Environmental Risk of Polymers and their Degradation Products

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Abstract

Polymer-based materials are found everywhere in the environment, but their impacts are yet to be fully understood. The degradation of different polymer types has been extensively investigated under specific laboratory conditions. However, only limited data are available on their degradation under environmentally relevant conditions, where a number of processes are assessed at once. This thesis therefore describes a series of outdoor aquatic microcosm studies and laboratory experiments to investigate the degradation of a case study polymer (natural rubber latex), to characterise the formation of degradation products, and to assess the effects these may have on aquatic organisms.

The outdoor microcosm studies showed that the exclusion of light and material thickness had a greater influence on degradation rate than media pH and sample movement. Analysis of the degradation solutions demonstrated that when the latex polymer degraded, there was an increase in the formation of microscopic latex particles; zinc (used to speed up the rate of curing processes) migrated from the latex polymer into the test solutions; and a mixture of dissolved substances that are potentially oxidised latex oligomers with additives residues were formed. Further analyses also showed that the atmosphere is a receiving environmental compartment for polymer degradates though the identification of a range of volatile substances produced during the degradation process.

Laboratory experiments were then conducted to investigate the direct toxicity of the formed degradate mixtures, using two freshwater organisms with different life cycle traits, the water column crustacean *Daphnia magna* and the sediment-dwelling larvae of *Chironomus riparius*. The results suggest that, to the organisms tested, there is limited environmental risk associated with latex degradation products.

Overall, environments receiving polymer debris are potentially exposed to a mixture of compounds that include the parent polymer, fragmented particles, leached additives, and subsequent degradation products; however at environmentally relevant concentrations this latex degradates pose little risk.

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Author's Declaration

The work in this thesis was undertaken as a PhD student in the EcoChemistry team at Fera, York, and the University of York. The research was funded jointly by DEFRA (Department for Environment, Food and Rural Affairs), through its Seedcorn fund, and an industrial sponsor.

I declare that this thesis is a presentation of my own original research and every effort has been made to indicate the contributions from others. The questionnaire survey and surface water modelling used to calculate the predicted surface water concentrations presented in Chapter 5 was performed as part of a Master's dissertation project, under the supervision of the PhD candidate.

Data from Chapters 2, 3 and 4 have been presented in internationally peer-review journals. These papers have been reworked, so they are presented in a consistent style for this thesis. A publication from research presented in Chapter 5 is currently being prepared (Table 0.1).

Table 0.1 Status of data presented in this thesis with respect to the publication process

Chapter	Title	Journal	Status
2	Occurrence, Degradation and Effects of Polymer-Based Materials in the Environment	Reviews of Environmental Contamination and Toxicology	Accepted for publication in volume 227
3	Effects of Environmental Conditions on Latex Degradation in Aquatic Systems	Science of the Total Environment	Published in 2013, Volume 447, pages 225-234
4	Characterisation of Substances formed during the Degradation of Latex	Environmental Science: Processes and Impacts	Accepted
5	Do Natural Rubber Latex Degradates Pose a Risk to Aquatic Systems?		In preparation

Chapter 1

Introduction

1.1 History of Polymer Development

Polymer-based materials (PBMs) are a complex mixture of their respective polymer/s and their various additive compounds. Today's major usage of these materials can be traced back to the 1800s with the development of rubber technology, where one of the key breakthroughs was the discovery of vulcanisation of natural rubber by Charles Goodyear (Stevenson *et al*., 2008). Throughout the 1800s a number of attempts were made to develop synthetic polymers (Table 1.1). Polystyrene (PS) and polyvinyl chloride (PVC) were discovered during this time, but were either too brittle to be commercially viable or would not keep their shape. The first synthetic polymer to enter mass production was Bakelite, developed by the Belgian chemist Leo Baekeland in 1909 (Vlachopoulos and Strutt, 2003). Later in the 1900s the modern form of PVC was created (1926), followed by polyurethane (PUR) (1937), a more processable polystyrene (1938), and high-density polyethylene (HDPE) and polypropylene (PP) (1951) (Hammer *et al*., 2012). More recently, technological advances have seen the development of polymers produced from the bacterial fermentation of sugars and lipids, and include polyhydroxyalkanoates (PHA), polylactides (PLA), aliphatic polyesters, and polysaccharides (Reddy *et al*., 2003).

There are many different types of polymers and what distinguishes one from another is the types of molecules used in their preparation and in the way they are joined together (Billmeyer, 2007). In general, polymers consist of a range of organic and inorganic molecules formed from elements, such as carbon, silicon, hydrogen, nitrogen (nylon), oxygen (polyester and polycarbonates) and chloride (polyvinyl choride) (Plastics Europe, 2010). These elements are joined together in different bond combinations to achieve the differences in polymer properties, for example, elasticity and the ability to be melted or remoulded into another form (Billmeyer, 2007). Polymers in their pure states are not generally usable as a commercially viable material (Mulder, 1998). Therefore, polymers are processed with a range of compounds to adjust their characteristics which make them suitable for their intended purpose.

Table 1.1 A brief profile of polymer development

Year	Polymer type	Notes
1839	Natural rubber latex	Charles Goodyear
1839	Polystyrene	Discovered by Eduard Simon
1862	Parkesine	Alexander Parkes
1865	Cellulose acetate	Paul Schützenberger
1869	Celluloid	John Wesley Hyatt
1872	Polyvinyl chloride	First created by Eugen Baumann
1894	Viscose Rayon	Charles Frederick Cross
1909	Bakelite	Leo Hendrik Baekeland
1926	Plasticized PVC	Walter Semon
1933	Polyvinylidene chloride	Ralph Wiley
1935	Low-density polyethylene	Reginald Gibson and Eric Fawcett
1936	Acrylic or polymethyl methacrylate	
1937	Polyurethane	Otto Bayer and co-workers
1938	Polystyrene	Made into a commercially viable polymer
1938	Polyethylene Terephthalte	John Whinfield and James Dickson
1942	Unsaturated polyester	John Whinfield and James Dickson
1951	High-density polyethylene	Paul Hogan and Robert Banks
1951	Polypropylene	Paul Hogan and Robert Banks
1953	Polycarbonate	Hermann Schnell
1954	Styrofoam	Ray McIntire
1960	Polylactic acid	Patrick Gruber is credited with inventing a commercially viable process for PLA
1978	Linear low-density polyethylene	DuPont

1.2 Polymers and the Environment

Today, PBMs are widely used in our daily lives for the manufacturing of consumable goods, building materials and medical applications, amongst a variety of other products. During their lifecycle PBMs can be intentionally or inadvertently released into the environment (Tharpes, 1989). The principal introduction routes of PBMs are likely to differ between geographical regions depending on infrastructure. The environmental occurrence of PBMs is identified as an important pollution related issue, because of the estimated volumes involved, and because they are difficult and time consuming to remove (Erren *et al*., 2009). Studies from around the world have now documented PBMs as a major component of marine and shoreline debris, including the Antarctic Peninsula (Benton, 1995; Convey *et al*., 2002), Australasia (Foster-Smith *et al*., 2007; Kusui and Noda, 2003), Europe and the North Atlantic (Galgani *et al*., 2000; Barnes and Milner, 2005; Liebezeit, 2008), Mediterranean (Koutsodendris *et al*., 2008; Turner and Holmes, 2001), and the Middle East (Abu-Hilal and Al-Najjar, 2009; Claereboudt, 2004); and as a pollutant of freshwater aquatic environments (Zbyszewski and Corcaran, 2011). The majority of work describing the environmental consequences of PBM debris has focused on marine settings, and identifies macro- (> 5 mm) and meso- (≤ 5 mm - ≥ 1mm) debris as presenting a hazard to marine mammals and birds as they can become entangled or mistake them as a food source (Bugoni *et al*., 2001; Hanni and Pyle, 2000; Page *et al*., 2004; Petry *et al*., 2009).

At present the majority of our understanding on the processes influencing the degradation of PBMs has been derived from artificial laboratory studies that investigate a single mechanism of degradation such as photodegradation (Nagai *et al*., 2005a, Nagai *et al*., 2005b), thermal degradation (Agostini *et al*., 2008; Cit *et al*., 2010), and biodegradation using microbial cultures (Cherian and Jayachandran, 2009; Cosgrove *et al*., 2007; Linos *et al*., 2000; Saad *et al*., 2010; Tsuchii *et al*., 1997). These studies have tended to focus on weight loss, changes in tensile strength, breakdown of the molecular structure and identification of specific microbial strains to utilise specific polymer types. However, there is limited information on the degradation of PBMs under environmentally relevant conditions, where a number of degradation mechanisms occur at once; and the potential for PBMs to form other chemical compounds during the degradation process and the effects these formed compounds may have on organisms has received little attention.

An understanding of the processes that determine how PBMs degrade under different environmental conditions, and the types and effects of degradation products formed would be valuable for understanding the environmental risks of these these materials

1.3 Aim of the Thesis

The overall aim of this thesis was to develop methods for assessing the potential risks of PBMs and their degradation products to the environment. This was undertaken using a case study Natural Rubber Latex (NRL) film used to make condoms, described as a mixture of *cis*,1-4 polyisoprene and a number of additive compounds. The specific objectives were to:

1. Review the current knowledge on the release, occurrence, degradation and effects of polymers and their associated chemical additives;
2. Develop environmental emission scenarios for polymer-based products;
3. Develop approaches to measure and characterise how polymers degrade and what they degrade into;
4. Develop methods to characterise the ecotoxicological effects of polymer degradation products.

1.4 Thesis overview

This thesis comprises six chapters. A description of each is given below.

Chapter 2 synthesises the existing knowledge on the environmental occurrence, degradation and effects of polymers and their associated chemical additives. This Chapter attempts to identify knowledge gaps and recommends areas for future research.

Chapter 3 describes the degradation of NRL in outdoor microcosms under a range of exposure treatments. Experiments were performed at the Food and Environment Research Agency. Outdoor microcosms were developed to investigate the degradation of NRL when exposed to natural cycles of sunlight and temperature. This Chapter describes weight loss, changes in chemical functionality, the formation of microscopic physical degradation products, and dissolved degradation products.

Chapter 4 describes the use of analytical methods to determine the migration of zinc from the NRL into solution, and for the characterisation of non-volatile, semi-volatile, and volatile substances. Chemo-metric techniques were also used to characterise the clustering of chromatographic data in principal component space.

Chapter 5 evaluates the risk of NRL condom degradation products. Laboratory experiments were performed to characterise the aquatic toxicity of degradation products, described in Chapters 3 and 4, using a range of acute and chronic endpoints to *Daphnia magna* and *Chironomus riparius*. The information generated from these ecotoxicity tests, was then compared to surface water concentrations calculated as part of a Master's dissertation project.

Chapter 6 discusses the main findings of the study in terms of the broader implications.

Chapter 2

Occurrence, Degradation and Effects of Polymer-Based Materials in the Environment

2.1 Introduction

At a global scale, increasing human population and associated economic growth has lead to an increase in the demand for consumable goods such as those made from PBMs (i.e., plastics and elastomers). During their lifecycle PBMs can be released into the environment from a variety of sources. Once in the environment, PBMs are exposed to a variety of mechanical and chemical weathering processes. This causes a change to the PBM structure and facilitates the disintegration of the PBM into increasingly smaller fragments (Andrady, 2011). Furthermore these materials are now thought to be contributing to the build-up of chemicals in the environment via the leaching of chemical additives that are used in the manufacturing process (Erren *et al*., 2009). The majority of physical effects data regarding bulk PBM items identifies them as presenting a hazard to mammals and birds as they can become entangled and/or mistake PBMs as a food source (Derraik, 2002; Laist, 1987). The majority of ecotoxicity data regarding PBM additives has focused on the effects of compounds that are generally referred to as having endocrine disruptive potential, such as the phthalates (Oehlmann *et al*., 2009). However, receiving environments are potentially exposed to a combination of both these physical and chemical components, as well as substances produced during degradation processes. Therefore, PBMs and their associated degradation products may compromise the viability of organisms at all trophic levels. At the base of the food chain primary producers may be more sensitive to substances that have a biological action. Non-selective and filter-feeding consumers could be susceptible to ingesting both bulk PBMs and fragmented particles, leading to the potential passage up the food chain to secondary and tertiary consumers. Despite this concern, PBMs are regarded under REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) as representing a low environmental concern because of their high molecular weight (ECHA, 2012). However, the occurrence of PBMs and their associated chemical additives in the aquatic environment have been recognized as an emerging worldwide problem, and their impacts are now gaining a wider scientific and social audience (Hammer, 2012; Thompson *et al*., 2009).

The purpose of this chapter is to provide a broad bibliographical review of the research that addresses the use, release, occurrence, degradation and effects of PBMs and their associated chemical additives in aquatic and terrestrial environments. Issues involving both the polymer component of PBMs and the additive component will be addressed.

2.2 Usage and Consumption

The PBMs used in society today are made from a broad class of materials that are both natural and synthetic in origin (Table 2.1). Natural polymers such as polyisoprene, derived from the tropical tree *Hevea brasiliensis*, are used to make NRL products (Agostini *et al*., 2008). Petrochemical-based polymers are manufactured through a thermal splitting process termed ‘cracking’, which separates oil and natural gas to produce different hydrocarbon monomers, such as ethylene and propylene (Chaudhuri, 2010). World demand for petroleum derived polymers is estimated at 230 million t annually (Plastics Europe, 2010), with annual consumption estimated to be 26 kg per person (CIPET, 2010). However, there are notable differences between geographic regions that result from differences in standards of living, live style and income (Table 2.2). Polyolefins (i.e., linear low density polyethylene (LLDPE), low density polyethylene (LDPE) and HDPE), and PP), account for ~ 60% of annual consumption followed by PVC and PS (Plastics Europe, 2010; Muth *et al*., 2006). Packaging represents the most important application for PBMs and accounts for 40.1% of overall consumption, followed by building and construction (20.4%), automotive (7%), electrical and electronic equipment (5.6%), and other market sectors including leisure and agriculture (26.9%) (Plastics Europe, 2010; Muth *et al*., 2006). PUR is a successful material for biomedical applications, where it is used to make artificial joints and flexible replacements for blood vessels and heart valves (Ghanbari *et al*., 2009). World demand for NRL is estimated at 10.97 million t annually; this demand is dominated by latex products (80.3%) such as medical and household products (NRS, 2011). Other natural rubber uses include tires (9.2%), general rubber goods (7.2%), industrial rubber goods (3.2%) and footwear (0.2 %) (NRS, 2011).

Technological advances have seen the development of polymers that have been altered to be more degradable. These polymers can be broadly divided into three categories. First, are those that have a biodegradable ingredient, such as starch, which is added to the polymer matrix to link short strands of a synthetic polymer chain together (Drimal *et al*., 2007; Reddy *et al*., 2003). Second, nano clay composites are used to provide a favorable environment for growing microorganisms that can utilize the polymer matrix as a food source; montmorillonite clay has been reported to promote microbial growth by stabilizing pH in the polymer matrix (Reddy *et al*., 2009). Third, are those produced from the bacterial fermentation of sugars and lipids that comprise a class of polymers that include PHA, PLA, aliphatic polyesters, polysaccharides, copolymers and/or blends of the above. Reddy *et al*., (2003) has described these as being the most promising technological advances, because the polymer matrix is thought to be fully utilized by microbial communities.

High-performance composites are also an important market segment. These consist of a polymeric matrix and fillers that are designed to provide improved mechanical properties. Carbon fibre composites have been an important innovation for the aircraft industry, and have reduced aircraft weight and thereby reduced fuel use (Mulder, 1998). Glass-fibre-reinforced polyester composites are used in shipping because of their impact resistance and light weight. PBMs are sometimes blended to improve the deficient properties of traditional single-chemical polymers. When the properties of two or more incompatible polymers are desired in one blend, compatibilizers are employed. For example, blends of PP and acrylonitrile butadiene rubber (NBR) are desirable to combine the oil resistance and elastic properties of NBR and the low density and chemical resistance properties of PP, although their individual physical, mechanical and chemical properties normally prevent this.

Table 2.1 Major polymer types and their uses

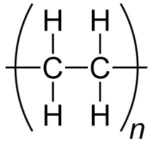
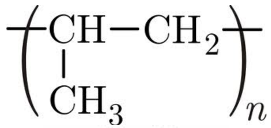
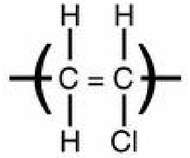
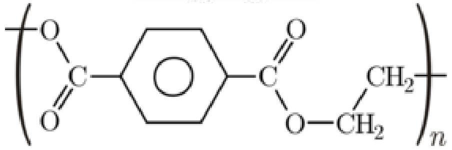
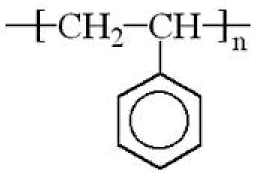
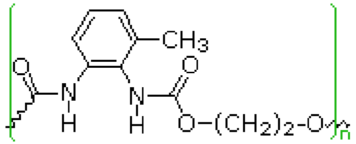
Polymer type	Uses	Structure
Polyethylene (PE)	Low density PE - Squeeze bottles, toys, carrier bags, high frequency insulation, chemical tank linings, heavy duty sacks, general packaging, gas and water pipes. High density - chemical drums, toys, picnic ware, household and kitchenware, cable insulation, carrier bags, and food wrapping material.	
Polypropylene (PP)	Food containers, microwavable meal trays, and in the auto industry	
Polylvinyl chloride (PVC)	Building, transport, packaging, electrical/electronic and healthcare applications	
Polyethylene terephthalate (PET)	Drinks bottles, oven-ready meal trays cable lining.	
Polystyrene (PS)	Food containers, take away boxes, vending cups, plastic cutlery, protective packaging, and CD boxes.	
Polyurethane (PUR)	Printing rollers, solid tyres, wheels, shoe heels, car bumpers, as foams in mattress and car seats, and in biomedical applications	

Table 2.1 Major polymer types and their uses (continued)

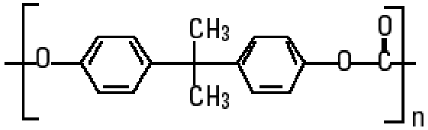
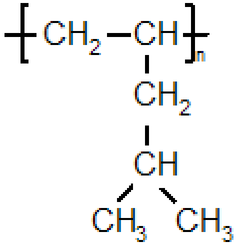
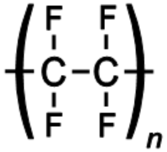
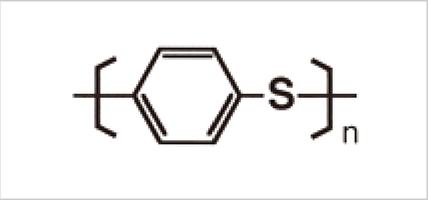
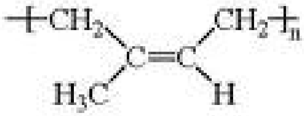
Polymer type	**Uses**	**Structure**
Polycarbonate (PC)	Bottles, utensils, containers, sheeting, electrical goods, and medical applications	
Polymethylpentene (PMP)	Medical ware, syringes, lamp covers, (good heat resistance), radar applications, encapsulation, and microwave food packaging.	
Polytetrafluoroethylene (PTFE)	Non-stick coating, gaskets, bearings, high and low temperature electrical and medical applications, laboratory equipment, pump parts, and thread seal tape.	
Polyphenylene Sulphide (PPS)	Electrical, automotive, cooking appliances, sterilizable medical, dental and laboratory equipment, hair dryer grills and components	
Polyisoprene (NR)	Gloves, tires, rubber boots, rubber bands, pencil erases, hoses, belts floorings, and medical applications	

Table 2.1 Major polymer types and their uses (continued)

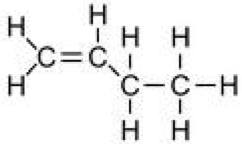
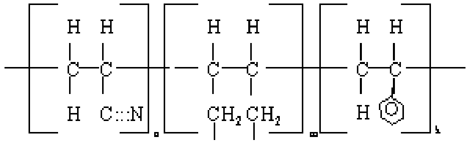
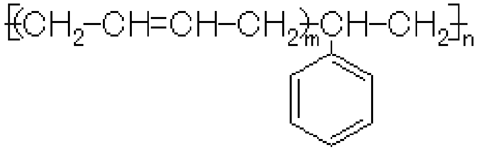
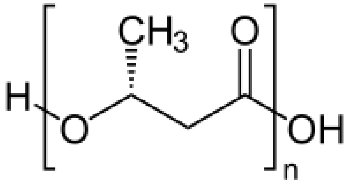
Polymer type	**Uses**	**Structure**
Polybutadiene	Tires, golf balls, and inner tubes.	
Acrylonitrile butadiene styrene (ABS)	Piping, musical instruments, golf club heads, automotive, medical devices for blood access, electrical devices, protective headgear, white-water canoes, small kitchen appliances, and toys	
Styrene-butadiene (SBR)	Tires, shoes, building applications and paper coating	
Polyhydroxyalkanoates (PHA)	Medical devices, such as cardiovascular patches, orthopedic pins adhesion barriers, stents, guided tissue repair/regeneration devices, articular cartilage repair devices bone implant material, drug release system, scaffold for tissue engineering, bulking and filling agents	

Table 2.2 Geographical overview of per capita consumption of polymers (data sourced from the Central Institute for Plastic Engineering and Technology, 2010)

Country	**Polymer consumption per capita (Kg)**
India	5
China	12
South East Asia	10
Latin America	18
North America	90
West Europe	65
East Europe	10
Worldwide average	26

2.3 Bulk PBMs and the Environment

2.3.1 Environmental Release

PBMs may enter the environment from both ocean- and land-based sources. We address each of these in more detail below.

2.3.1.1 Ocean-based Sources

Ocean-based sources include items lost or discarded from commercial fishing vessels, offshore oil or gas platforms and waste dumped by recreational boat users. Losses of cargo can also occur from shipping during bad weather events or accidents and items lost from improper loading, unloading or on-board storage (Tharpes, 1989). In the past, pre-production PE and PP pellets have reportedly being used on the decks of ships to reduce friction when moving large objects; as such, many of these pellets are washed from the deck and are dispersed by winds and ocean currents (Tharpes, 1989). The dumping of wastes at sea has long been seen as a major issue and was prohibited under international legislation in 1973 (MARPOL 73/78 Annex V), which came into force in 1988 and regulates the operational discharges from shipping (do Sul and Costa, 2007). One requirement of the MARPOL ruling is that under no circumstances are PBMs to be disposed of at sea, but the enforcement of this regulation is noted as being an issue (Ryan *et al*., 2009).

2.3.1.2 Land-based Sources

General and Accidental Littering

On land, general and accidental littering are important routes of environmental entry of PBM debris (Gregory, 2009). General littering is the direct dropping of litter, and dumping of items; for example, illegal dumping of waste that can then be transported by wind or from drainage and storm water runoff to ocean sinks (Tharpes, 1989). Littering at festival sites is noted as an issue; especially from sites that have inadequate waste management systems (Cierjacks *et al*., 2012). Accidental littering, by contrast, results from windblown debris from bins, or from recycling and landfill facilities (Tharpes, 1989). Littering on land in the UK is covered by section 18 of the Clean Neighbourhoods and Environment Act of 2005, which came into force on 7th June 2005 and makes it an offence to litter on all public and private land and land covered by water (DEFRA, 2012).

Landfills

Landfills are a major end-of-life disposal route for PBMs (Barnes *et al*., 2009). In most developed regions of the world, waste is collected, transferred to landfills and is typically covered with soil daily (Rayne, 2008). However, in many developing regions waste materials are often disposed of in areas lacking adequate infrastructure, and are rarely or inadequately covered with soil (Rayne, 2008). This increases the likelihood of windblown debris migrating from landfill sites. Rayne, (2008) has also identified the increasing urbanization of Africa as a potential future problem, because it will increase the stress on limited waste management systems in this area of the world.

Sewage-related Debris (SRD)

SRD also presents a source from which PBMs can enter the environment. In many countries, domestic inputs of household waste to the sewage system are largely uncontrolled. Therefore, PBMs associated with personal hygiene products, such as condoms, cotton buds (Ashley *et al*., 2005; Williams and Simmons, 1999), and microscopic PE beads found in some hand cleaners and facial scrubs (Fendall and Sewell, 2009), as well as microscopic fibres (acrylic) shed from cloths during washing (Zubris and Richards, 2005) can constitute a portion of this waste stream.

Larger items are generally removed by screening methods, but may enter the environment during sewage overflow events that occur during periods of heavy rainfall. The ability of sewage treatment works to process microscopic beads and fibres has now been questioned. Browne *et al*., (2011) recently sampled wastewater from domestic washing machines and demonstrated that a single garment can produce >1,900 fibres per wash. Microscopic beads and fibres can potentially pass through finer screening processes and enter the environment via sludge application and discharge of treated waters (Browne *et al*., 2011). Coarse screens, designed to remove large solids and debris items, typically have a mesh size of 6 mm, whereas fine screens typically have mesh sizes of 1.5 mm to 0.2 mm (EPA, 2004).

Industrial Sources

Industrial sources of PBM waste include air-blasting technologies that use microscopic beads to strip paint from metallic surfaces and for cleaning engine parts; when discarded, they enter the environment through foul-water, or via transfer through sewage treatment processes (Derraik, 2002). LDPE films constitute a large-volume use of PBMs in agricultural crop production, and consequently they have become an important agricultural emission (Xu *et al*., 2006). Their application is thought to be one of the most important sources of PBM contamination of soils, because they become brittle and easily disintegrate rendering their recovery difficult (Xu *et al*., 2006). Agriculture films can also contain light-sensitive additives, such as ferric and nickel dibutyldithio-carbamates, the ratio of which can be adjusted so that the film is usable during a specific growing season, after which the product begins to photo-degrade (Klemchuk, 1990). This ultimately results in disintegration of the material, and when coupled with successive precipitation events the disintegrated particles can be washed into the soil where they accumulate (Klemchuk, 1990).

2.3.1.3 Conclusion

The principal introduction routes of PBMs into the environment are most likely general littering, dumping of unwanted waste materials, migrations from landfill and during refuse collection (Gregory, 2009; Teuten *et al*., 2009; Tharpes, 1989). Routes of minor importance are potentially the weathering of polymer-based building materials. However, the importance of one particular source over another will depend on geographical location and infrastructure. For example, landfills are identified as a potential important source in areas of the world where infrastructure is lacking, but microscopic PE beads in facial scrubs are probably more important in more affluent regions. One must also be aware of the difficulties in determining the sources of PBM debris, because of the length of time it may have been in the environment and the distances it may have travelled.

2.3.2 Environmental Occurrence

Upon their release to the environment PBMs are transported and distributed to various environmental compartments. The distances that an individual item will travel depends on its size and weight. Lightweight materials can be readily transported long distances via a windblown route or carried by freshwater to eventually accumulate in the oceans. During heavy rainfall events, roadside litter can be washed into drains and gullies, and, where the topography is favourable for it, can be carried to the sea. In this section, we review the literature in which the occurrence of polymers globally has been quantified.

2.3.2.1 Macro PBMs in the Oceans

Large items of PBM debris are termed ‘macro-plastics’ and have been generally categorized as items >5 mm in diameter, because this size provides an opportunity to assess markings to trace an object to its origin. Marine habitats are highlighted as one of the most important sinks for macro PBMs (Browne *et al.,* 2011; Derraik, 2002; Thompson *et al.,* 2009). PBMs are believed to contribute up to 80% of all anthropogenic debris in the oceans (Derraik, 2002). A well documented example are pre-production PE and PP pellets that are transported from manufacturing plants to plastic injection factories, where they are melted and molded into consumer products. These pellets have been reported floating in coastal surface waters, and in the world’s oceans, later to be washed ashore in non-industrialized areas such as the South Pacific Islands (Derraik, 2002; Gregory, 1977; Moore, 2008; Morris, 1980). Lightweight items, such as PE bags, polystyrene foam items and polymer drinks bottles, inappropriately disposed of on land, can be readily transported long distances via a windblown route or carried by freshwater to eventually accumulate in the oceans (Ryan *et al*., 2009).

There are now a number of studies in which macro PBMs have been observed or collected floating on the ocean surface and laying on the seafloor (Table 2.3). These studies provide a snapshot, but do highlight PBMs as the dominate component of ocean debris. Geographical variability in ocean PBM debris has been highlighted by Barnes and Milner, (2005), in their extensive study on the occurrence of drifting PBM debris in the Atlantic Ocean. These authors identified the English Channel as having the greatest number of debris items (10 - >100 items/km^2), 66% of which were a form of PBM. This study also established PBM debris to be an order of magnitude lower in both the Polar Regions, but the authors do highlight that the tropics and the West Atlantic were poorly sampled. One of the only documented cases of decreasing litter densities in the literature comes from Kuriyama *et al.*, (2003), who reported a 45.3% decrease in the number of littered items on the seabed of Tokyo Bay between 1996 and 2000; the authors of this study hypothesized this to be a result of litter removal by bottom trawl fishing vessels.

2.3.2.2 Macro PBMs on Shorelines and on Land

Shorelines around the world have been found to accumulate debris, including island shorelines far from any centres of human activity (Table 2.4). Benton, (1991) surveyed beach litter on Ducia Atoll in the south Pacific and found 953 items of debris over a 1.5 mile survey transect. This is one of the world's most remote islands, being 293 miles from the nearest inhabited location of Pitcarin Island, which in 1991 had a population of ~50 people. Another example comes from remote tropical beaches of Brazil, where PBMs have been found at densities of 9.1 items/m^2, accounting for 76% of the litter items found (Santos *et al.*, 2009). Evidence of the increasing occurrence of PBMs is provided from Scotland, where Caulton and Mocogni, (1987) found 0.35 items of litter/m^2, with plastics accounting for 29% of items found. Ten years later the same area of beach was surveyed and the density of litter was found to have increased to 0.8 items of litter/m^2, with PBMs accounting for 37% of items found (Velander and Mocogni, 1998).

The amount of PBM debris in the freshwater environment is less well documented, but one recent study reported on the distribution of PBM debris along the freshwater beaches of Lake Huron, Canada. In this study, 2,986 polymer pellets, 108 polymer fragments and 117 pieces of Styrofoam were found (Zbyszewski and Corcoran, 2011). On land, urban littering is considered to be an important environmental and public issue (Seco Pon and Becherucci, 2012), but it less well documented in the available literature. One researcher conducted a study in Nairobi, Kenya in 2001 and collected 4,834 plastic bags from 6 sites that measured 20 m x 50 m in size (Njeru, 2006). A similar study was performed in Mar del Plata, Argentina, in which 20,336 items (14.27 items/m^2) of litter were recovered from study sites between April 2008 and March 2009; in this study, PBMs accounted for 22% of the recovered litter (Seco Pon and Becherucci, 2012).

2.3.2.3 Micro PBMs in the Oceans

Particles <5 mm, formed as a result of the breakdown of larger materials, are now found floating on the ocean surface, mixed into the water column, and embedded in bottom sediments and beach sands (Colton *et al*., 1974; Thompson *et al*., 2004). These smaller particles are generally termed ‘micro-plastics’ (Barnes *et al*., 2009; Moore 2008). However, it has recently been suggested that the term microplastics be redefined as items <1 mm to include particles only discernible by microscopy (Andrady, 2011; Browne *et al*., 2011), The term ‘meso-plastic’ should then be introduced to the scientific literature to account for items between 1 and 5 mm (Andrady, 2011).

Colton *et al*., (1974) found PBM particles in 62% of surface plankton samples taken from the Atlantic Ocean (247 samples in total). Archived plankton samples, collected along routes between Aberdeen and the Shetlands and from Sule Skerry to Iceland as part of the continuous plankton recorder (CPR) survey, have also shown the presence of PBM particles and fibres in samples dating back to the 1960s (Thompson *et al*., 2004). This highlights the long-term trends first identified by Carpenter *et al*., (1972), who found fragmented polymer particles in surface nets while sampling the *Sargassum* (free-floating seaweed) community in the western Sargasso Sea. Furthermore, Carpenter *et al*., (1972) predicted that the increasing use and production of PBMs would lead to an increase in concentrations of these particles in the environment. In 2004, the CPR survey, the longest running plankton monitoring program in the North Sea and North Atlantic, added micro-plastic as their first non-biological marine entity to their recordings (Richardson *et al*., 2006).

One area that has received particular attention is the subtropical accumulation zone in the North Pacific gyre. In this area, debris has accumulated at such high densities as a result of high atmospheric pressure and the clockwise rotation of ocean currents that forces debris into a central area where strong winds and currents diminish (Cooper and Corcoran, 2010). Neuston sampling at 11 sites, using a mantra trawl, estimated a mean PBM abundance of 334,271 pieces km^2 (Moore *et al*., 2001). Items identified were fragments ranging in size from 0.44 - > 4.76 mm, pellets, PP monofilament and Styrofoam pieces. In a study performed along Californian coastal waters, surface samples were collected with a manta trawl, mid-depth samples with a bongo net and bottom samples with an epibenthic sled, all having 333 µm nets; PBM debris density was found to be greatest near the bottom, and least in mid-depth zones (Lattin *et al*., 2004). This suggests that when measuring the occurrence of PBM debris it is important to establish whether the concentrations of a true sink or an intermediate pathway are being measured. A more recent study, focusing on the North Western Mediterranean Sea, found neuston PBM particles at an average abundance of 0.116 m^2 (Collignon *et al*., 2012). For an in-depth review on microplastics in the marine environment see Cole *et al*., (2011).

2.3.2.4 Micro PBMs on Shorelines and on Land

Infra-red spectroscopy techniques have been utilized to identify fragment PBMs in the microscopic range by comparing spectra to those in a database of common polymers. This technique was principally pioneered by Thompson *et al*., (2004), whose research identified synthetic fibres (PE, PP, PS, nylon and NBS) in samples of beach sand and sub-tidal sediments from around the UK. Microscopic PBM granules and fibres have now been found in sediment at world heritage sites, such as the East Frisain Islands, where a maximum of 496 granules/10 g sediment has been observed (Liebezeit and Dubaish, 2012). Further inland, sewage sludge application has been identified as a source of polymer fibres in agricultural soils. Zubris and Richards, (2005) found polymer fibres were still present in field soils 15 years after application, with fibres also found in soil horizons below the depth of ploughing, suggesting some potential for movement through the soil profile.

2.3.2.5 Conclusion

There have now been a number studies from around the world that have documented PBMs as the dominate component of shoreline, ocean, and terrestrial debris, although geographical differences in PBM occurrence have been noted. Research on microplastic as a component of beach sediments is also gaining increasing attention. However, microplastics as a component of freshwater sediments and soils are yet to be investigated. Lake and roadside habitats would seem a good place to start; items littered on lakes have less transportation potential, and the regular grass cutting roadsides receive in some countries would mean that littered items are quickly disintegrated by mowing equipment.

