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68

Qizhi Su

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Doctoral Thesis

2021

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I can still remember when I first arrived at this lab back in 2017, I was a bit excited since things were new for me, the faces, the culture, and everything. I had never lived, studied, or even traveled outside of China. I was looking forward to new life here in Spain, in Zaragoza, and in the new lab. At the same time, I was a bit worried about not being able to get adapt to the new environment due to the linguistic and cultural differences. At the time of writing this acknowledgement and recalling the time I spent here, I just realize that the worry was unnecessary as people here are quite nice and friendly. Time flies, and my study here is coming to an end. I just cannot imagine how I can complete my doctoral study without the help from many people and the support from my families. Here, I would like to express my deep and sincere gratitude to them.

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To my parents

To my sisters

To my wife Yang Min-Hua

To our daughter Juy

Table of Content

Glossary of Terms

AIF: All-Ion Fragmentation

ANOVA: Analysis of Variance

APCI: Atmospheric Pressure Chemical Ionization

APPI: Atmospheric Pressure Photo Ionization

ASE: Accelerated Solvent Extraction

ATR: Attenuated Total Reflectance

BPA: Bisphenol A

CAR: Carboxen

CCD: Central Composite Design

CCS: Collision Cross Section

CE: Circular Economy

CFM-EI: Competitive Fragmentation Modeling for Electron Ionization

CFR: Code of Federal Regulations

CLP: Classification, Labelling, and Packaging

CMR: Carcinogenic, Mutagenic, and Reprotoxic Chemicals

CPPdb: Plastic Packaging-Associated Chemicals Database

DBE: Double Bond Equivalent

DI-SPME: Direct Immersion-Solid Phase Micro-Extraction

DVB: Divinylbenzene

EC: European Commission

ECE: Elemental Composition Experiment

ECHA: European Chemicals Agency

EDC: Endocrine Disrupting Chemicals

EFSA: European Food Safety Authority

EI: Electron Ionization

EIC: Extracted Ion Chromatograms

EPA: Environmental Protection Agency

EPR: Extended Producer Responsibility

ESBO: Epoxidized Soybean Oil

ESI: Electrospray Ionization

EU: European Union

FBMN: Feature-Based Molecular Networking

FCCdb: Intentionally Used Food Contact Chemicals Database

FCM: Food Contact Material

FDA: Food and Drug Administration

FDR: False Discovery Rate

FID: Flame Ionization Detector

FTIR: Fourier Transform Infrared

FT-NIR: Fourier Transform Near Infrared

FWHM: Full Width at Half Maximum

GC-MS: Gas Chromatography Coupled to Mass Spectrometry

GMP: Good Manufacturing Practice

HCA: Hierarchical Clustering Analysis

HCD: Higher Energy Collisional Dissociation

HDPE: High-Density Polyethylene

HMDB: Human Metabolome Database

HRMS: High-Resolution Mass Spectrometry

HS: Headspace

HSD: Honestly Significant Difference

HS-SPME: Headspace-Solid Phase Micro-Extraction

IAS: Intentionally Added Substance

IM-MS: Ion Mobility Mass Spectrometry

LC-MS: Liquid Chromatography Coupled to Mass Spectrometry

LDPE: Low-Density Polyethylene

LLDPE: Linear Low-Density Polyethylene

LLE: Liquid-Liquid Extraction

LLME: Liquid-Liquid Microextraction

LOD: Limit of Detection

LOESS: Locally Estimated Scatterplot Smoothing

LogP: Octanol/Water Partition Coefficient

LOQ: Limit of Quantification

MAE: Microwave-Assisted Extraction

MALDI: Matrix-Assisted Laser Desorption/Ionization

MANOVA: Multivariate Analysis of Variance

MF: Molecular Formula

MOAH: Mineral Oil Aromatic Hydrocarbons

MoNA: Massbank of North America

MOSH: Mineral Oil Saturated Hydrocarbons

MS1: Low Energy Spectrum

MSGC: Monostearolglycerol

MW: Molecular Weight

ND: Not Detectable

NEIMS: Neural Electron-Ionization Mass Spectrometry

NIAS: Non-Intentionally Added Substance

NIST: National Institute of Standards and Technology

NMR: Nuclear Magnetic Resonance

NTS: Non-Target Screening

OPLS-DA: Orthogonal Partial Least Squares Discriminant Analysis

PA: Polyacrylate

PAH: Polyaromatic Hydrocarbons

PC: Polycarbonate

PCA: Principal Component Analysis

PCB: Polychlorinated Biphenyl

PDMS: Polydimethylsiloxane

PE: Polyethylene

PET: Polyethylene Terephthalate

PLS-DA: Partial Least Squares Discriminant Analysis

PP: Polypropylene

PS: Polystyrene

PU: Polyurethane

PVC: Polyvinylchloride

QC: Quality Control

QCEIMS: Quantum Chemical Electron Impact Mass Spectrum

R^2: Determination Coefficient

RDBE: Ring Double Bond Equivalent

rPET: Recycled Polyethylene Terephthalate

rPO: Recycled Polyolefins

RSM: Response Surface Methodology

S/N: Signal to Noise

SIM: Selected Ion Monitoring

SLE: Solid-Liquid Extraction

SML: Specific Migration Limit

SMILES: Simplified Molecular Input Line Entry System

SPME: Solid Phase Micro-extraction

SSP: Solid State Polycondensation

SUP: Single Use Plastics

SVHC: Substances of Very High Concern

SWATH: Sequential Windowed Acquisition of All THeoretical

T_g: Transition Temperature

T_m: Melting Temperature

TS: Target Screening

UAE: Ultrasound-Assisted Extraction

US: United State

UV: Ultraviolet

Presentation

The doctoral thesis present here is titled "**Safety Assessment of Recycled Polyolefins for Food Contact Applications: Non-Target Screening of Volatile and Non-Volatile Substances**". This work was carried out in the University Group of Analytical Research (GUIA) led by Dr. Cristina Nerín de la Puerta in the Department of Analytical Chemistry at the University of Zaragoza in Zaragoza, Spain. The group is integrated into the Aragón Institute of Engineering Research (I3A).

The thesis concentrates on determining volatile and non-volatile substances present in post-consumer polyolefins and on finding out chemicals of high-risk regarding consumer health when these recycled polyolefins are going to be used as food contact materials. For these purposes, sensitive, reliable, and ease-to-use sample pre-treatment as well as data processing methods for non-target screening of volatile and non-volatile compounds have been developed and optimized. In addition, a chemical prioritization strategy has been proposed to help us focusing on substances of high concern as the number of identified substances was huge.

The thesis consists of six sections:

Section I gives a general introduction about the need and potential of mechanical recycling post-consumer polyolefins for food contact uses. Moreover, non-target screening-based data processing has been discussed with the focus on compound identification making use of various open-source tools as well mass spectra libraries.

Section II presents the general objectives of the thesis as well as specific objectives in each experimental work.

Section III is the experimental part, which comprises of 5 chapters. Each chapter entails chapter-specific abstract, introduction, materials and methods, results and discussions, and conclusions. These chapters can be organized into 3 subsections:

Developing sensitive sample treatment and reliable data analysis methodologies for non-target screening of volatile compounds

- **Chapter 1:** Non-target Screening of (Semi-)Volatiles in Food-Grade Polymers by Comparison of Atmospheric Pressure Gas Chromatography Quadrupole Time-of-Flight and Electron Ionization Mass Spectrometry
- **Chapter 2:** Direct Immersion - Solid-Phase Micro-extraction Coupled to Gas Chromatography - Mass Spectrometry and Response Surface Methodology for Non-target Screening of (Semi-) Volatile Migrants from Food Contact Materials

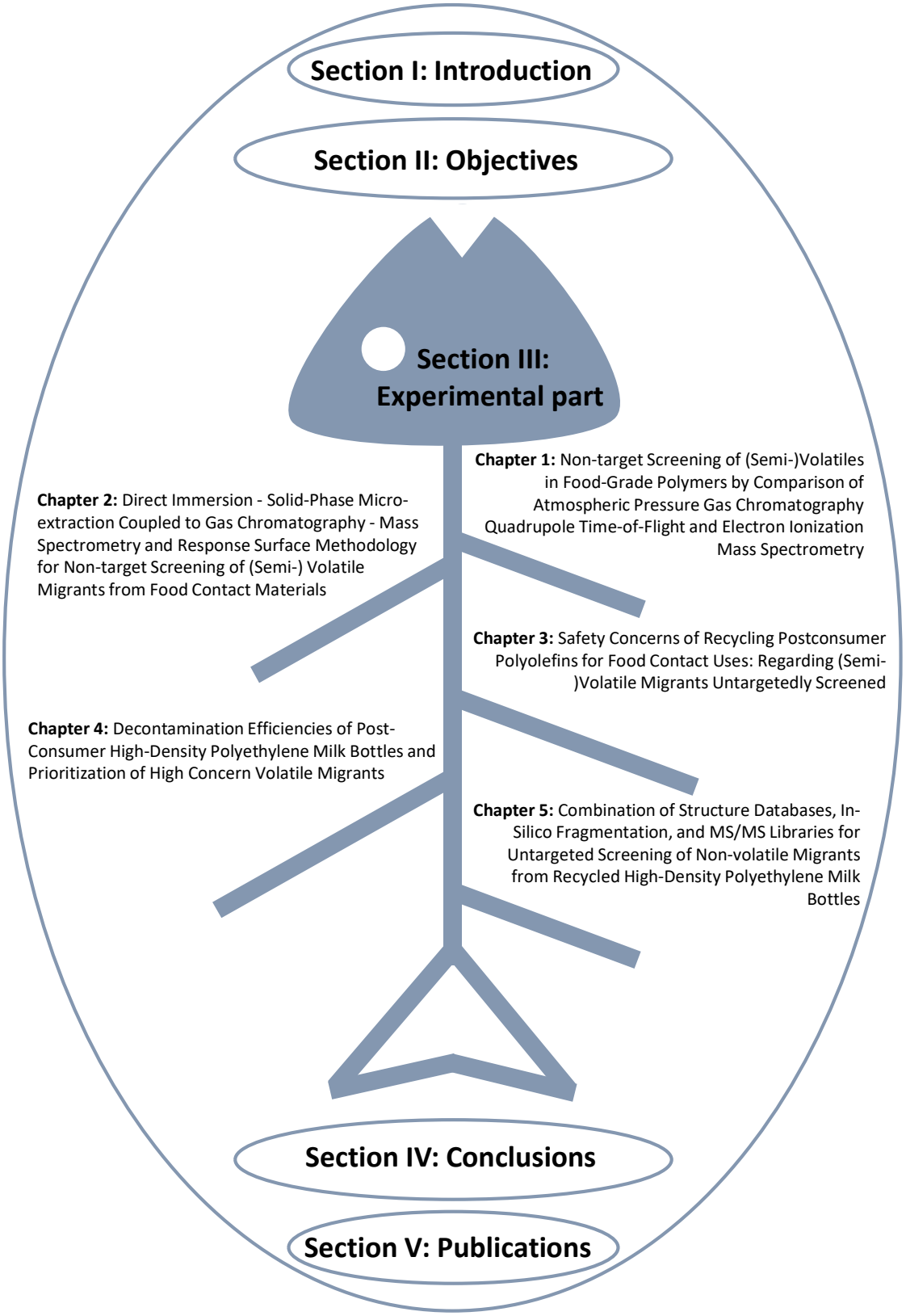
Non-target screening of volatile migrants from recycled polyolefins

- **Chapter 3:** Safety Concerns of Recycling Post-consumer Polyolefins for Food Contact Uses: Regarding (Semi-)Volatile Migrants Untargetedly Screened
- **Chapter 4:** Decontamination Efficiencies of Post-Consumer High-Density Polyethylene Milk Bottles and Prioritization of High Concern Volatile Migrants

Developing sensitive and reliable data analysis strategy for non-target screening of non-volatile compounds and its application to migrants coming from rHDPE

- **Chapter 5:** Combination of Structure Databases, In-Silico Fragmentation, and MS/MS Libraries for Untargeted Screening of Non-volatile Migrants from Recycled High-Density Polyethylene Milk Bottles

Section IV, **V**, and **VI** deal with general conclusions obtained, publications as well as bibliography. The diagram below displays the structure of this thesis.


Section I: Introduction
Section II: Objectives
Section III: Experimental part
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Chapter 3: Safety Concerns of Recycling Postconsumer Polyolefins for Food Contact Uses: Regarding (Semi-)Volatile Migrants Untargetedly Screened
Chapter 4: Decontamination Efficiencies of Post-Consumer High-Density Polyethylene Milk Bottles and Prioritization of High Concern Volatile Migrants
Chapter 5: Combination of Structure Databases, In-Silico Fragmentation, and MS/MS Libraries for Untargeted Screening of Non-volatile Migrants from Recycled High-Density Polyethylene Milk Bottles
Section IV: Conclusions
Section V: Publications

Session I: Introduction

Introduction

1. Plastic pollution

Plastics are ubiquitous in our daily life with a wide variety of applications, such as, packaging, automotive, electrical & electronic, and household products owing to their inexpensive, lightweight, durable, corrosion resistance, etc. properties (Hopewell et al., 2009; Ilyas et al., 2018). There are at least eight major types of polymers commonly used: high-density polyethylene (HDPE), low-density and linear low-density polyethylene (LDPE/LLDPE), polypropylene (PP), polystyrene (PS), polyvinylchloride (PVC), polyethylene terephthalate (PET), polyurethane (PUR) resins, as well as polyester, polyamide, and acrylic fibres (Geyer et al., 2017). However, in the last decades, plastic pollution has become an increasing concern to the environment because they are extremely durable and will persist for at least decades or even centuries in the environment (Hopewell et al., 2009). In addition, most raw materials for plastics come from non-renewable crude oil (Lithner et al., 2011) and therefore not sustainable.

Under linear economic, plastics are produced, consumed, and discarded. In this mode, there is continuous need for starting materials to produce new plastics. It is estimated that between 4-6% of oil and gas is used for plastics production in Europe (British Plastics Federation, 2019). Moreover, it was estimated that about 6300 million tonnes of plastics waste have been created between 1950 and 2015, of which only 9% were recycled, 12% incinerated, and leaving around 80% to accumulate in landfills or the natural environment (Geyer et al., 2017).

In 2010, around 8 million tonnes of plastic, which was 3% of the total plastic waste generated, entered the ocean (Jambeck et al., 2015). These plastics were known to negatively impact wildlife by entanglement and ingestion (Law, 2017). Particularly, microplastics (particles smaller than 5 mm) have recently drawn increasing attention as they can be easily ingested by organisms and thus may act as carriers for the transfer of pollutants within the food chain (Li et al., 2016; Teuten et al., 2009). Although not fully understood yet, there are some evidences of adverse effects of microplastics on fishes as well as mammal models (Yong et al., 2020). Compared to plastic pollution in aquatic

systems, plastic pollution in terrestrial environment remain largely unexplored, but further research are required considering the widespread presence and environmental persistence of plastic on the land (Chae and An, 2018; de Souza Machado et al., 2018). In a word, the large number of plastic pollution is posing a threat to our marine, freshwater, soil environment, and potentially the safety of our entire food system (Geyer et al., 2017; He et al., 2019).

2. Measures to mitigate plastic pollution

To address plastic pollution, various intervention policies were implemented across the world. Interventions (bans and levies) for plastic bags started since 1991, while policies (bans) to reduce microbeads, which are used as exfoliating materials in cosmetics, began in 2014 (Schnurr et al., 2018; Xanthos and Walker, 2017). Although not fully investigated yet, the effectiveness of these policies varied. For example, significant reductions (~ 90%) in plastic bag use have been seen in Ireland, Wale, and England (Schnurr et al., 2018; Xanthos and Walker, 2017), while plastic bags prevention in South Africa has been reported to be ineffective (Dikgang et al., 2012). On the other hand, strong interventions could give rise to negative social effects, for instance, a plastic manufacturer with 20000 employees went out of business soon after the government announced the plastic bag policy in 2008 (Xanthos and Walker, 2017).

Aside from the aforementioned intervention policies to reduce the consumption of single use plastics (SUP), there are several other ways viable to reduce negative impacts of continuously growing plastic pollution (EC, 2018; OECD, 2018).

- **Better product design:** such as using alternative materials, e.g., recycled, biobased or biodegradable plastics, designing light-weighting products, and designing products for recyclability.

- **Higher recycling rate:** such as increasing waste collection and recycling rates, improving sorting and decontamination abilities as well as boosting market for recycled plastics.

- **Clean up and remediation activities:** such as cleaning up plastics in the beaches and collecting plastics from rivers and oceans.

All these approaches could be beneficial but also come with certain risks and costs. Nevertheless, several life-cycle assessment based meta-analyses clearly concluded that plastics recycling has a significantly smaller greenhouse gas footprint than plastics incineration or landfilling and therefore making it relatively more desirable to displace virgin plastics by recycled equivalents (Hopewell et al., 2009; OECD, 2018).

In 2018, EU launched *A European strategy for plastics in a circular economy* as a key priority in a circular economy (CE). Fig. I-1 shows the scheme of CE. One of the ambitious targets established is that all plastics packaging placed on the EU market is either reusable or can be recycled in a cost-effective manner by 2030 further addressing the significance of plastic recycling.

Fig. I-1 Scheme of Circular Economy (EU-Parliment, 2015)

Extended Producer Responsibility (EPR) is a policy approach that gives a significant responsibility – financial and/or physical – to producers for the treatment or disposal of post-consumer products. Since the first implementation in the early 1990s for beverage packaging, EPR has significantly improved packaging recycling rates in EU, and it is thought to be a key instrument and one of the bases moving towards a CE (Leal Filho et al., 2019). However, to ensure the transition to a sustainable CE, the EPR

framework needs to be redesigned and to include ways to reduce plastic waste. There are many possibilities and new means to make it more efficient and effective, for example, increasing harmonization, extending to more products, improving separate collection and treatment of wastes, and so on (Leal Filho et al., 2019).

3. Plastic recycling

3.1. Mechanical recycling

The most common way to recycle plastic waste is mechanical recycling which typically involves collection, sorting, washing, and grinding of the materials (Al-Salem et al., 2009) as demonstrated in Fig. I-2. However, these steps can be applied in different order, multiple times or not at all, in the industry (Ragaert et al., 2017).

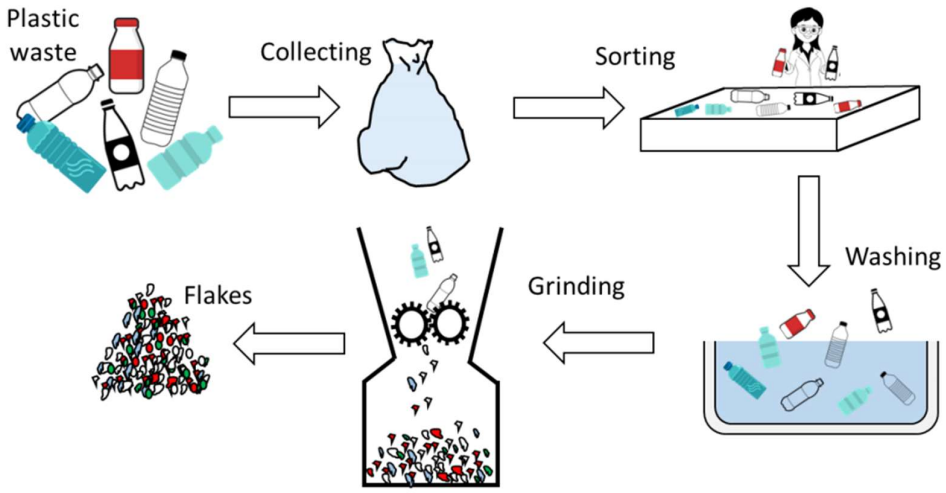


Fig. I-2 Typical mechanical recycling process (https://ubuntoo.com/blogs/mechanical-recycling-for-dummies, accessed on 26th March 2021)

3.1.1 Collection

Collection can be achieved by kerbside collection as well as deposit scheme. In kerbside collection systems, materials are comminglingly collected, but details about which materials can be placed in a same container could vary depending on the countries. For instance, in Spain, plastic materials are collected with TetraPak and metal cans in

yellow containers. The mixed materials are then sent to sorting plants for further separation by various sorting technologies. In contrast, in the deposit scheme, for example the well-known "Pfand" system, which was first launched in Germany in 2003, one-way beverage packaging with a filling volume of 0.1 to 3 litres is labelled with a uniform logo, a readable barcode, and the amount of deposit (€ 0.25) (Fig. I-3 A). Consumers can then return one-way with the deposit after consumption in any supermarket, petrol station or one of the almost 40000 special return machines (Fig. I-3 B) in Germany (Pfand, 2021). This way, everyone is incentivised to keep packaging in circulation, and out of the landfill. Moreover, materials collected by this way can be cleaner without contaminations from non-food grade materials, which is beneficial to recycle them into new food packaging, especially in the case of plastic packaging.

Fig. I-3 Labels in the packaging (A) and the return machine (B) in "Pfand" recycling system (from Internet)

3.1.2 Sorting

Depending on input materials as well as the sorting plants, various sorting schemes can be applied. An example is demonstrated below in Fig. I-4. In this example, the mixed waste (plastics, cans, and drink cartons) is firstly kerbside collected and delivered to the sorting plant. The waste is then sorted by a rotary sieve based on size, followed by a wind sifter to blow out loose paper etiquettes and plastic bags. Subsequently,

ferrous metal, carton, and non-ferrous metal are sorted by overhead magnet, optical sorter, and eddy current, respectively. From the large fraction, all foils are removed by another ballistic separator. The remaining mixed plastics are separated by Fourier Transform Near Infrared (FT-NIR) and optical colour recognition. Finally, manual sorting are applied to correct any automated mistakes by well-trained operators (Ragaert et al., 2017). In other cases, float-sink separation can be employed as well.

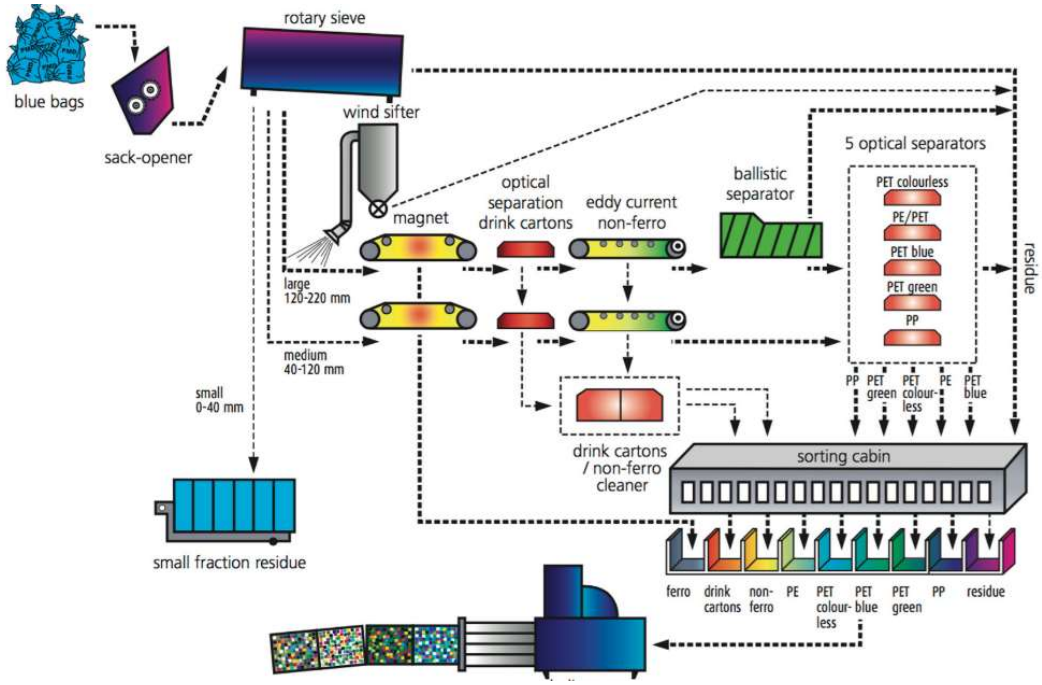


Fig. I-4 Sorting of plastics (bottles and fluid containers), metals (cans), and 'Drinkkarton' (TetraPak) waste (Ragaert et al., 2017)

3.1.3 Washing

Conventional recycling processes usually involve water-based washing to clean up surface contaminations such as dust, food residues, labels, and glue. Typical washing additives are caustic soda in concentrations of 2-3% as well as detergents. Washing can be applied directly to the recycled plastics before or after grinding into flakes. It can be either cold or hot washing, but anyway, the conditions applied are not high enough to depolymerise the polymer. Hence, the washing mainly happens on the surface of the polymer (Welle, 2011).

3.1.4 Closed- and open-loop recycling

Depending on whether the recycled plastics are used to produce their original products or not, mechanical recycling can be distinguished into closed- and open-loop recycling (Hahladakis and Iacovidou, 2019). Nevertheless, these terms are basically neutral and open-loop does not necessarily means that the new application is of lower "value", e.g., manufacturing textile fibres from recycled bottle-PET (Ragaert et al., 2017). Of course, that means we still need virgin PET for producing PET bottles.

3.2. Chemical recycling

Apart from energy recovery and mechanical recycling, chemical recycling has attracted growing interest. In this process, recycled plastics can be reverted to monomers or petrochemicals, which can then be used to re-manufacture virgin materials, by different types of technologies such as gasification, pyrolysis, fluid-catalysed cracking, hydrocracking, and so on (Ragaert et al., 2017). In 1991, the first chemical recycling process for post-consumer PET for direct food contact applications has been approved in US (Welle, 2011). While technically viable, it is less economic and has higher environmental profile compared to mechanical recycling (Hopewell et al., 2009; Shen and Worrell, 2014). Hence, chemical recycling has high potential for heterogeneous and contaminated polymers, where separation is either not economically feasible or not technically viable (Al-Salem et al., 2009; Ragaert et al., 2017).

4. Mechanical recycling of plastics for food contact applications

4.1. Plastics production and waste generation in packaging sector

As shown in Fig. I-5, 35.9% of primary plastics (virgin) produced were used for packaging and 46.7% of primary plastics waste came from packaging sector, which corresponded to 146 and 141 million tonnes, respectively, in 2015 at a global level (Geyer et al., 2017). This data illustrates the significance of plastics used in packaging in the context of CE. Moreover, most of the packaging are single-use meaning that they are disposed of very quickly after only a short period of use. As such, it is of great

importance to reduce and recycle plastic packaging, and therefore mitigate their negative effects on the environment and step forward to a CE.

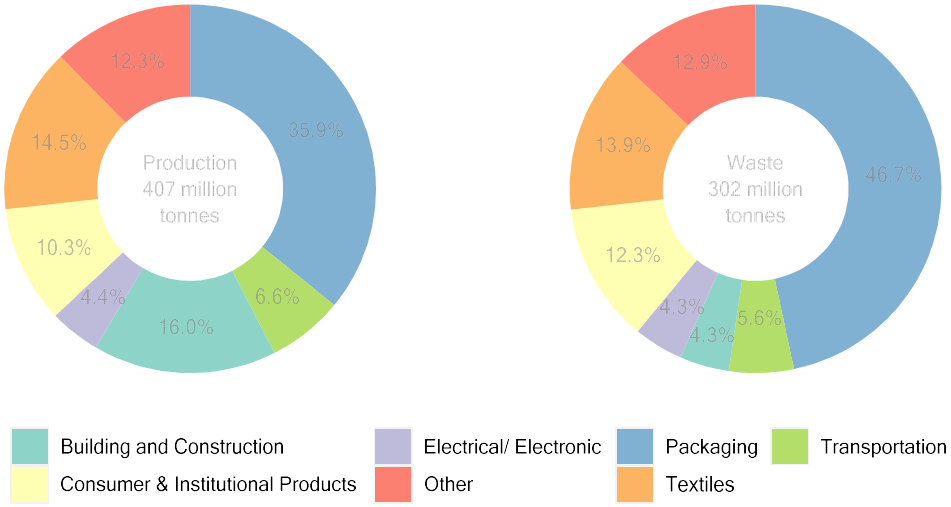


Fig. I-5 Global primary plastics production and primary waste generation in 2015 according to industrial use sector, data from (Geyer et al., 2017)

The main challenge of mechanical recycling arises from normally poorer mechanical properties of the recycled plastics because of thermal-mechanical and lifetime degradation as well as inclusion of immiscible plastics during the recycling process (Ragaert et al., 2017). In the last decades, there is significant progress in sorting technology though, it is still challenging to thoroughly separate a polymer from others in post-consumer waste. Chemical contamination in the recycled plastics represents another obstacle for their applications in some products as these chemicals might endanger consumer health. Especially for recycling food-grade plastics for new food packaging applications, contaminants present in recycled materials might not only alter the taste of the contacting food, but might also endanger human health by migrating into the food (Geueke et al., 2018).

4.2. Potential contaminants in recycled plastics

Additives are commonly used to improve the properties of the plastics during production, for instance, antioxidants, ultraviolet (UV) stabilizers, and processing aids,

which are also named intentionally added substances (IAS). Hence, it is not surprised to have some of them in the recycled plastics. Non-intentionally added substances (NIAS), for examples, oligomers unintentionally formed because of the incomplete reaction of monomers during plastic production, impurities and degradation products of IAS during use or recycling phases, can be present as well (Geueke, 2013; Geueke et al., 2018; Horodytska et al., 2020; Nerín et al., 2013). Food residues from previous uses like limonene, 1,8-cineole, γ-terpinene and p-cymene were frequently detected in recycled plastics (Dutra et al., 2011; Huber and Franz, 1997; Strangl et al., 2021). They might not pose risks to human health as they are widely used in food and can be efficiently removed by recycling process including additional cleaning steps (Geueke et al., 2018).

To recycle plastics for food-contact uses, it is of great importance to assure high purity of input materials. That is to say, very high percentage of the input materials must come from FCMs. In the case of recycled PET (rPET), after sorting, a minimum of 95% of PET originated from food-contact uses must be achieved (EC, 2008; FDA, 2006). However, post-consumer plastic wastes are normally collected in a commingle way, which are then separated in the sorting plants. As such, recycled plastics from previously food-contact uses can be contaminated by those from non-food-contact uses via cross-contamination and/or inclusion of those plastics in the final products due to insufficient sorting (EC, 2008; FDA, 2006). After consumption, plastic containers can be used to hold various stuffs, which is called misuse. The misuse could introduce a wide variety of contaminants, for example, pesticides or automotive chemicals, which is unpredictable. Moreover, diverse adjuvants can be added to offset any physical property loss during recycling process, but their uses have to comply with regulations for FCMs (EC, 2008; FDA, 2006).

4.3. Legislations and guidelines

To better protect consumers from potential harms caused by FCMs, different regulations have been enacted across the world stipulating the manufacture and application of FCMs. In Europe, there is a framework regulation (EC) No 1935/2004

served as a general principle for all types of food contact materials and a Commission Regulation (EU) No 10/2011 with 16 amendments nowadays that particularly deal with plastic FCMs. Whereas, in United States (US), FCMs are regulated together with food under the Code of Federal Regulations (CFR) Title 21, in which plastic additives in FCMs are regarded as indirect food additives. In China, there is a serial of regulations pertinent to FCMs, including but not limited to GB 4806.1-2016 as a general rule for FCMs, GB 9685-2016 on the use of additives, and GB 4806.7-2016 on plastic FCMs. Additionally, regulations on good manufacturing practice (GMP) were enacted to make sure consistent production and high-quality control in all the above-mentioned countries and regions, e.g., Commission Regulation (EC) No 2023/2006 in Europe.

Concerning the use of recycled plastic for food contact applications, the US FDA published a guideline named "Points to Consider for the Use of Recycled Plastics in Food Packaging: Chemistry Considerations." for the approval of post-consumer recycled plastics (in general) in 1992, which recommended to employ the so-called challenge test for the determination of cleaning efficiency of a recycling process using artificial contaminants (surrogates), considering the question of risk in a probabilistic way rather than on a compound-by-compound basis. The guideline was then updated as "Guidance for Industry: Use of Recycled Plastics in Food Packaging (Chemistry Considerations)." in 2006, which does not establish enforceable responsibility (FDA, 2006). However, plastic recyclers who intend to manufacture food grade plastics are invited to submit dossier about their recycling process based on the guidance to FDA for evaluation and comment. FDA will then give a positive opinion, the so-called no objection letter (NOL), or negative opinion depending on the assessment of characteristic of the input, sorting efficiency, the efficacy of decontamination, as well as intended applications of the recycled plastics (FDA, 2006).

The FDA suggests using substances of a variety of chemical and physical properties as surrogates considering both volatility and polarity. Below lists surrogates in each category, but one surrogate from each category is thought to be sufficient for testing (FDA, 2006):

- **Volatile polar substances:** chloroform, chlorobenzene, 1,1,1,-trichloroethane, diethyl ketone.
- **Volatile non-polar substances:** toluene.
- **Heavy metal:** copper(II) 2-ethylhexanoate, not for PET based on data from the last decade.
- **Non-volatile polar substances:** benzophenone, methyl salicylate.
- **Non-volatile non-polar substances:** tetracosane, lindane, methyl stearate, phenylcyclohexane, 1-phenyldecane, 2,4,6-trichloroanisole

In contrast, EC has enacted a Commission Regulation (EC) No 282/2008 to address this issue. Nevertheless, the principle is similar. Recyclers, who want to recycling plastics for food contact uses, have to submit dossier about their recycling processes in accordance with the guideline (EFSA, 2011) to European Food Safety Authority (EFSA). EFSA will then evaluate the whole process and give an either positive or negative opinion on the process. Based on the opinion given by EFSA, EC should then take the final decision on the authorization (EC, 2008). At the time of writing, EFSA has given positive safety assessments to over 140 recycling processes, but EC has not yet authorized any of them (De Tandt et al., 2021).

Similarly, both FDA guideline and EU regulation exempt cases of primary (closed and controlled chain) and tertiary recycling (chemical recycling), as well as using an effective barrier between contacting food and recycled plastics from mechanical recycling as they are thought to be of negligible risk (EC, 2008; FDA, 2006). For many other countries, there is still lack of regulation or guidance addressing this issue, for instance, China, Korea, and Thailand. Nevertheless, they may have measures in place in the foreseeable future under the global trend of CE.

4.4. Recycling PET beverage bottles as new FCMs

PET is a polyester manufactured from terephthalic acid and ethylene glycol by polycondensation reaction. Owing to high clarity, light-weight, and unbreakable properties as well as excellent barrier function against moisture and oxygen, PET has been widely used to make bottles for soft drinks, mineral water, and many other beverages (Welle, 2011).

4.4.1 Advantages of PET for recycling

In the early stage, as the demand of PET bottles and the recollection of post-consumer PET bottles continuously increased as well as growing ecological concerns on littering and carbon footprint, recycling PET bottles had become more and more viable. At the beginning, PET recyclates were mainly used for polyester fibre production. As recycling amount grew, however, traditional PET recycling markets could not consume all of them, which enforced the development of recycling processes for new PET beverage bottles. Except for the significant amount, there are additional reasons that make PET bottles to be nearly the ideal input material for bottle-to-bottle recycling, such as (Welle, 2011):

- Ease of separation from other waste.
- Easy removal of non-PET caps and labels as well as coloured PET.
- Few additives needed and thus no need to control additive status in the PET recyclates.
- Minimal contaminations from printing inks.
- Very inert nature of PET.
- Food grade quality of all PET raw materials for food and non-food packaging applications.

- Compensation of properties loss by rebuilding polymer chains with the help of solid state polycondensation (SSP) or similar reaction but requiring no additional catalysts as the remaining polymerisation catalysts are still active during recycling.
- Low absorption capacity of PET and thus, few contaminants trapped in the polymer.
- Low diffusion coefficients through PET.

4.4.2 Super-clean recycling processes

As it is pointed out in section 3.1.3, traditional washing processes are capable of removing contaminants from the surface of the polymers but not substances that have been absorbed into the polymer. Contaminants like flavour substances from soft drinks (e.g., limonene) are still detectable after conventional washing steps (Franz et al., 2004). Hence, recycled PET after traditional washing is still insufficient for direct food contact applications. To meet the high-quality requirements for food contact uses, several so-called super-clean technologies have been developed for deep cleansing the PET after conventional washing, achieving contamination levels similar to virgin PET. They typically involve high temperature treatment, vacuum or inert gas treatment, and surface treatment with non-hazardous chemicals, for example, PET super-clean recycling processes based on pellets/flakes (Welle, 2011). In Europe, the first commercial "super-clean" PET recycling plant was established in 1997 (Franz and Welle, 2020).

Until now, a number of PET super-clean recycling processes have been approved by EFSA or FDA (Bradley et al., 2008). In 2016, 59.8% of PET bottles and containers placed in the European market - in total 1.88 million tonnes - were collected, and 1.77 million tonnes were mechanically recycled (Hansen, 1997). According to Petcore Europe, about 25% of total rPET was used for producing new FCMs such as egg containers and other preformed plastic boxes. Other food contact applications of rPET include containers for water, soft drinks, juices (Petcore, 2021). The super-clean rPET can be mixed with virgin PET up to 100%.

4.5. Potential of recycling polyolefins as new FCMs

4.5.1 Polyolefins in packaging

Polyolefins is a family of polymers fabricated from unsaturated aliphatic hydrocarbons, which include PE with various density as well as PP. PEs have good processability and excellent water vapor barrier but low oxygen barrier property. LDPE and LLDPE are mainly produced as films as they are soft, flexible, and stretchable, with very good thermosealability at quite low temperatures. They are widely used for bakery products, frozen foods, stretching/cling films, heat-sealing layer of multilayer packaging, and so on (Novák et al., 2016). With higher crystallinity, HDPE provides good barrier against gas and water as well as high stiffness and hardness (Novák et al., 2016). Therefore, it can be used in many forms including bags, bottles, caps and containers. PP has relatively low transition temperature (T_g), moderate melting temperature (T_m), and good oil and chemicals resistance. It is used for a wide variety of food ranging from cold to heat-treated food, including microwaveable products in either flexible or rigid forms (Novák et al., 2016). As depicted in Fig. I-6, polyolefins are the most widely used polymers especially in the field of packaging. They account for more than a half of the plastic demand in the packaging sector in Europe.

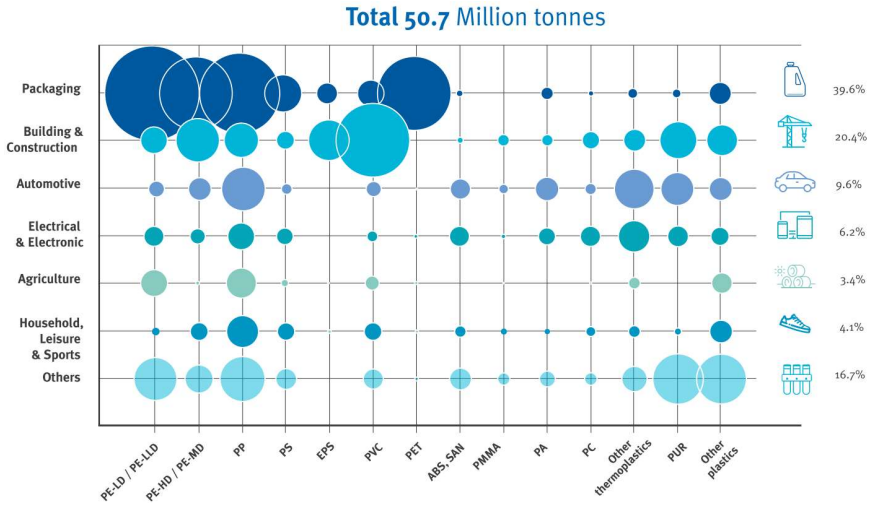


Fig. I-6 Plastic demand by segment and polymer type in 2019 (PlasticsEurope, 2020).

4.5.2 Challenges of recycling polyolefins as new FCMs

As discussed in 4.4, closed-loop recycling of PET beverage bottles has been available for decades owing to its excellent properties as well as the rapid development of the so-called super-clean recycling processes. However, other plastic FCMs are rarely recycled in a closed-loop manner. The majority of them can only be downcycled for non-food contact applications as they can be contaminated in an unpredictable way, and they are still difficult to be sufficiently decontaminated. Contaminants present in the recycled plastics may bring about unwanted organoleptic changes to the contacting food but may also pose potential risks to the consumers by migrating toxic substances. There are few recycled polyolefins (HDPE and/or PP crates/trays) approved for contact uses by EFSA (EFSA, 2012, 2013, 2014, 2018), but all of them have input materials under closed and controlled product loops meaning that they are not contaminated and therefore should not represent safety concerns.

The main difference between PET and polyolefins is that PET is a glassy polymer at room temperature and in most of the use conditions, while polyolefins are rubbery. For a given chemical, its diffusion coefficient is several orders of magnitude lower in PET than that in polyolefins (Palkopoulou et al., 2016). Higher diffusion means easier absorption of contaminants from previous uses/misuses and from other materials during the recycling processes. At the same time, it also means that these contaminants can migrate out easier into the contacting food. It is shown that for a given contaminant with a certain molecular weight (MW), its critical concentration is about two orders of magnitude larger for PET than that for PP (Palkopoulou et al., 2016). Moreover, stabilizers, e.g., Irgafos 168 and Irganox 1076, which are intentionally added to polyolefins to protect them from oxidation, result in several oxidized compounds. Hence, new reaction products, which are known as NIAS, can be formed from these stabilizers during polymer life cycle and recycling processes. Their migrations into the packaged food is well-known (Nerín et al., 2013; Vera et al., 2018). Taking all these into account, it can be concluded that the approaches used for PET cannot be extrapolated as such to polyolefins (Palkopoulou et al., 2016).

5. Analytical methods for determining chemicals present in FCMs

5.1. Target and non-target screening (NTS) analysis

5.1.1 Target analysis

As the name suggests, attention is paid to specific substances, which are the targets, in target analyses. The targets can be a class of chemicals or a series of different compounds that are of particular concerns to the analysts. For a given type of FCM, some compounds can be expected as they are common IAS in that material, e.g., phthalates esters in PVC, bisphenol A in polycarbonate, etc. Therefore, target analysis has been widely employed in the field of FCMs for the analysis of IAS. There were various analytical methods developed focused on both volatile and non-volatile chemicals, for example, phthalate esters (Yang et al., 2017) and epoxidized soybean oil (ESBO) (Choi et al., 2018) based on gas chromatography coupled to mass spectrometry (GC-MS), bisphenol-type contaminants applying liquid chromatography and fluorescence detector (Nerín et al., 2002), various antioxidants, UV stabilizers, phthalates as well as photo initiators employing liquid chromatography and photodiode array detector (Li et al., 2015), and aromatic amines based on liquid chromatography coupled to mass spectrometry (LC-MS) (Aznar et al., 2009; Pezo et al., 2012).

Since the targets are previous known and the number of targets is normally small in the context of a certain study, analysts are able to optimize the sample preparation as well as analytical parameters based on the properties of the analytes. Hence, higher sensitivity can be achieved such as using target-oriented selected ion monitoring (SIM) instead of scan mode in mass spectrometry. In a target analysis, reference standards are normally available, which enable accurate quantification of all targets. As the structures of the targets are already known, no structural elucidation is required in target analysis, and various detectors are suitable for this purpose. For instance, flame ionization detector (FID), which is cheaper than mass detector, can be coupled to gas chromatography for most of the volatile compounds like mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) (Pack et al.,

2020). For non-volatile compounds, UV, fluorescence, photodiode array detectors, etc., can be used (Li et al., 2015; Nerín et al., 2002).

5.1.2 Non-target screening analysis

NIAS are well-known to be present in almost all FCM. They can be impurities, degradation products, side products, and contaminants in the case of recycled materials (Geueke, 2013; Groh et al., 2019; Nerín et al., 2013). Some NIAS are already known to the community, e.g., oxidized Irgafos 168, 2,4-di-tert-butylphenol, and nonylphenol (Geueke, 2013), while some could be difficult or even not possible to be predicted, which pose quite a significant challenge to the analyst. In this sense, target analysis is insufficient since we do not even know what could be present in the materials. As a complementary, NTS was proposed, which aims to identify as many chemicals present as possible.

In contrast to target analysis, NTS requires structure elucidation. Mass spectrometry, which is normally coupled to a separation system such as GC and LC, is the most used technique for this purpose in the field of FCM (Sanchis et al., 2017). Fragment ions together with their relative intensities present in mass spectra provide rich information about the substructures and therefore enable us elucidating the structure of the detected compounds. Another method of choice is nuclear magnetic resonance (NMR) spectroscopy which is also generally used in metabolomics (Stanstrup et al., 2019) but not in FCM area. In most cases, we do not know which chemicals could be present. Consequently, it is quite challenging, if not impossible, to optimize sample preparation and instrumental conditions that work for all compounds. Chemicals with a wide variety of structures can have significantly different properties and therefore have distinct optimal conditions to be detected. Hence, there is still no NTS method that fits all situations. In practice, different laboratories could have their own preferences for this purpose. Moreover, many NIAS are not commercially available making it challenging to confirm their identifications and to accurately quantify them.

5.2. Ways of analysing chemical composition of plastic FCMs

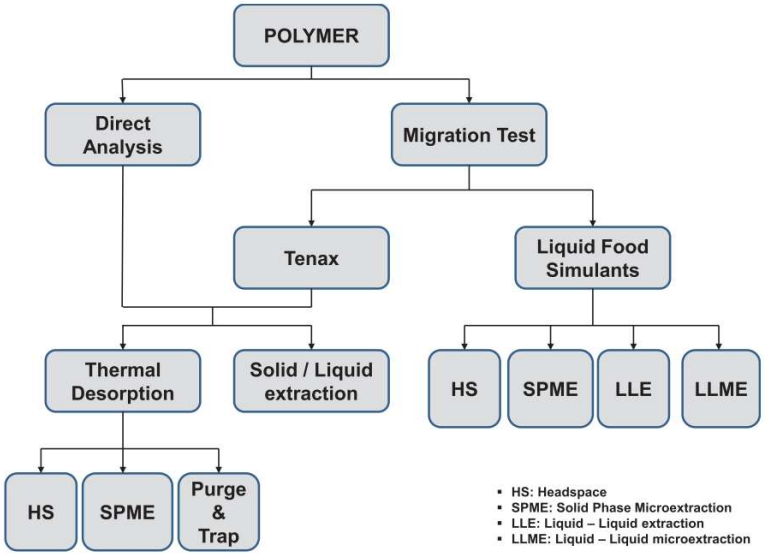


Fig. I-7 General scheme for sample treatment procedures (Nerín et al., 2013).

Plastic additives are generally uniformly dispersed in the polymer matrix (Zhang et al., 2020) but not chemically bonded to the polymer. The chemical composition of plastics can be analysed either by direct analysis or migration test as indicated in Fig. I-7.

5.2.1 Direct analysis

In direct analysis, samples are normally cut into small pieces or milled to powders prior to analysis. The smaller the size of samples, the better the extraction efficiency as smaller particles have higher surface to volume ratio and thus enhance the release of compounds from the samples. To this end, milling to powders, if available, can be a better option and it is recommended in order to increase the surface of the polymer and facilitates either the extraction or dissolution. In the case of plastics, which are quite tough, cryogenic treatment can be needed for the milling procedure.

Once the samples are ready, they can be heated up and the compounds could desorb from the polymer, which is called thermal desorption. It is noteworthy that the temperature cannot be too high because it might break down some additives and the

polymer, thus bringing new compounds which might not be present under normal use conditions. The released compounds can be then captured by various techniques such as headspace (HS) (Dutra et al., 2011; Franz et al., 2004), solid phase micro-extraction (SPME) (Nerín et al., 2009), and purge & trap (Skjevrak et al., 2005), and subsequently transferred to analytical instruments for determination. These procedures can be done automatically and are well-connected to GC-MS nowadays. The advantage of thermal desorption is that it requires no sophisticate sample treatment (Nerín et al., 2013). Nevertheless, the downside is that thermal desorption is mainly suitable for volatile compounds since semi-volatile and non-volatile compounds are difficult to escape from the polymer under the applied thermal conditions. As a result, the potential is limited and normally GC is used for the determination. For example, the GC-FID method developed for the determination of contaminants in post-consumer recycled PET by Franz R. was capable to detect chemicals with MW up to about 300 Da (Franz et al., 2004).

Unlike thermal desorption, solid-liquid extraction (SLE) is not limited for volatile compounds, but also suitable for non-volatile substances. Soxhlet (Fernandes et al., 2008), microwave-assisted extraction (MAE) (Camacho and Karlsson, 2000), accelerated solvent extraction (AES) (Li et al., 2015), and ultrasound-assisted extraction (UAE) (Moreta and Tena, 2014) can be used in this regard employing various solvents such as dichloromethane, hexane, and acetonitrile. Polymer structure is normally unchanged after the above-mentioned extractions. Alternatively, total dissolution can be applied as well. In this methodology, the polymer is firstly dissolved by a suitable solvent, which will release all compounds entailed in the polymer out. Afterward, another solvent is added to precipitate the polymer while the compounds remain in the supernatant liquid. Dissolution solvents vary depending on the type of polymer. For example, 1,1,1,3,3,3-Hexafluoro-2-propanol was used for PET (Ubeda et al., 2018) and hot o-xylene (Green et al., 2010), or hot toluene (Salafranca et al., 1999a) was applied for polyolefins while chloroform was employed for polycarbonate (PC) (Bignardi et al., 2014). Methanol was used for precipitation in all cases. Higher extraction efficacy was reported for total dissolution compared to UAE (Ubeda et al., 2018).

Direct MS analysis can be applied directly to polymer samples as well, for instance, atmospheric solids analysis probe (ASAP), directly analysis in real time (DART), and desorption electrospray ionization (DESI). Nonetheless, they are mainly used for confirmation but are not suitable for NTS because no separation steps are applied and therefore all substances are ionized together making it extremely difficult to interpretate based on merely MS fragments without having previous experience and knowledge of potential compounds (Nerín et al., 2013).

5.2.2 Migration test

Migration test using real food or various food simulants is less rigorous in comparison to SLE described above as it is not aimed to extract all substances present in the polymer. It uses less aggressive solvents which are well defined food simulants, and the polymer is not cut into tiny pieces. Thus, it is not unexpected that there will be more substances detected in SLE than in migration under the same analytical conditions. For this reason, it is a common practice to untargetedly screen compounds in the extract and then quantify those of interest in the migrates aiming to have higher sensitivity (García Ibarra et al., 2018; Hu et al., 2021; Ubeda et al., 2018). One of the disadvantages is that not all compounds detected in the extract will migrate into food simulants. It might not deserve to identify these non-migrate substances as there will not be exposure, especially in the case that no confident library hit can be achieved, and thus challenging and labour-intensive structural elucidation is required. Moreover, some compounds may only be present in migration since food simulants cover wider polarity range than extraction solvents (in most cases, only one solvent is selected for extraction), and some migrants may undergo transformations in particular food simulants. A good example is that some migrants will be hydrolysed and transform into new substances in water-based simulants (Singh et al., 2018; Úbeda et al., 2017). The neoformed substances are relevant to consumer health but they might not be detected in the extracts using organic solvents.

Food simulants used for the migration test as well as assay conditions (temperature and time of contact) are detailed in the Regulation 10/2011 (EC, 2011). After migration

test, the simulants are analysed using in each case the appropriate analytical techniques. Simulants 10% ethanol, 20% ethanol, 50% ethanol, 3% acetic acid, and 95% ethanol can be directly injected into LC systems but only the last one is compatible with GC instruments. To have higher sensitivity or to make it compatible (in the case of GC instruments), various extractions, including HS, SPME, liquid-liquid extraction (LLE), and liquid-liquid microextraction (LLME), can be applied to the liquid food simulants prior to instrumental analyses. Solid food simulant (Tenax) can be analysed in ways similar to the polymer as illustrated in Fig. I-7. As for real food migration, NTS is much more challenging because most of the food have complex constituents which further complicates sample treatments as well as data processing.

5.3. Identification by mass spectrometry

5.3.1 Volatile compounds

The most used technique for identifying volatile substances is GC-MS interfacing with electron ionization (EI). The application of a consensual ionization energy (70 eV) allows us to have reproducible mass spectra regardless of equipment applied and thus facilitates the comparison of generated spectra with library spectra. Nowadays, the latest commercial low resolution EI-MS libraries Wiley Registry 12th and NIST (National Institute of Standards and Technology) 2020 have more than one million nominal spectra covering 843000 unique compounds. These commercial libraries are powerful for the identification of volatile substances. There are also EI-MS libraries which are accessible and downloadable for the public, for example, the one compiled by RIKEN Centre for Sustainable Resource Science: Metabolome Informatics Research Team (http://prime.psc.riken.jp/compms/msdial/main.html#MSP) or by MassBank of North America (MoNA) (https://mona.fiehnlab.ucdavis.edu/downloads). These publicly available libraries encompass limited number of spectra though, they can be merged with existing library to expand the coverage, especially for those who do not have latest version of commercial library.

These experimental libraries are of great help for identifying the majority of volatile migrants, but there are still cases where existing libraries cannot help, for example, some emergent NIAS might not be present in any library. The coverage of the libraries can be expanded by predicting EI-MS spectra of existing molecules present in various structure database, e.g., Pubchem which has over 102 million compounds recorded (Wang et al., 2020). There are several commercial packages designed for this purpose including Mass Frontier (Thermo Scientific), MS Fragmenter (ACD Laboratories), and MOLGEN-MS (Kerber et al., 2006). They all produce so-called "bar-code" spectra, in which all predicted peaks have same height (Allen et al., 2016). In the last decade, EI-MS spectra prediction has evolved with various prediction models developed such as competitive fragmentation modelling for electron ionization (CFM-EI), quantum chemical electron impact mass spectrum (QCEIMS) (Spackman et al., 2018), and Neural electron-ionization mass spectrometry (NEIMS) (Wei et al., 2019) with the aim to have higher prediction accuracy and shorter prediction time. However, their potentials have not yet been widely evaluated in real applications and predicted EI-MS spectra of large dataset are still missing. Besides, library expansion by spectra prediction is limited by existing structure databases making it helpless for emergent NIAS.

Conversely, structure elucidation can also be done by the so-called "top-down" workflow, that is to predict molecular fingerprints, e.g., substructures, of unknowns from their EI-MS spectra, for instance, MetExpert (Qiu et al., 2018) and DeepEI (Ji et al., 2020). However, none of them have been applied to real sample analyses, possibly because they are still not accurate enough and further development is required.

A more popular approach to elucidate volatile unknowns is the use of soft ionization, mostly atmospheric pressure chemical ionization (APCI), and high-resolution mass spectrometry (HRMS), e.g., time-of-flight (TOF), Orbitrap, and ion trap, hyphenated to a quadrupole, e.g., APGC-QTOF-MS (Cherta et al., 2015; Onghena et al., 2015; Osorio et al., 2019). This approach enable us to obtain the molecular ion or the protonated molecule, as well as product ions with high mass accuracy, which is of great help for structural elucidation (Stettin et al., 2020). Data processing of this type of

data will be discussed below in 5.3.2 as they are essentially the same. With respect to NTS, this approach may not be used alone but as a complementary tool to normal GC-MS, since many of the volatile substances can be easily identified by library matching in GC-MS and thus require no structural elucidation which is still challenging and time-consuming. In this regard, the question turns out to be how to correlate peaks in both systems, because they do not have same retention behaviour even applying the same chromatographic conditions (Cherta et al., 2015; Onghena et al., 2015; Osorio et al., 2019). Moreover, the two systems can have significantly different chromatograms. HRMS normally have high sensitivity, but some compounds, such as hydrocarbons, are poorly ionized under soft ionization. The difficulty in correlating peaks in the two systems may hinder us from selecting truly unknowns that require subsequent structural elucidation. Once correspondence is found, APGC-HRMS can also be used to rank candidates in GC-MS library matching by evaluating precursor ions, which somehow add confidence to the identification, but still unable to differentiate isomers.

GC-MS/MS and GC-HRMS with EI source are thought to have notably higher sensitivity than conventional single quadrupole GC-MS due to their higher mass-analyser efficiency (Hernández et al., 2011). The latter one also enables us to partially resolve ambiguous annotation in library matching by evaluating elemental compositions of product ions with the help of specific software (Cherta et al., 2015). In terms of increasing identification confidence in GC-MS library matching, retention index (RI), also named Kovats index, is well-known and widely used in various research areas since it is cheap, easy-to-use, reproducible regardless of the equipment employed. There are a number of compounds that have experimental RI values based on different column polarities, but still, some of them are missing. To tackle this problem, various RI prediction models have been developed (Matyushin et al., 2019; Matyushin and Buryak, 2020; Vrzal et al., 2021). When a new NIAS, which normally does not have reference standard available, is tentatively identified, predicted RI provides an additional parameter for confirmation.

5.3.2 Non-volatile compounds

LC-MS is the most popular tool for determining non-volatile substances. There are several ion sources available for LC-MS systems, including electrospray ionization (ESI), APCI, atmospheric pressure photo-ionization (APPI), matrix-assisted laser desorption/ionization (MALDI), and direct-EI LC-MS interface, with ESI is currently the most common one (Segers et al., 2019). Under soft ionizations, precursor ions are preserved leaving minimized fragmentations. To have more substructure information from product ions, tandem mass (MS/MS) is implemented, in which collision-induced dissociation (CID) or higher energy collisional dissociation (HCD) of the even electron ions produced by soft ionizations will occur under high collision energy (Kind et al., 2018; Stettin et al., 2020). Either multiple or ramp collision energy can be applied. The former one give rise to multiple MS/MS spectra, while the latter one has only one spectrum.

Differing from GC-MS, LC-MS/MS spectra of a given compound vary significantly from instrument to instrument in terms of both fragment ion content and the relative abundance of ions formed, which makes creation of reproducible spectra libraries for all instrument types not a trivial task (Bristow et al., 2002). For this reason, there were no commercially available LC-MS/MS libraries for a long time until 2005 when NIST added a MS/MS library to the NIST 2005. The library was built on a variety of tandem quadrupole and ion trap mass spectrometers and contains 5191 spectra of 1943 unique compounds. The latest NIST 2020 library already contains 1320000 MS/MS spectra of 30000 unique compounds. Metlin and mzCloud libraries are also commercially available, but the former one is also searchable through an online platform (https://metlin.scripps.edu/landing_page.php?pgcontent=mainPage). LC-MS/MS library has two characteristics that are different from EI-MS library. Firstly, each MS/MS spectrum is linked to a precursor ion which is important for library matching. MS/MS library matching is precursor-oriented, which means that it compared and scores only MS/MS spectra that have a same precursor with a user-defined mass tolerance. Secondly, a MS/MS library can be either positive or negative based on the

polarity applied when acquiring the spectra. Hence, they should be chosen accordingly for library matching.

With the development of HRMS, LC-HRMS is becoming increasingly popular for NTS of substances that are not amenable to GC-MS. In the last decade, a number of downloadable high-resolution MS/MS libraries are accessible to the public, such as MoNA, RIKEN, GNPS (Wang et al., 2016), and so forth. Notably, all these libraries mainly focus on metabolomics and there is no FCM- or plastic-specific libraries available yet. As such, their value to the FCM area is still unknown but deserve to be further explored. In comparison to commercial libraries, these open libraries could have relatively lower quality as spectra uploaded by various contributors are not scrutinized, but anyway, false annotation rate of reference standards could be low. On the other hand, contributors are using various equipment under different conditions making the libraries feasible for more users with different equipment in place. Importantly, they are viable for all and thus are valuable sources for the public.

Although experimental MS/MS libraries are continuously growing, the number of compounds in the libraries is still limited compared to that in structure databases such as Pubchem and Chemspider. There are some in-silico MS/MS libraries that can be used directly for library matching, such as MoNA and human metabolome database (HMDB). Alternatively, structural elucidation can also be done by several tools based on tandem HRMS and in-silico MS/MS fragmentation. Examples of this type of tools include publicly available MAGMa (Ridder et al., 2014), CSI:FingerID (Dührkop et al., 2015), MetFrag (Ruttkies et al., 2016), MS-FINDER (Tsugawa et al., 2016), CFM-ID (Djoumbou-Feunang et al., 2019), SIRIUS4 (Dührkop et al., 2019), and MolDiscovery (Cao et al., 2020), to name a few. Proprietary tools from instrument manufacturers are also available accompanied with the equipment such as Masslynx and UNIFI from Waters, MassFrontier from ThermoFisher, and MassHunter Workstation from Agilent.

Molecular formula (MF) assignment is the first and a crucial step during structural elucidation (Ljoncheva et al., 2020). The precursor can be an adduct ion, such as $[M+H]^+$, $[M+Na]^+$, $[M+NH_4]^+$, $[M+K]^+$, $[2M+H]^+$, $[2M+Na]^+$, $[2M+NH_4]^+$, and

$[2M+K]^+$ in positive mode, and $[M-H]^-$ and $[2M-H]^-$ in negative mode. To have a correct MF assigned, we first need to determine which is the precursor ion and what is the adduct. In most cases, but not all, the most abundant ion in the low energy spectrum (MS1) is the precursor. Molecular ion can be determined, for example, by calculating mass differences between adducts when multiple adducts are formed simultaneously. In some cases, a compound can be ionized in both positive and negative modes, precursor ions in both modes are other useful information for determining molecular ion. With molecular ion assigned, MF can then be determined. Conventionally, all possible MFs for a given accurate mass are calculated with restrictions by user-defined element type, atom number, mass tolerance, and isotope pattern, which is a computationally intense procedure. In the field of FCM, C, H, N, O, and S can be the most common elements (Nerín et al., 2013), while P, Cl, F, and Br could be present as well. The higher the mass, the more possible candidates, but higher mass accuracy will decrease the number of candidates. Chemical rules such as double bond equivalent (DBE), ring double bond equivalent (RDBE), the nitrogen rule, LEWIS and SENIOR rules, and the element ratio check are most frequently applied to rule out chemically illogical candidates MFs (Ljoncheva et al., 2020). To further rank the candidates, fragmentation pattern from MS/MS spectrum (Dührkop et al., 2019; Tsugawa et al., 2016), heuristic rules such as the Seven Golden Rules (Kind and Fiehn, 2007) and hydrogen rearrangement rules (MS-FINDER) can be implemented as well.

Once MF is assigned, structure annotation can be done by one of the in-silico MS/MS tools mentioned above, which can be either rule-based such as MS-FINDER and MassFrontier (Ljoncheva et al., 2020) or machine learning-based (many others). As an example, MS-FINDER retrieves all molecules with the calculated MF, generates their in-silico spectra, and compares them to the experimental spectrum. It is able to predict fragments of compounds with known fragmentation rules, while relative intensities of the product ions are challenging to be predicted (Ljoncheva et al., 2020). Others, such as SIRIUS4 predict molecular fingerprints from the observed MS/MS spectrum, whose accuracies depend upon descriptiveness of the predicted molecular fingerprints (Ljoncheva et al., 2020).

There are several ways to prioritize and rank the candidates. The most commonly used one is the data source-related criteria. The presence of a compound in relevant databases and the number of references and patents from scientific literature illustrate the possibility of being an already identified relevant substance (Ljoncheva et al., 2020). Various structure databases, mainly metabolomics-related, have been integrated into the abovementioned tools. Depending on the subjects under investigation, various relevant databases can be selected, which will narrow down the number of candidates. This criterion is of great help for "known unknown" but of no help for "unknown unknown". In the field of FCM, there are two important structure databases compiled, namely "plastic packaging-associated chemicals" (CPPdb) (Groh et al., 2019) and "intentionally used food contact chemicals" (FCCdb) (Groh et al., 2021). Nevertheless, none of them have been embedded into these tools. The in-silico fragmentation tools often allow users to apply their own structure databases, but compounds in the two FCM-related databases lack of structural information such as InChIKey and Simplified Molecular Input Line Entry System (SMILES) which are vital for the tools. The CPPdb also includes compounds from two reviews on NIAS (Geueke, 2018; Nerín et al., 2013) though, the number of known NIAS could still be limited let alone emergent NIAS. It is noteworthy that many tentatively identified NIAS in literature, such as oligomers (Abe et al., 2016; Hoppe et al., 2016; Ubeda et al., 2018), have no structural information like SMILES and InChIKey, which makes it sophisticated to include them into structure database because many of them are not present in existing chemical repositories such as Pubchem and Chemspider.

Chromatographic retention-related criteria are other ways to rank the candidates. In the case of GC-based elucidation, experimental and predicted RI can be used as aforementioned. Nonetheless, retention behaviour in LC systems depends upon several parameters such as pH, temperature, buffer, solvents, and so on, making it difficult to construct experimental RI-like libraries that can be applied on various LC systems, and also difficult to build prediction models that adapt all LC systems. In spite of that, there are still efforts made on predicting retention time in LC (Bonini et al., 2020; Low et al., 2021; Stanstrup et al., 2015; Witting and Böcker, 2020; Yang et al., 2021). With respect

to NTS, each laboratory may have their own equipment settings. Thus, they can construct their own retention time library and use it to build a retention time prediction model under the same chromatographic conditions. As Bonini et al. (Bonini et al., 2020) suggested, as least 300 compounds should be used in order to have a good retention time prediction model.

LC coupled to ion mobility mass spectrometry (IM-MS) has been demonstrated to have several advantages over traditional technologies, including improved peak capacity, separation of isomers, and generation of multidimensional data to facilitate identification (Luo et al., 2020). Collision cross section (CCS) determined by IM-MS, attracts growing attention as an additional identification criterion in analytical approaches as it represents a unique physicochemical property of an ion (Mairinger et al., 2018). Consequently, various experimental CCS libraries as well as CCS prediction models such as MetCCS (Zhou et al., 2017), DeepCCS (Plante et al., 2019), and AllCCS (Zhou et al., 2020), have been built with the aim to facilitate identification. In a comparison study, high reproducibility (deviation < 1%) has been observed for most evaluated compounds measured by a drift tube IM-MS (DTIM-MS) and a traveling wave IM-MS (TWIM-MS) (Canellas et al., 2021), while some compounds showed deviations up to 6.2% indicating that CCS databases cannot be used without care independently from the instrument type (Hinnenkamp et al., 2018). Recently, IM-MS has been successfully employed to identify compounds in plastic FCM (Canellas et al., 2019, 2020; Vera et al., 2019).

5.4. Data acquisition

5.4.1 Data-dependent acquisition (DDA)

As illustrated in Fig. I-8 A, during DDA analysis, only precursor ions (from full scan MS1) that are higher than user-defined intensity threshold will trigger subsequent CID/HCD fragmentation and therefore have MS/MS spectra, while all other peaks are discarded. The advantage is that the acquired MS/MS spectra are relatively clean. Lowering the intensity threshold leads to more product ion peaks but also decrease the

purity of spectra (Kind et al., 2018). The main shortcoming is that MS/MS spectra are only available for a limited number of precursor ions (Samanipour et al., 2018). Hence, in the case of coeluted compounds, the one with lower intensity will be neglected.

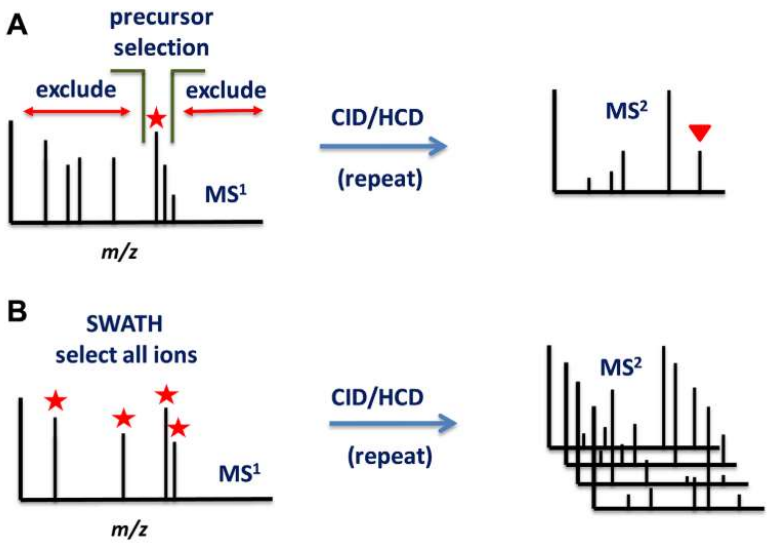


Fig. I-8 Data-dependent acquisition mode (A) and Data-independent acquisition mode (B) (Kind et al., 2018)

5.4.2 Data-independent acquisition (DIA)

In comparison to DDA, DIA is less common. During DIA, all precursor ions generated in MS1 are subjected to CID/HCD fragmentation (Fig. I-8 B). The benefit is that low intensity precursor ions are also fragmented even if they are coeluted with higher intensity precursor ions. However, the downside is also obvious, that is the direct link between a specific precursor ion and its corresponding product ions is broken, and thus resulting in mixed MS/MS spectra which are complex and difficult to process requiring adequate deconvolution algorithms (Kind et al., 2018; Samanipour et al., 2018). Both all-ion fragmentation (AIF), MS^E from Waters, and Sequential Windowed Acquisition of All THeoretical (SWATH) belong to DIA. With slight discrepancy, in SWATH, full scan mass range in MS1 will be divided into several isolation windows (20 Da or more), which will result in cleaner MS/MS spectra compared to AIF and MS^E.

5.5. Software for processing MS-based data

Proprietary software accompanied with analytical instruments are powerful for analysing instrument-specific data as they are designed for that purpose. In the last decades, open-source processing and analysis tools attract increasing interests in light of innovative, open and reproducible science (Stanstrup et al., 2019). A wide variety of open-source software have been developed providing publicly accessible and advanced processing and analysis approaches. Data acquired by equipment from various brands are stored in different proprietary formats, which are difficult to analyse outside the vendor software. Therefore, the first step to analyse them by open-source tools is to convert them into open data formats, such as XML-based formats (mzXML, mzData, mzML), netCDF (also known as ANDI-MS), and classical text files (JCMP-DX or txt) (Gorrochategui et al., 2016). Equipment manufactures offer specific tools to this end, for instance, Databridge and File converter from Waters and ThermoFisher, respectively.

One important step to deal with MS-based data is the deconvolution, which is a computational separation process of co-eluting components and therefore creates a pure spectrum for each component (Du and Zeisel, 2013). Regarding GC-MS data, Automated Mass Spectrometry Deconvolution and Identification System (AMDIS) is well-known. Improved automated deconvolution algorithms have to implemented into various open-source tools/platforms like XCMS (both web-based and in R environment) (Benton et al., 2004), Mzmine2 (Pluskal et al., 2010), MS-DIAL (Tsugawa et al., 2015), and GNPS (Aksenov et al., 2020), among others. These tools integrate peak detection based on extracted ion chromatograms (EIC), deconvolution, peak alignment across all samples, and peak filtration based on, for example fold change between sample groups and blanks, into well-connected pipeline workflows. The deconvoluted and filtered peaks with clean spectra are then able to be exported for identification by other software such as NIST MS Search Program. Library matching algorithm is also embedded into MS-DIAL. EI-MS library can be either downloaded from the abovementioned sources 5.3.1 or converted from commercial libraries. Moreover, automated RI calculation and

its use as an additional identification index are also integrated in MS-DIAL, which largely simplify GC-MS data processing.

XCMS, MZmine2, MS-DIAL, and GNPS are capable of handling LC-HRMS data as well. At the time of writing, only MS-DIAL is able to process DIA data, e.g., MS^E data from Waters thanks to it great DIA deconvolution algorithm (Tada et al., 2020). In comparison, LC-HRMS data are relatively more complicated to process. A widely known difficulty is the in-source fragments. Even under soft ionization, some compounds undergo fragmentation and multiple adducts can be formed. The large number of fragment ions and adducts are recognised as individual features (m/z and retention time pairs) during peak detection procedure, but they are not individual compounds. For this reason, several tools have been developed to group these fragment ions and adducts that come from a same compound, and to keep only representative features together with MS/MS spectra for subsequently annotation. These tools include CAMERA (Kuhl et al., 2012), CliqueMS (Senan et al., 2019), and MS-CleanR (Fraisier-Vannier et al., 2020). After annotation by library matching, remaining unknowns can then be sent to structural elucidation by tools mentioned above 5.3.2. Molecular networking, in which molecules differing by simple transformations such as glycosylation, alkylation, and oxidation/reduction are corelated as networks, is another powerful tool for the identification of unknowns. It is also well-connected to many open-source tools such as the Feature-based Molecular Networking (FBMN) in the GNPS platform (Nothias et al., 2020). The GNPS allows users to upload and analyse their data via the GNPS server and share them with others, which is excellent for reproducibility and further exploration of the data (Aron et al., 2020).

