ABSTRACT

Title of Dissertation:	MECHANISMS OF SORPTION OF PHARMACEUTICAL AND PERSONAL CARE PRODUCTS TO MICROPLASTICS
	Alterra Sanchez, Doctor of Philosophy, 2021
Dissertation directed by:	Professor Michael Gonsior, Center for Environmental Science; and Associate Professor Lance Yonkos, Environmental Science and Technology

Microplastics (MPs) are found in almost every ecosystem and in many commercially important seafood species. MPs have been found to be physically harmful to marine organisms, but also may act as vectors for organic pollutants and together have been shown to cause toxic effects in a variety of species. Wastewater effluent is a significant source of MPs to aquatic systems, as well as pharmaceutical and personal care product compounds (PPCPs).

The first research contribution was to create a reference material for secondary fragment type microplastics of the polymer’s high-density polyethylene (HDPE) and polypropylene (PP) that is standardized by surface area (SA) for use in sorption and toxicity studies. Standardization for both polymers was successful, with a SA coefficient of variation of ~3%. PP MPs had greater SA due to it being a less crystalline polymer than HDPE. This reference material may act as a more realistic material than purchased

powders or beads, as well as provide a more readily comparable material for fragment type MPs.

The second research contribution used the reference material to create an extraction methodology for measuring semi-polar PPCPs associated with MPs. Acetone was chosen as the best solvent for extraction and extraction efficiency for both polymers was ~88% (first extraction). This methodology was then used to measure the equilibrium of the antimicrobial triclocarban with PP and PE of different MP particle sizes. Smaller particles with greater SA sorbed more triclocarban, rather than larger particles. PP had greater sorption of triclocarban than PE. This was due to PP MPs having greater SA due to being a less crystalline polymer.

The third contribution created a framework for prioritizing study compounds based on environmental relevance for MP sorption using polyethylene as a model polymer, water solubility, and a correlation with the octanol water-distribution ratio, D_{OW}, rather than the octanol-water partition coefficient, K_{OW}. A possible threshold for polyethylene sorption was found for compounds with water solubility ~20-60mg/L and D_{OW} ~3.7.

This dissertation advances the understanding of MP reference materials, extraction methodology, sorption mechanisms, and modeling of sorption data of PPCPs.

MECHANISMS OF SORPTION OF PHARMACEUTICAL AND PERSONAL CARE PRODUCTS TO MICROPLASTICS

by

Alterra Ellen Sanchez

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Advisory Committee:
- Associate Professor Michael Gonsior, Chair
- Associate Professor Lance Yonkos, Co-Chair
- Professor Andrew Baldwin
- Associate Professor Jeremy Testa
- Professor Neil Blough, Dean’s Representative

Dedication

I dedicate this dissertation to my husband, Kevin “Drake” Zielinsky, who has stayed by my side for the last 12 years and never left, even when I made the crazy decision to go to graduate school. Your love, care, commitment, and patience helped make this a reality.

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Table of Contents

DEDICATION.....II
ACKNOWLEDGEMENTS.....III
TABLE OF CONTENTS.....IV
CHAPTER 1: INTRODUCTION.....1
  Relevance.....1
  What is a Microplastic? Definition and their Lifecycle......3
  MPs in Wastewater Effluent and the Urban Environment.....6
  MPs and Sorbed Organic Pollutants.....8
  Sorption Mechanisms and Influencing Factors.....10
    Hydrophobic and electrostatic interactions, and the effects of pH.....10
    Surface Area.....13
    Crystallinity.....14
  Pharmaceutical and Personal Care Products.....16
    Wastewater as a Source of PPCP Laden MPs.....16
    Polar and Ionic PPCPs.....19
  Toxicological Effects of Ingested MPs and Sorbed Organic Pollutants.....22
  MPs as Vectors for Organic Pollutants: the Need for Reference Materials.....26
CHAPTER 2: FABRICATING A MICROPLASTIC REFERENCE MATERIAL THAT IS STANDARDIZED BY SURFACE AREA.....28
  Background and Rational.....28
  Methods.....32
    Fabrication of Microplastic Reference Material.....32
    MP Surface Area Analysis.....34
  Results and Discussion.....37
    MP Characterization: Scanning Electron Microscopy (SEM) Imaging and Surface Area.....37
    Limitations of the Reference Material.....43
  Conclusions and Future Directions.....45
CHAPTER 3: SORPTION OF THE ENDOCRINE DISRUPTOR TRICLOCARBAN TO MICROPLASTICS: EXTRACTION METHODOLOGY AND EFFECTS OF PARTICLE SIZE.....49
  Background and Rational.....49
  Methods.....52
    Chemical Reagents.....52
    Extraction of Triclocarban from MPs.....53
    Kinetic Experiment.....55
    Analysis of Triclocarban.....55
    Sorption Isotherms of Triclocarban.....56
    Modeling of TCC Sorption Isotherms.....57
  Results and Discussion.....57
    Extraction Method.....57
    Kinetic Experiment.....58
    Sorption Isotherms.....60

Conclusions and Future Directions 66
CHAPTER 4: PRIORITIZING STUDY COMPOUNDS BASED ON ENVIRONMENTAL RELEVANCE 67
Rational and Background 67
PPCP Sorption to MPs and the Octanol-Water Partition Coefficient, K_{OW} 67
The octanol-water distribution ratio, D_{OW} 71
Methods 75
Chemical Reagents 75
Sorption isotherms of venlafaxine, diphenhydramine, and DEET 76
Analysis of PPCPs 77
Modeling of Dow and K_{dPE} 77
Results and Discussion 80
Sorption of venlafaxine, diphenhydramine, and DEET 80
K_{OW} and Water Solubility 81
D_{OW} and sorption of PPCPs to PE MPs 85
Prioritizing Future Work on PPCPs and MPs 88
Conclusions and Future Directions 89
CHAPTER 5: DISSERTATION CONCLUSIONS 91
APPENDICES 100
Appendix 1 100
Appendix 2 101
Fabricating Microplastics 101
SEM Images of MPs of All Size Classes 103
Appendix 3 112
Select BET Plots Using Nitrogen Adsorptive Gas 112
Select BET Plots Using Krypton Adsorptive Gas 114
Instrument Parameters for Surface Area Analysis with Krypton 116
Appendix 4 117
Triclocarban experiments 117
Venlafaxine, DEET, and diphenhydramine experiments 119
UHPLC-MS/MS settings 122
Appendix 5 125
BIBLIOGRAPHY 126
Chapter 1 126
Chapter 2 134
Chapter 3 138
Chapter 4 142
Chapter 5 146

This Table of Contents is automatically generated by MS Word, linked to the Heading formats used within the Chapter text.

Chapter 1: Introduction

Relevance

It was estimated that 8 million metric tons of plastic debris entered the ocean in 2010, and that estimate is expected to grow by an order of magnitude by 2025 (Jambeck et al., 2015). Plastic debris has deleterious effects on over 250 species of marine life via ingestion and entanglement, including about 50% of marine mammals and birds, and are found globally in almost every ecosystem compartment (Appendix 1; Gregory, 2009; GESAMP, 2015; IVL SWEDEN, 2016). Microplastics have been found in many commercially important seafood species, especially lower trophic level organisms (Rochman et al., 2015; Walkinshaw et al., 2020). Along with being physically harmful, plastics may act as vectors for organic pollutants (Rochman, 2016; Ma et al., 2020; Du et al., 2020). Plastics loaded with organic pollutants have been shown to cause liver toxicity, decreased growth (Rochman et al., 2013) and altered liver metabolism (Qu et al., 2018) in fish, as well as reduced growth rate in algae (Zhu et al., 2019). A growing body of literature has now deemed small plastic fragments most commonly defined as having a diameter of 5 mm or smaller, known as microplastics (MPs), as the subject of most concern when it comes to marine debris (GESAMP, 2015; IVL SWEDEN, 2016; Rochman, Cook, and Koelmans, 2016; Lambert and Wagner, 2016).

The planetary boundary concept was created to define limits within which humanity could survive without significantly disrupting the biosphere (Rockström, et al., 2009; Jahnke, et al., 2017) and MPs are currently discussed in the context of microplastics and sorbed pollutants. The planetary boundary framework has recently been applied to the potential for chemical contaminants to have unknown, but potentially catastrophic effects (Rockström, et al., 2009; Jahnke, et al., 2017). Currently, chemical contaminants are responsible for over half of the planetary boundaries (e.g., ozone, carbon dioxide) originally set by Rockström et al. (Jahnke, et al., 2017). Persson, et al., 2013 proposed that chemical contaminants are responsible for far more planetary boundaries, quoting "we are just unaware of them" (Jahnke, et al., 2017). Persson, et al. stated that there are three conditions that all must be met for a contaminant to be a planetary boundary threat: 1) the pollution must have an unknown disruptive effect on important biosphere processes, 2) the negative effect must not be discovered until it is a global issue, and 3) the effect must be poorly reversible (Jahnke, et al., 2017). Plastic debris is known to meet conditions two and three, thus, marine MPs would fulfill all conditions to be a planetary boundary threat if it also would meet condition one: that it is currently causing an unknown disruptive effect on biosphere processes (Jahnke, et al., 2017). Though plastics may cause harm to individual organisms, there is very little evidence for cascading effects caused by microplastics (Jungblut, et al., 2020); however, with the large quantity of plastics estimated to be floating on the ocean surface (~210,000 tons), and probably an order of magnitude more in sediments and the deep sea, it is imperative that we not make assumptions and continue to rigorously study this issue (Jahnke, et al., 2017; Appendix 1). Indeed, whether MPs are a significant source of organic pollutants to organisms in the environment and humans is still under debate (Rochman et al., 2013; Du et al., 2020; Campanale et al., 2020; Ma et al., 2020). The specific surface properties of plastics as vectors of different organic pollutants remain an active field of research (Li et al., 2018; Razanajatovo et al., 2018; Liu et al., 2019; Wu et al., 2019; Wu et al., 2020; Elizalde-Velázquez et al., 2020) and is the topic of this dissertation.

What is a Microplastic? Definition and their Lifecycle.

Before discussing MPs, plastic debris must first be defined. There has been much contention over the definition in the last few years, and the debates are still ongoing, Hartmann et al. 2019 has suggested four criterion that can be used to determine if litter is plastic debris: 1) the object consists of synthetic or heavily modified natural polymers as the essential or main ingredient, 2) has no intended function in the natural environment, and 3) & 4), is solid and insoluble at 20 °C. Under this definition cotton strings from clothing and cellophane from gift wrapping would not be considered plastic debris. However, many scientists consider bits of cellophane to be MPs as it is heavily modified from cellulose, though it can equally be argued that it is not a plastic (Hartman et al., 2019). This disagreement demonstrates the complexity of defining plastic debris, and as such, should be considered a “diverse contaminant suite” rather than a single type of material (Rochman et al. 2019). Thus, the term “MPs” is really a catch-all for a type of debris that can vary significantly in composition other than just polymer type, due to additives such as plasticizers or antimicrobials. Most importantly, these differences may alter their ecotoxicity (Rochman et al. 2019). However, MPs may be broadly categorized into primary and secondary types that help to define source, management, and mitigation. Primary MPs are manufactured to be of microscopic size, like microbeads from domestic personal care products or abrasives for industrial applications; these are the MPs that can be easily regulated (GESAMP, 2015; IVL SWEDEN, 2016). However, secondary MPs cannot be directly regulated because they are pieces that have fragmented off larger debris that were already in the environment (GESAMP, 2015; IVL SWEDEN, 2016). Plastic debris (using the above criterions) does not mineralize in the natural environment, but continually breaks down into smaller pieces; and thus, most plastics will likely become MPs if not removed (GESAMP, 2015; Ter Halle et al., 2016; IVL SWEDEN, 2016).

MPs degrade via photochemical, physical, and biological processes (biofilm formation and microbial degradation; the last of which is the least known (Muthukumar et al., 2011; Zettler et al., 2013; Jahnke et al., 2017). Shear and tensile stresses (e.g. abrasion, etc.), combined with photochemical degradation, cause fragmentation of most polymers (Ter Halle et al., 2016). Polyethylene (PE), for example, degrades through UV radiation via free radical reactions in the polymer chain and this is the main mechanism for its degradation (Guadagno, 2001). Wave action, fluctuating temperatures, and salinity also influence the weathering of MPs (Jahnke et al., 2017).

Plastics in the ocean are readily colonized by bacteria (Zettler et al., 2013; Muthukumar et al., 2011; Jacquin et al., 2019; Ma et al., 2020). The impact of bacterial and fungi communities on plastic fragmentation and degradation has been extensively studied and dozens of microorganisms have been found to biodegrade different polymers, including PE, PS, PET, and PP (Jacquin et al., 2019). There are four essential steps of biodegradation (described in detail by Dussud and Ghiglione, 2014): 1) biodegradation concerns the growth of biofilms on the plastic, this increases porosity and induces cracks, which weakens the plastic and/or the microorganisms release acid resulting in chemical degradation; 2) bio-fragmentation is the chemical breakdown of the polymer into smaller oligomers that can be taken up by cells via extracellular enzymes released by microorganisms; 3) oligomers less than 600 Daltons can be used as a carbon source by colonizing cells; and 4) mineralization, the last step in biodegradation of a plastic and results in the release of completely oxidized metabolites (e.g., CO_2, H_2O) (Jacquin et al., 2019). However, the rate at which microorganisms have been shown to metabolize plastics is quite slow, even under controlled conditions (Jacquin et al., 2019; Krueger et al., 2015; Harshvardhan and Jha, 2013). Indeed, most consumer plastics are resistant to biodegradation in the environment (Jacquin et al., 2019; Krueger et al, 2015). Unsuitable C/N ratios may be an important limiting factor for biodegradation of polymers in the ocean (Sauret et al., 2016; Jacquin et al., 2019). Consequently, there is almost no data on plastic mineralization rates in the ocean, and fate and identity of polymer degradation products are still largely unknown (Jacquin et al., 2019).

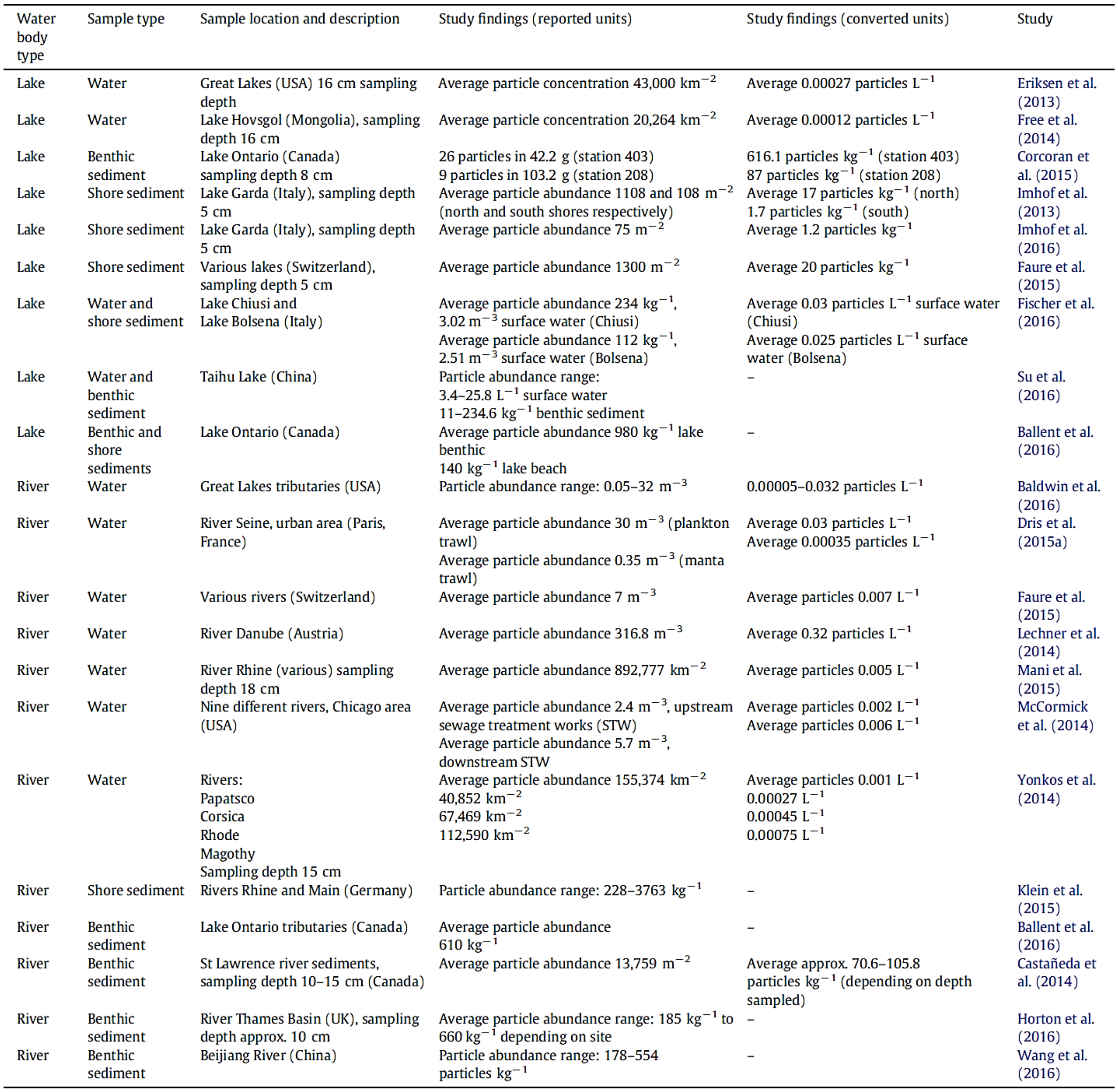
To date, almost any polymer can be found in the environment. A summary of 42 international studies found that marine MPs consist of PE, polypropylene (PP), and polystyrene, followed by polyamide, polyester, acrylic, polyoximethylene, polyvinyl alcohol, polyvinyl chloride (PVC), poly methylacrylate, polyethylene terephthalate (PET), alkyd, and polyurethane (PU) (IVL SWEDEN, 2016). These plastics enter the environment through sanitary wastewater and stormwater systems, accidental spills, littering, and through abrasion of outside materials (GESAMP, 2015; IVL SWEDEN, 2016). The last means is one of the reasons MPs are so challenging to regulate. In addition to littering, MPs are also made from intentional outside usage items: shoe soles, mailboxes, benches, tires, etc., all eventually degrade through exposure and release MPs during their lifetimes (GESAMP, 2015; Ter Halle et al., 2016; IVL SWEDEN, 2016). Plastic debris (using the above criterions) does not mineralize in the natural environment, but continually breaks down into smaller pieces; and thus most plastics will likely become MPs if not removed (GESAMP, 2015; Ter Halle et al., 2016; IVL SWEDEN, 2016).

MPs in Wastewater Effluent and the Urban Environment

MP distribution is prolific globally and is concentrated in densely populated areas where their use is highest (Table 1.1; Horton et al., 2017). Yonkos et al. 2014 found that MP concentration was positively correlated with urban land use in the Chesapeake Bay estuary system. A study of the Great Lakes found that downstream of metropolitan Detroit, particle concentration averaged ~370,000 particles km^{-2} (Eriksen et al., 2013; Horton et al., 2017). In Lake Huron, where the population is lower, particle concentration averaged ~3,400 particles km^{-2} (Eriksen et al., 2013; Horton et al., 2017). However, Lake Hovsgol in Mongolia was found to have MPs concentrations similar to the Great Lakes (Free et al., 2014; Horton et al., 2017). There is improper waste management and wastewater input into Lake Hovsgol, which contributes significantly to plastic pollution (Free et al., 2014). Indeed, wastewater effluent has been found to be an important source of MPs to aquatic environments (Magnusson and Norén, 2014; GESAMP, 2015; IVL SWEDEN, 2016; Murphy et al., 2016; Mason et al., 2016; Mintenig et al., 2017).

The most common types of MPs found in wastewater effluent are PP, PE (high density polyethylene, HDPE, and low density polyethylene, LDPE), polyester, and PVC: they are used for products such as disposable food containers, cutting boards, grocery bags, clothing, and construction materials, respectively (GESAMP, 2015; IVL SWEDEN, 2016; Mintenig et al., 2017). These polymers enter rivers and estuaries through storm runoff or wastewater effluent, where they eventually travel to the ocean (GESAMP, 2015; IVL SWEDEN, 2016). >98% of the MPs in wastewater are removed during the settling of sludge (Magnusson and Norén, 2014; Murphy et al., 2016). However, with wastewater treatment plants (WWTPs) treating, collectively, hundreds of billions of liters a day, <1% translates into millions of MPs traveling with effluent into rivers and bays. Mason et al., 2016 estimated that the output from WWTPs in the United States is 3-23 billion MPs/day for the United States.

Table 1.1. Summary of selected freshwater MP environmental sampling studies, covering a range of freshwater environments. From Horton et al., 2017.



Water body type	Sample type	Sample location and description	Study findings (reported units)	Study findings (converted units)	Study
Lake	Water	Great Lakes (USA) 16 cm sampling depth	Average particle concentration 43,000 km^{-2}	Average 0.00027 particles L^{-1}	Eriksen et al. (2013)
Lake	Water	Lake Hovsgol (Mongolia), sampling depth 16 cm	Average particle concentration 20,264 km^{-2}	Average 0.00012 particles L^{-1}	Free et al. (2014)
Lake	Benthic sediment	Lake Ontario (Canada) sampling depth 8 cm	26 particles in 42.2 g (station 403) 9 particles in 103.2 g (station 208)	616.1 particles kg^{-1} (station 403) 87 particles kg^{-1} (station 208)	Corcoran et al. (2015)
Lake	Shore sediment	Lake Garda (Italy), sampling depth 5 cm	Average particle abundance 1108 and 108 m^{-2} (north and south shores respectively)	Average 17 particles kg^{-1} (north) 1.7 particles kg^{-1} (south)	Imhof et al. (2013)
Lake	Shore sediment	Lake Garda (Italy), sampling depth 5 cm	Average particle abundance 75 m^{-2}	Average 1.2 particles kg^{-1}	Imhof et al. (2016)
Lake	Shore sediment	Various lakes (Switzerland), sampling depth 5 cm	Average particle abundance 1300 m^{-2}	Average 20 particles kg^{-1}	Faure et al. (2015)
Lake	Water and shore sediment	Lake Chiusi and Lake Bolsena (Italy)	Average particle abundance 234 kg^{-1}, 3.02 m^{-3} surface water (Chiusi) Average particle abundance 112 kg^{-1}, 2.51 m^{-3} surface water (Bolsena)	Average 0.03 particles L^{-1} surface water (Chiusi) Average 0.025 particles L^{-1} surface water (Bolsena)	Fischer et al. (2016)
Lake	Water and benthic sediment	Taihu Lake (China)	Particle abundance range: 3.4–25.8 L^{-1} surface water 11–234.6 kg^{-1} benthic sediment	–	Su et al. (2016)
Lake	Benthic and shore sediments	Lake Ontario (Canada)	Average particle abundance 980 kg^{-1} lake benthic 140 kg^{-1} lake beach	–	Ballent et al. (2016)
River	Water	Great Lakes tributaries (USA)	Particle abundance range: 0.05–32 m^{-3}	0.00005–0.032 particles L^{-1}	Baldwin et al. (2016)
River	Water	River Seine, urban area (Paris, France)	Average particle abundance 30 m^{-3} (plankton trawl) Average particle abundance 0.35 m^{-3} (manta trawl)	Average 0.03 particles L^{-1} Average 0.00035 particles L^{-1}	Dris et al. (2015a)
River	Water	Various rivers (Switzerland)	Average particle abundance 7 m^{-3}	Average particles 0.007 L^{-1}	Faure et al. (2015)
River	Water	River Danube (Austria)	Average particle abundance 316.8 m^{-3}	Average 0.32 particles L^{-1}	Lechner et al. (2014)
River	Water	River Rhine (various) sampling depth 18 cm	Average particle abundance 892,777 km^{-2}	Average particles 0.005 L^{-1}	Mani et al. (2015)
River	Water	Nine different rivers, Chicago area (USA)	Average particle abundance 2.4 m^{-3}, upstream sewage treatment works (STW) Average particle abundance 5.7 m^{-3}, downstream STW	Average particles 0.002 L^{-1} Average particles 0.006 L^{-1}	McCormick et al. (2014)
River	Water	Rivers: Papatsco Corsica Rhode Magothy Sampling depth 15 cm	Average particle abundance 155,374 km^{-2} 40,852 km^{-2} 67,469 km^{-2} 112,590 km^{-2}	Average particles 0.001 L^{-1} 0.00027 L^{-1} 0.00045 L^{-1} 0.00075 L^{-1}	Yonkos et al. (2014)
River	Shore sediment	Rivers Rhine and Main (Germany)	Particle abundance range: 228–3763 kg^{-1}	–	Klein et al. (2015)
River	Benthic sediment	Lake Ontario tributaries (Canada)	Average particle abundance 610 kg^{-1}	–	Ballent et al. (2016)
River	Benthic sediment	St Lawrence river sediments, sampling depth 10–15 cm (Canada)	Average particle abundance 13,759 m^{-2}	Average approx. 70.6–105.8 particles kg^{-1} (depending on depth sampled)	Castañeda et al. (2014)
River	Benthic sediment	River Thames Basin (UK), sampling depth approx. 10 cm	Average particle abundance range: 185 kg^{-1} to 660 kg^{-1} depending on site	–	Horton et al. (2016)
River	Benthic sediment	Beijiang River (China)	Particle abundance range: 178–554 particles kg^{-1}	–	Wang et al. (2016)

MPs and Sorbed Organic Pollutants

MPs, along with being a physical danger to wildlife, are also vectors for persistent organic pollutants; POPs (Endo et al., 2016; Horton et al., 2017). POPs are recalcitrant to degradation and accumulate in the environment and in organisms (Wania and Mackay, 1999). They effect the reproduction, development, immune system, and most other toxicological consequences in humans and wildlife, alike. Some well-known POPs include pesticides such as dichlorodiphenyltrichloroethane (DDT); polyaromatic hydrocarbons (PAHs) from the combustion of fossil fuels; and polybrominated diphenyl ethers (PBDEs), flame retardants commonly used in plastics. Due to the hydrophobicity of POPs, they either evaporate or sorb into organic matter in sediments, lipids of organisms, and in plastics.

The first experiment to document the sorption of POPs to plastics was conducted at beaches in the Sea of Japan and Tokyo Bay. All collected MPs were PP and had high amounts of PCBs, DDE (a degradation product of DDT), and nonylphenols and concentrations were comparable to that of the surrounding sediments (Mato et al., 2001). Teuten et al., 2007 examined the sorption of the PAH phenanthrene from seawater into MPs made of PE, PP, and PVC and compared to estuary sediments. They found that phenanthrene preferentially sorbed to plastics, as shown by the significantly higher distribution coefficients compared to sediments (Table 1.2), and that PE sorbed an order of magnitude more phenanthrene than PP and PVC (Teuten et al., 2007). Rochman et al., 2013 soaked HDPE, LDPE, PP, PVC, and polyethylene terephthalate (PET) production pellets, hanging off of a dock at various locations in San Diego Bay for 12 months. After a year, PAHs and PCBs concentrations on PE and PP were much greater than PET and PVC. The experiment also showed that PET and PVC reach equilibrium much faster than PE and PP, which explains their much higher concentrations (Rochman et al., 2013). Together, these experiments suggest that MPs made up of different types of plastics may have different associated risks to organisms (Teuten et al., 2007; Rochman et al., 2013). In addition, due to their long equilibrium time, MPs of PE and PP may concentrate POPs to orders of magnitude higher than sediments and particulate organic matter, posing a much larger risk if ingested.

Table 1.2. Isotherm parameters for sorption of phenanthrene to plastics and sediments. Adapted from Teuten et al., 2007.

Solid Phase	K_d[a]	K_{oc}[b]
	Polymers	
Polyethylene	38100 ± 5600	44500
polypropylene	2190 ± 170	2560
$\text{PVC}_{200\text{-}250}$	1650 ± 200	4340
PVC_{130}	1690 ± 310	4450
	Sediments	
Plym	135 ± 16	20100
Mothecombe	19 ± 3	10400

[a] Distribution coefficient ± SD (L kg^{-1}). [b] $K_{oc} = K_d$/fraction organic carbon (L kg^{-1}).

Sorption Mechanisms and Influencing Factors

Hydrophobic and electrostatic interactions, and the effects of pH

The mechanisms controlling the sorption process are dependent on the physical and chemical properties of the polymer and the associated substance (Tourinho et al., 2019). When speaking of microplastics and organic pollutants, hydrophobic interactions are the dominant mechanisms (Lee et al., 2014; Llorca et al., 2018; Tourinho et al., 2019; Wu et al., 2019). Common polymers, i.e., PE, PP, and PS, are hydrophobic and have no polar functional groups. Thus, hydrophobic organic contaminants, such as PAHs and PCBs, with large nonpolar structure (eg., aromatic rings) will be attracted to the nonpolar surface of the MP (Hüffer and Hofmann, 2016; Tourinho et al., 2019). This is considered one of the main mechanisms controlling sorption of hydrophobic organic contaminants (Mato et al., 2001; Lee et al., 2014; Wu et al., 2016; Hüffer and Hofmann, 2016;

Tourinho et al., 2019). For these compounds, the only interactions that they may undergo are van der Waals forces (specifically London Dispersion) and π-π stacking; and therefore only polymers such as PS, which has an aromatic functional group, experience π-π interactions (Huffer and Hofmann, 2016; Hartman et al., 2017; Huffer et al., 2018). Huffer and Hofmann, 2016 found that PAH sorbed to PA more than PE and PP. Xu et al., 2018 also found that tetracycline sorbed more to PS than PE, and that this may be attributed to the strong π-π interactions between tetracycline and PS. The a**d**sorption of non-planar molecules is weaker than for planar molecules (Huffer and Hofmann, 2016). This is because most atoms of a **planar** molecule may interact with the MP surface, while comparatively few atoms in a <u>non-planar</u> molecule may interact with the surface. This makes a**d**sorption stronger for **planar** substances (Smiciklas et al., 2000; Onjia et al., 2001; Huffer and Hofmann, 2016). In contrast, the a<u>b</u>sorption of <u>non-planar</u> molecules is stronger than the a<u>b</u>sorption of **planar** molecules due to lower cavity formation energy for <u>non-planar</u> compounds (Goss, 2004; Huffer and Hofmann, 2016). <u>Non-planar</u> molecules may change their conformation to fit in a smaller cavity, while a **planar** compound cannot contort (Goss, 2004; Huffer and Hofmann, 2016).

However, electrostatic interactions are also important for MP sorption, especially for more polar compounds such as pharmaceutical and personal care products (PPCPs) (Guo et al., 2018; Li et al., 2018; Liu et al., 2019). For example, the polymer polyamide (PA), which has a polar amide functional group (R-N-CO-R), seem to have higher sorption than PS, PE, and PP for more hydrophilic compounds such as antibiotics (Li et al., 2018; Tourhino et la., 2019). Here, hydrogen bonding played a role as the antibiotics amoxicillin and ciprofloxacin are ionic at environmental pH (both zwitterions), and greater sorption was attributed to hydrogen bonding with the proton donor amide group in PA (Li et al., 2019). Electrostatic interactions may also sometimes occur for nonpolar MPs, as negative surface charges may form on the surface of the MP due to the pH of point of zero charge (pH_{pzc}) being lower than most naturally occurring pHs, especially for PVC and PS (Li et al., 2018; Liu et al., 2019; Xu et al., 2018; Zhang et al., 2018). Therefore, positively charged substances may be attracted to the MP surface, where anionic chemicals will be repulsed (Guo et al., 2018; Liu et al., 2019 Razanajatovo et al., 2018; Xu et al., 2018).

The effect of changing pH on sorption depends on the pK_a of the substance. For example, Guo et al., 2018 found that the sorption of Tylosin decreased with decreasing pH (pH 3-11). PVC and PS have negative surface charges and interacted favorably with the Tylosin cations (weak base, pK_a of 7.73) at lower pH values (Guo et al., 2019). Zhang et al., 2018 also found that for the antibiotic oxytetracycline, sorption to PS was highest at pH 5, where the largest fraction would be its cationic form (Zhang et al., 2018; Tourhino et al., 2019). However, as the pH increased, the neutral fraction of Tylosin also increased (Guo et al., 2018). Thus, the electrostatic interaction at higher pH decreased due to the increase of hydrophobic forces (Guo et al., 2018). The effect of pH on PE and PP, however, was negligible (Guo et al., 2018). Tylosin is a relatively large antibiotic with hydroxyl and carboxyl groups spread throughout its structure. Therefore, no increase in sorption of Tylosin, despite an increase in the neutral fraction, may be attributed to the high polarity of these functional groups, and their structural density (Figure 1.1), not allowing favorable hydrophobic interactions to occur. The sorption of tetracycline, a zwitterion and polyprotic acid (pka 3.3, 7.7, 9.5), to PE, PP and PS was the highest at pH

6 (Xu et al., 2018). At pH 6, tetracycline would exist as a zwitterion, and thus have a neutral charge, while at higher and lower pH sorption would be dominated by electrostatic interactions (Xu et al.,2018; Tourhino, et al., 2019). The effect of pH, then, is quite complex due to hydrophobic forces also playing a role (Tourhino et al., 2019).

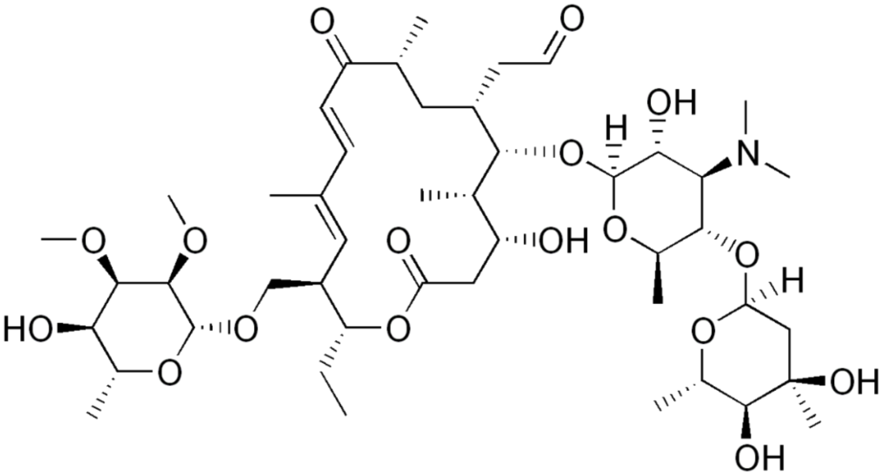


Figure 1.1. Structure of the molecule Tylosin, an antibiotic and bacteriostatic feed additive used in veterinary medicine. Tylosin cations (weak base, pKa of 7.73) was found to interact with the negative surface charges of PVC and PS microplastics only at lower pH values, while sorption decreased at higher pH due to an increase in the neutral fraction of Tylosin (Guo et al., 2018).