Table 2.3 Polymer based materials as a component of marine debris

Location	**Depth**	**Mean density of litter (items / Km^2)**	**% plastic items**	**Reference**
North Atlantic & Europe				
Baltic sea	Sea floor	0.12	35.7	1
North Sea	Sea floor	0.15	48.3	1
Bay of Biscaye	Sea floor	0.14	79.4	1
Celtic Sea	Sea floor	0.53	29.5	1
Adriatic Sea	Sea floor	0.38	69.5	1
English Channel	Surface	10 - 100	66	2
Sargasso Sea	Surface	3500	100	3
Gulf of Mexico	Sea floor	Not stated	204 pieces	4
Mediterranean				
Malta				
Greece	15 m - seafloor	0 - 437 items	~ 65	5, 6, 7
France	40 - 1448	0 - 78/ha	70.6	8
Pacific				
Central California (2007)	20 - 365 m	6900	95	9
Southern California (2002)	20 - 365 m	320	41	9
Southern Chile	Surface	1 - 250	80	10
SE Pacific (Chile)	Surface	0 - 1.8	86.9	11
Brazil	Sea floor	2.9/100 m^2		12

Table 2.3 Polymer based materials as a component of marine debris (continued)

Location	Depth	Mean density of litter (items / Km^2)	% plastic items	Reference
North Pacific Gyre	surface	334,271	100	15
Tokyo Bay (1996 & 2000)	Sea floor	338 & 185	90 & 90	13
Kodiak Island, Alaska (1994 - 1996)	Sea floor	Not stated	49 (1994), 59 (1995) & 47 (1996)	14
Middle East				
Jordan, Gulf of Aqaba	Coral Reef	2.8	42	16

References: 1 - Galgani *et al*., (2000); 2 - Barnes and Milner, (2005); 3 - Carpenter and Smith, (1972); 4 - Wei *et al*., (2012); 5 - Katsanevakis and Katsarou, (2004); 6 - Koutsodendris *et al*., (2008); 7 - Stefatos *et al*., (1999); 8 - Galgani *et al*., (1996); 9 - Watters *et al*., (2010); 10 - Hinojsa and Thiel, (2009); 11 - Thiel *et al*., (2003); 12 - Oigman-Pszczol and Creed, (2007); 13 - Kuriyama *et al*., (2003); 14 - Hess *et al*., (1999); 15 - Moore *et al*., (2001); 16 - Abu-Hilal and Al-Najjar, (2009)

Table 2.4 Polymer based materials as a component of shoreline debris

Location	Number of beaches	Mean density of litter (items / m^2)	% plastic items	Reference
Europe				
Scotland (Firth of Forth) (1999 & 2007)	16 & 37	6.2 (max. Density)	46	1, 2
Scotland (Cramond) (1987 & 1998)	1	0.35 & 0.8 respectively	29.37 & 37.12 respectively	3, 4
Wales	1	Not stated	> 50	5
Germany, Kachelotplate	1	Not stated	60.4	6
Mediterranean	32	36	Reported as most common item found	7
Russia	8	0.2	55.1	8
Inch Strand, Ireland	1	0.22	46	9
Mediterranian				
Malta	7	1.6 – 167 (max. 1462)	Counts of pellets	10
Australaisa				
Australia (Cable Beach)	1	0.5	14.65	11
Australia (Greater Sydney region)	6	0.2	89.8	12
Japan	18	3.4	72.9	8

Table 2.4 Polymer based materials as a component of shoreline debris (continued)

Location	Number of beaches	Mean density of litter (items / m^2)	% plastic items	References
Middle East				
Israel	6	0.03 - 0.88	70.6	13
Gulf of Oman	11	Ranged from 0.43 - 6.01, with a mean density of 1.79	61	14
North America				
West Indies	5	0.37	47	15
New Jersey, USA	1	728 items over 500 m transects (monthly mean)	~ 73	16
South America				
Chile	43	1.8	Reported as most common item found	17
Brazil	1 - 16	1 -10 items	~ 57	18, 19, 20
Canada				
Nova Scotia	1 (70 m)	2129 items collected	86	21

Table 2.4 Polymer based materials as a component of shoreline debris (continued)

Location	Number of beaches	Mean density of litter (items / m^2)	% plastic items	References
Antarctic Peninsula				
Scotia Arc Islands	4	0 – 0.3	> 70	22
Oeno Pitcarin	1	0.35	45	9
Ducia Atoll, South Pacific	1	0.12	38	23

References: 1 - Storrier *et al*., (2007); 2 - Velander and Mocogni, (1999); 3 - Caulton and Mocogni, (1987); 4 - Velander and Mocogni, (1998); 5 - Williams and Tudor, (2001); 6 – Liebezeit, (2008); 7 - Martinez-Ribes *et al*., (2007); 8 - Kusui and Nada, (2003); 9 – Benton, (1995); 10 - Turner and Holmes, (2011); 11 - Foster-Smith, (2007); 12 - Cunningham and Wilson, (2003); 13 - Bowman *et al*., (1998); 14 – Claereboubt, (2004); 15 - Nagelkerken *et al*., (2001); 16 – Ribic, (1998); 17 - Bravo *et al*., (2009); 18 - Santos *et al*., (2009); 19 - Silva-Cavalcanti *et al*., (2009); 20 - Oigman-Pszczol and Creed, (2007); 21 - Walker *et al*., (2006); 22 - Convey *et al*., (2002); 23 – Benton, (1991)

2.3.3 Environmental Degradation

Once in the environment PBMs are degraded through abiotic or biotic factors working together or in sequence; these processes cause the polymer matrix to disintegrate, resulting in the formation of fragmented particles of various sizes and leached additives (Fig. 2.1). There are now a number of studies whose authors have investigated the degradability of a range of PBMs under a range of exposure conditions (Table 2.5). In the following section we address the degradation of PBMs with a focus an studies that are environmentally relevant.

2.3.3.1 Factors Affecting Degradation

Polymer Characteristics

Polymer characteristics play an important role in the degradation rate of PBMs. Those PBMs that contain ester linkages (e.g., polyester polyurethanes) are reported to be readily biodegraded by the action of esterases (Albertsson and Karlsson, 1993). The molecular composition of a PBM also affects the hydrophobicity of the polymer surface, which in turn affects how easily microorganisms can attach themselves (Albertsson and Karlsson, 1993). Complexity of a specific polymer structure (cross-linked polymers that form highly ordered networks) and composition (co-polymers) can affect overall degradability by directly influencing the accessibility of enzymes (Artham and Doble, 2008). PBMs with short and regular repeating units that have high symmetries and strong inter-chain hydrogen bonding (e.g., PE, PP and PET**)**, often limit accessibility and are less susceptible to enzyme attack (Artham and Doble, 2008). Kumar *et al*., (2006) studied the degradability of ethylene-propylene co-polymers, and found biotic degradability to decrease with increased ethylene content over a 6-month time period. Composition also affects how sensitive a polymer is to photo-degradation. Kaczmarek *et al*., (2007) used blends of poly (ethylene oxide) and pectin and found that after 20 hours of exposure the blends most sensitive to UV irradiation were those with an equal weight-ratio of each polymer.

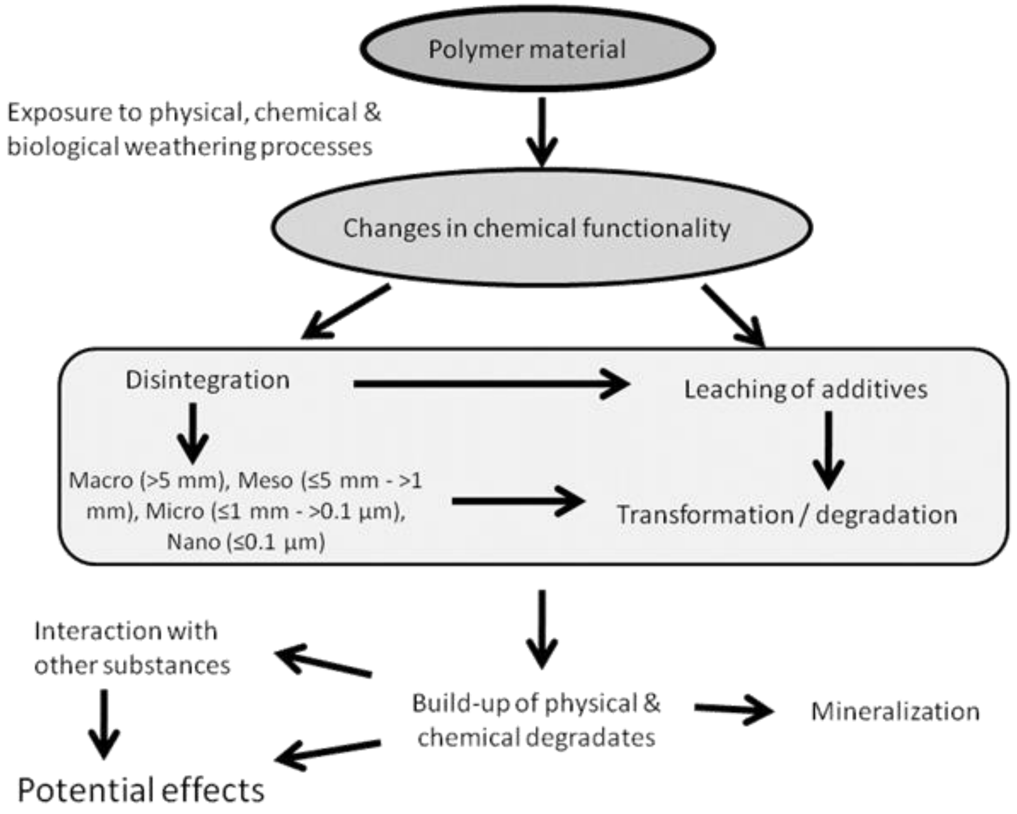


Figure 2.1 Conceptual model illustrating degradation pathways for polymer materials

Abiotic Degradation

Photo-degradation

Under ambient conditions, photo-degradation is one of the primary means by which PBMs are damaged (Klemchuk, 1990; Lucas *et al*., 2008). The main processes involved are chain scission and cross-linking reactions, when exposed to ultra-violet (UV) radiation (290-400 nm) or visible radiation (400-700nm) (Al-Salem, 2009). Most polymers tend to absorb high-energy radiation, which activates their electrons to higher reactivity and foments oxidation, cleavage, and other forms of degradation (Shah *et al*., 2008). The most damaging UV wavelength for a specific material depends on the bonds present; for polyethylene this is 300 nm and for polypropylene 370 nm (Singh and Sharma, 2008). When exposed to UV radiation PE and PP films lose their mechanical integrity and tensile strength, which is accompanied by a decrease in their average molecular weight (Singh and Sharma, 2008). UV absorption in PS has been found to occur at the benzene ring, causing loss of mechanical properties, chain scission, cross-linking reactions and is a precursor to oxidative degradation (Nagai *et al*., 1999). Nagai *et al*., (2005b) analyzed the photo-degradation of a polyether-polyester elastomer under laboratory conditions and found the degradation mechanism upon UV exposure was a selective degradation of the ether parts of soft segments in the polymer matrix, and resulted in the formation of ester, aldehyde, formate and propyl end groups.

Thermal Degradation

Thermal degradation is the molecular deterioration of a polymer as a result of over-heating, which causes bond scissions of the main polymer chain and results in a change in properties. This process affects the entire polymer and not just the polymer surface, and results in changes to molecular weight, loss of tensile strength, changes in crystallinity, reduced durability, embrittlement, changes in color and cracking (Arkatkar *et al*., 2009). Thermally pretreated PP has shown enhanced biodegradation, when compared to non-pretreated samples after 12 months (Arkathar *et al*., 2009). Thermal degradation of polyolefins (PP, LDPE & PET) at temperatures of 673, 773, 873 and 973 K were found to form tar-containing paraffinic structures in PP and LDPE, while aromatic structures were produced by pyrolysis of PET (Cit *et al*., 2010). The heat involved in the thermal degradation process also provides energy for the oxidation of carbon in the polymer backbone (Krzan *et al*., 2006).

Oxidative Degradation

Oxidation processes can be photo or thermally induced and are considered important, especially for non-hydrolyzable materials such as PE (Rutkowska *et al*., 2002a). The introduction of O_2 into the polymer matrix leads to the formation of OH and CO functional groups, which aid subsequent breakdown by biotic processes. The presence of O_3 in the atmosphere, even in small concentrations, accelerates the ageing process of PBMs, because O_3 attacks covalent bonds to produce cross-linking reactions and/or chain scissions producing free radicals (Lucas *et al*., 2008).

Hydrolytic Degradation

The rate of hydrolysis is dependent on the presence of hydrolyzable covalent bonds such as ester, ether, anhydride, amide, carbamide (urea) or ester amide (urethane) groups in the polymer (Lucas *et al*., 2008). PBMs with these functionalities are able to absorb moisture (e.g., polyesters), which then promotes hydrolytic cleavage of the polymer chain (Krzan *et al*., 2006). Hydrolytic degradation of polyester occurs when positively charged hydrogen ions in acidic or negatively charged hydrogen ions in alkaline media attack the ester linkage, thus breaking the polyester chain (Iskander and Hassan, 2001). This reduces the polymer chain length and alters its molecular weight distribution, which directly impacts the strength of the material. In addition to chain breakage, hydrolysis in alkaline media also causes surface erosion of polyesters, which is subsequently manifested by weight loss (Iskander and Hassan, 2001).

Mechanical Disintegration

Mechanical disintegration is the breakdown of the material through the application of shear forces. This process is distinguished from degradation as the materials molecular bonds remain unchanged. Under field conditions, polymers are exposed to several forms of mechanical degradation that include ageing and breakage from atmospheric weathering, water turbulences, freeze-thaw cycles, pressure due to burial under soil or snow, or damage inflicted by animals or birds.

Biotic Degradation (Biodegradation)

Abiotic processes act as an important first step in the degradation of PBMs as they result in a loss of mechanical properties and structural changes to the materials molecular bonds. These processes increase the surface area available for microbial colonization (Kijchavengkul *et al*., 2010; Lucas *et al*., 2008). The size of polymer molecules and their general lack of water solubility prevent microorganisms from transporting them into their cells, where most biochemical processes take place (Artham and Doble, 2008). Biological processes involved in PBM disintegration start outside the microbial cell, with the secretion of extracellular enzymes (Artham and Doble, 2008). These enzymes are too large to penetrate deep into the polymer, so act on the surface by cleaving the polymer chain via hydrolytic mechanisms (Palmisano and Pettigrew, 1992). Biological processes are further enhanced by the formation of the aforementioned utilizable functional groups in the polymer chain (Albertsson *et al*., 1987; Nagai *et al*. 2005a). Over time, abiotic and biotic factors work together to further the degradation process. Chain scission reduces the molecular weight of the polymer, which in turn provides greater accessibility for moisture and oxygen to induce crosslinking reactions that cause the polymer structure to further weaken and become more susceptible to microbial activity (Kijchavengkul *et al*., 2010; Roy *et al*., 2008). When the molar mass of the polymer is sufficiently reduced to generate oligomers and then monomers that are water soluble, the process of mineralization can begin. These substances are transported through the semi-permeable outer membrane of the microorganisms, where they are assimilated as a carbon or nitrogen source through the appropriate metabolic pathway.

2.3.3.2 Degradation in the Natural Environment

Aquatic Environment

In the aquatic environment the mechanical disintegration of PBMs is facilitated by wave action and grinding with sediment particles, whereas changes in chemical functionality are driven by UV exposure. Floating debris has a greater exposure to sunlight and the oxidative properties of the atmosphere, which act alongside the hydrolytic properties of water to cause the material to become brittle and fragment. Sudhakar *et al*., (2007) immersed sheets of LDPE, HDPE and PP of 1.5 mm thickness for 6 months in ocean waters of Bay of Bengal at a depth of 3 m, and found weight loss was greatest in LDPE sheets (2.5%), HDPE (0.75%), PP (0.5%). The authors of this study also found samples at sites with higher dissolved O_2 had increased oxidation. Rutkowska *et al*., (2002b) investigated the degradation of polyurethanes in the Baltic Sea over a period of twelve months and found the rate of degradation was dependent on the degree of cross-linking. In the deep ocean environment where sunlight and oxidative processes are missing, the rate of abiotic degradation is extremely low (Watters *et al*., 2010). Biodegradation in these environments is considered minimal, due to the reduced diversity and density of microbial communities (Browne *et al*., 2008; Watters *et al*., 2010). Therefore, PBMs do not readily biodegrade but rather disintegrate, breaking into smaller and smaller pieces (Barnes *et al*., 2009). In the absence of significant microbial degradation, the sediment compartment in both marine and freshwater environments could function as a continuing source of environmental exposure.

Soil Environment

Soil burial studies have been used as a method to evaluate the degradation of PBMs in the terrestrial environment. Soil type is an important factor affecting degradation; under laboratory conditions, polycaprolactone (PCL) degraded to a greater extent in clay soils than in sandy soils, owing to the great density of microbial communities associated with the clay soils (Cesar *et al.*, 2009). However, when compared to solar exposed samples, buried samples degraded at a much slower rate. Kijchavengkul *et al.*, (2010) buried polyester films in soil for 280 days and found minimal degradation when compared to solar exposed films. A similar result was also found by Williams and Simmons, (1996) for PE strips that had been buried for 4 months; these strips retained greater tensile strength than samples that had been exposed to sunlight for 4 months.

The combined effect of multiple degradation processes has also been studied. For example, several authors have evaluated the effects of UV exposure prior to conducting biodegradation studies under soil burial conditions. Saad *et al.*, (2010) used polyhydroxybutyrate (PHB) films with a 0.1 – 0.12 mm thickness and found samples exposed to 9 h UV radiation showed ~52% weight loss after 28 days soil burial, compared to ~32% weight loss for samples without pre UV exposure. Sadi *et al.*, (2010) also used PHB films (3 mm thickness) and found pre UV exposure increased the rate of degradation, but at a much slower rate due to the increased thickness of the film. These studies suggest that abiotic pre-treatment acts as a first step in weakening the polymer structure. This initiates the formation of oxygenated compounds and low molecular weight hydrocarbons, which are recognized by microbial communities and can be utilized as a food source (Roy *et al.*, 2008). In sea water media, Sudhaker *et al.*, (2008) also found thermal pre-treatment enhanced the biodegradation of LDPE and HDPE by two marine microbes, namely, *Baccillus sphericus* and *Bacillus cereus*. Thermal processes are considered to contribute minimally to marine environmental disintegration of plastics because of the prevailing low water temperatures.

Biodegradation studies have tended to deal with the use of concentrated microbial cultures, with the aim of assessing a particular strain’s ability to degrade a particular PBM. Actinomycetes are reported to be the main group of NRL degrading microbes, with *Bacillus* sp. SBS25 also reported as being capable of utilizing NRL as a sole carbon source (Cherian and Jayachandran, 2009). Tsuchii *et al*., (1997) studied strains of *Nocardia* and observed that they only slightly degraded strips of tread cut from truck tires, when used as a sole carbon source. However, degradation of the tire was enhanced by the addition of more easily accessible carbon sources in the form of latex glove and unvulcanized rubber materials, which were readily utilized by the bacteria (Tsuchii *et al*., 1997).

Biological processes are affected by the amount and type of microorganisms present, their sensitivity to associated environmental parameters and the adaptability of the microbiota (Krzan *et al*., 2006; Palmisano and Pettigrew, 1992). Koutny *et al*., (2009) isolated bacterial strains from forest soils, most belonging to different genera of the proteobacteria group and three *Rhodcoccus* strains, and showed that commonly found bacteria were capable of adhering to and growing on the surface of oxidized LDPE film.

PBMs with a starch component are effectively hollowed out when exposed to microbial communities; this increases the surface to volume ratio allowing for higher oxygen and moisture permeability, enhancing both oxidative and hydrolytic processes (Rutkowska *et al*., 2002b). In theory, the released polymer fragments will have a greater surface area than the original polymer, allowing them to be further degraded by the micro-biota. However, in the case of PE, microorganisms have been found to utilize the starch component, but are unable to utilize the remaining PE fragments, which remain non-degradable (Reddy *et al*., 2003). The starch is utilized by microorganisms, leaving behind a lace-like structure with reduced physical integrity. However, the molecular weight of the remaining material was not reduced sufficiently for permanent assimilation into the microbial biomass (Klemchuk, 1990). Therefore, the remaining polymer matrix was no more biodegradable than the untreated polymer. This causes the disintegration of the polymer matrix, which generates many smaller particles and produces a wider distribution of polymer particles in the environment (Palmisano and Pettigrew, 1992). PBMs, such as starch filled PE, rather than being biodegradable are only bio-disintegrated (Klemchuk, 1990). However, there are no studies that quantify particle concentrations or particle sizes formed during polymer disintegration and degradation.

2.3.3.3 Conclusion

There is a broad literature dealing with the degradation of various polymer types under various conditions. Most of these studies were performed in the laboratory and had a major focus on samples exposed to high energy UV irradiation. In the future, a focus is needed on test conditions that are more environmental relevant, such as degradation in marine water and freshwater microcosms, so that samples are exposed to natural cycles of sunlight and temperature. This approach should also include the use of microbial communities' that represent nature conditions (e.g., agricultural soils of different types, freshwater and marine water), rather than concentrated cultures. Attention is also needed on testing materials of different thicknesses and determining if degradation half-lives can be calculated for PBM films, foams and bulkier items. The identification of microscopic PBM particles in environmental matrices (section 2.3.2.4) highlights a need to establish whether nano-sized particles are also formed during the degradation of PBMs. This is a potentially important issue given the current concerns regarding the environmental behaviour and ecotoxicity of engineered nano materials.

Table 2.5 Selected polymer degradation studies in various environmental matrices

Type of material	Length of study	Main findings	Reference
Aquatic			
HDPE, LDPE & PP 1.5 mm think sheets	6 months	Samples were immersed in the Bay of Bengal at a depth of 3 m. Weight loss was greatest in LDPE sheets (2.5 %), followed by HDPE (0.75 %) and then PP (0.5 %). Sites with higher dissolved oxygen had increased oxidation.	16
PE glycols (PEGs) water soluble	135 days	Greater degradation was observed in freshwater media when compared to seawater media.	3
PHA	42 days	Speices of bacteria belonging to the phylogenetic groups of *Cytophaga-Flavobacterium-Bacteroides*, *g-Proteobacteria* and *b-Proteobacteria* were found to utilize PHA in a eutrophic reservoir.	22
PUR	12 months	The degree of cross-linking effects PUR degradability in sea water. PUR with a heavily cross-linked network was very resistant to degradation.	19
PHA	160 days	Film samples had 58% weight loss at a depth of 120 cm in the South China sea. Degrading microbes isolated from seawater were identified as *Enterobacter cloacae* sp. IBP_V001, *Bacillus* sp. IBP_V002, and *Gracilibacillus* sp. IBP_V003.	44
Soil			
PE	10 years	Photooxidation processes produced carbonyl groups which were utilised by microorganisms to degrade the shorter segments of the PE chain.	1
LDPE	12 months	After 12 months it was impossible to separate film residues from soil. No change in diversity of ammonia-oxidizing bacteria was detected.	12
LDPE	220 days	Higher molecular weight components declined, but lower molecular weight components remained at the same level over the study period.	13

Table 2.5 Selected polymer degradation studies in various environmental matrices (continued)

Type of material	**Length of study**	**Main findings**	**Reference**
LDPE starch blends	90 days	*P. chrysosporium* inoculated soils enhanced biodegradation. Uninoculated soil showed minimal biodegradation.	14
PE compost bags	36 weeks	PE with 9% starch, LDPE and HDPE had 36%, 2.1% and 1.3% weight loss respectively.	15
PP	12 months	Thermally pre-treated PP showed greater weight loss, greater loss of tensile strength and greater changes in crystallinity than non pre-treated PP.	31
PHB & PHB/PP blends	90 days	PHB/PP blends had higher degradation than PHB and samples degraded quicker under alkaline conditions	33
PUR	28 days	Photolysis prior to biodegradation increased the rate of degradation.	32
PUR	5 months	Sample had 95% loss in tensile strength. *Geomyces pannorum* and a *Phoma* sp. were the dominant species recovered from buried samples.	40
Polyester films	40 weeks	Degradation was slower for soil buried samples than for solar exposed samples.	39
PE & PCL	120 days	PE samples remained almost non-biodegradable; PCL was shown to be biodegradable.	34
Composting			
PE starch blends	125 days	Pure PE remained unchanged over the study period; PE with 40% starch had 25% surface erosion.	23
2 commercial biodegradable polymers	60 days	Starch based polymer degraded quicker than synthetic polymer with a biodegradable additive.	24
Ethylene-Propylene copolymers	6 months	Photo-oxidative aging enhanced biodegradation. The composition of monomers in co-polymers effects degradability and degradability decreased with increased ethylene content.	25
PVC	6 months	*Phanaerochaete chrsosporium* PV1 was able to utilize the PVC as a nutrient source.	43

Table 2.5 Selected polymer degradation studies in various environmental matrices (continued)

Type of material	Length of study	Main findings	Reference
Landfill			
PVC	28 months	Found biodegradation rather than leaching accounted for the loss of plasticisers from PVC in landfill simulation.	26
PHB, PCL & PVC	120 days	Plastic volume reduction was more effective under aerobic conditions than anaerobic conditions.	27
UV			
LLDPE	16 month outdoor study	UV stabiliser content increased resistance to weathering.	8
LDPE	100 hours	0.1 % content of photo-degrading additives caused embrittlement after 100 hours UV exposure.	9
Blends of poly(ethylene oxide) and pection	20 hours	Free radicals were formed under UV radiation (e.g. hydroxyl, alkoxyl, acyl radicals, or various macroradicals). Blends most sensitive to UV irradiation were those with an equal weight-ration of the blend.	28
PUR	1 year	PURs with lactic acid and ethylene glycol degradable chain extenders showed greater mass loss over the study period, when compared to pure PUR.	18
polyester elastomer	100 hours	Degradation occurred in the ether parts of the polymer chain.	29
degradation of nano & micro PLGA	100 days	Surface-associated poly vinyl alcohol (used as a stabiliser during formulation of particles) rather than particle size controlled degradation rate.	30
PP		The distribution of photo-degradation products is not dependent on the conditions of irradiation.	20
Thermal			
PE, PP & PS		The presence of PE increased alkane content, PS led to higher aromatic content, PP favoured alkene formation of end products.	21

Table 2.5 Selected polymer degradation studies in various environmental matrices (continued)

Type of material	Length of study	Main findings	Reference
NR		Thermal oxidation of the polyisoprene is demonstrated by the formation of CO groups and cleavage of C-C bonds.	36
Biodegradation			
PE	20 months	Samples with 5% and 8% starch were not susceptible to biodegradation in seawater.	17
LDPE		Bacterial strains isolated from forest soils, most belonging to different genera of the proteobacteria group and three Rhodcoccus strains, were able of adhering to the surface of oxidised LDPE film and were able to grow there.	42
LDPE	15 months	Abiotic degradation produces an increase in carbonyl compounds over-time while the opposite was observed in biotically aged samples.	2
LDPE	600 h UV & then 120 days bacterial culture	Oxygenated compounds and hydrocarbons are formed as a result of UV exposure making LDPE suitable for bacterial colonisation. Bacterial strains were able to secrete extracellular surfactants which made the LDPE more bio-available.	4
Linear LDPE & HDPE	60 days	Main degradation pathway was oxidative as shown by the addition of carbonyl group.	5
Clay filled PE		Growth of microbes was significantly greater on PE with nano-clay composite than those without and oxidation was an important process in aiding the utilization of PE by microorganisms.	6
PE starch blends	28 days	Demonstrated that fungal strains utilized starch in starch blends, but not the PE polymer.	7
PE	4 month	Buried samples retained greater tensile strength than those exposed to sunlight.	11
NRL	8 weeks	Tread from a truck tire was degraded only slightly when it was used as the sole growth substrate for a strain *of Nocardia*, but its degradation was enhanced by addition of latex was readily utilized as a growth substrate.	41

Table 2.5 Selected polymer degradation studies in various environmental matrices (continued)

Type of material	**Length of study**	**Main findings**	**Reference**
NRL		The biodegradation mechanism of *Gordonia* strains was described as the scission of the *cis*-1,4 double bond by oxygen attack to produce carbonyl groups with an aldehyde on the one side and a ketone on the other side of each molecule.	38
NRL and SBR		After incubation with *Nocardia* sp. DSMZ43191, *Streptomyces coelicolor*, *Streptomyces griseus*, bacterial isolate 18a, *Acinetobacter calcoaceticus*, and *Xanthomonas* sp. NRL gloves had 11-18% weight loss.	10
NRL	10 weeks	*Bacillus* sp. SBS25 used NR as the sole source of carbon and was able to produce low molecular weight degradation products.	35
NRL		Of the microbial strains investigated Actino-bacteria were able to degrade NR.	37

PE – polyehthylene; PP – polypropylene; PUR – polyurethane; PCL - Polycaprolactone ; PHB - poly (Hydroxybutyrate); PHA – polyhydroxyalkanoates; NR – natural rubber; SR – synthetic rubber. References: 1 – Albertsson *et al*., (1987); 2 - Albertsson *et al*., (1995); 3 - Bernhard *et al*., (2008); 4 - Roy *et al*., (2008); 5 - Agamuthu and Faizura, (2005); 6 - Reddy *et al*., (2009); 7 - Shang *et al*., (2009); 8 - Al-Salem, (2009); 9 - Magagula *et al*., (2009); 10 - Bode *et al*., (2001); 11 - Williams and Simmons, (1996); 12 - Kapanen *et al*., (2008); 13 - Xu *et al*., (2006); 14 - Orhan & Büyükgüngör, (2000); 15 – Orhan *et al*., (2004); 16 - Sudhakar *et al*., (2007); 17 - Rutkowska *et al*., (2002a); 18 - Tatai *et al*., (2007); 19 - Rutkowska *et al*., (2002b); 20 - Philippart *et al*., (1997); 21 - Pinto *et al*., (1999); 22 – Volova *et al*., (2007); 23 – Vieyra *et al*., (2013); 24 - Mohee and Umar, (2007); 25 - Kumar *et al*., (2006); 26 - Mersiowsky *et al*., (2001); 27 - Ishigaki *et al*., (2004); 28 - Kaczmarek *et al*., (2007); 29 - Nagai *et al*., (2005a); 30 - Panyman *et al*., (2003); 31 - Arkatkar *et al*., (2009); 32 - Saad *et al*., (2010); 33 - Pachekoski *et al*., (2009); 34 - Cesar *et al*., (2009); 35 – Cherian and Jayachandran, (2009); 36 – Agnostini *et al*., (2008); 37 – Rifaat and Yosery, (2004); 38 – Linos *et al*., (2000); 39 - Kijchavengkul *et al*., (2010) 40 – Cosgrove *et al*., (2007); 41 – Tsuchii *et al*., (1997); 42 – Koutny *et al*., (2009); 43 - Ali *et al*., (2009); 44 Volova *et al*., (2011)

2.3.4 Environmental Effects

2.3.4.1 Entanglement and Ingestion

Once they enter the environment PBMs have the potential to mimic natural food sources (Fig. 2.2). Laist, (1997) addressed this in one of the most frequently cited studies. This author identified 135 species of marine vertebrates and 8 species of invertebrates that are susceptible to entanglement, and 111 species of seabirds that are known to ingest plastic items. Hanni and Pyle, (2000) and Page *et al*., (2004) also reported PBM packing loops as a threat to sea lions in California and fur seals in Australia, respectively; Bugoni *et al*., (2001) identified plastic bags as the main debris type ingested by sea turtles.

Seabirds are identified as particularly sensitive to PBM debris intake, and are known to accumulate high numbers of items in their stomachs. Robards *et al*., (1995) found species-specific differences for PBM ingestion in a colony-based survey of multiple species; however, the authors highlight that these differences may be because of geographical differences in PBM pollution Surface-feeding and plankton-feeding divers are most at risk as they are more likely to confuse PBM items with their food source (Applegate *et al*., 2008). Petry *et al*., (2009) studied the stomach contents of 185 birds found dead during beach surveys from July 1997 and July 1998. They identified PBM items in 77% of the stomachs of Cory's Shearwater, *Calonectris diomedea*, a pelagic seabird that winters in the waters off the state of Rio Grande do Sul in Southern Brazil. The most significant causes of mortality were from ingesting large PBM items, such as syringes, cigarette lighters and toothbrushes (Petry et al. 2009). The ingestion of such items causes obstruction of the digestive tract and internal injury, leading to diminished food consumption, loss of nutrition and eventually starvation and death (Bugoni *et al*., 2001; Derraik, 2002).

Entanglement and ingestion of PBM debris in the terrestrial environment is not as well documented in the literature as it is in the marine environment; however, livestock are known to consume PBMs. In a recent study, PBMs were identified as the dominant foreign item consumed by livestock in Birjand, Iran (Omidi *et al*., 2012). Foreign bodies such as plastic bags have also been highlighted as one of the many animal husbandry problems experienced by farmers in Southern Africa (Dreyer *et al*., 1999).

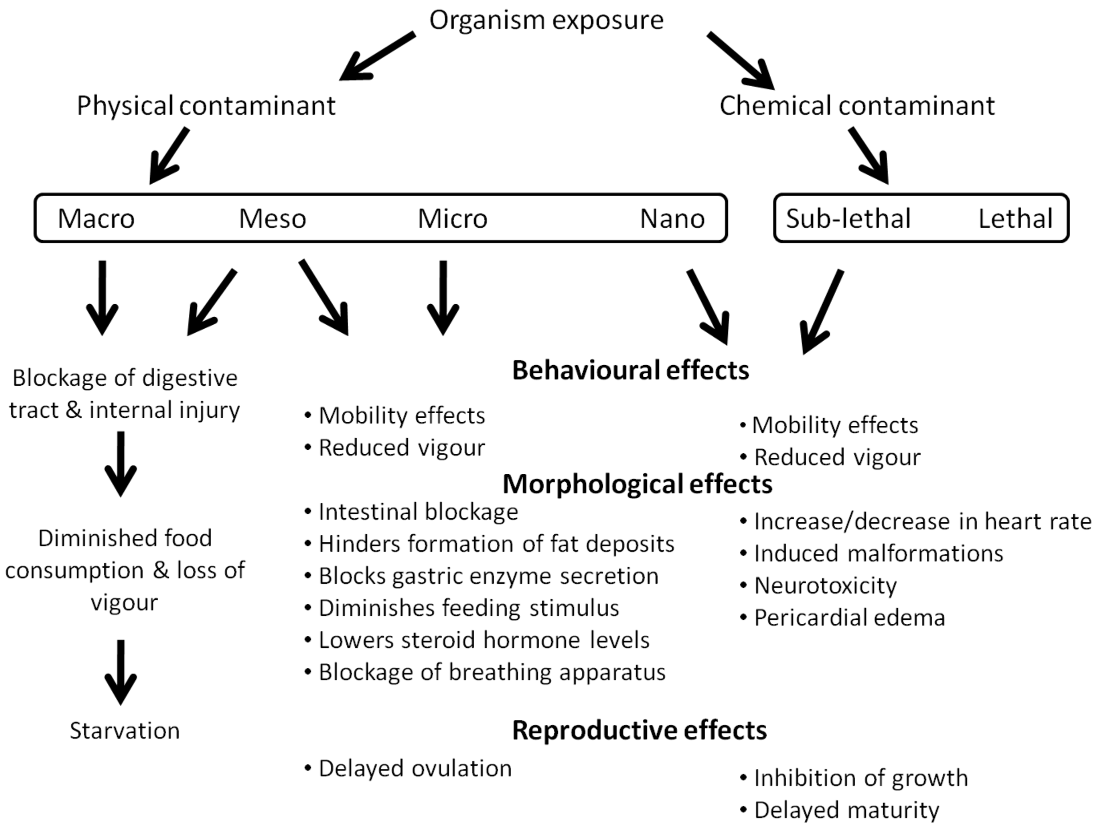


Figure 2.2 Conceptual model illustrating the potential effects of degradates produced during the degradation of polymer based materials

2.3.4.2 Ingestion of Fragmented Particles

The ingestion by a variety of organisms of micro size PBM particles has been reported (Fig. 2.2). Bern, (1990) found that the crustacean zooplankton, *Bosmina coregoni,* did not differentiate between polystyrene beads (2 mm and 6 mm) and algae when exposed to combinations of both objects. Thompson *et al*., (2004) exposed amphipods (detritivores), lugworms (deposit feeders), and barnacles (filter feeders) to microscopic plastic particles and found all three species ingested them within a few days. Browne *et al*., (2008) found microscopic polystyrene fragments (2 µm in diameter) were ingested by the mussel *Mytilus edulis* under laboratory conditions; these particles were then translocated from the gut to the circulatory system. Researchers have suggested that ingesting PBM particles could present a potential physical hazard leading to the following effects: intestinal blockage in fish, hindering formation of fat deposits, blocking gastric enzyme secretion, feeding stimulus diminution, lowering steroid hormone levels, and delaying ovulation that may cause reproductive failure (Ryan *et al*., 1988). The ingestion of micro-plastic particles by plankton-feeding species creates the potential for PBMs to pass up the food-chain. Evidence that this occurs is seen from PBM particles having been recovered from fur seal scats on Macquaire Island (Eriksson and Burton, 2003). It was hypothesized by the authors that these particles were consumed by a pelagic fish species, *Electrona subaspera*, which were then consumed by fur seals (Eriksson and Burton, 2003).

