Master's thesis

NTNU
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Department of Chemistry

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Non-target analysis of chemicals extracted and migrated from plastic food contact articles

Master's thesis in Environmental Toxicology and Chemistry
Supervisor: Martin Wagner
Co-supervisor: Sarah Stevens
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Trondheim, 30.05.2022
Hanna Sofie Skåland

Abstract

Plastic production is constantly increasing, and today plastics are used for all kinds of purposes, including food packaging and other food contact articles (FCA). To obtain desired properties of the plastics, numerous chemical additives are used in plastic production. In addition, non-intentionally added substances are present in plastics. These chemicals are normally not covalently bound to the polymer, and can thus migrate from the plastic products to the environment. For plastic FCA, chemicals can migrate to the foodstuff, and in that way constitute an important source of human exposure to chemicals. The research on the total number of chemicals present in plastics is limited, and little is known about their identity and toxicity. Knowledge is also missing on the extent of migration of plastic chemicals migrating to food. The aim of this project was therefore to increase the knowledge of the chemical composition of plastic FCA, tentatively identify the chemicals present in plastics and investigate the migration of plastic chemicals to food simulants. To investigate the chemical composition of the plastics, 39 plastic samples made of six different polymer types were cut into pieces, extracted with methanol (MeOH) and analyzed using a non-target approach. In addition, a migration study investigating the migration of plastic chemicals into 50% ethanol (EtOH) and water was performed for three of the samples, and the extent of migration into the two solvents was compared to MeOH extraction. The migration kinetics of the three samples were also assessed, focusing on the increasing and stabilizing features.

More than 16800 unique chemical features were detected in the MeOH extracts, and the number of features in each sample varied from 37 to 9936. The polyurethane (PUR) and polyvinyl chloride (PVC) samples had the highest numbers of features on average, while the polyethylene terephtalate (PET) samples contained the lowest number of chemical features. However, the variations were large within each polymer type, and no clear correlation was found between the number of features and the polymer type or the type of product. Only 16% of the detected features were tentatively identified, meaning that most of the features remained unknown. Of the identified compounds, several were known to be toxic, and some relatively potent toxicants were detected with high abundances in the samples. The number of chemical features migrating to 50% EtOH was considerably higher than to H2O. Compared to MeOH extraction, more and other features were migrating to 50% EtOH. This means that MeOH was not extracting all chemicals present in the plastic samples, and illustrates that the detected chemical composition is highly dependent on the solvent used for extraction or migration. The migration into 50% EtOH was also larger than previously known, and indicates that humans are exposed to many plastic chemicals through food intake. The assessment of migration kinetics revealed large variations between the samples and migration solvents. Several features did not reach an equilibrium after ten days, and further research on migration kinetics over longer periods of time is recommended to improve the test conditions.

Sammendrag

Produksjonen av plast er stadig økende, og i dag blir plast brukt til alle slags formål, deriblant til produkter som skal være i kontakt med mat (FCA). For å gi plasten ønskede egenskaper, blir mange tilsetningsstoffer brukt i plastproduksjon. I tillegg inneholder plast stoffer som ikke er tilsatt med vilje. Disse kjemiske stoffene er vanligvis ikke kovalent bundet til polymeren, og kan dermed migrere fra plastproduktet til omgivelsene. For FCA, kan stoffene migrere til matvarene og på den måten utgjøre en kilde til kjemikalier for mennesker. Forskningen på det totale innholdet av kjemiske stoffer i plast er begrenset, og lite er kjent om disse stoffenes identitet og toksisitet. Kunnskapen om migrasjon av kjemikalier til mat er også mangelfull. Målet med dette prosjektet var derfor å øke kunnskapen om den kjemiske sammensetningen av FCA, identifisere stoffene som finnes i plast og undersøke migrasjonen av kjemikalier til mat-simulanter. For å undersøke den kjemiske sammensetningen av plast, ble 39 plastprodukter laget av seks forskjellige polymertyper kuttet i biter, ekstrahert med metanol (MeOH) og analysert med en "non-target" tilnærming. I tillegg ble migrasjonen av plastkjemikalier til 50% etanol (EtOH) og vann undersøkt for tre av plastproduktene, og migratene ble sammenlignet med MeOH-ekstraktene. Migrasjonskinetikken ble også undersøkt, med fokus på forbindelsene som økte og stabiliserte seg i løpet av migrasjonstiden.

Mer enn 16800 forskjellige kjemiske forbindelser ble funnet i MeOH-ekstraktene, og antallet stoffer i hver prøve varierte mellom 37 og 9936. Polyuretan (PUR) og polyvinylklorid (PVC) inneholdt flest stoffer i gjennomsnitt, mens polyetylentereftalat (PET) inneholdt færrest stoffer. Variasjonen var imidlertid stor innenfor samme polymertype, og det ble ikke funnet noen klar sammenheng mellom antallet kjemiske forbindelser i en prøve og polymertypen eller typen produkt. Bare 16% av forbindelsene som ble funnet ble tentativt identifisert, noe som betyr at flesteparten av forbindelsene forble ukjente. Av de identifiserte forbindelsene var flere av dem kjente toksiske kjemikalier, og noen relativt potente kjemikalier ble funnet med høy konsentrasjon i prøvene. Antallet kjemiske forbindelser som migrerte til 50% EtOH var tydelig høyere enn til vann. Sammenlignet med MeOH-ekstraksjon var det flere og andre forbindelser som migrerte til 50% EtOH. Dette betyr at MeOH ikke ekstraherte alle kjemikaliene i plastproduktene, og illustrerer at den kjemiske sammensetningen som blir funnet avhenger av hvilken væske som blir brukt til ekstraksjon eller migrasjon. Migrasjonen til 50% EtOH var større enn tidligere kjent, hvilket indikerer at mennesker blir eksponert for mange plastkjemikalier gjennom maten de spiser. Undersøkelsen av migrasjonskinetikk viste at det var store variasjoner mellom prøvene og mellom 50% EtOH og vann. Mange forbindelser hadde ikke stabilisert seg etter ti dager, og videre forskning på migrasjons-kinetikk over lengre tidsperioder er anbefalt for å forbedre testbetingelsene.

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List of abbreviations

Abbreviation	Explanation
AA	Anti-androgenic
AR	Androgen receptor
DDA	Data dependent acquisition
DIA	Data independent acquisition
DMSO	Dimethyl sulfoxide
ERα	Estrogen receptor
ESI	Electro spray ionization
EtOH	Ethanol
FCA	Food contact articles
FCC	Food contact chemicals
FTIR	Fourier-transform infrared spectroscopy
GC	Gas chromatography
HPLC	High performance liquid chromatography
LC	Liquid chromatography
MeOH	Methanol
MS	Mass spectrometry
m/z	Mass to charge ratio
NIAS	Non-intentionally added substances
PB	Procedural blank
PE	Polyethylene
PE_HD	High density polyethylene
PE_LD	Low density polyethylene
PET	Polyethylene terephtalate
PP	Polypropylene
PPARγ	Peroxisome proliferator activated receptor gamma
PS	Polystyrene
PUR	Polyurethane
PVC	Polyvinyl chloride
PXR	Pregnane X receptor
QC	Quality control
QTOF	Quadrupole time-of-flight
SPE	Solid phase extraction
TTR	Time trend ratio
UPLC-QTOF-MS/MS	Ultra high pressure liquid chromatography quadrupole time-of-flight tandem mass spectroscopy
UPW	Ultra pure water

1 Introduction

1.1 Plastics

Plastics are widely used in many industrial sectors, and a world without them is hard to imagine. Plastics are versatile, cheap and lightweight materials that are beneficial for human health and the environment in several ways [1]. However, there are also multiple downsides associated with the use of plastic products, including extensive waste generation [1], potential shortage of resources in the future [2], and migration of hazardous chemicals, posing a threat to human health and the environment [3][4]. The global production of plastics is increasing, and today about 350 million tons of plastic are produced every year [5].

In Europe, the largest percentage of the plastics is used for packaging (39.9%) [5], and 60% of this is used for food contact purposes [4]. Food contact articles (FCA), as defined by Muncke et al. (2017), are finished products that are meant for food contact, consisting of one or more food contact materials, like plastics, coatings, adhesives and printing inks [6]. Food packaging and other plastic FCA can be important to the society, as they protect the food from physical damage and microbial growth, and delay the degradation of the foodstuff [6] [7]. However, FCA also contain food contact chemicals (FCC), that can be intentionally or non-intentionally added to the plastic [8].

1.1.1 Plastic chemicals

Plastics are complex mixtures of one or more polymers, fillers and chemical additives [1] [3] [9]. The plastic polymers are made by polymerisation of monomers. In order to make the polymerisation reaction occur, initiators and catalysts are often used in addition to the monomers [3]. The plastic polymer is further improved by the addition of chemical additives. These are added to give certain properties, and each of them is important for the functionality of the final product [1]. Commonly used additives include antioxidants, pigments, plasticizers, UV stabilizers, lubricants, slip agents and flame retardants [2] [10] [11].

Two recent studies aimed at providing an overview of chemicals present in plastics. Groh et al. (2019) found 906 chemicals that were likely associated with plastic packaging and 3377 chemicals that were possibly associated [4]. Wiesinger et al. (2021) found 10547 chemicals likely used as monomers, additives or other processing aids in plastic production in general, and of these, 8567 were identified as having high confidence in their use in plastics [11]. Both studies identified hazardous compounds, and Wiesinger et al. found over 2400 compounds that were considered potentially concerning, due to their persistant, bioaccumulative or toxic properties [11].

In addition to these known chemicals and intentionally added additives that are present in plastics, it is also well known that plastics contain non-intentionally added substances (NIAS) [4]

[12] [13]. NIAS can be impurities present in the raw materials or additives that have been used in the manufacturing of the plastic product [12]. Further, they can be byproducts or residual compounds from the polymerisation reactions during production, e.g. oligomers [14]. The polymerisation reaction is normally not complete, and unreacted monomers can therefore be found in the finished product as NIAS [3]. In addition, degradation of the polymer or the additives due to high temperature or irradiation can lead to formation of NIAS. These compounds are often smaller than the original compounds, and are therefore more likely to migrate from the plastic product [12]. NIAS are specifically mentioned in the European regulation on plastic materials and articles intended to come into contact with food [15], and this has increased the interest in the evaluation of NIAS [16].

1.2 Migration

Both the intentionally added and non-intentionally added compounds can migrate into food. The polymer itself is considered not to be bioavailable due to its large molecular size [3], but the additives, which are mostly not covalently bound to the polymer, may be released to the environment via migration to liquids or solids, e.g. foodstuffs [17]. Migration can also occur from the food to the packaging [18], but in the following, migration will be referred to as the mass transfer of compounds from the FCA to the food [19] [20]. Chemicals can migrate from the surface of the plastic product to the food that is in contact with the FCA, but it can also slowly migrate from the inside of the plastic to the surface [1] [19]. The basic principle behind the migration, is diffusion of a compound from an area with higher concentrations to an area with a lower concentration [14] [19].

The degree of migration from a FCA is dependent upon the chemical composition of the FCA and the foodstuff, and the properties of the migrating compounds. In addition, temperature, contact time and contact area between the FCA and the food is affecting the migration. [10] [21] [22]. A longer contact time will increase the chance for migration, and a larger direct contact area will give a higher migration potential [14] [21]. The latter implies that smaller containers have a higher migration potential than larger containers, as a larger part of the food will be in contact with the plastic in a smaller container. Higher temperatures will make the migration go faster, and big fluctuations in temperature (for example from freezer temperature to cooking temperature) also increases the migration considerably [10].

When it comes to characteristics of the migrating compound, it has been found that migration is dependent on size. Generally, small molecules with low boiling points have the highest diffusion coefficient and thus have a higher migration potential [12] [18]. The migration rate is also dependent on the solubility of the compound in the FCA compared to in the food, and the solubility of additives should therefore be high in the plastic and low in the foodstuff that is in contact with the plastic [1]. Regarding the properties of the foodstuff, the migration rate

normally increases with increasing fat content [1] [18], and the migration rates are also higher in acidic foods [21].

1.2.1 Regulatory migration testing guidelines

For plastic FCA, migration testing guidelines for the EU are given in Commission Regulation (EU) No. 10/2011 [15]. The guidelines provide information on the choice of food simulants, temperature and time periods for testing. The determination of migrating compounds from FCA to food is for many reasons not carried out under real conditions [16] [18]. For instance, the testing is normally not performed in food, as food has a very complex chemical and physical structure [18] [19]. The large number of interfering compounds in the food matrix thus makes it difficult to identify migrating compounds [16] [23]. Instead extractants or food simulants can be used to evaluate the chemical composition of the FCA. Extractants and food simulants are used to determine the total content of the FCA, and the migrating fraction of the chemicals respectively [16].

Food simulants are supposed to mimic the behaviour of real food, and simulate the interactions between the FCA and the food [16] [19]. The migration tests are constructed to slightly overestimate the migration into real food, to represent a worst case migration scenario [16] [24] [25]. To simulate aqueous foods, water or 10% ethanol (EtOH) in water is often used as a food simulant. If the food is acidic (pH lower than 4.5), 3% acetic acid in water is used [15] [18]. For foods with a more lipophilic character, 20% or 50% EtOH can be used. 20% EtOH is used for alcoholic foods with an alcohol content up to 20%, and food with a considerable amount of organic ingredients, e.g. fruit puree. 50% EtOH is used as a food simulant for oil in water emulsions, like dairy products, and for alcoholic foods with an alcohol content above 20%. For foods that contain free fats on the surface, vegetable oil is used, and to test migration into dry food, poly(2,6-diphenyl-p-phenylene oxide) is used [15].

The EU regulation also provide guidelines for the temperature and length of the migration testing. This should be decided based on the worst foreseeable conditions of time and temperature that could be used in actual application of the FCA [19]. To test the migration, migration tests are normally carried out over a shorter period of time and a higher temperature than the real conditions [18]. The migration tests used to simulate long time periods of contact are carried out for ten days, either at 20°C or 40°C. The former should be used for food stored in freezer or refrigerator, while the latter is for "storage at room temperature or below, including heating up to 70°C for up to two hours, or heating up to 100°C for up to 15 minutes" [15].

1.3 Non-target chemical analysis

To analyze and identify the migrating and extractable chemicals, target or non-target analysis are used. Previously, targeted analysis was by far the most common, and several studies have

focused on relatively few selected compounds or groups of compounds. These are often toxic compounds that are unwanted in FCA, and include bisphenol A [26] [27] and phthalates [28] [29] [30] among others. Target analysis of a few selected compounds has also been used to assess the safety of FCA recently [31]. This approach is problematic, as the targeted analysis is focusing on one or a few chemicals, and misrepresents the chemical complexity of the FCA, since other additives with unknown toxicity, or unknown NIAS will not be investigated [32]. When the total chemical mixture of migrating compounds are not taken into account, a conclusion about human health risk cannot be drawn with certainty [33].

The non-targeted approach has gained more popularity the last years, and has been used to analyze wastewater samples [33], organic contaminants in food, environmental and biological samples [34], chemicals in medical masks [35] and compounds migrating from plastic packaging [7]. This approach aims at detecting and identifying as many of the compounds in a sample as possible, and by this also identify the unknown compounds present in complex samples. In non-targeted analysis, compounds are first tentatively identified by searching in spectral libraries [33] [34], before selected features may be confirmed using standards [36]. The main differences between targeted and non-target analysis are given in figure 1.1.

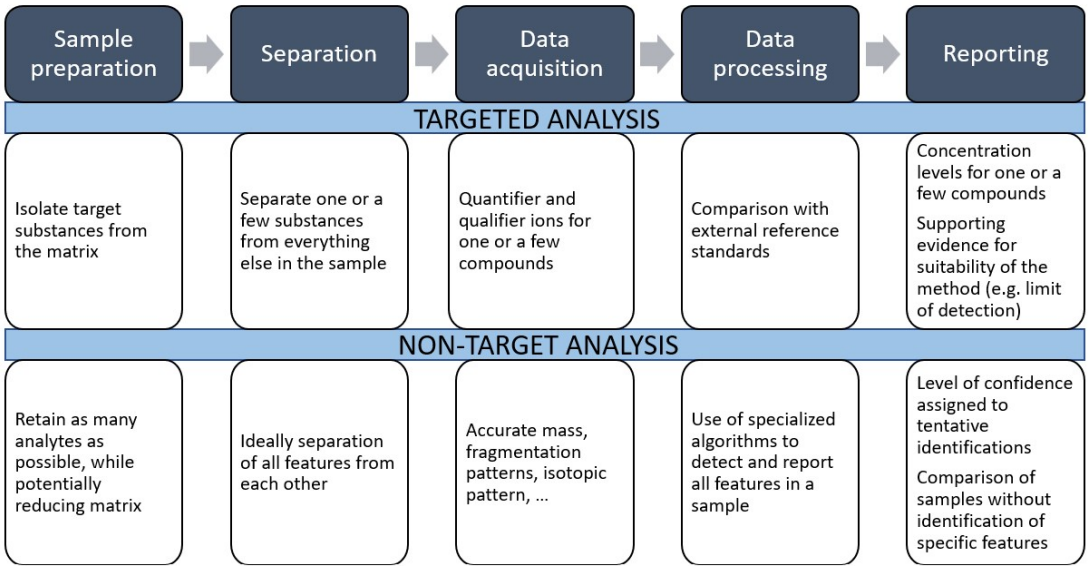


Figure 1.1: Overview of workflow steps in targeted and non-target analysis, and the main differences between the two approaches. Adapted from Schulze et al. (2020) [32].

1.3.1 Instrumental analysis

Mass spectrometry (MS) in combination with gas chromatography (GC) or liquid chromatography (LC) has been used for non-targeted analysis. GC is suitable for semi-volatile compounds, while non-volatile and more polar compounds should be separated by LC [7] [34] [37]. For analysis of migrates of plastic FCA, LC is the most commonly used separation technique [38]. The combination of LC and high resolution MS provides both separation of the

compounds in the sample and accurate mass measurements [39]. By that, it makes detection of a large number of compounds possible using a non-targeted approach. Also compounds that have not been previously identified, e.g. NIAS, can be detected with a high level of sensitivity [33].

A mass spectrometer consists of an ion source, a mass analyser and a detector [40]. The role of the ion source is to ionize the compounds eluting from the chromatographic column before entering the mass analyser [41]. In non-targeted analysis, the most commonly used ion source is electrospray ionization (ESI) in positive mode [38]. ESI is a weak ionization method, and it can be operated in both positive and negative ion mode, where the two polarities give complementary information [39].

In non-targeted analysis, a mass analyser with high resolution and full spectrum acquisition should be used to detect as many compounds as possible [34]. The most widely used mass analyser that provides this, is the quadrupole time-of-flight (QTOF) mass analyser [38]. This is a tandem MS, providing information about both the molecular ion and the fragmentation pattern. This is very useful when identifying compounds in complex samples where many compounds can have the same molecular formula [39]. The QTOF is similar to the triple quadrupole mass analyser, but the third quadrupole has been replaced by a time-of-flight tube [42]. The first quadrupole can be used as a mass filter to select ions with a specific mass to charge ratio (m/z), or it can let all ions pass through to the second quadrupole. The second quadrupole can be used as a collision cell, where the ions are bombarded with neutral gas molecules, resulting in fragmentation of the ions. It can also alternate between acting as a collision cell and letting all ions through, so that both molecular ions and fragmentation ions can be detected in the same run [38] [42]. After the second quadrupole, the ions go to the time-of-flight analyser where they are separated based on their mass [42].

The two quadrupoles can be operated with either data dependent acquisition (DDA) or data independent acquisition (DIA). In DDA, the data collection is done based on predetermined rules, determining when to switch between only detecting molecular ions and detecting fragment ions. In other words, when the second quadrupole acts as a collision cell and when it does not [38] [42]. The rules usually concern the intensity of the molecular ions observed, and the top n number of molecular ions are selected for fragmentation [39]. For these selected ions, information about both the molecular ion mass and the fragmentation pattern are obtained in the same run. DDA can be a suitable method for complex samples, where the probability of co-eluting compounds is high, and when the compounds with lower abundances are not important to detect or identify [39].

In DIA, all ions within a chosen m/z range are fragmented in the second quadrupole, and MS and MS/MS data for all the compounds are detected in the same run [38] [39]. This gives a tandem MS spectrum with fragmentation patterns for all the co-eluting molecular ions

at a given timepoint, regardless of their intensity [39]. This approach is advantageous for identifying unknown compounds, and is the most used acquisition method for non-targeted analysis [38]. However, for complex samples, sophisticated techniques are needed to link the fragmentation ions back to their respective molecular ions [34] [39]. MS^E acquisition is a commonly used DIA method, which thus collects data without any pre-selection [34] [38].

1.3.2 Data processing strategies

When a sample is analysed by high resolution MS, the number of detected peaks can be up to several thousand per sample. Consequently, processing and identification of non-targeted datasets is time consuming, and it is beneficial and time-saving to reduce the number of relevant peaks before attempting to identify the compounds [38]. The reduction is mainly done by prioritizing compounds that require further identification based on some criteria, and reducing potential false positive candidate compounds [39]. Today, there is no standard protocol describing how data from non-targeted analyses of plastic FCA should be processed [38], but Martínez-Bueno et al. (2019) [38] and Fisher et al. (2021) [39] have provided an overview of commonly used data processing strategies.

Firstly, when analyzing multiple samples, the detected peaks must be accurately aligned between samples to allow accurate and representative comparisons between them. This is important, as the retention times can shift during the sample analysis time [39] [43]. Further, all ions that correspond to the same compound should be grouped together into a "chemical feature", also called "feature" in a process called deconvolution. These ions originating from the same compound can be the fragmentation ions, isotopes and adducts [38] [39]. In the ESI, positive ions are most commonly formed by addition of a proton, however, ions can also be created by the formation of adducts [44], where other charged compounds are added to the molecule being ionized [45], for example Na^+, $NH_4{}^+$ and K^+. These ions can come from e.g. solvent impurities and glassware [41] [44].

After these steps, the data reduction process starts. A common way of reducing the amount of data is to compare the features detected in the samples to the features detected in procedural blanks, and remove background features that are not specific to the sample from further investigation [38] [39]. This can be done either by removing all the features that are present in the procedural blank from all the samples [46] [47], or by removing a feature only if it is below a set intensity ratio threshold in the sample compared to the blank [47]. Using a filter with area in sample/blank of more than ten [48] [49], or more than three [50] has been used previously. Other data reduction strategies include intensity thresholds and mass range restrictions. In many cases, a combination of more than one reduction strategy is necessary to reduce the number of ions to manageable levels, so that the most interesting ions can be identified and reviewed [38].

1.3.3 Identification

Identification of the features can be done by using all available information obtained from MS and MS/MS, such as molecular ions, isotope patterns and fragments. The fragments give a greater confidence in the identification when structural isomers with the same molecular weight are present in the sample [43]. In practice, the features are often tentatively identified by comparing the mass spectra to experimental or theoretical databases [38]. ESI is not a very stable and reproducible ion source, and standardized ESI mass spectra libraries are therefore not available. However, theoretical mass spectra libraries exist, and these can be used for the identification [38].

The software used for the identification gives a match score for the similarity between the experimental mass spectra and the database spectra. The real spectra do not always match very well with the theoretical spectra, for instance if co-eluting compounds have common fragment ions, and the spectra are interfering with each other [34]. Therefore, often a relatively low match score is used as the threshold to prevent loosing potential interesting compounds [34].

By comparing the mass spectra to library spectra, true unknown chemicals are omitted. In other words, chemicals that are not present in the databases can not be identified using this method. Therefore, some studies have also identified compounds without any information from databases. Instead, the compounds have been identified using a structural elucidation approach. Here, the molecular ion mass and the fragmentation pattern of the compound are investigated to be able to make an educated guess on the chemical structure of the unknown compounds [38].

When identifying compounds by comparing their spectra to database spectra, the compounds can be identified with a level 3 (tentative candidate) or 2 (probable structure) of confidence, as defined by Schymanski et al. (2014) [36]. However, the highest level of confidence, level 1 (confirmed structure), can not be obtained without confirming the structure using a reference standard [36] [51]. Reference standards are not always available, making it hard to reach a level 1 of confidence for all compounds [33] [38]. The large number of intentionally and non-intentionally added compounds present in plastics also hamper a prioritization of compounds.