Surface Area

The surface area of sorbents (e.g., organic matter) is well known to significantly affect the sorption of organic pollutants, metals, and biofilms in the environment (Endo et al., 2016; Rochman et al., 2016; Rist and Hartmann, 2018). It is also hypothesized that weathered MPs may be more effective vectors of organic pollutants than virgin production pellets, beads, powders, and other unweathered polymer materials mainly due to an increase in surface area, as weathering has been shown to modify surface properties (Endo et al., 2005; Teuten et al., 2007; Ogata et al., 2009; Hartmann et al., 2017; Zhang et al., 2018; Liu et al., 2019). Microplastics in the environment have irregular shapes (Rochman et al., 2019) and particles with irregular shapes are expected to have higher surface area, and thus have higher sorption of pollutants (Brennecke et al., 2016). This is due to the higher surface area-to-volume ration (SA:V) of smaller particles (Velzeboer et al., 2014; Li et al., 2019; Wang et al., 2019), and that this ratio decreases for rounder shapes. Li et al., 2019 found that sorption of triclosan to PS MPs increased with decreasing particle size, and that sorption was similar between differently sized particles with similar surface area. Wang et al., 2019 also found that the sorption of phenanthrene and nitrobenzene to PS MPs increased with decreasing particle size, and that surface area increased with decreasing particle size range. Unexpectedly, the sorption decreased for the two smallest particles sizes, and the authors explain that this is due to MP particle aggregation, which reduced the accessible surface area available for sorption (Wang et al., 2019). However, surface area may not always control sorption. Xu et al., 2018 found that tetracycline sorbed more to PS than PE, even though PE particles were smaller and measured to have greater surface area, and that this may be attributed to the strong π-π interactions between tetracycline and PS. The size of pores on the MP surface may also increase sorption, as glassy polymers such as PS have been observed to have numerous internal pores or “nanovoids,” which would increase interaction with the MP surface (Teuten et al., 2009, Pignatello et al., 2006; Seidensticker et al., 2018).

Crystallinity

Crystallinity is the degree of structural order in a solid. In crystalline regions or in a crystal, the atoms or monomers are arranged in a regular, periodic manner. Crystallinity significantly effects properties such as transparency of the solid and hardness. For polymers, the solid will be a mixture of amorphous (non-crystalline) and crystalline regions, and crystallinity will be reported as a percentage of the volume of the polymer that is crystalline (Jose et al., 2004; Dos Santos et al., 2013). Differential scanning calorimetry is widely used to determine the crystallinity of polymers (Jose et al., 2004; Dos Santo et al., 2013).

For absorption of organic pollutants, i.e., sorption with*in* the polymer, partly depends on the free volume within the matrix (Hartmann et al., 2017). The structural mobility of the polymer chains, the ability to contort into different conformations, also will affect absorption (Hartmann et al., 2017). Both properties influence the crystallinity and glass transition temperature (T_g) of a polymer (Hartmann et al., 2017; Tourhino et al., 2019), and is defined as the T at which 30-50 carbon chains begin to move (Shrivastava, 2018. Absorption occurs in amorphous regions of polymers and these regions may be "glassy" ($<T_g$) or "rubbery" ($\geq T_g$) (Hartmann et al., 2017). Glassy regions are "frozen" in their structural conformation and cannot contort to fit sorbed molecules, so glass polymers are expected to absorb comparatively little (Hartmann et al., 2017; Tourhino, et al., 2019). Crystalline regions of polymers are incapable of sorption (Hartmann et al., 2017). Wang and Wang, 2018 found that sorption was higher to PE than PS or PVC, as the these two polymers have large crystalline regions. While, PE, though more crystalline, is expected to have greater sorption than PP due to the greater distance between PE polymer chains, allowing for easier diffusion (Pascall et al., 2005; Rochman et al., 2013; Wilson et al., 2018). In contrast, Li et al., 2018 measured the sorption of 5 polar ($-$1.37-1.32 $\log K_{OW}$) antibiotics on PS, PE, PP, and PVC of similar particle size (75-180µm) and found that crystallinity did not explain the trend. Elizalde-Veláquez et

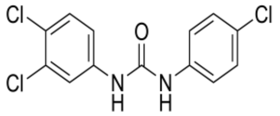
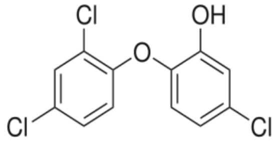
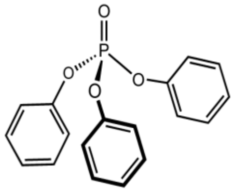
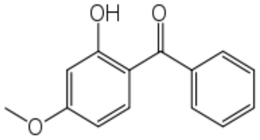
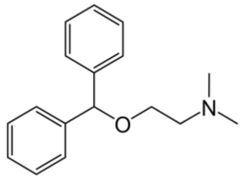
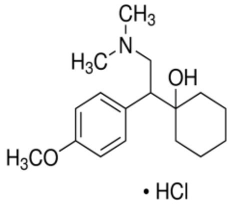
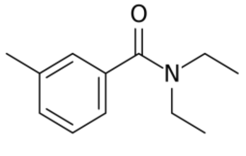
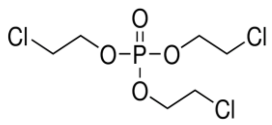
al., 2020 measured 5 polar antibiotics (-1.37 - $1.32\ \log K_{OW}$) sorbed to ultra-high molecular weight polyethylene (UHMWPE), average molecular weight medium density polyethylene (AMWPE), PS, and PP and also found that crystallinity did not explain sorption (crystallinity increases in the order of PP < UHMWPE < AMWPE < PS) (Elizalde-Veláquez et al., 2020). Sorption increased in the order of PP < PS < AMWPE < UHMWPE; however, the authors note that sorption increased with decreasing particle size and observed that their PP MPs had a "perfect spherical shape" (Elizalde-Veláquez et al., 2020). Unfortunately, surface area of the particles were not measured in this study. For more polar compounds, like antibiotics (Li et al., 2018; Guo et al., 2018), adsorption may be the dominant mechanisms, while absorption factors such as crystallinity and free volume may play smaller roles. Surface area (particle size), however, may heavily influence both MP sorption scenarios (Li et al., 2019; Wang et al., 2019).

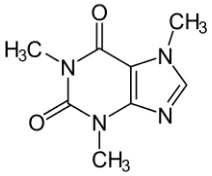
Pharmaceutical and Personal Care Products

Wastewater as a Source of PPCP Laden MPs

WWTPs are a major pathway to waterways for MPs, however they are also a significant source for sorption of organic pollutants. This is especially important for PPCPs, as they are concentrated (Table 1.3) in sanitary sewer influent (Ratola et al., 2012; Margot et al., 2015; Subedi and Loganathan, 2016). The distribution of PPCPs with plastics is still an active field of research, and have also not been directly measured from MPs found in wastewater effluent, to the best of my knowledge. PPCPs are structurally diverse and have various water solubilities (Table 1.3). However, though most PPCPs are less hydrophobic than hydrocarbons due to the presence of polar moieties, many have relatively high octanol-water partition coefficients (K_{OW}), a common measure of hydrophobic distribution tendency (e.g., into lipids, sediments, soils) in the environment (ECETOC-TR 123, 2013, p. 16). In consequence, some have been found to sorb onto MPs at concentrations magnitudes higher than the surrounding water (Lee et al., 2014; Teuten et al., 2007; Wu et al. 2016). Wu et al. 2016 examined the distribution of 4 PPCPs commonly found in wastewater (Margot et al., 2015) into PE MPs and found that there was larger sorption with increasing K_{OW} (Table 1.4). Increased sorption to MPs with increasing hydrophobicity has been frequently observed for PPCPs (Lee et al., 2014; Wu et al., 2016; Razanjatovo et al., 2018; Elizalde-Velázquez et al., 2020).

Table 1.3. Several PPCPs found in the influent of conventional WWTPs. Adapted from Margot et al., 2015.

Compound Structure	Compound Name	Use	*Log* K_{OW}	Typical Influent Concentration (ng/L)
	Triclocarban	Antimicrobial	4.90	700
	Triclosan	Antimicrobial	4.76	2,000
	Triphenyl phosphate	Fire retardant	4.70	140
	Oxybenzone	UV filter	3.52	2,700
	Diphenhydramine	Antihistamine	3.20	$350^{\#}$
	Venlafaxine hydrochloride	Antidepressant	3.27	220
	DEET (N, N-diethylmeta-toluamide)	Insect repellant	2.02	1840
	Tris(2-chloro-ethyl) phosphate	Fire retardant	1.51	390

	Caffeine	Stimulant	0.16	1,640

From Subedi and Loganathan, 2016.

Table 1.4. Sorption (mean ± se) of four PPCPs to PE particles using a linear model. From Wu et al., 2016.

Compound	**$LogK_{OW}$**	**K_d (L/kg)**	**r^2**
Carbamazepine (CBZ)	2.45	191 ± 6.4	0.991
17α-ethinyl estradiol (EE2)	3.67	312 ± 21.5	0.959
Triclosan (TCS)	4.76	5140 ± 290	0.973
4-methylbenzylidene camphor (4MBC)	5.10	53,225 ± 3,715	0.957

Polar and Ionic PPCPs

For MP sorption of polar PPCPs, such as antibiotics, the trend is not as straightforward as with more hydrophobic organic contaminants. Indeed, there seems to be very little relation between K_{OW} of PPCPs and their K_d with MPs (Table 1.5). To explain the trend here, the consideration of multiple types of interactions and forces are necessary, mainly pK_a, K_{OW}, and chemical structure. For example, ciprofloxacin and amoxicillin are both ionic at environmental pH and exist as zwitterions. Based on their K_{OW}, these substances would be expected to have very little sorption, but as zwitterions they have neutral charge and this may allow more sorption compared to compounds with a net charge (Guo et al., 2018; Xu et al., 2018; Tourhino, et al., 2019. Additionally, ciprofloxacin had higher sorption for both PE and PP than amoxicillin, despite having a slightly lower K_{OW}. (Li et al., 2018). However, ciprofloxacin has larger sections of neutral structure (Figure 1.2 a and b) that would allow for more interaction with the MP surface (Smiciklas et al., 2000; Onjia et al., 2001; Huffer and Hofmann, 2016). While, dibutyl phthalate has a K_{OW} of 4.5 and has significantly less sorption (Liu et al., 2019) than triclosan (Wu et al., 2016), whose K_{OW} (4.76) is similar. Again here, structure is needed to understand the differences. Dibutyl phthalate has more polar structure than triclosan (Figure 1.2 d and c), which would inhibit interaction with the hydrophobic polymer surface. Thus, K_{OW} alone is not enough to explain sorption trends for polar PPCPs.

Table 1.5. Selected physical parameters and average sorption distribution constants, logK_d (L/kg), of various PPCPs with polymers PE, PP, and/or PS.

PPCP	LogK_{OW}	pK_a	logK_d (L/kg) PE	PP	PS	Study
ciprofloxacin	0.28	6.09, 8.8	1.74	1.76	1.71	Li et al., 2018
amoxicillin	0.87	3.2, 11.7	0.92	1.24	na	Li et al., 2018
carbamazepine	2.45	13.9	2.28	na	na	Wu et al., 2016
diethyl phthalate	2.47	*nonionic*	-5.03	na	-4.79	Liu et al., 2019
propranolol	3.12	9.42	-2.64	na	na	Razanajatovo et al., 2018
naproxen	3.18	4.15	0.63	0.26	0.36	Elizalde-Veláquez et al., 2020
17α-Ethinyl estradiol	3.67	10.46	2.49	na	na	Wu et al., 2016
γ-hexachlorocyclohexane	3.72	*nonionic*	2.33	2.58	3.01	Lee et al., 2014
ibuprofen	3.97	4.91	1.11	0.40	1.00	Elizalde-Veláquez et al., 2020
sertraline	4.30	9.16	-2.48	na	na	Razanajatovo et al., 2018
dibutyl phthalate	4.50	*nonionic*	-3.03	na	-2.71	Liu et al., 2019
triclosan	4.76	7.90	3.71	na	na	Wu et al., 2016
4-methylbenzylidene camphor	5.10	*nonionic*	4.73	na	na	Wu et al., 2016

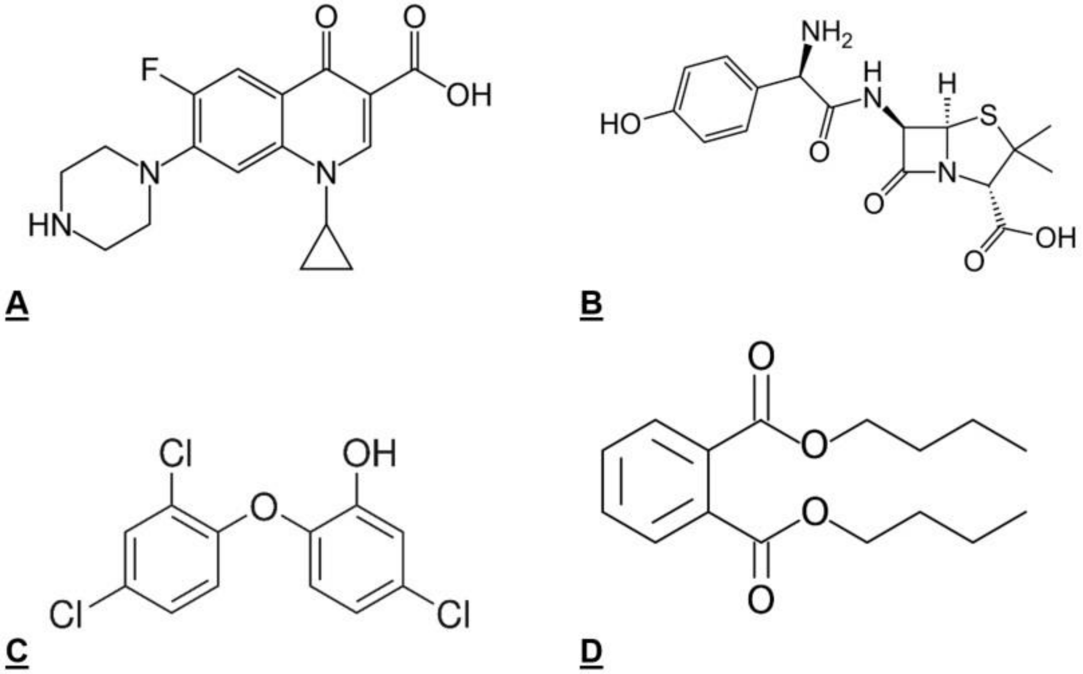


Figure 1.2. Structures of the antibiotics a) ciprofloxacin and b) amoxicillin, c) the antimicrobial triclosan, and d) the plasticizer dibutyl phthalate.

Caution should be taken, however, when generalizing from this data (Table 1.5) due to the high concentrations used in many of these studies that far surpassed levels detected in aquatic environments. Elizalde-Velázquez et al., 2020 reported low sorption of ibuprofen and naproxen under environmental realistic concentration scenarios, and only found high levels of sorption at pH 2 when the acid groups are protonated in their neutral form. Similarly, Seidensticker et al., 2018 conducted single batch sorption experiments for 19 PCPPs and found that for polar compounds, sorption could only be reliably detected when the solid-to-liquid ratio (MP: water) was 6-7 orders of magnitude higher than MP concentrations observed in the field. They additionally found that calculated partition coefficients deviated greatly from measured values of polar compounds and that this was independent of pH (Seidensticker et al., 2018). This may help to explain why ciprofloxacin and amoxicillin are reported to have much higher sorption than the plasticizers dibutyl and diethyl phthalate, despite that they have much lower K_{OW}'s and higher water solubility (Table 1.5). All four compounds were measured at concentrations 1-3 orders of magnitude higher than highest observed river concentration (400-15000 µg/L) and may have skewed results (NCBI, 2020). Many organic pollutants experience nonlinear sorption isotherms (Teuten et al., 2007; Bakir et al., 2012; Lee et al., 2014; Guo et al., 2018), so it should not be assumed that linear sorption coefficients at higher concentrations would be representative of behavior at environmentally relevant levels. Though more data is needed, especially toxicological data, it is hypothesized that due to their relatively low concentrations in aquatic systems, MPs may not be substantial vectors of highly polar and ionizable organic pollutants (Koelmans et al., 2016; Lohmann, 2017; Seidensticker et al., 2018).

Toxicological Effects of Ingested MPs and Sorbed Organic Pollutants

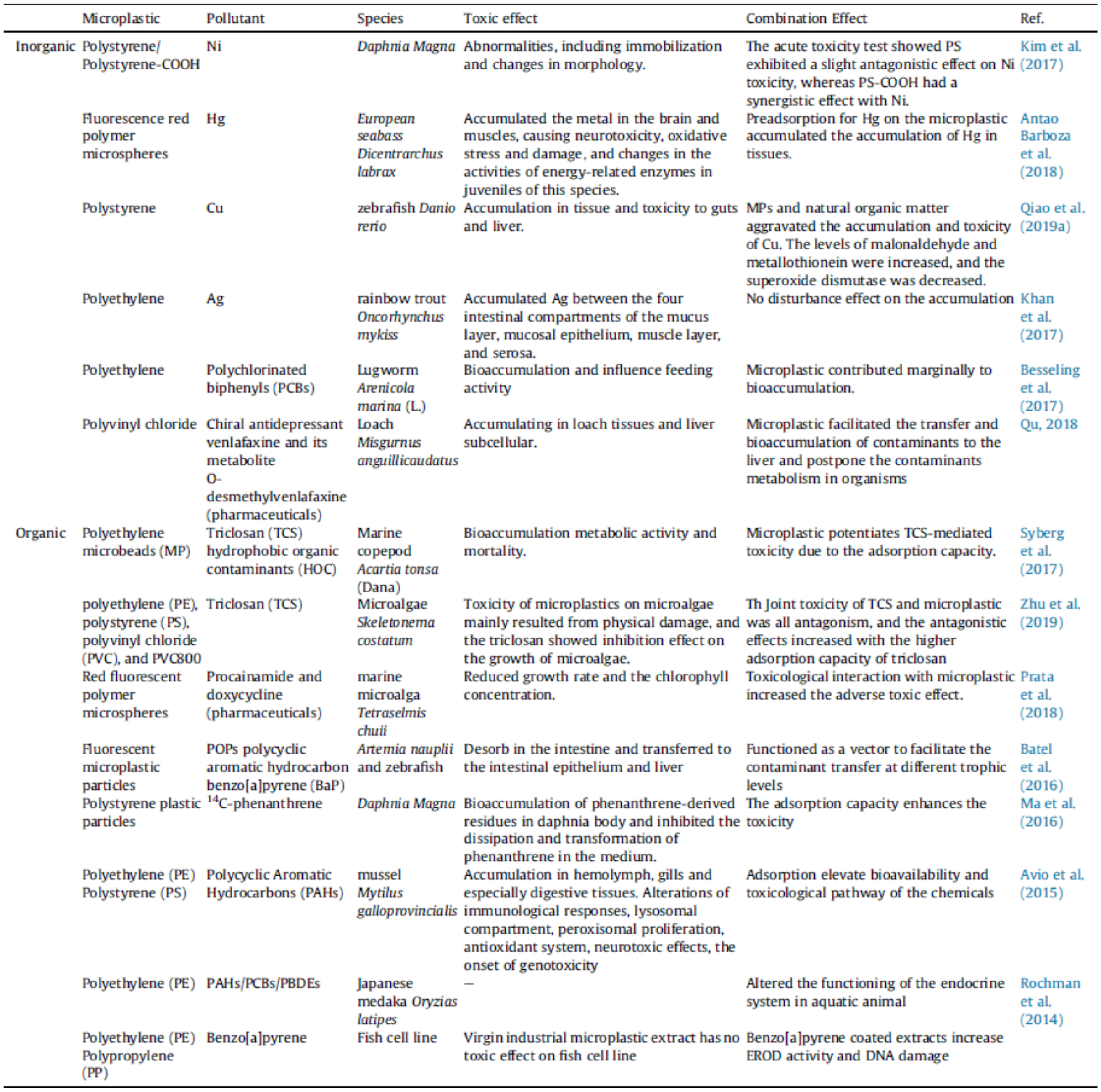
It is well known that organisms ingest MPs and those exert some toxicity, however, it is relatively unclear how big a role MPs play as a particle versus as an exposure route to POPs and PPCPs. It is not a question of whether plastics are vectors of organic contaminants to organisms, but if plastics are of ecological importance in comparison to other sources such as water, sediments, and prey (Rochman, 2016; Herzke et al. 2016; Rist and Hartmann, 2018). Current research has shown that the toxicological effects of ingesting MPs with sorbed organic pollutants depends on the species, chemical, exposure time, and polymer type (Rochman, 2016; Herzke et al. 2016; Rist and Hartmann, 2018; Rochman et al., 2019; Ma et al., 2020; Du et al., 2020). There have been hundreds of studies over the last five years, and there has been much criticism for environmentally irrelevant (very high) concentrations, unrealistic exposure time, use of unweathered MPs (i.e., pellets, microbeads), and the use of a large range of particle sizes (Table 1.6; Rochman, 2016; Beckingham and Gosh, 2017; Devriese et al., 2017; Horton et al., 2017; Rist and Hartmann, 2018; Ma et al., 2020). In addition, ecologically relevant experiments did not use a similar reference material or some form of fully characterized MP particles, and have shown mixed results (Table 1.6; Rochman et al., 2013; Chua et al., 2014; Rochman, 2016; Wardrop et al., 2016; Rist and Hartmann, 2018). A recent review (Ma et al., 2020) reported 13 studies that did find combined effects of the contaminant and the MP type (varied) studied (Table 1.7). At the same time however, other reviews have claimed that despite some evidence MPs present no risk to the aquatic environment (Besseling et al., 2017; Burns and Boxall, 2018; Adam et al., 2019; Ma et al., 2020). Along with the many issues discussed above, a lack of standardized methodology for the analysis of MPs and their associated pollutants, and thereby the ability to compare parallel experiments, has been listed as one of the leading reasons for this lack of consensus (Ma et al., 2020).

Table 1.6. Comparison of select studies on the toxic effects of MPs with sorbed organic pollutants.

Study and Approach	**Findings**	**Concerns**
2013 Rochman, et al. -Model organisms = marine fish, adult (7 months) Medaka, ~300mg. Pellets of LDPE were exposed to environmental contaminants via soaking in San Diego Bay, CA. -Pellets ground to <0.5mm and mixed with fish food for a 10% plastic diet. -Exposure time = 2 months.	-Differences only apparent after 2 month exposure. -No differences after 1 month, suggesting short-term exposures to 10% plastic diet not a significant source of POPs. -There were no significant differences in CYP1A enzyme transcription (important for contaminant metabolism in the liver). However, more glycogen depletion and cell necrosis in fish from plastic treatment.	-Very large range of particle sizes and shapes. -Cod liver oil was in fish food, and caused PAHs, PCBs, and PBDEs to be found in controls. -Fish are exposed for entire lifetimes, not just two months. -Many mechanisms may be involved when organisms are exposed to complex mixtures, so measuring one response may not be enough to say whether an effect exists or not (Rochman, et als.' own commentary).
2014 Chua, et al. -Model organism = amphipods. -Used amphipods, seagrass, and seawater from the field, i.e., not clean model organisms. -PE microbeads size = 11-700 µm. -Exposed amphipods in 1mL of seawater to MPs that were either soaked in 5ng/mL (environmentally relevant concentration) or 50 ng/mL PBDE solution. -Exposure time = 72 hours.	- In general, presence of MPs decreased body burden of PBDEs in all treatments. -Significant differences in congener uptake, with higher brominated BDEs sorbing more to tissue, especially BDE-99.	-Not long enough equilibrium time for BDE to sorb to plastics. -Very short exposure time. -Short elimination time from organisms (less than 12 hours). -Large range of MPs diameter. -Particles used of different diameters (surface are), thus probably varying concentrations of PBDEs. - Perhaps MP induced exposure of PBT is greatest for low/middle trophic organisms whose food-gut residency time is much longer than for organisms such as amphipods.
2016 Wardrop, et al. -Model organism = freshwater fish, juvenile, Murrary River Rainbow fish, age and weight not cited. -Microbeads extracted from facial scrub, listed and confirmed as PE. - PE microbeads size = 10-700 µm. -PBDE concentration of microbeads was ~200ng/g. -Fish food was 14% plastic diet. -Exposure time = 63 days.	-Plastic diet fish had significantly higher PBDE concentrations after 21 days, and continued exposure resulted in increased accumulation over the experiment. -Lower brominated congeners showed highest body burdens, whereas higher brominated congeners did not appear to transfer. -However, BDE-99 did not appear to bioaccumulate.	- Large range of MPs diameter. -Plastic diet 4% higher than other studies. - Contrary to previous studies that found plastics normally reduce body burden; authors used PBDE concentrations 2 orders of magnitude higher than two other studies, including one (2013 Rochman, et al.) that soaked pellets directly in marine water. - Out of 4 dozen papers cited for "relevant concentrations," only 3 reported concentrations of ~200 ng PBDE /g and they were for PP, not PE. The other papers did not list PBDEs.
2017 Devriese, et al. -Model organism = Norwegian lobster, average carapace length = 9-13 cm. -Purchased PE and PS microspheres. -PE and PS sphere size = 500-600 µm. -Fed 9 times over 3 weeks.	-Little bioaccumulation after ingestion of PE with PCBs. - Negligible bioaccumulation after ingestion of PS with PCBs. -Microspheres had no effect on nutritional state of lobsters. -No PCB desorption from lobster tissue to microspheres.	-Assumed PCB sorption to microspheres was 100% and any loss was due to inadequate recovery. - Microspheres loaded to environmentally irrelevant concentrations: ~6ppm. -Positive control had higher PCB concentrations than loaded PS. -Lobsters only fed 9 times over 21 days. -Reported no desorption from tissues, however, lipids from the tails were extracted when microspheres do not have access to this tissue for any desorption to occur. - Although all test organisms were fed with the same amount of PCBs through the PCB-spiked gelatin, these results revealed a significantly lower PCB accumulation in the tail tissues of experiment A (conducted in winter) than

		for experiment B and C (conducted in summer).
2017 Beckingham and Gosh -Used gut fluid method meant to mimic a marine polychaete, Arenicola marina. -Compared gut fluid solubilization potential between biochar, coal, wood, PP. -Bioaccumulation tests were conducted with a freshwater oligochaete, Lumbriculus variegatus. The choice of methods was "practical, due to availability of clean background sediment and L. variegatus for bioaccumulation studies, and the existence of pre-established in vitro methods for A. marina. Although the strength of the gut fluids is likely different, the principle solubilizing agents and digestive action of the two species, within the same phyla Annelida, are expected to be similar." - PP particle size = 35µm, all others <60-75µm.	-Surface area = 109, 2.4, 0.20, 0.19, for biochar, coal, wood, and PP, respectively. -Gut fluid solubilization potential: wood > biochar > PP > coal. -Biouptake in worms was lower by 76% when PCBs were associated with polypropylene compared to sediment.	- Used different gut mimic than test organisms in bioaccumulation test. -Clean sediment was used. -K_d for PCBs were not determined separately, but as a mixture. -PCB concentrations not environmental relevant (pppm range). -"Although a typical solid-to-gut fluid volume for Arenicola marina is ~0.3 g dry wt/mL, a ratio of 0.05 g dry wt/mL was used to ensure an adequate volume of fluid could be collected following incubation," is a whole magnitude lower than the suggested ratio. -Bioaccumulation tests were conducted to standard guidelines but were shortened to 7 days, which the authors state in the discussion: "equilibration with worm lipids is unlikely after only 7 days." -For bioaccumulation test, used biochar, sediment, and polypropylene, but didn't use wood or another environmentally relevant source of organic matter. - Compared weathered wood vs. *un*weathered plastics.

Table 1.7. List selected toxicity studies focusing on the combined effects of MP and adsorbed pollutants covering a range of polymer, pollutant, and species type. From Ma et al., 2020.



	Microplastic	Pollutant	Species	Toxic effect	Combination Effect	Ref.
Inorganic	Polystyrene/ Polystyrene-COOH	Ni	*Daphnia Magna*	Abnormalities, including immobilization and changes in morphology.	The acute toxicity test showed PS exhibited a slight antagonistic effect on Ni toxicity, whereas PS-COOH had a synergistic effect with Ni.	Kim et al. (2017)
	Fluorescence red polymer microspheres	Hg	*European seabass Dicentrarchus labrax*	Accumulated the metal in the brain and muscles, causing neurotoxicity, oxidative stress and damage, and changes in the activities of energy-related enzymes in juveniles of this species.	Preadsorption for Hg on the microplastic accumulated the accumulation of Hg in tissues.	Antao Barboza et al. (2018)
	Polystyrene	Cu	zebrafish *Danio rerio*	Accumulation in tissue and toxicity to guts and liver.	MPs and natural organic matter aggravated the accumulation and toxicity of Cu. The levels of malonaldehyde and metallothionein were increased, and the superoxide dismutase was decreased.	Qiao et al. (2019a)
	Polyethylene	Ag	rainbow trout *Oncorhynchus mykiss*	Accumulated Ag between the four intestinal compartments of the mucus layer, mucosal epithelium, muscle layer, and serosa.	No disturbance effect on the accumulation	Khan et al. (2017)
	Polyethylene	Polychlorinated biphenyls (PCBs)	Lugworm *Arenicola marina* (L.)	Bioaccumulation and influence feeding activity	Microplastic contributed marginally to bioaccumulation.	Besseling et al. (2017)
	Polyvinyl chloride	Chiral antidepressant venlafaxine and its metabolite O-desmethylvenlafaxine (pharmaceuticals)	Loach *Misgurnus anguillicaudatus*	Accumulating in loach tissues and liver subcellular.	Microplastic facilitated the transfer and bioaccumulation of contaminants to the liver and postpone the contaminants metabolism in organisms	Qu, 2018
Organic	Polyethylene microbeads (MP)	Triclosan (TCS) hydrophobic organic contaminants (HOC)	Marine copepod *Acartia tonsa* (Dana)	Bioaccumulation metabolic activity and mortality.	Microplastic potentiates TCS-mediated toxicity due to the adsorption capacity.	Syberg et al. (2017)
	polyethylene (PE), polystyrene (PS), polyvinyl chloride (PVC), and PVC800	Triclosan (TCS)	Microalgae *Skeletonema costatum*	Toxicity of microplastics on microalgae mainly resulted from physical damage, and the triclosan showed inhibition effect on the growth of microalgae.	Th Joint toxicity of TCS and microplastic was all antagonism, and the antagonistic effects increased with the higher adsorption capacity of triclosan	Zhu et al. (2019)
	Red fluorescent polymer microspheres	Procainamide and doxycycline (pharmaceuticals)	marine microalga *Tetraselmis chuii*	Reduced growth rate and the chlorophyll concentration.	Toxicological interaction with microplastic increased the adverse toxic effect.	Prata et al. (2018)
	Fluorescent microplastic particles	POPs polycyclic aromatic hydrocarbon benzo[a]pyrene (BaP)	*Artemia nauplii* and zebrafish	Desorb in the intestine and transferred to the intestinal epithelium and liver	Functioned as a vector to facilitate the contaminant transfer at different trophic levels	Batel et al. (2016)
	Polystyrene plastic particles	^{14}C-phenanthrene	*Daphnia Magna*	Bioaccumulation of phenanthrene-derived residues in daphnia body and inhibited the dissipation and transformation of phenanthrene in the medium.	The adsorption capacity enhances the toxicity	Ma et al. (2016)
	Polyethylene (PE) Polystyrene (PS)	Polycyclic Aromatic Hydrocarbons (PAHs)	mussel *Mytilus galloprovincialis*	Accumulation in hemolymph, gills and especially digestive tissues. Alterations of immunological responses, lysosomal compartment, peroxisomal proliferation, antioxidant system, neurotoxic effects, the onset of genotoxicity	Adsorption elevate bioavailability and toxicological pathway of the chemicals	Avio et al. (2015)
	Polyethylene (PE)	PAHs/PCBs/PBDEs	Japanese medaka *Oryzias latipes*	–	Altered the functioning of the endocrine system in aquatic animal	Rochman et al. (2014)
	Polyethylene (PE) Polypropylene (PP)	Benzo[a]pyrene	Fish cell line	Virgin industrial microplastic extract has no toxic effect on fish cell line	Benzo[a]pyrene coated extracts increase EROD activity and DNA damage	

MPs as Vectors for Organic Pollutants: the Need for Reference Materials

The heterogeneity of experimental approaches makes comparing studies and discovering whether there are consistent trends in MP toxicity challenging, if not impossible (Rochman et al., 2019; Cowger et al., 2020). There is a clear need for the development of standardized methodology for testing the toxicity of MPs and organic pollutants (Cowger et al., 2020). An integral part of this process will be the creation of a reference material for MPs of consistent surface area, as surface area is well known to affect the sorption of POPs (Endo et al., 2016; Rist and Hartmann, 2018; Cowger et al., 2020). In addition, there is a large knowledge gap for the distribution of PPCPs found in wastewater and that are associated with MPs, and thus whether they act as important vectors for these compounds in the environment is unknown. Thus, the focus of this dissertation was to create a MP reference material; develop methodology to extract PPCPs from MPs; measure the sorption equilibrium of one semi-polar but hydrophobic PPCP and three relatively hydrophilic PPCPs found in wastewater at environmentally relevant concentrations; and determine if there is a significant correlation between hydrophobicity (K_{OW}) of the four PPCPs and their sorption onto MPs.

Chapter 2: Fabricating a Microplastic Reference Material that is Standardized by Surface Area

Background and Rational

It is well known that organisms ingest MPs and those exert some toxicity, however, it is relatively unclear how big a role MPs play as a particle versus as an exposure route to POPs and PPCPs. It is not a question of whether plastics are vectors of organic contaminants to organisms, but if plastics are of ecological importance in comparison to other sources such as water, sediments, and prey (Rochman, 2016; Herzke et al. 2016; Rist and Hartmann, 2018). Current research has shown that the toxicological effects of ingesting MPs with sorbed organic pollutants depends on the species, chemical, exposure time, and polymer type (Rochman, 2016; Herzke et al. 2016; Rist and Hartmann, 2018; Rochman et al., 2019; Ma et al., 2020; Du et al., 2020). There has been much criticism for environmentally irrelevant (very high) concentrations, unrealistic exposure time, use of unweathered MPs (i.e., pellets, microbeads), and the use of a large range of particle sizes (Rochman, 2016; Beckingham and Gosh, 2017; Devriese et al., 2017; Horton et al., 2017; Rist and Hartmann, 2018; Ma et al., 2020). In addition, ecologically relevant experiments did not use a similar reference material or some form of fully characterized MP particles, and have shown mixed results (Rochman et al., 2013; Chua et al., 2014; Rochman, 2016; Wardrop et al., 2016; Rist and Hartmann, 2018). Additionally, a lack of standardized methodology for the analysis of MPs and their associated pollutants, and thereby the ability to compare parallel experiments, has been listed as one of the leading reasons for this lack of consensus (Ma et al., 2020).

