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Title: Towards a greater understanding of the presence, fate and ecological effects of microplastics in the freshwater environment
Issue Date: 2019-12-19

CHAPTER 1

Introduction

1. Plastics as an environmental pollutant

In today's society, people would struggle to live without plastics. Plastics are strong, waterproof, durable and cheap, making it the material of choice for manufacturers of many everyday items including packaging, electrical items and clothing, among others. However, these features of plastics also mean they now represent a significant proportion of our waste. Despite measures to reduce plastic consumption and disposal, or to recycle plastic items, the amount discarded as plastic waste is increasing year-on-year, with the potential for much of this waste to be mismanaged and enter the environment (Jambeck et al., 2015; PlasticsEurope, 2015). The longevity of plastics implies that plastic litter that ends up in the environment will persist to leave a legacy of our 'throw-away society' for hundreds, if not thousands of years to come. With fears that the mass of plastic in the oceans could equal or exceed the weight of fish in the sea by 2050 (World Economic Forum, 2016), the general public are becoming increasingly concerned about the effects of plastics on the environment. Within the last two to three years, plastics and microplastics have begun to attract significant academic and media attention, reflecting societal concerns about the issue of waste and environmental pollution.

While plastics are durable, they invariably degrade with age, with large items fragmenting to form multiple smaller pieces, with those < 5 mm in size defined as 'microplastics' (Arthur and Baker, 2009; Moore, 2008). Despite this degradation, the resulting fragments are estimated to last for hundreds or even thousands of years within the environment (Barnes et al., 2009). Microplastics fall within two categories: primary microplastics (manufactured specifically to be smaller than 5 mm, including cosmetic microbeads, glitter and nurdles) and secondary microplastics (derived from the breakdown and weathering of large-scale plastics or plastic-containing products, such as fragments of degraded litter or microfibers from synthetic textiles) (Hartmann et al., 2019). Microplastics are of particular concern as an environmental contaminant due to their potential for ingestion by organisms, with evidence to suggest they can cause harm to organisms and ecosystems. In addition to microplastics being a particulate pollutant, microplastics may act as a source of organic chemicals to the environment in the form of plasticisers leached from plastics as they degrade (Lohmann, 2017).

2. Importance of studying microplastics

Awareness of microplastics as a potential environmental contaminant first arose in the early 1970s, with the incidental discovery of small plastic particles in marine environmental samples (Buchanan, 1971; Carpenter and Smith, 1972). This led researchers to realise that plastic pollution consisted not just of the large-scale litter that is widely visible within the environment, but that plastics were also present at a much smaller scale. Since these first observations, many studies have since used environmental sampling as a means of assessing microplastic distribution and abundance across a wide range of environments. Due to the prevalence and widespread use of plastics in all aspects of daily life, sources and emissions of microplastics to the environment as a result of product use and degradation are varied and diverse. It is recognized that the majority of microplastic waste will originate on land as this is where plastics are primarily used and discarded. However, microplastics have the capability to become widely distributed from their original source by wind, water or human actions (Lebreton et al., 2017; Nizzetto et al., 2016; Zylstra, 2013).

The marine environment is, to date, the most widely studied environment with respect to microplastic pollution, with comparatively much less understood about the contamination of freshwater systems. This is despite the understanding that rivers represent the main link between the terrestrial and the marine environment, facilitating the movement of plastics from land-based sources to the sea (Jambeck et al., 2015; Lebreton et al., 2017). However, it is highly unlikely that all particles will pass through freshwater systems unimpeded; on their journey from land to sea, microplastics will encounter a wide range of complex interactions that will influence their behaviour, transport and fate. Thus not all microplastics will reach the ocean (Castañeda et al., 2014; Dris et al., 2015; Wagner et al., 2014). Whether accumulated within sediments or passing through the water column, microplastics within rivers can become bioavailable to organisms across a range of trophic levels (Sanchez et al., 2014; Windsor et al., 2019b). A huge variety of factors will influence the potential ecological effects of microplastics including (but not limited to) environmental conditions, type of polymer, associated chemicals and size and shape of particles (Windsor et al., 2019a; Wright et al., 2013b).