Statistical and multivariate analyses such as principal component analysis (PCA), hierarchical clustering analysis (HCA), partial least squares discriminant analysis (PLS-DA), and Orthogonal PLS-DA (OPLS-DA), among others, have been implemented into various open-source software or platforms, e.g., MetaboAnalyst (Chong et al., 2019). All these open-source tools as well as publicly available EI-MS and MS/MS libraries are powerful for the identification of small molecules and for further exploring of the

data. While they have been widely used in metabolomics, their potentials in the FCM area are still seldomly explored.

Session II: Objectives

1. General objectives

The thesis aims to establish sensitive and reliable sample pre-treatment methods as well as data analysis workflows to identify and quantify migrants from recycled polyolefins. Both volatile and non-volatile substances will be analysed in a non-target screening manner.

2. Specific objectives

The general objectives are accomplished by 5 chapters with specific objectives in each chapter.

Chapter 1:

- Investigating in depth the differences between GC-MS and APGC-QTOF-MS.
- Combining conventional GC-MS and advanced APGC-QTOF-MS to facilitate and improve the identification of volatile substances coming from polypropylene samples used as food contact materials.

Chapter 2:

- Developing and optimizing a sensitive and ease-to-use DI-SPME, which can be coupled to GC-MS and APGC-QTOF-MS, for the determination of volatile and semi-volatile migrants in various liquid food simulants (10% ethanol, 95% ethanol, 3% acetic acid, etc.).

Chapter 3:

- Determining volatile and semi-volatile migrants coming various mixed post-consumer polyolefins from China and Spain by previously established methods (Chapter 1 and 2).

- Studying chemical classes, molecular weight, and XLogP distributions as well as possible origins of the detected migrants.
- Establishing and applying prioritization strategy for the migrants based on compound toxicity, detection frequency, and chromatographic response.
- Quantification of the prioritized migrants in 95% ethanol and 3% acetic acid food simulants.

Chapter 4:

- Determining volatile and semi-volatile substances in various rHDPE milk bottle flake and pellet samples.
- Investigating compositional similarities (chemicals present and their intensities) of different batches of rHDPE milk bottle flake and pellet samples.
- Studying the efficacies of washing twice and an extra decontamination technique on the removal of volatile substances.
- Prioritizing and quantifying migrants coming from rHDPE milk bottle pellet samples based on the strategy established in Chapter 3.

Chapter 5:

- Establishing a comprehensive non-target screening workflow to process FCM-related LC-HRMS based data by integrating publicly accessible and in-house MS/MS spectra libraries, feature cleaning, in-silico fragmentation, chemicals associated with plastic packaging structure database, and pseudo-MRM.
- Determining non-volatile substances in rHDPE milk bottle flake and pellet samples.
- Quantifying non-volatile migrants coming from rHDPE milk bottle pellet samples.

Session III: Experimental part

Developing sensitive sample treatment and reliable data analysis methodologies for non-target screening of volatile compounds

Chapter 1: *Non-target Screening of (Semi-)Volatiles in Food-Grade Polymers by Comparison of Atmospheric Pressure Gas Chromatography Quadrupole Time-of-Flight and Electron Ionization Mass Spectrometry*

Chapter 2: *Direct Immersion - Solid-Phase Micro-extraction Coupled to Gas Chromatography - Mass Spectrometry and Response Surface Methodology for Non-target Screening of (Semi-) Volatile Migrants from Food Contact Materials*

Non-target screening of volatile migrants from recycled polyolefins

Chapter 3: *Safety Concerns of Recycling Post-consumer Polyolefins for Food Contact Uses: Regarding (Semi-)Volatile Migrants Untargetedly Screened*

Chapter 4: *Decontamination Efficiencies of Post-Consumer High-Density Polyethylene Milk Bottles and Prioritization of High Concern Volatile Migrants*

Developing sensitive and reliable data analysis strategy for non-target screening of non-volatile compounds and its application to migrants coming from rHDPE

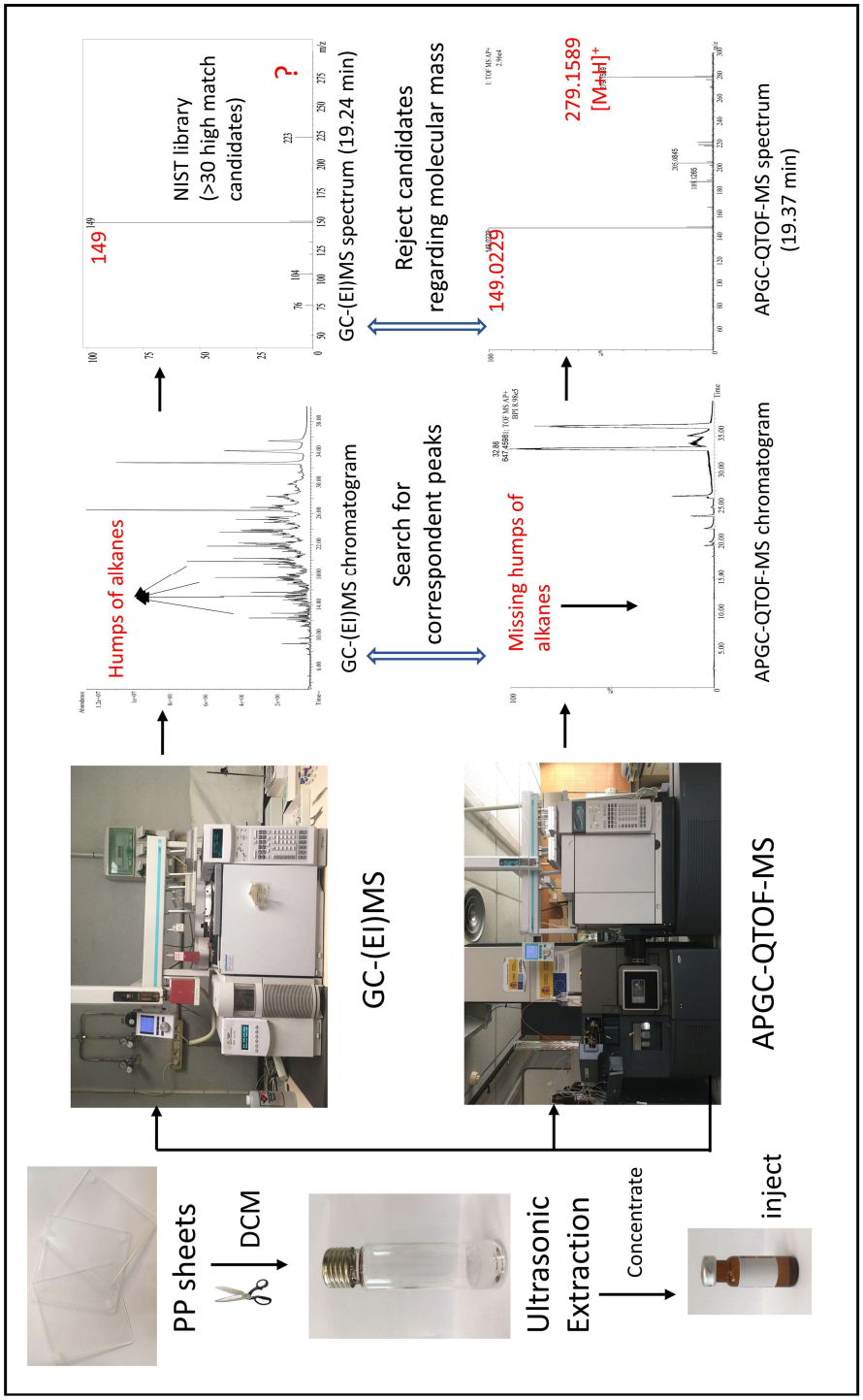
Chapter 5: *Combination of Structure Databases, In-Silico Fragmentation, and MS/MS Libraries for Untargeted Screening of Non-volatile Migrants from Recycled High-Density Polyethylene Milk Bottles*

Chapter 1

Non-target Screening of (Semi-)Volatiles in Food-Grade Polymers by Comparison of Atmospheric Pressure Gas Chromatography Quadrupole Time-of-Flight and Electron Ionization Mass Spectrometry

1. Abstract:

Atmospheric pressure gas chromatography (APGC) coupled to quadrupole time-of-flight (QTOF) and electron ionization mass spectrometry together with commercial library search are two complementary techniques for non-target screening of volatile and semi-volatile compounds. Optimization was first conducted to achieve easier search of correspondent peaks between the two systems. Analytical strategy for the determination of volatile and semi-volatile compound with different identification confidence levels was then proposed and applied to food contact grade polypropylene (PP) samples. Identification was found to be much easier and less time-consuming especially when correspondent peak was found in the two systems with the help of library search, exact mass of precursor and fragment ions as well as Retention Index (RI). The behaviour of APGC-QTOF-MS was also further investigated. Apart from the $M^{+\cdot}$ ion and the well-known adduct $[M+H]^+$, others such as $[M-3H+O]^+$, $[M-3H+2O]^+$ and $[M-H+3O]^+$ were also observed for n-alkanes. Besides, new reaction products, formed by diol compounds (1-Monostearoylglycerol, 2-Monostearoylglycerol and NX 8000K) and silanediol dimethyl which would be a transformation product of the silicone base septum or the methyl 5% phenyl polysiloxane based column, were found. These new compounds were only detected in APGC-MS-QTOF as EI-GC-MS was not enough sensitive for this purpose.


PP sheets
DCM
Ultrasonic Extraction
Concentrate
inject
GC-(EI)MS
APGC-QTOF-MS
Humps of alkanes
GC-(EI)MS chromatogram
Search for correspondent peaks
Missing humps of alkanes
APGC-QTOF-MS chromatogram
GC-(EI)MS spectrum (19.24 min)
NIST library (>30 high match candidates)
149
?
Reject candidates regarding molecular mass
149.0229
279.1589
APGC-QTOF-MS spectrum (19.37 min)

2. Introduction

Food safety concerns arising from FCM have attracted growing attention in recent years. Current legislations mainly focus on authorized substances and on those that could be present in specific foods. This is true in food (Kunzelmann et al., 2018) as well as FCM sector. In FCM, most of them are also known as IASs. Traditional targeted screening (TS) based on building ways to determine a list of known compounds (García Ibarra et al., 2018), normally IASs, is a typical strategy to check FCM compliance. However, apart from IASs, there are also NIAS coming from impurities of starting materials, degradation products from raw materials, unwanted side-products, and so on, which might endanger consumer health (Geueke, 2013; Nerín et al., 2013). Hence, to give consumers higher level of security, NIAS should also be considered in FCM safety evaluation before being launched into the market. Target screening is therefore insufficient, and generic analytical screening methods are required (Leeman and Krul, 2015).

Non-target screening (NTS) is a very good idea in order to have a comprehensive understanding of FCMs; however, it is not so easy without knowing even the origin of the unknowns. Fortunately, the development of hybrid quadrupole HRMS (Q-HRMS) like quadrupole-time of flight mass spectrometry (QTOF-MS) together with soft ionization techniques e.g., ESI and APCI allow obtaining exact mass of the molecule. Also, high energy collision cell can provide structural information from accurate masses of fragment ions. Taking the advantage of HRMS, soft ionization, and collision fragmentation, elucidation of the molecular structure is available (Canellas et al., 2014). LC coupled to Q-HRMS have been successfully applied for NTS of non-volatile compounds in various matrices including wastewater (Gago-Ferrero et al., 2015), olive oil (Kalogiouri et al., 2018), food (Kunzelmann et al., 2018; Wrona et al., 2016), FCM (Aznar et al., 2012; Isella et al., 2013; Vera et al., 2013, 2018), and so on.

For volatile compounds, conventional GC-MS in EI mode is a powerful tool for the identification as commercially available spectral libraries, such as NIST and Wiley, cover the spectra of several hundreds of thousands of compounds (Hollender et al., 2017). However, its shortage is also obvious when chemicals are not included in the

library. In this situation, soft ionization mode together with HRMS are in high demand. APGC-QTOF-MS is the combination to meet this requirement, which is merely available in the market since 2008 (Mullin et al., 2017). Until now, it has been successfully employed to detect some specific classes of chemicals in different samples, for example, organophosphorus pesticides in fruits and vegetables (Cheng et al., 2017), nitro-polyaromatic hydrocarbons in PM 2.5 (Y. Zhang et al., 2018), nitro and oxo polyaromatic hydrocarbons (PAH) (Carrizo et al., 2015; Domeño et al., 2012) and brominated flame retardants in food (Lv et al., 2017). To the best of our knowledge, it has not yet been widely used for NTS. A non-target analytical strategy based on GC-(EI)TOF-MS and APGC-QTOF-MS was proposed by Cherta et al. (Cherta et al., 2015). Library search of the GC-(EI)-TOF-MS spectrum was first conducted to generate positive matches list (library match>700). Then both molecular ion and protonated molecule of those candidates were extracted from APGC-QTOF-MS chromatogram at similar retention time to confirm or reject candidates. It has been used for identification of potential migrants from 4 composite FCMs into isooctane and Tenax food simulants. The number of candidates were reduced by approximately half with the help of APGC-QTOF-MS. However, the methodology did not deal with the case where no candidate can be obtained with satisfied match. In this case, the chemical would probably not be present in the library, and we would have no idea about the possible molecular ion neither protonated molecule. Hence, the potential of APGC-QTOF-MS for unknown elucidation is limited. Onghena (Onghena et al., 2015) developed a strategy for elucidation of unknown migrants from plastic FCM (baby bottles) based on GC-MS, GC-(EI)TOF-MS, and APGC-QTOF-MS. Extracted mass of molecular ion and protonated molecule were carried out to search for correspondent peak in APGC-QTOF-MS spectrum as well. If no correspondence could be found by this way, APGC-QTOF-MS spectra was manually examined for possible molecular ion or protonated molecule at the expected retention time. However, how to do this was not clarified. In addition, retention time distinction between both systems was not well defined. APGC-QTOF-MS was said to have about 2 min earlier retention time than GC-(EI)TOF-MS. However, the time difference is not fixed across the whole chromatogram. This would add difficulty in finding correspondent peak between both systems especially when the

chromatogram is complicated. Furthermore, the two abovementioned studies mainly focused on the power of APGC-QTOF-MS in non-target identification, but no effort have been made to see the difference between both systems.

The objective of this article is to further explore the potential of APGC-QTOF-MS for NTS using conventional GC-MS together with commercial library search as a complementary tool by analysing the extractables from food contact grade PP. Adjustment of chromatographic conditions to make the two systems more comparable has been made. Struggle has been made to further understand the distinction between these two complementary platforms, especially the behaviour of APGC, so that complementary information from both techniques can be realized to widen the scope and reduce the time of non-target volatile compounds screening.

3. Materials and methods

3.1. Reagent and samples

Dichloromethane (DCM) for GC residue analysis was bought from Scharlab (Barcelona, Spain). Ultra-pure water was obtained from a Wasserlab purification system (QUGR0011; Navarra, Spain).

Standards used were purchased from various suppliers: C7-C40 saturated alkane standard from Supelco (49452-U; Pennsylvania, USA); didecyl phthalate (84-77-5) from Riedel-de Haën (Bucharest, Romania); benzene propanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester (6386-38-5) from Activate Scientific (Ely, UK); benzofuran (271-89-6) and silane, cyclohexyldimethoxymethyl- (17865-32-6) from Alfa Aesar (Heysham, UK); Tinuvin 326 (3896-11-5) and Irgafos 168 (31570-04-4) from Ciba-Geigy (Barcelona, Spain); tridecane (629-50-5), octacosane (630-02-4), stearamide (124-26-5), palmitamide (629-54-9), benzaldehyde, 4-propyl- (28758-06-0), dioctyl phthalate (DOP; 117-81-7), butylhydroxytoluene (BHT; 128-37-0), 2,6-di-tert-butylbenzoquinone (719-22-2), 2,4-di-tert-butylphenol (96-76-4), 3,5-di-tert-butyl-4-hydroxybenzaldehyde (1620-98-0), diisobutyl phthalate (DIBP; 84-69-5), dibutyl phthalate (DBP; 84-74-2), palmitic acid (57-10-3), stearic acid (57-11-4), tributyl

acetylcitrate (77-90-7), 1-monostearolglycerol (123-94-4, abbreviated as 1-MSGC), 2-monostearolglycerol (621-61-4, abbreviated as 2-MSGC) and NX 8000K (882073-43-0) from Sigma-Aldrich (Madrid, Spain); Oxidized Irgafos 168 were self-manufactured in our lab. Two PP sheets intended for food contact use were supplied by a European company.

3.2. Solvent extraction from polypropylenes

PP samples were quickly cleaned with ethanol, dried in the air, and cut into small pieces (ca. 2 mm × 2 mm) with scissors. 1.00 g of sample was weighed into a 20 mL glass vial by a Mettler Toledo analytical balance (XS205; Ohio, USA). Three consecutive extractions with 2 mL DCM were applied in an ultrasonic bath for 1 hour (Brasonic 3510-MTH; Connecticut, USA). The extract was collected and concentrated by a nitrogen concentrator (Techne DB-3; Staffordshire, UK) at 40 ℃ until ca. 1mL and weighed. The concentrated extract was then filtered with a 0.2 μm Acodisc GHP syringe filter (Corporation, New York, USA), and injected in both GC-MS and APGC-QTOF-MS using the parameters described below. Three replicates were conducted for each sample. Also, two blank samples were prepared in the same manner as described above, with the exception that no sample was added.

3.3. GC-MS analysis

For GC-MS injection, a 7820A gas chromatography equipped with a 7693 autosampler, coupled to a 5977B mass spectrometry detector from Agilent (California, USA) was used. HP-5 MS column (30 m × 0.25 mm id, 0.25 μm film thicknesses) also from Agilent Technologies was employed. 2 μL injection volume was applied using splitless mode, and solvent delay was 3 min. Liner with 4 mm internal diameter and 10 μL syringe were used in both systems. The inlet temperature was set at 250 ℃. Helium (99.999%) was the carrier gas at a constant flow rate of 2.4 mL/min. In-line gas purifier (RMSH-2, Agilent) was used to remove oxygen, water, and hydrocarbons from He before entering GC in both systems. The total flow, however, was evenly divided into 2 fractions: one went into the mass detector and the other went into an olfactory even

though it was not used here. Scan mode with a mass range from 40-700 was applied. The temperature program was as follows: kept 50 ℃ for 3 min, increased to 300 ℃ at the rate of 10 ℃/min, and held for 12 min.

3.4. APGC-QTOF-MS analysis

For APGC-QTOF-MS injection, an Agilent A7890 gas chromatography equipped with a PAL autosampler, coupled to a high-resolution mass spectrometer Xevo G2 QTOF (> 20000 FWHM at 956 m/z and > 10000 FWHM at 152 m/z) from Waters (Massachusetts, USA) was employed. Atmospheric pressure chemical ionization (APCI) was used to interface the GC and QTOF-MS, which allows obtaining precursor ions.

SPB 5 column, which has the same dimension (30 m × 0.25 mm id, 0.25 μm film thickness) and stationary phase (methyl 5% phenyl polysiloxane) as HP 5 MS column was applied. The inlet temperature, oven temperature program and carrier gas were all as the same as that in GC-MS analysis. The flow rate here, however, was 3.5 mL/min. In this way, the chromatograms between GC-MS and APGC-QTOF-MS are more comparable.

The heated transfer line temperature was set at 280 ℃, and the auxiliary gas (N_2) flow rate 300 L/h. 150 ℃ source temperature and 1.0 μA corona current were applied. No humidity modifier was used. Cone and desolvation gas (99.999% N_2) flow were 20 and 175 L/h, respectively. The scan mass range was also from 40 to 700. MS^E acquisition mode which is designed for simultaneous acquisition of both precursor and fragmentation ions was used. Low energy (6 V) was set to keep more precursor ion while high energy (10-40 V) was applied in the collision cell to generate higher fragmentation. Positive ion mode was selected. Exact mass 281.0517 ($C_7H_{21}O_4Si_4$) from column bleed was used to correct the mass for every peak of interest after injection. The mass accuracy of another column bleed ion 355.0705 ($C_9H_{27}O_5Si_5$) was checked after correction to ensure the quality of correction. Acceptable mass distinction here was set at 5 ppm. If the mass correction is not good, which happens sometimes when there is interference near the used mass, 355.0705 can be used for correcting mass, and check 281.0517. If interference happens in both ions, which should not be a common case,

other column bleed ions for example, 207.0327 or 429.0893 can be considered as well. BHT was injected before and after the samples to make sure the equipment was in good condition.

3.5. Data processing

The workflow of identification using both GC-MS and APGC-QTOF-MS is shown in Fig. III-1.1. Identification confidence proposed by E. Schymanski et al. (Schymanski et al., 2014) was used here. It contains 5 levels. **Level 1: Confirmed structures** are those confirmed by a reference standard with MS, MS/MS and retention time match; **Level 2**: **Probable structure** indicates unambiguous spectrum-matching with library or literature information or diagnostic evidence including MS/MS fragments and/or ionization behaviour, parent compound information and the experimental context; **Level 3: Tentative candidate(s)** describes that evidence exist for possible structures but no one specific structure can be concluded as for lacking of sufficient information, for instance, positional isomers; **Level 4: clear molecular formula** means only one formula can be undeniable assigned by spectral information, e.g., adduct, isotope, and/or fragment information, but no further information can be obtained; **Level 5: Exact mass (m/z)** of interest for the investigation can be measured, but no sufficient information exists to assign even one specific formula.

The first step was to pick out all peaks that had a Signal to Noise (S/N) ratio higher than 10 in APGC-QTOF-MS by manually checking the total ion chromatogram (TIC) using Masslynx 4.1 from Waters. Peaks present also in blanks were excluded, except for the case where its height was over 10 times higher in samples than that in blanks (Cherta et al., 2015).

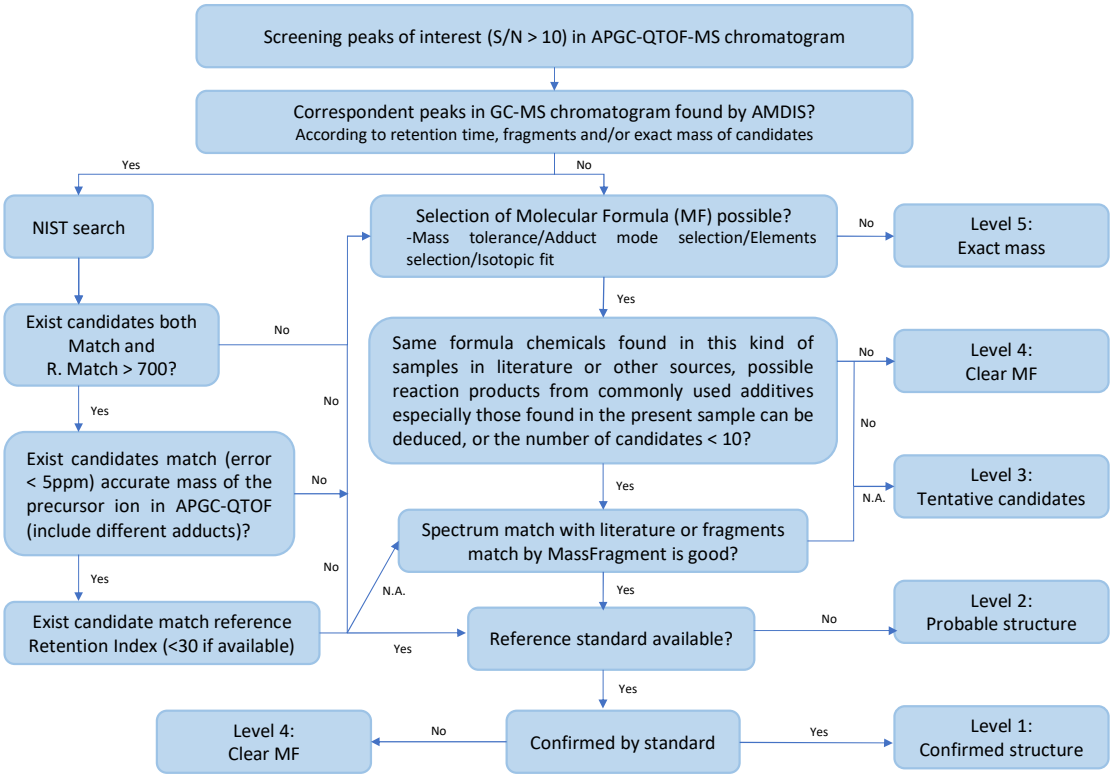


Fig. III-1.1 Flow chart of non-target screening of volatile and semi-volatile compounds by combing APGC-QTOF-MS and GC-MS

The second step was to find out their correspondent peak in GC-MS chromatogram. To achieve this goal, the spectra of peaks that had closed retention time (less than 0.5 min) in GC-MS were compared to the APGC-QTOF-MS spectrum in low and high energy modes. The comparison of chromatograms of the same sample in GC-MS and APGC-QTOF-MS were much easier when applying the optimized parameters described above, because of the smaller retention time difference between them. AMDIS from NIST (Maryland, USA) was employed for peak picking from GC-MS chromatogram. It was of great help to find out individual components by deconvoluting GC-MS file automatically even though some of them were very small or overlapped by others such as alkanes. In most cases, shared abundant ions were found in both systems which helped to find correspondence. However, there were also exceptions where the spectra in both systems were totally different as shown below 4.1.3 and also in a previous study by Onghena et al. (Onghena et al., 2015). In this case, exact mass of high

match candidates from possible peaks were considered. By this way, correspondence could be found for most peaks. If neither shared abundant ion(s) nor high match candidates could be found, which would happen but might not be a common case, it would be difficult to know if they were correspondent or not. Of course, not all peaks had correspondence in GC-MS, because APGC-QTOF-MS has much higher sensitivity than GC-MS. What is more, the inherent property of the two ionization techniques is not the same. It is possible that some compounds could only be detected by one of these two techniques.

The third step was to search candidates using NIST library once correspondence was found. When hits with both match and R. match higher than 700 were found, their exact mass were compared with the precursor ion obtained in APGC-QTOF-MS, considering the possible adducts, usually the molecular ion or the protonated ion (fourth step). Relatively low match value (700) was set here just to reduce false negative judgement, because it could be the result of relatively low concentration, peak overlap and so on. As additional confirmation, exact mass and RI match in the following steps were important to decrease false positive deduction. When the accurate mass error is lower than 5 ppm, RI was checked (<30) as well if it existed (fifth step). This way, taking the advantage of library search as well as the exact mass of the precursor ion, the number of tentative candidates could be largely reduced. Then, reference standards were injected in APGC-QTOF-MS to confirm it, if available. If only one candidate matched spectral library, accurate mass, and RI, it was set as probable structure even though no reference standard was available.

When no correspondent peak was found in GC-MS, or all candidates were ruled out by RI, accurate mass or even library match, another process was applied. This process is much more effortful and difficult, posing a great challenge in analytical chemistry. It involves accurate mass of the precursor ion, element selection, adducts and isotopic fit (Nerín et al., 2013). Even though unambiguous molecular formula can be generated, there could be, in most cases, a lot of chemicals fit this formula, making the work imaginably huge, especially when the number of candidates is huge. In this sense, information from samples, literature search, and structure deduction from

probable/confirmed chemicals are useful to narrow the range of candidates. MassFragment from Waters is a powerful tool to generate possible fragments from a given chemical based on the likelihood of breaking certain bonds (Canellas et al., 2014). Therefore, it allows us to evaluate if a candidate is good or not by comparing experimental spectrum with MassFragment result. According to the extent we obtain, candidates can be assigned to various identification confidence levels, from level 1 to level 5 as shown in Fig. III-1.1.

4. Results and discussions

4.1. Comparison between GC-MS and APGC-QTOF-MS

Soft ionization, in this case APCI, allows us to keep more precursor ions, while Q-HRMS helps to elucidate the structure of the molecular ion and/or protonated molecule. However, it is anyway difficult and time-consuming, as there are still huge possibilities, even with an accurate mass. What is worse, there is no commercially available library for APGC-HRMS up to now. This means that every peak of interest obtained in APGC-HRMS chromatogram needs to be annotated using the structure elucidation, which makes the work imaginable huge and effortful. Combining APGC-QTOF-MS and GC-MS, the advantage of library search, precursor ions conservation as well as accurate mass, can be well integrated, making the structure elucidation of the peaks largely reduced. However, chromatograms between these two systems are not comparable because of their system distinctions. Optimization is therefore required.

4.1.1 Alkanes in GC-MS and APGC-QTOF-MS

As can be seen in Fig. III-1.2 A, the chromatograms in APGC-QTOF-MS and GC-MS differ a lot. There are humps in GC-MS chromatogram, while the chromatogram in APGC-QTOF-MS looks much cleaner. With the help of NIST library search, the humps were identified as alkanes, which are not of high interest in terms of safety of food contact materials.

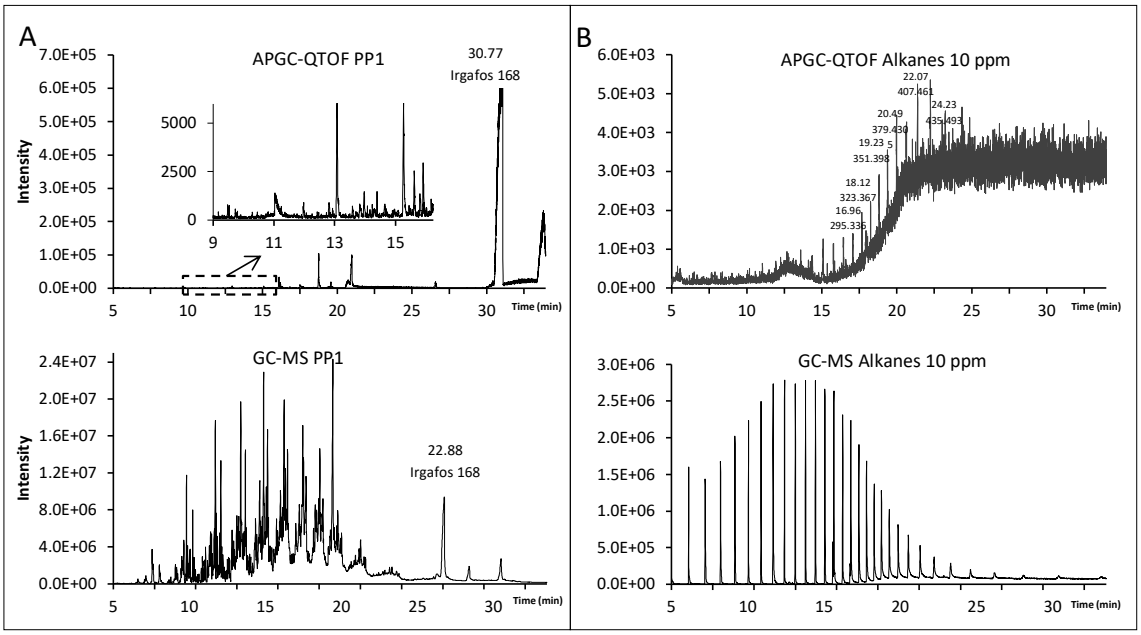


Fig. III-1.2 Chromatograms of PP1 sample (A) and alkanes at 10 µg/g (B) in both APGC-QTOF-MS and GC-MS

Alkane standard (C7-C40) was injected in both systems to see the differences. Compared to GC-MS, the peaks of alkanes in APGC-QTOF-MS were relatively low, some of which were even invisible at the used concentration (10 µg/g), while they were very high in GC-MS (Fig. III-1.2 B). This phenomenon suggested that APGC is not good for alkane ionization under the used conditions, which reduce the complexity of the chromatograms and allow us focusing on components of interest.

Fig. III-1.3 A shows the spectrum of octacosane. In agreement with the research by Hourani et al.(Hourani and Kuhnert, 2012) where dry nitrogen gas source APCI-QTOF-MS was used, $[M-H]^+$ ion was observed. Besides, $[M-H]^+$ was found in many studies using various reagents (dimethylether, methane, and nitric oxide) as Bell et al. summarized in the introduction as well as in their own study which used ^{63}Ni as a reagent(Bell et al., 1994). In addition, $[M-3H+O]^+$, $[M-3H+2O]^+$, and $[M-H+3O]^+$ were monitored. These ions were previously found for n-tridecane, n-pentadecane, and n-heptadecane using corona assisted direct analysis in real time (corona-DART) coupled to QTOF-MS(Sekimoto et al., 2016). The authors explained that they originated from hydride abstraction and oxidation reactions. Surprisingly, the relative intensity of [M-

$H]^+$ decreased while that of $[M-3H+O]^+$ increased with the reduction of carbon number in alkanes. As shown in Fig. III-1.3 B, $[M-H]^+$ of tridecane (183 m/z) was hardly visible, but $[M-3H+O]^+$, $[M-3H+2O]^+$ were very high. This phenomenon could cause confusion when choosing the precursor ion for an unknown peak. Fortunately, there were common fragments (85.1014, 71.0857, 57.0698, and so on) in all alkanes, which would be of great help when we encounter this kind of situation. However, $[M+N]^+$ mode was observed for alkanes in another research where much higher cone gas flow 150 L/h was applied while only 20 L/h herein (Wu et al., 2015). The authors investigated the effect of various experimental parameters on the formation of $[M+N]^+$ ion, and the result showed that $[M+N]^+$ ion could not be seen when the cone gas flow below 150 L/h.

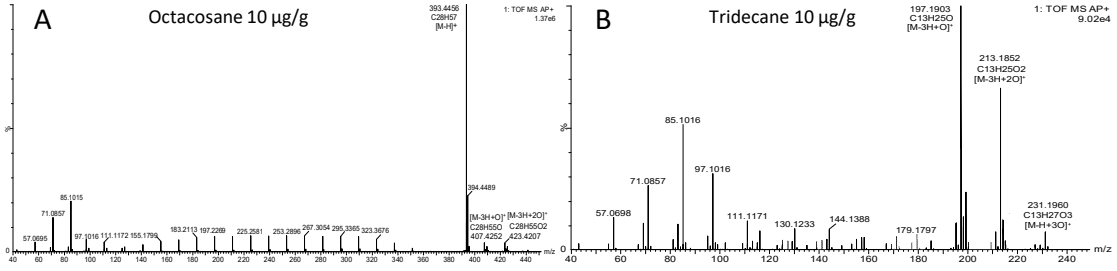


Fig. III-1.3 Spectrum of octacosane (A) and tridecane (B) in 10 µg/g level analysed by APGC-QTOF-MS

4.1.2 Retention time in GC-MS and APGC-QTOF-MS

From the same chart (Fig. III-1.2), we can notice that GC-MS had a shorter retention time than APGC-QTOF-MS under the same chromatographic conditions, leading it relatively troublesome to compare the two chromatograms directly. It is well known that EI works in a high vacuum environment, while APGC works in atmosphere. The high vacuum may generate a pulling force at the end of the column, driving the compounds within the column moving faster. This could be one of the reasons why compounds in APGC-QTOF-MS had longer retention time. It is interesting that shorter retention time was observed in APGC-QTOF-MS compared to GC-(EI)-TOF in previous studies (Cherta et al., 2015). In their studies, higher flow rate was applied in APGC-QTOF-MS than that in GC-(EI)-TOF (1.2 VS 1.0 mL/min). Longer retention

time in APGC-QTOF-MS, however, can be overcome by its ability to have higher flow rate than the usual level applied in EI source (Tienstra et al., 2015).

In order to make the two chromatograms more comparable, six standards (benzofuran, BHT, DOP, Tinuvin 326, didecyl phthalate and Irgafos 168) covering various molecular mass and retention times were injected under different flow rates (1.2, 1.5, 2.0, 2.5, 3.0, 3.5 mL/min) in APGC-QTOF-MS while 2.4 mL/min was kept in GC-MS. As can be seen in Fig. III-1.4, when the flow rate in APGC-QTOF-MS reached 3.5 mL/min, the retention time difference of all the six chemicals in the two systems is less than 0.5 min. This way, it is more convenient to find correspondent peaks in the two systems.

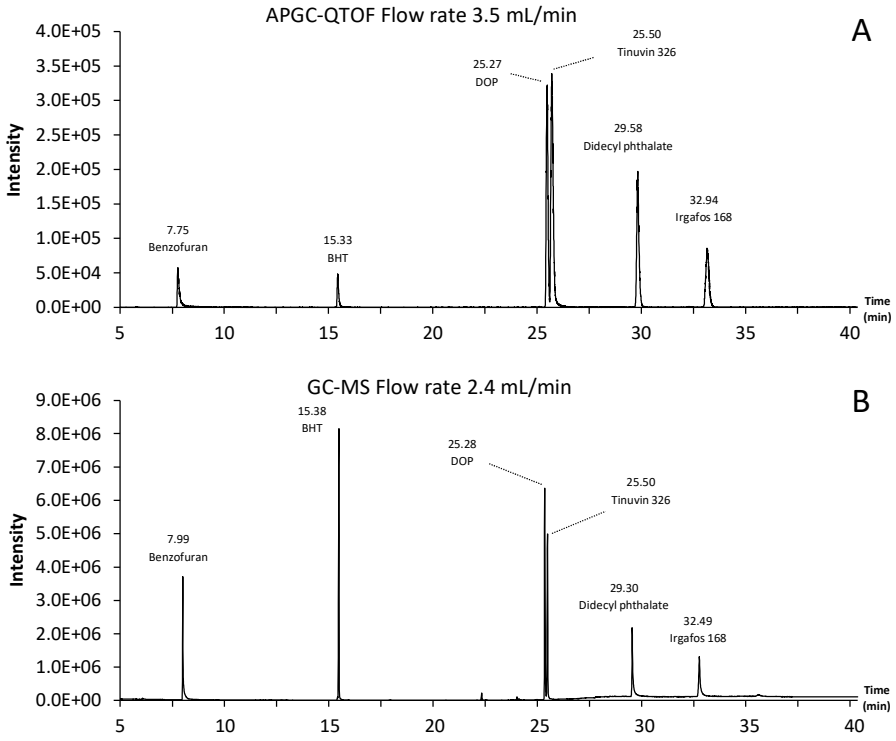


Fig. III-1.4 Chromatograms of six standards (20 μg/g) in APGC-QTOF-MS flow rate 3.5 mL/min (A) and GC-MS flow rate 2.4 mL/min (B)

4.1.3 Spectra in GC-MS and APGC-QTOF-MS

Same compound could show totally distinct fragmentation behaviour under EI and APGC ionization because the precursor ion to be fragmented is an odd electron radical ion in EI, while in APGC is an even electron protonated ion. Hence, fragmentation pathways as well as product ions are usually different. Even so, in most cases, same fragmentation ions could still be found in these two systems as shown in Table III-1.1, regarding those high abundant ions. These shared fragmentation ions together with close retention time could be helpful when finding corresponding peak between these two systems. However, there are components that have totally distinct fragmentation behaviours in these two systems, which would add difficulty to find corresponding peak. 7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione (RT 19.90 min in APGC-QTOF-MS in Table III-1.1) is one of these exceptions. Its spectra in APGC-QTOF-MS low energy, high energy, and in GC-MS are shown in Fig. III-1.5. The spectrum in GC-MS (A) looked totally different from that found in APGC-QTOF-MS, at both low and high energies (B and C). Therefore, adjusting gas flow rate in APGC-QTOF-MS to make them the same retention time is of great help.

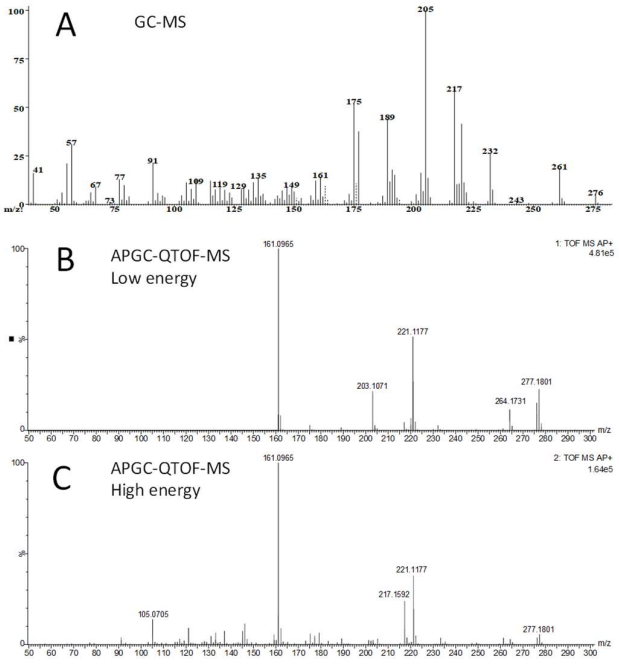


Fig. III-1.5 **7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione** spectra in GC-MS (A), APGC-QTOF-MS low energy (B) and high energy (C)

Table III-1.1 Compounds detected in PP samples by combination of APGC-QTOF-MS and GC-MS

APGC-QTOF-MS			Nº	GC-MS					Formula	MW (Monoiso)	Status	Adduct	Note
Fragment Ions	**Precursor Ion**	**RT**		**RT**	**Ions**	**Candidate Number >700**	**Candidates (mass error < 5ppm and Retention Index < 30 if it exists)**	**CAS /NIST**					
75.0266, 157.1052 105.0371	**189.1314**	10.56	**1**	10.62	105, 75, **188**	3	Silane, cyclohexyldimethoxymethyl-	17865-32-6	C9H20O2Si	188.1233	**Confirmed**	$[M+H]^+$	Catalyst in propylene polymerization
73.0470, 155.1252 143.0887, 89.0422	**173.1355**	11.32	**2**	11.34	89, 61, 143, **172**	0	-	-	C9H20OSi	172.1283	Clear MF	$[M+H]^+$	Small peak
91.0548, 119.0864	**149.0966**	12.44	**3**	12.35	91, 119, **148**	9	Benzaldehyde, 4-propyl-	28785-06-0	C10H12O	148.0888	Confirmed	$[M+H]^+$	Degradation product of clarified agent NX8000
134.0729, 119.0495 91.0545	**163.1123**	14.02	**4**	13.96	**163**, 134, 119, 91	26	-	-	C7H18NOP	163.1126	Clear MF	$M^{+\cdot}$	Small peak
							1-propanamine, 3-[(2-methylpropyl)phosphinyl]-	55359-13-2	C7H18NOP	163.1126	Tentative	$M^{+\cdot}$	Starting material of azaphospholanes (lubricating oil antioxidants or flame retardants in plastic)
165.0900, 193.1580	**221.1535**	14.87		-	-	-	-	-	C14H20O2	220.1463	Clear MF	-	Small peak
			5				2,6-di-tert-butylbenzoquinone	719-22-2	C14H20O2	220.1463	**Confirmed**	$[M+H]^+$	Transformation product of BHT; **$[M+NO]^+$** was also observed
							2,4-di-tert-butylphenol	96-76-4	C14H22O	206.1671	**Confirmed**	$M^{+\cdot}$	
191.1424, 163.1114	**206.1665**	15.25	**6**	15.27	191, **206**, 163	34	2,5-di-tert-butylphenol	5875-45-6	C14H22O	206.1671	Non-confirmed	-	
							3,5-di-tert-butylphenol	1138-52-9	C14H22O	206.1671	Non-confirmed	-	
							3,5-di-tert-butyl-4-hydroxybenzaldehyde	1620-98-0	C15H22O2	234.1620	**Confirmed**	$[M+H]^+$	
219.1379, 191.1067	**235.1698**	18.27	**7**	18.21	219, **234**, 191	9	2,6-ditert-butyl-4-(hydroxymethylene)-2,5-cyclohexadien-1-one	101100-38-3	C15H22O2	234.1620	Non-confirmed	-	
							3,5-di-tert-butylbenzoic acid	16225-26-6	C15H22O2	234.1620	Non-confirmed	-	
							3,5-di-tert-butyl-4-hydroxyacetophenone	14035-33-7	C16H24O2	248.1776	Tentative	-	
193.1230, 233.1542 205.1231	**249.1850**	18.90	**8**	18.77	233, **248**, 205	27	2-methyl-2-[2-(2,6,6-trimethyl-3-methylene-cyclohex-1-enyl)-vinyl]-[1,3]dioxolane	194769[a]	C16H24O2	248.1776	Tentative	-	
							2,4,6-triisopropylbenzoic acid	49623-71-4	C16H24O2	248.1776	Tentative	-	
88.0763, 74.0602	**256.2641**	19.09	**9**	-	-	-	-	-	C16H33NO	255.2562	Clear MF	$[M+H]^+$	Small peak

APGC-QTOF-MS			Nº	GC-MS					Formula	MW (Monoiso)	Status	Adduct	Note
Fragment Ions	**Precursor Ion**	**RT**		**RT**	**Ions**	**Candidate Number >700**	**Candidates (mass error < 5ppm and Retention Index < 30 if it exists)**	**CAS /NIST**					
							Palmitamide	629-54-9	C16H33NO	255.2562	Not	-	Rejected by standard
							Dodecanamide, N,N-diethyl-	3352-87-2	C16H33NO	255.2562	Tentative	-	Found in literature, MassFragment match
88.0762, 74.0604	**256.2641**	19.34	**1(**	-	-	-	-	-	C16H33NO	255.2562	Clear MF	$[M+H]^+$	Relatively big peak, isomer of RT 19.09 min
							Diisobutyl phthalate	84-69-5	C16H22O4	278.1518	**Confirmed**	$[M+H]^+$	
149.0229	**279.1589**	19.37	**1**	19.24	149	> 30 Match >850	1-butyl 2-isobutyl phthalate	17851-53-5	C16H22O4	278.1518	Non-confirmed	-	Catalyst
							Di-sec-butyl phthalate	4489-61-6	C16H22O4	278.1518	Non-confirmed	-	
221.1172, 203.1067 161.0965	**277.1802**	19.41	**1**	-	-	-	-	-	C11H14NP	276.1725	Clear MF	$[M+H]^+$	Small peak; Could be isomer of peak 19.90
							1-oxaspiro[4.5]deca-7, 9- diene-2, 6-dione, 7,9-bis(1, 1-dimethylethyl)	1783860-53-6	C17H24O3	276.1725	Tentative	-	Has similar structure to the compound (RT 19.90)
							1-oxaspiro[4.5] deca-7, 9-diene-2, 6-dione, 7,9-bis(1, 1-dimethylethyl)-, (5S)-	1399009-49-4	C17H24O3	276.1725	Tentative	-	Stereo structure with the former one
161.0965, 221.1177 203.1071	**277.1801**	19.90	**1**	19.76	205, 217, 175, 189, 220, **276**	1	7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	82304-66-3	C17H24O3	276.1725	Probable	$[M+H]^+$	Impurity of Irganox; Although most abundant fragments are not the same, mass match
178.0782, 165.0703 152.0624	**191.0862**	19.97	**1**	-	-	-	-	-	C15H10	190.0783	Clear MF	$M^{+\cdot}$	Big peak; $[M+H2O]^+$ was also observed, but very small
107.0491, 277.1807 147.0803	**292.2036**	20.12	**1**	19.95	277, **292**, 147	22	Metilox or Irganox 1300	6386-38-5	C18H28O3	292.2038	**Confirmed**	$M^{+\cdot}$	Antioxidant
87.0445, 239.2378 129.0914, 213.1850	**257.2474**	20.27	**1(**	20.08	73, 60, 129, 213, **256**	3	Palmitic acid	57-10-3	C16H32O2	256.2402	**Confirmed**	$[M+H]^+$	Lubricant; $[2M+H]^+$ (513.4880) was also observed
88.0764, 74.0604	**284.2956**	21.36	**1**	-	-	-	-	-	C18H37NO	283.2875	Clear MF	$[M+H]^+$	Small peak
							Stearamide	124-26-5	C18H37NO	283.2875	Not	-	Slip agent, but Rejected by Standard
							Tetracanamide, N,N-diethyl-	57303-20-5	C18H37NO	283.2875	Not	-	Found in literature, MassFragment match
87.0446, 267.2684 129.0915, 185.1540	**285.2793**	22.10	**1**	21.98	73, 60, 129, **284**, 241, 185	2	Stearic acid	57-11-4	C18H36O2	284.2715	**Confirmed**	$[M+H]^+$	Lubricant; $[2M+H]^+$ (569.5507) was also observed
129.0187, 185.0812 259.1545	**403.2327**	22.99	**1**	22.83	185, 129, 259	2	Tributyl acetylcitrate	77-90-7	C20H34O8	402.2253	**Confirmed**	$[M+H]^+$	Plasticizer

APGC-QTOF-MS				GC-MS									
Fragment Ions	**Precursor Ion**	**RT**	**N°**	**RT**	**Ions**	**Candidate Number >700**	**Candidates (mass error < 5ppm and Retention Index < 30 if it exists)**	**CAS /NIST**	**Formula**	**MW (Monoiso)**	**Status**	**Adduct**	**Note**
131.0525, 75.0260	**387.2929**	24.87	**2(**	-	-	-	-	-	C21H42O4Si	386.2852		$[M+H]^+$	reaction product during analysis
313.2734, 239.2370 257.2479	**331.2848**	25.03	**2**	24.83	98, 239, 134	3	1-monohexadecanoylglycerol	542-44-9	C19H38O4	330.2770	Tentative	$[M+H]^+$	
							2-monohexadecanoylglycerol	23470-00-0	C19H38O4	330.2770	Tentative	$[M+H]^+$	
131.0525, 75.0258 399.2932	**415.3234**	26.54	**2**	-	-	-	-	-	C23H46O4Si	414.3165		$[M+H]^+$	reaction product during analysis
341.3056, 267.2686 95.0855	**359.3161**	26.71	**2**	26.42	98, 134, 267, 285, 327	4	1-monostearoylglycerol	123-94-4	C21H42O4	358.3083	Tentative	$[M+H]^+$	They have same RT and spectra, thus cannot be differentiated
							2-monostearoylglycerol	621-61-4	C21H42O4	358.3083	Tentative	$[M+H]^+$	
441.2983	**647.4598**	32.86	**2**	32.56	441, **646**	1	Antioxidant Irgafos 168	31570-04-4	C42H63O3P	646.4515	**Confirmed**	$[M+H]^+$	Antioxidant
133.1018, 117.0371	**541.2986**	34.12	**2**	-	-	-	-	-	C31H44O6Si	540.2907		$[M+H]^+$	reaction product during analysis
133.1013, 147.0805 91.0543	**485.2911**	34.53	**2(**	34.20	147, 91, 483	0	NX 8000	882073-43-0	C29H40O6	484.2825	**Confirmed**	$[M+H]^+$	Clarified agent, confirmed by standard
495.2682, 647.4250	**663.4557**	35.93	**2**	35.45	316, 647, **662**	1	Oxidized Irgafos 168	95906-11-9	C42H63O4P	662.4464	**Confirmed**	$[M+H]^+$	Oxidized Irgafos 168

Note: "-" means not available; [a] means NIST number

4.2. Non-target screening of volatile and semi-volatile compounds from PP extracts

Using the method established above, the extracts from two PP samples used for food contact purpose were screened. The chemicals found in the samples with different identification confidence level are shown in Table III-1.1. A total of 27 compounds were found in all samples. They are ordered according to their retention time. Nine of them were detected only by APGC-QTOF-MS, probably due to the higher sensitivity in APGC-QTOF-MS than in GC-MS or their different ionization pattern. Thirteen of them were confirmed by reference standards. They are antioxidants, lubricants, catalysts as well as their transformation products. Besides, two of them were set to have one probable structure, five of them were assigned to have tentative structures, and seven of them were only found to have clear molecular formula. Case studies below will explain how the proposed strategy works in detail.

4.2.1 Reducing the number of candidates with the help of APGC-QTOF-MS

The peak with RT 19.37 min (unless otherwise specified, RT refers to retention time in APGC-QTOF-MS) was buried in the TIC chromatogram as shown in Fig. III-1.6 A. Correspondent peak, which was also hidden in TIC mode GC-MS chromatogram, was clearly found in GC-MS (Fig. III-1.6 B) thanks to AMDIS. However, there were quite a lot of candidates with match even higher than 850, which would cause trouble in identification. Fortunately, most of the candidates could be ruled out with the help of APGC-QTOF-MS. A precursor ion 279.1589 was observed in APGC-QTOF-MS, which suggested that the molecular weight could be 279 ($M^{+\cdot}$) or 278 ($[M+H]^+$) considering two very common adduct modes. Finally, only 4 isomers (diisobutyl phthalate, 1-butyl 2-isobutyl phthalate, dibutyl phthalate, and di-sec-butyl phthalate) out of more than 30 candidates matched the exact mass. Dibutyl phthalate was then rejected by RI. The rest are displacement isomers. Diisobutyl phthalate reference standard was then injected, and it matched very well the retention time and spectrum in APGC-QTOF-MS. For the others, no standards were available in our lab. However, considering that diisobutyl phthalate had a higher match in NIST search and it was more frequent

detected in FCM in the literature, this peak was confirmed as diisobutyl phthalate with high reliability. The other two were set as "non-confirmed" because there are displacement isomers which could have exactly the same retention time and spectra as those indicated for 1-monostearoylglycerol and 2-monostearoylglycerol below (4.2.3). If this phenomenon happens, the peak could be one of them or the mix of them. That is why they cannot be directly ruled out without the injection of reference standards but only "non-confirmed". The same rule was applied for the peaks RT 15.25 min and RT 18.27 min, which were confirmed as 2,4-di-tert-butylphenol and 3,5-di-tert-butyl-4-hydroxybenzaldehyde, respectively, but the rest as "non-confirmed".

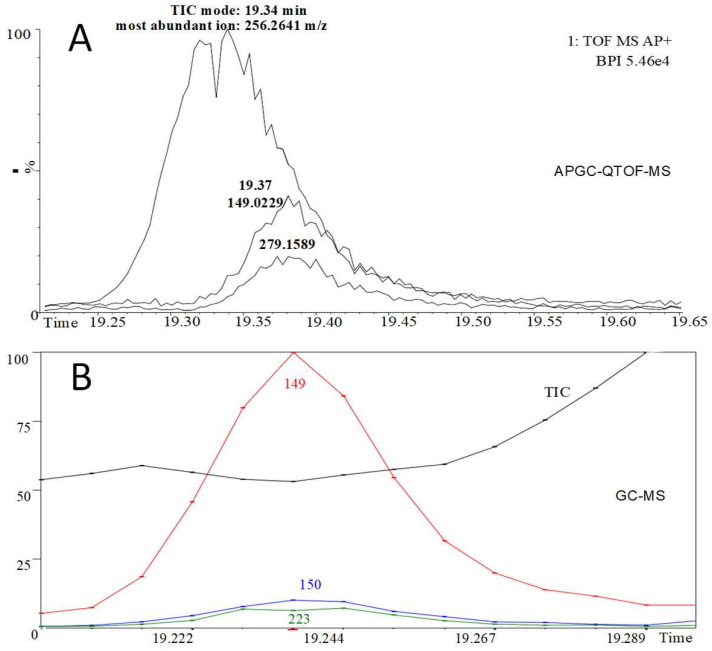


Fig. III-1.6 Chromatograms (TIC and extracted ions) in APGC-QTOF-MS low energy (RT 19.37 min, A) and GC-MS (RT 19.24 min, B)

4.2.2 Molecular formula selection with the aid of GC-MS

It is well known that different adducts could be formed when applying soft ionization like APGC, which would add difficulty in deducing molecular formula; while molecular ion rather than other adducts would exist in EI spectrum if it is not too fragile to break down totally. In this respect, once correspondence is found in GC-MS, effort could be made to find out the molecular ion if it exists. In many cases, it would be very

small even hardly visible. However, APGC-QTOF-MS spectrum could be a good reference telling us where to find it. This step is of great help, especially when correspondence is found but no acceptable candidates can be obtained in GC-MS.

Correspondence was found for the peak (RT 14.02 min), and 26 candidates were listed with match higher than 700. However, all of them were rejected by exact mass as well as RI telling that it would not be present in the NIST library. Precursor ion 163.1123 m/z was obtained in APGC-QTOF-MS. It is worth to mention that odd electron should be selected in Elemental Composition Experiment (ECE) when assuming the precursor ion is $M^{+\cdot}$ and the returned formula is exactly the MF. Whereas even electron should be used when it is assumed as a protonated ion or other adducts, the molecular formula (MF) is the returned formula minus the fixed formula, usually H, in APGC-QTOF-MS. 163 m/z was clearly found in the right end of the GC-MS spectrum (Fig. III-1.7 A) indicating that 163.1123 was the mass of the molecule and $C_7H_{18}NOP$ was generated as the MF by ECE. It is worth mentioning that except for C, H, O, N, which are common elements in polymer compositions, Si and P were chosen as well when conducting ECE. The reason is that these two elements were found in some confirmed components, and some of those unknowns could be related to them, for example reaction products from them. There were three and eleven hits in Chemspider and SciFinder, respectively. Most of them were found in the list of Precursor Chemicals of the Chemical Weapons Convention, and they were then rejected. One of them, 1-propanamine, 3-[(2-methylpropyl)phosphinyl]-, was found to be a starting material of azaphospholanes, which are lubricating oil antioxidants or flame retardants in plastic, and no relevant information was found for the others. Unfortunately, there was no commercial standard available and it was then set as tentative. When looking at RT 11.32 min, 173.1355 m/z was regarded as a protonated precursor ion because 172 m/z was monitored unambiguously in the GC-MS spectrum (Fig. III-1.7 B).

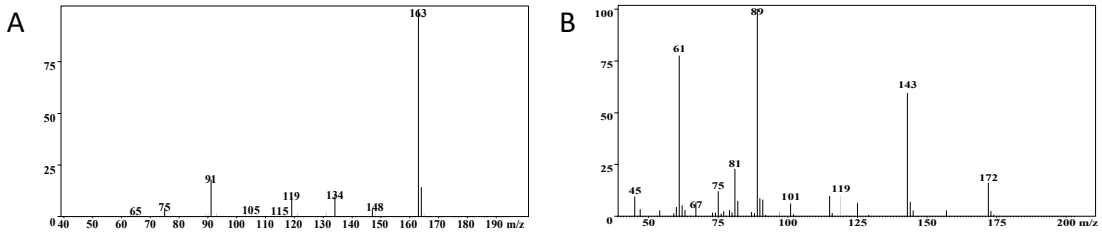


Fig. III-1.7 GC-MS spectrum of the peak at 13.96 min (A) and at 11.32 min (B)

4.2.3 New reaction products in APGC-QTOF-MS

As shown in Table III-1.1, for the compound with RT 26.71 min, correspondence was set at RT 26.42 min in GC-MS because high match candidates (1-MSGC and 2-MSGC) have molecular weight 358.3083, which suggested that 359.3153 m/z could be a protonated precursor ion. Besides, similar fragment 267 m/z was also observed in both systems. The fragment ion 267.2686 m/z in APGC-QTOF-MS corresponded to the formula $C_{18}H_{35}O$ (267.2688 m/z) which is a common fragment from the candidates 1-MSGC and 2-MSGC. Both standards (100 µg/g) were then injected in APGC-QTOF-MS. However, surprisingly, the two standards had exactly the same RT as well as spectra in both systems. Their GC-EI-MS spectra matched very good the library, confirming that those two standards were good. However, in APGC-QTOF-MS, instead of matching RT 26.71 min, they matched RT 26.54 min and had the precursor ion 415.3234 m/z rather than 359.3153 m/z (Fig. III-1.8 A).

This phenomenon was quite unexpected and unusual. It was believed that 415.3234 m/z (RT 26.54 min) might not be the precursor ion of 1-MSGC nor 2-MSGC because no correspondence could be found for that big peak (APGC-QTOF-MS RT 26.71 min, 359.3153 m/z) in the sample GC-MS chromatogram (Fig. III-1.8) if it were. There was no suspected peak at the near right side of the peak (RT 26.42 min) in the GC-MS chromatogram of the sample (Fig. III-1.8 B). It is noteworthy that the peak (RT 26.42 min in GC-MS) in the sample was much higher than that in the standards (100 µg/g), suggesting that its concentration in the sample could be very high. Therefore, this unusual phenomenon was suspected to be related to the high concentration.

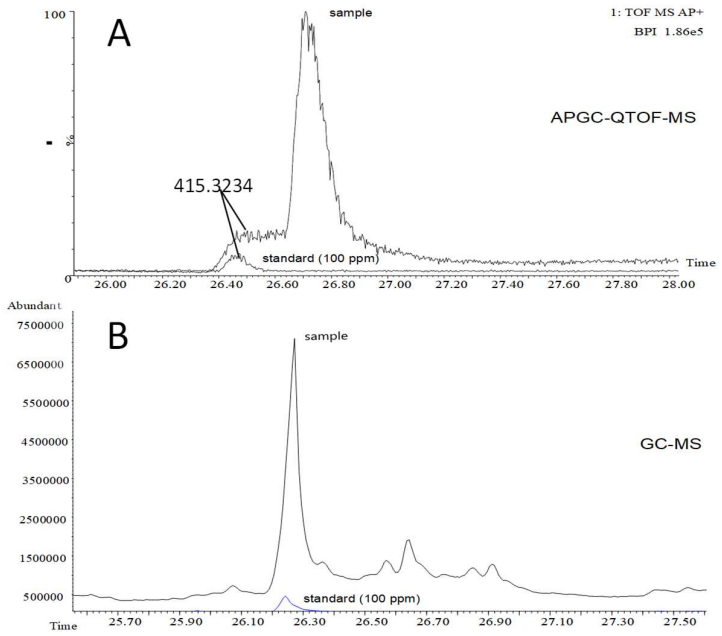


Fig. III-1.8 Sample and standard chromatograms in APGC-QTOF-MS (A) and GC-MS (B)

To further understand the phenomenon, higher concentration (200 µg/g 2-MSGC) of the standard was injected. Fig. III-1.9 illustrates that extracted mass 359.3161 was totally absent in 100 µg/g (A) but obviously present in 200 µg/g (B) standard chromatograms. As the concentration increased, extracted mass 359.3161 appeared. It suggested that 359.3161 (RT 26.71 min) could be the protonated ion of the standard, and 415.3234 could be a reaction product related to the standard. The reason for missing 359.3161 ion in 100 µg/g standard could be that all standards entering the column were totally consumed to produce a new product that had 415.3234 precursor ion. Hence, no protonated standard appeared in the chromatograms. When the concentration increased to 200 µg/g, the chemical that reacted with the standard was finished, so the rest of standard could be eluted out and protonated under APGC. Finally, RT 26.71 min was confirmed as 1-MSGC and 2-MSGC and had correspondence with RT 26.42 min in GC-MS.

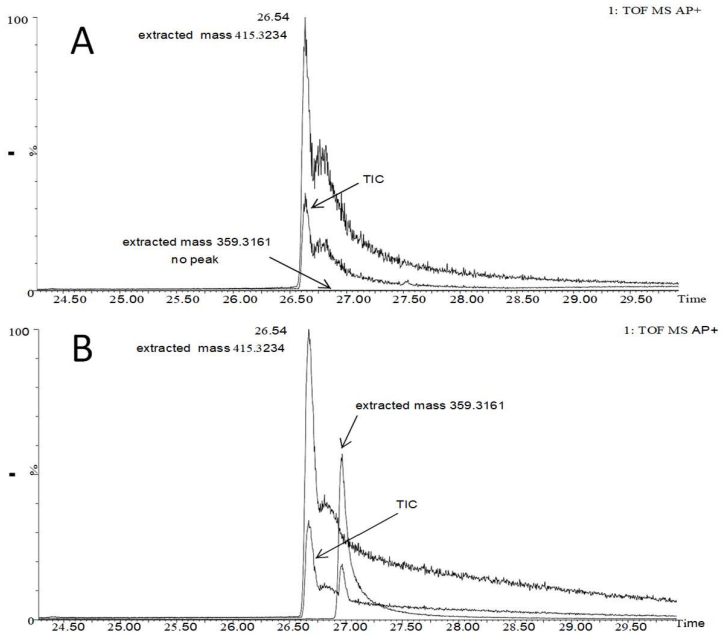


Fig. III-1.9 TIC and extracted masses of 2-monostearoylglycerol standard in APGC-QTOF-MS at 100 µg/g (A) and 200 µg/g (B)

To better understand the confusing peak that had a precursor ion 415.3234 (RT 26.54 min), ECE was then conducted and $C_{23}H_{47}O_4Si$ was unequivocally generated. The distinction between the generated formula and the standards is C_2H_5Si. It was interesting that silanediol, dimethyl (CAS 1066-42-8, MF $C_2H_8O_2Si$), which could be a transformation product of the silicone base septum or the methyl 5% phenyl polysiloxane based column, was witnessed in many of our daily injections in GC-EI-MS. This substance could react with the two near hydroxyl groups by losing two H_2O under certain conditions. Taking 1-MSGC as an example, the possible reaction is shown in Fig. III-1.10. The reaction product (MF $C_{23}H_{46}O_4Si$) was then protonated and detected by the detector. The absence of this reaction product in GC-MS chromatograms is possibly due to either the high electronic ionization energy breaking down the reaction product totally and/or the lower sensitivity of quadrupole mass spectrometer. In addition, similar columns were used in both systems though, their amount of column bleed could be a little different depending on their usage.

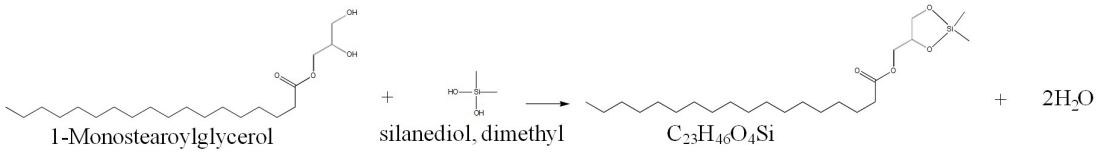


Fig. III-1.10 Possible reaction between 1-monostearoylglycerol and silanediol, dimethyls

Same phenomenon was witnessed for NX 8000K (MF $C_{29}H_{40}O_6$, 484.2825). Consequently, 485.2911 (RT 34.53 min) was set as its protonated molecular ions and 541.2986 (RT 34.12 min, MF $C_{31}H_{45}O_6Si$) as its protonated reaction product ion. For the peak with RT 24.83 min in GC-MS, 1-monohexadecanoylglycerol and/or 2-monohexadecanoyl-glycerol (MF $C_{19}H_{38}O_4$, 330.2770) were found to be candidates with high match (both match and R. match higher than 900). Finally, 331.2848 (RT 25.03 min) was set as its protonated ion and 387.2929 (RT 24.87 min, MF $C_{21}H_{43}O_4Si$) was set as its protonated reaction product ion, even though no standard was available in our lab because they have very similar structure with 1-MSGC, 2-MSGC and NX 8000K (Fig. III-1.11). They all have two hydroxyl groups near to each other which would allow them to react with silanediol dimethyl in a same manner.

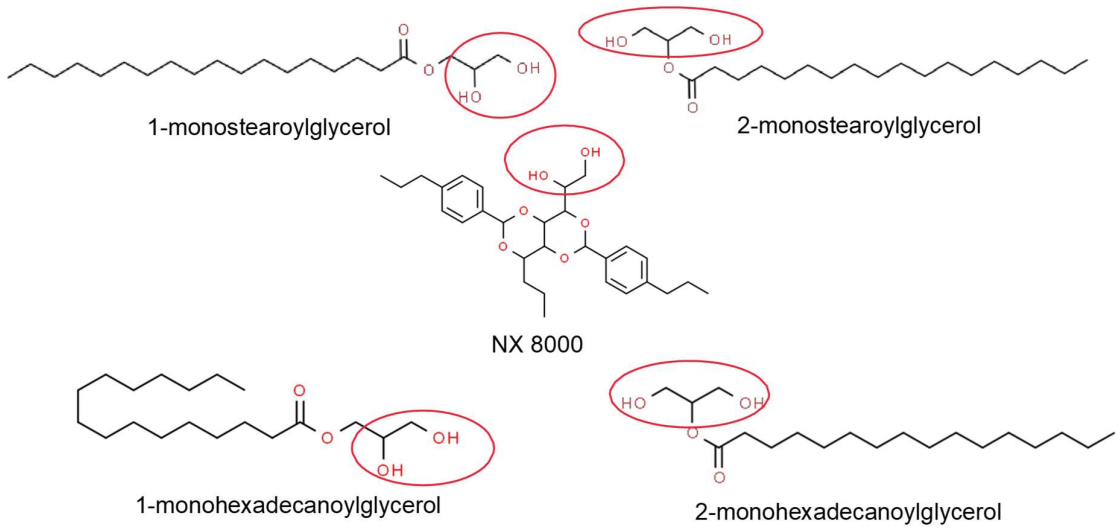


Fig. III-1.11 Structural similarity of 1-monostearoylglycerol, 2-monostearoylglycerol, NX 8000K, 1-monohexadecanoylglycerol and 2-monohexadecanoylglycerol

5. Conclusions

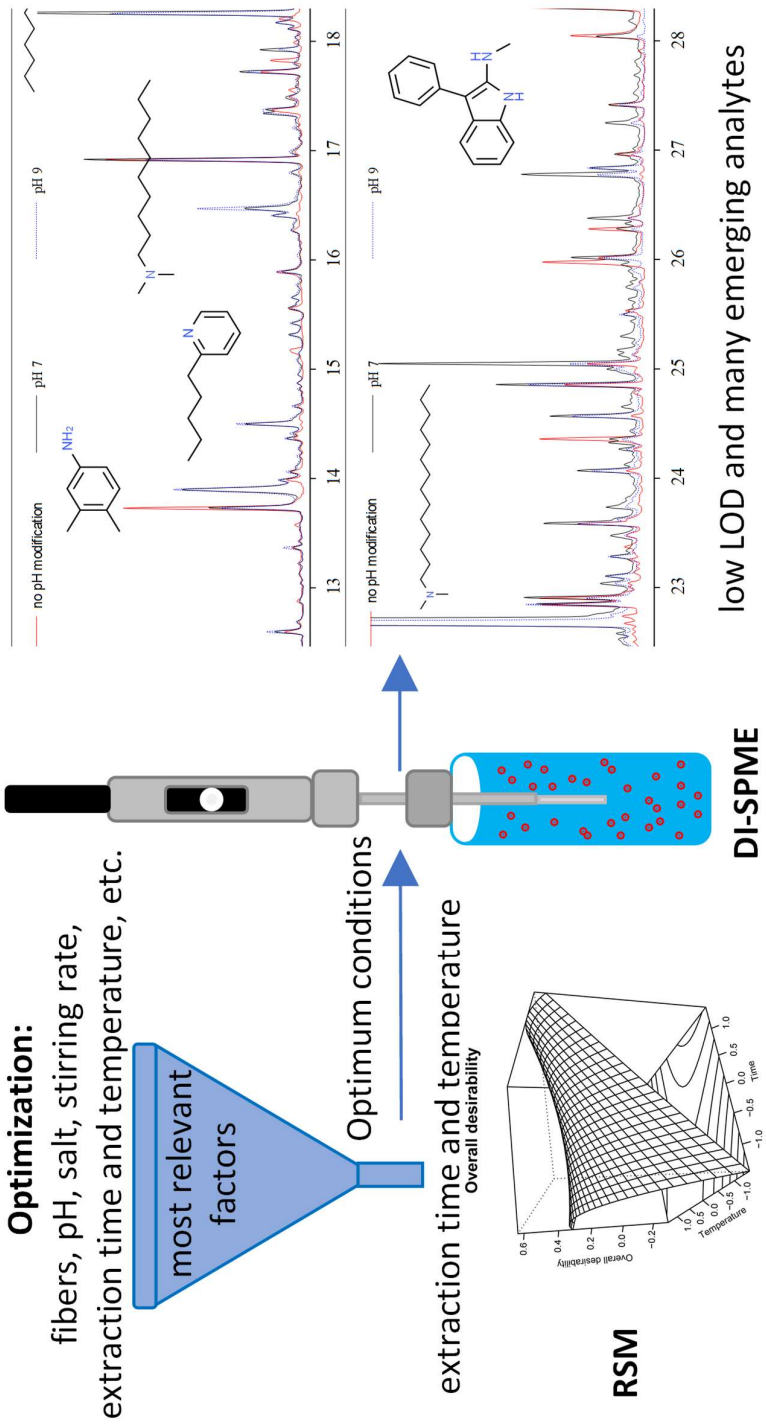
A non-target screening strategy using APGC-QTOF-MS and GC-EI-MS as complementary techniques have been established and successfully applied for the identification of two PP extracts. The methodology could be used in many other research fields regarding non-target screening of volatile compounds. Besides, comprehensive comparison of the two systems has been conducted which would be helpful when using these two techniques. $[M+H]^+$ or $M^{+\cdot}$ are two common precursor ions in APGC-QTOF-MS. However, $[M-H]^+$, $[M-3H+O]^+$, $[M-3H+2O]^+$, and $[M-H+3O]^+$ precursor ions were found for alkanes depending on the number of carbons. This phenomenon could add difficulty in deducing molecular formula. It is still unclear that if chemicals that have similar properties to alkanes would have similar ionization modes or not, so caution should be paid when deducing MF. Furthermore, new reaction products formed by diol compounds and silanediol dimethyl, which would be a transformation product of the silicone base septum or the methyl 5% phenyl polysiloxane based column, were found. It was quite unusual and has caused confusion during the identification process. In summary, APGC-QTOF-MS is a powerful tool for volatile chemicals identification. However, caution must be paid to avoid mistaken identification.