MPs must be clearly defined as a contaminant, which then leads to the development of standardized methods for analyzing environmental samples and conducting lab-based experiments (Horton et al., 2017; Cowger et al., 2020). Specifically, there is a clear need for the development of a standardized methodology for measuring the sorption chemistry, and ultimately toxicity, of MPs and sorbed organic pollutants (Horton et al., 2017; Rist and Hartmann, 2018; Cowger et al., 2020). Standardization will reduce uncertainty and finally make it possible to rigorously compare studies (Horton et al., 2017; Cowger et al., 2020). Rist and Hartmann, 2018 and Cowger et al., 2020 have suggested that in order for the field to progress, research should focus on the development of reference materials and thorough particle characterization in exposure and distribution studies.

An integral part of this process will be the creation of a reference material for MPs that can be made with cheap materials and commonly available laboratory equipment; as accessibility is integral to feasibility and, ultimately, widespread use. The MPs should also be standardized, or normalized, by surface area, as the surface area of sorbents (e.g., organic matter and plastics) is well known to significantly affect the sorption of organic pollutants, metals, and biofilms in the environment (Endo et al., 2016; Rochman et al., 2016; Rist and Hartmann, 2018). Microplastics in the environment have irregular shapes (Rochman et al., 2019) and particles with irregular shapes are expected to have higher surface area, and thus have higher sorption of pollutants (Brennecke et al., 2016). This is due to the higher surface area-to-volume ration (SA:V) of smaller particles (Velzeboer et al., 2014; Li et al., 2019; Wang et al., 2019), and that this ratio decreases for rounder shapes. Additionally, laboratory-made particles will not have uniform diameters, and instead will be defined by size *classes*. For example, to make particles of roughly 275 µm in diameter, MPs could be separated with mesh sieves of pore sizes 300 µm and 250 µm. Single particle diameter will vary, however, surface area per gram of MPs should not. Therefore, standardizing by surface area will allow for comparison of organic pollutant sorption between differing MP size classes, as well as between polymer types. A potential caveat remains in that it is still unclear if biofilm formation differs between different ways of manufacturing surface area-normalized size fractions, but biofilm formation and its influence on sorption is beyond the scope of this work. However, the reference MPs should have a surface morphology that takes into account weathered debris, i.e., they are pitted or roughly textured. It is hypothesized that weathered MPs may be more effective vectors of organic pollutants than virgin production pellets, beads, powders, and other pristine polymer materials; as weathering has been shown to modify surface properties, particularly an increase in surface area (Endo et al., 2005; Teuten et al., 2007; Hartmann et al., 2017; Zhang et al., 2018; Liu et al., 2019). This may be especially true for large, planar compounds (e.g., compounds with aromatic rings, like PCBs) that may a***d***sorb more strongly than a*b*sorb (Onjia et al., 2001; Smiciklas et al., 2000; Huffer and Hofmann et al., 2016). Therefore, a reference material that morphologically resembles weathered MPs may provide more environmentally realistic sorption behavior (Hartmann et al., 2017; Liu et al., 2019) and could be used in follow-up research to evaluate the effect of biofilm on sorption.

There are many types of plastic debris, and thus, there are many kinds of MPs (Rochman et al., 2019). Indeed, two MPs both composed mainly of polypropylene could, in truth, be two very different particles in terms of ecotoxicity, due to different properties such as shape, hardness, and chemical additives (Rochman et al., 2019). In consequence, unambiguously defining what *type* of MP (including potential additives) the reference material represents is essential for attributing its use (e.g., what hypotheses can be tested, what organisms might ingest them) and will, ultimately, allow for more accurate comparisons of research that uses similarly defined particles. Here, the goal was to create a reference material for secondary MPs of the “fragment” type of PE and PP. Fragment microplastics have a rigid structure and irregular shape and can be round or angular; plastic shavings fit this category (Rochman et al. 2019). PE and PP were chosen because they are amongst the most produced and consumed polymers worldwide (GESAMP, 2015; PlasticsEurope 2017). They are also frequently observed in wastewater and are the most prevalent MPs found in the marine environment (GESAMP, 2015; Magnusson et al., 2016; Murphy et al., 2016). The approach to make fragments were chosen because they are the easiest to make due to their varying structure and shape, which allows greater flexibility during the fabrication process. As PE and PP are widely used in consumer products, fragments also represent a large fraction of PE and PP secondary MPs, i.e., fragmentation from rigid/hard products such as reusable water bottles, cutting boards, twist caps, plastic utensils, etc. They do not, however, represent secondary MPs from flexible/soft items such as PE shopping bags or PP food wrapping, for example, which would be considered film type MPs, nor do they represent microbeads, pellets, or foams (for more information on microplastic types, please see Rochman et al. 2019).

The specific objectives of this study were to: 1) create a methodology to fabricate a reference material that consistently mimics (i.e., rough textured and irregularly shaped) fragment type secondary MPs of HDPE and PP of 9 size classes, using 10 mesh sieves of 45-600 µm – e.g., 45-75, 500-600 µm, etc.; and 2) measure the surface area of each size class to determine if standardization using surface area and mass basis is feasible, as particle diameter within size classes will vary, but surface area per gram should not. It was hypothesized that: 1) HDPE MPs would have greater surface area than PP, as PE has generally shown more sorption of organic pollutants (Teuten et al., 2007; Karapanagioti and Klontza, 2008; Lee et al., 2014; Rochman et al., 2013); 2) that smaller particles would have greater surface area; 3) that SA would increase non-linearly with smaller size classes due to the inverse relationship between surface area and volume; and lastly, 4) that variation in particle diameter and shape within size classes will not cause significant variation in surface area on a mass basis.

Methods

Fabrication of Microplastic Reference Material

Sorption and toxicity studies that have made MP material used either pristine production pellets, grated plastic labware, or microbeads from facial soap (Rochman et al., 2013; Chua et al., 2014; Lee et al., 2014; Beckingham and Gosh et al., 2017). These studies used a large range of MPs particle diameter and did not characterize the surface area to be able to standardize organic contaminant sorption. A study from China did successfully make MPs from pellets and sieved them to a small size range (Wu et al., 2016). However, production pellets are only accessible for those in the polymer industry in the U.S. and receiving samples is difficult (personal experience). Additionally, the analytical mill that was used (IKA A11 basic analytical mill; Staufen, Germany) only grinds very hard, spherical items well, while the plastic that is accessible in the U.S. comes in the form of production rods. Small, flat pieces do not grind in an analytical mill as they lodge between the blades, causing the mill to seize and stop; even with prior cooling using liquid nitrogen (personal observation). Instead, the method described below may be used with any plastic product but is ideal for cylindrical or rectangular rods. The tools used in this method are readily available to anyone with access to a hardware store and common lab equipment.

HDPE and isotactic PP production rods were purchased from Piedmont Plastics, USA. Production rods were submerged in liquid nitrogen for 5 minutes. Using a baked (500°C for 6 hours) stainless-steel wood file, the rods were shaved, and particles collected in a stainless-steel tray. Once the plastic rods were no longer sufficiently cold and brittle (indicated by the ice crust having melted), the rods were refrozen, and the filing process continued until sufficient material was created. A boar bristle brush and stainless-steel scoopula were used to transfer the material to glass jars and closed with Teflon lined caps. Next, to create MPs of the needed size classes, particles were rinsed through a stack of brass sieves of mesh sizes 45, 75, 150, 212, 250, 300, 350, 425, 500, and 600 µm in diameter, with the smallest mesh on the bottom. Shavings were placed in the top sieve and rinsed with a small hose connected to a tap water faucet for 5 min. MPs were rinsed using a gentle swirling motion, starting from the edges and spiraling inward. After 5 min, each sieve was gently lifted to allow water to flow through the sieves; this was especially important for the 3 smallest mesh sieves. This process was repeated for the top sieve until no further visible separation was observed. For each smaller sieve, starting with 500 µm, only one 5 min rinse was needed. Once separated, the MPs in each sieve were rinsed with ~200 mL of DI water. Next, to remove an organic contaminants that may be left over from production, MPs were transferred to 150 mL beakers (one for each sieve size) and

80mL of 50:50 v/v hexane: acetone added, covered with aluminum foil, and then sonicated for 20 minutes. Dirty solvent was removed by filtering the solution with MPs through a 45 µm brass sieve. MPs were transferred back to the beaker, and 80 mL of fresh 50:50 hexane: acetone added and sonicated a second time. This process was repeated a third time to ensure all organic contaminants in the plastics were removed. This process may also remove additives in the polymers and effect their structure, however, solubilization of the polymers was not an issue for PE and PP. Other polymers with high additive content may be adversely affected. After the third sonication, MPs were poured over a 45 µm sieve again, and then rinsed with ~10ml of acetone to remove residual potentially contaminated solvent. MPs were then transferred to new acetone rinsed beakers (one per size class of each polymer), covered with a Kim wipe (sealed by taping around the edges) to prevent dust contamination, and left to dry at room temperature for ≥ 24 hours. They were then further dried in a 45˚C oven for 8 hours. This additional drying step was required for the surface area analysis. See Appendix 2 for a step by step fabrication protocol. From this point on size classes will be referred to by their smallest length, e.g., the 45-75 µm MPs are classified as the 45 µm size class.

MP Surface Area Analysis

The Brunauer, Emmett, and Teller (BET) Theory was used to determine the surface area of the MP reference material. Using BET theory, the specific surface area of a powder is determined by the adsorption of a gas, usually nitrogen or krypton, as a monomolecular layer (N_2 is most commonly used). The total surface area is related to how much gas is adsorbed on the surface of a solid material. Adsorption results from weak van der Waals forces between the gas molecules and the surface of the powder. The data are analyzed using the BET adsorption isotherm equation:

$$\frac{1}{\left[V_a\left(\frac{P_0}{P}-1\right)\right]} = \frac{C-1}{V_m C} \times \frac{P}{P_0} + \frac{1}{V_m C} \qquad 1)$$

where P is partial vapor pressure of adsorbate gas in equilibrium with the surface at 77.4 K (boiling point of liquid nitrogen – reaction temperature used for no matter adsorbate gas identity), in pascals; P_0 is the saturation pressure of adsorbate gas, in pascals; V_a is the volume of gas adsorbed at standard temperature and pressure (STP) [273.15 K and atmospheric pressure (1.013×10^5 Pa)], in milliliters; V_m is the volume of gas adsorbed at STP to produce an apparent monolayer on the sample surface, in milliliters; and C is the dimensionless constant that is related to the enthalpy of adsorption of the gas on the powder. A value of V_a is measured at ≥3 values of P/P_0 and the BET value:

$$\frac{1}{\left[V_a\left(\frac{P_0}{P}-1\right)\right]} \qquad 2)$$

is plotted against P/P_0 according to equation 1. From the linear plot, the slope $C-1)/(V_mC)$, and the intercept $1/(V_m C)$, are found by linear regression analysis. The correlation should be linear in the P/P_0 range of 0.05 to 0.30: data are considered acceptable if the correlation coefficient, r^2, is ≥0.9975. From here, V_m is calculated as 1/(slope/intercept). C is calculated as (slope/intercept) +1. From the value of V_m, the specific surface area, S, in m^2g^{-1}, is calculated by the equation:

$$S = \frac{V_m N a}{[m \times 22400]} \qquad 3)$$

where, N is Avogadro's constant (6.022×10^{23} mol^{-1}); a is the effective cross-sectional area of one molecule of adsorbate gas, in square meters; m is the mass of the powder sample, in grams; and 22400 is the volume occupied by 1 mole of gas at standard temperature and pressure (STP) allowing for minor departures from the ideal, in milliliters.

Initially, nitrogen was used as the adsorptive gas, and was run at 77 K by an ASAP 2020 instrument (Micromeritics Instrument Co., GA, USA). However, the correlation coefficients, r^2, were not acceptable (r^2 = ~0.92, see Appendix 3 for plots); additionally, these data were not supported by the triclocarban (TCC) sorption equilibrium isotherms, i.e., sorption did not increase with surface area as expected (see Chapter 2). Therefore, it was concluded that the surface area data were likely not accurate; despite previous work using this method for analyzing MPs (Hüffer and Hofmann, 2016; Hu et al., 2017; Wang and Wang, 2018; Liu et al., 2019). The MPs were measured again, post hoc, but instead of nitrogen krypton was used as the adsorptive gas. This method was successful (BET plot r^2 >0.9975), corroborated the TCC sorption data, and hence was used for further analysis in this study. An in-depth explanation on this work and the effect of nitrogen gas can be found in the results and discussion below. Briefly, each size class of both polymers were analyzed in triplicate (and were independent, i.e., the portion used for surface area analysis was not replaced into the bulk sample before sampling the next aliquot of MPs). This was necessary to test the variation of the material to ensure that future aliquots used during experiments would have consistent surface area, i.e., that they are standardized on a mass basis. Samples were prepared from ~0.50 g of MPs. For degassing, samples were placed under vacuum at 60°C for 60 minutes. Normally, degassing would occur at much higher temperatures (>>100°C), but due to the melting points of HDPE and PP (~120°C and ~150°C, respectively), this was not possible. It was found that successful degassing at 60°C was possible if each sample was first dried at 45˚C for 8 hours (as previously mentioned). This also allowed for a relatively shorter degassing time, as degassing times for non-oven dried samples were ≥3 hours. An isothermal jacket was used, and sample backfill was with nitrogen. Krypton was used as the adsorptive gas and run at 77.3 K on a TriStar II Plus instrument (Micromeritics Instrument Co., GA, USA). Relative pressure (P/P_0) was kept between 0.05 - 0.30 during the analysis (See Appendix 3 for full instrument parameters).

Standardization on a surface area to mass basis was considered if the coefficient of variation of surface area within size classes was <5%. The total data set (all 9 size classes) for both polymers was modeled using the least squares method of regression analysis. After visually analyzing the data, non-linear functions (e^x, logx, lnx, and power – ax^b) were compared for best of fit of data. The models were compared using an ANOVA approach and the model with the lowest relative standard error (RSE) was chosen. Models were created and analyzed with R Statistical Computing software (R Core Team, 2019) and Microsoft Excel 2016.

^Explanation of theory adapted from http://particle.dk/methods-analytical-laboratory/surface-area-bet-2/

Results and Discussion

MP Characterization: Scanning Electron Microscopy (SEM) Imaging and Surface Area

The fabricated MPs of HDPE and PP have an irregular shape and can be described as fragments, however the two polymers produce distinctly different MPs

(Rochman et al. 2019). HDPE fragments are more angular than PP (Figure 2.1; SEM images of all size classes in Appendix 2). This is most likely indicative of the difference in hardness and crystallinity between the two polymers, with PP having a crystallinity of ~41%, while HDPE is ~62% (Jose et al., 2004; Dos Santos et al., 2013). PP, being a softer plastic, would bend and twist more before fragmenting than HDPE. This can be clearly seen in the SEM images, as PP has a curling and almost fluffy appearance, while PE is rigid and angular. In contrast to the initial hypothesis, it would then be expected that PP would have more surface area because of its highly textured appearance and, indeed, that is the case (Table 2.1).

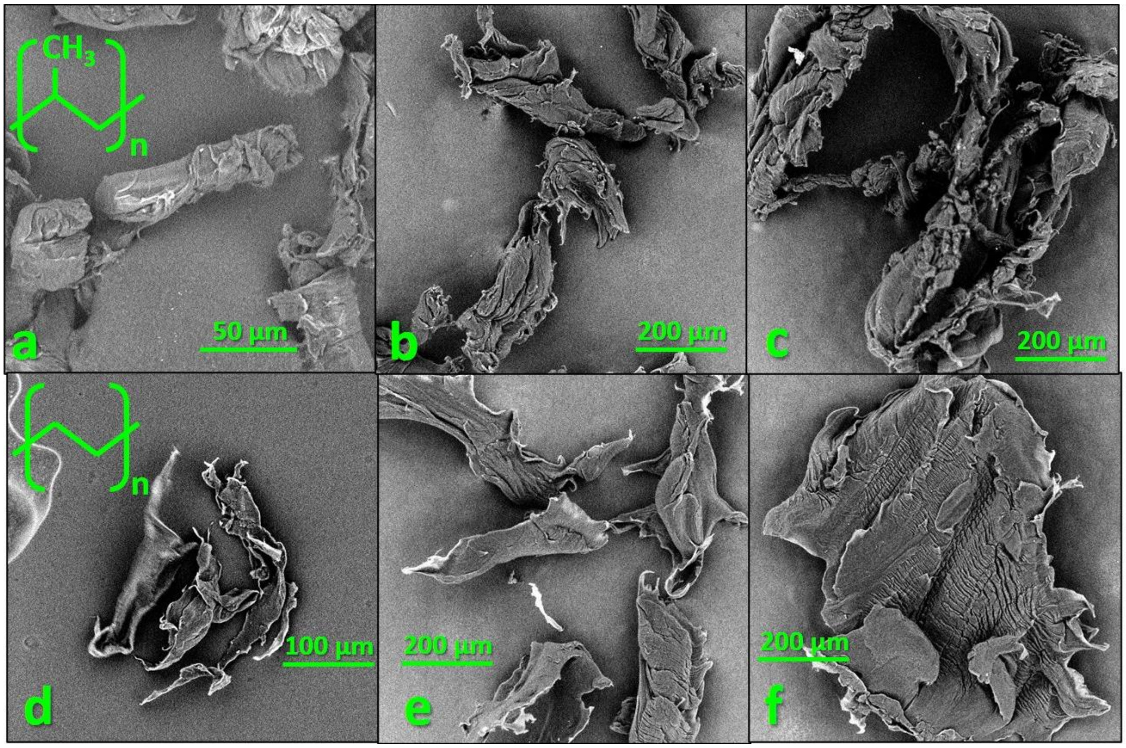


Figure 2.1. SEM images and molecular structures of select MPs: 45, 212, and 500 µm size classes of PP (a, b, and c, respectively), and the same size classes for HDPE (d, e, and f, respectively).

As previously stated, the BET surface area measurements using nitrogen were inaccurate. BET plots with nitrogen were non-linear (Appendix 3, Figures 1-4), and high linearity is required for the analysis (r^2>0.9975), which was achieved with krypton gas (Appendix 3, Figures 5-8). Krypton produced more accurate data because its saturation pressure is ~1/300 that of nitrogen at 77 K, and therefore the free space above the sample contains ~1/300 the number of krypton molecules at the same relative pressure (Webb and Orr, 1997). The consequence of using krypton instead of nitrogen, is that the error due to free space is lowered by ~300x, which makes krypton ideal for relatively low surface area materials (Webb and Orr, 1997). Of the nine size classes, only 150, 212, and 500 µm MPs were analyzed using nitrogen, and these will be used to discuss the measurement differences (Table 2.1). Measuring with krypton revealed that 150 µm HDPE MPs were largely overestimated by roughly a factor of nine, 212 µm HDPE MPs were underestimated by ~1.25 times, and 500 µm HDPE MPs were overestimated roughly 2 times when using nitrogen. For PP MPs, a similar trend was observed, with the smaller particles being underestimated. As far as the author is aware, there are no studies which have measured the surface area of PP MPs, and none that have compared different sized particles and measured their surface area accurately. Some studies have suggested that BET with nitrogen may underestimate surface area due to the capillary condensation and the diffusion-limited access of nitrogen at very low temperatures (Pignatello, 1998; Guo et al., 2012; Hüffer and Hofmann, 2016; Liu et al., 2019). However, Hüffer and Hofmann (2016) using nitrogen as the adsorptive gas found that PE and PS MPs of similar diameter had similar surface area; despite that it has been observed that glassy polymers have numerous internal pores or “nanovoids” (Teuten et al., 2009, Pignatello et al., 2006), which would significantly increase surface area (Hartmann et al., 2017). Others (Wang and Wang, 2018; Liu et al., 2019; Zuo et al., 2019) have also found that PE MPs had greater surface area than PS MPs. Underestimation of surface area may be a systematic error when using nitrogen as an adsorptive gas for BET analysis of some MPs.

Table 2.1. Characteristics of fabricated MPs. Surface area (SA) data presented use either krypton or nitrogen as the adsorptive gas during BET analysis; all means are calculated from triplicates unless otherwise noted. Nitrogen data is in italics to notate that the data are considered incorrect.

Polymer Type	**Crystallinity %**	**Diameter µm**	**SA using Kr** $m^2\,g^{-1}$	**SA using N₂** $m^2\,g^{-1}$
HDPE	62^	150-212	0.2991 ± 0.0076	*2.8076*
HDPE		212-250	0.2016 ± 0.0074	*0.1608*
HDPE		500-600	0.1315 ± 0.0071	*0.2571*
PP	41^	212-250	0.2953 ± 0.0074	*0.2632 ± 0.0276**
PP		500-600	0.2128 ± 0.0066	*0.2552 ± 0.0489**

Note: Error is standard deviation. ^Averaged from Jose et al., 2004 and Dos Santos et al., 2013. *Duplicates.

The major objective for this study was to determine if standardization of the reference material on a surface area to mass basis ratio was feasible. This was considered successful if the coefficient of variation (CV) <5% for individual size classes. This criterion was met by all size classes except 425 µm PP, and 425 and 500 µm HDPE; however, their variation can still be considered relatively low. The CV for 425 and 500 µm HDPE were 7.5 and 5.4%, respectively; while, 425 µm PP was 11%. If these can be considered outliers, the mean CV for PP was 3.1 ± 0.9%, and 3.2 ± 0.6% for HDPE. The 45 µm size class for both polymers only had one replicate due to insufficient material, however, given that the CV for all size classes below 425 µm had CV ~3%, the presented data seems a reasonably accurate representation of this size class's surface area. Therefore, standardization on a surface area to mass was achieved.

Out of the non-linear functions tested (e^x, logx, lnx, and power – ax^b), the power function (Figure 2.2; HDPE model: SA =$13.224x^{-0.764}$, $r^2 = 0.9704$; PP model: SA =$3.4909x^{-0.461}$, $r^2 = 0.9331$) was the best model fit for both PP and HDPE. As hypothesized, surface area increased nonlinearly with decreasing size class. As previously discussed, PP MPs had significantly greater surface area than HDPE. However, this trend stopped for the two smallest size classes, and PP and HDPE surface areas became more similar. At some particle size below 212 µm, PP MPs began to deform more under mechanical force during fabrication, giving them a more regular and rounder shape, while harder HDPE continued to fragment irregularly. This can be seen from the SEM images show (Figure 2.1, a and d) that the 45 µm MPs of PP are rounder and pellet like, while HPDE MPs are flatter, angular, and irregularly shaped (see Appendix 2 for more images). This makes sense because rounder shapes have less surface area because of the surface area to volume ratio of spheres (Figure 2.3). This trend may continue for MPs smaller than 75 µm, with PP becoming more spherical. Whether HDPE MPs continue to fragment irregularly, or also converge on a rounder shape, was not established in this study.

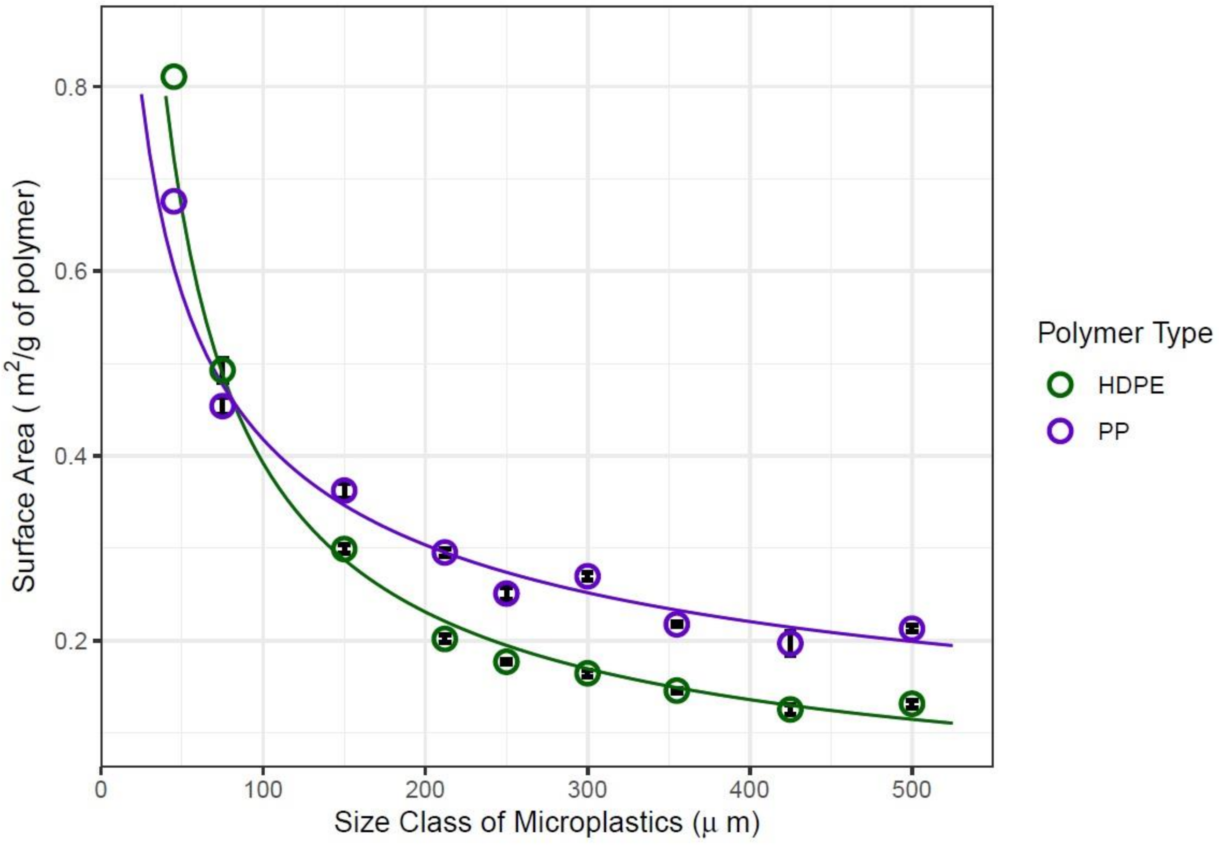


Figure 2.2. SA measured by BET with krypton as the adsorptive gas of all 9 sizes classes of HDPE (green) and PP (purple) MPs. All means calculated from triplicate and error is standard error; except HDPE 75 µm size class contains two replicates, and the 45 µm size class of both polymers has only 1 replicate, due to insufficient material. HDPE model (green line): SA $=13.224x^{-0.764}$, $r^2 = 0.9704$. PP model (purple line): SA $=3.4909x^{-0.461}$, $r^2 = 0.9331$.

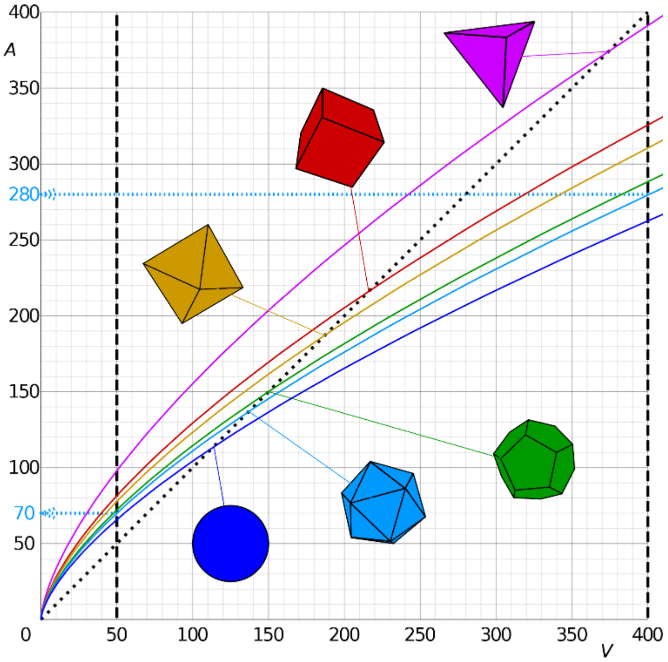


Figure 2.3. Graphs of surface area, A, against volume, V, of 5 regular solids a sphere; showing that surface area decreases for rounder shapes, and that the surface area: volume ratio decreases with increasing volume. As an example, the intercepts with the light blue dashed lines show that when the volume increases 8 (2^3) times, the surface area increases 4 (2^2) times. Image taken from https://en.wikipedia.org/wiki/Surface-area-to-volume_ratio, no changes were made to the image, license: https://creativecommons.org/licenses/by-sa/3.0/deed.en.

Limitations of the Reference Material

MPs of the PP reference material have many folds that create a curling and fluffy-looking texture; while HDPE MPs are rigid, angular, and have minimum folding (Figure 2.1). It would be expected that these differences would most likely be observed of PE and PP fragments found in the field. Though the MPs made here were cooled with liquid nitrogen before fabrication, the fundamental mechanical forces should still apply, i.e, lower crystallinity polymers should fold/bend more under physical pressure rather than fragmenting. However, most PE and PP MPs found in nature contain additives that will have modified the materials’ plasticity and other properties. LDPE, for example, has a similar crystallinity to PP (Pedroso and Rosa, 2005), and therefore would be expected to also create highly textured MPs unlike HDPE. Therefore, it cannot be expected that all PE and PP debris will fragment in the same way as described here.

The fragment reference material created here of PP and PE do not mimic rod like or other sub-round and round type fragments, except for the 45 and 75 µm size classes of PP (Rochman et al., 2019). They have not been treated with ultraviolet (UV) radiation or any other type of chemical degradation, nor have they been allowed to form a biofilm. UV radiation changes the physical structure of polymers. For example, PE, for example, degrades through UV radiation induced free radical reactions in the polymer chain and is the main mechanism for PE degradation (Guadagno, 2001). Therefore, unique surface microstructure due to these abiotic and biotic processes are absent from this study, but remain very important components in real environmental samples. In the same vein, microstructure and shape due to the mechanical and cleanup processes used to fabricate these particles may or may not be represented in the field. In consequence, their surface area may also be different when compared to PE and PP fragments found in the environment. Indeed, the reference material may have less surface area than those in the field due to not being exposed to the diverse array of biophysicochemical forces that occur in nature, especially UV radiation, as it is known to increase the surface area of polymers (Liu et al., 2019). A future investigation should be conducted to determine the structural (e.g., shape, size distribution, surface area) differences between the fabricated reference material described here and UV degraded material versus “natural” MPs found in the field.

The presented approach is not meant to be used as a standard operating procedure blindly. Instead, researchers using this reference material fabrication method may create slightly differently structured particles from using various mill files, smaller or larger force when grinding, angle of contact of the file with the polymer rod, etc. Therefore, MPs made using this method should always be analyzed for surface area and imaged to visualize shape and surface structure; i.e., standardization on a surface area to mass ratio should always be confirmed. This method of fabrication is somewhat labor intensive. Four volunteers working about 6 hours each can make ~30 grams of material; but smaller particles are less numerous, especially the 45 and 75 µm size classes. To overcome this, jewelers' files with much smaller grit were used, but the diamond coating that created the finer grit came off during grinding and contaminated the material (grit coating is glued onto the file). Regrinding larger MPs in a coffee grinder with dry ice was also tried, but the material only floated around the chamber and did not fragment. It is possible that an electric grinder may be successful if it was continuously cooled by liquid nitrogen, however, it should be noted that these systems are quite costly.

Conclusions and Future Directions

The fabrication method of reference MP material and standardization using the surface area: mass basis was successful. PP has lower crystallinity than HDPE, and therefore deformed more during fabrication, creating particles with more surface area. However, this trend did not continue for the two smallest size classes, 45 and 75 µm, because PP formed into more round, pellet-like particles, while HDPE continued to fragment into irregular, angular particles. Future work should aim to create a more continuous data set for surface area and MP particle size, especially for smaller particles

(<45 µm). Krypton gas should also be used when measuring surface area of MPs with BET method due to the possible underestimation of surface area when using nitrogen. More sophisticated modeling statistics could then be used, such as fitting the model with the maximum likelihood method. This method would provide uncertainty of the estimation/model and would be more helpful for further inference and application. Equilibrium sorption experiments with organic pollutants should then be conducted with this continuous data set of smaller particle size classes to determine if sorption will continue to increase with surface area and smaller particles sizes, or if there is a threshold for sorption (see Chapter 2 for more discussion on surface area and sorption). This may help determine relative ecological risk for different MP particle sizes and sorbed organic pollutants. Furthermore, particle size distribution within size classes should be analyzed, as a) size distribution may be additionally useful in predicting the surface area of environmental MPs, and b) may be helpful for toxicity experiments with organisms that have particle size restrictive feeding modes. In the same vein, though single particle diameter may vary, the average particle diameter per gram should not. It is worth investigating whether average particle diameter within individual size classes is significantly correlated with surface area. If average particle diameter is correlated well with surface area, there may be no need to measure surface for future experiments using this fabrication methodology as sorption should be inversely correlated with average particle diameter.

To enable experiments on smaller size classes, future work should aim at an automated process that could simply make more material to achieve more of the smaller size classes with as uniform as possible surface area. Robotics are increasingly being used in research, therefore another approach besides using costly internally cooled grinders could be to develop a robot that can grind/shave polymer rods using the fabrication method developed here. Even if the robot is not necessarily faster, it could be left on for days at a time, creating more material to work with. Eventually, these methods should also be tried with polyvinyl PVC and PS to determine if low surface area variation within size classes could also be achieved with these different polymers.

Future work should also aim to characterize the different MP shapes and surface morphologies of PE and PP MPs found in the field to inform the creation of a better reference material. There are many types of MP types such as fibers, spheres, pellets, films, and foams (Rochman et al., 2019). The relationship shown here (Figure 2.2) between surface area and particle class size may not hold for rounder (more sphere-like) MPs, such as spheres and pellets. Due to their rounder shape, spheres and pellets would be expected to have less surface area and would not sorb as many organic pollutants or support relatively large biofilm formation. While, films and fragments would be expected to have relatively larger surface area and may support a larger biofilm, along with greater pollutant load. Changes in surface area that affect biofilm formation may also change the bacterial communities (speciation) on MPs (Endo et al., 2016; Rochman et al., 2016; Rist and Hartmann, 2018; Muthukumar et al., 2011; Zettler et al., 2013; Jahnke et al., 2017; Rummel, 2017). This may be especially important for MPs in wastewater, where bacterial and contaminant load is significant. Therefore, the fragment type MP reference material made here only represents a single fraction of PE and PP MPs found in the environment. However, despite the discussed limitations, however, this reference material may: 1) act as a more realistic material than purchased powders or beads, 2) provide a more readily comparable reference material for fragment type MPs of PE and PP, and 3) serve as a clean starting material that can then be subject to exposure and/or biologicals prior to use in experiments.

Chapter 3: Sorption of the endocrine disruptor triclocarban to microplastics: extraction methodology and effects of particle size

Background and Rational

WWTPs are a significant pathway to waterways for MPs and PPCPs (Ratola et al., 2012; Margot et al., 2015; Subedi and Loganathan, 2016). Unfortunately, the equilibrium of PPCPs with plastic debris have not been well characterized. Additionally, as far as the author is aware, no survey of sorbed PPCPs on MPs from wastewater effluent has been conducted to date. However, there have been studies that have measured this relationship in the laboratory. A variety of PPCPs are less hydrophobic than hydrocarbons due to the presence of polar moieties, but still have relatively high octanol-water partition coefficients (K_{OW}); and have been found to sorb onto MPs to concentration magnitudes higher than the surrounding water (Wu et al. 2016). Wu et al., 2016 examined the distribution of 4 PPCPs into PE MPs from water (Table 3.1). Using a batch equilibrium approach, artificial seawater was spiked with standards of the 4 compounds and measured over 168 hours. Their distribution coefficients, K_d showed that the U\v filter 4-methylbenzylidene camphor had the highest affinity for PE, then triclosan, 17α-ethinyl estradiol, and carbamazepine . The K_d ’s presented here are higher than the K_d values for sediments presented by Teuten et al., 2007; evidence that PPCPs may preferentially bind to PE MPs in the environment. The K_d ’s were also correlated with their K_{OW} (r2=0.83), suggesting more hydrophobic PPCPs will accumulate to greater concentrations (Wu et al. 2016).

