Migration of Polycyclic Aromatic Compounds from Food Storage Containers to Food

by

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ABSTRACT

My thesis was geared towards understanding the migration of a suite of polycyclic aromatic compounds (PACs) from commercially available plastic containers. The literature is replete with examples of chemicals that can migrate from plastics (e.g., bisphenol-A, flame retardants) but to my knowledge a comprehensive study on PAC migration from different plastic types has not been reported. This is surprising considering that most of the plastics in commerce are derived from crude-oil and PACs are known to be one of the main contaminants in crude-oil. Furthermore, many PACs are known to cause negative health outcomes in wildlife. To understand PAC migration from plastics, I took four plastic-types and incubated them separately at 40^{o}C with dichloromethane and olive-oil. At prescribed time-points I sampled the incubation media and measured PACs using gas chromatography tandem mass spectrometry. I was able to detect several polycyclic aromatic hydrocarbons (PAHs) and their alkylated derivatives. I also was able to report the rate of migration of four PAHs from high-density polyethylene. My work is important because in addition to reporting the migration of many PACs from plastics for the first time, it also provides evidence for a poorly studied route of human exposure to these compounds and further impetus for investigation into biopolymers and other renewable plastic derivatives.

DEDICATION

I would like to express my heartfelt gratitude to my friends and family for their encouragement over the past two years. Without many of you, I would not have had the courage to pursue graduate studies. I am incredibly fortunate to have this special group of people who have provided unwavering support throughout my academic journey and continue to stand by me as I embark on my next endeavor. Thank you all for being my much-needed support system during this important stage of my life and career.

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Finally, thank you to the Department of Chemistry at the University of Manitoba and COGRAD for providing a welcoming space that was conducive to my learning and well-equipped facilities to conduct this research project to complete my degree.

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Chapter 1. Introduction

1.0 Plastics: Origin and Usage

Thermoplastics are ubiquitous in modern society ranging in usage from medical supplies, children's toys, to common household products and have come a long way since their inception in the early 1900s[1,2]. Because these plastics can be melted and reshaped, it makes them highly versatile. Furthermore, plastic versatility also arises from their ability to be lightweight, flexible, and low manufacturing costs. All these attributes contribute to why thermoplastics remain popular today.

Unsurprisingly, thermoplastics are dominant in the packaging industry and accounts for 44% of all plastics in use. From 1950s to 2017, it has been reported that 9.2 billion tonnes of plastic have been manufactured, with more than half of that all made only in the last 25 years[2].

Thermoplastics are repeating polymeric chains, most often derived from ethylene and propylene monomers, and can be synthesized by one of two methods:

1. Step Reaction Polymerization (Condensation): A heat driven reaction between monomers; sometimes with the help of a catalyst to produce a ratio between pairs of functional groups on monomers (Equation 1). Here, water is removed in this process to drive the reaction to the right to favour the polymerization of a monomer. In this step reaction polymerization, the longer monomers are exposed to heat, the longer the polymeric chain then becomes[3–5]:

Equation 1 – Reaction between monomers to produce a ratio between pairs of functional groups on monomers. This reaction may take place with the aid of a catalyst

$$H_2N(CH_2)_6NH_2 + xHOOC(CH_2)_4COOH \leftrightarrow (-HN(CH_2)_6)NHOC(CH_2)_4CO -)_x + 2xH_2O$$

2. Chain Reaction Polymerization (Addition): Here a monomer (possessing a C=C bond) forms a free radical through the process of reacting with an already growing polymer chain by breaking the double bond. The resulting action is then a new free radical valance that allows for further reaction with another monomer.

By the reaction mechanisms above, monomers then form polymers such as polyethylene, polyvinyl chloride, polystyrene, and polypropylene that are used in many commercial plastic goods. Figure 1 shows the global distribution usage of the different types of thermoplastics in commerce and Table 1 shows the different products they are used in.

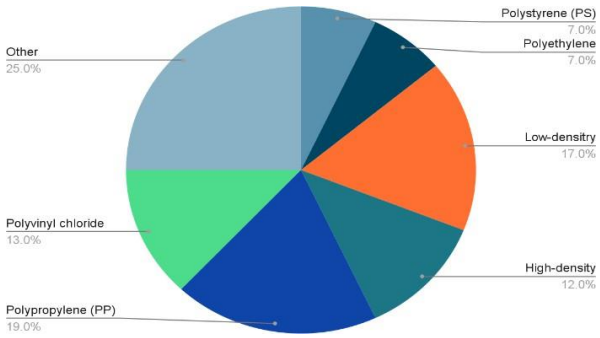


Figure 1 – Global Distribution of Various Plastic Types[2,3,6]

Table 1 - The Usage of Various Common Plastic Types[1–3].

Plastic Type	Usage
Polystyrene (PS)	Packaging material (packing peanuts), Styrofoam
Polyethylene terephthalate (PET)	Food packaging (water bottles), fabrics
Low-density polyethylene (LDPE)	Plastic bags (garbage bags, Ziploc), shrink wrap
High-density polyethylene (HDPE)	Plastic bottles (milk jugs, shampoo bottles)
Polypropylene (PP)	Storage containers (Tupperware)
Polyvinyl Chloride (PVC)	Construction (piping, wiring), medical devices

1.1 Bioplastics

Despite their first emergence 40 years ago [7], bioplastics are an emerging alternative to conventional plastics, derived from renewable biological sources such as plants, algae, and microorganisms. Unlike traditional plastics, which are primarily petrochemical-based and contribute to environmental pollution and resource depletion, bioplastics are designed to offer a more sustainable option. They can be categorized into two main types: those that are biodegradable and those that are bio-based but not necessarily biodegradable[7–9]. Biodegradable bioplastics, such as polylactic acid and polyhydroxyalkanoates, break down into natural components under specific conditions, potentially reducing their environmental impact. Bio-based bioplastics, like bio-polyethylene and bio-polypropylene, are produced from renewable resources but do not necessarily degrade any faster than their conventional counterparts[7]. The use of bioplastics aims to mitigate the reliance on fossil fuels, lower greenhouse gas emissions, and reduce plastic waste.

Current production is scaled at approximately 2 million tonnes per year and increasing as research and technology advances[8].

1.2 Chemicals intentionally added to plastics

Virgin plastics have little use on their own. After polymerization, they are brittle and do not have the functional characteristics needed for use. Additives are chemicals that are purposely added to plastics to make them more functional. The plastic additive market share value was estimated to be over $48 billion USD in 2020 and project to have an annual growth rate of 5.7% in the years 2021-2028. The Asian market is reported to hold a vital share in this industry accounting for over $21 billion USD; given their large stake in the plastic manufacturing industry (30%) this is then unsurprising that China corners the additive market as well[10].

There are many additives that can be introduced into a polymer. Pre-blending involves introducing the additives prior to the polymerization process. Additives can also be introduced by melting the polymer then mixing the additives. Finally, master batching is a technique that can be used when a concentrated amount of additive is introduced to the polymer via a carrier resin, and finally in-situ polymerization[11,12] .

Additives are classified by their function. Table 2 highlights common plastic additives and their intended purpose.

Table 2 - Common Plastic Additives Used by Industries [13].

Additive	Typical Concentration (%)	Description
Fillers	0-50	Improves the strength of thermoplastics
Colourants	≤ 17.5	Adds colour and makes thermoplastics light resistant
Plasticizer	10-70	Improves fluidity and flexibility during production
Lubricants	0.1-3	During molding, prevents the adhesion to molds and to each other
Flame retardants	12-18	Prevents combustion
Antioxidants	0.05-3	Minimize/prevent oxidation or discolouration when exposed to light/heat

Additives should be cost-effective while delivering maximum efficiency, stability, and be non-toxic [14,15]. In addition, their potential impact on environmental and human health should be carefully evaluated. Some additives are lipophilic in nature and when they leach from the plastic have the potential to bioaccumulate in living organisms. This bioaccumulation is influenced by the chemical properties of the additives and their interactions with other lipophilic substances in the environment. Consequently, the potential for environmental and health risks associated with these additives must be assessed, especially in the context of their long-term stability and persistence. The literature is replete with examples on the environmental occurrence of chemical additives used

in plastics[16–21] . The partitioning of additives from plastic have been previously studied by Teuten et al. The authors suggests that additives can enter the environment by diffusion from the plastic because of the physical properties of the plastic itself (porosity, polymer thickness, additive hydrophobicity etc.) [21,22]. This is because some additives are not covalently bound to the polymer backbone, which allows for migration out of plastics[23]. Most compounds that make up the leachate include organic compounds, metals [24], phenols, phthalates[25,26], and non-intentionally added substances (NIASs)[23]; compounds that are not directly added in the manufacturing process but are simply by-products of the process itself.

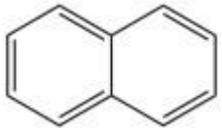
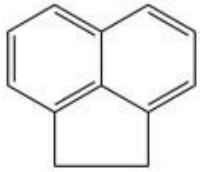
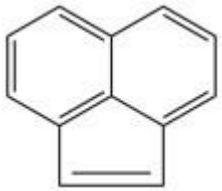
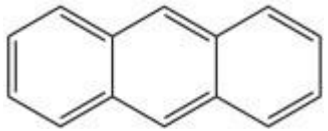
Many additives possess an octanol-water partition coefficient (K_{ow}) greater than 1000 (i.e., log K_{ow}>3) and, as such, the risk of bioaccumulation becomes a cause for concern. In fact, many additives are under review by the U.S Food and Drug Administration (US-FDA) and their international counterparts and those that have been identified as hazardous to humans are regulated by the Stockholm Covention[27]. Based on the persistence, bioaccumulation, and toxicity (PBT) properties, additives are inherently harmful to living organisms and stricter regulations are needed to ensure the safety of humans.

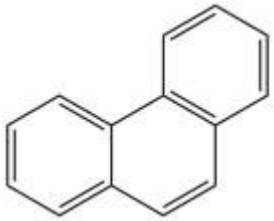
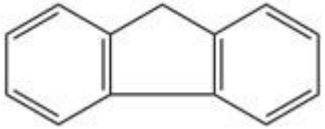
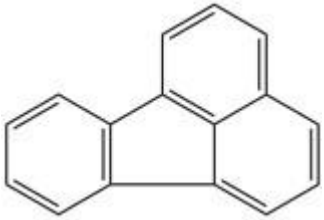
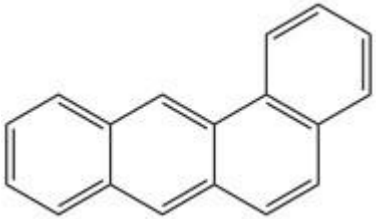
1.3 PACs and their inherent toxicity

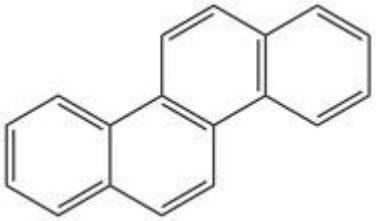
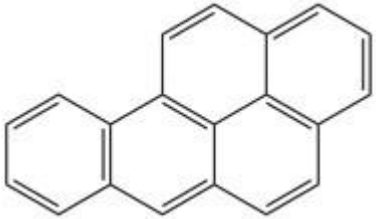
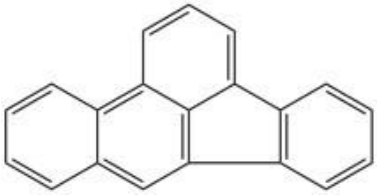
Polycyclic aromatic hydrocarbons (PAHs) and their alkylated derivatives (collectively addressed here as PACs, or polycyclic aromatic compounds) are known for being environmental pollutants. The former compound class has been extensively studied and is known for its toxicity. The U.S

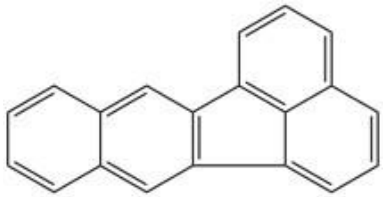
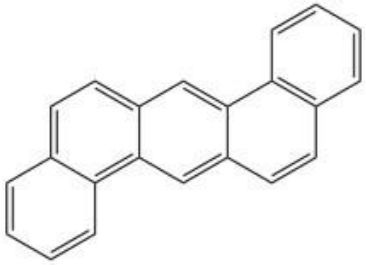
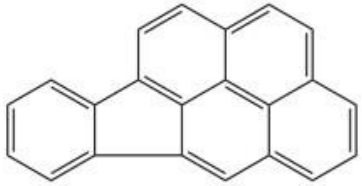
EPA has identified 16 “priority pollutants” described below in Table 3 where most toxicity studies have been focused on.

Table 3 - PAHs Identified by U.S EPA as "16 Priority Pollutants".