Chapter 2

Direct Immersion - Solid-Phase Micro-extraction Coupled to Gas Chromatography - Mass Spectrometry and Response Surface Methodology for Non-target Screening of (Semi-) Volatile Migrants from Food Contact Materials

1. Abstract

Towards a more rigorous inspection of food contact materials, the importance of sample preparation for non-target screening should be addressed. Direct immersion – solid-phase micro-extraction coupled to gas chromatography mass spectrometry (DI-SPME-GC-MS) was optimized for non-target screening of migrants in 3% acetic acid, 10% ethanol, and 95% ethanol food simulants by response surface methodology (RSM) in the present study. Optimum conditions were DVB/CAR/PDMS fibre, no pH adjustment for 10% and 95% ethanol simulant but pH adjustment to 7 for 3% acetic acid simulant, no salt addition, 5 min pre-incubation, 55 min extraction at 70 °C, 8 min desorption at 250 °C. Besides, 9.5 times dilution of 95% ethanol samples prior to extraction was required. pH modification of 3% acetic acid samples was found to be critical for the extraction of amines. The proposed methodology was then evaluated by determining the limit of detection (LOD) as well as repeatability of 35 food contact materials - related substances. Except for those amines and diols which have relatively high LOD, the LODs of the rest substances were 0.1 - 14.1 µg/kg with precision 1.9 - 23.0% in 10% ethanol and were 0.1 – 20.2 µg/kg with precision 2.5 - 19.6% in 3% acetic acid simulant. The LOD and precision in 95% ethanol simulant were 0.7 – 163.7 µg/kg and 1.4% - 26.8%, respectively. The proposed method can be applied for an overall screening of migrants from these 3 simulants at even trace level though, attentions should be paid to some specific analytes, e.g., diols and amines, which could have high LOD and toxicity.


Optimization:
fibers, pH, salt, stirring rate,
extraction time and temperature, etc.
most relevant factors
Optimum conditions
extraction time and temperature
Overall desirability
RSM
DI-SPME
low LOD and many emerging analytes
no pH modification
pH 7
pH 9
Overall desirability
Temperature
Time

2. Introduction

Food contact materials (FCM) are manufactured from raw materials and so-called IASs, such as antioxidants, lubricants, UV stabilizers, and so on (Peters et al., 2019). However, NIAS can be present in FCMs as well, e.g. reaction by-products, oligomers, degradation and/or impurities of raw materials, etc. (Geueke, 2013). Both of them could migrate into the contacting foodstuffs from FCMs and therefore post potential risks to human health.

Target analysis is a conventional way to check the compliance of FCMs with legislation. However, this strategy does not work for NIAS since we do not even know what they are. To further ensure consumers' health, NTS is drawing increasing attention in recent years (Martínez-Bueno et al., 2019; Peters et al., 2019). Nevertheless, to the best of our knowledge, most of the publications about NTS of migrants mainly focus on the strength of different techniques, especially the use of high resolution mass spectrometry, in the qualification of unknown compounds (Canellas et al., 2015; Gómez Ramos et al., 2019; Martínez-Bueno et al., 2017; Onghena et al., 2015; Su et al., 2019; Vaclavikova et al., 2016). Few attentions have been concentrated on the potential of various sample preparations in NTS. Sample preparation is one of the important aspects of NTS because it determines the capacity of the screening methodology, i.e., the number of substances that can be detected and their limit of the detection (LOD).

LC coupled to HRMS is powerful and commonly used for structure elucidation of unknown migrants. However, it is still time-consuming, laborious, and sophisticated, posing a great challenge to analysts since no FCM-related library is publicly available to date (Nerín et al., 2013). Interestingly, 47 out of 89 FCM-related chemicals (< 500 Da) tentatively identified by LC-Q-HRMS in 10 scientific articles (Aznar et al., 2015, 2012; Canellas et al., 2017, 2015; Gómez Ramos et al., 2019; Isella et al., 2013; Martínez-Bueno et al., 2017, 2016; Onghena et al., 2015; Pezo et al., 2012) were found in the NIST 14 library for GC-MS (Table III- 2.1). The fact suggests that the power of GC-MS with libraries can be further explored in terms of NTS. Moreover, retention index is well established to assist identification in GC-MS increasing the reliability of

identification when no reference standard is available, which is quite common for NIAS. Then, developing a sensitive GC-MS method towards a wide range of analytes can release the burden of structure annotation of many compounds using HRMS and help us focus on truly unknown analytes.

Table III- 2.1 Food contact materials - related compounds (< 500 Da) detected in 10 publications by LC-Q-HRMS and their presence/absence in NIST 14 library

N°	Name	CAS	Mass	NIST	Reference
1	diethylene glycol adipate	6607-34-7	216	N	(Aznar et al., 2015)
2	di(tetrahydrofurfuryl)adipate	105-02-2	314	N	
3	dibutyl pimelate	51238-94-9	272	Y	
4	diethyl sebacate	110-40-7	258	Y	
5	tributyl acetylcitrate	77-90-7	402	Y	
6	dipropyl sebacate	15419-91-7	286	N	
7	triethylene glycol monododecyl ether	3055-94-5	318	Y	
8	tetraethylene glycol monododecyl ether	5274-68-0	362	Y	
9	dibutyl sebacate	109-43-3	314	Y	
10	glycol ricinolate	106-17-2	343	N	
11	di-hexyl sebacate	122-62-3	426	Y	
12	bis(2-methoxyethyl)adipate	106-00-3	262	Y	
13	neopentyl glycol adipate	27925-07-1	214	N	
14	triphenyl phosphate	115-86-6	326	Y	
15	bis[1-(2-ethylbutoxy)-1-oxo-2-propanyl] 2-butenedioate	Not available	-	N	
16	oleamide	301-02-0	281	Y	
17	stearamide	124-26-5	283	Y	
18	behenic amide	3061-75-4	339	Y	
19	N,N-diethyldodecanamide	3352-87-2	255	Y	(Martínez-Bueno et al., 2017)
20	glyceryl palmitate	542-44-9	330	Y	
21	glyceryl monostearate	123-94-4	359	Y	
22	N-[(9Z)-9-octadecen-1-yl]acetamide	82448-16-6	310	N	
23	1,8-diazacyclotetradecane-2,9-dione	56403-09-9	226	N	(Gómez Ramos et al., 2019)
24	caprolactam	105-60-2	113	Y	
25	1,8,15-triazacycloheneicosane-2,9,16-trione	56403-08-8	339	N	
26	1,8,15,22-tetraazacyclooctacosane-2,9,16,23-tetrone	5834-63-9	452	Y	
27	1,8,15,22,29-pentaazacyclopentatriacontane-2,9,16,23,30-pentone	864-90-4	566	N	
28	3,4,6,7-tetrahydro-2,5,8-benzotrioxacycloundecin-1,9-dione	13988-26-6	236	N	
29	3,6,9,12,15-oxabicyclo(15,3)heneicosa-1(21),17,19-triene-2,16-dione	65745-83-7	324	Y	
30	1,6,11,16-tetraoxacycloicosane-2,5,12,15-tetrone	110365-01-0	344	N	
31	AA-MEG-AA-DEG	-	-	N	
32	diethyl 5-({[(2,4,5-trimethoxybenzoyl)oxy]acetyl}amino) isophthalate	-	-	N	
33	1,6-dioxacyclodecane-7,12-dione	777-95-7	200	Y	
34	1,6,13,18-tetraoxacyclotetracosane-2,5,14,17-tetrone	141850-18-2	400	N	
35	diglycerol	627-82-7	166	Y	(Aznar et al., 2012)
36	triglycerol	56090-54-1	240	N	
37	tetraglycerol	56090-54-1	314	N	
38	caffeine	58-08-2	194	Y	
39	dipropylene glycol monomethyl ether	34590-94-8	148	Y	
40	1,2-benzisothiazol-3(2H)-one	2634-33-5	151	Y	(Canellas et al., 2015)
41	3(2H)-isothiazolone, 2-methyl-	2682-20-4	115	Y	
42	2,4,7,9-tetramethyl-5-decyne-4,7-diol	126-86-3	226	Y	(Canellas et al., 2017)
43	2,4,6-triamino-1,3,5-triazine	108-78-1	126	Y	

N°	Name	CAS	Mass	NIST	Reference
44	(2E)-3-phenylprop-2-enal	104-55-2	132	Y	(Martínez-Bueno et al., 2016)
45	N,N-dimethyldecylamine	1120-24-7	185	Y	(Isella et al., 2013)
46	1,4-dioxacyclotridecane-5,13-dione	4471-27-6	213	N	
47	N,N-dimethyldodecylamine	112-18-5	213	Y	
48	1,1-(methanediyldibenzene-4,1-diyl)bis[3-(2-hydroxyethyl)urea]	7747-61-7	372	N	
49	N,N-dimethyllauramide	3007-53-2	227	Y	
50	N,N-dimethylpentadecanamine	17678-60-3	255	Y	
51	bis(3,4-dimethylbenzylidene)sorbitol	135861-56-2	415	N	(Onghena et al., 2015)
52	2,5-bis(5-tert-butyl-2-benzoxazolyl)thiophene	7128-64-5	430	Y	
53	benzoic acid, 4-ethoxy-,ethyl ester	23676-09-7	194	Y	
54	aminocaproic acid	60-32-2	131	Y	(Pezo et al., 2012)
55	L-leucine, N-L-leucyl-	3303-31-9	244	Y	
56	dimethyl phthalate	131-11-3	194	Y	
57	l-leucyl-l-leucyl-l-leucine	10329-75-6	357	N	
58	1,8-diazacyclotetradecane-2,9-dione	56403-09-9	226	N	
59	1,4,7,18,21-pentaoxa-11,14,25,28-tetraazacyclohentriacontane(9CI)	178472-45-2	448	N	
60	1,8,15-triazacycloheneicosane-2,9,16-trione	56403-08-8	339	N	
61	4H-imidazol-4-one,3-[(1-acetyl-4-piperidinyl)methyl]-2-amino-5-butyl-5-(cyclohexylmethyl)-3,5-dihydro-	856881-34-0	390	N	
62	1,3-bis(isocyanatomethyl)-cyclohexane	38661-72-2	194	N	
63	butanediamide,N4-hydroxy-N1-[(1S)-2-methyl-1-(1-pyrrolidinylcarbonyl)propyl]-2-pentyl-,(2R)-	54124-71-9	355	N	
64	N-cyclohexylurea	698-90-8	142	Y	
65	Diazenedicarboxamide,N,N’-dihexyl-(9CI)	18880-27-8	284	N	
66	4-piperidinol,1-hydroxy-2,2,6,6-tetramethyl-	3637-10-3	173	N	
67	triethylamine	121-44-8	101	Y	
68	1-cyanodecane	2244-07-7	167	Y	
69	1-cyclohexyl-3-methyl-urea	39804-96-1	156	N	
70	Tert-butyl N-(4-aminobutyl)-N-[4-(4-aminobutyl-tert-butoxycarbonyl-amino)butyl]carbamate	343247-50-7	430	N	
71	1,4-bis(isocyanatomethyl)-cyclohexane	10347-54-3	194	N	
72	naphtylethylenediamine	551-09-7	186	Y	
73	1,4-bis(2-hydroxypropyl)-2-methylpiperazine	94-72-4	216	N	
74	2-eicosane,1-diazo-	102376-62-5	322	N	
75	4(1H)-Quinazolinone,2,3-dihydro-2-phenyl-	954-91-6	224	N	
76	1,3,4-thiadiazole-2-acetic acid,5-amino-,ethyl ester	88124-55-4	187	N	
77	phenanthridine	229-87-8	179	Y	
78	2-(3-Tert-butyl-9-methyl-6,8-dioxo-7-propyl-6,7,8,9-tetrahydro[1,2,4]triazino[3,4-f]purin-1(4H)-yl)acetamide	-	375	N	
79	pyridine, 4,4'-trimethylenedi-	17252-51-6	198	Y	
80	acridine,1,2,3,4-tetrahydro-	3295-64-5	183	N	
81	9,10-dihydroxylysergic acid	5878-43-3	270	Y	
82	1-(cyclohexylcarbonyl)piperazine	27561-62-2	196	N	
83	1,8,15,22-tetraaza-2,9,16,23-cyclooctacosanetetrone	5834-63-9	452	Y	
84	morpholine, 4-(1-cyclohepten-1-yl)-	7182-08-3	181	Y	
85	l-lysyl-l-prolyl-l-valine	67727-97-3	342	N	
86	Urea, N'-cyclooctyl-N,N-dimethyl-	2163-69-1	198	Y	
87	3,6,9,12,15-pentaoxaheptadecane-1,17-diol,hexamethyl-	52794-80-6	366	N	
88	3,6,9,12,15,18,21-heptaoxaoctacosan-1-ol	39619-72-2	424	N	
89	1,3-dicyclohexylurea	2387-23-7	224	Y	

Migration test using different types of food simulants (3% acetic acid, 10% ethanol, 95% ethanol, etc.) (EC, 2011) is widely applied to assess the safety of FCM

because they are much simpler than foodstuffs, helping us focus more on those components coming out from FCM. Various strategies have been applied to extract migrants from aqueous simulants prior to GC analysis, for example, LLE (N. Zhang et al., 2018), rotatory evaporation and re-dissolution with GC-amenable solvents,(Carrero-Carralero et al., 2019), and HS-SPME (Alin and Hakkarainen, 2012). To our knowledge, only LLE has been optimized for NTS of migrants from 50% ethanol (a food simulant for milk) (Onghena et al., 2014). However, in comparison to LLE, SPME is simpler, solvent-free, and available in autosampler. It is a versatile and non-exhaustive sample preparation tool and has been successfully applied in a wide variety of fields, e.g., flavour and fragrance investigations, environmental studies, and diverse bioanalytical applications.(Reyes-Garcés et al., 2017)

In light of these advantages, the present work aims to develop and optimize direct immersion (DI) SPME (DI-SPME) for the extraction of migrants from different food simulants regarding un-targeted screening. As far as we know, it is the first attempt to optimize DI-SPME for untargeted migrants screening purposes. Experimental conditions that would affect the extraction efficiency of DI-SPME were first optimized using a central composite design (CCD) and response surface methodology (RSM). The optimization was conducted using migration solutions from recycled polyolefin sample instead of only a few selected standards since it would contain many polyolefin-related chemicals and would be more representative to screen polyolefin's migrants. In addition, the power of the optimized DI-SPME for untargeted screening of migrants from other FCMs was also evaluated by determining LOD and repeatability of 35 reference standards which are commonly found in FCMs. The present study is part of our work to assess the potential of using recycled polyolefins for food contact purpose and will offer us convenience, reliability, and robustness to comprehensively investigate potential human health-related compounds that are present in recycled polyolefins.

3. Materials and methods

3.1. Reagents and samples

Standards were purchased from Sigma-Aldrich (Madrid, Spain): triethylamine (121-44-8), p-xylene (106-42-3), caprolactam (105-60-2), α-methylstyrene (98-83-9), 2,6-diaminotoluene (823-40-5), allyl methacrylate (96-05-9), naphthalene (91-20-3), 2-naphthylamine (91-59-8), dipropylene glycol monomethyl ether (34590-94-8), eugenol (97-53-0), 1-dodecene (112-41-4), diphenyl ether (101-84-8), benzophenone (119-61-9), 2-ethylhexyl acrylate (103-11-7), dimethyl isophthalate (1459-93-4), ethylene glycol dimethacrylate (97-90-5), 2,6-diisopropylnaphthalene (24157-81-1), o-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine (PFBOA, 72915 -12-9), diphenyl carbonate (102-09-0), 2,4,7,9-tetramethyl-5-decyne-4,7-diol (126-86-3) , bisphenol A (80-05-7), diethyl sebacate (110-40-7), stearamide (124-26-5), dibutyl sebacate (109-43-3), Cyasorb UV 12 (131-54-4), Tinuvin 326 (3896-11-5), Chimassorb 81 (1843-05-6), glyceryl monostearate (123-94-4), octocrylene (6197-30-4), bis(2-ethylhexyl)adipate (DEHA, 103-23-1), dioctyl terephthalate (4654-26-6), tributyl acetylcitrate (77-90-7), dinonyl phthalate (84-76-4), di-hexyl sebacate (122-62-3), 2,5-bis(5-tert-butyl-2-benzoxazolyl)thiophene (7128-64-5). Stock solutions (more than 1000 μg/g) of each standard were prepared in methanol or ethanol except for Tinuvin 326 and 2,5-Bis(5-tert-butyl-2-benzoxazolyl)thiophene, which were prepared in dichloromethane and hexane, respectively. They were then grouped (ca. 8 standards per group), mixed and diluted until 10 μg/g in ethanol as working solutions. Tiny amount (0.018g) of working solutions was spiked into different food simulants (18 mL), that is 10 ng/mL in simulants, to evaluate the performance of the developed method. For less sensitive analytes, higher concentrations were prepared accordingly. All standards and solutions were under gravimetric control.

Recycled polyolefin pellets (cylinder-like, d = 5 mm, h = 2 mm, density = 971 kg/m^3) were supplied by a European company. Post-consumer polyolefin was collected, washed, and extruded without applying super clean process according to the company. Twenty grams of sample were immersed in 100 mL food simulant (3% acetic acid and

95% ethanol) for migration at 70 °C for 2 h. Afterwards, they were filtered (0.2 μm hydrophilic polypropylene filter) at room temperature and stored in the fridge at -25 and 4 °C for 95% ethanol and 3% acetic acid, respectively, to minimize any change over time. They were then used for optimization by DI-SPME-GC-MS. Blanks were simultaneously prepared to remove sample-irrelative features.

3.2. GC-MS analysis

A 6500 CTC autosampler mounted gas chromatography (6890N) coupled to a mass spectrometry (5975) was from Agilent (California, USA). The separation was performed on a DB-5 MS column from Agilent (30 m × 0.25 mm id, 0.25 μm film thicknesses). Agilent ultra-inert liners (id = 0.75 and 4 mm for SPME and liquid injection, respectively) were used and the inlet temperature was set at 250 ºC and the carrier gas flow (He) was 1.0 mL/min. Scan mode with a mass range from 40-700 Da was applied. Spitless mode and 5 min solvent delay were employed. 2 μL of injection volume was applied for liquid injection. The ramp of temperature was as follows: held 50 ºC for 5 min, increased to 300 ºC at the rate of 8 ºC/min, and held for 10 min. Grob mixture was used for quality control of the GC-MS.

3.3. Sample treatment for 95% ethanol samples

Two strategies were applied to process 95% ethanol samples. One was to concentrate 5 mL sample into 1 mL by a nitrogen concentrator (Techne DB-3; Staffordshire, UK) at 40 °C and directly injected in GC-MS. Another one was to use DI-SPME-GC-MS, which was to transfer an aliquot of 1.9 mL sample into an 18 mL glass vial following by adding 16.1 mL water (that is 10% ethanol). The obtained solution was mixed, extracted by DI-SPME (1 cm 50/30 μm DVB/CAR/PDMS fibre), and finally injected into GC-MS. The DI-SPME was accomplished by a 6500 CTC autosampler, and the conditions were: 600 rpm stirring rate, 5 min pre-incubation, 30 min extraction at both 40 and 80 °C, 8 min desorption in the GC inlet. Following desorption, the fibre was cleaned at 270 °C in a fibre cleaner for 2 min.

3.4. Selection of fibre coating

The extraction efficiencies of 5 SPME fibres were compared using the DI-SPME process described above except for the extraction temperature that was fixed at 80 °C. One-centimetre-long fibres including 50/30 μm DVB/CAR/ PDMS, 100 μm PDMS, 85 μm CAR/PDMS, 85 μm polyacrylate (PA), and 65 μm PDMS/DVB were purchased from Supelco (PA, USA). All fibers were conditioned prior to use according to the manufacturer's guide. **Identification of the most relevant DI-SPME factors** The selected fibre was then used for finding out the most important DI-SPME factors. Influences of salt (5% and 10% NaCl and 5% Na_2SO_4) and pH (pH =5, 7 (original), and 9) were first examined. It is worth mentioning that all DI-SPME optimizations including RSM were conducted using diluted 95% ethanol migration sample though, the final optimized parameters were applied for 3% acetic acid samples as well. The only exception was that the effect of pH (pH = 2 (original), 7, and 9) on the extraction efficiency of migrants from 3% acetic acid was examined independently since the pH of 3% acetic acid is very low and would have a significant influence on the extraction. NaOH pellets were first used to neutralize 3% acetic acid solutions in a large scale until ca. 6.5 since its high acidity. NaOH and acetic acid water solutions (both high and low concentrations) were then utilized for fine adjustment. Moreover, each group of comparative experiment was processed in a same batch to minimize any change (if there was) of the sample.

3.6. Response surface methodology: central composite design

Once the most relevant factors were identified, a response surface methodology was employed to optimize the best conditions. A blocked CCD including 14 experiments (7 for each block) was used. The first block consisted of 4 factorial points (-1, 1) and 3 central points (0, 0); the second block included 4 rotatable axis (star) points (-1.414, 1.414) and 3 central points. The studied factors and their levels are shown in Table III-2.2. The RSM and all statistical analyses across the study were processed by R programming (Team, 2019) using ***rsm*** (Lenth, 2009) and ***desirability*** (Kuhn, 2016) packages.

Table III-2.2 Noncoded levels of the factors analyzed by CCD

run.order	Temperature (°C)	Time (min)	Block
1 (C)	75	40	1
2	65	30	1
3 (C)	75	40	1
4	85	50	1
5	85	30	1
6	65	50	1
7 (C)	75	40	1
1	75	54.1	2
2 (C)	75	40	2
3	89.1	40	2
4	60.9	40	2
5 (C)	75	40	2
6 (C)	75	40	2
7	75	25.9	2

3.7. Evaluation of the strength of the proposed DI-SPME-GC-MS method for non-target screening of FCM migrants

The potential of the developed non-target screening method was evaluated by determining the LOD and repeatability of 35 reference standards that are possibly present in food contact materials. The LOD was calculated as the concentration that has a signal to noise ratio (S/N) of 3 using the least rather than the most abundance ion. Regarding NTS, detection of the least instead of the most abundance ion is the basis of reliable library match. The repeatability was calculated under 10 µg/kg when possible; if not, higher concentrations were used. The 35 standards (MW < 500 Da) were selected from the following lists considering their availability in the authors' laboratory as well: **1)** analytes that were detected in our recycled polyolefin sample and had Cramer III level; **2)** chemicals that are potentially present in FCM (Sanchis et al., 2017); **3)** substances that were identified in FCM by LC-QTOF-MS but are present in NIST 14 library; 4) substances that have specific migration limit lower than 0.05 mg/kg food in the Commission Regulation EU 10/2011 (EC, 2011). The long list of standards for validation covers a wide range of molecular weight and structures regarding FCMs.

4. Results and discussions

4.1. Sample treatment for 95% ethanol migration samples

For 95% ethanol simulant, it is convenient to inject it directly into GC-MS. However, higher sensitivity is of great interest for NTS of migrants regarding human health. A simple way to achieve higher sensitivity is to concentrate the sample by evaporating the solvent, although some volatile compounds could be vented as well. Another convenient way is to use SPME, since it is highly automated and well-connected to GC-MS thanks to the CTC autosampler. Unlike 3% acetic acid and 10% ethanol simulants, 95% ethanol samples cannot be extracted directly by DI-SPME because high ethanol content would damage or shorten the lifespan of the fibre. One of the compromises is to dilute it, and then it can be extracted by DI-SPME. The dilution decreases the concentration though, higher sensitivity could be obtained by DI-SPME thanks to its powerful extractability. This way, the loss of volatile compounds can be avoided.

In order to see the capability of these two methods (solvent evaporation and DI-SPME), 95% ethanol migration from recycled polyolefin sample was used instead of selecting a few standards because the results of the mentioned strategy could vary a lot depending on the standards selected. What is more, recycled polyolefins were thought to be much more complex than the virgin one and thus, it can be a good representative sample for developing a non-target migrant screening method for recycled polyolefin FCM.

The performance of these two strategies is shown in Fig. III-2.1. As can be seen, liquid injection had much better performance than DI-SPME at 40 °C in terms of the height of peaks at the right side of the chromatogram (more than c.a. 24 min, relatively big molecules), while it turned out to the opposite regarding peaks at the left hand (Fig. III-2.1 A). The reason could be that those small molecules (at the left hand) were lost during the concentration process. However, Fig. III-2.1 B depicts that liquid injection without concentration had much lower peak height across the whole chromatogram meaning that the concentration step did not have significant negative effects on volatile

substances. Another reason for worse performance for high molecular weight substances by DI-SPME could be that the used conditions were not appropriate to extract them. Considering that extraction temperature is a critical parameter for DI-SPME, higher temperature (80 ° C) for DI-SPME was applied. Fig. III-2.1 C demonstrates that DI-SPME at 80 °C had higher efficiency for almost all peaks. The results suggest that DI-SPME applying a high extraction temperature has higher potential for non-target screening of migrants in 95% ethanol simulant than liquid injection. Another advantage of using this strategy is that only one calibration curve is needed for each compound when quantifying migrants in 10% and 95% ethanol.

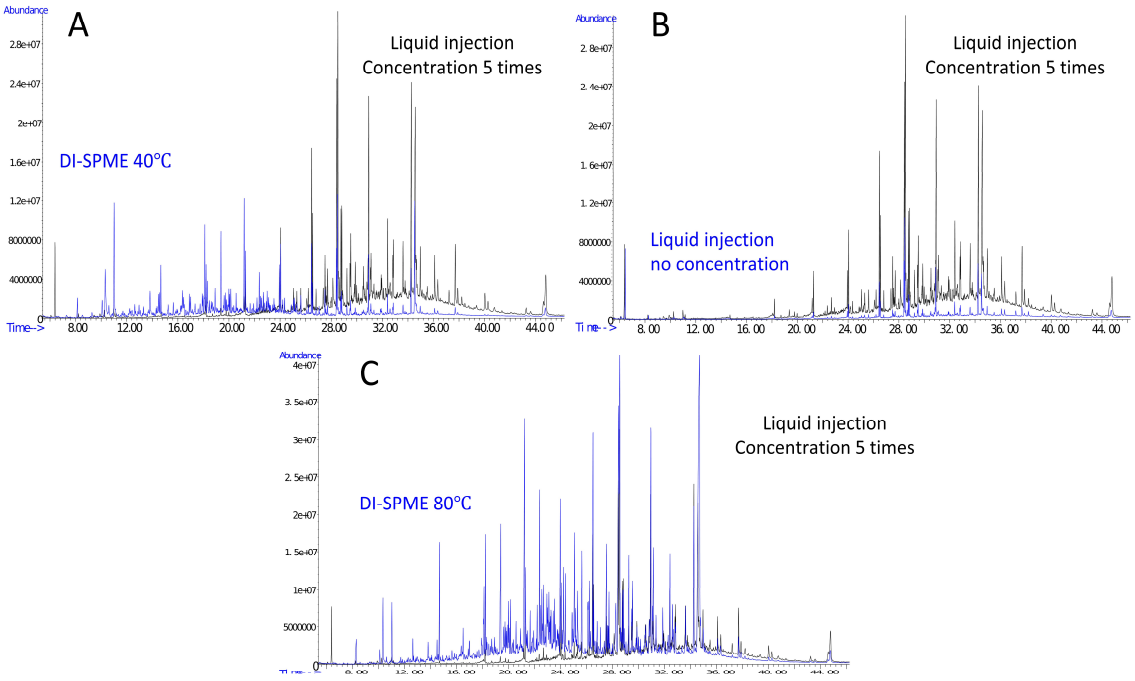


Fig. III-2.1 Comparison of liquid injection with DI-SPME at 40 °C (A) and with DI-SPME at 80 °C (B) using DVB/CAR/PDMS fibre

4.2. Selection of SPME fibre

Fibre coating plays an important role in SPME because the physicochemical properties of the coating greatly affect the distribution of analytes between the sample and the coating. As depicted in Fig. III-2. A, PA fibre showed much lower performance than the DVB/CAR/PDMS one across the whole chromatogram. The result was in

agreement with a previous study where different fibres were compared to extract volatile compounds in plain *sufu* by HS-SPME (Chen et al., 2019). As a polar coating, PA fibre was observed to extract much less of least polar analytes (Shirey and Mindrup, 1999). From a NTS point of view, migrants could be both polar and non-polar. As such, PA fibre was not suggested for NTS. As for the non-polar PDMS fibre, it was better than the PA one Fig. III-2. B though, when compared to the DVB/CAR/PDMS fibre, its limitation was apparent mainly for those components located at the left hand of the chromatogram Fig. III-2. C.

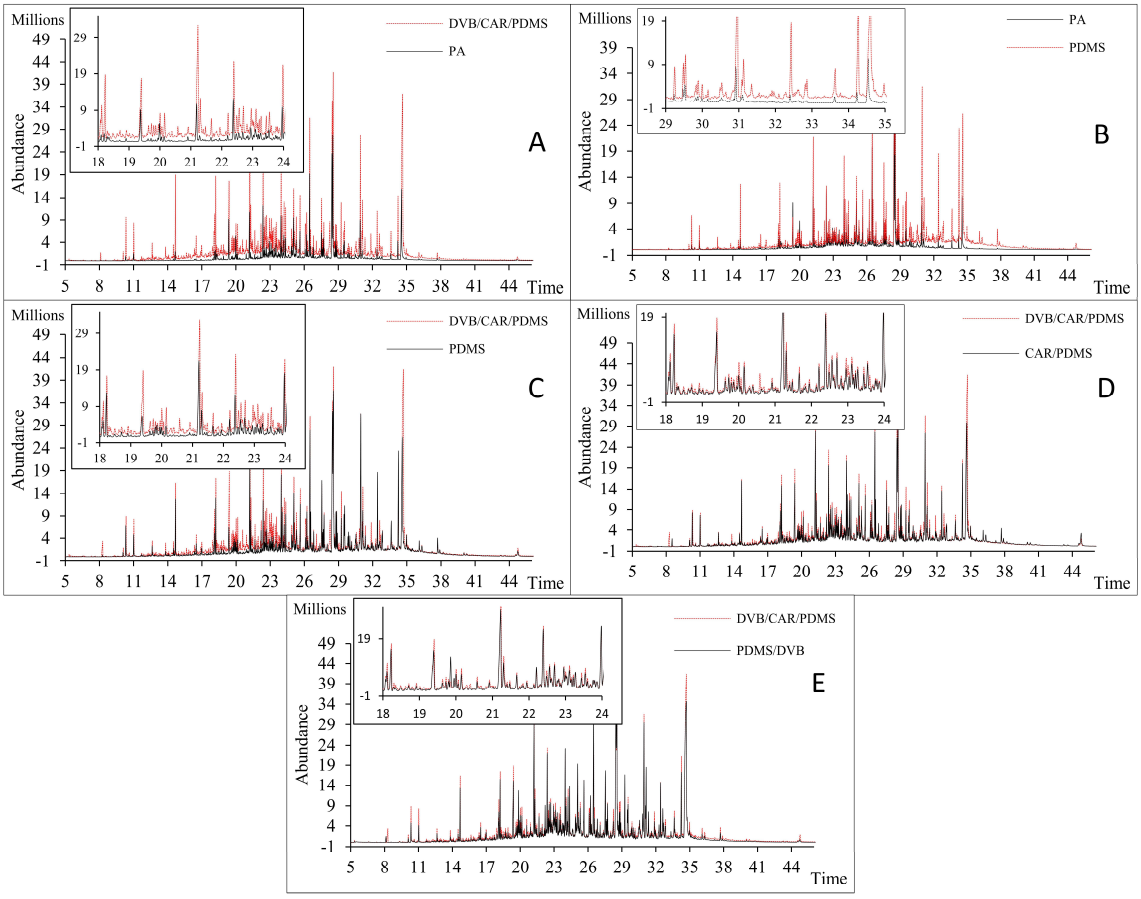


Fig. III-2.2 Comparison of DVB/CAR/PDMS and PA fibres

However, the discrepancies among DVB/CAR/PDMS, CAR/ PDMS, and PDMS/DVB fibres were not visually obvious (Fig. III-2. D and E) and the number of peaks (S/N > 10 and with clear spectrum) detected were the same (140). Nevertheless, the number of substances extracted from a commercial plain *sufu* by these 3 fibres

differed significantly in the research by Chen et al. (Chen et al., 2019) probably because they used HS-SPME instead of DI-SPME. The HS-SPME conditions applied, which were not specified in their study, might affect as well, since the conditions would influence their performances. Further examination was done in terms of peak area. Regarding total peak area, no significant difference was observed among them (ANOVA, $p = 0.197$). Nonetheless, the peaks were divided into 2 groups according to the order of magnitude of their peak areas Table III-2.3, namely Group 1 (47 peaks) and Group 2 (93 peaks), respectively.

Table III-2.3 Information of grouped compounds used for optimization

No.	RT	Names	Area	**No.**	RT	Names	Area
Group 1 (47 analytes)							
1	9.077	Benzene, propyl-	58616	**71**	28.630	Hexadecanamide	8378451
2	8.958	Camphene	84285	**72**	21.475	Tetradecanal	8702707
3	7.369	Unknown (91, 106)	122852	**73**	30.561	Oleamide	9064267
4	9.479	Benzene, 1,3,5-trimethyl-	141323	**74**	16.974	Nonane, 2,2,4,4,6,8,8-heptamethyl-	9064309
5	9.204	Cyclohexene, 4-ethenyl-1,4-dimethyl-	236334	**75**	28.897	Benzoic acid, tridecyl ester	9155463
6	12.929	Benzene, 1,2-dichloro-4-methyl-	375417	**76**	18.304	Diphenyl ether	9406857
7	6.656	p-Xylene	401616	**77**	14.508	1-Dodecene	9895503
8	11.091	Cyclohexane, butyl-	432567	**78**	20.065	Butylated Hydroxytoluene	10008654
9	10.831	Unknown (105, 120)	441554	**79**	23.214	Unknown (197, 212, 155)	10223889
10	7.629	n-Nonane	496654	**80**	27.597	Hexadecanoic acid, propyl ester	10507681
11	13.460	Unknown (69, 55, 83, 154)	498999	**81**	19.797	Pentadecane	10611167
12	7.280	Styrene	638505	**82**	24.158	Carbonic acid, bis(2-ethylhexyl) ester	10625540
13	9.590	Nonane, 3-methyl-	650560	**83**	27.664	Methyl linoleate	11219459
14	6.563	Octane, 4-methyl-	721542	**84**	22.575	1,3-di-iso-propylnaphthalene	12024754
15	11.767	Decane, 4-methyl-	728395	**85**	20.919	n-Tridecan-1-ol	12038892
16	15.481	Unknown (69, 55, 83)	739795	**86**	37.693	(Z)-Decyl icos-9-enoate	12456669
17	12.391	Cyclohexene, 1-methyl-4-(1-methylethylidene)-	848867	**87**	29.511	Tributyl acetylcitrate	12519215
18	13.059	Benzene, 1,2,4,5-tetramethyl-	937602	**88**	12.643	Undecane	12684016
19	11.626	Naphthalene, decahydro-, trans-	973225	**89**	23.036	Methyl tetradecanoate	13458623
20	11.990	Decane, 3-methyl-	1030269	**90**	19.730	2-Tridecanone	13776271
21	13.379	Cyclohexane, pentyl-	1061990	**91**	26.824	Isopropyl palmitate	13781346
22	32.506	Unknown (91, 129, 207)	1191683	**92**	30.814	Stearamide	14296141
23	40.256	Hexadecanoic acid, hexadecyl ester	1292228	**93**	16.504	Tridecane	14483465
24	15.689	Dodecane, 5-methyl-	1423285	**94**	31.349	Glycol stearate	14963824
25	14.040	Unknown (81, 95, 166, 151)	1462760	**95**	22.479	Unknown (120, 138, 191)	15330309
26	15.228	cis,trans-3-Ethylbicyclo[4.4.0]decane	1467926	**96**	26.557	Eicosane	15904189
27	13.304	Benzene, 1,3,5-trichloro-	1501121	**97**	21.669	Dodecanoic acid, 1-methylethyl ester	17175994
28	13.958	Undecane, 2-methyl-	1560058	**98**	33.629	Octocrylene	17678789
29	14.292	Nonanenitrile	1607637	**99**	23.445	Octanal, 2-(phenylmethylene)-	17863165
30	17.286	Heptylcyclohexane	1692385	**100**	28.771	1-Docosene	17866677
31	14.768	2-Dodecene, (Z)-	1794757	**101**	28.763	Octadecanoic acid, ethyl ester	17993286

No.	RT	Names	Area	No.	RT	Names	Area
32	13.876	2,3-Dimethyldecane	1877847	**102**	29.447	trans,trans-9,12-Octadecadienoic acid, propyl ester	18190315
33	44.542	Irganox 1076	2017660	**103**	31.081	Butyl 9,12-octadecadienoate	19857038
34	13.238	Naphthalene, decahydro-2-methyl-	2076487	**104**	31.861	Unknown (55, 41, 69, 122, 136)	21490483
35	15.979	Dodecane, 3-methyl-	2178348	**105**	22.561	Dodecanoic acid, propyl ester	22436416
36	10.615	Heptane, 3-(chloromethyl)-	2181282	**106**	27.731	Methyl elaidate	22438493
37	12.881	trans-Decalin, 2-methyl-	2264361	**107**	18.118	Decanoic acid, ethyl ester	22901735
38	14.367	Naphthalene	2356798	**108**	22.702	2-Pentadecanone	23276110
39	14.939	Undecane, 2,6-dimethyl-	2378049	**109**	20.154	Dodecanoic acid, methyl ester	23476114
40	14.092	Undecane, 3-methyl-	2592053	**110**	22.211	Octane, 1,1'-oxybis-	24925834
41	32.091	Benzoic acid, pentadecyl ester	2736767	**111**	22.954	Ethanol, 2-(dodecyloxy)-	25022498
42	14.582	Octanoic acid, ethyl ester	2793635	**112**	29.536	cis-9-Octadecenoic acid, propyl ester	25487259
43	19.368	2,6-Di-tert-butylquinone	2997417	**113**	28.830	Docosane	25595856
44	22.145	Unknown (92, 196, 105)	3298010	**114**	23.541	Benzene, 1,1'-(1,2-cyclobutanediyl)bis-, cis-	27349236
45	20.674	Unknown (155, 170)	3460824	**115**	25.309	Homosalate	27674310
46	18.378	Naphthalene, 2,6-dimethyl-	3481720	**116**	26.223	Ethyl 9-hexadecenoate	28377205
47	16.900	Unknown (71, 118, 160)	3622867	**117**	24.061	Octadecane	28805833
Group 2 (93 analytes)				**118**	24.901	Galaxolide	30271297
48	21.818	Benzophenone	4426077	**119**	23.110	2,6-Diisopropylnaphthalene	30423416
49	16.372	1-Tridecene	4777803	**120**	11.024	D-Limonene	33365344
50	23.162	Unknown (197, 212, 155)	4871235	**121**	10.340	Decane	36313186
51	29.038	Ethyl 9.cis.,11.trans.-octadecadienoate	4925562	**122**	21.305	Hexadecane	37706460
52	18.623	Naphthalene, 1,7-dimethyl-	5010273	**123**	24.373	Isopropyl myristate	38082049
53	23.489	Unknown (135)	5162880	**124**	25.643	Hexadecanoic acid, methyl ester	44472762
54	20.629	Unknown (155, 170)	5327894	**125**	32.418	Diisooctyl phthalate	45268668
55	33.336	Unknown (73, 55)	5334367	**126**	31.133	Unknown (55, 41, 69, 83, 98, 264)	45853243
56	32.277	Oleic acid, 3-hydroxypropyl ester	5779374	**127**	29.254	Glycol palmitate	52969823
57	19.953	α-Farnesene	6100800	**128**	24.239	2-Ethylhexyl salicylate	53118756
58	20.385	Naphthalene, 1,6,7-trimethyl-	6148843	**129**	27.530	1-Octadecanol	57315090
59	25.190	Tetradecanoic acid, propyl ester	6313226	**130**	25.086	1-Hexadecanol	61968170
60	36.086	Hexadecanoic acid, dodecyl ester	6317511	**131**	34.246	Bis(2-ethylhexyl) isophthalate	75090636
61	18.712	Caryophyllene	6417162	**132**	14.694	Dodecane	76357216
62	30.153	2-Ethylhexyl trans-4-methoxycinnamate	6673091	**133**	22.397	9-Octadecenoic acid (Z)-, 2-hydroxyethyl ester	88916731
63	19.857	1-Dodecanamine, N,N-dimethyl-	6846298	**134**	19.403	1-Dodecanol	91178207
64	10.088	Unknown (57, 41)	7107799	**135**	23.979	Tetradecanoic acid, ethyl ester	91769056
65	29.997	Benzoic acid, tridecyl ester	7228597	**136**	30.947	Hexanedioic acid, bis(2-ethylhexyl) ester	142309372
66	28.680	Hexadecanoic acid, butyl ester	7270795	**137**	26.498	Hexadecanoic acid, ethyl ester	153977625
67	18.348	Dodecanal	7272372	**138**	28.459	Linoleic acid ethyl ester	162354105
68	18.920	Phenol, 2,6-bis(1,1-dimethylethyl)-	7908672	**139**	21.231	Dodecanoic acid, ethyl ester	169288061
69	26.305	Oleanitrile	8212854	**140**	28.548	Ethyl elaidate	174620507
70	24.960	Unknown (149)	8351873				

Note: RT stands for retention time in min; Area is the mean chromatographic peak area.

The total peak area of each group was then calculated, and multivariate analysis of variance (MANOVA) showed significant difference ($p = 0.01939$) among the 3 fibres. Univariate one-way ANOVAs depicted that the distinction mainly came from the Group 1 substances ($p = 0.005047$). Fig. III-2. shows the pairwise mean comparisons regarding Group 1 (Tukey HSD multiple comparisons). As can be seen, both DVB/CAR/PDMS

and CAR/PDMS fibres had better performance than the PDMS/DVB fibre with respect to Group 1 substances. It is noteworthy that the total peak area of Group 1 substances accounted for only ca. 2.5% of the total peak area of all peaks and it was even smaller than the standard deviation of that of Group 2. Therefore, the distinctions in Group 1 were hidden when using the total peak area of all peaks as a measure. In addition, most of the Group 1 compounds had retention time lower than 20 min, which means that most of the Group 1 are small molecules or more volatile compounds. This fact suggests that the DVB/CAR/PDMS and CAR/PDMS fibres had better extractability over small molecules than the PDMS/DVB fibre. The result can be explained by the fact that Carboxen (CAR) has a much higher percentage of micropores which are good for small molecules extraction than the Divinylbenzene (DVB). As for CAR/PDMS and DVB/CAR/PDMS fibre, no significant difference was found though, DVB/CAR/PDMS was selected for NTS of migrants from recycled polyolefins considering its smaller standard deviation over the two groups.

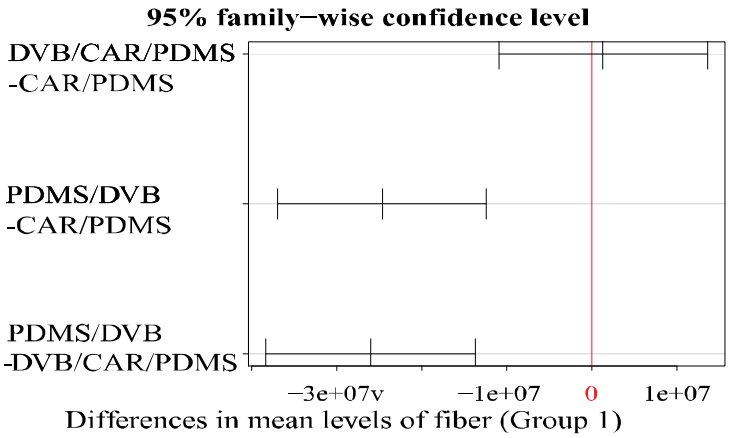


Fig. III-2.3 Tukey HSD pairwise mean comparisons regarding Group 1 substances

4.3. Identification of the most relevant factors

There are many factors that could affect the efficacy of DI-SPME including stirring rate of the agitator, pre-incubation time, addition of organic solvent, dilution of samples, addition of salt, sample pH, extraction temperature, and extraction time. ***Agitator stirring*** rate was found to be positive for all classes of analytes in the research by Zhang et al. (L. Zhang et al., 2018) because it enhances mass transport of the analytes.

As such, 600 rpm was chosen herein according to their research to enable fast agitation without causing mechanical damage to the fibre. ***Pre-incubation time*** was deemed to be more important for HS-SPME, since equilibrium between the sample and the headspace is critical; while for DI-SPME, pre-incubation is employed to control sample temperature prior to extraction (L. Zhang et al., 2018); thereby, short pre-incubation time (5 min) was applied in the present study**.** ***Addition of organic solvent*** may promote the release of analytes bound to the matrix. However, considering the simplicity of food simulants, organic solvent addition will not be beneficial, but will act as a competitor of analytes. Therefore, no organic solvent was added herein. ***Sample dilution*** with water can minimize matrix effect and increase the release of analytes bound to the matrix (Souza-Silva and Pawliszyn, 2015). Again, food simulants are simple, and dilution would not be profitable but decrease the concentration of analytes. It is necessary to dilute 95% ethanol samples as mentioned above and 10% ethanol food simulant was well tested and found not to negatively impact the life span of SPME fibres. Therefore, 95% ethanol samples were diluted 9.5 times, which is 10% ethanol. ***Salt addition*** was found to promote the extraction of certain analytes thanks to the salting-out effect (L. Zhang et al., 2018). In addition, Na_2SO_4 was reported to offer better extraction efficiency as well as better repeatability as compared to NaCl (Souza-Silva and Pawliszyn, 2015). Surprisingly, 5%, 10% NaCl, and 5% Na_2SO_4 did not improve extraction efficiency but negatively affect the baseline of the chromatograms in the present study (Fig. III-2.4). As a result, no salt was added in the present study.

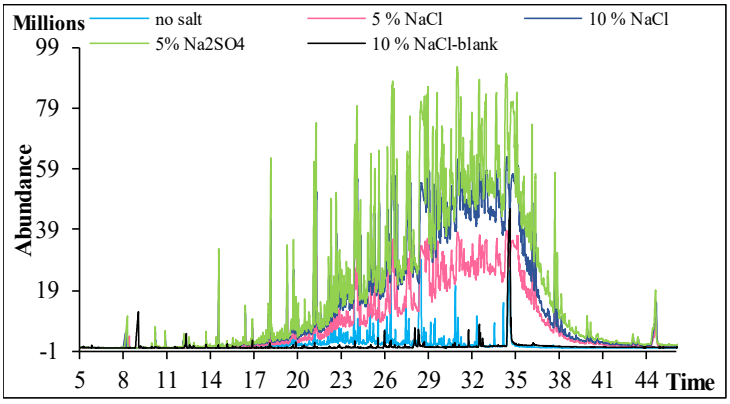


Fig. III-2.4 Effect of salt addition on the extraction efficiency from diluted 95% ethanol samples

Sample pH is critical for some kinds of analytes, since only nonionized form of analytes is extracted by SPME fibres. However, no significant differences were found under pH 5, 7, and 9 (ANOVA, p = 0.36 and 0.26 for Group 1 and Group 2 substances, respectively). From a NTS perspective, the pKa of migrants would vary a lot and pH 7 could be a good balance. Therefore, no pH modification (pH 7) was made for 10% or diluted 95% ethanol samples. Regarding 3% acetic acid samples, pH modification did greatly impact the extraction. After modifying the sample to pH 7 and 9 (the original was 2), there were many emerging peaks (cycled in red) across the chromatograms (Fig. III-2.). Most of them were found to be amines as shown in the figure. As it is known, amines are bases and the nitrogen lone pair of amines can take a hydrogen ion from a hydroxonium ion and form ammonium ions (Clark, 2019). As a consequence, they are difficult to be extracted by DI-SPME under acidic environment and neutralization of pH is necessary. Some compounds showed higher peaks in the original sample though, the number of them was limited and they could be detected in pH 7 sample as well with slightly lower intensities. When further increased the pH to 9, some peaks disappeared, for example, stearic acid (cycled in blue), which is very common in FCM as a slip agent, was totally absent in the pH 9 sample. This behaviour could be expected, as at pH 9 stearic acid (pKa = 10.15) is in dissociated (anionic) mode while at pH 7 it is in its molecular form. Thus, 3% acetic acid was modified to pH 7 prior to extraction.

Extraction temperature is critical for DI-SPME. On the one hand, higher temperature increases analyte diffusivity in the sample and thus increases the extracted amount in pre-equilibrium conditions. On the other hand, increasing extraction temperature has a negative effect on the partition coefficient between the fibre coating (stationary phase) and the sample (Souza-Silva and Pawliszyn, 2015). As such, extraction temperature was further optimized using RSM. ***Extraction time*** did significantly influence the extraction as well. Considering the possible interactions between extraction temperature and time, extraction time was selected for further optimization as well.

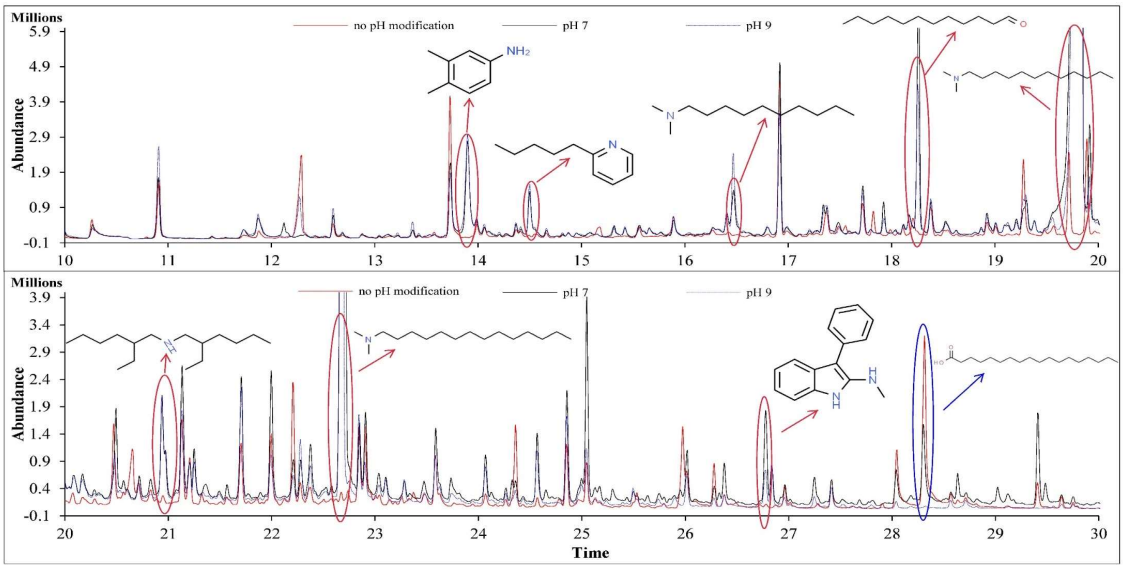


Fig. III-2.5 Effect of pH modification on the extraction efficiency from 3% acetic acid sample

4.4. Response surface methodology: central composite design

Unlike one-variable-at-a-time method, response surface methodology enables us to evaluate not only individual influence of significant factors but also their interactive effects. By applying proper experimental designs, a suitable prediction mathematic model can be obtained based on the fit of a polynomial equation to the experimental data, which allows us to determine the outcome inside the range studied for each factor (Bezerra et al., 2008; L. Zhang et al., 2018). For this purpose, a central composite design (CCD) was employed to optimize the extraction temperature and extraction time.

From a non-target screening point of view, lowering the limit of detection across the whole chromatogram is preferred; that is, the higher the peaks the better the outcome. Total peak area seems to be a good measure of the yield; however, using total peak area would bury the information from those small peaks as abovementioned. It is important to balance the outcome of these two groups of analytes. From this perspective, dividing them into two groups as described above is a good compromise. Doing so, the size of analytes as well as magnitudes of peak areas were taken into account. To maximize the overall outcome of these two groups, the prediction mathematic models of each group were first built through RSM. A multiple-response approach namely Derringer &

Suich's desirability function was then applied to obtain the optimized set of values for each factor that has the maximum overall desirability. The overall desirability was calculated by the geometric mean of the desirability of each group and scale for each group can be set according to their relative importance to the overall desirability. Group 2 had a higher weight (double) than Group 1 because the number of peaks in Group 2 was twice as that in Group 1 (93 VS 47). The response surface plots for Group 1, Group 2, and overall desirability are shown in Fig. III-2.6. The determination coefficient (R^2) for Group 1 and Group 2 were 0.9299 and 0.9304, respectively; and the lack of fit were 0.7896 and 0.1303, respectively, indicating a good fit for the two groups. As can be seen, temperature negatively affected the extraction of Group 1 substances but positively influenced the Group 2 chemicals. Increasing temperature enhances the mobility of chemicals but also decreases the partition of them between the fibre and the simulant. Group 1 might reach equilibrium easily since they were relatively small and had low intensities as abovementioned. As a result, high temperature would reduce the amount of Group 1 chemicals attached to the fibre. On the other hand, extraction time was beneficial for both groups especially for Group 2 whose total peak area increased remarkably over time. Therefore, a compromised temperature and longer time would give the highest throughput as it is evidenced by the overall desirability response surface plot. The optimum conditions to have the highest overall desirability were determined as 70 °C. Extraction temperature and 55 min extraction time. Experimental responses for Group 1 and Group 2 under the optimum conditions matched well the predicted values attained by the mathematical models (t-test, n = 4, p > 0.05).

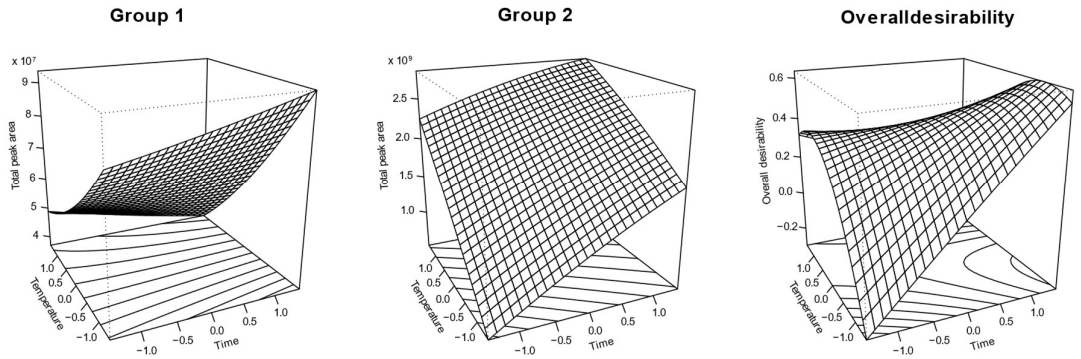


Fig. III-2.6 Response surface plots for Group 1, Group 2, and overall desirability

4.5. Evaluation of the strength of the proposed DI-SPME-GC-MS method for non-target screening of FCM migrants

As evidenced above, the optimal DI-SPME conditions gave the best extraction of migrants from migration samples of recycled polyolefins. However, the capability of the proposed method for a more generic non-target screening towards different FCMs was evaluated by determining LOD, and repeatability of 35 standards covering a wide variety of molecular weights and structures. Among the 35 standards evaluated, most of them had very low level of LODs (Table III-2.4, <10 µg/kg in 10% ethanol and 3% acetic acid but a little bit higher in 95% ethanol) suggesting that the proposed method is powerful for non-target screening of most of the analytes at even trace level. However, there were also exceptions. Some chemicals, e.g., triethylamine, 2-naphthylamine, and BPA, had relatively high LOD (60.6, 217.2 and 319.1 µg/kg, respectively in 10% EtOH) in comparison to others; but they are still in ppb level. In addition, there were 4 analytes that could not be detected even at 1 mg/kg. Among them, 2-naphthylamine, triethylamine, caprolactam, and 2,6-diaminotoluene are amines. As was previously reported by Ning et. al. (Ning et al., 2005), most of the aliphatic and heterocyclic amines can be strongly adsorbed on the column and injector during GC analysis; hence, low concentration cannot be detected without derivatization. Interestingly, many amines were detected in 3% acetic acid migration sample after pH modification, which suggests that their concentration could be high. Their high concentration in 3% acetic acid simulant could be expected due to the alkaline nature of amines, which will be protonated and thus, increase the migration from the plastic. For the other three (dipropylene glycol monomethyl ether, BPA, and Cyasorb UV12), they are diols or diol ether. As was pointed out, substances containing more than one alcohol functional group could have low volatility, thus derivatization may be needed to promote volatility (Ether, 2002).

Table III-2.4 LOD (μg/kg) and repeatability (n = 3) of the 35 analytes in 10% ethanol, 95% ethanol, and 3% acetic acid food simulants

Chemicals	CAS	MF	XlogP	Mass (Da)	10% ethanol		95% ethanol		3% acetic acid	
					LOD	RSD	LOD	RSD	LOD	RSD
Triethylamine	121-44-8	$C_6H_{15}N$	1.4	101	60.6	20.9	711.9	n.a.	13.1	10.2
p-xylene	106-42-3	C_8H_{10}	3.2	51	2.1	11.4	24.9	9.4	2.8	12.4
Caprolactam	105-60-2	$C_6H_{11}NO$	-0.1	42	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
α-Methylstyrene	98-83-9	C_9H_{10}	3.5	51	1.2	12.8	14.2	13.2	1.0	13.4
2,6-Diaminotoluene (2,6-TDA)	823-40-5	$C_7H_{10}N_2$	0.9	77	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Allyl methacrylate	96-05-9	$C_7H_{10}O_2$	1.7	111	14.1	6.9	163.7	14.8	20.2	17.5
Naphthalene	91-20-3	$C_{10}H_8$	3.3	102	0.4	13.6	5.2	7.0	0.4	7.1
2-Naphthylamine	91-59-8	$C_{10}H_9N$	2.3	89	217.2	7.7	2524.0	n.a.	21.2	14.8
Dipropylene glycol monomethyl ether	34590-94-8	$C_7H_{16}O_3$	0.7	104	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2-Methoxy-4-(prop-2-en-1-yl)phenol (Eugenol)	97-53-0	$C_{10}H_{12}O_2$	2.0	55	4.6	9.9	53.0	4.1	2.5	9.6
1-Dodecene	112-41-4	$C_{12}H_{24}$	6.8	168	0.8	11.3	9.0	9.5	0.7	10.3
Diphenyl ether	101-84-8	$C_{12}H_{10}O$	4.2	65	0.4	11.1	4.7	7.3	0.4	5.5
Benzophenone	119-61-9	$C_{13}H_{10}O$	3.4	51	0.7	5.6	8.1	8.0	1.0	12.7
2-Ethylhexyl acrylate	103-11-7	$C_{11}H_{20}O_2$	3.8	112	0.3	17.7	3.5	14.0	0.1	12.0
Dimethyl isophthalate	1459-93-4	$C_{10}H_{10}O_4$	2.2	50	2.0	12.0	22.9	17.9	1.3	3.7
Ethylene glycol dimethacrylate	97-90-5	$C_{10}H_{14}O_4$	1.9	113	1.6	12.8	18.2	11.9	0.6	11.7
2,6-Diisopropylnaphthalene	24157-81-1	$C_{16}H_{20}$	5.8	141	0.1	6.2	0.7	3.3	0.2	2.5
o-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine (PFBOA)	72915-12-9	$C_7H_4F_5NO$	1.3	117	10.6	8.0	123.1	6.3	8.5	14.5
Diphenyl carbonate	102-09-0	$C_{13}H_{10}O_3$	3.3	94	4.7	7.9	54.2	16.1	2.2	18.5
2,4,7,9-Tetramethyl-5-decyne-4,7-diol	126-86-3	$C_{14}H_{26}O_2$	2.7	169	22.4	17.8	259.8	2.9	5.9	17.7
4,4'-(Propane-2,2-diyl)diphenol (Bisphenol A)	80-05-7	$C_{15}H_{16}O_2$	3.3	119	319.1	13.5	3707.8	7.8	81.0	19.6
Diethyl sebacate	110-40-7	$C_{14}H_{26}O_4$	3.5	158	2.8	15.6	32.8	14.6	0.3	10.4
bis(2-hydroxy-4-methoxyphenyl) methanone (Cyasorb UV12)	131-54-4	$C_{15}H_{14}O_5$	3.3	124	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Stearamide	124-26-5	$C_{18}H_{37}NO$	6.8	283	1.3	14.6	14.9	9.7	1.6	5.1
Dibutyl sebacate	109-43-3	$C_{18}H_{34}O_4$	5.3	214	0.8	12.9	9.2	12.4	0.6	4.6
2-tert-Butyl-6-(5-chloro-2H-benzotriazol-2-yl)-4-methylphenol (Tinuvin 326)	3896-11-5	$C_{17}H_{18}ClN_3O$	5.6	91	0.4	14.2	4.8	14.9	0.6	16.9
[2-Hydroxy-4-(octyloxy)phenyl](phenyl)methanone (Chimassorb 81)	1843-05-6	$C_{21}H_{26}O_3$	6.8	197	5.9	18.1	68.7	26.8	4.0	13.9
Glyceryl monostearate	123-94-4	$C_{21}H_{42}O_4$	7.4	327	11.0	23.0	127.9	7.9	31.7	3.8
2-Ethylhexyl 2-cyano-3,3-diphenylprop-2-enoate (Octocrylene)	6197-30-4	$C_{24}H_{27}NO_2$	7.1	165	3.0	15.8	34.5	1.4	1.0	6.3
Bis(2-ethylhexyl) adipate	103-23-1	$C_{22}H_{42}O_4$	6.8	241	0.2	8.6	2.0	16.4	0.4	12.1
Dioctyl terephthalate	4654-26-6	$C_{24}H_{38}O_4$	9.9	57	2.1	18.7	24.8	13.7	1.1	15.7
Tributyl acetylcitrate	77-90-7	$C_{20}H_{34}O_8$	3.3	329	2.0	1.9	23.4	12.7	0.6	12.4
Dinonyl phthalate	84-76-4	$C_{26}H_{42}O_4$	10.1	167	2.9	14.4	33.7	18.4	3.8	16.8
Di-hexyl sebacate	122-62-3	$C_{26}H_{50}O_4$	9.0	297	1.8	10.5	20.7	21.6	0.8	10.3
2,5-Bis(5-tert-butyl-2-benzoxazolyl)thiophene	7128-64-5	$C_{26}H_{26}N_2O_2S$	8.0	105	132.0	12.6	1533.7	n.a.	194.4	9.7

Note: Mass is the least abundance ion (Da) used for calculating LOD; RSD (%) represents repeatability calculated under 10 μg/kg when possible, for less sensitive compounds, higher concentrations were used accordingly. n.d. stands for not detected.

The main difficulty in untargeted screening analysis when using chromatography is to select the peaks to be identified. A common proposal is to focus the effort only on the highest peaks and neglect all those below a certain size, assuming that they correspond to very low concentration level (Martínez-Bueno et al., 2019). However, this is not necessarily true, because the response of analytes varies a lot. For example, many of the amines and diols analytes could have relatively low responses in GC-MS analysis. Their peaks could be small even in relatively high concentrations, which would be of high human health concerns. In addition, many of them are included in the NIST 14 library. Once they are detected in GC-MS, they can be easily identified with the help of libraries. Maybe they can be readily detected in LC, with or without previous concentration, but the identification is still challenging regarding untargeted screening. As such, when conducting untargeted screening, many of the small peaks can be easily checked if they are amines or diols with the help of libraries; while in LC-MS analysis without the library, this task will be more challenging.

5. Conclusions

For the first time, direct immersion – solid-phase micro-extraction coupled to gas chromatography mass spectrometry has been optimized for untargeted screening of volatile and semi-volatile migrants from 3% acetic acid, 10% ethanol, and 95% ethanol food simulants by response surface methodology together with central composite design. The optimization was based on the recycled polyolefin samples though, it is thought to be suitable for virgin polyolefins and other types of food contact materials considering the complexity of post-consumer recycled polyolefins as well as the method evaluation, which assessed the LOD and repeatability of 35 chemicals that could come from different types of FCM.

The proposed method can extract most of the tested analytes at very low concentrations (< 10 μg/kg, which is the specific migration limit (SML) for the non-listed substances in the Regulation 10/2011/EU). However, many amine and diol compounds were found to have relatively high LOD or even not detected at 1 mg/kg even though they are included in the NIST library. The fact could be due to their GC-

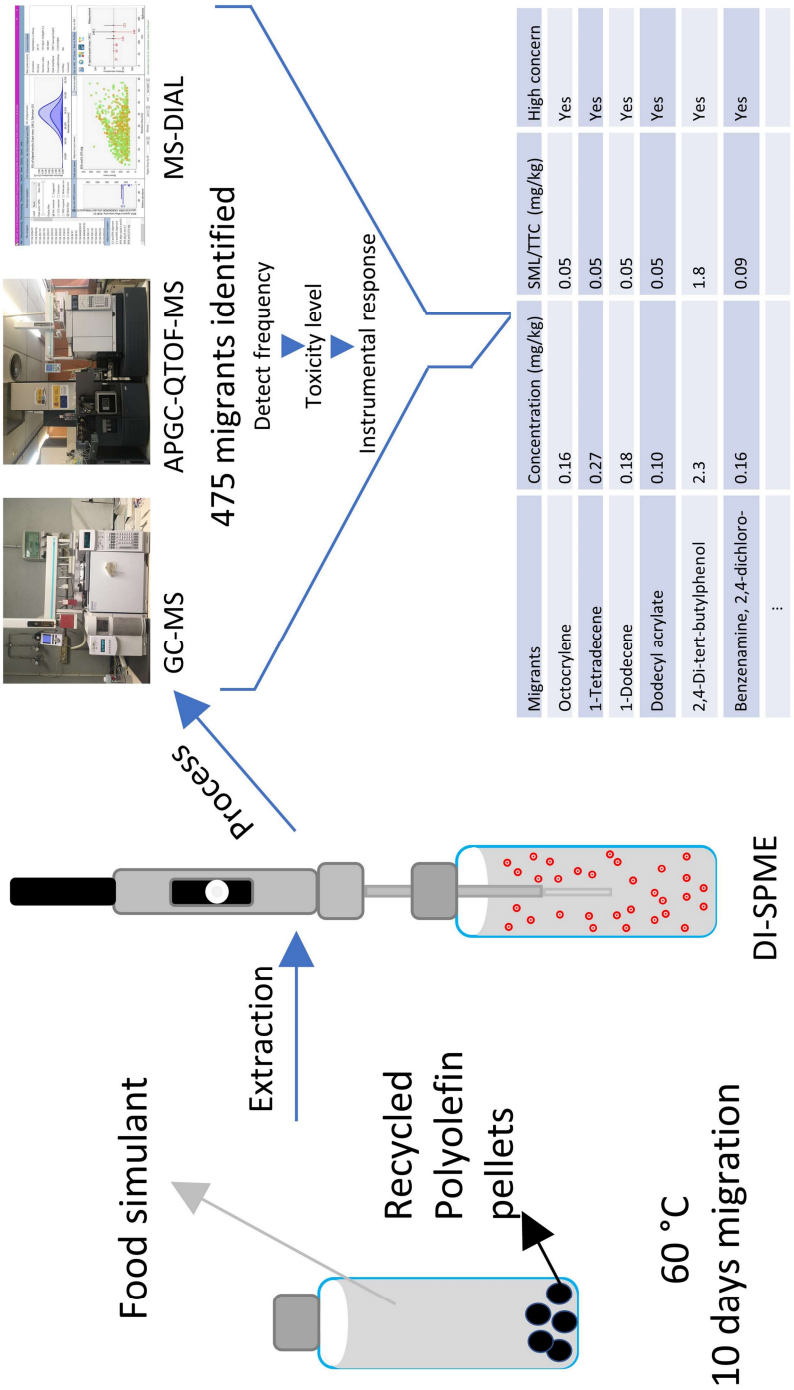
unamenable properties rather than the DI-SPME process and demonstrates once again that the size of peaks in GC-MS is not always indicative of low concentration and this criterion cannot be applied to any migrant. As such, we recommend doing NTS based on qualifiable features instead of on size of peaks. For 3% acetic simulant samples, pH adjustment to 7 is of great importance to detect many amines substances. It is quite difficult, if not impossible, to develop an analytical method for all types of analytes. Non-target screening does not necessarily mean comprehensive because of the limitation of the analytical approach applied. In this sense, knowledge and experience about the strength of the employed analytical method in untargeted screening as well as information about the sample would be helpful for comprehensive FCM safety assessment. For example, knowing the low response of many amine and diol analytes in GC-MS suggests that small peaks can be important as well if they can be easily identified as amine or diol chemicals with the help of library search.

Chapter 3

Safety Concerns of Recycling Post-consumer Polyolefins for Food Contact Uses: Regarding (Semi-)Volatile Migrants Untargetedly Screened

1. Abstract

Plastic recycling is one of the important ways to mitigate plastic pollution. However, chemicals present in recycled plastics is one of the key qualities affecting their potential uses and deserves more attention. 475 migrants coming from 15 post-consumer recycled polyolefins were identified by direct immersion-solid-phase micro-extraction gas chromatography mass spectrometry (DI-SPME-GC-MS) and atmospheric pressure-gas chromatography-quadrupole-time of flight-mass spectrometry (APGC-QTOF-MS). About 60% of them might not be of human risk because they were food additives/components or they are saturated hydrocarbons, fatty acyls, or prenol lipids. Most of them had molecular weight (MW) between 150 and 210 Da, though, high concern substances with high MW (e.g., octocrylene) implied that high MW surrogates are required to study the efficiency of recycling processes for polyolefins. The mean predicted octanol/water partition coefficient (XLogP) was about 6.5 and 3.5 for 95% ethanol and 3% acetic acid food simulants, respectively. Octocrylene, 1-tetradecene, 1-dodecene, dodecyl acrylate, 2,4-di-tert-butylphenol, 1,4-benzenedicarboxylic acid, diethyl ester, benzenamine, 2,4-dichloro-, and diethyl phthalate were of high concern depending on the potential food contact use of the materials. The results presented are informative and can be of great help for recyclers and law makers to recycle polyolefins for safe food contact use.


Food simulant
Extraction
Recycled Polyolefin pellets
60 °C
10 days migration
DI-SPME
Process
GC-MS
APGC-QTOF-MS
MS-DIAL
475 migrants identified
Detect frequency
Toxicity level
Instrumental response
Migrants
Concentration (mg/kg)
SML/TTC (mg/kg)
High concern
Octocrylene
0.16
0.05
Yes
1-Tetradecene
0.27
0.05
Yes
1-Dodecene
0.18
0.05
Yes
Dodecyl acrylate
0.10
0.05
Yes
2,4-Di-tert-butylphenol
2.3
1.8
Yes
Benzenamine, 2,4-dichloro-
0.16
0.09
Yes

2. Introduction

Environmental issues posted by extensive accumulation of plastic wastes in oceans, landfills, and other terrestrial compartment as a price of the current linear economy, have been reported to deteriorate the ecosystems and impact wildlife and possibly human health (Lithner et al., 2011). Packaging accounted for 39.9% of plastic demand in 2018 (PlasticsEurope, 2019). The European Plastic Strategy (EC, 2018) recommends recycling most of the plastic packaging and in this frame, polyolefins, which constitute about 70% of plastic packaging (PlasticsEurope, 2019), occupy an important place to mitigate environmental pollution and to reduce raw materials input to the packaging sector.

The main challenge to recycle packaging waste into new food packaging is that chemical migration, from the recycled materials to the food in contact with them, can be higher compared to virgin materials, and therefore pose potential risks to human health (Geueke et al., 2018). For example, additives accumulated and their degradation products (Coulier et al., 2007), oligomers of the raw materials (Ubeda et al., 2018), printing inks (Clemente et al., 2016), adhesives (Canellas et al., 2017) from labels and multilayers as well as products resulting from misuse of plastic packaging (Biedermann and Grob, 2013), etc. could be present in the recycled materials. Within EU, the use of recycled plastics for food contact is subjected to various regulations, e.g., the framework Regulation No 1935/2004 (EC, 2004) and Commission Regulation 10/2011 (EC, 2011). Food contact use of recycled PET (rPET) bottles has been well established in the last decades with the use of so-called super-clean recycling (Welle, 2011). However, the rich data available for rPET cannot be simply extrapolated to polyolefins in terms of safety issues (Palkopoulou et al., 2016). For a given substance, the diffusion coefficient is orders of magnitude higher in polyolefins than in PET (Dole et al., 2006). Hence, the absorption of chemical substances into polyolefins and their migration from the materials can be higher, and the decontamination step will be much more challenging. As a consequence, decontamination, challenge test, and quality control test should be tailored for recycled polyolefins (rPO) based on careful scientific studies (Palkopoulou et al., 2016).