1.4 Aim of the project

Until now, the plastic research has mainly focused on a few toxic compounds, and little is known about the total amount of chemicals present in FCA, their identity and toxicity. A closer investigation of these chemicals and their migration potential are therefore needed to determine the exposure of plastic chemicals to humans. Thus, the aim of this master project was to investigate the total amount of extractable and migratable chemicals present in plastic FCA. The chemical analysis of the plastics was the main focus of the thesis, and was done using a non-targeted approach. *In vitro* bioassays was also conducted in parallel by Sarah Stevens

(Department of Biology, NTNU). The project was rather exploratory, and as a consequence, no hypotheses was formulated. The goal of the project was to determine:

1. The number of extractable chemicals in the FCA,
2. Differences in chemical composition between polymer types,
3. The identity of the most prevalent chemicals extracted from the FCA,
4. The fraction of chemicals migrating into water and 50% ethanol (food simulants),
5. The migration kinetics in water and 50% ethanol over a ten days migration period.

2 Materials and methods

2.1 Plastic products

39 plastic FCA were used in this project (table 2.1). 17 of the plastic samples were in contact with food when bought, while the rest (22 samples) did not have any previous food contact. The products were selected to give a representative picture of the food contact plastics that are in use today. Therefore, the samples were covering the seven polymer types with the highest market share and global waste generation; polypropylene (PP), low density polyethylene (PE_LD), high density polyethylene (PE_HD), polystyrene (PS), polyethylene terephtalate (PET), polyvinyl chloride (PVC) and polyurethane (PUR) [5] [52]. They were obtained from four countries with high waste generation per capita in 2016 [53]; USA (105.3 kg/yr), UK (98.7 kg/yr), South Korea (88.1 kg/yr) and Germany (81.2 kg/yr), in addition to Norway (46.8 kg/yr) [54] [55].

The samples from South Korea, USA, Germany and the UK were purchased by partners in the respective countries (except sample PVC_4, PUR_12 and PVC_35 which were bought online). Any content was removed by gently washing with tap water in the countries of origin, before the samples were shipped to Norway in PE plastic bags. The PE bags used was sent from Norway in advance, to assure that the samples from the different countries were treated as similar as possible. After arrival in Norway, the samples with previous food contact, and the items that are normally washed before use, were washed with cold tap water and ultrapure water (UPW) before they were air dried.

The polymer type of all samples were confirmed using Fourier-Transform Infrared Spectroscopy (FTIR) (Agilent, Cary 610-FTIR microscope). Sample PS_33, PS_41 and PE_LD_44 were analyzed by Transmission-FTIR, while the rest were analyzed on the inside and outside by Attenuated Total Reflection-FTIR (table 2.1). Due to difficulties in distinguishing between PE_LD and PE_HD spectra, the PE samples were not divided into low and high density when this was not stated on the packaging. Two of the samples were composites of two different polymers, PE_PET_21b and PUR_PE_30.

2.2 Quality assurance measures

As far as possible, the use of plastic equipment was avoided in the experiments, and equipment made of glass or metal were used instead. An exception from this was the use of micro pipettes for transfer of small volumes. All scissors and glassware that were used for storing, and in the extraction and migration experiments went through a cleaning process before use, consisting of washing in washing machine, rinsing with UPW and acetone, and heating at 200°C for $>$ 2 h. For glass pipettes, the washing machine step was replaced by soaking the pipettes in hydrochloric acid (HCl, 1 M) for 24 h.

To avoid cross-contamination between samples, the scissors and clean-bench where the cutting took place were cleaned with ethanol between each sample. All other equipment used was also cleaned with ethanol before use. A cotton lab coat was always worn when cutting, to avoid microfiber contamination and keep the contamination sources similar for all the samples.

Table 2.1: Overview of the plastic products used in the project

Nr.	Product	Polymer	Previous food contact	Color	Country
1	Buttermilk	PS	Yes	White	Germany
3	Yoghurt cup	PP	Yes	White	Germany
4	Drinking tube	PVC	No	Transparent	Germany
5	Chewing gum box	PE_HD	Yes	Blue transparent	Germany
6	Couscous container	PP	Yes	Black	Germany
7	Food container	PE	No	White	Germany
8	Lid food container	PE_LD	No	Green	Germany
9	Oven bag	PET	No	Transparent	Germany
10	Freezer bag	PE_LD	No	Transparent	Germany
11	Dessert bowl	PS	No	Black	Germany
12	Drinking bladder	PUR	No	Blue	Germany
13	Cup	PS	No	Transparent	South Korea
14	Freezer bag	PE_HD	No	Transparent	South Korea
15	Zip lock bag	PE_LD	No	Transparent	South Korea
16	Bowl	PP	No	White	South Korea
17	Coffee cup	PP	Yes	Black	South Korea
18	Waterbottle	PET	Yes	Transparent	South Korea
21a	Sausage package	PE	Yes	Transparent	South Korea
21b	Sausage package	PE_PET	Yes	Orange	South Korea
25	Yoghurt cup	PET	Yes	Transparent	UK
26	Drinking bladder tube	PVC	No	Blue	Germany
27	Oven bag	PET	No	Transparent	UK
28	Freezer bag	PE	No	Transparent	UK
29	Cling film	PVC	No	Transparent	UK
30	Cheese package	PUR_PE	Yes	Transparent	UK
33	Styrofoam cup	PS	No	White	USA
34	Cling film	PE	No	Transparent	USA
35	Cling film	PVC	No	Transparent	USA
36	Flavored water bottle	PET	Yes	Transparent	USA
37	Frozen blueberries bag	PE	Yes	White	USA

Table 2.1: (continued)

Nr.	Product	Polymer	Previous food contact	Color	Country
38	Milk bottle	PE	Yes	Transparent	USA
39a	Yoghurt, big container	PP	Yes	White	USA
39b	Yoghurt lid, big container	PP	No	White	USA
40	Empty food container	PET	No	Transparent	Norway
40a	Food container	PET	Yes	Transparent	Norway
41	Empty plate	PS	No	Black	Norway
41a	Plate	PS	Yes	Black	Norway
43	Drinking bladder	PUR	No	Blue	Norway
44	Fruit netting	PE_LD	Yes	White	Norway

2.3 Extraction

13.5 g of each plastic product was cut into pieces of approximately 0.5-0.8 cm x 2.0 cm, and equal amounts (6.75 g in each) was divided between two 60 ml glass vials. The 13.5 g of plastic was extracted with 90 ml methanol (anhydrous, 99.8%, Sigma-Aldrich, 322415-1L). An exception was made for sample PE_LD_44, where 6.5 g plastic were exacted with 135 ml methanol, giving a three times lower concentration of plastic. The samples were extracted in a ultrasonic bath (VWR Ultrasonic Cleaner USC-TH) for 1 h at room temperature. On average, eight samples and one procedural blank (PB) consisting of only the solvent, methanol, were extracted at a time.

After extraction, an aliquot of 1 ml of the extract was taken out for the chemical analysis, and stored at -20°C until the analysis. In addition, 60 ml of the extract (30 mL from each vial) was taken out and concentrated under a gentle nitrogen stream (40°C, Reacti-vap III, Thermo Scientific). The wall of the vial was washed with the remaining solution several times during the evaporation process to keep most of the chemicals dissolved in the solution rather than precipitated on the walls of the vial. When the volume reached 1 ml, the extracts were transferred to 1.1 ml HPLC vials, and the evaporation was continued. When there was enough space in the vials, 600 µl dimethyl sulfoxide (DMSO) was added, and the remaining methanol was evaporated. The extracts in DMSO were then 100 times more concentrated than the initial extracts. They were stored at -20°C and later used in *in vitro* bioassays conducted by Sarah Stevens (Department of Biology, NTNU) screening for antiandrogenic activity (AA) and activation of the estrogen receptor alpha (ERα), the peroxisome proliferator activated receptor gamma (PPARγ) and the preganane X receptor (PXR). The DMSO extracts were also used in the migration experiment.

The extraction procedure is summarized in figure 2.1.

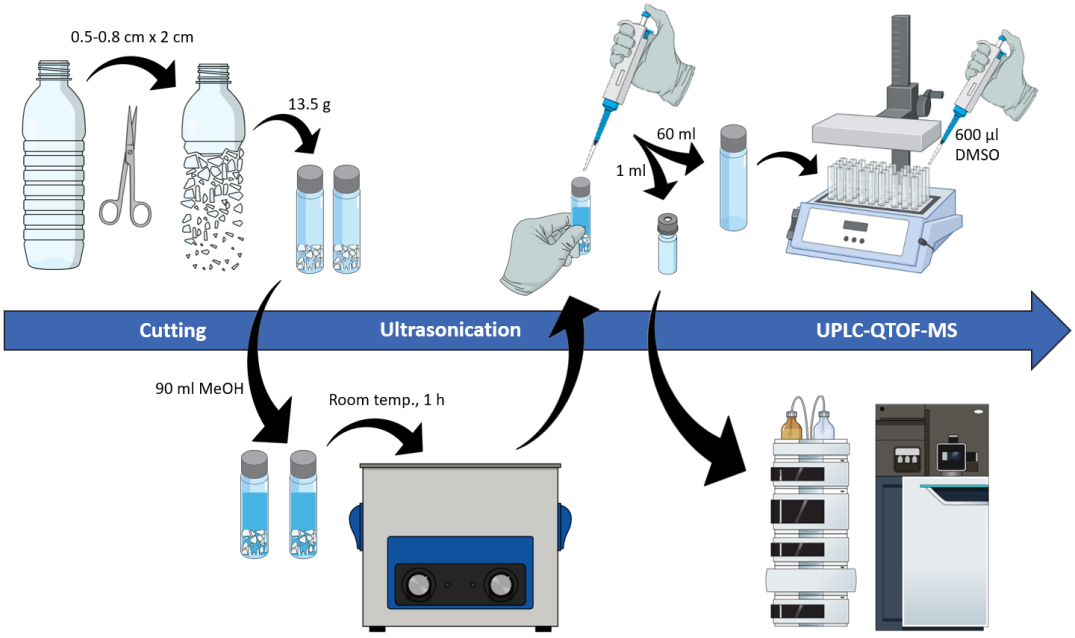


Figure 2.1: Procedure for extracting plastic chemicals before analysis. Figures retrieved from Biorender.com and Mindthegraph.com.

2.4 Migration

To analyse the migration of chemicals into water and 50% ethanol as food simulants, sample PVC_4, PE_LD_10 and PE_HD_14 were used. These were chosen to represent samples with different numbers of extractable chemical features that showed activation of several of the receptors used in the bioassays. The samples were first cut into pieces of approximately 0.5-0.8 cm x 2.0 cm. 78 g of each item was cut, and distributed into three glass bottles. The bottles were filled with 160 or 180 ml solvent, resulting in a plastic concentration of 0.15 g/ml in each bottle. One of the bottles were filled with 50% ethanol (EtOH, absolute for analysis, Supelco, 1.00983.2500) and 50% UPW, while the remaining two were filled with UPW only. Three PBs were also prepared and treated the same way as the samples; one containing 50% EtOH and two containing UPW.

All the bottles were then covered with aluminium foil and placed in a heating chamber (40°C, Termaks) together with a bowl of water for saturation of the air. Here, the migration into water and 50% EtOH was carried out for ten days, as in [56], and recommended by the European Commission regulation on plastic food contact materials [15].

2.4.1 Solid phase extraction of migrates

After ten days, the migrates and PBs were transferred into 1 l glass schott bottles and prepared for solid phase extraction (SPE) (blue part, figure 2.2). The two water migrates of the same sample were combined before further treatment.

160 ml of the 50% EtOH migrates were taken out and diluted with 640 ml UPW. The EtOH-percentage was thereby decreased to 10%. This was done to minimize the effect of EtOH in the SPE and thereby increase the comparability of the results of the migration experiment with water and 50% ethanol. To check how the EtOH content affected the SPE, 160 ml of the water migrate was diluted by adding 560 ml UPW and 80 ml EtOH, to obtain an EtOH content of 10% in this one as well. The rest of the water migrate (160 ml) was diluted with 640 ml UPW only. After this, all the migrates had a plastic concentration of 0.03 g/ml. In addition to the migrates, also the MeOH-extracts and extraction PBs dissolved in DMSO from the extraction experiment were analyzed (green part, figure 2.2). Therefore, 100 µl of the extracts and PBs was redissolved with 9.9 ml UPW to get a plastic concentration of 0.15 g/ml. This was further diluted with UPW to a final volume of 50 ml, and a plastic concentration of 0.03 g/ml; the same as in the migrates. Further, the pH of all migrates, extracts and PBs were adjusted to 2.5 using sulphuric acid (H_2SO_4, 3.5 M).

The SPE was carried out using C18 silica gel SPE cartridges (TELOS C18(EC)/ENV, 700 mg, 6 ml, 697-70M-006Z, Kinesis, Wertheim). These were placed on a manifold and conditioned with 2 ml n-heptane (for GC-ECD and GC-FID, Supelco, 1.04360.1000) followed by 2 ml acetone (HPLC grade (>99.9%), Sigma-Aldrich 270725-1L), 6 ml methanol (anhydrous, 99.8%, Sigma-Aldrich 322415-1L), and 8 ml UPW by gravity. The cartridges were accordingly loaded with the diluted migrates (800 ml) and diluted extracts (50 ml) with a constant vacuum flow of approximately 2-5 ml/min. Afterwards, the cartridges were dried under a nitrogen stream and stored at -20°C. Two days later, they were eluted with 5 ml acetone and 5 ml methanol by gravity. Vacuum was applied at the end.

The samples were then concentrated to 1 ml under a gentle nitrogen stream (40°C, Reacti-vap III, Thermo Scientific) and transferred to HPLC vials. The evaporation was continued, and as soon as it was space in the vials, 200 µl DMSO was added (100 µl DMSO to the extracts). The remaining acetone and methanol was then evaporated. For the chemical analysis, the migrates and extracts dissolved in DMSO were diluted with equal amounts of H_2O and MeOH to a plastic concentration of 0.15 g/ml.

To be able to evaluate the effect of the SPE, the migrates and PBs were also analyzed directly, without any pretreatment (top part, figure 2.2). These samples were taken from the glass bottles at day ten, before anything else was done. The extracts were also analyzed directly. 10 µl of the initial extracts dissolved in DMSO was therefore diluted with equal amounts of H_2O

and MeOH to a final volume of 1 ml, and a plastic concentration of 0.15 g/ml.

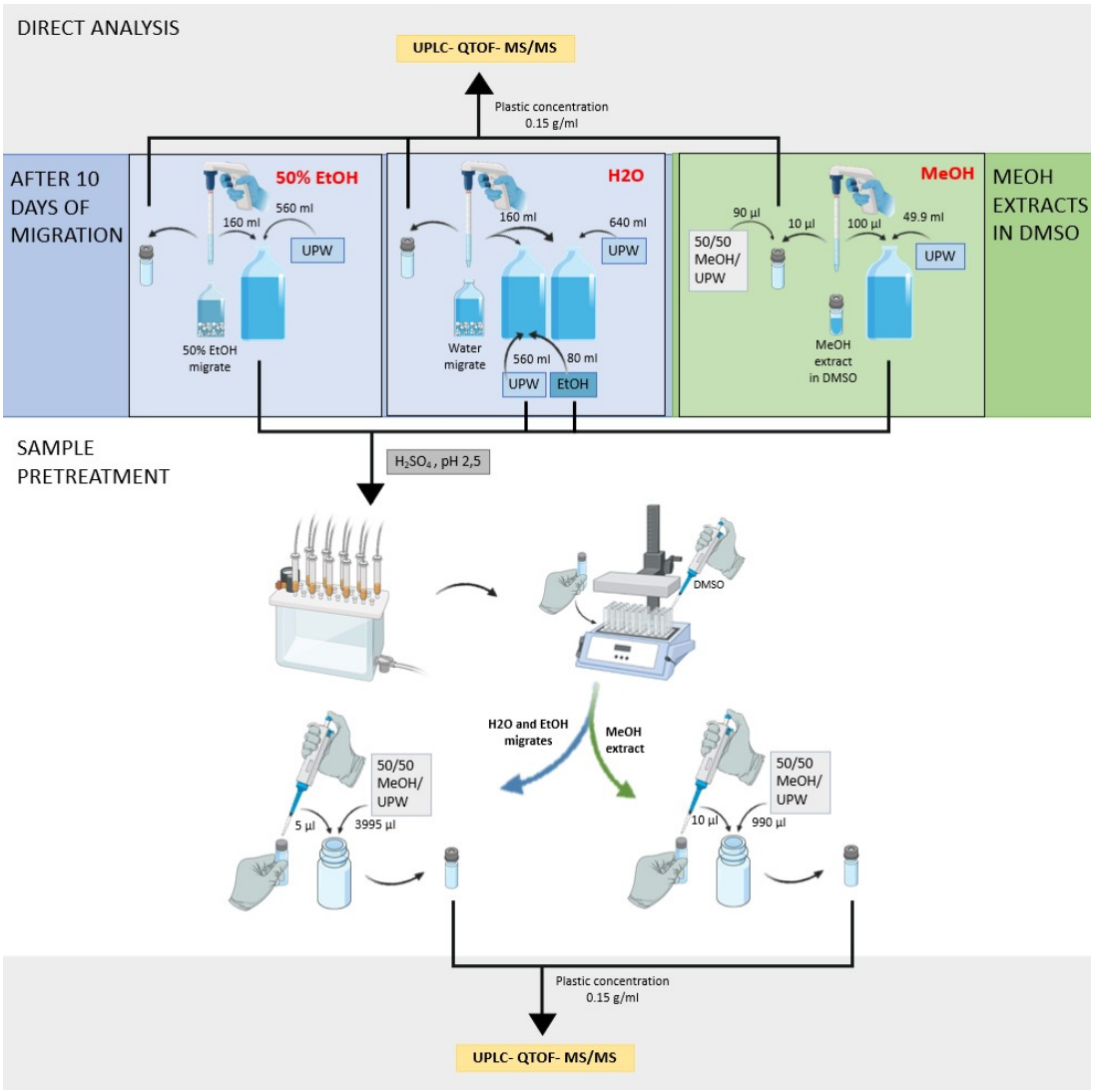


Figure 2.2: Overview of the samples and pretreatment methods used in the migration experiment. Figures retrieved from Biorender.com and Mindthegraph.com.

2.4.2 Migration kinetics

To assess the migration kinetics, samples of 0.1 ml were taken from each glass bottle (three for each sample + three PBs) at 3 h, 6 h, 12 h, one day, and two, four, six, eight and ten days after start. The sample taken from the two water PBs were combined in one vial. This was also done for the water-migrate samples containing the same plastic sample. The solvent was mixed before taking the samples, to ensure that the samples were representative, and that all plastic pieces were submerged in the solvent. The samples were transferred into HPLC vials and frozen at -80°C right after sampling. Later, the samples were analyzed directly by UPLC-QTOF-MS/MS, as described in section 2.5.2.

2.5 Non-target chemical analysis

All the non-target chemical analyses were performed using an ultra-high performance liquid chromatography system (Acquity UPLC I-Class, Waters) coupled to a quadropole time-of-flight mass spectrometer (Synapt G2-S HDMS, Waters).

2.5.1 Extracts and migrates

The analysis of the MeOH extracts and the water- and EtOH-migrates were similar, with some exceptions that will be mentioned. The separation was performed using an Acquity UPLC BEH C18 column (2.1 x 150 mm, 1.7 µm, Waters), at a column temperature of 55°C. Water was used as mobile phase A and methanol as mobile phase B, both containing 0.01% formic acid. A gradient elution program started at 20% B. This was kept constant for 0.5 min, before B was linearly increased to 95% in 28.5 min and held for 7 min. B was then increased to 100% and held for 2 min. After the analysis of the extracts, an extra washing step with 100% B at a flow rate of 0.350 ml/min for 7 min was included. Afterwards, the initial conditions were returned to, and this was held for 2 min prior to the next injection. The total run duration, including the washing, was 48 and 40 min for the extracts and migrates respectively. The injection volume was 2 µl for the methanol extracts and 3 µl for the migrates, and the mobile phase flow rate was 0.2 ml/min.

The extracts were analyzed according to polymertype, with the PS samples first, then PP, PE, PET, PUR and PVC in the end. A polymer-QC sample containing equal amounts of all samples that were made of the same polymer was analyzed before and after the samples of this specific polymer. In addition, a QC sample containing all of the samples was injected regularly, every 9th to 12th run, followed by a PB injection. Along with this, also solvent blanks (the mobile phase and MeOH used in extraction) were analyzed.

In the analysis of the migrates, the solvent blanks (the mobile phase, 50/50 MeOH/H_2O, and DMSO) were analyzed first. Then the migrates and PBs (after SPE) were analyzed, followed by the migrates and PBs that had not been enriched by SPE. Lastly, the MeOH extracts in DMSO before and after SPE were analyzed, with a washing step included in between each run. In addition, four QC samples were analyzed, containing the migrates before and after SPE and the MeOH extracts before and after SPE respectively.

The mass spectrometer was equipped with an ESI ionization source operated in positive mode. Data were acquired using the MS^E data acquisition mode allowing simultaneous acquisition of full scan MS and MS/MS spectra. The MS was operated in high resolution mode (resolution of 35000) for the methanol extracts, and resolution mode (resolution of 20000) for the migrates, since the concentration of compounds was lower in the migrates compared to the extracts, and resolution mode yields higher sensitivity than high resolution.

The m/z range was set to 50-1200, and the scan time was 0.3 s. The collision energy ranged from 15-45 V. Further information about the MS-settings are given in table 2.2.

Table 2.2: Mass spectrometer setup

Capillary (kv)	2.5
Source temperature (°C)	120
Sampling cone	30
Source offset	60
Desolvation temperature (°C)	350
Cone gas flow (L/Hr)	100
Desolvation gas flow (L/Hr)	800
Nebuliser Gas Flow (Bar)	6

2.5.2 Migration kinetics

The chemical analysis of the migrates at different timepoints was done using the instrumentation described in section 2.5 and 2.5.1. The MS detection settings are given in table 2.2.

The separation was performed using a Acquity UPLC BEH C18 column (2.1 mm x 50 mm, 1.7 µm). The mobile phase was composed of water (A) and methanol (B), both with 0.01% formic acid. The injection volume was 3 µl for the water migrates and 2 µl for the 50% ethanol migrates. The flow rate was 0.25 ml/min and the column temperature was 40°C. A gradient program was used, as shown in table 2.3. The total run duration was 10.5 min.

Table 2.3: Mobile phase gradient

Time (min)	**% A**	**% B**	**Curve**
0	80	20	-
0.5	80	20	6
5.0	10	90	5
7.0	5	95	6
7.5	0	100	6
9.0	0	100	6
9.1	80	20	1
10.5	80	20	6

The water samples were analyzed first, starting with the PB at the 9 timepoints, followed by sample PVC_4, PE_LD_10 and PE_HD_14. A QC sample consisting of equal parts of all the water migrates at the last timepoint (t9) was injected after the last timepoint for each sample. Afterwards, the 50% ethanol migrates were analyzed in the same order as the water migrates. Solvent blanks (the mobile phase, UPW and EtOH) were also analyzed.

The MS with ESI was operated in positive mode with a resolution of 20000 for the water migrates and 35000 for the 50% ethanol migrates, due to different concentrations of chemicals

in the samples. The m/z range was 99.00-1200.00, the scan time was 0.2 s and the collision energy ranged from 15-45 V.

2.6 Data analysis

2.6.1 Extraction of chemical features

The software Progenesis QI (version 3.0, Nonlinear Dynamics) was used to analyse and process the UPLC-QTOF-MS/MS data. Samples, QCs, PBs and solvent blanks were analyzed together in one project. When analyzing the MeOH extracts, the PUR and PVC- samples were imported to one project, while the PP, PE, PET and PS-samples were imported to another. This was done because the chemical composition of the PVC and PUR samples differed markedly from the other samples, and as a result, all samples did not align with the QC sample containing all the samples. Both projects followed the same data treatment steps. For the migration experiment, all data were imported to the same project file. For the migration kinetics-experiment, one project was made for each water-migrate sample, while the EtOH-migrates were processed together in the same project. Corresponding PBs and solvents were also included in the projects.