The regulatory trend with microplastics is increasingly moving towards the precautionary principle of banning products without full evidence of harm (e.g. banning of microbeads in personal care products in various countries globally). However, while microbeads are relatively easy to regulate as they are usually an additional, rather than a core ingredient in products, many other applications of (micro)plastic will be far less easy to eliminate. While the public are increasingly calling for bans or restrictions on certain plastic products, we must be certain to provide evidence of environmental release and harm in instances where banning specific plastic products may lead to a regrettable substitution, where products are replaced by potentially more harmful, and less well understood, products. This thesis aims to address the significant gaps remaining in our knowledge surrounding the sources, fate and ecological effects of microplastics in the context of these complex environmental factors.

3. Microplastics in the freshwater environment

Worldwide, humans rely heavily on freshwater systems for drinking water resources, in addition to food sources (fish and shellfish), irrigation and leisure activities. Clean water is essential for maintaining life, both aquatic and terrestrial. Contamination of freshwater systems by particulate or chemical contaminants can have significant implications for water quality, ecosystem health and function, and human health. It is therefore essential to understand how rivers may act as not only a transport pathway, but as a sink of microplastics, and the implications this may have on freshwater ecosystems and water quality.

Despite the comparative lack of research on microplastics in freshwater systems compared to the marine environment, the studies carried out to date imply that freshwaters may be equally, if not more, contaminated with microplastics than the oceans, with the highest ever concentrations of microplastics found recently in a UK river, and with flooding seen to significantly reduce sediment concentrations (Hurley et al., 2018). It is therefore critical that the scientific community works towards a greater understanding of the factors influencing microplastic accumulation and transport in freshwater environments, in addition to understanding the ecological effects, to better inform policy, industry and public decision-making.

4. Ecological impacts of microplastics

It has been observed in many studies that organisms across various trophic guilds will ingest microplastics. Microplastic ingestion may be either intentional (ingesting particles that resemble food) or unintentional (particles eaten incidentally in association with other food). It has been observed that many higher trophic organisms, including sea turtles, birds, marine mammals and fish contain (micro)plastics within their guts, likely as a result of food-chain transfer (Campbell et al., 2017; Eriksson and Burton, 2003; Lusher, 2015). Trophic transfer is therefore likely to lead higher trophic organisms to become exposed to microplastics when otherwise they may not have done (Eriksson and Burton, 2003; Nelms et al., 2018). Ingestion by lower trophic organisms could lead to a bioaccumulation within the predators, and even (size-dependent) translocation to body tissues (Mattsson et al., 2017; Moore, 2008; Watts et al., 2014).

While microplastics have been found widespread throughout the environment, including within organisms, there is still insufficient understanding of the ecological and toxicological implications of this exposure. Physical harm may include blockage of the gut following ingestion, internal or external abrasion or inflammation, or blockage of gills leading to suffocation (Moore, 2008; von Moos et al., 2012; Wright et al., 2013b). The potential for a particle to cause harm depends on a huge variety of factors including the size and shape of the particle, concentration of plastic particles or associated chemicals (discussed in section 5), environmental conditions and also particle behaviour within the environment, determining whether an organism is likely to encounter it. Different traits of organisms will also influence their susceptibility to harm resulting from microplastic exposure. Therefore, it is also highly likely that different species will be affected in different ways by exposure to microplastics, depending on feeding behavior, metabolism, life-history and physiological characteristics (Galloway et al., 2017; Setälä et al., 2016; Wright et al., 2013b).

Microplastic exposure, in some instances, has been shown to have detrimental effects on health, metabolism, reproduction and immunity (Besseling et al., 2014; Wright et al., 2013a). However, these studies often represent very highly polluted or unrealistic scenarios and are therefore not necessarily representative of the likely exposure conditions that these organisms will encounter in the environment. Lower (more realistic) concentrations tend not to induce significant effects on commonly observed endpoints such as survival, behavior and reproduction in the short term (Lenz et al., 2016). There is not yet sufficient evidence to accurately determine the long-term impacts of microplastic contamination on organisms and ecosystems, although recent research suggests that chronic sublethal effects on the less-frequently investigated traits such as gene expression, metabolism or hormone production may have protracted but potentially significant long-term impacts on populations and the ecosystems that depend on them (Galloway et al., 2017; Jaikumar et al., 2019).