Knowledge about the chemical compositions of post-consumer polyolefins is crucial for the design of appropriate recycling systems to improve the quality of rPO regarding safety (Welle, 2005). As far as we know, research data on this topic is limited. Existing studies mainly focused on the screening of (semi-)volatile extractables from recycled HDPE milk bottles by Soxhlet extraction and/or headspace - solid-phase micro-extraction gas chromatography mass spectrometry (Devlieghere et al., 1998; Dutra et al., 2011; Nerín et al., 1998; Welle, 2005). Recently, odorant compositions of post-consumer bags and films have been investigated as well (Cabanes et al., 2020; Strangl et al., 2020). However, chemical migration from these materials is rarely studied. Migration test from recycled materials can be an interesting topic as human exposure is more related to chemical migration rather than extraction.

The present study aims to evaluate the potential of using rPOs for food contact uses regarding safety concerns. (Semi-)volatile compounds migrating from various post-consumer polyolefins into two simulants (3% acetic acid and 95% ethanol) were untargetedly screened by a sensitive analytical method, namely DI-SPME-GC-MS. APGC-QTOF-MS together with MS-FINDER (Tsugawa et al., 2016) were applied to characterize peaks that cannot be simply identified by normal GC-MS as well as to improve identification confidence by confirming molecular formulas of the tentatively identified substances where no experimental RI is available. Chemical classes, MW, predicted toxicities (Cramer rules) in the absence of experimental toxicity data and octanol/water partition coefficient (XLogP) distributions of identified substances were analysed. Their possible origins were investigated by searching food related, plastic packaging related, as well as cosmetic related databases. In addition, a strategy was proposed to prioritize chemicals of higher concern obtained by nontargeted screening. Quantification of some prioritized migrants was done when available.

3. Materials and methods

3.1. Reagents and samples

Authentic standards including diphenyl ether (101-84-8), 2,6-diisopropylnaphthalene (24157-81-1), octocrylene (6197-30-4), 2,4-diphenyl-4-methyl-1-pentene (6362-80-7), benzophenone (119-61-9), benzenamine, 2,4-dichloro- (554-00-7), UV 531 (1843-05-6), decane, 1-chloro- (1002-69-3), 1,1'-biphenyl, 3-methyl- (643-93-6), 2,4-di-tert-butylphenol (96-76-4), 2-ethylhexyl salicylate (118-60-5), hexanedioic acid, bis(2-ethylhexyl) ester (103-23-1), bis(2-ethylhexyl) phthalate (117-81-7), dodecanoic acid, ethyl ester (106-33-2), hexadecanoic acid, methyl ester (112-39-0), diethyl phthalate (84-66-2), 1-octadecanol (112-92-5), 2,2,4-trimethyl-1,3-pentanediol diisobutyrate (6846-50-0), isoborneol (124-76-5), 9-octadecenoic acid, methyl ester, (E)- (112-62-9), benzaldehyde, 4-propyl- (28785-06-0), 1,4-benzenedicarboxylic acid, diethyl ester (636-09-9), caryophyllene (87-44-5), d-Limonene (5989-27-5), 2-tridecanone (593-08-8), 1-tetradecanamine, N,N-dimethyl- (112-75-4), benzenamine, 2,4-dimethyl- (95-68-1), morpholine, 4-octadecyl- (16528-77-1), 1,1'-biphenyl, 2,4-dichloro- (2050-67-1), biphenyl (92-52-4), 1-tetradecene (1120-36-1), 1-dodecene (112-41-4), dodecyl acrylate (2156-97-0), 2-ethylhexyl acrylate (103-11-7), isobornyl acrylate (5888-33-5), Tinuvin 326 (3896-11-5), and octadecanamide (124-26- 5) were purchased from Sigma-Aldrich (Madrid, Spain).

There were 6 and 9 post-consumer recycled polyolefin pellets from Spain and China, respectively (Table III-3.1). For Spanish samples, recycled plastics were collected from the yellow bin that contains only packaging. Flexible PO fraction was then separated from the collected materials, cut, washed with cold water containing detergent, and dried to obtain the flakes Fig. III-3.1. The flakes were then fed into an extruder to produce the corresponding pellets under vacuum. S1 to S6 pellet samples were collected at different time, which means that they were various batches from the same company. Company 1 and 2 locate in Qingyuang and Suzhou, China, respectively. According to the companies, C1 was made from HDPE bottles and turnover box, C2 was from bottles and LDPE films, C3 comprised of 90% bottles and LDPE films and

10% virgin PE, C9 consisted of 70% HDPE bottles and turnover box and 30% virgin PE, and C4 was made of mixed PE materials. The rest (C5 to C8) were purchased from company 3 which is a distributor but not a recycler and details about these samples remain unknown due to commercial confidentiality.

Table III-3.1 Detail information of samples (n=5)

Company	Spanish company	Chinese company 1				Chinese company 2	Chinese company 3			
Sample name	S1, S2, S3, S4, S5, S6	C1	C2	C3	C9	C4	C5	C6	C7	C8
Diameter (mm)	5.2 ± 0.2	3.8 ± 0.2	3.3 ± 0.3	6.2 ± 0.2	5.0 ± 0.1	3.7 ± 0.2	3.2 ± 0.3	3.5 ± 0.3	4.7 ± 0.3	3.5 ± 0.3
Height (mm)	2.3 ± 0.2	3.4 ± 0.2	4.1 ± 0.2	2.3 ± 0.3	2.8 ± 0.3	3.0 ± 0.1	3.1 ± 0.2	3.3 ± 0.4	2.1 ± 0.2	3.0 ± 0.1

Note: diameter and height were expressed as mean ± standard deviation (n=5)

Fig. III-3.1 Mixed flexible flake sample

3.2. Migration test

Migration tests were conducted following the EU regulation (No 10/2011) on plastic materials and articles intended to come into contact with food (EC, 2011). Based on the conventional assumption of 6 dm^2 surface area in contact with 1 kg of food, the amount of each plastic used for total immersion migration test (18 mL food simulant) was calculated. Both 95% ethanol and 3% acetic acid food simulants were used to simulate fatty and acidic food, respectively. All migration tests were carried out for 10

days at 60 °C as an accelerated standard test for long time storage (> 6 months) at room temperature according to the EU regulation. Samples and procedural blanks were simultaneously prepared and only migrants that had peak area 10 times higher in samples than that in blanks were counted.

3.3. Direct-immersion solid-phase micro-extraction (DI-SPME)

DI-SPME was optimized by response surface methodology (RSM) in our previous study (Su et al., 2020). The optimized conditions were as follows: 95% ethanol samples were diluted 9.5 times into 10% ethanol to avoid damage to the SPME fibre while 3% acetic acid samples were neutralized by NaOH prior to DI-SPME. Samples were pre-incubated in an agitator (70 °C) for 5 min, extracted for 55 min by a DVB/CAR/PDMS fibre Supelco (PA, USA), and finally thermally desorbed in the GC inlet (250 °C) for 8 min. Subsequently, the fibre was cleaned in a needle heater (270 °C) for 2 min prior to the next extraction. All DI-SPME processes were automatically done by a 6500 CTC autosampler connected to both GC-MS and APGC-QTOF-MS.

3.4. GC-MS analysis

GC-MS profiles were obtained from a gas chromatography (6890N) coupled to mass spectrometry (5975). A semi-polar DB-5 MS column (30 m × 0.25 mm id, 0.25 μm film thicknesses) from Agilent (California, USA) was employed for separation. The temperature ramp was as follows: initiated 50 ºC for 3 min, followed by increasing to 300 ºC at the rate of 3 ºC/min, and finally remained for 2 min. Helium (99.999%) was the carrier gas running at a constant flow rate of 1.0 mL/min. The inlet temperature was set at 250 ºC and splitless mode was employed. Mass scan range was from 40-700 Da. Test mixture for apolar capillary columns according to Grob (Sigma Aldrich) was injected every time prior to sample analysis for the control of the system.

3.5. Identification of migrants by MS-DIAL

Recycled polyolefins are quite complex and the number of migrants coming from each sample was huge. Therefore, manual interpretation of those GC-MS profiles one

by one can be considerably time-consuming and tedious. The use of MS-DIAL (Tsugawa et al., 2015) can facilitate such kind of data interpretation by automatic peak detection (chromatographic deconvolution), alignment, blank subtraction, and identification. MS-DIAL parameters were minimum peak height of 2000, sigma window of 0.5 and EI spectra cut off of 1 for deconvolution and identification was done before alignment by comparing spectra against NIST 14 library with score cut off of 85% to reduce false positive. Alignment was done with 0.1 min retention time tolerance and 70% EI similarity. Features with sample max / blank average fold change lower than 10 were removed. The generated list of compounds was then manually checked to assure identification (retention index with tolerance of 30 when available) and to mark down which samples did really contain that migrant.

3.6. APGC-QTOF-MS analysis

Gas chromatography (A7890; Agilent, California, USA) and high-resolution mass spectrometer Xevo G2 QTOF (Waters, Massachusetts, USA) were interfaced with an atmospheric pressure soft ionization, namely APGC. The setting of gas chromatography was the same as that of GC-MS, except for the gas flow rate which was 1.8 mL/min in APGC-QTOF-MS to achieve high comparability of chromatograms between GC-MS and APGC-QTOF-MS. Source temperature, corona current, cone and auxiliary gas flow were 150 ℃, 1.0 μA, 20 and 175 L/h, respectively. Mass range for both MS1 and MS2 function was from 40 to 700 Da. MS^E positive acquisition mode was applied with low energy at 6 V and high energy at 10-40 V. APGC-QTOF-MS data interpretation was explained in our previous article (Su et al., 2019) with one exception, where MS-FINDER (version 3.42) was used for in-silico fragmentation herein. Unlike LC-QTOF-MS, no mass correction in real time is available in the APGC-QTOF-MS. Masses of each spectra have to be manually corrected as previously detailed (Su et al., 2019). The corrected spectra were then imported into MS-FINDER for structural elucidation.

3.7. Data processing and prioritization of important migrants

Hierarchical clustering of samples and their correlations were calculated by MetaboAnalyst (Xia and Wishart, 2016) using peak areas of tentatively identified migrants. If two samples have similar migrants and intensities, then they will be in a same cluster and have high correlation (red). InChIKey, MW, molecular formula, and XLogP, of all identified migrants were retrieved from PubChem using ***webchem*** (Eduard Szöcs et al., 2020) package in R. Classification of migrants was done by ClassyFire developed by Fiehn lab (Djoumbou Feunang et al., 2016). Databases namely "Substances added to food", "EU cosmetic ingredients inventory", and "Colorants dyes and pigments" were downloaded from EPA as well. Moreover, "Chemicals associated with plastic packaging" database which contain chemicals likely (List A) and possibly (List B) associated with plastic packaging was downloaded (Groh et al., 2019) as well. "Food database" containing a long list of food components was downloaded from FooDB (http://foodb.ca, accessed on 20/02/2020). The list of migrants was then searched against these 5 databases by matching InChIKey characters to get a general idea about their possible origins. Besides, the list of carcinogenic, mutagenic, and reprotoxic chemicals (CMR, category 1A, 1B, and 2) was extracted from the Table 3 of Annex VI to the CLP Regulation (European Union, 2008) (accessed on 20/05/2020). Substances of very high concern (SVHC) database was downloaded from ECHA (https://echa.europa.eu/candidate-list-table, accessed on 20/05/2020). The list of endocrine disrupting chemicals (EDC) was extracted from the UN review report II (IPCP, 2017) about EDC. The SML of the migrants were obtained by consulting the EU10/2011 regulation (last updated on 29.08.2019). The toxicity of a migrant, if it is not in CMR, SVHC, or EDC lists and does not have SML value, was estimated by Toxtree (version 3.1.0.1851) based on Cramer rules (Patlewicz et al., 2008).

In an attempt to prioritize the migrants based on their toxicities, each migrant was assigned to a toxicity level with the following rules: chemicals that have SML as ND (not detectable at 0.01 mg/kg) or be included in CMR, SVHC, or EDC list have level V; chemicals that have Cramer III or have SML between 0.01 and 0.1 mg/kg obtain level IV; chemicals that have Cramer II or have SML between 0.1 and 1 mg/kg have

level III; chemicals that have Cramer I or have SML between 1 and 60 mg/kg get level II; chemicals that have SML equal to 60 mg/kg get level I. Once toxicity levels were designated, the migrants were prioritized first by detected frequency (from high to low separated by 60%), followed by toxicity level (from level V to I), and finally by average S/N calculated by MS-DIAL.

3.8. Quantification of migrants

Prioritized migrants were quantified by authentic standards (external calibration) when available. Calibration curves were prepared in 10% ethanol solution for migrants that had maximum response in 95% simulant, and the final concentration was calculated considering the dilution of sample (9.5 times); while prepared in pre-neutralized (pH = 7) 3% acetic acid for migrants that had maximum response in 3% acetic acid sample. The standard solutions were then analysed by DI-SPME-GC-MS as the samples did and procedural blanks have been subtracted.

4. Results and discussions

4.1. Identification by APGC-QTOF-MS

APGC-QTOF-MS allows for structural elucidation by monitoring the exact masses of precursor and product ions. As an example, the peak at 47.682 min (RI 1896) in GC-MS matched quite well to nimorazole in MS-DIAL (total spectrum similarity 90.8). However, nimorazole has reference RI 1803, which did not match to this peak. Therefore, this identification was ruled out (Fig. III-3.2 A). When carefully inspected the APGC-QTOF-MS chromatogram of the same sample, a corresponding peak was found at 47.50 min with precursor ion 256.2641 m/z (Fig. III-3.2 B). The correspondence was evidenced by a shared major fragment (100 m/z) in both systems and by a tolerable retention time shift. The structure of this unknown was then elucidated by MS-FINDER Fig. III-3.2 C.

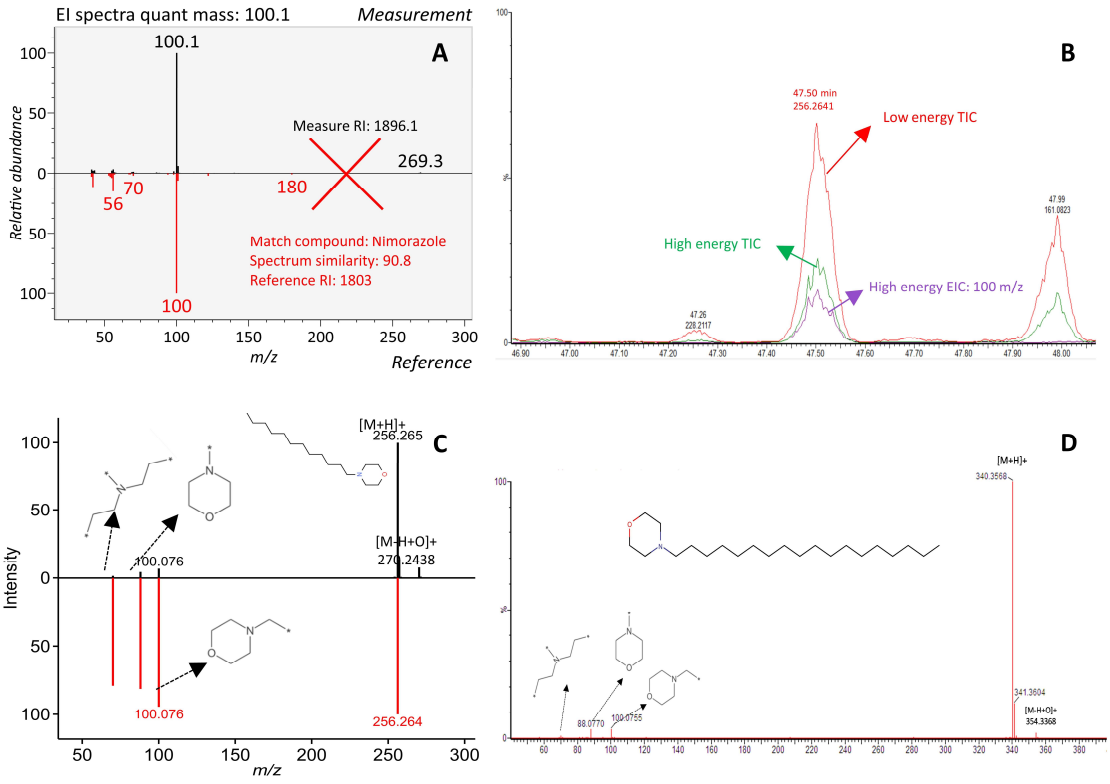


Fig. III-3.2 Structural elucidation of 4-dodecylmorpholine by APGC-QTOF-MS: misidentification by matching mass spectral library in GC-MS (A); total ion chromatogram and extracted ion chromatogram of the unknown peak in APGC-QTOF-MS (B); identification by MS-FINDER (C); indirect confirmation by 4-octadecylmorpholine in APGC-QTOF-MS high energy

The GC-MS spectrum of the unknown was predominated by 100 m/z. Looking in depth, numerous compounds have quite similar spectra in NIST 14 library and all of them have the same substructure (marked in Fig. III-3.3). The finding implies that the unknown could have this substructure as well. There were many compounds that had in silico MS/MS spectra matched well (score > 6 with 10 in total) to the unknown though, some of them have considerably different EI spectra compared to the unknown, e.g., palmitamide which has a predominant ion of 59 m/z and must not be the right identification. Among them, only one, namely 4-dodecylmorpholine contain this substructure. Moreover, predicted RI of 4-dodecylmorpholine using a deep convolutional neural network (Matyushin et al., 2019) was 1890, which is quite close to the experimental RI (1896). 4-dodecylmorpholine is an indirect additive used in food

contact substances. Hence, it is reasonable to be present in post-consumer rPO. The identification was indirectly confirmed by 4-octadecylmorpholine which is a homolog of 4-dodecylmorpholine with longer alkyl chain. They have same fragments and precursor patterns ($[M+H]^+$ and $[M-H+O]^+$) as shown in Fig. III-3.2 D.

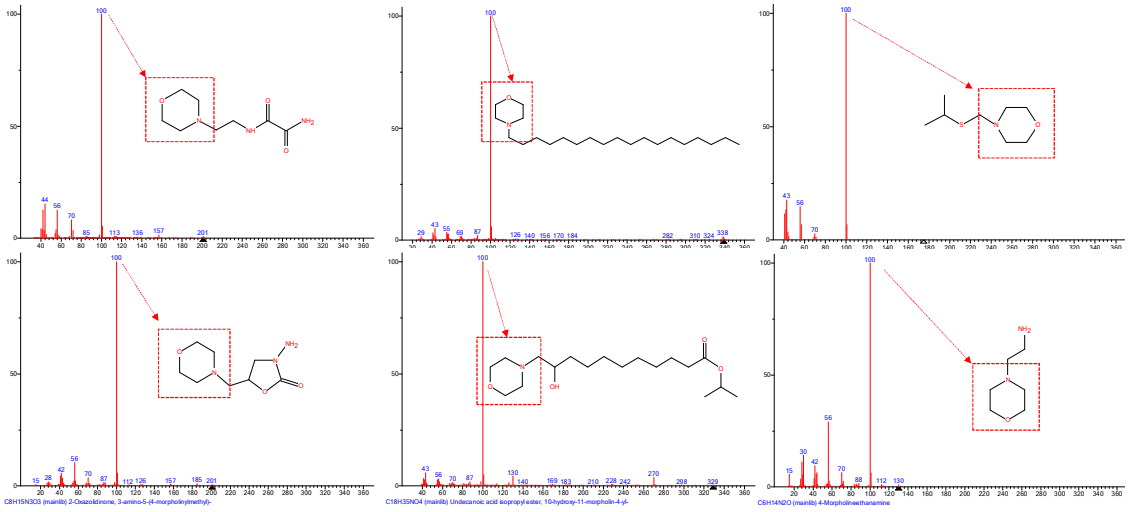


Fig. III-3.3 Compounds that have similar spectra to the unknown (47.682 min in GC-MS)

4.2. Tentatively identified migrants, their classification, and possible origins

There were 1893 features detected by MS-DIAL though, the result should be interpreted carefully, since a feature does not necessarily mean an individual compound. Many of the features can be artefacts, duplicates of other features based on the parameters used for data processing, e.g., sigma window used for automatic deconvolution. For this reason, using all detected features as the total number of chemicals present in samples could be misleading and exaggerated. This situation can be even worse in LC-MS/MS profiling considering that there could be plenty of adducts and in-source fragments. With the help of MS-DIAL, NIST 14 library, and APGC-QTOF-MS, 474 migrants coming from these 15 post-consumer rPO were tentatively identified in total with high confidence and 34 of them were confirmed by authentic standards. The whole list and their detailed information are shown in Table III-3.2. The number of migrants detected in each sample ranged from 150 (C6) to 251 (C9) which indicates the complexities of the samples regarding chemicals present.

Table III-3.2 Tentatively identified compounds and their detailed information

RT	Name	Score	MW	LogP	Cram	Tox	FA	CM	DP	PPA	PPB	Fdb	Fill	S/N	High	Presence
5.59	Propane, 2-isothiocyanato-2-methyl-	*92.3*	115	2.6	III	IV							7	72	HC8	HC8
7.27	Heptanal	99.7	114	2.3	I	II	Y				Y	Y	60	33	HS2	HS1, HS2, HS3, HS4, HS5, HS6, HC3, HC8, HC9
8.48	(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	94.8	136	2.8	I	II		Y				Y	20	42	EC9	EC1, EC2, EC9
9.11	Camphene	99.6	136	3.3	I	II	Y	Y				Y	40	101	HC6	EC1, EC3, EC6, EC7, EC8, EC9, HC6, HC7, HC9
9.47	Benzaldehyde **(SML 60)**	**99.9**	106	1.5	-	I	Y	Y			Y	Y	80	67	HS2	HS1, HS2, HS3, HS4, HS5, HS6, HC1, HC2, HC3, HC6, HC8, HC9
10.19	alpha-Pinene **(SML 60)**	99.3	136	3.1	-	I	Y				Y	Y	20	101	EC9	EC1, EC8, EC9
10.37	Aniline **(CMR)**	**95.2**	93	0.9	-	V			Y		Y	Y	7	14	HC8	HC8
10.57	2,3-Octanedione	94.3	142	1.5	III	IV	Y					Y	40	16	HS2	HS1, HS2, HS3, HS4, HS5, HS6
10.84	2-Octanone	96.8	128	2.4	II	III	Y	Y				Y	40	19	HS2	HS1, HS2, HS3, HS4, HS5, HS6
10.84	Heptane, 2,2,4,6,6-pentamethyl-	98.6	170	5.6	I	II		Y			Y		60	8	EC9	EC1, EC2, EC3, EC4, EC5, EC6, EC7, EC8, EC9
10.92	1-Decene **(SML 0.05)**	92.8	140	5.7	-	IV					Y		47	14	EC8	EC8, HS1, HS2, HS3, HS4, HC1, HC4
11.26	Hexanoic acid, ethyl ester	97.2	144	2.4	I	II	Y	Y				Y	40	10	ES3	ES1, ES2, ES3, ES4, ES5, ES6
11.33	Pyridine, 2,4,6-trimethyl-	95.8	121	1.9	III	IV							20	9	HC9	HC2, HC3, HC9
11.38	Octanal	96.1	128	2.7	I	II	Y	Y				Y	100	37	HS6	HS1, HS2, HS3, HS4, HS5, HS6, HC1, HC2, HC3, HC4, HC5, HC6, HC7, HC8, HC9
11.67	3-Carene	92.3	136	2.8	I	II	Y	Y				Y	7	15	EC9	EC9
11.87	7-Oxabicyclo[2.2.1]heptane, 1-methyl-4-(1-methylethyl)-	98.5	154	2.5	III	IV	Y	Y				Y	13	29	HC9	HC8, HC9
12.29	Benzene, 1-methyl-3-(1-methylethyl)-	98	134	4	I	II						Y	60	54	EC9	EC1, EC2, EC3, EC4, EC5, EC6, EC7, EC8, EC9
12.37	2,2,7,7-Tetramethyloctane	92.2	170	5.8	I	II							7	7	EC4	EC4
12.51	D-Limonene	*	136	3.4	I	II	Y	Y		Y		Y	100	118	EC9	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC2, EC3, EC4, EC5, EC6, EC7, EC8, EC9
12.57	1-Hexanol, 2-ethyl- **(SML 30)**	99.7	130	3.1	-	II	Y	Y		Y		Y	40	512	ES3	ES1, ES2, ES3, ES4, ES5, ES6
12.81	Octane, 6-ethyl-2-methyl-	*94.1*	170	5.6	I	II							20	4	EC9	EC4, EC8, EC9
13.06	Indene	93.2	116	2.9	III	IV		Y					40	27	HS4	HS1, HS2, HS3, HS4, HS5, HS6
13.08	1-Hexanol, 5-methyl-2-(1-methylethyl)-	*85.6*	158	3.4	I	II							7	4	EC9	EC9
13.34	Furan, tetrahydro-2,2-dimethyl-5-(1-methyl-1-propenyl)-	97.2	154	2.7	III	IV							13	8	HC1	HC1, HC9
13.86	1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	93.6	136	2.8	I	II	Y	Y			Y	Y	33	39	EC9	EC1, EC, 2, HE 3, EC6, EC7, EC8, EC9, HC9
14.07	Acetophenone	**97.5**	120	1.6	I	II	Y	Y			Y	Y	67	38	HS2	HS1, HS2, HS3, HS4, HS5, HS6, HC3, HC6, HC8, HC9
14.38	Aniline, N-methyl-	96.1	107	1.7	I	II						Y	20	29	HS1	HS1, HS6, HC3
14.50	1-Octanol **(SML 60)**	99.9	130	3	-	I	Y	Y		Y	Y	Y	40	108	ES4	ES1, ES2, ES3, ES4, ES5, ES6
14.51	7-Octen-2-ol, 2,6-dimethyl-	99.2	156	2.9	III	IV		Y			Y		67	87	HC9	HS1, HS2, HS3, HS4, HS5, HC1, HC2, HC3, HC8, HC9
14.71	Decane, 2,3,8-trimethyl-	*91.5*	184	6.4	I	II							27	7	EC9	EC4, EC6, EC8, EC9
14.88	Octanenitrile	91.7	125	2.8	III	IV							27	18	HS1	HS1, HS3, HS4, HS6
14.92	1-Decene, 2,4-dimethyl-	*92.1*	168	6	I	II							13	3	EC9	EC3, EC9
15.12	Fenchone	96.1	152	2.3	III	IV		Y				Y	7	28	HC9	HC9
15.24	2,4-Dimethylstyrene	96.6	132	3.4	I	II							20	40	HC9	HC1, HC6, HC9
15.28	4,7-Methano-1H-indene, octahydro-	93.7	136	3.8	I	II							7	20	EC1	EC1
15.43	2-Nonanone	99.4	142	3.1	II	III	Y	Y				Y	93	57	HS1	HS1, HS2, HS3, HS4, HS5, HS6, HC1, HC2, HC3, HC4, HC5, HC7, HC8, HC9
15.52	1-Undecene	98.2	154	6.2	I	II						Y	13	5	EC3	EC3, EC5
15.74	3-Octanol, 3,7-dimethyl-	94.7	158	3.3	III	IV	Y	Y			Y	Y	53	217	HC9	HS1, HS3, HS4, HC1, HC3, HC5, HC8, HC9
15.88	Undecane	99.6	156	5.6	I	II					Y	Y	100	19	EC9	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC2, EC3, EC4, EC5, EC6, EC7, EC8, EC9
16.11	Bicyclo[2.2.1]heptan-2-ol, 1,5,5-trimethyl-	97.8	154	2.3	I	II							53	22	EC9	ES1, ES3, ES4, ES6, EC8, EC9, HC6, HC7, HC9
16.24	Phorone	88.8	138	2.8	I	II							7	35	HC3	HC3
16.27	Rose oxide	95.4	154	2.9	III	IV	Y					Y	20	20	HC9	HC1, HC8, HC9
16.33	Fenchol	94.7	154	2.5	I	II	Y	Y				Y	27	68	HC9	HC1, HC6, HC8, HC9
16.48	Decane, 3,4-dimethyl-	*96.3*	170	6.1	I	II							7	16	EC9	EC9
16.56	Isophorone **(CMR)**	93.6	138	1.6	-	V	Y	Y			Y	Y	27	155	HC6	HC3, HC5, HC6, HC7
16.67	Decane, 2,5-dimethyl-	96.5	170	6.1	I	II							40	5	EC9	EC3, EC4, EC6, EC7, EC8, EC9
16.86	Naphthalene, decahydro-2-methyl-	94.6	152	4.9	III	IV							47	28	EC9	ES1, ES3, ES4, ES6, EC1, EC8, EC9
17.30	3-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-	98.3	154	2	III	IV	Y					Y	13	31	HC9	HC8, HC9
17.31	Decane, 3,3,4-trimethyl-	*91.7*	184	6.5	I	II						Y	7	10	EC6	EC6
17.35	Cyclohexane, pentyl-	93.4	154	5.7	I	II							73	5	EC9	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC2, EC3, EC8, EC9

RT	Name	Score	MW	LogP	Cram	Tox	FA	CM	DP	PPA	PPB	Fdb	Fill	S/N	High	Presence
17.67	(+)-2-Bornanone	99.9	152	2.2	III	IV	Y						67	192	HC1	EC1, EC8, HS2, HC1, HC2, HC3, HC4, HC5, HC6, HC7, HC8, HC9
17.88	6-Methyl-1-octanol	*97.9*	144	3.2	I	II							40	60	HS5	HS1, HS2, HS3, HS4, HS5, HS6
18.21	Acetic acid, 2-ethylhexyl ester	99.7	172	3.2	I	II		Y			Y		100	100	HC6	HS1, HS2, HS3, HS4, HS5, HS6, HC1, HC2, HC3, HC4, HC5, HC6, HC7, HC8, HC9
18.31	Isoborneol	*	154	2.7	I	II	Y	Y				Y	60	279	HC6	EC6, HC1, HC2, HC3, HC4, HC5, HC6, HC7, HC8, HC9
18.38	Benzene, pentyl-	88.9	148	5	I	II						Y	7	13	ES4	ES4
18.49	Undecane, 5-methyl-	93.5	170	6.4	I	II							40	4	EC9	EC1, EC2, EC3, EC6, EC8, EC9
18.56	Bornyl chloride	*92.8*	173	4	III	IV							7	8	EC9	EC9
18.67	Undecane, 4-methyl-	91.4	170	6.4	I	II							27	6	EC9	ES3, EC1, EC4, EC9
18.71	Benzenamine, 2,4-dimethyl-	*	121	1.7	I	II					Y		53	240	HS2	HS1, HS2, HS3, HS4, HS5, HS6, HC3, HC9
18.72	endo-Borneol	96.3	154	2.7	I	II	Y	Y				Y	33	26	HC1	HC1, HC2, HC4, HC6, HC9
18.86	Undecane, 2-methyl-	93.3	170	6.4	I	II					Y		20	7	EC9	EC1, EC6, EC9
18.99	Benzoic acid, ethyl ester **(SML 60)**	91.3	150	2.6	-	I	Y	Y			Y	Y	7	8	ES6	ES6
19.12	Menthol	99.9	156	3	I	II		Y				Y	93	155	HC8	EC8, HS1, HS2, HS3, HS4, HS5, HS6, HC2, HC3, HC4, HC5, HC6, HC7, HC8, HC9
19.24	Benzenamine, 4-methoxy-	**92.7**	123	0.9	I	II						Y	40	41	HS2	HS1, HS2, HS3, HS4, HS5, HS6
19.27	dl-Menthol	99.8	156	3	I	II	Y				Y	Y	7	982	HC1	HC1
19.36	5-Undecene, 5-methyl-	*92.9*	168	5.9	I	II							20	4	EC9	EC2, EC8, EC9
19.38	Naphthalene, decahydro-2,3-dimethyl-	*95.5*	166	5.1	III	IV							67	7	EC1	ES1, ES3, ES4, ES6, EC1, EC2, EC3, EC4, EC8, EC9
19.43	4-Undecene, 5-methyl-	*94.5*	168	5.9	I	II							20	4	EC9	EC2, EC8, EC9
19.55	Ethanone, 1-(3-methylphenyl)-	95.7	134	2.3	I	II							73	40	HC9	HS1, HS2, HS3, HS4, HS5, HS6, HC1, HC3, HC6, HC8, HC9
19.60	Nonanenitrile	98.8	139	3.1	III	IV							40	87	HS1	HS1, HS2, HS3, HS4, HS5, HS6
19.64	Isomenthol	91.9	156	3	I	II						Y	7	15	HC1	HC1
19.66	1-Methyl-4-(1-hydroxy-1-methylethyl)benzene	87.9	150	2	I	II	Y	Y				Y	13	11	HC9	HC8, HC9
19.76	1,11-Dodecadiene	92	166	6.3	I	II							7	7	EC3	EC3
19.83	Butanoic acid, 3-hexenyl ester, (Z)-	93.9	170	2.7	I	II	Y	Y				Y	13	21	HC9	HC8, HC9
19.84	2-Undecene, 9-methyl-, (E)-	95.4	168	5.6	I	II							60	8	EC9	EC1, EC2, EC3, EC4, EC5, EC6, EC7, EC8, EC9
19.91	2-(4-Methyl-3-cyclohexen-1-yl)-2-propanol	99.3	154	1.8	III	IV	Y	Y		Y		Y	87	40	HC9	HS1, HS2, HS3, HS4, HS5, HS6, HC2, HC3, HC4, HC5, HC7, HC8, HC9
20.10	2-Decanone	99.2	156	3.7	II	III	Y					Y	93	113	HS1	ES4, HS1, HS2, HS3, HS4, HS5, HS6, HC2, HC3, HC4, HC5, HC6, HC7, HC8, HC9
20.13	1-Dodecene **(SML 0.05)**	*	168	6.8	-	IV					Y	Y	100	89	EC9	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC2, EC3, EC4, EC5, EC6, EC7, EC8, EC9, HS1, HS2, HS3, HS4, HS5, HS6, HC2, HC3, HC4, HC5, HC6, HC8, HC9
20.18	Pyridine, 2-pentyl-	94.6	149	2.9	III	IV	Y					Y	40	382	HS4	HS1, HS2, HS3, HS4, HS5, HS6
20.33	Benzene, 1-ethyl-2,4,5-trimethyl-	**93.1**	148	3.8	I	II							13	25	EC1	EC1, EC6
20.37	Octanoic acid, ethyl ester	99.5	172	3.5	I	II	Y	Y				Y	60	145	ES3	ES1, ES2, ES3, ES4, ES5, ES6, EC4, EC6, EC8, HS1, HS2, HS3, HS4, HS5, HS6
20.54	Dodecane	99.9	170	6.1	I	II		Y			Y	Y	100	71	EC9	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC2, EC3, EC4, EC5, EC6, EC7, EC8, EC9
20.70	Decanal	99.6	156	3.8	I	II	Y	Y			Y	Y	87	24	HC9	HS1, HS2, HS3, HS4, HS5, HS6, HC1, HC2, HC3, HC4, HC5, HC8, HC9
20.77	2-Dodecene, (Z)-	97.2	168	6.5	I	II							60	7	EC9	EC1, EC2, EC3, EC4, EC5, EC6, EC7, EC8, EC9
20.85	Cyclohexanol, 4-(1,1-dimethylethyl)-	99.5	156	3	I	II	Y	Y			Y		53	62	HC9	HC1, HC2, HC3, HC4, HC5, HC7, HC8, HC9
20.91	Benzaldehyde, 2,5-dimethyl-	99.5	134	2.1	I	II							100	1121	HC9	EC9, HS1, HS2, HS3, HS4, HS5, HS6, HC1, HC2, HC3, HC4, HC5, HC6, HC7, HC8, HC9
21.10	1-Heptanol, 2-propyl-	*97.6*	158	3.8	I	II							93	38	HC9	HS1, HS2, HS3, HS4, HS5, HS6, HC1, HC2, HC3, HC4, HC5, HC6, HC8, HC9
21.38	Decane, 3-ethyl-3-methyl-	*85.4*	184	6.8	I	II							20	4	EC1	EC1, EC6, EC9
21.41	Cyclohexanone, 4-(1,1-dimethylethyl)-	91.1	154	2.6	II	III		Y			Y		13	31	HC9	HC8, HC9
21.44	cis,trans-3-Ethylbicyclo[4.4.0]decane	92.8					Y	Y	Y	Y	Y		80	15	EC1	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC2, EC3, EC4, EC8, EC9
21.84	2-Ethylhexyl acrylate **(SML 0.05)**	*	184	3.8	-	IV					Y		33	26	EC6	EC3, EC5, EC6, EC7, HC4, HC5, HC6, HC7
21.90	cis, cis-2-Ethylbicyclo[4.4.0]decane	*89.8*	166	5.4	III	IV							40	11	EC9	ES1, ES3, ES4, EC1, EC4, EC9
22.09	Benzaldehyde, 4-(1-methylethyl)-	98.4	148	2.7	I	II	Y	Y				Y	67	52	HC9	HS1, HS2, HS3, HS4, HS5, HS6, HC1, HC3, HC8, HC9
22.14	Cyclohexane, hexyl-	95.3	168	6.2	I	II						Y	100	10	EC1	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC2, EC3, EC4, EC5, EC6, EC7, EC8, EC9
22.28	Carvone	95.9	150	2.4	II	III	Y					Y	80	42	HC9	HS1, HS2, HS3, HS4, HS5, HS6, HC1, HC4, HC5, HC6, HC7, HC9
22.51	2,2-Dimethylindene, 2,3-dihydro-	**91.5**	146	3.4	I	II							7	13	EC1	EC1, HC1
22.63	2-Propanol, 1-(2-butoxy-1-methylethoxy)- **(SML 60)**	*88.9*	134	-0.7	-	I	Y	Y			Y	Y	7	16	HC9	HC9
22.67	1-Butanone, 1-phenyl-	89.3	148	2.5	I	II		Y				Y	13	35	HS2	HS2, HS3
22.70	2-Isopropenyl-5-methylcyclohexyl acetate	85.1	196	3.5	II	III							7	12	HC9	HC9
22.85	Benzoic acid, 4-chloro-, methyl ester	91	171	2.8	III	IV							7	8	EC9	EC9
23.11	1H-Indene, 2,3-dihydro-1,6-dimethyl-	*92.6*	146	3.5	I	II							7	13	HC1	HC1
23.21	Dodecane, 4-methyl-	93.4	184	7	I	II							27	8	EC9	EC1, EC2, EC4, EC9
23.40	Cinnamaldehyde, (E)-	95.8	132	1.9	I	II	Y	Y				Y	33	273	HS5	HS2, HS3, HS4, HS5, HS6

RT	Name	Score	MW	LogP	Cram	Tox	FA	CM	DP	PPA	PPB	Fdb	Fill	S/N	High	Presence
23.43	Dodecane, 2-methyl-	95.6	184	7	I	II		Y				Y	80	4	EC9	ES1, ES3, ES4, EC1, EC2, EC3, EC4, EC5, EC6, EC7, EC8, EC9
23.52	Benzaldehyde, 4-propyl-	*	148	3	I	II							100	138	HC9	HS1, HS2, HS3, HS4, HS5, HS6, HC1, HC2, HC3, HC4, HC5, HC6, HC7, HC8, HC9
23.74	1-Decanol **(SML 60)**	97.9	158	4.6	-	I	Y	Y		Y	Y	Y	80	94	HC1	HS1, HS2, HS3, HS4, HS5, HS6, HC1, HC2, HC3, HC4, HC8, HC9
23.90	Benzene, pentamethyl-	93.8	148	4.6	I	II							13	54	EC1	EC1, EC6, HC1, HC6
23.92	Benzene, 1,3-bis(1-methylethenyl)-	**85.6**	158	4.9	I	II							7	48	EC3	EC3, HC3
24.03	Phenol, 2-(1-methylpropyl)-	99.7	150	3.4	I	II							20	72	HC1	HC1, HC4, HC8
24.22	Anethole	94.5	148	3.3	I	II	Y	Y					40	77	HS3	HS1, HS2, HS3, HS4, HS5, HS6
24.22	Decanenitrile	91.8	153	3.7	III	IV		Y					40	25	HS1	HS1, HS2, HS3, HS4, HS5, HS6
24.30	Isobornyl acetate	99.8	196	3.3	I	II	Y	Y			Y	Y	100	161	HC9	EC6, EC7, EC8, EC9, HS1, HS2, HS3, HS4, HS5, HS6, HC1, HC2, HC3, HC4, HC5, HC6, HC7, HC8, HC9
24.62	Cyclohexanol, 2-(1,1-dimethylethyl)-	*94.1*	156	3	I	II		Y					100	186	HC1	EC1, EC8, EC9, HS1, HS2, HS3, HS4, HS5, HS6, HC1, HC2, HC3, HC4, HC5, HC6, HC7, HC8, HC9
24.65	Thymol	92.8	150	3.3	I	II	Y	Y				Y	40	92	HC8	HS1, HC3, HC4, HC5, HC7, HC8
24.65	1-Tridecene	99.6	182	7.3	I	II						Y	67	19	EC3	ES1, ES5, ES6, EC2, EC3, EC4, EC5, EC6, EC7, EC8
24.69	Phenol, p-tert-butyl- **(SML 0.05)**	89.7	150	3.3	-	V	Y			Y		Y	7	283	HC9	HC9
24.81	Pyridine, 2-hexyl-	**93.6**	163	3.4	III	IV							47	156	HS4	HS1, HS2, HS3, HS4, HS5, HS6, HC9
24.85	Nonanoic acid, ethyl ester	94	186	4	I	II	Y	Y				Y	40	34	ES3	ES1, ES2, ES3, ES4, ES5, ES6
24.85	Benzaldehyde, 2,4,6-trimethyl-	**99.7**	148	2.5	I	II					Y	Y	53	82	HC6	HC1, HC2, HC3, HC4, HC5, HC6, HC7, HC9
25.04	Tridecane	99.7	184	6.6	I	II		Y			Y	Y	100	44	EC9	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC2, EC3, EC4, EC5, EC6, EC7, EC8, EC9
25.05	Phenol, 2-methyl-5-(1-methylethyl)-	94.9	150	3.1	I	II	Y	Y				Y	33	73	HC9	HS2, HS4, HC6, HC8, HC9
25.11	Naphthalene, 2-methyl-	97.6	142	3.9	III	IV		Y				Y	73	63	EC1	ES2, ES3, ES5, EC1, EC2, EC3, EC4, EC6, EC7, EC8, EC9
25.16	4-(t-Butyl)benzaldehyde	*99.2*	162	3.1	I	II							100	524	HC1	EC8, HS1, HS2, HS3, HS4, HS5, HS6, HC1, HC2, HC3, HC4, HC5, HC6, HC7, HC8, HC9
25.50	Cyclohexanol, 2-(1,1-dimethylethyl)- like	*95.4*						Y					60	52	HC1	EC1, EC8, EC9, HS5, HC1, HC2, HC3, HC4, HC5, HC7, HC8, HC9
25.56	Naphthalene, 1,2,3,4-tetrahydro-2,6-dimethyl-	89.7	160	3.9	I	II							27	23	ES1	ES1, ES2, ES3, ES6
25.58	2,3,6-Trimethylacetophenone	**92.1**	162	2.7	I	II							27	50	HC1	HC1, HC3, HC7, HC8
25.78	benzenamine, 4-(2-methylbutyl)-	**85.2**	163	3.4	I	II							20	47	HS4	HS2, HS3, HS4
25.90	Benzenamine, 2,6-diethyl-	92.4	149	2.7	I	II						Y	7	29	HC8	HC8
25.93	Nonane, 2,2,4,4,6,8,8-heptamethyl-	99.7	226	7.3	I	II							60	20	EC9	ES1, ES2, ES3, ES4, ES5, ES6, EC2, EC8, EC9
26.04	2-Isopropyl-5-methyl-1-heptanol	*90*	172	3.9	I	II							13	13	EC3	EC3, EC9
26.05	Benzenamine, 2,4-dichloro-	*	162	2.9	III	IV							87	349	HC3	EC3, HS1, HS2, HS3, HS4, HS5, HS6, HC1, HC2, HC3, HC4, HC5, HC8, HC9
26.17	Benzenamine, 2,6-dichloro-	87.6	162	2.8	III	IV							7	97	HC6	HC6
26.36	Cyclohexanol, 4-(1,1-dimethylethyl)-, acetate, trans-	99.2	198	3.4	II	III		Y			Y	Y	40	104	HC1	EC1, EC8, EC9, HC1, HC2, HC3, HC4, HC8, HC9
26.37	2-Propenoic acid, 1,7,7-trimethylbicyclo[2.2.1]hept-2-yl ester, exo- like 1	88.6											33	96	HC6	EC3, EC5, EC6, EC7, HC6, HC7, HC9
26.75	Heptylcyclohexane	96.7	182	6.8	I	II							100	16	EC1	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC2, EC3, EC4, EC5, EC6, EC7, EC8, EC9
26.79	Benzoic acid, 2,4,6-trimethyl-, methyl ester	91.3	178	2.9	I	II							7	66	HC6	HC6
26.97	Benzenepropanoic acid, ethyl ester	89.9	178	2.7	I	II	Y					Y	33	39	HS2	HS1, HS2, HS3, HS4, HS6
27.04	4-Acetylanisole	92.6	150	1.7	I	II	Y	Y				Y	40	212	HS3	HS1, HS2, HS3, HS4, HS5, HS6
27.08	Pentanoic acid, 2,2,4-trimethyl-3-hydroxy-, isobutyl ester	*87.5*	216	3	II	III							40	64	HS2	HS1, HS2, HS3, HS4, HS5, HS6
27.09	Longipinene	91	204	4.6	I	II							20	19	EC8	EC3, EC8, EC9
27.24	Tridecane, 6-methyl-	88.9	198	7.5	I	II							27	5	EC9	ES1, EC1, EC4, EC9
27.31	Phenol, 2-(1,1-dimethylethyl)-4-methyl-	98.1	164	3.6	I	II		Y					93	39	HC9	HS1, HS2, HS3, HS4, HS5, HS6, HC1, HC2, HC3, HC4, HC5, HC6, HC8, HC9
27.38	Tridecane, 5-methyl-	94.8	198	7.5	I	II							20	6	EC1	EC1, EC2, EC9
27.46	Benzenemethanol, 4-(1,1-dimethylethyl)-	92.6	164	2.9	I	II							20	21	HC1	HC1, HC8, HC9
27.46	2(3H)-Furanone, dihydro-5-pentyl-	94	156	2.2	II	III	Y	Y				Y	40	96	HS6	HS1, HS2, HS3, HS4, HS5, HS6
27.59	Tridecane, 4-methyl-	87.4	198	7.5	I	II						Y	20	3	EC1	EC1, EC4, EC9
27.71	Phenol, 2-(1,1-dimethylethyl)-5-methyl-	99.8	164	3.6	I	II					Y		93	175	HC8	HS1, HS2, HS3, HS4, HS5, HS6, HC1, HC2, HC3, HC4, HC5, HC7, HC8, HC9
27.79	Ethanone, 1-[4-(1-methylethenyl)phenyl]-	98.5	160	3.1	I	II							33	132	HC3	HC2, HC3, HC4, HC5, HC9
27.89	4-tert-Butylcyclohexyl acetate	90.1	198	3.4	II	III		Y			Y	Y	93	17	HC1	EC9, HS1, HS2, HS3, HS4, HS5, HS6, HC1, HC2, HC3, HC4, HC5, HC7, HC8, HC9
27.93	Longicyclene	88.4	204	5	I	II							13	21	EC7	EC4, EC7
28.05	Propanoic acid, 2-methyl-, 3-hydroxy-2,2,4-trimethylpentyl ester	95	216	3.1	II	III				Y			67	117	HS2	HS1, HS2, HS3, HS4, HS5, HS6, HC1, HC2, HC3, HC8
28.08	Tridecane, 3-methyl-	95	198	7.5	I	II						Y	80	8	EC9	ES1, ES3, ES4, ES5, ES6, EC1, EC2, EC3, EC4, EC5, EC7, EC9
28.10	1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-buten-1-one	94.3	192	3.2	I	II							20	94	HC9	HC1, HC8, HC9
28.12	Biphenyl	*	154	4	III	IV	Y			Y		Y	53	46	EC3	ES2, ES3, ES4, ES5, ES6, EC2, EC3, HS1, HS2, HS3, HS4, HS5, HS6
28.22	Copaene	94.3	204	4.5	I	II							67	45	EC9	ES1, ES2, ES3, ES4, ES6, EC1, EC2, EC5, EC7, EC9

RT	Name	Score	MW	LogP	Cram	Tox	FA	CM	DP	PPA	PPB	Fdb	Fill	S/N	High	Presence
28.29	Isobornyl acrylate	*	208	3.9	II	III					Y		60	548	EC6	EC1, EC2, EC3, EC4, EC5, EC6, EC7, EC8, EC9, HC1, HC2, HC3, HC4, HC5, HC6, HC7, HC8, HC9
28.41	2-Propenoic acid, 3-phenyl-, methyl ester	91.5	162	2.6	I	II	Y	Y				Y	13	37	HC9	HC1, HC9
28.42	4,7-Methanoazulene, 1,2,3,4,5,6,7,8-octahydro-1,4,9,9-tetramethyl-	91.2	204	4.3	I	II		Y					20	50	EC9	EC1, EC8, EC9
28.47	Isobornyl propionate	99.1	210	3.8	I	II	Y	Y				Y	13	53	HC6	EC6, HC6, HC7
28.57	Vinyl decanoate	*88.5*	198	4.7	I	II							7	13	HC9	HC9
28.57	Geranyl acetate	98.7	196	3.5	I	II	Y	Y					7	12	EC1	EC1, HC1
28.65	1,13-Tetradecadiene	96.7	194	6.8	I	II							40	17	EC3	EC1, EC2, EC3, EC4, EC5, EC8
28.71	Tridecane, 3-methylene-	93.3	196	7.3	I	II							27	28	EC6	EC2, EC5,EC6, EC7
28.72	Hexanoic acid, hexyl ester	98.4	200	4.4	I	II	Y	Y				Y	7	120	EC9	EC9, HC9
28.73	Naphthalene, 1,2,3,4-tetrahydro-5,6-dimethyl-	86.1	160	4	I	II							27	17	EC1	ES1, ES2, ES6, EC1
28.99	1-Tetradecene **(SML 0.05)**	*	196	7.9	-	IV					Y	Y	100	140	EC8	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC2, EC3, EC4, EC5, EC6, EC7, EC8, EC9
29.06	Phenol, 4-(1,1-dimethylpropyl)- **(SVHC)**	85.6	164	3.9	-	V					Y		47	1068	HC7	HS1, HS2, HS3, HS4, HS5, HC7, HC8
29.12	1-Decanol, 2-ethyl-	*94.8*	186	4.8	I	II							20	29	EC8	ES2, ES6, EC8
29.12	Diphenyl ether	*	170	4.2	III	IV	Y	Y			Y	Y	93	693	HC9	EC2, EC3, EC5, EC6, EC7, EC9, HS1, HS2, HS3, HS5, HS6, HC1, HC2, HC3, HC4, HC5, HC6, HC7, HC8, HC9
29.14	Decanoic acid, ethyl ester	99.5	200	4.6	I	II	Y	Y				Y	47	852	ES3	ES1, ES2, ES3, ES4, ES5, ES6, EC8, HS1, HS2, HS3, HS5
29.35	Tetradecane	99.8	198	7.2	I	II		Y			Y	Y	100	110	EC9	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC2, EC3, EC4, EC5, EC6, EC7, EC8, EC9, HS1, HS2, HS3, HS5, HS6, HC1, HC4, HC5, HC9
29.36	2-Dodecanol	87.2	186	5.1	II	III							7	46	HC1	HC1
29.40	Longifolene	98	204	5.1	I	II		Y					100	131	EC8	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC2, EC3, EC4, EC5, EC6, EC7, EC8, EC9
29.54	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]-	87.3	204	4.4	I	II		Y					13	23	EC9	EC1, EC9
29.64	Dodecanal	99	184	4.9	I	II	Y	Y				Y	87	717	HS1	ES1, ES2, ES4, ES5, ES6, EC1, EC2, EC3, EC4, EC5, EC6, EC7, EC8, HS1, HC1, HC2, HC3, HC4, HC5, HC6, HC7
29.70	1H-3a,7-Methanoazulene, 2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-, [3R-(3??,3a.beta.,7.beta.,8a??)]-	98	204	4.6	I	II		Y				Y	93	93	EC1	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC2, EC4, EC5, EC6, EC7, EC8, EC9, HS5, HC1, HC5, HC7
29.75	Naphthalene, 1,3-dimethyl-	98.7	156	4.4	III	IV							100	90	EC1	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC2, EC3, EC4, EC5, EC6, EC7, EC8, EC9, HC1, HC9
29.86	2-Propenoic acid, 1,7,7-trimethylbicyclo[2.2.1]hept-2-yl ester, exo- like												27	75	HC6	EC6, HC1, HC5, HC6, HC9
29.95	7-Tetradecene	96.9	196	7.9	I	II					Y	Y	47	7	EC6	EC1, EC3, EC4, EC5, EC6, EC8, EC9
30.04	Caryophyllene	*	204	4.4	I	II	Y						87	120	EC9	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC3, EC4, EC5, EC7, EC8, EC9
30.15	Pyridine, 5-hexyl-2-methyl-	96.5	177	4.2	III	IV							33	45	HS4	HS2, HS3, HS4, HS5, HS6
30.15	Cyclohexanepropanoic acid, 2-propenyl ester	88.3	196	3.8	II	III	Y	Y				Y	7	12	HC1	HC1
30.33	alpha-Ionone	97.5	192	3	I	II	Y	Y			Y	Y	60	29	HC9	HS1, HS2, HS3, HS5, HS6, HC1, HC2, HC8, HC9
30.37	Diphenylmethane	92.3	168	4.1	III	IV		Y					60	29	HC9	EC3, EC9, HS1, HS2, HS3, HS5, HS6, HC1, HC3, HC4, HC9
30.46	Dodecane, 1-methoxy-	95.9	200	5.7	I	II							20	301	EC9	EC1, EC8, EC9
30.48	cis-Thujopsene	98.7	204	4.8	I	II							20	54	EC1	EC1, EC8, EC9
30.55	Naphthalene, 1,4-dimethyl-	96.5	156	4.4	III	IV						Y	60	37	EC1	ES1, ES6, EC1, EC2, EC3, EC4, EC6, EC8, HC1, HC8, HC9
30.64	Longifolene-I2	*90.6*	204	5.7	I	II							13	15	EC3	EC3, EC5
30.66	2-Methyl-1-undecanol	94.7	186	5	I	II		Y					27	12	HS2	ES3, ES6, HS2, HS3, HS6, HC4
30.68	Naphthalene, 1,5-dimethyl-	85.4	156	4.4	III	IV							7	15	HC1	HC1
30.71	Quinoline, 2,3-dimethyl-	96.7	157	3	III	IV							20	34	HC9	HC3, HC8, HC9
30.77	Phenol, 2,6-bis(1,1-dimethylethyl)-	87.2	206	4.9	II	III				Y			87	152	EC8	ES1, ES2, ES3, ES4, ES5, ES6, EC2, EC3, EC4, EC7, EC8, HS1, HS3, HS4, HS6, HC2, HC3, HC4, HC5, HC6, HC7, HC8
30.79	2-Butanone, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	97.8	194	2.7	I	II	Y	Y				Y	60	15	HC1	HS1, HS2, HS3, HS4, HS5, HS6, HC1, HC8, HC9
30.85	(1S,4S,7R)-1,4-Dimethyl-7-(prop-1-en-2-yl)-1,2,3,4,5,6,7,8-octahydroazulene	92	204	4.6	I	II							20	19	EC9	EC1, EC8, EC9
30.86	Acenaphthylene	94.4	152	3.7	III	IV				Y			13	43	HS1	HS1, HC8
30.90	Seychellene	88.5	204	5.1	I	II							13	72	EC1	EC1, EC9
31.06	Naphthalene, 1-methoxy-	99.4	158	3.6	III	IV							93	419	HC8	ES2, ES3, ES6, EC8, EC9, HS1, HS2, HS3, HS4, HS5, HS6, HC2, HC3, HC4, HC5, HC6, HC7, HC8
31.15	Cyclohexane, octyl-	94.9	196	7.3	I	II							40	17	EC9	EC1, EC2, EC4, EC6, EC7, EC9
31.18	1-(4-tert-Butylphenyl)propan-2-one	**99.6**	190	3.2	I	II							93	859	HC1	EC1, HS2, HS3, HS4, HS5, HS6, HC1, HC2, HC3, HC4, HC5, HC6, HC7, HC8, HC9
31.25	Cyclopentane, nonyl-	92.8	196	7.3	I	II							7	12	EC6	EC6
31.41	5,9-Undecadien-2-one, 6,10-dimethyl-, (E)-	95.7	194	3.7	I	II	Y	Y					100	57	HS2	HS1, HS2, HS3, HS4, HS5, HS6, HC1, HC2, HC3, HC4, HC5, HC6, HC7, HC8, HC9

RT	Name	Score	MW	LogP	Cram	Tox	FA	CM	DP	PPA	PPB	Fdb	Fill	S/N	High	Presence
31.42	Humulene	81.4	204	4.5	I	II							7	43	EC9	EC9
31.46	Tetradecane, 6,9-dimethyl-	*85.4*	226	8.3	I	II							7	4	EC6	EC6
31.50	1H-3a,7-Methanoazulene, 2,3,6,7,8,8a-hexahydro-1,4,9,9-tetramethyl-, (1α,3aα,7α,8aβ)-	92.1					Y	Y	Y	Y	Y		13	45	EC1	EC1, EC8
31.53	Diisopropyl adipate	98.4	230	2.2	I	II	Y	Y				Y	40	84	HS3	HS1, HS2, HS3, HS4, HS5, HS6
31.55	8,8,9-Trimethyl-deca-3,5-diene-2,7-dione	**85.7**	208	2.7	III	IV							67	41	HC8	HS2, HS4, HS6, HC2, HC3, HC4, HC5, HC6, HC7, HC8
31.64	3-(4-Isopropylphenyl)-2-methylpropionaldehyde	**90.3**	190	3.3	I	II	Y	Y				Y	7	40	HC1	HC1
31.66	cis-.beta.-Farnesene	94.2	204	6.2	I	II						Y	7	22	EC9	EC9
31.90	2(3H)-Furanone, 5-hexyldihydro-	92.3	170	2.7	II	III	Y	Y				Y	13	195	HC9	HS3, HC9
31.93	2,6-Di-tert-butylbenzoquinone	**92.8**	220	3.4	II	III				Y		Y	27	2030	EC8	EC8, HC2, HC3, HC4
32.15	Dodecane, 1-chloro-	99.5	205	6.9	III	IV							67	116	ES4	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC3, EC8, EC9
32.16	Butanoic acid, 1,7,7-trimethylbicyclo[2.2.1]hept-2-yl ester, endo-	90	224	4.1	I	II	Y					Y	7	12	EC6	EC6
32.33	1-Dodecanol	99.8	186	5.1	I	II	Y	Y		Y		Y	100	363	EC4	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC2, EC3, EC4, EC5, EC6, EC7, EC8, EC9, HC4
32.35	trans-anti-trans-Tetra-decahydroanthracene	*86.6*	192	6	III	IV							7	45	EC4	EC4
32.39	.gamma.-Muurolene	93.6	204	4.3	I	II							53	51	EC9	ES2, ES3, ES4, ES6, EC3, EC5, EC7, EC9
32.46	quinoline, 4,5,8-trimethyl-	90.2	171	3.5	III	IV							27	233	HC9	HS2, HS3, HS4, HC9
32.48	4-(2,6,6-Trimethyl 2-cyclohexen-1-yl)-3-methyl-3-buten-2-one	99.6	206	3.3	I	II	Y	Y				Y	87	378	HC1	EC1, EC8, EC9, HS1, HS2, HS3, HS4, HC1, HC2, HC3, HC4, HC5, HC6, HC7, HC8, HC9
32.51	1,1'-Biphenyl, 3-methyl-	*	168	3.9	III	IV							73	28	EC7	ES1, ES2, ES3, ES5, ES6, EC1, EC2, EC4, EC6, EC7, EC8
32.71	3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-, (E)-	99.2	192	2.9	I	II	Y	Y				Y	80	548	HC9	HS1, HS2, HS3, HS4, HS5, HS6, HC1, HC2, HC3, HC5, HC8, HC9
32.76	8-Dodecen-1-ol, (Z)-	**92.1**	184	4.2	I	II							27	14	EC3	EC3, EC4, EC5, EC8
32.84	Cyclopropane, 1-methyl-1-(1-methylethyl)-2-nonyl-	*90.6*	224	7.7	II	III							7	12	EC6	EC6
32.86	1,1'-Biphenyl, 4-methyl-	93.8	168	4.6	III	IV	Y					Y	13	22	EC7	EC6, EC7
32.97	3-Tridecanone	98.6	198	5.1	II	III							33	58	EC6	EC3, EC4, EC5, EC6, EC7, HC6
33.02	Decanoic acid, propyl ester	98.1	214	5.1	I	II							33	147	ES3	ES1, ES2, ES3, ES4, ES6
33.04	Valencen	97.8	204	5.2	I	II	Y	Y					73	111	EC9	ES1, ES2, ES3, ES4, ES5, ES6, EC3, EC5, EC7, EC8, EC9
33.11	1-Pentadecene	99.6	210	8.4	I	II						Y	60	22	EC3	ES1, EC2, EC3, EC4, EC5, EC6, EC7, EC8, EC9
33.16	4H-Inden-4-one, 1,2,3,5,6,7-hexahydro-1,1,2,3,3-pentamethyl-	90.8	206	3.3	III	IV		Y					20	70	HC1	HC1, HC8, HC9
33.19	2-Tridecanone	*	198	5.2	II	III	Y	Y				Y	47	83	ES3	ES1, ES2, ES3, ES4, ES5, ES6, EC6, HC6
33.20	Undecanoic acid, ethyl ester	93	214	5.1	I	II	Y					Y	27	59	ES3	ES2, ES3, ES4, ES5
33.24	Naphthalene, 1,4,6-trimethyl-	89.1	170	4.8	III	IV							60	30	EC1	ES2, ES3, ES4, ES5, EC1, EC2, EC4, EC8, EC9
33.31	2,4,5,5,8a-Pentamethyl-6,7,8,8a-tetrahydro-5H-chromene	**95.7**	206	3.4	III	IV							27	143	HC1	EC1, HS1, HC1, HC8, HC9
33.35	alpha-Muurolene	89.8	204	4.1	I	II							73	49	EC3	ES2, ES3, ES4, ES5, ES6, EC2, EC3, EC5, EC6, EC7, EC9
33.43	Pentadecane	99.5	212	7.7	I	II		Y			Y	Y	100	27	EC9	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC2, EC3, EC4, EC5, EC6, EC7, EC8, EC9
33.61	1-Dodecanamine, N,N-dimethyl-	86.2	213	5.9	I	II		Y			Y		80	9345	HS1	HS1, HS2, HS3, HS4, HS5, HS6, HC1, HC2, HC3, HC7, HC8, HC9
33.72	.beta.-Bisabolene	98.5	204	5.2	I	II							47	87	EC9	ES1, ES2, ES3, ES4, ES6, EC7, EC9
33.75	Tridecanal	94.6	198	5.4	I	II	Y	Y				Y	33	18	EC5	EC1, EC3, EC4, EC5, EC7
33.88	Benzoic acid, 4-(1,1-dimethylethyl)-, ethenyl ester	*92.2*	204	4.7	I	II							7	477	HC1	HC1
33.92	2,4-Di-tert-butylphenol	*	206	4.9	I	II				Y			93	19693	EC8	ES1, ES2, ES3, ES4, ES5, ES6, EC2, EC3, EC4, EC5, EC6, EC7, EC8, EC9, HC4, HC9
33.99	2-Phenoxyethyl isobutyrate	86.1	208	2.8	I	II	Y	Y			Y	Y	7	138	HC3	HC3
34.04	Ethyl 4-t-butylbenzoate	*96.6*	206	4.3	I	II							40	415	ES3	ES1, ES2, ES3, ES4, ES5, ES6
34.09	1-Penten-3-one, 1-(2,6,6-trimethyl-2-cyclohexen-1-yl)-	95.2	206	3.4	I	II		Y			Y	Y	27	66	HC1	EC1, EC8, EC9, HC1, HC2, HC8, HC9
34.11	Naphthalene, 2-ethoxy-	98.7	172	3.8	III	IV	Y	Y				Y	20	285	HC9	EC9, HS1, HC8, HC9
34.22	Naphthalene, 1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-, (1S-cis)-	93.9	202	5.1	I	II						Y	60	93	EC9	ES2, ES3, ES4, ES5, ES6, EC2, EC5, EC7, EC9
34.27	Naphthalene, 1,6,7-trimethyl-	86.1	170	4.8	III	IV							27	98	EC1	ES1, ES6, EC1, EC4
34.30	Lilial	99	204	3.9	I	II		Y			Y		47	116	HC1	EC1, HS4, HC1, HC2, HC4, HC6, HC7, HC9
34.35	Dodecanoic acid, methyl ester	99.3	214	5.8	I	II	Y	Y				Y	100	548	EC1	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC2, EC3, EC4, EC5, EC6, EC7, EC8, EC9
34.59	Isoamyl salicylate	91.4	208	4.6	I	II	Y	Y				Y	67	254	EC9	ES2, ES3, EC1, EC9, HS1, HS2, HS3, HS4, HS5, HS6, HC1, HC2, HC6, HC9
34.63	Benzenamine, 3, 4,5-trichloro-	-	197	3.3	III	IV							27	11	HC3	HS3, HS4, HS6, HC3
34.71	1-Dodecanol, 2-methyl-, (S)-	*93.1*	200	5.6	I	II							87	12	EC9	ES2, ES3, ES6, EC4, EC9, HS1, HS2, HS3, HS4, HS5, HS6, HC2, HC3, HC4, HC5, HC6, HC7, HC9
34.80	2-Butenedioic acid, dibutyl ester	**85**	228	2.7	I	II		Y			Y		7	18	HC9	HC9
34.88	Naphthalene, 1,4,5-trimethyl-	95.6	170	4.9	III	IV							93	47	EC9	ES1, ES2, ES3, ES4, ES5, ES6, EC2, EC3, EC5, EC6, EC7, EC8, EC9, HS1, HC1
34.96	.alpha.-Calacorene	85.7	200	4.4	I	II							13	36	ES4	ES4, EC3
34.99	Naphthalene, 2,3,6-trimethyl-	96.7	170	4.7	III	IV							80	50	EC1	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC3, EC4, EC7, EC8, EC9, HC1

RT	Name	Score	MW	LogP	Cram	Tox	FA	CM	DP	PPA	PPB	Fdb	Fill	S/N	High	Presence
35.05	Cyclohexene, 4-[(1E)-1,5-dimethyl-1,4-hexadien-1-yl]-1-methyl-	89	204	5.2	I	II		Y					7	26	EC9	EC9
35.49	(Trichloromethyl)phenylcarbinyl acetate	92.1	268	3.5	III	IV		Y					13	52	HC1	HC1, HC2
35.66	Quinoline, 6-methoxy-4-methyl-	*87.1*	173	2.6	III	IV							7	32	ES3	ES3
35.69	Butanedioic acid, dibutyl ester	96.6	230	2.9	I	II							47	91	HS1	HS1, HS2, HS3, HS4, HS5, HS6, HC3
35.75	Ethanone, 2-(formyloxy)-1-phenyl-	*89.3*	164	1.5	I	II							7	22	HC6	HC6
36.06	2(3H)-Furanone, 5-heptyldihydro-	96.3	184	3.3	II	III	Y	Y			Y	Y	73	118	HC1	HS1, HS2, HS3, HS4, HS5, HS6, HC1, HC2, HC3, HC8, HC9
36.09	Pentanoic acid, 1,7,7-trimethylbicyclo[2.2.1]hept-2-yl ester, endo-	*96.1*	238	4.7	I	II							7	21	EC6	EC6, HC6
36.11	1-Pentadecene, 2-methyl-	87	224	8.4	I	II							7	14	EC8	EC8
36.14	Salicylic acid, pentyl ester	**97.8**	208	5.2	I	II	Y	Y			Y	Y	93	153	EC9	ES1, ES2, ES3, ES6, EC1, EC2, EC3, EC4, EC5, EC9, HS1, HS2, HS3, HS4, HS5, HS6, HC1, HC2, HC3, HC4, HC5, HC6, HC8, HC9
36.31	n-Tridecan-1-ol	99.6	200	5.7	I	II		Y				Y	87	48	EC4	ES1, ES2, ES3, ES4, ES5, ES6, EC2, EC3, EC4, EC5, EC7, EC8, EC9, HC4
36.33	1-Penten-3-one, 1-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	97.9	206	3.4	I	II	Y	Y				Y	20	296	HC1	EC1, HC1, HC8, HC9
36.39	Glutaric acid, di(isobutyl) ester	96.1	244	2.9	I	II							60	103	HS6	HS1, HS2, HS3, HS5, HS6, HC2, HC3, HC5, HC7
36.65	3,3'-Dimethylbiphenyl	94.8	182	4.3	III	IV							33	30	EC1	EC1, EC4, EC7, EC8, EC9, HC1
36.68	1,15-Hexadecadiene	90.8	222	7.9	I	II							20	209	EC3	EC3, EC4, EC5
36.75	Butyl caprate	95.7	228	5.4	I	II						Y	40	29	ES4	ES1, ES2, ES3, ES4, ES5, ES6
36.91	Diethyl Phthalate **(EDC)**	*	222	2.5	-	V		Y		Y			100	413	HC1	HS1, HS2, HS3, HS4, HS5, HS6, HC1, HC2, HC3, HC4, HC5, HC6, HC7, HC8, HC9
36.96	Phenol, 3,5-bis(1,1-dimethylethyl)-	90.7	206	4.9	I	II							47	626	HC9	HS1, HS2, HS5, HS6, HC1, HC8, HC9
37.03	Cetene	99	224	8.9	I	II						Y	47	97	EC9	EC2, EC3, EC5, EC6, EC7, EC8, EC9
37.11	Dodecanoic acid, ethyl ester	*	228	5.6	I	II	Y	Y				Y	100	1554	ES3	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC2, EC3, EC4, EC5, EC6, EC7, EC8, EC9, HS1, HS2, HS5, HS6
37.11	Dibutyl itaconate	**99.6**	242	3	I	II							40	625	HS3	HS1, HS2, HS3, HS4, HS5, HS6
37.13	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate **(SML 5)**	*	286	4.7	-	II		Y		Y			100	319	ES3	ES1, ES2, ES3, ES5, ES6, EC1, EC2, EC3, EC5, EC7, HS1, HS2, HS3, HS4, HS5, HS6, HC1, HC2, HC3, HC4, HC5, HC6, HC7, HC8, HC9
37.13	2-Tetradecanone	94	212	5.7	II	III						Y	7	60	EC6	EC6
37.15	Phenyl cyclohexyl ketone	97	188	3.7	I	II							7	66	HC6	HC6
37.20	Benzene, (1-methylnonyl)-	90	218	6.8	I	II							33	157	EC9	EC1, EC5, EC7, EC8, EC9
37.31	Hexadecane	99.4	226	8.3	I	II		Y			Y	Y	100	69	EC1	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC2, EC3, EC4, EC5, EC6, EC7, EC8, EC9, HS1, HS2, HS3, HS4, HS5, HS6, HC1, HC2, HC3, HC4, HC5, HC6, HC7, HC8, HC9
37.32	Phenol, 4-(1,1,3,3-tetramethylbutyl)- **(SHVC, EDC)**	86.9	206	5	-	V	Y			Y		Y	13	158	HS1	HS1, HC3
37.39	2-Hexyl-1-octanol	*90.7*	214	5.9	I	II							27	50	EC9	ES2, ES3, ES6, EC9
37.40	2-Naphthyl methyl ketone	96.7	170	3.2	III	IV	Y	Y				Y	13	29	HC9	HC1, HC9
37.51	Pyridine, 2-nonyl-	*87.4*	205	5.1	III	IV							40	39	HS4	HS1, HS2, HS3, HS4, HS5, HC4
37.65	Lauryl acetate	95.3	228	5.6	I	II	Y	Y				Y	27	30	EC9	ES2, ES3, EC1, EC9
37.69	Tetradecanal	99.8	212	6	I	II	Y	Y				Y	100	54	EC4	ES3, ES4, ES5, ES6, EC1, EC2, EC3, EC4, EC5, EC6, EC7, EC8, EC9, HS1, HS2, HS3, HS4, HS5, HS6, HC1, HC2, HC3, HC4, HC5, HC6, HC7, HC8, HC9
37.77	Diphenylamine	90.6	169	3.5	III	IV				Y		Y	7	21	HC3	HC3
37.94	1,7-Trimethylene-2,3-dimethylindole	**88.2**	185	3.1	III	IV							7	70	HC3	HC3
38.02	Benzophenone **(SML 0.6)**	*	182	3.4	-	III	Y	Y		Y		Y	100	355	HC1	HS1, HS2, HS3, HS4, HS5, HS6, HC1, HC2, HC3, HC4, HC5, HC6, HC7, HC8, HC9
38.06	1-Acenaphthenol	93.4	170	2.4	III	IV							7	46	HC8	HC8
38.32	Dodecanoic acid, 1-methylethyl ester	99.1	242	6.1	I	II		Y			Y		87	88	EC5	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC2, EC3, EC5, EC6, EC7, EC9
38.51	N,N-Dimethyldodecanamide	**95.7**	227	4.1	III	IV							47	496	HC1	HS2, HS4, HC1, HC2, HC3, HC5, HC8
38.75	Phenol, 4,6-di(1,1-dimethylethyl)-2-methyl-	82.9	220	5.3	I	II							7	34	HC1	HC1
38.81	1,4,5,8-Tetramethylnaphthalene	**90**	184	4.6	III	IV							67	49	EC1	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC3, EC4, EC5
38.90	Heptanal, 2-(phenylmethylene)-	99.2	202	4.2	I	II	Y	Y			Y		47	43	EC9	EC9, HS1, HS2, HS3, HS5, HC1, HC8, HC9
38.93	Benzene, 1,1'-(1,3-propanediyl)bis-	96	196	3.4	III	IV						Y	20	88	ES3	ES3, ES6, EC3
38.98	1-Octanamine, N-methyl-N-octyl-	**86.1**	256	7.1	I	II							20	56	HC9	HC1, HC8, HC9
39.09	Benzoic acid, phenyl ester	*95.6*	198	3.6	I	II		Y					7	46	HC6	EC6, HC6
39.12	1,4-Benzenedicarboxylic acid, diethyl ester	*	222	3.8	I	II					Y		80	127	ES1	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC2, EC3, EC4, EC7, EC8
39.16	Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester	97.2	226	2.7	II	III	Y	Y			Y		87	132	HC1	HS1, HS2, HS3, HS4, HS5, HS6, HC1, HC2, HC3, HC4, HC5, HC8, HC9
39.24	Cyclopentane, undecyl-	88.3	224	8.4	I	II							33	22	EC6	EC2, EC3, EC4, EC5, EC6
39.44	Glutaric acid, butyl 2-methylbutyl ester	95.1	258	3.2	I	II							60	197	HC3	EC3, HS4, HS5, HS6, HC2, HC3, HC4, HC5, HC7, HC8
39.53	Ar-tumerone	87.9	216	4	I	II						Y	7	1342	HC8	HC8
39.68	Octane, 1,1'-oxybis-	99.3	242	6.9	I	II		Y					87	92	ES4	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC2, EC3, EC4, EC5, EC7, EC8, HC4
39.72	(7a-Isopropenyl-4,5-dimethyloctahydroinden-4-yl)methanol	*86.3*	222	5	I	II							20	198	EC9	EC1, EC8, EC9, HC1, HC8, HC9