The first step in the data treatment was the import of the raw data files to Progenesis QI. Lock-mass correction with leucine enkephalin was done in the program. Next, the retention times of the runs were automatically aligned to one of the QC-chromatograms, chosen by the program. For the migration kinetics, all runs for one plastic sample were aligned to one of the samples, chosen by the program. Afterwards, peak picking was performed with automatic sensitivity to detect "fewer" peaks. The minimum chromatographic peak width was set to 0.05 min, and for the extracts and migrates, peaks eluting earlier than 1.5 min and after 36 min were ignored. In the migration kinetics experiment, peaks eluting between 0.82 and 8.5 min were included in the analysis. The fragment sensitivity was set to 0.2% of the base peak. Further, a search for common adducts (M+H, 2M+H, M+2H, M+H-H_2O, M+Na and 2M+Na) was done, and the mass spectra were deconvoluted.

2.6.2 Data reduction process for MeOH extracts

The resulting feature list from Progenesis QI was exported to Microsoft Excel for Windows. The raw abundance of each feature in the samples was compared to the maximum abundance of the same feature among the PBs and solvents. This maximum abundance was multiplied by ten, and then subtracted from the raw abundance of the feature in the samples. Only the features that had an abundance higher than zero in at least one of the samples after the subtraction were kept. This was done to exclude features that did not belong to the sample, but were originating from the solvents, or from fluctuations in the instrument itself [32].

2.6.3 Data reduction method for migrates

For the migrates, the feature list was also exported to Microsoft Excel. Like for the extracts, the raw abundance for each feature in each sample was compared to the maximum abundance of the same feature among the PBs and solvents. However, for the migrates, the samples had different corresponding blanks based on which pre-treatment the samples had gone through prior to the chemical analysis. Therefore, the abundance in each sample was only compared to the maximum abundance of the feature in the corresponding blanks and solvents. Further, the maximum abundance in the blanks was multiplied by ten, and then subtracted from the raw abundance of the feature in the samples. Only the features that had an abundance higher than zero in at least one of the samples after the subtraction were kept. In addition, the features that did not have any isotopic distribution were removed, as this is typical for "ghost peaks".

2.6.4 Data reduction process for migration-kinetics experiment

In the migration-kinetics experiment, the goal was to observe how the concentrations of the features changed over the migrating period. The focus was on the increasing features, and whether these features reached a plateau within ten days.

First, blank correction of the corresponding blanks (PB at the same timepoint) was performed as described in section 2.6.3. Negative numbers were then replaced with 0 before further data treatment. Next, the data was filtered for only features that were present in at least 3 of the timepoints [50]. Subsequently, features that increased over the time period were found by using a similar strategy as developed by Plassmann et al. [57] [58]. For each feature, the "time trend ratio" (TTR) was calculated, by dividing the average peak area at the three last timepoints (t7-t9) by the average peak areas at the three first timepoints (t1-t3) +1 to avoid dividing by zero. The features with TTR > 2 were kept. Then, Spearman's correlation (ρ) was calculated in IBM SPSS Statistics for Windows [59], where the features were compared to a linearly increasing time profile. The features with $\rho > 0.7$ and TTR > 2 were defined as increasing over the time period. To find the portion of the increasing features that reached a plateau within ten days, the average peak area at the last three timepoints (t7-t9) was divided by the peak area at the last timepoint (t9). When this number was higher than one, the feature was defined as reaching a plateau in less than ten days.

2.7 Tentative identification of features in MeOH- extracts

2.7.1 Databases

After removing features that were not present in the samples at a 10 fold higher concentration than in the blanks, the remaining features were attempted tentatively identified (confidence level 3 [36]) using the Metascope algorithm in Progenesis QI. From here on out, tentative identifications/ tentatively identified features will also be called "identifications" for simplicity.

The experimental spectra were compared with empirical spectra from MassBank (14 788 unique compounds, release version 2021.03) and theoretical *in silico* fragmentation spectra. For the *in silico* fragmentation, four databases were used. Three of the databases were previously constructed, and used by Zimmermann et al. (2021) [56]. These databases covered the chemicals present in plastic packaging (CPPdb, 2680 compounds, including the compounds on the positive list of the European plastic regulation 10/2011), the chemicals registered under the REACH regulation in 2020 (ECHAdb, 7092 compounds), and the chemicals (pre)registered under REACH in 2017, as provided by the NORMAN Suspect List Exchange (NORMANdb, 65 738 compounds). See the supporting info of [56] for more details. For this experiment, a fourth database was also constructed from the results of Wiesinger et al. (2021) (WIESINGERdb, 6414 compounds) [11].

The Wiesinger database was made by first extracting the CASRNs (n=10 547) from the paper's supporting information. These were put into Comptox epa batch search [60], and the SMILES-codes (n= 6631) were retrieved. The SMILES-codes were then converted to CIDs using PubChem Identifier Exchange service [61]. The CIDs (n= 6423) were uploaded to PubChem [62], and the structures (n= 6414) were downloaded as a sdf file. The sdf file was subsequently uploaded to Progenesis QI and the *in silico* fragmentation search was done there.

2.7.2 Identification method

The search for tentative identifications was done in Progenesis QI by comparing the spectra of each sample to the spectra in the databases. Progenesis QI searched for hits with a precursor ion tolerance of 5 ppm and a fragment ion tolerance of 10 ppm. The results of the identification were then filtered for hits with a score higher than or equal to 40 (based on fragmentation, mass, and isotope similarity, max. 60). If a feature had multiple identifications with a score higher than 40, the one with the highest score was picked, and if a feature had two identifications with the same highest score, one of them was chosen randomly. This tentative identification equals confidence level 3, as described by Schymanski et al. (2014) [36].

2.7.3 Search for usage and toxicity-information for the identified features

For all identified features with a score of at least 40 (n= 2146), the InChlKeys were found using the PubChem Identifier Exchange Service [61]. The list of ToxCast and Tox21 chemicals (INVITRODB_V3_20181017, 29403 unique compounds) were then downloaded [63], and the InChlKeys, IUPAC names and common names of the identified features were cross-referenced with the data in the ToxCast list. 290 of the identified features were found in ToxCast, and associated ToxCast assays for these compounds were downloaded [60]. In addition, a ToxCast summary database (INVITRODB_V3_SUMMARY) was downloaded from the US EPA [64], containing the file "oldstyle_ac50_Matrix_180918.csv" with the concentrations at 50% of the

maximum activity in the Toxcast assays (AC50) for 9213 compounds in 1409 assays.

Further, 45 ToxCast assays for the androgen receptor (AR), ERα, PPARγ and PXR were chosen, as these were used in the bioassays performed by Sarah Stevens (Department of Biology, NTNU) (table B.1). Information about the identified features' activity in these bioassays was found by cross-referencing the identified features with the ToxCast assays data. There were several assays for each receptor, and both agonist and antagonist assays were included. For the compounds that were active in more than one of the relevant assays targeting the same receptor, the number of agonist assays was compared to the number of antagonist assays (table B.4), and the compound was said to be an agonist only if it was active in more agonist assays than antagonist assays. If the number of active agonist and antagonist assays were the same, the compound was not determined to be either of them. For AR activity, this was the case for two compounds, for ERα it was ten compounds, for PPARγ it was four, and for PXR it was three compounds being active in the same number of agonist and antagonist assays. Afterwards, the number of compounds interfering with the AR, ERα, PPARγ and PXR receptors in each sample was counted, and compared to the activity detected in the samples in the experimental bioassays (table B.2).

After this, "oldstyle_ac50_Matrix_180918.csv" was used to extract information about the AC50 values of the identified features in the AR antagonist assays and the ERα, PPARγ and PXR agonist assays. Inspired by Zimmermann et al. (2019), the ratio of the lowest AC50 value for the feature and the largest peak area across the samples having the feature was then calculated for each of the four receptors (table B.3) [17], to identify the toxicologically most interesting features.

For the identified features among the ten features with the highest abundance in each sample, an additional search for toxicity and usage information was done. The search was done in PubChem [62], and in the supplementary information from Wiesinger et al. (2021) [11] and Groh et al. (2019) [4].

2.8 Statistical analysis

To get a visual impression of the chemical composition of the plastic extracts, clustered heatmaps were made using R studio [65]. This was done separately for the PVC and PUR samples in one heatmap, and the PP, PE, PET and PS samples in another. Both the features and the samples were clustered together in the heatmaps, so that similar features and similar samples appeared close to each other. Heatmaps were also made to examine the effect of the SPE on the chemical composition of the migrates. In this case, only the features were clustered together, and the corresponding samples with and without SPE were kept next to each other.

To make the heatmaps, the code in section F was used. The initial data-sets contained the features that were left after the data reduction strategies described in section 2.6.2 and 2.6.3,

for extracts and migrates respectively. Before generating the heatmaps, the abundances smaller than one were changed to zero, while the rest were log transformed with a base of ten.

Venn diagrams were made using Python, version 3.7, to visualize the differences and similarities in chemical composition of the MeOH-extracts, 50%EtOH-migrates and water-migrates of the same plastic sample. The code in section E was used for this. The dataset that were used contained the features after the data reduction strategies described in section 2.6.3.

3 Results

3.1 Number of extractable chemical features

To get an overview of the chemical composition and complexity of the plastic samples, methanol extracts were analyzed. The data was reduced considerably by the blank subtraction (on average, 71% in each sample, table A.1), and after blank subtraction, 8831 unique chemical features were found in the PP, PE, PS and PET samples (n = 32) in total. In the PVC and PUR samples (n = 7), 16846 unique chemical features were found. The number of features in the PUR and PVC samples was almost twice as high as in the other polymers, even though these samples comprised only 18% of the plastic samples. The plastic extracts were analyzed in two separate sets (set 1: PP, PE, PS and PET samples and set 2: PVC and PUR samples), and as a consequence of this, there is no information about common features across the two sets.

On individual sample level, the number of features ranged between 37 and 9936, and between 9.8 and 38.0% of the features were identified (table 3.1). Overall, the PUR samples had the highest number of features, with an average of 8247 features in each sample when including the PUR_PE sample. On the other end of the spectrum, there is PET, with an average of only 335 features in each sample when disregarding the PE_PET sample.

Table 3.1: Number of features and tentatively identified compounds in each sample. The samples are marked with color according to their number of features, with colors ranging from green = low numbers, to red = high numbers.

Sample	Features	Tentatively identified features (% of total features)	
PP_3	1220	240	(19.7%)
PP_6	194	28	(14.4%)
PP_16	90	12	(13.3%)
PP_17	751	128	(17.0%)
PP_39a	1327	273	(20.6%)
PP_39b	1464	289	(19.7%)
PS_1	326	67	(20.6%)
PS_11	124	17	(13.7%)
PS_13	264	46	(17.4%)
PS_33	1752	243	(13.9%)
PS_41	504	108	(21.4%)
PS_41a	1516	268	(17.7%)
PE_7	37	7	(18.9%)
PE_21a	2317	542	(23.4%)
PE_28	1243	410	(33.0%)

Table 3.1: (continued)

Sample	Features	Tentatively identified compounds (% of features)	
PE_34	366	94	(25.7%)
PE_37	3474	798	(23.0%)
PE_38	297	45	(15.2%)
PE_HD_5	164	16	(9.8%)
PE_HD_14	260	51	(19.6%)
PE_LD_8	74	12	(16.2%)
PE_LD_10	1588	559	(35.2%)
PE_LD_15	1211	460	(38.0%)
PE_LD_44	1504	386	(25.7%)
PE_PET_21b	3203	684	(21.4%)
PET_9	528	70	(13.3%)
PET_18	640	183	(28.6%)
PET_25	140	20	(14.3%)
PET_27	231	55	(23.8%)
PET_36	379	94	(24.8%)
PET_40	174	32	(18.4%)
PET_40a	251	37	(14.7%)
PVC_4	2283	328	(14.4%)
PVC_26	769	121	(15.7%)
PVC_29	9936	1521	(15.3%)
PVC_35	8946	1265	(14.1%)
PUR_12	8762	1022	(11.7%)
PUR_43	9175	985	(10.7%)
PUR_PE_30	6803	1097	(16.1%)

3.1.1 Chemical composition of methanol extracts

To characterize the chemical composition of the samples, a cluster analysis was performed based on the presence and abundance of the chemical features (figure 3.1). The PP, PE, PS and PET samples did not cluster based on polymer type. Instead, several samples with similar uses clustered together, e.g. PE_LD_10, PE_LD_15 and PE_28 (freezer bags), PE_7 and PE_LD_8 (food container and lid) and PP_3 and PP_39a (yoghurt cups) (figure 3.1a). The variation between samples was large, both in number of features and composition of features in each sample. No features were present in all the PP, PE, PS and PET samples, and more than half of the features were present in only one or two samples (table A.2).

The clustering in the PUR and PVC samples corresponded to their polymer type to a larger extent (figure 3.1b). However this was also more likely for this set, as the number of samples was low. The PUR and PVC samples also clustered based on type of plastic article, and clustering of the two drinking bladders (PUR_43 and PUR_12), the two drinking tubes (PVC_4 and PVC_26) and the two cling films (PVC_29 and PVC_35) was found. The differences between the PUR and PVC samples were not quite as prominent as for the other polymers. In this case, 75 features were present in all of the samples, but still more than half of the features were present in only one or two samples (table A.3).

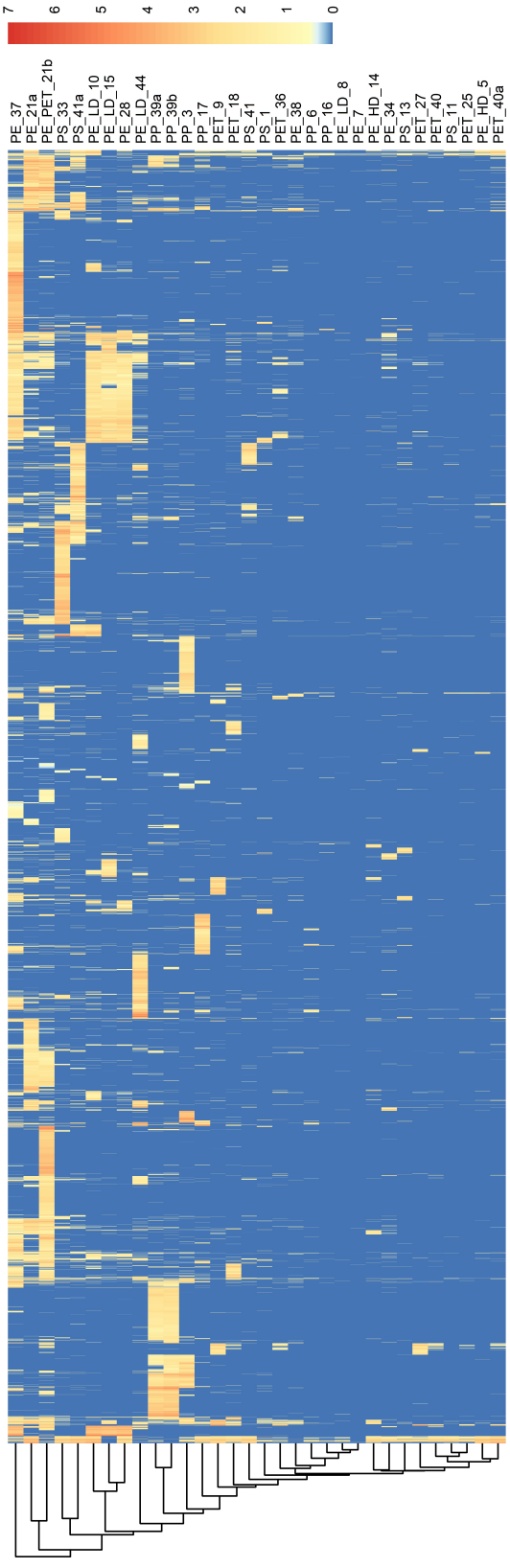
Looking at each polymer type at a time, the PUR samples were most similar to each other, and shared the most features (table A.4). The PUR sample with the fewest features (PUR_PE_30) shares 42.8% of its features with the two other PUR samples. The PS samples were also quite similar, and the PS sample with the fewest features (PS_11) shared 33.1% of its features with all the other PS samples. The polymer type with the most variation between the samples, was PE, with no shared features between all the samples. This was also the polymer type with the most samples (n=12). The PP samples (n=6) also comprised a quite heterogeneous group, and only one feature was present in all of the samples.

3.1.2 Tentatively identified compounds in methanol extracts

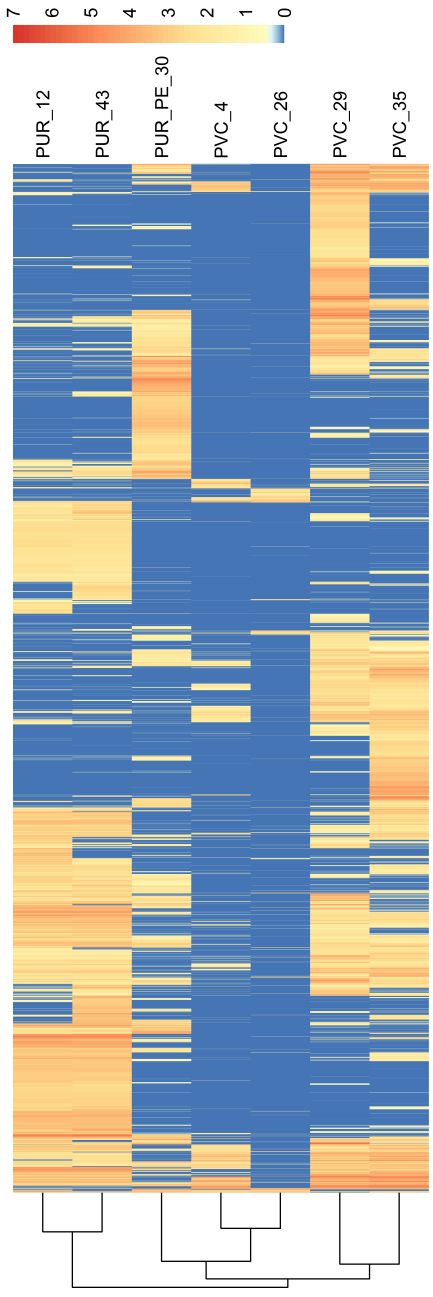
A search for identifications was also done, and the amount of data was reduced by data filtering steps (table A.1). In the PP, PE, PS and PET samples, a total of 1760 features (20%) were identified with a score > 40, while 2377 features (14%) were identified in the PVC and PUR samples. In total, 4137 features were identified, however only 2146 unique identifications were found (table A.5), meaning that multiple features were identified as the same compound.

Most of the 4137 features that were identified with a score > 40 were identified using the NORMAN_db (77% in the PP, PE, PET and PS samples and 91% in the PVC and PUR samples). The full list of identified compounds can be found in the supplementary excel file. Of the identified compounds among the ten features with the highest abundance in each sample (n = 69), 31 were known plastic related compounds, and 38 were not (table 3.2). For 13 of the compounds, no information about neither uses nor toxicity was found in the information sources used ([4] [11] [60] [62]).

The known plastic associated chemicals were used for many different purposes, ranging from colorants and plasticizers to flame retardants, antioxidants and "other processing aids". With regard to toxicity, several of the features with high abundance in the PUR samples were identified as toxic chemicals. To give an example, both PUR_12 and PUR_43 contained triphenyl phosphate, which is bioaccumulative, persistant and an endrocrine disrupting chemical (EDC). Triphenyl phospate is also found to interfere with all the receptors used in the bioassays. Both PUR_12 and PUR_43 were active in the AR, PPARγ and PXR bioassays (table B.2).



(a) PP, PE, PET and PS samples



(b) PVC and PUR samples

Figure 3.1: Heatmaps showing the chemical composition of the PP, PE, PET and PS samples (a), and PVC and PUR samples (b). The features are clustered, and shown as vertical lines, while the samples are clustered on the y-axis. The colors correspond to logarithmic peak area.

Despite having few features, a toxic compound was also identified among the most abundant features in sample PET_36; ethyl 4-(dimethylamino) benzoate. This is a persistant and bioaccumulative, carcinogenic, mutagenic and reprotoxic compound. It is also an ERα, PPARγ and PXR agonist.

It is also noteworthy that some samples share many features among the ten most abundant features. PE_21a and PE_PET_21b have three identified compounds in common, two of which are used as plasticizers. PE_LD_10, PE_LD_15 and PE_28, three freezer bags, also have two abundant features in common, identified as plastic chemicals used as colorants, fillers and lubricants. They are not classified as of potential concern by Wiesinger et al. [11]. Lastly, ethylene terepthalate cyclic trimer, a known NIAS in PET [4], was found with high abundances in five of the PET samples.

Information on toxicity for all identified compounds, was retrieved from ToxCast [60]. Of the 2146 unique identified compounds, there was information about 290 of them in ToxCast, and 181 were active in at least one of the AR, ERα, PPARγ or PXR bioassays listed in table B.1. 34 of the 39 plastic samples contained compounds reported to interfere with at least one of the receptors. However, not all of these samples were active in the experimental bioassays (table B.2).

Regarding the identified compounds, 63 were antiandrogenic (active in more androgen antagonist assays than androgen agonist assays), 70 were ERα agonists, 46 were PPARγ agonists and 112 were PXR agonists (table B.4). For the compounds with associated information about AC50 values, the AC50 value was compared to the compound's highest peak area, and the identified compounds with a peak area above 100 were ranked based on the AC50/area ratio, from lowest to highest (table B.3). Some known plastic chemicals with high rank for several receptors include tributyl 2-acetyloxypropane1,2,3-tricarboxylate (PubChem CID 6505) and octrizole (PubChem CID 62485).

According to the ToxCast data (table B.3), the most potent antiandrogen was cyclopentanol (PubChem CID 7298), with an AC50 value of 0.27 µM, and the most potent ERα agonist was 3-allyloxy-1,2-propanediol (PubChem CID 78950), with an AC50 of 0.06 µM. Neither of these are known plastic chemicals. The most potent PPARγ agonist was tetraethylene glycol (PubChem CID 8200), having an AC50 value of 0.06 µM. This compound was identified in eight samples covering all six polymer types. The most potent PXR agonist was triethylene glycol (PubChem CID 8172) with an AC50 value of 0.11 µM. This compound was identified in ten samples made of PE, PET, PP and PS. Both tetraethylene glycol and triethylene glycol are known plasticizers, and were also identified as androgen antagonists.

Table 3.2: Identified chemicals with the highest abundance (top ten) in each sample.

PubChem CID	Name	High abundance in sample	Known plastic chemical?	Uses	Toxicity
62485	Octrizole	PUR_12	Yes [11] [62]	UV light absorber, food contact articles (FCA) [4] [62].	Irritant, environmental hazard [62]. AR antagonist, ERα and PXR agonist [60].
8289	Triphenyl phosphate	PUR_12, PUR_43	Yes [11] [62]	Plasticizer, other processing aids, FCA [4] [62].	Environmental hazard, toxic to aquatic life [62]. Bioaccumulative, persistant, and EDC [11]. Metabolism disrupting chemical [66]. AR antagonist, ERα, PPARγ and PXR agonist [60].
586744	Bis(1,2,2,6,6-pentamethyl piperidin-4-yl)decanedioate	PUR_43	Yes [11] [62]	UV light absorber, stabilizer [62].	Toxic to aquatic life, irritant, persistant and bioaccumulative [11] [62].
157881	Methyl 1,2,2,6,6-penta methyl-4-piperidyl sebacate	PUR_43	Yes [11] [62]	Antioxidant, stabilizer [62].	Toxic to aquatic life, irritant, persistant and bioaccumulative [11] [62].
93817	Ethyl 4-[(N-methylanilino) methylideneamino]benzoate	PUR_43	Yes [11]	Colorant, filler, light stabilizer, other processing aids [11].	Toxic to aquatic life, irritant [62]. Not classified as substance of potential concern [11].
105778	Octane-1,8-diyl bis(2,2,6,6-tetramethylpiperidine-4-carboxylate)	PUR_43	Yes [62]	Polyester compositions [62].	-
79912	1,8,15,22-Tetrazacyclo octacosane-2,9,16,23-tetrone	PUR_PE_30, PE_21a	Yes [4] [11]	-	-
283592	1,8-Diazacyclotetradecane-2,7-dione	PUR_PE_30	Yes [11]	Flame retardant, stabilizer [62].	Not classified as of potential concern [11].