It is important to note that even at high concentrations plastics may not always be harmful; some studies suggest that microplastics may be ingested and egested without consequence (Beiras et al., 2018; Jovanović et al., 2018; Kaposi et al., 2014; Weber et al., 2018), while others show that some organisms can eat and metabolise plastic. For example, waxworms have been found to digest polyethylene, specifically due to the polymer-degrading bacteria *Enterobacter asburiae* YT1 and *Bacillus* sp. YP1 within the gut (Yang et al., 2014). A similar study was carried out which discovered that mealworms can digest and depolymerise polystyrene foam due to the gut bacterium *Exiguobacterium* sp. strain YT2, remaining as healthy over a one month test as mealworms that were fed a normal diet (Yang et al., 2015a, b). In addition to acting as a food source, plastics have also been shown to act as a microbial habitat, with the potential to acquire a distinct microbial community that is different in composition and less diverse than the surrounding environment (McCormick et al., 2014; Oberbeckmann et al., 2018; Zettler et al., 2013). While this novel substrate can be beneficial to the microbial communities which associate with plastic, the presence of plastics may also detrimentally alter the bacterial community structure within specific environments, changing the ecosystem structure by leading to the dominance of certain species. It is recognised that in order to ascertain any likely consequences of the widespread microplastic presence under realistic environmental conditions, it is important to understand the ecological impacts of microplastics not only at concentrations that are representative of those found within the environment, but also under representative timescales of exposure and with the heterogeneous mix of particles (and chemicals) to which organisms will be exposed (Lenz et al., 2016; Rist and Hartmann, 2018).

While there is a wide gap between our knowledge of the presence and abundance of microplastics in the marine environment compared to freshwaters, including rivers and lakes, our comparative understanding of organism interactions between these two systems is yet more unbalanced. While many ecological studies have focused on the presence of microplastics with wild-caught marine fish and invertebrates, far fewer address freshwater organism exposure or interactions. Further, considering our knowledge of rivers as carriers of microplastics, receiving and transporting microplastics from diverse sources and inputs, little emphasis has been put on research investigating the environmental factors influencing freshwater organism exposure, for example proximity to sources or differential exposure as a result of life history traits. This thesis therefore aims to investigate how specific sources and inputs such as wastewater effluent can be linked to organism exposure, in addition to how intraspecific differences might influence ingestion. This will significantly increase our understanding of the factors influencing organism exposure and thus the potential for harm.

5. Plastics as a carrier of toxic chemicals

In addition to causing physical harm, there are two ways in which microplastics may impose a chemical hazard to organisms, either as a result of incorporated plasticiser chemicals, or the sorption of organic chemicals from the environment. Plastics are manufactured containing a variety of different plasticiser chemicals (e.g. phthalates, bisphenol A, dyes) which are added to plastics during manufacture, including plasticisers, flame retardants and dyes to give them different properties, for example to improve flexibility and durability (Lithner et al., 2009; Lithner et al., 2012). These chemicals are not chemically bound to the polymer structure and thus can leach out of plastic as the product ages, a process which can be accelerated by environmental conditions such as high temperatures or UV exposure (Bandow et al., 2017). This release of plasticisers allows these (potentially harmful) chemicals to become freely available within the environment and to organisms (Huang et al., 2013; Lithner et al., 2009). It has also been suggested that gut surfactants and an increased temperature within the stomach (compared to within the external environment) can facilitate plasticiser leaching from particles following ingestion (Bakir et al., 2014).

Microplastics are hydrophobic, with a large surface area to volume ratio, and so will associate with hydrophobic organic chemicals (HOCs, e.g. pesticides, polychlorinated biphenyls, polybrominated diphenyl ethers) within the environment (Ašmonaitė et al., 2018; Mato et al., 2001; Rochman et al., 2013b). This may lead to the alteration of these chemicals' toxicity and bioavailability to organisms (Rochman et al., 2013a; Teuten et al., 2009). There is widespread scientific debate as to whether plastics facilitate the uptake and bioaccumulation of these chemicals within organisms, or whether binding to plastics makes the chemicals less available, thereby reducing uptake (Bakir et al., 2016; Koelmans et al., 2016). Some studies have shown that plastics can increase bioaccumulation of HOCs within organisms. For example, PCBs have been observed to significantly accumulate within marine worms exposed to PCBs in the presence of polystyrene (Besseling et al., 2013) and fish exposed to plastics with sorbed contaminants have been seen to suffer increased hepatic stress compared to exposure to virgin uncontaminated plastics (Rochman et al., 2013a). Conversely, other studies have shown that microplastics do not change the toxicity of HOCs (Beiras and Tato, 2019) or that microplastics may in fact reduce the bioavailability of HOCs due to strong chemical binding (Beckingham and Ghosh, 2016; Zhu et al., 2019). There is even the suggestion of ingested microplastics binding and removing HOCs that had previously been accumulated, although there is insufficient evidence to support this hypothesis (Gouin et al., 2011; Rummel et al., 2016). Recent studies have suggested that while microplastics may have an influence on bioavailability of HOCs, within a realistic environmental scenario, plastics will likely be a negligible route of transport for uptake of these chemicals compared to other modes of uptake, including ingestion of organic matter and dermal uptake directly from the water (Bakir et al., 2016; Grigorakis and Drouillard, 2018; Koelmans et al., 2016). This contrasting evidence highlights the importance of further research in this field to better understanding these microplastic-chemical associations and dynamics. An important factor to note is that the majority of these results are based on modelling exercises; therefore further experimental studies are required to verify these results (Bakir et al., 2016; Gouin et al., 2011; Koelmans et al., 2016). This need to provide comprehensive and relevant ecotoxicological data to inform and feed into models is discussed in section 7.

