case study. Food Addit Contam Part A. 29:1489–1500.

International Life Sciences Institute Europe. 2015. Guidance on best practices on the risk assessment of non intentionally added substances (NIAS) in food contact materials and articles [Internet]. [cited 2016 Apr 14]:1–19. Available from: http://www.ilsi.org/Europe/Pages/ViewItemDetails.aspx?WebId=84D7FA4A-0FD5-40CD-A49A-2DA6FCDFD654&ListId=0348EB34-DF85-49DD-9ADE-77ED136643F1&ItemID=434

Koster S, Boobis AR, Cubberley R, Hollnagel HM, Richling E, Wildemann T, Wurtzen G, Galli CL. 2011. Application of the TTC concept to unknown substances found in analysis of foods. Food Chem Toxicol. 49:1643–1660.

Koster S, Rennen M, Leeman W, Houben G, Muilwijk B, van Acker F, Krul L. 2014. A novel safety assessment strategy for non-intentionally added substances (NIAS) in carton food contact materials. Food Addit Contam Part A. 31:422–43.

Lund KH, Petersen JH. 2002. Safety of food contact silicone rubber: Liberation of volatile compounds from soothers and teats. Eur Food Res Technol. 214:429–434.

Maragou NC, Makri A, Lampi EN, Thomaidis NS, Koupparis MA. 2008. Migration of bisphenol A from polycarbonate baby bottles under real use conditions. Food Addit Contam Part A. 25:373–383.

Matériaux au contact des denrées alimentaires, Produits de nettoyage de ces matériaux: Arrêté du 25 novembre 1992. Les éditions des Journaux Off français. brochure N.

McCombie G, Harling A, Biedermann M, Biedermann-Brem S, Eicher A, Suter G, Morandini M, Pechstein S, Schmaschke G, Lauber U, Grob K. 2015. Survey of plasticizers migrating from the gaskets of lids into oily food in glass jars: The second European enforcement campaign shows poor compliance work. Food Control. 50:65–71.

Mertens B, Simon C, Van Bossuyt M, Onghena M, Vandermarken T, Van Langenhove K, Demaegdt H, Van Hoeck E, Van Loco J, Vandermeiren K, et al. 2016. Investigation of the genotoxicity of substances migrating from polycarbonate replacement baby bottles to identify chemicals of high concern. Food Chem Toxicol. 89:126–137.

Meuwly R, Brunner K, Fragnière C, Sager F, Dudler V. 2005. Heat stability and migration from silicone baking moulds. Mitteilungen Aus Leb Und Hyg. 96:281–297.

Meuwly R, Sager F, Brunner K, Dudler V. 2007. Migration of Siloxane Oligomers in Foodstuffs from Silicone Baking Moulds. Dtsch Leb Rundschau. 103:561–568.

Nerin C, Alfaro P, Aznar M, Domeño C. 2013. The challenge of identifying non-intentionally added substances from food packaging materials: a review. Anal

Chim Acta. 775:14–24.
Onghena M, van Hoeck E, Vervliet P, Scippo ML, Simon C, van Loco J, Covaci A. 2014. Development and application of a non-targeted extraction method for the analysis of migrating compounds from plastic baby bottles by GC-MS. Food Addit Contam Part A. 31:2090–102.
Simoneau C, Van Den Eede L, Valzacchi S. 2012. Identification and quantification of the migration of chemicals from plastic baby bottles used as substitutes for polycarbonate. Food Addit Contam Part A. 29:469–480.
Simoneau C, Valzacchi S, Morkunas V, Van Den Eede L. 2011. Comparison of migration from polyethersulphone and polycarbonate baby bottles. Food Addit Contam Part A. 28:1763–1768.
Thermo Scientific. 2015. Q Exactive GC – A New Chapter in GC-MS [Internet]. [cited 2016 Feb 12]. Available from: http://www.thermoscientific.com/content/dam/tfs/ATG/CMD/cmd-documents/sci-res/pres/ms/events/acs2015/PP-GC-MS-Q-Exactive-New-Chapter-ACS2015-EN.pdf
Waters Corporation. 2015. Vion IMS QToF [Internet]. [cited 2016 Jan 15]:food. Available from: http://www.waters.com/waters/en_CZ/Vion-Ion-Mobility-Quadrupole-Time-of-Flight-Mass-Spectrometry/nav.htm?cid=134845751&locale=en_CZ
WHO, EFSA. 2016. Review of the Threshold of Toxicological Concern (TTC) approach and development of new TTC decision tree [Internet]. [cited 2016 Mar 17]. Available from: http://www.efsa.europa.eu/sites/default/files/corporate_publications/files/1006e.pdf
Zhang K, Wong JW, Begley TH, Hayward DG, Limm W. 2012. Determination of siloxanes in silicone products and potential migration to milk, formula and liquid simulants. Food Addit Contam Part A. 29:1311–1321.

Summary

Summary

Bisphenol-A (BPA) has been used for many years as a monomer for polycarbonate (PC) polymers (water and infant feeding bottles) and epoxy resins (canned food packaging) from which it can be released into the food, the major exposure source of BPA to humans. Since BPA has endocrine disrupting properties, its use was prohibited for the production of polymers for food contact materials for children younger than 3 years old (European Commission, regulation No. 10/2011). Furthermore, in a recent opinion, the Superior Health Council of Belgium expressed its concern regarding the possible risks associated with the used alternatives to PC (No. 8697, 11.03.2010). Consequently, alternatives to PC food contact materials (FCMs) for infants, such as polypropylene (PP), polyethersulphone (PES), polyamide (PA), Tritan™ or silicone baby bottles, have appeared on the market.

Migration of BPA from PC has already been extensively studied. Unfortunately, the nature and amounts of substances migrating from the polymeric alternatives other than PC is much less known. The principal aim of this PhD was the identification and quantification of the major and most toxic compounds migrating from baby bottles, in the frame of a Belgian governmental project (ALTPOLYCARB) involving several Belgian universities.

The first experimental chapter of this thesis (**Chapter 3**) describes the possible alternatives to PC FCMs for children under 3 y old on the Belgian market. These articles were documented by an initial market survey in baby-shops, supermarkets and pharmacies. 24 baby bottle types from different manufacturers were encountered here. The polymers used in the manufacture were, in order of importance, PP, PES, PA, Tritan™ and silicone. Some PC baby bottles have also still been encountered. Baby cups, teats, dinnerware and other infant FCMs were studied as well. However, for the latter items, a major percentage of the polymers used for their production could generally not be identified. Given the lack of information for the other FCMs categories than baby bottles, and considering the major importance of the latter for infant feeding, the migration tests were started on a selection of representative baby bottles.

For migration testing, the use of simulants is prescribed in the legislation to mimic the testing of real foods. Specifically, a mixture of water-EtOH (50:50, v/v) is recommended as a simulant for milk. After sterilisation of the bottle during ten minutes with boiling water, three migrations were performed during 2h at 70 °C.