2.3.4.3 Sorption of POPs to Particle Fragments

The ingestion of PBMs could provide a novel route of expose for chemicals that adsorb to the PBM surface. Persistent organic pollutants (POPs) (e.g., polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), some pesticides and polybrominated diphenyl ethers (PBDE)) have been shown to biomagify in food webs, mimic natural hormones to cause reproductive disorders, and possibly increase the risk of disease (Ryan et al. 1988). Carpenter and Smith, (1972) were the first to predict that PBM particles could be a factor to help explain the presence of PCBs in oceanic communities. They hypothesized that as polymers disintegrate into smaller particles, the surface area of the PBM would increase providing an increased surface for absorbing hydrophobic chemicals (Fig. 2.1). If the PBM particles are then taken up by organisms, the polymer-associated chemicals would also be transported into the organisms, possibly leaching into tissues and leading to long-term toxicity issues. Since then, polymer particles have increasingly been investigated as a vector for hydrophobic contaminants to enter the food-web (Saal *et al*., 2008). Mato *et al*., (2001) found that PE and PP pellets (1–5 mm diameter) accumulated PCBs at concentrations up to 106 times that of the surrounding environment, while Ryan *et al*.. (1988) found a positive correlation between ingested PBMs and PCB tissue concentration in seabirds, indicating transfer of these contaminants to organisms. Teuten *et al*., (2007) found that the pollutant phenanthrene (used to make dyes, plastics, pesticides, explosives and drugs), was transmitted to the lugworm, *Arenicola marina*, by contaminated PE particles absorbed from seawater that were mixed into sediments inhabited by the worm. Other POPs such as the chlordanes, dichlorodiphenyltrichloroethane (DDT), dichlorodiphenyldichloroethylene (DDE) and metals such as mercury, zinc and lead have also been found to absorb to PBMs (Endo *et al*., 2005; Van *et al*., 2012). It has also recently been suggested that sorption behaviour of POPs to polymer surfaces is driven by polymer characteristics such as polymer type and density (Fries and Zarfl, 2012). In their study, Fries and Zarfl, (2012) found LDPE had higher diffusion coefficients than did HDPE, meaning shorter equilibrium times for low density polymers. The knowledge that chemical contaminants adsorb to PBM particles creates the potential for novel uptake exposure routes, with the potential for indirect effects on PBM debris consumption.

2.3.4.3 Spread of Alien Species

It has been emphasized that PBM debris may provide a substrate for fouling organisms to be transported long distances, thereby contributing to species dispersal (Derraik, 2002; Gregory, 2009). Barnes and Milner, (2005) reported sightings of an exotic species of barnacle, *Elminius modestus*, on debris in the northern Pacific, and Aliani and Molcard, (2003) documented benthic invertebrates living on marine debris transported by wind and surface currents over the western Mediterranean Sea. PBM pellets (2–1.5 mm diam.) have also been identified as providing an oviposition site for the ocean-skater insect *Halobates,* and show that PBM debris may affect the dispersion of this species (Majer *et al*., 2012). However, Majer *et al*., (2012) do highlight temperature as a limiting factor with the geographical range of this species, as low water temperatures would prevent their full development. Barnes and Milner, (2005) also tentatively suggested that the differences in water temperature could be a limiting factor in species dispersal.

2.3.4.5 Conclusion

Bulk PBMs are well documented as entanglement and ingestion hazards. The effects of microplastics are less well understood but research on uptake into aquatic organisms is starting to emerge. Microplastic uptake and effects on terrestrial organisms are yet to be investigated. Given that soils are highlighted as a potential sink (section 2.3.2.4), it is likely that if soil dwelling organisms can ingest soil particles, they can also ingest microplastic particles. Research questions regarding the interaction of microplastics with POPs are also starting to emerge, but these issues are focused primarily on the aquatic environment. The terrestrial environment also needs to be considered, because the sorption of pesticides to microplastics in soil may also present an exposure route for pesticides to soil organisms.

2.4 Polymer Additives and the Environment

The types and functions of additives used in the production of PBMs are wide ranging. Some of the most important regarding their environmental impact are those that have an endocrine disruptive potential. These include chemicals or chemical classes such as phthalates, brominated flame retardants (BFRs) and bisphenol A (BPA) (Moore, 2008). Phthalate esters are primarily used as plasticizers to impart flexibility to the polymer matrix, and are also used in other products such as inks, lubricating oils, and as solvents in perfumes, paints and additives in hair-sprays, insect repellents and home furnishings (Fatoki et al. 2010; Julinova and Slavik 2012; Teil *et al*., 2006; Yuan *et al*., 2002). In the past, the most important phthalate representative was di-(2-ethylhexyl) phthalate (DEHP), but due to restrictions on its use, others such as di-isodecyl phthalate (DIDP), di-isononyl phthalate (DINP) and di-n-butyl phthalate (DBP) are now commercially important (Clara *et al*., 2010; Tickner *et al*., 2001).

PVC resins are the most important polymer in terms of phthalate usage. PVC can be produced in two forms; the first is a plasticized from that makes the PVC flexible and the second is an unplasticised form (uPVC) used for the production of rigid materials. In the plasticized form, phthalates can account for 50% of total polymer weight (Mulder, 1998; Oehlmann *et al*., 2009). Other PBMs that can incorporate phthalates include PET, polyvinyl acetates, cellulosic and PUR (Teil *et al*., 2006).

BFRs are a diverse group of chemical mixtures that contain brominated organic compounds (Zhang *et al*., 2009). BFRs are commonly used in a variety of polymer products, such as computers, televisions, kitchen appliance casings, car trimmings, electrical insulation, polyurethane foams, as well as textiles to improve fireproof properties (de Wit, 2002). There are approximately 80 different mixtures of BFRs used commercially, and until recently PBDEs were the most widely used (Hu *et al*., 2009). In Europe and North America, restrictions on the use of PBDEs have lead to terra-brominated bisphenol A (TBBPA) and hexabromocyclododecane (HBCD) becoming more commercially important (Hu *et al*., 2009).

BPA is widely used as a monomer in the production of commercial polycarbonate (Duong *et al*., 2010), and as an antioxidant and stabilizing material for polymer products (Yamamoto *et al*., 2001). Other additives that are used in PBMs include antimicrobial agents (used in food packaging to preserve shelf-life), and dyes and pigments (often used to improve aesthetic properties of the material) (Saron and Felisberti, 2006). Recently, silver nano-particles have been utilized as an antimicrobial agent in plastic food packaging materials. Nanosilver damages bacterial cells by weakening cell membranes and destroying enzymes that transport cell nutrients, therefore prolonging the shelf life of food stuffs (Silvestre *et al*., 2011). Stabilizer technology has the aim to extend the service life of PBMs used in outdoor environments, especially in regions of the world that have high temperatures and long summer seasons (Al-Salem, 2009). Solvents may also be applied to coat objects with plastic layers or to clean plastics before printing (Mulder, 1998).

2.4.1 Fate of Additives

The phthalates are generally considered to be chemically stable over a wide temperature range and are easily dissolved in water (Clara *et al*., 2010), so tend to adsorb to inorganic and organic particles such as plankton in the water column, before being deposited onto sediments (Larsson *et al*., 1986). The phthalates are not chemically bonded to the polymer matrix, and, hence, migrate from the products in which they are used by volatilization and enter the atmosphere. Once in the atmosphere they can undergo oxidative or photolytic reactions, followed by wet or dry deposition (Teil *et al*., 2006). The hydrolytic metabolites of DEHP have been identified as mono methylhexyl phthalate (MEHP) and 2-ethanohexanol (Tickner *et al*., 2001).

BFRs are stable and resist degradation, but studies have shown that the higher brominated PBDEs will undergo degradation via de-bromination to more persistent lower-brominated compounds (Birnbaum and Staskal, 2004). Such degradation occurs in sand, sediments, and soils under laboratory conditions (Birnbaum and Staskal, 2004; Soderstrom *et al*., 2004). The half life of deca-BDE in sediments is estimated to be <30 min under UV light, 53 h under natural sunlight, and 150 – 200 h in soils (Soderstrom *et al*., 2004). The leach-ability of PBDEs from TV housings was found to be enhanced by the presence of dissolved organic matter in landfill leachate, but degradation rates of PBDEs varied from congener to congener (Kim *et al*., 2006).

TBBPA is reactively bonded to the polymer matrix and requires cleavage of covalent bonds before migration can take place. Photo- and bio-degradation occurs with TBBPA, and the breakdown products have been identified as tri-, di-, and mono-BBPA, as well as BPA (Debenest *et al*., 2010). In water, TBBPA derivatives are produced from the photochemical degradation and decomposition of the PBMs (Eriksson *et al*., 2004), whereas thermal degradation also leads to the formation of the above mentioned brominated species (Barontini *et al*., 2004). TBBPA is reported to have a half life of 7 – 81 days in water, depending on season, and 2 months under both aerobic and anerobic conditions in soils and sediments (Birnbaum and Staskal, 2004). HBCD has low water solubility and has been shown to persist in sediments (Remberger et al. 2004). Analysis of BFR residues in harbor seals sampled from the northwest Atlantic identified 16 congeners of PBDE at concentrations ranging from 35 to 19,500 ng/g lipid wt (Shaw *et al*., 2012). Shaw *et al*., (2012) also identified tissue-specific concentrations of an α-HBCD isomer that displayed significantly higher concentrations in the liver (2 – 279 ng/g lipid wt) than in the blubber (2 – 29 ng/g lipid wt).

The migration of BPA from commercially available polycarbonate baby bottles has been shown to range from 2.4-14.3 µg/kg, when bottles were filled with boiling water and left at ambient temperatures for 45 min, mainly during the first eight cycles of such use (Maragou *et al*., 2007). To put this into context the estimated dietary exposure for infants aged up to 1 year old ranges between 0.2-2.2 µg/kg-bwt/day, which is below the recently established tolerable daily intake (Maragou *et al*., 2007). Polycarbonate PBMs were also shown to exhibit accelerated leaching velocity of the BPA when exposed to salts in sea water (1.6 ng/mL/day at 20 ^{o}C and 11 ng/mL/day at 37 ^{o}C) compared river water (0.2 ng/ml/day at 20 ^{o}C and 4.8 ng/ml/day at 37 ^{o}C) (Sajiki and Yonekubo, 2003). The estimated half-life of BPA is up to 14 days in seawater (Robinson and Hellou 2009), with aerobic degradation of BPA constituting the most dominant degradation pathway, except when it is present in the atmosphere, and thereby susceptible to reaction with hydroxyl radicals (Staples *et al*., 1998).

2.4.2 Occurrence of Associated Additives

Chemical additives are used in the polymer manufacturing process to improve a materials performance, and such additives are dispersed within the three-dimensional porous structure of the polymer. These additives can be released to the environment during the manufacturing process, throughout a PBMs lifecycle and during subsequent PBM degradation processes. The rate at which additives are leached depends on the pore diameter of a particular polymer structure and the molecular size of the additives used; lower molecular weight additives move more easily through a polymer matrix that display larger pore size (Gopferich, 1996). Various environmental samples have been analyzed for the presence of these additives, and they have been detected at various concentrations ranging from ng/L to mg/L (Table 2.6).

Phthalates have been described as one of the most abundant and ubiquitous man-made chemicals in the environment (Liao *et al*., 2009; Martin and Voulvoulis, 2009). Because they are not chemically bound to the polymer resin in which they are used, they tend to slowly migrate to the surface of the product and leach or evaporate from the end-product to the surrounding environment, both during and after the useful life of a specific product (Martin and Voulvoulis, 2009). DEHP and DBP are the most commonly occurring phthalates. Residues of both have been detected in multiple environmental compartments: surface waters (Kelly *et al*., 2010), river sediments (Huang *et al*., 2008), sewage sludge, wastewater effluent and untreated wastewater (Clara *et al*., 2010), rainwater (Teil *et al*., 2006), stormwater (Bjorklund *et al*., 2009) and agricultural soils (Hu *et al*., 2003). The other phthalates are generally considered to be of minor importance.

The concentration of phthalates reported to exist in surface waters have ranged from sub µg/L (e.g., Kelly *et al*., 2010) to high mg/L levels in contaminated hotspots (e.g., Fatoki *et al*., 2010), and to mg/kg in sediments (e.g., Kelly *et al*., 2010). Concentrations reported for agricultural soils in China (23 locations; 0.89 – 10.03 mg/kg) (Hu *et al*., 2003), were much higher than those found in agricultural soils in Denmark (2 locations; 0.3-1,900 µg/kg) (Vikelsoe *et al*., 2002). The concentrations observed for China were thought to be influenced by the use of agricultural films containing phthalates (Hu *et al*., 2003). The maximum concentration of DEHP in final effluent from a European Waste Water Treatment Plant (WWTP) was reported to be 182 µg/L, and was derived from a review of studies of estrogenic compounds that had a median concentration of 5.3 µg/L (Martin and Voulvoulis, 2009). In the Venda region of South Africa, it has been reported by Fatoki *et al*., (2010) that PBMs are indiscriminately disposed of as a common practice. This has caused river water pollution by phthalates at levels ranging from 0.16 mg/L to 10.17 mg/L (Fatoki *et al*., 2010). This is noted by Fatoki *et al*., (2010) as an issue of concern, because water from these rivers and their associated dams are the primary sources of potable water. This poses a risk to human health, because people who drink water contaminated with such levels exceed the USEPA established safe limit for phthalates (<6 µg/L) over many years, and may develop liver and reproductive problems (USEPA, 2012).

BPA can be released into the environment through sewage treatment effluent, landfill leachate (Wintgens *et al*., 2003) or degradation of polycarbonate polymers (Mohapatra *et al*., 2010). BPA residues are most commonly reported in surface waters and wastewater effluents, where they display concentrations up to 213.6 µg/L; sediments have been identified as being modest sinks (Wang *et al*., 2011) (Table 2.6). PBMs in landfills are thought to be a possible source of BPA in groundwater; in Japan, median concentration of 269 mg/L have been detected in sampled leachates (Yamamoto *et al*., 2001). The maximum concentration of BPA in final effluent from a European WWTP was reported to be 40.09 µg/L, with a median value of 0.36 µg/L (Martin and Voulvoulis, 2009).

A review of the literature has indicated PBDEs, TBBPA and HBCD to be the most commonly occurring BFRs detected in environmental samples. PBDE and TBBPA differ in that PBDEs are generally used as an additive flame retardant, and thereby are not chemically bonded to the polymer matrix; in contrast, TBBPA is primarily used as a reactive flame retardant and is covalently bonded to the polymer matrix (Alaee *et al*., 2003). Flame retardants, when used as additives (rather than a reactive compound), exhibit leaching and evaporation behavior similar to those displayed by the phthalates (Debenest *et al*., 2010). Levels in sediments are generally highest from urban and industrial areas, particularly downstream from WWTPs or from product manufacturing sites. Sellström and Jansson, (1995) found high concentrations of TBBPA in sediments sampled downstream from plastic manufacturing factories (270 µg/kg dwt), in comparison to upstream sediments (34 µg/kg dwt), indicating the factory as the source. Harrad *et al*., (2009) reported similar concentrations of HBCD and TBBPA in water, sediments and fish (see Table 6) from nine English lakes that had no major point-source inputs (i.e., from WWTP), with minimal seasonal variations and found aqueous concentrations were significantly correlated, but no common source was identified. Hites, (2004) also provided a review of PBDE concentrations present in human samples (0.03 – 193 ng/g for milk; 0.44 – 6.03 ng/g for blood, lipid wt), outdoor and indoor air (82.6 – 1,780 pg/m^3), marine mammals (0.42 – 4,950 ng/g lipid wt), birds (124 – 7,510 ng/g lipid wt for gull eggs) and fish (6.31 – 7,200 ng/g lipid wt).

Table 2.6 Concentrations of compounds associated with the manufacturing of polymer products detected in various environmental matrices

Compound	**Country**	**Concentration reported (min: max)**	**Reference**
Plasticisers	***Surface water***		
DEHP	Chi, Ger, Ire, Jap, Neth, SA, Tai, U.S	n.d. – 2.18 mg/L	1, 2, 3, 4, 5, 6, 7, 8
DBP	Chi, Neth, Tai	0.04 – 13.5 µg/L	2, 3, 5
DEP	SA	0.16 - 3.56 mg/L	7
DINP	Ire	0.14 – 1.89 µg/L	4
bis(2-ethylhexyl) adipate	U.S	10 ug/L (max)	8
triphenyl phosphate	U.S	0.22 ug/L (max)	8
Phthalic anhydride	U.S	1 ug/L (source - plastic manufacturing)	8
	River sediments		
DEHP	Can, Chi, Ger, Ire, Jap, SA, Tai	0.014 – 25.27 mg/kg	1, 2, 3, 4, 6, 7, 10
DBP	Can, Chi, Ire, SA, Tai	n.d. – 0.89 mg/kg	2, 3, 4, 7, 9, 10
DINP	Can, Ire	n.d. - 6.16 mg/kg	4, 10
DIDP	Ire	0.1 - 7.46 mg/kg	4
BBP	Can, Tai	< 0.3 – 220 µg/L	9, 10
	Sewage sludge		
DEHP	Aus	20 - 29 mg/kg	11
DMP	Aus	n.d. - 89 µg/kg	11
DEP	Aus	< 40 - 130 µg/kg	11
BBP	Aus	120 - 380 µg/kg	11
DOP	Aus	58 - 180 µg/kg	11
	Wastewater effluent		
DEHP	Aus, Fra	ng - 5.02 µg/L	11, 12
DEP	Aus	n.d. - 1.1 ng/L	11
DMP	Aus	n.d. - 0.19 ng/L	11
DBP	Aus	n.d. - 2.4 ng/L	11
BBP	Aus	0.088 - 1.4 ng/L	11
DOP	Aus	n.d. - 0.26 ng/L	11
	Untreated wastewater		
DEHP	Aus	3.4 - 34 ng/L	11
DEP	Aus	0.77 - 9.2 ng/L	11
DMP	Aus	n.d. - 2.4 ng/L	11

Table 2.6 Concentrations of compounds associated with the manufacturing of polymer products detected in various environmental matrices (continued)

Compound	**Country**	**Concentration reported (min: max)**	**Reference**
DBP	Aus	n.d. - 8.7 ng/L	11
BBP	Aus	0.31 - 3.2 ng/L	11
DOP	Aus	n.d. - 1.1 ng/L	11
	Soil		
DEHP	Chi, Den	0.012 – 7.11 mg/kg	13, 14
DBP	Chi, Den	n.d. - 1.56 mg/kg	13, 14
DEP	Chi	n.d. - 2.61 mg/kg	13
	Stormwater		
DEHP	Aus, Swe	0.45 - 24 µg/L	11, 15
DEP	Aus	n.d. - 0.27 µg/L	11
DMP	Aus, Swe	n.d. - 0.3 µg/L	11, 15
DBP	Aus, Swe	< 0.02 - 0.27 µg/L	11, 15
DIDP	Aus, Swe	n.d. - 17 µg/L	11, 15
DINP	Aus, Swe	0.005 - 85 µg/L	11, 15
BBP	Aus, Swe	n.d. - 0.33 µg/l	11, 15
DOP	Aus	n.d. - 0.37 µg/L	11
	Rainwater		
DEHP	Fra	423 ng/L (mean)	16
DMP	Fra	116 ng/L (mean)	16
DEP	Fra	333 ng/L (mean)	16
DBP	Fra	592 ng/L (mean)	16
BBP	Fra	81 ng/L (mean)	16
DOP	Fra	10 ng/L (mean)	16
	Other		
Japan (aquatic vegetation)	DEHP	20 - 2000 ug/kg	6
Taiwan (fish)	DEHP	2.4 - 253.9 mg/kg (dwt)	9
Bisphenol-A	***Surface water***		
BPA	Aust, Chi, Ita, Jap, Kor, Port, Swit, Tai, U.S	n.d. – 39.4 µg/L	8, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27
	Sediments		
BPA	Ita	< 2 - 118 µg/kg (dwt)	26

Table 2.6 Concentrations of compounds associated with the manufacturing of polymer products detected in various environmental matrices (continued)

Compound	Country	Concentration reported (min: max)	Reference
	Sewage sludge		
BPA	Can, Ger	0.001 - 1.36 mg/kg (dwt)	1, 28
	Wastewater effluent		
BPA	Aus, Aust, Gre, Port, Kor, Spa	0.0026 – 213.6 µg/L	20, 25, 26, 27, 28, 29, 30
	Landfill leachate		
	Jap	1.3 - 17200 µg/L	31
Flame retardents	***Surface water***		
PBDE	Arg	n.d.	33
TBBPA	U.K	140 - 3200 pg/L	32
HBCD	U.K	80 - 270 pg/L	32
tri(dichlorisopropyl) phosphate	U.S	0.16 ug/L	8
tri(2-chloroethyl)phosphate	U.S	0.54 ug/L	8
	River sediments		
PBDE	Bel, Swit	0.14 – 8413 ng/g (dwt)	34, 35
TBBPA	U.K, Jap, Swe	< 0.2 – 270 µg/kg (dwt)	32, 36, 37
HBCD	U.K, Jap, Swe	880 - 4800 pg/g (dwt)	32, 34, 36
	Marine sediment		
TBBPA	Jap	5.5 ng/L	36
HBCD	Jap	< 2 - 860 ng/L	36
	Sewage sludge		
TBBPA	Swe	31 - 56 µg/kg	37
	Landfill leachate		
TBBPA	Jap	0.3 - 540 ng/L	36
HBCD	Jap	< 2 - 8 ng/L	36
	Soil		
PBDE	Arg	n.d.	33

Table 2.6 Concentrations of compounds associated with the manufacturing of polymer products detected in various environmental matrices (continued)

Compound	**Country**	**Concentration reported (min: max)**	**Reference**
	Other		
PBDE	Can (Crab, Sole and Porposie)	4 - 2300 ng/g (lipid weight; lwt)	38, 39
TBBPA	U.K (fish)	< 0.29 - 270 pg/L (lwt)	32
HBCD	U.K (fish)	14 - 290 ng/g (lwt)	32

n.d. – not detected; DEHP - Di(2-ethylhexyl)phthalate; DEP - Di ethyl phthalate; DMP - Di methyl phthalate; DBP - Di-n-butyl phthalate; DIDP - Di-isodecyl phthalate; DINP - Di-isononyl phthalate; BBP - Butyl Benzyl Phthalate; DOP - Dioctyl phthalate; BPA – Bisphenol-A; PBDE - Polybrominated diphenyl ethers; TBBPA - Tetrabromobisphenol A; HBCD – Hexabromocyclododecane; Arg – Argentina; Aus – Austria; Aust – Australia; Bel – Belgium; Can – Canada; Chi – China; Den – Denmark; Fra – France; Ger – Germany; Gre – Greece; Ire – Ireland; Jap – Japn; Kor – Korea; Neth – Netherlands; Port – Portugal; SA – South Africa; Spa – Spain; Swe – Sweden; Swit – Switzerland; Tai – Taiwan; UK – United Kingdom; U.S – United States. References: 1 – Fromme *et al*., (2002); 2 - Yuan *et al*., (2002); 3 - Zeng *et al*., (2008); 4 – Kelly *et al*., (2010); 5 – Peijneuburg and Struijs, (2006); 6 – Yuwatini *et al*., (2006); 7 – Fatoki *et al*., (2010); 8 – Kolpin *et al*., (2002); 9 – Huang *et al*., (2008); 10 – McDowell and Metcalfe, (2001); 11 – Clara *et al*., (2010); 12 – Dargnat *et al*., (2009); 13 – Hu *et al*., (2003); 14 –Vikelsoe *et al*., (2002); 15 – Bjorklund *et al*., (2009); 16 – Teil *et al*., (2006); 17 – Wang *et al*., (2011); 18 – Liu *et al*., (2011); 19 – Ribeiro *et al*., (2009); 20 – Ying *et al*., (2009); 21 – Zhao *et al*., (2009); 22 – Voutsa *et al*., (2006); 23 - Duong *et al*., (2010); 24 - Chen *et al*., (2010); 25 – Pojana *et al*., (2007); 26 - Arditsoglou and Voutsa, (2010); 27 - Fernandez *et al*., (2009); 28 - Stasinakis *et al*., (2008); 29 - Ko *et al*., (2007); 30 - Furhacker *et al*., (2000); 31 - Yamamoto *et al*., (2001); 32 – Harrad *et al*., (2009); 33 – Fontana *et al*., (2009); 34 – Covaci *et al*., (2005); 35 – Kohler *et al*., (2008); 36 – Suzuki and Hasegawa, (2006); 37 – Sellstrom and Jansson, (1995); 38 – Ikonomou *et al*., (2002); 39 – Luross *et al*., (2002)

2.4.3 Toxicity of Chemical Additives

Once released from the degraded polymer matrix, chemical additives may become available for uptake by living organisms. The phthalates and BPA have been found to cause a range of effects on fish, crustacean, amphibian and bacteria species; effects include mortality, delayed maturity, reduced vigor, induce morphological deformations and reduce reproduction (Table 2.7). DEHP represents the most widely studied phthalate and is regarded to be one of the most toxic of the class (Jonsson and Baun 2003). However, its metabolite MEHP, which is considered to be itself toxic, has not been widely studied. DEHP has displayed toxicity to rats through impaired testis development at high doses (Table 2.6). Other important phthalates, such as DMP, DEP, DBP and BBP, also exhibit similar toxic effects to DEHP. The toxicity of some of the minor phthalates (i.e., DIDP, DNIP and DOP) is less well researched, possibly because there concentrations in most aquatic environments are reported at low µg/L or less (Table 2.6). Oehlmann *et al*., (2009) published a comprehensive review of the effects of phthalates and BPA on wildlife, and highlighted the lack of long-term exposure or toxicity data at environmentally relevant concentrations, particularly in complex mixtures. Human exposure can occur through ambient environmental concentrations (Tickner *et al*., 2001). DEHP containing PVC, since the 1960s, has been used to produce a range for medical devices and in the construction industry (Rossi and Lent, 2006; Tickner *et al*., 2001). Rossi and Lent, (2006) have proposed the phasing out and replacement of PVC, and recommend a preference towards PBMs that do not contain hazardous additives such as PP and PE as means of reducing phthalates exposure.

BFRs exposure has been found to inhibit growth of plankton and algae colonies and reduce zooplankton reproduction (Debenest *et al*., 2010). Mice and rat studies have shown liver disturbances, nervous system damage and decreased thyroxine levels; pentaBDE has been found to accumulate in certain predatory birds and mammals that are at the top of the food chain (Rhee *et al*., 2002). Another toxic compound that is associated with polymer manufacturing is zinc, which has been identified as the dominant toxicant in wastewater from rubber manufacturing factories (Park *et al*., 2008). Exposure of *Daphnia magna* to accelerators (e.g., zinc diethyl dithiocarbamate (ZDEC) and zinc mercaptobenzothiazole (ZMBT)) that are used to produce rubber and latex products gave 48 h EC_{50} values that were lower than those reported for DMP, DEP, DBP, BBP and MEHP (Jonsson and Baun, 2003) (Table 2.7). This higher toxicity level indicates that the risks associated with other additives compounds used in PBM manufacturing are also important.

2.4.4 Conclusion

The phthalates, BPA and BFRs are considered to be the most important PBM additives, because these are considered to be biologically active. To be effective these chemicals often have properties that make them resistant to photo- and bio-degradation. These properties imply a potential for accumulation and persistence in the environment, and as such there is a growing body of literature dealing with the environmental occurrence and effects of these compounds. However, there are many other PBMs that incorporate an even greater number of additive compounds, and the risks of these compounds also need to be evaluated. An example is the class of chemicals termed the halogen-free flame retardants, which are of growing interest as replacements for the more traditional BFRs, and are the subject of an interesting and in-depth review by Waaijers *et al.*, (2013). These authors highlight that for many of these compounds their environmental behaviour and ecotoxicological properties are only known to a limited extent.

Table 2.7 Selected studies in which the effects of commonly used polymer additives was reviewed

Compound	Organism	Findings	Reference
Phthalate	***Aquatic***		
DEHP	*Daphnia magna*	24 h EC_{50} 71.07 mg/L based on immobilization	1
	Chironomus tentans	24 h LC_{50} 438.96 mg/L based on death of individuals	1
	Danio rerio	5 g/kg after 10 day exposure via intraperitoneal injection caused increase in hepatosomatic index and levels of hepatic vitellogenin transcript, and a decrease in fertilisation success of oocytes	2
	Salmo salar	1500 mg/kg dosed via diet resulted in small incidence of juvenile intersex	3
	Oryzias latipes	No evidence of oestrogenic effects at tested concentrations	4
	Cyprinus carpio	Disrupted synthesis of testoterone	5
	Mytilus galloprovincialis	21 day expose to 500 µg/L increased catalaseand acyl-CoA oxidase activity & inhibition of superoxide & manganate superoxide dismutase	6
	Lumbriculus variegatus	Not acutely toxic	7
	Pseudokirchneriella subcapitata	72 h EC_{50} > 0.003 mg/L based on inhibition of growth	8
	Vibrio fischeri	15 min EC_{50} > 0.003 mg/L	8
	Hyalella azteca	No effect at concentrations tested	7
	Folsomia fimetaria	EC_{50} > 5000 mg/kg based on adult survival & reproduction, > 1000 mg/kg based on juvenile survival, growth & number of cuticles	16
	Escherichia coli & *Bacillus subtilis*	24 h low doses (<150 µg/ml) stimulated growth, doses >300 µg/mL showed morphological deformations	17
	Caenorhabditis elegans	24 h LC_{50} 22.55 mg/L based on mortality	18
	Adult Wistar rats	90 day dose of 500 mg/kg/d decreased weight & volume of testis	19
	Adult rats	Dose of 1 g/kg decreased testis weight & was linked with oxidative stress within testis	21

Table 2.7 Selected studies in which the effects of commonly used polymer additives was reviewed (continued)

Compound	**Organism**	**Findings**	**Reference**
DMP	*Daphnia magna*	48 EC_{50} 284 mg/L based on immobility	8
	Chironomus tentans	10 day LC_{50} 68.2 mg/L	7
	Pseudokirchneriella subcapitata	72 h EC_{50} 228 based on inhibition of growth	8
	Vibrio fischeri	15 min EC_{50} 26.3 mg/L	8
	Hyalella azteca	10 day LC_{50} 28.1 mg/L	7
	Lumbriculus variegatus	10 day LC_{50} 246 mg/L	7
DEP	*Daphnia magna*	48 EC_{50} 90 mg/L based on immobility	8
	Chironomus tentans	10 day LC_{50} 31 mg/L	7
	Pseudokirchneriella subcapitata	72 h EC_{50} 70.4 based on inhibition of growth	8
	Cyprinus carpio	96 h LC_{50} 48 mg/L based on induced testicular atrophy	10
	Vibrio fischeri	15 min EC_{50} 143 mg/L	8
	Hyalella azteca	10 day LC_{50} 4.21 mg/L	7
	Lumbriculus variegatus	10 day LC_{50} 102 mg/L	7
DBP	*Daphnia magna*	48 EC50 6.78 mg/L based on immobility	8
	Chironomus tentans	10 day LC_{50} 2.64 mg/L	7
	Cyprinus carpio	Disrupted synthesis of testoterone	10
	Oncorhynchus mykiss	No significant vitellogenin response	11
	Vibrio fischeri	15 min EC_{50} > 7.4, mg/L	8
	Pseudokirchneriella subcapitata	72 h EC_{50} 2.52 based on inhibition of growth	8
	Xenopus laevis	96 h LC_{50} 14.5 mg/L based on mortality, 96 h EC50 0.98 mg/L based on number of surviving tadpoles with a least 1 malformation	14
	Rana rugosa	Development of ovaries in 17% of males gonads exposed to 10 uM during days 19 - 23 after fertilization	15

Table 2.7 Selected studies in which the effects of commonly used polymer additives was reviewed (continued)

Compound	Organism	Findings	Reference
	Folsomia fimetaria	EC$_{50}$ 305 mg/kg based on adult survival, 19.4 mg/kg juvenile survival, 68 mg/kg reproduction, > 10 mg/kg growth & number of cuticles	16
	Adult rats	2 week dose of 500 mg/kg/d by oral gavage decrease in body and testicular weight, 250 and 500 mg/kg/d decreased sperm count	20
	Hyalella azteca	10 day LC$_{50}$ 0.63 mg/L respectively	7
	Lumbriculus variegatus	10 day LC$_{50}$ 2.48 mg/L respectively	7
BBP	*Daphnia magna*	48 EC$_{50}$ 2.43 mg/L based on immobility	8
	Danio rerio	6 µg/L found to induce changes in sperm mortility. No effect on number of eggs spawned & viability of embryos at 8 h post fertilisation.	9
	Oncorhynchus mykiss	500 mg/kg dosed via intraperitoneal injection resulted in 3-fold increase in vitellogenin in males	11
	Pimephales promelas	No evidence of oestrogenic effects at tested concentrations	12
	Vibrio fischeri	15 min EC$_{50}$ > 1.3 mg/L	8
	Hyalella azteca	10 d LC$_{50}$ 0.46 mg/L	7
	Pseudokirchneriella subcapitata	72 h EC$_{50}$ 0.96 mg/L based on inhibition of growth	8
	Lumbriculus variegatus	10 d LC$_{50}$ 1.23 mg/L	7
	Gasterosteus aculeatus	31 day exposure to 100 µg/L caused behavioural alterations	36
DHP	*Lumbriculus variegatus*	Not acutely toxic	7
	Hyalella azteca	No effect at concentrations tested	7
DINP	*Oryzias latipes*	1 µg/g fish/day had no effect on reproduction or development at tested concentrations	13
DIDP	*Oryzias latipes*	1 µg/g fish/day had no effect on reproduction or development at tested concentrations	13
DOP	*Escherichia coli & Bacillus subtilis*	24 h low doses (< 150 µg/ml) stimulated growth, doses > 300 µg/ml showed morphological deformations	17

Table 2.7 Selected studies in which the effects of commonly used polymer additives was reviewed (continued)

Compound	**Organism**	**Findings**	**Reference**
MEHP	*Daphnia magna*	48 EC$_{50}$ 3.47 mg/L based on immobility	8
	Pseudokirchneriella subcapitata	51.9 mg/L based on inhibition of growth	8
	Vibrio fischeri	15 min EC$_{50}$ 45.4 mg/L	8
	Mouse fetal oocytes	24 h 60 % mortility in oocyte survival at 500 uM and 32 % at 125 uM	22
BPA	*Chironomus tentans*	24 h LC$_{50}$ 3.264 mg/L & 96 h EC50 2.7 mg/L based on mortality	1, 23
	Chironomus riparius	chronic LOEC 1.0 mg/L based on reduction of larval wet weight and delay in moulting	24
	Daphnia magna	24 h EC$_{50}$ 0.237 mg/L based on immobilization, 21 day NOEC $\geq$ 3.16 mg/L based on reproduction rate, EC$_{50}$ 16 mg/L based on reproductive tests, 48 h EC$_{50}$ 10 mg/L based on immobilization	1, 25, 26, 27
	Hyalella azteca	42 day LOEC 1.1 mg/L based on number of offspring per female	23
	Marisa cornuarietis	96 h LC$_{50}$ 2.24 mg/L (25 degrees), > 4.03 mg/L (22 degrees) and no effect on fecundity, egg hatching, juvenile growth & reproduction at concentrations up to 0.64 mg/L. A significant decrease in female growth was observed at 0.64 mg/L.	23, 29
	Oncorhynchus mykiss	50 mg/kg dosed by injection increased basal vitellogenin concentration by a factor of 700 after 6 days & 48 h EC$_{50}$ 15 mg/L	11, 28
	Pimephales promelas	96 h LC$_{50}$ 4.7 mg/L	27
	Acartia tonsa	LOEC 300 µg/L based on egg production	9
	Potamopyrgus antipodarum	4 week EC$_{50}$ 5.67 µg/kg embryo reproduction via sediment exposure	35
	Mytilus hemocytes	24 h EC$_{50}$ 34.486 uM expressed as % lysosomal destabilisation	34
	Menidia menidia	96 h LC$_{50}$ 9.4 mg/L	27
	Mysidopsis bahia	96 h LC$_{50}$ 1.1 mg/L	27
	Skeletonema cotatum	96 h EC$_{50}$ 1.0 mg/L based on cell count	27