RT	Name	Score	MW	LogP	Cram	Tox	FA	CM	DP	PPA	PPB	Fdb	Fill	S/N	High	Presence
39.94	1,3-di-iso-propylnaphthalene	85.0	212	5.9	III	IV							53	247	EC2	ES1, ES2, ES4, ES5, ES6, EC2, EC3, EC5
40.01	n-Hexyl salicylate	90.4	222	5.7	I	II		Y			Y		93	733	EC1	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC2, EC3, EC4, EC5, EC6, EC8, EC9, HS1, HS2, HS3, HS6, HC1, HC9
40.03	1,1'-Biphenyl, 2,2',5,5'-tetramethyl-	85.3	210	5	III	IV							27	439	EC4	ES3, ES4, EC4, EC7
40.10	1-Tetradecanol	98.7	214	6.2	I	II	Y	Y		Y	Y	Y	87	165	EC1	ES2, ES3, ES4, ES5, ES6, EC1, EC2, EC3, EC4, EC5, EC6, EC7, EC8
40.11	1,7-di-iso-propylnaphthalene	94.5	212	5.9	III	IV					Y		53	535	EC2	ES2, ES3, ES4, ES5, ES6, EC2, EC5, EC8
40.12	Phenol, 2,4-bis(1,1-dimethylpropyl)-	**85.3**	234	6	I	II					Y		7	519	EC7	EC7, HC7
40.29	Methanone, (1-hydroxycyclohexyl)phenyl-	84.7	204	2.6	I	II					Y		20	220	HC6	EC6, HC5, HC6, HC7
40.36	Hexanedioic acid, bis(2-methylpropyl) ester	90.9	258	3.2	I	II	Y	Y		Y		Y	13	33	HS6	HS5, HS6
40.49	1,4-di-iso-propylnaphthalene	86.5	212	5.9	III	IV							20	84	EC2	ES4, EC2, EC5
40.68	3-Pentadecanone	93.3	226	6.2	II	III							67	246	EC6	ES1, ES2, ES3, ES4, ES6, EC2, EC3, EC5, EC6, EC7
40.70	Dodecyl acrylate **(SML 0.05)**	*	240	6.2	-	IV					Y		40	28	EC1	ES4, EC1, EC2, EC3, EC4, EC8
40.87	2-Pentadecanone	98.6	226	6.3	II	III	Y					Y	100	342	EC8	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC2, EC3, EC4, EC5, EC6, EC7, EC8, EC9
40.97	Benzene, (1-methyldecyl)-	92.9	232	7.4	I	II							27	207	EC9	EC1, EC2, EC8, EC9
41.12	1,1'-Biphenyl, 2,2',5,5'-tetramethyl- like	89.5											33	345	EC4	EC4, EC5, EC6, EC7, EC8
41.12	1-Tetradecanamine, N,N-dimethyl-	*	241	6.9	I	II		Y					33	4578	HS4	HS1, HS2, HS3, HS4, HC9
41.29	1,7-Trimethylene-2,3,5-trimethylindole	**85.4**	199	3.5	III	IV							7	77	HC9	HC9
41.42	Pentadecanal-	95.4	226	6.5	I	II						Y	40	59	EC7	EC1, EC3, EC4, EC6, EC7, EC9, HC6
41.45	2,6-Diisopropylnaphthalene like	92											53	180	ES1	ES1, ES2, ES3, ES4, ES5, ES6, EC2, EC3
41.49	Ethanol, 2-(dodecyloxy)-	**99.2**	230	5.1	I	II		Y					67	111	EC9	ES2, ES5, EC1, EC4, EC9, HS1, HS2, HS3, HS4, HS5, HS6, HC1, HC4, HC8, HC9
41.54	Acridine, 1,2,3,4,5,6,7,8-octahydro-	85.5	187	3.3	III	IV							7	50	HC3	HC3
41.71	2,6-Diisopropylnaphthalene	*	212	5.8	III	IV							100	920	EC2	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC2, EC3, EC4, EC5, EC6, EC7, EC8, EC9
41.85	Methyl tetradecanoate	97.2	242	6.8	I	II	Y	Y				Y	93	533	EC1	ES2, ES3, ES4, ES5, ES6, EC1, EC2, EC3, EC4, EC5, EC6, EC7, EC8, EC9
41.87	1,3-di-iso-propylnaphthalene like	91.6											47	157	EC2	ES1, ES4, ES5, EC2, EC3, EC5, EC7
42.00	1-Octanamine, N-methyl-N-octyl- like	99.3											53	368	HC8	HS1, HS2, HS3, HS4, HS5, HS6, HC8, HC9
42.51	Octanal, 2-(phenylmethylene)-	99	216	4.8	I	II	Y	Y				Y	80	496	EC9	ES2, ES3, ES6, EC1, EC2, EC3, EC4, EC5, EC6, EC7, EC8, EC9, HC1, HC2, HC4, HC5, HC8, HC9
42.68	Glutaric acid, ethyl 4-methylhept-3-yl ester	85.7	272	3.8	I	II							13	85	EC6	EC6, HC56
42.93	1,1'-Biphenyl, 3,3'-dichloro-	93	223	5.3	III	IV							33	51	EC3	ES2, ES4, ES5, EC3, EC9
43.03	Benzyl Benzoate	99.7	212	4	I	II	Y	Y		Y		Y	33	146	HC1	EC1, HS3, HS4, HS5, HC1, HC9
43.09	3,5-Di-tert-butylbenzoic acid	**94.5**	234	4.8	I	II							100	1113	HC8	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC2, EC3, EC4, EC5, EC8, EC9, HS1, HS2, HS3, HS4, HS5, HS6, HC1, HC2, HC3, HC4, HC5, HC6, HC7, HC8, HC9
43.11	Naphthalene, 1,2,3,4-tetrahydro-1-phenyl-	*86.6*	208	4.8	III	IV							7	39	EC3	EC3
43.23	Phenanthrene	87.3	254	6	III	IV							7	73	EC8	EC8
43.24	Dibutyl adipate	96.7	258	3.1	I	II		Y		Y			40	42	HS5	HS1, HS2, HS3, HS4, HS5, HS6
43.37	Octanal, 2-(phenylmethylene)- like	98.6											40	180	EC9	EC1, EC2, EC3, EC4, EC8, EC9, HC1, HC2, HC8, HC9
43.47	Benzene, 1,1'-(3-methyl-1-propene-1,3-diyl)bis-	*88.5*	208	4.8	III	IV							13	56	EC1	EC1, EC8, HC1
43.52	n-Dodecyl methacrylate	95.7	254	7.2	I	II		Y			Y		7	12	EC4	EC4
43.67	n-Pentadecanol	95.6	228	6.8	I	II		Y				Y	87	22	EC9	ES1, ES2, ES3, ES5, ES6, EC1, EC2, EC4, EC5, EC7, EC8, EC9, HS1, HS2, HS5, HC1, HC2, HC3
43.78	2,4-Diphenyl-4-methyl-1-pentene	*	236	6	III	IV					Y		60	470	EC7	ES1, ES2, ES3, EC1, EC2, EC3, EC4, EC5, EC7
43.82	Ethyl 9-tetradecenoate	93.9	254	5.8	I	II							40	48	ES4	ES1, ES2, ES3, ES4, ES5, ES6
43.99	Glutaric acid, ethyl 6-methylhept-2-yl ester	98.6	272	3.6	I	II							7	52	EC6	EC6, HC6
44.02	1,7-Trimethylene-2,3,5-trimethylindole like	**91.3**											47	69	HC3	HS1, HS2, HS3, HS4, HS5, HS6, HC3
44.26	1-Octadecene	99.2	253	10	I	II					Y		87	27	ES6	ES1, ES2, ES3, ES4, ES5, ES6, EC2, EC3, EC4, EC5, EC7, EC8, EC9
44.26	Benzene, 1,1'-(1,2-ethenediyl)bis[2-methyl-	*85.9*	208	4.9	III	IV							7	100	EC1	EC1, HC1
44.41	Tetradecanoic acid, ethyl ester	99.3	256	6.7	I	II	Y	Y				Y	93	5102	ES4	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC3, EC4, EC5, EC6, EC7, EC8, EC9, HS2, HS3, HS4
44.52	Octadecane	99.2	255	9.3	I	II		Y			Y	Y	100	56	ES3	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC3, EC4, EC5, EC6, EC7, EC8, EC9, HS1, HS2, HS3, HS4, HS5, HS6, HC1, HC2, HC3, HC4, HC5, HC6, HC7, HC8, HC9
44.56	Benzene, (1-methylundecyl)-	85.3	246	7.9	I	II							20	59	EC8	EC2, EC5, EC8
44.64	2-Ethylhexyl salicylate	*	250	5.7	I	II		Y					67	4957	ES4	ES1, ES2, ES3, ES4, ES5, ES6, EC2, EC3, EC7, EC9, HS1, HS2, HS3, HS4, HS5, HS6
44.79	Carbonic acid, bis(2-ethylhexyl) ester	86.9	286	6.8	I	II							47	34	EC9	ES1, ES2, ES3, ES4, ES5, ES6, EC9
44.88	E-11(12-Cyclopropyl)dodecen-1-ol	*87.9*	224	5.4	II	III							7	50	EC9	EC9, HC9
44.89	3,5-di-tert-Butyl-4-hydroxyacetophenone	**84.8**	248	4.6	II	III							40	160	HC8	HS2, HS3, HS6, HC2, HC4, HC8
44.96	Hexadecanal	99.7	240	7.1	I	II						Y	100	25	EC5	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC2, EC3, EC4, EC5, EC6, EC7, EC8, EC9
45.57	Isopropyl myristate	99.8	271	7.2	I	II	Y	Y		Y		Y	87	137	EC1	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC3, EC4, EC6, EC7, EC8, EC9, HC1, HC8, HC9

RT	Name	Score	MW	LogP	Cram	Tox	FA	CM	DP	PPA	PPB	Fdb	Fill	S/N	High	Presence
45.59	Benzene, (1-butylnonyl)-	null	261	8.5	I	II							7	67	EC9	EC9
45.95	Isoamyl laurate	85.6	271	7	I	II	Y	Y				Y	7	15	ES6	ES6
46.06	Naphthalene, 1-phenyl-	88.8	204	4.8	III	IV							7	28	EC3	EC3
46.09	Galaxolide	95.7	258	4.8	III	IV		Y			Y		67	270	EC8	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC2, EC8, EC9
46.20	Phenol, 4-(1-methyl-1-phenylethyl)- **(SML 0.05)**	87.3	212	3.7	-	IV					Y		7	30	HC9	HC9
46.39	2-Pentenoic acid, 3-methyl-5-(2,6,6-trimethyl-1-cyclohexenyl)	88.5	236	4	I	II							7	497	EC6	EC6
46.54	Benzoic acid, 2-hydroxy-, phenylmethyl ester	95.6	228	3.2	I	II	Y	Y			Y	Y	20	982	HC1	HS4, HC1, HC9
46.55	Tonalid	**86.1**	258	5.3	I	II		Y				Y	7	302	EC9	EC9
46.55	2-Propenenitrile, 3,3-diphenyl-	91.7	205	4	III	IV							13	35	HS3	HS1, HS3
46.58	Diisobutyl phthalate **(CMR, SVHC, EDC)**	97.3	278	4.1	-	V				Y		Y	67	527	HC9	ES2, ES4, ES5, ES6, EC2, EC4, EC5, EC9, HS1, HS2, HS3, HS4, HS5, HS6, HC4, HC5, HC9
46.66	Ethyl 13-methyl-tetradecanoate	**95**	271	7	I	II							40	104	ES4	ES1, ES2, ES3, ES4, ES5, ES6
46.90	Cyclopenta[g]-2-benzopyran, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-	95	258	4.8	III	IV		Y			Y		27	193	EC1	ES1, EC1, EC8, EC9, HC1, HC9
46.91	Hexadecanal, 2-methyl-	**98.5**	255	7.6	I	II							7	32	EC6	EC6
47.38	1-Hexadecanol **(SML 60)**	99.7	242	7.3	-	I	Y	Y		Y		Y	60	242	EC1	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC8, EC9
47.47	Tetradecanoic acid, propyl ester	97.8	271	7.3	I	II							33	156	ES3	ES1, ES2, ES3, ES4, ES6
47.59	Adipic acid, ethyl 2-ethylhexyl ester	**95.7**	286	4.1	I	II							33	52	ES1	ES1, ES2, ES3, ES4, ES6
47.61	Pentadecanoic acid, ethyl ester	98.2	271	7.3	I	II						Y	40	229	ES4	ES1, ES2, ES3, ES4, ES6, EC8
47.63	3-Heptadecanone	93	255	7.3	II	III							27	310	EC6	EC2, EC3, EC5, EC6
47.68	4-Dodecylmorpholine	-	255	5.5	III	IV							93	187	HC9	HS1, HS2, HS3, HS4, HS5, HS6, HC1, HC2, HC3, HC4, HC5, HC7, HC8, HC9
47.71	Hexadecanenitrile	96.5	237	6.9	III	IV							33	17	EC4	EC1, EC4, EC5, EC8, EC9
47.82	Methyl n-hexadecyl ketone	90.1	269	7.9	II	III							80	172	EC8	ES1, ES2, ES3, ES4, ES6, EC2, EC3, EC4, EC5, EC6, EC7, EC8
47.95	Decanoic acid, 2-ethylhexyl ester	91.3	285	7.3	I	II							7	63	EC4	EC4
47.95	Dimethyl palmitamine	93.3	270	8	I	II		Y			Y		60	709	HS2	HS1, HS2, HS3, HS4, HS5, HS6, HC1, HC3, HC9
48.02	Benzene, (1-methyldodecyl)-	94	261	8.5	I	II							53	70	EC9	ES1, ES2, ES3, ES6, EC1, EC2, EC5, EC9
48.05	Diphenyl sulfone **(SML 3)**	**98.1**	218	2.4	-	II					Y		20	34	HS3	HS1, HS3, HS6
48.35	Heptadecanal	**99.1**	255	7.6	I	II						Y	40	15	EC7	EC1, EC2, EC3, EC4, EC5, EC7
48.42	Ethanol, 2-(tetradecyloxy)-	*95.4*	258	6.2	I	II		Y					73	11	EC9	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC2, EC4, EC8, EC9, HS1, HS2, HS3, HS4, HS5, HS6, HC1, HC4, HC9
48.54	Oxacycloheptadec-8-en-2-one, (8Z)-	**85.6**	252	5.5	I	II		Y				Y	7	13	EC1	EC1
48.63	Hexadecanoic acid, methyl ester	*	271	7.9	I	II		Y				Y	100	1358	EC9	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC2, EC3, EC4, EC5, EC6, EC7, EC8, EC9
48.68	Fenpropidin	**86.5**	274	5.5	III	IV							7	215	HS4	HS4
49.18	7-Acetyl-6-ethyl-1,1,4,4-tetramethyltetralin like												20	307	EC7	EC2, EC5, EC7
49.63	Dibutyl phthalate **(CMR, SVHC, EDC, SML 0.3)**	93.7	278	4.7	-	V		Y		Y		Y	100	587	HC1	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC2, EC3, EC4, EC5, EC6, EC7, EC8, EC9, HS1, HS2, HS3, HS4, HS5, HS6, HC1, HC2, HC3, HC4, HC5, HC6, HC7, HC8, HC9
49.75	7-Acetyl-6-ethyl-1,1,4,4-tetramethyltetralin like 2												67	597	EC7	ES1, ES2, ES3, ES4, ES5, ES6, EC2, EC5, EC7. EC9
50.13	Ethyl 9-hexadecenoate	99.8	283	6.9	I	II							40	147	ES3	ES1, ES2, ES3, ES4, ES5, ES6
50.16	n-Hexadecanoic acid **(SML 60)**	98.8	256	6.4	-	I	Y	Y			Y	Y	93	555	EC3	ES1, ES2, ES3, ES4, ES5, ES6, EC2, EC3, EC4, EC5, EC6, EC7, EC8, EC9
50.18	4b,8-Dimethyl-2-isopropylphenanthrene, 4b,5,6,7,8,8a,9,10-octahydro-	94	256	6.7	I	II							33	74	EC7	EC2, EC3, EC5, EC7, EC8
50.22	Glutaric acid, butyl 2-propylpentyl ester	93.5	300	4.6	I	II							13	88	EC3	EC3, EC6
50.38	n-Heptadecanol-1	96.4	257	7.8	I	II						Y	20	17	EC1	EC1, EC5, EC7
50.54	Butyl myristate	92.4	285	7.6	I	II		Y					20	17	ES4	ES2, ES3, ES4
50.55	Chlorpyrifos	95.4	351	5.3	III	IV							7	369	EC1	EC1
50.57	Manoyl oxide	91.1	291	5.9	III	IV						Y	67	34	EC7	ES1, ES2, ES3, ES4, ES5, ES6, EC2, EC3, EC5, EC7
50.68	Diethylene glycol monododecyl ether	**96.3**	274	4.9	I	II							7	25	ES1	ES1
50.78	13-Octadecenal, (Z)-	*87.2*	267	7.2	I	II							13	17	EC3	EC3, EC5
51.00	Hexadecanoic acid, ethyl ester	99.1	285	7.8	I	II	Y	Y			Y	Y	100	6413	ES3	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC2, EC3, EC4, EC5, EC6, EC7, EC8, EC9, HS1, HS2, HS4, HS6, HC8, HC9
51.02	1H-Pyrazole, 4,5-dihydro-1,3-diphenyl-	**85.6**	222	3.5	III	IV							40	1191	HS3	HS1, HS2, HS3, HS4, HS5, HS6
51.26	Epimanoyl oxide	98.1	291	5.9	III	IV							67	66	EC2	ES1, ES2, ES3, ES4, ES5, ES6, EC2, EC3, EC5, EC7
51.34	1-Hexadecanol, acetate	95.2	285	7.7	I	II		Y					13	8	EC1	EC1, EC9
51.70	1-Decanamine, N-decyl-N-methyl-	**89.6**	162	0.8	I	II							7	39	HC9	HC9
51.96	Isopropyl palmitate	98.5	299	8.2	I	II	Y	Y		Y		Y	60	105	EC1	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC8, EC9

RT	Name	Score	MW	LogP	Cram	Tox	FA	CM	DP	PPA	PPB	Fdb	Fill	S/N	High	Presence
52.09	10,18-Bisnorabieta-8,11,13-triene	**89.8**	242	6.1	I	II							87	205	EC7	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC2, EC3, EC5, EC7, EC8, EC9
52.29	Fluoranthene **(SVHC)**	87.0	202	5.2	-	V					Y		7	30	EC8	EC8
52.54	Phenanthrene, 1,2,3,4,4a,9,10,10a-octahydro-1,1,4a-trimethyl-7-(1-methylethyl)-, (4aS-trans)-	96.7	271	7.1	I	II						Y	60	66	EC7	ES3, ES4, ES5, ES6, EC2, EC3, EC5, EC7, EC8
52.71	Adipic acid, 2-decyl ethyl ester	88.7	315	5.3	I	II							7	111	ES5	ES5
52.73	9-Octadecen-1-ol, (Z)- **(SML 60)**	99.6	269	7.4	-	I	Y	Y		Y			7	81	ES4	ES4
52.75	Ethyl 15-methyl-hexadecanoate	91.8	299	8.1	I	II							20	25	ES3	ES2, ES3, ES6
53.03	Heptadecanoic acid	94	271	6.9	I	II					Y	Y	13	28	EC9	EC4, EC9
53.05	Terephthalic acid, isobutyl butyl ester	**85.8**	278	5.6	I	II							13	53	EC3	EC3, EC5
53.33	Oleanitrile	**95.5**	264	7.1	III	IV					Y		73	12	EC8	ES2, ES3, ES4, ES5, EC2, EC3, EC4, EC5, EC6, EC8, EC9
53.35	cis-10-Heptadecenoic acid	86.6	268	6.9	I	II							47	31	ES3	ES1, ES2, ES3, ES4, ES5, ES6, EC9
53.53	1-Octadecanol	*	271	8.4	I	II	Y			Y		Y	93	409	EC8	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC2, EC3, EC4, EC5, EC7, EC8, EC9
53.56	Phthalic acid, ethyl hept-2-yl ester	88.8	292	4.9	I	II							33	58	ES5	ES1, ES2, ES3, ES5, ES6
53.76	Hexadecanoic acid, propyl ester	94.2	299	8.3	I	II							33	197	ES3	ES1, ES2, ES3, ES5, ES6
53.82	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	96.3	295	6.9	I	II		Y					53	92	ES3	ES1, ES2, ES3, ES5, ES6, EC1, EC7, EC9
53.88	Heptadecanoic acid, ethyl ester	97.9	299	8.4	I	II							33	55	ES3	ES1, ES2, ES3, ES5, ES6
53.96	9-Octadecenoic acid, methyl ester, (E)-	*	297	7.6	I	II		Y		Y			80	149	ES2	ES1, ES2, ES3, ES5, ES6, EC1, EC2, EC3, EC5, EC7, EC8, EC9
54.06	Heneicosane	97.8	297	11	I	II		Y				Y	47	9	ES1	ES1, ES2, ES3, ES5, ES6, EC3, EC5
54.14	Octadecanenitrile	**91.3**	266	8	III	IV							13	15	EC8	EC1, EC8
54.15	Dodecanoic acid, isooctyl ester	91.5	313	8.4	I	II							13	23	EC4	EC4, EC7
54.73	Hexadecanoic acid, 2-methylpropyl ester	94.1	313	8.8	I	II		Y					33	32	ES4	ES1, ES2, ES3, ES4, ES6
54.84	Methyl stearate	99	299	9	I	II		Y				Y	87	182	EC1	ES1, ES2, ES3, ES5, ES6, EC1, EC2, EC3, EC4, EC5, EC6, EC7, EC8
54.93	1-Naphthalenamine, N-phenyl-	89.8	219	4.4	III	IV					Y		7	13	EC8	EC8
55.33	cis-13-Octadecenoic acid	98.9	283	7.2	I	II							40	105	ES5	ES1, ES2, ES3, ES4, ES5, ES6
55.33	Naphthalene, 1-(phenylmethoxy)-	**89.8**	234	4.7	III	IV							33	209	EC5	ES5, ES6, EC2, EC5, EC7
55.98	Linoleic acid ethyl ester	99.2	309	7.3	I	II		Y					67	826	ES3	ES1, ES2, ES3, ES4, ES5, ES6, EC3, EC4, EC6, EC8
56.25	Ethyl Oleate	99.3	311	8	I	II	Y	Y					100	266	ES4	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC2, EC3, EC4, EC5, EC6, EC7, EC8, EC9, HS1, HS2, HS3, HS4, HS6
56.42	Hexadecanamide	94.9	255	6.8	III	IV		Y		Y		Y	47	784	ES4	ES4, ES6, EC2, EC3, EC5, EC7, EC9
56.55	Hexadecanoic acid, butyl ester **(SML 60)**	99.4	313	8.7	-	I					Y	Y	100	808	EC4	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC2, EC3, EC4, EC5, EC6, EC7, EC8, EC9
56.67	Succinic acid, di(2-propylpentyl) ester	91.5	343	6.1	I	II							13	18	EC5	EC2, EC5
56.86	Octadecanoic acid, ethyl ester	99	313	8.9	I	II	Y	Y				Y	87	1385	ES4	ES1, ES2, ES3, ES4, ES5, ES6, EC2, EC3, EC4, EC5, EC6, EC7, EC8
56.93	Docosane	88.1	311	11.5	I	II		Y			Y	Y	100	177	EC1	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC2, EC3, EC4, EC5, EC6, EC7, EC8, EC9
56.94	Terephthalic acid, ethyl 2-ethylhexyl ester	**94.5**	306	5.1	I	II							33	345	ES3	ES1, ES2, ES3, ES5, ES6
57.04	Retene	88.6	234	6.5	III	IV							27	32	EC3	EC2, EC3, EC5, EC7
57.07	1-Phenyldibenzofuran	**92**	244	5.3	III	IV							7	122	EC8	EC8
57.24	9,12-Octadecadienoic acid, ethyl ester	**97.2**	309	7.3	I	II		Y					40	27	ES2	ES1, ES2, ES3, ES4, ES5, ES6
57.84	2-Butenedioic acid (E)-, bis(2-ethylhexyl) ester	*99.4*	341	6.5	I	II					Y		27	14	EC7	EC2, EC3, EC5, EC7
58.00	Hexadecanoic acid, 2-hydroxyethyl ester	**98**	301	6.8	I	II		Y					60	579	ES4	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC3, EC9
58.44	N,N-Dimethylhexadecanamide	**85.9**	284	6.3	III	IV							13	175	EC1	EC1, EC6
58.55	n-Propyl 9,12-octadecadienoate	**97.5**	323	7.8	I	II							40	67	ES4	ES1, ES2, ES3, ES4, ES5, ES6
58.59	Dodecanoic acid, 2-phenylethyl ester	88	305	7.2	I	II							7	74	EC1	EC1
58.75	(E)-9-Octadecenoic acid propyl ester	*96.1*	325	8.5	I	II							40	36	ES3	ES1, ES2, ES3, ES4, ES5, ES6
58.98	1-Eicosanol	89.4	299	9.5	I	II		Y			Y	Y	13	6	EC6	EC3, EC6
59.49	Glutaric acid, di(2-propylpentyl) ester	99.6	357	6.5	I	II							47	103	EC6	EC2, EC3, EC5, EC6, EC7, EC8, EC9
59.49	iso-Propyl 9-.cis.,11-.trans.-octadecadienoate	90.2					Y	Y	Y	Y	Y		40	16	ES4	ES1, ES2, ES3, ES4, ES5, ES6
59.53	Octadecanoic acid, propyl ester	90.6	327	9.4	I	II							13	26	ES3	ES2, ES3
59.78	Tricosane	95.4	325	12.1	I	II					Y	Y	80	15	ES1	ES1, ES2, ES3, ES4, ES5, ES6, EC2, EC3, EC4, EC5, EC7, EC8
60.27	N-Methyl-N-benzyltetradecanamine	**90.6**	318	8.4	I	II							13	671	HC1	HC1, HC9
60.30	2-Ethylhexyl trans-4-methoxycinnamate **(EDC)**	86.1	290	5.3	-	V		Y					40	13689	EC1	ES1, ES5, EC1, EC4, EC8, EC9
60.48	5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (all-Z)-	*89.1*	317	5.9	I	II							33	8	EC1	ES1, ES2, ES3, ES4, EC1
60.59	Ethanol, 2-(octadecyloxy)-	**94.7**	315	8.4	I	II		Y			Y		13	29	EC6	EC1, EC6
60.85	Benzyl butyl phthalate **(SML 30)**	96.5	312	4.9	-	V				Y			13	25	EC9	ES5, EC9
61.35	Butyl 9-octadecenoate or 9-18:1	**95.2**	339	8.8	I	II		Y		Y			40	12	ES4	ES1, ES2, ES3, ES4, ES5, ES6

RT	Name	Score	MW	LogP	Cram	Tox	FA	CM	DP	PPA	PPB	Fdb	Fill	S/N	High	Presence
61.45	9-Octadecenamide, (Z)- **(SML 60)**	88.5	282	6.6	-	I		Y		Y			13	59	EC9	EC2, EC9
61.61	cis-11-Eicosenoic acid	87.5	311	7.6	I	II						Y	33	5	ES4	ES1, ES3, ES4, ES5, ES6
62.12	Triphenyl phosphate **(EDC)**	87	326	4.6	-	V				Y			13	270	EC6	EC3, EC6
62.34	1-Heneicosanol	98.5	313	10	I	II							47	22	EC8	EC2, EC3, EC4, EC5, EC6, EC8, EC9
62.46	Hexanedioic acid, bis(2-ethylhexyl) ester **(SML 18)**	*	371	6.8	-	II		Y		Y		Y	100	3679	EC3	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC2, EC3, EC4, EC5, EC6, EC7, EC8, EC9
62.48	Tetracosane	99.4	339	12.6	I	II					Y	Y	100	39	EC1	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC2, EC3, EC4, EC5, EC6, EC7, EC8, EC9
62.65	Isophthalic acid, butyl 2-ethylhexyl ester	**89.7**	334	6	I	II							13	16	EC9	EC1, EC9
62.69	Butyl 9,12-octadecadienoate like	92.3											40	66	ES3	ES1, ES2, ES3, ES4, ES5, ES6
62.84	9-Octadecenoic acid (Z)-, 2-hydroxyethyl ester like	97.6											40	81	ES3	ES1, ES2, ES3, ES4, ES5, ES6
63.51	Octadecanoic acid, 2-hydroxyethyl ester	96.8	329	7.8	I	II	Y					Y	47	199	ES3	ES2, ES3, ES4, ES5, ES6, EC1, EC4
64.41	Behenic alcohol	91.5	327	10.5	I	II		Y				Y	13	9	ES1	ES1, EC6
64.42	Hexanoic acid, 2-ethyl-, hexadecyl ester	**88.7**	369	10.7	I	II		Y					13	18	ES1	ES1, ES6
64.45	Bifenthrin **(CMR)**	**94.5**	423	6	-	V							7	378	EC8	EC8
65.06	Pentacosane	98.9	353	13.1	I	II					Y	Y	87	14	EC4	ES1, ES2, ES3, ES4, ES5, ES6, EC2, EC3, EC4, EC5, EC6, EC7, EC8
65.86	9-Octadecenoic acid (Z)-, 2-hydroxyethyl ester	96.3	327	6.9	I	II		Y			Y		33	8	ES4	ES1, ES2, ES3, ES4, ES6
65.94	Tinuvin 326 **(SML 30)**	*	316	5.6	-	II				Y			27	377	EC9	EC3, EC5, EC8, EC9
66.16	Bis(2-ethylhexyl) phthalate **(CMR, SVHC, EDC, SML 1.5)**	*	391	7.4	-	V				Y			100	1573	ES2	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC2, EC3, EC4, EC5, EC6, EC7, EC8, EC9
66.55	Tinuvin 329	**88.5**	323	7.3	III	IV		Y			Y		7	213	EC2	EC2
67.55	Hexacosane	99.5	367	13.7	I	II					Y	Y	100	19	EC7	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC2, EC3, EC4, EC5, EC6, EC7, EC8, EC9
69.13	Octocrylene **(SML 0.05)**	*	362	7.1	-	IV		Y		Y			60	519	ES4	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC8, EC9
69.96	Heptacosane	98.8	381	14.2	I	II					Y	Y	73	7	ES6	ES1, ES2, ES3, ES4, ES5, ES6, EC2, EC3, EC4, EC7, EC8
70.15	Cyclopropanecarboxylic acid, 3-(2,2-dichlorovinyl)-2,2-dimethyl-, (3-phenoxyphenyl)methyl ester, (1R-trans)-	**99.3**	391	6.5	III	IV							20	22	EC1	EC1, EC8, EC9
70.95	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	99.5	391	7.4	I	II							93	165	ES4	ES1, ES2, ES3, ES4, ES5, ES6, EC1, EC2, EC3, EC5, EC6, EC7, EC8, EC9
71.26	UV 531 **(SML 6)**	*	326	6.8	-	II		Y		Y			60	247	EC5	ES2, ES3, ES4, ES5, EC2, EC3, EC5, EC8, EC9
71.42	Phthalic acid, nonyl 2-propylpentyl ester	*85.1*	405	8.3	I	II							33	11	ES4	ES2, ES3, ES4, ES6, EC7
72.71	Phthalic acid, 5-methylhex-2-yl nonyl ester	*92.5*	391	8.1	I	II							60	20	ES6	ES1, ES2, ES3, ES4, ES5, ES6, EC2, EC3, EC7
72.89	Squalene	92.4	411	11.6	I	II		Y					40	16	ES4	ES1, ES2, ES3, ES4, ES5, ES6
73.08	Phthalic acid, bis(7-methyloctyl) ester	**89.8**	419	9.6	I	II				Y			80	59	EC3	ES1, ES2, ES3, ES4, ES5, ES6, EC2, EC3, EC5, EC7, EC8, EC9
74.48	Dinonylphthalate	90.8	419	10.1	I	II					Y		40	19	EC7	ES3, ES6, EC2, EC3, EC7, EC9
74.53	Nonacosane	98.7	409	15.3	I	II						Y	33	4	ES6	ES5, ES6, EC2, EC7, EC8
76.00	Hexadecanoic acid, dodecyl ester	97	425	13	I	II		Y					33	43	EC4	ES1, ES2, ES3, ES4, EC4
76.70	Triacontane	96.7	423	15.8	I	II						Y	27	5	ES6	ES5, ES6, EC6, EC7

Note: In the **Name** column, content inside the bold parentheses **()** shows if the compound is a CMR, SVHC, or EDC, and its SML value (mg/kg) if there is.

The **Score** column depicts the library matching score given by MS-DIAL; * means that the identification was confirmed by standard; *value* in italic form means that there is no experimental RI from the library and the peak was not found in APGC-QTOF-MS, therefore, MF confirmation is not possible; **value** in bold form means that the MF was confirmed by APGC-QTOF-MS.

The **LogP** column is the XLogP value retrieved from Pubchem.

The **Cram** column is the Cramer rule-based toxicity level predicted by Toxtree. When a compound is CMR, SVHC, EDC, or have SML, prediction is not suitable.

The **Tox** column is the toxicity level assigned in the present study based on the rules proposed in 3.7.

Columns **FA, CM, DP, PPA, PPB, Fdb** indicate if the compound is present in "Substances added to food", "EU cosmetic ingredients inventory", "Colorants dyes and pigments", "Chemicals associated with plastic packaging List A", and "Chemicals associated with plastic packaging List B", and "FoodDB", respectively. Y is the abbreviation of yes.

Columns **Fill** and **S/N** are calculated by MS-DIAL. Fill (%) = (number of samples that have the compounds detected / total number of samples) * 100. S/N is the average S/N.

The **High** column is the name of migrate that have the highest intensity.

The **Presence** column shows the compound was detected in which migrates. E stands for 95% ethanol migration, while H means 3% acetic acid migration.

As classified by ClassyFire, there were mainly 9 classes of chemicals (Fig. III-3.4). As the highest hit class (24.6%), fatty acyls compounds are mainly fatty acid/acid ester/alcohol and have toxicity level II, which might not be risky concerning human health. Benzene and substituted derivatives together with naphthalenes account for 22.4% of the migrants. Many of them have class V or IV, e.g., phthalates and chlorobenzenamines, which can be toxic and deserve attention. There were many alkyl benzenes/naphthalenes though, no typical chromatograms of MOAH were found and the presence of MOAH can be excluded. Prenol lipids was the third largest class but most of them (58.8%) were found to be food components or food additives.

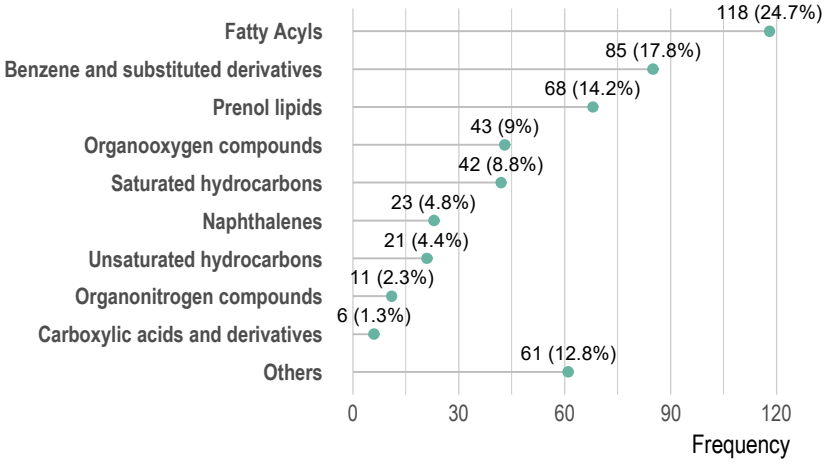


Fig. III-3.4 Chemical classes distribution of detected migrants. Absolute frequency with relative frequency (%) in bracket

Aiming to understand possible origins of those migrants, they were searched against the 5 aforementioned databases to check if they are food/plastic packaging/cosmetic related. Fig. III-3.5 shows the number of migrants found in each group. There were 186 migrants (39.2%) found to be food related (either in food additives or food component database). They might not be of safety concern but their migration could change the organoleptic properties of the contacting food (Vera et al., 2020). Among them, fatty acid esters merit specific attentions for their high intensities and frequencies detected. Moreover, 55 compounds were found to be plastic packaging related (either in List A or B of Chemicals associated with plastic packaging database)

omitting those already defined as food related, e.g., bis(2-ethylhexyl) phthalate and 2,2,4-trimethyl-1,3-pentanediol diisobutyrate, which are used as plasticizer in plastics. Leaving out those regarded as food/plastic packaging related, 30 migrants might come from cosmetic. For example, 2-ethylhexyl trans-4-methoxycinnamate which is used as sunscreen in cosmetics, was detected in 40% of the samples and had high average intensity. Finally, only 4 migrants were found in the colorant, dye, and pigments database, and all of them were food related as well.

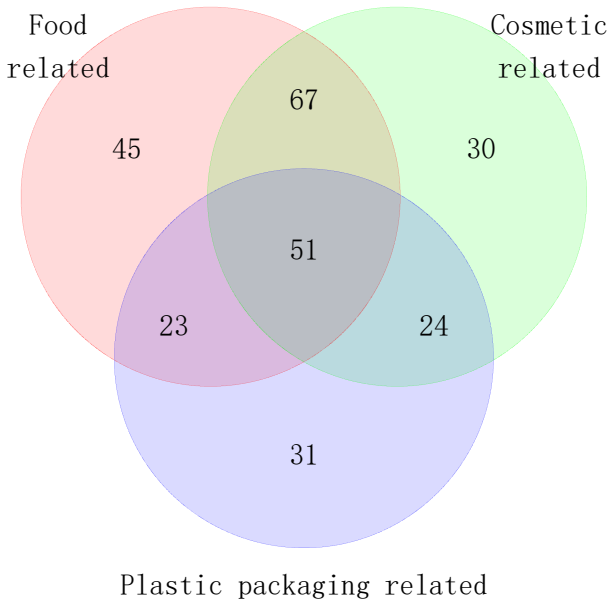


Fig. III-3.5 Possible origins of detected migrants

4.3. Correlations among samples and regional differences

By comparing tentatively identified migrants in both 95% ethanol and 3% acetic acid food simulants by GC-MS, one can get a general idea about how different the samples were. Fig. III-3.6 shows the correlation found. Blue region represents the significant differences, while red colour indicates high similarity in GC profile between samples. It is not unexpected that 95% ethanol and 3% acetic acid had significantly different migration profiles (blue region in Fig. III-3.6), as the two simulants have diverse polarities and affinities to various chemical structures. There were 251 migrants only detected in 95% ethanol simulant and 134 migrants only in 3% acetic acid, while

only 91 migrants were detected in both simulants. With respect to distinctions within the same simulant, certain patterns can be seen. It is clear that samples from Spain were quite different from those from China, but they were remarkably similar to each other, possibly because they all came from the same company but only from different lots. The results suggest that chemical compositions of post-consumer rPO from this company are uniform to some extent. It is good for the company since compositional uniformity is vital for controlling the quality of recycled materials. The result could come from its relatively steady input of recycled materials. For a well-developed recycling company, the input of recycled materials can be consistent to some degree once it has a fixed area to collect the post-consumer materials, because the consumption structure in a particular area can be steady in a certain stage and thus, chemicals coming from related pre-consumer plastic and residues from foods can be similar in a large scale. However, huge shifts of collection area might provide different inputs.

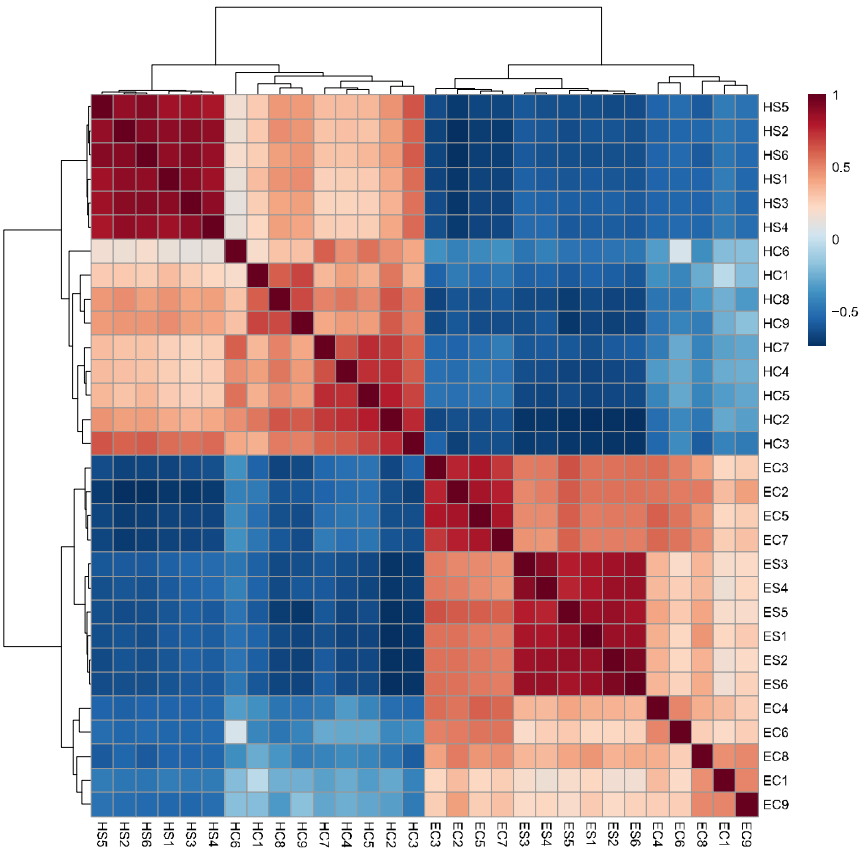


Fig. III-3.6 Hierarchical clustering of samples (marginal diagram) and their correlations using peak areas tentatively identified migrants

As for samples from China, they were not consistent to each other as they came from 3 different companies and possibly several polymer types. As can be seen, C1, C8 and C9 were classified into a same cluster while sample C2, C3, C5, and C7 were in another cluster in both simulants, possibly because these two clusters represented two types of polyolefins. As it is known, C1 and C9 were mainly consisted of HDPE while C2 and C3 comprised of LDPE. Interestingly, there were 219 (46.1%) migrants detected in both Spanish and Chinese samples, suggesting that they are probably common in rPO. However, there were 192 (40.4%) and 67 (14.1%) migrants that were only present in Chinese and Spanish samples, respectively. The result depicts that some compounds might be region related, e.g., isoborneol (detail in 4.5).

4.4. Molecular weight distribution of detected migrants.

In light of the correlation analysis, samples were divided into 5 groups for the evaluation of MW and predicted octanol/water partition coefficient (XLogP). Spanish samples were group 1; C1, C8, and C9 were group 2; C2, C3, C5, and C7 were group 3; C4 and C6 were designated as group 4, and group 5, respectively. Interestingly, MW concentrated on around 150 - 210 Da in all groups (Fig. III-3.7). As it is known, the smaller the molecules the easier they can be absorbed into and released from the polymers (Fang and Vitrac, 2017). At this point, common surrogates with MW ranging from 92 to 298 Da for rPET challenge test (EFSA, 2011) seems sufficient to check the ability of a recycling procedure to remove the majority of contaminants. However, this is not the case of polyolefins, where there were also high concern substances with higher MW detected. For example, octocrylene has MW 361.5 Da. It was detected in 60% of samples (both Spanish and Chinese samples) and its highest concentration was ca. 0.17 mg/kg, which is 3 times higher than its SML (0.05 mg/kg). Further, less high MW compounds detected can also result from the limitation of the analytical techniques (GC-MS in this case) (Palkopoulou et al., 2016). It was shown that decontamination efficiency strongly decreases with increasing MW regardless of the investigated technologies (Palkopoulou et al., 2016). Therefore, decontamination of polyolefins should be carefully optimized to remove all chemical substances of concern including high MW substances.

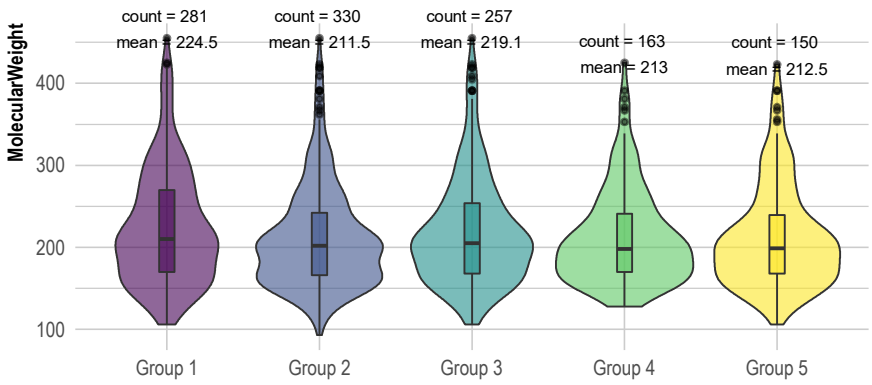
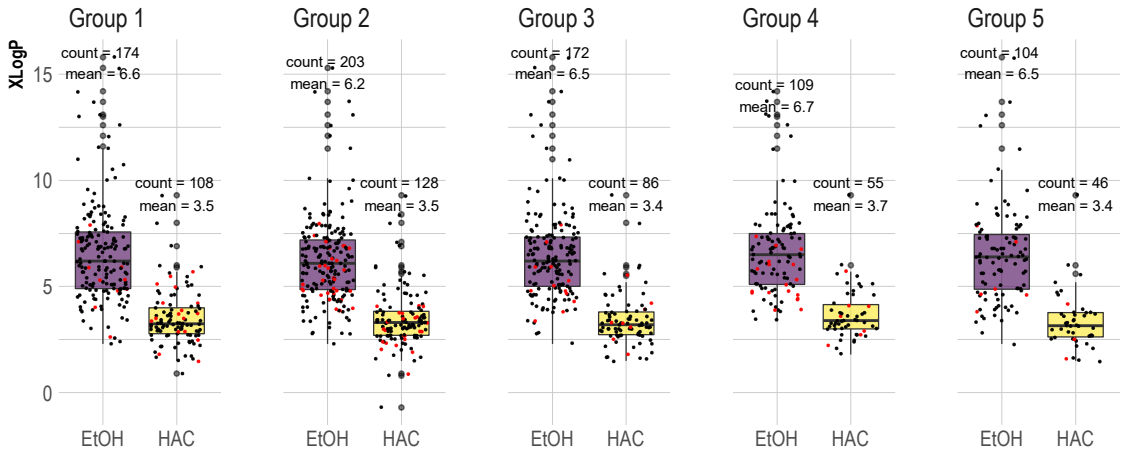


Fig. III-3.7 Molecular weight distribution of detected migration by groups

4.5. Distribution of predicted octanol/water partition coefficient (XLogP) of migrants

Regarding XLogP distribution (Fig. III-3.8), the differences between the two simulants were significant. The XLogP of migrants focused on 6.5 in 95% ethanol while on 3.5 in 3% acetic acid in all groups. The result can be expected because of the polarity difference between the two simulants as above mentioned. Hence, high XLogP compounds have higher potential to migrate into 95% ethanol while low XLogP substances to 3% acetic acid. Further, polyolefins are nonpolar polymers and have a good affinity to apolar chemicals while they have low affinity to polar molecules (Palkopoulou et al., 2016). As such, the absorption of more polar contaminants into polyolefins during their entire lives could be low. This could be one of the reasons why the number of migrants (less polar) in 95% ethanol is about twice higher than that in 3% acetic acid (251 vs 134) as above mentioned (4.2). Therefore, based on the potential uses (in contact with fatty or aqueous food) of rPO, various decontamination techniques can be developed. Moreover, this result can also be informative for the optimization of LC-ESI-HRMS for non-targeted screening of migrants in different food simulants since hydrophobicity (LogP) is one of the key factors that affecting ionization efficiency in electrospray (Liigand et al., 2014).



Note: EtOH and HAC are the migration into 95% ethanol and 3% acetic acid food simulants, respectively. Dots in red are chemicals that have toxicity level V and IV.

Fig. III-3.8 XLogP distribution of detected migrants by groups and simulants

4.6. Prioritization to high concern migrants and their concentrations

The huge number of detected migrants in these samples is informational for understanding classes, MW, and XLogP distribution of rPO contaminants, which can be instructive for developing appropriate rPO decontamination techniques. However, it might also distract us from focusing on key migrants regarding human health. The prioritized migrants including their highest concentration in samples are presented in Table III-3.3. Regarding the possible origin of a component, it is not easy to know the true one. Here, the priority was given to food related, followed by plastic related and cosmetic related, since once it is food related, it is more acceptable to be detected in food contact materials and it might not be of safety concern. Food additives do not necessarily mean safe though, they were regarded as acceptable here, as their migration into the contacting food (normally in ppb level) could be much lower in comparison to the amount of addition as food additives. When no such relationship was found, functional uses described in Pubchem was marked down.

Table III-3.3 Prioritized important migrants, their concentrations, and quantification details

Name	Fill	Tox	S/N	Mass	Range	R^2	LOQ	Con (μg/kg)	Note (SML unit: mg/kg)
Bis(2-ethylhexyl) phthalate	100	V	1573	149	1-50	0.9995	0.4	47.6 ± 38.1	CMR; SVHC; EDC; SML: 1.5
Dibutyl phthalate	100	V	587						CMR; SVHC; EDC; SML: 0.3
Diisobutyl phthalate	67	V	527						CMR; SVHC; EDC
Diethyl Phthalate	100	V	413	149	10-500	0.9880	1.8	315.8 ± 34.7	EDC
Diphenyl ether	93	IV	693	170	5-100	0.9629	0.1	69.2 ± 5.7	food related
Octocrylene	60	IV	519	249	1-100	0.9931	5.7	166.6 ± 22	SML: 0.05
2,4-Diphenyl-4-methyl-1-pentene	60	IV	470	119	1-20	0.9834	0.4	< 10	plastic related
Benzenamine, 2,4-dichloro-	87	IV	349	161	20-500	0.9599	4.5	158.9 ± 5.6	intermediate for other chemicals
Galaxolide	67	IV	270						plastic related
4-Dodecylmorpholine	93	IV	187	100	10-100	0.9969	0.1	*21.5 ± 0.7*	additive in food contact substances
1-Tetradecene	100	IV	140	55	1-100	0.9990	4.0	272.0 ± 17.8	SML: 0.05
Dodecane, 1-chloro-	67	IV	116	91	1-20	0.9969	4.5	*23.7 ± 0.3*	intermediate
1-Dodecene	100	IV	89	55	1-100	0.9985	6.1	181.8 ± 9.9	SML: 0.05
Diphenylmethane	60	IV	29						cosmetic related
1,1'-Biphenyl, 3-methyl-	73	IV	28	168	1-20	0.9719	3.0	18.0 ± 0.2	
Oleanitrile	73	IV	12						plastic related
Benzophenone	100	III	355	105	1-100	0.9937	0.6	68.1 ± 5.2	SML: 0.6
Phenol, 2,6-bis(1,1-dimethylethyl)-	87	III	152						plastic related
Propanoic acid, 2-methyl-, 3-hydroxy-2,2,4-trimethylpentyl ester	67	III	117						plastic related
2,4-Di-tert-butylphenol	93	II	19693	191	50-500	0.9967	0.8	2257.8 ± 284.8	plastic related
1-Dodecanamine, N,N-dimethyl-	80	II	9345	58	10-100	0.9621	0.1	*43.1 ± 0.6*	plastic related
Hexadecanoic acid, ethyl ester	100	II	6413	88	1-50	0.9751	0.6	*355.6 ± 41.2*	food related
Tetradecanoic acid, ethyl ester	93	II	5102	88	1-50	0.9751	0.6	*192.4 ± 24.7*	food related
2-Ethylhexyl salicylate	67	II	4957	120	1-50	0.9878	1.2	143.8 ± 0.3	cosmetic related
Hexanedioic acid, bis(2-ethylhexyl) ester	100	II	3679	129	1-50	0.9995	0.6	122.1 ± 59.9	SML: 18
Benzaldehyde, 2,5-dimethyl-	100	II	1121	134	5-100	0.9624	0.7	*125.5 ± 10.1*	

Name	Fill	Tox	S/N	Mass	Range	R^2	LOQ	Con (µg/kg)	Note (SML unit: mg/kg)
1-(4-tert-Butylphenyl)propan-2-one	93	II	859	147	5-100	0.9624	0.7	*120.4 ± 5*	
Linoleic acid ethyl ester	67	II	826	67	1-100	0.9856	1.8	*774.5 ± 270.6*	cosmetic related
n-Hexyl salicylate	93	II	733	120	1-50	0.9878	1.2	*377.6 ± 9.6*	plastic related
Dimethyl palmitamine	60	II	709						plastic related
Hexadecanoic acid, 2-hydroxyethyl ester	60	II	579						cosmetic related
4-(t-Butyl)benzaldehyde	100	II	524	147	5-100	0.9624	0.7	*59 ± 0.5*	
2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	100	II	319	71	1-50	0.9960	1.2	182.1 ± 16.2	SML: 5
Isoborneol	60	II	279	95	50-1000	0.9487	0.3	615.9 ± 26.3	food related
Ethyl Oleate	100	II	266	55	1-100	0.9856	1.8	*926.2 ± 57*	food related
UV 531	60	II	247	213	1-50	0.9850	9.0	184.8 ± 80.5	SML: 6
1,4-Benzenedicarboxylic acid, diethyl ester	80	II	127	177	5-100	0.9669	44.8	70055.4 ± 2399.1	plastic related
D-Limonene	100	II	118	68	50-500	0.9812	7.6	2022.4 ± 155.5	food related
Phthalic acid, bis(7-methyloctyl) ester	80	II	59						plastic related
2-Ethylhexyl trans-4-methoxycinnamate	40	V	13689						EDC
Phenol, 4-(1,1-dimethylpropyl)-	47	V	1068						SVHC
1H-Pyrazole, 4,5-dihydro-1,3-diphenyl-	40	IV	1191						
1,7-di-iso-propylnaphthalene	53	IV	536	191	1-20	0.9962	2.5	*14.3 ± 0.3*	plastic related
N,N-Dimethyldodecanamide	47	IV	496						slip agent
1,1'-Biphenyl, 3,3'-dichloro-	33	IV	51	222	1-20	0.9883	2.8	*4.4 ± 0.1*	Polychlorinated biphenyl in PCB
Dodecyl acrylate	40	IV	28	55	1-100	0.9974	0.8	102.3 ± 3.9	SML: 0.05
2-Ethylhexyl acrylate	33	IV	26	55	1-50	0.9982	8.1	33.5 ± 4.5	SML: 0.05
Isobornyl acrylate	60	III	548	67	1-500	0.9984	10.1	7422.4 ± 39.6	plastic related
1-Tetradecanamine, N,N-dimethyl-	33	II	4578	58	10-100	0.9621	0.1	11.7 ± 1.2	cosmetic related
Tinuvin 326	27	II	377	300	1-100	0.9069	10.3	319.2 ± 138.7	SML: 30
Terephthalic acid, ethyl 2-ethylhexyl ester	33	II	345	177	5-100	0.9669	0.4	*2870.5 ± 352.9*	
Benzenamine, 2,4-dimethyl-	53	II	240	121	20-500	0.9664	13.3	451.8 ± 35.2	plastic related
Aniline, N-methyl-	20	II	29						food related

Note: Columns **Fill**, **Tox**, and **S/N** are the same as Table III-3.2. The **Mass** column depicts mass used for quantification. The Range column shows the concentration range of the calibration curves. Both range and LOD are expressed in µg/kg. The **Con (µg/kg)** column is the highest concentration found in the samples and *values* in italic forms were obtained from semi-quantification.

With respect to regulation compliance, there were several compounds that exceeded their SML and should be emphasized. As a photostabilizer, octocrylene was detected in 60% of samples with highest concentration at ca. 0.17 mg/kg which is three times higher than its SML (0.05 mg/kg) in the regulation (EU 10/ 2011), while another commonly used photostabilizer UV 531 had migration much lower than its SML (6 mg/kg). Octocrylene can also be the result of contamination from cosmetic packaging as it is widely used as UV filter in cosmetics as well. It is not surprising to detect 1-tetradecene and 1-dodecene as they are two olefin monomers. However, they were observed in all samples and their highest migrations were 3-5 times higher than their SML (0.05 mg/kg). A similar result was observed by a previous study where 2 out of 5 recycled PP samples had 1-dodecene migration ca. 3 times higher than its SML (Coulier et al., 2007). As it is known, acrylates are common monomers of plastics, adhesives, and paints. There were few of them mainly found in Chinese samples. Dodecyl acrylate and 2-ethylhexyl acrylate were detected in ca. 40% of samples, but the former one exceeded its SML (twice) while the latter one did not. Besides, isobornyl acrylate got quite high migration (7.4 mg/kg). Other migrants, e.g., benzophenone, that have SML value in the regulation, were below the limit and should not be of human health concern.

2,4-Di-tert-butylphenol, likely coming from the degradation of antioxidant tris(2,4-ditert-butylphenyl) phosphite (Irgafos 168) or [3-[3-(3,5-ditert-butyl-4-hydroxyphenyl)propanoyloxy]-2,2-bis[3-(3,5-ditert-butyl-4ydroxyphenyl)propanoyloxymethyl]propyl]3-(3,5-ditert-butyl-4-hydroxyphenyl)propanoate (Irganox 1010), was found in all samples except for EC1, and its highest migration even reached 2.26 mg/kg, which is higher than the TTC value for Cramer I components. As a NIAS, 2,4-Di-tert-butylphenol has been reported to increase with increasing recycling steps (Coulier et al., 2007). According to Pubchem, isoborneol is used as a flavouring, fragrance, to make other chemicals, and in traditional Chinese medicine. It had relatively high migration level (maximum 0.62 mg/kg) as well. However, it is interesting that this component was detected in all samples coming from China but not from Spain which is a good example for region related contaminants. Besides, high level of migration was recorded for d-limonene. Limonene is widely used as flavouring in food and fragrance in perfume.

Hence, it can be residue from the previous uses as it was in rPET (Nerín et al., 2003). Its high migration can be problematic as well regarding the organoleptic properties of the contacting food.

As plasticizers and additives, phthalates are commonly used in many consumer products and they have been reported for endocrine-disrupting and reproductive effects in animal studies (Wang et al., 2019). Moreover, many of them are in the CMR, SVHC, and/or EDC lists and therefore deserve attentions. There were ten phthalates detected in this set of samples. Among them, dibutyl phthalate (DBP), diisobutyl phthalate (DiBP), bis(2-ethylhexyl) phthalate (DEHP), diethyl phthalate (DEP), phthalic acid, bis(7-methyloctyl) ester (DINP branched), and phthalic acid, 5-methylhex-2-yl nonyl ester were detected in more than 60% of samples. Compared to the former four phthalate, the latter two were less commonly detected in recycled plastics (Devlieghere et al., 1998; Geueke et al., 2018; Huber and Franz, 1997). DEHP had concentration lower than their SML while DEP hit 0.32 mg/kg and has no SML and thus merits more attention. Furthermore, three terephthalates and one isophthalate were identified. They, especially 1,4-Benzenedicarboxylic acid diethyl ester, were present in 80% of samples at high concentration (7.0 mg/kg), which is much higher than the TTC value for Cramer II compounds. This compound can be formed by a transesterification reaction between PET chain and ethanol or be a side product formed during PET polymerization (Alin and Hakkarainen, 2013). Its high concentration in rPO could be the result of cross contamination from PET and/or inclusion of PET in these recycled plastics.

There were sixteen amines detected and five of them were listed in Table III-3.3. As we explained in our previous article (Su et al., 2020), amines could have very low response in GC-MS because many of them can be strongly adsorbed on the column or injector. Hence, their detection in the samples might suggest relatively high concentration. For example, benzenamine, 2,4-dichloro- was detected in 86.7% of samples with concentration as high as 0.16 mg/kg, which is twice higher than the TTC value for Cramer III compounds. It is commonly used as intermediates for pesticides, dyes, etc., and thus can be counted as NIAS as well. As far as we know, it is the first time to report their migration from recycled plastics. 1-tetradecanamine, N,N-dimethyl-,

had high average S/N though, its concentration was not that high possibly because of its high response factor. As expected, most of amines were only detected in 3% acetic acid simulant. In consequence, they might be risk when in contact with acidic but not fatty food. Furthermore, there were one pesticide named cyclopropanecarboxylic acid, 3-(2,2-dichlorovinyl)-2,2-dimethyl-, (3-phenoxyphenyl)methyl ester, (1R-trans)-, and two insecticides named chlorpyrifos and bifenthrin detected, but these compounds were only observed in C1, C8, and C9 samples implying that these samples might contain plastic flow from agricultural field. This could be also the reason why these three samples were grouped together in 4.2.

Last but not least, couples of chlorine-containing compounds were detected. For example, dodecane, 1-chloro-, which is used as intermediate for many other basic organic chemical manufacturing, was detected in 66.7% of samples with 0.024 mg/kg as the highest migration, which is lower than the TTC value for Cramer III compounds. One polychlorinated biphenyl (PCBs), 1,1'-Biphenyl, 3,3'-dichloro- was found in 33.3% of samples (both Spanish and Chinese samples), but its maximum concentration was lower than 0.01 mg/kg. Although the production of PCBs was banned in 1970s across most of the world, their residues in the environment are still present in some regions (Song et al., 2018). For this reason, this compound was assumed to be an environmental contaminant.

5. Conclusions

Among the 474 migrants detected in various recycled polyolefins, 39.2% were food related and 24.1% were found as saturated hydrocarbons, fatty acyls, or prenol lipids, which might not be human risk. Molecular weight distribution analysis shows that most migrants have MW between 150-210 Da. However, using surrogates similar to PET with MW up to 300 Da is insufficient for challenge test of recycled polyolefins as evidenced by high migration of octocrylene (MW 361.5 Da) and heavier compounds such as octocrylene, hexadecanoic acid dodecyl ester and triacontane with molecular weight of 361.5, 424.7, and 422.8, respectively, among others, could be used as surrogates as well. Predicted octanol/water partition coefficient (XLogP) distribution

illustrates that chemicals which can migrate into different food simulants vary a lot. Therefore, the decontamination strategy for recycled polyolefins can be driven by their intended uses (e.g., for fatty or acidic food).

Looking in depth into particular migrants, octocrylene, 1-tetradecene, 1-dodecene, and dodecyl acrylate exceeded their SML. Besides, 2,4-di-tert-butylphenol and 1,4-benzenedicarboxylic acid, diethyl ester were of high concern in 95% ethanol (fatty food) migration concerning their detected frequency and highest concentration. For 3% acetic acid simulant (acidic food), benzenamine, 2,4-dichloro- and diethyl phthalate deserve more attention.

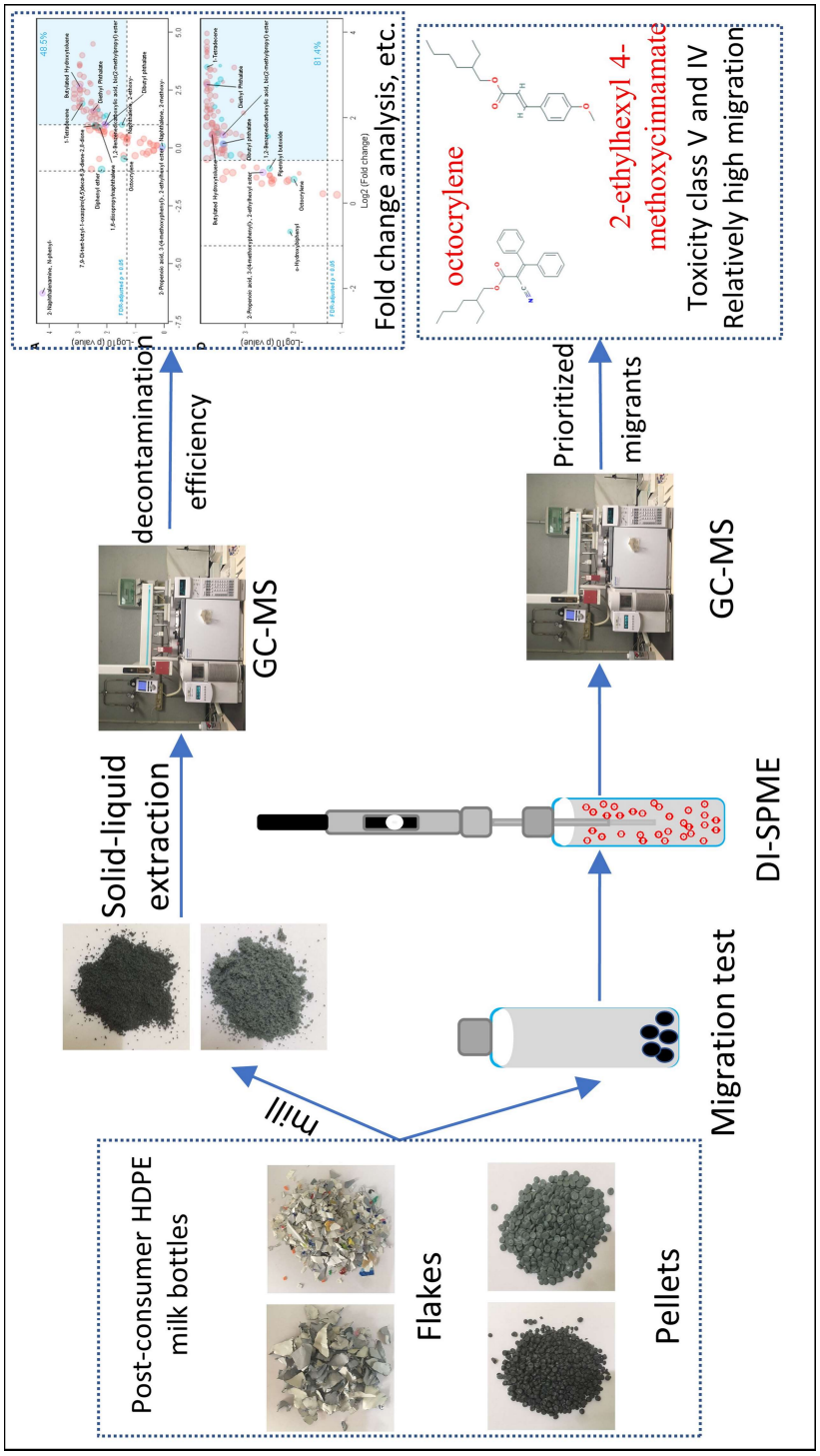
This study presents a fundamental input of chemicals that can migrate out from post-consumer recycled polyolefins as well as their MW and XLogP distribution. The prioritization strategy helps us concentrate on higher risk migrants. This database as well as the data analysis is beneficial for developing sufficiently clean recycled polyolefins for food contact and thus closing the loop.

Chapter 4

Decontamination Efficiencies of Post-Consumer High-Density Polyethylene Milk Bottles and Prioritization of High Concern Volatile Migrants

1. Abstract:

High-density polyethylene (HDPE) milk bottles are well-distinguished from other plastics in the mix-collected plastic waste and have potential to be closed-loop recycled. To evaluate this option, volatile substances present in various recycled HDPE (rHDPE) pellets and flakes from post-consumer milk bottles were analysed for similarities between different industrial recycling companies and batches. All substances found were classified in five different levels based on toxicity, from level I to level V (high toxicity). Chemicals present in the samples from different recyclers varied considerably, while those from different batches of a given recycler gave similar results. However, the study of rHDPE stream mixed with high volume of non-milk-bottles provided significant differences between batches. Washing the rHDPE twice and applying extra decontamination techniques reduced to a half the intensities for most chemicals detected, including two toxicity level V substances, butylated hydroxytoluene and diethyl phthalate. Nevertheless, other two high concern compounds, octocrylene, and 2-ethylhexyl-4-methoxycinnamate were not significantly reduced and thus deserve special attention when decontaminating rHDPE and evaluating its feasibility for food contact uses. Extra decontamination was able to reduce the intensities of 1-dodecene and 1-tetradecene. In total, 265 substances were detected in migration tests (95% ethanol and 3% acetic acid) and 58 of them were prioritized by toxicity. Regarding volatile migrants, rHDPE with low content of non-milk-bottle could be safe for direct contact with low-fat content food. For high-fat foods, the main concerns came from 1-tetradecene, octocrylene, and 2-ethylhexyl-4-methoxycinnamate.


Post-consumer HDPE milk bottles
Flakes
Pellets
mill
Migration test
DI-SPME
GC-MS
Solid-liquid extraction
decontamination efficiency
Prioritized migrants
GC-MS
Fold change analysis, etc.
octocrylene
2-ethylhexyl 4-methoxycinnamate
Toxicity class V and IV
Relatively high migration

2. Introduction

Plastic recycling is one of the important topics in the European plastic strategy in a circular economy (EC, 2018). Globally, it is also well accepted as an essential way to tackle increasingly prominent environmental issues posed by plastic pollution. Mechanical recycling, as one of the well-established and widely used approaches, only accounts for 14 – 18% plastic waste recycling rate at global level (OECD, 2018) and 31% in Europe (d'Ambrières, 2019) and requires further improvements.