Table 3.2: (continued)

PubChem CID	Name	High abundance in sample	Known plastic chemical?	Uses	Toxicity
3024584	2-O-decyl 1-O-nonyl benzene-1,2-dicarboxylate	PVC_26	Yes [62]	Plasticizer [62].	-
22833495	(Z)-Dianhydro-D-mannitol, mono-9-octadecenoate	PVC_29	No [62]	-	-
92028	2,3-Diacetyloxypropyl docosanoate	PVC_29	No [62]	-	-
14420741	10-Dodecoxy-10-oxodecanoic acid	PVC_35	No [62]	-	-
14900	1,3-Dihydroxypropan-2-yl hexadecanoate	PE_LD_44	No [62]	Cosmetics [62].	Toxic to aquatic life [62].
3020637	2-O-(2-Ethylhexyl) 1-O-undecyl benzene-1,2-dicarboxylate	PE_HD_5, PET_40a, PE_21a	No [62]	Adhesive [62].	-
114222	2-Hydroxy-5-nonylbenzaldehyde	PE_HD_14	No [62]	Mining activities [62].	Corrosive, irritant, health hazard [62].
6505	Tributyl 2-acetyloxypropane-1,2,3-tricarboxylate	PE_LD_10, PS_41a	Yes [11] [62]	Plasticizer, other processing aids, FCA [4] [11].	Persistant, bioaccumulate, carcinogenic, mutagenic [11]. PPARγ and PXR agonist [60].
5365371	Erucamide	PE_LD_10, PE_LD_15, PE_28	Yes [11] [62]	Colorant, filler, lubricant, FCA [11].	Irritant [62]. Not classified as of potential concern [11].
547891	Icos-11-enamide	PE_LD_10	Yes [11]	FCA [4] [11].	Not classified as of potential concern [11].

Table 3.2: (continued)

PubChem CID	Name	High abundance in sample	Known plastic chemical?	Uses	Toxicity
31292	Octadecanamide	PE_LD_10, PE_LD_15, PE_28, PS_1	Yes [11]	Antistatic agent, colorant, filler, lubricant, FCA [11].	Not classified as of potential concern [11].
42981	Laurocapram	PE_LD_15	No [62]	Used for transdermal delivery of sex hormones [62].	Irritant [62]. AR antagonist, PPARγ, ERα and PXR agonist [60].
25076636	4-(4,7-Dimethyl-2,3,3a,4,5,6,7,7a-octahydro-1H-inden-5-yl)- 2-methylcyclohexan-1-ol	PE_LD_15	No [62]	-	-
12131	2,3-Di(hexanoyloxy)propyl hexanoate	PE_21a, PE_PET_21b	No [62]	Lubricant, textile manufacturing [62].	-
169212	2,3-Diacetyloxypropyl dodecanoate	PE_21a, PE_PET_21b	Yes [11] [62]	Biocide, colorant, filler, lubricant, plasticizer, other processing aids, cling films [11].	Not classified as of potential concern [11].
256388	2,3-Diacetyloxypropyl octadecanoate	PE_21a, PE_PET_21b	Yes [11]	Plasticizer in PVC [11].	Not classified as of potential concern [11].
92028	2,3-Diacetyloxypropyl docosanoate	PE_21a	No [62]	-	-
5322095	Trilinolein	PE_28	No [62]	Solvent, cosmetics [62].	-
117474	2-Hexadecylphenol	PE_28	Yes [62]	Polycarbonate resins [62].	-
3024290	8-(3,7-Dimethyloct-6-enoxy)-8-methoxy-2,6-dimethyloctan-2-ol	PE_34	No [62]	-	-

Table 3.2: (continued)

PubChem CID	Name	High abundance in sample	Known plastic chemical?	Uses	Toxicity
66989	N-octadecyloctadecan-1-amine	PE_37, PE_PET_21b, PP_3	No [62]	Lubricant, hydrocarbon resin emulsion, sunscreen [62].	Irritant, toxic to aquatic life [62].
84218	Docosan-1-amine	PE_38, PS_41	No [62]	Hair cosmetics [62].	-
5460026	Gentiobiose	PE_38	No [62]	Biological molecule [62].	-
88404	N-benzyloctadecan-1-amine	PE_38	Yes [62]	Thermoplastic elastomer [62].	-
5148036	Gibberellin A7	PE_38	No [62]	Fungicide [62].	-
22019755	2-(Dimethylamino)-2-[(4-methylphenyl)methyl]-1-(4-morpholin-4-ylphenyl)butan-1-one	PP_3	Yes [4] [11]	UV light absorber, paints and coatings [62].	Reproductive toxicity, harmful to aquatic life [62].
175585	Ditrimethylolpropane tetraacrylate	PP_3, PP_39a, PP_39b	Yes [11] [62]	Colorant, monomer [11].	Irritant [62]. Not classified as of potential concern [11].
352309	Lauryldiethanolamine	PP_16, PS_13	Yes [62]	Flame retardant, PUR resin forming composition, propylene resin composition [62].	Irritant, toxic to aquatic life, genotoxic [62]. AR antagonist, ERα , PPARγ and PXR agonist [60].
74429	2-[2-Dodecoxyethyl(2-hydroxyethyl)amino]ethanol	PP_16, PS_13	No [62]	Antistatic agent, surfactant, cosmetics [62].	-
61494	2-[2-Hydroxyethyl (octadecyl)amino]ethanol	PP_17	Yes [4][11]	Antistatic agent, emulsifier, lubricant, processing aids [11].	Irritant, corrosive, toxic to aquatic life [62]. Persistant and bioaccumulative [11].

Table 3.2: (continued)

PubChem CID	Name	High abundance in sample	Known plastic chemical?	Uses	Toxicity
104214	2-[2-Hydroxyethyl (octadecyl)amino]ethyl octadecanoate	PP_17	Yes [11] [62]	Antistatic agent, FCA [11] [62].	Corrosive, irritant [62]. Not classified as of potential concern [11].
12942137	2,2'-(Tetradecylimino) diethanol	PP_17	Yes [62]	Antistatic agent, PUR resin-forming composition, PS resin particles [11]. [62].	Not classified as of potential concern [11]. AR antagonist, ERα, PPARγ and PXR agonist [60].
84238	Dibenzo-24-crown-8	PP_17	No [62]	Adhesive, polymers for use as anion exchange membranes [62].	Irritant [62].
90236	Tris(4-anilinophenyl) methanol	PP_39a, PP_39b	No [62]	Purification of triphenyl-methane compounds [62].	-
122825	2,4-Diethylthioxanthen-9-one	PP_39a, PP_39b	Yes [11] [62]	Adhesive, colorant, filler, other processing aids [11].	Not classified as of potential concern [11].
111970	(4-Aminophenyl)-bis(4-anilinophenyl)methanol	PP_39a, PP_39b	No [61]	-	-
92212	Bisphenol a diglycidyl ether diacrylate	PP_39a, PP_39b	Yes [11]	Monomer [11].	Not classified as of potential concern [11].
122441	N-[5-[2-(2,5-Dioxopyrrolidin-1-yl) ethyl-ethylamino]-2-[(4-nitrophenyl)diazenyl]phenyl] acetamide	PP_39a, PP_39b	No [62]	-	-

Table 3.2: (continued)

PubChem CID	Name	High abundance in sample	Known plastic chemical?	Uses	Toxicity
3034861	2-[[4-[(4-Aminophenyl)-(4-anilinophenyl)methylidene] cyclohexa-2,5-dien-1-ylidene]amino] benzenesulfonic acid	PP_39a	No [62]	-	-
86171	2-Benzyl-2-(dimethylamino)-1-(4-morpholin-4-ylphenyl)butan-1-one	PP_39b, PP_39a	Yes [62] [11]	Colorant, catalyst, filler, lubricant, other processing aids [11].	Persistant, bioaccumulating, carcinogenic, mutagenic and reprotoxic [11]. AR antagonist, ERα, PPARγ and PXR agonist [60].
6437194	(3-Hydroxy-2,2-dimethyl propyl) (Z)-octadec-9-enoate	PS_1	No [62]	Lubricant and lubricant additives [62].	-
96408	2-[2,4-Bis(2-methylbutan-2-yl)phenoxy]ethyl prop-2-enoate	PS_13	No [62]	-	-
4867	2-[2-[2-[2-[2-[2-[2-[2-(2-Hydroxyethoxy)ethoxy] ethoxy]ethoxy]ethoxy] ethoxy]ethoxy]ethanol	PS_33	Yes [62]	Processing aids and additives, coating, PE-plastic [62].	Irritant, of low concern according to EPA safer choice [62].
11917	1,3-Dimethyl-1,3-diphenylurea	PS_33	No [62]	-	Irritant, toxic to aquatic life [62].
79718	Heptaethylene glycol	PS_33	No [62]	Processing aids and additives [62].	Of low concern according to EPA safer choice [62].

Table 3.2: (continued)

PubChem CID	Name	High abundance in sample	Known plastic chemical?	Uses	Toxicity
87168	2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-Hydroxyethoxy)ethoxy] ethoxy]ethoxy]ethoxy] ethoxy]ethoxy]ethoxy] ethoxy] ethoxy]ethanol	PS_33	No [62]	Cosmetics [62].	-
68995	O-cresolphthalein	PS_33	No [62]	-	-
10465	Heptadecanoic acid	PS_41	Yes [11] [62]	Surfactant, lubricant, other processing aids [11] [62].	Verified to be of low concern (EPA Safer choice) [62].
24762	2-Hydroxyethyl octadecanoate	PS_41	Yes [11] [62]	Emulsifier, plasticizer, food additive [11] [62].	Verified to be of low concern (EPA Safer choice) [62]. ERα agonist [60].
2724775	N-(fluorenylmethoxy-carbonyl)-L-glutamine	PS_41	No [62]	Peptide [62].	-
18934204	(2-Ethyl-2-methyl-1,3-dioxolan-4-yl)methyl prop-2-enoate	PE_PET_21b	No [62]	Ink, adhesives [62].	Irritant [62].
62354	Scilliroside	PE_PET_21b	No [62]	Little used rodenticide [62].	-
15089684	Ethylene terephthalate cyclic trimer	PET_9, PET_25, PET_27, PET_36, PET_40	Yes [4]	NIAS in PET [4]	-
6010	Methyltestosterone	PET_18	No [62]	Drug, steroid [62].	AR antagonist, ERα, PPARγ and PXR agonist [60].

Table 3.2: (continued)

PubChem CID	Name	High abundance in sample	Known plastic chemical?	Uses	Toxicity
21124472	(8S,9S,10S,13S,14S)-10,13-Dimethyl-4,5,6,7,8,9,11,12, 14,15,16,17-dodecahydro-3H-cyclopenta[a]phenanthren-1-ol	PET_18	No [62]	Pharmaceutical [62].	-
112492	Nona-2,4-dien-1-ol	PET_18	No [62]	Flavoring agent in food, ink [62].	-
135454	Cascarin	PET_27	No [62]	-	-
25127	Ethyl 4-(dimethylamino) benzoate	PET_36	Yes [11] [62]	Colorant, filler, other processing aids [11].	Persistant and bioaccumulative, carcinogenic, mutagenic and reprotoxic, toxic to aquatic life [11]. [62]. ERα, PPARγ and PXR agonist [60].
6763	Phenanthrene-9,10-dione	PET_36	No [62]	Production of herbicides, pharmaceuticals, adhesives etc. [62].	Irritant, cytotoxic [62].
337778	Methyl 3-amino-4-methylbenzoate	PET_36	No [62]	-	Irritant [62].
1821	5-Fluorouracil arabinoside	PET_36	No [62]	Pharmaceutical [62].	-

3.2 Migration into water and 50% EtOH

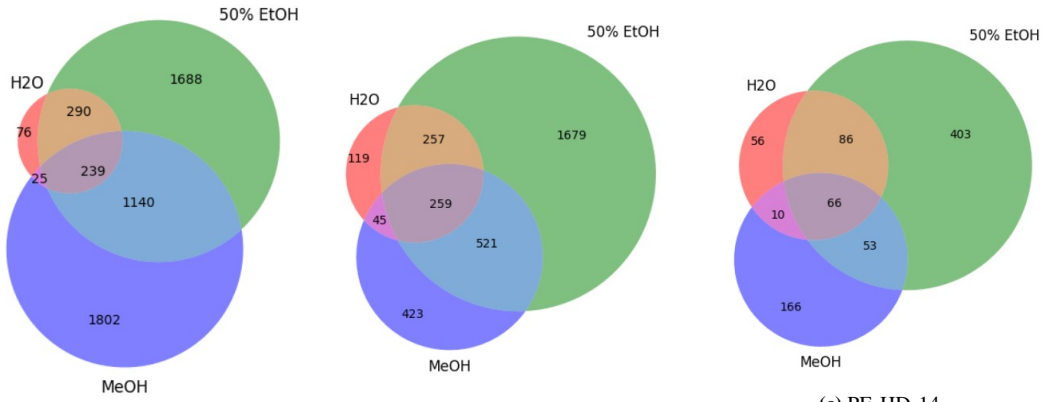
The solid phase extracted and concentrated migrates and MeOH extracts in DMSO were analyzed together with the migrates and extracts that had not undergone SPE. The sample pretreatment method was assessed, and the effect of the SPE was found to be acceptable (section C). The data presented in the following results are therefore coming from the chemical analysis of the solid phase extracted samples.

The number of detected features in the water migrates ranged from 218 to 680 (table 3.3). The 50% ethanol migrates had a considerably higher number of features, ranging from 608 to 3357. The number of features in the EtOH migrates was also higher than in the MeOH extracts in DMSO for all three samples. Sample PE_HD_14 had the lowest numbers of features across all solvents.

Table 3.3: Number of features migrating to water and 50% EtOH in the three samples compared to the number of features extracted with MeOH.

Sample	Features in H_2O	Features in 50% EtOH	Features in MeOH extracts
PVC_4	630	3357	3206
PE_LD_10	680	2716	1248
PE_HD_14	218	608	295

To examine the features' ability to leach into the three solvents, a venn diagram was made for each sample. All three samples had some shared features, that migrated into both H_2O and 50% EtOH, and were extracted by MeOH. These were 239, 259 and 66 features in sample PVC_4, PE_LD_10 and PE_HD_14 respectively (figure 3.2). There were also features that migrated exclusively into only one of the solvents for all the three samples.



(a) PVC_4 **(b)** PE_LD_10 **(c)** PE_HD_14

Figure 3.2: Venn diagrams showing the number of chemicals migrating into one or more of the solvents used (MeOH, 50% EtOH and H_2O) for sample PVC_4, PE_LD_10 and PE_HD_14.

Further, the abundances of the features in the migrates and MeOH extracts were investigated and compared. Features with a migrate/extract abundance ratio < 0.1 were defined as being retained in the plastic, and showing low migration. Features having a ratio > 0.1 were defined as readily leachable. The portion of migrating features were considerably higher in the 50% EtOH migrates than in the water migrates (figure 3.3). In sample PVC_4, the number of features migrating to 50% EtOH was quite similar to the number of features showing no or low migration (2377 and 2271 features respectively). For sample PE_LD_10 and PE_HD_14, the number of features migrating to 50% EtOH was clearly higher than the number of features showing no or low migration.

The migration to water was less prominent than to 50% EtOH, and a smaller fraction of the features was leaching to water, that is, being readily leachable or only present in the water migrate (left side of figure 3.3). In sample PE_HD_14 and PE_LD_10, 38% and 34% of the features were leaching to water respectively. For sample PVC_4, only 11% of the total features were leaching to water.

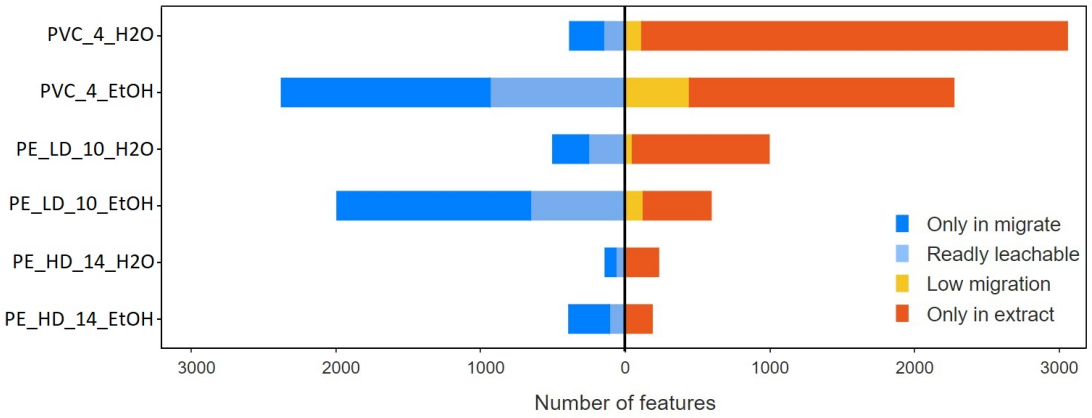
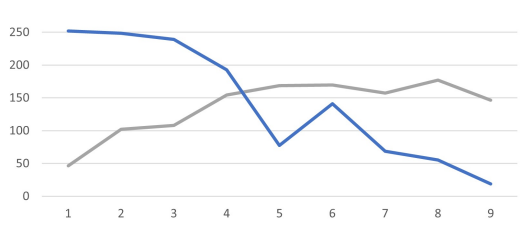


Figure 3.3: Number of features that are migrating and retained in the plastics when using H_2O and 50% EtOH as migrants. The left side is showing features that are only detected in migrates, or are present in the migrates with an abundance of at least 10% of the abundance in the extracts. The right side of the graph is showing features having no, or low migration (more than 10 fold higher concentration in the extracts than in the migrates).

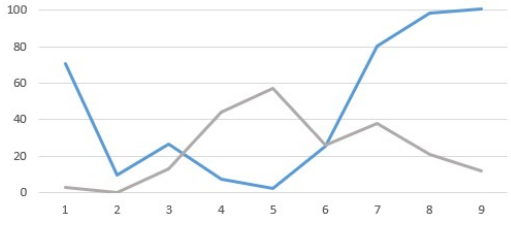
3.2.1 Migration kinetics in water and 50% ethanol

In the migration-kinetics experiment, the goal was to inspect how the concentrations of the features changed over the migrating time period, and the focus was on the increasing features. Therefore, features with a TTR lower than 2 were excluded, as these features were stable or decreasing over the time period, or were not increasing as much as two fold from the three first to the three last timepoints (see figure 3.4a for examples). This filtering step reduced the number of features by 49% on average. The reduction was larger in the 50% EtOH migrates than the water migrates, and the most features were excluded in sample PE_LD_10.

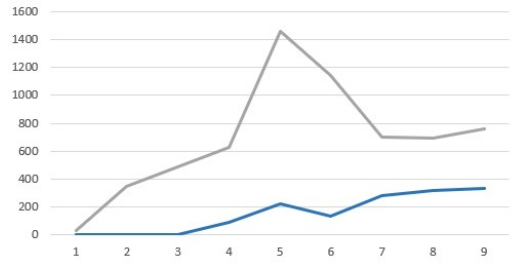
Next, the features with a Spearman's correlation, ρ, lower than 0.7 were also excluded. This filtering step removed features not showing a quite linear increase over the time period (figure 3.4b), and reduced the number of features further, by 16% on average. This time, the reduction was largest for the water migrates. The resulting features were defined as the "increasing features" (figure 3.4c), and accounted for between 15% and 58% of the features in the water migrates of each sample, and between 4% and 36% of the features in the 50% EtOH migrates (table 3.4). Of the increasing features, between 13% and 92% were reaching a plateau (figure 3.4d), and the variations were large between the samples and solvents (table 3.4).



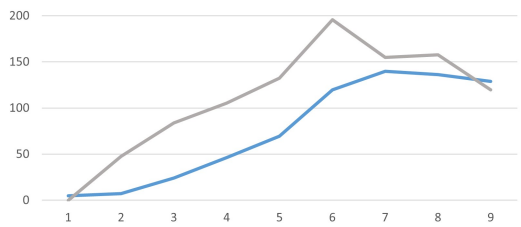
(a) Features excluded due to a TTR < 2 (grey: 1.85, blue: 0.19).



(b) Migration curves with TTR > 2, but excluded due to a $\rho < 0.7$ (grey: 0.33, blue: 0.55).



(c) Features included due to a TTR > 2 and a $\rho > 0.7$ (grey: ρ=0.72, blue: ρ=0.97).



(d) Migration curves with TTR > 2 reaching a plateau.

Figure 3.4: Migration curves showing examples of what the excluded and included features could look like.

Table 3.4: Number of increasing features detected in the H_2O- and EtOH- migrates

Sample	# Features	# Features increasing over the time period (Share of all features)	# Features reaching a plateau (Share of increasing features)
PVC_4_H_2O	122	71 (58%)	23 (32%)
PE_LD_10_H_2O	476	170 (36%)	156 (92%)
PE_HD_14_H_2O	54	8 (15%)	1 (13%)
PVC_4_EtOH	731	261 (36%)	94 (36%)
PE_LD_10_EtOH	905	39 (4%)	20 (51%)
PE_HD_14_EtOH	555	162 (29%)	60 (37%)

4 Discussion

4.1 Number of extractable features

In total, more than 16846 unique chemical features were found in the MeOH extracts, and the number of features in each sample spanned from 37 (PE_7, food container) to 9936 (PVC_29, cling film) (table 3.1). In a previous study using a similar method, between 269 and 15656 features were found in MeOH extracts of each plastic sample [56]. The mentioned study used both food contact plastics and plastic products not intended for food contact. Bearing that in mind, it was hoped that the number of features was lower in our study, as there are stricter regulations for food contact plastics [15]. The numbers were slightly lower in our study, but the results did not differ clearly from the results of the previous study [56].

By reviewing publicly available lists of chemicals used in FCA provided by industry, legislatures and scientific literature, more than 4000 chemicals has been found to be likely or possibly associated with plastic packaging [4], and more than 10000 compounds have been found to be used in plastic production in general [11]. In addition to these, it is known that many unknown NIAS are present in plastics [4], and it is therefore not surprising that the number of chemical features detected in our samples was higher than the number of known plastic associated compounds. Several compounds intentionally or non-intentionally used in plastics are still not known to the public. The lack of transparency in the plastic industry and missing information about the use of chemicals in plastic products are challenges that have previously been addressed [4] [11]. More openness in the industry and continued research on the "known unknown" compounds in plastics are needed to enable conduction of proper risk assessments of FCA.

The number of detected chemical features in the methanol extracts is not necessarily the same as the number of compounds in the plastics. In the migration experiment in our study, it was found that MeOH did not extract all the features present in the plastic samples, since several features migrated exclusively to 50% EtOH (figure 3.2). This demonstrates that some compounds in our samples were not extracted by MeOH.

Further, the number of detected chemical features is also not necessarily the same as the number of extracted compounds. This is because the number of chemical features detected is dependent on MS settings and the software used for data treatment [51]. Only charged species can be detected in the MS [41] [67]. Neutral species must consequently first be ionized, either in positive or negative mode. The two modes provide complementary information, and both modes should therefore be used if the goal is to find the total number of chemicals in a sample [39] [68]. In our study, the samples were only ionized in positive mode, and it is therefore reasonable to believe that the total number of compounds in the samples are higher than the number of features found in our study.

In addition, the peak picking algorithm in Progenesis QI is not perfect, and some peaks could be missed due to this. Then again, peaks that are not actually peaks can also be picked by the algorithm, leading to more features than actual compounds in the samples. The software is also deconvoluting the mass spectra, since positive ions from ESI can be formed either by addition of one or two protons, by formation of multimers such as $[2M+H]^+$, or by addition of other charged compounds, e.g. Na^+ [45]. The deconvolution process groups together ions that originate from the same compound by searching for compound ions that have approximately the same retention times, and ion masses that differ by the mass difference between experimental adducts [69]. If all charged species that come from the same compound are not deconvoluted, the number of features will be higher than the number of compounds in the sample. On the other hand, if the ions are incorrectly grouped together, fewer features will be detected. The impact of the latter source of error was attempted minimized by manual inspection of the deconvoluted ions. However, it is hard to predict in which direction the errors caused by the software have influenced the results.