Table 2.7 Selected studies in which the effects of commonly used polymer additives was reviewed (continued)

Compound	**Organism**	**Findings**	**Reference**
	Oryzias latipes	LOEC 10 µg/L based on testis-ova	4
	Heteromyenia sp.	abnormal growth observed at 16 mg/L, at 80 mg/L complete germination failure	33
	Eunapius fragilis	abnormal growth observed at 16 mg/L	33
	Lemna minor	EC_{50} 20 mg/L based on frond density	23
	Vibrio fischeri	15 min EC_{50} 6.2 mg/L	28
	Pseudokirchneriella subcapitata	72 hr EC_{50} 2.2 mg/L	28
	Thamnocephalus platyurus	24 h LC_{50} 19.9 mg/L	28
	Selenastrum capricornutum	96 h EC_{50} 2.7 mg/L based on cell count (3.1 mg/L for cell volume)	27
	Xenopus laevis African frog)	No effect found at concentrations tested	9
	Tigriopus japonicus	48 h EC_{50} 4.32 mg/L based on adult motility at 25 degrees	32
	Brachionus calyciflorus	48 h LOEC 3.6 mg/L based on intrinsic rate of increase in offspring	23
	Brachydanio rerio	48 h EC_{50} 8.91 mg/L based on immobilization	32
	Hydra vulgaris	72 h LOEC 0.460 mg/L based on sub-lethal toxicity to polyps & 96 h EC_{50} 6.9 mg/L based on polyp survival	32
	Cnidarian test (Hydra attenuata assay)	96 h EC_{50} 19.9 mg/L	28
Flame retardants			
TBBPA	*Vibrio fischeri*	15 min EC_{50} 56.9 mg/L	28
TBBPA	*Pseudokirchneriella subcapitata*	72 h EC_{50} >250 mg/L	28
TBBPA	*Thamnocephalus platyurus*	24 h LC_{50} 8.3 mg/L	28
TBBPA	Cnidarian test (Hydra attenuata assay)	96 h EC_{50} 0.2 mg/L	28

Table 2.7 Selected studies in which the effects of commonly used polymer additives was reviewed (continued)

Compound	**Organism**	**Findings**	**Reference**
TBBPA	*Rainbow trout*	48 h EC_{50} 13.9 mg/L	28
TBBPA	*Daphnia magna*	48 h EC_{50} 0.69 mg/L based on immobilization	30
TBBPA	*Oncorhynchus mykiss*	no significant vitellogenin response	11

DEHP - Di(2-ethylhexyl)phthalate; DEP - Di ethyl phthalate; DMP - Di methyl phthalate; DBP - Di-n-butyl phthalate; DIDP - Di-isodecyl phthalate; DINP - Di-isononyl phthalate; BBP - Butyl benzyl phthalate; DOP - Dioctyl phthalate; DNP - Di-nonyl phthalate; MEHP - Mono(2-ethylhexyl) phthalate; BPA – Bisphenol-A; TBBPA - Tetrabromobisphenol A. References: 1 - Park and Choi, (2007); 2 - Urern-Webster *et al*., (2010); 3 - Norman *et al*., (2007); 4 - Metcalfe *et al*., (2001); 5 - Thibaut and Porte, (2004); 6 - Orbea *et al*., (2002); 7 - Call *et al*., (2001); 8 - Jonsson and Baun, (2003); 9 - Oehlmann *et al*., (2009); 10 - Barse *et al*., (2007); 11 - Christiansen *et al*., (2000); 12 - Harries *et al*., (2000); 13 - Patyna *et al*., (2006); 14 - Lee *et al*., (2005); 15 - Ohtani *et al*., (2000); 16 - Jensen *et al*., (2001); 17 - Sandy *et al*., (2010); 18 - Roh *et al*., (2007); 19 - Dorostghoal *et al*., (2010); 20 - Zhou *et al*., (2010); 21 - Kasahara *et al*., (2002); 22 - Bonilla and Mazo, (2010); 23 - Mihaich *et al*., (2009); 24 - Watts *et al*., (2003); 25 – Caspers, (1998); 26 - Mu *et al*., (2005); 27 - Alexander *et al*., (1988); 28 - Debenest *et al*., (2010); 29 - Forbes *et al*., (2008); 30 - Liu *et al*., (2007); 31 - Pascoe *et al*., (2002); 32 - Marcial *et al*., (2003); 33 - Hill et al., (2002); 34 - Canesi *et al*., (2007); 35 - Duft *et al*., (2003); 36 – Wibe *et al*., (2004)

2.5 Recommendations for Future Research

Considerable information is now available on the environmental effects of PBMs. As described previously in this review, there are several emerging areas of interest that need future research attention. These include research to:

1. Better understand the sources and sinks for microscopic polymer particles (as highlighted by Browne *et al*. 2011); this research should address both terrestrial and freshwater sinks.
2. Establish appropriate degradation test strategies consistent with realistic environmental conditions, because the complexity of environmental systems is lost when only one process (e.g., hydrolysis) is assessed in isolation.
3. Establish appropriate analytical methods to characterize the formation and ecotoxicity of both the physical and chemical constituents formed during PBM degradation.
4. Evaluate the uptake and the long-term effects of very small polymer particles in both aquatic and terrestrial compartments.
5. Evaluate the extent to which different polymer characteristics (i.e., the molecular bonds present in different materials) influence sorption behavior of anthropogenic compounds, and how these characteristics influence ecotoxicity.

2.6 Summary

There now a great number of PBMs on the market, because of the increasing demand for cheaper consumable goods, and light weight industrial materials. Each PBM constitutes a mixture of their representative polymer/s and their various chemical additives. The major polymer types are polyethylene, polypropylene and polyvinyl chloride, with natural rubber and biodegradable polymers becoming increasingly more important. The most important additives are those that are biologically active, because to be effective such chemicals often have properties that make them resistant to photo- and bio-degradation. During their lifecycle PBMs can be released into the environment form a variety of sources. The principal introduction routes being general littering, dumping of unwanted waste materials, migration from landfills and emission during refuse collection. Once in the environment, PBMs are primarily broken down by a photodegradation processes, but due to the complex chemical makeup of PBMs, receiving environments are potentially exposed to a mixture of macro, meso and micro size polymer fragments, leached additives and subsequent degradation products. In environments where sunlight is absent (i.e., soils and the deep sea) degradation for most PBMs is minimal. The majority of literature to date that has addressed the environmental contamination or disposition of PBMs has focused on the marine environment. This is because the oceans are identified as the major sink for macro PBMs, where they are known to present a hazard to wildlife via entanglement and ingestion. The published literature establishes the occurrence of microplastics in marine environment and beach sediments, but is inadequate as regards contamination of soils and freshwater sediments. The uptake of microplastics for a limited range of aquatic organisms has also been established, but there is a lack of information regarding soil organisms, and the long-term effects of microplastic uptake are also less well understood. There is currently a need to establish appropriate degradation test strategies consistent with realistic environmental conditions, because the complexity of environmental systems is lost when only one process (e.g., hydrolysis) is assessed in isolation. Enhanced methodologies are also needed to evaluate the impact of PBMs to soil and freshwater environments.

Chapter 3

Effects of Environmental Conditions on Latex Degradation in Aquatic Systems

3.1 Introduction

As described in Chapter 2, a number of studies have now documented PBMs as a major component of ocean and shoreline debris (Barnes *et al*., 2010; Barnes and Milner, 2005; Oigman-Pszczol and Creed, 2007; Santos *et al*., 2009), and as a component of debris in freshwater environments (Zbyszewski and Corcaran, 2011). The environmental degradation of PBMs could involve disintegration of the PBM into increasingly smaller polymer fragments, including microscopic and nano sized particles; chemical transformation of the PBM and polymer fragments; degradation of the PBM and polymer fragments into non-polymer organic molecules; transformation/degradation of these non-polymer molecules into other compounds; and ultimate mineralisation to carbon dioxide and water. Due to the many degradation processes that occur, environmental systems receiving PBMs will potentially be exposed to a complex mixture of the parent material, polymer fragments of different sizes and polymer degradation and transformation products. Each of these could be taken up by and affect aquatic and terrestrial organisms. For example, there is increasing concern over the impacts of nanoparticles (NPs) on organisms as particle size is recognised as an important property in determining their interaction with living systems. Desai *et al*., (1997) showed that 100 nm sized particles of a polylactic polyglycolic acid co-polymer had a 10-fold higher intracellular uptake in an *in-vitro* cell culture when compared to 10 µm sized particles made of the same material. NPs have also been shown to produce cytotoxic, genotoxic, inflammatory and oxidative stress responses in mammalian and fish systems (Dhawan *et al*., 2011).

The majority of our current understanding on the processes influencing polymer degradation has been derived from artificial laboratory studies that investigate a single mechanism of degradation such as photodegradation (Nagai *et al*., 2005a, Nagai *et al*., 2005b), thermal degradation (Agostini *et al*., 2008; Cit *et al*., 2010), and biodegradation using microbial cultures (Cherian and Jayachandran, 2009; Cosgrove *et al*., 2007; Linos *et al*., 2000; Saad *et al*., 2010; Tsuchii *et al*., 1997). There is limited information on the degradation of polymers under environmentally relevant conditions, where a number of degradation mechanisms occur at once, and where information is available, the focus has been on understanding degradation in marine systems (O'Brine and Thompson, 2010; Sudhakar *et al*., 2007; Rutkowska *et al*., 2002a; Rutkowska *et al*., 2002b). These studies have tended to focus on weight loss, changes in tensile strength, breakdown of the molecular structure and identification of specific microbial strains to utilise specific polymer types. The potential for PBMs to form other chemical compounds and nano-sized polymer particles has received little attention.

The aim of this chapter was therefore to characterise the degradation of a case study polymeric NRL film under realistic conditions. To do this, outdoor microcosms were used so that the formation and subsequent degradation of the polymer transformation products could be monitored over time under natural cycles of sunlight and temperature. Experiments were initiated at different times of the year to cover different seasons. The specific objectives were to: i) explore the effects of season on the degradation rate of the NRL film, ii) explore differences in degradation rates in freshwater and marine water, iii) investigate the importance of temperature and light for latex degradation, iv) characterise molecular changes to the NRL film during the degradation process, and v) characterise to what extent particles in the nano-meter size range are formed following degradation of a PBM.

3.2 Methods

3.2.1 General study design

The degradation studies were undertaken using a NRL film which is used in the manufacture of NRL condoms (0.08 mm thickness, provided by a leading UK manufacturer). For all studies, NRL samples (approximately 25 cm^2) were placed individually into clear glass vessels (volume 250 ml) and spread out in 200 ml of test media. For each time point individual samples were established in triplicate and control samples without NRL samples, were also established. To expose the NRL to natural cycles of sunlight and temperature, test vessels were then placed outdoors on a platform, under non-sterile conditions, and covered with a non ultra-violet filtering perspex sheet (B&Q, UK) to prevent flooding by rainfall (Fig 3.1). Evaporation was dealt with by regularly replacing lost water with the respective media, except for the marine water experiment where demineralised water was used to prevent the build-up of salts. Weather conditions for the entire study were recorded using a weather station located next to the experimental site (Delta-T Devices Ltd, UK).

Figure 3.1 Outdoor microcosm study set-up.

3.2.1.1 Semi-field degradation over different seasons

To understand the effects of season on the degradation of NRL, two experiments were initiated at different times of the year: one in August 2010 and one in November 2010. The degradation medium used was demineralised water. For the August study, samples were removed for analysis after 10, 20, 35, 50, 90, 120 and 250 days of exposure and for the November study, samples were removed after 30, 60, 90, 120, 150 and 200 days.

A water/sediment system was also investigated during this time period. The sediment used in this experiment was collected from a local stream, but the results were inconclusive as the sample become overgrown with algae.

3.2.1.2 Importance of selected environmental variables and thickness on latex degradation

To assess the effects of different environmental variables on NRL degradation, a series of studies were undertaken, starting in June 2011, using an artificial freshwater media (pH 7.9; containing $CaCl_2$ 294 mg/L; $MgSO_4$ 123.25 mg/L; $NaHCO_3$ 64.75 mg/L and KCl 5.75 mg/L). The variables investigated were; an additional pH value (pH 5.5); presence/absence of sunlight; and water movement. Studies were also performed using artificial marine water (Red Sea Salt mix, Red Sea Aquatics Ltd, UK, batch number 26 04 0915; pH 8.4), and a thicker latex film (1.5 mm). Media pH was adjusted using either NaOH or HCl accordingly. The exclusion of light was achieved by covering the test vessels with foil and the effect of water movement was simulated by shaking the relevant vessels once a week for 16 h at 80 rpm. For all of these manipulations a more rigorous sampling regime was applied with samples being removed and taken for analysis after 1, 3, 7, 14, 28, 56, 112 and 200 days exposure. Throughout the study pH was recorded (Appendix, Table A.1).

3.2.2 Analytical methods

3.2.2.1 Weight loss

Upon collection, samples were filtered under vacuum using pre-dried and weighed, 1.6 µm pore diameter, glass fibre filter papers (Whatman, UK). The filter papers were then dried at 40 ^{o}C to a constant weight and the weight recorded. A sample (20 ml) of the filtered test media was taken at this stage and stored at 5°C for characterization in terms of NP concentration and size distribution and dissolved organic carbon (DOC) concentration. For material from the marine media, it was necessary to wash the salts off the filtered sample. To do this, samples were immersed in demineralised water for 24 hrs and filtered. This process was repeated until a constant weight was measured. After weighing, latex samples were stored at 5^{o}C until the chemical functionality of the latex sample could be assessed.

3.2.2.2 Changes in chemical functionality

Changes in chemical functionality of the NRL samples were characterized using Fourier Transform Infra-Red spectroscopy (FTIR). The infra-red absorption spectra of the filtered NRL samples were measured using an Attenuated Total Reflection (ATR)-FTIR. ATR makes use of an evanescent wave to collect the absorption spectrum of a studied sample as radiation is passed through a crystal at an angle in which total reflection occurs on the top surface where the sample is located. The FTIR spectra were recorded using a Bruker spectrometer model Vertex 70 (Bruker, Germany) in the 400 – 4000 cm^{-1} wave number range. All spectra were the average of 16 scans recorded at a resolution of 4 cm^{-1} and peak height was used to represent the IR intensity, which is expressed as absorbance.

3.2.2.3 Particle analysis

Nanoparticle tracking analysis (NTA) was used to determine the number and size distribution of particles in the size range 30 nm to 2000 nm. Analysis was performed using a NanoSight LM 10 (NanoSight Ltd, UK). To characterise each individual sample and control in a representative manner, nine video images of each sample were taken. The focus of the camera was judged by eye and was adjusted so the majority of particles on the screen were in focus at the start of video capturing. Video image length was set at 60 s and all images were performed at room temperature. The processing of video images was performed using NTA 2.2 software. The detection threshold was set to automatic; this determines the minimum grey scale value of any particle in the image necessary for it to qualify as a particle to be tracked. A blur (smoothing setting) of 5 x 5 was then used following the recommendation in the operating manual that if automatic threshold detection is used, the blur setting should be increased by one level higher than normally used. The minimum expected particle size was set at 30 nm for all samples due to the unknown nature of the samples being analysed. The minimum track length, which defines the minimum number of steps a particle must take before its size is calculated and included in the analysis, was set to automatic allowing the software to calculate this based on the number of particles in the video. To verify the filtering process was not affecting the distribution profiles, a mixture of mono-dispersed 500 nm and 1000 nm (12:1 ratio) polystyrene beads were characterised, then filtered through a 1.6 µm filter paper and then characterised again (Fig. 3.2). This shows the presence of two distinct size populations indicating NanoSight is indeed suitable for poly-dispersed samples and the filtering process has not interfered with the sample.

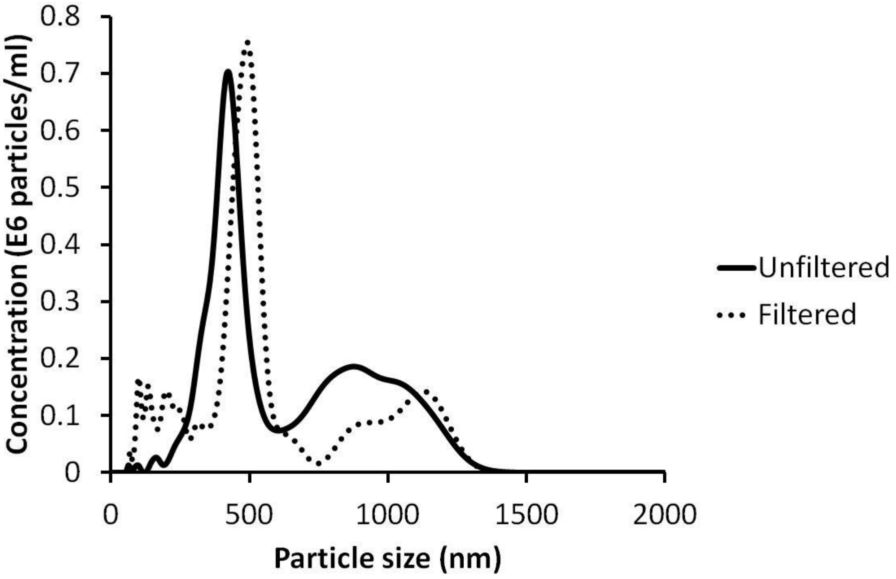


Figure 3.2 Distributions of a poly-dispersed mixture of 500 and 1000 nm particles before and after filtering through a 1.6 µm glass filter paper. The distributions are the average of three replicate measures.

3.2.2.4 Dissolved organic carbon analysis

The degradation media was analysed with a LiquiTOC combustion analyzer (Elementar, Germany). Subsamples of the degradation media were diluted 10-fold with demineralised water to bring them within the calibration range. The diluted sample was then filtered using a 0.45 µm glass fibre filter paper (Whatman, UK) to separate the dissolved fraction from the solid fraction. A range of potassium hydrogen phthalate and sodium carbonate standards (1–50 mg/L) was used for making a standard curve from which DOC was calculated. Results for the NRL treatments were corrected using the corresponding control DOC values.

3.2.3 Data analysis (The modelling approach used in this study is outlined below. For and alternative modelling approach please see Appendix 2)

3.2.3.1 Degradation rate and modeling approach

Degradation rates were calculated using a series of models that describe bi-phasic degradation (FOCUS, 2006). The results for the August and November experiments were best described by a hockey-stick model. This assumes degradation initially occurs according to first-order kinetics, which is described by a constant fractional rate of degradation (k_1) (Equation 3.1). Then at a certain point in time, known as the breakpoint, the rate constant changes to a different value (k_2) that declines with time (Equation 3.2).

$$M = M_0\, e^{-k_1 t} \text{ for } t \le t_b \qquad \text{Equation 3.1}$$

$$M = M_0\, e^{-k_1 t_b}\, e^{-k_2(t-t_b)} \text{ for } t > t_b \qquad \text{Equation 3.2}$$

Where: M is the % material recovery at time t (days); M_0 is the % material recovery at the start of the study; k_1 is the rate constant for $t \le t_b$, k_2 is the rate constant for $t > t_b$, and t_b is the time (days) at which the constant changes.

The results for the second set of experiments appeared to be multi-phasic in nature as an increase in weight was seen at the beginning of the exposure period. To include this phase in the modeling process an exponential growth equation was fitted to this part of the measured data (Equation 3.3). Then at the point in time where weight loss starts to occur a breakpoint was applied and a revised version of a bi-exponential model (described as the sum of two first order equations (FOCUS, 2006) was used to describe the remaining measured data (Equation 3.4).

$$M = M_0\, e^{k_1 t} \text{ for } t \le t_b \qquad \text{Equation 3.3}$$

$$M = M_1\, e^{-k_2(t-t_b)} + M_2\, e^{-k_3(t-t_b)} \text{ for } t > t_b \qquad \text{Equation 3.4}$$

Where M_1 is the amount of material (%) applied to compartment 1 at time t = *breakpoint*, M_2 is the amount of material (%) applied to compartment 2 at time t = *breakpoint*, k_2 is the rate constant in compartment 1, and k_3 is the rate constant in compartment 2. $M_1 + M_2$ must be equal to the weight of material at which the breakpoint is applied.

Model parameters were fitted by applying a trial and error approach (Appendix, Table A.2). Estimation of the time taken for 50 % (DT_{50}) of the latex to degrade for the August and November treatments was derived based on equations 3.5 and 3.6. Model fits for the remaining treatments were derived from a modified version of a bi-exponential model. As there is no analytical equation to calculate degradation endpoints for this model, DT_{50} values were derived from a table of calculated concentrations. Measurements used to assess goodness of fit for the optimised parameters were carried out using, sum of square residuals (SSRes), root mean square error (RMSE), chi-square test, model error and coefficient of determination (r^2 value) (table 1); descriptions for these indices can be found in FOCUS, (2006) guidance document.

$$DT_x = \frac{1}{k_1} \ln\left(\frac{M_0}{M}\right) \text{ if } DT_x \leq t_b$$

Equation 3.5

$$DT_x = t_b + \frac{1}{k_2} \ln\left(\frac{M_0}{M}\right) - \left(\frac{k_1}{k_2}\right) \text{ if } DT_x > t_b$$

Equation 3.6

3.2.3.2 Relating degradation of the parent material to weather parameters

The weather data was used to estimate the amount of solar radiation (MJ m^{-2}) was responsible per unit of degradation. This was achieved by calculating the difference in weight loss between consecutive time points, and dividing through by the amount of solar radiation received between sampling intervals; from this an overall average was determined. Sampling intervals with measured weight gain were excluded with calculations adjusted appropriately.

3.2.3.3 Calculation of particle size distribution & particle mass

To determine the particle size distribution in the samples that had contained NRL, data on the particle size distribution and number concentration of the equivalent control treatment was subtracted from the treated sample data. This corrected for the presence of any naturally occurring particles and particles that may have entered into the sample during the filtering process.

The mass of particles present was estimated using the distribution data by calculating the volume of particles present in the sample (Equation 3.7), and then multiplying by the density of the solid which was taken to be 920 mg/cm^3 for polyisoprene (Equation 3.8). It should be noted that for this calculation it was assumed all particles were solid spheres following Gillespie *et al*., (2011).

$$\text{Volume} = \frac{\pi}{6}d^3 \text{ (cm) x concentration (no. particles / ml)} \qquad \text{Equation 3.7}$$

$$\text{Mass (mg/ml)} = \text{Volume x } \rho \qquad \text{Equation 3.8}$$

3.2.3.3 Mass balance

To assess losses to the atmosphere (e.g. through mineralisation of the polymer to CO_2 and H_2O or volatilisation of organic transformation products or additives), a mass balance analysis was performed using the weight of material collected on the 1.6 µm filter paper, the DOC concentrations, (corrected for controls), and the mass of particles in the filtered media above 450 nm (particles below 450 nm were excluded as these will be included in the DOC measurements). The combined weight was then compared to the material starting weight.

3.2.3.4 Statistical analysis

The generated datasets were identified as either non-normally distributed and of unequal variance using the Shapiro-Wilk test. Therefore, overall differences between treatments were evaluated using a Friedman test, which reports the test result as a chi square value. All statistical tests were followed by a *post-hoc* Tukey multiple comparisons test applied to the different combinations. Stepwise regression was used to identify which of mean temperature (^{o}C) or mean intensity of solar radiation (MJ m^{-2}) each sampling interval received during its exposure, best describes degradation rate for each treatment investigated. All statistical tests were performed using SigmaPlot version 12 and a significance level of 0.05.

3.3 Results and discussion

3.3.1 Seasonal effects on degradation rate

The effect of season on degradation rate was pronounced. When exposure was initiated in August, NRL samples started to disintegrate within 10 d, compared to the study initiated in November where degradation was negligible until the onset on spring (Fig. 3.2). The breakpoint (t_b) at which the second rate constant is used to describe the degradation kinetics, for the August treatment, was modelled as 44 d. Over this 44 d period samples were exposed to an average daily temperature of 13.9 ^{o}C and a total of 421.01 MJ m^{-2} of solar radiation was received; at the end of this treatment material recovery was 19.65 % (± 1.62). The breakpoint for the November treatment was modelled as 105 d, over this time period the latex samples were exposed to a lower average daily temperature (2.18 ^{o}C) and received far less solar radiation (184.79 MJ m^{-2}) than the August treatment. Material recovery for this treatment after 200 d was 17.12 % (± 6.25).

The total solar radiation receive at the study site, over the duration of each treatment was 1188 MJ m^{-2} for the August treatment and 1172 MJ m^{-2} for the November treatment. Solar radiation was identified as the weather variable that best described the seasonal effect on weight loss, with similar solar intensities responsible for one percent weight loss (Table 3.1). Previous studies have also demonstrated the enhanced field degradation of PE films when exposed to increased levels of sunlight intensity and higher temperatures in summer seasons (Al-Salem, 2009).

3.3.2 Effects of environmental conditions and polymer thickness on degradation of latex

When the effects of a range of environmental conditions on degradation were assessed over 200 d (Fig. 3.3) greatest degradation was seen in the lower pH treatment (DT_{50} = 75 d), this was followed by the marine water treatment (DT_{50} = 87 d) and the higher pH freshwater treatment (DT_{50} = 158 d; fig. 3.2) (Table 3.1). Samples subjected to water movement gave a much longer DT_{50} (220 d). The use of movement appeared to cause any fragmented particles to congeal, reducing the impacts of weathering. Limited degradation was seen in the samples where sunlight was excluded and in the thicker NRL samples, with material recovery after 200 d measured at 97.73 % (± 0.38) and 88.57 % (± 4.39), respectively; therefore it was not possible to model the degradation kinetics for these treatments. Overall, differences in degradation rate between these six treatments were identified (χ^2 = 12.762, p = 0.026); however, *post-hoc* analysis indicates only the thicker material and exclusion of light treatments to be significantly different from the other treatments.

The total solar radiation and average daily temperature received at the study site over the duration of this experimental period was 1651 MJ m^{-2} and 12.22 ^{o}C respectively. Solar radiation was again identified as the weather variable that best described weight loss under each degradation scenario (Table 3.1). The corresponding sunlight intensities responsible for one percent degradation ranged from 30.42 MJ m^{-2} for the lower pH treatment to 144.33 MJ m^{-2} for the thicker NRL treatment (Table 3.1). Other studies have also demonstrated solar radiation as the driving variable for the rate of polymer degradation. Saad *et al*., (2010) investigated the effect of ultra violet (UV) radiation on the biodegradation rate of PHB films 0.1 – 0.12 mm thickness, and found samples exposed to 9 h UV radiation had ~ 52 % weight loss after 28 days soil burial compared to ~32 % weight loss for samples with no pre UV exposure. PE samples (0.08 mm thickness) immersed in the Baltic Sea for 20 months were also found to have no measureable weight changes because of the lack of sunlight in this environment (Rutkowska *et al*., 2002a).

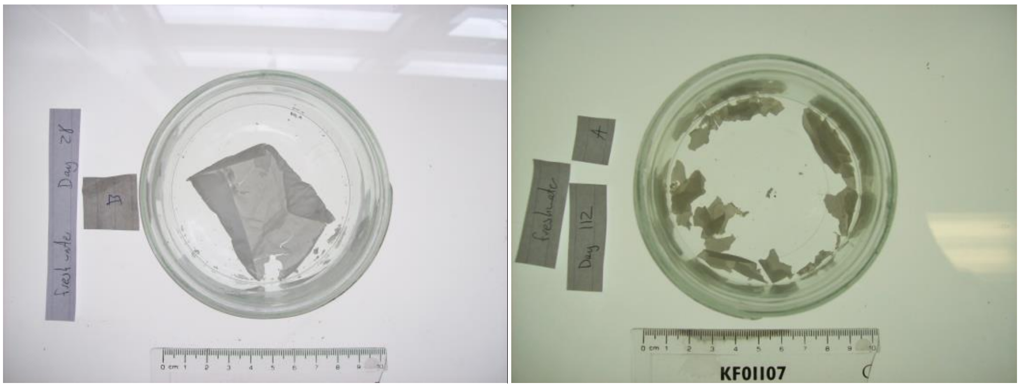


Figure 3.3 NRL samples at day 28 and day 112 in freshwater media

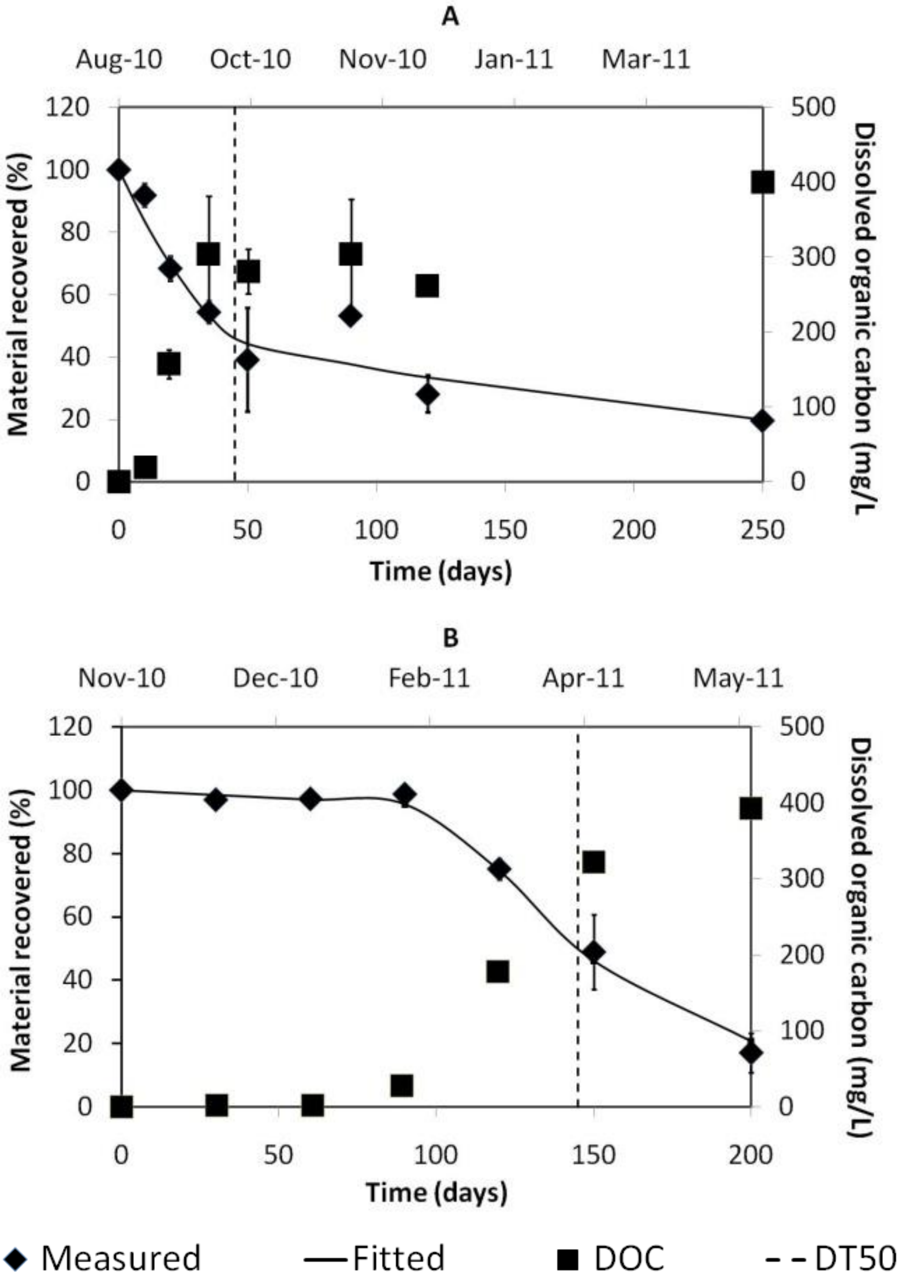


Figure 3.4 Degradation of NRL over time in, a) August 2010 treatment; b) November 2010 treatment. Diamond shaped data points represent mean material recovery; the solid line dissecting these data points represents the best fit model; the vertical dashed line represents the point at which 50 % degradation is reached, and the squares represent dissolved organic carbon. Error bars displayed were obtained by the standard deviation of the different measurements for each sample; where not visible, bars fall within the symbols.

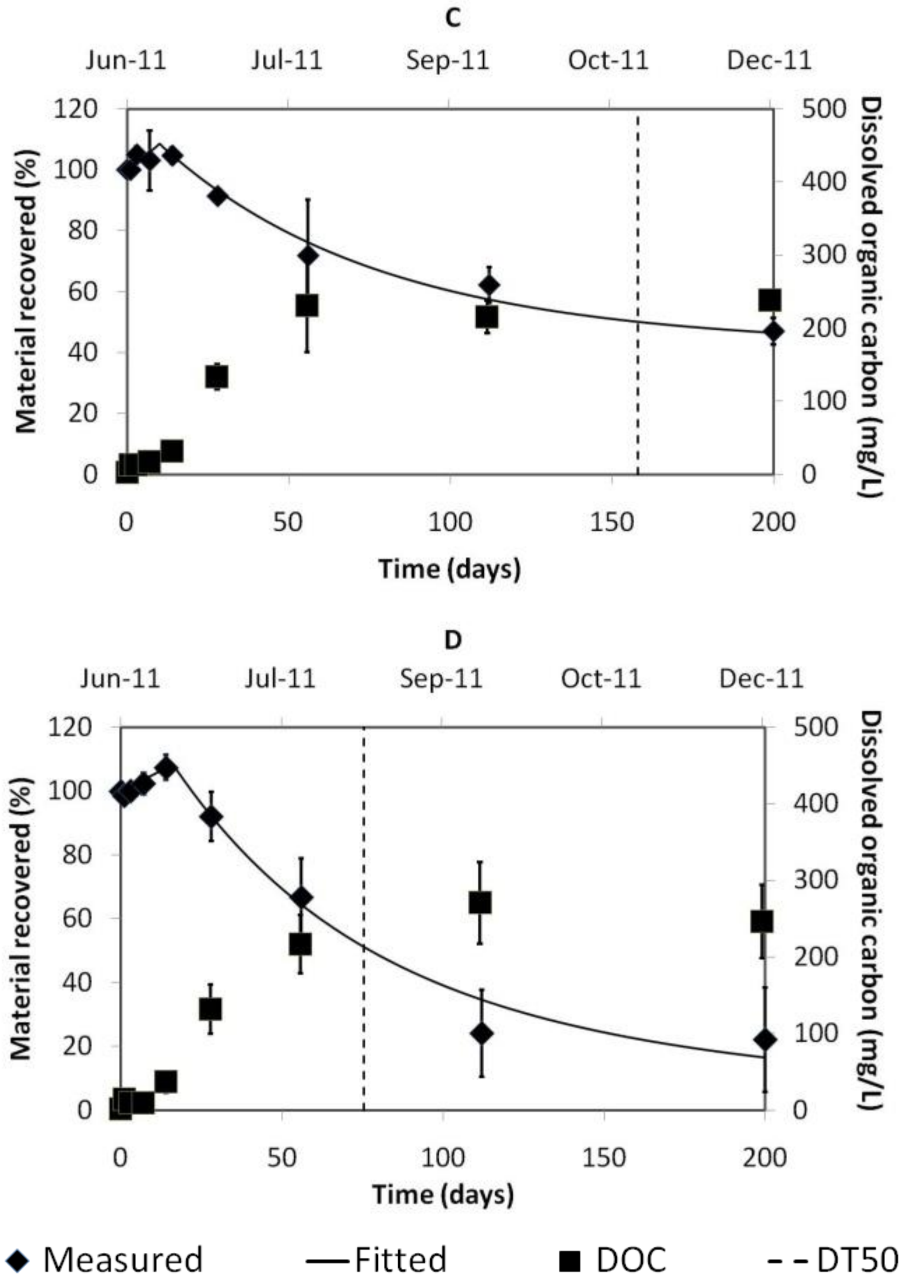


Figure 3.4 Degradation of NRL over time (continued) in c) artificial freshwater (pH 7.9); d) artificial freshwater (pH 5.5). Diamond shaped data points represent mean material recovery; the solid line dissecting these data points represents the best fit model; the vertical dashed line represents the point at which 50 % degradation is reached, and the squares represent dissolved organic carbon. Error bars displayed were obtained by the standard deviation of the different measurements for each sample; where not visible, bars fall within the symbols.