Firstly, a liquid-liquid extraction (LLE) of the simulant with a mixture of common organic compounds (**Chapter 4.1**) was optimised. To develop a robust and general method, a mixture of 17 chemicals (identified in the literature as possible migrants from FCMs) covering a wide variety in polarity and chemical functionality was chosen to evaluate the

extraction efficiency of n-hexane, iso-octane, ethyl acetate (EtOAc)-n-hexane (1:1 and 1:3), MTBE and dichloromethane (DCM)-n-hexane (1:1 and 1:3). The extracts resulting from the LLE step were analysed on GC-(EI)MS by monitoring specific ions for each analyte and for the internal standard. EtOAc-n-hexane (1:1) and DCM-n-hexane (1:1) were the most efficient extraction solvents. Consequently, there was opted for the non-chlorinated solvents and EtOAc-n-hexane (1:1) was selected for the application to real samples.

We have assessed the possible release of unknown chemicals from PP, PES, PA, Tritan™ and silicone baby bottles. The migration solutions from the baby bottles were extracted and analysed on GC-(EI)MS performing an untargeted database search using Wiley® and NIST® libraries. Although the concentrations observed were rather low, various compounds, such as alkanes, phthalates, amides, etc. were detected based on this library search. In **Chapter 4.2**, unidentified peaks were further investigated by advanced mass spectrometric techniques, such as GC-(EI)TOF-MS and GC-(APCI)QTOF-MS, to specifically elucidate the structure of these unknown compounds. The expected presence of the accurate mass molecular ion and/or protonated molecule in APCI together with the fragmentation pattern observed in both techniques were used for elucidation purposes. By developing an identification strategy based on the combination of these analytical techniques, compounds (e.g. dicyclopentyl-(dimethoxy)silane, Irganox 1010, etc.) that could not be identified before were elucidated here.

Additionally, the same extracts were analysed also by LC-QTOF-MS under MS^E mode. The full-spectrum accurate mass data of both (de)protonated molecule and fragment ions were acquired simultaneously. Data were automatically processed using a home-made database containing around 1200 chemicals present on the list provided by the EU Regulation No. 10/2011 and expected migrating compounds, such as anti-oxidants, plasticisers, etc. When a peak was detected, and its reference standard was not available in the lab, a tentative identification was performed using the accurate mass of the observed fragment ions. Several compounds which were previously not identified by GC-MS analysis were elucidated.

In parallel with the identification process, the genotoxicity and endocrine activity of the identified migrants were evaluated by the partner universities participating in the ALTPOLYCARB project using a battery of *in vitro* assays. **Chapter 5.1** describes the optimisation of a LLE method for a number of migrating compounds that were selected based on the outcomes of this toxicity screening/scoring and the migrating abundances observed in **Chapter 4.1**. Monitoring and quantification of these compounds was done using GC- and LC-QqQ-MS methods, for which several validation parameters were determined (sensitivity, selectivity, linearity, accuracy, precision, recoveries and matrix

effects). Analysis of the 3rd migration step of the standard migration conditions (3 migrations, 2 h at 70°C)
applied on the baby bottles (which has to comply with the EU legislative migration limits) showed that for some baby bottles, several not authorised compounds exceeded the generally adapted "no-detection limit" of 10 µg kg^{-1}. Substances, such as 2,4-di-*tert*-butylphenol (up to 118 µg kg^{-1}), 2-butoxyethyl acetate (up to 945 µg kg^{-1}) and 4-propylbenzaldehyde (up to 27 µg kg^{-1}) were detected in several bottles, as well as some phthalates. The silicone bottle even exhibited concentrations of 2,2,4-trimethyl-1,3-pentanediol diisobutyrate (TXIB) around 350 µg kg^{-1}. For all detected compounds authorised by the EU Regulation No. 10/2011 with a specific migration limit (SML), such as benzophenone (600 µg kg^{-1}, found up to 97 µg kg^{-1}), concentrations in the migration solutions were below the SMLs.

In **Chapter 5.2**, an evaluation of the effect of several "real-life use conditions" by means of duration tests such as microwave, sterilisation and dishwasher treatment on the profile of the different migrants was determined and compared with a reference treatment (30 min at 40°C) and the standard EU "repetitive use conditions". Analysis of the extracts from the microwave experiments showed a modest increase in the concentrations of the observed migrants (e.g. azacyclotridecan-2-one from the PA bottle: 124 µg kg^{-1} after the first microwave heating vs. 70 µg kg^{-1} in the first reference-experiment). Moreover, a prolonged release of the target compounds was also observed whereas these migrants disappeared significantly faster in the reference experiment. The dishwasher treatment resulted also in a slight increase of some of the target compounds, whilst others exhibited lower concentrations than the reference experiment. This was most probably due to the fact that they were already partially washed away during dishwashing.

Steam sterilisation showed a quick removal of the monitored compounds and the detected concentrations were lower (e.g. TXIB from silicone bottle 28 vs. 118 µg kg^{-1}) or similar (di(iso)butyl phthalate) to those seen after the reference treatment. The tendency observed for the steam sterilisation indicated a clear advantage of performing this treatment in order to eliminate residual chemicals that might still be present in the polymer. For the cooking sterilisation, generally the same observations as for the steam sterilisation were done, since also here a preliminary removal of the chemicals was seen. However, an increased release of some target compounds was seen for the silicone bottle, suggesting that this treatment was not suitable for this material.

For all duration tests, a downwards tendency of the measured concentrations was observed through the subsequent cycles. The target compounds observed after the different duration tests were in accordance with those seen before in the EU repetitive

use experiment; however here the observed concentrations were significantly higher for most compounds. Although the repetitive use experiment therefore seemed to overestimate the actual migration from baby bottles under real-life use conditions, it has to be mentioned that all migration tests were performed individually, and that the combination thereof might result in a higher release.

Chapter 5.3 presents the results of a fingerprinting study that was made on the initial samples and on those exposed to a high number of duration tests. To this end, not only the influence of real-life treatments on migration of the target compounds, but also on the rest of possible migrants (e.g. degradation products) was investigated. It was shown that under these real-life use treatments no degradation of the polymers took place, although the steam sterilisation resulted after 10 cycles in an increased release of an antioxidant (BHT) from the PP bottles compared to the first cycle.

In **Chapter 6**, a critical discussion was done on the outcomes of the presented research. Special focus was made on the detection of multiple non-intentionally added substances in the baby bottles. Moreover, suggestions were made on how the presence of these compounds could be safeguarded in future studies.

Samenvatting

Bisfenol-A (BPA) werd gedurende vele jaren gebruikt als monomeer voor de productie van polycarbonaat (PC) polymeren en epoxyharsen. Toepassingen hiervan zijn respectievelijk water- en babyflessen en conserven en verpakkingen waaruit het kan vrijkomen in het voedsel, de belangrijkste blootstellingsbron van BPA voor de mens. Aangezien BPA hormoon verstorende eigenschappen bezit, werd het gebruik ervan verboden bij de productie van polymeren gebruikt voor voedselcontactmaterialen (FCMs) voor kinderen jonger dan 3 jaar (Europese Commissie, verordening Nr. 10/2011). Bovendien heeft de Hoge Gezondheidsraad van België in een recent advies haar bezorgdheid geuit over de mogelijke risico's verbonden aan het gebruik van de alternatieven voor PC (Nr 8697, 11.03.2010). Als gevolg van dit verbod zijn alternatieven voor PC FCMs voor zuigelingen op de markt gekomen, zoals polypropyleen (PP), polyethersulfon (PES), polyamide (PA), Tritan™ of silicone babyflesjes.