Name	Formula	Structure	Molecular Weight	Log K_{OW}	Log K_{OC}
Naphthalene	$C_{10}H_8$		128.17	3.29	2.97
Acenaphthene	$C_{12}H_{10}$		154.21	3.98	3.66
Acenaphthylene	$C_{12}H_{18}$		152.20	4.07	1.40
Anthracene	$C_{14}H_{10}$		178.23	4.45	4.15

Phenanthrene	$C_{14}H_{10}$		178.23	4.45	4.15
Fluorene	$C_{13}H_{10}$		166.22	4.18	3.86
Fluoranthene	$C_{16}H_{10}$		202.26	4.90	4.58
Benzo (a) -anthracene	$C_{20}H_{12}$		228.29	5.61	5.30

Chrysene	$C_{18}H_{12}$		228.29	5.9	N/A
Pyrene	$C_{16}H_{10}$		202.26	4.88	4.58
Benzo(a)pyrene	$C_{20}H_{12}$		252.32	6.06	6.74
Benzo(b)fluoranthene	$C_{20}H_{12}$		252.32	6.04	5.74

Benzo(k)fluora-nthene	$C_{20}H_{12}$		252.32	6.06	5.74
Dibenz(a,h)-anthracene	$C_{22}H_{14}$		278.35	6.84	6.52
Benzo(g,h,i)-perylene	$C_{22}H_{12}$		276.34	6.50	6.20
Indeno[1,2,3-cd] pyrene	$C_{22}H_{12}$		276.34	6.58	6.20

PAHs specifically have been extensively studied and like the additives discussed in Section 1.3, also exhibit PBT properties. Under the Canadian Environmental Protection Act (CEPA, 1999), the PAHs in Table 4 are classified as either carcinogenic (Group 1) or probably carcinogenic (Group 2)[28]. This could be due to the dense π electrons on the aromatic rings of PAHs that cause these compounds to be more resistant to nucleophilic attack thus a contributing factor to PAHs' persistence and toxicity[29] Additives are found to contain PAHs in materials such as carbon black and extender oils [30,31].

There have been several reports highlighting the presence and toxicity of alkylated PAHs (APAHs)[32]. Alkylated PAHs are present in greater amounts in crude oil than their unsubstituted counterparts and thus a more significant contribution to the overall toxicity of PACs[33,34]. This can also pose concern for biota, namely aquatic organisms as photochemical and microbial transformation products of APAHs are thought to be soluble in water, leading to increased inherent toxicity and bioaccumulation[35]. Unfortunately, regulatory requirements surrounding APAHs are lacking and require more investment into their PBT studies from government and regulatory bodies.

1.4 Thesis Hypothesis

My thesis hypothesis is that petrochemicals used in the production of thermoplastics migrate from plastics into the surrounding environment i.e., food.

1.5 Hypothesis Testing

To test my hypothesis, I designed experiments to measure the amount of PACs migrating from plastics into a food simulant i.e., olive oil. First, I purchased plastic containers that were readily available commercially. I purposely selected four popular plastic-types used in the food industry namely high-density polyethylene (HDPE), low density polyethylene (LDPE), polyethylene terephthalate (PET), and polypropylene (PP). Second, a known volume of food simulant was incubated with each plastic at 40˚C in a water-bath and a known volume of food simulant was withdrawn on days 0, 2, 4, 6, 8 and 10. Using the same experimental design, I also incubated containers with a strong solvent, dichloromethane. This allowed me to compare differences in the type and amount of chemicals migrating under the two-exposure scenarios. Finally, analytes in my samples were detected and measured using gas chromatography tandem mass spectrometry.

1.6 Thesis Structure

This thesis is divided into five (5) sections. *Chapter 1* introduces the concept of plastics which include the market share of plastics commercially available today, formation of plastics from their origin in crude oil products to final market ready product, and the nature of PACs as well as their inherent toxicity. In *Chapter 2*, I discuss the methodology employed to execute this project. This section also provides some background information on the instrumentation that was routinely used for sample preparation. *Chapter 2* also provides insight on the materials and experimental methods used. Here I go into detail on the chemicals and reagents used, as well as the specifications on the experimental design. In *Chapter 3* I present the results gathered from the study to show the migration of PACs from plastic to food contact substance (FCS) under various environmental

conditions. Finally, *Chapter 4* provides the conclusion of the study as well as suggestions on further directions for this project.

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Chapter 2. Methodology

The aim of this chapter is to describe the sample preparation and analysis used in my research.

An overview of the workflow I used is shown in Figure 2.

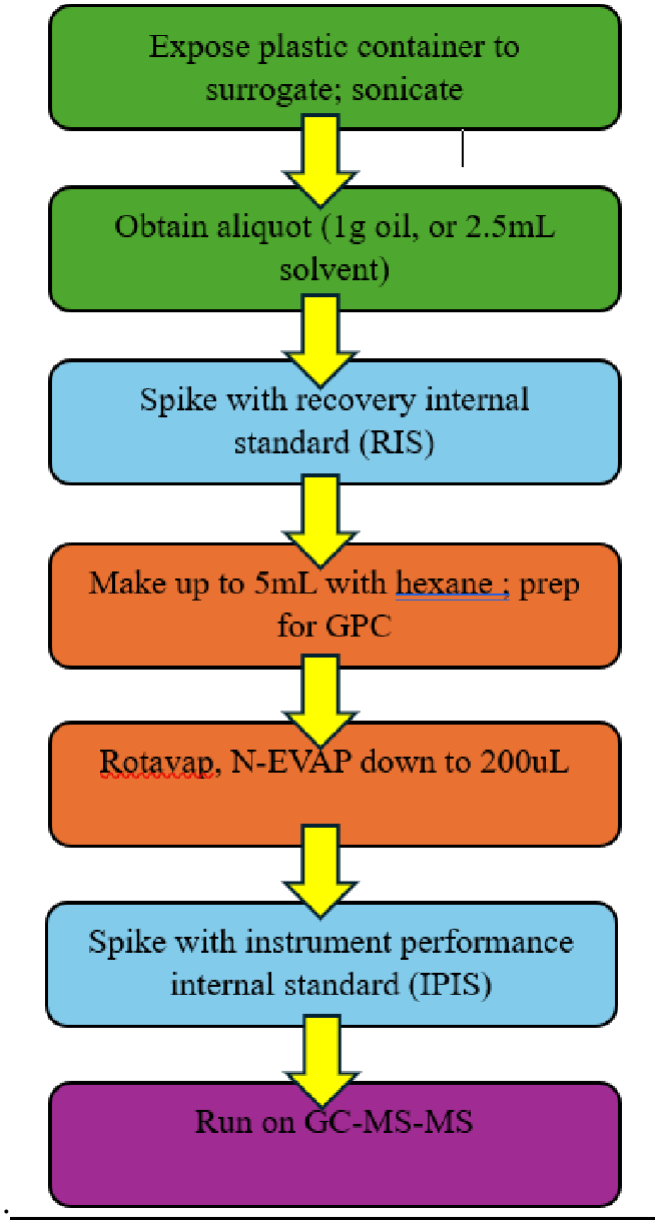

Figure 2 – Workflow of my Project

2.0 Sampling & Sample Preparation

While there is no official consensus on terms, generally sample preparation is associated with the chemical modification of a sample such as dissolving samples in solvent, extracting analytes from a matrix, separation of interferences from analytes, and reacting analytes with a reagent to transform the analyte into measurable signals on the instrument of choice[36].

Sample preparation is the most critical step in any analysis. This includes sample collection, isolation of target analytes, detection of targets, quantification and data interpretation. Typically, this step is regarded as the most time-consuming sequence in any analytical method with some estimates stating that this takes up about two-thirds of total analysis time[37]. This could possibly be due to the manual and laborious nature of this process and so some consideration must be taken to ensure successful analysis[36,38,39]. When determining the best approach to sample preparation, one must consider the nature of analytes, matrix and final separation method [38]. Sample preparation is a necessary function for the instrumentation used to detect the analyte[40].

2.1 Analyte Extraction

Common analytical extraction methods include: liquid- liquid extraction (LLE), solid-phase extraction (SPE), and solid-liquid extraction (SLE). In my project, I used both LLE and a modified form of SPE.

Every extraction method involves forcing the mass-transfer of analyte(s) from the sample matrix into a different phase more suited for the instrument used for detection. A broad overview of LLE can be described as exposing a liquid sample containing the analyte(s) to a solvent in which the analyte is soluble in. The migration of analytes can be encouraged into the liquid phase by agitation (e.g., sonication). The process of LLE can be summarized in three steps: the penetration of solvent in the liquid matrix, the diffusion of analytes to the outer surface of the liquid, and finally the solubility of analytes in the solvent[41]. For SPE, which typically uses a column to support the solid adsorbent, I used an in situ dispersive SPE method to purify my samples. The principle of this approach is that interferences will be preferentially adsorbed onto the dispersant leaving the analytes of interest in the solution.

2.2 Quality Control

Balance and syringes were calibrated daily prior to use. All glassware was rinsed with water, acetone and hexane before being baked at 250˚C overnight prior to use. All samples (1.00 g oil, 2.50 mL solvent) were fortified with a known amount of recovery internal standard (RIS) to account for any losses incurred during sample processing. Mass labelled d_{10} anthracene was added to sample extracts prior to GC-MS/MS analyses to account for any fluctuations in the performance of the system. For my studies, five procedural blanks were used at each time-point.

I performed a control spike study to examine any re-adsorption onto the plastic under realistic conditions (oil). Here, 20 ng of PAHs were spiked into the FCS (i.e., solvent and olive oil) and

sampled at days 0,5, and 10. Sample collection and processing were carried out to the same nature as my blanks and samples.

2.3 Chemicals and Reagents

High purity (Optima grade) organic solvents were purchased from Fisher Scientific (Ottawa, Ontario, Canada). Eleven (11) individual APAHs that include 1,7- dimethylphenanthrene, 1,8-dimethylphenanthrene, 1-methylnaphthalene, 1-methylphenanthrene, 2,6-dimethylphenanthrene, 2-methylnaphthalene, 2-methylphenanthrene, 3,6-dimethylphenanthrene, 3-methylphenanthrene, 9/4-methylphenanthrene, and retene were all purchased from Accustandard Inc. (New Haven, Cincinnati, USA) and Caledon laboratory Chemicals (Georgetown, Onatrio, Canada). Cold-pressed extra virgin olive oil was acquired from Loblaws (Brampton, Ontario). Sixteen (16) unsubstituted PAHs as a native mix and deuterium mass labelled d_{10}-anthracene was also purchased from Accustandard Inc. All standards were of >98% purity. The APAH and PAH standards were prepared in-house in hexane in varying concentrations. Labelled internal standards used for this project include d_8-acenaphthylene, d_9-acenaphthene, d_{12}-benz(a)anthracene, d_{12}-benzo(a)pyrene, d_{12}-benzo(b)fluoranthene, d_{12}-benzo(ghi)perylene, d_{12}benzo(k)fluoranthene, d_{12}-chrysene, d_{14}-dibenz(ah)anthracene, d_{10}-fluoranthene, d_{10}-fluorene, d_{12}-indeno(123-cd)pyrene, d_8-naphthalene, d_{10}-phenanthrene, and d_{10}-pyrene. Labelled anthracene (d_{10}-anthracene) was used as an instrument performance internal standard (IPIS). Size-exclusion SX-3 Biobeads used for GPC sample cleanup was purchased from Bio-Rad Laboratories (Mississauga, Ontario, Canada). Finally, materials used for dSPE include silica gel (923 grade, 100-200 mesh), alumina (60-325 mesh), and anhydrous sodium sulphate dispersant were all purchased from Fisher Scientific (Ottawa, ON, Canada).

2.4 Exposure Design

An assortment of plastic containers consisting of HDPE, LDPE, PET, and PP were all purchased from Amazon Canada. Under the direction of a guidance document by the US-FDA[42], a total of 10 containers per plastic type was assigned to two FCS: olive oil to mimic fatty foods and a mixture of 50/50 DCM and hexane (n=5). Each container was filled and maintained to three-quarters of its total volume with the assigned FCS. The containers were exposed to the appropriate FCS for a period of 10 days as per US-FDA guidelines to maximize exposure rates. The containers were sonicated for 3 hours per day of exposure (Fisher Scientific, Model FS220D) in a 40˚C water bath. At days 0, 2, 4, 6, 8, and 10, either 2.5mL of solvent or 1g of oil was withdrawn from the sample containers. Control samples consisted of FCS in glass containers (n=3 at each time point) held under identical conditions to the plastic samples. Samples and procedural blanks were stored in the fridge (~7˚C) prior to processing.

2.5 Gel Permeation Chromatography

Gel permeation chromatography (GPC) has been employed in the field since the 1960s[43] and uses cross-linked polydextran gels of various pore sizes as the stationary phase[44] . Over time, the development of other packing materials such as polyacrylamide agarose and silica derivatized with glycidylpropylsilane[45] has emerged. This type of chromatography can be used for a variety of sample types including biological samples[46,47], sediments[48] and polymers[49]. The premise of GPC is that molecules are separated based on their size due to ability to act as a type of sieve[45,50,51].

The basic operating principle of GPC is that under a steady flow of solvent, there is a preferential penetration of smaller analytes into the packing material of the column and analytes smaller in size have a relatively shorter distance to the end of the column than larger ones. Molecules that are larger in size, are unable to penetrate the pores of the packing material, consequently, these are the compounds that typically elute first out of the column. The smaller analytes diffuse into the gel material of the column where they eventually migrate through the column and elute with later retention times (Figure 3)[50] .