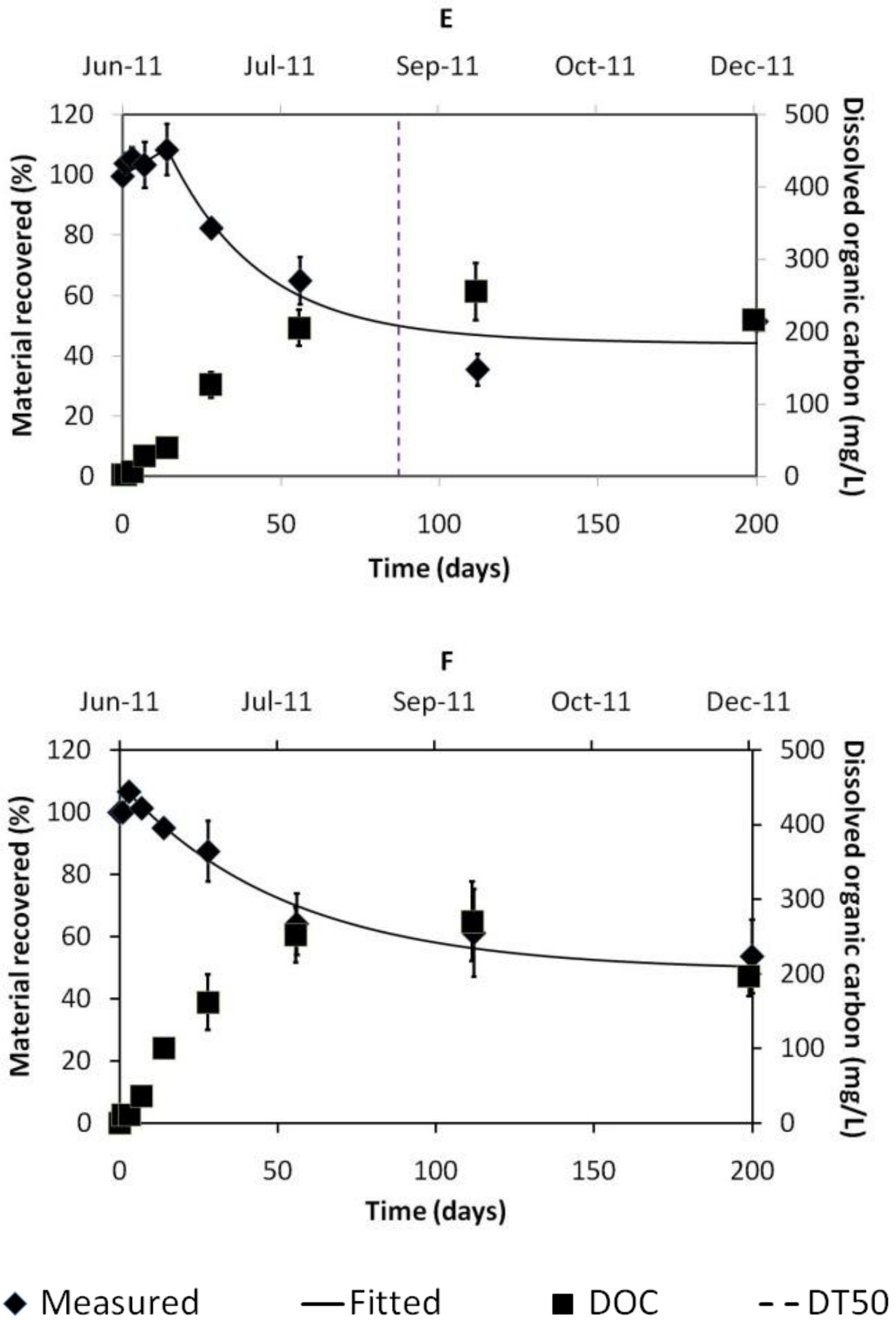


Figure 3.4 Degradation of NRL over time (continued) in e) artificial marine water; f) simulated water movement. Diamond shaped data points represent mean material recovery; the solid line dissecting these data points represents the best fit model; the vertical dashed line represents the point at which 50 % degradation is reached, and the squares represent dissolved organic carbon. Error bars displayed were obtained by the standard deviation of the different measurements for each sample; where not visible, bars fall within the symbols.

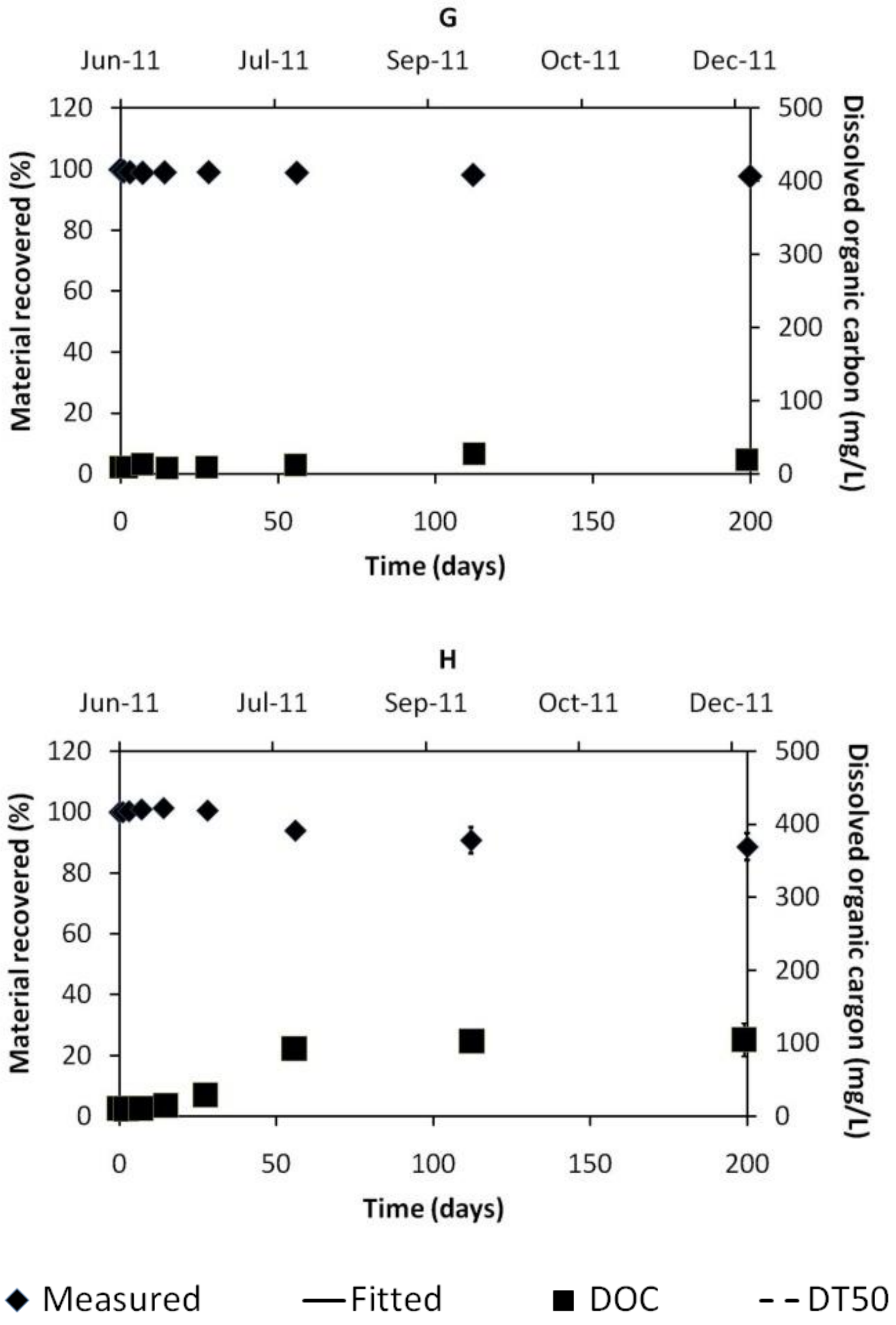


Figure 3.4 Degradation of NRL over time (continued) in g) absence of light; and h) the thicker latex sample. Diamond shaped data points represent mean material recovery; the solid line dissecting these data points represents the best fit model; the vertical dashed line represents the point at which 50 % degradation is reached, and the squares represent dissolved organic carbon. Error bars displayed were obtained by the standard deviation of the different measurements for each sample; where not visible, bars fall within the symbols.

Table 3.1 Statistical indices derived for modelling of weight loss and values derived for explaining sunlight intensity as the driving factor.

	Summer 2010	Winter 2010	Freshwater	Lower pH	With movement	Marine water
Statistical indices						
DT_{50}	38.51	145.04	158	75.5	220	87
SSRes	368.43	32.79	59.3	158.39	80.59	240.17
RMSE	3.26	1.43	1.46	2.01	1.60	1.56
χ^2 (tabulated χ^2)	8.75 (9.49)	0.92 (7.81)	0.83 (5.99)	5.25 (5.99)	1.24 (5.99)	4.74 (5.99)
Model error	10.96	2.69	3.16	6.49	4.29	7.55
r^2	0.91	0.98	0.90	0.94	0.88	0.93
Sunlight intensity as a predictor for mass loss	$r^2 = 0.60$ ($p = 0.002$)		$r^2 = 0.87$ ($p = 0.001$)	$r^2 = 0.81$ ($p = 0.002$)	$r^2 = 0.74$ ($p = 0.006$)	$r^2 = 0.87$ ($p = 0.001$)
MJ m^{-2} responsible for 1 % weight loss*	13.65 (± 5.75)	14.05 (± 5.03)	36.64 (± 20.82)	30.42 (± 23.29)	36.22 (± 21.05)	29.85 (± 13.56)

DT_{50} estimated time for 50 % of the latex to degrade; *SSRes* Sum of square residuals; *RMSE* Root mean square error. *Thicker material = 144.33 (23.34)

3.3.3 Changes in chemical functionality

The FTIR spectra of unexposed NRL contained several peaks characteristic of vibration modes attributed to *cis*-1,4 polyisoprene (Agostini *et al*., 2008; Nor and Ebdon, 2000; Fig 3.4). Absorption bands were observed at 2960 cm^{-1} which arise from C-H asymmetric stretching in CH_3 groups and a strong absorption band was also seen at 1450 cm^{-1} characteristic of angular deformation of $–CH_2$- groups (Nor and Ebdon, 2000). Exposure to natural sunlight and temperatures resulted in the reduced vibrations at these absorbance bands ($\chi^2 = 27.571$, $p < 0.001$ and $\chi^2 = 22.143$, $p < 0.001$ respectively), but only treatments where sunlight was excluded and a thick material used were significantly different from other treatments.

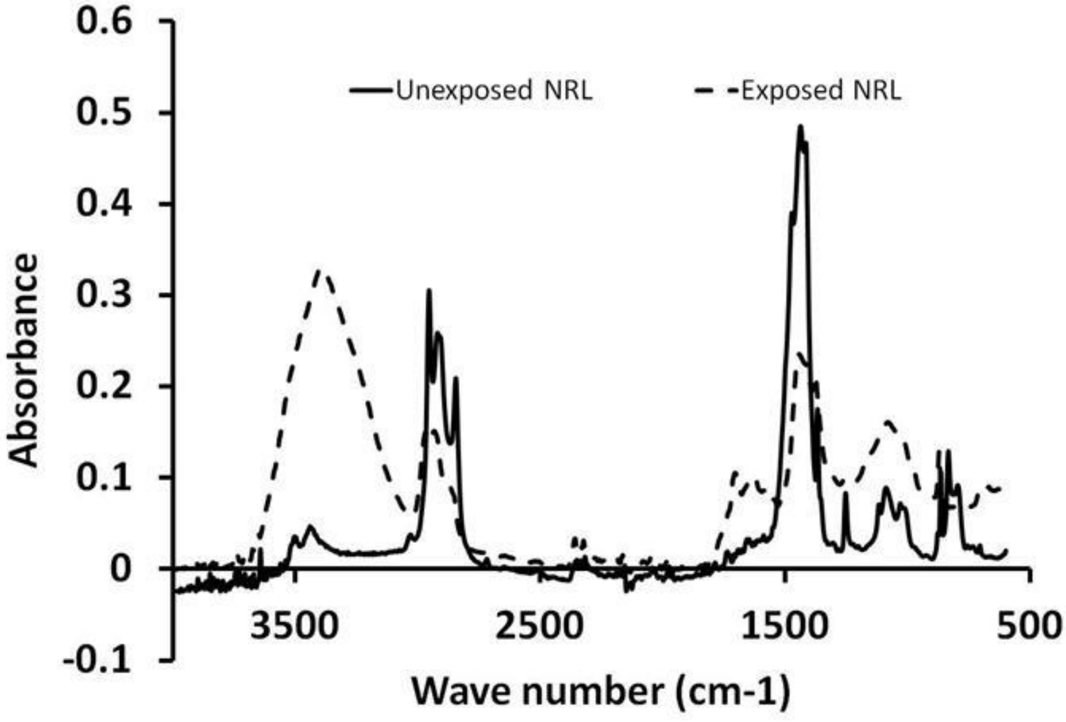


Figure 3.5 FTIR-ATR spectra of unexposed NRL and an NRL sample after exposure to natural cycles of sunlight and temperature.

The observed weakening of characteristic absorbance bands was accompanied by the formation of a broad OH peak in the region 3000 - 3600 cm^{-1} and a low intensity CO band at 1715 cm^{-1} (Fig. 3.3). OH and CO groups are oxy-generated products that attach themselves to polymer molecules and are formed by photo-oxidation processes (Albertsson *et al*., 1987). In the excluding light treatment these functional groups were only observed at very low absorbencies and were shown to be significantly different to other treatments (χ^2= 31.286, $p < 0.001$ and $\chi^2 = 26$, $p < 0.001$ respectively).

Under ambient conditions, photo-degradation is considered one of the primary sources of damage for polymeric materials (Klemchuk, 1990; Lucas *et al*., 2008). The lack of change observed in the FTIR spectra when light is excluded indicates photo-oxidation as the major mechanism involved in degradation process. A possible mechanism for the photodegradation of NRL is shown in Fig. 3.7. This initially involves the light-driven oxidative scission of the C=C bond in the *cis*-1,4 polyisoprene chain as described by Tsuchii *et al*., (1985) (Fig. 3.4, structure I) forming end chain CO groups. The formation of OH end groups (structure VII) is preceded by the UV induced change in the chemical structure of the latex molecules which occurs through free radical intermediates (structure III). These reactive intermediates are formed when the latex absorbs light and oxygen which easily undergo photolysis into intermediate hydroperoxide groups (structure IV) as described by Mailhot *et al*., (2000) and Kaczmarek *et al*., (2007) in photo-degradation experiments of PS and poly(ethylene oxide) respectively. Further CO groups may also be produced by photo-oxidation processes (structure VI) (Kaczmarek *et al*., 2007). The appearance of CO and OH groups is also noted by Agostini *et al*., (2008) and Riyajan *et al*., (2003) in thermal degradation and biodegradation studies of NRL, respectively.

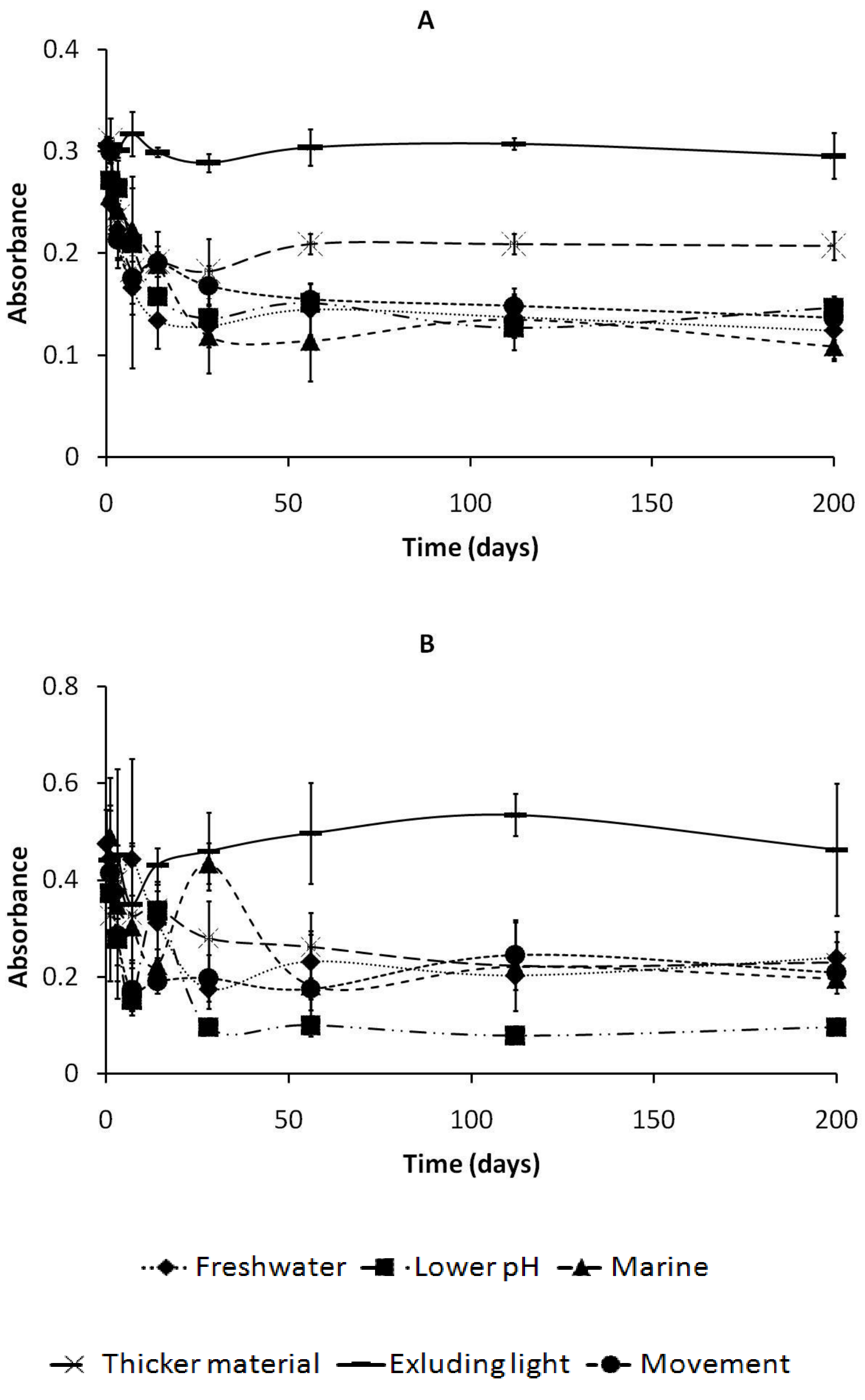


Figure 3.6 Changes in FTIR-ATR peak intensities during exposure to natural cycles of sunlight and temperature at, a) 2960 cm^{-1}; b) 1450 cm^{-1}. Diamonds = freshwater treatment; squares = lower pH treatment; triangles = marine water treatment; stars = thicker material; circles = simulation of movement; solid line = exclusion of light.

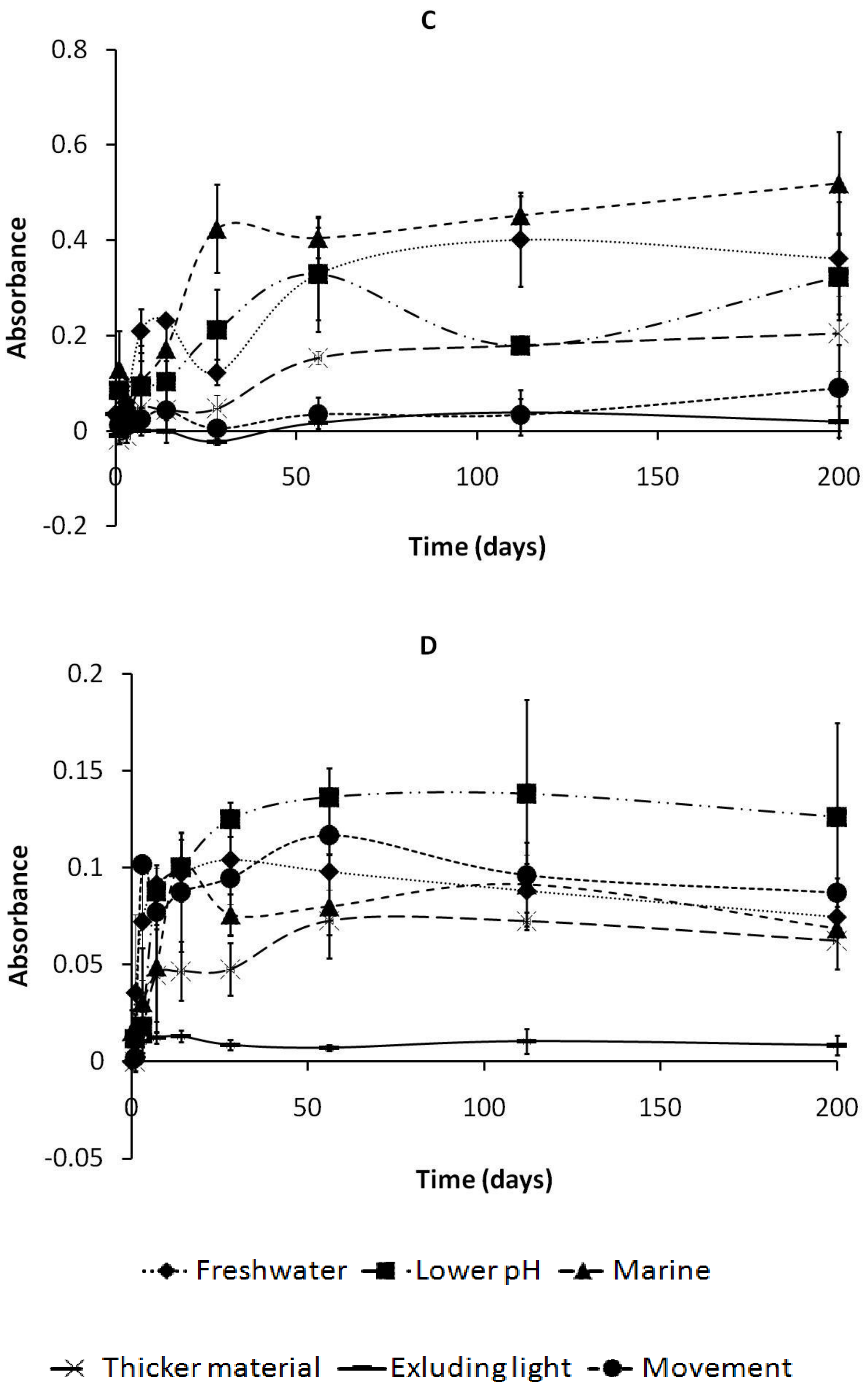


Figure 3.6 Changes in FTIR-ATR peak intensities during exposure to natural cycles of sunlight and temperature at, c) 3000 - 3600 cm^{-1} and d) 1715 cm^{-1}. Diamonds = freshwater treatment; squares = lower pH treatment; triangles = marine water treatment; stars = thicker material; circles = simulation of movement; solid line = exclusion of light (continued).

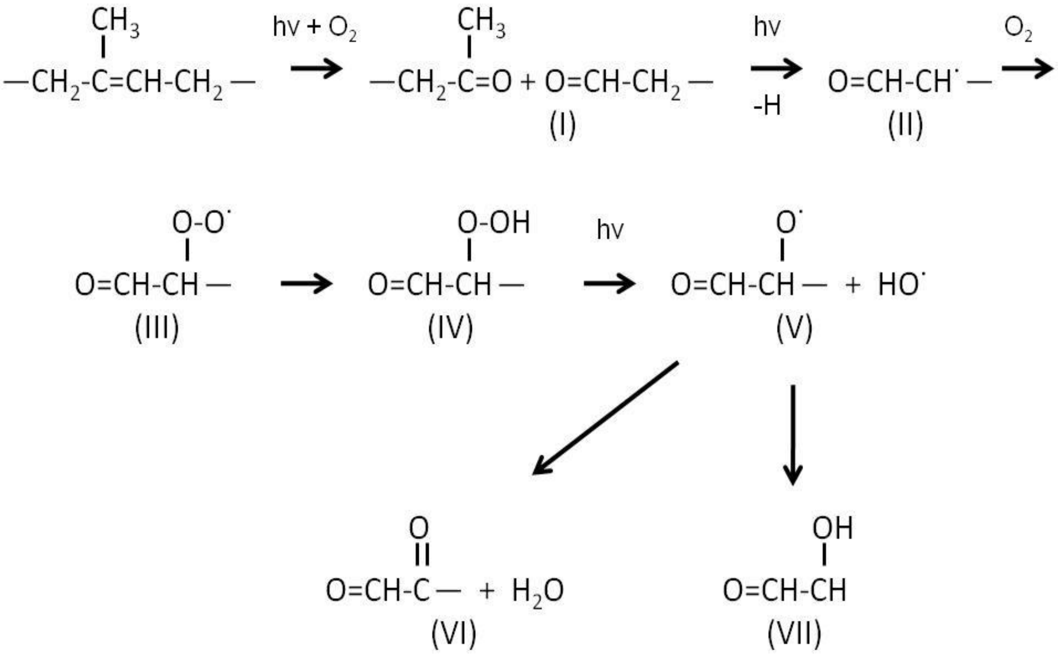


Figure 3.7 Proposed route for the breakdown of NRL in the aquatic environment

3.3.4 Formation of NPs

NTA was used to characterise both the concentration and size distribution of particles < 1.6 µm formed during the degradation of NRL (Fig. 3.5). Previous studies have shown this technique to be suitable for characterisation of samples with heterogeneous distributions of NPs, as it does not give bias towards larger particles (Filipe *et al.*, 2010; Montes-Burgos *et al.*, 2010). The results show particles < 1.6 µm were formed from the NRL over the course of the exposure period when compared to concentrations in the controls. After 200 d the concentration of particles in the freshwater treatment was 3.30 x 10^8 / ml (± 0.79), this compares to 0.2 x 10^8 / ml (± 0.13) in the control for the same time point (Fig. 3.5). Particles in the control samples most likely originate from atmospheric dust entering the sample during the exposure period.

The water movement and thicker material treatments generated the highest concentration of particles after 200 d (4.89 x 10^8 / ml ± 1.28 and 4.80 x 10^8 / ml ± 1.38, respectively), even though these two treatments displayed slower degradation rates than the other treatments. Overall differences in particle concentrations in the second set of experiments were identified (χ^2 = 256.359, $p < 0.001$), with the exclusion of light and marine water treatments found to have significantly lower concentrations of particles < 1.6 µm than the other treatments.

Mean particle diameter was the same across exposure treatments (Fig. 3.4; χ^2 = 9.929, $p > 0.05$). After 200 d mean particle diameter ranged from 196.52 nm (± 89.48) in the freshwater treatment to 207.33 nm (± 100.29) in the lower pH treatment. Overall, the size of particles formed were mainly in the 100 – 400 nm range, with few larger particles (> 400 nm) being measured. One possible explanation is that larger particles precipitate over time and only become re-suspended when they themselves further breakdown or they could potentially aggregate with time and therefore be caught by the filtering process.

The mass of NRL particles present in the test media was also estimated, based on the particle size distribution and number concentration, and was generally seen to increases in line with particle concentration. The largest mass of particles present after 200 d was 3.83 µg/ml (± 0.29) in the lower pH scenario, which accounts for 0.54 % (± 0.06) of the material starting weight; this compares to a mass of 2.13 µg/ml (± 0.48) after 200 d in the freshwater experiment, which accounts for 0.3 % (± 0.06) of the material starting weight.

3.3.5 DOC

An increase in the formation of DOC over time was observed for all treatments, with the exception of the exclusion of light treatment (Fig. 3.2). The formation of DOC was closely related to the latex weight loss results with the faster periods of latex degradation corresponding with the rapid increase in DOC levels. Slower phases of degradation were associated with a stabilisation in measured DOC levels and can be attributed to the reduced sunlight intensities experienced throughout the autumn and winter months. DOC formation in the treatment where light was excluded and in the thick latex treatment was significantly lower than in all other treatments (χ^2 = 66.907, $p < 0.001$).

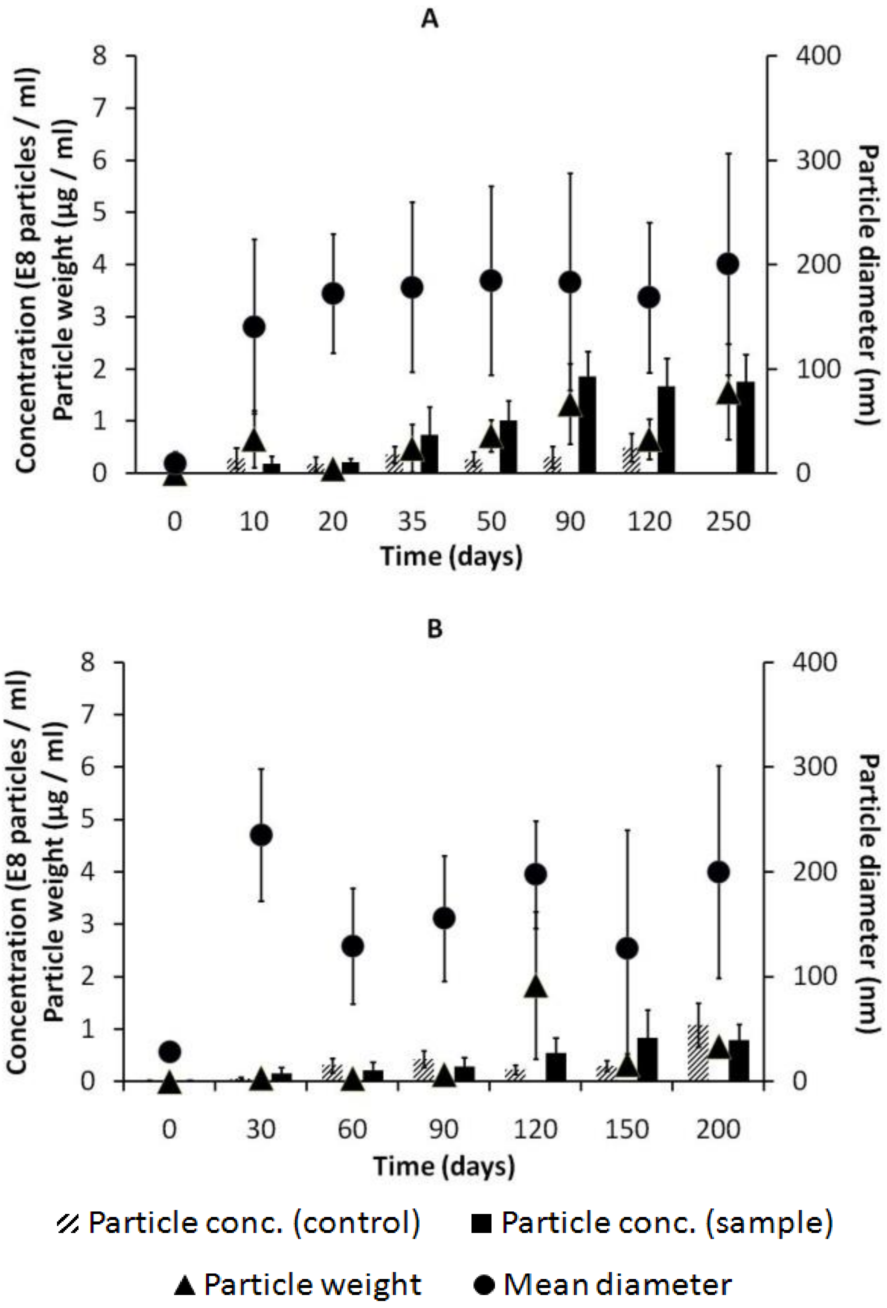


Figure 3.8 Characterisation of nano sized particles formed during the degradation of NRL in, a) August 2010 treatment; b) November 2010 treatment. Solid bars represent particle concentration in the sample filtrate; bars with diagonal lines represent particle concentration in the controls; circles represent mean particle concentration in the samples and triangles represent particle weight. Error bars displayed were obtained by the standard deviation of the different measurements for each sample; where not visible, bars fall within the symbols.

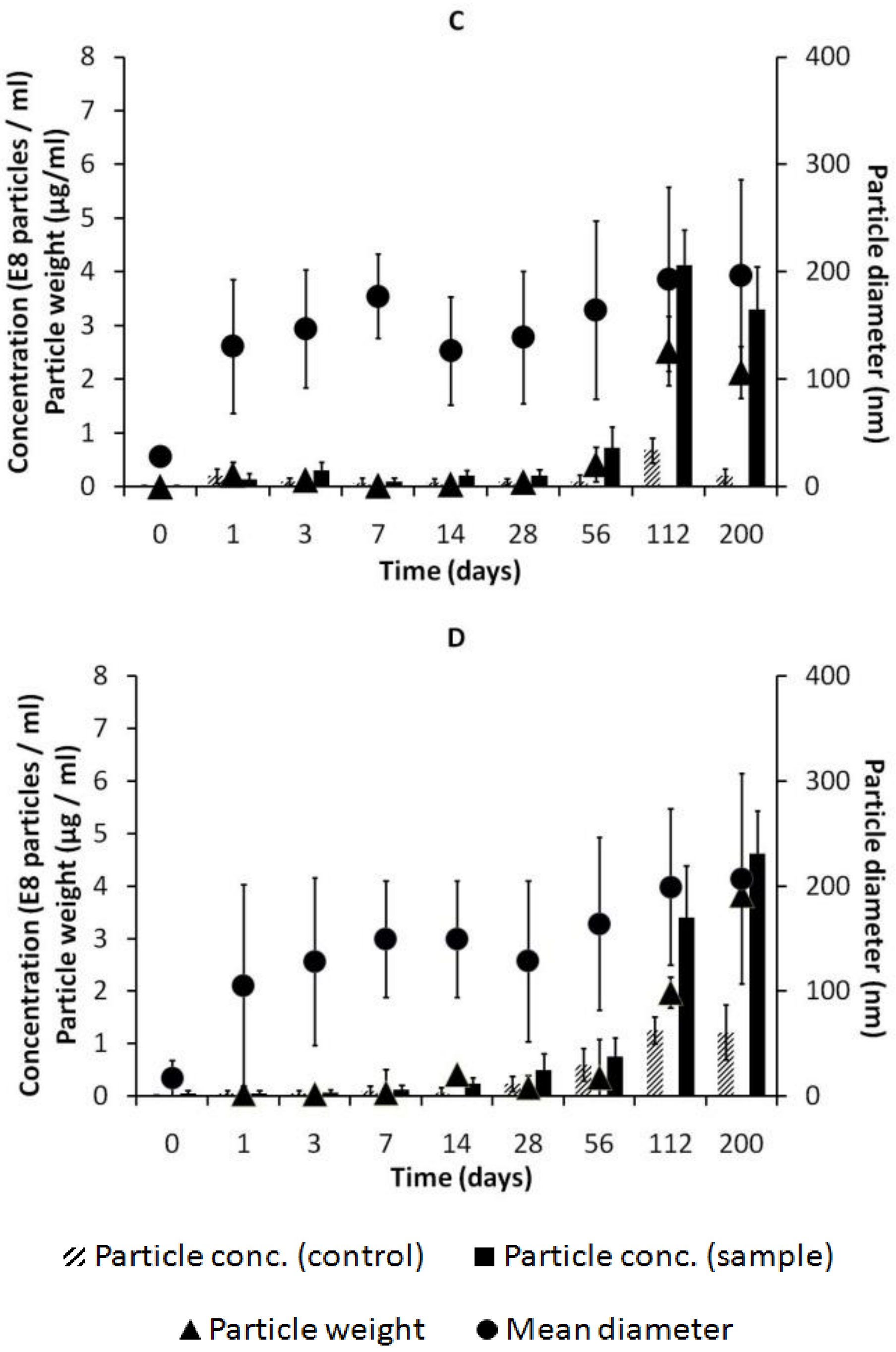


Figure 3.8 Characterisation if nano sized particles formed during the degradation of NRL in, c) artificial freshwater (pH 7.9); d) artificial freshwater (pH 5.5). Solid bars represent particle concentration in the sample filtrate; bars with diagonal lines represent particle concentration in the controls; circles represent mean particle concentration in the samples and triangles represent particle weight. Error bars displayed were obtained by the standard deviation of the different measurements for each sample; where not visible, bars fall within the symbols (continued).