Migratie van BPA uit PC werd reeds uitgebreid bestudeerd. Helaas zijn de aard en de hoeveelheid van de stoffen die migreren uit de PC alternatieven veel minder gekend. Het hoofddoel van dit doctoraat was de identificatie en kwantificatie van de belangrijkste componenten die migreren uit babyflessen. Dit onderzoek werd uitgevoerd in het kader van een Belgisch overheidsproject (ALTPOLYCARB) met verschillende Belgische universiteiten.

Het eerste experimentele hoofdstuk van dit proefschrift (**Hoofdstuk 3**) beschrijft de mogelijke alternatieven voor PC FCMs voor kinderen jonger dan 3 jaar oud, op de Belgische markt. Deze artikelen werden in kaart gebracht door een marktonderzoek in babywinkels, supermarkten en apotheken. 24 soorten zuigflessen van verschillende fabrikanten werden aangetroffen. De polymeren die gebruikt werden voor de fabricatie waren, gerangschikt volgens marktaandeel, PP, PES, PA, Tritan™ en siliconen. Ook werden nog altijd enkele PC babyflessen aangetroffen. Baby kopjes, fopspenen, eetgerei en andere FCMs voor zuigelingen werden ook bestudeerd. Een belangrijk percentage van de polymeren die gebruikt worden voor de productie van laatstgenoemde items kon doorgaans echter niet worden geïdentificeerd. Gezien het gebrek aan informatie voor de andere categorieën van FCMs dan babyflessen, en gezien het grote belang van deze laatste voor zuigelingenvoeding, werden de migratie proeven gestart met een selectie van representatieve babyflesjes.

Voor de migratie testen wordt het gebruik van simulanten voorgeschreven in de wetgeving om echt voedsel na te bootsen. Specifiek wordt een mengsel van water en EtOH (50:50, v/v) aanbevolen als simulant voor melk. Na sterilisatie van de fles gedurende tien minuten met kokend water werden drie migraties uitgevoerd gedurende 2 uur bij 70 °C. Allereerst werd een vloeistof-vloeistof extractie (LLE) met een mengsel

van organische solventen geoptimaliseerd om de extractie van de migranten uit het simulant te verbeteren (**Hoofdstuk 4.1**). Om een robuuste en algemene methode te ontwikkelen werd een mengsel van 17 chemicaliën (in de literatuur voorkomend als mogelijke migranten uit FCMs) met een brede variëteit in polariteit en chemische functionaliteit uitgekozen om de extractie-efficiëntie van de verschillende solventen te evalueren. De extracten van de LLE stap werden geanalyseerd via GC-(EI)MS door monitoring van de specifieke ionen voor elk analiet en de interne standaard. Ethyl acetaat (EtOAc)-n-hexaan (1:1) en Dichloormethaan (DCM)-n-hexaan (1:1) waren de meest efficiënte extractiesolventen. Bijgevolg werd geopteerd om te kiezen voor het niet gechloreerde solvent en EtOAc-n-hexaan (1:1) te selecteren voor de applicatie op de echte stalen.

De ontworpen methode werd toegepast om de mogelijke vrijstelling van ongekende chemicaliën uit PP, PES, PA, Tritan™ en siliconen babyflessen te bestuderen. Voor de identificatie van de migrerende componenten werden Wiley® en NIST® massaspectra bibliotheken gebruikt. Hoewel de waargenomen concentraties vrij laag waren werden verschillende verbindingen, zoals alkanen, ftalaten, amiden, enz. gedetecteerd op basis van deze bibliotheek zoekopdracht.

In **Hoofdstuk 4.2** werden niet geïdentificeerde pieken verder onderzocht met geavanceerde massaspectrometrische technieken, zoals GC-(EI)TOF-MS en GC-(APCI)QTOF-MS, om specifiek de structuur van deze onbekende verbindingen op te helderen. De verwachte aanwezigheid van het accurate massa moleculair ion en/of de geprotoneerde molecule in APCI werd samen met het fragmentatiepatroon waargenomen in beide technieken, gebruikt voor de structuuropheldering. Door het ontwikkelen van een identificatie strategie die berust op de combinatie van deze analytische technieken werden verbindingen (bijvoorbeeld dicyclopentyl-(dimethoxy)silaan, Irganox 1010, etc.) die voorheen niet konden worden geïdentificeerd hier opgehelderd.

Daarna werden dezelfde extracten geanalyseerd met LC-MS-QTOF onder MS^E modus. De full-spectrum accurate massa gegevens van zowel de ge(de)protoneerde molecule als fragment ionen werden hierbij gelijktijdig gedetecteerd. De gegevens werden automatisch verwerkt met behulp van een zelfgemaakte database met ongeveer 1200 chemische stoffen gebaseerd op de lijst gedefinieerd in de Europese Verordening Nr. 10/2011 en verwachte migrerende verbindingen, zoals antioxidanten, weekmakers, enz. Wanneer een piek werd gedetecteerd en de referentiestandaard niet beschikbaar was in het laboratorium werd een poging tot identificatie uitgevoerd met behulp van de accurate massa van de waargenomen fragment ionen. Verscheidene verbindingen die niet eerder werden geïdentificeerd door GC-MS analyse werden zo opgehelderd.

Parallel met het identificatieproces werden de genotoxiciteit en hormonale activiteit van de geïdentificeerde migranten geëvalueerd met behulp van een batterij *in vitro* testen. Dit werd uitgevoerd bij de partneruniversiteiten die deel uitmaakten van het ALTPOLYCARB project. **Hoofdstuk 5.1** beschrijft de optimalisatie van een LLE methode voor een aantal migrerende verbindingen die werden geselecteerd op basis van de resultaten van deze toxiciteit screening/scoring en de migrerende abundanties waargenomen in **Hoofdstuk 4.1**. Monitoring en kwantificering van deze verbindingen werd uitgevoerd met behulp van GC en LC-QqQ-MS methoden, waarbij verschillende validatie parameters werden bepaald (gevoeligheid, selectiviteit, lineariteit, nauwkeurigheid, precisie, recoveries en matrixeffecten). Analyse van de 3^{e} migratiestap onder de standaardmigratie condities (3 migraties, 2 uur bij 70 °C) toegepast op de zuigflessen (die moet voldoen aan de wettelijke EU migratielimieten) toonde aan dat uit sommige babyflessen verschillende niet toegelaten verbindingen migreerden en de algemeen aangenomen "no-detectiegrens" van 10 µg kg^{-1} overschreden. Stoffen, zoals 2,4-di-*tert*-butylfenol (tot 118 µg kg^{-1}), 2-butoxy-ethylacetaat (tot 945 µg kg^{-1}) en 4-propylbenzaldehyde (tot 27 µg kg^{-1}) werden gedetecteerd in een aantal flessen, evenals sommige ftalaten. De siliconen fles vertoonde zelfs concentraties van 2,2,4-trimethyl-1,3-pentaandioldiisobutyraat (TXIB) rond 350 µg kg^{-1}. Voor alle gedetecteerde verbindingen waarvoor de Europese Verordening Nr. 10/2011 een specifieke migratielimiet (SML) oplegt, zoals benzofenon (600 µg kg^{-1}, gedetecteerd tot 97 µg kg^{-1}), waren de concentraties in de migratie oplossingen onder de SMLs.