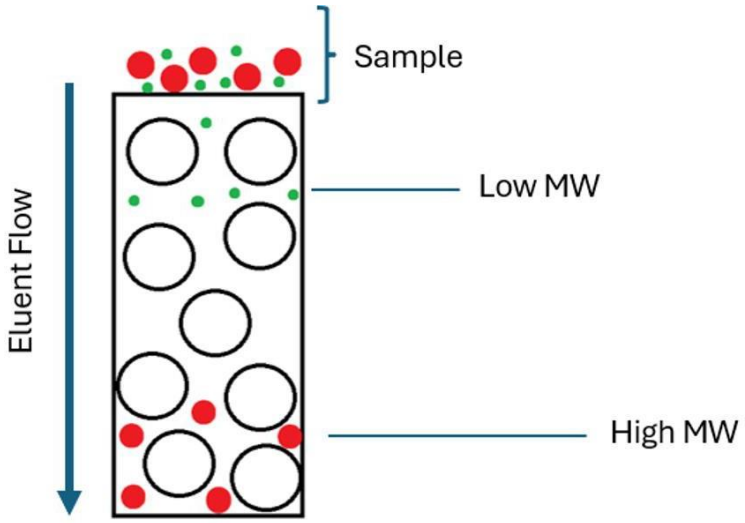

Figure 3 – Diagram Showing General Operation of GPC

For my project there was a need for a clean-up method to separate the analyte of interest from lipid and extraneous solvent during sample collection to minimize damage to the liners of the GC column. As such, a J2 scientific PrepLinc GPC was utilized for sample clean up. The column was packed with 60g of SX-3 Biobeads that was made into a slurry when combined with hexane:DCM (50:50, v/v). This slurry was transferred into the column to form a long bed. The column was then

allowed to settle overnight prior to the application of moveable plungers to create the pressure needed to pack the column.

Figure 4 – J2 Scientific PrepLinc GPC

Due to the nature of the long bed of the column, a maximum of 1g of lipid was allowed to be introduced onto the column making this an ideal situation for the samples used in my study. Samples were eluted from the column using a 50:50 mixture of hexane: DCM (v/v) as a mobile phase with a set pressure and flow rate of 6psi and 5mL/min, respectively. In this system, the first 140mL was discarded while the subsequent lipid-free 120mL that eluted from the column was collected in a 250mL round-bottom flask that was rinsed and baked at 250˚C overnight prior to collection.

2.6 Dispersive Solid Phase Extraction (dSPE)

Dispersive solid phase extraction (dSPE) is a sample preparation technique first proposed in 2003 by Anastassiades et al[52]. This simplified SPE method allows for multiple samples to be analyzed at one time, is rapid, and uses minimal solvent[53]. It is employed in my project in which the sample in an organic phase is combined with a mixture of salt and a solid phase sorbent such as silica, C18, or other functional particle that is tailored to extract the analyte of interest[52]. It is important to choose an organic solvent to maximize isolation of analytes as the polarity of the analytes must also be considered when choosing an organic solvent[52]. In addition, salts also play an important role as they are often used to remove water from the system which forces analytes to remain in the non-polar organic phase[52] . Finally, the role of the dispersive sorbent is important as one must chose a sorbent as it to retain the components of the matrix while not retaining the analytes and allowing them to remain in the organic solvent[52]. Common sorbents include amines, carbon black, C_{18}, and alumina. Subsequently, the dispersive mixture undergoes a manual process such as vortex or handshaking. This promotes separation of the sorbent from the sample matrix so all that remains is a purified, more concentrated analyte of interest[52].

For my project, my extracts required further processing by dSPE. This involved transferring the plastic leachate from the GPC to a 125mL round bottom flask with multiple 20mL rinses of DCM and hexane (70:30 v/v). The round bottom flasks contained silica (8g), anhydrous sodium sulphate (2g) and 5% deactivated alumina (1g). The flasks were allowed to sit for approximately 30 minutes with periodic swirling to allow maximal sorbent material interaction with the sample. The extracts were then transferred to 60mL ASE vials by glass wool funnel to separate sorbent from the analyte of interest. Afterwards, the extract was transferred to 125mL round-bottom flasks with 3 hexane

rinses. The contents of the round-bottom flasks were concentrated to approximately 1mL by way of rotary evaporation (Heidolph North America, Wood Dale, Illinois, USA). Concentrated extracts were then transferred to glass vials with 3 hexane rinses to be concentrated down to a final volume of 200µL under a stream of nitrogen. The samples were then ready for analysis and quantification by GC-MS/MS.

2.7 Gas Chromatography

Gas chromatography (GC) has significantly improved and evolved since it was first developed by Martin and James 1952 to provide improved resolution and sensitivity towards the analysis of a variety of mixtures[54]. All chromatography methods operate on the same purpose of transporting a sample through the length of an analytical column. The GC method operates on the premise of partition chromatography where analytes in a sample are partitioned between a stationary and mobile phase[55]. In more modern uses, GC in principle vapourizes a sample through heat and injected into the head of the chromatographic column by way of a carrier gas, typically helium (inert gas) or nitrogen (non-reactive gas). Like many other chromatographic techniques, factors such as sample volatility, sample polarity, column temperature, column packing polarity, and column length can influence the separation of a sample into its various components[56]. In addition, this instrument is highly efficient and has good separation efficiency, they are also easily interfaced to a mass spectrometer. GCs are also valued for their sensitivity, high accuracy for quantitative analysis, requiring small sample sizes (µL), relative simplicity, and the fact that they are also inexpensive compared to other analytical instrumentation[57].

My project used an Agilent 7890B gas chromatograph (Agilent Technologies) coupled with a 7000C triple quadrupole mass spectrometer that was fitted with an electron ionization (EI) source for the MS/MS acquisition. The analytes of interest were injected on a 30m Agilent J&W DB-5ms Ultra Inert column (30 m × 0.25 mm × 0.25 µm). As a carrier gas, helium was used at a constant flow rate of 1.2mL/min. As part of the injection process, 1µL of sample extract was injected in a spitless injector maintained at 250˚C. The GC oven temperature program was set to as follows: from an initial temperature of 60˚C held for 1 min, it was then increased to 210˚C at 35 ˚C/min, then increased to 260˚C at 2˚C/min, ramped up to 300 ˚C at 10 ˚C/min where it was held for 5 min, and a final increase at 50 ˚C/min to 325 ˚C where it was held for 5.5 min. In addition, other set parameters on the GC includes the transfer line maintained at 320 ˚C, gas saver at 20mL/min at 3 min, split flow at 50 mL/min and septum purge at 5mL/min. Nitrogen was used as the collision gas at a pressure of 60 psi.

2.8 Mass Spectrometry

Mass spectrometry can be used for a variety of purposes like structural analysis for organic compounds, or when coupled with a GC, is primarily used for quantitative analysis. Due to their relative low cost, GC-MS is an ideal setup for research purposes. Simply, MS operates on the principle of ionization, separation, and detection of analyte ions based on their mass-to-charge ratio (m/z)[58]. Since its inception there have been several technologies developed that has resulted in a multitude of mass spectrometers that are capable of ionization and ion analysis. Several of these technologies have been reported for optimal usage in the analysis of petroleum products and environmental pollution monitoring in general [59–61].

2.8.1 Ionization Techniques

Ionization is the first step where neutral analytes are converted into gas phase ions. Previously this has been done where the sample is first volatilized then ionized, but this would limit this type of analysis to those of low molecular weight that are thermally stable[62]. Over the course of the last several decades, there has been significant development in ionization technology to be more inclusive of larger, more complex molecules that are more representative of organic pollutants in the environment. Developments include matrix-assisted laser desorption- ionization (MALDI) [63], Electrospray Ionization (ESI)[64], Atmospheric Pressure Chemical Ionization (APCI)[64], and electron ionization (EI); each having the capacity to ionize molecules of various sizes and relative hardness or softness of the ionization phase[58]. Electron ionization (EI) was used in this research project and will be further detailed in the subsequent section.

2.8.1.1 Electron Ionization

This method of hard ionization results in fragmentation of analyte molecular ions[58]; has been in use since mass spectrometry has been in use in the early 20th century[58]. In EI, electrons that are emitted from a hot filament interact with incoming neutral analyte molecules where a portion of these molecules become ionized.

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

By way of a repeller plate at the ion source maintained at a high voltage, ions are repelled out of the ion source and toward the analyzer tube. [58,65] It is here that potential energy is converted into kinetic energy that can be described by:

$$eV = \frac{1}{2}mv^2$$

Typically, this will yield 70eV, however, only 10eV is needed to ionize most organic compounds. Any excess energy that remains results in extensive fragmentation that often leads to providing further information on the structural composition.

2.8.2 Mass Analyzers

The quadrupole mass analyzer has been popular since the 1970s for GC-MS and liquid chromatography mass spectrometry (LC-MS) driven by its low cost and ease of operation. Here, mass separation occurs because of the ion motion in dynamic electric field rather than a kinetic energy being directly imposed on the ions. A quadrupole is comprised of 4 metal rods (Figure 5) that allow for ions to move between them.

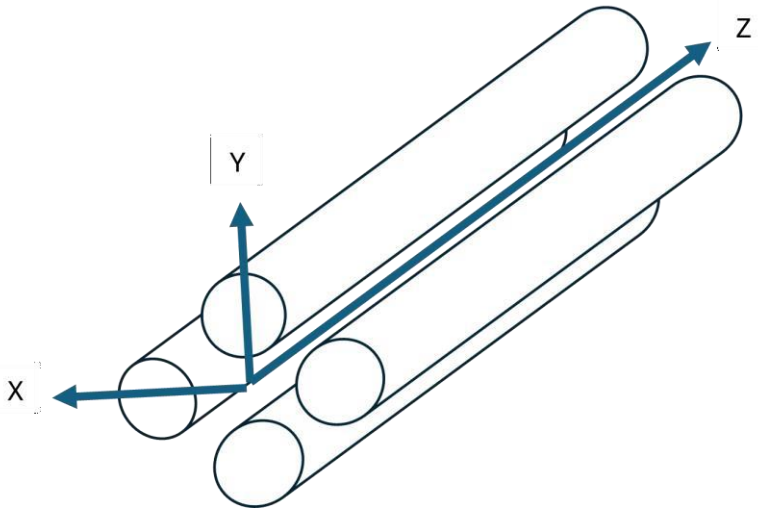

Figure 5 – Rod Orientation in a MS Quadrupole

Each rod is comprised of a direct current (DC) and alternating current (AC) that creates an electric field. The motion of an incoming ion can be described by Mathieu functions:

$$\frac{d^2x}{d\tau^2} + (a_x + 2q_x cos2\tau)x = 0$$

Equation 2 – Equation of Motion in the x Direction

$$\frac{d^2y}{d\tau^2} + (a_y + 2q_y cos2\tau)y = 0$$

Equation 3 – Equation of Motion in the y Direction

Where the parameters of a and q can be obtained by:

$$a_x = -a_y = \frac{4qU}{m_i {r_0}^2 \omega^2}$$

Equation 4 – Definition of 'a' Parameter in Equation 4 and 5

$$q_x = -q_y = \frac{2qV}{m_i {r_0}^2 \omega^2}$$

Equation 5 – Definition of 'q' Parameter in Equation 4 and 5

$$\tau = \frac{\omega t}{2}$$

Equation 6 – Definition of τ in Equation 4 and 5

Optimizing the U (DC voltage), V (radiofrequency voltage), and ω (frequency) parameters of the equations above, will result in a stable trajectory for certain m/z values to reach the detector[58,62,66]. The m/z range of quadrupoles is between 300 to 4000 m/z[67].

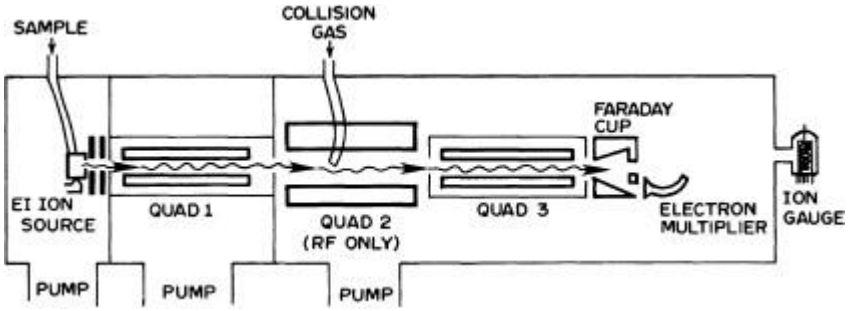

Figure 6 – Basic Layout of a Triple Quadrupole MS

Despite the name, triple quadrupoles (QqQ), it has only 2 true quadrupole mass analyzers in Q1 and Q3, while Q2 is simply a collision cell (see Figure 6). Here ions of interest are selected in Q1 before being transferred to the collision cell (q2). In the collision cell, they interact with the neutral atoms argon (or other suitable collision gas such as helium or nitrogen) to form what are known as product ions. The product ions are then transferred, in part due to the fact only a radio frequency (rf) field is present in Q2 allowing seamless transfer into Q3 without separation. It is in Q3 that a mass spectrum of the ions is derived from the selected precursor ion in what is known to be the product ion scanning mode in this type of mass analyzer[58,66–68].

In practice, Q1 is typically set to filter for a specific mass (precursor ions) as they enter from the ion source. Once transferred to Q2, the ions interact with neutral atoms of argon (or other suitable gas such as helium or nitrogen) to form product ions. From here they are transferred, in part due to the rf only field present in the collision cell, which allows for seamless transfer from Q2 to Q3

without any separation occurring. Finally, in Q3, a final mass filter present filters for a specific fragment(s). This arrangement is typical of the scan method known as multiple reaction monitoring (MRM). This scanning method is typically valued for its ability to be highly specific when quantitating multiple precursor ions.

2.8.3 MS Conditions for Analysis

Prior to establishing MRM conditions, a full-scan EI mass spectra (m/z 50-600) of each analyte was required. This process entails determining the most abundant precursor ion which was then singled out and fragmented in the collision cell of the MS. The collision energies for the acquisition of maxima product ions can be found in Table 4 below along with the MRM precursor to product ion transition. This is a condensed version to that originally published by Idowu et al[69]. Conditions for this scan are like that described in Chapter 2.6.2.