Comprising 39.6% of plastic demand and 46.7% of global primary plastic waste generation (PlasticsEurope, 2012, 2020), the plastics used in packaging sector are vital in the way to a circular economy. Before being authorized for food contact uses, recycled materials should comply with Framework Regulation EC 1935/2004 (EC, 2004) and EU 10/2011 (EC, 2011) requiring that they may not pose risk to human health. Currently, only a few of post-consumer polyolefins are closed-loop recycled for food contact uses (Silano et al., 2018a), and most of the recycled plastics for food contact are referred to polyethylene terephthalate (PET), thanks to their promising high purity and low levels of contaminants (Strangl et al., 2019). Compared to PET, polyolefins, which represent 70% of plastic packaging (PlasticsEurope, 2019), are more challenging to be closed-loop recycled as they have much higher chemical sorption capacity, faster diffusion of chemicals through them, and thus higher migration potential than PET (Palkopoulou et al., 2016). Consequently, cleaning procedures that work well on PET, e.g., the so-called super-clean PET recycling system, cannot be simply extrapolated to polyolefins (Palkopoulou et al., 2016). Further developments and investments in innovative recycling systems are required to satisfy the high quality demands of industry (Strangl et al., 2019).

Polyolefins are widely used in food packaging in various forms, for example, PE films or thermal sealing layers in multilayer packages, PP crates/trays, HDPE milk bottles, etc. Among them, HDPE milk bottles could be the first candidate for closed-loop recycling (Welle, 2005) since they could be easier to collect and sort from a kerbside collection system and might have less contaminants compared to others.

Bottle-to-bottle recycling (separated collection of post-consumer HDPE milk bottles) might come back with minimum contamination, but it requires a major update of the whole recycling systems since HDPE milk bottles are currently mix-collected with other plastics. Similar to PET bottles, HDPE milk bottles are well-distinguished from others in the mixed plastic waste collection (Silano et al., 2011a, 2011b) and therefore could have less contamination from non-food grade plastics. However, knowledge about the compounds present and their concentration in post-consumer plastics, and the capabilities of various recycling processes to remove them, are crucial for the design of efficient HDPE recycling process (Welle, 2005). As far as we know, research studies on this topic are limited. Some of them mainly focused on the odorants, which is also important for food contact uses, but chemicals of high safety concern were not considered (Demets et al., 2020; Strangl et al., 2018, 2019). Extraction as well as migration from various rHDPE were investigated (Coulier et al., 2007; Devlieghere et al., 1998; Dutra et al., 2011; Huber and Franz, 1997; Welle, 2005). However, these studies are outdated as some of them are more than 20 years old. Technological development in analytics allows generating value-added information. There could be progress made in the plastic industry as well. Recently, a highly sensitive direct immersion – solid-phase microextraction coupled to gas chromatography – mass spectrometry (DI-SPME-GC-MS) method was developed for the untargeted screening of (semi-)volatile migrants in different food simulants (Su et al., 2020). This analytical procedure enabled getting a deeper insight into chemicals present in rHDPE that might endanger human health.

Consistency of chemicals present between different batches of sorted HDPE bottles in the recycling plant could be one of the key points for the quality control of recycled materials. Thus, the first objective of this work was to evaluate the batch effect in the recycling industry (samples collected at different time) on the chemicals present in flakes and pellets from rHDPE milk bottles by hierarchical clustering (HCA). The second objective was to evaluate the efficiency of two cleaning processes (washing twice and extra decontamination) on the removal of chemicals present in the rHDPE samples and to deeply understand the factors largely affecting the cleaning efficiency.

Thirdly, with the aim to find out the most concerning chemicals in rHDPE from milk bottles samples, a highly sensitive DI-SPME-GC-MS method was employed for the untargeted screening of migrants coming from these rHDPE samples in both 95% ethanol (v/v) and 3% (w/v) acetic acid food simulants. The large number of migrants identified was then prioritized and quantified when available. Finally, several substances with prioritized concern were listed and addressed with particular attention for rHDPE samples with the aim to provide useful information for developing effective decontamination techniques and establishing legislation to assure high quality rHDPE.

A schematic overview of the analytical strategy applied in this study is shown in Fig. III-4.1. The research has been distributed in two sections, one deals with the first and second objectives by employing sample extraction from both flakes and pellets, HCA, and fold change analysis, while the other one concerns the third objective via migration study from pellets, prioritization, and quantification of high concern substances.

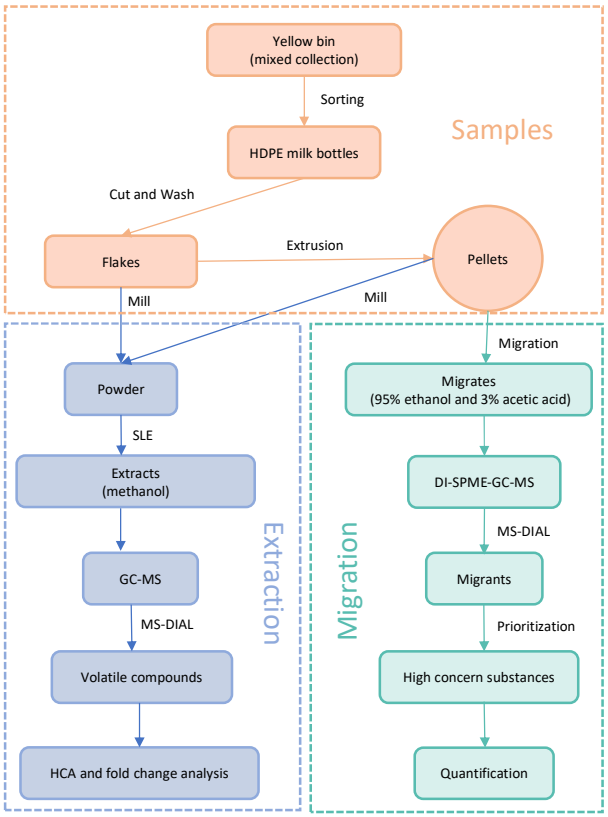


Fig. III-4.1 Schematic overview of the analytical strategy applied in this study.

3. Materials and methods

3.1. Reagents and samples

Butylated hydroxytoluene (CAS 128-37-0), diethyl phthalate (84-66-2), naphthalene (91-20-3), diisobutyl phthalate (84-69-5), 2-ethylhexyl-4-methoxycinnamate (5466-77-3), diphenyl ether (101-84-8), 1-dodecene (112-41-4), alpha-terpineol (98-55-5), 2,6-diisopropylnaphtalene (24157-81-1), 1-tetradecene (1120-36-1), 1-methyl-naphthalene, (1321-94-4), octocrylene (6197-30-4), 3-methyl-1,1'-biphenyl, (643-93-6), 1-chloro-decane, (1002-69-3), 7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione (82304-66-3), 2,4-dichloro-benzenamine (554-00-7), biphenyl (92-52-4), 2,4-dichlorobiphenyl (33284-50-3), 3-phenyltoluene (643-93-6), o-hydroxybiphenyl (90-43-7), pyrimethanil (53112-28-0), (+)-2-bornanone (464-49-3), 2-tridecanone (593-08-8), benzophenone (119-61-9), 2-ethylhexyl salicylate (118-60-5), ethyl dodecanoate (106-33-2), 1-octadecanol (112-92-5), 1,1'-oxybis-octane, (629-82-3), 1-octadecanol (112-92-5), d-limonene (5989-27-5), dl-menthol (89-78-1), isoborneol (507-70-0), alpha-terpinene (99-86-5), N,N-dimethyltetradecylamine (112-75-4), diisooctyl phthalate (27554-26-3) and 2,4-dimethyl-benzenamine, (95-68-1) were from Sigma-Aldrich (Madrid, Spain).

Post-consumer HDPE milk bottles in flakes and pellets (abbreviated as F and P, respectively) forms were provided by 3 Spanish plastic recyclers located in different provinces and autonomies. According to the recyclers, rHDPE milk bottles were kerbside collected (yellow container in Spain) and separated from other plastics in sorting plants. HDPE milk bottles (white with slightly black colour inside, see Fig. III-4.2, first 3 bottles) can be well-distinguished from other PE bottles (totally white, see Fig. III-4.2, last bottle). Besides, most of the collected bottles still keep their labels. Thus, they are visually distinguishable and therefore can be manually sorted. They were then cut up and washed with water to attain the flake samples. Pellets were then obtained by directly extruding the flakes without additional decontamination steps, except otherwise specified. Detailed information of the samples is depicted in Fig. III-4.3. Samples P1.3’ and F1.3’ were obtained directly from P1.3 and F1.3, respectively, by

applying an extra decontamination technique which is a non-destructive deodorization process by heating. The appearance of both pellets and flakes did not change after this step. However, no more details are available for extra decontamination due to confidential reasons. Samples P2.1, P2.2, P2.3, F2.4, F2.5, and F2.6 were washed twice with water. However, samples were collected on various days as specified in Fig. III-4.3. Notably, although P2.2, P2.3, F2.4, F2.5, F2.6 were collected on the same day, there were no direct correspondences, e.g., P2.2 was not related to F2.4, F2.5, nor F2.6. Furthermore, to explore the possible origin of octocrylene and 2-ethylhexyl-4-methoxycinnamate, 3 bottles of milk and 1 bottle of liquid yogurt packaged in HDPE bottles were bought from the local supermarkets (Zaragoza, Spain). The samples collected are shown in Fig. III-4.2. The bottles were then cleaned with water and dried for the extraction as described below.

Fig. III-4.2 Self-collected HDPE bottles (the last one is the package of liquid yogurt while the others are the packages of milk)

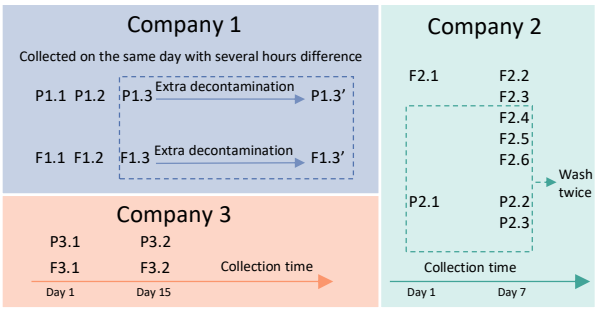


Fig. III-4.3 Detailed sample information including collection time and additional processes applied

3.2. Sample extraction

Samples of both flakes and pellets were milled into powders by an ultra-centrifugal mill (Retsch ZM 200; Haan, Germany) using a perforated plate sieve with aperture size of 0.5 mm. The milled samples (1.00 g) were then extracted with 5 mL of dichloromethane for 1 h by ultrasonic bath (Brasonic 3510-MTH; Connecticut, USA). Three consecutive extractions were applied by adding fresh dichloromethane in each case, and the extracts were then mixed and evaporated to dryness with a gentle nitrogen flow at 40 °C (Techne DB-3; Staffordshire, UK). Subsequently, 0.4 mL of methanol was added to re-dissolve the extract under ultrasonic bath (5 min). Finally, the extract was vortexed for 30 s and filtered by a 0.2 μm Acrodisc GHP syringe filter (Waters, New York, USA) prior to GC-MS analysis. Owing to instrumental capacity limitation, samples from each company were grouped and processed under the same lot to minimize the potential batch effect in the extraction process. Samples and procedural blanks were simultaneously prepared in triplicate. Quality control (QC) sample pooled from the filtered extracts (50 μL from each sample) was employed for sample alignment and normalization to minimize the effect of instrumental variation during injections and thus to have more robust statistical analysis.

3.3. Migration tests

For pellet samples, the protocol proposed in our previous article (Su et al., 2021a) was used. In short, the surface area of each pellet was estimated based on their cylinder-like shape. The size of the pellet samples is shown in Table III-4.1. The number of pellets needed for 18 mL food simulant (the size of the migration container) was then calculated accordingly. For flakes, weight method (average weight of the pellets used for migration) was utilized owing to the difficulty to calculate the corresponding surface area. Two food simulants, namely 95% ethanol (v/v) and 3% (w/v) acetic acid were used as fatty and acidic food surrogates, respectively, as the worst-case scenarios. The migration test was conducted under 60 °C for 10 days according to the Commission Regulation EU No. 10/2011 (EC, 2011). Samples including procedural blanks were prepared in duplicate.

Table III-4.1 The size of pellet samples (n=5)

Company	Company 1	Company 2	Company 3
Sample name	P1.1, P1.2, P1.3, P1.3'	P2.1, P2.2, P2.3	P3.1, P3.2
Diameter (mm)	4.7 ± 0.3	5.3 ± 0.4	4.7 ± 0.4
Height (mm)	2.8 ± 0.3	1.5 ± 0.5	2.0 ± 0.1

Note: diameter and height were expressed as mean ± standard deviation (n=5)

3.4. Fourier-transform infrared (FTIR) spectroscopy analysis

The flakes from company 2 contained many non-milk bottle plastics. To evaluate the types of polymer present, the flakes (ca. 50 g) were manually separated into 5 fractions (Fig. III-4.4) and the polymer types (5 pieces from each fraction) were measured by an FTIR spectrometer (Cary 630, Agilent, USA). Attenuated total reflectance (ATR) sampling was used in all the cases. The FTIR absorbance spectrum from 4000 to 650 cm^{-1} was measured in the samples with a resolution of 4 cm^{-1} and 64 scans. Identity of polymer in each case was carried out by comparison of the FTIR spectra obtained to the spectra in the commercial polymer libraries.

Fig. III-4.4 Manually separated fractions from company 2 flakes

3.5. Direct-immersion solid-phase micro-extraction (DI-SPME)

The DI-SPME procedure was optimized and used in our previous studies (Su et al., 2020, 2021). Briefly, 95% ethanol samples were 9.5 times diluted to prevent SPME fibre damage, while neutralization with NaOH was applied to 3% acetic acid samples prior to DI-SPME. SPME conditions were as follows: pre-incubation at 70 °C for 5 min, extraction for 55 min by a DVB/CAR/PDMS fibre Supelco (PA, USA), desorption in the GC inlet (250 °C) for 8 min, and fibre cleaning at 270 °C for 2 min. The DI-SPME processes were automatically achieved by a CTC Analytics CombiPAL autosampler (Zwingen, Switzerland) connected to the GC-MS.

3.6. Quantification of prioritized migrants

Quantification of prioritized migrant was done under the same conditions as mentioned in section 3.5. Calibration plots were done from each pure standard corresponding to each identified compound. Substances found in 95% ethanol migration were quantified in 10% ethanol by DI-SPME-GC-MS as above described, in order to avoid any damage to the SPME fibre. The final concentration was recalculated considering the dilution factor of the samples. In the case of 3% acetic acid migration, pre-neutralized 3% acetic acid was used instead.

3.7. GC-MS analysis

A gas chromatography Agilent 6890N coupled to a mass spectrometer Agilent 5975 was used for this purpose. The separation was carried out in an Agilent DB-5 MS capillary column (30 m × 0.25 mm id, 0.25 µm film thickness) with the following temperature program: started at 50 ºC, it increased to 100 ºC at 15 ºC/min, then slowly rose to 200 ºC at 2 ºC/min, and finally climbed to 300 ºC at 15 ºC/min. Carrier gas was helium (99.999%) at 1.0 mL/min. The inlet temperature was set at 250 ºC and splitless mode was employed. Mass scan range was 40-700 Da. Test mixture 2 for apolar capillary columns according to Grob (Sigma Aldrich) was injected prior to each sequence of samples to control the correct performance of the system. The QC sample

(section 3.2) was injected twice at the beginning and the end of the sequence as well as every 10 injections.

3.8. Data analysis

All GC-MS data were processed by MS-DIAL version 4.36 (Tsugawa et al., 2015) by applying the following settings: minimum peak height of 1000, sigma window of 0.5 and EI spectra cut-off of 1 for deconvolution; alignment was done with 10 retention index (RI) tolerance and 85% EI similarity; features with sample max / blank average fold change lower than 10 were removed. NIST 14 spectral library in NIST MS search format (*.MSP) including RI information was used for identification. Experimental semi-polar RI was retrieved, averaged, and assigned to each spectrum when available. When no experimental RI is available, predicted RI using a deep convolutional neural network (Matyushin et al., 2019) was calculated. Identification was done before alignment with 80% spectrum similarity and 85% total score cut-off to reduce false positive. The identified table list was manually curated to assure identification, alignment, peak area integration, and to check the presence of a certain substance in each sample. Locally estimated scatterplot smoothing (LOESS) algorithm was then utilized to normalize batch or amplitude drifts within MS-DIAL. Subsequently, the normalized peak area table was exported for further multivariate analysis. Missing values were replaced with 1/10 of minimum peak area over all samples.

Multivariate analysis including hierarchical clustering and fold change analysis was carried out by MetaboAnalyst (Chong et al., 2019). Data transformation (log or cube root transformation) and scaling (mean, auto, pareto, or range scaling) were selected for each subset of analysis by visually assessing how Gaussian the data distribution appeared according to the MetaboAnalyst tutorial. False discovery rate (FDR) adjusted p-values based on Benjamini-Hochberg procedure was used. Data visualization was accomplished by ggplot2 package (Hadley Wickham, 2016) in R programming. Chemical classification was done by ClassyFire (Djoumbou Feunang et al., 2016). Migrants were prioritized as previously proposed in our study (Su et al., 2021a). In short, migrants listed as carcinogenic, mutagenic, and reprotoxic chemicals

(CMR, categories 1A, 1B, and 2 in the classification, labelling, and packaging (CLP) regulation) (European Union, 2008), substances of very high concern (SVHC) from European Chemicals Agency (ECHA) (https://echa.europa.eu/candidate-list-table), endocrine disrupting chemicals (EDC) (IPCP, 2017), and/or having specific migration limit (SML) as ND (not detectable at 0.01 mg/kg) in the positive list of EU regulation (EC, 2011) (positive list for short below) obtained toxic **level V**. The CMR, SVHC and positive list were last updated on 15th of September 2020. Toxtree (version 3.1.0.1851) based on Cramer rules (Patlewicz et al., 2008) was utilized for toxicity estimation when the migrant is not present CMR, SVHC, EDC or positive list. Cramer class III or SML between 0.01 and 0.1 mg/kg migrant was **level IV**; Cramer class II or SML between 0.1 and 1 mg/kg attained **level III**; Cramer class I or SML between 1 and 60 mg/kg got **level II** and migrants having SML equal to 60 mg/kg constituted **level I**.

4. Results and discussions

The number of pellets or the weight of flakes, respectively, can be employed for migration tests as an approximation method. However, the contact surface in each sample could vary because of the irregular shape of samples. Consequently, it is difficult to normalize the chromatographic response of each chemical in the migration samples either by weight or by contact surface. As it is known, the contact surface to food simulant volume ratio in migration test, which is set in Europe to 6 dm^2 to 1 kg food simulant, is vital and could have great effect on chemical migration. Hence, using migration results for the following multivariate analysis might, to some extent, add uncertainties to the results. In this sense, multivariate analysis described below using extraction data will be more robust. For extraction, all samples were milled into powders to have identical shape and LOESS normalization was employed to minimize instrumental variation during analysis.

4.1. Compositional similarity among samples

Compositional similarities (number of chemicals present and their corresponding intensities) of the rHDPE samples provided by 3 different recyclers were evaluated. In

addition, samples from a same company were collected at different time (from hours to weeks). Therefore, differences between batches of waste HDPE bottles could be assessed as well. The compositional similarities were evaluated by hierarchical clustering of the chemicals detected in the extracts using their normalized chromatographic peak areas. As illustrated in Fig. III-4.5, various batches of samples (both pellets and flakes) from company 1 were quite similar, and for company 3 alike. The batch difference was even smaller than the distinction between replicates. Samples from company 1 were collected on the same day with only few hours of difference. However, samples from company 3 were collected within 15 days. Although the composition from these 2 companies varied considerably, batches within the same company showed consistency, which is very positive for the industries. As expected, P1.3' and F1.3' were from company 1, but they were not clustered into the same group with other samples because they had been cleaned by the so-called extra decontamination technique.

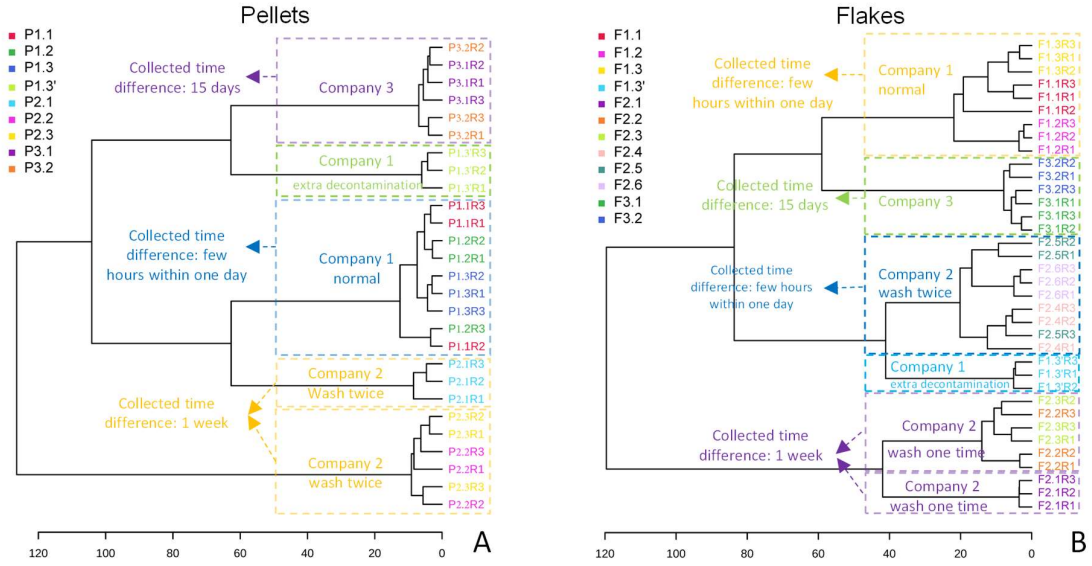


Fig. III-4.5 Hierachical clustering of pellet (A) and flake (B) samples

In contrast, the situation was more complicated for company 2 samples. Sample P2.1 was quite different from P2.2 and P2.3. Sample F2.1 was dissimilar from F2.2 and F2.3 as well. The main difference among them was that P2.1 and F2.1 were collected 7

days before the others. Sample F2.4, F2.5, and F2.6 were collected on the same day, and they were similar to each other. The results suggest that for company 2, samples from the same day were somehow consistent, while those from different days could vary considerably. The phenomenon is interesting and deserves further evaluation. Looking in depth into the sample difference from each company (Fig. III-4.6), we found that samples from companies 1 and 3 were rather clean (mainly from milk bottles) while company 2 samples contained many colour pieces e.g., various films with printing inks. Apart from HDPE flakes, isotactic PP films and flakes were found in company 2 samples by FTIR analysis (Fig. III-4.6). Obviously, these colour pieces did not come from milk bottles per se. Their presence in the so-called recycled milk bottles is most likely the consequence of poor separation capability during the recycling process. Hence, the significant chemical variation of company 2 samples collected on different days could be explained by the complexity of the samples because of the non-milk-bottle plastic contamination.

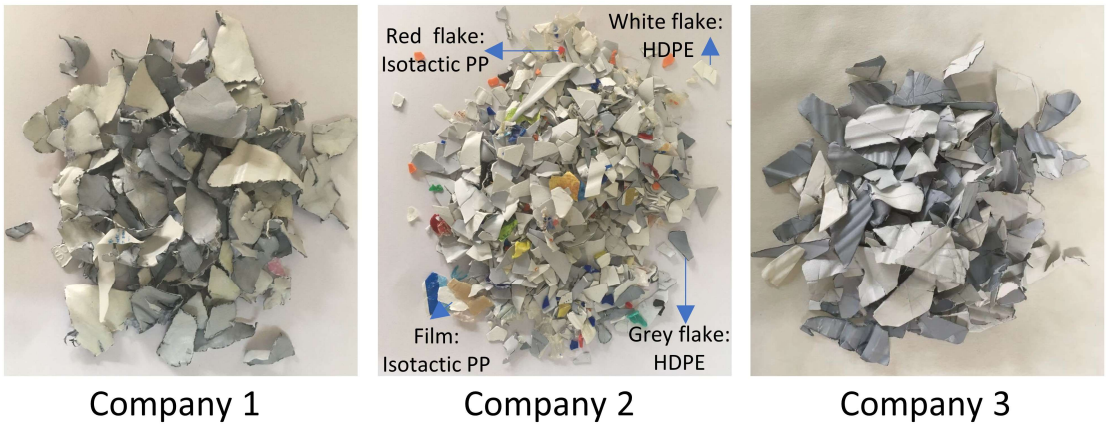
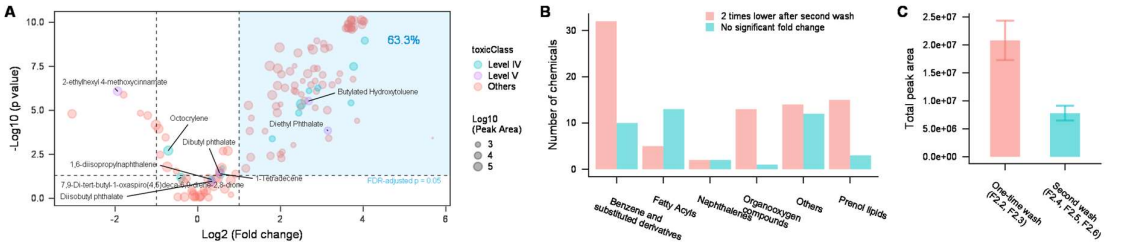


Fig. III-4.6 Flake samples from each company

4.2. Efficiency of washing twice on the removal of chemicals

Washing with water is a simple way to clean up contaminants attached on the surface of the recycled plastics. However, one-time washing might not be sufficient. For this reason, we have tested if washing the rHDPE twice with the same procedure would provide additional chemical removal capability. Sample F2.1 was excluded for this comparison since it was not collected on the same day than others and showed a

different GC-MS profile as aforementioned in section 4.1. Smaller peak size was observed for the majority of peaks after employing second wash. More specifically, the chromatographic peak area of 63.3% of chemicals including two level V substances, butylated hydroxytoluene (BHT) and diethyl phthalate (DEP) was halved after washing the flakes twice with water (Fig. III-4.7 A). Benzene and substituted derivatives, organooxygen compounds, and prenol lipids were the 3 classes of chemicals more easily removed (Fig. III-4.7 B). Besides, the total chromatographic peak area dropped more than half (Fig. III-4.7 C).



Note: Fold change is expressed as one-time wash versus twice wash; the size of the circles is mapped to the average peak area of the samples that applied second wash

Fig. III-4.7 Efficiency of washing twice: fold change analysis by volcano plot (A), chemical classes distribution (B), and total chromatographic peak area (C)

As aforementioned, flakes from company 2 contained high proportion of non-milk-bottle plastics, e.g., coloured films, coloured flakes, etc. Each type of plastic might have different contaminants. For example, coloured films might have more contaminants (chemicals from printing inks) than milk bottle flakes. To assess whether the percentage of each type of plastics has changed after the second wash, flakes from company 2 were manually separated into 5 fractions, namely, milk bottle flakes, transparent flakes, coloured flakes, coloured films, and transparent films (Fig. III-4.4), and the weight of each fraction was calculated. As shown in Table III-4.2, the percentage of milk bottle fraction significantly increased from 54% to 84% after the second wash, which means that high amount of non-milk bottle plastics was removed in this step. This could be one of the important reasons why much less and lower peaks were observed for samples subjected to a second wash. However, we speculate that more cleaning processes, e.g., sink float separation, might have been applied in the so-

called second wash as those non-milk bottle fractions might not be easily removed by simply washing with water.

Table III-4.2 The percentage of each fraction of polymer before and after second wash

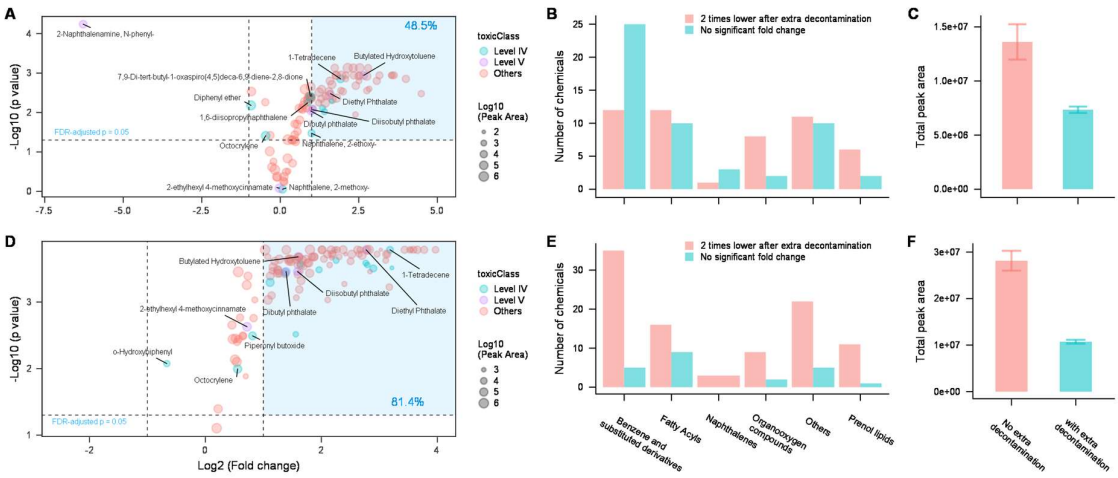
Polymer fractions	One time wash (%)			Wash twice (%)		
	F2.1	F2.2	F2.3	F2.4	F2.5	F2.6
transparent film (PE, PP)	2.4	3.2	2.7	0.3	0.4	0.3
transparent flake (PE)	32.1	36.8	37.3	4.1	4.7	4.2
colour film (PP and others)	3.5	3.6	3.3	1.3	1.0	1.2
colour flake (PE, PP)	5.8	3.1	3.0	12.0	8.8	8.2
milk bottle (HDPE)	56.3	53.6	54.2	82.3	85.4	86.3

Unlike DEP, two heavier phthalates, dibutyl phthalate (DBP) and diisobutyl phthalate (DiBP) were more difficult to remove. Moreover, 2-ethylhexyl-4-methoxycinnamate, which is an EDC, as well as octocrylene and 1-tetradecene, which had been reported to have excess migration values (SML 0.05 mg/kg) in recycled polyolefins (Su et al., 2021a) were not easy to clean neither (Fig. III-4.7 A). The compound 2-ethylhexyl-4-methoxycinnamate had significantly higher intensities after second wash probably because of sample heterogeneity. Albeit sample collected on the same day had similar GC-MS profiles (as mentioned in 4.1), it does not mean that they were exactly the same. In fact, several chemicals varied their chromatographic intensities.

4.3. Efficiency of the extra decontamination technique

To examine the effectiveness of the extra decontamination step, P1.3 and F1.3 were processed by this technique, and corresponding cleaned samples P1.3’ and F1.3’ were obtained, respectively. Many peaks had lower intensities after extra decontamination. Explicitly, 48.5% and 81.4% compounds in flakes (Fig. III-4.8 A) and pellets (Fig. III-4.8 D), respectively, got less than half chromatographic response after being treated with the extra decontamination technique. Interestingly, much higher cleaning efficiency was observed for pellets, as evidenced by their total peak area decrease (Fig. III-4.8 C and F). Firstly, there were more substances (118 vs 103)

detected and higher total peak area observed ($2.8 \cdot 10^7$ VS $1.4 \cdot 10^7$) in pellets than in flakes before applying extra decontamination. Therefore, it is reasonable to have higher cleaning efficiency in the most contaminated samples (pellets). For example, most of the benzene and substituted derivatives were sufficiently cleaned (fold change higher than 2) in pellets but not in flakes (Fig. III-4.8 B and E). By plotting only this class of chemicals (Fig. III-4.9 A and B), we can understand that 37.8% of benzene and substituted derivatives had fold change between 1.5 and 2 in flakes with low p-values, which means that their intensities were actually reduced. However, their intensities in flakes were already small (Fig. III-4.9 C) making it more difficult to be further reduced. Secondly, higher efficiency in pellets could also be the result of polymer degradation during extrusion which is well known in plastic recycling (Schyns and Shaver, 2021; Singh et al., 2017). Because of the thermal conduction and viscous shearing applied to polymers with an extruder, polymer chain length and mechanical properties are reduced (Schyns and Shaver, 2021), causing increased diffusion of chemicals within the polymer. As such, chemicals in the degraded polymer (pellet) could be more easily extracted and cleaned, which also explains why there were more substances and higher intensities detected in pellet samples.



Note: Fold change is expressed as no extra decontamination versus extra decontamination; the size of the circles is mapped to the average peak area of the samples that applied extra decontamination

Fig. III-4.8 Efficiency of extra decontamination: fold change analysis by volcano plot on flakes (A), chemical classes distribution on flakes (B), and total chromatographic peak area

on flakes (C), fold change analysis by volcano plot on pellets (D), chemical classes distribution on pellets (E), and total chromatographic peak area on pellets (F)

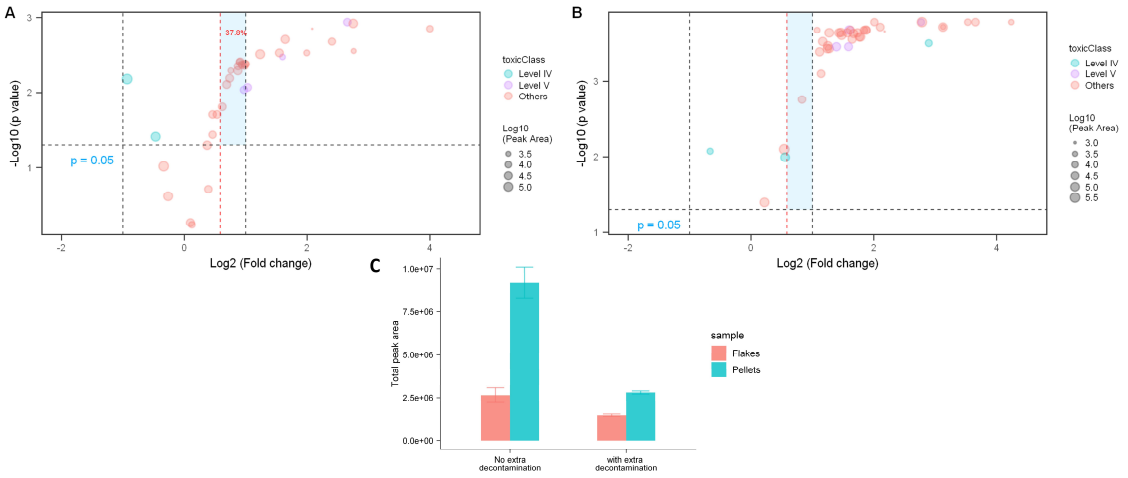


Fig. III-4.9 Efficiency of extra decontamination on benzene and substituted derivatives: fold change analysis by volcano plot on flakes (A) and pellets (B), total peak area before and after extra decontamination in flakes and pellets (C)

Washing twice was effective for reducing the concentration of DEP and BHT, whereas DBP, DiBP as well as 1-tetradecene remained almost constant. On the other hand, extra decontamination demonstrated to be effective for these compounds. Nevertheless, extra decontamination did not sufficiently decrease the content of octocrylene and 2-ethylhexyl-4-methoxycinnamate neither. Quite significantly, N-phenyl-2-naphthalenamine, which is a suspected carcinogen, had much higher response in flakes after extra decontamination (Fig. III-4.8 A left-top). As shown in Fig. III-4.10, this compound was actually detected in both F1.3' and P1.3', which were the only two samples subjected to extra decontamination. However, it was only identified in F1.3' by MS-DIAL because it had too low intensity to have a representative spectrum in P1.3'. Since it was not observed in samples F1.3 and P1.3, it is speculated to be a contaminant or a newly formed substance during the extra decontamination process. As far as we know, this is the first time to report the presence of this compound in recycled plastics after certain treatments.

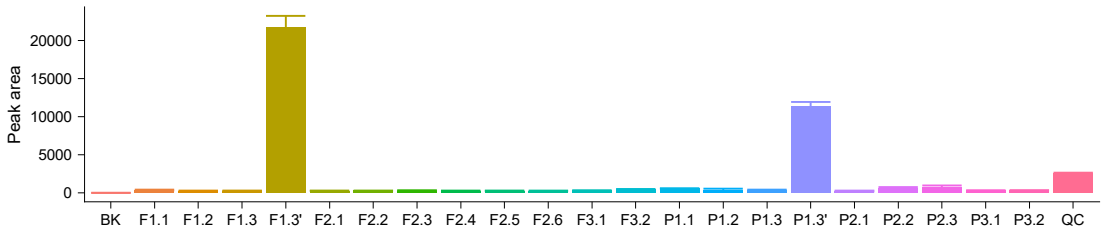


Fig. III-4.10 Bar chart of N-phenyl-2-naphthalenamine across samples

It is worth mentioning that the cleaning efficiencies of the two methods were not directly comparable, as they were not applied to the same samples, and the degree of contamination in samples was also different. In contrast to company 1 samples, those from company 2 were more contaminated. The numbers of chemicals considered in Fig. III-4.7 A and Fig. III-4.8 A were 128 and 103, respectively. Moreover, total peak areas of the flakes before applying second wash and extra decontamination were $2.1 \cdot 10^7$, and $1.4 \cdot 10^7$, respectively.

4.4. (Semi-)quantification of prioritized migrants

The use of a sensitive DI-SPME screening method enhances the capability of finding compounds of human health concern. On the other hand, it also increases the number of noise substances which are sensitive and of low toxicity, e.g., alkanes, and therefore distracts us from focusing on migrants of real concern. Hence, the long list of migrants detected was prioritized by toxicity class, detection frequency, and maximum response as proposed in our previous work (Su et al., 2021a). There were 265 migrants detected overall (Table III-4.3). In agreement with previous studies (Dutra et al., 2011; Huber and Franz, 1997; Welle, 2005), commonly used plastic additives (BHT, DEP, DBP, etc.), degradation products (7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione), various saturated/unsaturated oligomers, aliphatic esters, fatty alcohol/aldehyde, as well as some favour compounds (galaxolide, camphor, 1,8-cineole, etc.) were detected in these samples.

Table III-4.3 Detailed information of tentatively identified migrants

RT	Name	mRI	rRI	Score	Cram	Tox	LogP	MW	Fill	S/N	Presence
3.50	Meta-xylene	967	991.2	85.5	I	II	3.2	106	33	24	HP3.1, HP3.2, HF2.1, HF2.2, HF2.3, HF2.5, HF2.6
3.56	Bicyclo[4.2.0]octa-1,3,5-triene	966	948.8	93.5	III	IV	1.8	104	43	41	HP1.1, HP1.2, HP1.3, HP2.1, HP2.3, HP3.1, HP3.2, HF2.1, HF2.6
3.66	Bicyclo[2.2.1]hept-2-ene, 2,7,7-trimethyl-	970	972.9	93.6	I	II	2.9	136	10	8	EP2.1, EP2.2
4.06	Alpha-pinene **(SML 60)**	979	970.0	98.1	-	I	2.8	136	52	81	EP1.1, EP1.2, EP1.3, EP3.1, EP3.2, EP1.3', EF2.1, EF2.2, EF2.3, EF2.4, EF2.5
4.36	Benzene, 1,2,4-trimethyl-	991	990	98.6	I	II	3	120	19	21	EP2.2, EP2.3, EP3.1, HP2.2, HP2.3, HP3.1, HP1.3'
4.43	Phenol **(CMR, SML 3)**	993	980	91	-	V	1.5	94	14	17	HP2.1, HP2.2, HP2.3
4.70	Benzene, 1,2,3-trimethyl-	1003	1013	96.4	I	II	3.6	120	19	68	EP2.2, HP2.2, HP2.3, HP3.1, HP3.2
4.71	Octanal	1003	1003	99.3	I	II	2.7	128	81	11	HP1.1, HP1.2, HP1.3, HP2.1, HP2.3, HP3.1, HP3.2, HP1.3', HF1.2, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.1, HF3.2, HF1.3'
4.95	Alpha-terpinene	1022	1017	99.2	I	II	2.8	136	57	30	EP2.1, EP2.2, EP2.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3'
4.99	1,4-cineole	1020	1016	99	III	IV	2.5	154	100	96	HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3', HF1.1, HF1.2, HF1.3, HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.1, HF3.2, HF1.3'
5.06	Benzene, 1-methoxy-4-methyl-	1024	1021	97.9	I	II	2.7	122	48	27	HP2.1, HF1.1, HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.2, HF1.3'
5.10	1-Hexanol, 2-ethyl- **(SML 30)**	1027	1030	90.3	-	II	3.1	130	100	13	EP2.2, EP2.3, HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3', HF1.1, HF1.2, HF1.3, HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.1, HF3.2, HF1.3'
5.12	p-Cymene	1028	1025	98.6	I	II	4.1	134	86	83	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', HP2.1, HP3.1, HP3.2, HF2.1, HF2.2, HF2.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3'
5.20	D-Limonene	1033	1030	99.9	I	II	3.4	136	100	311	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3', HF1.3, HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.1, HF3.2, HF1.3', EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3'
5.26	Eucalyptol (1,8-cineole)	1036	1032	99.5	III	IV	2.5	154	95	54	EP1.3, EP2.1, HP1.1, HP1.2, HP1.3, HP2.1, HP2.3, HP3.1, HP3.2, HP1.3', HF1.1, HF1.2, HF1.3, HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.1, HF3.2, HF1.3', EF2.1, EF2.2, EF2.3, EF2.5, EF2.6
5.44	Furan, tetrahydro-2,2-dimethyl-5-(1-methyl-1-propenyl)-	1047	1035.8	95.1	III	IV	2.7	154	76	28	HP1.1, HP1.2, HP1.3, HP2.1, HP3.1, HP3.2, HP1.3', HF1.1, HF1.2, HF1.3, HF2.1, HF2.2, HF2.5, HF2.6, HF3.1, HF3.2
5.45	3-(Trifluoromethyl)-benzenamine	1048	1032.3	91.7	III	IV	2.3	161	10	288	HP2.2, HP2.3
5.53	Cyclohexanol, 3,3,5-trimethyl-, cis-	1053	1073	92	I	II	2.6	142	57	434	HP1.1, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF1.3'
5.67	.gamma.-Terpinene	1060	1060	99.8	I	II	2.8	136	100	57	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', HP2.1, HP3.2, HF2.1, HF2.2, HF2.3, EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3'
5.66	Benzene, 1-ethyl-3,5-dimethyl-	1060	1058	96	I	II	3.4	134	24	33	EP2.2, EP2.3, EP3.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3'
5.82	7-Octen-2-ol, 2,6-dimethyl-	1069	1064	98.9	III	IV	2.9	156	100	526	HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3', HF1.1, HF1.2, HF1.3, HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.1, HF3.2, HF1.3'
5.88	Benzenamine, 3-methyl-	1073	1075	96	I	II	1.4	107	29	253	HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3
6.03	Benzene, 4-ethyl-1,2-dimethyl-	1082	1085	91.5	I	II	3.4	134	10	14	EP2.2, EP2.3
6.11	P-tolualdehyde	1090	1079	97.7	I	II	2.1	120	14	44	HP2.1, HP2.2, HP2.3
6.19	2-Nonanone	1092	1092	99.8	II	III	3.1	142	76	111	HP1.1, HP1.2, HP1.3, HP2.3, HP3.1, HP3.2, HP1.3', HF1.1, HF1.2, HF1.3, HF2.4, HF2.5, HF2.6, HF3.1, HF3.2, HF1.3'
6.21	Cyclohexene, 1-methyl-4-(1-methylethylidene)-	1093	1088	98.8	I	II	2.8	136	76	162	HP1.1, HP1.2, HP1.3, HP2.3, HP3.1, HP3.2, HP1.3', HF1.1, HF1.2, HF1.3, HF2.4, HF2.5, HF2.6, HF3.1, HF3.2, HF1.3'
6.28	2-Methyl-2-nonanol	1097	1097.3	96.7	III	IV	3.6	158	24	131	HP2.1, HP2.2, HP2.3, HP3.1, HF1.3'
6.32	3-Octanol, 3,7-dimethyl-	1100	1098	99.7	III	IV	3.3	158	100	477	EP2.1, HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3', HF1.1, HF1.2, HF1.3, HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.1, HF3.2, HF1.3'
6.62	(2S,4R)-4-Methyl-2-(2-methylprop-1-en-1-yl)tetrahydro-2H-pyran	1112	1111	99.8	III	IV	2.9	154	86	64	HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3', HF1.1, HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.2, HF1.3'
6.74	Fenchol	1117	1106.5	97	I	II	2.5	154	76	43	HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3', HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.2
6.79	1,2,4,5-tetramethylbenzene	1119	1116	98.9	I	II	4	134	62	29	EP2.2, EP2.3, EP3.1, EP3.2, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3', HF2.4, HF3.1, HF3.2, HF1.3', EF2.1, EF2.2, EF2.3, EF2.4, EF2.6, EF3.1, EF3.2, EF1.3'

RT	Name	mRI	rRI	Score	Cram	Tox	LogP	MW	Fill	S/N	Presence
6.88	Benzene, 1,2,3,5-tetramethyl-	1123	1117	97.3	I	II	4	134	91	46	EP2.2, EP2.3, EP3.1, EP3.2, HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3', HF1.3, HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.1, HF3.2, HF1.3', EF2.1, EF2.2, EF2.3, EF3.1, EF3.2, EF1.3'
6.98	2,4,6-Octatriene, 2,6-dimethyl-, (E,Z)-	1127	1131	99.4	I	II	4.2	136	24	8	EP2.1, EP2.2, EP2.3, EP3.1, EP3.2
7.14	1-Methyl-4-(1-methylethyl)-3-cyclohexen-1-ol	1133	1136	98.9	III	IV	2	154	81	45	HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3', HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.2, HF1.3'
7.03	o-Chloroaniline	1133	1126	98.1	III	IV	1.9	128	29	119	HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3
7.29	2,4,6-Octatriene, 2,6-dimethyl-	1140	1144	99	I	II	4.2	136	38	13	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2
7.37	4-Chlorobenzonitrile	1143	1135.1	91.5	III	IV	2.6	138	10	165	HP2.2, HP2.3
7.39	Benzene, 2,4-dimethyl-1-(1-methylethyl)-	1143	1140.1	91.4	I	II	3.8	148	10	7	EP2.2, EP2.3
7.41	Cyclohexanemethanol, .alpha.,.alpha.,4-trimethyl-	1147	1136.4	91.3	III	IV	2.8	156	29	42	HP1.1, HP1.2, HF1.2, HF2.2, HF2.4, HF2.6
7.47	(+)-2-Bornanone (camphor) **(SML 60)**	1147	1142	99.4	-	I	2.2	152	100	144	HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3', HF1.1, HF1.2, HF1.3, HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.1, HF3.2, HF1.3'
7.58	1H-Indene, 2,3-dihydro-4-methyl-	1152	1145	91.9	I	II	3.1	132	19	18	HP2.2, HP2.3, HP3.1, HP3.2
7.62	Benzene, 1,2,3,4-tetramethyl-	1153	1146	94.7	I	II	4	134	19	35	EP2.2, EP2.3, EP3.2, HP2.2, HP2.3, HP3.1, HP3.2
7.66	l-Menthone	1154	1158.7	99.3	II	III	2.7	154	95	44	HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3', HF1.1, HF1.2, HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.1, HF3.2, HF1.3'
7.76	Isoborneol	1159	1168	98.1	I	II	2.7	154	100	216	HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3', HF1.1, HF1.2, HF1.3, HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.1, HF3.2, HF1.3'
7.96	Benzenamine, 2,4-dimethyl-	1167	1167	99.1	I	II	1.7	121	19	357	EP2.1, HP2.1, HP2.2, HP2.3, HP3.2
7.98	endo-Borneol	1168	1168	99.6	I	II	2.7	154	43	52	HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.1, HF3.2, HF1.3'
8.04	2,4-Dichlorophenol	1170	1168	96.8	III	IV	3.1	163	10	565	HP2.2, HP2.3
8.08	3,5,5-Trimethylhexyl acetate	1172	1180	96.9	I	II	3.5	186	14	11	EP2.1, HP2.1, HP3.1, HP3.2
8.11	Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1.alpha.,2.beta.,5.alpha.)-(.+/-.)-	1173	1169	99.5	I	II	3	156	100	202	HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3', HF1.1, HF1.2, HF1.3, HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.1, HF3.2, HF1.3'
8.26	Cyclohexanol, 2-(1,1-dimethylethyl)-	1180	1190.1	96.6	I	II	3	156	71	24	HP1.1, HP1.2, HP1.3, HP2.1, HP1.3', HF1.1, HF1.2, HF1.3, HF2.1, HF2.4, HF2.5, HF2.6, HF3.1, HF3.2, HF1.3'
8.42	Ethanone, 1-(3-methylphenyl)-	1186	1182	98.4	I	II	2.3	134	48	72	HP1.1, HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.1, HF3.2, HF1.3'
8.43	Cyclohexanol, 3,3,5-trimethyl-, acetate, cis-	1187	1196	95.7	I	II	3.2	184	24	27	EP1.2, HP1.1, HP1.2, HP1.3, HP3.2, HP1.3'
8.47	Naphthalene **(CMR)**	1188	1182	98.4	-	V	3.3	128	91	158	EP2.2, EP2.3, HP1.1, HP1.2, HP1.3, HP2.2, HP2.3, HP3.1, HP1.3', HF1.1, HF1.2, HF1.3, HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.1, HF3.2, HF1.3', EF1.3'
8.54	1-Dodecene **(SML 0.05)**	1191	1190	99.7	-	IV	6.8	168	100	47	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', HP1.1, HP1.2, EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3'
8.58	Alpha-terpineol	1193	1190	99.1	III	IV	1.8	154	100	152	HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3', HF1.1, HF1.2, HF1.3, HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.1, HF3.2, HF1.3'
8.64	Octanoic acid, ethyl ester	1195	1196	92.3	I	II	3.5	172	10	29	HP2.2, HP2.3
8.71	Methyl salicylate **(SML 30)**	1198	1192	98.1	-	II	2.3	152	43	63	HP2.1, HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.1, HF3.2
8.81	Cyclohexanol, 4-(1,1-dimethylethyl)-, cis-	1202	1220.2	92.7	I	II	3	156	100	18	HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3', HF1.1, HF1.2, HF1.3, HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.1, HF3.2, HF1.3'
9.28	Benzaldehyde, 2,5-dimethyl-	1217	1208	98	I	II	2.1	134	29	950	HP1.1, HP1.2, HP1.3, HP2.1, HP2.2 HP2.3, HP1.3'
9.62	Cyclohexanone, 4-(1,1-dimethylethyl)-	1226	1221.8	99.2	II	III	2.6	154	38	106	HP1.1, HP1.2, HP1.3, HP2.1, HF2.1, HF2.2, HF2.3 ,HF3.2, HF1.3'
9.88	Fenchyl acetate	1234	1223	96.5	I	II	3.1	196	5	22	HP2.1
9.88	1-(2-Chlorophenyl)-ethanone	1234	1241	90	III	IV	2.1	155	10	23	HP2.2, HP2.3
10.04	Benzaldehyde, 4-(1-methylethyl)-	1239	1239	97.5	I	II	2.7	148	33	61	HP1.1, HP1.2, HP1.3, HP2.1, HP2.2 HP2.3, HP3.1, HP1.3'
10.11	2-Butanone, 3-phenyl-	1241	1244	96.8	I	II	2.1	148	29	7	HP1.1, HP1.2, HP1.3, HP2.1, HP2.2 HP2.3, HF2.6
10.15	D-Carvone	1242	1242	96.6	II	III	2.4	150	19	35	HP1.1, HP1.2, HP1.3, HP2.1
11.06	Benzoic acid, 2-hydroxy-, ethyl ester	1269	1270	91.9	I	II	3	166	19	14	EF1.2, EF2.1, EF2.5, EF1.3'
11.05	Benzene, 1-(1,1-dimethylethyl)-3-ethyl-5-methyl-	1269	1255.9	90.7	I	II	4.8	176	5	14	EP2.1
11.08	1-Decanol **(SML 60)**	1270	1273	99.4	-	I	4.6	158	38	42	HP1.1, HP1.2, HP1.3, HP2.1, HP2.2 HP2.3, HP3.1, HP3.2, HP1.3'
11.14	Benzaldehyde, 4-propyl-	1273	1260.3	95.8	I	II	3	148	38	77	HP1.1, HP1.2, HP1.3, HP2.1, HP2.2 HP2.3, HP3.1, HP3.2, HP1.3'

RT	Name	mRI	rRI	Score	Cram	Tox	LogP	MW	Fill	S/N	Presence
11.26	Tricyclo[4.2.1.1(2,5)]dec-3-en-9-ol	1275	1269.5	94.5	III	IV	1.7	150	100	131	HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3', HF1.1, HF1.2, HF1.3, HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.1, HF3.2, HF1.3'
11.42	Benzene, pentamethyl-	1280	1259	89.8	I	II	4.6	148	10	21	EP2.2, EP2.3
11.58	Anethole	1285	1284	98.2	I	II	3.3	148	86	80	HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HF1.1, HF1.3, HF2.1, HF2.2, HF2.3, HF2.4, HF2.6, HF3.1, HF3.2, HF1.3'
11.80	2-Undecanone	1291	1294	99.6	II	III	4.1	170	100	117	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3', HF1.1, HF1.2, HF1.3, HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.1, HF3.2, HF1.3', EF1.2, EF1.3, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2
11.89	O-t-butylcyclohexyl acetate (trans)	1294	1286.4	97.5	II	III	3.6	198	95	413	EP1.1, EP1.2, EP1.3, EP2.1, EP3.1, EP3.2, EP1.3', HP1.1, HP1.2, HP1.3, HP2.1, HP2.3, HP3.1, HP3.2, HP1.3', HF1.1, HF1.2, HF1.3, HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.1, HF3.2, HF1.3', EF1.1, EF1.2, EF1.3, EF2.2, EF2.3, EF3.2
11.90	2-Methyl-naphthalene	1294	1298	98.7	III	IV	3.9	142	10	393	EP2.2, EP2.3, HP2.2, HP2.3
12.09	Tridecane	1300	1285.5	96	I	II	6.6	184	91	34	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EF2.6, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3'
12.42	4-(t-Butyl)benzaldehyde	1308	1290.9	93.1	I	II	3.1	162	86	337	EP2.1, HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HF1.1, HF1.3, HF2.1, HF2.2, HF2.3, HF2.4, HF2.6, HF3.1, HF3.2, HF1.3'
12.50	1-Methyl-naphthalene	1310	1307	99.4	III	IV	3.9	142	71	238	EP2.2, EP2.3, EP3.1, EP3.2, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3', HF1.1, HF1.2, HF1.3, HF2.1, HF2.3, HF2.4, HF2.6, HF3.1, HF3.2, HF1.3', EF1.3, EF2.1, EF2.3, EF3.1, EF3.2, EF1.3'
12.65	2-tert-butylcyclohexyl acetate	1313	1309.3	98.2	II	III	3.6	198	100	42	EP2.1, EP1.3', HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3', HF1.1, HF1.2, HF1.3, HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.1, HF3.2, HF1.3'
12.79	Naphthalene, 1,2,3,4-tetrahydro-2,6-dimethyl-	1317	1304	91	I	II	3.9	160	19	18	EP2.2, EP2.3, EF2.4, EF3.1
12.98	Decanoic acid, methyl ester	1322	1325	93.7	I	II	4.7	186	5	27	EP2.1
13.05	Nonane, 2,2,4,4,6,8,8-heptamethyl-	1323	1322	99.8	I	II	7.3	226	57	59	EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3'
13.23	2,4-Dichlorobenzenamine	1328	1324	95.6	III	IV	2.9	162	19	99	HP1.1, HP2.1, HP2.2, HP2.3
13.38	4-tert-Butylcyclohexyl acetate	1331	1330	99.5	II	III	3.4	198	91	63	EP1.1, EP1.2, EP1.3, EP2.1, EP3.1, EP3.2, EP1.3', HP1.1, HP1.2, HP1.3, HP2.1, HP2.3, HP3.1, HP3.2, HP1.3', HF1.1, HF1.2, HF1.3, HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.2, HF1.3', EF1.1, EF1.3, EF2.1, EF2.2
13.71	Methyl anthranilate	1339	1343	97.2	I	II	1.9	151	14	17	HP2.1, HF2.1, HF2.2
13.75	Heptylcyclohexane	1340	1346	96.9	I	II	6.8	182	33	35	EP1.1, EP1.2, EP1.3, EP2.1, EP3.1, EP3.2, EP1.3'
14.01	.alpha.-Terpinyl acetate	1347	1350	99	I	II	2.4	196	48	13	HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF1.3'
14.07	4-Acetylanisole	1348	1350	94.4	I	II	1.7	150	10	82	HP2.2, HP2.3
14.17	Phenol, 2-(1,1-dimethylethyl)-4-methyl-	1350	1354	98.8	I	II	3.6	164	33	156	HP1.1, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3'
14.22	Tricyclo[5.4.0.0(2,8)]undec-9-ene, 2,6,6,9-tetramethyl-, (1R,2S,7R,8R)-	1351	1353	97.9	I	II	4.6	204	10	10	EF2.1, EF2.2
14.46	2(3H)-Furanone, dihydro-5-pentyl-	1357	1363	95.3	II	III	2.2	156	14	20	HP1.3, HF1.2, HF3
14.52	Phenol, 2-(1,1-dimethylethyl)-5-methyl-	1358	1366	96.9	I	II	3.6	164	5	16	HP2.1
14.61	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)-	1361	1364	97.4	I	II	3.5	196	14	7	HP1.2, HP1.3, HP1.3'
14.85	Para-tert-butylcyclohexanol	1366	1374.6	92.7	I	II	3	156	100	93	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP1.3', HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3', HF1.1, HF1.2, HF1.3, HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.1, HF3.2, HF1.3', EF1.1, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF1.3'
15.01	1-undecanol	1370	1371	98.9	I	II	4.6	172	43	16	HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3'
15.02	Propanoic acid, 2-methyl-, 2-ethyl-3-hydroxyhexyl ester	1371	1373	95.7	II	III	2.9	216	33	45	HP2.3, HP3.1, HP3.2, HP1.3', HF1.3, HF3.1, HF3.2
15.06	1,2,4-Methenoazulene, decahydro-1,5,5,8a-tetramethyl-, [1S-(1.alpha.,2.alpha.,3a.beta.,4.alpha.,8a.beta.,9R*)]-	1371	1374	98.4	I	II	5	204	24	11	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2
15.08	2-Buten-1-one, 1-(2,6,6-trimethyl-3-cyclohexen-1-yl)-	1372	1377.2	96.8	I	II	3.4	192	71	72	HP1.1, HP1.2, HP1.3, HP3.2, HP1.3', HF1.1, HF1.2, HF1.3, HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.2
15.19	Biphenyl	1375	1381	98.2	III	IV	4	154	19	22	EP2.2, EP2.3, HP2.2, HP2.3, HP3.1, HP3.2
15.26	Copaene	1376	1376	98.4	I	II	4.5	204	48	9	EP1.1, EP1.2, EP1.3, EP1.3', EF1.3, EF2.1, EF2.2, EF2.3, EF3.1, EF3.2
15.50	4,7-Methanoazulene, 1,2,3,4,5,6,7,8-octahydro-1,4,9,9-tetramethyl-, [1S-(1.alpha.,4.alpha.,7.alpha.)]-	1382	1381	97.2	I	II	4.3	204	24	12	EP1.1, EP1.2, EP1.3, EP2.1, EP1.3'

RT	Name	mRI	rRI	Score	Cram	Tox	LogP	MW	Fill	S/N	Presence
15.65	(-)-.beta.-Bourbonene	1386	1393	95.5	I	II	4.7	204	14	10	EF2.1, EF2.2, EF2.3
15.91	1-Tetradecene **(SML 0.05)**	1392	1392	99.7	-	IV	7.9	196	81	86	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', HF1.3, EF1.1, EF1.2, EF1.3, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2
15.95	Propanoic acid, 2-methyl-, 2-phenylethyl ester	1393	1396	92.7	I	II	3.3	192	5	20	HP2.1
16.03	Decanoic acid, ethyl ester	1395	1396	98.3	I	II	4.6	200	14	77	EP2.1, EP2.2, EP2.3
16.20	Diphenyl ether	1399	1400	99.7	III	IV	4.2	170	105	6913	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3', HF1.1, HF1.2, HF1.3, HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.1, HF3.2, HF1.3', EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3'
16.31	Naphthalene, 2,7-dimethyl-	1401	1401	99.6	III	IV	4.3	156	10	123	EP2.2, EP2.3, HP2.2, HP2.3
16.49	Benzoic acid, 2-(methylamino)-, methyl ester	1405	1408	97.9	I	II	2.3	165	57	56	HP1.1, HP1.2, HP1.3, HP2.1, HP3.1, HP3.2, HF1.3, HF2.1, HF2.2, HF2.6, HF3.1, HF3.2
16.57	Dodecanal	1407	1409	99.7	I	II	4.9	184	43	51	EP3.1, EP1.3', HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3'
16.62	Longifolene	1408	1405	97.7	I	II	5.1	204	33	24	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EF2.2
16.67	Benzene, 1,2,3,4-tetramethyl-5-(1-methylethyl)-	1409	1399.4	91.4	I	II	5.3	176	33	258	HP2.2, HP2.3, HP3.1, HP3.2, HF2.4, HF3.1, HF3.2
16.88	1H-3a,7-Methanoazulene, 2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-, [3R-(3.alpha.,3a.beta.,7.beta.,8a.alpha.)]-	1413	1411	98.6	I	II	4.6	204	91	36	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF3.1, EF1.3'
16.93	Naphthalene, 1,3-dimethyl-	1414	1416	99.2	III	IV	4.4	156	43	103	EP1.1, EP1.2, EP2.3, HP2.3, HP3.1, HP3.2, EF1.1, EF1.2, EF1.3, EP2.3, EF2.4
17.06	1,6-dimethyl-naphthalene	1417	1420	99.4	III	IV	4.4	156	38	55	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2
17.21	Salicylic acid, 1-methylpropyl ester	1421	1417	95	I	II	3.7	194	14	42	HP1.1, HP1.2, HP1.3
17.31	3,4-Dichlorobenzenamine	1423	1421	99.2	III	IV	2.7	162	10	664	HP2.2, HP2.3
17.41	.alpha.-Ionone	1425	1426	99.6	I	II	3	192	100	92	HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3', HF1.1, HF1.2, HF1.3, HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.1, HF3.2, HF1.3'
17.51	Diphenylmethane	1427	1434	92.9	III	IV	4.1	168	19	44	HP2.2, HP2.3, HP3.1, HP3.2
17.54	Dodecane, 1-methoxy-	1427	1424	99.9	I	II	5.7	200	86	106	EP1.2, EP2.1, EP2.2, EP3.1, EP3.2, EP1.3', EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3'
17.76	Naphthalene, 1,4-dimethyl-	1432	1436	99	III	IV	4.4	156	62	52	EP2.2, EP2.3, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3', EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF3.1, EF3.2
17.91	Phenol, 2,6-bis(1,1-dimethylethyl)-	1435	1440	98.3	II	III	4.9	206	19	81	EP1.3, HF2.1, EF2.1, EF2.2, EF2.3
17.92	2,7-Dimethyl-quinoline	1435	1422	95.6	III	IV	3	157	10	186	HP2.2, HP2.3
17.99	.beta.-Humulene	1437	1440	96	I	II	4.8	204	38	8	EP1.1, EP1.2, EP1.3, EP2.1, EP1.3', EF2.1, EF2.2, EF1.3'
18.19	Seychellene	1441	1459	91.4	I	II	5.1	204	10	28	EP2.1, EP1.3'
18.29	2-Methoxy-naphthalene	1443	1450.1	99.1	III	IV	3.5	158	100	3602	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP3.1, EP3.2, HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3', HF1.1, HF1.2, HF1.3, HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.2, HF1.3', EF1.1, EF2.1, EF2.2, EF2.3, EF3.1, EF3.2, EF1.3'
18.31	1-(4-tert-Butylphenyl)propan-2-one	1444	1458.0	96.5	I	II	3.2	190	95	539	HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3', HF1.1, HF1.2, HF1.3, HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.2, HF1.3'
18.39	2-n-Heptylcyclopentanone	1445	1452.1	98.7	II	III	4	182	33	65	HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF1.3', EF2.1, EF2.2, EF2.3
18.43	1,2-Dimethyl-naphthalene	1446	1452	92.6	III	IV	4.3	156	10	29	EP2.2, EP2.3, HP2.2, HP2.3
18.56	2-Phenyl-pyridine	1449	1466	88.9	III	IV	2.6	155	10	59	HP2.2, HP2.3
18.67	Humulene	1451	1454	96.8	I	II	4.5	204	33	14	EP1.1, EP1.2, EP1.3, EP2.1, EF2.1, EF2.2, EF2.3
18.67	Diisopropyl adipate	1451	1448.4	98	I	II	2.2	230	10	61	HP2.2, HP2.3
18.76	Decahydro-1,1,4a,5,6-pentamethylnaphthalene	1454	1472	88.6	I	II	6.2	208	24	34	EP2.2, EP2.3, EP3.1, EP3.2, EF2.5
18.82	(E)-.beta.-Famesene	1454	1457	93.7	I	II	6.2	204	19	21	EP3.2, EF2.1, EF2.2, EF2.3
18.85	3-(4-Isopropylphenyl)-2-methylpropionaldehyde	1455	1455.3	99.4	I	II	3.3	190	76	32	HP1.1, HP1.2, HP1.3, HP3.1, HP3.2, HP1.3', HF1.1, HF1.2, HF1.3, HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.2
19.06	Benzene, (1-ethylheptyl)-	1459	1468.9	94.7	I	II	6.3	204	14	8	EF2.1, EF2.2, EF2.3
19.42	1-Chlorododecane	1467	1469	97	III	IV	6.9	205	38	9	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2
19.51	2,5-cyclohexadien-1-one, 2,6-bis(1,1-dimethylethyl)-4-hydroxy-4-methyl-	1469	1478	95.2	III	IV	3.5	236	57	207	HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3', HF1.1, HF1.3, HF2.4
19.65	1-Dodecanol	1472	1473	99.9	I	II	5.1	186	43	222	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', HP1.2, HP1.3, HP2.1

RT	Name	mRI	rRI	Score	Cram	Tox	LogP	MW	Fill	S/N	Presence
19.86	.alpha. Isomethyl ionone	1476	1480	99.2	I	II	3.3	206	100	317	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3', HF1.1, HF1.2, HF1.3, HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.2, HF1.3', EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3'
19.95	3-Methyl-1,1'-biphenyl	1478	1486	97.3	III	IV	3.9	168	52	31	EP1.1, EP1.2, EP1.3, EFP2.2, EP2.3, EP3.1, EP3.2, HP2.2, HP2.3, EF1.3, EF3.1, EF3.2
20.14	3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	1482	1491	98.2	I	II	2.9	192	100	368	EP1.1, HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3', HF1.1, HF1.2, HF1.3, HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.1, HF3.2, HF1.3'
20.28	Dodecanenitrile	1485	1490	94.4	III	IV	4.7	181	33	6	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EF2.2
20.33	1,1'-Biphenyl, 4-methyl-	1486	1492	96.7	III	IV	4.6	168	10	20	EP2.2, EP2.3, HP2.2, HP2.3
20.35	Butanoic acid, 1,1-dimethyl-2-phenylethyl ester	1487	1488	99.5	I	II	3.5	220	71	31	HP1.2, HP1.3, HP1.3', HF1.1, HF1.2, HF1.3, HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.1, HF3.2, HF1.3'
20.53	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,7.beta.,8a.alpha.)]-	1491	1492	98.4	I	II	5.2	204	10	14	EP3.1, EP3.2
20.67	2-Tridecanone	1494	1505	96.3	II	III	5.2	198	86	45	EP1.1, EP1.2, EP1.3, EP3.1, EP3.2, EP1.3', EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3'
20.89	.alpha.-Muurolene	1498	1499	98.2	I	II	4.1	204	24	6	EP1.2, EP1.3, EF2.1, EF2.2, EF2.3
21.09	Benzene, 1-methyl-4-(1,2,2-trimethylcyclopentyl)-, (R)-	1502	1505	97.9	I	II	5.5	202	14	40	EP2.1, EF2.1, EF2.3
21.16	1-Dodecanamine, N,N-dimethyl-	1504	1519	82.5	I	II	5.9	213	43	5607	HP2.1, HP3.2, HF1.1, HF1.2, HF1.3, HF2.4, HF2.5, HF2.6, HF1.3'
21.19	Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,7.alpha.,8a.beta.)]-	1504	1503.6	98.7	I	II	4.6	204	43	18	EP1.1, EP1.2, EP1.3, EP2.1, EP1.3', EF2.2, EF2.3, EF2.5, EF1.3'
21.41	o-Hydroxybiphenyl	1509	1506	96.4	III	IV	3.1	170	10	260	HP2.2, HP2.3
21.40	Tridecanal	1509	1512	97.6	I	II	5.4	198	48	9	EP1.1, EP3.1, EP3.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6
21.48	Butylated Hydroxytoluene **(EDC, SML 3)**	1510	1513	99.2	-	V	5.3	220	100	1070	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3'
21.86	Naphthalene, 2-ethoxy-	1518	1521.5	99.5	III	IV	3.8	172	100	759	EP1.1, EP1.2, EP1.3, EP2.1, EP3.1, EP3.2, HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3', HF1.1, HF1.2, HF1.3, HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.1, HF3.2, HF1.3', EF2.1, EF2.2, EF2.3, EF3.1, EF3.2, EF1.3'
21.88	1,4,6-Trimethyl-naphthalene	1518	1505.9	92.4	III	IV	4.8	170	38	74	EP2.2, EP2.3, EP1.3', EF1.1, EF1.2, EF1.3, EF3.1, EF3.2
21.97	trans-Calamenene	1520	1528	90.7	I	II	5.1	202	19	43	EP1.1, EP1.2, EF2.2, EF2.3
22.05	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	1522	1524	97.9	I	II	3.8	204	43	39	EP1.1, EP1.2, EP1.3, EP3.1, EF2.1, EF2.2, EF2.3, EF3.1, EF3.2
22.06	1,1'-Ethylidenebis-benzene	1521	1527	92.6	III	IV	3.6	182	10	81	EP2.2, EP2.3, HP2.2, HP2.3
22.08	Benzenepropanal, 3-(1,1-dimethylethyl)-.alpha.-methyl-	1522	1519.2	97.5	I	II	3.9	204	86	334	HP1.1, HP1.2, HP1.3, HP3.1, HP3.2, HP1.3', HF1.1, HF1.2, HF1.3, HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.1, HF3.2, HF1.3', EF2.2, EF2.3
22.12	Dodecanoic acid, methyl ester	1523	1526	99.4	I	II	5.8	214	91	200	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', EF1.1, EF1.2, EF1.3, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3'
22.19	2-(2-Methylpropyl)-quinoline	1524	1538.6	86.4	III	IV	3.8	185	10	78	HF2.2, HF2.3
22.43	Isoamyl salicylate	1529	1542	96.6	I	II	4.6	208	95	261	EP1.1, EP1.2, EP1.3, EP2.1, EP3.1, EP3.2, EP1.3', HP2.2, HP2.3, HP3.1, HP3.2, HP1.3', HF1.2, HF1.3, HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.1, HF3.2, HF1.3'
22.69	Benzene, (1-butylhexyl)-	1534	1535	98.2	I	II	6.8	218	100	117	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3'
22.87	Naphthalene, 2,3,6-trimethyl-	1538	1540	99.3	III	IV	4.7	170	62	65	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, HP2.2, HP2.3, EF1.1, EF1.2, EF1.3, EF3.1, EF3.2
23.10	Benzene, (1-propylheptyl)-	1542	1546	92.8	I	II	6.8	218	100	113	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3'
23.56	2,2,2-Trichloro-1-phenylethyl acetate	1552	1560.6	97.8	III	IV	3.5	268	38	46	HP1.3, HP3.1, HP3.2, HF2.2, HF2.3, HF2.4, HF2.5, HP1.3'
23.68	1,6,7-Trimethyl-naphthalene	1554	1568	95.1	III	IV	4.8	170	10	31	EP2.2, EP2.3
23.87	Butanedioic acid, dibutyl ester	1558	1557.8	96.7	I	II	2.9	230	14	36	HP2.1, HP2.2, HP2.3
23.98	Benzene, (1-ethyloctyl)-	1560	1568	96	I	II	6.8	218	67	122	EP1.1, EP1.2, EP1.3, EP2.1, EP3.1, EP3.2, EP1.3', EF1.1, EF1.3, EF2.1, EF2.2, EF2.3, EF2.6, EF1.3'
24.32	2(3H)-Furanone, 5-heptyldihydro-	1567	1574	99	II	III	3.3	184	95	181	HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3', HF1.1, HF1.2, HF1.3, HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.1, HF3.2, HF1.3'