In **Hoofdstuk 5.2** werd het effect van verschillende "echte gebruiksomstandigheden" door middel van duurtesten op het profiel van de verschillende migranten geëvalueerd en vergeleken met een referentie behandeling (30 min bij 40 °C) en de standaard EU "repetitieve gebruiksomstandigheden". Analyse van de extracten uit de microgolfoven experimenten toonden een lichte toename van de concentratie van de waargenomen migranten (bv. azacyclotridecan-2-on uit de PA fles: 124 µg kg^{-1} na de eerste microgolfopwarming versus 70 µg kg^{-1} in het eerste referentie experiment). Bovendien werd ook een verlengde vrijgave van de gemonitorde verbindingen waargenomen terwijl deze migranten beduidend sneller verdwenen in het referentie experiment. De vaatwasser behandeling resulteerde ook in een lichte toename van een aantal van de doelcomponenten, terwijl andere migranten lagere concentraties dan het referentie experiment vertoonden. Dit was waarschijnlijk te wijten aan het feit dat deze componenten al gedeeltelijk verwijderd werden gedurende het was proces. Stoomsterilisatie toonde een snelle verwijdering van de gemonitorde verbindingen en de waargenomen concentraties waren lager (bijvoorbeeld TXIB uit de siliconen fles 28 vs. 118 µg kg^{-1}) of gelijkaardig (di(iso)butyl ftalaat) vergeleken met die na de

referentiebehandeling. De tendens waargenomen voor de stoomsterilisatie gaf een duidelijk voordeel aan van het uitvoeren van deze behandeling om residuele chemicaliën die nog in het polymeer zouden kunnen aanwezig zijn te elimineren. Voor de kooksterilisatie werden algemeen dezelfde waarnemingen als voor de stoomsterilisatie gedaan, aangezien ook hier een initiële verwijdering van de chemicaliën werd waargenomen. Een verhoogde afgifte van bepaalde doelverbindingen werd echter gezien voor de siliconen fles, wat suggereert dat deze behandeling niet geschikt is voor dit materiaal.

Voor alle duurtesten werd een neerwaartse tendens van de gemeten concentraties waargenomen doorheen de opeenvolgende cycli. De gemonitorde verbindingen waargenomen na de verschillende testen waren kwalitatief overeenkomstig met degene die eerder werden gedetecteerd in het EU repetitieve gebruik experiment; in deze studie waren de waargenomen concentraties echter significant hoger voor de meeste verbindingen. Hoewel het repetitieve gebruik experiment daarom de werkelijke migratie van babyflessen onder reële gebruiksomstandigheden leek te overschatten, moet rekening worden gehouden met het feit dat alle migratieproeven afzonderlijk werden uitgevoerd, en dat de combinatie van behandelingen zou kunnen leiden tot een hogere afgifte.

Hoofdstuk 5.3 stelt de resultaten voor van een “fingerprinting” studie die werd gemaakt op zowel de initiële stalen als de stalen die werden onderworpen aan duurtest cycli. Zodoende werd niet alleen de invloed van echte gebruiksomstandigheden op de gemonitorde componenten bepaald, maar tevens ook op de rest van de mogelijke migranten (bv. afbraakproducten). Er werd aangetoond dat de polymeren geen afbraak vertoonden onder deze echte gebruiksomstandigheden, maar de stoomsterilisatie zorgde na 10 cycli wel voor een toegenomen vrijgave van een antioxidant (BHT) uit de PP flesjes in vergelijking met de eerste cyclus.

In **Hoofdstuk 6** werden de resultaten van het gepresenteerde onderzoek kritisch besproken. Er werd met name speciaal gefocust op het feit dat verschillende “non-intentionally added substances” werden gedetecteerd in de babyflesjes. Daarnaast werden ook suggesties gemaakt naar hoe de aanwezigheid van deze componenten verder kan worden gecontroleerd in toekomstige studies .

Objetivos y Plan de Trabajo

Esta Tesis Doctoral forma parte de ALTPOLYCARB, Proyecto financiado por el Ministerio de Salud Pública de Bélgica, cuyo principal objetivo es evaluar los posibles riesgos derivados de la migración de productos químicos presentes en FCMs para niños menores de 3 años. Esta tesis pretende abordar este problema, respondiendo a las siguientes cuestiones:

1. ¿Qué alternativas al PC se están usando actualmente en FCMs en recipientes de plástico para alimentos de niños en Bélgica?
2. ¿Qué materiales se usan como FCMs o pueden llegar a estar en contacto con alimentos para niños menores de 3 años?
3. ¿Qué sustancias pueden migrar desde estos materiales (cuestiones 1 y 2) a los alimentos (o simulantes)?
4. ¿Bajo qué circunstancias y en qué cantidades migran estos compuestos desde dichos materiales?

En paralelo a esta Tesis, varios otros centros de investigación han colaborado en el proyecto para responder las preguntas relacionadas con la toxicidad y la actividad biologica de las substancias migrantes. Los centros participantes han sido: *University of Antwerp (UA), Scientific Institute of Public Health (WIV-ISP), University of Liège (ULg), Free University of Brussels (VUB) y Veterinary and Agrochemical Research Centre (CODA-CERVA),* cada uno de ellos especializado en diferentes ámbitos en el campo de FCMs. En paralelo a la identificación química, se evaluó la toxicidad de la disolución total de migrantes en términos de genotoxicidad, mutagenecidad y actividad disruptora endocrina. Debido a que la extrapolación de las disoluciones de migrantes a líneas celulares *in vitro* no es evidente por la incompatibilidad de éstas con el simulante seleccionado (H_2O-EtOH), se evaluó en una primera aproximación la toxicidad de los componentes puros. Este trabajo fue realizado por los otros centros colaboradores y no se discute en esta Tesis. Por otro lado, estos centros también evaluaron los riesgos de la exposición a las sustancias que migran de los materiales en contacto con alimentos para niños menores de 1 año y entre 1 y 3 años.

Para completar el primer objetivo de esta tesis, se ha realizado una detallada revisión bibliográfica sobre las alternativas al PC en biberones. Se realizó un estudio de campo para documentar la presencia de estos materiales alternativos en el mercado belga. Además, se investigaron materiales plásticos que están en contacto con alimentos para niños menores de 3 años con el fin de dar respuesta al objetivo 2. El **Capítulo 3** discute estos datos obtenidos en el estudio de mercado en colaboración con la ULg. Aunque se documentaron numerosos FCMs para niños menores de 3 años, el trabajo de la Tesis se centró en la identificación química y cuantificación de sustancias que pueden migrar en materiales para biberones alternativos al PC. La amplia variedad de productos

encontrados diferentes a los biberones hizo que no se plantease la realización de test de migración para todos estos materiales, por exceder los límites de la Tesis. Además, los biberones son, con gran diferencia, los FCMs más usados en bebés, por lo que resultan ser el principal producto objeto de estudio. Sin embargo, los resultados del estudio de mercado realizado sobre otros tipos de FCMs podrían ser la base de futuros proyectos de investigación.