Table 4 - MS/MS Ion Transitions, RT Windows and CE for Selected PACs.

Target Analyte	Quantitation/Confirmation ion transitions	RT Window (min)	CE (eV)
Naphthalene	127.9/102.1; 127.8/77.0	4.00-6.80	40
Naphthalene d8	136.0/108.0; 136.0/134.0	4.00-6.80	35
Acenaphthylene	151.9/150.0; 151.9/151.0	6.80-7.00	45
Acenaphthylene-d8	160.0/158.1; 160.0/156.1	6.80-7.00	40
Acenaphthene	152.9/152, 152.9/151.0	7.00-7.88	35
Acenaphthene-d10	162.0/160.2; 162.0/158.1	7.00-7.88	35
Fluorene	164.9/163, 164.9/115.0	7.88-9.40	40
Fluorene-d11	176.0/174.2; 176.0/172.1	7.88-9.40	40
Anthracene	177.9/176.1; 177.9/151.0	9.40-11.72	40
Anthracene-d10	188.0/160.1; 188.0/184.1	9.40-11.72	40
Fluoranthene	201.9/200.1; 201.9/201.1	11.72-12.20	40
Fluoranthene-d10	212.0/208.2; 212.0/210.1	11.72-12.20	40
Pyrene	201.9/200.1; 201.9/201.1	12.20-12.58	45

Pyrene-d10	212.0/208.1; 212.0/210.2	12.20-12.58	45
Chrysene	227.9/226.1; 227.9/202.1	16.30-21.30	40
Chrysene-d12	240.0/236.2; 240.0/212.2	16.30-21.30	40
Benzo(b)fluoranthene	251.9/250.1; 251.9/226.1	21.30-23.00	35
Benzo(b)fluoranthene -d12	264.0/260.2; 264.0/236.1	21.30-23.00	45
Benzo(a)pyrene	251.9/250.1; 251.9/226.1	23.00-29.50	40
Benzo(a)pyrene-d12	264.0/260.2; 264.0/236.2	23.00-29.50	45
Indeno(1,2,3-c,d)pyrene	275.9/275.9; 274.1/274.1*	29.50-31.00	15
Indeno(1,2,3-c,d)pyrene-d12	286.2/286.2; 288.0/288.0*	29.50-31.00	15
Dibenz(a,h)anthracene	277.9/277.9**; 276.1/276.1*	29.50-31.00	15
Dibenz(a,h)anthracene-d14	292.0/292.0**; 288.2/288.2*	29.50-31.00	15
Benzo(ghi)perylene	275.9/275.9**; 275.1/275.1*	31.00-32.00	15
Benzo(ghi)perylene-d12	288.0/288.0**; 286.2/286.2*	31.00-32.00	15
2-Methylnaphthalene	141.8/141.1	5.40-5.63	15
1-Methylnaphthalene	141.8/141.1	5.77-5.83	15
3-Methylphenanthrene	191.8/191.1	10.46-10.62	15

2-Methylphenanthrene	191.8/191.1	10.51-10.70	15
9/4- Methylphenanthrene	191.8/191.1	10.58-10.89	15
1-Methylphenanthrene	191.8/191.1	10.72-10.89	15
3,6-Dimethylphenanthrene	205.8/191.1	11.41-11.59	15
2,6-Dimethylphenanthrene	205.8/191.1	11.49-11.69	15
1,7-Dimethylphenanthrene	205.8/191.1	11.70-11.88	15
1,8-Dimethylphenanthrene	205.8/191.1	12.00-12.11	15
Retene	219.0/204.1	13.51-13.62	15

*Pseudo-MRM confirmation ion transitions , **Pseudo-MRM quantitation ion transitions

2.9 Method Validation

The parameters to validate our method were done so according to Eurachem Guidelines for analytical methods[70]. Method characteristics that were assessed include accuracy, limits of detection (LOD), limits of quantitation (LOQ), linear dynamic range, precision, repeatability, and selectivity and were determined to have fell within the acceptable range[70]. GC-MS/MS data acquisition and processing was conducted using GCQQQR Software (Agilent) and Quantitative Analysis 12.1(Agilent) respectively.

2.10 Statistical Analysis

Microsoft Office Excel 2016© was used to calculate parameters such as standard deviation (SD) and relative standard deviation (RSD). Statistical analysis that includes the measurement of goodness of fit in regression models (R^2) and Student's t-test were modeled using SigmaPlot 15.0 (Systat Software Inc. San Jose, CA, USA); for the purpose of the Student's t-test, a $p \leq 0.05$ was considered to be statistically significant result representing a real relationship between dependent and independent variables. Analysis of Variance (ANOVA) testing was also conducted to determine if there was any significance among replicates. Again, where $p \leq 0.05$ was statistically significant result, representing a real relationship between dependent and independent variables.

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Chapter 3. Results and Discussion

Before I present my results, I would like to first describe the sources of my experimental uncertainty, bias, and the steps I took to mitigate them. After, I would like to discuss how I used my procedural blanks to ascribe positive detects of my analytes. Finally, I will discuss my experimental findings.

3.0 Sources of Experimental Uncertainty and Bias

Despite utilizing the experimental approach outlines by the US-FDA testing guidelines there always remains the possibility of analyte loss because of the analytical protocol. To monitor this, each extract (1g oil, 2.5mL solvent) was intentionally spiked with 15 isotopically labelled recovery internal standards which was then carried throughout the analytical method.

The results of the recovery for the procedural blanks are shown in Table 5. Overall, the bias of the RIS ranged from 81.72% to 91.36% and my precision ranged from 3.76 to 14.59 %. Similar recoveries for my internal standards were observed in my real samples suggesting that losses due to sample processing were minimal and repeatable.

Fluctuations in the instrument performance between injections may also lead to inaccurate results. To account for this, mass labelled anthracene was added as our instrumental performance internal standard (IPIS) to the extracts prior to injection on the GC-MS/MS. The variability of the IPIS in

the extracts was less than 20% and can be interpreted as being a small contribution on the overall experimental uncertainty (Table A1 in appendix).

I also assessed the potential for PACs in solution to re-adsorb onto plastic. The amount of mass labeled PAH measured in oil on day 0, 5 and 10 expressed as a percent is shown in Figure A1. While there were a few instances in which the amount of mass labeled PAHs were statistically different between time points (Student t-test, $p<0.05$), these differences were small and implies that re-adsorption of analytes onto plastic is not a major mechanism of loss. Furthermore, this indicates that the analytes prefer to remain in solution and thus will not reach equilibrium over the duration of my migration experiments.

Finally, it is unreasonable to assume that there is homogenous distribution of my analytes in each of my plastic replicates. In fact, Kuzmicz and Ciemniak (2017) noted that PAHs in plastic packaging materials can vary between manufacturers and between production batched from the same manufacturer[71]. Unfortunately, there is no way to account for this variability and it is likely this is the source of the variance seen in the frequency of detection (f_D) in my analyte replicate measurements and some of the high uncertainty values (i.e., standard error of the mean) in my replicates. If PACs were evenly distributed throughout the plastics I used in my studies, I would expect the amounts of PACs in both media to increase continuously with time according to Fick's law of diffusion until reaching equilibrium.

As highlighted earlier in this thesis, the focus of this study was to develop the foundational framework for more in depth study in the future in to the migration of PACs. While providing this framework to highlight the serious impacts of PAC migration, this study also calls attention to the limitations of the experimental design. Given that plastics are not all made equally, we cannot assume that each of the plastics I studied contain the same number of PACs. Despite my best efforts to draw out meaningful trends, such as observing the role of steric hinderance; the fact that there is significant variability between individual plastic containers could not be ignored. As a result, the interpretation of my results are restrictive in nature, but aims to provide a high level contextual summary of the migration of PACs out of the plastics themselves.

Table 5 - Arithmetic Mean Amounts (pg) and Standard Deviation (σ) of Replicate Measurements of Polycyclic Aromatic Hydrocarbons in Procedural Blanks (n=5). Recoveries are the Arithmetic Means ± Standard Deviation of Corresponding Mass-Labeled Internal Standard in all Samples.

Compound	**Mass (pg)**	**Mean + $3\sigma^1$ (pg)**	**Recovery (%)**
Acenapthene	121.77±21.39	224.87	86.72 ±12.13
Acenapthylene	30.54±3.40	56.88	85.26 ±11.91
Benz[a]anthracene	38.39±7.81	71.60	85.65 ±14.59
Benzo[a]pyrene	32.45±5.31	61.04	84.49 ±9.73
Benzo[b]fluoranthene	34.54±6.84	68.67	87.44 ±6.26
Benzo[g,h,i]perylene	135.50±39.46	252.19	81.72 ±6.67
Benzo[k]fluoranthene	39.95±7.50	73.35	84.80 ±3.76

Chrysene	42.03±5.59	76.83	84.12 ±8.86
Dibenzo[a,h]anthracene	45.57±11.21	84.69	89.60 ±5.65
Fluoranthene	35.39±6.10	67.59	86.95 ±8.32
Fluorene	246.58±47.08	442.41	87.83 ±8.23
Indeno[1,2,3-c,d]pyrene	97.22±25.36	173.09	86.36±6.86
Naphthalene	720.62±132.46	1383.62	91.36 ±9.81
Phenanthrene	111.16±19.82	210.83	84.04 ±10.91
Pyrene	66.83±11.64	133.58	85.84 ±9.27

[1] If our analytes in samples were smaller than their respective mean+3σ amounts then it was considered a non-detect (n.d)

3.1 Treatment of Procedural Blanks

Procedural blanks were used in all my migration studies. In total there were 5 blanks (n=5) for each FCS (solvent, oil) at each prescribed time point. Respective amounts of each analyte detected in FCSs are presented in Table 6 along with the mean ± 3 times the standard deviation. Positive identification of my analytes in test samples was determined if the mean value of 5 replicate sample values were greater than the mean ± 3 SD determined in the blanks. Naturally, this led to more conservative detection frequencies, but we felt this approach to be intuitively satisfying.

3.2 Results of Solvent Study

The rationale for using a strongly non-polar solvent as an FCS, is that it would maximize the number and amounts of analytes detected. In addition to the selection criteria described above, I also chose to report a positive detection only if the f_D value exceeded 60%.

Overall, I was able to detect the greatest number of analytes in HDPE (23) and LDPE (19). Only 9 of my analytes were detected in PET while only 1 analyte was measurable in PP (Table 6 to Table 12). Lower MW compounds (acenaphthene, acenaphthylene, anthracene, fluoranthene, fluorene, naphthalene, phenanthrene) were detected in higher amounts compared to their higher weighted counterparts. For the polyethylene-based plastics, there a preference for PAHs.

The following sections will discuss the chemicals I detected in solvent and their respective amounts in each of the four plastics studied.

3.2.1.1 Migration of Polycyclic Aromatic Compounds from HDPE

Fourteen of the sixteen PAHs I screened for were detected in at least one time point with 5 PAHs consistently detected at all sampling points (see Table 6). The amounts of PACs that migrated from HDPE ranged from 49 pg to 19 ng. Based on the data presented below, compounds with fewer rings were detected more consistently with high f_D values of at least 60%. In addition, low molecular weighted (LMW, < 400 g/mol) compounds were detected more at higher amounts and more consistently than high molecular weighted (HMW, ≥400g/mol) compounds.

Table 6 - Arithmetic Mean ± Standard Error and frequency of detection of the 16 PAHs measured in solvent incubated with HDPE Plastic.

Compound	Time					
	0	**2**	**4**	**6**	**8**	**10**
Acenaphthene	2899.18±61.24 80	6217.02±1423.79 60	3069.92±521.84 100	3847.16±2076.68 100	n.d	n.d
Acenaphthylene	363.53±43.66 80	871.15±244.43 60	1249.34±426.52 100	1333.87±450.86 100	3419.16±595.18 100	1901.68±770.43 100
Anthracene	n.d	256.98 ± 67.76 60	481.33±195.81 100	524.37±133.74 100	1307.33±236.75 100	797.26±339.16 100
Benz[a] anthracene	n.d	n.d	n.d	n.d	212.75±63.90 80	177.35±71.18 60
Benzo[a] pyrene	n.d	n.d	n.d	n.d	140.66 ±8.71 60	n.d
Benzo[b] fluoranthene	n.d	n.d	n.d	n.d	246.95±108.39 100	177.36±78.97 60

Benzo[g,h,i] perylene	n.d	n.d	n.d	n.d	n.d	n.d
Benzo[k] fluoranthene	n.d	n.d	n.d	n.d	229.10±83.92 60	100.46±46.47 60
Chrysene	n.d	n.d	n.d	298.50±242.67 60	965.53±630.01 100	919.90±297.32 60
Dibenzo[a,h] anthracene	n.d	49.02±14.70 60	n.d	n.d	142.20±7.42 60	147.87±27.07 80
Fluoranthene	127.51±19.22 80	892.94±341.38 60	2687.96±1820.75 100	2060.58±558.62 100	3940.00±892.09 100	2735.77±909.17 100
Fluorene	753.85±69.54 80	961.41±156.41 60	3058.05±1198.70 100	3168.19±1152.44 100	3377.21±286.78 100	1751.38±219.79 100
Indeno[1,2,3-c,d]pyrene	n.d	n.d	n.d	n.d	n.d	n.d
Naphthalene	n.d	n.d	1279.87±254.63 60	2789.38±676.55 60	9104.43±3396.94 100	6308.62±1935.70 100

Phenanthrene	1294.82±283.27 100	3750.09±758.88 60	7049.86±2659.92 100	6914.33±1607.02 100	19154.57±4555.6 100	12339.94±5107.48 100
Pyrene	126.73±19.44 60	1357.96±623.56 60	5300.88±3319.21 100	4402.99±1334.48 100	4417.51±703.73 100	3095.75±487.44 100

As shown in Table 7 all 11 APAHs that I screened for were detected at various time points, with over 50% of the compounds detected at all time points. The minimum f_D was no less than 80%. The amounts of APAHs detected ranged from 128 pg to 5624 ng.