4.2 Chemical composition of extracts

As shown by the data, there is a large variation in the number of chemical features detected in the plastic samples. The number of features seems to be connected to the polymer type to some degree. The PUR samples had the highest number of features on average, followed by PVC. PVC is well known for containing a lot of additives, and is the polymer type containing the most additives [70] [71] [72]. Many additives have also been found in PUR in a previous study [56], and it has been ranked as one of the most hazardous polymer types based on monomer composition [3]. Our results are in that way consistent with previous research.

At the same time, the number of features varies sizably between samples of the same polymer type, especially for the PE and PVC samples. Even though PVC is known for having many additives, sample PVC_26 (drinking bladder tube) contained only 769 features (table 3.1). Except from PET, there were samples of all the other polymer types containing more than 769 features. The number of features in a sample can therefore not be predicted by the polymer type, and the differences are larger among samples of the same polymer than between the polymer types. However, from our data, the probability of finding many features seems to be higher in PUR and PVC samples than in products made of e.g. PET or PS. It is thus positive that PUR and PVC are less used in FCA than the other tested polymers [5]. It is also promising that three samples, PP_16 (bowl), PE_7 (food container) and PE_LD_8 (food container lid), had less than 100 features. This means that it is possible to produce plastic products containing relatively few chemicals.

When clustering together chemically similar samples (figure 3.1), the clustering was not based on polymer type, but to a larger extent based on the type of plastic article. Examples include

clustering of two drinking bladders (PUR_12 and PUR_43), three freezer bags (PE_LD_10, PE_LD_15 and PE_28), and clustering of two yoghurt cups and a lid (PP_3, PP_39a and PP_39b). However, sample PE_HD_14, a freezer bag, was not clustering together with the three others, and one yoghurt cup (sample PET_25) was not chemically similar to the other yoghurt cups. It is worth noting that PET_25 is made of another polymer type than the other yoghurt cups, and this might contribute to the differences in chemical composition. To sum up, it can not be concluded that products with the same use are necessarily chemically similar. The data still indicates that samples made for the same use and by the same polymer type could be chemically similar. However, the quantity of samples with the same usage is too small to say anything with a large degree of certainty.

Plastics are diverse substances, with different chemical composition [73], and it has been argued that "plastic is not plastic" [74]. This was also confirmed by our study. It was found that around 30% of the detected features in the PP, PE, PS and PET samples were present in only one sample, and only 62 features (0.7%) were present in more than 20 of the 32 samples (table A.2). This illustrates that there are large differences in the chemical composition of the samples. The PUR and PVC samples had more features in common than the other polymers, and 75 features (0.4%) were found in all of the PVC and PUR samples (table A.3). The fact that more features were in common was expected, as the number of features was higher and the number of samples were lower in the set of PVC and PUR samples.

Looking more into the shared features in samples made from the same polymer type, it can be seen that there were differences also within one polymer type (table A.4). There was no features in common in all of the PE samples, and the PE samples might therefore be quite different when it comes to chemical composition. It should however be noted that as many as 12 samples were made of PE, and the PE sample with the smallest number of features had only 37 features (table 3.1). Taking this into consideration, it is not that surprising that the PE samples did not have any features in common. In addition, both the PE_LD and PE_HD samples were included in the same group, and this may have made the PE group more diverse than if the PE_LD and PE_HD were separated.

Further, six samples of both PP and PS were used in this project, but the number of common features within the two polymers was fairly different. For the PP samples, only one feature was present in all the samples, and this corresponded to 1.1% of the possible number of common features (= the number of features in the PP sample with the fewest features). For PS, on the other hand, 41 features (33.1%) were in common. This indicates that the PS samples used in this project were more similar to each other than the PP samples were. The polymer type with the biggest similarity between samples was PUR, with 2914 common features (42.8%) in the three samples.

4.3 Tentatively identified compounds in methanol extracts

In total, 16% of the detected features in the methanol extracts were tentatively identified, and between 9.8% and 38.0% of the features were identified in each sample. A recent study using the same databases for identification of plastic chemicals, with the exception of WIESINGER_db, managed to identify between 2% and 17% [56]. The identification rate was a bit higher in our study, but the results demonstrates that most of the compounds present in plastics are still unknown. More openness in the plastic industry [4] [11] and focus on identifying "true unknown" chemicals that has not yet been documented [43] by investigating the fragmentation pattern in the MS [38] are ways forward to increase the identification rate.

In this study, the identification rate was quite low, and the number of correctly identified compounds are probably even lower, as it is reasonable to believe that a part of the features has been incorrectly identified. This because the features were just *tentatively* identified, corresponding to confidence level 3 as defined by Schymanski et al. [36]. Accordingly, no reference standards were used, and more uncertainty is associated with the identifications. The choice of databases will influence the proportion of correct identifications [39]. When selecting the databases, the goal is to find the balance between identifying as many compounds as possible, and avoiding false positive identifications. A larger, more generalized database, like NORMAN_db, is not always preferable, as it may generate many false positive identifications. On the other hand, to identify all of the chemicals present in plastics, a quite comprehensive database should be used.

In our study, 77% of the tentative identifications in the PP, PE, PET and PS samples and 91% in the PVC and PUR samples were found in NORMAN_db. Most of the identifications were also found in NORMAN_db in a previous study by Zimmermann et al. (2021) [56]. As this database is not selective for known plastic associated compounds, this might indicate that a big part of the compounds present in plastics are non intentionally added, or that several false identifications have been found. Among the ten most abundant features in each sample (table 3.2), 45% of the identified chemicals are known to be used in plastics. The remaining identified compounds among the ten most abundant features (55%) are either implausible, like pharmaceuticals and biological molecules, or have little or no associated information about uses. These chemicals could come from the food or other substances that have been in contact with the plastics, they could be NIAS that were formed in the production of the plastic, or they could be chemicals that are used in plastic production but are not known to the public, which is a known challenge [11]. The compounds could also originate from reactions occurring in the mass spectrometer. It is known that co-eluting compounds can react with each other or with background contamination, and this can result in formation of new ions that were not present in the plastic extract [45].

In total, 4137 features were tentatively identified in this study, but they were identified as only

2146 different compounds (table A.5). This means that some features with different retention times were identified as the same compound. When two compounds have different retention times and the same mass, they could either be isomers or different compounds with the same mass, and should thus not be identified as the same compound. Retention time prediction could be a way forward in reducing the number of features identified as the same compound. It also provides an additional verification step in the identification workflow [43] [75]. However, to be able to confirm the identifications, analytical standards must be introduced [36].

Regarding the 31 high-abundance features that were identified as compounds known to be used in plastics (table 3.2), it can be noted that multiple compounds were present in more than one sample, and a few were also present in samples made of different polymer types. These common compounds could be additives that are often used in several polymers, or common impurities coming from the manufacturing process, like ethylene terephthalate cyclic trimer, which is a NIAS in PET.

4.3.1 Toxicity

Information about both usage and toxicity were obtained for the identified compounds among the ten most abundant features in each sample (table 3.2). The toxic effects of the identified compounds ranged from "irritant" and "harmful to aquatic life" to more worrying effects seen from a human health perspective, like "carcinogenic", "reprotoxic", "bioaccumulative" and "persistant". These effects were listed for chemicals not known to be used in plastic production, but more interestingly, also some of the known plastic chemicals had these effects. The tentatively identified compounds that are known to be used in plastics are rather plausible identifications, and consequently, it is probable that toxic compounds are present with high abundance in the plastic samples.

Many (n=33) of the identified compounds with high abundances did not have any associated information about toxicity. Some of these could be NIAS, since hazard data for NIAS is incomplete in public domain sources, as toxicological studies are mostly conducted on intentionally added substances [19]. It is concerning that hazard data is lacking for several abundant features in the plastic samples, and more research is needed to characterize the toxicity of these. It should however be noted that the high-abundance features are not necessarily the features with the highest concentration in the plastics, as the peak area in the mass spectrum is dependent upon the ionization efficiency of the compound, and is connected to the properties of the compound and the instrument. The peak area in the mass spectra may therefore not be linearly related to concentration [56] [76].

The toxic effect of a chemical is also dependent on how potent it is (how low the AC50 value is). By comparing the AC50 values of the identified compounds with the highest peak areas (table B.3), it was found that several quite potent compounds were present at relatively high

levels in the plastics. One interesting compound is octrizole (PubChem CID 62485), a known plastic associated chemical used as a UV light absorber. It was both one of the most abundant features in PUR_12 (drinking bladder), the PXR agonist with the highest AC50/area, the antiandrogen with the second highest AC50/area and had the fifth highest AC50/area value among the ERα agonists. An other known plastic chemical that deserves some attention, is tributyl 2-acetyloxypropane1,2,3-tricarboxylate (PubChem CID 6505). This had the highest AC50/area among the PPARγ agonists, and was also the PXR agonist with the second highest AC50/area value. In addition it was one of the most abundant compounds in both PE_LD_10 (freezer bag) and PS_41a (plate) (table 3.2). Both PE_LD_10 and PS_41a were active in all the experimental bioassays (table B.2), while PUR_12 was active in all except ERα.

Of the 39 samples, 36 were active in at least one of the bioassays (table B.2). The three freezer bags, PE_LD_10, PE_LD_15 and PE_28, all interfered with all four receptors tested. These samples were also sharing several chemical features (figure 3.1a). In addition to these, sample PVC_4 (tube), PE_34 (cling film), PVC_35 (cling film), PE_37 (frozen blueberries packaging), PS_41 (empty plate) and PS_41a (plate) were active in all of the bioassays. The three PUR samples were all showing antiandrogenic, PPARγ and PXR activity, but no ERα activation. As previously mentioned, the variation in the number of features was quite large between the PVC samples. Interestingly, the toxicity in the bioassays also varied, but the variation was not correlated with the number of features. PVC_4 (drinking tube) and PVC_26 (drinking bladder tube), which had the lowest numbers of features (2283 and 769 respectively), activated four and three of the receptors used in the bioassays respectively. On the other hand, PVC_29 (cling film) with 9936 features, was only active in two of the bioassyas, and PVC_35 (cling film) with 8946 features was active in all four assays. This illustrates that there is no absolute link between the number of features and the activity in these bioassays, and chemical analysis should therefore not be used to predict toxicity.

In a similar study by Zimmermann et al. (2019), the PVC and PUR samples induced the highest toxicity. This is consistent with what was found in our study. The mentioned study also found that the least toxic samples were the PET and PE_HD samples [17]. In our project, there was a large variation between the PE samples, ranging from no activity to activation of all four receptors. The least toxic samples were PE_7 (food container), PE_LD_8 (food container lid) and PP_16 (bowl), which were not active in any of the assays. These three samples were also the ones containing less than 100 features, as mentioned earlier.

With regards to the toxicity reported in ToxCast, it was observed that some samples had tentatively identified compounds that were active in many ToxCast assays, but the samples were not active in the experimental bioassays (table B.2). This applies to for example sample PVC_29, that contained 18 tentatively identified antiandrogenic compounds and 14 ERα agonists, but did not show any activation of these receptors in the bioassays. In contrast, sample PVC_35

was active in all bioassays, and contained similar numbers of active compounds according to ToxCast (21 antiandrogenic and 16 ERα agonists). There were also examples of the opposite case; samples that were active in the bioassays, but contained few or no tentatively identified agonists according to ToxCast. Examples are sample PS_1 (buttermilk), which was showing ERα activity, but had no known ERα agonists, and samples PET_9 (oven bag), PE_HD_14 (freezer bag) and PE_34 (cling film), which were all activating the PPARγ receptor, but contained only one, one and zero PPARγ agonists respectively. A reason for this is that the Toxcast data do not describe all the features present in the samples. Accordingly, the samples being active in the bioassays, but not containing any active compounds can be explained by that the active compounds were not (correctly) identified, or not found in ToxCast. Only 10-38% of the features in each sample were tentatively identified with a score > 40 (table A.1). Of these features, several were tentatively identified as the same compound (table A.5) and of these compounds, only 14% were found in ToxCast. The mixture toxicity (synergism or antagonism) could also play an important role in the toxicity of the plastic extracts, and the ToxCast data offer information about the toxicity of individual compounds only. Further, the toxicity depends on concentration and potency of the compounds. With the low identification rates that were obtained in this study, and the lack of quantitative information, it is not advisable to predict the toxicity from the identified compounds. A better approach is to combine chemical and toxicological analyzes to identify toxic chemicals of concern.

4.4 Migration into water and 50% EtOH

In the water migrates, between 218 and 680 features were detected (table 3.3). In comparison, a recent study found between 203 and 12783 features migrating to water from plastic consumer products [56]. Some of these consumer products were not intended for food contact, but the FCA with the most features had 10796 features in the water migrate. Compared to these results, our results are clearly in the lower part of the scale. However, it should be mentioned that the plastic concentration was 0.24 g/ml in the mentioned study [56], while the plastic concentration in our study was 0.15 g/ml. A lower number of detected features is therefore expected in our study.

The number of migrating features were significantly higher in 50% EtOH, and ranged between 608 and 3357 (table 3.3). The number of features detected in the 50% EtOH migrates was also higher than in the MeOH extracts for all three samples. This was not expected, as extracts are supposed to show the chemical content of the plastics [16], and by that give a "worst case scenario" in the context of human exposure to chemicals leaching from plastics. From our results, this seems to not be the case. However, regarding toxicity, it is not yet known if the most toxicity is migrating to 50% EtOH or to MeOH. Which chemicals that are extracted by a solvent, is dependent on the solubility of the individual chemicals in this extraction solvent [16]. The selection of the optimal extraction solvent to extract most of the chemicals from

plastic products is also dependent upon the polymer type of the sample. Nerin et al. (2022) suggested to use 95% EtOH for PS samples, and acetonitrile/dichloromethane as extraction solvents for PET and PVC [16]. Perhaps more chemicals would be extracted by these solvents than by MeOH.

Sample PVC_4 (drinking tube) was the sample with the most extractable features, more than twice as many as PE_LD_10 (freezer bag). In the 50% EtOH migrates, on the other hand, the difference between PVC_4 and PE_LD_10 was smaller, and in the water migrates, fewer features were found in PVC_4 than in PE_LD_10. Different surface area of the two samples may play a role here. Migration is dependent on surface area, and the migration rates are higher in thinner products than in thicker products (with less surface area per gram) [77]. Both sample PE_LD_10 and PE_HD_14 were thin freezer bags, while PVC_4 was a tube with approximately 1 mm wall thickness. In the migration experiment, the same mass of each of the samples was used, leading to a higher surface area of PE_LD_10 and PE_LD_14 compared to PVC_4. Normally, migration is measured per surface area and not by weight [18] [19], so the use of equal masses of the samples can have lead to less comparable results, and an underestimation of the migration from PVC_4 relative to the other samples. In extractions, on the other hand, surface area is not expected to have a very big importance. Therefore, the extraction experiment was carried out using equal masses of each plastic product for simplicity reasons. To be able to compare the migrates to the extracts in the next step, the migration was also done based on (the same) mass.

As stated, more features were migrating to 50% EtOH than what was extracted with MeOH. When also inspecting the number of shared features between the two solvents (figure 3.2), it was made clear that the overlap between 50% EtOH and MeOH was not very big for any of the samples. The number of features only migrating to 50% EtOH, or only to MeOH was higher than the number of features leaching to both solvents (expect for sample PE_LD_10). This implies that the complete chemical composition of the samples cannot be found in neither of the solvents, and the fraction of chemicals leaching to the two solvents are quite different.

With regards to the features migrating to water, it was observed that the number of features migrating to both water and 50% EtOH was bigger than the number of features migrating to both water and MeOH. This may be related to the polarity of the different solvents. The polarity index for water is 10, while 50% EtOH has 7.1, and 100% MeoH has 5.1 [78]. This means that H_2O and EtOH are more similar with regards to polarity, and hence will extract chemicals with more similar properties than H_2O and MeOH. It can also be noted that some features were found exclusively in the water migrate. In theory, there can be toxic compounds migrating to water that are not extracted by MeOH, and more focus should therefore be given to migration studies in the future, as these have a higher relevance for human exposure than extraction studies.

Figure 3.2 also show that some features are shared between all three solvents. These seem to be very leachable features, and humans are probably exposed to them when eating foods packed in these plastic products. Further research should focus on these features, and assess the toxicity of the very leachable fraction of the migrating features.

Regarding the abundances of the shared features in the migrates and extracts, most of the shared features were "readily leachable" (figure 3.3). For sample PE_HD_14, almost no features were showing "low migration" neither in water nor 50% EtOH. For the other two samples, some of the shared features had a more than 10 fold higher abundance in the MeOH-extracts than in the migrates, and were thus showing "low migration". However, the number of "readily leachable" features were higher than the number of features mainly being in the extracts for all three samples.

Migration experiments are more realistic than extraction [16], and 50% EtOH is supposed to simulate alcoholic foods and oil in water emulsions [15]. However, it has been suggested that the use of 50% EtOH as a food simulant overestimates the migration into foods [79]. This because ethanol-water mixtures have been found to cause swelling of the plastics [79] [80] [81], and a higher degree of swelling will lead to a higher concentration of compounds in the migrate [82]. The swelling effect is smaller when plastics chemicals are migrating into real food instead of ethanol. In a study using samples made of the acrylonitrile butadiene styrene (ABS) polymer, it was found that migration into 50% EtOH caused swelling of the polymer (4% weight increase after ten days at 40°C), while migration into milk or cream showed a significantly lower swelling (less than 2% weight increase after 90 days at 40°C) [79]. These findings indicate that the migration to 50% EtOH is overestimating the migration into food and is not completely realistic, at least for the tested polymer type. There are also other issues related to the use of ethanol as a migration solvent. According to Nerin et al. (2022), some components can react with ethanol via transesterification and form new compounds. Especially migration studies with PUR samples should therefore not be performed in EtOH food simulants, but rather in 3% acetic acid or water [16]. While migration into 50% EtOH is overestimating the migration, migration into water is thought of as giving a more realistic picture of the migration to aquatic environments, or underestimating the real migration to some degree [56].

In our study, the plastic products were cut into pieces to make the conditions similar in the extraction and migration experiments. This is not fully reflecting the real usage conditions, as it is not that common to have food contact on both sides of the plastic packaging in real life, at least not at the same time. Further research should focus on more realistic migration conditions, adapted to the uses of the plastic items. Two recent studies, by Pack et al. (2021) [13] and Zangmeister et al. (2022) [83] designed their methods based on how the plastic items were normally used in real-life situations, and are recommended for inspiration.

4.5 Migration kinetics in water and 50% ethanol

Between 4% and 58% of the detected features in the migrates were defined as increasing, and of these, between 13% and 92% (median of 34%) were stabilizing. The features were defined as increasing if they were present in at least 3 timepoints, had a TTR > 2 and a $\rho > 0.7$ in the Spearman's correlation. The proportion of increasing and stabilizing features were varying a lot, both between the samples and between the solvents (table 3.4), and no obvious pattern was observed. Migration is dependent on, among other things, the size and solubility of each compound in the migrating solvent [1] [18], and this can be a reason for the variation between the samples.

For sample PVC_4 (drinking tube) and PE_LD_10 (freezer bag), the proportion of features having a TTR > 2 was higher in the water than in 50% EtOH. This may be related to the migration rate, since features with high abundances already at the first timepoints may increase a lot in absolute abundance over the migration period, but not in relative abundance compared to the first timepoints. Therefore, the features with a TTR > 2 might be over-represented by small features, and slowly migrating features that are not present at the first timepoints. It is known that migration is faster into 50% EtOH than into water [1], and this could therefore be a reason for a lower proportion of increasing features in the 50% EtOH migrates.

The migration kinetics in sample PE_LD_10 (freezer bag) in 50% EtOH were notably different from the other samples (table 3.4), with only 4% increasing features. This may point towards a particularly fast migration for many of the compounds in this sample, resulting in high concentrations of many of the features already at the first timepoint (after three hours), giving a TTR lower than 2. Blanco et al. (2021) found that some photoinitiators, phthalates and plasticizers reached equilibrium after 60 minutes of migration into 50% EtOH at room temperature [84]. This might also be the case for many of the features in sample PE_LD_10. To test this fast migration hypothesis, more timepoints closer to the start of the migration should be included in further research. Our results indicated that for this particular sample in 50% EtOH, it was not necessary to carry out the migration study for as long as ten days to find a stabilized abundance for most of the features. However, more research should be done on migration kinetics in the future, as our study is not very comprehensive.

According to the EU migration testing guidelines, all features should have reached a plateau after ten days of migration [15], but this seems to not be the case for most of the samples in our study. For five of the six samples, less than 52% of the increasing features were stabilizing. However, in the last sample, PE_LD_10 (freezer bag), 92% of the increasing features were stabilizing, making this sample stand out. Migration carried out over longer timeperiods than ten days should be tested to investigate if more features stabilize after a longer period of time. Some features were also decreasing again at the last timepoints. This could be caused by degradation of the compounds, leading to formation of new compounds during the migration

period [85].

4.6 Limitations of quality control

In this project, no standard mixtures were used to test neither the solid phase extraction procedure nor the instrument performance of the UPLC-QTOF-MS/MS. For SPE, it is recommended to validate the method and evaluate the recovery by using a group of internal standards having different structures and a wide range of physio-chemical properties [86] [87]. The recoveries are often different for the different compounds and a chosen method will not be ideal for all compounds. For instance, Kirchnawy et al. (2014) used four standards to evaluate the SPE recovery, and found recoveries between 50% and 95% for these standards [88]. This also means that the recovery of a few compounds can not predict the recovery of all other chemicals present in the samples. Recoveries are especially important for quantification, but low recoveries can also affect the detectability of the compounds. Validation of sample pretreatment methods used for non-target analysis is challenging, due to the amount of unknown chemicals present in the samples, and the diversity in chemical composition. The identities of the chemicals present in the samples are also unknown, making it hard to choose internal standards that are similar to the chemicals in the samples [87]. In this project, the QC-testing was therefore instead done by analyzing the samples both before and after SPE, what allowed for an inclusion of all detected features. However, since standards were not used, it is not known which properties the chemicals that were recovered in the SPE had compared to the ones that were not recovered. In addition, the concentrations of the compounds are not known. Internal standards could also be used to control the reproducibility of the chromatographic method, in addition to the pooled QC samples. Multiple injections of the same sample could also be a way to assess the instrument stability [32] [39]. It would also have been preferable to have independent replicates (typically 3 is recommended [16]), to check for repeatability of the method. This was not done due to time constrains.

5 Conclusion

The number of features detected in the plastic extracts was higher than the number of compounds known to be associated with plastics, and similar to a previous study. The number and type of chemical features were varying greatly between samples, both of different polymer types and within one polymer type. This demonstrates that plastic products have a large chemical complexity, and that the chemical composition or number of chemicals in a plastic product can not be predicted based on the polymer type or type of product. This makes further research on the effect of plastic chemicals more difficult. One generalization that can be made however, is that PUR and PVC samples have more extractable features than the other four polymer types tested. This was found both in this project and previous studies. Conveniently, these two polymer types are also less used in packaging and FCA than the others.

Most of the features (84%) present in the plastic samples were not identified, and some uncertainty is also associated with the tentative identified compounds. More openness in the plastic industry, more comprehensive databases and more focus on identifying "true unknown" chemicals could contribute towards determining more of the chemicals present in plastics. Of the identified compounds, several were known to be toxic, including some present with high abundances in the samples. The known plastic related compounds with documented toxicity should not be used in production of plastic FCA, but rather be substituted with non-toxic alternatives. A general reduction in the number of additives used in plastic production would also make it more manageable to get an overview of the health hazard related to plastic FCA. In our study, it was found that plastic products containing less than 100 extractable features are on the market, which implies that a reduction of additives is possible. Today, many of the chemicals present in plastics are not tested for toxicity, and it is therefore unknown which effect they have on humans. More research is needed to assess the toxicity of both the additives and NIAS present in plastic FCA.

The migration of features was more prominent into 50% EtOH than to water. The number of features detected in 50% EtOH was also higher than the number of features present in the MeOH extracts, indicating that MeOH extraction does not represent the "worst case scenario" with regards to human exposure to plastic chemicals. The chemical composition in the water, 50% EtOH and MeOH samples was quite different, illustrating that the choice of migration or extraction solvent has a great impact on the results. Further research should focus on features that are migrating to both water and 50% EtOH, since these are readily leachable and probably present in foodstuff. Further studies could also focus on realistic use conditions adapted to the individual plastic products. Here, also the migration kinetics should be taken into account, which differed largely between the samples and migration solvents used in this project.