Posteriormente, se seleccionaron los plásticos a evaluar en cuanto a la potencial migración de compuestos no deseados. Las condiciones de migración se establecieron sobre la base de las actuales regulaciones europeas (EU No. 10/2011) **(Capítulo 4)**.

Previamente a los trabajos realizados en esta Tesis, no existía experiencia en el *Toxicological Centre* en lo relativo a determinación de migrantes en plásticos usados para alimentos de niños. Por ello, fue necesario desarrollar y optimizar nuevas metodologías analíticas para abordar este tema. Esta tarea fue posible gracias a una intensa colaboración con el *Scientific Institute of Public Health*. El hecho de que los contaminantes estén presentes a muy bajos niveles de concentración (ng ml^{-1}), hace necesaria la aplicación de técnicas muy sensibles y selectivas. Para responder al objetivo 3, se aplicó una extracción genérica líquido-líquido (LLE), que permitió extraer un amplio espectro de compuestos químicos de la disolución de migrantes, con diferentes polaridades. Los extractos se analizaron en primer lugar por cromatografía de gases acoplada a espectrometría de masas (GC-MS) (**Capítulo 4.1**) y por cromatografía líquida acoplada a MS con cuadrupolo-tiempo de vuelo (LC-QTOF-MS). Se procedió a la identificación de los picos más abundantes, asumiendo que correspondían posiblemente a los compuestos que migran en mayor proporción de los materiales alternativos. Los picos que no pudieron identificarse por GC-MS o en el *screening* inicial por LC-QTOF MS, se investigaron con más detalle mediante GC acoplada a HRMS y un análisis más exhaustivo por LC-QTOF MS. Esta parte del trabajo se hizo en colaboración con la Universidad Jaume I de Castellón (España). Los resultados relativos a la identificación de los compuestos detectados en las muestras se presentan en el **Capítulo 4.2**.

Los compuestos detectados deben cumplir con la legislación vigente en cuanto a los límites de migración con el fin de garantizar el uso seguro y saludable de los biberones, para lo cual es necesario proceder a su cuantificación. Para llevar a cabo este trabajo, se procedió a una selección de compuestos a cuantificar, sobre la base de las experiencias previas y los resultados obtenidos en cuanto a abundancia del agente migrante y toxicidad estimada, todo ello con el fin de responder al objetivo 4. El procedimiento LLE previamente aplicado se optimizó y se evaluaron las características analíticas del método cuantitativo. La cuantificación de compuestos seleccionados a partir de los encontrados en biberones del mercado belga, se llevó a cabo mediante métodos validados basados en GC-MS/MS QqQ y LC-MS/MS QqQ. Los resultados obtenidos en el

estudio cuantitativo se muestran y discuten en el **Capítulo 5.1**. Además, aplicando estos métodos cuantitativos validados, se procedió a evaluar el efecto de las condiciones de uso práctico de los biberones, tales como calentamiento en microondas, uso de lavavajillas y esterilización, con el fin de poder estimar de forma más detallada el grado de exposición a estas sustancias por parte del consumidor. El **Capítulo 5.2** muestra los resultados obtenidos en esta parte del estudio. Finalmente, en el **Capítulo 5.3** se encuentran los cromatogramas obtenidos antes y después de cada tratamiento específico. Mediante aplicación de software especializado, se realizó una comparación de los datos, lo que permitió evaluar no sólo la presencia de compuestos seleccionados sino también la posible formación de productos de degradación/transformación de los polímeros después de cada tratamiento específico. Para realizar este estudio, se aplicó GC-TOF MS. En el futuro, el trabajo se podría ampliar con análisis realizados por LC-QTOF MS.

Los resultados cualitativos y cuantitativos obtenidos en la Tesis se discuten de modo crítico en el **Capítulo 6**, en donde se resaltan los aspectos más destacables y las conclusiones más relevantes. Finalmente, sobre la base de los datos encontrados en esta Tesis, se hacen algunas recomendaciones para trabajos futuros.

Resumen

El Bisfenol-A (BPA) se ha usado durante muchos años como monómero en polímeros de policarbonato (PC) (botellas de agua y comida para niños-biberones) y en resinas tipo epoxi (botes de comida preparada), a partir de los cuales puede liberarse este compuesto a la comida y al agua, lo que constituye la principal fuente de exposición a BPA de los seres humanos. Como consecuencia de sus propiedades como disruptor endocrino, se prohibió su uso en la producción de polímeros para materiales en contacto con comida para niños menores de 3 años (*European Commission, Regulation No. 10/2011*). Recientemente, el *Superior Health Council of Belgium* expresó su preocupación por los posibles riesgos asociados a las alternativas usadas para fabricación de PC (No. 8697, 11.03.2010). Los productos alternativos al PC para fabricación de materiales en contacto con alimentos (*food contact materials (FCMs)*) para niños, tales como polipropileno (PP), polietersulfona (PES), poliamida (PA), Tritan™ o silicona, han aparecido en el mercado en los últimos años.

La migración de BPA a partir de PC ha sido ampliamente estudiada. Sin embargo, la naturaleza y las cantidades de sustancias liberadas de los materiales poliméricos usados como alternativa al PC son mucho menos conocidas. Por ello, el principal objetivo de esta Tesis ha sido la identificación y cuantificación de los compuestos mayoritarios y de mayor toxicidad que pueden migrar desde los biberones en el marco de un proyecto del gobierno belga (ALTPOLYCARB) en el que han participado varias Universidades de Bélgica.

El primer capítulo experimental de esta Tesis (**Capítulo 3**) describe las posibles alternativas al PC en FCMs para niños menores de 3 años en el mercado belga. Este trabajo se documentó con un estudio inicial de mercado en tiendas de productos para bebés, supermercados y farmacias. Se encontraron hasta 24 biberones de diferentes fabricantes. Los polímeros usados en la fabricación fueron, en orden de importancia, PP, PES, PA, Tritan™ y silicona, aunque también se encontraron algunos recipientes de PC. Se estudiaron también otros productos usados en FCMs para niños, tales como tazas, tetinas, cubiertos. Sin embargo, en estos últimos materiales no se pudieron identificar la mayoría de polímeros usados en su producción. Dada la falta de información para las otras categorías de FCMs distintas a los biberones, y considerando la mayor importancia que estos tienen en la alimentación para bebés, los test de migración se realizaron solamente para una selección representativa de biberones. Para la realización de test de migración, en la legislación se establece el uso de simulantes con el fin de asemejar los resultados a los test en alimentos reales. Específicamente, se recomienda una mezcla de agua-EtOH (50:50, v/v) como simulante para leche. Para ello, después de la esterilización de la botella durante 10 minutos con agua hirviendo, se realizan tres experiencias repetidas de migración, durante 2h cada una, a 70°C.