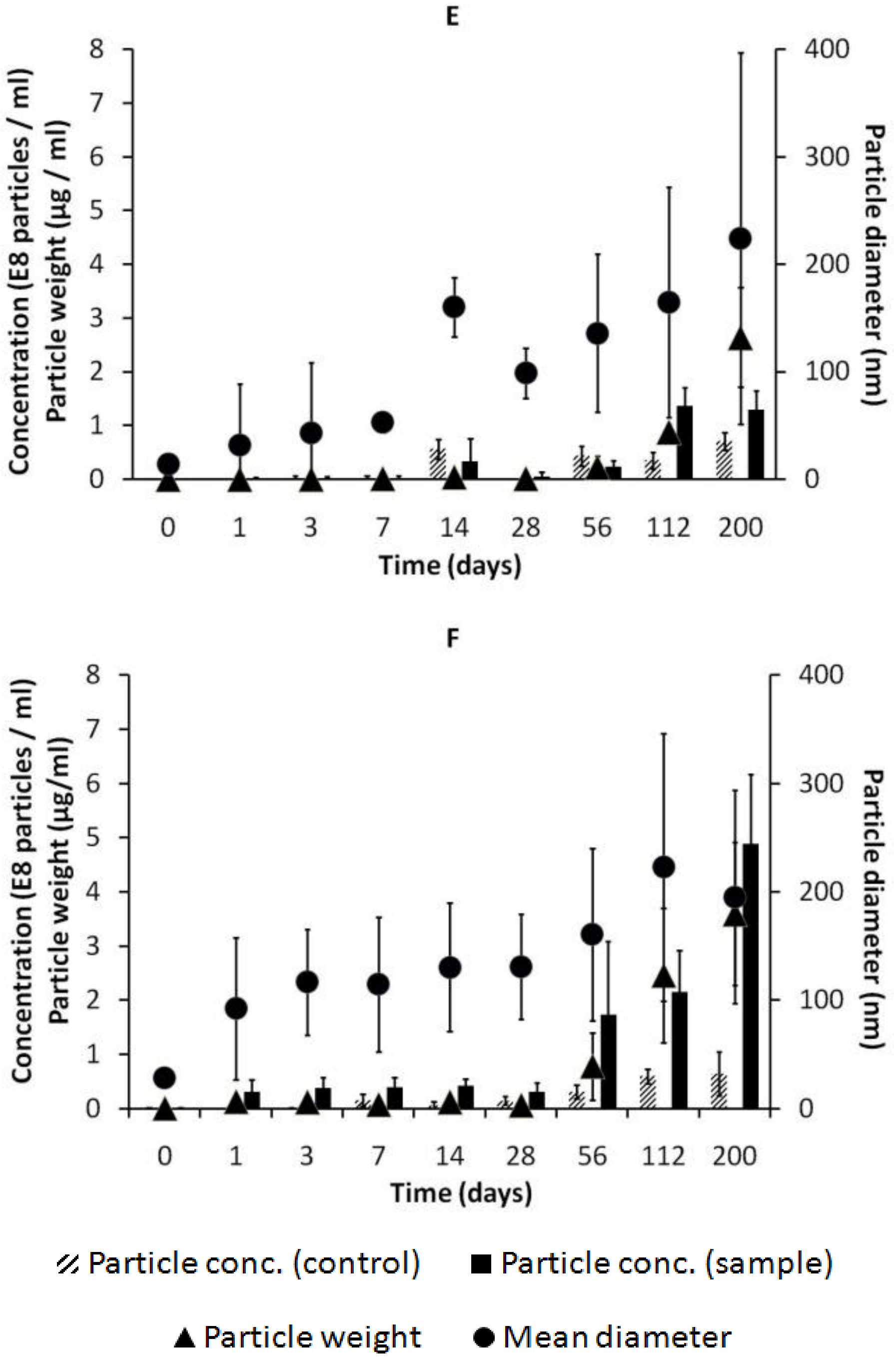


Figure 3.8 Characterisation if nano sized particles formed during the degradation of NRL in, e) artificial marine water; f) simulated water movement. Solid bars represent particle concentration in the sample filtrate; bars with diagonal lines represent particle concentration in the controls; circles represent mean particle concentration in the samples and triangles represent particle weight. Error bars displayed were obtained by the standard deviation of the different measurements for each sample; where not visible, bars fall within the symbols (continued).

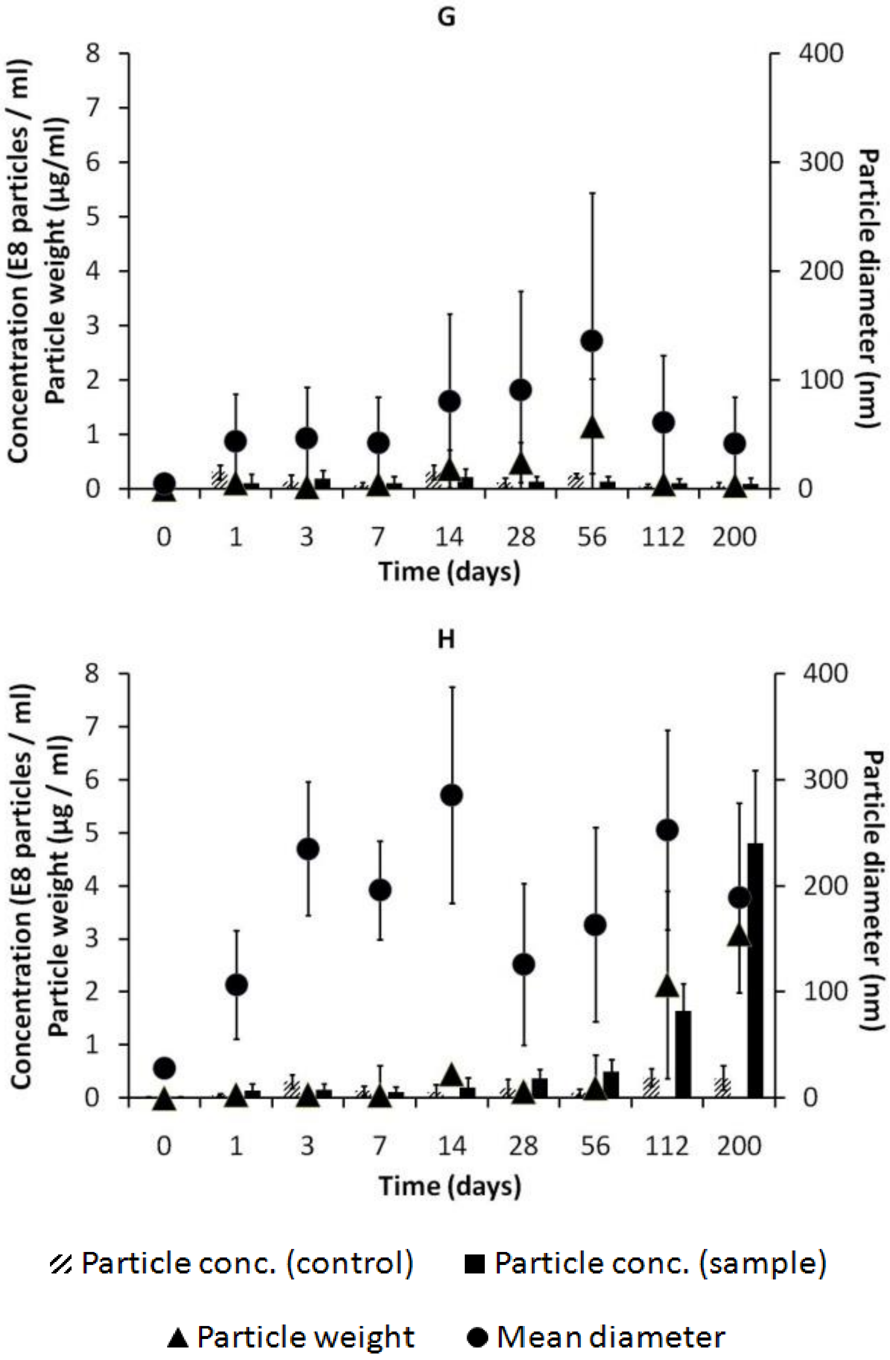


Figure 3.8 Characterisation if nano sized particles formed during the degradation of NRL in, g) absence of light; and h) a thicker latex sample. Solid bars represent particle concentration in the sample filtrate; bars with diagonal lines represent particle concentration in the controls; circles represent mean particle concentration in the samples and triangles represent particle weight. Error bars displayed were obtained by the standard deviation of the different measurements for each sample; where not visible, bars fall within the symbols (continued).

3.3.6 Mass balance

Data from the weight loss, particle and DOC measurements were combined to determine whether the mass loss of latex could be explained by particle formation and formation of soluble organic transformation products alone (Fig. 3.6). For the exclusion of light and thicker material treatments it was possible to develop a mass balance for all sampling points, however, this was not the case for the remaining treatments. For these remaining six treatments an increase in total mass was observed as the NRL starts to break apart and degrade. The mass increases may be due to a combination of processes which include the formation of microbial biomass on the NRL, as biodegradation is a mechanism of degradation for some polymers (Linos *et al.*, 2000). As microbiological analysis was not undertaken mass increase due to absorption of oxygen cannot be ruled out. The addition of oxygen due to photo-oxidation processes may also contribute to increases in mass. As time progresses it was not possible to develop a full mass balanced indicating that other loss mechanisms might be occurring. This might be explained by volatilization processes that come into effect as the latex degrades (Agostini *et al.*, 2008). The decreasing absorbance in the FTIR spectra indicates degradation of the macromolecules occurs. This degradation could be due to chain breakage as proposed in Fig. 3.3 coupled with the evolution of low-molecular volatile products.

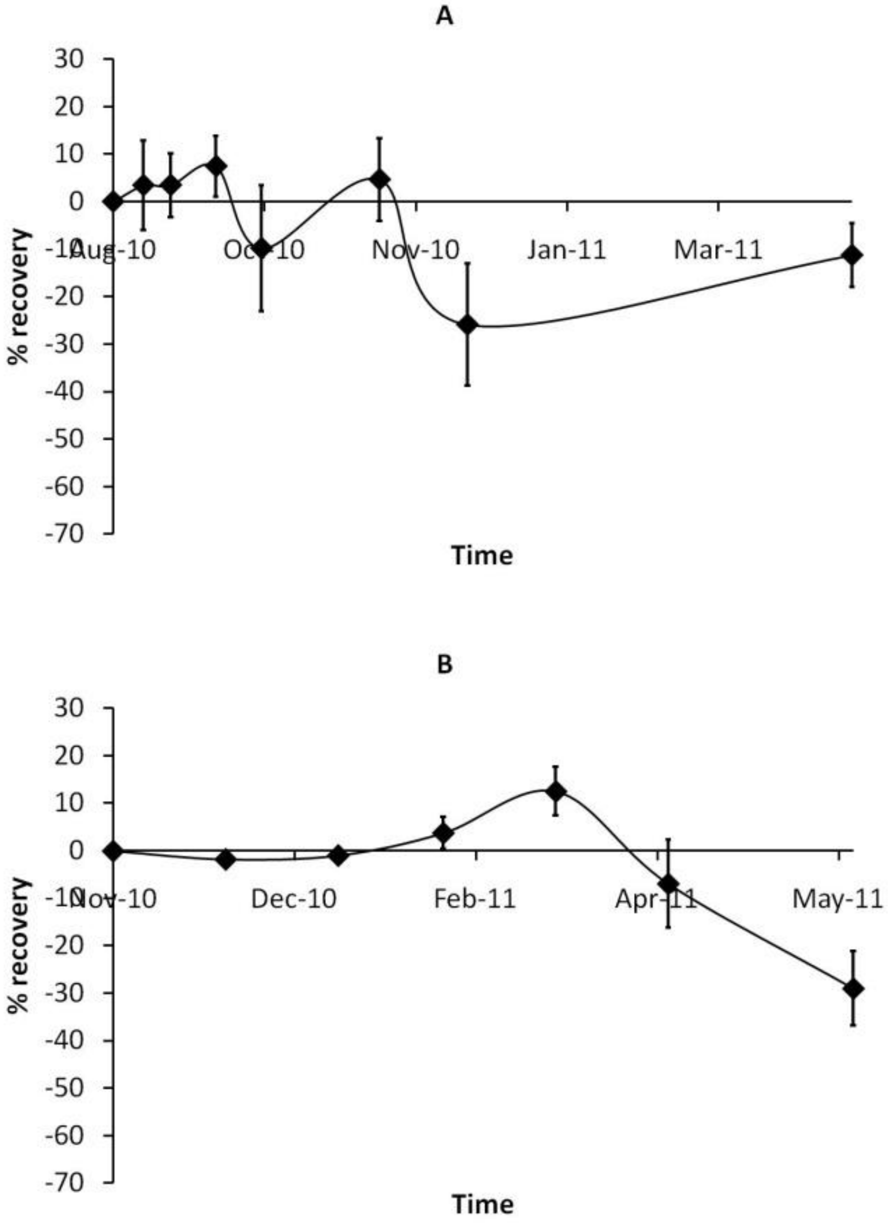


Figure 3.9 Changes in the mass balance for the degradation of NRL during exposure to natural cycles of sunlight and temperature in, a) August 2010 treatment; b) November 2010 treatment. Error bars displayed were obtained by the standard deviation of the different measurements for each sample; where not visible, bars fall within the symbols.

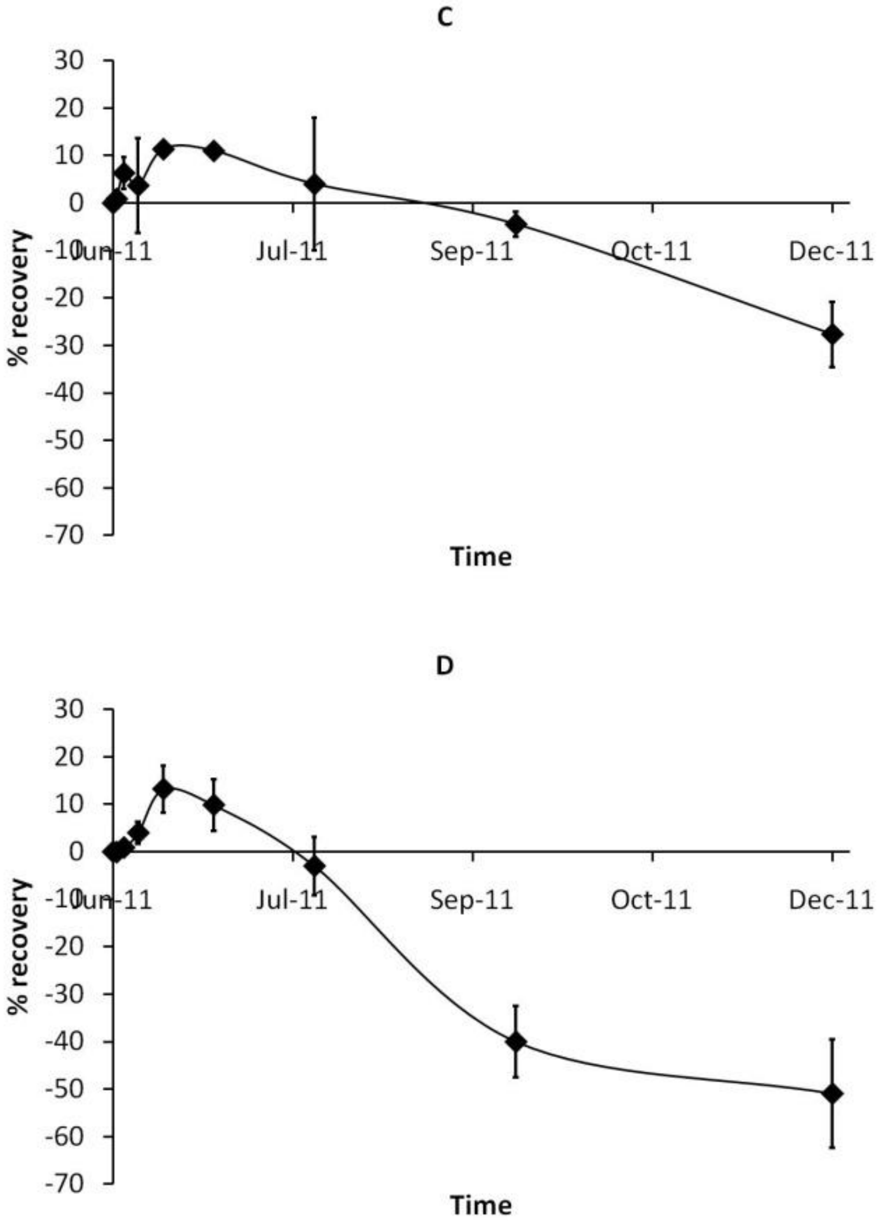
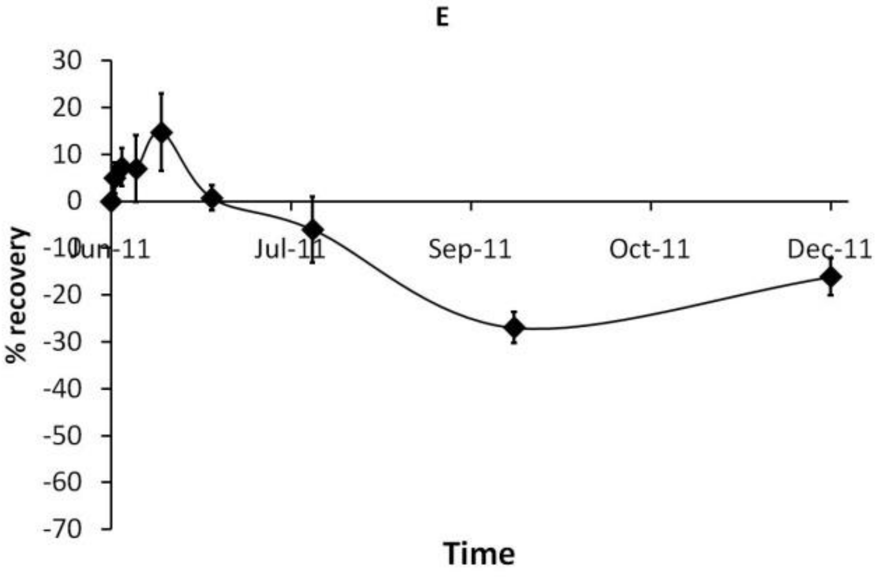


Figure 3.9 Changes in the mass balance for the degradation of NRL during exposure to natural cycles of sunlight and temperature in, c) artificial freshwater (pH 7.9); d) artificial freshwater (pH 5.5). Error bars displayed were obtained by the standard deviation of the different measurements for each sample; where not visible, bars fall within the symbols (continued).



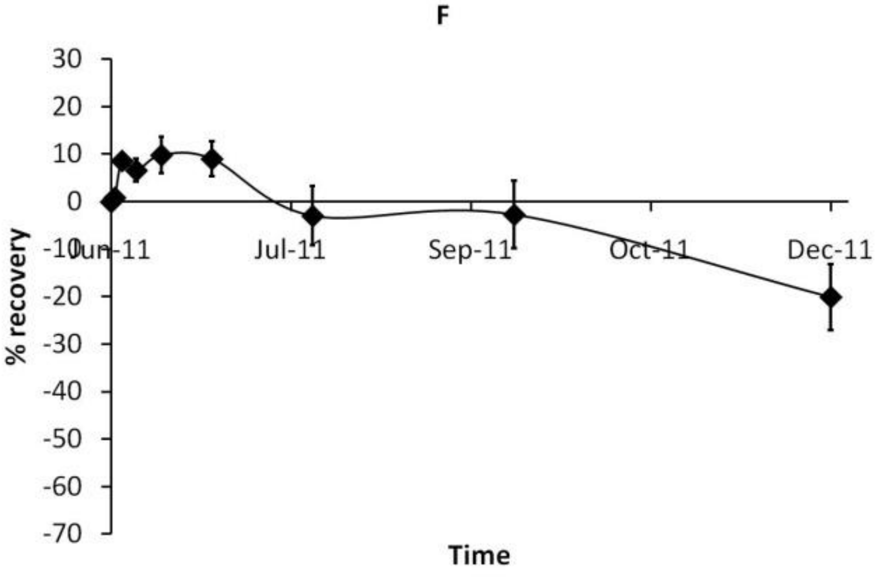
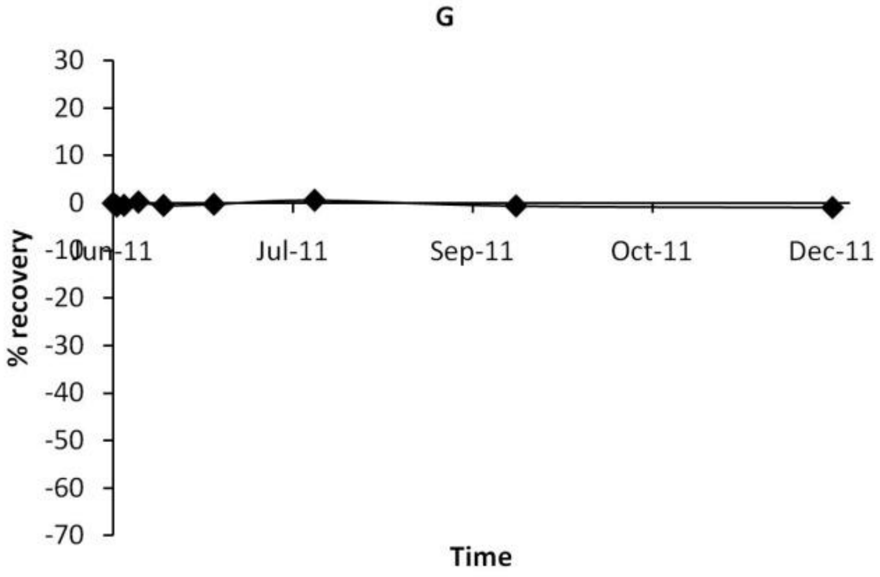


Figure 3.9 Changes in the mass balance for the degradation of NRL during exposure to natural cycles of sunlight and temperature in, e) artificial marine water; f) simulated water movement. Error bars displayed were obtained by the standard deviation of the different measurements for each sample; where not visible, bars fall within the symbols (continued).



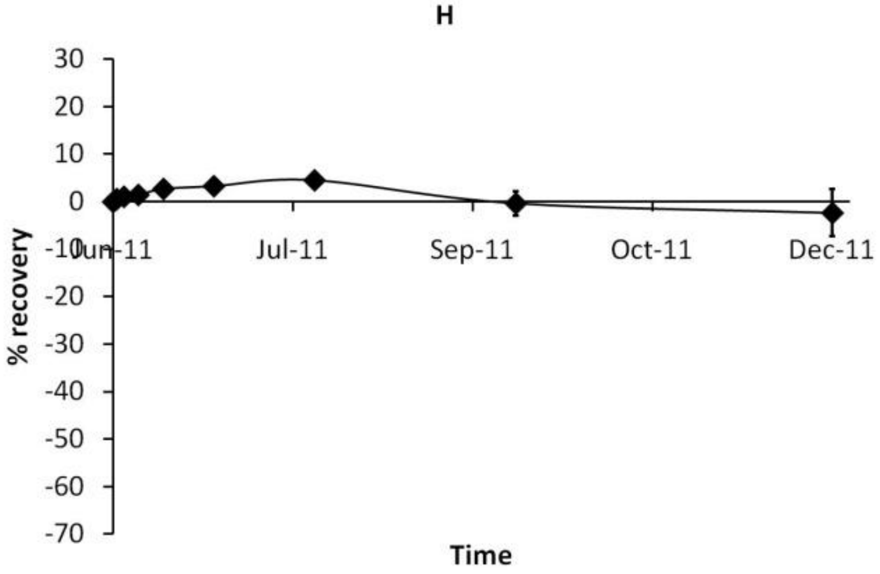


Figure 3.9 Changes in the mass balance for the degradation of NRL during exposure to natural cycles of sunlight and temperature in, g) absence of light; and h) a thicker latex sample. Error bars displayed were obtained by the standard deviation of the different measurements for each sample; where not visible, bars fall within the symbols (continued).

3.4. Conclusion

The outdoor microcosms used in this work have attempted as far as possible to mimic field conditions. The results show that the modelled degradation kinetics are characterised by multi-phasic degradation patterns, and degradation rate is affected by sunlight intensity and polymer thickness. The degradation mechanism is that of photo induced weakening of the NRL material as indicated by the FTIR analysis, coupled with the formation of oxidation products; these structural changes then result in the disintegration of the NRL film.

The major finding of this study was to identify and quantify the formation of particles in the nano size range. Andrady, (2011) has theorised the potential for particles in the nano range to be formed during polymer degradation. This study provides the first insight into the temporal formation of microscopic particles in aquatic systems, including particles in the nano range.

The results show that as PBMs degrade, receiving environments are potentially exposed to a mixture of dissolved polymer degradation products (DPs). The mass balance also indicates that losses to the atmosphere may occur. Therefore, in the next chapter analytical methods to characterisation the chemical make-up of this dissolved component were explored.

Chapter 4

Characterisation of Substances formed during the Degradation of Natural Rubber Latex

4.1 Introduction

In the previous chapter, the degradation of the NRL samples was shown to produce a substantial dissolved component, as well as highlighting potential losses to the atmosphere. Analytical screening methods to identify potential compounds of interest in unknown mixtures have been developed. These approaches utilise gas chromatography – mass spectrometry (GC-MS) with both headspace and liquid injection analysis to characterise volatile and semi-volatile compounds, as well as liquid chromatography-time-of-flight mass spectrometry (LC-TOF-MS) to characterise non-volatile compounds (Bradley *et al*., 2008). The identities of chromatography peaks produced by GC-MS analysis are determined by comparison of the generated mass spectra with that of a library match (Bradley *et al*., 2008). LC-TOF-MS data is processed using the relevant software that extracts chromatographic peaks, and produces accurate mass data for those peaks, which can be compared to a molecular formula database to predict probable formula (Bradley *et al*., 2009). Multiple variable statistical techniques have also been developed for the characterisation of complex mixtures of compounds; these approaches allow multiple samples and variables to be resolved and visualised (Bro, 2003; Christensen *et al*., 2005; Tas and Vandergreef, 1994).

The chemical diversity of PBMs makes the assessment of their environmental fate a major challenge. In addition to this, environmental systems are dynamic with numerous processes occurring together. Therefore, the physicochemical and biological changes that PBMs are potentially subjected to, greatly complicate our understanding with regards the extent of transformations that take place in receiving environments. The manufacturing of NRL products often includes the addition of sulphur as a vulcanising agent to link together polyisoprene chains. Zn compounds are incorporated into the NRL mixture to provide a range of properties, such as heat and light stabilization, and Zn oxide also provides an effective activator to speed up the rate of curing processes. These compounds make the NRL material usable and fit for purpose. The aim of this chapter was to further characterise the dissolved component identified in Chapter 3, and to investigate the formation of volatile compounds.

4.2 Methods

4.2.1 Material

The NRL was described as a combination of vulcanised *cis*-1,4-polyisoprene (Fig. 4.1), and various other compounds including a Zn based accelerator, Zn oxide, an antimicrobial agent, an antioxidant, stabilizing agents, surfactants, and various other pigments and solvents.

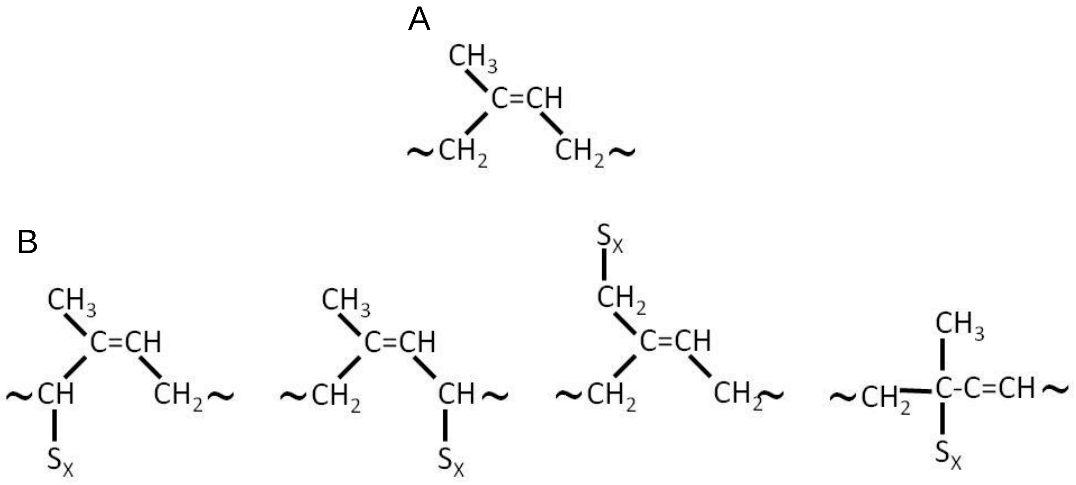


Figure 4.1 (a) *cis*-1,4-polyisoprene unit and (b) sulphur links upon vulcanisation of *cis*-1,4-polyisoprene following Buzare *et al*., (2001).

4.2.2 Study design

To investigate the effect of different environmental variables on the formation of substances during the degradation of latex in the aquatic environment, a series of studies were undertaken in June 2011 using artificial freshwater media ($CaCl_2$ 294 mg/L; $MgSO_4$ 123.25 mg/L; $NaHCO_3$ 64.75 mg/L and KCl 5.75 mg/L). Effects of a range of variables were investigated: pH values of 7.9 and 5.5: presence/absence of sunlight; sample movement (to simulate water turbulence) and material thickness (1.5 mm). Studies were also performed using an artificial marine water (Red Sea Salt mix, batch number 26 04 0915; pH 8.4). For the pH study, the medium was adjusted using NaOH or HCl. Exclusion of light was achieved by covering the test vessels with foil; and the effect of sample movement was simulated by shaking the relevant vessels once a week for 16 h at 80 rpm.

For all manipulations, NRL samples (approximately 25 cm^2) were placed individually into clear glass vessels (volume 250 ml) and spread out in 200 ml of test medium. For each time point, individual samples were established in triplicate and control samples without NRL were also established. The test vessels were then placed outdoors on a platform, covered with a non ultra-violet filtering perspex sheet (B&Q, UK) to prevent flooding by rainfall, and then exposed to natural sunlight and temperature. Samples were removed after 1, 3, 7, 14, 28, 56, 112 and 200 days of exposure. Evaporation was dealt with by adding the respective medium to the desired level, except for the marine water experiment, where demineralised water was used to prevent the build-up of salts. Upon collection, the NRL was recovered by filtering and the samples were then stored in Nalgene wide mouth PP bottles and frozen at -22 ^oC. Corresponding freshwater (at both pH values), marine water and demineralised water blanks were also prepared and stored in the same way.

To determine the loss of volatile substances to the atmosphere during degradation, NRL samples (~ 25 cm^2) were placed in 20 ml glass headspace vials with 10 ml freshwater medium and sealed using aluminium crimp caps with PTFE/Si septa. The sampling regime was as described above, with five replicate samples prepared and duplicate control samples without NRL. Upon collection, corresponding blanks were prepared in the same way, one with and one without the addition of latex. Headspace vials were then stored at -22 ^oC until analysis could be preformed.

4.2.3 Analytical methods

To characterise any potential degradation products the following methods were used (a) Atomic absorption spectrometry (AAS) was used to determine the migration of zinc from the latex into solution, (b) solid phase extraction (SPE) followed by LC-TOF-MS was used to investigate formation of non-volatile substances, (c) liquid extraction followed by GC-MS was used for the determination of semi-volatile substances, and (d) headspace GC-MS was used to characterise any volatile substances.

AAS anaylsis

AAS analysis was performed using an AA-6300, Shimadzu (Duisburg, Germany). Subsamples were diluted 10-fold with demineralised water to bring them within the calibration range. A standard solution of zinc nitrate was used for making a standard curve (0.01, 0.05, 0.1, 0.5, 1, 2, 3 mg/L) from which the total zinc concentration was calculated. The results presented were corrected by subtracting the corresponding control values.

LC-TOF-MS analysis

Prior to analysis, samples were extracted using SPE with 100 ml of sample and a C18 ODS cartridge (500 mg bed weight and 6 ml volume, Agilent, UK) at a flow rate of 3 ml/min. The cartridge was first conditioned with 5 ml MeOH, followed by 5 ml HPLC grade water. The sample was then eluted with 5 ml MeOH and the extract concentrated to 1 ml under nitrogen flow. LC separation was facilitated using an Agilent ZORBAX Eclipse XDB-C18 2.1 x 100 mm, 3.5 µm column. Positive mode electrospray was utilised, for this the mobile phase consisted of 0.1 % acetic acid (channel A) and MeOH/H_20 (9:1) (channel B). The mobile phase gradient at time t minutes for channel B was 0 (30 %), 10 (50 %), 20 (50 %), 30 (100 %), 32.5 (100 %), and 35 (30 %). The flow rate was 0.3 ml/min and the injection volume was 5 µl. TOF-MS analysis was carried out with a nebulizer pressure of 45 psi, capillary of 4000 V, gas temperature of 350^{o}C, drying gas flow at 9 L/min, skimmer of 65 V, fragmentor of 150 V, and octopole RF voltage of 500 V. The mass range measured was 100-1100 m/z. The TOF-MS data produced were processed using Agilent Molecular Feature Editor software. Given the complexity of data generated, the analysis focused on the characterisation of the most intense peaks. The approach taken was to characterise only the peaks which fulfilled the following criteria; those with a mass error < 5 ppm, a software score of ≥ 70, and peaks for which identification was consistent between replicates. The predicted formulae generated were then compared to online databases Chemspider and the National Institute of Standards and Technology (NIST).

GC-MS of semi volatiles

Samples were extracted by adding 10 ml dichloromethane (DCM) to 50 ml of sample in glass vials. These samples were then shaken overnight at room temperature on an orbital shaker. The DCM layer was then taken and concentrated to 0.5 ml under nitrogen flow. The extracts were analysed using an Agilent 6890 gas chromatography (Agilent, Palo Alto, California, USA) equipped with an Agilent 5973 inert mass selective detector. Splitless injection of 1 µl was carried out into a Phenomen ZB-5MS capillary column at a pressure of 9.03 psi. Following injection, the oven was held at 80 ^{o}C for 3 min and then raised by 10^{o}C / min to 320^{o}C, where it was held for 3 min giving a total run time of 30 min. The injector was held at 250^{o}C. Helium was used as the carrier gas with a flow rate of 1 ml/min. The MS was operated in electron impact ionization mode with scanned monitoring between 40 and 600 amu.

GC-MS of volatiles

The formation of volatile substances, following incubation for 30 min at 90^{o}C, was assessed using an Agilent 6890 gas chromatography (Agilent, Palo Alto, California, USA) equipped with a Agilent G1888 headspace sampler and an Agilent 5973 inert mass selective detector. The initial inlet temperature was 120^{o}C and 1 µl was injected in split mode (ration 0.1:1) with a pressure of 16.66 psi into a DB-VRX capillary column (0.25 mm x 60 m x 1.4 µm). Helium was used as the carrier gas with a flow rate of 1 ml/min. Following injection, the oven was held at 40^{o}C for 3 min and then raised by 10^{o}C per min to 180 ^{o}C, where temperature was held for 3 min, giving a total run time of 20 min. The MS was operated in electron impact ionization mode with scanned monitoring between 30 and 450 amu. The approach taken to the data analysis was to characterise only the peaks which fulfilled the following criteria; those with a software score of $\geq$ 70, and peaks for which identification was consistent between replicates.