Table 3.1. Characteristics of fabricated MPs. Surface area (SA) data presented use either krypton or nitrogen as the adsorptive gas during BET analysis; all means are calculated from triplicates unless otherwise noted. Nitrogen data is in italics to notate that the data are considered incorrect.

Compound	**K_d (L/kg)**	**r^2**
Carbamazepine (CBZ)	191 ± 6.4	0.991
17α-ethinyl estradiol (EE2)	312 ± 21.5	0.959
4-methylbenzylidene camphor (4-MBC)	53,225 ± 3715	0.957
Triclosan (TCS)	5140 ± 290	0.973

To further fill in knowledge gaps, the endocrine disruptor and antimicrobial triclocarban (TCC), was chosen for these sorption experiments. TCC is found in soaps, plastics (e.g., children's toys), and lotions (Rochester et al., 2017). Antimicrobials are endocrine disruptors that can cause adverse immune responses and negatively affect the human reproductive system (Ahn et al., 2008, Boberg et al., 2010, Dann and Hontela, 2011, Smith et al., 2013; Vimalkumar et al., 2019). TCC is often studied with its analog antimicrobial, triclosan, however, TCC is not as well researched for its toxicity (Vimalkumar et al., 2019), and even less is known about its interaction with MPs in the environment. TCC is found at the ppb level in wastewater, which is a major source of TCC to the aquatic environment (Margot et al., 2015). Environmental concentrations have also been observed at ppt level in surface waters (Vimalkumar et al., 2019), and has been observed as high as 5.6 ppb in river water in the U.S. (Halden and Paull, 2005). TCC (Table 3.2) is hydrophobic and is easily sorbed in natural sediments, suggesting that it may also preferentially bind to PE and PP MPs (Venkatesan et al., 2012, Tamura et al., 2013; Vimalkumar et al., 2019). The U.S Food and Drug Administration has banned 19 antimicrobials, including TCC, in over-the-counter consumer wash products (e.g., body wash) in 2016 (FDA, 2016). However, the ban in hand sanitizers has only just passed in April 2019 and TCC is still being used globally, especially in Asia (Fan et al., 2019).

Another understudied aspect of MP sorption of organic pollutants is the effect of particle size, or surface area, on the distribution of these compounds. The surface area of sorbents (e.g., organic matter and plastics) is well known to significantly affect the sorption of organic pollutants, metals, and biofilms in the environment (Endo et al., 2016; Rochman et al., 2016; Rist and Hartmann, 2018). It is also hypothesized that weathered MPs may be more effective vectors of organic pollutants than virgin production pellets, beads, powders, and other unweathered polymer materials mainly due to an increase in surface area, as weathering has been shown to modify surface properties (Endo et al., 2005; Teuten et al., 2007; Ogata et al., 2009; Hartmann et al., 2017; Zhang et al., 2018; Liu et al., 2019). Microplastics in the environment have irregular shapes (Rochman et al., 2019) and particles with irregular shapes are expected to have higher surface area, and thus have higher sorption of pollutants (Brennecke et al., 2016). This is due to the higher surface area-to-volume ration (SA:V) of smaller particles (Velzeboer et al., 2014; Li et al., 2019; Wang et al., 2019), and that this ratio decreases for rounder shapes. Additionally, many aquatic organisms (e.g. larvae, filter feeders) will only consume particles in a certain size range, and therefore will not consume MPs above or below a certain particle diameter (Setälä et al., 2018). Quantifying how particle size affects the sorption of organic pollutants to MPs is therefore important to understand what MPs are of most concern, and in consequence what organisms may be more at risk. The sorption chemistry of PE MPs and organic pollutants, such as polyaromatic hydrocarbons and polychlorinated biphenyls, has been well researched, and has largely been observed to be the better sorbent of hydrophobic organic pollutants out of the most common polymers that are observed in nature and in wastewater, e.g., PE, PP, polystyrene (PS), and polyvinyl chloride (PVC) (Teuten et al., 2007; Karapanagioti and Klontza, 2008; Lee et al., 2014; Rochman et al., 2013). However, PP is found at equal amounts when compared to PE in the environment and is much less studied, especially for PPCPs.

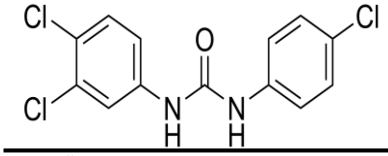
Currently, as far as the author is aware, there is no methodology for measuring semi-polar compounds, like TCC and many PPCPs, sorbed to MPs. Though extraction from MPs is not necessary for isotherm experiments, it is currently unknown whether MPs from wastewater effluent contain environmentally relevant concentrations of PPCPs, though it is highly suspected. The methodology presented here could then be used to extract PPCPs from MPs found in wastewater effluent. Thus, the objectives of this study were to: 1) create an extraction method for PPCPs from MPs; 2) validate the extraction method with a highly replicated, single batch sorption study; and 3) determine the differences in sorption of TCC to HDPE and PP MPs of two different size classes (MPs made using the method were discussed in chapter 1). It was hypothesized that: 1) MPs with larger SA, rather than size class, would sorb more TCC; and 2) that HDPE would have greater SA then PP, and thus sorb more, as polyethylene had generally been found to sorb more organic contaminants (Teuten et al., 2007; Karapanagioti and Klontza, 2008; Lee et al., 2014; Rochman et al., 2013).

Methods

Chemical Reagents

TCC (Table 3.2) was purchased from Sigma-Aldrich, USA. ^{13}C-labeled TCC was used as a surrogate for recovery and was purchased from Wellington Laboratories, Canada. Deuterium-labeled TCC-d4 was used as an internal standard for ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC/MS/MS) analysis and was purchased from CDN Isotopes, Canada. All reagents had >98% purity. All solvents used for UHPLC/MS/MS were HPLC grade and purchased from Fisher Scientific International, Inc. All deionized (DI) water used during the sorption experiments was filtered through a Hydro Analyzer Water System 200, which produces water with a resistivity of >18Mohms. All stock solutions were made in methanol, stored at -30˚C, and kept in the dark. MPs used in these experiments were previously characterized for surface area, please see Chapter 2 for more details.

Table 3.2. Structure and selected properties of the antimicrobial and endocrine disruptor, triclocarban.

Compound Structure	**Compound Name**	**Use**	$\log K_{OW}$	pK_a	**Typical Sanitary Wastewater Influent Conc. (ng/L)**
	Triclocarban (TCC)	Antimicrobial	4.90[#]	12.7 *	700^

[#]Moffat et al., 2004; *Loftsson et al., 2005; ^Margot et al., 2015.

Extraction of Triclocarban from MPs

Acetone was used as an extraction solvent as TCC and many PPCPs are highly soluble in acetone; additionally, acetone swells polymers, which is needed for extraction from MPs. However, acetone is usually not preferred as a mobile phase in UHPLC columns, and extracts would need to be evaporated and reconstituted in methanol. Methanol was compared to acetone to determine if it was suitable in order to avoid the reconstitution step. However, methanol was found to extract 30% less TCC than acetone, thus acetone was used for extraction. Next, the number of acetone extractions was determined by three sequential extractions of PP and HDPE loaded with TCC. Specifically, five replicates each of 10 ± 1 mg of the 150 µm size class of HDPE and PP MPs were added to 60 mL amber glass vials, along with 50 mL of DI water and 5µg of TCC, and covered with aluminum foil lined Teflon caps. To minimize the cosolvent effect, the methanol/water v/v was kept <0.01%. The vials were placed on an ATR Rotamix Rotator, which inverts samples so that MPs were evenly exposed to TCC. Rotating speed was set at 50 rpm and held at 25 ± 2 ˚C for five days to allow equilibrium to occur. After five days, a modified method from Lee et al. (2014) was used where the MPs were removed by filtering through a pre-weighed 0.45 µm Whatman GF/C glass microfiber filter using vacuum filtration. About 5 mL of DI water was used to rinse the MPs of test solution, as well as the sides of the Buchner funnel. Filters were then placed in pre-weighed aluminum pans and covered in aluminum foil to dry for ≥48 hours at room temperature. Once dry, the filters were carefully folded closed with stainless-steel forceps (Appendix 4 Figure 1) and transferred to 20 mL amber glass vials. 10 mL of acetone was added to the vial, and a portion of this volume was used to rinse the aluminum pan of any stray particles. 600 ng of ^{13}C-labeled TCC was added to calculate recoveries. The samples were then placed in an ultra-sonicator for 30 min. After sonication, a 5 mL aliquot was taken from the sample, and 5 mL of fresh acetone was replaced. This 2nd extract was also sonicated for 30 min. The process was then repeated a third time. All three, 5 mL aliquots taken from the sequential extractions were evaporated to near dryness under a gentle nitrogen stream at 5 psi and then reconstituted with 2 mL of methanol. Mass extracted during each sequential extraction was calculated, and it was determined that almost all TCC was recovered during the first extraction (extraction efficiency >87%). The TCC concentration in MPs were then measured using only one extraction for the remainder of the experiments.

Kinetic Experiment

The sorption kinetics of TCC was measured to establish the minimum time required for equilibrium to occur. This experiment allowed the verification of the feasibility (i.e., variation) of using fabricated reference MPs for sorption experiments. A batch sorption experiment was conducted with 100 µg TCC/L in DI water over 5 days with 150 µm size class HDPE. Smaller particles than those used for the sorption isotherms (212 and 500 µm) were used for the kinetic experiments to determine a conservative estimate of equilibrium time. Samples were taken in triplicate at 2, 4, 8, 12, 20, 24, 48, 72, and 120 hours. Specifically, 10 ± 1 mg of HDPE was added to 60 mL amber glass vials, along with 50 ml of DI water and 5 µg of TCC. Experimental conditions were the same as during the sequential extraction study. After the 120 hours, water samples were taken before MPs were removed from the test solution. A glass syringe was used to take a 1 mL aliquot of water that was transferred to a 1.5 mL HPLC autosampler vial. Extraction of MPs was the same as described above.

Analysis of Triclocarban

A Shimadzu Nexera X2 ultra high-performance liquid chromatograph (UHPLC) coupled with a Shimadzu 8040 triple quadrupole mass spectrometer (MS/MS) (Shimadzu North America, Columbia, MD, USA) equipped with an ESI- source was used for the analysis of TCC. For full method information see Appendix 4, but briefly, the UHPLC was equipped with an ACQUITY UHPLC HSS T3 C18 reverse phase column (1.8 µm, 2.1 x 100 mm; Waters Corporation, Milford, MA, USA) and run isocratically using a mobile phase of 10 mM ammonium acetate in a solution of methanol: acetonitrile: water (60:15:25 v/v). Water samples were injected as is for the kinetic experiment. Both extracts and water samples received 120 ng TCC-d4/mL internal standard immediately before analysis. The regression coefficients, r^2, of the standard curves for TCC and ^{13}C-labeled TCC were all >0.995. Loss of TCC to the vials was determined to be ~9%. All recoveries for TCC extracted from MPs were between 111-122%. Data were not recovery corrected. For the 212 µm PP isotherm, the nitrogen evaporator was very briefly turned on >5 psi, resulting in some sample loss and having recoveries <50%. Due to this known problem, outliers were removed, but data was no longer appropriate for modeling a sorption isotherm of 212 µm PP and TCC; however, this did not change the overall trend (Figure 3.2).

Sorption Isotherms of Triclocarban

The isotherms were made of five points (concentrations): 20, 40, 60, 80, and 100 µg TCC/L, at three replicates each, for HDPE and PP MPs of sizes 212 and 500 µm size classes. The isotherms were at lower concentrations than previous work on organic carbon sorption to determine distribution in a more environmentally relevant concentration scenario. The experiment was conducted using the batch equilibrium approach described above, and held for 48 hours (the equilibrium time found in the kinetic experiment, Figure 9). For a step by step protocol of extraction and isotherm experiments, please see Appendix 4.

Modeling of TCC Sorption Isotherms

TCC sorption was modeled and parameters estimated using R Statistical Computing software (R Core Team, 2018) and the “SorptionAnalysis” package (Chattopadhyay, 2017). A linear model and the Freundlich equation were used to model sorption. The linear model is described as:

$$C_{MP} = K_{MPw} \times C_w \tag{1)}$$

where K_{MPw} (L water /Kg plastic) is the distribution coefficient of TCC from water to plastic, and C_{MP} (mg TCC/Kg of MP) and C_w (mg TCC/L of water) are the concentrations at equilibrium. The Freundlich equation is described as:

$$C_{MP} = K_F \times C_w^{\frac{1}{n}} \tag{2)}$$

where K_F (L water /Kg plastic) is Freundlich’s constant and n is the exponent of non-linearity. The value of C_{MP} was determined via UHPLC/MS/MS, while C_w for the isotherms were calculated by mass balance. Data were not recovery corrected. All error is reported as standard error.

Results and Discussion

Extraction Method

The purpose of the kinetic experiment was not only to determine equilibrium time, but also to validate the created methodology used to extract TCC from MPs. Extraction efficiency determined during development was high for both PP and HDPE, 88.4 ± 0.776% and 88.3 ± 0.147% (error is standard deviation), respectively. The variation of extracted TCC in the kinetic experiment was low and consistent between samples of MP extracts (Figure 3.1a). If this extraction were to be used for wastewater samples (or any other field application), it should not be used for compounds that are either a) highly soluble in water and/or b) are ionic at environmental pH. For example, triclosan is considered hydrophobic, however, due to its pK_a (~8) a large fraction would be ionic in wastewater effluent and many natural waters. This methodology also requires multiple rinsing steps with DI water, and appreciable loss in recovery may result if less hydrophobic compounds are quantified. Before attempting field measurements, it is additionally recommended that a spike and recovery test be conducted for each analyte of interest. Additionally, MPs should not be peroxide treated or otherwise solvent cleaned before extraction, as this could remove analytes of interest. Pollutant load will therefore reflect not just the MP, but also what was contained in any biofilm layer that is present. Currently, as far as the author is aware, there is no way of separating organic pollutants of interest in the biofilm from the MP. However, it can be argued that the MP is a vector for the biofilm, and therefore any organic pollutants contained in the biofilm should be included with the measurement.

Kinetic Experiment

The equilibrium time found for TCC and 150 μm HDPE MPs was ≤48 hours (Figure 3.1). The kinetics of PP were not measured because PE had previously been observed to sorb more organic pollutants (of similar $\log K_{OW}$) than PP (Teuten et al., 2007; Karapanagioti and Klontza, 2008; Lee et al., 2014; Rochman et al., 2013). Additionally, the 150 μm HDPE MPs were initially shown to have 9x more SA (2.8076 $m^2\ g^{-1}$) than the other two sizes of both polymers (SA of the MPs were originally determined using nitrogen as the adsorptive gas; discussed in chapter 2, Table 2.1).

However, after analysis of the sorption isotherms it was apparent that the SA data were not accurate and so krypton was used instead, despite previous work using the nitrogen method (Hüffer and Hofmann, 2016; Wang and Wang, 2018; Liu et al., 2019). Consequently, using 150 µm HDPE MPs to determine equilibrium time was thought to have been a conservative approach. However, Wu et al. (2016) showed that the equilibrium time of four PCPPs, logK_{OW} 2.45 – 4.76, with HDPE MPs were all ≤48 hours. Zuo et al. (2019) found for phenanthrene (logK_{OW} 4.52) that the sorption to MPs of the non-crystalline polymer adipate coterephtalate was 3x higher than PE, yet both had the same equilibrium time (~12 hours). The equilibrium, then, of TCC and PP should not have been affected, but the overall trend in distribution would not have changed as PP had higher sorption than HDPE for both sizes of MPs.

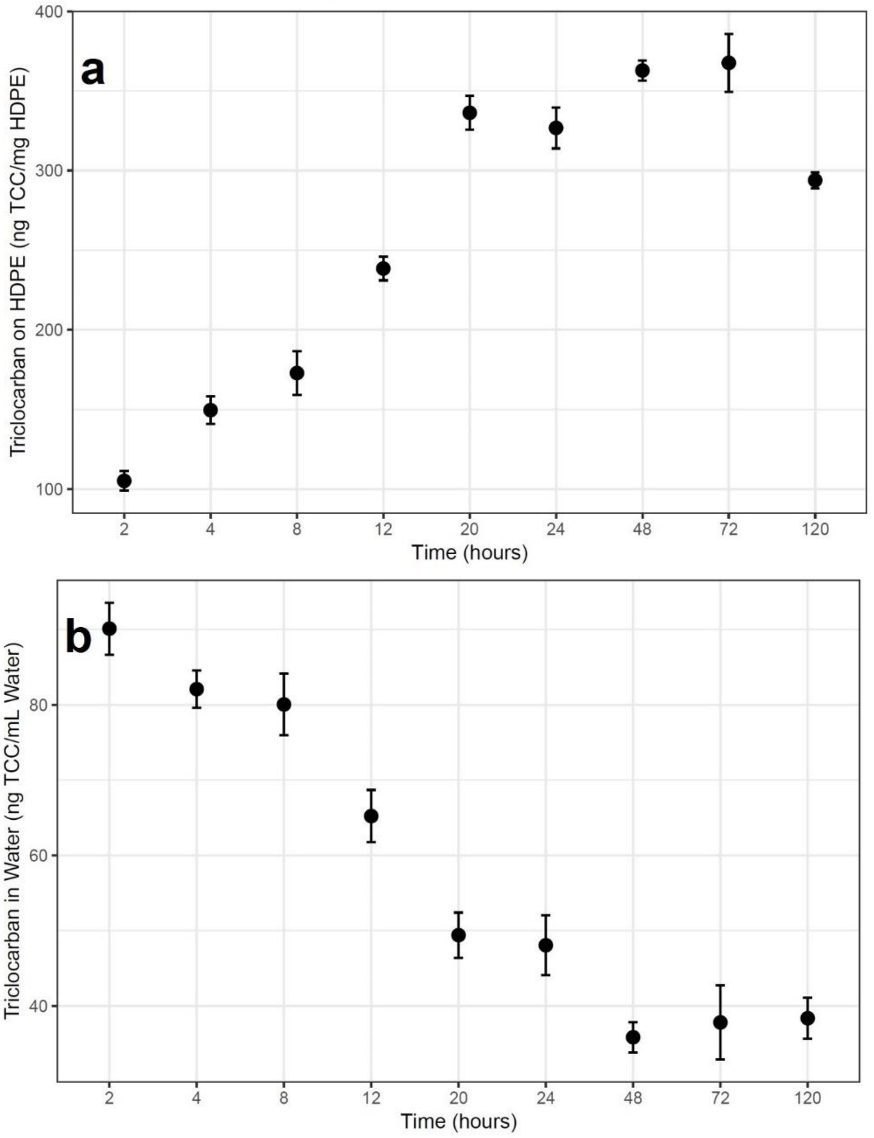


Figure 3.1. Kinetics of TCC sorbing onto 150 µm size class HDPE MPs from water over 120 hours. A) is the TCC concentration in HDPE and B) is the concentration in water over time. Error is standard error.

Sorption Isotherms

The isotherms were non-linear, therefore only the Freundlich curves are shown (Figure 3.2 and Table 3.2). PP MPs of both sizes had significantly more sorption than HDPE. Although the 212 µm PP isotherm could not be modeled due to insufficient data (loss during N_2 evaporation), the overall trend was not affected. Interestingly, despite the losses during evaporation, 212 µm PP had the greatest sorption of TCC. This result was surprising, as HDPE was hypothesized to sorb more TCC. PE is expected to have greater sorptive capacity than PP because of the greater distance between the polymer chains (C-H vs. C-CH_3 chains for HDPE and PP, respectively), which allows molecules to diffuse more easily into the polymer matrix (Pascall et al., 2005; Rochman et al., 2013; Hartman et al., 2017). The greater sorption can then be explained by the higher SA of 212 µm PP MPs (Table 3.3), but also the fact that PP is ~20% less crystalline than HDPE. Both polymers are only capable of non-specific Van der Waals interactions, therefore the only major difference, other than SA, is the amount of amorphous regions within the polymer where nearly all a**b**sorption takes place (Huffer and Hofmann., 2016; Hartman et al., 2017; Tourhino, et al., 2019). This is evident when comparing the 212 µm HDPE and 500 µm PP MPs, because although similar in SA, 500 µm PP had greater sorption. Lee et al. (2014) observed the opposite trend, with planar polycyclic aromatic hydrocarbons (PAHs) and chlorinated benzenes (CBs) sorbing most to LDPE, and non-planar hexachlorocyclohexanes (HCHs) sorbing more to PP. However, it should be noted that low-density polyethylene has a similar crystallinity to PP (42%, Pedroso and Rosa, 2005). In this case, crystallinity was the same, and the volume within the polymer matrix differed. Non-planar HCHs were able to sorb more to PP because they could conform to fit within the polymer chains of PP, while planar PAHs and CBs could not.

Table 3.2. Estimated Freundlich and linear models for triclocarban sorption to MPs. Error is standard error.

MPs	**Freundlich**			**Linear**	
Size & Polymer Type	***log K_F* (L kg^{-1})**	***n***	***r^2***	***log K_d* (L kg^{-1})**	***r^2***
212 µm HDPE	5.17 ± 0.239	1.87 ± 0.152	0.915	4.17 ± 3.18	0.845
500 µm HDPE	6.53 ± 0.616	2.85 ± 0.396	0.796	4.25 ± 3.40	0.715
212 µm PP*	na	na	na	na	na
500 µm PP	4.71 ± 0.232	1.40 ± 0.133	0.895	4.22 ± 3.18	0.880

*Insufficient data to model; please see the section *"Analysis of Triclocarban"* and the discussion.

Table 3.3. Characteristics of fabricated MPs. Surface area (SA) data use krypton as the adsorptive gas during BET analysis; all means are calculated from triplicates. *See Chapter 2 for more detail on SA characterization.*

Polymer Type	**Crystallinity %**	**Diameter µm**	**SA using Kr *m^2 g^{-1}***
HDPE	62^	212-250	0.2016 ± 0.0074
HDPE		500-600	0.1315 ± 0.0071
PP	41^	212-250	0.2953 ± 0.0074
PP		500-600	0.2128 ± 0.0066

Note: Error is standard deviation. ^Averaged from Jose et al., 2004 and Dos Santos et al., 2013.

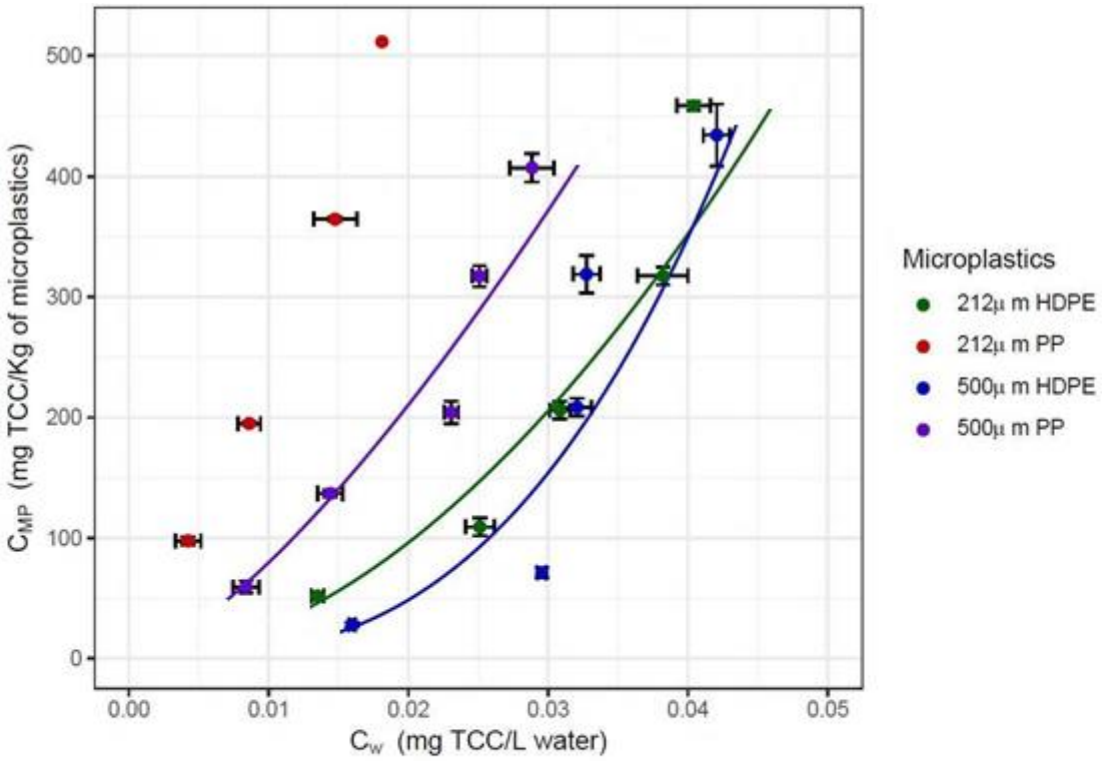


Figure 3.2. Freundlich isotherm model fitting curves for TCC into HDPE and PP MPs. For Freundlich coefficients, see Table 3.2. Error is standard error. 212 μm PP sorption is likely significantly underestimated due to loss during nitrogen evaporation (see methods for discussion).

TCC is a planar molecule, and is therefore expected to both a<u>b</u>sorb and a*d*sorb. A*d*sorption of planar molecules is stronger than for non-planar molecules because more atoms may interact with the surface of the MP and may also be the dominant mechanism for more polar compounds (Onjia et al., 2001; Smiciklas et al., 2000; Huffer and Hofmann et al., 2016; Li et al., 2018; Guo et al., 2018). In addition, the fabricated MPs have a heterogeneous surface, and thus will have different a*d*sorption sites with different energies (i.e., $\Delta E_{adsorption}$) (Huffer and Hofmann, 2016). Frequently what is observed with organic pollutant sorption onto MPs, or other organic sorbents, is that lower energy sites are quickly filled first (Huffer and Hofmann et al., 2016; Razanajotovo et al., 2018). Once all of the lower energy sites are filled, sites with higher $\Delta E_{adsorption}$ are then occupied (Huffer and Hofmann et al., 2016; Razanajotovo et al., 2018). This behavior creates a concave isotherm, where sorption slows at higher concentrations of the organic pollutant as all sites become filled. However, the opposite is seen here and sorption of TCC becomes more favorable at higher concentrations. This may be a type of co-solvent effect, where at low concentrations $\Delta E_{adsorption}$ is relatively high, but as [TCC] increases on the surface, it begins to interact favorably with itself; creating the convex isotherm observed. At environmentally relevant concentrations, PPCPs with polar moieties like TCC may not reach a saturation concentration that is observed with more hydrophobic organic pollutants, like PAHs or PCBs.

It should be noted, however, that the Freundlich equation is restrictive and does not fully explain the observations. The Freundlich isotherm explains a*d*sorption, while a**b**sorption is also taking place, which may explain the less-than-ideal non-linear fits. A less restrictive model fit will likely be much more accurate and the justification for better non-linear fits should be explored in future studies.

The isotherms were also calculated on a SA basis (Figure 3.3 and Table 3.4) to determine if SA was responsible for differences in sorption within polymer groups. 212 µm PP now shows less sorption, but this is due to loss during N_2 evaporation which was discussed earlier. Sorption for the other sizes follow their surface area trends (Table 3.3)., with 500 µm PP having the most sorption per unit surface area, and then 212 µm and 500 µm HDPE.

Table 3.4. Estimated Freundlich models for triclocarban sorption to MPs on a surface area basis instead of mass. Error is standard error.

MPs	**Freundlich**		
Size & Polymer Type	***log*** K_F **(L m^{-2})**	***n***	r^2
212 µm HDPE	2.82 ± 0.239	1.87 ± 0.152	0.915
500 µm HDPE	3.93 ± 0.615	2.85 ± 0.396	0.797
212 µm PP*	na	na	na
500 µm PP	2.38 ± 0.232	1.40 ± 0.133	0.895

*Insufficient data to model; please see the section *"Analysis of Triclocarban*" and the discussion.

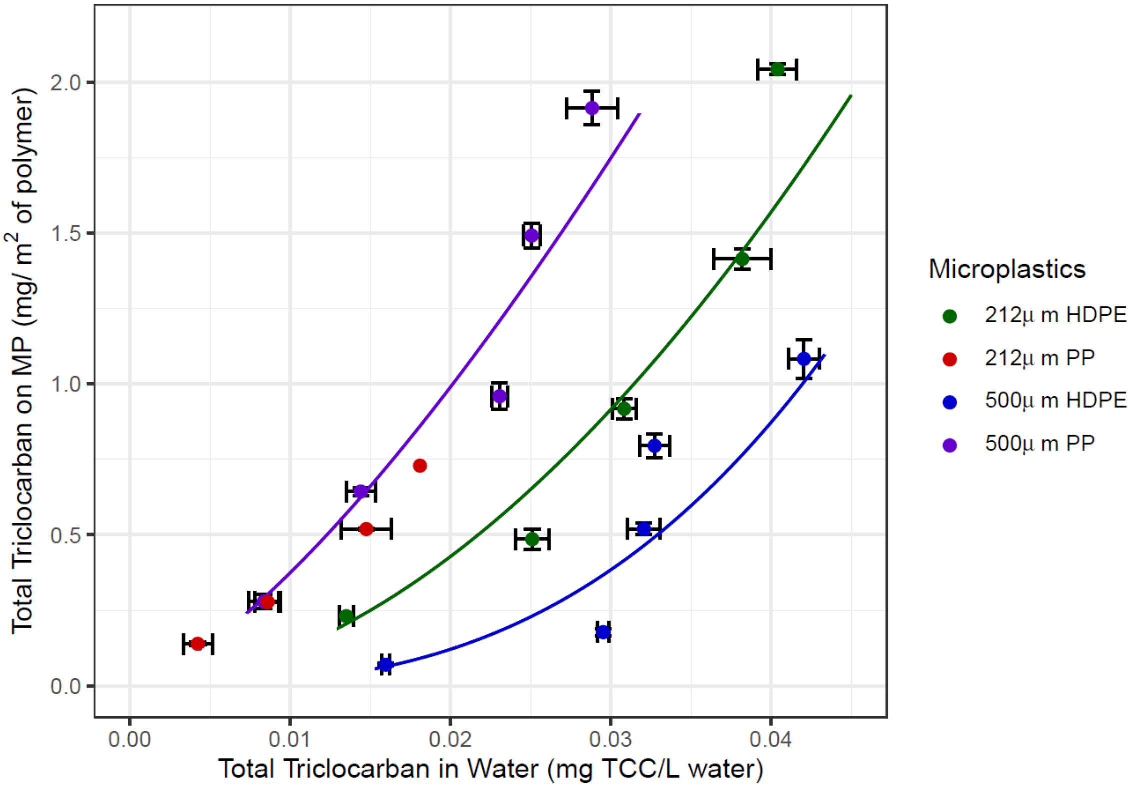


Figure 3.3. Freundlich isotherms for TCC sorption to MPs on a surface area basis instead of mass. For SA data, see Table 3.3. Error is standard error. 212 µm PP sorption is likely significantly underestimated due to loss during nitrogen evaporation (see methods for discussion).

Conclusions and Future Directions

A main objective of this study was to validate an extraction method for PPCPs from MPs that may be used for field measurements. The variation of the sorption of the antimicrobial TCC in a single batch (kinetic) experiment was low and consistent among samples; thus, the extraction method was successful. This method could then be used for monitoring hydrophobic PPCPs associated with MPs found in the environment; but should not be used for more hydrophilic PPCPs. The sorption of TCC to HDPE and PP MPs of two different size classes of known SAs was also investigated. The results showed that PP had significantly greater sorption than HDPE, due not only to its higher SA, but also because PP is a less crystalline polymer. These data suggest that MPs may be a transport vector for TCC from wastewater effluent. Future studies on the sorption of organic pollutants to MPs should control for particle size and measure the SA of MPs studied, as well as exploring non-linear models other than the Freundlich Isotherm. Additionally, surveys of MPs from effluents should include extractions of select samples to begin monitoring of associated PPCPs into aquatic environments.

Chapter 4: Prioritizing study compounds based on environmental relevance

Rational and Background

PPCP Sorption to MPs and the Octanol-Water Partition Coefficient, K_{OW}

PPCP's, such as the antimicrobial triclocarban, may sorb to microplastics (MPs) from sanitary wastewater effluent (Ratola et al., 2012; Lee et al., 2014; Margot et al., 2015; Subedi and Loganathan, 2016; Wu et al., 2016). PPCP laden particles may then travel in the receiving water to estuaries or the open ocean, where they can be ingested by wildlife (GESAMP, 2015; IVL SWEDEN, 2016). However, whether consumed MPs will transfer their sorbed contaminants to organisms and cause harm is still being investigated and is actively debated (Rochman, 2016; Herzke et al. 2016; Rist and Hartmann, 2018; Rochman et al., 2019). Therefore, the next goal would be to design toxicity tests to measure the harmful effects of ingesting MPs with sorbed PPCPs. Given the immense array of pharmaceutical active ingredients (APIs) and cosmetic ingredients (CIs), it is not feasible to measure them all. The testing of APIs and CIs for MP sorption is also a relatively new field of MP research, and toxicity assessments are currently still a future goal (Rochman, 2016; Herzke et al. 2016; Rist and Hartmann, 2018; Rochman et al., 2019). In order to advance the field, and ultimately move toward data that may inform ecotoxicity evaluations, MP sorption of APIs and CIs needs to be evaluated.

It has been shown that, in general, PAHs and chlorinated hydrocarbons will sorb to MPs according to their increasing K_{OW} values (Rochman et al., 2013; Lee et al., 2014). For MPs of PE, PP, and PS it was found that their plastic partition coefficients (equation

1) in seawater (K_{PEsw}, K_{PPsw}, and K_{PSsw}) for PAHs and chlorinated benzenes were within an order of magnitude of their K_{OW} (Lee et al., 2014). K_{MPsw} is defined as

$$C_{MP} = K_{MPsw} \times C_w \qquad 1)$$

where K_{MPsw} (L seawater /Kg microplastic) is the distribution coefficient of an organic pollutant from seawater to a MP of some polymer, and C_{MP} (mg pollutant /Kg of MP) and C_w (mg pollutant/L of water) are the concentrations at equilibrium. Hexachlorohexanes (HCHs), on the other hand, had K_{MPsw} values much smaller (multiple orders of magnitude smaller) than their K_{OW} values (Lee et al., 2014). This is because HCHs have relatively high polarity while the plastic polymers are non-polar (Goss et al., 2008). Wu et al. 2016 examined the distribution of 4 PPCPs commonly found in wastewater (Margot et al., 2015) into PE MPs. Like HCHs, the more polar carbamazepine and 17α-ethinyl estradiol had much lower sorption than the more hydrophobic compounds, triclosan (TCS) and 4-4-methylbenzylidene camphor (Wu et al., 2016). However, the general trend for both studies was that sorption increased with increasing $K_{OW,}$ which is expected.