RT	Name	mRI	rRI	Score	Cram	Tox	LogP	MW	Fill	S/N	Presence
24.41	Benzoic acid, 2-hydroxy-, pentyl ester	1568	1570.4	99.7	I	II	5.2	208	100	576	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3', HF1.1, HF1.2, HF1.3, HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.1, HF3.2, HF1.3', EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3'
24.64	1-Penten-3-one, 1-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	1573	1608.2	78.3	I	II	3.4	206	71	115	HP1.1, HP1.2, HP1.3, HP2.1, HP3.1, HP3.2, HP1.3', HF1.1, HF1.2, HF1.3, HF2.1, HF2.2, HF2.3, HF2.5, HF3.2
24.74	Diethyltoluamide	1575	1561.1	91.7	I	II	2	191	10	87	HP2.2, HP2.3
25.25	Butyl caprate	1585	1589	89.8	I	II	5.4	228	10	6	EP2.2, EP2.3
25.35	2-(Methylmercapto)benzothiazole	1587	1607	90.4	III	IV	3.1	181	10	151	HP2.2, HP2.3
25.40	3,3'-Dimethylbiphenyl	1588	1589	97.9	III	IV	4.3	182	10	146	EP2.2, EP2.3
25.42	Diethyl Phthalate **(EDC)**	1589	1594	99.1	-	V	2.5	222	100	320	HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3', HF1.1, HF1.2, HF1.3, HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.1, HF3.2, HF1.3'
25.55	4,4'-Dimethylbiphenyl	1591	1608	92.8	III	IV	5.1	182	10	61	EP2.2, EP2.3
25.59	Cetene	1592	1592	99.6	I	II	8.9	224	100	60	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3'
25.69	Dodecanoic acid, ethyl ester	1594	1595	99.6	I	II	5.6	228	100	643	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3'
25.75	Quinoline, 6-(1-methylpropyl)-	1595	1577.8	90.1	III	IV	3.9	185	24	61	HF1.1, HF1.2, HF2.1, HF2.2, HF2.3
25.79	Benzene, (1-methylnonyl)-	1596	1607	94.8	I	II	6.8	218	100	174	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3'
26.08	2-Tetradecanol	1602	1593	90.5	II	III	6.1	214	5	20	EP2.1
26.09	2-Naphthyl methyl ketone	1602	1604	99.2	III	IV	3.2	170	24	26	HP2.1, HF1.1, HF2.1, HF2.2, HF2.3
26.24	2-Octanone, 1-phenyl-	1605	1611.2	90.8	I	II	4	204	5	28	HP2.1
26.46	Lauryl acetate	1609	1607	95.7	I	II	5.6	228	10	20	EP1.3, EP2.1
26.56	Diphenylamine	1611	1621	93.1	III	IV	3.5	169	5	178	HF3.1
26.58	Di-(p-tolyl)methane	1612	1614.6	96.9	III	IV	4.6	196	10	65	EP2.2, EP2.3
26.85	Benzophenone **(SML 0.6)**	1617	1635	92.7	-	III	3.4	182	100	280	HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3', HF1.1, HF1.2, HF1.3, HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.1, HF3.2, HF1.3'
27.39	Dodecanoic acid, 1-methylethyl ester	1628	1617	96.1	I	II	6.1	242	100	21	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3'
27.63	Benzene, (1-butylheptyl)-	1632	1632	95.8	I	II	7.4	232	76	177	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4
27.91	1-Methyl-7-(1-methylethyl)-naphthalene	1638	1627	92.3	III	IV	5.2	184	10	32	EP2.2, EP2.3
28.26	1-Hexanamine, 2-ethyl-N-(2-ethylhexyl)-N-methyl-	1645	1638.0	97.2	I	II	6.5	256	24	47	HP1.1, HP1.2, HP1.3, HP2.1, HP1.3'
28.49	Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester	1649	1649	98.6	II	III	2.7	226	100	141	HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3', HF1.1, HF1.2, HF1.3, HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.1, HF3.2, HF1.3'
28.62	1-Methyl-3-[(4-methylphenyl)methyl]-benzene	1652	1638.0	89.2	III	IV	4.6	196	10	70	EP2.2, EP2.3
28.79	Hexanoic acid, 3,5,5-trimethyl-, 2-ethylhexyl ester	1655	1660	98.7	I	II	6.2	271	43	14	EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EF2.4, EF2.5, EF2.6, EF3.1, EF3.1
29.10	Benzene, (1-ethylnonyl)-	1661	1667	96	I	II	7.4	232	81	129	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EF1.1, EF1.2, EF1.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3'
29.20	Octane, 1,1'-oxybis-	1664	1654.8	98.3	I	II	6.9	242	100	44	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', HP2.1, HP2.2, HP2.3, EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3'
29.62	Hexyl salicylate	1672	1683	97.6	I	II	5.7	222	100	754	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3', HF1.1, HF1.2, HF1.3, HF2.2, HF2.3, EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3'
29.77	1-Tetradecanol	1675	1676	99.8	I	II	6.2	214	100	26	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3'
29.80	Methanone, (1-hydroxycyclohexyl)phenyl-	1675	1687	91.9	I	II	2.6	204	10	35	HP1.2, HF1.2
30.15	2-Methyl-9H-fluorene	1682	1673	95.6	III	IV	4.5	180	10	42	EP2.2, EP2.3
30.88	2-Pentadecanone	1697	1698	99.3	II	III	6.3	226	100	150	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3'

RT	Name	mRI	rRI	Score	Cram	Tox	LogP	MW	Fill	S/N	Presence
30.96	Benzene, (1-methyldecyl)-	1698	1708	94.9	I	II	7.4	232	100	248	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3'
31.42	2,3-dihydro-1,1,3-trimethyl-3-phenyl-1H-Indene	1707	1714	94.2	III	IV	5.6	236	95	60	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3'
31.56	1,6-diisopropylnaphthalene	1715	1707	95.3	III	IV	5.4	212	91	82	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', EF1.1, EF1.2, EF1.3, EF2.1, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3'
32.26	Cyclohexyl salicylate	1724	1714.1	96.4	I	II	4.2	220	86	46	HP1.1, HP1.2, HP1.3, HP2.1, HP3.1, HP3.2, HP1.3', HF1.1, HF1.2, HF1.3, H4, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.1, HF3.2, EF1.1, EF1.2, EF1.3, EF2.6, EF3.2
32.27	Methyl tetradecanoate	1724	1725	99.2	I	II	6.8	242	62	230	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', EF2.1, EF2.2, EF2.3, EF2.4
32.37	Benzene, (1-pentylheptyl)-	1726	1726	97.7	I	II	7.9	246	43	91	EP1.1, EP1.2, EP1.3, EP2.1, EP1.3', EF1.1, EF2.1, EF2.2, EF2.3
32.61	Benzene, (1-butyloctyl)-	1731	1730	98.1	I	II	7.9	246	38	104	EP1.1, EP1.2, EP1.3, EP2.1, EP1.3', EF2.1, EF2.2, EF2.3
33.10	Octanal, 2-(phenylmethylene)-	1741	1750	95.8	I	II	4.8	216	100	56	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3', HF1.1, HF1.2, HF1.3, HF2.1, HF2.2, HF2.3, HF3.1, HF3.2, HF1.3', EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3'
33.24	Ambrox	1744	1766	87	III	IV	4.7	236	10	41	HP2.1, HF2.1
33.64	3,3'-dichloro-1,1'-biphenyl	1752	1755	97.9	III	IV	5.2	223	14	184	EP2.1, EP2.2, EP2.3
33.79	Benzyl Benzoate	1755	1762	97	I	II	4	212	19	32	HP1.3, HP2.1, HP2.2, HP2.3
33.80	3,5-di-tert-Butyl-4-hydroxybenzaldehyde	1755	1772	93.6	II	III	4.4	234	43	537	EP2.2, EP2.3, HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3'
34.20	Benzene, (1-ethyldecyl)-	1763	1766	99.3	I	II	7.9	246	52	81	EP1.1, EP1.2, EP1.3, EP2.1, EP3.1, EP3.2, EP1.3', EF1.1, EF2.1, EF2.2, EF2.3
34.27	Dibutyl adipate	1765	1766	99.6	I	II	3.1	258	19	70	HP1.3, HP2.1, HP2.2, HP2.3
34.91	Octyl octanoate	1777	1779	99.4	I	II	6.5	256	48	47	EP2.1, EP2.2, EP2.3, EP3.1, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3'
35.22	Pyrimethanil	1783	1792	91.7	III	IV	2.9	199	10	25	HP2.2, HP2.3
35.39	Dodecyl butyrate	1787	1780	93.9	I	II	6.4	256	19	28	EP3.1, EP3.2, EF3.1, EF3.2
35.57	2-(2-Methylphenyl)-1-phenyl-, (Z)-1-propene	1790	1783.8	90.4	III	IV	5.2	208	10	10	EP2.2, EP2.3
35.73	1-Octadecene	1794	1793	99.6	I	II	10	253	95	24	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF1.3'
35.78	Tetradecanoic acid, ethyl ester	1795	1794	99.1	I	II	6.7	256	100	356	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3'
36.09	2-ethylhexyl salicylate	1801	1810	98.7	I	II	5.7	250	100	1237	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', HP2.2, HP2.3, HP3.1, EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3'
36.08	Benzene, (1-methylundecyl)-	1801	1808	96.9	I	II	7.9	246	48	177	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP1.3', EF2.1, EF2.2, EF2.3
36.47	Carbonic acid, bis(2-ethylhexyl) ester	1809	1804.3	98.8	I	II	6.8	286	62	29	EP1.1, EP1.2, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EF2.1, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2
37.39	Isopropyl myristate	1828	1827	99.9	I	II	7.2	271	100	33	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3', EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3'
38.15	Galaxolide	1843	1850	90	III	IV	4.8	258	100	36	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', HP2.2, HP2.3, EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3'
38.15	Benzene, (1-propyldecyl)-	1843	1840	91.5	I	II	8.5	261	5	50	EP2.1
38.83	Tonalid	1857	1843	94.1	I	II	5.3	258	100	213	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', HP3.1, HP3.2, HP1.3', EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3'
38.82	Benzyl salicylate	1857	1869	93.4	I	II	3.2	228	43	25	EP1.1, EP1.2, EP1.3, HP1.1, HP1.2, HP1.3, HF1.2, HF1.3, HF2.1, HF2.2, HF2.3, HF3.1, EF2.2
38.99	Diisobutyl phthalate **(CMR, EDC, SVHC)**	1861	1870	94	-	V	4.1	278	43	177	EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3'
39.74	Homosalate	1876	1904	86.4	I	II	5	262	5	645	EF2.5
40.68	Hexadecanenitrile	1895	1914.2	91.2	III	IV	6.9	237	14	7	EP2.1, EP2.2, EP2.3
40.85	2-Heptadecanone	1899	1902	98.2	II	III	7.3	255	100	38	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3'
41.07	Benzene, (1-methyldodecyl)-	1903	1914	94.4	I	II	8.5	261	57	53	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', EF2.1, EF2.2, EF2.3
41.33	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	1909	1923	96.8	III	IV	3.8	276	33	125	EP2.2, HP1.2, HP1.3, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3'

RT	Name	mRI	rRI	Score	Cram	Tox	LogP	MW	Fill	S/N	Presence
42.07	Hexadecanoic acid, methyl ester	1924	1926	99.8	I	II	7.9	271	100	440	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', HP2.1, HP2.2, HP2.3, EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3'
42.58	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	1935	1943	95.8	II	III	5	292	91	90	EP1.1, EP1.2, EP1.3, EP2.1, EP2.3, EP1.3', HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3', HF1.1, HF1.2, HF1.3, HF2.1, HF2.2, HF2.4, HF2.6, HF3.1, HF3.2, EF1.2, EF2.1, EF2.2, EF2.3, EF2.4
43.00	Benzyl decanoate	1943	1940.9	96.2	I	II	5.7	262	10	14	EP2.2, EP2.3
43.42	Dibutyl phthalate **(CMR, EDC, SVHC, SML 0.3)**	1953	1965	91	-	V	4.7	278	43	55	HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3'
43.91	n-Hexadecanoic acid **(SML 60)**	1963	1968	98.7	-	I	6.4	256	67	18	EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', HF1.1, HF1.2, HF2.1, HF3.1, HF3.2, EF1.3, EF2.2, EF1.3'
43.99	Metolachlor	1961	1968.3	98	III	IV	3.1	284	10	22	HP2.2, HP2.3
44.61	Chlorpyrifos	1975	1973	94	III	IV	5.3	351	10	9	EP3.1, EP3.2
45.29	Hexadecanoic acid, ethyl ester	1991	1993	99.7	I	II	7.8	285	100	567	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3'
46.72	Isopropyl palmitate	2020	2023	99.2	I	II	8.2	299	43	22	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3'
47.52	3-Methylbutyl tetradecanoate	2039	2051	92.5	I	II	8	299	14	7	EP3.1, EP3.2, HP3.1, HP3.2, HP1.3'
49.23	1-Octadecanol	2080	2082	99.6	I	II	8.4	271	100	38	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', HP3.1, HP3.2, HP1.3', EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3'
49.67	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	2090	2092	99.7	I	II	6.9	295	48	12	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', EF3.2
51.22	Methyl stearate	2127	2128	99.8	I	II	9	299	52	60	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', EF2.1, EF2.3
52.60	Linoleic acid ethyl ester	2161	2162	98.9	I	II	7.3	309	52	7	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', EF3.1, EF3.2
53.89	Succinic acid, di(2-ethylhexyl) ester	2192	2181.8	93.6	I	II	6.1	343	14	17	EP2.1, EP2.2, EP2.3
54.02	Octadecanoic acid, ethyl ester	2195	2194	99.3	I	II	8.9	313	81	91	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2
54.96	2-Butenedioic acid (E)-, bis(2-ethylhexyl) ester	2237	2223.4	94.7	I	II	6.5	341	19	12	EP1.1, EP1.2, EP1.3, EP1.3'
55.23	3-Methylbutyl hexadecanoate	2251	2253	98.2	I	II	9.1	327	19	9	EP3.1, EP3.2, EF3.1, EF3.2
55.42	Dodecanoic acid, 2-phenylethyl ester	2261	2264	93	I	II	7.2	305	24	29	EP1.1, EP1.2, EP1.3, EP2.1, EP1.3'
55.52	benzoic acid, 4-(dimethylamino)-, octyl ester	2266	2266	96.8	I	II	5.8	277	5	16	EP2.1
55.65	Tributyl acetylcitrate **(SML 60)**	2272	2250	90.2	-	I	3.3	403	57	614	EP3.1, EP3.2, HF1.2, HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.1, HF3.2, HF1.3', EF2.2, EF2.3, EF2.4, EF3.1, EF3.2
56.37	2-ethylhexyl 4-methoxycinnamate **(EDC)**	2314	2339	87.8	-	V	5.3	290	33	283	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', EF3.1, EF3.2
56.61	Methyl dehydroabietate	2333	2336	95.6	I	II	5.9	315	33	31	EP1.1, EP1.2, EP1.3, EP1.3', EF1.1, EF1.3, EF1.3'
57.39	Hexanedioic acid, bis(2-ethylhexyl) ester **(SML 18)**	2397	2398	99.8	-	II	6.8	371	76	127	EP1.1, EP1.2, EP1.3, EP2.1, EP3.1, EP3.2, EP1.3', HP3.1, HP3.2, HP1.3', EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF3.2, EF1.3'
57.57	9-Octadecenoic acid (Z)-, pentyl ester	2416	2422	96.9	I	II	9.4	353	19	13	EP3.1, EP3.2, EF3.1, EF3.2
58.13	Hexanoic acid, 2-ethyl-, hexadecyl ester	2476	2482.4	98.1	I	II	10.7	369	52	12	EP1.3, EP2.1, EP3.1, EP3.2, EF1.1, EF2.1, EF2.2, EF2.3, EF3.1, EF3.2, EF1.3'
58.72	Diisooctyl phthalate	2549	2542	98.6	I	II	8.5	391	29	102	EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, HP3.1, HP3.2, HP1.3'
59.62	Octocrylene **(SML 0.05)**	2679	2693.3	95.9	-	IV	7.1	362	57	414	EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', EF1.1, EF1.3, EF2.2, EF2.3, EF3.1, EF3.2, EF1.3'

Note: In the **Name** column, content inside the bold parentheses **()** shows if the compound is a CMR, SVHC, or EDC, and its SML value (mg/kg) if there is.
The **mRI** column means measured RI value, while the **rRI** column stands for reference RI value. Integer rRI values are experimental RI from NIST 14 library while others are predicted values calculated by a deep convolutional neural network (Matyushin et al., 2019).
The **Score** column depicts the library matching score given by MS-DIAL.
The **Cram** column is the Cramer rule-based toxicity level predicted by Toxtree. When a compound is CMR, SVHC, EDC, or have SML, prediction is not suitable.
The **Tox** column is the toxicity level assigned in the present study based on the rules proposed in 3.8.
The **LogP** column is the XLogP value retrieved from Pubchem.
Columns **Fill** and **S/N** are calculated by MS-DIAL. Fill (%) = (number of samples that have the compounds detected / total number of samples) * 100. S/N is the average S/N.
The **Presence** column shows the compound was detected in which migrates. E stands for 95% ethanol migration, while H means 3% acetic acid migration.

At the end, 58 prioritized migrants (all toxicity level V and IV compounds) and their concentrations in all pellet samples were quantified/semi-quantified where available (Table III-4.4) since such samples are intended to be used for the manufacture of final products, thus being more representative. Some level IV substances (Cramer class III) were excluded from this list because they are commonly used as flavouring ingredients and have low migration values (μg/kg level) compared to their uses as food additives and therefore should not pose threats to consumers. The quantification detail including standard used for quantification, limit of detection (LOD), limit of quantification (LOQ), and coefficient of determination (R^2) in each food simulant are included in Table III-4.5.

Among the prioritized migrants, many of them were only found in company 2 samples, for example metolachlor (herbicide), 2-(methylmercapto)benzothiazole (fungicide), and pyrimethanil (fungicide). Moreover, one polychlorinated biphenyl (3,3'-dichloro-1,1'-biphenyl) and many chemical intermediates (for dyes or pesticides) including 4-chloro-benzonitrile, 2,4-dichloro-phenol, 3,4-dichloro-benzenamine, and hexadecanenitrile were unique in company 2 samples as well. Their presence could be the result of the inclusion of many non-milk-bottle-origin rHDPE owing to the poor separation of the input in the recycling plant (Fig. III-4.6). These pesticides imply the presence of plastic waste from agricultural field or from bottles used for pesticides in the rHDPE flow, including misuses. Furthermore, two alkenes, 1-dodecene and 1-tetradecene, had migration values higher than their SML (50 μg/kg).

Table III-4.4 Prioritized migrants and their concentrations (μg/kg) from each company's pellet samples into 95% ethanol and 3% acetic acid food simulants

Name	Company 1								Company 2						Company 3				Remarks
	EP 1.1	EP1.2	EP1.3	EP1.3'	HP1.1	HP1.2	HP1.3	HP1.3'	EP2.1	EP2.2	EP2.3	HP2.1	HP2.2	HP2.3	EP3.1	EP3.2	HP3.1	HP3.2	
Butylated hydroxytoluene	4.1±0.2	4.2±0.2	4.9±0.1	2.1±0.1	n.d.	n.d.	n.d.	n.d.	12.2±0.1	2.3± 0.3	1.9±0.1	n.d.	n.d.	n.d.	< 1	< 1	n.d.	n.d.	V
Diethyl phthalate	n.d.	n.d.	n.d.	n.d.	8.7±3.9	11.4±2.5	11.9±3.7	2.3±0.2	n.d.	n.d.	n.d.	47.4±3.1	25.5±1.7	28.2±1.3	n.d.	n.d.	13.7±1.6	13.3±1.8	V
Naphthalene	n.d.	n.d.	n.d.	n.d.	< 0.1	< 0.1	< 0.1	< 0.1	n.d.	14.6±1.8	12.1±0.1	n.d.	3±0.03	2.9±0.1	n.d.	n.d.	0.5±0.1	n.d.	V
Diisobutyl phthalate	n.d.	n.d.	8.8±1.1	n.d.	0.6±0.1	0.8±0.1	0.8±0.2	0.4±0.03	10.7±0.2	24.4±0.5	30.6±4.2	0.8±0.02	1±0.3	0.9±0.1	10.5±0.7	12.4±0.3	0.9±0.1	0.9±0.1	V
Dibutyl phthalate	n.d.	n.d.	n.d.	n.d.	0.1±0.04	0.1±0.03	0.2±0.1	0.1±0.01	n.d.	n.d.	n.d.	0.3±0.01	0.2±0.01	0.3±0.1	n.d.	n.d.	0.2±0.02	0.2±0.01	V
2-ethylhexyl-4-methoxycinnamate	43.4±25	43.9±17	39.5±17	71.9±25	n.d.	n.d.	n.d.	n.d.	82.8±10	212±74	309±13.4	n.d.	n.d.	n.d.	64.9±22	70.7±6.6	n.d.	n.d.	V
Diphenyl ether	104.8±1	51±1.9	58.2±1.9	9.5±0.7	3.7±0.7	3.9±0.5	4.3±0.4	1.8±0.2	596±15	76.7±16	62.3±0.8	20.3±0.2	3.8±0.9	3.6±0	123.1±10	151.7±12	9.4±0.1	9.2±0.3	IV; Flavouring ingredient
Galaxolide	-	-	-	-	n.d.	n.d.	n.d.	n.d.	-	-	-	n.d.	-	-	-	-	n.d.	n.d.	IV; Fragrance in soaps
1-Dodecene	21.2±2.1	33.5±8.8	27.1±1.2	< 1	< LOQ	< LOQ	-	-	57.8±3.8	105.9±1	90±5.1	-	-	-	36±7.6	46.5±11	-	-	IV; SML 50 μg/kg
Tricyclo[4.2.1.1(2,5)]dec-3-en-9-ol	n.d.	n.d.	n.d.	n.d.	-	-	-	-	n.d.	n.d.	n.d.	-	-	-	n.d.	n.d.	-	-	IV
2-Methoxy-naphthalene	-	-	-	n.d.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	IV; Emulsifier, stabilizer
Alpha-terpineol	n.d.	n.d.	n.d.	n.d.	32.8±0.5	31.2±0.1	32±2	9.5±1.5	n.d.	n.d.	n.d.	62.9±0.6	133.7±3	119.1±9	n.d.	n.d.	67.7±5	80.6±12	IV; Flavouring ingredient
2,3-dihydro-1,1,3-trimethyl-3-phenyl-1H-Indene	-	-	-	n.d.	n.d.	n.d.	n.d.	n.d.	-	-	-	n.d.	n.d.	n.d.	-	-	n.d.	n.d.	IV
1,6-diisopropylnaphthalene	4.8±0.2	4.8±0.2	5.4±0	3.8±0.3	n.d.	n.d.	n.d.	n.d.	3.6±0.2	2.1±0.1	2.1±0.1	n.d.	n.d.	n.d.	3.7±0.1	4.3±0.3	n.d.	n.d.	IV
1-Tetradecene	52.2±4.2	52.6±3.9	52.9±5.5	12.5±0.5	-	-	-	-	58.6±1.1	78.3±2.2	64.7±0.4	-	-	-	68.7±17	84.1±16	-	-	IV; SML 50 μg/kg
1-Methyl-4-(1-methylethyl)-3-cyclohexen-1-ol	n.d.	n.d.	n.d.	n.d.	< LOQ	< LOQ	< LOQ	< LOQ	n.d.	n.d.	n.d.	5.7±0.3	10.9±0.1	9.3±1.1	n.d.	n.d.	< LOQ	< LOQ	IV; Flavouring ingredient
1-Methyl-naphthalene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	< 0.1	n.d.	44.4±7.9	40.7±1.1	n.d.	2.6±0.2	2.5±0.04	< 1	< 1	< 0.1	< 0.1	IV; Flavouring ingredient
2,5-cyclohexadien-1-one, 2,6-bis(1,1-dimethylethyl)-4-hydroxy-4-methyl-	n.d.	n.d.	n.d.	n.d.	-	-	-	-	n.d.	n.d.	n.d.	-	-	-	n.d.	n.d.	-	-	IV; NIAS
Octocrylene	39.4±28	44.6±27	35.7±18	57.5±26	n.d.	n.d.	n.d.	n.d.	99.6±20	407±277	770±180	n.d.	n.d.	n.d.	160.7±27	195.2±31	n.d.	n.d.	IV; SML 50 μg/kg
3-Methyl-1,1'-biphenyl	< 1	< 1	< 1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	8.9±0.7	8.4±0.3	n.d.	0.1±0.01	0.1±0.01	< 1	< 1	n.d.	n.d.	IV
Bicyclo[4.2.0]octa-1,3,5-triene	n.d.	n.d.	n.d.	n.d.	-	-	-	n.d.	n.d.	n.d.	n.d.	-	n.d.	-	n.d.	n.d.	-	-	IV
1,4,6-Trimethyl-naphthalene	n.d.	n.d.	n.d.	< 1	n.d.	n.d.	n.d.	n.d.	n.d.	46.9±4	43.4±1.4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	IV
1-Chlorododecane	< 1	< 1	1.1±0.1	n.d.	n.d.	n.d.	n.d.	n.d.	15.7±2.2	13.1±1.2	10.3±0.1	n.d.	n.d.	n.d.	2.4±0.3	2.7±0.3	n.d.	n.d.	IV
2,2,2-Trichloro-1-phenylethyl acetate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-	-	IV
1,6-dimethyl-naphthalene	< 1	< 1	< 1	n.d.	n.d.	n.d.	n.d.	n.d.	< 1	31.2±2.7	30.7±1.2	n.d.	n.d.	n.d.	< 1	< 1	n.d.	n.d.	IV
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	n.d.	n.d.	n.d.	n.d.	n.d.	30.3±26	55.4±21	13.5±1.5	n.d.	< LOQ	n.d.	n.d.	39.9±4.6	45.9±0.5	n.d.	n.d.	15.3±1.2	13.4±1.1	IV; NIAS
Dodecanenitrile	-	-	-	n.d.	n.d.	n.d.	n.d.	n.d.	-	-	-	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	IV
o-Chloroaniline	n.d.	n.d.	n.d.	n.d.	2.9±0.1	< LOQ	< LOQ	n.d.	n.d.	n.d.	n.d.	2.1±0.01	24.5±0.6	21.8±2.3	n.d.	n.d.	n.d.	n.d.	IV; Intermediate
2-Methyl-2-nonanol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-	-	-	n.d.	n.d.	-	n.d.	IV
Diphenylmethane	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	< 0.1	< 0.1	n.d.	n.d.	< 0.1	< 0.1	IV
2,4-Dichlorobenzenamine	n.d.	n.d.	n.d.	n.d.	< LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3.5±0.1	10.5±0.5	12±0.4	n.d.	n.d.	n.d.	n.d.	IV; Intermediate
3,3'-dichloro-1,1'-biphenyl	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	4.6±0.1	8.9±0.1	9.1±0.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	IV; PCB 11
Hexadecanenitrile	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-	-	-	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	IV; Intermediates
2-Methyl-naphthalene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	181±29	142±10	n.d.	4.4±0.6	4.2±0	n.d.	n.d.	n.d.	n.d.	IV
Di-(p-tolyl)methane	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-	-	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	IV
3,4-Dichlorobenzenamine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	118±18	124.8±8	n.d.	n.d.	n.d.	n.d.	IV; Intermediate
4,4'-Dimethylbiphenyl	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	16.8±0.7	16.5±0.3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	IV
3,3'-Dimethylbiphenyl	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	12.9±1	12.6±0.7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	IV
2-Methyl-9H-fluorene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-	-	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	IV
1,6,7-Trimethyl-naphthalene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	65±4.6	64.1±3.3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	IV
2,7-Dimethyl-quinoline	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-	-	n.d.	n.d.	n.d.	n.d.	IV
2,4-Dichlorophenol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-	-	n.d.	n.d.	n.d.	n.d.	IV; Intermediate
1,1'-Ethylidenebis-benzene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-	-	n.d.	-	-	n.d.	n.d.	n.d.	n.d.	IV
1-Methyl-7-(1-methylethyl)-naphthalene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	15.2±0.5	15.1±0.4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	IV

Name	Company 1								Company 2						Company 3				Remarks
	EP 1.1	EP1.2	EP1.3	EP1.3'	HP1.1	HP1.2	HP1.3	HP1.3'	EP2.1	EP2.2	EP2.3	HP2.1	HP2.2	HP2.3	EP3.1	EP3.2	HP3.1	HP3.2	
1,2-Dimethyl-naphthalene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	12.4±1.1	11.6±0.4	n.d.	0.5±0.02	0.5±0.01	n.d.	n.d.	n.d.	n.d.	IV
1-Methyl-3-[(4-methylphenyl)methyl]-benzene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-	-	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	IV
o-Hydroxybiphenyl	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	31.7±1.8	33.9±0.9	n.d.	n.d.	n.d.	n.d.	IV; Flavouring ingredient
Metolachlor	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-	-	n.d.	n.d.	n.d.	n.d.	IV; Herbicide
2-(Methylmercapto)benzothiazole	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-	-	n.d.	n.d.	n.d.	n.d.	IV; Fungicide
2-(2-Methylphenyl)-1-phenyl-, (Z)-1-propene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-	-	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	IV
4-Chlorobenzonitrile	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-	-	n.d.	n.d.	n.d.	n.d.	IV; Intermediate
2-Phenyl-pyridine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-	-	n.d.	n.d.	n.d.	n.d.	IV
Ambrox	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	IV
Pyrimethanil	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	4.3±0.1	4.4±0.3	n.d.	n.d.	n.d.	n.d.	IV; Fungicide
2-(2-Methylpropyl)-quinoline	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-	-	n.d.	n.d.	n.d.	n.d.	IV
1-(2-Chlorophenyl)-ethanone	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-	-	n.d.	n.d.	n.d.	n.d.	IV
Chlorpyrifos	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-	-	n.d.	n.d.	IV; Insecticide
3-(Trifluoromethyl)-benzenamine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-	-	n.d.	n.d.	n.d.	n.d.	IV

Note: the values are expressed as mean±sd in duplicates; E represents 95% ethanol migration while H stands for 3% acetic acid migration; roman numerals in the Remarks column are the toxic classes assigned for the corresponding migrants, detailed in Appendix C; - means detected but not quantified, while n.d. stands for not detected.

Table III-4.5 Quantification detail including R^2, LOD, and LOQ in both simulants

Name	Standard used for quantification	95% ethanol migration				3% acetic acid migration			
		Range	R^2	LOD	LOQ	Range	R^2	LOD	LOQ
Butylated hydroxytoluene	Butylated yydroxytoluene	0.1-9	0.9996	0.004	0.01				
Diethyl phthalate	Diethyl phthalate					0.8-42	0.999	0.13	0.44
Naphthalene	Naphthalene	0.1-9	0.9888	0.01	0.05	0.1-11	0.9964	0.01	0.04
Diisobutyl phthalate	diisobutyl phtalate	0.1-20	0.9939	0.01	0.03	0.1-10	0.9975	0.01	0.03
Dibutyl phthalate	diisobutyl phtalate	0.1-20	0.9939	0.01	0.03	0.1-10	0.9975	0.01	0.03
2-ethylhexyl-4-methoxycinnamate	2-ethylhexyl-4-methoxycinnamate	5-41	0.9992	0.75	2.49				
Diphenyl ether	Diphenyl ether	0.1-11	0.9952	0.03	0.08	0.1-20	0.9989	0.01	0.03
1-Dodecene	1-Dodecene	1-40	0.9966	0.06	0.18	0.1-19	0.9863	0.005	0.02
Alpha-terpineol	Alpha-terpineol					5-98	0.9846	1.10	3.62
1,6-diisopropylnaphthalene	2,6-diisopropylnaphtalene	0.1-10	0.9993	0.003	0.01				
1-Tetradecene	1-Tetradecene	0.1-18	0.9942	0.02	0.05				
1-Methyl-4-(1-methylethyl)-3-cyclohexen-1-ol	alpha-terpineol					5-49	0.9972	1.10	3.62
1-Methyl-naphthalene	1-Methyl-naphthalene	0.1-12	0.9942	0.01	0.03	0.1-15	0.9896	0.02	0.06
Octocrylene	Octocrylene	4-78	0.9996	1.16	3.83				
3-Methyl-1,1'-biphenyl	3-Methyl-1,1'-biphenyl	0.1-10	0.9961	0.01	0.05	0.1-11	0.9997	0.02	0.08
1,4,6-Trimethyl-naphthalene	1-methylnaphtene	0.1-12	0.9942	0.01	0.03	0.1-15	0.9896	0.02	0.06

Name	Standard used for quantification	95% ethanol migration				3% acetic acid migration			
		Range	R^2	LOD	LOQ	Range	R^2	LOD	LOQ
1-Chlorododecane	1-Chlorodecane	0.1-9	0.9996	0.20	0.50				
1,6-dimethyl-naphthalene	1-Methyl-naphthalene	0.1-12	0.9942	0.01	0.03	0.1-15	0.9896	0.02	0.06
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	5-87	0.9946	1.66	5.49	5- 48	0.9901	1.28	4.22
o-Chloroaniline	2,4-Dichlorobenzenamine					5-108	0.9942	0.54	1.78
Diphenylmethane	Biphenyl					0.1-11	0.9995	0.01	0.03
2,4-Dichlorobenzenamine	2,4-Dichlorobenzenamine					5-108	0.9942	0.54	1.78
3,3'-dichloro-1,1'-biphenyl	2,4-Dichlorobiphenyl (PCB-7)	0.1-10	0.9999	0.02	0.06				
2-Methyl-naphthalene	1-Methyl-naphthalene	0.1-12	0.9942	0.01	0.03	0.1-15	0.9896	0.02	0.06
3,4-Dichlorobenzenamine	2,4-Dichlorobenzenamine					5-108	0.9942	0.54	1.78
4,4'-Dimethylbiphenyl	3-Methyl-1,1'-biphenyl	0.1-10	0.9961	0.01	0.05				
3,3'-Dimethylbiphenyl	3-Methyl-1,1'-biphenyl	0.1-10	0.9961	0.01	0.05				
1,6,7-Trimethyl-naphthalene	1-Methyl-naphthalene	0.1-12	0.9942	0.01	0.03	0.1-15	0.9896	0.02	0.06
1-Methyl-7-(1-methylethyl)-naphthalene	1-Methyl-naphthalene	0.1-12	0.9942	0.01	0.03				
1,2-Dimethyl-naphthalene	1-Methyl-naphthalene	0.1-12	0.9942	0.01	0.03	0.1-15	0.9896	0.02	0.06
o-Hydroxybiphenyl	o-Hydroxybiphenyl					10-486	0.9936	2.66	8.78
Pyrimethanil	Pyrimethanil					5-106	0.9867	0.99	3.27

Samples from companies 1 and 3 were much cleaner than those from company 2 in terms of appearances of flakes as well as the detected migrants. The compound 1-dodecene had migration ranged from 21 to 106 μg/kg, which is similar to a previous study (Coulier et al., 2007) where migration test was carried out with iso-octane at 20°C for 2 days. For company 3 samples, one insecticide, chlorpyrifos, was detected in 95% ethanol migration test as well. Additionally, octocrylene and 1-tetradecene migrated about 170 and 70 μg/kg in 95% ethanol, respectively, which is higher than their SML (50 μg/kg). The EDC UV filter, 2-ethylhexyl-4-methoxycinnamate had relatively high migration (about 70 μg/kg) as well. As for 3% acetic acid migration, the main concern comes from the three phthalates for their possible EDC properties. DiBP and DBP had very low migration (< 1 μg/kg) and should not be problematic. However, DEP has relatively high migration (> 10 μg/kg, which is the SML for the non-listed substances in EU 10/2011). Fortunately, the concentration of this compound can be reduced several times by either washing twice or extra decontamination as previously discussed (Fig. III-4.7 and Fig. III-4.8). DEP and DBP were previously detected in the extracts of rHDPE (Dutra et al., 2011; Huber and Franz, 1997), however, their higher potential to migrate into acidic food simulants was not demonstrated.

No pesticides were detected in the migration from company 1 samples though, slightly high migration values were observed for 1-tetradecene and DEP in the samples without applying extra decontamination, which is similar to company 3 samples. However, their migrations were much lower after applying extra decontamination (P1.3') and do not represent a human risk. It is worth noting that these 2 compounds could also be reduced by washing twice the post-consumer flakes. Further, many other prioritized compounds, such as BHT, 1-dodecene, and 1,6-diisopropylnaphthalene had much lower migration or were even not detected after extra decontamination. Similar to company 3 samples, the two UV filters octocrylene and 2-ethylhexyl-4-methoxycinnamate had relatively high migration, 57.5 and 71.9 μg/kg, respectively. Unfortunately, extra decontamination did not work well on these two compounds as above discussed. Nevertheless, they were not detected in 3% acetic acid migration implying that they prefer to migrate into fatty foods. The results show that these rHDPE

could not be used for high-fat content food packaging, but it could be adequate for acidic foods in terms of migration of (semi-)volatile compounds. It is interesting that octocrylene and 2-ethylhexyl-4-methoxycinnamate are common UV filters in cosmetics, but they were found in all rHDPE samples originated from milk bottles. The result suggests that they could be incorporated in the formulation of rHDPE milk bottles to protect fat matter in milk from light oxidation which causes bitter taste. However, as far as we know, this is the first time that these UV filters are reported in rHDPE. To evaluate whether they came from the HDPE milk bottles or from contamination, 4 HDPE milk bottles from various brands were purchased from the local supermarkets and tested. Neither octocrylene nor 2-ethylhexyl-4-methoxycinnamate were detected in any sample. Therefore, they might be common contaminants from cosmetic packaging waste but are not intentionally added to the HDPE milk bottles. This fact demonstrates that better classification of the input is required to get only post-consumer milk (or food in general) bottles. Considering their high concern and the difficulty to remove them from rHDPE, measures to mitigate cross contaminations from cosmetic/personal care packaging could be of great help to get high quality rHDPE. Additionally, these two compounds had much lower sensitivities (LOD) than many other migrants (Table III-4.5), and thus could be easily overlooked when manually picking peaks of concern in chromatograms based on peak size.

5. Conclusions

In the present work, hierarchical clustering analysis was employed to investigate whether the chemical compositions of various batches of rHDPE milk bottles from 3 recyclers vary considerably. As anticipated, the composition from the 3 recyclers were different. In addition, well classified samples from companies 1 and 3 (with negligible non-milk-bottle rHDPE contamination) showed rather consistent chemical composition between batches, while poorly classified samples from company 2 (containing many non-milk-bottle rHDPE) varied remarkably among batches. The chemical removal efficiencies of two cleaning procedures were evaluated by fold change analysis. Both washing twice and extra decontamination techniques showed comparable efficiencies. About 50% of chemicals had half chromatographic responses after applying the two

techniques. However, the two techniques are not directly comparable since they were not applied to the same samples. A noticeable discrepancy was that extra decontamination was able to reduce the migration of 1-tetradecene to a safe level while washing twice did not.

Quantitative analysis of the prioritized migrants showed that impure milk bottle rHDPE samples (from company 2) were contaminated with several pesticides and one polychlorinated biphenyl, which are of very high concern. Besides, many prioritized migrants were only detected in this set of samples and some of them had migration values several times higher than their SML. Consequently, this type of rHDPE might not be suitable for food contact uses. Samples from companies 1 and 3 had negligible non-milk-bottle rHDPE contamination and much less prioritized chemicals were detected in migration tests. However, there were still migrants of high concern, with relatively high migration, such as 1-dodecene, 1-tetradecene, octocrylene, and 2-ethylhexyl-4-methoxycinnamate. The former two can be sufficiently lowered by extra decontamination, while the latter two cannot be reduced by either washing twice or extra decontamination. Octocrylene, and 2-ethylhexyl-4-methoxycinnamate have molecular weight 360 and 290 Da, respectively. As it is reported (Palkopoulou et al., 2016), decontamination yield in polyolefins strongly drops with increasing molecular weight.

The present work contributes to the scientific knowledge by demonstrating that fold change analysis constitutes a good way to examine the efficacy of decontamination techniques applied to recycling of plastics, allowing the evaluation of intensity changes for every detected compound, and focusing on the changes of high concern substances combined with toxicity data.

From a practical point of view, we have pointed out that special attention should be paid for octocrylene and 2-ethylhexyl-4-methoxycinnamate when recycling HDPE from milk bottles for new food contact uses in terms of decontamination as well as legislation. Deeper studies are required to know whether they are common in rHDPE milk bottles samples or not. In the worst cases, while they are not easy to remove,

additional decontamination procedures at industrial level should be considered, although we can suggest enhancing sorted collection as the most interesting and simple option to avoid cross contamination, which constitutes the main limitation of the present system for collecting and recycling HDPE.

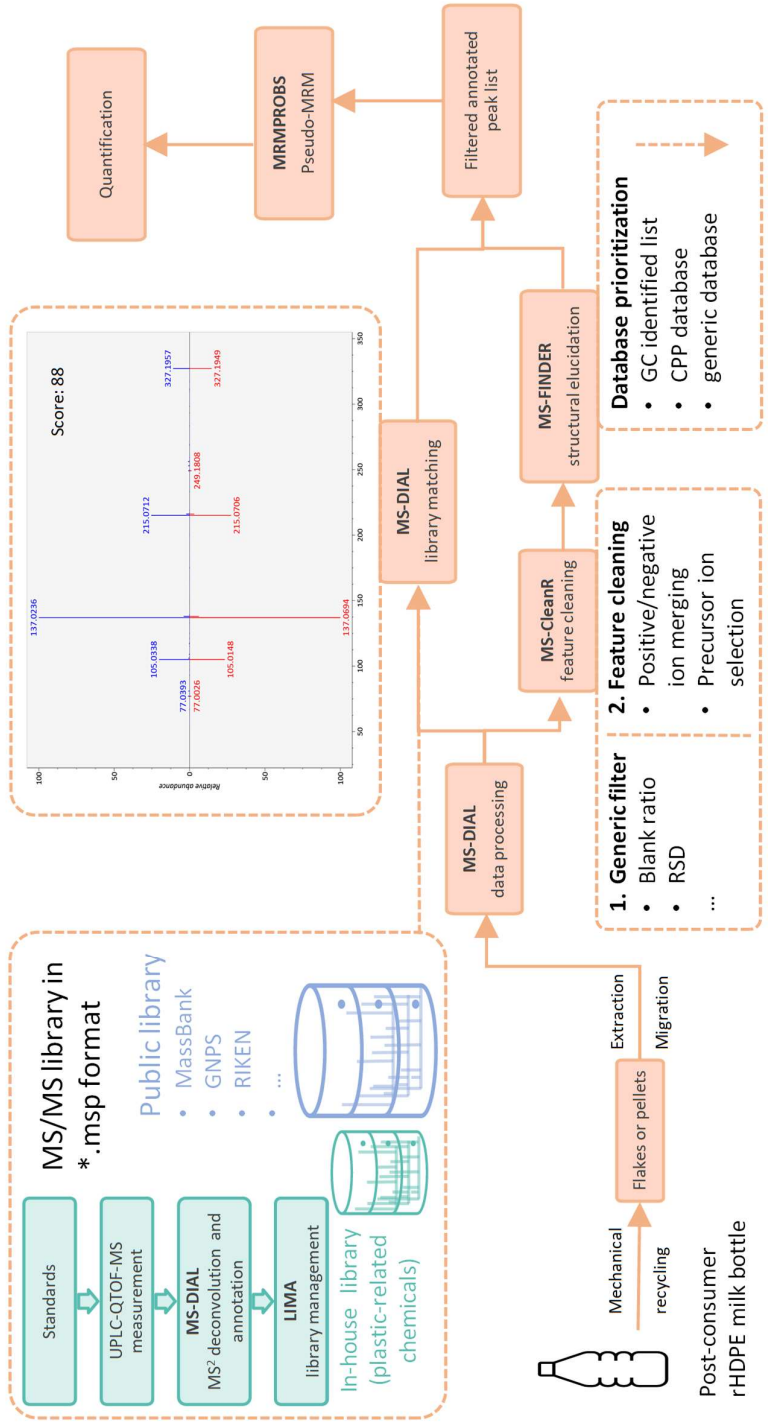
To better understand the main factors affecting the decontamination efficacy, accessible process decontamination conditions will be helpful. It has to be mentioned that the present work used pellets for migration tests, which would overestimate the results. In this sense, further research focused on migration tests by using real bottles manufactured from rHDPE will surely provide more realistic results. Despite the huge amount of information generated in this study, it has been limited to the determination of volatile compounds. A similar work with focus on non-volatile organic compounds as well as metals, would give an overall knowledge conducing to an optimum harnessing of HDPE bottle. This will be the subject or further research.

Chapter 5

Combination of Structure Databases, In-Silico Fragmentation, and MS/MS Libraries for Untargeted Screening of Non-volatile Migrants from Recycled High-Density Polyethylene Milk Bottles

1. Abstract

Non-volatile compounds present in recycled high-density polyethylene were untargetedly screened for the first time, by a comprehensive ultra-high performance liquid chromatography-quadrupole-time-of-flight mass spectrometry. There were 83 compounds identified in total and 66 of them were easily annotated by MS-DIAL making use of publicly available and in-house MS/MS libraries. Moreover, MS-CleanR was employed to clean up features exported from MS-DIAL, keeping merely precursor ions truly coming from the samples for subsequent structural elucidation. An *in-silico* fragmentation tool (MS-FINDER) combing chemicals preciously identified in the same set of samples by gas chromatography coupled to mass spectrometry as well as compounds associated with plastic, facilitated and improved the identification of remained unknowns. Once chemicals were identified, a pseudo multiple reaction monitoring method was applied for sensitive target screening of their presence in the samples. Quantification results demonstrated that well separated rHDPE milk bottle samples (with very limited amount of non-milk-bottle plastics) after mechanical recycling could be used for contacting acidic food. However, removal or reduction of octocrylene and 2-ethylhexyl-4-methoxycinnamate from these samples is vital for their safety uses for high fatty foodstuff.


Post-consumer rHDPE milk bottle
Mechanical recycling
Flakes or pellets
Extraction
Migration
Standards
UPLC-QTOF-MS measurement
MS-DIAL MS² deconvolution and annotation
LIMA library management
In-house library (plastic-related chemicals)
MS/MS library in *.msp format
Public library
• MassBank
• GNPS
• RIKEN
• ...
MS-DIAL data processing
1. Generic filter
• Blank ratio
• RSD
...
MS-CleanR feature cleaning
2. Feature cleaning
• Positive/negative ion merging
• Precursor ion selection
MS-FINDER structural elucidation
Database prioritization
• GC identified list
• CPP database
• generic database
MS-DIAL library matching
Score: 88
Filtered annotated peak list
MRMPROBS Pseudo-MRM
Quantification

2. Introduction

Closed-loop recycling of food contact plastics is attracting increasing interest under the context of circular economy. According to EFSA, to recycle polyethylene terephthalate (PET) for food contact uses, a minimum of 95% recycled PET should come from food packaging (EFSA, 2011). This number can be even higher for high-density polyethylene (HDPE) (De Tandt et al., 2021). In Spain, plastic packaging is mixed collected with other packaging such as metal and cartons in yellow containers. Among them, HDPE milk bottles can be easily and automatically sorted thanks to the intermediate carbon black containing layer which acts as a protection against UV light. This intermediate layer enables HDPE milk bottles to be separated from other HDPE packaging making use of near-infrared spectroscopy (NIR). Therefore, high purity recycled HDPE (rHDPE) milk bottle materials are attainable at an industrial level, making it possible to be closed-loop recycled.

To closed-loop recycle food contact plastics, recycled materials may not cause negative effect on consumer health regarding chemical migration according to European Commission (EU) regulations EC 1935/2004 (EC, 2004) and EU 10/2011(EC, 2011). Hence, safety assessment of the recycled materials, which serves as a base for the regulatory framework in many countries (Cecon et al., 2021), is paramount with respect to consumer health. Some studies have investigated migratable substances from rHDPE in the last decades (Coulier et al., 2007; Dutra et al., 2011; Huber and Franz, 1997; Salafranca et al., 1999a, 1999b; Welle, 2005). Recently, odorants were examined (Cabanes et al., 2020; Strangl et al., 2018, 2019, 2021) and volatile migrants were untargetedly screened using sensitive direct immersion - solid-phase microextraction coupled to gas chromatography - mass spectrometry (GC-MS) (Su et al., 2021a). These studies are mainly focused on volatile substances with well-developed GC-MS and commercial libraries. However, as far as we know, non-targeted screening of non-volatile

compounds in recycled polyolefins, which is more sophisticated and requires expensive high-resolution mass spectrometry (HRMS) and more expertise, is still missing.

Identification of non-volatile substances in FCM employing HRMS together with vendor software has been well documented (Martínez-Bueno et al., 2019; Nerín et al., 2013; Peters et al., 2019). In the last decade, various open-source tools like XCMS (Benton et al., 2004), MZmine 2 (Pluskal et al., 2010), MS-DIAL (Tsugawa et al., 2015) and *in-silico* fragmentation tools, e.g., MetFrag (Ruttkies et al., 2016), MS-FINDER (Tsugawa et al., 2016), and SIRIUS 4 (Dührkop et al., 2019), have been developed to facilitate and improve the handling of HRMS data in the metabolomics community. Nonetheless, they are, in principle, applicable to the identification of any small molecule (Ljoncheva et al., 2020). These tools are of major interest in light of innovative, open and reproducible science (Stanstrup et al., 2019). In contrast to vendor software, they are able to leverage publicly available MS/MS libraries like MassBank, RIKEN and GNPS libraries, and can connect to advanced tools, for example, CAMERA (Kuhl et al., 2012), MS-CleanR (Fraisier-Vannier et al., 2020), and CliqueMS (Senan et al., 2019) for feature (mass-retention time pair) cleaning.

Complementing our previous study on volatile compounds in rHDPE (Su et al., 2021b), the present study aims to untargetedly screen non-volatile migrants, which come from rHDPE milk bottles, in 2 food simulants (95% ethanol and 3% acetic acid) by ultra-high performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS) and advanced data processing workflow. Various publicly accessible and in-house MS/MS libraries were firstly compiled and utilized for the identification in MS-DIAL. MS-CleanR was then employed to clean up redundant features which include multiple adducts and a large number of in-source fragments. An *in-silico* fragmentation tool (MS-FINDER) was finally applied to identify the remaining unknowns, taking advantage of the list of chemicals previously identified in the same set of samples by GC-MS, as well as a list of chemicals associated with plastic packaging compiled by Groh et al (Groh et al., 2019). After the chemicals were annotated, *pseudo* multiple reaction monitoring (*pseudo*-MRM) using parent-product ion pairs exported from MS-DIAL was employed as a sensitive targeted

analysis to determine the presence of each identified substance in the samples by MRMPROBS program. Finally, the concentrations of the annotated compounds in the simulants were quantified.

3. Materials and methods

3.1. Standards and samples

The following analytical standards were purchased from Sigma-Aldrich (Madrid, Spain): aminophenazone (58-15-1), o-anisidine (90-04-0), 2,4-dimethylbenzenamine (95-68-1), caprolactam (105-60-2), caffeine (58-08-2), N,N-bis (2-hydroxyethyl)dodecylamine (1541-67-9), dimethyldibenzylidene sorbitol (135861-56-2), N-[3-(dimethylamino)propyl]dodecanamide (3179-80-4), N,N-dimethyltetradecylamine (112-75-4), pyrimethanil (53112-28-0), N,N-dimethylhexadecylamine (112-69-6), N-methyldidecylamine (7396-58-9), 3,3'-dichlorobenzidine (91-94-1), tebuconazole (80443-41-0), 7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione (82304-66-3), lauric acid diethanolamide (120-40-1), diflufenican (83164-33-4), tributyl citrate (77-94-1), tributyl acetylcitrate (77-90-7), octocrylene (6197-30-4), avobenzone (70356-09-1), 2-stearoylglycerol (621-61-4), Chimassorb 81 (1843-05-6), dioctyl phthalate (117-84-0), bis(2-ethylhexyl) adipate (103-23-1), erucamide (112-84-5), Irgafos 168 (31570-04-4), Irganox 1010 (6683-19-8), tris(2,4-ditert-butylphenyl)phosphate (95906-11-9), Irganox 1076 (2082-79-3), glycerol dihexanoate (502-52-3), ethyl 4-(dimethylamino)benzoate (10287-53-3), oxybenzone (131-57-7), 2-ethylhexyl-4-methoxycinnamate (83834-59-7), palmitamide (629-54-9), palmitic acid (57-10-3), propanil (709-98-8), oleic acid (112-80-1), thiabendazole (148-79-8), 1-octylpyrrolidin-2-one (2687-94-7), docosanamide (3061-75-4), diisodecyl phthalate (89-16-7), 1,2,3-trideoxy-4,6:5,7-bis-o-[(4-propylphenyl)methylene]-nonitol (NX 8000 K, 882073-43-0), and 2,5-bis(5-tert-butylbenzoxazol-2-yl)thiophene (7128-64-5). 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionic acid (20170-32-5) was from Enamine (Riga, Latvia).

Descriptions about the samples were previously detailed in our previous article (Su et al., 2021b). In brief, rHDPE milk bottles in flakes (F) and pellets (P) forms were

supplied by 3 Spanish companies. Samples F1.1, F1.2, F1.3, F1.3', P1.1, P1.2, P1.3, and P1.3' were from company 1; samples F2.1, F2.2, F2.3, F2.4, F2.5, F2.6, P2.1, P2.2, and P2.3 were from company 2; samples F3.1, F3.2, P3.1, and P3.2 were from company 3.

3.2. Solid-liquid extraction

All rHDPE samples were grounded into fine powders by an ultra-centrifugal mill (Retsch ZM 200; Haan, Germany). Five millilitres of dichloromethane were then employed to extract the sample (1.00 g) assisted by ultrasonic bath (Brasonic 3510-MTH; Connecticut, USA) for 1 h. The extraction was consecutively conducted 3 times with fresh dichloromethane used in each. Subsequently, the 3 extracts were collected together in an 18 mL vial and evaporated to dryness by a gentle flow of nitrogen (Techne DB-3; Staffordshire, UK) at 40 °C. Then, the extract was re-dissolved with 0.4 mL methanol for 5 min under ultrasonication and 30 s vortex mixing. Prior to UPLC-QTOF-MS analysis, the extract was filtered through a 0.2 μm Acodisc GHP syringe filter (Corporation, New York, USA). Samples and procedural blanks were simultaneously prepared in triplicate. Quality control (QC) sample was pooled from filtered extracts (50 μL from each sample).

3.3. Migration test

Neither the pellets nor the flakes are the final food contact articles. According to the regulation EU 10/2011, 6 dm^2/kg contact surface to volume ratio should be used for migration test. However, it is difficult to measure the surface area of the pellets and flakes because of their irregular shapes. Therefore, a *pseudo*-migration (leachable) or extractability test was carried out, in which estimated surface area was used. Pellets were cylinder-like, and their surface area were approximated by measuring their diameters and heights (data has already been shown in our previous study (Su et al 2021). For flakes, the weight approach (average weight of the pellets used for migration) was employed. Only 95% ethanol and 3% acetic acid food simulants were used as the worst-case scenario emulating fatty and acidic foods. To simulate the long-term storage

(> 6 month) at room temperature, 10 days at 60 °C was applied. Samples as well as procedural blanks were simultaneously prepared in duplicate.

3.4. UPLC-QTOF-MS analysis

A Waters Acquity UPLC equipped with an Atlantis™ premier BEH C18 AX column (2.1 × 100 mm) of 1.7 μm particle size (Milford, MA, USA) was employed for the separation. Column temperature was set at 40 °C under the flow of 0.3 mL/min. Water and methanol, both spiked with 0.1% formic acid, were the mobile phase A and B, respectively, for both positive and negative modes. A 13 min run was used with the following gradient elution: initial mobile phase A/B 95/5 was shifted to A/B 100/0 in 7 min, kept for 4 min, then dropped to the initial mobile phase in 0.1 min, and maintained for additional 1.9 min to get the system ready for the next injection. Injection volume was 10 μL.

The high-resolution mass spectrometer (QTOF-MS) was coupled to the UPLC by an electro spray ionization (ESI) probe. The conditions employed were as follows: resolution mode, capillary voltage 3.0 kV, sampling cone voltage 45 V, extraction cone 4.0 V, source temperature 150 V, desolvation temperature 350 °C, cone gas flow rate of 40 L/h, and the desolvation gas flow rate of 600 L/h. Data independent analysis (DIA), named MS^E, was used for data acquisition. In the MS^E, low energy (6 V) in the ionization chamber provided the precursor information (MS^1), while ramp high energy in the collision cell (10 - 30V) enabled the acquirement of fragment ions (MS^2), which is critical for structural elucidation. Mass ranged from 50 to 1200 Da in both functions. Leucine enkephalin (CAS 58822-25-6) at 2 ng/mL was employed for on-line mass correction. Test-mix from Waters was injected every 20 injections to ensure the accuracy of the measurements.

3.5. UPLC-QTOF-MS data processed by MS-DIAL

The UPLC-QTOF-MS data was processed by MS-DIAL (version 4.38) (Tsugawa et al., 2015). The settings were as follows: MS^1 and MS^2 tolerances were 0.01, and 0.025 Da, respectively; maximum charged number of 2 and considering Cl and Br

elements for isotope recognition; minimum peak height of 3000 and mass slice width of 0.1 Da; sigma window value of 0.5 and MS^2 abundance cut-off of 10 for the deconvolution; adducts in negative mode were $[M-H]^-$, $[M+FA-H]^-$, $[M+Hac-H]^-$, $[2M-H]^-$, $[2M+FA-H]^-$, and $[2M+Hac-H]^-$; adducts in positive mode were $[M+H]^+$, $[M+NH_4]^+$, $[M+Na]^+$, $[M+K]^+$, $[2M+H]^+$, $[2M+NH_4]^+$, $[2M+Na]^+$, and $[2M+K]^+$; retention time tolerance of 0.05 min and MS^1 tolerance of 0.015 Da for alignment; features (mass-retention time pairs in MS^1) that had sample max/blank average fold change lower than 5 were removed.

3.6. Compiling MS/MS libraries for non-targeted screening

The MS-DIAL developer has compiled a wide range of publicly available MS/MS spectra, for example, MassBank, MassBank-EU, Fiehn/Vaniya natural product library, etc., into a united library for positive mode, and negative mode alike. In addition, many other libraries, e.g., FDA libraries, NIH clinical collections, and pesticides were downloaded from GNPS (https://gnps.ucsd.edu/ProteoSAFe/libraries.jsp, accessed on 08/09/2020). The GNPS libraries, which are in *.mgf format, were not directly feasible for MS-DIAL. Therefore, they were firstly converted to the *.msp format and then combined with the MS-DIAL library accordingly in R programming (example R code shown below).

```
getmgf_GNPS_pos <- function(file){ # define a function  to convert *.mgf library to *.msp format
  msp <- readLines(file) # read msp as lines, and then manipulate it
  ncomp <- grep('^BEGIN IONS', msp, ignore.case = TRUE) # ncomp stands for number of compounds
  splitFactorTmp <- rep(1:length(ncomp), diff(c(ncomp, length(msp) + 1))) # determine the positions to separate each compound
  li <- split(msp, f = splitFactorTmp) # put each compound as a list element
  getmsp <- function(x){ # define a function to extract the content of each entry
    namet <- x[grep('^NAME=', x, ignore.case=TRUE)] # extract the entry that includes Name information
    name <- gsub('^NAME=', '', namet, ignore.case=TRUE) # extract Name information
    premzt <- x[grep('^PEPMASS=',  x, ignore.case=TRUE)] # extract the entry that includes Precursor ion information
    premz <- gsub('^PEPMASS=', '', premzt, ignore.case=TRUE) # extract Precursor ion information
    pretype <- str_extract(name, "\\[?[0-9]?M(\\+|-).*$") # extract the entry that includes Precursor type (adduct)
    if(grepl('\\[', pretype)){
      pretype <- paste0(pretype, "+")
    }else{
      pretype <- paste0("[", pretype, "]+")}
    name <- gsub(' \\[?[0-9]?M(\\+|-).*$', '', name, ignore.case=TRUE) # extract Precursor type information
    spetypet <- x[grep('^MSLEVEL', x, ignore.case = TRUE)] # extract the entry that includes Spectrum type Information
    spetype <- gsub('^MSLEVEL=', '', spetypet, ignore.case = TRUE) # extract Spectrum type information
    ionmodet <- x[grep('IONMODE=', x, ignore.case = TRUE)] # extract the entry that includes Ion mode information
    ionmode <- gsub('IONMODE=', '', ionmodet, ignore.case = TRUE) # extract Ion mode information
    smilest <- x[grep('^SMILES=', x, ignore.case = TRUE)] # extract the entry that includes Smiles information
    smiles <- gsub('^SMILES=', '', smilest, ignore.case = TRUE) # extract Smiles information
    instrut <- x[grep('SOURCE_INSTRUMENT=', x, ignore.case = TRUE)] # extract the entry that includes Instrument type
    instru <- gsub('SOURCE_INSTRUMENT=', '', instrut, ignore.case = TRUE) # extract Instrument type information
    np <- length(which(grepl('^[0-9]', x))) # determine the number of product ions in the spectrum

    if(as.numeric(np) > 0){
      # matrix of masses and intensities
      massIntIndx <- which(grepl('^[0-9]', x) & !grepl(': ', x)) #give the index of mass values
      massesInts <- unlist(strsplit(x[massIntIndx], '\t| ')) # change mass list into a vector
      massesInts <- as.numeric(massesInts[grep('^[0-9].*[0-9]$|^[0-9]$', massesInts)])
      mz <- massesInts[seq(1, length(massesInts), 2)]
      ins <- massesInts[seq(2, length(massesInts), 2)]
      spectra <- cbind.data.frame(mz=mz,ins=ins)
      return(list(Name=name, PrecursorMZ=premz, PrecusorType=pretype, SpectrumType=spetype,
                  IonMode=ionmode, Formula=NA, SMILES=smiles, InChIKey=NA,
                  Ionization=NA, InstrumentType=instru, CollisionEnergy=NA,
                  RetentionTime=NA, CCS=NA, Ontology=NA, Comment=paste(PI, DataColletor, Submituser, LibraryQuality, sep = "; "),
                  'Number of peaks' = np, Spectra=spectra))
    }else{
      return(list(Name=name, PrecursorMZ=premz, PrecusorType=pretype, SpectrumType=spetype,
                  IonMode=ionmode, Formula=NA, SMILES=smiles, InChIKey=NA,
                  Ionization=NA, InstrumentType=instru, CollisionEnergy=NA,
                  RetentionTime=NA, CCS=NA, Ontology=NA, Comment=paste(PI, DataColletor, Submituser, LibraryQuality, sep = "; "),
                  'Number of peaks' = np))}
  }
  li <- lapply(li,getmsp) # apply the define function (getmsp) to the li list
  return(li)
}

# download positive libraries into a folder, and process them all together with the previously defined function
GNPSsite <- list.files(path = "data folder", pattern = "*.mgf", full.names = TRUE)
GNPScollect_pos <- do.call(c, lapply(GNPSsite, getmgf_GNPS_pos))
```

Moreover, a home-built MS/MS library, which contains 449 and 172 mainly food packaging associated chemicals in positive and negative mode, respectively, were merged as well. The building of MS/MS libraries followed the strategy proposed by Tada et al. (Tada et al., 2019). Briefly, standard solutions with various concentrations were injected by UPLC-QTOF-MS under the same conditions used in this study. MS/MS spectra of the standards were then deconvoluted by MS-DIAL and managed by a library manager named MS-LIMA (Tada et al., 2019). A screenshot of the in-house library is shown in Fig. III-5.1. The compiled positive and negative MS/MS libraries were then employed for the identification in MS-DIAL. MS^1 and MS^2 mass tolerance

for identification were 0.01 and 0.05 Da, respectively. Further, the identification score cut-off was 80%.

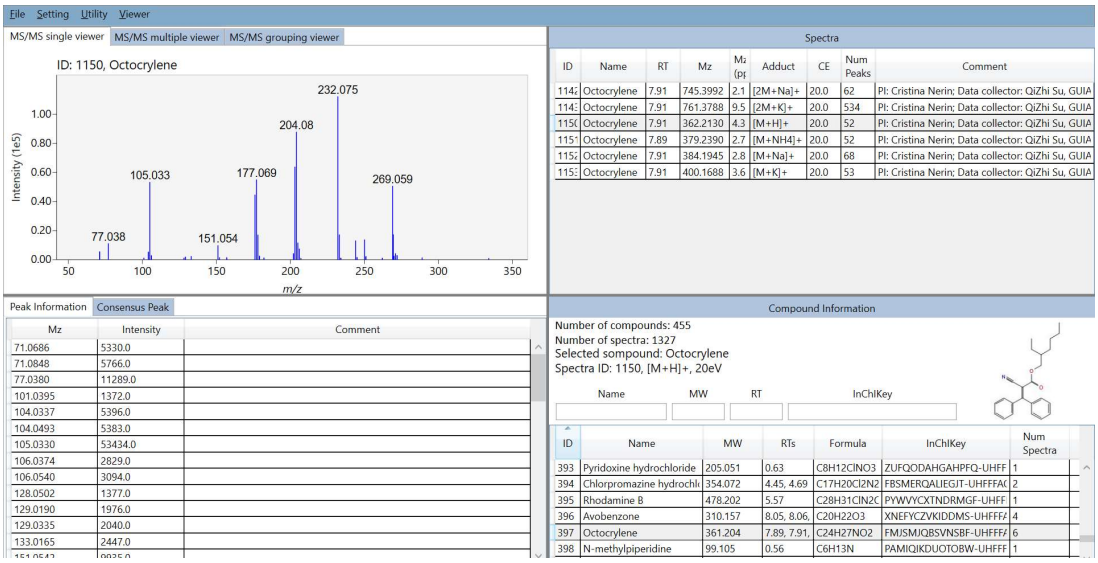


Fig. III-5.1 Screenshot of the in-house library (positive mode)

3.7. MS-CleanR feature cleaning and structural elucidation by MS-FINDER

MS-CleanR, a R package (Fraisier-Vannier et al., 2020), was applied to clean up the feature table generated by MS-DIAL. The MS-CleanR is a shiny R package which is easy to use and requires no coding. It was developed to seamlessly work with the MS-DIAL data. The first step in MS-CleanR was to remove features that had blank ratio (blank/QC) and relative standard deviation (in QC) higher than 0.5 and 30, respectively. Subsequently, the features were grouped into clusters based on the MS-DIAL pre-calculated links, Pearson correlation, and so on. The minimum Pearson correlation was 0.8 at the maximum p-value of 0.05. The maximum mass and retention time difference for Pearson correlation and positive/negative mode merging were 0.005 and 0.025, respectively. It deserves to be mentioned that the same mobile phases must be used in order to correlate peaks in positive and negative modes since the pH value of mobile phases is well-known to have great impact on the retention behaviour of some compounds. Besides, adducts were corrected based on the links previously found. In theory, one cluster represents an individual substance and features within the same cluster are different forms of the substance, e.g., adducts or in-source fragments. Both

the most intense and most connected features were kept for the following structural elucidation in MS-FINDER, which is a hydrogen rearrangement rules based *in-silico* MS/MS fragmentation software (Tsugawa et al., 2016).

In MS-FINDER, 0.005 and 0.025 Da tolerances were employed for MS^1 and MS^2, respectively. Relative abundance cut-off was 1%. Elements C, H, O, N, P, S, F, and Cl were selected. LEWIS and SENIOR valence rules were used, and isotopic ratio tolerance was 20% to reduce the number of formula candidates. Advance settings for AIF (all ions fragmentation) were checked with all adducts described in section 3.5 selected, which will consider the fragmentations of various adducts as well. Three structure databases were applied for the identification, namely: volatile and semi-volatile substances identified by GC-MS in the same set of samples in our previous study (**volDB**) (Su et al., 2021b), chemicals associated with plastic packaging (**cppDB**) compiled by Groh K. J. etc. (Groh et al., 2019), and a generic database (**genDB**) integrated in MS-FINDER. The **genDB** includes only FoodDB (Food), PlantCyc (Plant), T3DB (Toxin), STOFF (Environment), NPA (Natural Products Atlas), KNApSAcK (Natural product), NANPDB (Natural product), and UNPD (Natural product) because they could be contaminants in recycled plastics. Weights given to the three structure databases in MS-CleanR were 2, 1.5, and 1, respectively.

In theory, every feature kept by MS-CleanR represents a single compound. However, some of them did not have representative MS/MS and are meaningless for structural elucidation. Therefore, they were eliminated from the final results when visually checking the identification of each remained feature even though they had "good match" candidates.

3.8. Pseudo-multiple reaction monitoring (Pseudo-MRM) by MRMPROBS

MRMPROBS (multiple reaction monitoring based probabilistic system) is an open-source software launched by the same developer as MS-DIAL. It was initially designed for large-scale targeted metabolomics with the aim to overcome the time-consuming, often subjective and makeshift manual data assessment by automated posterior probabilistic (Tsugawa et al., 2013). In the present study, MRMPROBS was

used as a pseudo-MRM approach to determine the presence of an identified compound in each sample. Firstly, the top 5 product ions of identified compounds were exported as a MRMPROBS library (transitions in MRM) in MS-DIAL. Secondly, this set of transitions was used for MRM in MRMPROBS to automatically detect and identify the presence of these compounds in the samples. In contrast to conventional MRM analysis, no re-acquisition of MRM data by triple quadrupole mass spectrometry (QqQ/MS) is required here. The MRMPROBS program used directly the DIA data set (used for nontargeted screening in MS-DIAL) for the analysis. That is why it is called pseudo-MRM. In MRMPROBS, smoothing level of 1 scan, minimum peak width of 5 scans, and minimum peak height of 100 were used for peak detection. For the identification, retention time tolerance, amplitude tolerance, and minimum posterior score were 0.1 min, 15%, and 60%, respectively. Finally, only when a peak had peak area 3 times higher than the blanks was counted as being present in the corresponding sample.

4. Results and discussions

4.1. Identification of substances by matching libraries

Identification of substances was firstly done by matching the compiled MS/MS libraries (section 3.6) in MS-DIAL. As shown in Table III-5.1, there were 66 compounds identified either in extracts or migrates by library search (score > 80). Few compounds were included regardless of their relatively low scores as they had only few product ions and the large number of tiny and noisy signals negatively affect the match scores, for example, N-[3-(dimethylamino)propyl]dodecanamide and dimethyldibenzylidene sorbitol. However, they were confirmed by standards. The open libraries are not plastic-specific though, recycled plastic might contain contaminants from environment and food residues, etc. In the present study, many pesticides, e.g., propanil and pyrimethanil, were easily identified by matching the libraries. In addition, some common plastic-relevant chemicals, e.g., caprolactam, 7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione, and Irgafos 168, were characterized with the help of our in-house library.