6. The value of field studies to inform our understanding of ecosystem exposure

Given the discrepancies between concentrations found within the field and those used within ecotoxicological tests, further field studies are essential in order to understand not only the types and concentrations of microplastics present within the environment, and temporal changes in these, but where microplastics derive from and where they accumulate. It is also important to understand how the concentrations of microplastics at different sites are affected by environmental factors, for example weather or water currents and anthropogenic factors such as urbanisation, sewage or litter input, so that we can better understand the environments that are most susceptible to microplastic accumulation and organism exposure. It is essential that we understand the presence and sources of microplastic pollution across a variety of locations and environments worldwide, in addition to presence within biota as a result of ingestion and inhalation. Without this knowledge we would be unable to determine the extent and likely effects of microplastic pollution at current or predicted future levels of environmental contamination (Adam et al., 2019; de Souza Machado et al., 2018). This information will allow for better prediction and understanding of likely interactions between plastics and organisms, and the possible impacts of these interactions, in addition to understanding which regions and ecosystems are most at risk.

Despite a growing number of studies in this area over the last few years, robust and consistent methodologies are only now starting to emerge. This lack of consistency extends even as far as the definition of microplastics, with most studies defining these as plastic particles < 5 mm, while others use < 1 mm as a working definition (Claessens et al., 2013; Frias and Nash, 2019; Hartmann et al., 2019). It is therefore recognised that there is a need for standardisation, or at least harmonisation, of methods used for microplastic analysis across studies, to allow for accurate comparison of data (Besley et al., 2016; Rochman et al., 2017). This is especially important given the growing requirements of industries and governments for reliable and reproducible data, with the ultimate aim of using these data to inform policies, regulations and business strategies. With the understanding that all researchers will continue to use different techniques based on the samples, the research question(s) being asked and the resources available to them, it is essential to come to a consensus that data should be presented and reported in such a way that is repeatable by others, also allowing them to be interpreted correctly and compared to other relevant studies. This should include information such as (but not limited to): mesh size of sampling nets, depth and/or volume sampled, sample storage, density of separation solutions, temperature and pH for digestion protocols and polymer analysis technique (Helm, 2017; Mai et al., 2018; Rochman et al., 2017).

7. The need for realistic conditions in ecotoxicological assessments of microplastics

While field studies provide valuable information on the levels of environmental contamination, this information is not useful in itself, unless it can be put into context of environmental or ecological implications: the question of ‘so what?’. Laboratory experiments are therefore a vital tool for helping us understand the toxicological mechanisms, and biological and chemical associations, which cannot be observed purely by environmental sampling or field observations. Spatial and temporal variability in the environment are such that it can be impossible to tease apart cause and effect across biotic and abiotic variables. Many questions around the factors influencing fate, bioavailability and toxicity of microplastics (and other chemicals) cannot be answered without running specific and targeted studies under controlled conditions (Rist and Hartmann, 2018). Such controlled testing allows for small adjustments of variables to determine the impacts of subtle changes within the system, for example different types, sizes and concentrations of plastic particles (Rist and Hartmann, 2018).