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Appendix A Supplementary information about methanol extracts

Table A.1: Number of features and identifications found in the plastic extracts before and after different data filtering steps.

Sample	Features	Features after subtracting 10x blank	Possible identifications found	Identifications with score > 40	Identified features with score > 40	% of features identified with score > 40
PS_1	3035	326	3639	1300	67	21
PP_3	3920	1220	14822	5419	240	20
PVC_4	5560	2283	21048	8848	328	14
PE_HD_5	2664	164	1186	128	16	10
PP_6	2789	194	1197	410	28	14
PE 7	2623	37	490	222	7	19
PE_LD_8	2704	74	696	132	12	16
PET_9	3239	528	3305	446	70	13
PE_LD_10	4145	1588	32868	18832	559	35
PS_11	2619	124	877	93	17	14
PUR_12	10001	8762	53402	17120	1022	12
PS_13	2774	264	2530	506	46	17
PE_HD_14	2939	260	3251	787	51	20
PE_LD_15	3912	1211	27865	16235	460	38
PP_16	2681	90	590	109	12	13
PP_17	3507	751	6258	1584	128	17
PET_18	3539	640	10247	4803	183	29
PE_21a	5174	2317	32741	14023	542	23
PE_PET_21b	5970	3203	39935	17359	684	21
PET_25	2864	140	890	137	20	14
PVC_26	3467	769	7066	1672	121	16
PET_27	2793	231	2773	962	55	24
PE_28	3954	1243	23961	14321	410	33
PVC_29	10000	9936	89238	35436	1521	15
PUR_PE_30	10000	6803	55088	20584	1097	16
PS_33	4575	1752	12702	3706	243	14
PE_34	3193	366	6582	3334	94	26
PVC_35	10002	8946	72498	28549	1265	14
PET_36	3315	379	4544	2227	94	25
PE_37	6049	3474	43529	20108	798	23
PE_38	3194	297	2310	689	45	15
PP_39a	4128	1327	12512	3819	273	21
PP_39b	4258	1464	13980	4283	289	20
PET_40	2864	174	1558	525	32	18
PET_40a	2747	251	1864	273	37	15
PS_41	3188	504	7347	2191	108	21
PS_41a	4279	1516	13486	4572	268	18
PUR_43	12252	9175	49204	13712	985	11
PE_LD_44	4240	1504	23909	12936	386	26

Table A.2: Distribution of the features among the PP, PE, PET and PS samples

Number of features that are present in exactly 1 sample	2876
Number of features present in exactly 2 samples	1769
Number of features present in exactly 3 samples	1280
Number of features present in exactly 4 samples	895
Number of features present in exactly 5 samples	527
Number of features present in exactly 6 samples	380
Number of features present in exactly 7 samples	295
Number of features present in exactly 8 of the samples	210
Number of features present in exactly 9 of the samples	144
Number of features present in exactly 10 of the samples	111
Number of features present in exactly 11 of the samples	79
Number of features present in exactly 12 of the samples	40
Number of features present in exactly 13 of the samples	37
Number of features present in exactly 14 of the samples	34
Number of features present in exactly 15 of the samples	15
Number of features present in exactly 16 of the samples	26
Number of features present in exactly 17 of the samples	20
Number of features present in exactly 18 of the samples	5
Number of features present in exactly 19 of the samples	13
Number of features present in 20 or more of the samples	62
Number of features present in all of the samples (n=32)	0

Table A.3: Distribution of the features among the PVC and PUR samples

Number of features that are present in exactly 1 sample	3659
Number of features that are present in exactly 2 samples	4827
Number of features that are present in exactly 3 samples	3269
Number of features that are present in exactly 4 samples	2583
Number of features that are present in exactly 5 samples	1901
Number of features that are present in exactly 6 samples	532
Number of features that are present in all (n=7) samples	75

Table A.4: Number of common features in samples of the same polymer. PE_PET_21b is counted as a PET sample.

Polymer	Number of samples	Common features in all samples	Share of common features	Share of common features in the sample with the fewest features [%]
PE	12	0	0	0
PP	6	1	0.035	1.1
PS	6	41	1.4	33
PET	8	23	0.59	26
PUR	3	2914	22	43
PVC	4	178	1.4	7.8

A.1 Number of unique identifications found

Table A.5: Identified features versus unique identifications with a score > 40.

Polymer group	Number of features identified with a score > 40	Number of unique identifications with a score > 40
PP, PE, PET, PS	1760	1182
PVC, PUR	2377	1371
In total	4137	2146

Appendix B Toxicity assessment

Table B.1: Selected ToxCast assays used to retrieve information about estrogenic, antiandrogenic, PXR and PPARγ activity.

Receptor	Assay Name in Toxcast	Mode
AR	ATG_AR_TRANS_dn	Antagonist
	NVS_NR_hAR	Antagonist
	OT_AR_ARELUC_AG_1440	Antagonist
	OT_AR_ARSRC1_0480	Antagonist
	OT_AR_ARSRC1_0960	Antagonist
	TOX21_AR_BLA_Antagonist_ratio	Antagonist
	TOX21_AR_LUC_MDAKB2_Antagonist_10nM_R1881	Antagonist
	TOX21_AR_LUC_MDAKB2_Antagonist_0.5nM_R1881	Antagonist
	ACEA_AR_antagonist_80hr	Antagonist
	ATG_AR_TRANS_up	Agonist
	TOX21_AR_BLA_Agonist_ratio	Agonist
	TOX21_AR_LUC_MDAKB2_Agonist	Agonist
	TOX21_AR_LUC_MDAKB2_Agonist_3uM_Nilutamide	Agonist
	ACEA_AR_agonist_80hr	Agonist
ERα	ACEA_ER_80hr	Agoniost
	ATG_ERE_CIS_up	Agonist
	ATG_ERa_TRANS_up	Agonist
	NVS_NR_hER	Agonist
	OT_ER_ERaERa_0480	Agonist
	OT_ER_ERaERa_1440	Agonist
	OT_ERa_EREGFP_0120	Agonist
	OT_ERa_EREGFP_0480	Agonist
	TOX21_ERa_BLA_Agonist_ratio	Agonist
	TOX21_ERa_LUC_VM7_Agonist	Agonist
	TOX21_ERa_LUC_VM7_Agonist_10nM_ICI182780	Agonist
	ATG_ERa_TRANS_dn	Antagonist
	ATG_ERE_CIS_dn	Antagonist
	TOX21_ERa_BLA_Antagonist_ratio	Antagonist
	TOX21_ERa_LUC_VM7_Antagonist_0.5nM_E2	Antagonist
	TOX21_ERa_LUC_VM7_Antagonist_0.1nM_E2	Antagonist
PPARγ	ATG_PPRE_CIS_up	Agonist
	ATG_PPARg_TRANS_up	Agonist
	NVS_NR_hPPARg	Agonist
	OT_PPARg_PPARgSRC1_0480	Agonist
	OT_PPARg_PPARgSRC1_1440	Agonist
	TOX21_PPARg_BLA_Agonist_ratio	Agonist
	TOX21_PPARg_BLA_antagonist_ratio	Antagonist
	ATG_PPARg_TRANS_dn	Antagonist
	ATG_PPRE_CIS_dn	Antagonist
PXR	ATG_PXRE_CIS_up	Agonist
	ATG_PXR_TRANS_up	Agonist
	NVS_NR_hPXR	Agonist
	TOX21_PXR_Agonist	Agonist
	ATG_PXRE_CIS_dn	Antagonist
	ATG_PXR_TRANS_dn	Antagonist

Table B.2: Number of detected features, and identified compounds that interfere with the AR, ERα, PPARγ and PXR receptors. Samples interfering with the receptors in the experimental bioassays conducted by Sarah Stevens (Department of Biology, NTNU) are marked with *.

Sample	**Detected features**	**AR antagonists/ agonists**	**ERα agonists/ antagonists**	**PPARγ agonists/ antagonists**	**PXR agonists/ antagonists**
PS_1	326	2/0	0/1 *	1/0	2/0 *
PP_3	1220	5/2	8/4 *	5/0	12/0 *
PVC_4	2283	4/3 *	6/0 *	2/0 *	9/0 *
PE_HD_5	164	0/0	0/0	0/0	0/0 *
PP_6	194	0/0	1/0	0/0	0/0 *
PE_7	37	0/0	0/0	0/0	0/0
PE_LD_8	74	0/0	0/0	1/0	1/0
PET_9	528	5/1	0/1	1/0 *	4/0 *
PE_LD_10	1588	2/1 *	3/1 *	2/0 *	9/0 *
PS_11	124	0/0	0/0	0/0	0/0 *
PUR_12	8762	13/8 *	16/3	4/1 *	25/2 *
PS_13	264	3/0	2/2 *	3/0	4/0 *
PE_HD_14	260	1/0 *	2/0	1/0 *	3/0 *
PE_LD_15	1211	2/1 *	2/1 *	4/0 *	8/0 *
PP_16	90	2/0	0/1	1/0	2/0
PP_17	751	4/1	3/2	0/0	4/0 *
PET_18	640	2/1	5/0 *	3/0 *	7/1 *
PE_21a	2317	9/4	8/4 *	5/0 *	19/0 *
PE_PET_21b	3203	7/5	12/2	8/0 *	20/1 *
PET_25	140	0/0	0/0	0/0	0/0 *
PVC_26	769	2/2 *	3/0	2/0 *	3/0 *
PET_27	231	1/0	0/0	2/0	1/0 *
PE_28	1243	1/0 *	1/1 *	2/0 *	5/0 *
PVC_29	9936	18/6	14/6	10/3 *	26/4 *
PUR_PE_30	6803	14/4 *	10/8	9/2 *	30/6 *
PS_33	1752	8/3	7/4 *	4/0 *	12/1 *
PE_34	366	3/1 *	1/2 *	0/0 *	4/0 *
PVC_35	8946	21/7 *	16/10 *	9/3 *	26/6 *
PET_36	379	3/0	2/0	1/0	3/0 *
PE_37	3474	8/3 *	14/4 *	6/0 *	24/0 *
PE_38	297	2/0	0/1	0/0	1/0 *
PP_39a	1327	6/3	5/4 *	5/0 *	9/0 *
PP_39b	1464	5/3	4/3 *	5/0 *	6/0 *
PET_40	174	0/0	0/0	0/0	0/0 *
PET_40a	251	1/0	1/0	0/0	2/0 *
PS_41	504	3/0 *	3/1 *	3/0 *	5/0 *
PS_41a	1516	3/0 *	4/1 *	4/0 *	7/1 *
PUR_43	9175	15/6 *	12/5	4/2 *	27/1 *
PE_LD_44	1504	4/1	5/1 *	5/1 *	8/0 *

Table B.3: Overview of known AR antagonists, ERα agonists, PPARγ agonists and PXR agnonist tentatively identified in the plastic extracts, sorted from the lowest to highest AC50/area for each receptor. Identified compounds with no AC50 value, or a peak area below 100 are not included in the table.

Rank	PubChem CID	Lowest AC50 [µM]	Highest area	AC50/ area	Detected in samples
AR antagonists					
1	7517	0.69	19746.94	3.50E-05	PUR_12, PUR_PE_30, PVC_35
2	62485	31.39	607969.62	5.16E-05	PUR_12, PUR_43,PET_18
3	42981	42.55	191304.30	2.22E-04	PUR_12, PUR_PE_30, PVC_29, PVC_35, PE_LD_44, PE_28, PE_37, PE_LD_10, PE_LD_15, PE_PET_21b
4	12942137	12.48	39595.25	3.15E-04	PE_37, PP_17
5	2337	6.07	17968.95	3.38E-04	PUR_12, PUR_43,PVC_29, PVC_35, PE_21a
6	62556	3.15	6559.35	4.80E-04	PP_3, PP_39a, PP_39b, PS_1
7	66030	16.22	31243.80	5.19E-04	PUR_12, PUR_PE_30, PVC_29, PVC_35, PE_LD_44, PE_PET_21b
8	135	0.60	1101.66	5.48E-04	PVC_4, PVC_26
9	177	1.15	1506.02	7.66E-04	PVC_29, PVC_35
10	7298	0.27	304.81	8.72E-04	PUR_PE_30, PVC_29
11	6540	0.89	983.92	9.00E-04	PE_21a
12	10313	40.58	40616.79	9.99E-04	PUR_12, PUR_43
13	86171	31.86	31444.62	1.01E-03	PE_21a, PE_37, PP_3, PP_39a, PP_39b
14	656641	5.86	5544.11	1.06E-03	PUR_PE_30
15	51392	16.36	15416.24	1.06E-03	PUR_PE_30
16	164877	1.38	1127.57	1.23E-03	PP_39a, PP_39b
17	53232	3.57	2162.54	1.65E-03	PVC_4
18	352309	28.60	13092.12	2.18E-03	PE_37, PP_16, PS_13
19	8663	1.75	767.41	2.29E-03	PP_3, PS_13,PS_33, PS_41, PS_41a
20	3893	14.01	3872.28	3.62E-03	PUR_PE_30, PVC_29, PVC_35, PE_21a, PE_28, PE_37, PE_LD_10, PE_LD_15, PE_PET_21b
21	7991	1.58	386.07	4.10E-03	PVC_29, PVC_35
22	7612	1.28	284.58	4.50E-03	PUR_PE_30, PVC_4, PVC_29, PVC_35
23	9444	3.06	531.40	5.77E-03	PUR_PE_30, PVC_29
24	5281	12.14	2043.33	5.94E-03	PVC_29, PVC_35, PE_LD_44
25	5192	2.44	369.03	6.61E-03	PUR_43
26	62142	53.92	8028.63	6.72E-03	PUR_PE_30, PVC_4, PVC_29, PVC_35, PE_37, PS_33
27	87293	4.19	504.41	8.31E-03	PP_3
28	12977	23.70	2386.49	9.93E-03	PUR_PE_30, PVC_29, PVC_35
29	27756	40.53	2636.04	1.54E-02	PVC_4, PVC_29, PVC_35
30	196	7.43	429.41	1.73E-02	PUR_12, PUR_PE_30, PVC_35
31	4128060	36.09	1925.87	1.87E-02	PE_LD_44, PE_PET_21b, PP_39a, PP_39b
32	10789	32.25	1528.98	2.11E-02	PVC_4, PVC_26, PE_21a, PE_34, PE_PET_21b, PP_3, PP_39a, PS_33, PS_41, PS_41a
33	8172	55.41	2626.47	2.11E-02	PE_21a, PE_37, PE_HD_14,PE_LD_10, PE_PET_21b, PET_9, PET_36, PP_17, PS_33, PS_41a
34	403	12.94	597.37	2.17E-02	PUR_43,PVC_29, PVC_35
35	28693	42.73	1730.29	2.47E-02	PVC_35
36	3441	45.69	1495.97	3.05E-02	PUR_12, PUR_43,PVC_29, PVC_35
37	4641	20.06	576.83	3.48E-02	PUR_12, PUR_43,PVC_29, PVC_35
38	62459	81.48	1863.61	4.37E-02	PE_LD_44, PE_21a, PE_28, PE_34, PE_37, PE_LD_10, PE_LD_15, PE_PET_21b, PS_33
39	2775	13.13	294.03	4.47E-02	PET_9, PET_18, PET_27, PET_36, PET_40
40	445354	47.08	952.00	4.95E-02	PE_21a, PE_LD_15, PE_PET_21b

Table B.3: (continued)

Rank	PubChem CID	Lowest AC50 [μM]	Highest area	AC50/ area	Detected in samples
41	8200	39.99	745.12	5.37E-02	PUR_PE_30, PVC_35, PE_LD_44, PE_21a, PE_37, PE_PET_21b, PP_39a, PS_41a
42	21059	57.41	976.38	5.88E-02	PUR_12, PUR_43,PUR_PE_30, PVC_35
43	7495	32.31	395.05	8.18E-02	PUR_12, PUR_43
44	2346	48.21	512.16	9.41E-02	PE_21a, PE_34, PE_37, PE_38, PE_PET_21b, PET_9, PET_36, PET_40a, PP_16, PP_17, PP_39a, PS_1, PS_33, PS_41, PS_41a
45	89440	42.99	299.23	1.44E-01	PVC_29, PVC_35
46	7017	64.40	395.49	1.63E-01	PUR_12, PUR_43
47	5280450	93.09	363.86	2.56E-01	PVC_4, PVC_29, PVC_35
48	3293	29.85	101.45	2.94E-01	PP_3
ERα agonists					
1	66030	0.08	31243.80	2.70E-06	PUR_12, PUR_PE_30, PVC_29, PVC_35, PE_LD_44, PE_PET_21b
2	10634	0.08	11309.18	7.12E-06	PUR_12, PVC_4, PVC_26, PVC_29, PVC_35
3	78950	0.06	3839.09	1.67E-05	PUR_PE_30, PVC_29, PVC_35
4	86171	0.96	31444.62	3.06E-05	PE_21a, PE_37, PP_3, PP_39a, PP_39b
5	62485	31.55	607969.62	5.19E-05	PUR_12, PUR_43,PET_18
6	117549	0.06	204.69	3.01E-04	PE_37
7	5281	2.30	2043.33	1.12E-03	PVC_29, PVC_35, PE_LD_44
8	53232	7.09	2162.54	3.28E-03	PVC_4
9	5541	39.31	7900.33	4.98E-03	PUR_12, PVC_29, PVC_35
10	6215	4.83	864.43	5.59E-03	PP_3, PP_39a, PP_39b
11	243	69.32	11144.64	6.22E-03	PUR_12, PVC_26, PE_PET_21b, PP_39b, PS_33, PS_41, PS_41a
12	8663	5.70	767.41	7.43E-03	PP_3, PS_13,PS_33, PS_41, PS_41a
13	802	13.96	1842.87	7.58E-03	PUR_PE_30
14	21059	8.83	976.38	9.05E-03	PUR_12, PUR_43,PUR_PE_30, PVC_35
15	24321	3.98	428.04	9.29E-03	PUR_12
16	92877	18.53	1686.66	1.10E-02	PE_21a, PE_28, PE_34, PE_37, PE_LD_10, PE_LD_15, PE_PET_21b, PP_3, PP_39a, PP_39b, PS_33
17	89440	4.80	299.23	1.60E-02	PVC_29, PVC_35
18	31307	6.61	345.44	1.91E-02	PUR_PE_30, PVC_29, PVC_35
19	656583	10.59	469.29	2.26E-02	PE_37
20	7017	11.96	395.49	3.02E-02	PUR_12, PUR_43
21	7675	38.88	1126.78	3.45E-02	PUR_12, PUR_43,PUR_PE_30, PVC_4, PVC_29, PVC_35, PE_37
22	7495	13.65	395.05	3.46E-02	PUR_12, PUR_43,
23	985	37.51	1028.73	3.65E-02	PVC_26, PVC_29, PVC_35, PE_LD_44, PE_21a, PE_PET_21b, PP_6
24	15624	33.88	850.07	3.99E-02	PUR_12, PUR_43,PE_37, PP_39a, PP_39b
25	379	22.10	547.67	4.04E-02	PUR_12, PUR_43,PVC_4, PVC_29, PVC_35
26	135	57.01	1101.66	5.17E-02	PVC_4, PVC_26
27	189821	38.27	735.56	5.20E-02	PE_21a, PE_PET_21b
28	13690	48.37	836.03	5.79E-02	PE_LD_44, PE_21a, PE_28, PP_39a, PP_39b, PE_37, PE_LD_10, PE_LD_15, PE_PET_21b
29	31256	37.63	634.91	5.93E-02	PUR_PE_30
30	6998	7.08	111.26	6.36E-02	PE_21a, PE_37, PE_HD_14,
31	5280450	25.78	363.86	7.09E-02	PVC_4, PVC_29, PVC_35
32	66357	55.52	352.98	1.57E-01	PE_LD_15
33	87293	91.07	504.41	1.81E-01	PP_3
34	8105	82.56	433.23	1.91E-01	PUR_12, PUR_43,PUR_PE_30
35	3293	20.07	101.45	1.98E-01	PP_3
36	8158	94.52	357.16	2.65E-01	PVC_29, PVC_35

Table B.3: (continued)

Rank	PubChem CID	Lowest AC50 [μM]	Highest area	AC50/ area	Detected in samples
37	7716	57.26	149.45	3.83E-01	PUR_PE_30, PVC_4, PVC_29, PVC_35
PPARγ agonists					
1	6505	7.39	184095.63	4.01E-05	PUR_PE_30, PE_LD_10, PS_41a
2	42981	7.87	191304.30	4.12E-05	PUR_12, PUR_PE_30, PVC_29, PVC_35, PE_LD_44, PE_28, PE_37, PE_LD_10, PE_LD_15, PE_PET_21b
3	8200	0.06	745.12	8.40E-05	PUR_PE_30, PVC_35, PE_LD_44, PE_21a, PE_37, PE_PET_21b, PP_39a, PS_41a
4	62556	1.78	6559.35	2.71E-04	PP_3, PP_39a, PP_39b, PS_1
5	14871	13.01	25093.68	5.19E-04	PUR_12, PUR_43,PUR_PE_30, PVC_29, PVC_35, PE_21a, PET_18
6	61014	92.22	99277.68	9.29E-04	PVC_29
7	86171	42.25	31444.62	1.34E-03	PE_21a, PE_37, PP_3, PP_39a, PP_39b
8	2337	40.96	17968.95	2.28E-03	PUR_12, PUR_43,PVC_29, PVC_35, PE_21a
9	3893	10.05	3872.28	2.60E-03	PUR_PE_30, PVC_29, PVC_35, PE_21a, PE_28, PE_37, PE_LD_10, PE_LD_15, PE_PET_21b
10	62142	21.51	8028.63	2.68E-03	PUR_PE_30, PVC_4, PVC_29, PVC_35, PE_37, PS_33
11	66030	89.04	31243.80	2.85E-03	PUR_12, PUR_PE_30, PVC_29, PVC_35, PE_LD_44, PE_PET_21b
12	23173	63.93	17348.65	3.68E-03	PUR_12, PUR_43,PUR_PE_30, PVC_29, PVC_35, PE_LD_44, PE_PET_21b, PET_18, PS_41a
13	352309	66.65	13092.12	5.09E-03	PE_37, PP_16, PS_13
14	51392	85.37	15416.24	5.54E-03	PUR_PE_30
15	164877	9.38	1127.57	8.32E-03	PP_39a, PP_39b
16	4128060	17.29	1925.87	8.98E-03	PE_LD_44, PE_PET_21b, PP_39a, PP_39b
17	27756	24.07	2636.04	9.13E-03	PVC_4, PVC_29, PVC_35
18	802	17.74	1842.87	9.63E-03	PUR_PE_30
19	11005	118.18	11156.74	1.06E-02	PUR_12, PUR_PE_30, PVC_29, PE_LD_44, PE_28, PE_37, PE_LD_10, PE_LD_15, PS_41a
20	91656	11.78	914.85	1.29E-02	PUR_43,PVC_35
21	25127	68.41	4823.10	1.42E-02	PUR_PE_30, PE_37, PET_36
22	5280450	6.05	363.86	1.66E-02	PVC_4, PVC_29, PVC_35
23	445354	21.01	952.00	2.21E-02	PE_21a, PE_LD_15, PE_PET_21b
24	2244	44.93	1972.14	2.28E-02	PUR_12, PUR_43,PVC_4, PVC_26, PVC_29, PE_PET_21b, PET_27
25	637759	27.91	867.63	3.22E-02	PP_3
26	7789	12.95	372.58	3.48E-02	PE_LD_44, PE_PET_21b, PP_6, PP_17
27	87293	20.51	504.41	4.07E-02	PP_3
28	6540	43.37	983.92	4.41E-02	PE_21a
29	8840	17.50	270.09	6.48E-02	PE_LD_44, PE_28, PE_37, PE_HD_14,PE_LD_8, PE_LD_10, PE_LD_15, PET_27, PP_3, PS_13,PS_33, PS_41, PS_41a
30	66357	23.90	352.98	6.77E-02	PE_LD_15
31	7984	87.48	1262.75	6.93E-02	PE_21a, PE_PET_21b, PS_41, PS_41a
32	8158	25.43	357.16	7.12E-02	PVC_29, PVC_35
33	8593	35.11	409.94	8.57E-02	PUR_43,PUR_PE_30
34	189821	67.22	735.56	9.14E-02	PE_21a, PE_PET_21b
35	3293	10.26	101.45	1.01E-01	PP_3
36	66636	21.79	193.70	1.13E-01	PUR_PE_30
37	17891	72.09	465.14	1.55E-01	PUR_12, PUR_43,PVC_29
38	41270	105.91	675.56	1.57E-01	PUR_12, PVC_4, PVC_26, PVC_29, PVC_35