Since this initial work, the sorption of several more polar PPCPs to PE MPs has been measured (Table 4.1). There seems to be only a very weak correlation between K_{OW} of these PPCPs and their K_{dPE} (the equilibrium distribution coefficient for polyethylene) with PE MPs (Figure 4.1). It can be deduced that other factors besides hydrophobic forces alone are controlling sorption and that K_{OW} alone is not sufficient to explain the sorption of the PPCPs to PE. Knowledge of chemical structure, acid/base behavior, water solubility, and in some cases other salts in solution is also required. TCS has significantly higher sorption than dibutyl phthalate, despite having similar K_{OW}, because TCS has a more hydrophobic carbon backbone, i.e., has amphiphilic tendencies or surfactant-like structure, which would increase interaction with the hydrophobic surface of PE (Smiciklas et al., 2000; Onjia et al., 2001; Huffer and Hofmann, 2016). The same can be said for the sorption of the antibiotics ciprofloxacin and amoxicillin, as ciprofloxacin had higher sorption despite having a lower K_{OW}.

Table 4.1. Selected physical parameters and average sorption distribution constants, logK_{dPE} (L/kg), of various PPCPs with PE. Parentheses indicate that the substance is neutral at most environmental pH conditions. "A" or "B" for polyprotic PPCPs indicated whether the pK_a is for an acid or base group.

PPCP	**LogK_{OW}**	**pK_a**	**log$K_{d\mathrm{PE}}$**	**Study**
ciprofloxacin	0.28	6.10A, 9.2B	1.74	Li et al., 2018
amoxicillin	0.87	2.67A, 7.11A, 9.55B	0.92	Li et al., 2018
carbamazepine	2.45	(13.9)	2.28	Wu et al., 2016
diethyl phthalate	2.47	*nonionic*	-5.03	Liu et al., 2019
propranolol	3.12	9.42	-2.64	Razanajatovo et al., 2018
17α-Ethinyl estradiol	3.67	10.46	2.49	Wu et al., 2016
γ-hexachlorocyclohexane (lindane)	3.72	*nonionic*	2.33	Lee et al., 2014
sertraline	4.30	9.16	-2.48	Razanajatovo et al., 2018
dibutyl phthalate	4.50	*nonionic*	-3.03	Liu et al., 2019
triclosan	4.76	7.90	3.71	Wu et al., 2016
triclocarban	4.90	(12.7)	4.17	*Chapter 3 Results*
4-methylbenzylidene camphor	5.10	*nonionic*	4.73	Wu et al., 2016

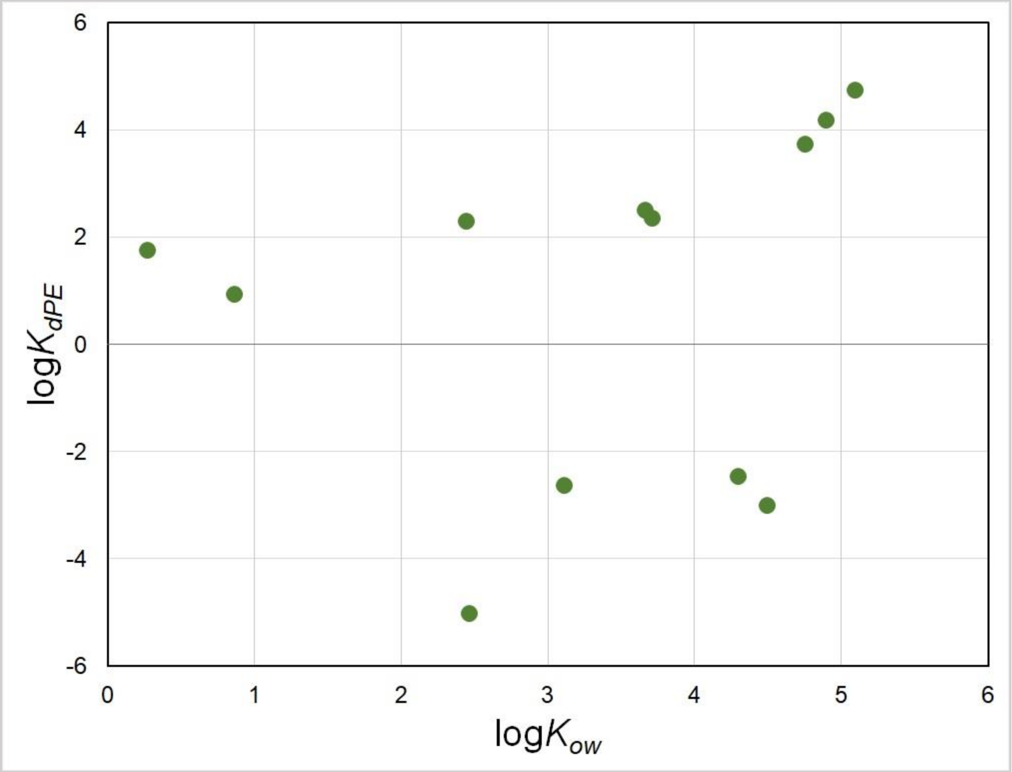


Figure 4.1. The relationship between log K_{OW} and log K_{dPE} (L/Kg) for MP sorption of various PPCPs; data taken from the studies in Table 4.1.

PPCPs with relatively high polarity do not seem to follow the linear correlation between MP sorption and K_{OW} that more hydrophobic substances appear to have (Lee et al., 2014; Wu et al., 2016). This does make sense, however, given that the correlation (logK_{OW} vs. logK_d) is a linear free energy relationship (LFER). LFERs may provide weak correlations for compounds of different polarities between two systems whose phases display very different properties (e.g., octanol/water and polymer/water partitioning) (Schwarzenbach et al., 2003, p. 89). However, despite this limitation, useful LFERs may be created for predicting organic pollutant distribution in the environment (Table 4.2) (Schwarzenbach et al., 2003, p. 91; ECETOC-TR 123, 2013, p. 16). Therefore, a linear model describing the distribution of PPCPs with plastics using K_{OW} should be possible,

$$logK_{MPw} = \alpha logK_{OW} + \beta \qquad 2)$$

where, K_{MPw} (L/Kg) is the sorption (distribution) coefficient of a PPCP with a MP in an aqueous solution, K_{OW} is the octanol-water partition coefficient, and coefficients α and β are constants that may be calculated using experimental data and linear regression analysis.

Table 4.2. Examples of one-parameter LFERs for relating partition constants and/or partition coefficients in different two-phase systems. From Schwarzenbach et al., 2003, p. 91.

Partition Constants/Coefficients Correlated	LFER
Octanol–water partition constant and aqueous solubility of the pure liquid compound	$\log K_{iow} = -a \cdot \log C_{iw}^{sat} + b$
Natural organic carbon–water partition coefficient and octanol–water partition constant	$\log K_{ioc} = a \cdot \log K_{iow} + b$
Lipid–water partition coefficient and octanol–water partition constant	$\log K_{ilipw} = a \cdot \log K_{iow} + b$
Air–solid surface partition constant and vapor pressure of the pure liquid compound	$\log K_{ias} = a \cdot \log p_{iL}^{*} + b$
Air–particle partition coefficient and air–octanol partition constant	$\log K_{iap} = a \cdot \log K_{iao} + b$

The octanol-water distribution ratio, D_{OW}

PPCPs vary widely in water solubility and many API's are acids or bases and are ionic at environmental conditions, i.e., pH 5-9 (ECETOC-TR 123, 2013; p. 57-58). The pH would then affect sorption by controlling the neutral fraction of the compound (Guo et al., 2018; Zhang et al., 2018). MP sorption may than increase or decrease depending on the pK_a of the substance and whether it is an acid or a base. Compounds that have several pKa value complicate the matter. Therefore, a more successful and broader model of K_{OW} and MP sorption may be possible, that is one that can include substances with a range of polarity, if the effects of pH are accounted for in the K_{OW} term. This may be done by estimating the octanol-water distribution ratio, D_{OW}:

$$logD_{OWpH_i} = logK_{ow} - \log\left(1 + 10^{(pH_i - pK_a)\Delta n}\right) \tag{3}$$

where, Δn = 1 for acids and – 1 for bases, D_{OWpHi} is the octanol-water distribution ratio at pH i, and pK_a is the acid dissociation constant of the substance. D_{OW} is a form of K_{OW} that accounts for the pH dependent fraction of the ionizable form of the substance (ECETOC-TR 123, 2013, p. 16, 42). Then, equation 2 would change to:

$$logK_{MPw} = \alpha logD_{OWpH_i} + \beta \tag{4}$$

where, K_{MPw}, D_{OWpHi}, α, and β are as defined above.

The European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) used D_{OW} to better understand partitioning of ionic organic pollutants in sanitary sewage sludge (ECETOC-TR 123, 2013, p. 16, 56). They used organic carbon-water partition co-efficient (K_{OC}) as a representation of the organic matter in sludge, and measured the K_{OC} of 66 ionic compounds (38 pharmaceuticals, 7 industrial chemicals, and 3 biocides) with D_{OW} was modeled using the programs SPARC (SPARC Performs Automated Reasoning in Chemistry) and ACD (Advanced Chemistry Development)/LogP, both of which predict octanol-water partition coefficients from chemical structure (ECETOC-TR 123, 2013, p. 34). Both SPARC and ACD/logP predict K_{OW}, and then calculate D_{OW} according to equation 3. Using equation 4, except with K_{OC} and not K_{MPw}, they found a moderate correlation between D_{OW} and K_{OC} of the ionic organic pollutants (Figure 4.2). The regression coefficient ($r^2 = 0.64$) does not explain all variance, but this is expected as other factors besides hydrophobicity, such as structural differences (see “other cationics” in Figure 4.2), also influence partitioning, as previously discussed here and in Chapter 1. However, the model ($\log K_{OC} = 0.31 D_{OW} + 2.78$, Figure 4.2) was proposed to replace the default assumption that there is zero partitioning into sludge ($K_{OC} = 0$) when evaluating the fate and elimination of discharged organic pollutants using the WWTP assessment tool SimpleTreat 3.1(Franco et al., 2013; ECETOC-TR 123, 2013, p. 59). This resulted in a revision of the tool (now SimpleTreat 4.0; Struijs, 2014; Struijs, 2015), which is routinely applied as part of the European Union System for the Evaluation of Substances regulatory program REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) and is still being used today (Franco and Egmond, 2019). Other agencies that use $\log D_{OW}$ in evaluating bioconcentration potential include the United States Food and Drug Administration (FDA) and the European Medicines Agency (EMA) (ECETOC-TR 123, 2013, p. 19).

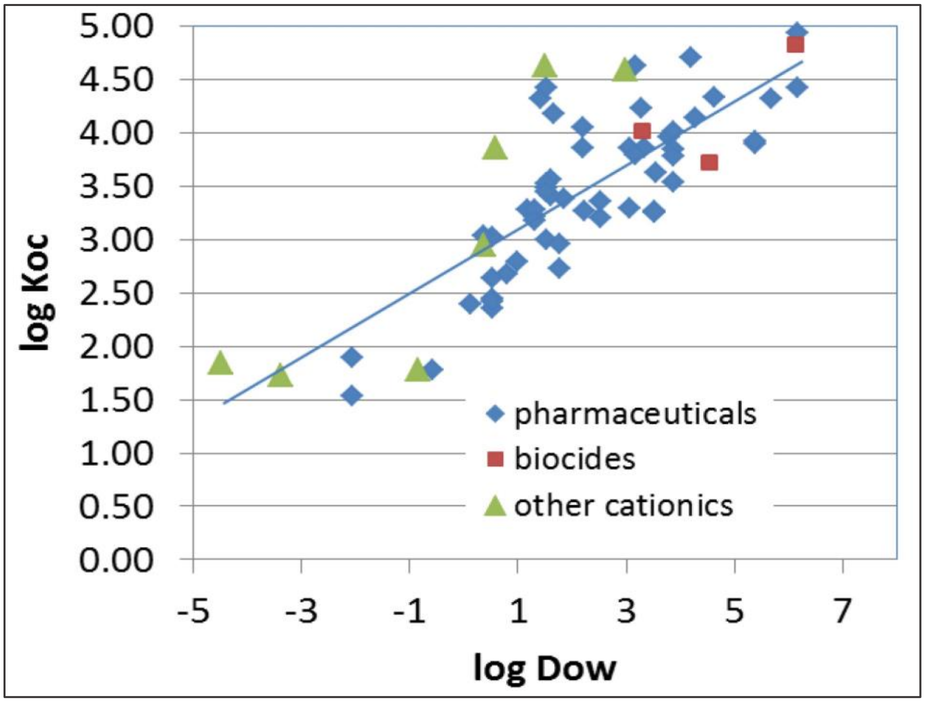


Figure 4.2. D_{OW} was modeled with SPARC or ACD and modeled with measured K_{OC} values at pH 7 of 38 pharmaceuticals, 7 industrial chemicals (labeled as "other cationics"), and 3 biocides; $\log K_{OC} = 0.31 D_{OW} + 2.78$, $r^2 = 0.64$. From ECETOC-TR 123, 2013, p. 59.

A better model for PPCP sorption to MPs may be possible if D_{OW} is used instead of K_{OW} and may help to inform future studies on the toxicity of PPCP laden MPs. Thus, the objectives of this study were to 1) measure the K_d for the polar API's N, N-Diethyl-meta-toluamide (DEET), venlafaxine, and diphenhydramine ($\log K_{OW}$ 2.02, 3.20, and 3.27, respectively) at an environmentally realistic concentration scenario and at pH 7 for HDPE and PP MPs; 2) calculate D_{OW} at pH 7 for these substances and those in Table 4.1 at a range of environmental pH (i.e., pH 5-9); 3) use this data to create a model for PE MP sorption using D_{OW} and measured K_{dPE} values, and the same for K_{OW}; and 4) use these models to make suggestions on how to begin prioritizing future work on PPCP sorption to MPs. It was hypothesized that: 1) sorption to PE and PP MPs for the three compounds from objective 1 would be lower than their log K_{OW}; and 2) MP sorption would be positively correlated with D_{OW} in the calculated model.

Methods

Chemical Reagents

Venlafaxine HCl and diphenhydramine were purchased from Sigma-Aldrich, USA. DEET was purchased from SPEX CertiPrep, USA. D11 venlafaxine HCl (Santa Cruz Biotechnology, USA), deuterated diphenhydramine-d3, and DEET-d6 (Santa Cruz Biotechnology, USA) were used as a surrogate for recovery. Venlafaxine-d6 HCl, diphenhydramine-d5, and DEET-d4/d3 were used as internal standards for ultra-high performance liquid chromatograph-tandem mass spectrometry (UHPLC/MS/MS) analyses and were purchased from CDN Isotopes, Canada. All reagents had >98% purity. See Figure 4.3 for chemical structures, K_{OW}, and pK_a values. All solvents used for UHPLC/MS/MS were HPLC grade, and solvents used during solid phase extraction (SPE) were reagent grade, and were purchased from Fisher Scientific International, Inc. All deionized (DI) water used during the sorption experiments was filtered through a Hydro Analyzer Water System 200, which produces water with a resistivity of >18Mohms. All stock solutions were made in methanol, stored at -30°C, and kept in the dark. MPs used were previously characterized, please see Chapter 1 for more details. Briefly, HDPE and PP were made by grinding production rods (Piedmont Plastics, USA) with a stainless steel mill file after cooling with liquid nitrogen. Shavings were sieved through 212, 150, and 75 µm brass sieves to obtain the needed sizes. MPs were cleaned with 50:50 v/v acetone: hexane and sonicated for 30 minutes. MPs were then rinsed with clean acetone to remove dirty solvent and left to dry at room temperature for 48 hours, and finally dried in a 45˚C oven for 8 hours.

Sorption isotherms of venlafaxine, diphenhydramine, and DEET

The isotherms were originally conducted of 20 - 100 µg PPCP/L, however, no sorption was observed. In order to rigorously evaluate the potential sorption of these compounds, the isotherm points were changed to 200, 400, 600, 800, and 1000µg/L for HDPE and PP MPs of the 150 µm size class. The experiment was conducted using the batch equilibrium approach and held for an equilibrium time of 5 days (120 hours) (Wu et al., 2016; Razanajatovo et al., 2018; Liu et al., 2019). For a step by step protocol of isotherm experiments, please see Appendix 4. Briefly, three replicates each of 30 - 50 ± 1 mg of the 150 µm size class of HDPE and PP MPs were added to amber glass vials, and 20mL of pH 7.5-phosphate buffer and 30 mL DI water added to control for pH; final pH was 7.48 ± 0.02. To minimize the cosolvent effect, the methanol/water v/v was kept ≤0.02%. The vials were placed on a vertical shaker (ATR Rotamix Rotator, USA), rotating speed set at 50 rpm, and held at 25 ± 2 ˚C for 5 days. After the equilibrium time, a 1 mL aliquot of experimental solution was taken with a 5 mL glass syringe. Modified from EPA Method 542, to concentrate analytes and remove salts from phosphate buffer, the solution was passed through an HLB SPE cartridge, 6cc (200mg) (Waters Oasis Cat. No. WAT106202). A SPE cartridge was conditioned with 2 × 5 mL DI water and 2 × 2.5 mL 50:50 v/v methanol: acetone. PPCPs were then eluted from the cartridge with 2 × 2.5 mL 50:50 v/v methanol: acetone. Eluent was evaporated to near dryness under a gentle nitrogen stream at 7 psi (~40 min) at 50˚C, and then reconstituted with 1 mL of methanol and analyzed by UHPLC/MS/MS.

Analysis of PPCPs

A Shimadzu Nexera X2 ultra high-performance liquid chromatograph (UHPLC) coupled with a Shimadzu 8040 triple quadrupole tandem mass spectrometer (MS/MS) (Shimadzu North America, Columbia, MD, USA) equipped with an ESI- source. For full method information see Appendix 4, but briefly, the UHPLC was equipped with a Phenomenex Synergi^{TM} ether-linked phenyl polar reverse phase column (2.5 µm, 2 x 100 mm; Phenomenex Incorporated, USA) and run isocratically using a mobile phase of 10 mM ammonium acetate and 0.1% formic acid in a solution of methanol: water (76:24 v/v). Extracts received 120ng/mL of the appropriate isotope labeled internal standard immediately before analysis. The regression coefficients, r^2, of the standard curves were all >0.995. No loss to the vials was detectable. Extraction recoveries for all compounds were between 73-95%. Sorption to MPs was calculated via mass balance.

Modeling of Dow and K_{dPE}

Using K_{OW} and pK_a values of compounds in Table 4.1 (structures in Figure 4.3), D_{OW} was calculated using equation 3. For nonionic PPCPs, D_{OW} is equivalent to their K_{OW} (ECETOC-TR-123, 2013, p. 16). However, for the zwitterions ciprofloxacin and amoxicillin, D_{OW} calculation is not straightforward due to multiple pK_a's. A correction factor for each ionic species must be considered, as well as the effects of counter ions such as K^+, Na^+, and Cl^- in solution (Csizmadia, et al., 1997; Tsantili-Kakoulidou, et al., 1999; ECETOC-TR-123, 2013, p. 42). Therefore, advanced predictive software such as ACD/logP or SPARC, plus detailed knowledge of experimental conditions (e.g., temperature, pH), is required to accurately calculate D_{OW} for polyprotic compounds and was out of the scope of this dissertation (Csizmadia, et al., 1997; Tsantili-Kakoulidou, et al., 1999; ECETOC-TR-123, 2013, p. 42). However, in order to estimate D_{OW} for illustrative modeling purposes, the zwitterions were calculated as either an acid or a base, depending on pH, that would create the largest fraction of ionized species possible. Amoxicillin was calculated as an acid (pK_a 2.67). While, ciprofloxacin was calculated as a base (pK_a 9.2) for pH 5-7, and as an acid (pK_a 6.1) for pH 8 and 9. Models of MP sorption with K_{OW} and D_{OW} were calculated with equations 2 and 4, respectively. PPCPs with negative $\log K_{d\mathrm{PE}}$ values and compounds with nondetectable sorption were removed in order for any relationship to be made between $K_{d\mathrm{PE}}$ and K_{OW}/ D_{OW}, and thus were not included in these analyses (See also Appendix 5).

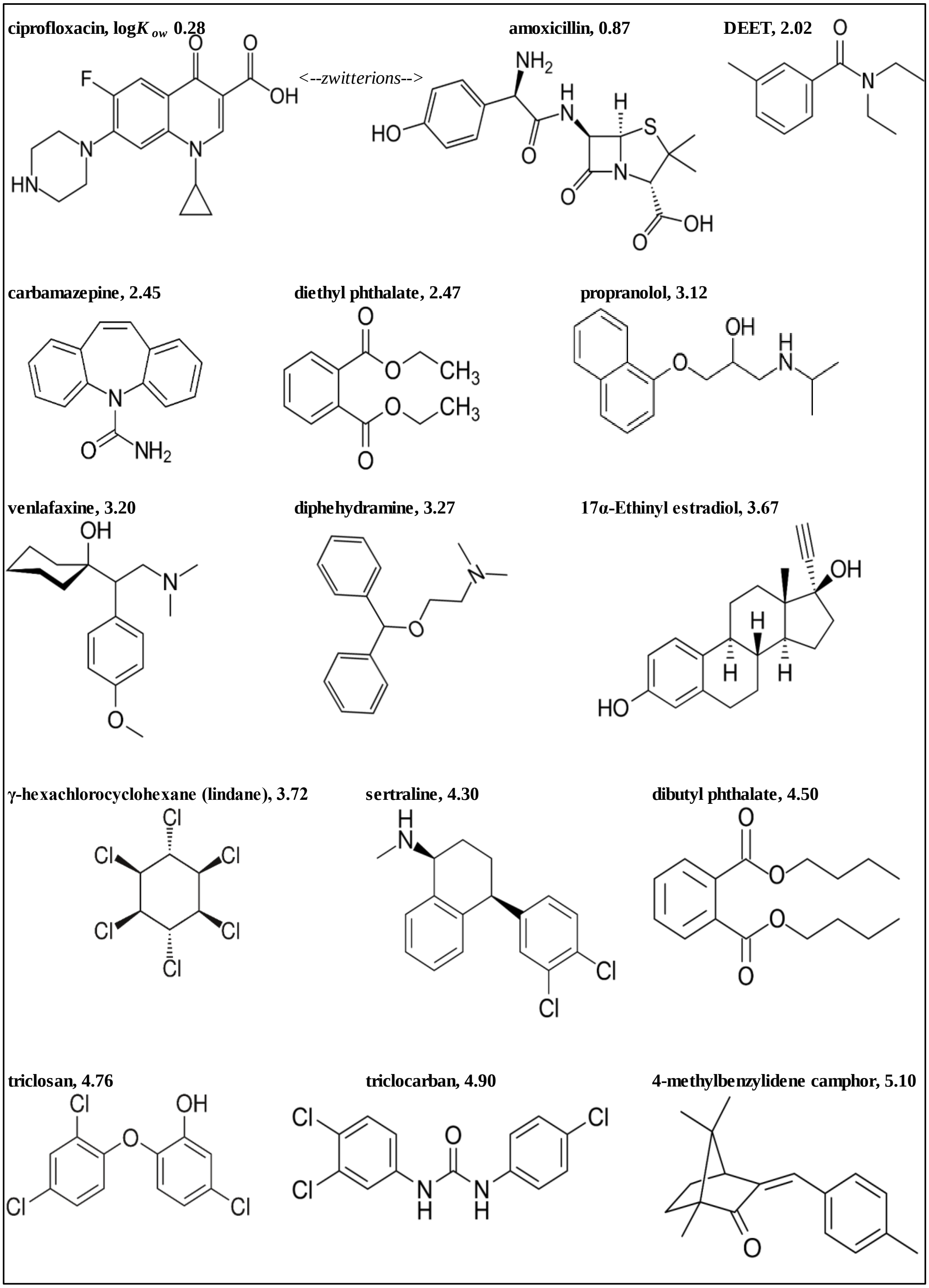


Figure 4.3. Structures of compounds from used in modeling analyses. Left to right, top to bottom in order of increasing logK_{OW}.

Results and Discussion

Sorption of venlafaxine, diphenhydramine, and DEET

Sorption to HDPE and PP was not detected for all three compounds: venlafaxine, diphenhydramine, and DEET. Due to their pK_a's, it is not surprising that diphenhydramine (8.98) and venlafaxine (10.09) had negligible sorption, as they are both entirely ionic at pH 7.4. As such, there would be no favorable interaction with the surface of either HDPE or PP, both of which can only interact through London Dispersion forces (Huffer and Hofmann, 2016; Hartman et al., 2017; Huffer et al., 2018). While, DEET, though not polar and may be theoretically capable of associating with HDPE and PP, has a relatively high water solubility of 912 mg/L, a magnitude higher than what was used in the isotherm experiments. Similarly, Seidensticker et al., 2018 could not detect sorption of polar and ionic PPCPs under environmentally relevant conditions and required MP: water ratios 6-7 orders of magnitude higher than what is observed in the field. Ibuprofen and naproxen were also reported to have very low sorption under realistic concentration scenarios, and high levels of sorption was only observed at pH 2, a condition not frequently encountered in nature (Elizalde-Velázquez et al., 2020). While, Razanajatovo, et al., 2018 despite having similarly low isotherm concentration ranges as the experiment here, was able to detect a minimal amount of sorption for two ionic PPCPs, propranolol and sertraline (Table 4.3). However, their PE MPs were significantly smaller in size (45 vs. 150 µm), and thus would have larger surface area (Chapter 2 results) and greater sorption (Chapter 3 results; Velzeboer et al., 2014; Li et al., 2019; Wang et al., 2019). Additionally, ultra-high molecular weight polyethylene (UHMWPE) MPs were used in Razanajatovo, et al., 2018 , and UHMWPE is a slightly softer and less crystalline polymer than HDPE, and is used more for specialty industrial purposes, rather than for consumer products. The lower crystallinity of UHMWPE would also increase sorption (Chapter 3 results; Hartmann et al., 2017). Therefore, it is not expected that sertraline and propranolol would be preferentially sorbing to PP and HDPE in the environment. The same may be said for DEET, diphenhydramine, and venlafaxine.

K_{OW} and Water Solubility

There are two distinct trends in PE sorption (logK_{dPE}) of PPCPs of varying polarity and logK_{OW} (Figure 4.4). Though it is a limited dataset, it appears that MP sorption to PE may be expected to significantly increase for substances with logK_{OW} > ~3.7. This does make sense, however, given that the correlation (logK_{OW} vs. logK_d) is a LFER, and LFERs usually do not provide strong correlations for compounds of very different structure (here, polarity) (Schwarzenbach et al., 2003, p. 89). The model for substances of logK_{OW} > ~3.7 is significantly better than for those of logK_{OW} < ~3.7 (i.e., lower hydrophobicity) ($r^2 = 0.96$ vs. 0.63). This difference may be attributed to the fact that for PPCPs with lower logK_{OW}, there are different forces (e.g., electrostatic) that contribute to intermolecular interactions other than hydrophobic interactions (Tourinho et al., 2019). Lee et al., 2014 also observed a weaker trend for logK_{OW} and MP sorption for hexachlorohexanes, in comparison to the more hydrophobic PAHs and chlorinated benzenes. This may be especially true for zwitterions, as they are ionic but of neutral charge, and thus may sorb more than other charged PPCPs (Li et al., 2018; Xu et al.,2018; Tourhino, et al., 2019).

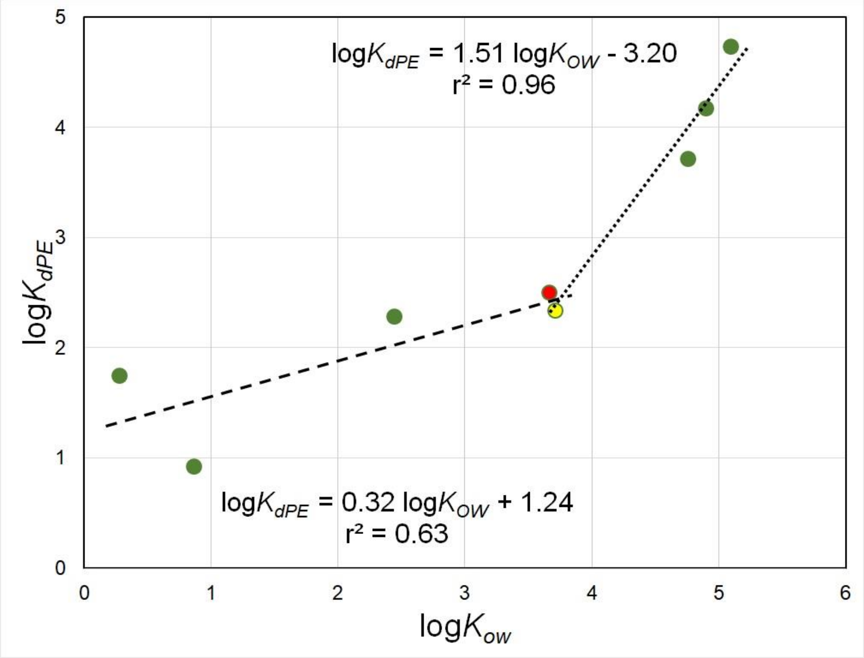


Figure 4.4. The relationship between $\log K_{OW}$ and $\log K_{dPE}$ (L/Kg) for MP sorption of various PPCPs ; data taken from the studies in Table 4.1 and results from Chapter 2 (triclocarban sorption to HDPE). 17α-ethinyl estradiol (red) and lindane (yellow) are colored to indicate they are included in both models.

To obtain the relationship described above (Figure 4.4), however, PPCPs with negative $\log K_{d\mathrm{PE}}$ values and compounds with nondetectable sorption (Table 4.3) were removed. It is clear that $\log K_{OW}$ alone cannot explain PE MP sorption of polar PPCPs. Sorption to PE does, however, generally increase with a decrease in water solubility (Table 4.3). The PPCPs that do not follow this trend are sertraline, dibutyl phthalate, ciprofloxacin, and amoxicillin.

Table 4.3. Selected physical parameters and average sorption distribution constants, logK_{dPE} (L/kg), of various PPCPs with PE, in the order of increasing water solubility. Underlining indicates substances that appear to go against the trend of increased sorption with decreasing water solubility. While, propranolol is in bold to notate the existence of a potential threshold for MP sorption between ~20-60 mg/L water solubility. -ND = not detected.

PPCP	Water Solubility (mg/L)	LogK_{OW}	log$K_{d\mathrm{PE}}$	Study
4-methylbenzylidene camphor	0.001	5.10	4.73	Wu et al., 2016
triclocarban	0.11	4.90	4.17	Chapter 3 Results
lindane	0.20	3.72	2.33	Lee et al., 2014
sertraline	3.8	4.30	-2.48	Razanajatovo et al., 2018
triclosan	10	4.76	3.71	Wu et al., 2016
dibutyl phthalate	11.2	4.50	-3.03	Liu et al., 2019
17α-ethinyl estradiol	11.3	3.67	2.49	Wu et al., 2016
carbamazepine	18	2.45	2.28	Wu et al., 2016
propranolol	**61.7**	**3.12**	**-2.64**	**Razanajatovo et al., 2018**
venlafaxine	572	3.20	-ND	Chapter 4 Results
DEET	912	2.02	-ND	Chapter 4 Results
diethyl phthalate	1080	2.47	-5.03	Liu et al, 2019
diphenhydramine	3060	3.27	-ND	Chapter 4 Results
amoxicillin	3430	0.87	0.92	Li et al., 2018
ciprofloxacin	30000	0.28	1.74	Li et al., 2018

Sertraline is a bulky hydrocarbon that has polar moieties (R–N, R–Cl), which make it less soluble in water than similarly structured hydrocarbons such as carbamazepine, which contains a carbonyl group and is a smaller molecule (See Figure 4.3 for structures). Sertraline has a high logK_{OW} because it is not only a bulky hydrocarbon, but also contains polar moieties that increases its solubility in octanol due to similar intermolecular forces shared between the compounds (i.e., polarity). So, though sertraline has a high logK_{OW}, PE sorption is very low because PE has no polar groups and would not have the same favorable interactions as octanol, in addition to the fact that sertraline is ionic at environmental pH. While, dibutyl phthalate has a higher water solubility than sertraline, despite being neutral and also having a higher logK_{OW}. The long hydrophobic tail (butyl group) make it less soluble in water than similar compounds such as diethyl phthalate, but more soluble in octanol than sertraline because of more similar intermolecular forces shared with octanol. Dibutyl phthalate, then, has very low sorption to PE for the same reasoning as sertraline. On the other hand, carbamazepine and 17α-ethinyl estradiol have lower logK_{OW} *and* higher water solubility than sertraline and dibutyl phthalate, and yet have significantly higher sorption to PE. However, both have larger uninterrupted sections of neutral structure, i.e., the polar moieties are on the edges or at one side of the molecule. This surfactant-like structure may allow more interaction with the MP surface for adsorption (Smiciklas et al., 2000; Onjia et al., 2001; Huffer and Hofmann, 2016). Indeed, the sorption of the widely used surfactant nonylphenol (Figure 4.5; acidic pK_a 10.31) to PE did not significantly decrease even at pH 10 (Seidensticker et al., 2018). Additionally, the organophosphorus insecticide diazinon (Figure 4.5; logK_{OW} 3.3) also had relatively high sorption to PE MPs compared to other polar compounds, despite containing a very polar functional group (S=phosphate–R) (Seidensticker et al., 2018).

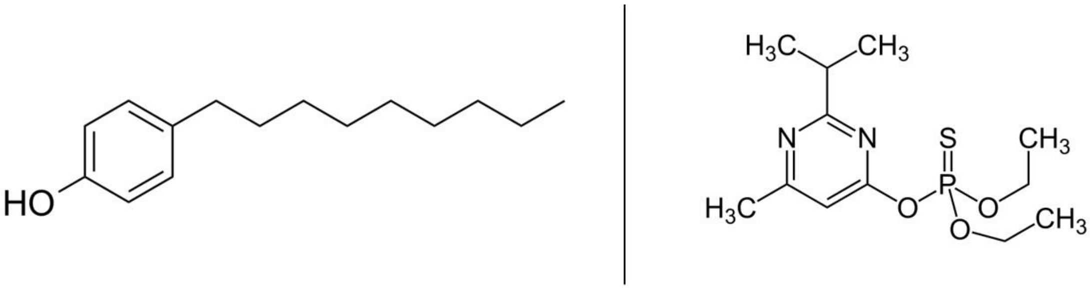


Figure 4.5. The structures of the surfactant 4-nonylphenol (left) and the insecticide diazinon (right).