As with other pollutants, the fundamentals of environmental risk assessment can also be applied to microplastics. This requires evaluating the likelihood of exposure combined with the potential hazard (Rand, 1995; Suter, 1995). Microplastics are much more complex to risk assess compared to many chemical contaminants, as they are composites of multiple chemicals in association with a polymer (Rochman et al., 2019). Despite the importance of understanding the impacts of these chemical mixtures, assessing the impacts of individual compounds and polymers is essential first and foremost. Our understanding of the physical and chemical harm posed by microplastics of varying polymer types, sizes, and shapes, is still limited. Therefore the common approach of toxicity testing using single particle types (or simple mixtures) at high concentrations is valuable for understanding mechanisms of hazard, thresholds and modes of toxicity for microplastics with differing characteristics, in addition to informing predictive models of mixture toxicity (Au et al., 2017; Backhaus and Faust, 2012; Faust et al., 2003). While studies carried out at high concentrations exceeding the concentrations to which the organisms would currently be exposed are often met with criticism, it must be noted that environmental concentrations will inevitably increase as a combined result of increased usage and disposal of plastics, alongside degradation of existing plastic debris (Geyer et al., 2017; Thompson, 2015). Once within the environment, microplastics are difficult if not impossible to remove (Brandon et al., 2016; Lusher et al., 2014), therefore exposures at high concentrations are valuable to determine possible 'worst-case' future scenarios which may occur as a result of increasing environmental contamination (Huvet et al., 2016; SAPEA, 2019). These data are especially useful when combined with process-based models to determine large-scale or long-term ecological impacts of microplastics and their chemical associations (Ashauer et al., 2006; Jager et al., 2006; Kimball and Levin, 1985). Developing this knowledge on the ecotoxicological effects of different types and concentrations of microplastics to organisms of different sensitivities, under different environmental conditions, is essential for informing environmental risk assessment and regulation of microplastics (Backhaus and Faust, 2012; Huvet et al., 2016).

The majority of microplastic studies to date have used concentrations of microplastics that far exceed those found in environmental samples (Koelmans et al., 2015; Lenz et al., 2016). It is therefore often impossible to determine whether the effects seen are representative of likely consequences within real-world scenarios without considering these data in line with exposure data. A recent review by Adam et al. (2019) assessed the likelihood of environmental risk by carrying out a meta-analysis of existing microplastic exposure and hazard data. They compared measured environmental concentrations (and therefore probability distributions of exposure) with predicted no effect concentrations (PNEC). While their analysis showed that the majority of PNECs are lower than the likely exposure, leading to little likelihood of hazard, there were a few incidences where organisms may be exposed to concentrations of microplastics above the PNEC and therefore hazard may occur (Adam et al., 2019). This applies, for example, to sensitive species in highly polluted regions. Such an assessment cannot be carried out without sufficient data on environmental concentrations and toxicity to organisms. An earlier review paper published when slightly fewer data were available did not find any likelihood of hazard when comparing exposure to toxicity (Burns and Boxall, 2018), thus highlighting the need for further research to determine where and to what extent these overlaps may occur.

This thesis aims to tackle some of the challenges in ecotoxicological microplastic research, considering that the term 'microplastics' covers a complex heterogeneous range of materials and particle types that do not exist in isolation from other environmental contaminants (Rochman, 2015; Rochman et al., 2019). Specifically, the ecotoxicological chapters of this thesis (chapters 5 and 6) address the ongoing uncertainties surrounding the interactions of microplastics with hydrophobic organic chemicals, and how these interactions may impact on different biological endpoints including mortality, chemical bioaccumulation and microbiome change. Chapter 5 also addresses the pressing need to incorporate data into models, using microplastic and associated chemical toxicology data to run a process-based survival model (Chapter 5). Using different organisms, polymers and chemicals across multiple studies provides a greater understanding of how microplastics, alone and in combination with other chemical stressors, can affect freshwater invertebrates.