Table 7 - Arithmetic Mean ± Standard Error and frequency of detection of the 11 APAHs measured in solvent incubated with HDPE Plastic.

Compound	Time				
	0	**2**	**4**	**6**	**8**
1,7-Dimethylphenanthrene	n.d	660.57±209.73 100	2170.43±1789.14 100	1341.90±495.12 100	761.92±342.40 100
1,8-Dimethylphenanthrene	n.d	n.d	n.d	n.d	191.25±57.38 100
1-Methylnaphthalene	302.43±58.86 80	850833.53±237586.10 100	5624565.82±1889071.00 100	1948726.07±229618.6 100	3405.06±1383.83 100
1-Methylphenanthrene	141.3±22.69 80	587.76±252.75 100	2465.75±1690.99 100	1338.38±240.05 100	1600.93±693.13 100

2,6- Dimethylphenanthrene	n.d	263.48±152.19 80	611.23±463.41 100	643.78±136.90 100	730.36±314.60 100
2-Methylnaphthalene	202.31±40.81 80	584796.43±164450.00 100	4195622.49±1363478.00 100	1589530.37±190432.60 100	2542.68±1020.03 100
2-Methylphenanthrene	300.11±40.80 80	803.13±253.00 100	2861.46±1674.04 100	1765.84±207.79 100	1762.57±756.07 100
3,6- Dimethylphenanthrene	n.d	238.40±110.90 100	931.72±709.55 100	541.73±142.19 100	943.45±412.48 100
3-Methylphenanthrene	225.58±33.16 80	687.35±192.68 100	2600.55±1435.50 100	1679.20±151.69 100	1813.46±773.98 100
9/4- Methylphenanthrene	128.74±23.09 80	651.85±305.14 100	3014.58±2137.91 100	1633.89±310.86 100	1281.60±593.50 100

Retene	n.d	n.d	n.d	203.16±16.35 80	770.52±256.07 100

3.2.1.2 Migration of Polycyclic Aromatic Compounds from LDPE

Ten (10) of the sixteen (16) screened PAHs were detected at varying time points in this study (see Table 8). The amounts the migrated out of the LDPE plastic ranged from 52 pg to 9 ng. Overall, there was less PAH migration from LDPE relative to HDPE; which may be attributed to the difference in branching between the polyethylene-based plastics I studied. Like HDPE, small PAHs were generally favoured over larger PAHs than contained 4+ rings (i.e. anthracene being readily present in 5 of 6 sampling points while benzo(b)fluoranthene was not detected).

Table 8 - Arithmetic Mean ± Standard Error and frequency of detection of the 10 PAHs measured in solvent incubated with LDPE Plastic.

Compound	Time					
	0	**2**	**4**	**6**	**8**	**10**
Acenaphthene	n.d	860.00±135.92 100	979.13±107.94 100	1340.73±451.48 80	1256.14±233.87 60	758.85±162.16 60
Acenaphthylene	n.d	1084.42±103.57 100	1123.09±87.24 100	1497.96±387.21 80	1661.91±285.29 60	847.17±121.81 80
Anthracene	n.d	292.15±30.34 100	433.31±98.02 100	441.68±136.23 80	480.98±78.89 60	337.52±41.63 60
Benz[a]anthracene	n.d	62.97±7.66 60	n.d	n.d	n.d	52.41±0.94 60
Chrysene	n.d	74.28±7.97 80	n.d	n.d	n.d	n.d
Fluoranthene	n.d	1291.25±260.44 100	1254.23±123.41 100	1817.46±422.74 80	1739.50±262.24 60	1347.45±144.23 80

Fluorene	n.d	2667.89±70.92 100	5183.16±249.65 100	6826.73±1917.80 80	6572.56±510.87 60	4594.17±1320.21 80
Naphthalene	n.d	1441.11±59.08 60	n.d	2025.91±489.05 60	3016.63±199.81 60	n.d
Phenanthrene	n.d	6363.76±497.04 100	7121.83±748.18 80	8819.23±2348.55 80	8057.48±974.05 60	7617.17±855.47 80
Pyrene	n.d	1878.55±298.75 100	2749.45±287.09 100	4147.65±814.98 80	4006.12±474.27 60	3084.44±448.79 80

As shown in Table 9, all 11 APAHs that I screened for were detected at various time points. Interestingly, all compounds were detected with high f_D at all 5 time points. Given the overwhelming presence of APAHs, there seems to be an affinity for this type of PAC in the FCS. The minimum f_D here was no less than 60%. The amounts of APAHs detected ranged from 3 ng to 38 ng.

Table 9 - Arithmetic Mean ± Standard Error and frequency of detection of the 11 APAHs measured in solvent incubated with LDPE Plastic.

Compound	Time				
	0	**2**	**4**	**6**	**8**
1,7-Dimethylphenanthrene	3689.34±182.69 100	3574.10±410.31 100	3397.29±402.02 100	3555.64±405.70 80	3201.16±296.79 60
1,8-Dimethylphenanthrene	4416.82±253.66 100	4674.97±790.21 100	3964.24±450.70 100	4472.92±790.51 80	4389.33±359.19 60
1-Methylnaphthalene	21787.03±1390.72 100	20346.47±663.02 100	20260.09±1254.28 100	21364.43±1940.32 80	23218.77±1369.32 60
1-Methylphenanthrene	20675.42±2232.63 100	19772.54±557.47 100	22716.93±1471.19 100	20519.18±870.11 80	19552.33±1252.44 60
2,6-Dimethylphenanthrene	6268.12±322.69 100	4622.11±637.10 100	4173.26±108.49 100	5076.89±316.72 80	4573.14±379.82 60
2-Methylnaphthalene	27933.72±1713.37 100	18737.57±961.17 100	19346.65±1846.65 100	19592.51±1574.80 80	19314.88±1810.65 60

2-Methylphenanthrene	23345.26±1028.19 100	18822.89±1244.87 100	20063.78±642.50 100	19394.20±912.15 80	16808.41±1735.66 60
3,6-Dimethylphenanthrene	7038.18±399.21 100	5174.18±782.03 100	4451.59±91.26 100	4887.12±210.62 80	4529.42±106.25 60
3-Methylphenanthrene	36460.17±1386.45 100	35732.39±2396.5 100	38236.59±1740.53 100	35169.49±1111.02 80	35465.54±4699.19 60
9/4-Methylphenanthrene	24852.87±1090.71 100	20055.53±1320.56 100	21371.87±681.57 100	20661.58±967.61 80	17918.56±3189.04 60
Retene	3440.56±392.81 100	6014.81±419.16 100	5843.22±417.08 100	6747.76±273.29 80	6863.52±494.29 60

3.2.1.3 Migration of Polycyclic Aromatic Compounds from PET

Four (4) of the sixteen (16) screened PAHs were detected at varying time points in this study (see Table 10). The amounts that migrated out of the PET plastic ranged from 64 pg to 6 ng. Based on the data presented here, there may be a preference for PACs to migration from polyethylene compounds given the lack of detection in the solvent for most of the PAHs (4 PAHs in PET compared to 10 PAHs detected in LDPE). However, this is not a definitive conclusion given the extreme variability seen between plastic batches that is documented by the variability in the f_D of individual PACs.

Table 10 - Arithmetic Mean ± Standard Error and frequency of detection of the 4 PAHs measured in solvent incubated with PET Plastic.

Compound	Time					
	0	**2**	**4**	**6**	**8**	**10**
Anthracene	5142.94±409.77 80	6627.79±1433.66 100	1247.10±231.66 100	887.67±162.81 80	n.d	2244.39±612.84 80
Benz[a]anthracene	n.d	n.d	424.78±76.20 100	n.d	n.d	n.d
Benzo[a]pyrene	64.73±14.27 60	n.d	n.d	n.d	n.d	n.d
Fluoranthene	107.05±11.23 60	210.87±91.10 60	n.d	n.d	n.d	n.d

As shown in Table 11, only 5 of the 11 APAHs that I screened for were detected. Despite the infrequent detection, the replicates within each time point were detected with at least 60% frequency. The amounts detected ranged from 79 pg to 835 pg.

Table 11 - Arithmetic Mean ± Standard Error and frequency of detection of the 5 APAHs measured in solvent incubated with PET Plastic.

Compound	Time				
	0	**2**	**6**	**8**	**10**
1,7-Dimethylphenanthrene	n.d	150.17±29.10 60	n.d	n.d	n.d
1,8-Dimethylphenanthrene	n.d	134.86±7.74 60	n.d	n.d	n.d
1-Methylphenanthrene	823.64±132.68 80	586.63±176.51 100	n.d	231.08±63.90 60	707.17±28.02 80
2,6-Dimethylphenanthrene	835.91±93.02 80	603.35±94.05 100	n.d	n.d	333.03±43.00 80
9/4-Methylphenanthrene	n.d	79.20±6.84 60	n.d	n.d	n.d

3.2.1.4 Migration of Polycyclic Aromatic Compounds from PP

For PP, only one APAH and none of the PAHs were detected. The 3 time points where 2-methylphenanthrene was detected had an f_D of at least 60 percent. Amounts of 2-methylphenanthrene ranged from 149 pg to 654 pg. Again, the lack of data available makes it difficult to make definitive conclusions but clearly PP contains smaller amounts and number of PACs relative to the other plastic types.

Table 12 - Arithmetic Mean ± Standard Error and frequency of detection of the single APAHs measured in solvent incubated with PP Plastic.

Compound	Time					
	0	**2**	**4**	**6**	**8**	**10**
2-Methylphenanthrene	n.d	n.d	149.74 ±20.38 60	275.73 ±21.60 100	654.69 ±104.00 80	n.d

3.2.2 Results of Oil Study

3.2.2.1 Migration of Polycyclic Aromatic Compounds from HDPE

Twelve (12) PAHs were detected at varying time points in this study with 4 PAHs detected at all sampling points (see Table 13). The amounts the migrated out of the HDPE plastic ranged from 59 pg to 43 ng, which was greater than that found in solvent. It is interesting to note that there more PAHs in oil relative to solvent, in terms of frequency (in many instances, the PAHs that were

detected were detected at nearly every sampling point), particularly with LMW PAHs (benz[a]anthracene, benzo[k]fluoranthene etc.). Many PAHs were not detected at day 8 of sampling, which was not the case in the solvent. Of the PAHs found at day 8, there is a variety of PAHs detected (acenaphthylene, benzo[a]pyrene, fluorene and pyrene) with varying number of rings and MW. Based on my findings, it is reasonable to suggest that PAHs with more stacked rings are more susceptible to migration. In the case of benzo[a]pyrene and dibenzo[a,h]anthracene, this trend is apparent as the former was detected in the oil while the latter was undetectable. While comparing the average migration amounts of anthracene and phenanthrene, there is a notable difference between the two with the latter having nearly 27 times greater presence per sampling point over the former.

Table 13 - Arithmetic Mean ± Standard Error and frequency of detection of the 12 PAHs measured in oil incubated with HDPE Plastic.

Compound	Time				
	0	**2**	**4**	**6**	**8**
Acenaphthene	n.d	1226.57 ±138.68 100	n.d	n.d	n.d
Acenaphthylene	3585.36±397.16 100	4317.22±576.95 100	3511.37±162.68	3970.06±510.48 100	499.02±154.91 80
Anthracene	1066.30±131.99 100	1198.91±219.78 100	1013.84±93.83 100	986.80±112.57 100	n.d
Benz[a]anthracene	248.53±63.13 100	182.10±55.43 100	157.84±32.28 100	43579.47±26453.03 100	n.d
Benzo[a]pyrene	168.10±34.32 100	114.31±24.50 100	119.38±20.94 100	112.0±21.93 100	59.17±7.21 60
Benzo[b]fluoranthene	890.16±19.41 100	157.05±29.10 100	163.19±20.77 60	100.93±22.15 100	n.d

Benzo[k]fluoranthene	149.61±44.57 100	122.29±55.53 60	165.55±52.94 100	107.99±27.03 100	n.d
Chrysene	1040.62±184.20 100	746.60±236.18 100	754.01±229.46 100	667.58±133.34 100	n.d
Fluoranthene	3009.81±645.33 100	2827.17±656.88 100	2636.43±296.69 100	2553.72±568.48 100	n.d
Fluorene	2335.78±276.46 100	2761.54±494.31 100	2166.94±207.51 100	2526.94±316.55 100	660.14±129.11 100
Phenanthrene	20797.08±2808.97 100	20473.61±3516.31 100	18098.13±1219.44 100	18595.53±2435.85 100	n.d
Pyrene	2761.95±662.51 100	2584.95±740.56 100	2695.44±349.78 100	2836.98±622.49 100	2940.66±778.85 100

As shown in Table 14, 10 of 11 APAHs that I screened for were detected at various time points. The minimum f_D here was no less than 60%. The amounts of APAHs detected ranged from 176 pg to 380 ng, which was less than what was seen in solvent. In this plastic type, migration may be molecular weight-dependent, as fewer APAHs were detected here compared to the PAHs (Table 13). Here, only 9/4 methylphenanthrene and retene were detected. In addition, there may be a preference for substituted compounds over multi-substituted ones. One instance that can highlight this trend is 1-methylphenanthrene where it is present in oil at almost six times the amount of 1,8-dimethylphenanthrene.