En primer lugar, se optimizó la extracción líquido-líquido (LLE) con una mezcla de compuestos orgánicos más habituales (**Capítulo 4.1**). Con el fin de desarrollar un método robusto y genérico, se probó una mezcla de 17 contaminantes, seleccionados sobre la base de datos previos reportados en la literatura científica, cubriendo un amplio rango de polaridades y grupos funcionales. Se evaluó la eficiencia de extracción de varios disolventes: n-hexano, iso-octano, acetato de etilo (EtOAc)-n-hexano (1:1 y 1:3), metilterbutiléter (MTBE) y diclorometano (DCM)-n-hexano (1:1 y 1:3). Los extractos resultantes de LLE se analizaron mediante GC-(EI)MS monitorizando iones específicos (modo SIM) para cada analito y para el patrón interno. EtOAc-n-hexano (1:1) y DCM-n-hexano (1:1) fueron los solventes más eficientes para la extracción. Finalmente, se seleccionó un disolvente no clorado, por lo que la elección correspondió a EtOAc-n-hexano (1:1) con el fin de ser aplicado al análisis de muestras reales.

Se evaluó la posible liberación de compuestos químicos desconocidos de diversos materiales usados en biberones, como PP, PES, PA, Tritan™ y silicona. Las disoluciones de migrantes obtenidas de los biberones se extrajeron mediante LLE y se analizaron por GC-(EI)MS en búsqueda de compuestos desconocidos usando las librerías de espectros de Wiley® y NIST®. Aunque las concentraciones presentes parecieron ser bajas, se pudo identificar varios compuestos, como alcanos, ftalatos, amidas, etc. En el **Capítulo 4.2**, se investigaron picos que no pudieron ser identificados haciendo uso de técnicas más avanzadas, como GC-(EI)TOF-MS y GC-(APCI)QTOF-MS, con el fin elucidar la estructura de estos compuestos desconocidos. Sobre una base amplia de datos, se hizo una búsqueda de la masa exacta del ion molecular y de la molécula protonada en APCI. La presencia de un pico cromatográfico, junto con la fragmentación observada en ambas técnicas, sirvió para fines de elucidación. La estrategia aplicada, basada en la combinación de información suministrada por ambas técnicas, permitió identificar varios compuestos que no pudieron ser identificados previamente (e.g. diciclopentil-(dimetoxi)silano, Irganox 1010, etc.).

Adicionalmente, los mismos extractos se analizaron también por LC-QTOF-MS en modo MS^E. Ello permitió obtener simultáneamente el espectro completo medido en masa exacta, a baja y alta energía de colisión, suministrando información relevante sobre la molécula (de)protonada y los iones fragmentos. Los datos fueron procesados automáticamente, usando una base de datos propia que contenía unos 1200 compuestos químicos presentes en las listas de la *EU Regulation No. 10/2011* y agentes migrantes esperados, tales como anti-oxidantes, plastificantes, etc. Cuando se detectó un pico cromatográfico pero el patrón de referencia no estaba disponible en el laboratorio, se llevó a cabo la identificación tentativa usando las masas exactas de los iones fragmentos observados en los espectros. De este modo, se pudieron elucidar varios compuestos que no pudieron ser identificados mediante GC-MS.

En paralelo al proceso de identificación, se evaluó la genotoxicidad y la actividad endocrina de los compuestos migrantes. Este trabajo se llevó a cabo en las universidades que colaboraron en el proyecto ALTPOLYCARB mediante la realización de ensayos *in vitro*. El **Capítulo 5.1** describe la optimización de un método basado en LLE para compuestos migrantes seleccionados en base a los resultados obtenidos en los test de toxicidad y a las abundancias observadas en las experiencias de emigración del **Capítulo 4.1**. La detección y cuantificación de estos compuestos se hizo mediante métodos basados en GC- y LC-QqQ-MS/MS, los cuales fueron previamente validados (sensibilidad, selectividad, linealidad, exactitud, precisión, recuperaciones y efecto matriz). Los análisis de la 3ª etapa de migración en las condiciones estándar de migración aplicadas en biberones (la cual tiene que cumplir con los límites de migrantes establecidos en la legislación de la UE) mostraron que, en algunos tipos de biberones, varios compuestos no autorizados superaron el límite de no-detección, de 10 μg kg^{-1}, generalmente establecido para compuestos no autorizados. Compuestos como 2,4-di-*tert*-butilfenol (hasta 118 μg kg^{-1}), 2-butoxietil acetato (hasta 945 μg kg^{-1}) y 4-propilbenzaldehído (hasta 27 μg kg^{-1}) fueron encontrados en varias muestras, así como algunos ftalatos. Los biberones de silicona llegaron a mostrar concentraciones de 2,2,4-trimetil-1,3-pentanodiol diisobutirato (TXIB) de hasta 350 μg kg^{-1}. Para todos aquellos compuestos autorizados en la *EU Regulation No. 10/2011*, con límites específicos de migración (specific migration limit, SML), tales como benzofenona (600 μg kg^{-1}, encontrado hasta 97 μg kg^{-1}), las concentraciones encontradas en las disoluciones de migración fueron inferiores a los SMLs establecidos por la legislación.

En el **Capítulo 5.2** se evaluó el efecto de las condiciones de uso real de los biberones, como son el microondas, la esterilización y el lavado en lavavajillas con test de duración, sobre el perfil de los compuestos que migran, y se comparó con el tratamiento de referencia (30 min a 40°C) y con las condiciones de uso repetido en la UE (3 migraciones, 2 h a 70°C). El análisis de los extractos resultantes de las experiencias en microondas mostró un ligero aumento en las concentraciones de los compuestos que migran (e.g. azaciclotridecan-2-ona del biberón de PA: 124 μg kg^{-1} después del primer calentamiento en microondas frente a 70 μg kg^{-1} en el primer experimento de referencia). Además, se observó una liberación prolongada de los compuestos seleccionados, mientras que en las condiciones de referencia la liberación de migrante se produjo de manera notablemente más rápida. El tratamiento en lavavajillas también condujo a un ligero aumento de algunos de los compuestos seleccionados, mientras otros presentaron concentraciones inferiores a las del experimento de referencia. Esto podría explicarse porque estos compuestos pudieron ser parcialmente lavados y eliminados durante el tratamiento en lavavajillas.

La esterilización mediante vapor de agua mostró una rápida eliminación de los compuestos seleccionados, ya que sus concentraciones fueron menores a las encontradas en los tratamientos de referencia (e.g. TXIB en biberón de silicona 28 μg kg^{-1}, frente a 118 μg kg^{-1}) o similares (di(iso)butil ftalato). El comportamiento observado en la esterilización por vapor de agua mostró claras ventajas en cuanto a la eliminación de residuos químicos que podrían liberarse por los polímeros. En cuanto a la esterilización por calentamiento en agua hirviendo, se observó un comportamiento general semejante al de la esterilización por vapor de agua, ya que se eliminaron la mayoría de contaminantes químicos liberados por el biberón. Sin embargo, se observó un ligero aumento de algunos compuestos en los recipientes de silicona, lo que sugiere que este tratamiento no es adecuado para dicho material.