8. Model freshwater organisms

In order to answer a variety of ecologically-relevant questions within this thesis, a range of organisms have been selected to study the interactions and impact of microplastics in the freshwater environment: the common roach *Rutilus rutilus*, the water flea *Daphnia magna* and the great pond snail *Lymnaea stagnalis*. These organisms are all very different in terms of morphology, life history, habitat (e.g. water column or benthic) and feeding behaviour. The species have been selected as representative of prolific freshwater families within Europe, with a wealth of available data and/or experimental protocols available including OECD-recommended guidelines on culturing and toxicity testing (OECD, 2004, 2012, 2016). These species span different functional feeding groups and trophic levels, including lower trophic level species daphnia and pond snails, and a tertiary consumer (roach). This difference in feeding habits between species could affect their susceptibility to ingest microplastics. For example, omnivorous roach will have an additional route of microplastic exposure due to the potential for trophic transfer from both plants and invertebrates (Vasek and Kubecka, 2004), while generalist pond snails may be more likely to ingest microplastics (especially those associated with organic matter) than the more selective roach and daphnia (Elger and Lemoine, 2005; Hartmann and Kunkel, 1991; Lammens and Hoogenboezem, 1991). There are also likely intraspecific differences which will affect individual susceptibility to ingestion and possible harm, such as age, size and gender (based on possible behavioural differences). Additionally, sediment concentrations are likely higher than pelagic microplastics concentrations as microplastics sink and accumulate, leading benthic species to be more highly exposed (Leslie et al., 2017; Rodrigues et al., 2018). The type (and thus density) of polymers will also affect their availability to different organisms. For example, snails will only ingest particles that are dense enough to sink (or whose density is affected by the particle's interaction or aggregation with organic material), whereas fish and daphnia may also ingest buoyant particles that float or reside within the water column. *Daphnia magna* are the mostly widely studied species with respect to microplastic ingestion and effects (Besseling et al., 2014; Jemec et al., 2016; Ogonowski et al., 2016; Rehse et al., 2016; Rosenkranz et al., 2009), whereas no data are available for the other species.

9. Aim of the thesis

The results of field and laboratory studies can be used to help direct future research, develop our understanding of environmental and ecological processes and variation, and ultimately inform environmental policy and risk assessment. With this in mind, this thesis combines field and laboratory studies to address some of the most pressing questions in the field of microplastic research. Given the comparative lack of research on microplastics in freshwater systems, especially in the UK, this thesis therefore has the following overarching aims: to identify abundance, types and sources of microplastics in freshwater systems in the UK, and to investigate how organisms and chemicals interact with microplastics and the potential ecological effects on a range of freshwater organisms from different functional feeding groups and trophic levels.

These aims can be fulfilled within sub-objectives:

1. To identify the gaps within the state-of-the-art on the sources, distribution, fate and behaviour of microplastics and their effects on species and ecosystems;
2. To determine the presence, abundance and types of microplastics, as well as their sources, within tributaries of the River Thames (UK);
3. To establish whether fish ingest microplastics in their natural environment, focussing on the River Thames (UK);
4. To experimentally determine whether high versus low Kow (a measure of hydrophobicity based on octanol-water partition coefficient) compounds interact differently with microplastics, potentially altering toxicological effects to *Daphnia magna*;
5. To experimentally assess whether the presence of microplastics reduces uptake of flame retardant chemicals (polybrominated diphenyl ethers, PBDEs) and alters the microbiome in the pond snail *Lymnaea stagnalis*

10. Outline of the thesis

Based on the above objectives, this thesis consists of the following chapters:

Chapter 1: Introduction to the topic and thesis aims (this chapter)

Chapter 2: A literature review to examine the state of the scientific knowledge on microplastics within freshwater and terrestrial environments, and to identify research gaps that should be addressed by subsequent chapters in this thesis.

Chapter 3: An environmental study to establish the extent of microplastic pollution within sediments of tributaries of the River Thames, to quantify and identify particles and to determine the sources of environmental particles.

Chapter 4: An environmental study to quantify microplastics from the guts of fish (*Rutilus rutilus*) within the non-tidal (freshwater) River Thames and to determine whether presence and quantity of plastic particles can be linked to environmental factors: exposure to microplastics based on distance from the source of the river, and biological factors: size and gender of fish.

Chapter 5: A laboratory study to experimentally determine whether the presence of microplastics (1 μm polystyrene beads) affects toxicity and sublethal effects of pesticides (based on hydrophobicity and therefore binding to plastics) to *Daphnia magna* using pesticides with high and low log Kows.

Chapter 6: A laboratory study to assess how the presence or absence of microplastics (nylon fragments) may alter the accumulation of PBDEs at various concentrations within the great pond snail *Lymnaea stagnalis* and whether any effect of PBDEs (with or without microplastics) can be observed on the microbiome.

Chapter 7: A discussion to bring together the findings across all chapters of the thesis, and the scientific implications of these. This chapter includes recommendations for future research and concluding remarks.

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