For ciprofloxacin and amoxicillin, though ionic at environmental pH, both are neutrally charged. This may explain the unexpected sorption given their relatively high water solubility (Li et al., 2018; Xu et al.,2018; Tourhino, et al., 2019). Therefore, high water solubility may not be indicative of negligible MP sorption. It should be noted, though, that both antibiotics compounds were measured at high concentrations (500-1500 µg/L) for their isotherms, a magnitude more than what is observed in nature (Li et al., 2018). While, plasticizers dibutyl and diethyl phthalate were measured at similarly high concentrations, but significantly lower sorption was reported (Liu et al., 2019). Therefore, caution should be taken when making statements about the sorption of similar zwitterion antibiotics to PE MPs. There is little work on the sorption of zwitterions (Tourhino, et al., 2019), and more data will be needed to confirm whether neutrally charged compounds also sorb to the reported levels (Table 4.3) in aquatic environments.

It appears that there may be a threshold for MP sorption between ~20-60 mg/L water solubility, but only if the compounds just previously discussed are excluded (Table 4.3). Like any LFER, there will be compounds with unexpected distribution behavior (Schwarzenbach et al., 2003, p. 89). This may be an indication of chemical groups that should be considered separately and in their own model of *Kow*, such as surfactants and surfactant-like substances, or they may be outliers in truth. Larger data sets of polar PPCPs are needed to better understand how water solubility and structure influence MP sorption.

D_{OW} and sorption of PPCPs to PE MPs

The two positively correlated trends for PPCPs of logK_{OW} > ~3.7, and those of logK_{OW} < ~3.7, and MP sorption remained when using D_{OW} instead of K_{OW} (Figure 4.6).

The main difference when using D_{OW} is the significantly higher correlation ($r^2 \cong 0.95$ vs. 0.63) with logK_{dPE} for more polar PPCPs (log$K_{OW} <$ ~3.7) than with K_{OW}. This may be attributed to the fact that D_{OW} may be a more accurate representation of distribution due to hydrophobic forces for ionic compounds because D_{OW} controls for the speciation, ie., the ionic fraction, of the molecules ciprofloxacin and amoxicillin (ECETOC-TR 123, 2013; p. 57-58; Guo et al., 2018; Zhang et al., 2018). As hypothesized, however, the lowest pH, 5, does not have a stronger correlation ($r^2 = 0.63$ vs. 0.55). And at pH 6, r^2 is also markedly lower, though still higher than for logK_{OW} ($r^2 = 0.63$ vs. 0.74). Some error is because logK_{dPE} values were measured only at pH 7 (or close to neutral). However, the weaker correlation is mostly due to large changes in D_{OW} for ciprofloxacin resulting from the attempt to estimate D_{OW} without considering the effects of multiple pK_a's. Advanced predictive software, plus detailed knowledge of experimental conditions, is required to accurately calculate D_{OW} for zwitterions and was out of the scope of this dissertation (Csizmadia, et al., 1997; Tsantili-Kakoulidou, et al., 1999; ECETOC-TR-123, 2013, p. 42). Future work should aim to collect more data on polyprotic compounds under realistic concentration scenarios and use environmental modeling programs, such as ACD/logP or SPARC, to create a better model for MP sorption of ionic and other polar PPCPs.

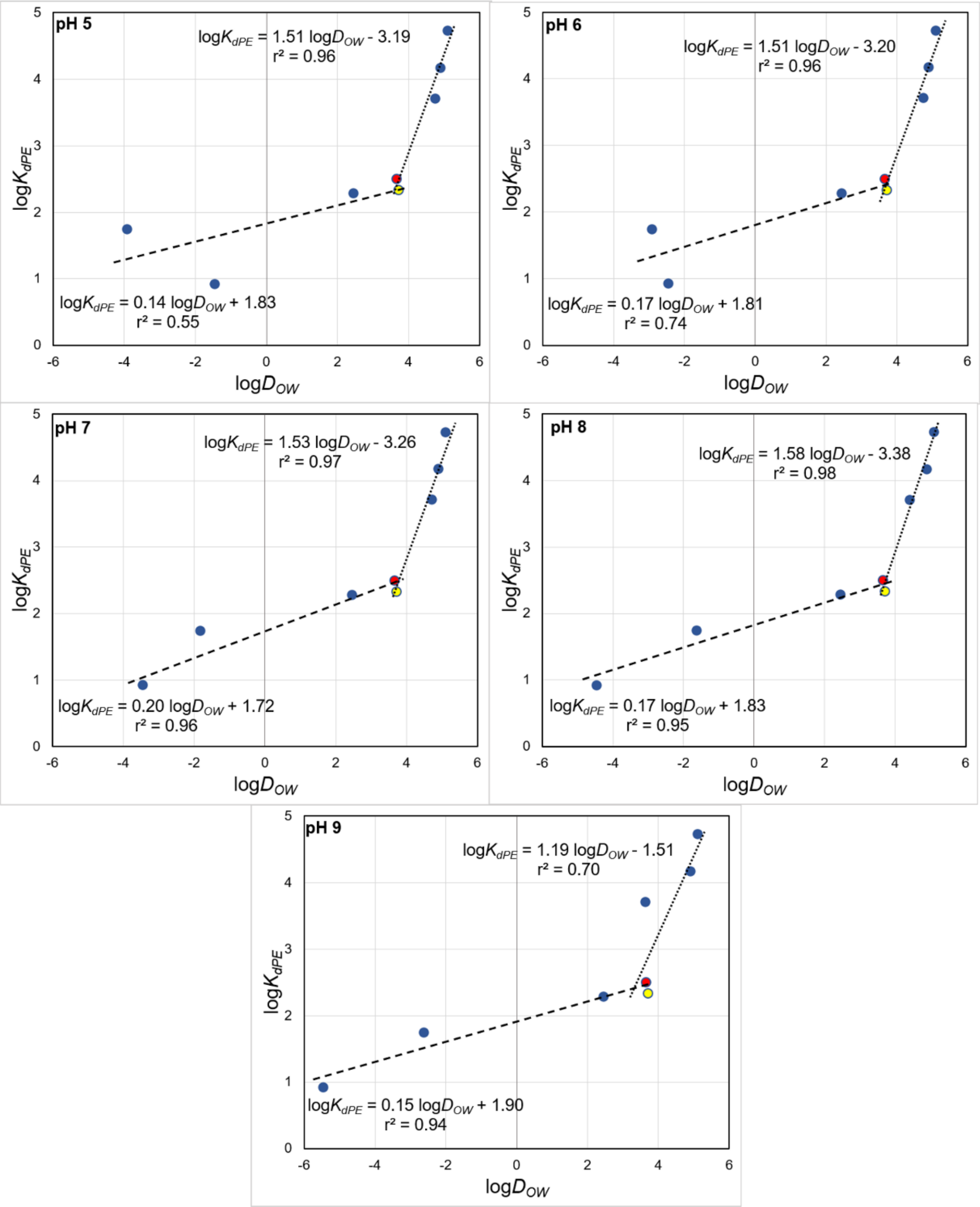


Figure 4.6. The relationship between logD_{OW} and logK_{dPE} (L/Kg) for MP sorption of various PPCPs at pH 5 – 9; data taken from the studies in Table 4.1 and results from Chapter 2 (triclocarban sorption to HDPE). 17α-ethinyl estradiol (red) and lindane (yellow) are colored to indicate they are included in both models shown for each pH.

The correlation for PPCPs with logK_{OW} > ~3.7 and logK_{dPE} did not change significantly when logD_{OW} is used instead ($r^2 \cong 0.95$, D_{OW} vs. 0.96, K_{OW}) (Figure 4.6).

This is not surprising, as the only dominant force controlling sorption for higher logK_{OW} compounds is hydrophobic interactions (Tourhino et al., 2019). Lee et al., 2014 also observed a stronger trend for logK_{OW} and MP sorption for hydrophobic PAHs and chlorinated benzenes, as compared to the more polar hexachlorocyclohexanes. However, at pH 9 the correlation with D_{OW} is significantly lower ($r^2 \cong 0.95$ vs. 0.70). This is due to the weak acid TCS, which has a pK_a of 7.90 and is largely ionic at pH 9, and thus the MP sorption would be expected to be significantly less than at pH 7. At marine conditions (pH 8.1 at ocean surface (Ocean Acidification, 2020)), TCS would then also not be expected to be associated with MPs and would instead largely be in the dissolved phase; however, ocean acidification will change this. From pH 8 to 7, the logD_{ow} for TCS increases by 0.30, which translates into a 50% increase of the neutral fraction of TCS associated with the hydrophobic phase. Current projections predict that the pH of the ocean surface will decrease from 8.1 to 8.05, and potentially as low as 7.75, by the end of this century (Ocean Acidification, 2020). Future models of acidic PPCPs with pK_a's equivalent to marine conditions will need to be revaluated, as the potential for MP sorption to PE and other hydrophobic polymers will increase due to climate change induced ocean acidification.

Prioritizing Future Work on PPCPs and MPs

The FDA, REACH, EMA, and the VICH (the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products) all use regulatory trigger values of logK_{OW} (between 3 - 4.5) to indicate if a substance may require chronic toxicity testing (ECETOC-TR 123, 2013, p. 19). Though much more work is needed to gain a level of understanding that is required for creating regulatory guidelines (Du et al., 2020; Ma et al., 2020), water solubility, in conjunction with logD_{OW}, may be used similarly for prioritizing future research on the sorption of PPCPs to MPs. For example, many APIs are weak monoprotic bases, and would not be expected to significantly sorb to PE or PP. While, some CIs, such as plasticizers found in nail polish, are neutral polar compounds, and thus predicting MP sorption is less straight forward. However, even ionic and strongly polar PPCPs may sorb to MPs if they have a) surfactant-like structure (Smiciklas et al., 2000; Onjia et al., 2001; Huffer and Hofmann, 2016; Seidensticker et al., 2018), and/or b) the polymer involved has similar intermolecular forces, such as pi-pi stacking with PS, or highly polar groups, such as carbonyls in polyamides (Huffer and Hofmann, 2016; Li et al., 2018). For the same reasoning, polyester, the most prevalent MP found in sanitary wastewater effluent (Mason et al., 2016; Murphy et al., 2016), may be expected to sorb highly polar PPCPs due to having both aromatic and ester groups. However, to the best of my knowledge, no study has been conducted on the sorption of PPCPs to polyester MPs. Future studies should focus analytes of interest to compounds whose water solubility and logD_{OW} suggest sorption. In addition, special focus should be made for substances with unique structure, such as zwitterions or surfactants and surfactant-like compounds, that may have unexpected sorption behavior.

Conclusions and Future Directions

Sorption was not detectable for PPCPs DEET, diphenhydramine, and venlafaxine to PP and HDPE MPs. PPCP sorption to PE increased with increasing log$K_{OW,}$ but only if compounds with negative logK_{dPE} values are excluded from the correlation. Therefore, logK_{OW} alone cannot explain PE MP sorption of polar PPCPs. Water solubility explains much of the trend for PPCP sorption to PE, and there appears to be a threshold for sorption between ~20-60 mg/L water solubility. However, some compounds due to their structure may have unique sorption behavior, such as dibutyl phthalate (high K_{OW}, low sorption) and amoxicillin (low K_{OW}, reported to sorb).

D_{OW} may be a more accurate representation of MP sorption of ionic compounds, and offer a better model for sorption than K_{OW} for polar PPCPs. Future work should aim to collect more data on polyprotic compounds under realistic concentration scenarios and over a more continuous pH range using programs such as ACD/logP or SPARC to accurately estimate D_{OW}. More specifically, the hypothesis that a continuous pH model of D_{OW} would better fit extreme pH's (i.e., 5 and 9) should be tested. Additionally, the behavior of acidic PPCPs, such as TCS, with pK_a's equivalent to aquatic conditions (pH 5-9) will need to be revaluated under climate change conditions, as the potential for sorption to hydrophobic polymers will increase with ocean acidification.

Future research on MP sorption should prioritize study compounds by those whose water solubility and $\log D_{OW}$ suggest potential polymer distribution. In addition, special focus should be made for substances with unique structure, such as zwitterions or surfactants and surfactant-like compounds, and polar PPCPs with high $\log K_{OW}$ but without large amphiphilic structure (not surfactant-like), that may have unexpected sorption behavior. Additionally, work should begin on understanding the potential for sorption of polar PPCPs by polyester, the most common MP in wastewater effluent, as polyester has both aromatic and ester groups that may favorably interact with a variety of polar substances. Together, this approach may help to prioritize MP sorption research in a way that will create data sets that will better inform future toxicological testing.

Chapter 5: Dissertation Conclusions

The first ideas for this work began in 2015 when I reviewed the research in preparation for a grant. At the time, my literature review was a couple dozen papers on MPs and associated organic pollutants, many of which did not contain characterization studies and most focused only on PAHs and PCBs. Since then, the field of MPs has grown exponentially. Over 8,000 papers were published in 2020 (https://app.dimensions.ai/, keyword “microplastic”), and over 1,400 sorption papers (keywords “microplastic”, “sorption”). However, 6 years later, researchers still have not come to a consensus on whether MPs with sorbed organic pollutants pose a significant threat to aquatic ecosystems (Tourhino et al., 2019; Du et al., 2020; Ma et al., 2020). A large part of this issue is a lack of standardized methodology, which I address in Chapters 2-3, and an incomplete mechanistic understanding of the sorption process, especially for polar PPCPs, which I address in Chapters 3-4.

In Chapter 1, I reviewed the literature up until early 2020, with a special focus on MP sorption mechanisms and influencing factors. The sorption process for organic pollutants is controlled by a variety of forces, including hydrophobic and electrostatic interactions, and pH. Sorption of organic pollutants are also affected by physical factors, such as surface area, and has been shown to be positively correlated with decreasing particle size/increasing surface area (Li et al., 2019; Wang et al., 2019). This is an important consideration for studying MPs, as MPs found in the environment are irregularly shaped, and irregular particles have higher surface area (Brennecke et al., 2016). For the sorption of PPCPs, the general trend is that sorption increases according to logK_{OW} (Lee et al., 2014; Wu et al., 2016; Li et al., 2018). This trend does not hold for all

PPCPs, especially for highly polar compounds (Liu et al., 2019) and those with pK_a's in the natural aquatic range (pH 5-9) (Li et al., 2018); knowledge of structure is needed to help explain the differences. However, some studies used concentrations for their equilibrium isotherms that were 1-3 magnitudes higher than what is seen in the environment (Li et al., 2018; Liu et al., 2019) and did not use similarly sized particles when comparing MPs of different polymers (Elizalde-Velázquez et al., 2020). It is argued that due to their low concentrations in aquatic systems, polar PPCPs will not sorb to MPs (Seidensticker et al., 2018; Elizalde-Velázquez et al., 2020), but much more data is needed, especially from field sampling, to be certain (Tourhino, et al., 2019). Similarly, two recent toxicological reviews (Du et al., 2020; Ma et al., 2020) have concluded that the effects of ingestion of MPs with sorbed organic pollutants on aquatic organisms is remains unclear. Both reviews attribute this to lack of comparable methodology used in the reviewed studies and inconsistency with regard to environmentally realistic conditions.

To further the field, an attempt was made to create methodology for a reference material of PP and PE fragment type microplastics of consistent (standardized) surface area (Chapter 2). The methodology was successful and is currently the only method for creating MP reference materials. PP particles generally had greater surface area, as it is lower in crystallinity than HDPE, and therefore deformed more during fabrication and created higher surface area (Brennecke et al., 2016). However, this was not the case for the two smallest size classes, because PP formed into more round, pellet-like particles (less surface area), while HDPE continued to fragment into irregular, angular particles (more surface area). This reference material may: 1) act as a more realistic material than purchased powders or beads, 2) provide a more readily comparable reference material for fragment type MPs of PE and PP, and 3) serve as a clean starting material that can then be subject to exposure and/or biologicals prior to use in experiments. Future work should aim to create a more continuous data set that may be used to create a better predictive model for MP particle size and surface area. Particle size distribution within size classes should be analyzed, as it may be useful in predicting the surface area of environmental MPs and may inform the creation of a better reference material for toxicity experiments. It is also worth investigating whether average particle diameter within individual size classes is significantly correlated with surface area. If average particle diameter is correlated well with surface area, there may be no need to measure surface area for future experiments using this fabrication methodology, as pollutant sorption to MPs should be inversely correlated with average particle diameter. For these reasons, future work should aim to determine the structural (e.g., shape, size distribution, surface area) differences between the fabricated reference material described here and UV degraded material versus “natural” MPs found in the field. Additionally, the effects of degradation and biofilm formation on fragmentation and MP shape/structure should also be studied. Lastly, PVC and PS MPs should be made with this or a similar method to determine if creating a reference material can be expanded to other polymers.

An objective of Chapter 3 was to create and validate an extraction method for acetone soluble compounds from MPs that may be used for field measurements. The variation of the sorption of the antimicrobial TCC in the single batch (kinetic) experiment was low and consistent among samples; thus, the extraction method created was successful. This method may then be used for monitoring hydrophobic PPCPs associated with MPs found in the environment; but should not be used for more hydrophilic PPCPs, as the method has multiple DI water rinsing steps. This same extraction method was then used to measure the equilibrium of TCC to HDPE and PP MPs of two different size classes (made with the methodology created in Chapter 2). The results showed that PP had significantly greater sorption than HDPE, due not only to its higher SA, but also because PP is a less crystalline polymer. These data suggest that MPs may be a vector for TCC from wastewater effluent. Future studies on the sorption of organic pollutants to MPs should control for particle size and measure the SA of MPs studied, especially when comparing MPs of different polymer types. For data analysis, less restrictive model fits other than the Freundlich Isotherm should be explored to better fit sorption data. Additionally, surveys of MPs from wastewater effluent and rivers should include extractions of select samples to begin monitoring of MP associated organic pollutants in aquatic environments.

To better understand the sorption of polar and ionic PPCPs, the equilibrium of DEET, diphenhydramine, and venlafaxine to PP and HDPE MPs were measured in an environmentally realistic scenario (Chapter 4). No sorption was detected for any of the three compounds. Similarly structured PPCPs, such as sertraline and propranolol (Razanajatovo et al., 2018), are not expected to sorb to PE and PP MPs in the field. This data, in conjunction with studies on 12 other PPCPs (Table 12), were used to understand the relationship between logK_{OW} and sorption to PE MPs. PPCP sorption to PE increased with increasing log$K_{OW,}$ but only when compounds with negative logK_{dPE} values were excluded from the correlation. Therefore, logK_{OW} alone cannot explain PE MP sorption of polar PPCPs. However, sorption to PE generally increases with decreasing water solubility, and there appears to be a threshold for sorption between ~20-60 mg/L water solubility. But for some compounds, sorption behavior may not be predicted by either water solubility or logK_{OW}, such as dibutyl phthalate (high K_{OW}, high water solubility, very low sorption; Liu et al., 2019) and amoxicillin (very low K_{OW}, high water solubility, reported to sorb; Li et al., 2018). While other compounds (e.g, carbamazepine and 17α-ethinyl estradiol; Wu et al., 2016) have surfactant-like structure (i.e., polar moieties are on the edges or on one side of the molecule) and will have larger sorption, despite a lower logK_{OW} (Seidensticker et al., 2018).

The main objective of Chapter 4 was to determine if a better model could be made for PE MP sorption of polar and ionic PPCPs with logD_{OW} instead of log*Kow*. Log*Dow* did create a stronger correlation for PPCPs with log*Dow* < ~3.7. However, estimation errors for the *Dow* of the zwitterion ciprofloxacin caused weaker correlations at pH 5 and 6. Future work should aim to collect more data on polyprotic compounds under realistic concentration scenarios, with a more continuous pH range, using programs such as ACD/logP or SPARC to more accurately estimate D_{OW} (Csizmadia, et al., 1997; Tsantili-Kakoulidou, et al., 1999; ECETOC-TR-123, 2013, p. 42). More specifically, the hypothesis that a continuous pH model of D_{OW} would better fit extreme pH's (i.e., 5 and 9) should be tested. The correlation for PE MP sorption of PPCPs with log*Dow* >3.7 was not significantly different from the log*Kow* model, except at pH 9, due to the larger ionic fraction of the antimicrobial TCS (pK_a 7.90). While at lower pH, TCS fits the model because it is mostly neutral at pH ≤ 8. Thus, the behavior of acidic PPCPs, such as TCS, with pK_a's equivalent to aquatic conditions (pH 5-9) will need to be revaluated under climate change conditions, as the potential for sorption to hydrophobic polymers will increase with ocean acidification.

New research on MP sorption should prioritize study compounds by those whose water solubility and logD_{OW} suggest potential polymer distribution. In addition, special focus should be made for substances with unique structure, such as zwitterions or surfactants and surfactant-like compounds, and polar PPCPs with high logK_{OW} but without amphiphilic structure (not surfactant-like), that may have unexpected sorption behavior. Additionally, work should begin on understanding the potential for sorption of polar PPCPs by polyester MPs in wastewater and the environment. Polyester has both aromatic and ester groups that may favorably interact with a variety of substances and is the most prevalent MP found in sanitary wastewater effluent (Mason et al., 2016; Murphy et al., 2016). Together, this approach may help to prioritize MP sorption research in a way that will better inform future toxicological experiments and eventually a risk assessment.

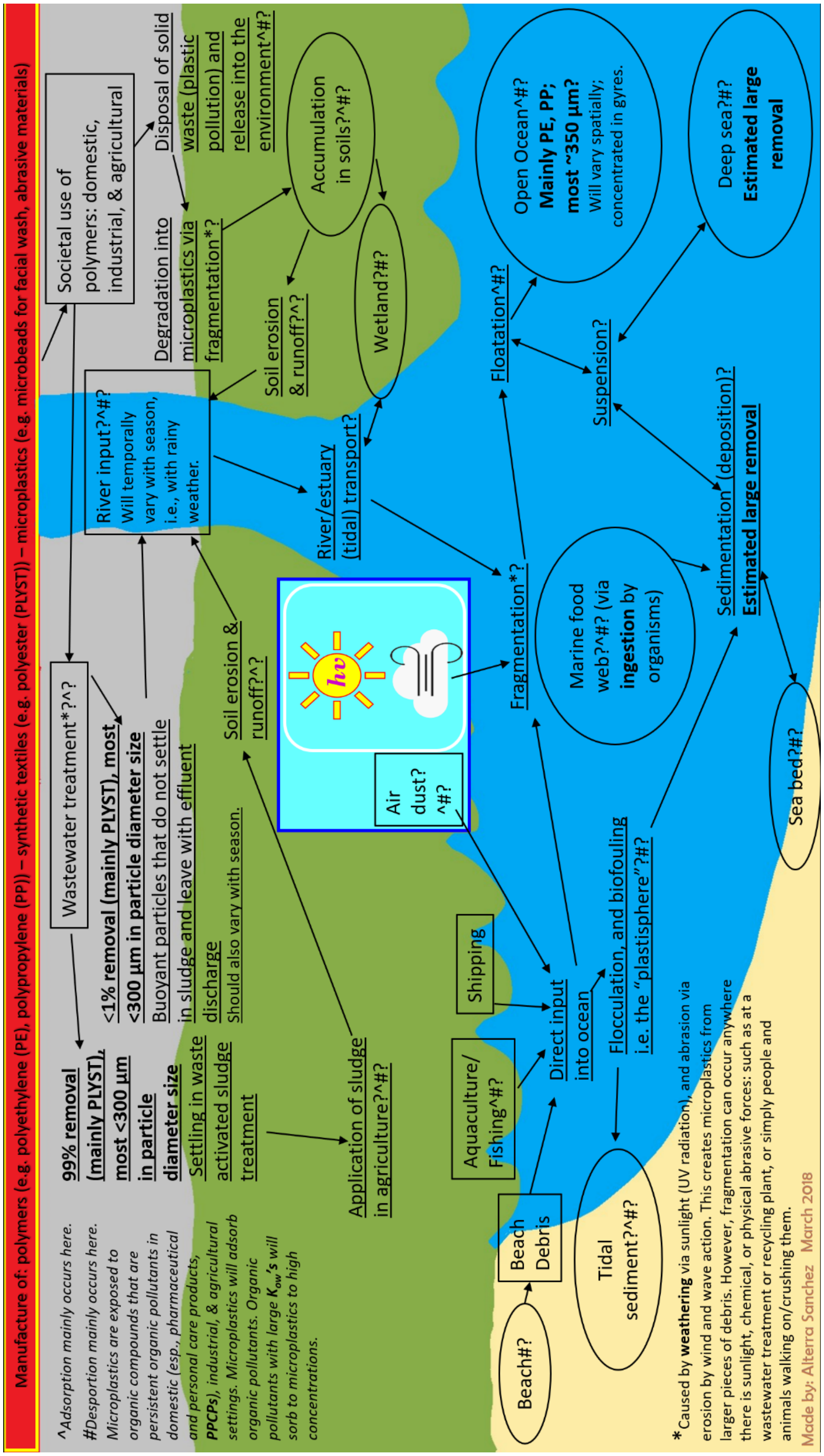
This work has inspired many new questions. The most salient of which are the mechanisms of multiple-compound sorption, i.e., the controlling factors of partitioning when different molecules are interacting with the MP surface, but also with each other. In Chapter 3 it was hypothesized that TCC sorption to PE and PP increased with increasing concentration due to a type of co-solvent effect, where TCC facilitates the adsorption (and possibly absorption) of itself. This raises intriguing possibilities for the sorption of polar and ionic PPCPs. Will the presence of compounds like TCC create a more favorable environment for the sorption of substances that, on their own, may not sorb to PE and PP MPs? If so, would this interaction be even more favorable for polymers such as PA and PS, as they are capable of more than just hydrophobic interactions? Additionally, though some PPCPs may sorb at neutral pH's, they may *desorb* at extremely acidic pH's such as in the gut of aquatic organisms. Compounds that fall into this category should be studied for risk assessment purposes. For biofilms, will they enhance the sorption of polar and ionic PPCPs, and in turn make MPs a better vector for organic pollutants from wastewater and other urban effluents? This may have implications for toxicity, as complex mixtures of organic pollutants have been shown to be more harmful than a single chemical alone (Rochman, et al., 2013; Echeveste, et al., 2016; Petersen, et al., 2017; Puckowski, et al., 2017).

Another interesting question is the possibility of sorption-desorption in the environment due to diel-scale pH changes. Daily-scale pH variability can be high if (1) a system is eutrophic or where high-density primary producers produce and consume CO_2 at high rates over the daily cycle; and (2) where tidal exchange with wetlands is extensive and low pH/high CO_2 is exported from marsh creeks with tides, or where episodic freshwater inputs deliver low pH water. For compounds with short equilibrium times (≤48 hrs) such as TCS (pK_a 7.90) (Wu et al., 2016), and that have a pK_a near environmental pH, this may mean sorption-desorption is a continuous process and equilibrium with MPs is never achieved. This may have implications for toxicity if relative load of PPCPs on MPs is lower or higher at certain parts of the day in these habitats and could affect certain species differently depending on their foraging modes, i.e., the time of day that they eat.

There are many other areas of research that could not be explored here that are important for sorption of organic pollutants (see Appendix 1). Treated wastewater sludge is routinely applied to agricultural lands as fertilizer, and with it all of the MPs (with sorbed PPCPs) that have settled during waste activated sludge treatment (Murphy et al., 2016). Which PPCPs will desorb into the soil and which will be retained by MPs? Will desorbed PPCPs increase contaminant load of crops and/or the toxicity of agricultural runoff? The same type of questions can be applied to MPs in stormwater that reach wetlands and rivers. Will MPs travel to bays and the ocean loaded with PPCPs or will they desorb along the way? Could there then be a gradient of toxicity of MPs following the watershed out into the open ocean as they desorb PPCPs during their travel? These questions have significant implications for aquaculture and fishing, as these activities tend to be densest at the end of the watershed and may especially be important for highly populated areas where there is untreated stormwater and wastewater runoff.

This dissertation explored a small part of MP research that is concerned with the class of organic pollutants known as PPCPs, and their potential to associate with polymers in the environment. Specifically, methodologies and materials were designed that may aid in harmonizing the field, so that experiments eventually may become more comparable and repeatable on a global scale. A larger and more nuanced understanding of the underlying mechanisms of PPCP sorption to MPs was also gained. After over 10 years of cumulative research, this is still but a beginning to understanding the implications of plastic pollution on the biosphere. Currently, sorbed organic pollutants to MPs seem to be a minor problem for aquatic systems when compared to other anthropogenic stressors, but with unclear and unquantified consequences (Jahnke, et al., 2017; Ma et al., 2020). With over 200,000 tons of plastic on the ocean surface, and possibly over 200,000,000 tons in the deep sea, it is imperative that we not make assumptions, or we may owe future generations an answer for why we ignored this problem (Jahnke, et al., 2017; Ma et al., 2020).

Appendices



Appendix 1

MP Global Distribution Conceptual Framework

Schematic representation of sources, sinks, and pathways of MP transport in the environment: from manufacture to the ocean. Rectangle shapes represent major sources, oval shapes represent major sinks, if underlined it represents a major transport pathway, and black arrows show the fluxes between the different compartments. "*" signifies degradation of MP via fragmentation. "^" and "#" signifies sorption and desorption of organic pollutants from MP, respectively. "?" represents an area where there is a significant knowledge gap. "?" are put after what is unknown, i.e., if sorption data is missing, the "?" will follow the "^" or "#". Schematic modified from: Figure 1 of: Zhang, Hua. "Transport of MPs in Coastal Seas." Estuarine, Coastal and Shelf Science, vol. 199, 2017, pp. 74–86.Figure 2 of: Horton, Alice A., et al. "MPs in Freshwater and Terrestrial Environments: Evaluating the Current Understanding to Identify the Knowledge Gaps and Future Research Priorities." Science of The Total Environment, vol. 586, 2017, pp. 127–141.

Appendix 2

Making Microplastics and SEM Images

Fabricating Microplastics

1. Using acetone rinsed steel forceps to hold a 2.5 cm diameter rod of high-density polyethylene, completely submerge in liquid nitrogen for 5 minutes.
2. Using an acetone rinsed and baked (at 450 °C for 4 hours) stainless steel wood file, shave the end of the rod and collect shavings in an acetone rinsed stainless-steel tray.
3. Once plastic rod is no longer freezing (ice crust has melted), repeat step 1.
4. Repeat step 2.
5. Continue process of freezing and shaving until ~15 grams of MPs have been made.
6. In another acetone rinsed shallow (2.5cm height) stainless-steel tray, set a stack of DI water rinsed brass sieves of mesh No. 35, 40, 45, 50, 60, 70, 100, 200, and 325, with the smallest sieve on the bottom. The sieve openings are 45, 75, 150, 212, 250, 300, 355, 425 and 500 µm in diameter, respectively. Place the stack in the tray in a clean sink.
7. Pour shavings into the sieve top sieve (i.e., 500 µm sieve), rinsing the shavings into the sieve with a tap water hose connected to the faucet.
8. Rinse the shavings thoroughly with tap water, making sure to rinse MPs off the sides of the sieve. Using a spiral motion, starting from the rims and working in. The sieve will start to fill, when this occurs stop rinsing and gently lift an edge of the sieve slightly to break the vacuum to allow water to flow through the sieve.
9. Repeat step 5 for five minutes.
10. After 5 minutes, stop rinsing and gently lift each sieve to break vacuum, being careful not to lose any of the rinse, i.e., only lift enough to break the vacuum. Keep the sieves over one another.
11. Repeat steps 5-7, five more times, for a total of 180 minutes of rinsing.
12. Tilt the 500 um sieve over the stack of sieves to drain the excess tap water, and so that the MPs are in one pile next to the wall of the sieve.
13. Gently rinse the MPs with 200 mL of DI water. Set sieve on a paper towel to drain.
14. Repeat steps 5-8 for the rest of the sieves, however only do one iteration of rinsing, i.e., 5 minutes.
15. Remove MPs from all sieves with an acetone rinsed steel scoopula and place in separate, acetone rinsed, and baked glass 150 mL beakers (one for each sieve size).
16. Rinse sieves vigorously with tap water to remove left over MPs.
17. Add 80mL of 50:50 hexane:acetone, cover with tinfoil, and sonicate for 30 minutes.
18. Over an acetone rinsed 45µm sieve, pour out solvent and MPs, catching the solvent in a metal tray/bowl for disposal.

19. Rinse MPs on sieve with ~10mL of acetone using a squirt bottle to remove dirty solvent.
20. Using an acetone rinsed metal scoopula, transfer MPs into another clean 150mL beaker.
21. Add 80 mL of fresh 50:50 hexane:acetone, cover with tinfoil, and sonicate a final time for 30 minutes.
22. Repeat steps 18-20
23. Cover beakers with a Kim wipe, and tape to secure, to prevent dust contamination.
24. Let dry at room temperature for ≥ 48 hours and then bake at 45°C for 4-6 hours.

SEM Images of MPs of All Size Classes

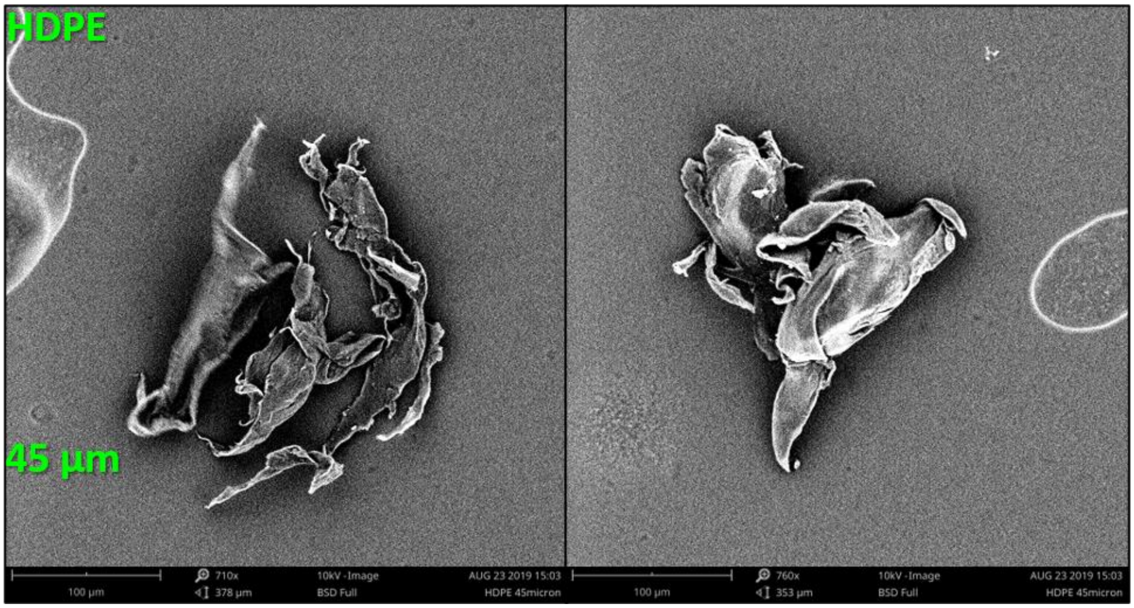


Figure 1. SEM images of the HDPE fragment type MPs reference material for size class 45 µm.