4.2.4 Statistical analysis

The AAS data was identified as non-normally distributed, using the Shapiro-Wilk test. Therefore, overall differences for zinc concentrations between treatments were evaluated, using Friedman Repeated Measures ANOVA on Ranks. Statistical tests were followed by a *post-hoc* Tukey multiple comparisons test applied to the different combinations using SigmaPlot 12, and a significance level of 0.05.

To interpret and analyse GC-MS chromatographic data (Fig. 4.2a), chemo-metric evaluation techniques as described by Christensen *et al*., (2005) and Christensen & Tomasi, (2007) were adopted. Chromatographic data files were first exported in csv file format for use in MS Excel. Once in Excel, a number of processing steps were undertaken prior to multivariable data analysis. First, a retention time window between 5 and 18 minutes (corresponding to 2024 data points) was used to exclude solvent peaks. Second, chromatograph baselines were removed by calculating the first derivative (difference between consecutive data points); this procedure aids comparison between samples where the baselines are distorted because of incompletely resolved peaks (Fig. 4.2b) (Christensen *et al*., 2005). Third, baseline removal is often associated with a decrease in the signal-to-noise ratio (S/N). To improve this, Christensen & Tomasi, (2007) recommend a smoothing algorithm. Here, the Savitzky-Golay algorithm was applied with a width of 9 data points and a first order fit (Fig. 4.2c). Fourth, a normalisation step is applied to compensate for concentration effects and sensitivity changes using the following Christensen *et al*., (2005) (equation 4.1, Fig. 4.2d).

$$Normalisation = \frac{First\ derivative}{\sqrt{\sum First\ derivative\ ^\wedge 2}} \qquad \text{Equation 4.1}$$

The GC-MS chromatographic data were then analysed using principal component analysis (PCA). PCA reduces the complexity of a multivariable dataset into a graphical summary. The goal of this approach was to provide an overview of the generated data to identify homogeneity or heterogeneity between groups of samples in principal component space. PCA was performed using Minitab 15.

To characterise the headspace GC-MS data in terms of the most important compounds identified, a significance measure following Lundgren *et al*., (1999) was applied. First, a frequency index is calculated as the percentage fraction of time points that have a particular compound present. The significance measure is then calculated by multiplying the frequency index with the overall median peak area for that compound (Lundgren *et al*., 1999).

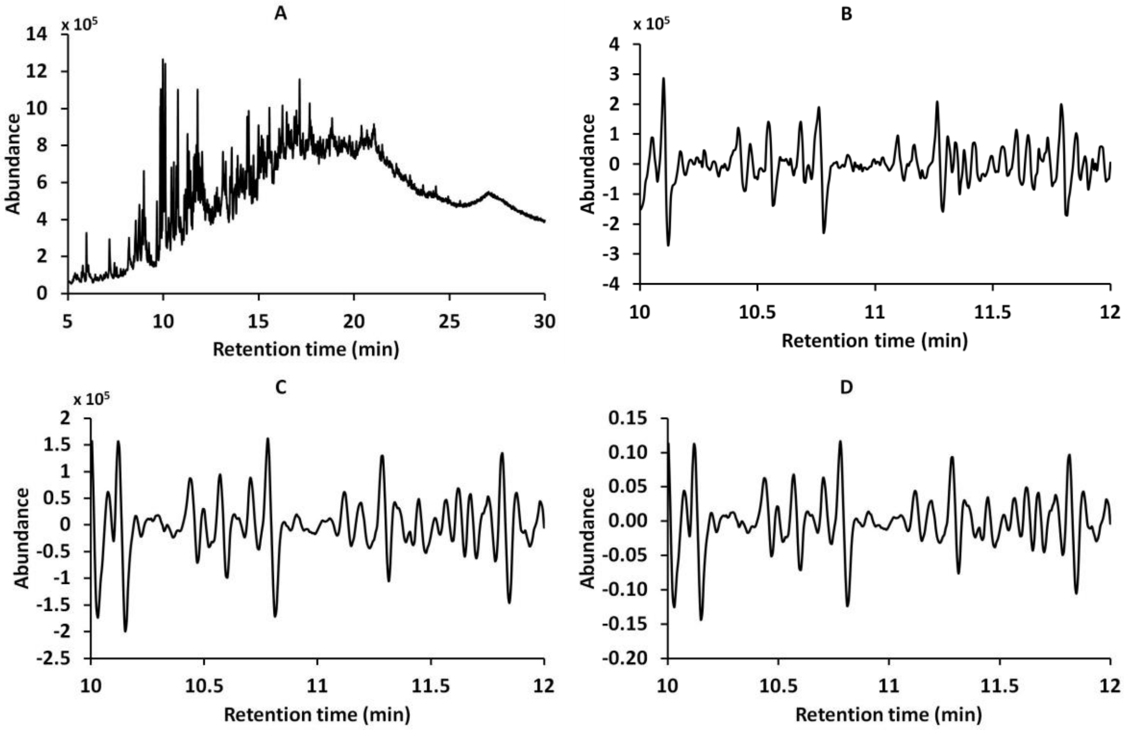


Figure 4.2 GC-MS chromatographic data processing, (a) an unresolved complex mixture, (b) first derivative, (c) after the application of a smoothing, and (d) a normalised chromatogram.

4.3 Results

4.3.1 Migration of zinc from latex into solution

The concentrations of Zn migrating from the NRL into solution were similar for each exposure treatment with the exception of the exclusion of light treatment ($\chi^2 = 26.967$, $p < 0.001$). Zn was not detected in the exclusion of light treatment when concentrations were corrected for those in the controls. The concentrations measured suggest that in a static environment concentrations steadily increase over time, with the highest concentrations measured in the freshwater lower pH treatment (Fig. 4.3). However, when movement was simulated a peak Zn concentration was achieved at day 56, after which concentrations declined.

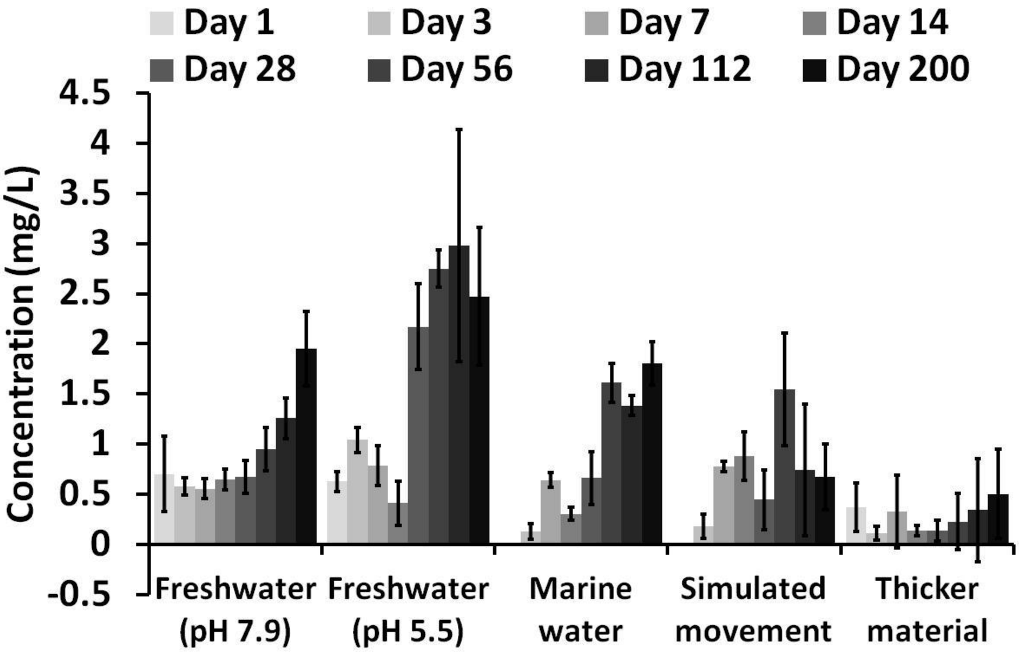


Figure 4.3 Concentrations of Zn migrating from the NRL over time in the different exposure treatments.

4.3.2 Characterisation of media soluble non-volatile substances

LC-TOF-MS analysis with electrospray ionization in positive mode was used to monitor the formation of NRL degradates. Structural matches for the predicted formulas generated were investigated using the NIST and Chemspider library searching. However, potential matches were too numerous for an exact prediction to be made. This is due to the different potential isomeric forms possible for the predicted formulae.

Analysis of the chromatographic peaks indicated a degree of similarity between the different pH treatments. Proposed DPs $C_{10}H_{14}O_2$ and $C_{10}H_{18}O_3$ are observed in all three media types at a retention time of approximately 3.6 min and 5.2 min respectively (Fig. 4.4 and table 4.1). A number of the chromatographic peaks in all three media types are proposed as $C_{12}H_{18}O_3$ and $C_{14}H_{18}O_4$, although differences between the three media types used indicated generation at different time stages (moments in time). The proposed predicted formulas indicated that the degradates produced largely consist of oxygen containing compounds (Table 4.1).

The temporal peak shape suggests the majority of degradates increase in concentration over time, and then they themselves undergo transformation processes, and decrease in concentration (Fig. 4.4). This continuous process of transformation is highlighted by shifts in peak retention time and changes in predicted formulae (Table 4.1). This is potentially a result of water loss which causes peak identification to change from $C_{14}H_{18}O_4$ to one with a low molecular mass ($C_{14}H_{16}O_3$). The transformation processes may also indicate cross-linking reactions are occurring with the transformation of $C_{14}H_{18}O_4$ to a predicted formula with a high molecular mass ($C_{15}H_{24}O_4$).

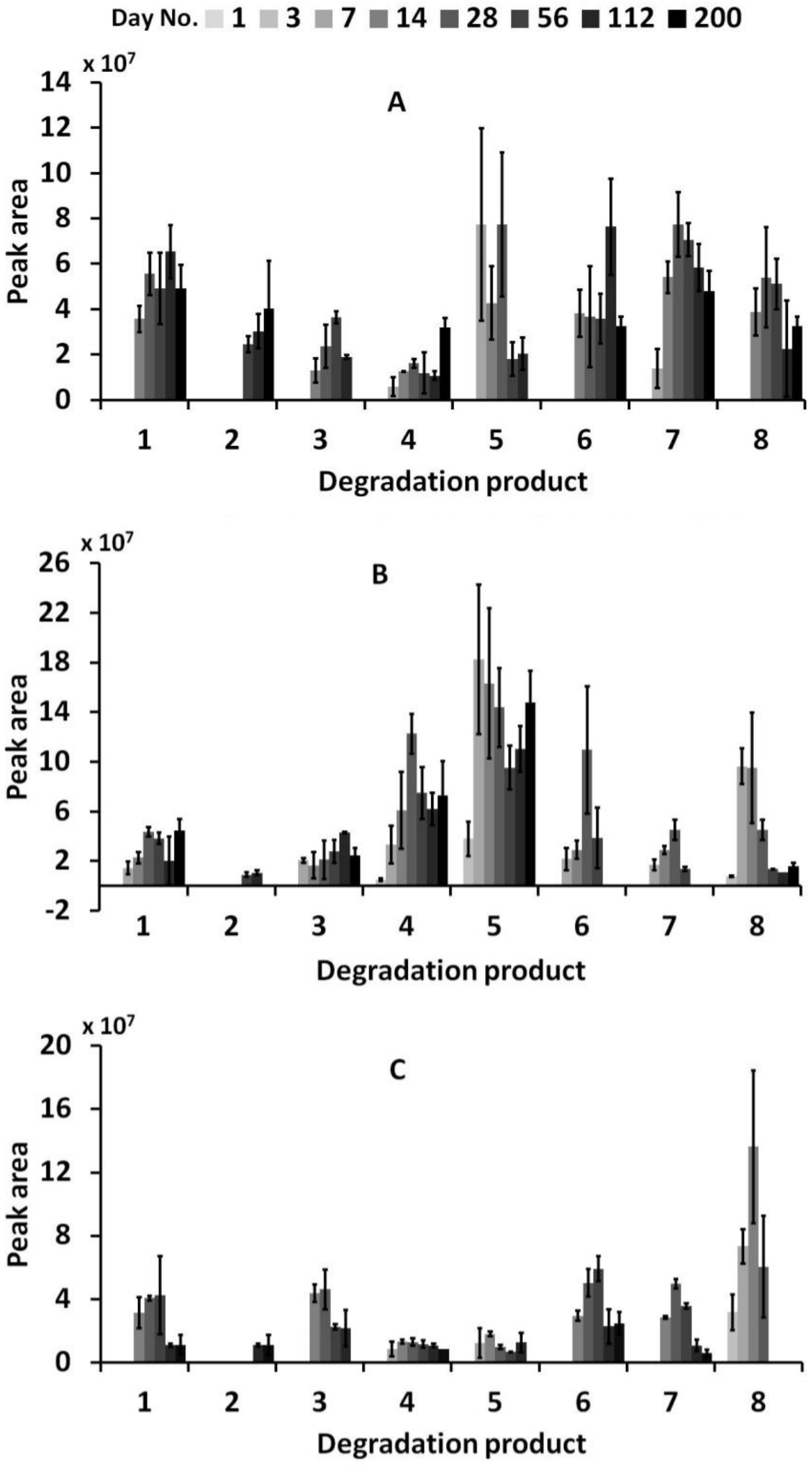


Figure 4.4 LC-TOF-MS profile of changes in peak area of NRL degradates over time in a) artificial freshwater (pH 7.9); b) artificial freshwater (pH 5.5); and c) artificial marine water (pH 8.9). Collection time points refer to 1, 3, 14, 28, 56, 112 and 200 days.

Table 4.1 Predicted formula and accurate mass for substances formed during the degradation of latex.

Degradation product	**Predicted formula**	**Calculated Mass**	**Treatment**	**Behaviour**
1	$C_{10}H_{14}O_2$	166.1008	Freshwater (pH 7.9)	Early formation with peak not fully achieved.
			Freshwater (pH 5.5)	Early formation and its peak are not achieved over the exposure duration.
			Marine water	Early formation with peak achieved and then declines.
2	$C_{10}H_{18}O_3$	186.1255	Freshwater (pH 7.9)	Late formation and its peak are not achieved over the exposure duration.
			Freshwater (pH 5.5)	Late formation with small peak area then dissipates.
			Marine water	Late formation with small peak area.
3	$C_{12}H_{18}O_2$	196.1466	Freshwater (pH 7.9)	Early formation then dissipates.
			Freshwater (pH 5.5)	Early formation.
			Marine water	Early formation then dissipates. PQMs* are achieved throughout.
4	$C_{12}H_{18}O_3$	210.1273	Freshwater (pH 7.9)	Early formation and its peak is not achieved over the exposure duration.
			Freshwater (pH 5.5)	Early formation and then declines. Best quality match changes over time to $C_{13}H_{22}O_4$ & $C_{15}H_{22}O_4$.
			Marine water	Early formation.
5	$C_{12}H_{18}O_3$	210.1273	Freshwater (pH 7.9)	Early rapid formation and then dissipates. Peak identification changes to $C_{14}H_{16}O_3$ after at day 56.
			Freshwater (pH 5.5)	Early rapid formation. $C_{14}H_{16}O_3$ may be possible after day 56
			Marine water	Early formation.

Table 4.1 Predicted formula and accurate mass for substances formed during the degradation of latex (continued).

Degradation product	Predicted formula	Calculated Mass	Treatment	Behaviour/notes
6	$C_{14}H_{18}O_4$	250.1208	Freshwater (pH 7.9)	Early formation with peak achieved and then declines. The predicted formula given is identified throughout, but with PQMs. $C_{14}H_{16}O_3$ may also be possible after day 28.
			Freshwater (pH 5.5)	Early formation with peak achieved and then dissipates. The predicted formula given is identified throughout, but with PQMs.
			Marine water	Early formation with peak achieved and then declines.
7	$C_{14}H_{18}O_4$	250.1208	Freshwater (pH 7.9)	Early formation with peak achieved and then declines.
			Freshwater (pH 5.5)	Early formation with peak achieved and then dissipates.
			Marine water	Early formation with peak achieved and then declines. The predicted formula given is identified up until day 28; $C_{14}H_{16}O_3$ may be possible at day 14. $C_{12}H_{18}O_3$is identified from day 56 onwards.
8	$C_{14}H_{18}O_4$	250.1208	Freshwater (pH 7.9)	Early formation with peak achieved and then declines. The predicted formula given is identified from day 28 onwards. $C_8H_{12}N_6O$ is identified as the best quality match at day 3, and for days 7 and 14 $C_{15}H_{24}O_4$ may be possible.
			Freshwater (pH 5.5)	Early formation with peak achieved and then declines. The predicted formula given is identified throughout, but at early sampling times $C_9H_{17}NOS$ may be possible.
			Marine water	Early formation with peak achieved and then dissipates.

*PQM = poor quality match

4.3.3 Characterisation of media soluble semi-volatile substances

Sample analysis by GC-MS produced an unresolved complex mixture (UCM) of substances (Fig. 4.2a). The term UCM describes a raised baseline hump that is often observed in gas chromatograms of petroleum samples (Mommaerts *et al*., 2012). The characterisation of such datasets is often undertaken by visualisation of the chromatographic data in principal component space (Christensen *et al*., 2005). PCA results were explored using the first two principal components (Fig. 4.5). The resulting two-component model of the each treatments profile visualises the temporal variation in sample spread, for each replicate sample taken, from each sampling time point, plus corresponding controls. PCA revealed the most complex dataset as the treatment where movement was stimulated, because of the low variance accounted for by the first two principle components (37.5 %; Fig. 4.5-d). For this treatment the samples can be separated into 5 clusters; the day 1 samples and the controls are grouped together, and the remaining are then grouped temporally as follows, day 3 and 7, 14 and 28, 56, and 112 and 200. The least complex dataset was the exclusion of light treatment, for this treatment the first two PC accounted for 70.2 % of the total variance (Fig. 4.5-f). Here the control samples do not cluster together, but are inter-dispersed with the degraded NRL samples, indicating negligible leaching of additive compounds into solution.

When comparing the remaining treatment groups (Fig. 4.5) a general pattern of clustering emerges largely based on their distribution in time. The control samples are either grouped with the day 112 and 200 samples, as with the freshwater (pH 7.9) treatment (Fig. 4.5-a), or on their own (marine water treatment). This type of sample distribution shows the formation of the complex mixture by clustering the day 14, 28 and 56 samples away from the controls. The subsequent dissipation of the complex mixture is then indicated by the later time points been identified as acting more like the controls. This type of pattern would agree with peak shapes identified in of the TOF data, in that a continuous process of degradate formation and transformation is occurring.

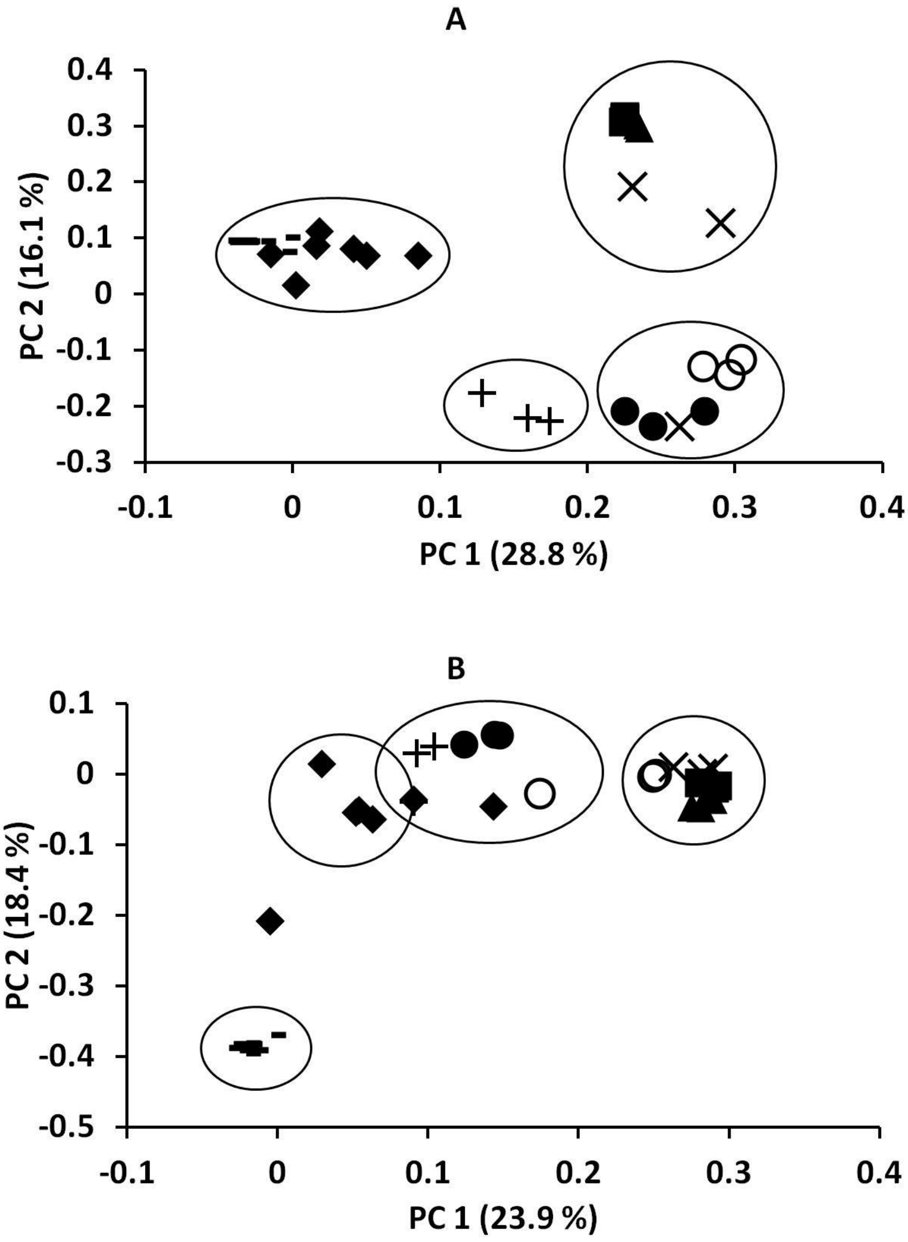


Figure 4.5 Projection of extracted degradates in principal component space resulting from GC-MS analysis of NRL degraded under different environmental conditions in a) freshwater (pH 7.9), b) freshwater (pH 5.5). Diamonds = control sample, squares = day 1 samples, triangles = day 3, cross = day 7, circle = day 14, solid circles = day 28, addition sign = day 56, short dash = day 112, and long dash = day 200. Percentage values indicate the variance explained by each principal component.

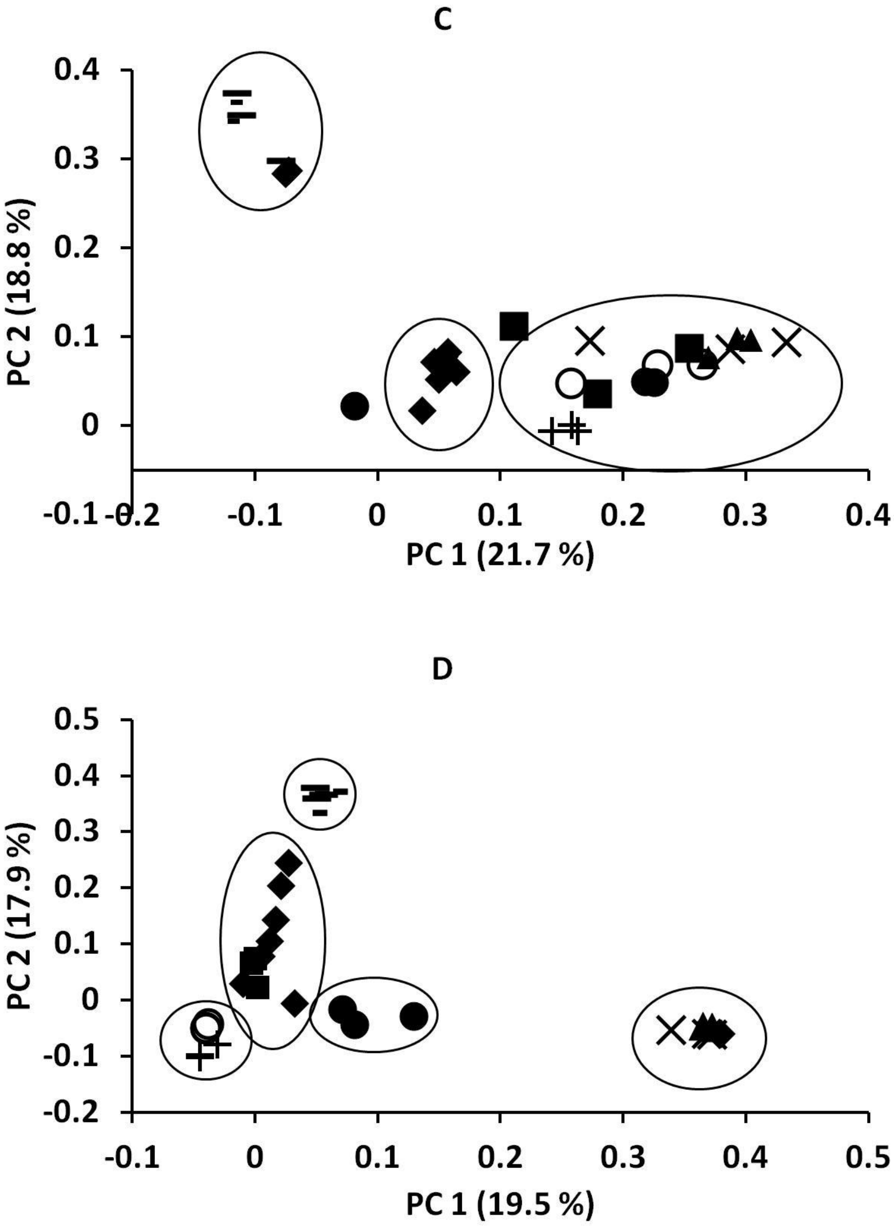
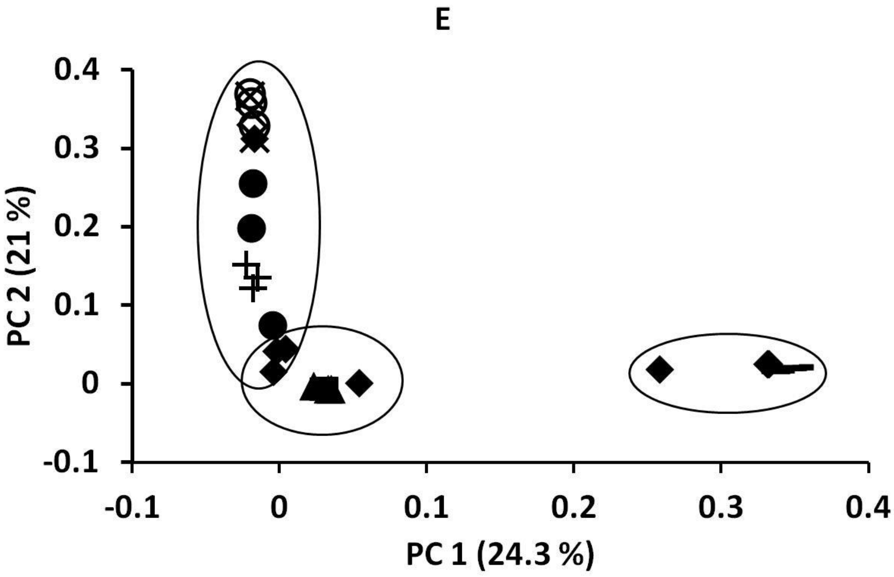


Figure 4.5 Projection of extracted degradates in principal component space resulting from GC-MS analysis of NRL degraded under different environmental conditions in c) marine water, d) with movement simulated. Diamonds = control sample, squares = day 1 samples, triangles = day 3, cross = day 7, circle = day 14, solid circles = day 28, addition sign = day 56, short dash = day 112, and long dash = day 200. Percentage values indicate the variance explained by each principal component (continued).



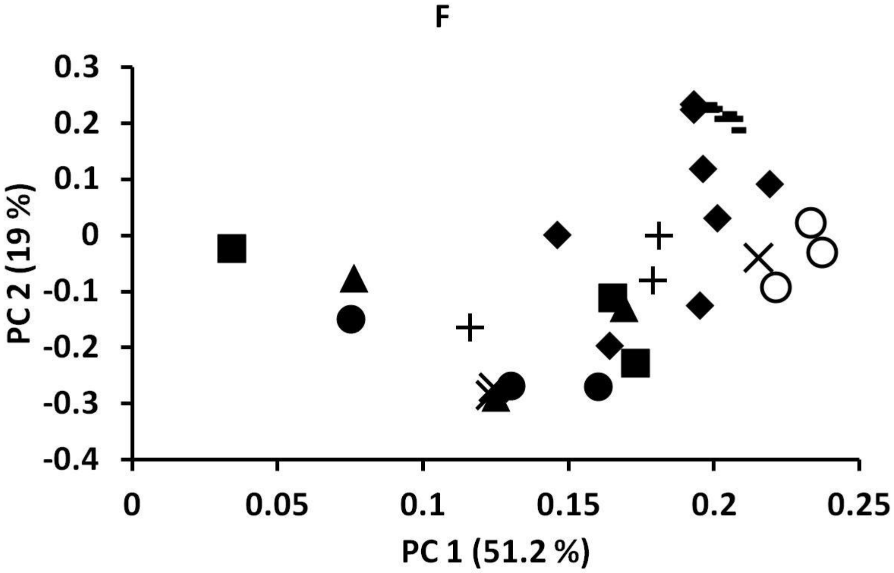


Figure 4.5 Projection of extracted degradates in principal component space resulting from GC-MS analysis of NRL degraded under different environmental conditions in, e) thicker material, and e) excluding light. Diamonds = control sample, squares = day 1 samples, triangles = day 3, cross = day 7, circle = day 14, solid circles = day 28, addition sign = day 56, short dash = day 112, and long dash = day 200. Percentage values indicate the variance explained by each principal component (continued).

4.3.4 Characterisation of volatile substances

The chromatographic separation and identification of volatile degradates were monitored using headspace GC-MS with comparison of the generated mass spectra with that of a library match. A total of 11 volatile compounds were identified (Table 4.2). The identified substances are shown to be generated in different stages of the exposure and are characterised by how they differ in their formation and decline. The most important substances are identified as butanal and pentanal, which have a presence frequency of 75 %, these substances generate large peak areas, then decline and are not identified after day 56. Hexanal and 2-heptanone are observed at all time points, but are considered as less important because of their relative smaller peak area. Substances of minor significance are 3-Octanone and 4-Nonanone, as they are characterised by their appearance after 28 days and subsequence disappearance from the mixture, whereas Butanal-3-methyl is characterised by its late formation.

Table 4.2. Volatile chemical compounds produced during latex degradation in freshwater.

Chemical compound	Formula	Overall frequency (%)	Overall median peak area (x 10^{-6})	Significance measure
Butanal	C_4H_8O	75	9.59	7.19
Butanal-3-methyl	$C_5H_{10}O$	25	0.40	0.10
Furan,2,5-dimethyl	C_6H_8O	75	0.81	0.60
Furan,2-pentyl	$C_9H_{14}O$	50	0.31	0.15
Heptanal	$C_7H_{14}O$	75	0.34	0.25
2-heptanone	$C_7H_{14}O$	100	1.10	1.10
Hexanal	$C_6H_{12}O$	100	4.59	4.59
4-Nonanone	$C_9H_{18}O$	25	0.12	0.03
3-Octanone	$C_8H_{16}O$	25	0.14	0.03
Pentanal	$C_5H_{10}O$	75	13.21	9.91
1-pentanol	$C_5H_{12}O$	25	6.87	1.71

4.4 Discussion

The NRL used in this study was vulcanised *cis*-1,4-polyisoprene linked together with sulphur bonds, and with the addition of a number of additive compounds. To date, a number of studies have investigated the degradation of mainly polyolefins in the aquatic environment (O'Brine and Thompson, 2010); Rutkowska *et al*., 2002; Sudhakar *et al*., 2007). These studies focused on weight loss, and changes in tensile strength and chemical functionality, but the environmental degradation pathways of PBMs are yet to be fully understood. Our understanding of rubber latex degradation comes mainly from laboratory investigations into the oxidation mechanisms (Bevilacqua, 1955; Colin *et al*., 2007; Mayo, 1960; Tobolsky *et al*., 1950); the degradation of sulphur links between isoprene bonds (Bredberg *et al*., 2001; Kim and Park, 1999; Rooj *et al*., 2011; Sato *et al*., 2009); and the ability of microorganisms to degrade compounded natural and synthetic rubber tires (Linos *et al*., 2000; Tsuchii *et al*., 1985; Tsuchii *et al*., 1997; Tsuchii and Tokiwa, 2006).

The present study followed the degradation process of NRL in outdoor microcosms, under a variety of exposure treatments. Each of the treatments underwent decomposition, with the exception of the exclusion of light treatment, and shows photodecomposition is the most crucial factor affecting the fate of NRL. The analyses of the degraded NRL solutions indicate the possible formation and continuous transformation of substances form a non-volatile state to semi-volatile state and then volatile state, and from semi-volatile state and then volatile state. The replicate measures for both the Zn migration and the formation of non-volatile substances are shown to be quite variable. This may be due to the specific circumstances of the study conditions which cause different patterns of NRL decomposition. The increasing presence and distributional differences of fragmented NRL particles, Zn, and dissolved substances in the water column may influence degradation rates. Light absorption by dissolved materials may enhance their degradation/transformation, while decreasing irradiance for degrading the parent NRL. Whereas, light absorbed by fragmented NRL particles will enhance photo induced degradation of the parent NRL.

The breakdown of the polyisoprene matrix that binds the Zn based compounds to the material causes the Zn to become mobilised and released into the solution. The metabolic products from microbial activity are thought to enhance the leaching of Zn from car tires by lowering the pH of the localised environment (Councell *et al*., 2004). This may account for the elevated Zn concentrations observed in the lower pH freshwater experiment (Fig. 4.3). The pH value of a degrading solution is known to play an important role in degradation process (Despotovic *et al*., 2012), and is a possible indication that Zn migration is pH dependent. The decrease in Zn concentrations observed when movement was simulated, suggests that water movement causes Zn to precipitate, or facilitate its sorption and recombination with NRL fragments. The treatment were movement was stimulated also has the most complex set of GC-MS data as determined by PCA analysis. Water movement has the potential to stimulate a more complex degradation pathway than static conditions, and is potentially a more realistic scenario.

The observation of proposed degradation products $C_{12}H_{18}O_3$ and $C_{14}H_{18}O_2$ at different time stages in the three media types could be due to the different chemical environments of the three pH treatments. The chemical additives are incorporated into the liquid NRL mixture during manufacturing process, and are mostly likely strongly bound within the polyisoprene matrix. Therefore, the proposed compounds are a potential mixture of oxidized polyisoprene oligomers and additive residues.

Under ambient conditions environmental fate processes for PBMs are primarily photo induced (Al-Salem, 2009; Lambert *et al*., 2013). In this study the samples existed near the air-water interface, therefore degradation processes include direct photolysis and reactions with OH radicals, oxygen, nitrogen oxides and other photo-chemically generated radicals (Sinkkonen and Paasivirta, 2000). The bond energy of different NRL molecular bonds are; C=C 621 kJ/mol, C-H 416 kJ/mol, C-C 348 kJ/mol, C-S 273 kJ/mol, and S-S 227 kJ/mol (Rooj *et al*., 2011). Therefore, environmental fate processes potentially begin with the photo induced cleavage of S-S and C-S bonds (Fig. 4.6), as these are deemed to have lower bond energy. In NRL devulcanization studies, chain scission is thought to occur first at the S-S and C-S linkages due to their lower bond strength (Rooj *et al*., 2011). Once sulphide bonds are broken, sulphur atoms are theoretically available to react with water molecules or atmospheric and dissolved oxygen to produce sulphur dioxide. This degradation pathway would explain why sulphur containing compounds are not effectively identified in the degradation solutions.