Table III-5.1 Migrants identified in 95% ethanol or 3% acetic acid food simulants

N°	RT	Precursor	Adduct	Name	Score	Fill	Matrix	Cram	Tox	Remark	Presence
1	**0.83**	341.1081	$[M-H]^-$	sucrose	Lmatch (86)	14.3	95EtOH	-	I	SML 60	EF2.1, EF2.2, EF2.3
2	**0.95**	232.1456	$[M+H]^+$	**aminophenazone**	genDB (6.2)	9.5	95EtOH	III	IV	drug	EP2.2, EP2.3
3	**1.28**	124.0759	$[M+H]^+$	**o-anisidine**	Lmatch (86)	14.3	3HAC	-	V	Intermediate; CMR; SVHC	EP2.1, EP2.2, EP2.3, HP2.1, HP2.2, HP2.3
4	**1.65**	158.0972	$[M+H]^+$	quinoline, 2,7-dimethyl-	volDB (5)	28.6	3HAC	III	IV		HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3
5	**1.77**	122.0967	$[M+H]^+$	**2,4-dimethylbenzenamine**	Lmatch (88)	42.9	3HAC	I	II	intermediate	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3'
6	**2.61**	202.0432	$[M+H]^+$	**thiabendazole**	Lmatch (81)	9.5	extract	III	IV	drug	HP2.2, HP2.3
7	**3.11**	114.0915	$[M+H]^+$	**caprolactam**	Lmatch (86)	23.8	3HAC	-	II	nylon 6 monomer; SML 15	EP2.1, EP2.2, EP2.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2
8	**3.46**	195.0885	$[M+H]^+$	**caffeine**	Lmatch (87)	47.6	extract	III	IV		HF1.1, HF1.2, HF1.3, HF3.1, HF3.2, HP1.1, HP1.2, HP1.3, HP2.2, HP2.3
9	**3.92**	142.0427	$[M+H]^+$	3-chloro-o-toluidine	cppDB (5.4)	14.3	3HAC	III	IV	intermediate	EP2.1, EP2.2, EP2.3, HP2.1, HP2.2, HP2.3
10	**4.78**	274.2754	$[M+H]^+$	**N,N-bis (2-hydroxyethyl)dodecylamine**	Lmatch (97)	61.9	3HAC	I	II		EF1.1, EF2.1, EF2.2, EF2.3, EF2.5, EF1.3', EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP1.3', HF1.1, HF2.1, HF2.2, HF2.3, HF2.5, HF1.3', HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP1.3'
11	**4.83**	214.2535	$[M+H]^+$	N,N-dimethyldodecylamine	cppDB (6.2)	100	3HAC	I	II	antistatic	EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3', EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', HF1.1, HF1.2, HF1.3, HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.1, HF3.2, HF1.3', HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3'
12	**4.83**	200.2371	$[M+H]^+$	N-methyldodecylamine	Lmatch (87)	90.5	extract	III	IV		EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3', EP1.1, EP1.2, EP1.3, EP3.1, EP3.2, HF1.1, HF1.2, HF1.3, HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.1, HF3.2, HF1.3', HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP3.1, HP3.2
13	**4.90**	285.2917	$[M+H]^+$	**N-[3-(dimethylamino)propyl]dodecanamide**	Lmatch (72)	33.3	3HAC	III	IV	antistatic	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP1.3', HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP1.3'
14	**5.02**	230.2482	$[M+H]^+$	lauramine oxide	Lmatch (92)	33.3	extract	III	IV	surfactants	EF1.1, EF1.2, EF2.1, EF2.2, EF2.3, EF2.4, EF3.1, HF1.2
15	**5.02**	150.0912	$[M+H]^+$	N-(2,4-dimethylphenyl)formamide	Lmatch (84)	14.3	extract	I	II	Insecticides	EP2.1, EP2.2, EP2.3
16	**5.04**	288.2896	$[M+H]^+$	2-aminoheptadecane-1,3-diol	Lmatch (87)	14.3	extract	II	III		EP1.1, EP1.2, EP1.3
17	**5.33**	202.0854	$[M+H]^+$	simazine	Lmatch (90)	19	extract	-	V	CMR; herbicide	EF3.1, EF3.2, EP3.1, EP3.2
18	**5.34**	242.2852	$[M+H]^+$	**N,N-dimethyltetradecylamine**	Lmatch (86)	100	95EtOH	I	II	antistatic	EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3', EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', HF1.1, HF2.1, HF3.1, HF3.2, HF1.3', HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3'
19	**5.40**	266.0975	$[M+H]^+$	albendazole	Lmatch (80)	9.5	extract	III	IV	drug	HF3.1, HF3.2
20	**5.70**	200.1191	$[M+H]^+$	**pyrimethanil**	Lmatch (92)	28.6	3HAC	III	IV	fungicide	EP2.2, EP2.3, HF3.1, HF3.2, HP2.2, HP2.3, HP3.1, HP3.2
21	**5.74**	242.1439	$[M+H]^+$	prometryn	Lmatch (88)	9.5	extract	III	IV	herbicide	EP2.2, EP2.3, HP2.2, HP2.3
22	**5.79**	242.1435	$[M+H]^+$	terbutryn	Lmatch (78)	9.5	extract	III	IV	herbicide	EP2.2, EP2.3, HP2.2, HP2.3
23	**5.86**	192.1384	$[M+H]^+$	diethyltoluamide	Lmatch (93)	61.9	95EtOH	I	II	insect repellent	EF1.1, EF3.1, EF3.2, EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, HF1.1, HF2.3, HF2.6, HF3.1, HF3.2, HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2
24	**5.88**	270.3173	$[M+H]^+$	**N,N-dimethylhexadecylamine**		95.2	95EtOH	I	II	antistatic	EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3', EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', HP1.1, HP1.2
25	**5.95**	312.3632	$[M+H]^+$	**N-methyldidecylamine**	Lmatch (92)	42.9	95EtOH	I	II	intermediate	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3'
26	**6.14**	404.1255	$[M+H]^+$	azoxystrobin	Lmatch (89)	9.5	extract	III	IV	fungicide	EP2.2, EP2.3, HP2.2, HP2.3
27	**6.18**	253.0307	$[M+H]^+$	**3,3'-dichlorobenzidine**	Lmatch (84)	14.3	95EtOH	-	V	intermediate	EP2.1, EP2.2, EP2.3, HP2.1, HP2.2, HP2.3
28	**6.37**	230.1168	$[M+H]^+$	sebuthylazine	Lmatch (87)	28.6	extract	III	IV	herbicides	EF3.1, EF3.2, EP2.2, EP2.3, EP3.1, EP3.2
29	**6.43**	215.9981	$[M-H]^-$	**propanil**	Lmatch (90)	9.5	95EtOH	III	IV	herbicides	EP2.2, EP2.3, HP2.2, HP2.3
30	**6.43**	182.0097	$[M+H]^+$	2-(methylsulfanyl)-1,3-benzothiazole	Lmatch (81)	9.5	extract	III	IV	fungicides	EP2.2, EP2.3
31	**6.48**	194.1175	$[M+H]^+$	**ethyl 4-(dimethylamino)benzoate**	Lmatch (91)	28.6	extract	I	II	paint additives	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3
32	**6.71**	332.0665	$[M+H]^+$	piroxicam	genDB (5.9)	9.5	3HAC	III	IV	drug	EP2.2, EP2.3, HP2.2, HP2.3
33	**6.75**	284.1428	$[M+H]^+$	metolachlor	Lmatch (86)	9.5	extract	III	IV	herbicide	EP2.2, EP2.3, HP2.2, HP2.3
34	**6.76**	415.2133	$[M+H]^+$	**dimethyldibenzylidene sorbitol**	Lmatch (64)	23.8	extract	-	I	plastic additive; SML 60	EP1.1, EP1.2, EP1.3, EP2.2, EP2.3
35	**6.83**	293.1735	$[M-H]^-$	**3-(3,5-Di-tert-butyl-1-hydroxy-4-oxocyclohexa-2,5-dien-1-yl)propanoic acid**	cppDB (6.4)	100	3HAC	III	IV	NIAS	EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3', HF1.1, HF1.2, HF1.3, HF2.1, HF2.2, HF2.3, HF2.4, HF2.5, HF2.6, HF3.1, HF3.2, HF1.3', HP1.1, HP1.2, HP1.3, HP2.1, HP2.2, HP2.3, HP3.1, HP3.2, HP1.3'
36	**6.86**	229.0862	$[M+H]^+$	**oxybenzone**	Lmatch (85)	33.3	extract	-	V	UV filter; EDC; SML 6	EF3.1, EF3.2, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2
37	**6.91**	198.1856	$[M+H]^+$	**1-octylpyrrolidin-2-one**	cppDB (5.9)	23.8	95EtOH	III	IV		EF2.1, EF2.2, EF2.3, EP2.2, EP2.3, HF2.1, HF2.2, HF2.3, HP2.2, HP2.3
38	**6.94**	308.1541	$[M+H]^+$	**tebuconazole**	Lmatch (83)	9.5	95EtOH	-	V	Fungicides; CMR; EDC	EP2.2, EP2.3, HP2.2, HP2.3

N°	RT	Precursor	Adduct	Name	Score	Fill	Matrix	Cram	Tox	Remark	Presence
39	**6.97**	305.1081	$[M+H]^+$	diazinon	Lmatch (86)	9.5	extract	III	IV	insecticide	EF3.1, EF3.2
40	**7.00**	342.078	$[M+H]^+$	propiconazole	Lmatch (81)	9.5	95EtOH	-	V	Fungicides; CMR	EP2.2, EP2.3, HP2.2, HP2.3
41	**7.06**	277.1817	$[M+H]^+$	**7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione**	Lmatch (83)	47.6	95EtOH	III	IV	NIAS	EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1
42	**7.14**	406.074	$[M+H]^+$	difenoconazole	Lmatch (85)	9.5	extract	III	IV	fungicide	EP2.2, EP2.3, HP2.2, HP2.3
43	**7.19**	200.202	$[M+H]^+$	lauramide	Lmatch (85)	9.5	95EtOH	III	IV		EP2.2, EP2.3, HP2.2, HP2.3
44	**7.20**	310.2367	$[M+Na]^+$	**lauric acid diethanolamide**	Lmatch (86)	14.3	95EtOH	-	II	antistatic; SML 5	EP1.1, EP1.2, EP1.3, EP1.3', HP1.1, HP1.2, HP1.3, HP1.3'
45	**7.22**	395.0808	$[M+H]^+$	**diflufenican**	genDB (6.3)	9.5	95EtOH	III	IV	herbicides	EP2.2, EP2.3
46	**7.24**	220.1125	$[M+H]^+$	N-phenyl-2-naphthylamine	volDB (5.4)	9.5	95EtOH	-	V	Lubricant; CMR	EF1.3', EP1.3'
47	**7.30**	383.2042	$[M+Na]^+$	**tributyl citrate**	cppDB (6.3)	85.7	95EtOH	III	IV	plasticizer	EF1.1, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3', HF2.1, HF2.2, HF2.3
48	**7.32**	421.2326	$[M+Na]^+$	tris(2-butoxyethyl) phosphate	Lmatch (89)	42.9	extract	III	IV	plasticizer	EF1.1, EF3.1, EF3.2, EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, HF1.1, HF3.1, HF3.2
49	**7.37**	277.1812	$[M-H]^-$	**3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionic acid**	Lmatch (88)	28.6	extract	II	III	NIAS	EF1.2, EF1.3, EP1.1, EP2.1, EP2.2, EP2.3
50	**7.40**	273.1853	$[M+H]^+$	galaxolidone	Lmatch (83)	28.6	extract	III	IV		EF1.3, EF2.1, EF1.3', EP2.1, EP2.2, EP2.3
51	**7.47**	179.0701	$[M+H]^+$	3-methoxycinnamic acid	Lmatch (86)	33.3	extract	I	II		EF1.1, EF3.1, EF3.2, EP2.2, EP2.3, EP3.1, EP3.2
52	**7.48**	599.1155	$[M+Na]^+$	ethylene terephthalate cyclic trimer	cppDB (5.5)	9.5	95EtOH	III	IV	PET oligomer	EP1.2, EP2.1
53	**7.54**	322.1454	$[M+H]^+$	pyriproxyfen	Lmatch (84)	14.3	extract	III	IV	Insecticide	EF1.3', EP2.2, EP2.3
54	**7.54**	425.2149	$[M+Na]^+$	tributyl acetylcitrate	Lmatch (81)	81	95EtOH	-	I	plasticizer; SML 60	EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EP1.1, EP1.2, EP1.3, EP3.1, EP3.2, EP1.3'
55	**7.55**	199.1328	$[M+H-H2O]^+$	sebacic acid monomethyl ester	Lmatch (80)	19	extract	I	II		EF2.4, EP2.1, EP2.2, EP2.3
56	**7.63**	255.1746	$[M+H]^+$	4-methylbenzylidene camphor	Lmatch (84)	38.1	extract	-	V	UV filter; EDC	EF2.5, EF3.1, EF3.2, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2
57	**7.67**	295.2272	$[M-H]^-$	9-hydroxy-10,12-octadecadienoic acid	Lmatch (88)	76.2	extract	II	III		EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3', EP1.1, EP1.2, EP1.3, EP2.2
58	**7.77**	507.2737	$[M+Na]^+$	**1,2,3-trideoxy-4,6:5,7-bis-o-[(4-propylphenyl)methylene]-nonitol (NX8000)**	Lmatch (82)	33.3	extract	-	II	plastic additive; SML 5	EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP1.3'
59	**7.88**	384.1934	$[M+Na]^+$	**octocrylene**	Lmatch (90)	100	95EtOH	-	IV	UV filter; SML 0.05	EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3', EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3'
60	**8.00**	259.2065	$[M+H]^+$	galaxolide 1	volDB (5.8)	28.6	95EtOH	III	IV		EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3
61	**8.04**	311.1645	$[M+H]^+$	**avobenzone**	Lmatch (91)	100	95EtOH	III	IV	UV filter	EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3', EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3'
62	**8.06**	291.1998	$[M+H]^+$	**2-ethylhexyl 4-methoxycinnamate**	Lmatch (83)	61.9	extract	-	V	UV filter; EDC	EF1.1, EF3.1, EF3.2, EF1.3', EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3'
63	**8.13**	443.3351	$[M+H]^+$	1,2,3-Propanetriol 1-stearate 2,3-bisacetate	cppDB (6)	100	95EtOH	I	II		EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3', EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3'
64	**8.20**	256.2647	$[M+H]^+$	**palmitamide**	Lmatch (85)	42.9	extract	III	IV		EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3'
65	**8.47**	381.298	$[M+Na]^+$	**2-stearoylglycerol**	Lmatch (92)	76.2	95EtOH	I	II	lubricant	EF1.1, EF1.2, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF1.3', EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP1.3'
66	**8.50**	327.1963	$[M+H]^+$	**Chimassorb 81**	Lmatch (88)	42.9	95EtOH	-	II	UV absorber; SML 6	EF2.2, EF2.3, EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP1.3'
67	**8.52**	437.3062	$[M-H]^-$	2,4-di-tert-butylphenyl 3,5-di-tert-butyl-4-hydroxybenzoate (UV 120)	cppDB (5.7)	9.5	95EtOH	-	I	plastic additive; SML 60	EP2.2, EP2.3
68	**8.57**	803.5445	$[2M+Na]^+$	**dioctyl phthalate**	Lmatch (88)	19	95EtOH	-	V	Plasticizer; EDC	EF1.2, EF3.2, EP2.2, EP2.3
69	**8.63**	393.2969	$[M+Na]^+$	**bis(2-ethylhexyl) adipate**	Lmatch (93)	66.7	95EtOH	-	II	Plasticiser; SML 18	EF1.1, EF1.2, EF1.3, EF2.4, EF2.5, EF3.2, EF1.3', EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3'
70	**8.81**	255.2318	$[M-H]^-$	**palmitic acid**	Lmatch (91)	71.4	95EtOH	-	I	SML 60	EF1.1, EF1.3, EF2.2, EF2.3, EF2.4, EF2.5, EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3'
71	**8.81**	360.324	$[M+Na]^+$	**erucamide**	Lmatch (85)	61.9	95EtOH	-	I	lubricant; SML 60	EF1.1, EF1.2, EF2.1, EF2.2, EF2.3, EF2.4, EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP1.3'
72	**8.86**	647.4585	$[M+H]^+$	**Irgafos 168**	Lmatch (85)	100	95EtOH	-	I	antioxidant; SML 60	EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3', EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3'
73	**8.86**	281.2475	$[M-H]^-$	**oleic acid**	Lmatch (85)	100	95EtOH	-	I	SML 60	EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3', EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3'
74	**9.18**	340.3574	$[M+H]^+$	**docosanamide**	cppDB (5.9)	61.9	95EtOH	-	I	processing aid; SML 60	EF1.1, EF2.1, EF2.2, EF2.3, EF2.4, EF2.6, EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP1.3'
75	**9.25**	469.3289	$[M+Na]^+$	**diisodecyl phthalate**	Lmatch (86)	33.3	95EtOH	-	V	Plasticizer; EDC	EF1.1, EF3.1, EF3.2, EP1.1, EP2.1, EP2.2, EP2.3

N°	RT	Precursor	Adduct	Name	Score	Fill	Matrix	Cram	Tox	Remark	Presence
76	**9.36**	1175.776	$[M-H]^-$	**Irganox 1010**	Lmatch (89)	100	95EtOH	-	I	antioxidant; SML 60	EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3', EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3'
77	**9.39**	431.1784	$[M+H]^+$	**2,5-bis(5-tert-butyl-benzoxazol-2-yl)thiophene**	Lmatch (86)	71.4	95EtOH	-	III	plastic additive; SML 0.6	EF1.1, EF2.3, EF2.4, EF2.6, EF3.1, EF3.2, EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3'
78	**9.61**	199.0157	$[M-H]^-$	(4-chloro-2-methylphenoxy)acetic acid	Lmatch (73)	9.5	3HAC	III	IV	herbicides	EP2.2, EP2.3, HP2.2, HP2.3
79	**10.24**	663.454	$[M+H]^+$	**oxidized Irgafos 168**	Lmatch (98)	100	95EtOH	III	IV	NIAS	EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3', EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3'
80	**10.27**	385.3471	$[M+H]^+$	(+)-4-cholesten-3-one	Lmatch (71)	90.5	extract	III	IV		EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3', EP1.1, EP1.2, EP1.3, EP2.2, EP3.1, EP3.2, EP1.3'
81	**11.44**	553.4598	$[M+Na]^+$	**Irganox 1076**	Lmatch (85)	100	95EtOH	-	II	antioxidant; SML 6	EF1.1, EF1.2, EF1.3, EF2.1, EF2.2, EF2.3, EF2.4, EF2.5, EF2.6, EF3.1, EF3.2, EF1.3', EP1.1, EP1.2, EP1.3, EP2.1, EP2.2, EP2.3, EP3.1, EP3.2, EP1.3'
82	**11.60**	591.4948	$[M+Na]^+$	**glycerol dihexanoate**	Lmatch (69)	14.3	95EtOH	I	II	emollient	EP2.1, EP2.2, EP2.3
83	**11.73**	533.529	$[M+H]^+$	(Z)-octadec-9-enyl oleate	cppDB (5.1)	9.5	95EtOH	I	II		EP2.2, EP2.3

Note: Chemicals **Name** in bold font were confirmed by reference standard.

Lmatch in the **Score** column represents library match, and the number in the bracket were the scores (full mark 100) given by MS-DIAL, while others column were the three structure databases that the compounds were finally identified, and the number in the bracket were the scores (full mark 10) given by MS-FINDER.

The **fill** is the percentage of samples that detected the chemical in all samples (21 in total).

The column **Matrix** tells where the chemicals were identified. Some compounds were initially identified in the extracts by matching libraries, and their determination in the simulants were achieved by pseudo-MRM in MRMPROBS.

The **Cram** column is the Cramer rule-based toxicity level predicted by Toxtree. When a compound is CMR, SVHC, EDC, or have SML, prediction is not suitable.

Tox column gives the toxicity level of the compounds based on the method previous proposed by our group (Su et al., 2021a, 2021b).

In the **Remark** column, SML has unit of mg/kg

The **Presence** column shows the compound was detected in which migrates. E stands for 95% ethanol migration, while H means 3% acetic acid migration.

It should be noted that some compounds had different retention time (RT) in 95% ethanol and 3% acetic acid food simulants (Fig. III-5.2). The phenomenon is understandable as we were using a reverse phase column and had initial mobile phase of 5% ethanol. For aqueous sample, e.g., 3% acetic acid, sample injection should have no significant influence on the composition of mobile phase since the sample solvent and mobile phase are almost the same. However, for organic samples, in this case of 95% ethanol, 10 µL injection volume is significant and it takes time to mix with the mobile phase. During this time, the mobile phase contains more organic solvent which has stronger elution power. As such, analytes can move faster and have shorter retention time. In the field of food contact materials, various food simulants can be used, and a compound could have significantly different retention time in these simulants and hence could be misidentified as different compounds. Besides, we are addressing that retention time information in MS/MS libraries or from prediction models should be used with cautions for identifying compounds in aqueous food simulants, as organic solvents are commonly used to prepare standard solutions for building MS/MS libraries or retention time databases for retention time prediction.

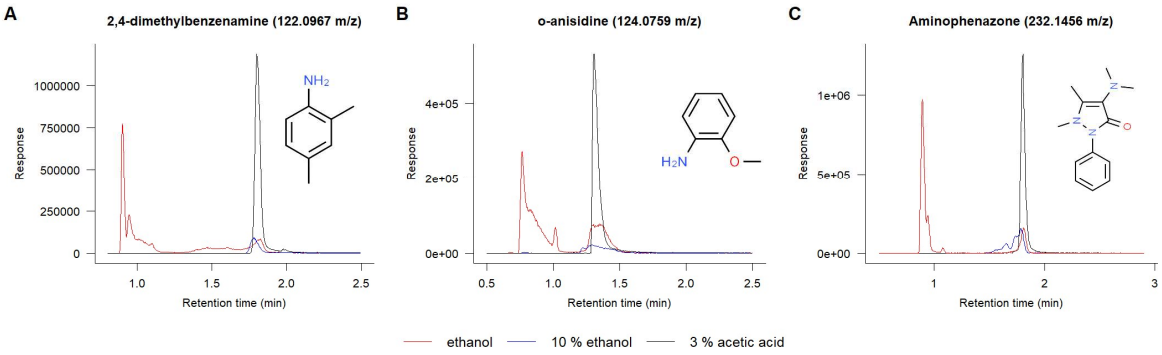


Fig. III-5.2 Chromatograms of 2,4-dimethylbenzenamine (A), o-anisidine (B), and aminophenazone (C) in 3 solvents. The 10% ethanol standards were obtained by directly diluting the ethanol standards 10 times.

4.2. MS-CleanR feature cleaning and structural elucidation by MS-FINDER

Despite some features were annotated with high library matching scores, they were finally removed by MS-CleanR as they were in-source fragments of others but not individual compounds. For instance, the feature 106.0866 m/z at 7.20 min was

identified as diethanolamine. However, it was actually an in-source fragment of the feature 288.2544 m/z at 7.20 min, which was identified as lauric acid diethanolamide. This in-source fragment was automatically removed by MS-CleanR despite of its high library match. After MS-CleanR, the remained features were considered as individual compounds and therefore subjected to MS-FINDER for structural elucidation. Below, we take few examples to explain the structural elucidation step making use of some chemical structure databases.

4.2.1 Integrating results from GC-MS

MS-FINDER allows users to access the whole Pubchem chemical structure database though, it will be quite slow, and the number of candidates would be large, since Pubchem archives tens of millions of compounds. Even for a given molecular formula, the number of candidates would be huge, and *in-silico* fragmentation of all these candidates would be time-consuming and requires lots of computational resources. However, it is not necessary to search the whole Pubchem for every peak. For a specific type of sample, many compounds might have been detected or known to be present in the same type of samples. Therefore, it is more reasonable to detect the same chemical in a similar sample. That is the rationale of using various structure databases for structural elucidation. It works like manually checking identifications, comparing fragmentation patterns or *in-silico* fragmentation, of a given precursor ion/formula in the literature in similar samples. This manual way is tedious and requires a lot of expertise. In contrast, MS-FINDER will computationally fragment all structures with the same molecular formula in the selected databases and rank them based on *in-silico* fragmentation probability, the frequency of a candidate in the databases, and so on. Hence, using various structure databases relevant to the samples under investigation could be of great help.

There were more than 200 compounds previously identified by GC-MS in the same set of samples by our group (Su et al., 2021b). Most of them are volatile chemicals and might not be detected in liquid chromatography. However, many semi-volatile compounds could be detectable in liquid chromatography as well. If a compound has

already been identified in GC-MS, then it is not necessary to elucidate it again in liquid chromatography - HRMS (LC-HRMS). The problem is how to correlate an unknown peak in LC-HRMS with any GC-MS identified compound. A common practice is to calculate the exact mass of all identified compounds and their common adducts, for example, $[M+H]^+$ and $[M+Na]^+$ in positive mode and $[M-H]^-$ in negative mode, and then compare them with peaks in LC-HRMS. Identical mass (within mass tolerance) possibly implies the same compound. However, it is not necessarily true and relying only on exact mass might not be sufficient.

Herein, we present a more convenient and reliable way to correspond peaks in LC-HRMS to GC-MS identified compounds. That is, to make the GC-MS identified chemicals into a structure database and use them in MS-FINDER as a database for structural elucidation. In MS-FINDER, based on the precursor ion and adduct type, molecular formulas are rated by mass errors, isotopic ratio, product ions, neutral losses (Tsugawa et al., 2016). Subsequently, the software will predict the MS/MS of all chemicals that have the same formula in the selected database and compare them to the acquired MS/MS spectra, which is called precursor oriented spectral search. Combining not only exact mass but also in-silico MS/MS spectrum, the correspondence can be found with high confidence. For example, the peak 214.2535 m/z at 4.826 min matches well (score 6.1) to N,N-dimethyldodecylamine when selecting the volDB (Fig. III-5.3 A). It means that N,N-dimethyldodecylamine was previously detected in the same set of samples and had theoretical MS/MS spectrum that matched to the unknown. In addition, its distributions in GC-MS and LC-HRMS among the samples were consistent (Fig. III-5.3 B) illustrating good correspondence. Although there were two more chemicals, namely medelamine A, tetradecylamine, that had higher score than N,N-dimethyldodecylamine when selecting the genDB, the latter one is more reliable as its identification in GC-MS was based on high library match and close retention index compared to the reference. Moreover, the MS/MS spectrum of N,N-dimethyldodecylamine shared the same pattern with its homolog N,N-dimethyltetradecylamine, which was confirmed by reference standard (Fig. III-5.4). Based on this similar pattern, the peak 270.3173 m/z at 5.884 min was identified and

confirmed as N,N-dimethylhexadecylamine. Many correspondences were found by this way and their identifications were of high confidence combining library and retention index match in GC-MS as well as good *in-silico* MS/MS spectra in LC-HRMS.

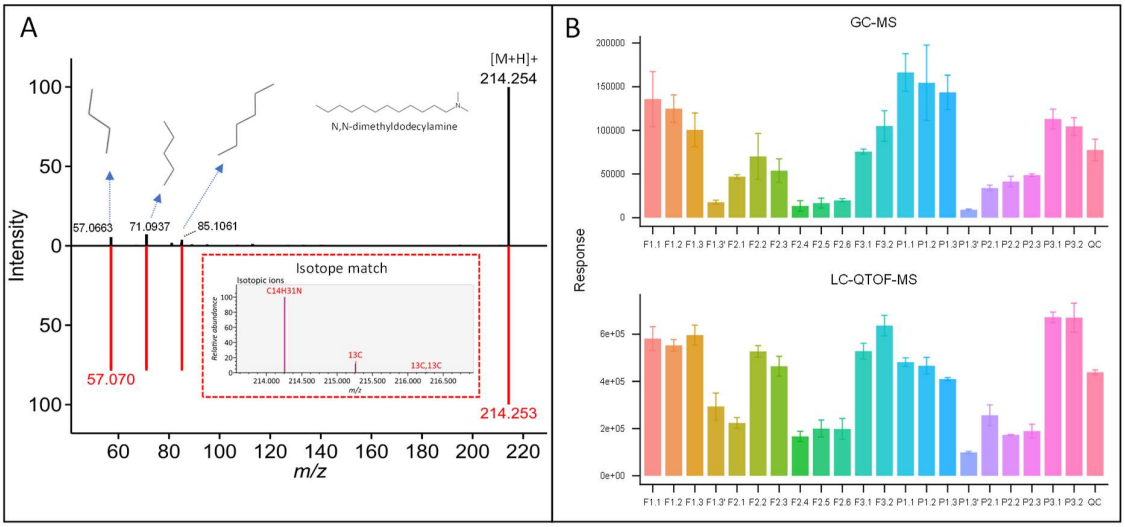


Fig. III-5.3 Identification of N,N-dimethyldodecylamine: In-silico fragmentation match by MS-FINDER (A) and the distribution of this compound among samples in GC-MS and LC-QTOF-MS (B)

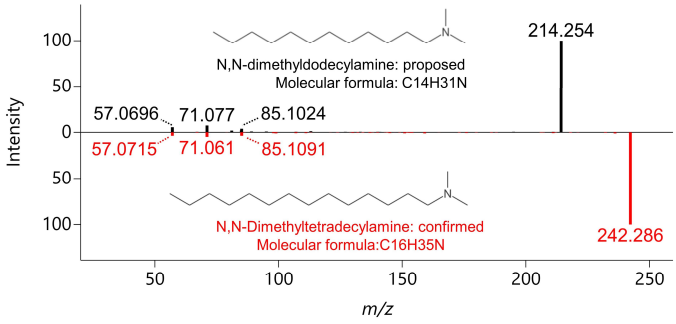


Fig. III-5.4 MS/MS spectra of the two homologs N,N-dimethyltetradecylamine (confirmed by standard) and N,N-dimethyldodecylamine

4.2.2 Chemicals associated with plastic packaging database (cppDB) as a useful structure database

MS-FIDNER has integrated 24 structure databases mainly related to metabolites or natural products as it was initially developed for the metabolomics community.

However, MS-FINDER allows user-defined structure database as well. Regarding plastic materials, the chemicals associated with plastic packaging database (cppDB) compiled by Groh, et al. (Groh et al., 2019), could be of great help for characterizing plastic-related chemicals. The initial version did not include structural information, e.g., Smiles and InChIKey, which are vital for *in-silico* fragmentation. Hence, this key information was added and the cppDB was re-organized into the form that is compatible with MS-FINDER (downloadable in https://zenodo.org/record/4454648).

To illustrate the merit of the cppDB, the identification of 3-(3,5-Di-tert-butyl-1-hydroxy-4-oxocyclohexa-2,5-dien-1-yl)propanoic acid (compound 35) is shown below as an example. The peak 293.1735 m/z at 6.832 min in negative mode had a formula $C_{17}H_{26}O_4$ with tolerable mass error < 5 ppm and good isotope match (Fig. III-5.5 A). There were many candidates that the *in-silico* MS/MS spectra matched well to the unknown (scores > 6) when selecting the genDB. However, when using the cppDB, compound 35 matched to the peak (Fig. III-5.5 B), which implied that this plastic-related compound could be the candidate as well. Although many candidates had slightly higher scores than compound 35 in MS-FINDER, compound 35 is a more suitable candidate because it has been reported as a hydrolysate of 7,9-Di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione (compound 41) which is a degradation product of a common polyolefin antioxidant, Irganox 1010 (Beißmann et al., 2013; Singh et al., 2018). Moreover, compound 41 and Irganox 1010 have been confirmed in this set of samples. In MS-CleanR, identifications using volDB, cppDB, and genDB were merged considering various weights set to each database (see section 3.7). Therefore, the unknown was automatically identified as compound 35 as it is in the cppDB, which had a weight of 1.5, while genDB candidates had a weight of 1 in MS-CleanR. The identification was finally confirmed by a home-made standard following the strategy proposed by Singh et al (Singh et al., 2018). Briefly, compound 35 was obtained by partially hydrolysis of compound 41 at 70 °C for 1 h.

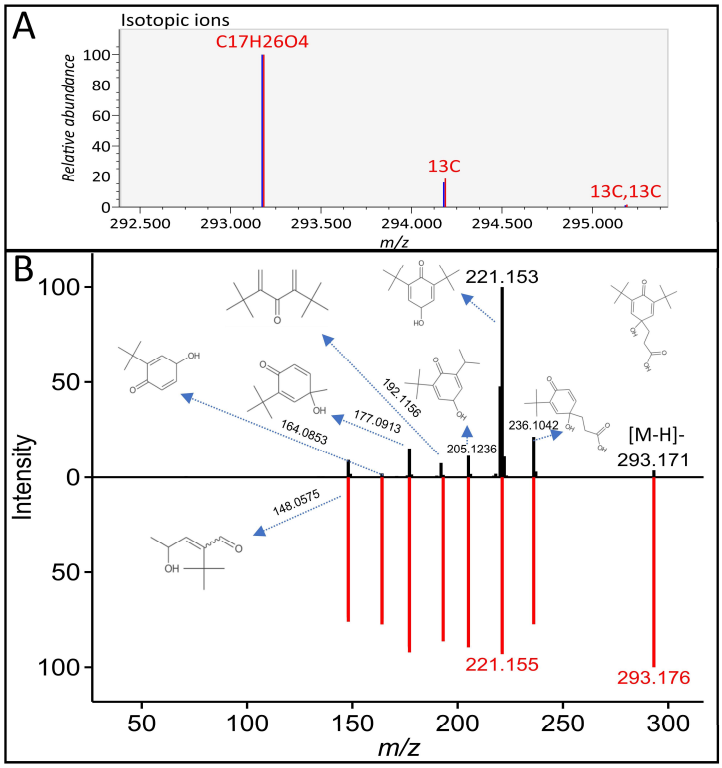


Fig. III-5.5 Identification of 3-(3,5-Di-tert-butyl-1-hydroxy-4-oxocyclohexa-2,5-dien-1-yl)propanoic acid: isotope match (A), in-silico fragmentation match by MS-FINDER (B)

In the same manner, many other compounds were identified, for example, lauric acid diethanolamide. Some commonly used polymer additives, e.g., Irgafos 168, Irganox 1010, and Chimassorb 81 were identified by our in-house library in the present study. Nevertheless, they can be easily and automatically identified by the abovementioned strategy as well, without the use of in-house library. Of course, confirmation by reference standard is the only gold standard for unambiguous identification, but when reference standards are not available, combining *in-silico* MS/MS and the cppDB would somehow increase the confidence level of identification in plastic materials.

4.2.3 Use of generic databases (genDB)

Aside from plastic-related chemicals, environmental contaminants as well as food residues might be present in the recycled plastics as well. As a consequence, only food, environment, and natural product related databases were used as the generic database (section 3.7). There were several compounds identified by this way and some of them were confirmed by reference standards. For instance, $C_{19}H_{11}F_5N_2O_2$ was found to be the best formula for the peak 395.0808 m/z at 7.215 min in positive mode (Fig. III-5.6 A). Within the genDB, diflufenican was the only candidate that had *in-silico* MS/MS explainable to the experimental spectrum of the unknown (Fig. III-5.6 B). There were some pesticides/drugs identified in this set of samples, especially in sample P2.2 and P2.3. Therefore, it is not unexpected to detect other pesticides in these samples. Looking in depth into the distribution of this unknown in the samples, it was only found in P2.2 and P2.3 as well (Fig. III-5.6 C). Diflufenican is an herbicide and thus it was thought to be a good candidate. The identification was finally confirmed by certificated standard. Similarly, there were some other pesticides/drugs identified, e.g., pyrifenox and piroxicam. They were only detected in samples P2.2 and P2.3 and had good in-silico MS/MS match to the experimental spectra. For this reason, their identification can be relatively more reliable. However, many other compounds were only determined by in-silico MS/MS spectra match (score > 5) and the identification confidence level could be lower. Furthermore, there were 13 compounds that remained unknown. These compounds were merely identified by *in-silico* fragmentation and were not thought as tentatively identified in the present study.

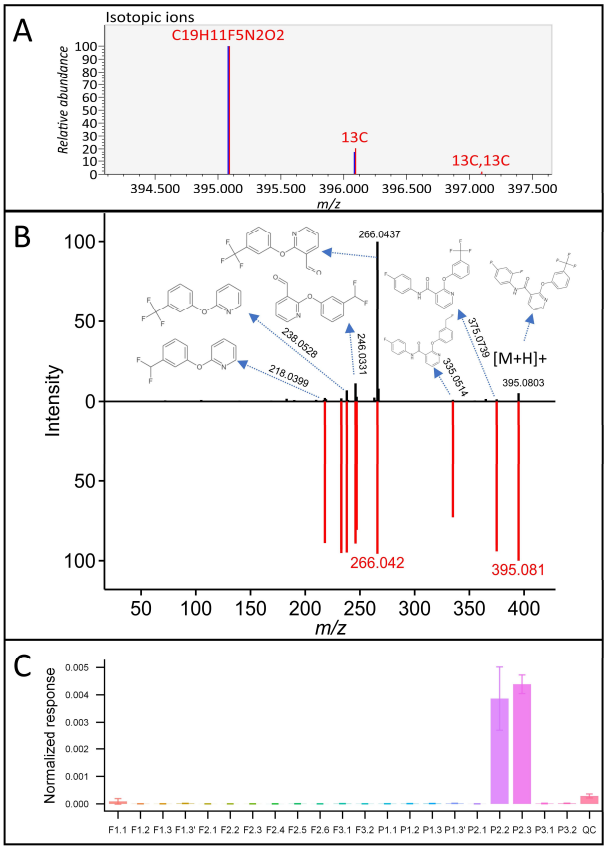


Fig. III-5.6 Identification of diflufenican: isotope match (A); in-silico fragmentation match by MS-FINDER (B); distribution of this compound across samples

4.3. Pseudo-MRM by MRMPROBS

For an identified substance, it is important to check which samples contained the substance. MS-DIAL allows users to check the annotation in each sample. However, some samples were not annotated, possibly because they were too low to have representative MS/MS spectra, or they were simply noise. It is not easy to judge using EIC/TIC. Furthermore, the MS-DIAL approach does not work for substances that were not identified by library match, since there will be no annotation information for each sample. As such, we used a pseudo-MRM (MRMPROBS) method for this purpose. The presence/absence of a substance in each sample were automatically and more precisely determined by posterior probabilistic integrating peak intensity and retention time, precursor-product ion ratio, shape, and coelution similarity (Tsugawa et al., 2013). For instance, 2,2'-methylenebis(6-tert-butyl-4-methylphenol) was not identified in samples

F2.1 and F2.2 by MS-DIAL. Nevertheless, they were automatically identified in F2.1 and F2.2 by pseudo-MRM using the precursor-product ion pairs (339.2321-163.0938 m/z) generated by MS-DIAL (Fig. III-5.7). Moreover, the absence of this compound in sample F2.4 was verified as well. Consequently, the *pseudo*-MRM provided an automated and sensitive way to detect the presence/absence of an identified compound with low false positive rate.

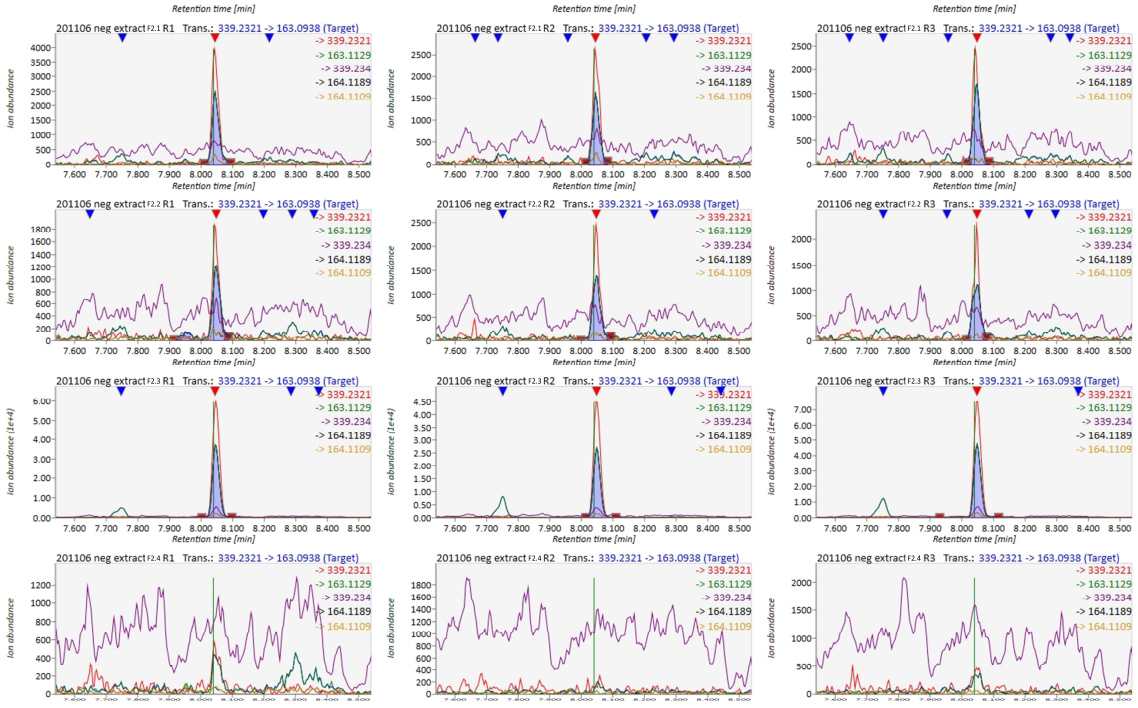


Fig. III-5.7 Evaluating the presence/absence of 2,2'-methylenebis(6-tert-butyl-4-methylphenol) (Antioxidant 2246) in each sample by MRMPROBS (pseudo-MRM)

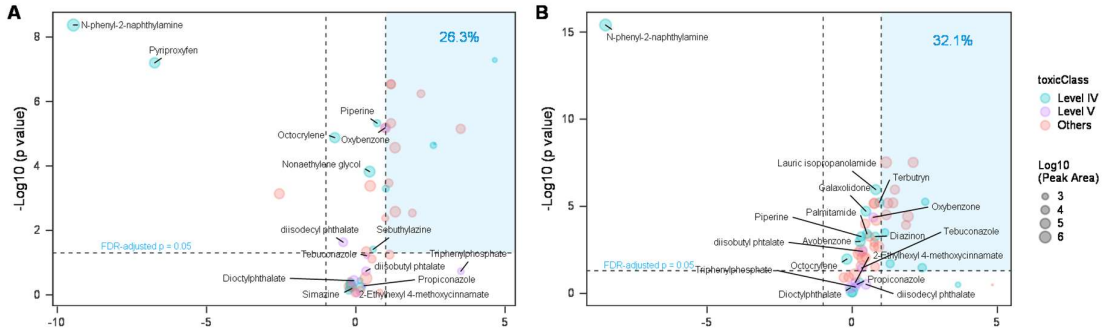
For the migration samples, except for chemicals characterized in the migrates, compounds identified (matching libraries) in the extracts were included for pseudo-MRM as well. They might not have representative spectra for structural elucidation though, some of them migrated into the simulants and were detected by the pseudo-MRM using the transitions established in the extracts, for example, azoxystrobin and ethyl 4-(dimethylamino)benzoate (Table III-5.1). The *pseudo*-MRM worked like a sensitive targeted analysis in this context.

4.4. Efficiency of extra decontamination on chemical removal

In our previous study, the efficiency of extra decontamination on chemical removal was evaluated by volatile profiles (Su et al., 2021b). As a complement, the non-volatile profiles acquired by UPLC-QTOF-MS were evaluated herein. To have a more robust analysis, extracts were used for this purpose as previously explained (Su et al., 2021b). The number of features detected in the extracts was huge and most of them were unknown. Therefore, only chemicals that were confirmed or tentatively identified by matching well (match > 80) to libraries were used to have a meaningful evaluation. To evaluate the efficacy of extra decontamination, chemicals identified in company 1 samples F1.1, F1.2, F1.3, P1.1, P1.2, and P1.3 (no extra decontamination) as well as F1.3' and P1.3' (with extra decontamination) were then subset for the fold change analysis.

In agreement to our previous study (Su et al., 2021b), N-phenyl-2-naphthylamine got much higher intensity after extra decontamination in both flakes and pellets samples (Fig. III-5.8), and it was suspected as a contaminant or reaction product during the extra decontamination process. Pyriproxyfen, an insecticide, grabbed our attention for its higher intensity after extra decontamination as well. However, high intensity was only observed in F1.3' but not in P1.3'. Therefore, in contrast to N-phenyl-2-naphthylamine, pyriproxyfen was speculated as an accidental contaminant in F1.3'. Furthermore, two UV filters (octocrylene, 2-ethylhexyl-4-methoxycinnamate) and 3 heavy phthalates (dioctylphthalate, diisobutyl phthalate, and diisodecyl phthalate) were not significantly reduced by extra decontamination as previously discussed (Su et al., 2021b). In contrast to the GC-MS profiles, there was a lower percentage of compounds that had less than half intensities after extra decontamination in the UPLC-QTOF-MS profiles. This result was expected, as non-volatile compounds are more difficult to remove. It is noteworthy that many pesticides (sebuthylazine, propiconazole and so on) were found in the extracts of company 1 samples (Fig. III-5.8). However, none of them were found in the migration (neither 95% ethanol nor 3% acetic acid) from these samples (Table III-5.1). The reason was that they had too low responses in the extracts. Taking propiconazole as an example, it had quite high response in P2.2 and P2.3 (Fig. III-5.9), from which the

representative MS/MS spectrum derived for matching libraries. Nevertheless, it was also found in other samples by the sensitive *pseudo*-MRM. Therefore, they were included in the examination of the efficiency of extra decontamination, but they might not be human risks since they were not found in migration.



Note: Fold change is expressed as no extra decontamination versus extra decontamination; the size of the circles is mapped to the average peak area of the samples that applied extra decontamination

Fig. III-5.8 Efficiency of extra decontamination: fold change analysis by volcano plot on flakes (A); fold change analysis by volcano plot on pellets (B)

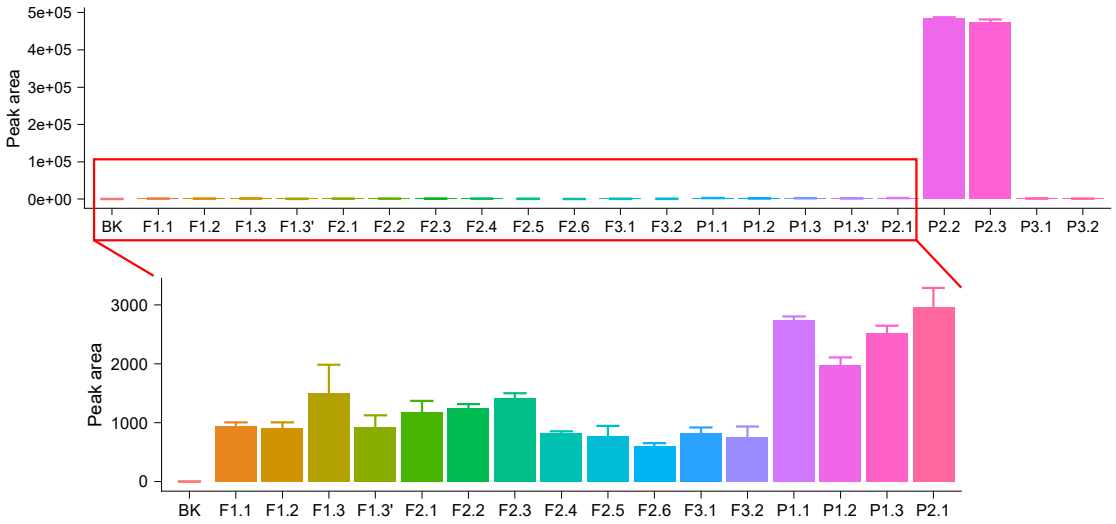


Fig. III-5.9 Bar chart of propiconazole across samples (extracts)

4.5. Quantification of the migrants in two simulants

Agreeing to the GC-MS results (Su et al., 2021b), many high concern substances (level V and IV) were unique in company 2 samples (Table III-5.2), since these samples

contain a large portion of non-milk-bottle-origin rHDPE possibly from agriculture field. Therefore, better separation of the recycled materials to exclude those non-milk-bottle plastics is vital to have less contaminated rHDPE. In addition, quantification details including linear range, determine coefficient (R^2), LOD, and LOQ are shown in Table III-5.3.

With respect to company 3 samples, the main risks came from octocrylene and 2-ethylhexyl-4-methoxycinnamate (an endocrine disruptor), which agreed to the previous study (Su et al., 2021b). Oxybenzone has SML of 6 mg/kg, whereas it was thought to be an EDC (IPCP, 2017). Its migration was low (ca. 0.003 mg/kg in 95% ethanol). Another UV filter, avobenzone, which is classified as Cramer class III, gave a migration value of ca. 0.03 mg/kg in 95% ethanol. Similar to the other companies, the Cramer III substance oxidized Irgafos 168 had about 0.2 mg/kg migration in 95% ethanol, which was slightly higher than its migration from virgin polyethylene (Vera et al., 2019).

Regarding company 1 samples, octocrylene and 2-ethylhexyl-4-methoxycinnamate were the primary risks as well and they were not significantly reduced by the extra decontamination. In contrast to company 3, the two UV filters oxybenzone and avobenzone had much lower migration (or not detected). For other migrants with SML in the European regulation EU 10/2011, they all had migration values lower than their SML. Moreover, only few compounds migrated to 3% acetic acid, and their migration were quite low and might not be risky for human health.

Table III-5.2 Quantification of the migrants in 95% ethanol and 3% acetic acid

Name	Company 1								Company 2						Company 3				Remarks
	EP1.1	EP1.2	EP1.3	EP1.3'	HP1.1	HP1.2	HP1.3	HP1.3'	EP2.1	EP2.2	EP2.3	HP2.1	HP2.2	HP2.3	EP 3.1	EP3.2	HP3.1	HP3.2	
Aminophenazone	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	89 ± 3	98.2 ± 4.7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	IV
o-Anisidine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	41.6 ± 3.4	10.6 ± 5.2	7 ± 2.1	47.2 ± 3.1	25.8 ± 3.7	27 ± 4.3	n.d.	n.d.	n.d.	n.d.	V
2,4-Dimethylbenzenamine	1.6 ± 0.04	1.5 ± 0.02	< LOQ	n.d.	1.3 ± 0.1	1 ± 0.2	0.6 ± 0.3	< LOQ	71.6 ± 0.7	21.8 ± 0.8	19 ± 0.2	89.5 ± 4.8	39.5 ± 0.4	38.2 ± 0.8	< LOQ	< LOQ	< LOQ	< LOQ	II
Thiabendazole	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3.4 ± 0.4	3.3 ± 0.1	n.d.	n.d.	n.d.	n.d.	IV
Caprolactam	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	280 ± 24	957 ± 33	933 ± 12	320 ± 0.4	943 ± 8	940 ± 14	n.d.	n.d.	10.6 ± 1.5	9 ± 10.5	II; SML 15
Caffeine	n.d.	n.d.	n.d.	n.d.	3.1 ± 0.1	3 ± 0.5	2.8 ± 0.6	n.d.	n.d.	n.d.	n.d.	n.d.	5.5 ± 0.3	5.1 ± 0.1	n.d.	n.d.	n.d.	n.d.	IV
N,N-bis (2-hydroxyethyl)dodecylamine	1.8 ± 0.01	1.2 ± 0.04	1.1 ± 0.01	0.2 ± 0.1	< LOQ	< LOQ	< LOQ	< LOQ	12.2 ± 0.4	1.7 ± 0.3	1.3 ± 0.2	4.1 ± 0.3	< LOQ	< LOQ	n.d.	n.d.	n.d.	n.d.	II
N-[3-(Dimethylamino)propyl]dodecanamide	2.5 ± 0.2	2.2 ± 0.3	2 ± 0.2	1.2 ± 0.1	0.3 ± 0.2	0.2 ± 0.05	0.3 ± 0.01	< LOQ	< LOQ	4.1 ± 0.5	3.9 ± 0.1	0.1 ± 0.01	1.3 ± 0.1	1.4 ± 0.1	n.d.	n.d.	n.d.	n.d.	IV
N,N-Dimethyltetradecylamine	8.9 ± 0.04	9.1 ± 0.5	8.2 ± 0.3	2.5 ± 0.04	6.1 ± 0.2	5.2 ± 0.2	5 ± 0.3	1.8 ± 0.1	2.3 ± 0.3	3.2 ± 0.3	3.1 ± 0.4	2.9 ± 0.2	3.1 ± 0.2	3.1 ± 0.1	10 ± 0.4	11.8 ± 1.8	4.6 ± 0.4	4.8 ± 0.8	II
Pyrimethanil	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	15.7 ± 1.3	13.4 ± 2.5	n.d.	12.4 ± 2.1	12.3 ± 0.4	n.d.	n.d.	< LOQ	< LOQ	IV
N,N-Dimethylhexadecylamine	10.3 ± 0.3	12.7 ± 1.8	10.1 ± 0.1	6.4 ± 0.4	29 ± 1.3	29.6 ± 0.1	n.d.	n.d.	3 ± 0.2	6.5 ± 0.4	6.6 ± 0.4	n.d.	n.d.	n.d.	2.8 ± 0.1	3.1 ± 0.2	n.d.	n.d.	II
N-methyldidecylamine	10.7 ± 0.2	9.1 ± 0.01	9.5 ± 0.5	8.3 ± 0.05	n.d.	n.d.	n.d.	n.d.	7.3 ± 0.6	3 ± 0.6	2.8 ± 0.1	n.d.	n.d.	n.d.	0.2 ± 0.1	0.2 ± 0.01	n.d.	n.d.	II
3,3'-dichlorobenzidine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	209 ± 7	74.9 ± 0.8	73.6 ± 0.1	46.5 ± 0.7	16.8 ± 0.7	17.4 ± 2	n.d.	n.d.	n.d.	n.d.	V
Propanil	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	861 ± 46	739 ± 44	n.d.	519 ± 9	518 ± 31	n.d.	n.d.	n.d.	n.d.	IV
Ethyl 4-(dimethylamino)benzoate	1 ± 0.1	1 ± 0.1	1 ± 0.2	n.d.	n.d.	n.d.	n.d.	n.d.	1.5 ± 0.1	1.6 ± 0.2	1.4 ± 0.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	II
Dimethyldibenzylidene sorbitol	13.4 ± 0.3	13.8 ± 0.8	13.3 ± 0.8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	12.5 ± 0.1	12 ± 1.3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	I; SML 60
Oxybenzone	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	17.7 ± 1.8	21.4 ± 0.1	19.6 ± 1.6	n.d.	n.d.	n.d.	11.6 ± 1.3	12.4 ± 0.1	n.d.	n.d.	V; SML 6
1-octylpyrrolidin-2-one	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	42.4 ± 3.4	41.5 ± 2.1	n.d.	52.3 ± 2.2	52.3 ± 1	n.d.	n.d.	n.d.	n.d.	IV
Tebuconazole	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	12.2 ± 0.6	12.4 ± 0.9	n.d.	12.5 ± 0.3	12.5 ± 0.1	n.d.	n.d.	n.d.	n.d.	V
Lauric acid diethanolamide	31.2 ± 2.8	34 ± 2.1	40.2 ± 1.9	4.9 ± 0.4	0.9 ± 0.1	1.2 ± 0.4	1.1 ± 0.03	< LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	II; SML 5
Diflufenican	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	38 ± 10.9	42.2 ± 3.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	IV
tributyl citrate	2.2 ± 0.6	2.9 ± 0.4	2.3 ± 0.2	1.2 ± 0.3	n.d.	n.d.	n.d.	n.d.	4.5 ± 0.6	2 ± 0.2	1.6 ± 0.1	< LOQ	< LOQ	< LOQ	4.4 ± 0.4	4.3 ± 0.71	n.d.	n.d.	IV
3-(3,5-Di-tert-butyl-4-hydroxyphenyl)propionic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	III
1,2,3-trideoxy-4,6:5,7-bis-o-[(4-propylphenyl)methylene]-nonitol (NX 8000 K)	21.8 ± 1.6	23.5 ± 1.1	15.2 ± 4.5	19 ± 2.3	n.d.	n.d.	n.d.	n.d.	32.6 ± 11	39.2 ± 13	27.7 ± 2.3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	II; SML 5
Octocrylene	28.8 ± 0.3	33.4 ± 1.6	28.2 ± 0.6	22.3 ± 4.9	n.d.	n.d.	n.d.	n.d.	79.5 ± 2.1	503 ± 6	456 ± 33	n.d.	n.d.	n.d.	118 ± 5	122 ± 0.1	n.d.	n.d.	IV; SML 0.05
Avobenzone	< LOQ	< LOQ	< LOQ	< LOQ	n.d.	n.d.	n.d.	n.d.	16.6 ± 0.6	199 ± 12	210 ± 14	n.d.	n.d.	n.d.	30.6 ± 2.6	33.7 ± 0.4	n.d.	n.d.	IV
2-Ethylhexyl 4-methoxycinnamate	47.8 ± 0.2	45.8 ± 0.7	46.7 ± 0.6	49.5 ± 1.6	n.d.	n.d.	n.d.	n.d.	58.2 ± 1.8	198 ± 8	193 ± 8	n.d.	n.d.	n.d.	52.2 ± 0.7	50.3 ± 0.6	n.d.	n.d.	V
Palmitamide	< LOQ	< LOQ	< LOQ	< LOQ	n.d.	n.d.	n.d.	n.d.	< LOQ	< LOQ	< LOQ	n.d.	n.d.	n.d.	< LOQ	< LOQ	n.d.	n.d.	IV
2-stearoylglycerol	296 ± 24	247 ± 22	277 ± 1	135 ± 0.4	n.d.	n.d.	n.d.	n.d.	630 ± 29	297 ± 22	262 ± 13	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	II
Chimassorb 81	1.7 ± 0.04	1.2 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	n.d.	n.d.	n.d.	n.d.	1.6 ± 0.1	5.4 ± 0.3	5.2 ± 0.6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	II; SML 6
Dioctyl phthalate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	49.2 ± 2.8	57.4 ± 2.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	V
bis(2-ethylhexyl) adipate	74.6 ± 18	76.9 ± 24	66.4 ± 29	106 ± 1	n.d.	n.d.	n.d.	n.d.	189 ± 8	120 ± 12	79.9 ± 19	n.d.	n.d.	n.d.	31.1 ± 6.4	22.3 ± 1.1	n.d.	n.d.	II; SML 18
Palmitic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	I; SML 60
Erucamide	362 ± 85	279 ± 80	223 ± 86	229 ± 26	n.d.	n.d.	n.d.	n.d.	742 ± 140	917 ± 97	600 ± 271	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	I; SML 60
Irgafos 168	462 ± 151	395 ± 57	365 ± 115	203 ± 200	n.d.	n.d.	n.d.	n.d.	236 ± 98	176 ± 3	374 ± 284	n.d.	n.d.	n.d.	125 ± 38	95.2 ± 0.4	n.d.	n.d.	I; SML 60
Oleic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	I; SML 60
Docosanamide	< LOQ	< LOQ	< LOQ	< LOQ	n.d.	n.d.	n.d.	n.d.	< LOQ	< LOQ	< LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	I; SML 60
diisodecyl phthalate	< LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	< LOQ	6 ± 1.6	4 ± 2.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	V
Irganox 1010	210 ± 41	161 ± 18	147 ± 19	89.5 ± 9.3	n.d.	n.d.	n.d.	n.d.	429 ± 32	124 ± 10	195 ± 0.1	n.d.	n.d.	n.d.	184 ± 24	118 ± 35	n.d.	n.d.	I; SML 60
2,5-Bis(5-tert-butyl-benzoxazol-2-yl)thiophene	8.8 ± 0.4	6 ± 0.1	6.3 ± 0.7	6.7 ± 0.5	n.d.	n.d.	n.d.	n.d.	9.2 ± 0.3	5.9 ± 0.6	5.5 ± 0.4	n.d.	n.d.	n.d.	7.6 ± 0.7	7.8 ± 0.1	n.d.	n.d.	III; SML 0.6
Oxidized Irgafos 168	168 ± 0.1	147 ± 0.1	176 ± 9	208 ± 55	n.d.	n.d.	n.d.	n.d.	348 ± 3	360 ± 10	367 ± 4	n.d.	n.d.	n.d.	198 ± 1	195 ± 4	n.d.	n.d.	IV
Irganox 1076	632 ± 138	549 ± 28	511 ± 17	452 ± 3	n.d.	n.d.	n.d.	n.d.	196 ± 19	153 ± 4	163 ± 6	n.d.	n.d.	n.d.	94 ± 3	107 ± 6	n.d.	n.d.	II; SML 6
Glycerol dihexanoate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	61.2 ± 1.3	52.1 ± 0.7	50 ± 4.7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	II

Note: n.d. stands for Not Detected.

Table III-5.3 Quantification detail in 95% ethanol and 3% acetic acid

Name	95% ethanol				3% acetic acid			
	Range	R^2	LOD	LOQ	Range	R^2	LOD	LOQ
Aminophenazone	1-114	0.9955	0.6	2.0				
o-Anisidine	1-103	0.9985	0.3	1.0	1-94	0.9975	0.4	1.2
2,4-Dimethylbenzenamine	1-114	0.9947	0.2	0.6	1-117	0.9947	0.1	0.4
Thiabendazole					1-43	0.997	0.2	0.6
Caprolactam	24-2395	0.9948	3.9	12.8	5-938	0.9989	0.4	1.4
Caffeine					1-95	0.9965	0.2	0.6
N,N-bis (2-hydroxyethyl)dodecylamine	0.1-44	0.9989	0.02	0.1	0.1-54	0.9982	0.01	0.02
N-[3-(Dimethylamino)propyl]dodecanamide	1-108	0.9975	0.2	0.7	0.1-48	0.9933	0.02	0.1
N,N-Dimethyltetradecylamine	0.1-48	0.9969	0.01	0.04	1-98	0.9992	0.01	0.0
Pyrimethanil	1-109	0.996	0.1	0.2	1-98	0.9968	0.1	0.3
N,N-Dimethylhexadecylamine	1-127	0.9975	0.1	0.2	8-841	0.9973	0.6	1.9
N-methyldidecylamine	0.1-104	0.9982	0.02	0.1				
3,3'-dichlorobenzidine	10-2301	0.9964	1.1	3.7	1-890	0.9928	0.4	1.4
Propanil	5-965	0.9999	0.5	1.6	11-953	0.9991	0.2	0.6
Ethyl 4-(dimethylamino)benzoate	1-52	0.9942	0.3	0.9				
Dimethyldibenzylidene sorbitol	1-100	0.9954	0.3	1.0				
Oxybenzone	5-105	0.9953	1.5	5.0				
1-octylpyrrolidin-2-one	1-119	0.9968	0.1	0.3	1-96	0.9935	0.1	0.4
Tebuconazole	1-95	0.9973	0.04	0.1	1-91	0.9986	0.2	0.6
Lauric acid diethanolamide	1-111	0.9989	0.3	1.1	1-109	0.9996	0.1	0.2
Diflufenican	1-98	0.9987	0.3	0.9				
tributyl citrate	1-48	0.995	0.3	1.1	1-93	0.9943	0.8	2.7
1,2,3-trideoxy-4,6:5,7-bis-o-[(4-propylphenyl)methylene]-nonitol (NX 8000 K)	10-989	0.9944	3.7	12.1				
Octocrylene	12-109	0.9996	0.3	1.0				
Avobenzone	10-1754	0.9987	1.6	5.4				
2-Ethylhexyl 4-methoxycinnamate	49-1947	0.9949	5.6	18.5				
Palmitamide	20-1878	0.9926	179.8	593.2				
2-stearoylglycerol	11-970	0.9945	2.3	7.6				
Chimassorb 81	1-101	0.9998	0.3	0.9				
Dioctyl phthalate	21-2121	0.9985	10.1	33.2				
bis(2-ethylhexyl) adipate	12-1221	0.9974	0.8	2.7				
Erucamide	22-1987	0.9916	51.8	171.1				
Irgafos 168	54-1838	0.9845	7.3	24.2				
Docosanamide	2-191	0.9949	16.1	53.0				
diisodecyl phthalate	4-381	0.994	1.0	3.3				
Irganox 1010	49-4535	0.9976	3.4	11.2				
2,5-Bis(5-tert-butyl-benzoxazol-2-yl)thiophene	1-101	0.9916	0.4	1.3				
Oxidized Irgafos 168	12-1155	0.9905	0.7	2.3				
Irganox 1076	11-976	0.9914	0.7	2.4				
Glycerol dihexanoate	11-1056	0.9934	3.2	10.6				

5. Conclusions

To the best of our knowledge, it is the first time to characterize non-volatile compounds present in rHDPE by non-targeted screening. The publicly available MS/MS libraries and our in-house libraries were of great help to identify significant number of substances, especially those pesticides in the public libraries and plastic related chemicals in our libraries. The large number of features found in MS-DIAL were cleaned by MS-CleanR keeping only precursor ions for the subsequent structural elucidation in MS-FINDER. However, manual examination of MS/MS spectra of the remained features is recommended, as some of them might not have representative spectra, and structural elucidation using these spectra might be erroneous and meaningless. Pseudo-MRM of all identified compounds (both in extracts and migrates) using precursor-product ion pairs exported from MS-DIAL provided a more sensitive and precise pseudo targeted analysis of these compounds in the samples.

Taking the list of chemicals identified in GC-MS as a structure database for MS-FINDER or any other *in-silico* fragmentation tool, e.g., MetFrag (Ruttkies et al., 2016) and Sirius (Dührkop et al., 2019), correspondences of some unknowns (in LC-QTOF-MS) to the identified compounds (in GC-MS) were easily found integrating both exact mass, *in-silico* fragmentation as well as their distributions among samples in the two systems. It helped us avoid spending plenty of time on elucidating compounds that were already known in the samples. The cppDB was valuable for identifying plastic related compounds in the recycled plastics, and it is expected to be helpful for other investigations associated with plastic packaging. As the scientific knowledge of plastic packaging is continuously growing, there is a need to keep the database up to date, for example, to include many (newly) identified/suspected NIAS. The proposed strategy is valuable for the identification of known unknown, but not for unknown unknown, which is much more challenging, as they might not be present in any existing structure databases.

It is sure that company 2 samples with high amount of non-milk-bottle plastics were of high risk for food contact uses, as a number of high-risk substances (e.g.,

pesticides) were detected in either 95% ethanol and/or 3% acetic acid migration with relatively high migration. For well-separated rHDPE milk bottles, octocrylene and 2-ethylhexyl-4-methoxycinnamate were the main obstacles for their uses for contacting fatty food, but their uses for acidic food might not be problematic.

Session IV: Conclusions

At the end, several conclusions can be drawn from the studies conducted in this thesis. These conclusions are organized in a chapter-by-chapter fashion below:

Chapter 1:

- Conventional GC-MS and APGC-QTOF-MS have been successfully combined to facilitate and improve non-target screening of volatile and semi-volatile substances in two PP samples.
- Comparable retention time was optimized in the two systems by adjusting carrier gas flow in APGC-QTOF-MS.
- Hydrocarbons were not ionized well in APGC-QTOF-MS, which give rise to much simpler chromatograms compared to GC-MS.
- Hydrocarbons were detected in high concentration (around 10 μg/g), uncommon adducts such as $[M-H]^+$, $[M-3H+O]^+$, $[M-3H+2O]^+$, and $[M-H+3O]^+$ have been found for alkanes, which might complicate molecular formula deduction during structural elucidation.
- Diol compounds have been found to react with silanediol dimethyl, which would be a transformation product of the silicone-based septum or the methyl 5% phenyl polysiloxane-based column. The reaction happens before the ionization and will give an additional peak in the chromatograms.

Chapter 2:

- A solvent free, ease-to-use, and sensitive DI-SPME-GC-MS method has been established and optimized for non-target screening of volatile and semi-volatile migrants in 3% acetic acid, 10% and 95% ethanol food simulants employing response surface methodology together with central composite design.

- The method can be extended to other liquid food simulants such as 20% and 50% ethanol by simply diluting the simulants to solutions with low organic solvent content, e.g., 10% ethanol.

- For 3% acetic acid simulant, pH adjustment to 7 is paramount for detecting many amines substances.

- The power of the proposed method has been evaluated by LOD and repeatability of 35 food contact materials-related chemicals. Most of them have very low LOD (< 10 μg/kg, which is the SML for non-listed substances in EU 10/2011).

- Many amine and diol compounds were found to have relatively high LOD or to be non-detectable at 1 mg/kg, which is thought to be the result of their GC-unamenable properties. Significantly different LODs of the assessed compounds also suggest that a small peak in GC-MS does not necessarily mean low concentration. Therefore, we recommend focusing on all qualifiable features rather than on only "big" peaks regarding non-target screening of migrants.

Chapter 3:

- 474 migrants have been identified from 15 post-consumer polyolefin pellets after migration to 3% acetic acid and 95% ethanol by connecting DI-SPME to both GC-MS and APGC-QTOF-MS.

- Among them, 39.2% were food components or additives and 24.1% were saturated hydrocarbons, fatty acyls, or prenol lipids, which might not be human risk.

- Most of the migrants have MW between 150-210 Da. Nevertheless, high migration of octocrylene (361.5 Da) suggests that MW of surrogates up to 300 Da, which is the case of PET, is insufficient for challenge test of recycled polyolefins and high MW substances such as octocrylene, hexadecenoic acid dodecyl ester and triacontane could be added.

- Chemicals with high XLogP had higher potential to migrate to 95% ethanol, while those with low XLogP tended to migrate more to 3% acetic acid.

- Combining toxicity, detection frequency, and S/N, we were able to prioritize and then focus on migrants of high concern. Octocrylene, 1-tetradecene, 1-dodecene, and dodecyl acrylate had migration higher than their SML. In addition, 2,4-di-tert-butylphenol and 1,4-benzenedicarboxylic acid,diethyl ester were of high concern in 95% ethanol (fatty food) migration concerning their detection frequency and highest concentration. For 3% acetic acid simulant (acidic food), benzenamine, 2,4-dichloro- and diethyl phthalate deserve more attention.

Chapter 4:

- MS-DIAL enabled fast and reliable GC-MS data processing by integrating automatic peak detection, deconvolution, alignment, filtration, and identification (combining NIST EI-MS library, experimental and predicted retention index).

- Chemicals present in the samples from different recyclers varied considerably, while those from different batches of a given recycler gave similar results. However, the study of rHDPE stream mixed with high volume of non-milk-bottles provided significant differences between batches.

- Both washing twice and the extra decontamination technique showed comparable cleaning efficiency. However, high efficiency of washing twice could be the result of additional steps, which is unknown to the author, to remove non-milk-bottle rHDPE contamination. A noticeable discrepancy was that extra decontamination was able to reduce the migration of 1-tetradecene to a safe level while washing twice did not.

- Quantification of prioritized migrants showed that sever contaminated samples (high non-milk-bottle rHDPE fraction) contained many high concern substances such as pesticides, and therefore are not feasible for contact applications. For samples with negligible non-milk-bottle contamination, high migration of high

concern substances, e.g., 1-dodecene, 1-tetradecene, octocrylene, and 2-ethylhexyl-4-methoxycinnamate were also found. The former two substances could be sufficiently lowered by extra decontamination, while the latter two could not as they high much higher MW. Octocrylene and 2-ethylhexyl-4-methoxycinnamate could be cross contaminants from cosmetic packaging, which suggests that measures to mitigate cross contaminations from cosmetic/personal care packaging could be of great help to get high quality rHDPE.

Chapter 5:

An in-house MS/MS library (in both positive and negative mode), which entails more than 300 food packaging-related substances, has been built. Publicly available MS/MS libraries and the in-house library were able to annotate a significant number of non-volatile compounds in rHDPE milk bottle samples with the help of MS-DIAL.

After feature cleaning by MS-CleanR, MS-FINDER together with several sample-related structure databases were able to identify many remaining unknowns and a total of 83 non-volatile compounds have been identified in rHDPE milk bottle samples.

A *pseudo*-MRM analysis enable us to detect the identified compounds automatically and sensitively in each sample.

This comprehensive LC-HRMS data processing workflow (library matching, feature cleaning, *in-silico* fragmentation of sample related structure databases, and *pseudo*-MRM) was successfully applied to the rHDPE milk bottle samples, and it can be extended to other FCMs as well.

Session V: Publications

Publications:

Su, Q.Z., Vera, P., Van de Wiele, C., Nerín, C., Lin, Q.B., Zhong, H.N., 2019. Non-target screening of (semi-)volatiles in food-grade polymers by comparison of atmospheric pressure gas chromatography quadrupole time-of-flight and electron ionization mass spectrometry. Talanta 202, 285–296. https://doi.org/10.1016/j.talanta.2019.05.029

Su, Q.Z., Vera, P., Nerín, C., 2020. Direct immersion-solid-phase microextraction coupled to gas chromatography-mass spectrometry and response surface methodology for nontarget screening of (semi-) volatile migrants from food contact materials. Anal. Chem. 92, 5577–5584. https://doi.org/10.1021/acs.analchem.0c00532

Nerín, C., **Su, Q.Z.**, Vera, P., Mendoza, N., Ausejo, R., 2020. Influence of nonylphenol from multilayer plastic films on artificial insemination of sows. Anal. Bioanal. Chem. 412, 6519–6528. https://doi.org/10.1007/s00216-020-02698-2

Liu, Y.Q., Wrona, M., **Su, Q.Z.**, Vera, P., Nerín, C., Hu, C.Y., 2021. Influence of cooking conditions on the migration of silicone oligomers from silicone rubber baking molds to food simulants. Food Chem. 347, 128964. https://doi.org/10.1016/j.foodchem.2020.128964

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Session VI: Bibliography

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