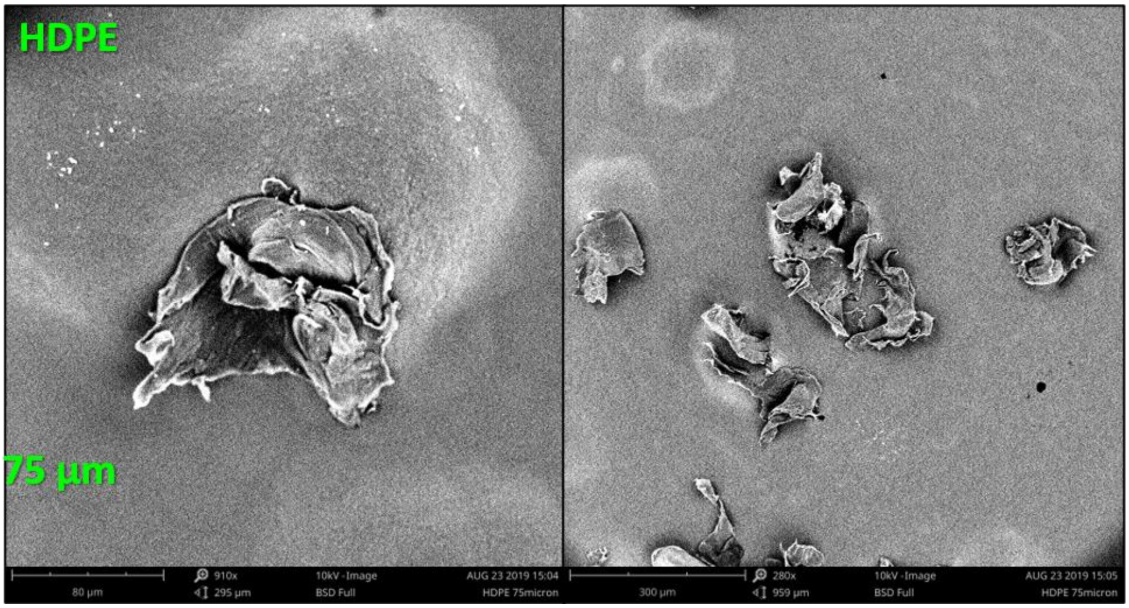


Figure 2. SEM images of the HDPE fragment type MPs reference material for size class 75 µm.

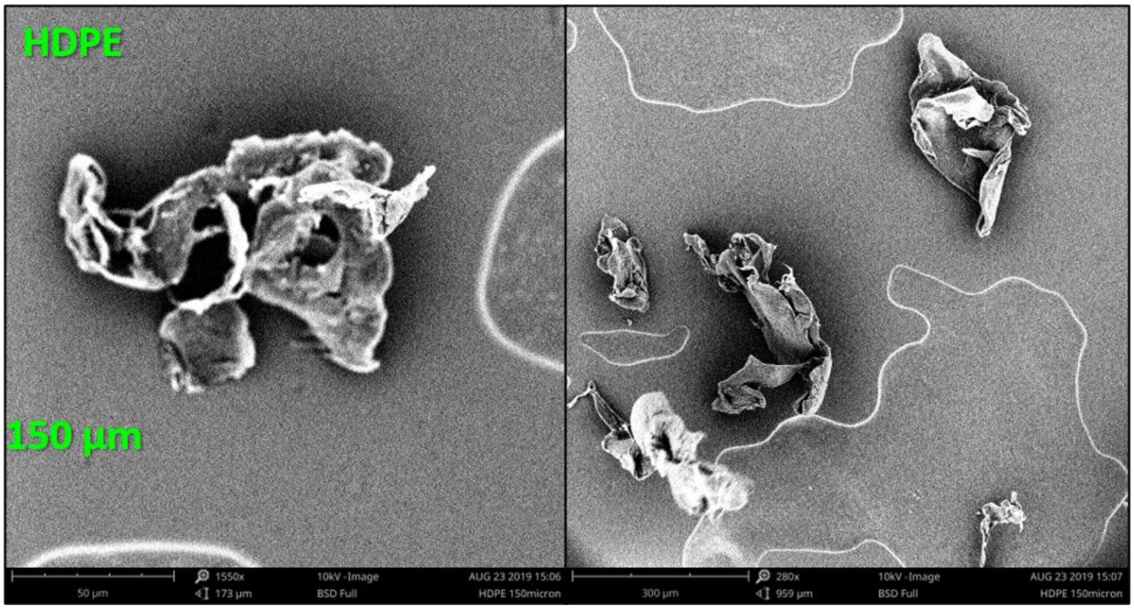


Figure 3. SEM images of the HDPE fragment type MPs reference material for size class 150 µm.

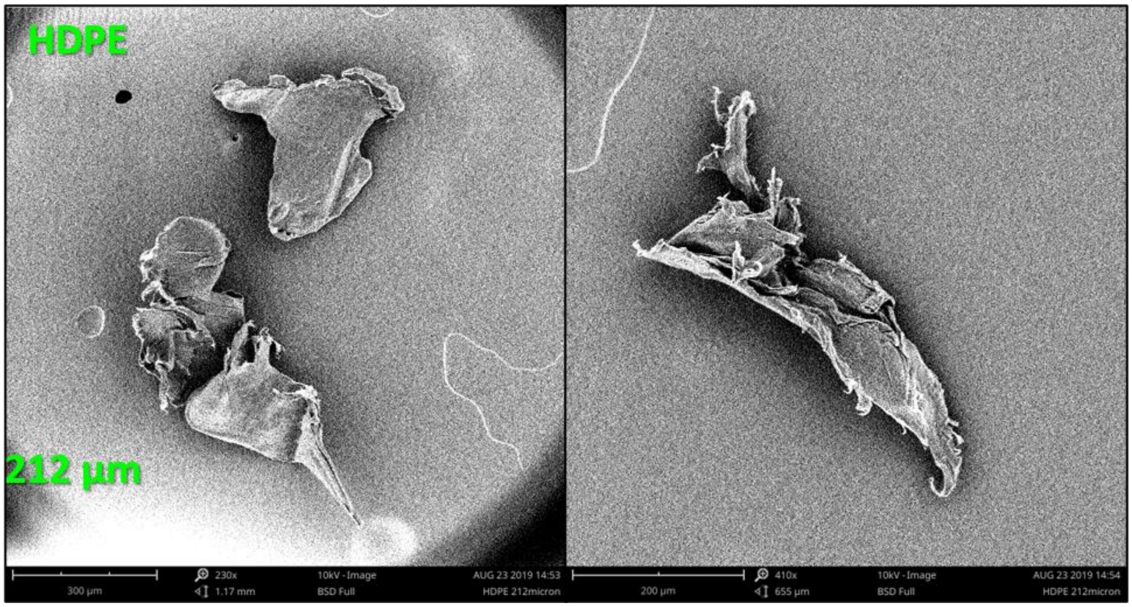


Figure 4. SEM images of the HDPE fragment type MPs reference material for size class 212 µm.

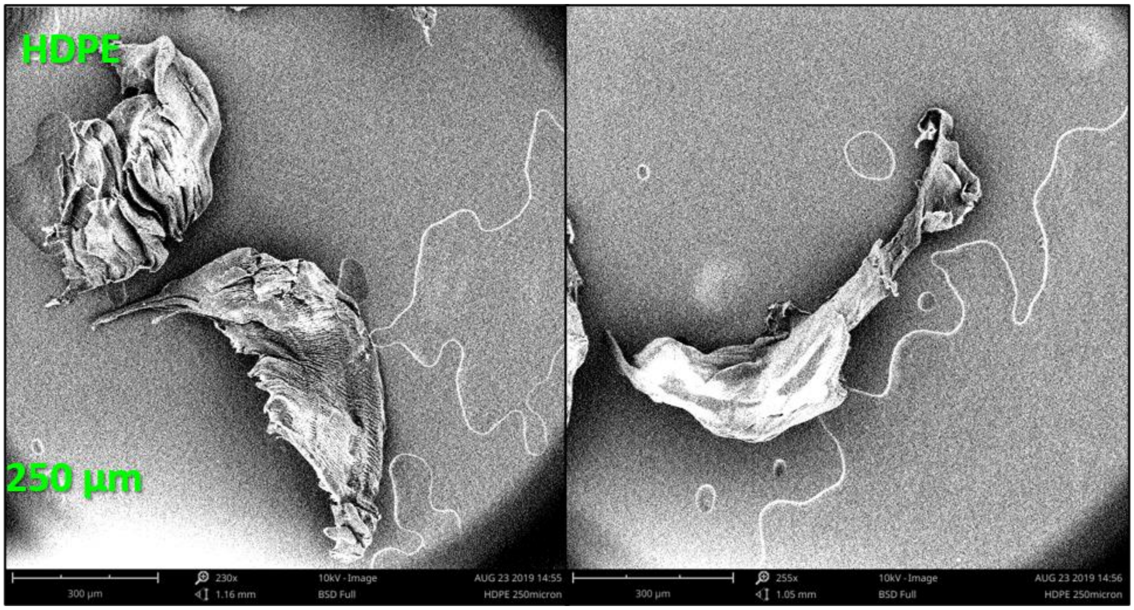


Figure 5. SEM images of the HDPE fragment type MPs reference material for size class 250 µm.

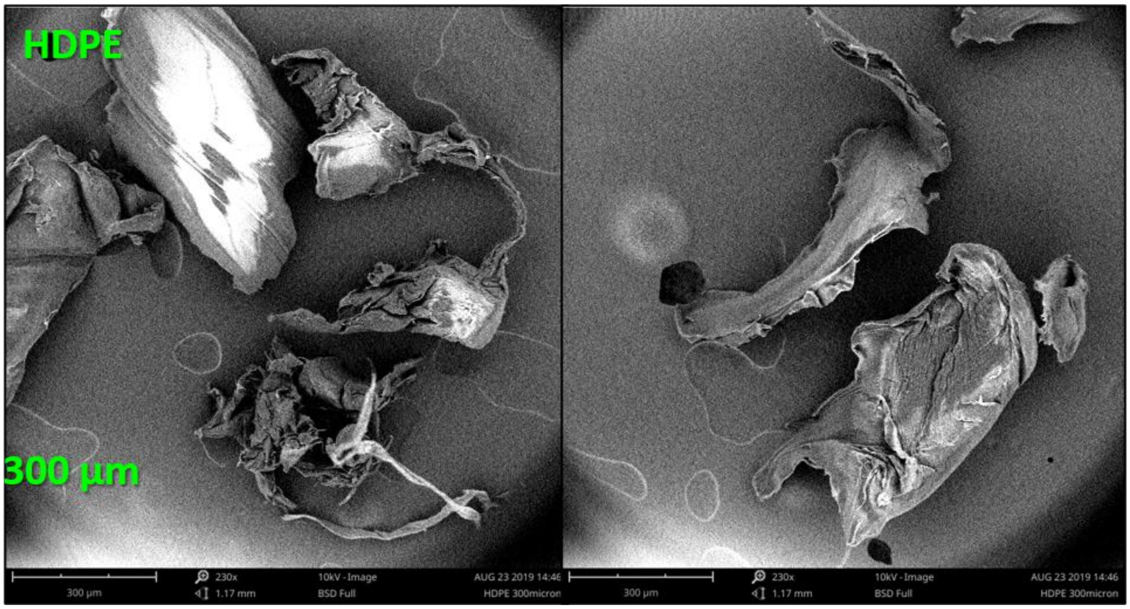


Figure 6. SEM images of the HDPE fragment type MPs reference material for size class 300 µm.

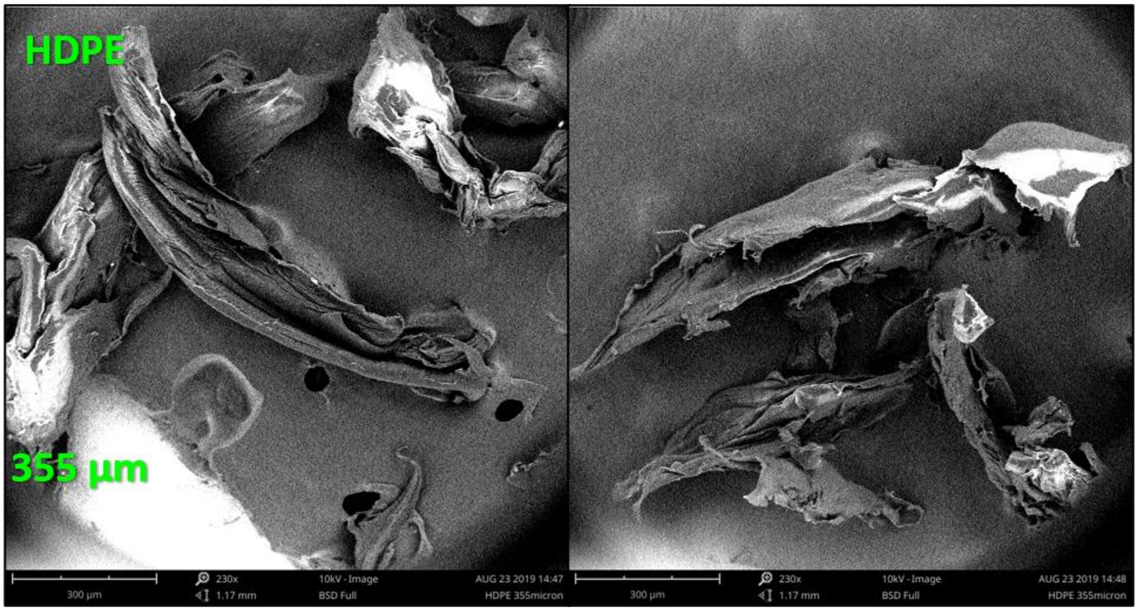


Figure 7. SEM images of the HDPE fragment type MPs reference material for size class 355 µm.

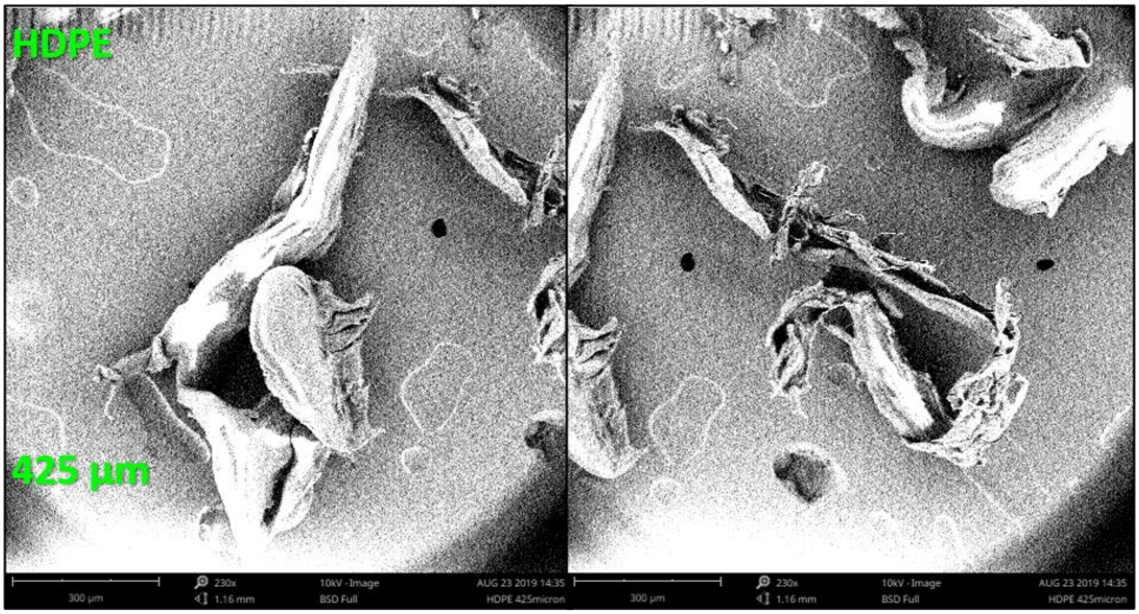


Figure 8. SEM images of the HDPE fragment type MPs reference material for size class 425 µm.

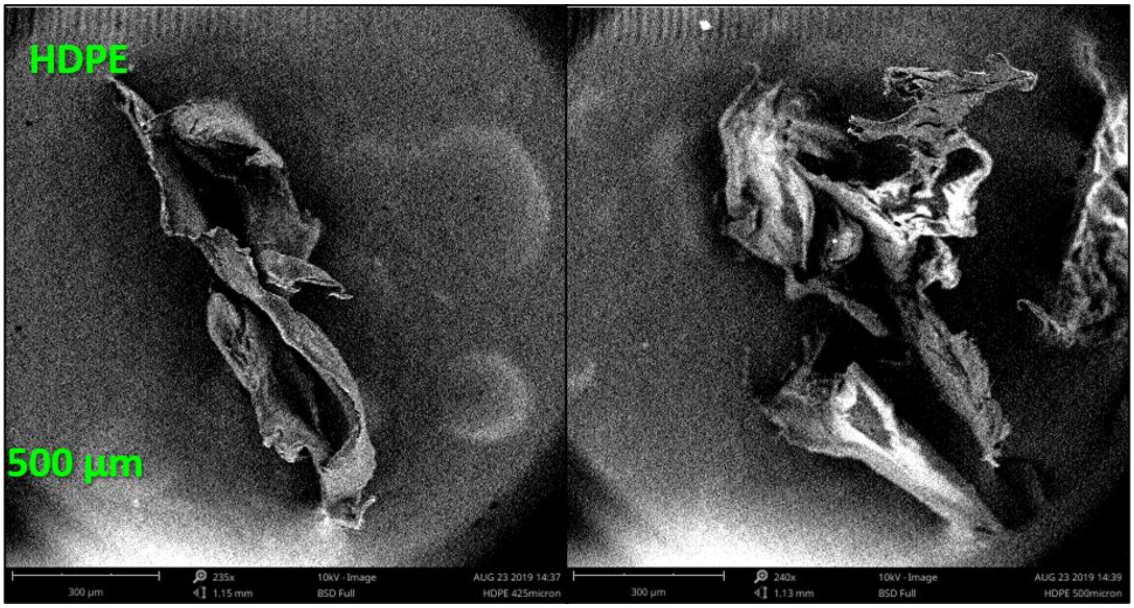


Figure 9. SEM images of the HDPE fragment type MPs reference material for size class 500 µm.

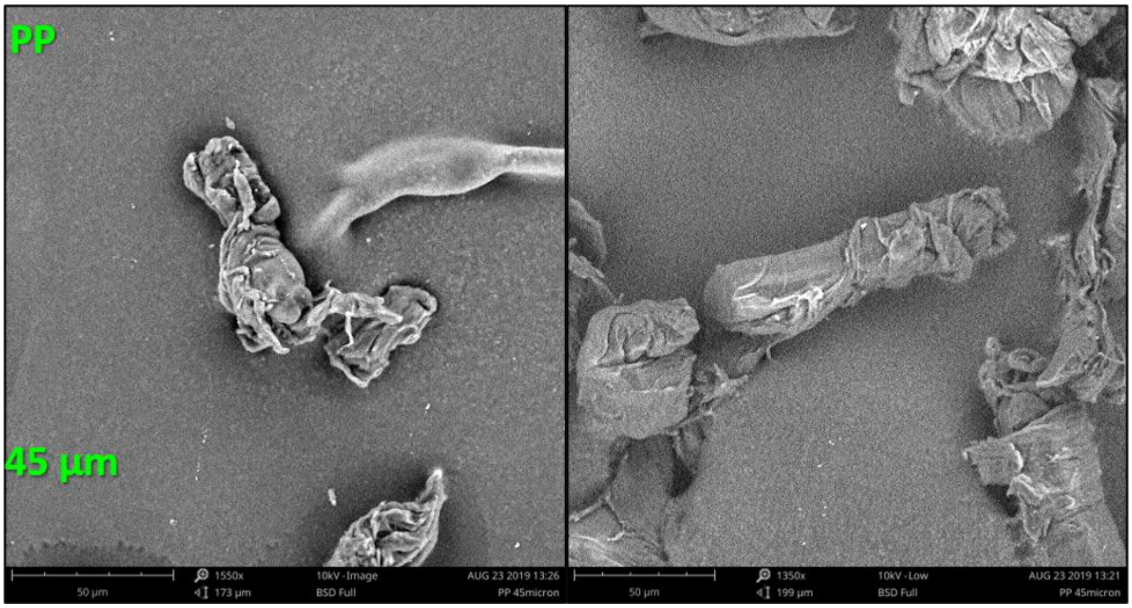


Figure 10. SEM images of the PP fragment type MPs reference material for size class 45 µm.

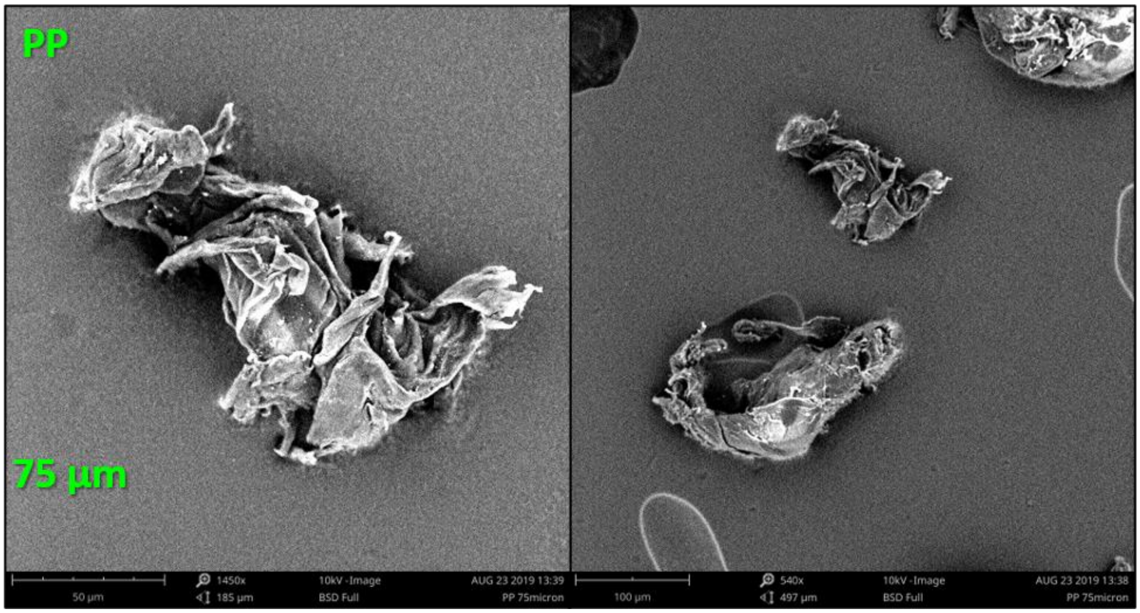


Figure 11. SEM images of the PP fragment type MPs reference material for size class 75 µm.

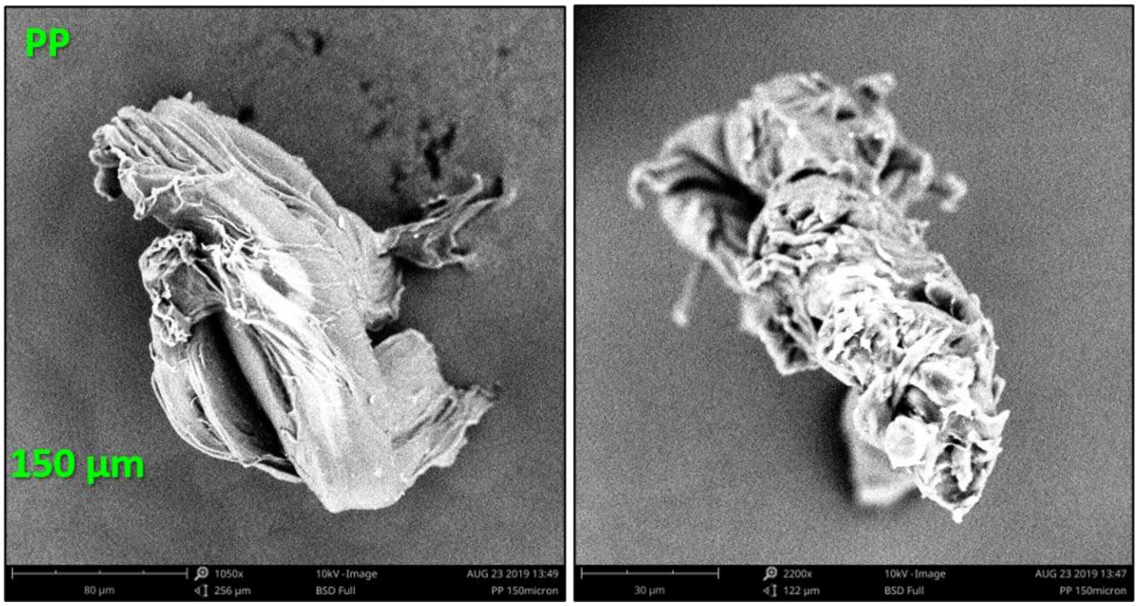


Figure 12. SEM images of the PP fragment type MPs reference material for size class 150 µm.

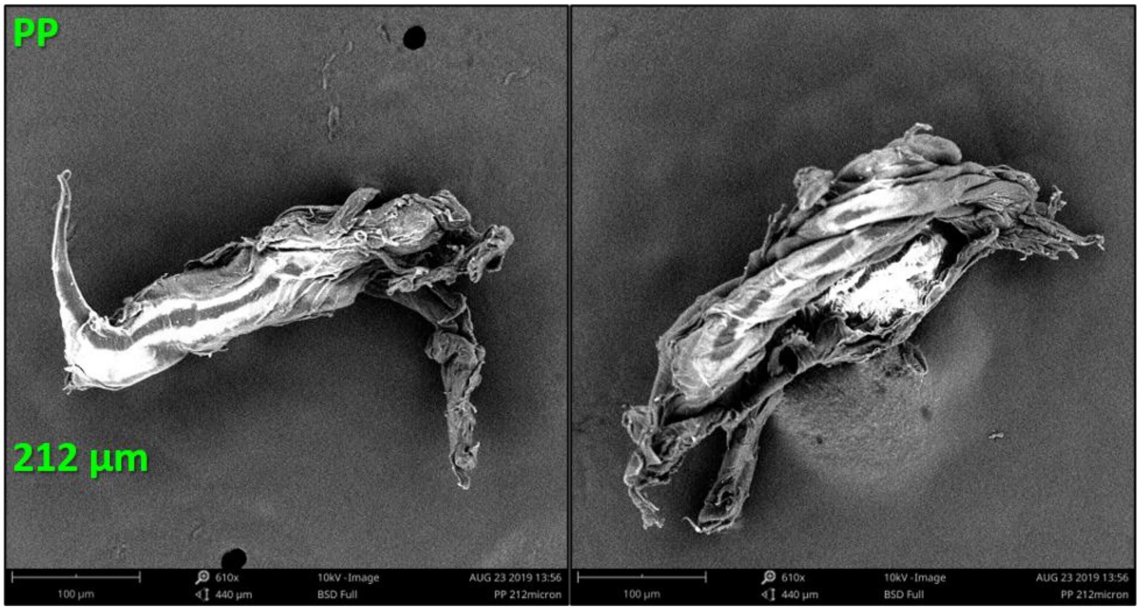


Figure 13. SEM images of the PP fragment type MPs reference material for size class 212 µm.

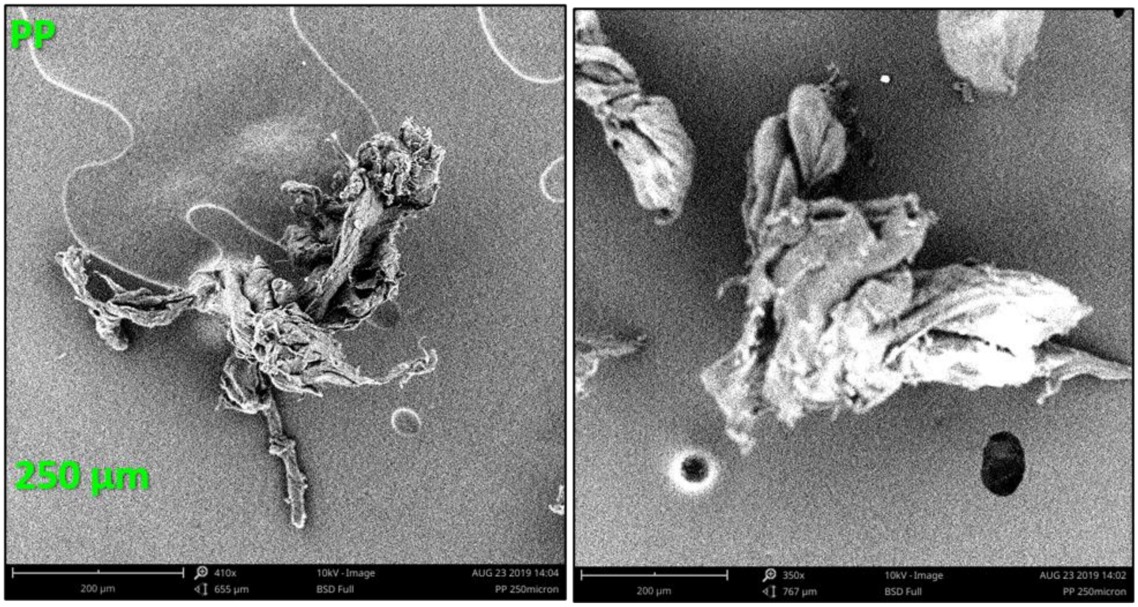


Figure 14. SEM images of the PP fragment type MPs reference material for size class 250 µm.

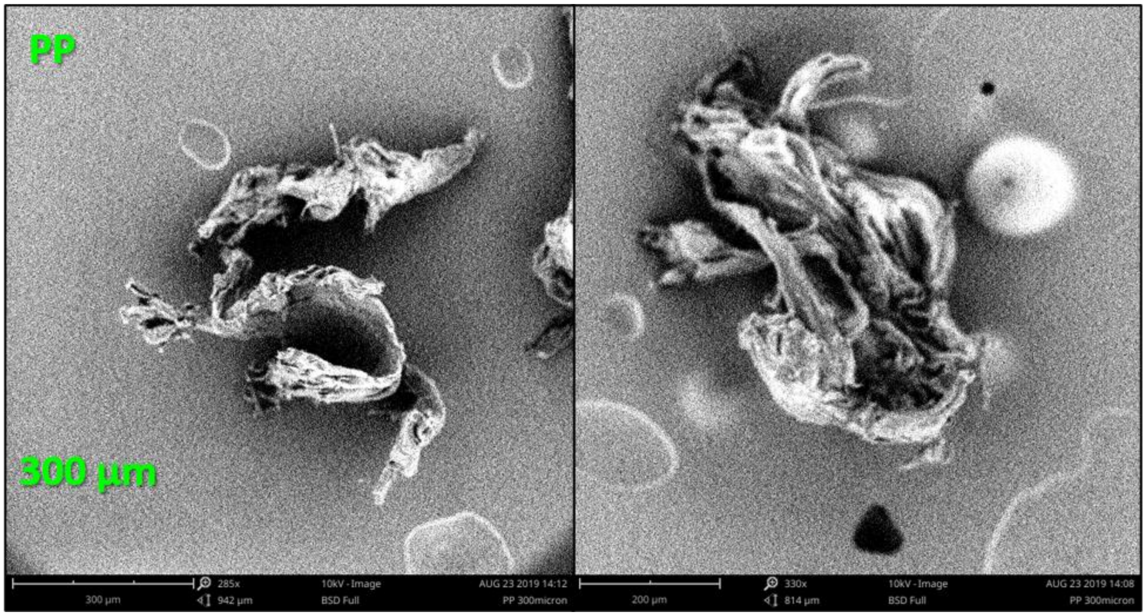


Figure 15. SEM images of the PP fragment type MPs reference material for size class 300 µm.

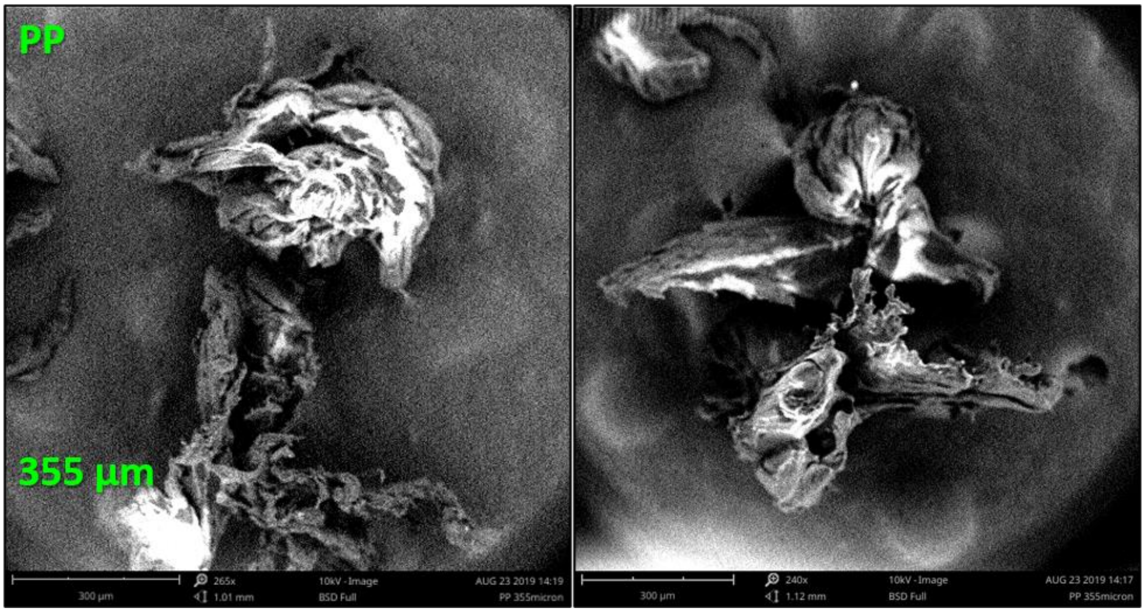


Figure 16. SEM images of the PP fragment type MPs reference material for size class 355 µm.

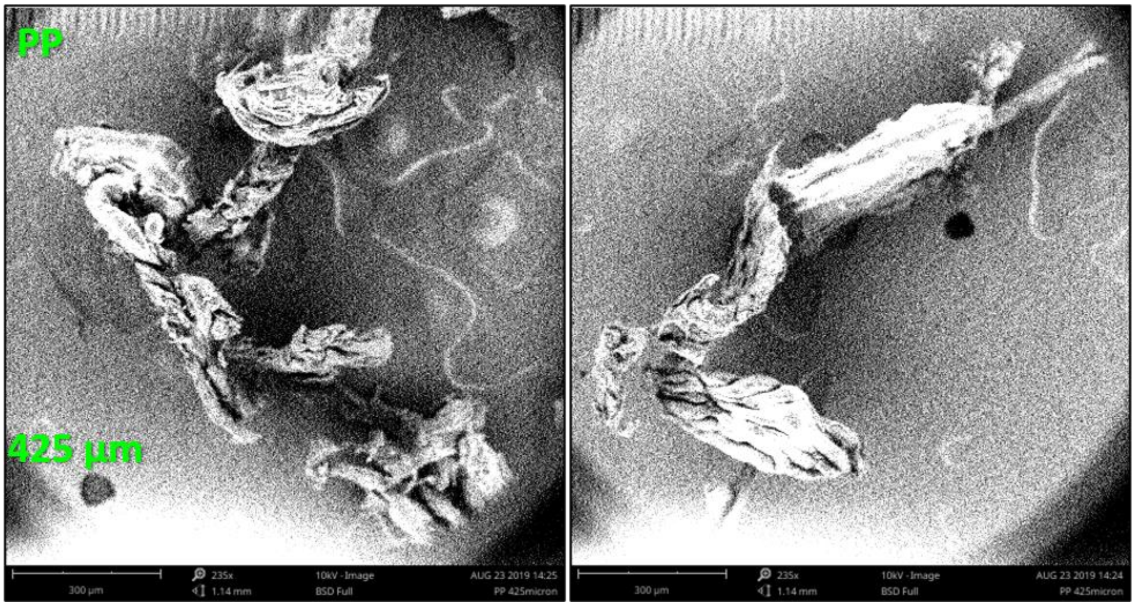


Figure 17. SEM images of the PP fragment type MPs reference material for size class 425 µm.