Table B.3: (continued)

Rank	PubChem CID	Lowest AC50 [µM]	Highest area	AC50/ area	Detected in samples
39	117549	32.31	204.69	1.58E-01	PE_37
40	35785	64.45	318.68	2.02E-01	PUR_PE_30, PVC_29, PE_21a, PE_28, PE_37, PE_LD_10, PE_LD_15
41	2775	86.39	294.03	2.94E-01	PET_9, PET_18, PET_27, PET_36, PET_40
PXR agonists					
1	62485	1.04	607969.62	1.71E-06	PUR_12, PUR_43,PET_18
2	6505	0.85	184095.63	4.63E-06	PUR_PE_30, PE_LD_10, PS_41a
3	13097	0.70	132896.28	5.28E-06	PUR_12, PUR_43,PUR_PE_30, PP_39a, PP_39b
4	42981	1.41	191304.30	7.36E-06	PUR_12, PUR_PE_30, PVC_29, PVC_35, PE_LD_44, PE_28, PE_37, PE_LD_10, PE_LD_15, PE_PET_21b
5	8172	0.11	2626.47	4.37E-05	PE_21a, PE_37, PE_HD_14,PE_LD_10, PE_PET_21b, PET_9, PET_36, PP_17, PS_33, PS_41a
6	66030	1.45	31243.80	4.64E-05	PUR_12, PUR_PE_30, PVC_29, PVC_35, PE_LD_44, PE_PET_21b
7	86171	1.63	31444.62	5.18E-05	PE_21a, PE_37, PP_3, PP_39a, PP_39b
8	12942137	2.22	39595.25	5.60E-05	PE_37, PP_17
9	62556	0.64	6559.35	9.81E-05	PP_3, PP_39a, PP_39b, PS_1
10	379	0.09	547.67	1.68E-04	PUR_12, PUR_43,PVC_4, PVC_29, PVC_35
11	51392	4.15	15416.24	2.69E-04	PUR_PE_30
12	445354	0.30	952.00	3.14E-04	PE_21a, PE_LD_15, PE_PET_21b
13	638024	1.26	1989.11	6.34E-04	PE_21a, PE_37, PE_PET_21b, PET_40a
14	53232	1.66	2162.54	7.67E-04	PVC_4
15	6540	0.99	983.92	1.00E-03	PE_21a
16	91656	1.16	914.85	1.27E-03	PUR_43,PVC_35
17	4128060	2.83	1925.87	1.47E-03	PE_LD_44, PE_PET_21b, PP_39a, PP_39b
18	23173	33.24	17348.65	1.92E-03	PUR_12, PUR_43,PUR_PE_30, PVC_29, PVC_35, PE_LD_44, PE_PET_21b, PET_18, PS_41a
19	62459	4.01	1863.61	2.15E-03	PE_LD_44, PE_21a, PE_28, PE_34, PE_37, PE_LD_10, PE_LD_15, PE_PET_21b, PS_33
20	27756	7.05	2636.04	2.67E-03	PVC_4, PVC_29, PVC_35
21	442021	36.02	12489.49	2.88E-03	PUR_12, PUR_43,PUR_PE_30, PVC_4, PVC_29, PVC_35, PE_21a, PE_37, PS_41a
22	25127	21.37	4823.10	4.43E-03	PUR_PE_30, PE_37, PET_36
23	13690	4.16	836.03	4.98E-03	PE_LD_44, PE_21a, PE_28, PE_37, PE_LD_10, PE_LD_15, PE_PET_21b, PP_39a, PP_39b
24	8028	38.75	6827.71	5.68E-03	PUR_12, PUR_43,PUR_PE_30, PVC_4, PVC_29, PVC_35
25	89440	2.00	299.23	6.68E-03	PVC_29, PVC_35
26	8914	30.38	3982.91	7.63E-03	PUR_12, PUR_PE_30, PVC_4, PVC_26, PS_33
27	6215	7.23	864.43	8.36E-03	PP_3, PP_39a, PP_39b
28	7439	29.58	3160.45	9.36E-03	PUR_PE_30, PVC_4, PVC_29, PVC_35, PE_21a, PE_28, PE_37, PE_LD_10, PE_LD_15, PE_PET_21b, PET_18, PET_36, PP_3, PP_39a, PP_39b, PS_33
29	35785	3.00	318.68	9.40E-03	PUR_PE_30, PVC_29, PE_21a, PE_28, PE_37, PE_LD_10, PE_LD_15
30	87293	5.92	504.41	1.17E-02	PP_3
31	24321	5.19	428.04	1.21E-02	PUR_12
32	15624	11.47	850.07	1.35E-02	PUR_12, PUR_43,PE_37, PP_39a, PP_39b

Table B.3: (continued)

Rank	PubChem CID	Lowest AC50 [µM]	Highest area	AC50/ area	Detected in samples
33	5280450	5.04	363.86	1.39E-02	PVC_4, PVC_29, PVC_35
34	3293	1.51	101.45	1.49E-02	PP_3
35	1268142	1.83	110.47	1.66E-02	PUR_12, PUR_43,PE_21a, PET_9
36	2244	39.63	1972.14	2.01E-02	PUR_12, PUR_43,PVC_4, PVC_26, PVC_29, PE_PET_21b, PET_27
37	802	37.37	1842.87	2.03E-02	PUR_PE_30
38	189821	15.79	735.56	2.15E-02	PE_21a, PE_PET_21b
39	637759	18.68	867.63	2.15E-02	PP_3
40	7675	26.93	1126.78	2.39E-02	PUR_12, PUR_43,PUR_PE_30, PVC_4, PVC_29, PVC_35, PE_37
41	7017	9.80	395.49	2.48E-02	PUR_12, PUR_43
42	7979	67.70	2357.48	2.87E-02	PUR_12, PUR_43,PUR_PE_30, PVC_29, PVC_35
43	7622	19.27	655.72	2.94E-02	PE_37
44	6010	9.94	317.23	3.13E-02	PE_37, PE_LD_10, PE_PET_21b, PET_18
45	7529	9.77	297.55	3.28E-02	PP_3
46	6836	14.40	419.21	3.43E-02	PUR_43,PUR_PE_30, PVC_35
47	5362	17.75	495.62	3.58E-02	PE_21a, PE_PET_21b
48	41270	24.75	675.56	3.66E-02	PUR_12, PVC_4, PVC_26, PVC_29, PVC_35
49	5755	24.09	552.39	4.36E-02	PE_21a, PE_PET_21b
50	656583	23.74	469.29	5.06E-02	PE_37
51	6544	48.66	880.19	5.53E-02	PUR_12, PUR_PE_30, PVC_4, PVC_29, PVC_35
52	31307	19.18	345.44	5.55E-02	PUR_PE_30, PVC_29, PVC_35
53	17891	39.03	465.14	8.39E-02	PUR_12, PUR_43,PVC_29
54	117549	18.11	204.69	8.85E-02	PE_37
55	31256	57.18	634.91	9.01E-02	PUR_PE_30
56	8840	27.69	270.09	1.03E-01	PE_LD_44, PE_28, PE_37, PE_HD_14,PE_LD_8, PE_LD_10, PE_LD_15, PET_27, PP_3, PS_13,PS_33, PS_41, PS_41a
57	2566	15.44	147.76	1.04E-01	PUR_12, PUR_43,PVC_35
58	11357	70.05	657.18	1.07E-01	PUR_43,PUR_PE_30, PVC_35
59	676486	53.86	266.55	2.02E-01	PUR_12, PUR_43
60	81278	70.19	335.30	2.09E-01	PUR_12, PUR_43,PVC_29
61	8105	90.93	433.23	2.10E-01	PUR_12, PUR_43,PUR_PE_30
62	7335	74.22	349.52	2.12E-01	PE_LD_44, PE_21a, PE_PET_21b, PS_33, PS_41a
63	66357	78.64	352.98	2.23E-01	PE_LD_15
64	11173	55.79	101.66	5.49E-01	PUR_12, PUR_43,PUR_PE_30, PVC_4, PVC_29, PVC_35
65	6998	74.76	111.26	6.72E-01	PE_21a, PE_37, PE_HD_14

Table B.4: Overview of the tentatively identified compounds with known toxicity, and which samples they are present in (x). The toxicity is given as X/Y, where X is the number of assays in which the compound is acting as an AR antagonist/ ERα agonist/ PPARγ agonist/ PXR agonist, while Y is the number of assays where the compound is showing the opposite behaviour. Samples PE_HD_5, PE_7, PS_11, PET_25 and PET_40 were left out of the table as they did not contain any of the listed compounds.

PubChem CID	Active in # assays	AA	ERα	PPARγ	PXR	PUR_12	PUR_43	PUR_PE_30	PVC_4	PVC_26	PVC_29	PVC_35	PE_21a	PE_28	PE_34	PE_37	PE_38	PE_HD_14	PE_LD_8	PE_LD_10	PE_LD_15	PE_LD_44	PE_PET_21b	PET_9	PET_18	PET_27	PET_36	PET_40a	PP_3	PP_6	PP_16	PP_17	PP_39a	PP_39b	PS_1	PS_13	PS_33	PS_41	PS_41a
66030	25	6/1	11/3	1/0	3/0	x	-	x	-	-	x	x	-	-	-	-	-	-	-	-	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6010	20	4/5	7/1	0/0	3/0	-	-	-	-	-	-	-	-	-	-	x	-	-	-	x	-	-	x	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-
12942137	18	6/0	3/4	1/1	3/0	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-
62556	17	3/1	3/4	2/1	2/1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	x	x	x	-	-	-	-
445354	16	6/0	2/2	2/1	3/0	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	x	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8663	13	4/0	7/1	0/0	1/0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	x	x	x	x
51392	12	4/0	1/4	1/0	2/0	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
86171	12	3/0	3/2	1/0	3/0	-	-	-	-	-	-	-	x	-	-	x	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	x	x	-	-	-	-	-
31307	11	2/3	5/0	0/0	1/0	-	-	x	-	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5280450	11	1/0	2/0	4/1	2/1	-	-	-	x	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3293	11	2/0	3/0	3/0	3/0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-
4128060	11	3/1	1/1	2/1	2/0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	-	-	-	-	-	-	-	-	-	x	x	-	-	-	-	-
656583	11	1/4	3/0	0/0	3/0	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5280934	10	1/0	2/0	3/1	2/1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-
7626	10	1/1	1/1	1/1	3/1	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28803	10	3/1	1/3	1/1	0/0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-
352309	9	3/1	0/3	1/0	1/0	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	x	-	-	-
87293	9	2/1	2/0	1/0	3/0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-
89440	9	3/0	3/0	0/0	3/0	-	-	-	-	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
42981	9	4/0	0/1	1/0	3/0	x	-	x	-	-	x	x	-	x	-	x	-	-	-	x	x	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
62485	9	3/0	2/1	0/0	3/0	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-
177	8	3/0	0/3	0/1	1/0	-	-	-	-	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table B.4: (continued)

PubChem CID	Active in # assays	AA	ERα	PPARγ	PXR	PUR_12	PUR_43	PUR_PE_30	PVC_4	PVC_26	PVC_29	PVC_35	PE_21a	PE_28	PE_34	PE_37	PE_38	PE_HD_14	PE_LD_8	PE_LD_10	PE_LD_15	PE_LD_44	PE_PET_21b	PET_9	PET_18	PET_27	PET_36	PET_40a	PP_3	PP_6	PP_16	PP_17	PP_39a	PP_39b	PS_1	PS_13	PS_33	PS_41	PS_41a
62459	8	3/0	0/2	0/0	3/0	-	-	-	-	-	-	-	x	x	x	x	-	-	-	x	x	x	x	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-
53232	8	2/0	2/1	0/0	3/0	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6540	8	1/0	1/2	1/0	3/0	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
656641	8	3/0	0/3	0/1	1/0	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6215	7	1/2	2/0	0/0	2/0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	x	x	-	-	-	-	-
92877	7	1/2	3/0	0/0	1/0	-	-	-	-	-	-	-	x	x	x	x	-	-	-	x	x	-	x	-	-	-	-	-	x	-	-	-	x	x	-	-	x	-	-
403	7	3/0	0/2	0/1	1/0	-	x	-	-	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7219	7	0/0	3/0	1/0	3/0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	x	x	x	x
189821	7	0/2	2/0	1/0	2/0	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
86132	7	3/0	2/0	0/0	2/0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-
7495	7	2/0	3/2	0/0	0/0	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1539	6	2/2	1/0	0/0	1/0	-	-	-	x	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25127	6	0/0	2/0	1/0	3/0	-	-	x	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-
6505	6	1/0	0/0	2/0	3/0	-	-	x	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x
7789	6	0/0	1/1	3/0	1/0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	-	-	-	-	-	x	-	x	-	-	-	-	-	-	-
6867	6	2/1	1/0	1/0	1/0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-
164877	6	2/0	1/2	1/0	0/0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	-	-	-	-	-
2337	6	1/0	1/1	1/0	1/1	x	x	-	-	-	x	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
91656	6	1/0	0/1	1/0	3/0	-	x	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
638024	6	0/0	1/0	1/1	3/0	-	-	-	-	-	-	-	x	-	-	x	-	-	-	-	-	-	x	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-
14871	6	0/0	1/1	2/1	0/1	x	x	x	-	-	x	x	x	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-
5978	6	2/1	0/2	0/0	1/0	x	x	x	-	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5281	5	1/0	4/0	0/0	0/0	-	-	-	-	-	x	x	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7896	5	0/0	3/0	0/0	2/0	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table B.4: (continued)

PubChem CID	Active in # assays	AA	ERα	PPARγ	PXR	PE_21a	PE_28	PE_34	PE_37	PE_38	PE_HD_14	PE_LD_8	PE_LD_10	PE_LD_15	PE_LD_44	PE_PET_21b	PET_9	PET_18	PET_27	PET_36	PET_40a	PP_3	PP_6	PP_16	PP_17	PP_39a	PP_39b	PS_1	PS_13	PS_33	PS_41	PS_41a	PUR_12	PUR_43	PUR_PE_30	PVC_4	PVC_26	PVC_29	PVC_35
6998	5	0/	4/	0/	1/	x	-	-	x	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5755	5	0/	0/	0/	2/	x	-	-	x	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7707	5	0/	3/	0/	2/	x	-	-	x	-	-	-	-	-	x	x	-	x	-	-	-	x	-	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-
802	5	0/	2/	2/	1/	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	x	-	-	-	-	-	-
3893	5	1/	0/	2/	0/	x	x	-	x	-	-	-	x	x	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4641	5	3/	1/	1/	0	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	-	-	x	x	-	-
8200	5	1/	0/	0	0/	x	-	-	x	-	-	-	-	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	x	x
16821	5	0/	1/	1/	1/	x	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7017	5	2/	2/	0/	1/	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	-	-	x	x	-	-
27756	5	1/	0/	1/	3/	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x
9444	5	3/	0/	0/	1/	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-
21800	5	1/	2/	0/	0/	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	-	-	-	-	-	-
10634	5	1/	2/	0/	0/	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	x	x
21059	4	1/	1/	0/	0/	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x
8158	4	0/	1/	2/	0/	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	x	x
15624	4	0/	2/	0/	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	-	-	-	-	x	x	-	-	-	-	-	-
28693	4	2/	0/	0/	1/	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x
117549	4	0/	1/	1/	2/	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x
3830	4	0/	1/	0/	1/	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	x
62142	4	1/	0/	2/	1/	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	x	-	-	x
442021	4	0/	1/	0/	2/	x	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	-	x	-	-	-	x
3385	4	1/	2/	0/	1/	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-
35785	4	0/	0/	1/	3/	x	x	-	x	-	-	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	x	-	-	-	-
13690	4	0/	1/	0/	3/	x	x	-	x	-	-	-	x	x	x	x	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-
		0	0	0	0																																		

Table B.4: (continued)

PubChem CID	Active in # assays	AA	ERα	PPARγ	PXR	PUR_12	PUR_43	PUR_PE_30	PVC_4	PVC_26	PVC_29	PVC_35	PE_21a	PE_28	PE_34	PE_37	PE_38	PE_HD_14	PE_LD_8	PE_LD_10	PE_LD_15	PE_LD_44	PE_PET_21b	PET_9	PET_18	PET_27	PET_36	PET_40a	PP_3	PP_6	PP_16	PP_17	PP_39a	PP_39b	PS_1	PS_13	PS_33	PS_41	PS_41a
17891	4	1/0	0/0	1/0	2/0	x	x	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24321	4	0/0	3/0	0/0	1/0	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
66357	4	0/0	2/0	1/0	1/0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10789	3	1/0	0/1	0/0	1/0	-	-	-	x	x	-	-	x	-	x	-	-	-	-	-	-	-	x	-	-	-	-	-	x	-	-	-	x	-	-	x	x	x
8172	3	1/0	0/0	0/0	2/0	-	-	-	-	-	-	-	x	-	-	x	-	x	-	x	-	-	x	x	-	-	x	-	-	-	-	x	-	-	-	x	-	x
7517	3	1/0	0/0	0/0	1/1	x	-	x	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2346	3	2/0	0/0	0/0	1/0	-	-	-	-	-	-	-	x	-	x	x	x	-	-	-	-	-	x	x	-	-	x	x	-	-	x	x	x	-	x	-	x	x
7675	3	0/0	2/0	0/0	1/0	x	x	x	x	-	x	x	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8105	3	0/0	2/0	0/0	1/0	x	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
443946	3	2/0	0/1	0/0	0/0	-	-	-	-	-	-	-	x	-	-	x	x	-	-	-	-	-	-	x	-	-	-	-	x	-	-	x	-	-	x	x	-	-
16837	3	0/1	1/1	0/0	0/0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-
1268142	3	1/0	0/0	0/0	2/0	x	x	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-
135	3	1/0	2/0	0/0	0/0	-	-	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6836	3	1/0	0/0	0/0	2/0	-	x	x	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2566	3	0/0	1/0	0/0	2/0	x	x	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5362	3	0/0	0/0	0/0	3/0	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7966	3	0/0	2/0	0/1	0/0	-	-	x	-	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31256	3	0/0	2/0	0/0	1/0	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13097	3	0/0	0/0	0/0	3/0	x	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	-	-	-	-	-
10313	3	1/0	0/1	0/1	0/0	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
985	3	0/0	2/0	0/0	0/1	-	-	-	-	x	x	x	x	-	-	-	-	-	-	-	-	x	x	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-
6714002	3	1/2	0/0	0/0	0/0	x	x	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11005	3	0/0	0/0	2/0	1/0	x	-	x	-	-	x	-	-	x	-	x	-	-	-	x	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x
2775	3	2/0	0/0	1/0	0/0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	x	x	-	-	-	-	-	-	-	-	-	-	-

Table B.4: (continued)

PubChem CID	Active in # assays	AA	ERα	PPARγ	PXR	PUR_12	PUR_43	PUR_PE_30	PVC_4	PVC_26	PVC_29	PVC_35	PE_21a	PE_28	PE_34	PE_37	PE_38	PE_HD_14	PE_LD_8	PE_LD_10	PE_LD_15	PE_LD_44	PE_PET_21b	PET_9	PET_18	PET_27	PET_36	PET_40a	PP_3	PP_6	PP_16	PP_17	PP_39a	PP_39b	PS_1	PS_13	PS_33	PS_41	PS_41a
637759	3	0/0	0/1	1/0	1/0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-
8840	3	0/0	0/1	2/0	1/0	x	-	-	-	-	-	-	-	x	-	x	-	x	x	x	x	-	-	-	x	-	-	-	x	-	-	-	-	-	-	-	x	x	x
2244	3	0/0	0/0	0/0	0/0	x	x	-	x	x	x	-	-	-	-	-	-	-	-	-	-	-	x	-	x	-	-	-	-	-	-	-	-	-	-	-	x	x	x
11112	2	0/0	1/0	0/0	1/0	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41270	2	0/0	0/0	1/0	1/0	x	-	-	x	x	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
175956	2	0/0	1/0	0/0	0/0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	-	-	-	-	-	-
12629	2	0/0	0/1	0/0	0/0	-	-	x	-	x	x	x	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3441	2	1/0	1/0	0/0	0/0	x	x	-	-	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
243	2	0/0	2/0	0/0	0/0	x	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13282	2	0/0	0/0	0/0	0/0	x	x	x	-	-	x	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
66636	2	0/0	0/0	1/0	1/1	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6734	2	0/0	1/0	0/0	1/1	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	x	x	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11357	2	0/0	0/1	0/0	1/0	-	x	x	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
379	2	0/0	1/0	0/0	1/0	x	x	-	x	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7946	2	0/0	0/0	0/0	0/0	-	-	x	-	-	x	x	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	x
24405	2	2/0	0/0	0/0	0/1	-	-	x	-	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
443958	2	0/0	1/0	0/0	0/0	-	-	x	-	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-
12977	2	1/0	0/0	0/0	0/0	-	-	x	-	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8593	2	0/0	0/0	1/0	1/1	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23173	2	0/0	0/0	1/0	1/0	x	x	x	-	-	x	x	-	-	-	-	-	-	-	-	-	x	-	x	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-
9920342	2	0/0	0/0	0/0	0/0	-	-	x	-	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12530	2	0/1	1/0	0/0	0/0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8892	2	0/0	0/0	0/0	0/0	-	-	x	-	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7298	2	1/0	0/0	0/0	2/1	-	-	x	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table B.4: (continued)

PubChem CID	Active in # assays	AA	ERα	PPARγ	PXR	PUR_12	PUR_43	PUR_PE_30	PVC_4	PVC_26	PVC_29	PVC_35	PE_21a	PE_28	PE_34	PE_37	PE_38	PE_HD_14	PE_LD_8	PE_LD_10	PE_LD_15	PE_LD_44	PE_PET_21b	PET_9	PET_18	PET_27	PET_36	PET_40a	PP_3	PP_6	PP_16	PP_17	PP_39a	PP_39b	PS_1	PS_13	PS_33	PS_41	PS_41a
196	2	1/0	0/0	0/0	0/1	x	-	x	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5541	2	0/0	2/0	0/0	1/0	x	-	x	-	-	x	x	-	-	-	x	-	-	-	x	-	x	x	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-
7071	2	0/0	0/0	0/0	1/0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11173	2	1/0	0/0	0/0	2/0	x	x	x	x	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13849	1	0/0	1/0	0/0	0/0	-	-	x	-	-	-	-	-	x	-	x	-	-	-	x	x	x	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	x
8028	1	0/0	0/0	0/0	1/0	x	x	x	x	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
736331	1	1/0	0/0	0/0	0/0	x	x	-	x	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
88069	1	0/0	0/0	0/0	1/0	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-
19309	1	0/0	0/0	0/0	1/0	-	-	x	-	-	x	x	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
212930	1	0/0	0/0	0/0	0/0	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
75557	1	1/0	0/0	0/0	1/0	x	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3275	1	0/0	0/0	0/0	1/0	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	x	x	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-
78950	1	0/0	1/0	0/0	0/0	-	-	x	-	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
73791	1	0/0	0/0	0/0	0/0	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
75472	1	0/0	0/0	0/0	1/0	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7360	1	0/0	0/0	0/0	0/0	-	-	x	x	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6544	1	1/0	0/0	0/0	1/0	x	-	x	x	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8095	1	0/0	0/0	0/0	1/0	-	-	x	-	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5578	1	0/0	1/0	0/0	0/0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-
91604	1	0/0	1/0	0/0	0/0	-	-	-	-	-	-	-	x	x	-	x	x	-	-	x	x	x	-	-	-	x	x	-	x	-	-	-	x	x	-	x	-	-	-
61014	1	0/0	0/0	0/0	0/0	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7735	1	0/0	1/0	0/0	0/0	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34359	1	0/0	1/0	0/0	0/0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-
20330	1	0/0	1/0	0/0	0/0	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	x	x	-	-	-	-	-	-	x	-	-	-	-	-	-	x	x	x	x
		0	0	0	0																																		