Table 14 - Arithmetic Mean ± Standard Error and frequency of detection of the 10 APAHs measured in oil incubated with HDPE plastic.

Compound	Time				
	0	**2**	**4**	**6**	**8**
1,8-Dimethylphenanthrene	176.42±82.76 60	n.d	n.d	n.d	n.d
1-Methylnaphthalene	1619.11±777.58 60	n.d	n.d	n.d	n.d
1-Methylphenanthrene	1069.26±255.67 60	994.09±536.05 60	n.d	793.18±597.15 80	n.d
2,6-Dimethylphenanthrene	324271.69±323926.0 0 80	n.d	n.d	509.83±274.29 60	n.d
2-Methylnaphthalene	n.d	n.d	n.d	7735.41±4075.19	n.d
2-Methylphenanthrene	1600.66±411.16 60	1640.34±1174.64 60	n.d	1313.12±901.73 80	n.d

3,6-Dimethylphenanthrene	380073.73±379626.20 80	n.d	n.d	n.d	n.d
3-Methylphenanthrene	1072.59±195.48 60	n.d	n.d	860.30±414.68 80	n.d
9/4-Methylphenanthrene	1618.38±506.93 60	1408.71±570.57 60	1726.31±1403.93 60	2095.54±1219.57 80	n.d
Retene	n.d	416.83±51.09 100	536.04±30.08 100	609.68±44.16 100	365.75±87.01 100

3.2.2.2 Migration of Polycyclic Aromatic Compounds from LDPE

Two of the sixteen PAHs, namely acenaphthene and naphthalene, were readily detected at all sampling time-points with f_D of 100% (see Table 15). The total amounts of these two compounds ranged from 1180 pg to 7885 pg, which was smaller than the amount observed in solvent where 10 PAHs migrated into the FCS. Given that naphthalene was 5 times greater than acenaphthene suggests that migration favours LMW compounds. A similar observation was made for migration into solvent.

Table 15 - Arithmetic Mean ± Standard Error and frequency of detection of the 2 PAHs measured in oil incubated with LDPE Plastic.

Compound	Time				
	0	**2**	**4**	**6**	**8**
Acenaphthene	1252.30±41.49 100	1180.81±99.23 100	1573.09±195.77 100	1190.84±108.51 100	1380.75±172.18 100
Naphthalene	6041.69±924.41 100	6842.90±1493.87 100	7885.00±722.89 100	5291.82±660.23 100	6100.76±669.55 100

As shown in Table 16, only retene was readily detected in oil. Despite being the lone compound detected, the f_D remained high with 100% for days 2 through 8 with amounts ranging from 584 pg to 1168 pg with both the number of compounds detected as well as the range of amounts seen in the leachate being less than what was seen in solvent where all 11 compounds were being detected at nanogram amounts. Although, the lack of detection of APAHs, indicate that PAHs may be preferred as they typically have lower MWs, which as noted previously may affect migration. It is interesting to note that the single APAH detected here peaks at Day 4 (168.96 pg) at which point slight decreases in the leachate begin to occur until Day 8 (1100.37 pg).

Table 16 - Arithmetic Mean ± Standard Error and frequency of detection of the single APAHs measured in oil incubated with LDPE Plastic.

Compound	**Time**				
	0	**2**	**4**	**6**	**8**
Retene	583.98±276.23 60	1150.75±103.24 100	1168.96±85.52 100	1021.26±48.19 100	1100.37±37.18 100

3.2.2.3 Migration of Polycyclic Aromatic Compounds from PET

Eleven (11) of the sixteen (16) screened PAHs were detected at varying time points in this study with 5 PAHs detected at all sampling points. The amounts that migrated out of the PET plastic ranged from 95 pg to 33 ng with phenanthrene being the dominant PAH. There was also ca. 5 times greater amount of APAHs measured in oil than solvent. From the information presented in Table 17, I can infer some structural influences on migration. Most notably, linear PAHs tend to

exhibit more migration over compounds with identical MW but have a stacked ring configuration (e.g acenaphthene, not detected). In this scenario, I also noted that chrysene and pyrene exhibit a similar relationship. However, I noted that there was a difference in the number of sampling points where the PAHs were detected and their respective f_D. For pyrene, only 2 of the 4 sampling points had detectable amount of pyrene, whereas chrysene had 3 of the 4 points with detectable levels and 2 of the 3 detection points had an f_D of 100%.

Table 17 - Arithmetic Mean ± Standard Error and frequency of detection of the 11 PAHs measured in oil incubated with PET Plastic.

Compound	Time			
	0	**2**	**6**	**8**
Anthracene	960.30±180.98 100	1228.90±3633.01 100	19955.08±7460.99 100	1255.56±350.17 100
Benz[a]anthracene	234.51±26.69 60	n.d	n.d	n.d
Benzo[a]pyrene	150.78±70.36 80	119.88±12.26 80	133.23±46.29 60	123.13±40.33 60
Benzo[b]fluoranthene	n.d	172.12±49.16 60	n.d	n.d
Benzo[g,h,i]perylene	n.d	255.72±114.94 60	n.d	n.d
Chrysene	904.96±250.07 100	592.58±258.56 100	n.d	579.39±155.00 80

Dibenzo[a,h]anthracene	n.d	n.d	95.26±18.25 100	n.d
Fluoranthene	2562.16±486.76 100	2123.68±1311.899 80	1576.32±832.38 60	1999.55±638.39 80
Fluorene	744.73±376.55 100	1089.85±587.88 100	639.32±311.48 100	763.84±401.38 60
Phenanthrene	5536.64±2066.28 100	8237.43±4570.38 100	6884.47±2736.42 80	33048.72±1482.46 100
Pyrene	1372.68±439.01 80	n.d	n.d	790.74±250.79 80

As shown in Table 18, only retene was readily detected in oil. Despite being the lone compound detected, the f_D was at least 80% throughout with amounts ranging from 987 pg to 1769 pg. While more compounds were detected in solvent, the amount of retene that migrated here was more than any other compound that migrated in solvent. Furthermore, retene was not detected in solvent. Again, it is worth noting that I was not able to detect any APAHs in oil which may indicate that PAHs may be preferred as they typically have lower MWs, which as noted previously may affect migration. It is interesting to note that the single APAH detected here also peaks at Day 4 (1769.07 pg) at which point slight decreases in the oil occur on day 6 (987.37 pg) and day 8 (1570.83 pg).

Table 18 - Arithmetic Mean ± Standard Error and frequency of detection of the single APAHs measured in oil incubated with PET Plastic.

Compound	Time				
	0	**2**	**4**	**6**	**8**
Retene	n.d	1440.96±66.36 100	1769.07±98.22 100	987.37±224.74 80	1570.83±66.67 100

3.2.2.4 Migration of Polycyclic Aromatic Compounds in PP

Twelve (12) of the sixteen (16) screened PAHs were detected at varying time points in this study (see Table 19). The amounts the migrated out of the PP plastic ranged from 79 pg to 16 ng. Of the PAHs that were detected, it appears that there was a peak that occurred at day 4. For example, phenanthrene had a peak at day 4 of 16.76±4.14 ng which steadily decreased to 8.31±2.65 ng and 10.00±3.42 ng for day 6 and day 8, respectively. The infrequent detection of benz[a] anthracene (day 4, 199.48±23.6 pg), benzo[a] pyrene (day 10, 79.04±6.26 pg), and benzo[k]fluoranthene (day 4, 146.05±27.51 pg) further suggests that larger MW compounds with 5+ rings are not readily migrating into either FCS. Furthermore, the abundance of PAHs present in the oil FCS (12 detected PAHs) may indicate a preference for PP plastic in oil over a harsh solvent system where there were not any PAHs detected.

Table 19 - Arithmetic Mean ± Standard Error and frequency of detection of the 12 PAHs measured in oil incubated with PP Plastic.

Compound	Time					
	0	**2**	**4**	**6**	**8**	**10**
Acenaphthene	n.d	652.87±328.78 60	754.98±181.97 80	n.d	n.d	362.42±117.29 100
Acenaphthylene	600.60±267.47 60	2041.19±1327.37 60	2821.53±744.22 80	1660.74±492.11 80	n.d	1530.07±604.23 100
Anthracene	n.d	1031.13±499.34 60	1290.15±565.80 80	730.02±95.17 60	n.d	863.68±27.45 80
Benz[a]anthracene	n.d	n.d	199.48±23.55 60	n.d	n.d	n.d
Benzo[a]pyrene	n.d	n.d	n.d	n.d	n.d	79.04±6.26 80
Benzo[k]fluoranthene	n.d	n.d	146.05±27.51 60	n.d	n.d	n.d
Chrysene	n.d	859.91±394.67 60	958.45±231.72 80	496.04±168.90 80	n.d	640.95±226.75 80

Fluoranthene	n.d	2640.04±1597.15 60	3303.67±827.84 80	1638.91±481.41 100	n.d	1974.16±692.63 100
Fluorene	n.d	1317.55±649.12 60	1554.23±410.23 80	n.d	n.d	1220.12±99.48 60
Naphthalene	n.d	7780.33±2702.54 60	660.87±2155.62 80	n.d	n.d	n.d
Phenanthrene	n.d	13514.60±8058.14 60	16759.51±4140.73 80	8308.16±2654.11 80	n.d	9976.05±3444.42 100
Pyrene	709.25±189.20 60	2902.00±1390.05 60	3119.40±770.45 80	1375.05±414.20 100	n.d	2281.81±621.88 80

As shown in Table 20, 8 APAHs were detected out of the 11 I screened for with a range in amount of 411 pg to 2649 pg which was about 4 time more than what migrated for the lone compound (2-methlyphenanthrene) found in solvent. Among the 11 APAHs detected, none of the compounds were detected in all 6 sampling time points. However, f_D was at least 60% with many compounds having a f_D of 80% or more. Like previous plastics, there appears to be a dependence on MW for leaching to occur as denoted by the non-detect of 1,7-dimethylphenanthrene, 1,8-dimethylphenanthrene, and 3,6-dimethylphenanthrene. It was surprising to observe that APAHs in PP plastic had more of a presence in oil than any other plastic studied here with 7 of the APAHs in PP exhibiting at least 3 sampling points of detection compared to HDPE where just 4 APAHs had 3 points of detection. Finally, the positioning of an alkyl group may also influence migration. This is most notable between 1-methylnaphthalene (on average 658 pg was in the leachate over 10 days) and 2-methylnaphthalene (where 1108 pg was in the leachate over 10 days, on average).

Table 20 - Arithmetic Mean ± Standard Error and frequency of detection of the 8 APAHs measured in oil incubated with PP Plastic.

Compound	Time					
	0	**2**	**4**	**6**	**8**	**10**
1-Methylnaphthalene	n.d	1704.20±399.24 80	n.d	689.18±33.56 60	n.d	1552.00±112.86 60
1-Methylphenanthrene	913.55±488.56 60	1080.46±329.99 100	1045.35±38.90 60	633.89±197.20 80	n.d	777.89±266.31 100
2,6-Dimethylphenanthrene	n.d	411.42±81.39 80	n.d	n.d	n.d	581.34±75.33 60
2-Methylnaphthalene	n.d	2481.43±577.33 80	1726.58±485.01 60	n.d	n.d	2445.46±281.18 60
2-Methylphenanthrene	n.d	1272.07±424.44 100	1165.81±431.01 60	790.76±244.76 80	n.d	1183.68±308.47 80
3-Methylphenanthrene	820.32±387.87 60	1077.11±422.20 100	1096.81±383.08 60	680.82±249.51 100	n.d	1034.26±321.00 100

9/4-Methylphenanthrene	n.d	728.20±280.49 100	872.19±297.69 60	464.2±159.96 100	n.d	758.95±220.94 100
Retene	n.d	2649.45±232.71 80	2043.22±176.50 80	1877.12±187.13 100	2037.23±574.19 60	2236.57±137.59 100

3.4 Rates of migration of PACs from plastic

I assessed the rate of migration of PACs from each plastic in both media. Based on my spiking experiments, migration of PACs never reached equilibrium for either of the plastic-types. Plotting the mass of PACs detected vs time allowed me to examine the rate at which PACs migrated from each plastic. The lone criterion I used was that an analyte had to have a detection frequency of 60% at each time-point. Anthracene, acenaphthylene, fluoranthene and phenanthrene all showed linear migration from HDPE when DCM was used as the incubating medium. No other discerning trends were evident. For all four of these analytes, there were no statistical differences between the amounts detected on days 8 and 10 (ANOVA, $p>0.05$) and so I performed my linear regression analysis from day 0 to 8. The rank order for the rates of migration were phenanthrene (1944.19±560.19 pg/day), fluoranthene (439.6±101.01 pg/day), acenaphthylene (301.7±81.62 pg/day) and anthracene (144.1 ± 32.94 pg/day).

Studies have shown that polymer thickness can significantly influence the diffusion of additives throughout the polymer matrix[72–74]. The limited kinetic data can be partly attributed to this as plastic thickness will act as a barrier to migration and the depth at PACs are embedded within the polymer matrix remains uncertain.