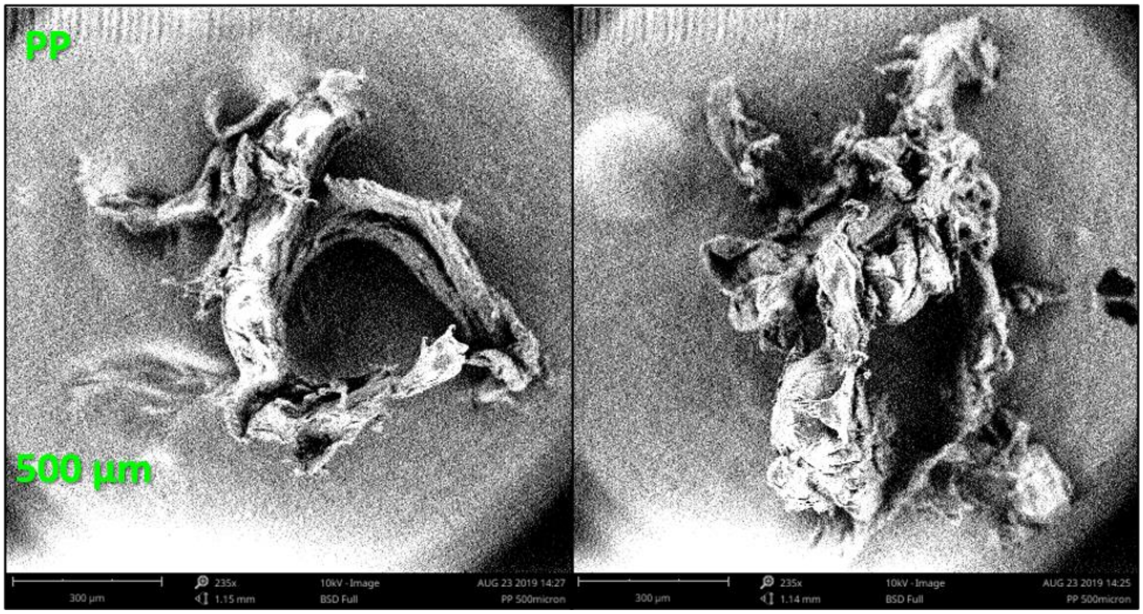


Figure 18. SEM images of the PP fragment type MPs reference material for size class 500 µm.

Appendix 3

BET Plots and Method for Surface Area Analysis

Select BET Plots Using Nitrogen Adsorptive Gas

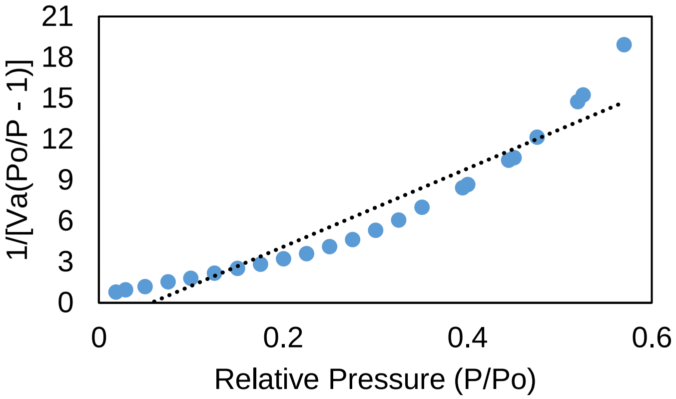


Figure 1. BET surface area plot for high-density polyethylene, 212-250 µm; $R^2 = 0.9124$. Calculated surface area is 0.1608 m²/g.

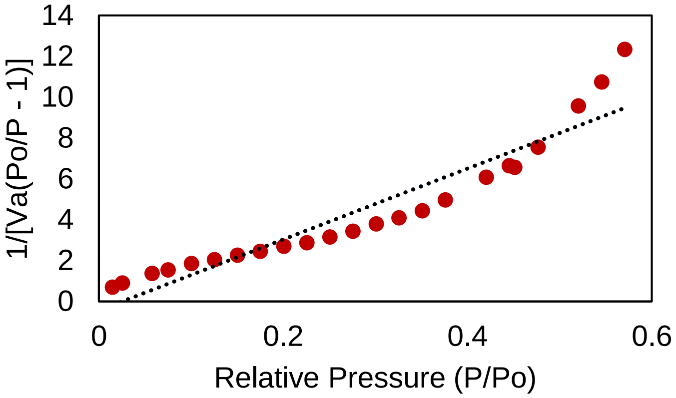


Figure 2. BET surface area plot for high-density polyethylene, 500-600 µm; $R^2 =$ 0.8936. Calculated surface area is 0.2571 m²/g

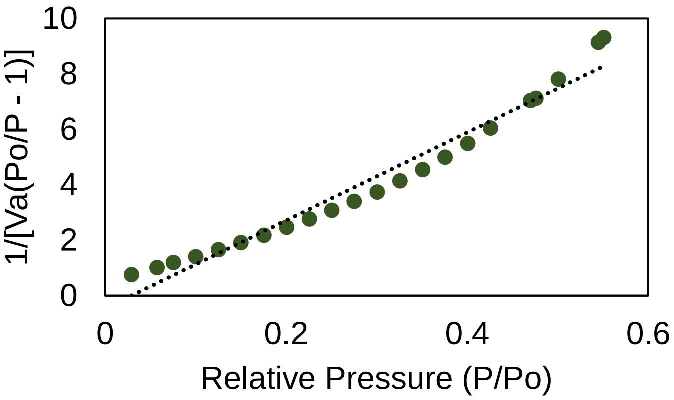


Figure 3. BET surface area plot for polypropylene, 212-250 µm; $R^2 = 0.9637$. Calculated surface area is 0.2826 m²/g.

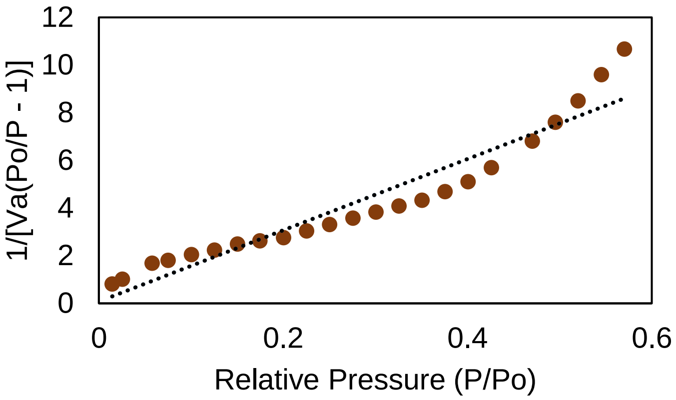


Figure 4. BET surface area plot for polypropylene, 500-600 µm; $R^2 = 0.9133$ Calculated surface area is 0.2897 m²/g.

Select BET Plots Using Krypton Adsorptive Gas

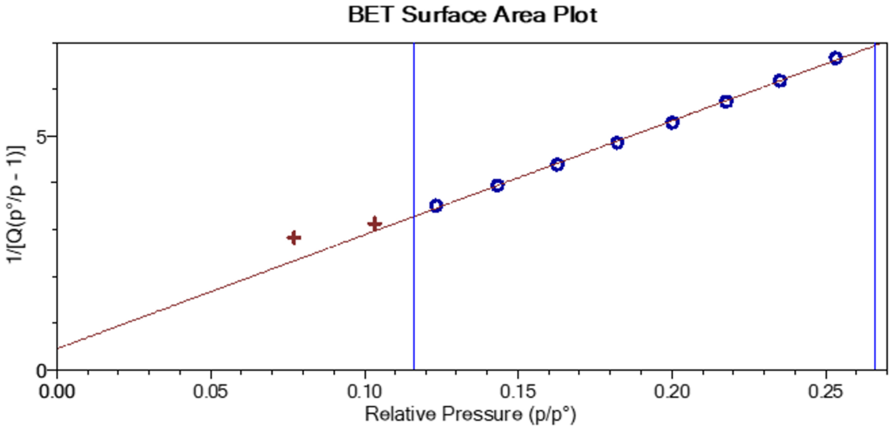


Figure 5. BET surface area plot using the TriStar II Plus for high-density polyethylene, 212-250 µm; R^2 = 0.9993. Calculated surface area is 0.2271 m²/g.

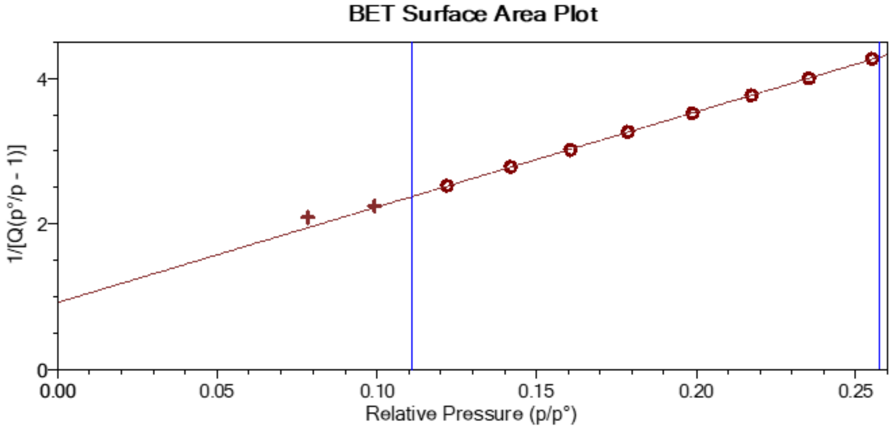


Figure 6. BET surface area plot using the TriStar II Plus for high-density polyethylene, 500-600 µm; R^2 = 0.9999. Calculated surface area is 0.4025 m²/g.

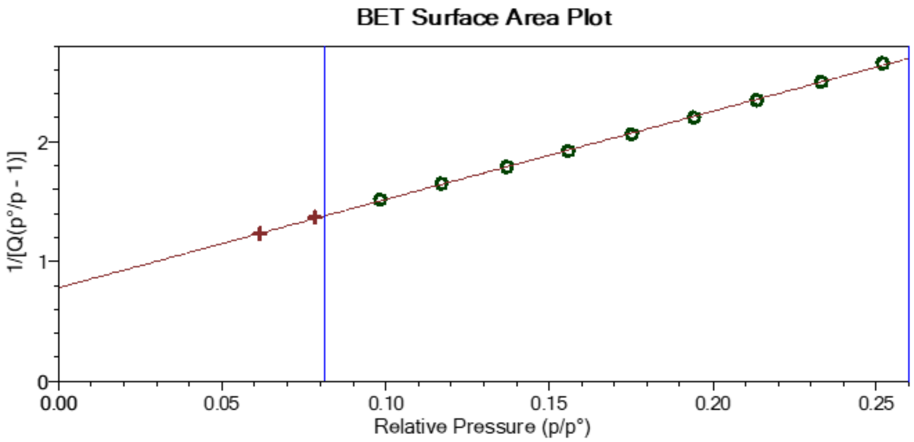


Figure 7. BET surface area plot using the TriStar II Plus for polypropylene, 212-250 µm; $R^2 = 0.9997$. Calculated surface area is 0.6918 m²/g.

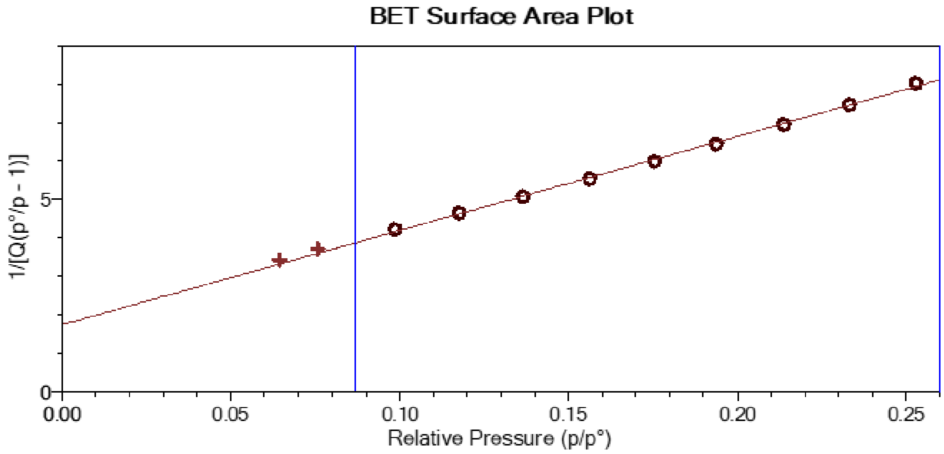


Figure 8. BET surface area plot using the TriStar II Plus for polypropylene, 500-600 µm; $R^2 = 0.9994$ Calculated surface area is 0.2149 m²/g.

Instrument Parameters for Surface Area Analysis with Krypton

Table 1. TriStar II settings for MP BET analysis of surface area with krypton as the adsorptive gas.

Mass of sample (g)	~0.05
Isothermal jacket	Yes
Filler Rod	No
Adsorptive	Krypton
Degass Conditions (evacuation was conducted on an ASAP 2420)	
Evacuation rate (mmHg/sec)	5
Unrestricted evac from (mmHg)	5
Vacuum level (µmHg)	10
Evacuation time (min)	60
Evacuation temp (°C)	60
Temp ramp rate (°C/min)	10
Analysis Conditions	
Preparation	
Fast evacuation	no
Evacuation rate (mmHg/sec)	5
Evacuation time (hr)	0.1
Free Space	
Measured before analysis	
Lower dewar for evac	Yes
Evacuation time (hr)	0.5
p^0 and Temperature	
p^0 type	Calculated from analysis temp
Temperature entered (K)	77.3
Equilibration interval (sec)	10
Sample Backfill	
Backfill at start of analysis	Yes
Backfill at end of analysis	Yes
Backfill gas	Nitrogen

Appendix 4

Protocol for Sorption Experiments

Triclocarban experiments

Setting up

1. Bake 60 mL amber glass vials at 550 °C for 4 hours.
2. Rinse foil lined caps with acetone.
3. Into vials, weigh out 10 ± 1 mg of chosen MPs.
4. To all vials, add 50 mL of DI water using an acetone rinsed graduated cylinder.
5. To vials add PPCP to concentrations according to experimental requirements.
6. To the lab blanks, add 50 µL of methanol.
7. Cap with foil lined caps.
8. Place vials on a rotating shaker at 50 rpm for 48 hours.

Collecting time points

9. Turn of rotating shaker and place vials on rack to allow plastics to settle for 15 minutes.
10. For all samples, take a 1 mL aliquot of the sample using an acetone rinsed, glass syringe, without disturbing the plastic particles as much as possible.
    a. Find a space in the floating plastic layer to insert the needle of the syringe.
    b. Pull water sample from far below the floating plastics, as to not pull MPs into the syringe.
    c. Take 2 mL of sample to ***rinse the syringe. Discard rinse.***
    d. Repeat step a and b, however now only take a 1 mL aliquot of sample water.
    e. Rinse syringe needle with DI water to remove plastics before transfer of aliquot. Rinse directly into 50mL vial.
    f. Transfer aliquot to a 2-mL amber, glass, autosampler vial.
    g. Store at 4°C until analysis by HPLC-MS.

For all samples, will isolate MPs (including lab blanks), **immediately** *after* aliquot of water is taken, filter sample:

    h. Using a baked, pre-weighed 0.45µm glass filter in an acetone rinsed ceramic Buchner funnel connected to a vacuum flask with gentle suction.
        i. Turn on vacuum.
        ii. Prime the filter using ~2mL of DI water before pouring in sample.
        iii. Swirl the vial to suspend particles and slowly pour over filter.
        iv. Rinse vial to remove stray particles 3x, using ~2 mL for each rinse.
        v. Use as little DI water as possible to rinse stray particles from the sides of the funnel (5-8mL).
        vi. Vacuum to dryness for 25 seconds.
        vii. Turn of vacuum.
    i. Carefully remove filter with acetone rinsed, metal forceps and replace back into labeled pan.

   j. Loosely cover pan with tin foil and allow filter to air dry for 48 hours.
   k. Measure mass of filter with MPs.
11. Immediately before analysis on the UHPLC-MS: spike each vial of water sample to a final concentration of 120 ng/mL of appropriate isotopic labeled PPCP for the internal standard. Inject water as is into the UHPLC-MS.

Extraction from MPs

12. After drying, using metal forceps, fold filter with MPs inward, then fold the long sides in, and place into the bottom of a baked 20 mL amber vial. (see Figure 1, below, for graphic explanation).
13. Add 10 mL of acetone to the vial.
14. Spike 3 vials with 600 ng of appropriate isotopic labeled PPCP.
15. Place in an ultra-sonicator for 30 minutes.
16. Without disturbing plastics, transfer a 5 mL aliquot of extract to a baked 20mL amber vial using a glass syringe.
17. Evaporate extracts under gentle nitrogen stream at 5psi to near dryness.
18. Reconstitute extracts with 2 mL methanol.
19. Vortex for 10 seconds.
20. Store at -20°C until analysis by HPLC-MS.
21. Immediately before analysis by UHPLC-MS: transfer 1mL into a 2-mL amber glass autosampler-vial and spike each vial to a final concentration of 120 ng/mL of internal standard.

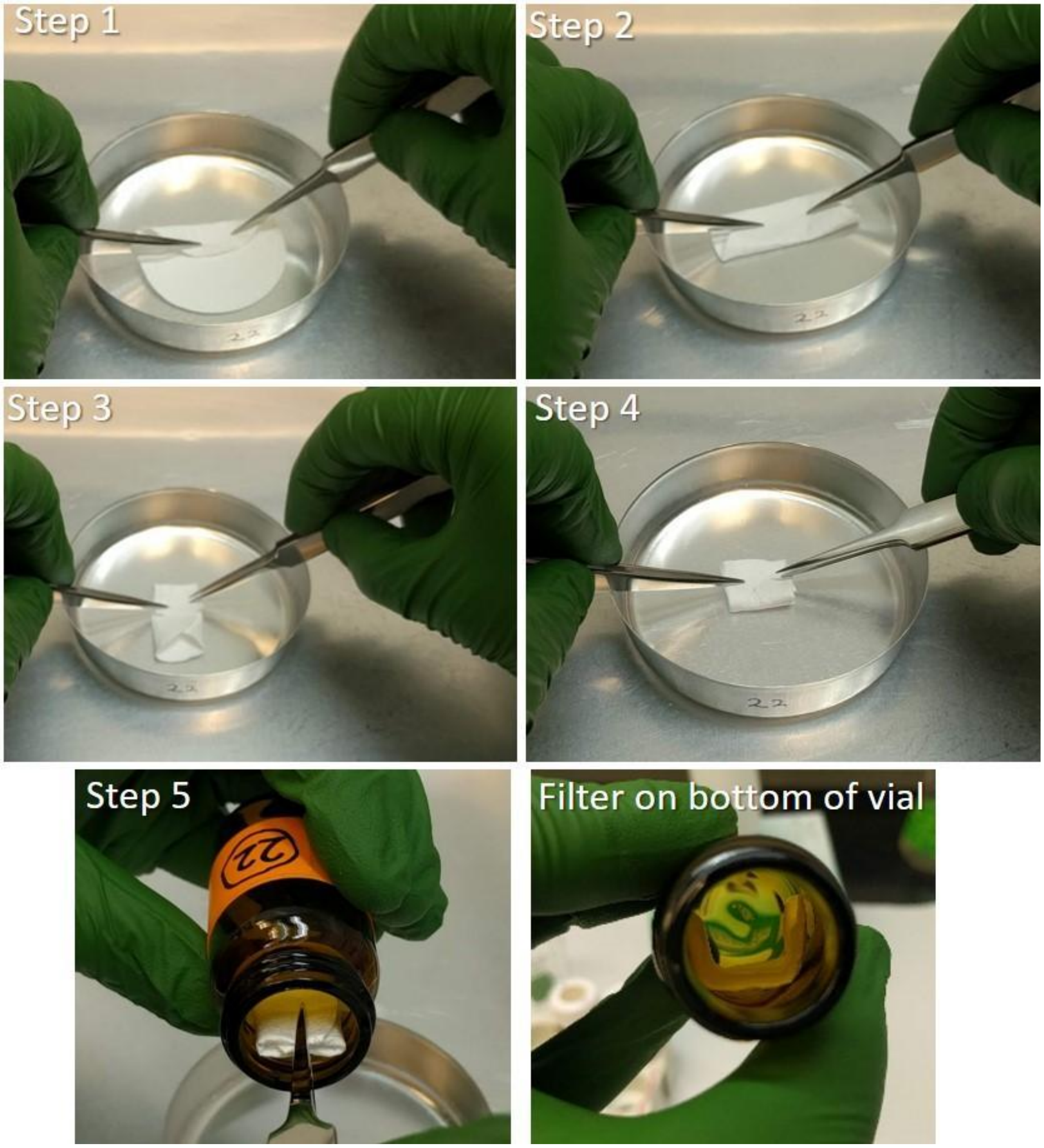


Figure 1. Folding procedure for MPs on glass filter for triclocarban extraction.

Venlafaxine, DEET, and diphenhydramine experiments

1. Bake 75 60 mL amber glass vials at 550 °C for 4 hours.
2. Rinse 3x, 76 aluminum lined caps with acetone.
3. Label vials, ensuring that labeling tape is in the *middle* of the vial – this makes the vials stable on the rotamix.
4. Into 16 vials, weigh out 30 ± 1 mg of 150-212 µm HDPE. Into another 16 vials, weigh out 10 ± 1 mg of 355-425 µm HDPE.
    a. Before each scoop, *stir microplastics well* so they are mixed to ensure each aliquot is a homogenous and representative sample.

5. Repeat step 3 for PP for both sizes.
6. To all vials add 20mL of pH 7.5-phosphate buffer and 30 mL DI water (final pH 7.4 ± 0.02), for a total volume of 50 mL, using an acetone rinsed graduated cylinder.
7. Excluding the HDPE and PP blanks, add the appropriate PPCP to a concentration of 1000, 800, 600, 400, and 200 µg/L into 12 vials *each* (i.e., 3 replicates and 4 treatments, so each isotherm point will have a total of 12 vials).
8. For duplicate water spikes (to calculate losses to vial), repeat step 7 but only for 2 vials for each concentration.
9. To the HDPE and PP blanks, add 100 µL of methanol.
10. Cap with aluminum lined caps.
11. Place vials on a rotating shaker at 50 rpm for ≥120 hours.

Collecting and Processing Samples:

12. Turn of rotating shaker, take off vials and wait for plastics to settle (≥15 min or the length of time required to set up the SPE assembly).
13. For water samples, conducted HLB SPE:
    a. Assemble extraction cartridge assembly with HLB SPE cartridge, 6cc (200mg) (Waters Oasis Cat. No. WAT106202), and the vacuum SPE manifold:
        i. Install the SPE cartridge and then attach pre-labeled 60ml sample reservoirs tubes to them; this is where samples will be loaded.
        ii. Attach vacuum pump to manifold.
        iii. Adjust pressure to 5-7.5 in Hg.
        iv. Open vacuum manifold valves (where SPE cartridges attach) a quarter turn.
    b. Cartridge cleaning:
        i. Add a 5 mL aliquot of methanol/acetone (1:1) and draw through the cartridge until dry.
            a. During these steps, ensure, and adjust if necessary, flow is ~10 mL/min (3-5 drops per second); mark adjustment on manifold valves.
        ii. Add another 5 mL aliquot of methanol/acetone (1:1) and draw through the cartridge until dry.
    c. Cartridge conditioning: the conditioning step is critical for recovery of analytes and can have a marked effect on method precision and accuracy:
        i. Conditioning with methanol/acetone
            a. Add 2.5 mL of methanol/acetone (1:1) to each cartridge.
            b. Pull 1–2 mL of solvent through the cartridge, and then turn off the vacuum temporarily.
            c. Allow the sorbent to soak for about one minute.
            d. Add another 2.5 mL of methanol/acetone (1:1) and draw ~1 mL of solvent through the cartridge, leaving a thin layer of solvent on the surface of the sorbent. **The cartridge must <u>not</u> be allowed to go dry from this point until the end of the sample extraction.**
        ii. Conditioning with water

        a. Add 2.5 mL of DI water to each cartridge and draw through, leaving a thin layer of liquid on the surface of the sorbent.
        b. Follow this with another 2.5 mL aliquot of DI water. Draw the water through each cartridge, **again being careful to keep the water level above the sorbent surface.**
        c. Turn off the vacuum.

d. Taking aliquot from sample
    i. Using a 5 mL syringe that has been acetone rinsed, find a space in the floating plastic layer to insert the needle of the syringe.
    ii. Find a space in the floating plastic layer to insert the needle of the syringe.
    iii. Pull water sample from far below the floating plastics, as to not pull microplastics into the syringe.
    iv. Take ~1 mL of sample solution to sample rinse the syringe up to the 20 mL line. Dispose of rinse.
    v. Using the same technique as described in steps 1-3, take 1 mL of sample solution.
    vi. Transfer this aliquot to the appropriately labeled 60 mL reservoir.
    vii. Pour the rest of sample into a 45 µm sieve, over a container to catch sample solution, to capture plastics.
        a. Dispose of microplastics as solid waste.
    viii. Dispose of sample solution as liquid waste.

e. Loading samples into cartridges/extraction:
    i. Add 300ng of the appropriate recovery surrogate to each sample.
        a. Venlafaxine: 150 µL of 2ppm.
    ii. Open manifold valves and turn on the vacuum to 5-7.5 in. Hg. Extract samples at a flow rate of ~10 mL/min, or 3-5 drops per second**. Ensure flow does not stop temporarily from sample reservoir to the cartridge**, as it should not go dry before entire sample volume is extracted.
    iii. When the sample has been drawn through the cartridge, add a 10 mL aliquot of DI water to the sample reservoir and pull the rinsate from the container through the cartridge using vacuum.
    iv. Pull air through the cartridge by maintaining full vacuum until no liquid appears to pass through the cartridge. Complete drying of the cartridge is not necessary for this method.

f. Cartridge Elution
    i. Release the vacuum from the extraction manifold so the top can be removed.
    ii. Clean out manifold.
    iii. Insert 15 mL conical glass collection tubes into the manifold to catch the extracts as they are eluted from the cartridge. The collection tube must extend above the drip tip beneath the cartridges to ensure collection of all the eluate.
    iv. Replace the top to the manifold, and resume vacuum.

    v. Add 2.5 mL of methanol/acetone (1:1) to the cartridge and, with vacuum, pull enough methanol/acetone into the cartridge to soak the sorbent leaving a small amount of methanol/acetone above the surface and turn off vacuum.
    vi. Allow the sorbent to soak for about one minute.
    vii. Add another 2.5 mL methanol/acetone (1:1) to the cartridge and, using vacuum, pull the remaining solvent through the cartridge until dry: after last drop wait 1 minute.
    viii. Vent the vacuum manifold, remove the top, and then remove collection tubes.

14. Evaporate water extracts under gentle nitrogen stream at 15 psi to near dryness (~30 min).
    a. Reconstitute extracts with 1 mL methanol in glass collection tubes.
    b. Vortex for 10 seconds.
    c. Store at -20°C until analysis.
    d. Immediately before analysis by UHPLC-MS/MS: spike each vial to a final concentration of 120 ng/mL of the appropriate internal standard.
    e. Vortex for 10 seconds.
    f. Transfer sample into a 2-mL amber glass autosampler-vial for UHPLC-MS/MS measurement.

UHPLC-MS/MS settings

i. Triclosan/triclocarban method
    a. A Shimadzu Nexera X2 Ultra High Performance Liquid Chromatograph (UHPLC) coupled with a Shimadzu 8040 triple quadrupole mass spectrometer (MS/MS) (Shimadzu North America, Columbia, MD, USA) equipped with an ESI- source was used for the analysis of TCS and TCC. The UHPLC was run isocratically using a mobile phase of 10 mM ammonium acetate in a solution of methanol: acetonitrile: water (60:15:25 v/v) at a flow of 0.5 mL/min and equipped with a ACQUITY UPLC HSS T3 C18 reverse phase column (1.8 µm, 2.1 x 100 mm) (Waters Corporation, Milford, MA, USA). Column oven temperature was set to 45 °C and the heat block is set to 400 °C. The nebulizing gas flow (argon) is 3 L/min and the drying gas flow (nitrogen) is 10 L/min. The desolvation line temperature is 225 °C. The total run time per sample will be 3.25 min. MS acquisition will occur in multiple reaction monitoring (MRM).

Transitions, Voltages, and Mobile phase				
Compound	**Precursor → Product Ion**	**Q1 prebias (V)**	**CE (eV) collision energy**	**Q3 prebias (V)**

TCS	288.00 → 34.95	17	11	13
TCC	314.90 → 162.00	19	12	17
$^{13}C_{12}$ TCS	299.00 → 35.00	19	10	13
$^{13}C_{13}$ TCC	327.90 → 167.85	20	13	17
D_3 TCS	289.90 → 35.00	18	9	13
D_4 TCC	318.90 → 162.00	19	14	30
Mobile Phase	10mM ammonium acetate in methanol: acetonitrile: water (60:15:25 v/v)			

ii. DEET, diphenhydramine, and venlafaxine method:
    a. A Shimadzu Nexera X2 Ultra High Performance Liquid Chromatograph (UHPLC) coupled with a Shimadzu 8040 triple quadrupole mass spectrometer (MS/MS) (Shimadzu North America, Columbia, MD, USA) equipped with an ESI- source was used for the analysis. A binary gradient was used using mobile phases: A, 0.1% formic acid and 0.1mM ammonium acetate in 95:5 v/v water/methanol, and B, same additives as A but in 100% methanol at a flow rate of 0.3 mL/min.; using a Phenomenex Synergi™ column (2.5 µm Polar-RP 100 Å, LC Column 100 x 2 mm) (Phenomenex Inc., Torrance, CA, USA). Column oven temperature was set to 50 °C and the heat block is set to 400 °C. The nebulizing gas flow (argon) is 3 L/min and the drying gas flow (nitrogen) is 15 L/min. The desolvation line temperature is 250 °C. The total run time per sample was 5.00 min. MS acquisition occurred in multiple reaction monitoring (MRM).

Transitions, Voltages, and Mobile phase				
Compound	**Precursor → Product Ion**	**Q1 prebias (V)**	**CE (eV) collision energy**	**Q3 prebias (V)**
DEET	192.00 → 91.10	-12	-30	-15
D_3 D_4 DEET	199.10 → 125.95	-18	-20	-24
D_6 DEET	198.10 → 119.10	-13	-18	-20
Diphenhydramine	257.05 → 168.0	-12	-12	-16
D_5 Diphenhydramine	261.10 → 172.10	-12	-15	-16

D_3 Diphenhydramine	295.05 → 167.00	-17	-14	-16
Venlafaxine	287.25 → 58.10	-18	-21	-23
D_{11} Venlafaxine	289.40 → 57.85	-15	-22	-10
D_6 Venlafaxine	284.15 → 64.05	-19	-22	-24
Mobile Phases	A: 0.1% formic acid and 0.1mM ammonium acetate in 95:5 v/v water/methanol. B: same additives as A but in 100% methanol.			

Appendix 5

LogD_{OW} vs logK_{dPE} with negative logK_{dPE} values (orange markers) at neutral or close to neutral pH.

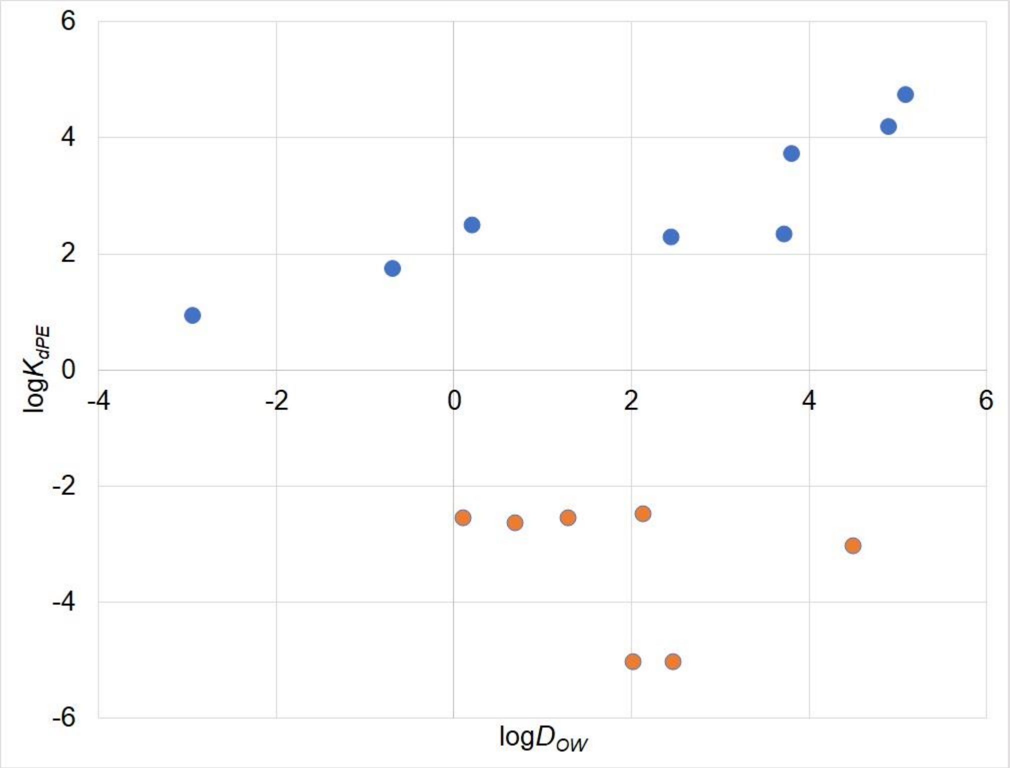


Figure 1. For visualization purposes ONLY, not used for analysis: venlafaxine and diphenhydramine were given an average value using the logK_{dPE} of propranolol and sertraline, as they have similar structures and pK_a's; and the same was done for DEET using diethyl phthalate's logK_{dPE}, as these chemicals are similar in structure and logK_{OW}. Calculation at other pH's (5, 6, 8, & 9) had similar looking relationships with negative logK_{dPE} values and did not resolve in any meaningful correlations.

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