En los test de duración se observó en general una disminución de las concentraciones a lo largo de los ciclos sucesivos. Los compuestos encontrados en los tratamientos realizados a lo largo del tiempo estuvieron de acuerdo con los observados previamente en los experimentos de uso repetido de la UE. Sin embargo, las concentraciones encontradas en este último caso fueron notablemente superiores para la mayoría de compuestos. Las experiencias de uso repetido parecen conducir a una sobreestimación de los niveles de migración en biberones en comparación con las condiciones reales de uso. Sin embargo, debe tenerse en cuenta que los test de duración se realizaron de forma individual, por lo que la combinación de varios tratamientos podría conducir a una liberación mayor a la observada en las experiencias individuales con un único tratamiento.

El **Capítulo 5.3** presenta los resultados obtenidos en un detallado estudio sobre muestras expuestas a numerosos test de duración. Con este fin, no solo se investigó la influencia de tratamientos realizados en la vida real sobre la migración de compuestos seleccionados, sino también sobre el resto de posibles migrantes (e.g. productos de degradación). Los resultados obtenidos indicaron que, bajo condiciones reales de uso, no se producía degradación relevante de los polímeros, aunque la esterilización con vapor de agua resultó, después de 10 ciclos, en una mayor liberación de antioxidantes de los recipientes de PP en comparación con el primer ciclo.

Finalmente, en el **Capítulo 6** se realiza una discusión crítica de los resultados más relevantes obtenidos en esta investigación, con especial énfasis en la detección de las sustancias no añadidas intencionalmente en biberones. Además, se sugieren varias acciones que podrían llevarse a cabo para prevenir la presencia de estos compuestos en el futuro.

Curriculum vitae

Personal data

Name	Matthias Onghena
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Education

1999-2005	Sint-Jozef-Klein Seminarie, Latin-Mathematics (6h)
2005-2009	Master in Industrial Sciences: Chemistry (industrial engineer), graduated “cum laude”, Karel de Grote Hogeschool, Antwerp, Belgium
2010-2011	Additional 18 credits of the master “Environmental chemistry”, Universidad de Valencia, Spain
2012-present	PhD studies in Pharmaceutical Sciences, Toxicological Centre, University of Antwerp

Peer-reviewed publications

Onghena M, Geens T, Goossens E, Wijnants M, Pico Y, Neels H, Covaci A, Lemiere F. Analytical characterisation of mannosylerythritol lipid (MEL) biosurfactants produced by biosynthesis based on feedstock sources from agro-food industry. *Analytical and Bioanalytical Chemistry* 400: 1263-1275 (2011)

Vazquez-Roig P, Andreu V, **Onghena M**, Blasco C, Picó Y. Assesment of the occurence and distribution of pharmaceuticals in a Mediterranean wetland (L’Albufera, Valencia , Spain). *Analytical and Bioanalytical Chemistry* 400: 1287-1301 (2011)

Blasco C, Vazquez-Roig P, **Onghena M**, Masia A, Picó Y. Analysis of insecticides in honey by liquid chromatography-ion-trap mass spectrometry: Comparison of different extraction procedures. *Journal of Chromatography A* 1218: 4892-4901 (2011)

Onghena M, Moliner-Martinez Y, Picó Y, Campíns-Falcó P, Barceló D. Analysis of 18 perfluorinated compounds in river waters: Comparison of HPLC-MS/MS, UPLC-MS/MS and capillary LC-MS. *Journal of Chromatography A* 1244: 88-97 (2012)

Onghena M, Van Hoeck E, Vervliet P, Scippo ML, Simon C, Van Loco J, Covaci A. Development and application of a non-targeted extraction method for the analysis of migrating compounds from plastic baby bottles by GC-MS., *Food Additives and Contaminants*. Part A 31: 2090–102 (2014)

Onghena M, Van Hoeck E, Van Loco J, Ibáñez M, Cherta L, Portolés T, Pitarch E, Hernandéz F, Covaci A. Identification of substances migrating from plastic baby bottles using a combination of low and high resolution mass spectrometric analyzers coupled to gas and liquid chromatography. *Journal of Mass Spectrometry* 50: 1234-1244 (2015)

Schymanski EL, Singer HP, Slobodnik J, Ipolyi IM, Oswald P, Krauss M, Schulze T, Haglund P, Letzel T, Grosse S, Thomaidis N, Bletsou A, Zwiener C, Ibáñez M, Portolés T, De Boer R, Reid MJ, **Onghena M,** Kunkel U, Schulz W, Guillon A, Noyon N, Leroy G, Bados P, Bogialli S, Stipaničev D, Rostkowski P, Hollender J, Non-target screening with high resolution mass spectrometry: Critical review using a collaborative trial on water analysis, *Analytical and Bioanalytical Chemistry* (2015). 407: 6237-6255 (2015)

Onghena M, Negreira N, Van Hoeck E, Quirynen L, Van Loco J, Covaci A. Quantitative determination of migrating compounds from plastic baby bottles by validated GC-QqQ-MS and LC-QqQ-MS methods. *Food Analytical Methods.* (2016) *Published online*: DOI 10.1007/s12161-016-0451-4

Onghena M, Negreira N, Quirynen L, Van Loco J, Covaci A. Quantitative determination of migrating compounds from plastic baby bottles by validated GC-QqQ-MS and LC-QqQ-MS methods. *Food Additives and Contaminants Part A. In press*

Vives JM, Farre M, Blasco C, **Onghena M**, Picó Y, Barceló D. Perfluoroalkyl Substances in Breast Milk, Infant Formula and Baby Food from Valencian Community (Spain). Submitted to *International Dairy Journal*

Simon C, **Onghena M**, Van Hoeck E, Van Loco J, Vandermarken T, Van Langenhove K, Demaegdt H, Mertens B, Vandermeiren K, Covaci A, Scippo ML. Screening of endocrine disrupting activity of migration products from plastic baby bottles using a multi-receptor panel of in vitro bioassays. Submitted to *Toxicology in Vitro*

Mertens B, Simon C, Van Bossuyt M, **Onghena M**, Vandermarken T, Van Langenhove K, Demaegdt H, Ost N, Schüürman G, Van Hoeck E, Van Loco J, Vandermeiren K, Covaci A, Scippo M, Elskens M, Verschaeve L. Investigation of the genotoxicity of

substances migrating from polycarbonate replacement baby bottles to identify chemicals of high concern. *Food and Chemical Toxicology* 89: 126-137 (2016)

Oral presentations

Analysis of perfluorinated compounds in water and sediments. **Onghena M**, Picó Y. Understanding effects of global change on water quantity and quality in river basins, ICRA-IDAEA-CSIC, Girona, Spain, December 2010 (Presenter)

Study of the endocrine disrupting activity of migration products from plastic food contact materials. Simon C, **Onghena M**, Covaci A, Van Hoeck E, Van Loco J, Elskens M, Demaegdt H, Mertens B, Scippo ML. Séminaires FARAH, Pôle "santé publique vétérinaire", Liège, April 2013 (Co-author)

Analysis of migrating compounds from plastic baby bottles with GC-QQQMS and LC-QTOFMS: Development and application of a non-targeted extraction method. **Onghena M**, Van Hoeck E, Vervliet P, Van Loco J, Covaci A. 6th International Symposium on Recent Advances in Food Analysis (RAFA 2013), Prague, Czech Republic, 5 - 8 November 2013 (Presenter)