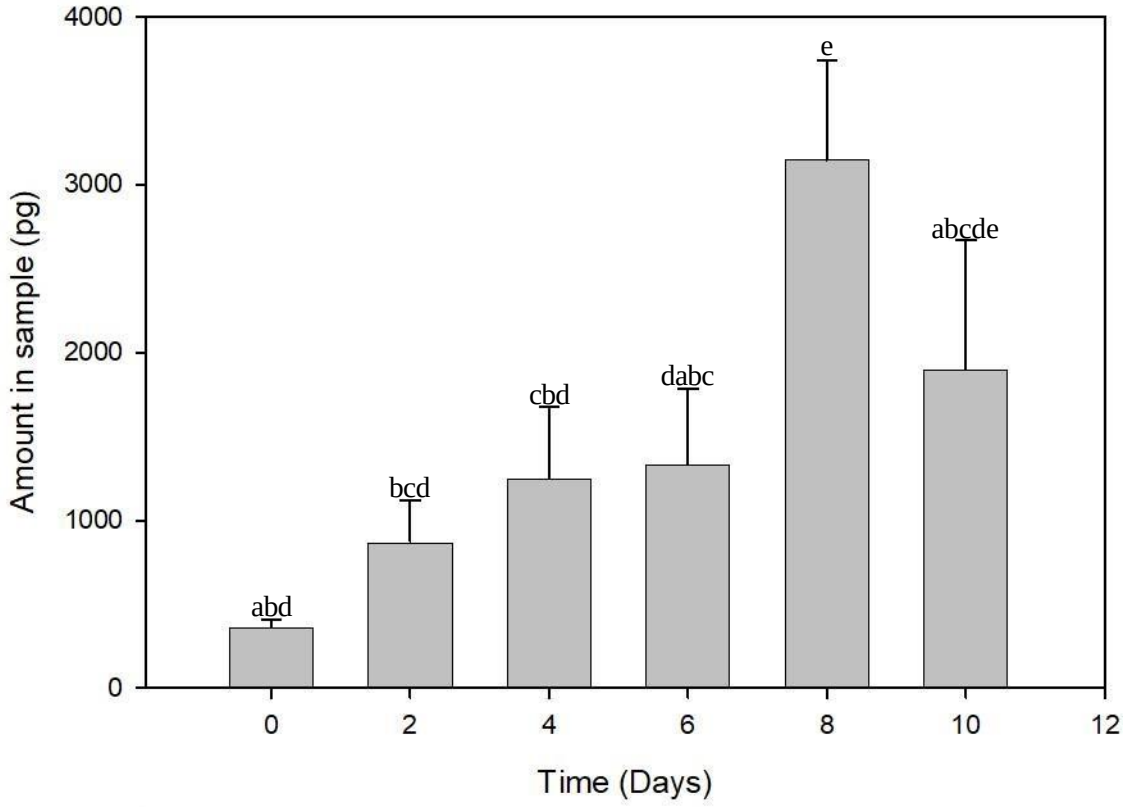

Figure 7 – Amount of acenaphthylene (pg, arithmetic mean ± standard error, n=5) migrating from HDPE into DCM. Similar letters imply no statistical difference (ANOVA, $p>0.05$) between the mean values.

Table 21 - Arithmetic Mean ± standard error at each time-point, the detection frequency (%) and rate of migration of acenaphthylene from HDPE.

Time point (Days)	Amount in leachate (pg)
0	363.53±43.66, 80
2	871.25±244.427, 60
4	1249.34±426.517, 100
6	1333.87±450.86, 100

8	3149.16±595.181, 100
10	1901.68±770.43, 100
Rate of migration (Days 0-8):	301.69±81.62 pg/day

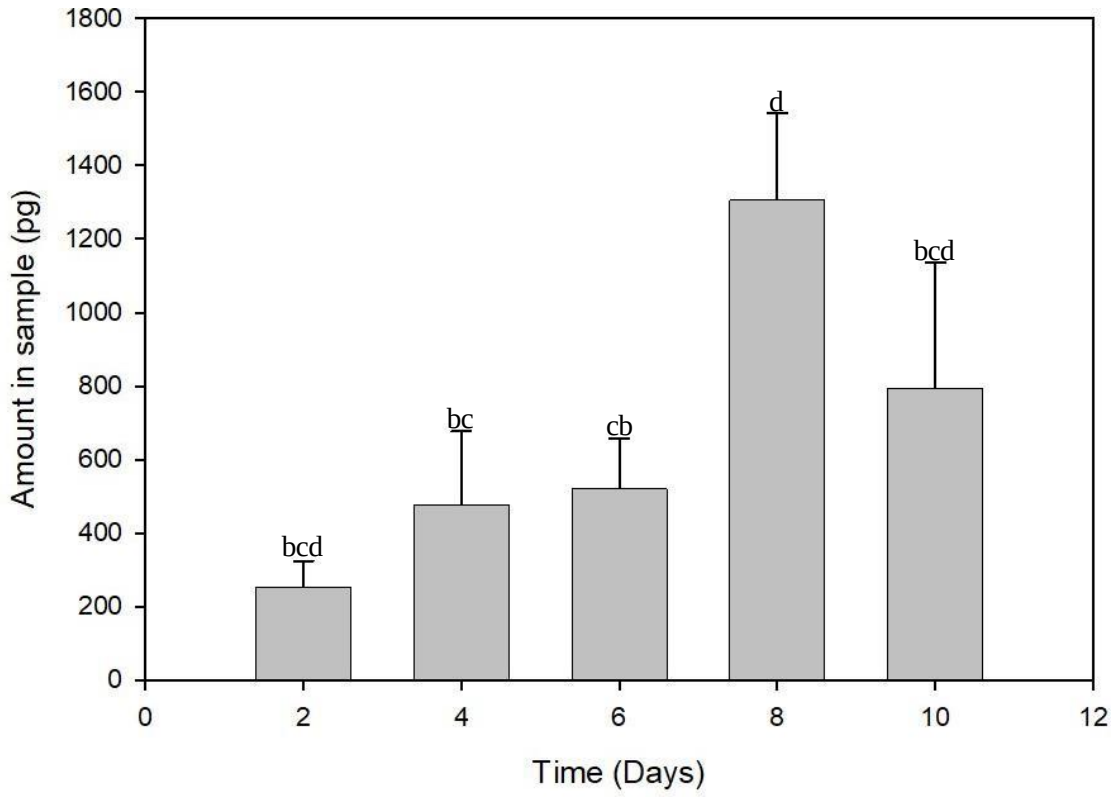

Figure 8 – Amount of anthracene (pg, arithmetic mean ± standard error, n=5) migrating from HDPE into DCM. Similar letters imply no statistical difference (ANOVA, $p>0.05$) between the mean values.

Table 22 - Arithmetic Mean ± standard error at each time-point, the detection frequency (%) and rate of migration of anthracene from HDPE.

Time point (Days)	Amount in leachate (pg)
0	n.d
2	256.98±67.76, 60
4	481.33±195.81, 100
6	524.37±133.74, 100
8	1307.33±236.75, 100
10	797.26±339.16, 100
Rate of migration (Days 0-8)	144.10±32.94 pg/day

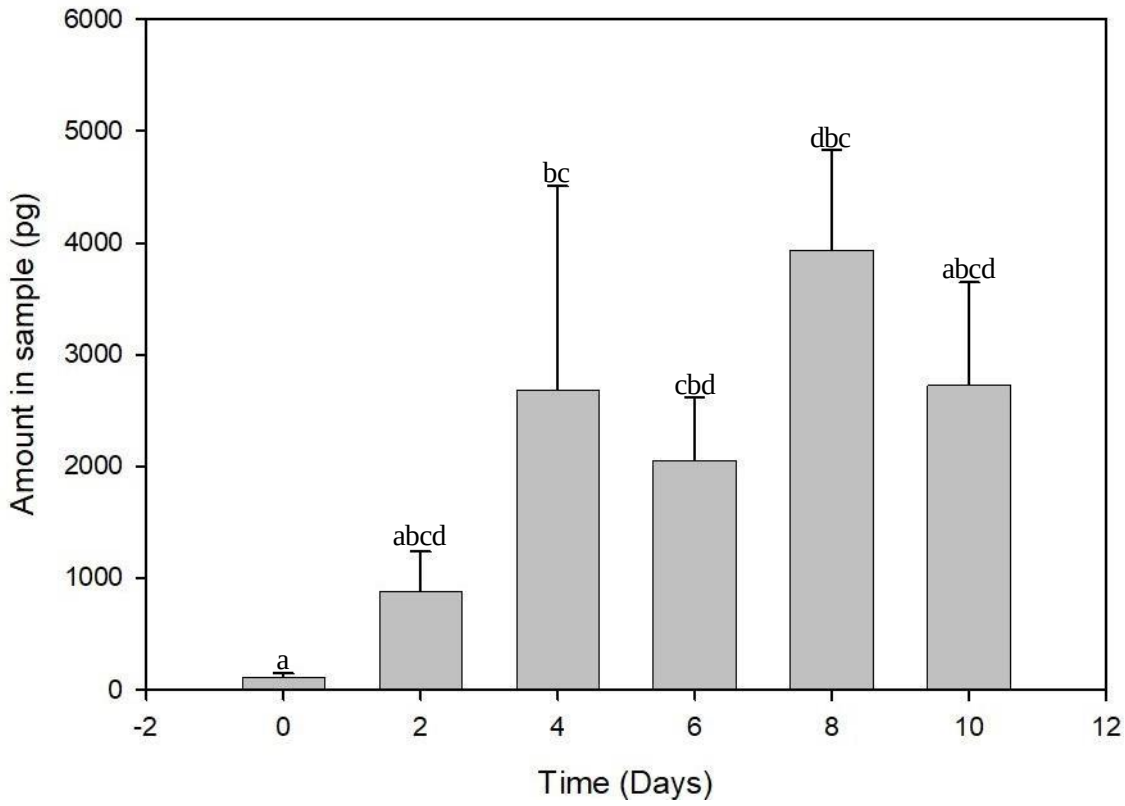

Figure 9 – Amount of fluoranthene (pg, arithmetic mean ± standard error, n=5) migrating from HDPE into DCM. Similar letters imply no statistical difference (ANOVA, $p>0.05$) between the mean values.

Table 23 - Arithmetic Mean ± standard error at each time-point, the detection frequency (%) and rate of migration of fluoranthene from HDPE.

Time point (Days)	Amount in leachate (pg)
0	127.51±19.22, 80
2	892.94±341.38, 60
4	2687.96±1820.75, 100
6	2060.58±558.62, 100

8	3940.00±892.09, 100
10	2735.77±909.17, 100
Rate of migration (Days 0-8)	439.63±101.01 pg/day

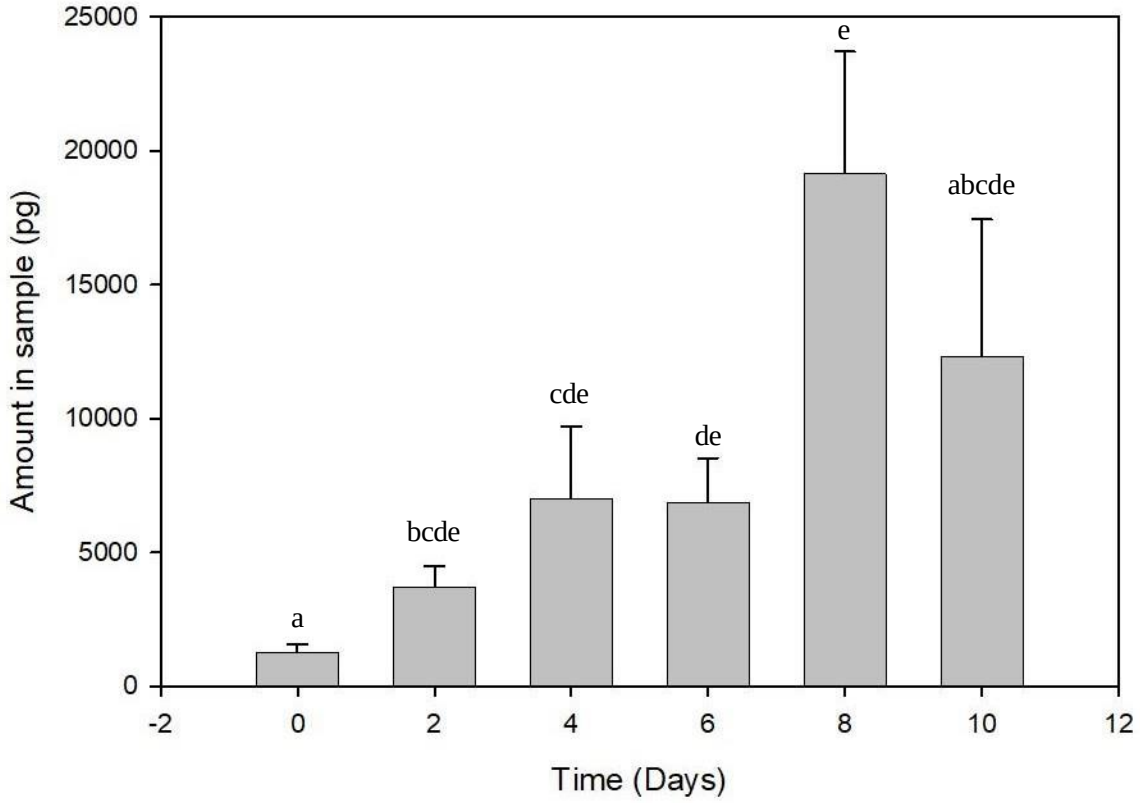

Figure 10 – Amount of phenanthrene (pg, arithmetic mean ± standard error, n=5) migrating from HDPE into DCM. Similar letters imply no statistical difference (ANOVA, $p>0.05$) between the mean values.