Table B.4: (continued)

PubChem CID	Active in # assays	AA	ERα	PPARγ	PXR	PUR_12	PUR_43	PUR_PE_30	PVC_4	PVC_26	PVC_29	PVC_35	PE_21a	PE_28	PE_34	PE_37	PE_38	PE_HD_14	PE_LD_8	PE_LD_10	PE_LD_15	PE_LD_44	PE_PET_21b	PET_9	PET_18	PET_27	PET_36	PET_40a	PP_3	PP_6	PP_16	PP_17	PP_39a	PP_39b	PS_1	PS_13	PS_33	PS_41	PS_41a
7505	1	0/0	1/0	0/0	0/0	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8082	1	0/0	1/0	0/0	0/0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-
7622	1	0/0	0/0	0/0	1/0	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8914	1	0/0	0/0	0/0	1/0	x	-	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-
7570	1	0/0	0/0	0/0	1/0	-	x	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28207	1	0/0	0/0	0/0	1/0	-	x	x	-	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7335	1	0/0	0/0	0/0	1/0	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	x	x	x	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-
81278	1	0/0	0/0	0/0	1/0	x	x	-	x	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
676486	1	0/0	0/0	0/0	1/0	x	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6481	1	0/0	0/0	0/0	1/0	x	x	x	x	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4054	1	1/0	0/0	0/0	0/0	x	x	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
91805	1	1/0	0/0	0/0	0/0	x	x	x	-	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7612	1	1/0	0/0	0/0	0/0	-	-	x	x	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5192	1	1/0	0/0	0/0	0/0	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7979	1	0/0	0/0	0/0	1/0	x	x	x	-	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24762	1	0/0	1/0	0/0	0/0	x	-	x	-	-	x	-	-	-	-	-	-	-	-	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	x
2893	1	0/0	0/0	0/0	0/0	x	x	x	x	x	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-
66571	1	0/0	0/0	0/0	1/0	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7529	1	0/0	0/0	0/0	1/0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-
7439	1	0/0	0/0	0/0	1/0	-	-	x	x	-	x	x	x	x	-	x	-	-	-	x	x	-	-	-	x	-	-	x	-	-	-	x	-	x	-	-	-	-	-
3681	1	0/0	0/0	0/0	0/0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
87329	1	0/0	0/0	0/0	1/0	x	x	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7296	1	0/0	0/0	1/0	0/0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7710	1	0/0	0/0	0/0	1/0	-	-	-	-	-	-	-	x	-	-	x	-	-	-	x	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table B.4: (continued)

PubChem CID	Active in # assays	AA	ERα	PPARγ	PXR	PVC_4	PVC_26	PVC_29	PVC_35	PE_21a	PE_28	PE_34	PE_37	PE_38	PE_HD_14	PE_LD_8	PE_LD_10	PE_LD_15	PE_LD_44	PE_PET_21b	PET_9	PET_18	PET_27	PET_36	PET_40a	PP_3	PP_6	PP_16	PP_17	PP_39a	PP_39b	PS_1	PS_13	PS_33	PS_41	PS_41a
7984	1	0/0	0/0	1/0	0/1	-	-	-	-	x	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x
109029	1	0/0	0/0	0/0	0/0	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8199	1	0/0	1/0	0/0	0/0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
62551	1	0/0	0/0	0/0	0/0	-	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	x
5366546	1	0/0	0/0	0/0	1/1	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5502	1	0/0	0/0	0/0	0/0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
564	1	1/0	0/0	0/0	0/0	-	-	-	-	x	-	-	-	-	-	-	x	x	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
62907	1	0/0	0/0	0/0	1/0	-	-	-	-	x	x	x	x	-	-	-	x	x	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7174	1	0/0	1/0	0/0	0/0	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	x	-	-	-	-	-	-
26098	1	0/0	1/0	0/0	0/0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-
753	1	0/0	0/0	0/0	0/0	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7716	1	0/0	1/0	0/0	0/0	-	x	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7991	1	0/1	0/0	0/0	0/0	-	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20039	1	0/0	0/0	0/0	1/0	-	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
66377	1	0/0	1/0	0/0	0/0	-	-	x	x	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Appendix C Migration method validation

C.1 Features detected in procedural blanks

To investigate how the number of features in the migrates changed in the solid phase extraction procedure, first the number of features in the procedural blanks before and after SPE were compared (table C.1).

Table C.1: Number of features in the procedural blanks. The "Number of common features" shows how many features that are present both before and after SPE.

Blank	Number of features	Number of common features
50% EtOH PB before SPE	9274	8519
50% EtOH PB after SPE	9210	
H_2O PB before SPE	9004	8096
H_2O PB after SPE	9234	
H_2O PB with 10% EtOH after SPE	9181	
MeOH extracts in DMSO PB before SPE	8829	8333
MeOH extracts in DMSO PB after SPE	9640	

In the 50% EtOH procedural blank a few more features were detected before than after the SPE, while in the water and DMSO blanks more features were detected after the enrichment. The largest differences were found in the DMSO blank, where 800 additional features were detected after the SPE compared to prior to the enrichment. SPE cartridges can contain impurities [89] [90], and chemical impurities in the cartridges may be extracted with organic solvents that are commonly used in the elution step of SPE [91]. The additional features detected after SPE may therefore originate from the SPE cartridge, or from the solvents used [90]. The volume of MeOH extracts in DMSO (50 ml) was considerably lower than the volume of the 50% EtOH and H_2O migrates (800 ml), and this may have impacted the results. The small volume can have caused fewer impurities in the SPE- cartridge to be washed away during the sample loading, and more of these impurities can consequently have been eluted and included in the sample.

When looking at the number of common features, it can be seen that around 90% of the features in each solvent were present both before and after SPE. The remaining features were either removed or added in the SPE. In targeted analysis, solid phase extraction is normally used to extract the target analytes of interest, and reduce the matrix [92], so it is expected that some features are removed in the SPE.

C.2 Effect of 10% EtOH in the SPE

The 50% EtOH migrates were diluted to 10% EtOH before SPE. This has also been done previously in targeted analyses, with SPE recoveries between 50% and 95% for the target compounds [88]. To investigate if the presence of ethanol in the migrates affected the performance of the SPE in our study, 10% EtOH was added to half of the water migrates. After enrichment, the number of features in the water migrates with and without 10% EtOH was compared (figure C.1). The results showed that all three samples had more features in the

migrates with 10% EtOH than in the pure water migrates after SPE. However, the size of this difference varied between samples. The greatest effect of the addition of EtOH to the water migrates was found in sample PE_LD_10, where the number of features in the migrate with 10% EtOH comprised 123% of the number of features in the water migrate without EtOH. Overall, the difference in SPE performance in water migrates and 10% EtOH migrates was relatively small, and the water migrates and ethanol migrates were comparable.

Figure C.1: Effect of 10% EtOH in the SPE.

C.3 Effect of SPE

To investigate how the chemical composition of the migrates was changed in the SPE procedure, the abundances of the features before and after SPE were compared (figure C.2). In all samples, some features were lost in the SPE. This was expected, since SPE is a selective technique that is not able to extract all compounds in samples containing compounds with a wide range of polarities [92][93].

In the 50% EtOH migrate of sample PE_LD_10, a large number of features was lost in the SPE. For this sample, a loss of several features was also expected, since a layer of precipitates was visible on top of the migrate after diluting it with water to 800 ml, prior to the SPE. A part of the compounds migrating to 50% EtOH were in other words not very soluble in water. Some of these precipitates remained on the walls of the glass bottle, and this might be a reason for the loss of chemical features after enrichment by SPE. The MeOH-extract of sample PVC_4 clearly had a changed chemical composition after SPE compared to before. A part of the features detected in the extract that had not been enriched (4_MeOH) were not present after SPE (4_MeOH_SPE, figure C.2). This may be because these compounds had a low affinity to the SPE-cartridge, and were therefore not extracted [92]. Typically, compounds with a higher affinity to water will be extracted to a smaller extent by C18 cartridges, since these compounds will rather stay in the water-based solvent than adsorb to the SPE cartridge. However, when comparing the chromatograms of the methanol extracts of PVC_4 before and after SPE (figure C.3), it can be seen that there were some additional early eluting peaks after SPE that were not present in the initial MeOH-extract. Since the chromatographic separation was done using a reversed phase method, polar compounds will elute first, and this indicates that there were more polar, water-soluble compounds after SPE than before. In other words,

the chromatograms did not show that polar compounds were lost in the SPE.

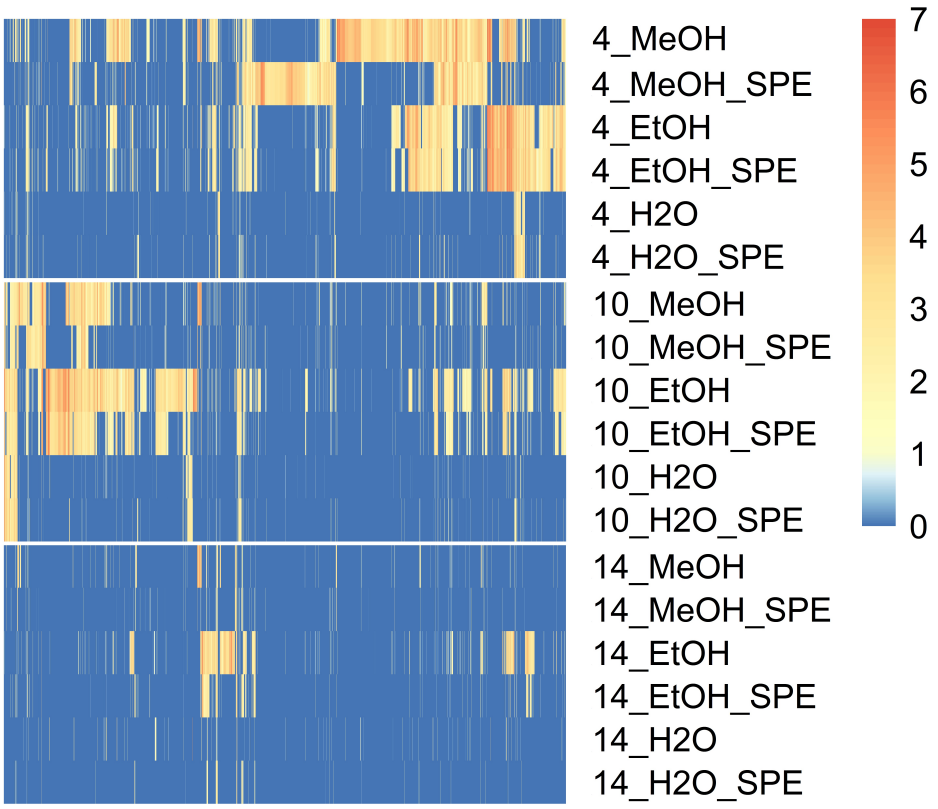


Figure C.2: Heatmap showing the chemical composition of the migrates before and after SPE. Samples marked "SPE" have undergone SPE. The features in the samples are shown as vertical lines and colored according to their logarithmic peak area.

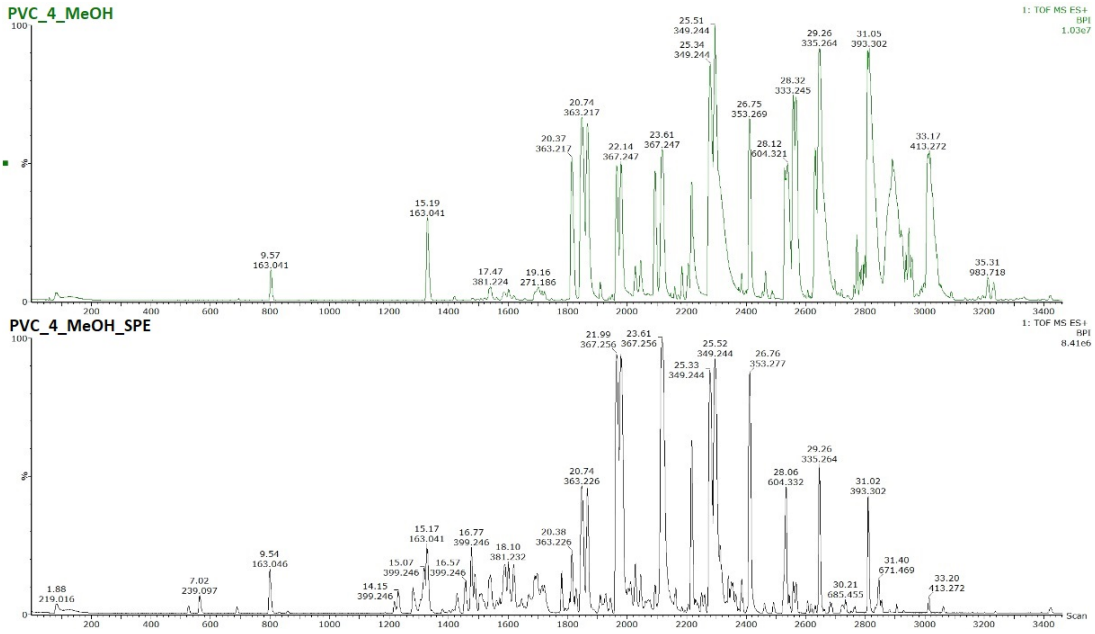


Figure C.3: Chromatograms showing the detected peaks in the MeOH extract of sample 4 before SPE (4_MeOH), and after SPE (4_MeOH_SPE).

The differences in chemical composition before and after the SPE could also be affected by the acidification of the samples. The directly injected samples were not acidified, so this could be a source of differences between the directly injected samples and the samples that have undergone SPE. Acidification may lead to degradation of specific chemicals in the migrate by hydrolysis [94], or change the compounds' ability to be ionized by ESI in positive mode [95]. Only charged ions can be detected by the MS, and therefore only compounds that are ionizable in positive mode will be visible in the mass spectra [67].

Signal suppression in the ESI can be a problem when many compounds elute at the same time. There is a limited concentration of excess charges in ESI droplets, and there is also limited space on the surface of the droplet [96][97]. Due to this, the co-eluting analytes must compete to get ionized, and this may reduce the number of analyte ions released into the gas phase [98]. The amount of co- eluting compounds could be larger in the directly analysed migrates than after a sample cleanup step like SPE, and lead to more signal suppression in the directly analysed migrates. This can also be a possible explanation for the features that are more abundant or present only after SPE.

From a chemical point of view, the choice of a SPE column is often based on the column's selectivity for target compounds [99]. With a non-target approach, the goal is to extract as many compounds as possible [32]. SPE can also be used to concentrate the samples. However, if the concentrations are high enough in the original sample, an alternative approach to analyzing as many compounds as possible, is to analyze the migrates directly, without SPE [93]. This can be a less time consuming approach avoiding the negative sides with SPE, like contamination from the cartridge and bad recovery. However, the migrates in our study were also analysed in bioassays, requiring higher concentrations and another solvent than in the initial migrates. Enrichment by SPE was therefore done both to preconcentrate the sample, and to change the solvent [90]. The SPE was in that way convenient in order to be able to conduct the bioassays, and the losses in the SPE were therefore acceptable.

In addition, from a toxicological perspective, it is not that important to recover all compounds in the migrates in the SPE, as long as the compounds inducing a toxic effect are extracted. In a previous study investigating different SPE cartridges' ability to extract the toxicity of water samples, Telos C18/ENV was found to recover the most toxicity among the tested SPE cartridges [100]. For this reason, this cartridge was also used in our project.

Appendix D Migration kinetics method validation

Throughout this project, the abundances of features after 10 fold blank subtraction was used in all calculations and figures. However, when assessing the migration kinetics, it was investigated if the subtraction of 10 times the blank abundance would affect the results. Therefore, the number of features present in > 3 timepoints and the number of increasing features were compared after subtraction of the 10 fold and 1 fold abundance of features detected in the blank (table D.1). In both cases, features were excluded if they had an abundance equal to or smaller than the 10 fold abundance of the respective feature in the blank. This comparison showed that the differences between the two approaches were only minor (table D.1). Therefore, the numbers after a 10 fold blank subtraction was used, to be consistent within the study.

It was also investigated how the number of increasing features changed if only the last and the first timepoints (t9 and t1) were used to determine if a feature was increasing or not. The TTR was therefore compared to t9/(t1+1) (table D.1). All the migrates, except PE_HD_14 in water, had an equal or higher number of features defined as increasing when comparing the last and first timepoints rather than the three last and three first (TTR). However, by manual inspection, it was found that the t9/t1 approach resulted in several "false" increasing features. The TTR approach was more conservative and stable, and was therefore used.

Table D.1: Comparison of the number of features in the data filtering steps after subtraction of the 10 fold and 1 fold abundance of the features in the corresponding blank.

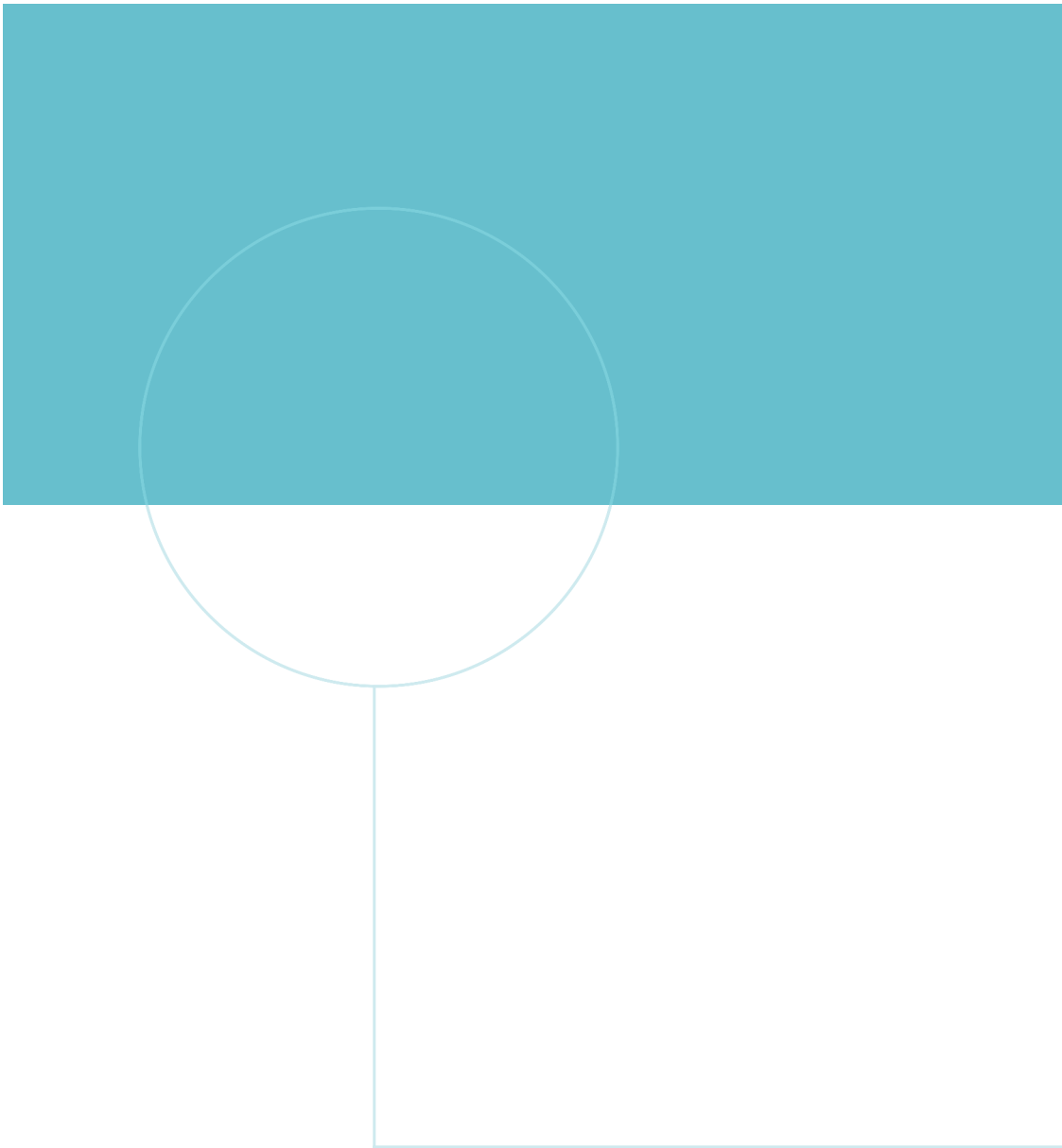
Sample	Features $>$ 10x blank	**Subtraction of 10x blank** Present in > 3 timepoints	TTR > 2	t9/t1 > 2	**Subtraction of 1x blank** Present in > 3 timepoints	TTR > 2	t9/t1 > 2
PVC_4 H_2O	122	98	81	81	144	92	98
PVC_4 EtOH	731	667	271	336	676	268	332
PE_LD_10 H_2O	476	444	214	233	476	183	233
PE_LD_10 EtOH	905	870	46	54	877	45	51
PE_HD_14 H_2O	54	14	11	9	33	18	15
PE_HD_14 EtOH	555	380	173	215	391	160	211

Appendix E Python-code venn diagrams

```
from matplotlib_venn import venn3, venn3_circles
from matplotlib import pyplot as plt

import csv

count = 0

with open("After subtr of 10x blank.csv","r") as csvfile:
for linje in csvfile.readlines():
        linje = linje.strip().split(";") # linje is a line in the csv.file
        IVDMSO = float(linje[8]) #number in parenthesis is the column number (0 is the first
column)
        IVEtOHt9 = float(linje[9])
        IVEtOH10 = float(linje[10])
        IVEtOH50 = float(linje[11])
        IVH2O = float(linje[12])
        IVH2Ot9 = float(linje[13])
        IVMeOH = float(linje[14])
        XDMSO = float(linje[15]) # sample 10
        XEtOHt9 = float(linje[16])
        XEtOH10 = float(linje[17])
        XEtOH50 = float(linje[18])
        XH2O = float(linje[19])
        XH2Ot9 = float(linje[20])
        XMeOH = float(linje[21])
        XIVDMSO = float(linje[22]) # sample 14
        XIVEtOHt9 = float(linje[23])
        XIVEtOH10 = float(linje[24])
        XIVEtOH50 = float(linje[25])
        XIVH2O = float(linje[26])
        XIVH2Ot9 = float(linje[27])
        XIVMeOH = float(linje[28])
        if XH2O > 0 and XEtOH50 > 0 and XMeOH <= 0 :
                count+=1 #counts how many features that meets the requirements set above.
print(count)

# The inequality signs are changed to find features that are in common for all combinations of
the 3 samples. Then the numbers of features are put in the venn3-function.

venn3(subsets=(119,1679,257,423,45,521,259),set_labels=("Water", "50% EtOH", "MeOH"),alpha=0.5)
# order of numbers: (A,B,AB,C,AC,BC,ABC)
plt.title("Compounds in water, 50% EtOH and MeOH, PE_LD_10")
plt.show()
```

Appendix F R-code heatmaps

```
install.packages("pheatmap")
install.packages("dplyr")
library(pheatmap)
library(dplyr)

global <-NULL

giveHeatMap <- function(data){
    global <<- data

col_breaks = c(
    seq(0, 0.5, length=52),
    seq(0.51, 7, length=48)
    )

return(
    pheatmap(
        data,
        cluster_rows=T,
        treeheight_col = 0,
        treeheight_rows = 0,
        cluster_cols = T,
        show_rownames= T,
        show_colnames = F,
        legend=T,
        border_color = "grey95",
        breaks = col_breaks,
        clustering_distance_cols = "euclidean",
        clustering_distance_rows = "euclidean",
        clustering_method = "complete",
        cex = 0.8
        ))
}

Heatmapdata <- read.csv("Heatmap_PVCPUR.csv",sep=";")

giveHeatMap(t(Heatmapdata))

save_pheatmap_png <- function(x, filename, width=12000, height=4000, res = 1200, point-
size=12) {
        png(filename, width = width, height = height, res = res, pointsize = pointsize)
        grid::grid.draw(x$gtable)
        dev.off()
}

save_pheatmap_png(giveHeatMap(t(Heatmapdata)),"Heatmap_PVC,PUR.png")
```



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