Analysis of migrating compounds from plastic baby bottles with GC-MS, GC-(Q)TOF-MS and LC-QTOF-MS. **Onghena M**, Van Hoeck E, Van Loco J, Ibáñez M, Cherta L, Portolés T, Pitarch E, Hernandéz F, Covaci A. International Symposium on Environmental Analytical Chemistry, Lausanne, Switzerland, 17-20 June 2014 (Presenter)

EDCs & Plastic baby bottles. Simon C, **Onghena M**, Covaci A, Van Hoeck E, Van Loco J, Elskens M, Vandermarken T, Van Langenhove K, Demaegdt H, Mertens B, Scippo ML. BDS conference, Turin, Italy, September 2014 (Co-author)

In vitro study toward the endocrine activity and the genotoxic potential of migration products from plastic baby bottles. Simon C, **Onghena M**, Covaci A, Van Hoeck E, Van Loco J, Elskens M, Vandermarken T, Van Langenhove K, Demaegdt H, Mertens B, Scippo ML. BelTox Annual Scientific Meeting, Geel, 4 December, 2014 (Co-author)

Identification of substances migrating from plastic baby bottles using a combination of low and high resolution mass spectrometric analyzers coupled to gas and liquid chromatography. **Onghena M**, Van Hoeck E, Van Loco J, Ibáñez M, Cherta L, Portolés T, Pitarch E, Hernandéz F, Covaci A., Waters symposium on extractables and leachables, Berlin, Germany, 15-16 June 2015 (Invited speaker)

Identification of substances migrating from plastic baby bottles using a combination of low and high resolution mass spectrometric analyzers coupled to gas and liquid chromatography. **Onghena M**, Van Hoeck E, Van Loco J, Ibáñez M, Cherta L, Portolés T, Pitarch E, Hernandéz F, Covaci A., MS in food and Feed, Ghent, 15 September 2015 (Presenter)

Identification of substances migrating from plastic baby bottles using a combination of low and high resolution mass spectrometric analyzers coupled to gas and liquid chromatography. **Onghena M**, Van Hoeck E, Van Loco J, Ibáñez M, Cherta L, Portolés T, Pitarch E, Hernandéz F, Covaci A., Waters MS Technology days, Utrecht-Brussels, 23-24 September 2015 (Invited speaker)

Identification of substances migrating from plastic baby bottles using a combination of low and high resolution mass spectrometric analyzers coupled to gas and liquid chromatography. **Onghena M**, Van Hoeck E, Van Loco J, Ibáñez M, Cherta L, Portolés T, Pitarch E, Hernandéz F, Covaci A., VII Reunión Nacional de la Sociedad Española de Espectrometría de Masas (SEEM2015), Castellon, Spain 27 October 2015 (Presenter)

Quantitative determination of migrating compounds from plastic baby bottles by validated GC-QqQ-MS and LC-QqQ-MS methods. **Onghena M**, Negreira N, Quirynen L, Van Loco J, Covaci A., Recent Advances in Food Analysis, Prague, Czech Republic, 3-6 November 2015 (Presenter)

Poster presentations

Analytical characterisation of mannosylerythritol lipid (MEL) biosurfactants produced by biosynthesis based on feedstock sources from agro-food industry. **Onghena M**, Geens T, Goossens E, Wijnants M, Pico Y, Neels H, Covaci A, Lemiere F. 28th International Symposium on Chromatography. Valencia Conference centre, Spain, September 2010 (Presenter)

Study of the estrogenicity of migration products from plastic food contact materials. Simon C, **Onghena M**, Covaci A, Van Hoeck E, Van Loco J, Elskens M, Demaegdt H, Mertens B, Scippo ML. Bioforum, Liège, April 2013 (Co-author)

Analysis of migrating compounds from plastic baby bottles with GC-QqQ-MS and LC-QTOF-MS: Development and application of a non-targeted extraction method. **Onghena M**, Van Hoeck E, Vervliet P, Van Loco J, Covaci A. Trends in Food Analysis VII, Ghent, 19 September, 2013 (Presenter)

Measurement of the estrogenic activity of migration products from plastic contact materials. Simon C, **Onghena M**, Covaci A, Van Hoeck E, Van Loco J, Elskens M, Demaegdt H, Mertens B, Scippo ML. FMV scientific day, Liège, Oktober 2013 (Co-author)

In vitro model to study the endocrine disrupting activity of migration products from plastic food contact materials. Simon C, **Onghena M**, Covaci A, Van Hoeck E, Van Loco J, Elskens M, Demaegdt H, Mertens B, Scippo ML. Beltox, Louvain La Neuve, December 2013 (Co-author)

Analysis of migrating compounds from plastic baby bottles with GC-QqQ-MS and LC-QTOF-MS: Development and application of a non-targeted extraction method. **Onghena M**, Van Hoeck E, Vervliet P, Van Loco J, Covaci A. ChemCYS symposium, Blankenberge, 17-18 February 2014 (Presenter)

In vitro model to study the endocrine disrupting activity of migration products from plastic food contact materials. Simon C, **Onghena M**, Covaci A, Van Hoeck E, Van Loco J, Elskens M, Vandermarken T, Van Langenhove K, Demaegdt H, Mertens B, Scippo ML. Bioforum, Liège, May 2014 (Co-author)

Identification of substances migrating from plastic baby bottles with GC-MS, GC-(Q)TOF-MS and LC-QTOF-MS. **Onghena M**, Van Hoeck E, Van Loco J, Ibáñez M, Cherta L, Portolés T, Pitarch E, Hernandéz F, Covaci A. Food Analysis Congress, Barcelona, 29-30 October 2014 (Presenter)

Identification of substances migrating from plastic baby bottles with GC-MS, GC-(Q)TOF-MS and LC-QTOF-MS. **Onghena M**, Van Hoeck E, Van Loco J, Ibáñez M, Cherta L, Portolés T, Pitarch E, Hernandéz F, Covaci A. Food Contact Materials Symposium, Brussels, 20 November, 2014 (Presenter)

In vitro study toward the endocrine activity and the genotoxic potential of migration products from plastic baby bottles. Simon C, **Onghena M**, Covaci A, Van Hoeck E, Van Loco J, Elskens M, Vandermarken T, Van Langenhove K, Demaegdt H, Mertens B, Scippo ML. Food contact material symposium, Brussels, 20 November 2014 (Co-author)

Identification of substances migrating from plastic baby bottles with GC-MS, GC-(Q)TOF-MS and LC-QTOF-MS. **Onghena M**, Van Hoeck E, Van Loco J, Ibáñez M, Cherta L,

Portolés T, Pitarch E, Hernandéz F, Covaci A. BelTox Annual Scientific Meeting, Geel, 4 December, 2014 (Presenter)

Quantitative determination of migrating compounds from plastic baby bottles by validated GC-QqQ-MS and LC-QqQ-MS methods. **Onghena M**, Negreira N, Quirynen L, Van Loco J, Covaci A. MS in Food and Feed, Ghent, 15 September, 2015 (Presenter)

Reviewer for:

Environmental pollution
MethodsX
Food Chemistry
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