Table 24 - Arithmetic Mean ± standard error at each time-point, the detection frequency (%) and rate of migration of phenanthrene from HDPE.

Time point (Days)	Amount in leachate (pg)
0	1294.82±283.27, 100
2	3750.09±758.88, 60
4	7049.86±2659.92, 100
6	6914.33±1607.02, 100
8	19154.57±4555.6, 100
10	12339.94±5107.48, 100
Rate of migration (Days 0-8)	1944.19±560.19 pg/day

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Chapter 4. Conclusion and Future Research Direction

Despite their widespread presence in nature, PACs are known to negatively affect living organisms. In humans, PACs have been reported to disrupt the endocrine system and possess carcinogenic properties. My research, based on the recommendations of the U.S FDA, provides evidence that PACs can migrate from various commercial plastic food containers, many of which are labeled as "food grade." The variability I observed in detection frequencies suggest that there is batch-to-batch variability in the manufacturing process and/or that PACs are not uniformly distributed within the plastic.

I observed that HDPE had the most number of PACs migrate out of both FCSs with 23 PACs for solvent, 22 PACs for oil. Interestingly, 19 total PACs in LDPE were detected in solvent, while 20 PACs in PP were detected in oil making up the second most abundant plastics in both FCSs. A similar trend was observed when analyzing the total amount of PACs present in the leachate of all plastic types. This translated to the highest amounts of PACs in HDPE for both solvent and oil while LDPE and PP were the second most abundant in amounts for solvent and oil, respectively. Given the abundance of PACs in HDPE it is unsurprising that this plastic also was the only plastic that exhibited tangible migration rates. Unfortunately, there were no discernable trends (i.e trends based on ring size) that could be identified based on the information that I obtained.

Further research should explore how PAC migration is affect by exposure to microwaves with food grade plastics as this study only observed migration under room temperature with commercially available plastics from unknown manufacturing sources.

This study focused solely on virgin plastics and did not account for the impact of scratches and cracks seen with repeated use, which may influence PAC migration patterns by increasing the surface area. In my experiments, I explored migration at 40˚C and at elevated temperatures I would expect greater migration of PACs. Additionally, investigating migration in different FCS, particularly under acidic conditions, could be valuable, as pH is known to affect migration.

Bioplastics are becoming increasingly popular due to their natural origins and offer similar functionality to synthetic plastics[75]. The findings of this study may encourage further investigation and adoption of bioplastics as an alternative to traditional thermoplastics that currently saturate the market.

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Appendix.

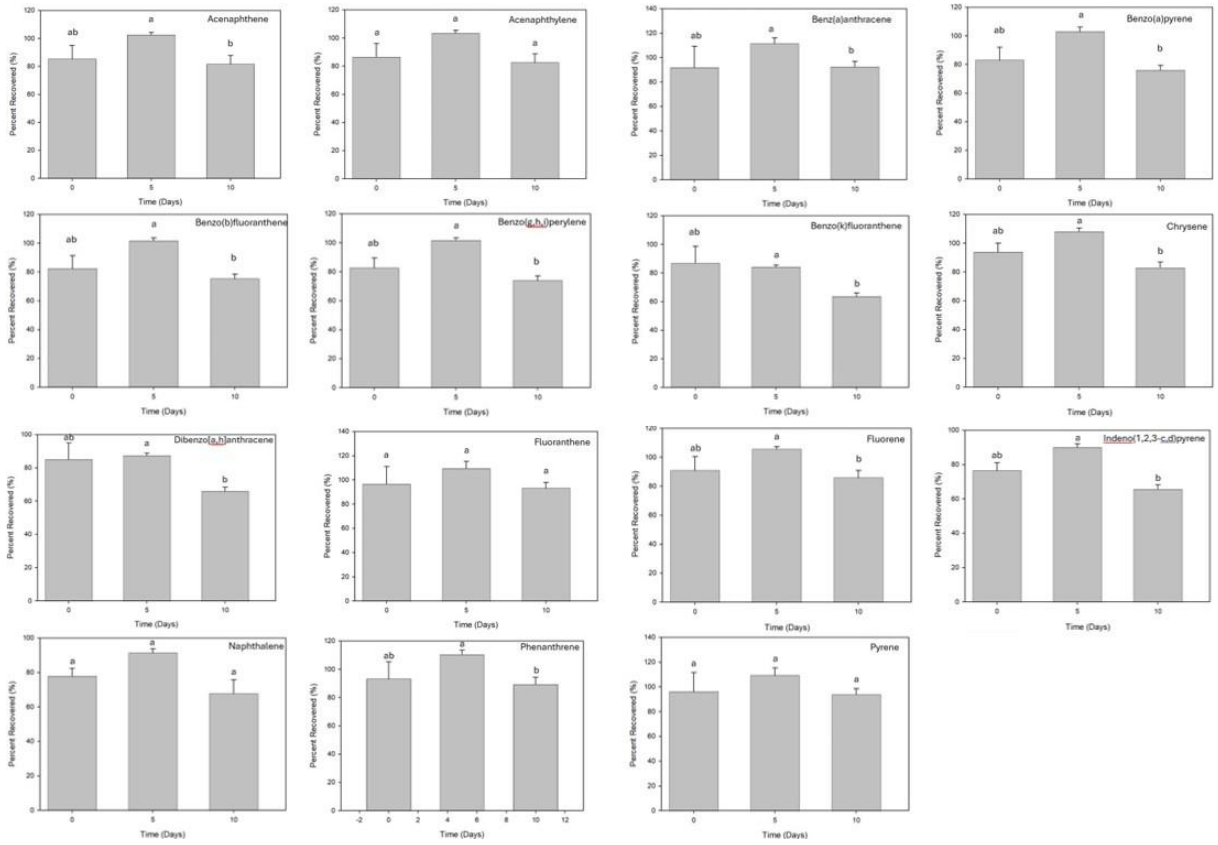

Figure A1 – Arithmetic mean ± standard error around the mean of mass labelled polycyclic aromatic hydrocarbons measured in oil on days 0,5 and 10. Where bars that share similar letters denote no statistical differences ($p>0.05$).

Table A1 - IPIS recoveries (%RSD) with extracts prior to injection containing isotopically labelled IPIS with the four plastic types studied (HDPE, LDPE, PET, PP). The cumulative average is displayed.

Plastic type				
Compound (IPIS)	**HDPE**	**LDPE**	**PET**	**PP**
Acenapthene (d)	13.65	17.57	17.47	17.33
Acenapthylene (d)	15.14	15.87	18.00	15.01
Benz[a]anthracene (d)	16.97	10.81	14.96	12.07
Benzo[a]pyrene (d)	11.86	16.98	19.35	17.85
Benzo[b]fluoranthene (d)	17.02	17.12	15.93	15.78
Benzo[g,h,i]perylene (d)	16.31	21.54	13.62	19.00
Benzo[k]fluoranthene (d)	17.70	19.08	13.69	16.43
Chrysene (d)	17.91	14.07	15.78	15.03
Dibenzo[a,h]anthracene (d)	13.45	19.66	12.20	13.61
Fluoranthene (d)	16.65	14.74	15.60	15.79
Fluorene (d)	15.99	16.59	15.21	16.40
Indeno[1,2,3-c,d]pyrene (d)	15.75	19.24	12.64	16.09
Naphthalene (d)	10.42	18.90	15.37	18.79
Phenanthrene (d)	12.11	14.15	16.22	16.28
Pyrene (d)	12.70	12.99	14.63	17.18
AVERAGE	**15.77**			

Table A2 - Average PAH migration value and corresponding standard error (pg) for each of the four plastic types studied over the course of the study period, in solvent[1,2].

Compound	HDPE	LDPE	PET
Acenaphthene	2672.21 680.59	865.81 181.9	n.d
Acenaphthylene	1478.12 421.85	1035.76 164.19	n.d
Anthracene	561.21 162.20	330.94 64.185	2691.64 475.12
Benz[a]anthracene	65.02 22.51	19.23 1.43	70.80 12.70
Benzo[a]pyrene	23.44 1.45	n.d	10.79 2.38
Benzo[b]fluoranthene	70.72 31.23	n.d	n.d
Benzo[g,h,i]perylene	n.d	n.d	n.d
Benzo[k]fluoranthene	54.93 21.73	n.d	n.d
Chrysene	363.99 195.00	12.38 1.33	n.d
Dibenzo[a,h]anthracene	56.515 8.20	n.d	n.d

Fluoranthene	2074.13 756.87	1241.65 202.18	52.99 17.06
Fluorene	2178.35 513.94	4307.42 678.24	n.d
Indeno[1,2,3-c,d]pyrene	n.d	n.d	n.d
Naphthalene	3247.05 1043.97	1080.61 124.66	n.d
Phenanthrene	8417.27 2495.36	6329.91 387.31	n.d
Pyrene	n.d	n.d	n.d

[1]Standard error is shown under average

[2] PAHs were not detected in PP

Table A3 - Average APAH migration value and corresponding standard error (pg) for each of the four plastic types studied ocer the course of the study period, in solvent[1].

Compound	HDPE	LDPE	PP	PP
1,7-Dimethylphenanthrene	986.96 567.28	3483.51 1702.51	30.03 5.82	n.d
1,8-Dimethylphenanthrene	38.25 11.48	4383.66 447.85	26.97 1.55	n.d
1-Methylnaphthalene	1685566.60 93729.48	21395.36 5124.694	n.d	n.d
1-Methylphenanthrene	1226.84 579.92	20647.28 1276.768	469.70 80.21	n.d

2,6-Dimethylphenanthrene	449.77 213.42	4942.70 352.97	354.46 46.01	n.d
2-Methylnaphthalene	1274538.90 343884.30	20984.94 1581.30	n.d	n.d
2-Methylphenanthrene	1498.62 586.34	19686.91 1112.67	n.d	180.03 24.33
3,6-Dimethylphenanthrene	531.06 275.02	5216.10 317.874	n.d	n.d
3-Methylphenanthrene	1401.23 517.40	36212.84 2266.738	n.d	n.d
9/4-Methylphenanthrene	1342.13 674.10	20972.08 1449.90	15.84 1.37	n.d
Retene	194.74 54.48	5781.97 399.33	n.d	n.d

[1]Standard error is shown under average

Table A4 - Average PAH migration value and corresponding standard error (pg) for each of the four plastic types studied over the course of the study period, in oil[1].

	HDPE	**LDPE**	**PET**	**PP**
Acenaphthene	2672.21 680.59	1315.56 123.44	n.d	295.05 104.67
Acenaphthylene	1478.12 421.85	n.d	n.d	1442.36 572.57

Anthracene	561.21 162.20	n.d	5849.96 2906.29	652.50 197.96
Benz[a]anthracene	65.02 22.51	n.d	58.6275 6.6725	33.25 3.93
Benzo[a]pyrene	23.44 1.45	n.d	131.755 42.31	13.17 1.04
Benzo[b]fluoranthene	70.72 31.23	n.d	43.03 49.16	n.d
Benzo[g,h,i]perylene	n.d	n.d	63.93 28.74	n.d
Benzo[k]fluoranthene	54.93 21.73	n.d	n.d	24.34 4.59
Chrysene	363.99 195.00	n.d	519.23 165.91	492.56 170.34
Dibenzo[a,h]anthracene	56.515 8.20	n.d	23.82 4.56	n.d
Fluoranthene	2074.13 756.87	n.d	2065.43 817.36	1592.80 599.84
Fluorene	2178.35 513.94	n.d	809.44 419.32	681.98 193.14
Indeno[1,2,3-c,d]pyrene	n.d	n.d	n.d	n.d
Naphthalene	3247.05 1043.97	6432.43 894.19	n.d	1406.87 809.69

Phenanthrene	8417.27 2495.36	n.d	13426.82 2713.89	8093.05 3049.57
Pyrene	n.d	n.d	540.86 172.45	1731.25 564.30

[1]Standard error is shown under average

Table A5 - Average APAH migration value and corresponding standard error (pg) for each of the four plastic types studies over the course of the study period, in oil[1].

	HDPE	**LDPE**	**PP**	**PP**
1,7-Dimethylphenanthrene	n.d	n.d	n.d	n.d
1,8-Dimethylphenanthrene	35.28 16.55	n.d	n.d	n.d
1-Methylnaphthalene	323.82 155.52	n.d	n.d	657.56 90.95
1-Methylphenanthrene	571.31 277.77	n.d	n.d	741.86 220.16
2,6-Dimethylphenanthrene	64956.30 64840.06	n.d	n.d	165.46 12.79
2-Methylnaphthalene	1547.08 815.04	n.d	n.d	1108.91 223.92
2-Methylphenanthrene	910.82 497.51	n.d	n.d	735.39 234.78

3,6-Dimethylphenanthrene	76014.75 75925.24	n.d	n.d	n.d
3-Methylphenanthrene	386.58 122.03	n.d	n.d	784.89 293.94
9/4-Methylphenanthrene	1369.79 740.20	n.d	n.d	470.6033 159.85
Retene	385.66 42.47	1005.06 110.07	1442.06 114.00	1807.25 218.02

[1]Standard error is shown under average