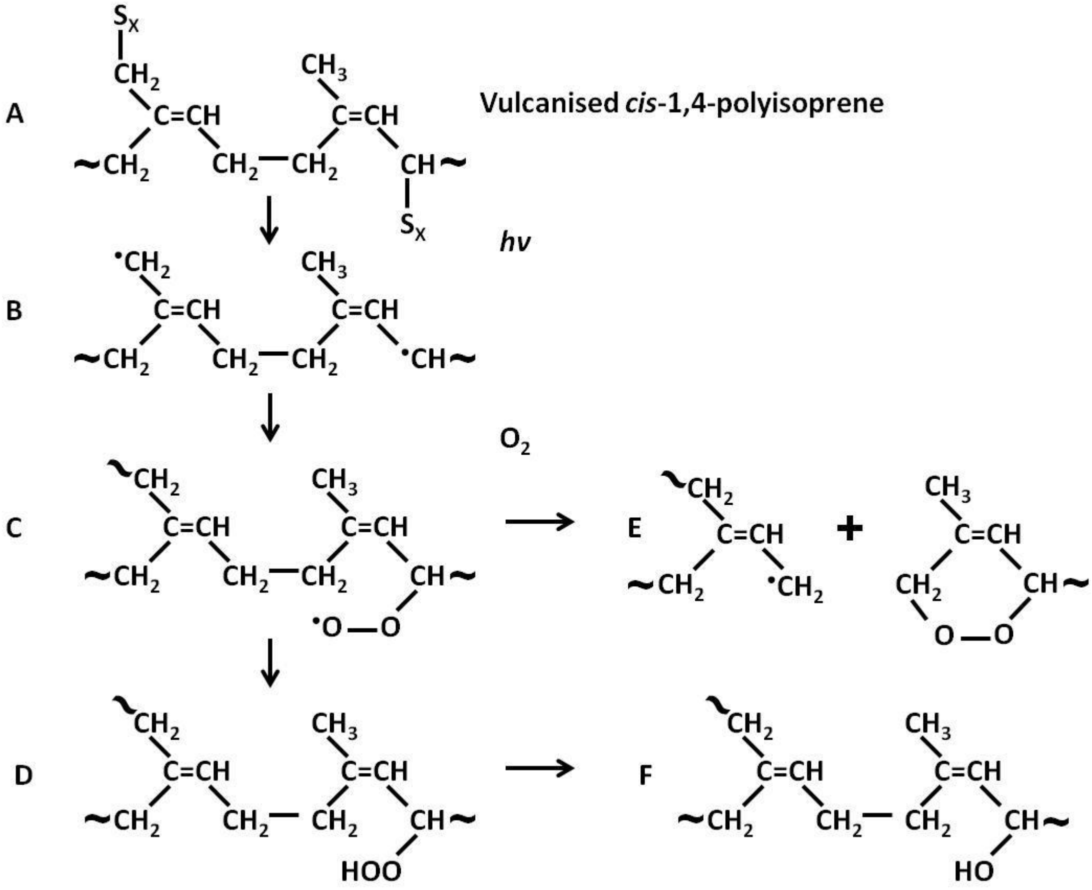


Figure 4.6 The cleavage of C-S links in NRL followed by oxidation reactions.

The breakage of S-S and C-S linkages results in a reduction in crosslink density, and the formation of intermediate hydroperoxide groups following Lambert *et al*., (2013) (Fig. 4.6 structures C and D). As the crosslink density is decreased it would be expected that chain scissions of the C-C bonds also take place. This can potentially cause the formation of cyclic links (Fig. 4.6 structure E) as described by Bevilacqua, (1955) and Colin *et al*., (2007), when discussing the oxidation of polyisoprene. Further chain scissions may occur by the activation of the double bond (Fig. 4.7 structure B) (Lambert *et al*., 2013; Linos *et al*., 2000), and by side branch formation induced by the presence of active hydrogen molecules in water because of N-dealkylation and hydrogen abstraction (Collin *et al*., 2012). An increase in chemical reactions causes the material to fragment at areas of high stress. This causes new surfaces to open up for further degradation to occur. An increasing number of chain scissions potentially lead to an increase in chain mobility, which in turn leads to a recombination of radicals, and cross-linking processes (Collin *et al*., 2012). Collins *et al*., (2012) have noted chain scissions are predominant for short irradiation times and cross-linking reactions are predominant for longer irradiation times, for polycarbonate degraded under UV light at 60 ^oC. There are various reactions that can lead to the formation of cross-links in NRL (Fig. 4.7). The absorption of light provokes the formation of radical species that can abstract a hydrogen atom on the methyl group and form a macro-radical (Fig. 4.7 structure C). This can then combine with oxygen, or a similar radical to form a cross-link with a $–CH_2-CH_2-$ bridge, via the combination of species that potentially originated from different polymer chains, as described by Collin *et al.*, (2012). Reactions such as isomerisation through chemical shifts could also yield further combinations (Fig. 4.7 structure D) (Buzaré *et al.*, 2001).

As the NRL degrades, volatile substances are also produced during the transformation of non- and semi- volatile substances, or directly from the parent material. Hexanal has previously been identified as a minor emission evolving from PVC flooring materials (Lundgren *et al.*, 1999), and is a major compound associated with the oxidative degradation of food stuffs, formed from intermediate hydroperoxides (Cognat *et al.*, 2012; Zhou *et al.*, 2000). Furans are also identified in the volatile mixture. Literature data indicates potential sources of furan formation come from the degradation of certain amino acids and poly-saturated fatty acids (Vranova and Ciesarova, 2009). The isolation of amino acids derived by hydrolysis from proteins of latex has also previously been described (Whitby and Greenberg, 1941). Given the complex nature of the parent material furan formation from the photooxidation of chemical additives also presents a potential degradation pathway.

The chemical diversity of the parent material and the many degradation processes that occur, mean that environmental systems receiving PBMs are potentially exposed to a complex mixture of the parent material as well as degradation and transformation products. This presents concerns over the ecotoxicity of degradate mixtures as they have the potential to be more biologically active than their parent compounds, as shown by Fenner *et al.*, (2002) whom identified nonylphenol monethoxylate, a DP of nonylphenol ethoxylate, to pose a greater risk than its parent compound.

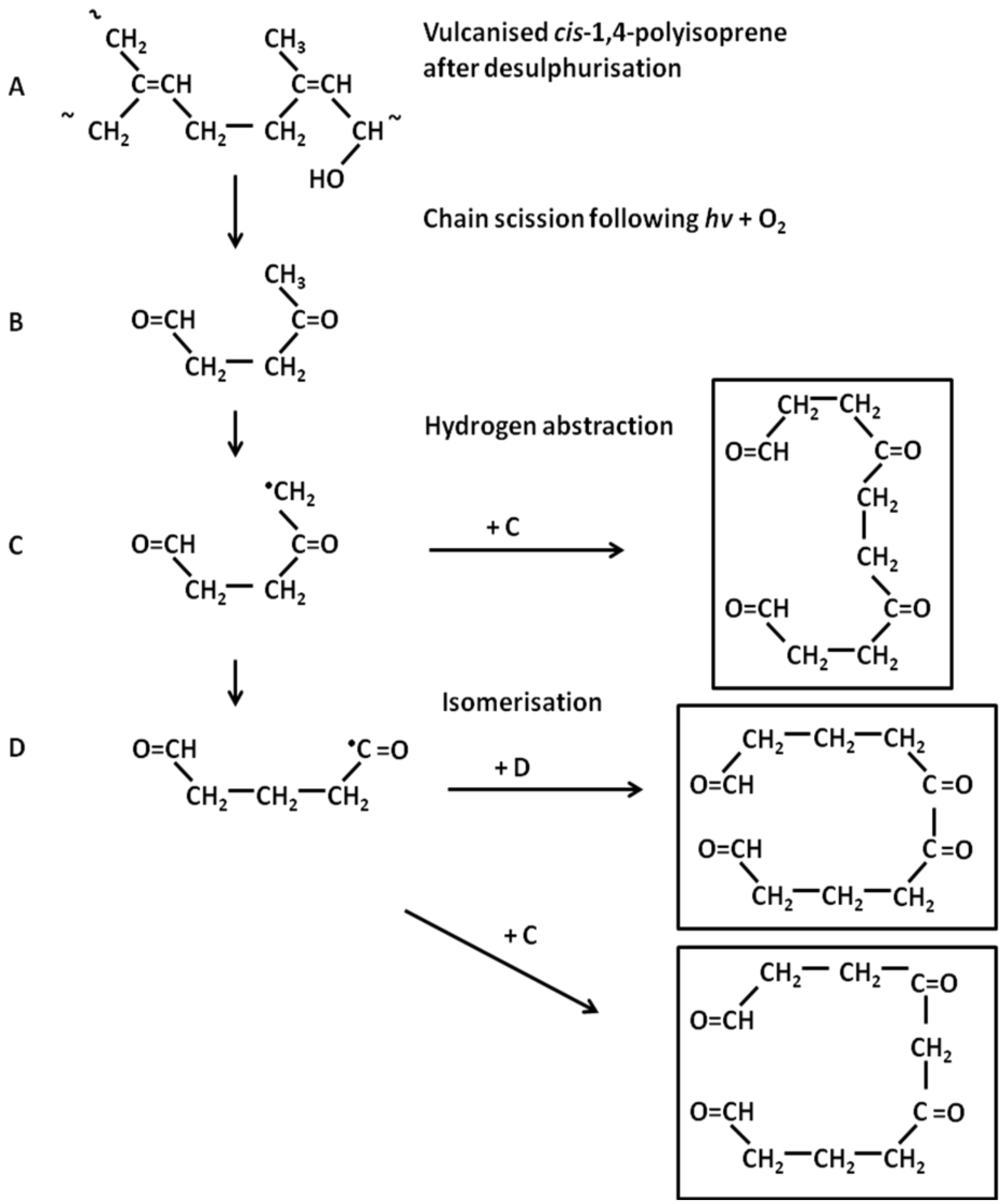


Figure 4.7 Photodecomposition of NRL with cross-linking reactions, adopted from Colin *et al*., (2007).

4.5 Conclusion

The chemical diversity of the parent material and the multiple degradation pathways that potentially co-exist, leads to the formation of a complex mixture of substances, which made identification of specific compounds challenging. This is because each reaction that takes place potentially leads to a further reaction that alters the molecular structure of the NRL, and also alters the structure of any formed degradates. The continuing process of transformation is highlighted by the PCA analysis. The obtained results show that the absence of light had a greater effect on the formation of degradates than material thickness. As the experiments were performed under natural sunlight irradiation, this can be explained by the absence of activation energy when light is excluded, and indicates the importance of photodegradation as the driving mechanism. The ecotoxicological risk of these complex mixtures should also not be ignored, given the concern as to the accumulation of PBMs in the environment. Therefore, in the next chapter laboratory studies were preformed to investigate the ecological effects of the NRL degradate mixture.

Chapter 5

Do NRL degradation products pose a risk to aquatic systems?

5.1 Introduction

In chapters 3 and 4, outdoor microcosms were combined with a range of analytical techniques to characterise the formation of NRL degradates. The results from this experimental work indicate that receiving environments are potentially exposed to a mixture of the parent item, microscopic particles, leached additives, and polymer and additives degradation and subsequent transformation products. As reviewed in Chapter 2, macro PBMs are known to present risks to wildlife via entanglement and ingestion, and the available data on the ecotoxicity of PBM additives focuses largely on those compounds that have a biological action. Microscopic PBM particles have been reported as floating on the ocean surface, mixed into the water column, and embedded in bottom sediments and beach sands, and their uptake has previously been reported for a range of marine biota (Browne *et al*., 2008; Cole *et al*., 2011; Graham and Thompson, 2009; Thompson *et al*., 2004). Nano-sized particles can also be derived from PBM debris and their formation has been quantified in Chapter 3. NPs are of particular concern as they are within the size range of viruses that can enter cells by endocytosis (Andrady, 2011). Once released or formed in the environment PBM particles are known to provide a surface for adsorbing and concentrating POPs (Endo *et al*., 2005; Fries and Zarfl, 2012; Mato *et al*., 2001; Teuten *et al*., 2007). This is thought to provide a potential pathway for POP uptake into organisms via the ingestion of polymer particles (Ryan *et al*., 1988). However, there are no studies currently that have investigated the uptake of POP loaded polymer particles to organisms (Andrady, 2011).

Presently, there is a growing body of research documenting the environmental occurrence of PBMs, but the environmental release of such materials is difficult to quantify. The domestic household is a significant contributor of PBMs to the waste stream with items disposed of through rubbish collection, and polymer-based personal hygiene products, such as condoms, disposed of to the sewage waste stream (Fig. 5.1) (Ashley *et al*., 2005; Williams and Simmons, 1999). Condoms in particular are an important mainstay for birth control, the reduction of sexually transmitted infections and HIV reduction (Cassell *et al*., 2006). It is estimated that 1.5-2 billion sanitary protection products are disposed of to the sewage system every year in the UK (MEMG, 2005). Screens at WWTPs have varied efficiencies at retaining solid items (Balmforth, 1990). Larger items are generally removed by coarse screens that typically have a mesh size of 6 mm and 5 mm, with estimated screen capture ratios (SCRs) of between 50-80 % depending on the screen type in use (e.g. spiral screens, slot screens), while fine screens typically have mesh sizes of 3-1 mm with estimated removal for flushed items of > 80 %, based on SCR (Thompson, 2012). Items disposed of via the sewage waste stream have the potential to decompose during transportation to the WWTPs. Microscopic particles and fibres can potentially pass through WWTPs and enter the environment via discharges of treated waters (Browne *et al*., 2011); whereas, bulk items may be released during overflow events during storm conditions.

An understanding of the release pathways of PBMs and the ecological effects of their degradation products is essential to understanding the risks of PBMs to environmental systems. The aim of this chapter was therefore to i) establish the extent to which NRL condom degradates affect the viability of aquatic organisms; ii) to evaluate the environmental risks of the NRL degrades; and iii) explore the extent to which a POP (fluoranthene) will influence toxicity of NRL degradates.

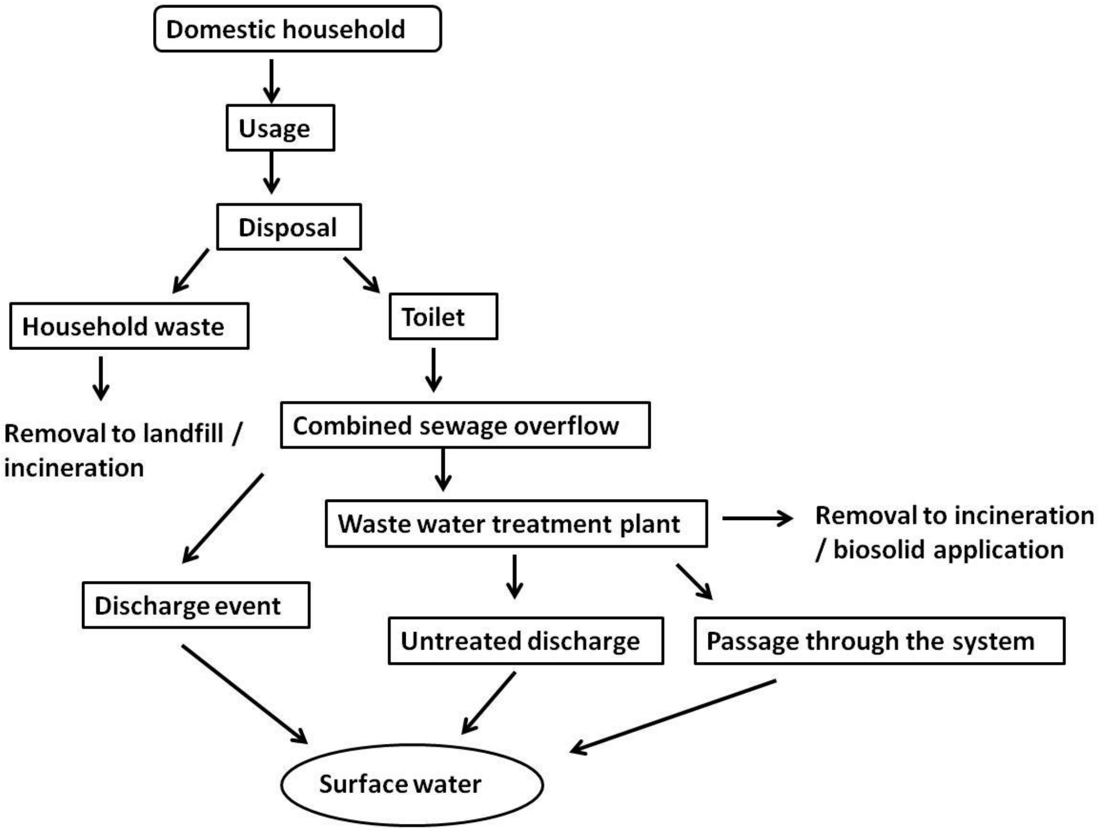


Figure 5.1 Fate of polymer-based personal care products from the domestic household to the environment

5.2 Methods

5.2.1 Materials

The NRL material is described in section 4.2.1. Analytical grade fluoranthene was purchased from Sigma-Aldrich (Gillingham, UK).

5.2.2 Generation of NRL degradates

To conduct ecotoxicity tests NRL degradates were generated using outdoor microcosms. Test solutions were aged for t = 1, 3, 7, 14, 28, 56, 112 and 200 days starting from June 2011. Each time point consisted of two treatment groups; one with the presence of NRL material at 0.75 g / L demineralised water, and the second without the presence of NRL (effectively an aged water control). This ratio was used to mirror the NRL-to-water ratio used in Chapters 3 and 4. Microcosms were established using stainless steel containers and covered with a non ultra-violet filtering perspex sheet (B&Q, UK) to prevent flooding by rainfall. Evaporation was dealt with by adding demineralised water to the desired level. Upon collection, the bulk latex material was removed by sieving through a 2 mm sieve. The samples were then further filtered using a 1.6 µm pore diameter, glass fibre paper (Whatman, UK).

5.2.3 Analysis of degradate solutions

NTA was used to determine the concentrations of NRL particles formed in the generation of NRL degradates mixtures. The procedure used is described in section 3.2.2.3. The mass of particles present in the NRL degradate mixtures was estimated using the procedure described in section 3.2.3.3.

5.2.4 Test organisms and their cultivation

The test organisms were chosen to represent freshwater aquatic species with different life cycle traits. The freshwater crustacean *Daphnia magna* lives in the water column and as a filter-feeding organism can catch suspended particles, and may inadvertently ingest foreign materials from surrounding water (Gillis *et al*., 2005). The second test organism was the sediment larvae of the aquatic midge *Chironomus riparius*. Both organisms hold an important position in ecological food webs, are distributed globally, and are used extensively to assess both acute and chronic toxicities of sediment and water pollutants (Ha and Chio, 2008; Hartmann *et al*., 2012).

D. magna were cultured in M4 medium, at a constant temperature (21 ± 0.5 ^{o}C) with a 16-8 h light-dark photoperiod. Cultures consisted of 2 L glass beakers containing 1.5 L of culture media and 20 – 25 daphnids. Neonates were removed three times a week and the culture medium was renewed once a week. Daphnids were fed with the green algae *Desmodermus subspicatus* three times a week (0.2 mg carbon per individual per day). *C. riparius* were cultured in an insect breeding and rearing cage aquarium (BugDrom) using artificial freshwater medium ($CaCl_2$ 294 mg/L; $MgSO_4$ 123.25 mg/L; $NaHCO_3$ 64.75 mg/L and KCl 5.75 mg/L), at a constant temperature (20 ± 0.5 ^{o}C) with a 16-8 h light-dark photoperiod, and constant aeration. Cultures were fed with fish flake food (Tetramin, Tetrawerke, Melle, Germany) at a rate of 0.5 mg per larva per day.

5.2.5 Direct and indirect toxicity of NRL degradates

5.2.5.1 Direct toxicity of NRL degradates

The aquatic ecotoxicity of NRL degradates was tested using both acute and chronic endpoints, by comparing the effect of those solutions with the presence of NRL degradates to those without, at various dilutions.

Acute toxicity tests

Daphnid acute toxicity tests were performed under the same conditions used for the cultures. All sampling time points were tested and involved six test dilutions (0, 10, 25, 50, 100, 200, 500 ml / L M4 media). Each test dilution was made up of five replicates, and each replicate consisted of five *D. magna* neonates (< 24 h) in 20 ml glass vessels with 10 ml test solution. The test vessels were covered with a glass lid and the number of immobile organisms was counted after 48 h.

Chronic toxicity tests

Daphnid reproduction and growth were assessed in a semi-static test design, using t = 56 and 112 day NRL degradate samples only. The tests were performed under the same conditions to those under which the culture was kept. Daphnids aged < 24 h at the start of the test were exposed for a period of 21 days (Fig. 5.2) to a set of seven test dilutions (0, 10, 50, 100, 200, 350 and 500 ml/L M4 medium). Each test dilution consisted of 5 test organisms held individually in 80 ml of test solution. Test solutions were renewed once a week and pH was measured. To assess impacts on reproduction, neonates were removed from test vessels and counted daily. To measure impacts on growth, body length was measured at the start and end of the exposure period using an incident light scanner (Canon, CanoScan 8800F) at a resolution of 1200 dpi. To do this, individual organisms were transferred to a Petri dish and the media was removed until organisms were observed to display minimal movement. The images were then analysed with purposely designed software (T.G. Preuss, Institute for Environmental Research, Aachen, Germany). Body length was defined as from the top of the eye to the base of the spine (Agatz *et al.*, 2012).

C.riparius emergence was assessed using a static test design based on t = 200 day NRL degradate sample only. The test was performed under the same conditions to those under which the culture was kept. Egg masses were obtained from cultures and transferred into vessels containing culture medium. First instar larvae (1-4 days post hatching) were used in the test. Hatched larvae were exposed for 28 days to a set of six test concentrations (1, 10, 50, 100, 200, 500 ml/L) made up with artificial freshwater medium. Each treatment consisted of 20 larvae held together in 500 ml beakers with sediment added to a depth of 3 cm. Sediment was prepared according to OECD 219. Test vessels were capped with 473 ml plastic food containers and aeration was provided using an aquarium pump through a Pasteur pipette via suitable tubing and placed so as not to disturb the sediment. Media was replenished daily to avoid desiccation. To assess impacts on emergence, organisms were removed from test vessels and counted daily.

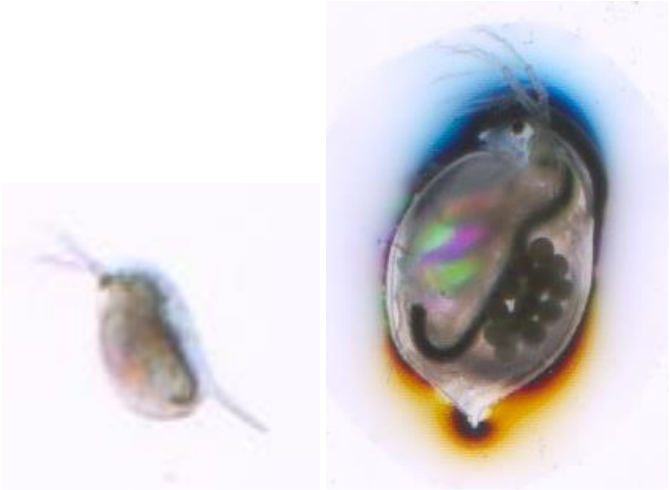


Figure 5.2 *D. magna* at $t = 0$ d (left) and $t = 21$ d.

5.2.5.2 Indirect toxicity of NRL degradates

A test was devised to investigate some of the current concerns regarding polymer particles providing an exposure route for POPs. Initially, the acute EC_{50} of fluoranthene, to *D. magna*, was established after 48 h exposure to test concentrations of 0, 60, 80, 100, 120, 140, 160 and 180 µg/L of fluoranthene in M4 media, including a solvent control at 1 %. Then various dilutions of the test solutions, with and without NRL degradates aged $t = 200$ days, were then spiked with fluoranthene at its calculated EC_{50}. The immobility of *D. magna* between the two treatment groups was then compared. A solvent control and M4 media control were also use to verify the test conditions.

5.2.5.3 Statistical analysis

A two-way ANOVA was utilised to compare immobility, reproduction, growth and emergence across concentrations and treatment groups. All pairwise multiple comparisons were conducted using Tukey test. Prior to all tests, data were tested for normality and equal variance by utilising the Shapiro-Wilk and Levene-Mediane tests, respectively. When comparing particle concentration between the NRL degradates mixture and aged water controls, both normality and equal variance tests failed, so Friedman Repeated Measures Analysis of Variance on Ranks was used. For the calculation of EC-values and confidence intervals a Sigmoidal, 3 Parameter model was used. All statistical analysis was performed using SigmaPlot version 12 and a significance level of 0.05.

5.2.6 Risk characterisation

Predicted no-effect concentrations (PNECs) were derived from the chronic no-observed effect concentrations (NOECs) by applying an assessment factor (AF) of 100 (TGD, 2003). Predicted environmental concentrations (PECs) for parent NRL condom material was taken from Catherine Johnson’s Master’s project. The project modelled emissions of a variety of polymer-based personal care products to the Rivers Ouse and Derwent catchment surface waters. For the purposes of the model, it was assumed that a certain percentage of condom material would flow through the sewage treatment process based on SCR described in section 5.1. Furthermore, the NRL were classed as a conservative waste type with no in-stream removal, assuming all items are transported downstream with no degradation. The risk ratio of PEC/PNEC was then calculated.

5.3 Results

5.3.1 Environmental fate of latex condom material

NTA was used to characterise the concentration and size distribution of particles < 1.6 µm formed during the generation of NRL degradates (Fig. 5.3). The concentration of NRL particles < 1.6 µm in the degradate mixture was shown to increase over time when compared to the control samples ($\chi^2 = 6.75$, $p = 0.009$). Generally, the mean particle diameter remains constant, but variability increases over time. Overall, the sizes of particles formed were mainly in the 100 – 500 nm range. The mass of NRL particles present in the degradate mixtures was also estimated, based on the particle size distribution and concentration, and was seen to increase in line with particle concentration ($r^2 = 0.974$, $p < 0.001$).

In chapters 3 and 4, the degradation of NRL in outdoor freshwater microcosms (pH 7.9) has highlighted the degradation solutions as consisting of a complex mixture of dissolved substances (Fig. 5.4). The samples for this work were generated during the same time period as the samples characterised in chapter 3 and 4.

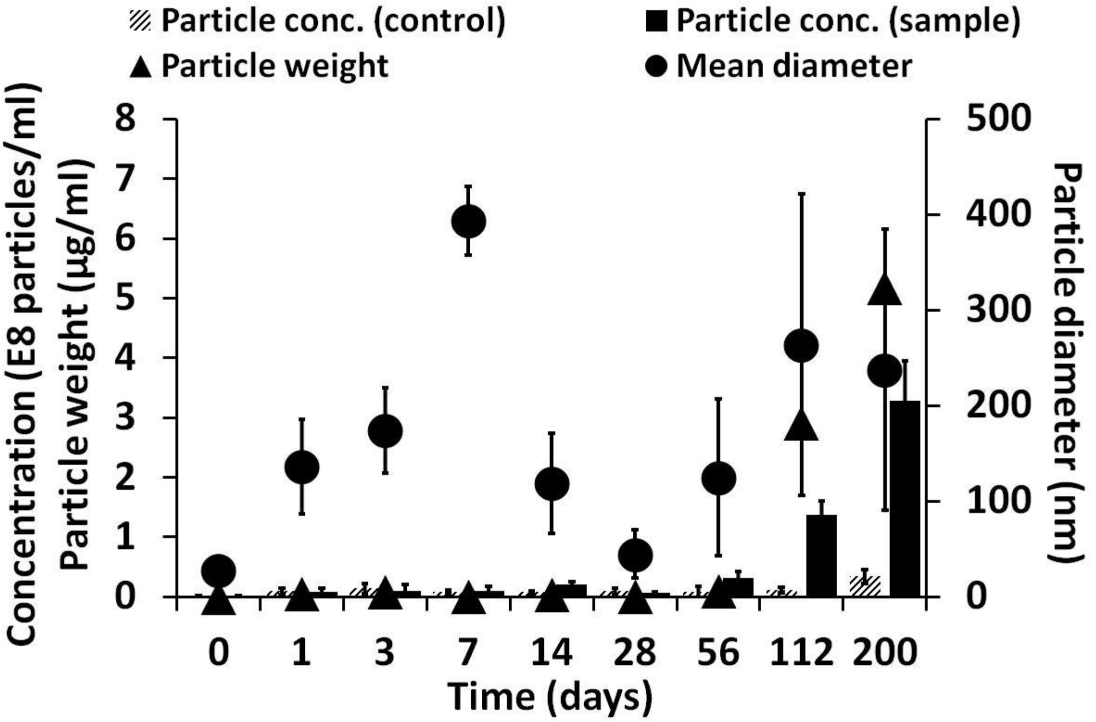


Figure 5.3 Characterisation of nano-sized particles formed during the degradation of NRL in outdoor microcosms. Solid bars represent particle concentration in the sample filtrate; bars with diagonal lines represent particle concentration in the controls; circles represent mean particle concentration in the samples and triangles represent particle weight. Error bars displayed were obtained by the standard deviation of the different measurements for each sample. The standard deviation presented is the standard deviation between the six replicate analyses for the sample.

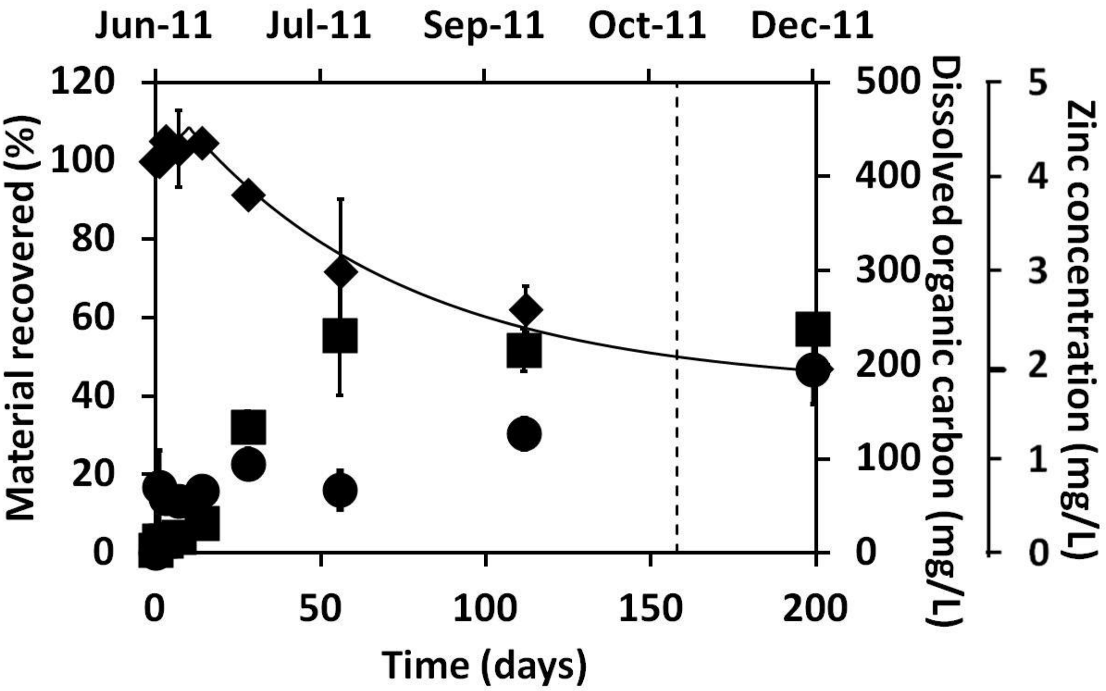


Figure 5.4 Summary of data taken from the artificial freshwater microcosm experiment (pH 7.9) where NRL was exposed under the same conditions as the degradate mixture. Diamond shaped data points represent mean material recovery of three replicate measures; the solid line represents modelled data; the vertical dashed line represents the point at which 50 % degradation is reached, the squares represent dissolved organic carbon and the circles represent Zn. Error bars displayed were obtained by the standard deviation of the different measurements for each sample; where not visible, bars fall within the symbols.

5.3.2 Direct NRL degradate toxicity

The concentrations tested can be expressed as their equivalent DP concentrations (mg/L), based on Fig 5.4 (Table 5.1). The generated treatment groups were not acutely toxic to *D. magna* when immobility in the presence and absence of latex DPs, at the various test concentrations, was compared for each time point (Table 5.2). The chronic toxicity tests showed no differences in the growth of *D. magna* when the presence and absence of latex DPs was compared for the time points tested (t = 56, p = 0.73, F = 0.121 and t = 112, p = 0.188, F = 1.792; Fig. 5.5b). Reproduction was identified to be greater in the presence of degradation products for t = 56 days ($p < 0.001$, F = 33.742; Fig. 5.4a). This is potentially due to the increased concentration of organic carbon in this mixture, originating from the degraded latex (Table 5.1). It is assumed that the organisms use this additional carbon as a food source, enhancing their reproductive output. For samples aged t = 112 days, no differences in reproduction between the two treatment groups were identified (p = 0.483, F = 0.501; Fig. 5.5). In view of the results no other samples were tested.

There was no concentration-dependent decrease in emergence of *C. riparius* after 28 days exposure to the two treatment groups (t = 112 days, p = 0.495, F = 0.561; Fig. 5.5c). Due to the results obtained, EC_{50} values for reproduction and growth (*D. magna*), and emergence (*C. riparius*) could not be calculated. Therefore, investigation did not proceed further than these initial tests and other generated samples were not tested.

5.3.3 Indirect NRL degradate toxicity

The acute EC_{50} for fluoranthene was shown to be 136.3 µg/L (Fig. 5.6), and compares well with previous published EC_{50} values for fluoranthene of 117 µg/L (Spehar *et al.*, 1999). No significant differences in *D. magna* immobility were identified when the test organism was exposed to fluoranthene in the presence and absence of NRL degradates (F = 1.894, p = 0.172; Fig. 5.7).

Table 5.1 The test concentrations used in the acute and chronic *D. magna* and *C. riparius* tests, and their equivalent concentration of NRL degradates based on Fig. 5.4.

	Equivalent concentration of degradation products (mg/L)							
Test concentration (ml/L)	*t* = 200 d	*t* = 112 d	*t* = 56 d	*t* = 28 d	*t* = 14 d	*t* = 7 d	*t* = 3 d	*t* = 1 d
10	2.39	2.16	2.32	1.34	0.32	0.17	0.13	0.14
25	5.99	5.419	5.80	3.36	0.80	0.44	0.34	0.35
50	11.98	10.83	11.61	6.72	1.61	0.89	0.68	0.71
100	23.97	21.67	23.23	13.45	3.22	1.78	1.36	1.42
200	47.95	43.35	46.46	26.90	6.44	3.57	2.72	2.84
350	83.92	75.87	81.31	47.08	11.28	6.26	4.77	4.98
500	119.89	108.38	116.16	67.25	16.11	8.94	6.82	7.12

Table 5.2 The NOEC for acute and chronic toxicity of NRL degradates to *D. magna* and *C. riparius*.

	Acute endpoint	Chronic endpoint		
Time (days)	Immobility *D. magna* NOEC based on DP conc. (ml/L)	Reproduction *D. magna* NOEC based on DP conc. (ml/L)	Growth *D. magna* NOEC based on DP conc. (ml/L)	Emergence *C. riparius* NOEC based on DP conc. (ml/L)
1	> 500	—	—	—
3	> 500	—	—	—
7	> 500	—	—	—
14	> 500	—	—	—
28	> 500	—	—	—
56	> 500	> 500	> 500	—
112	> 500	> 500	> 500	—
200	> 500	—	—	> 500

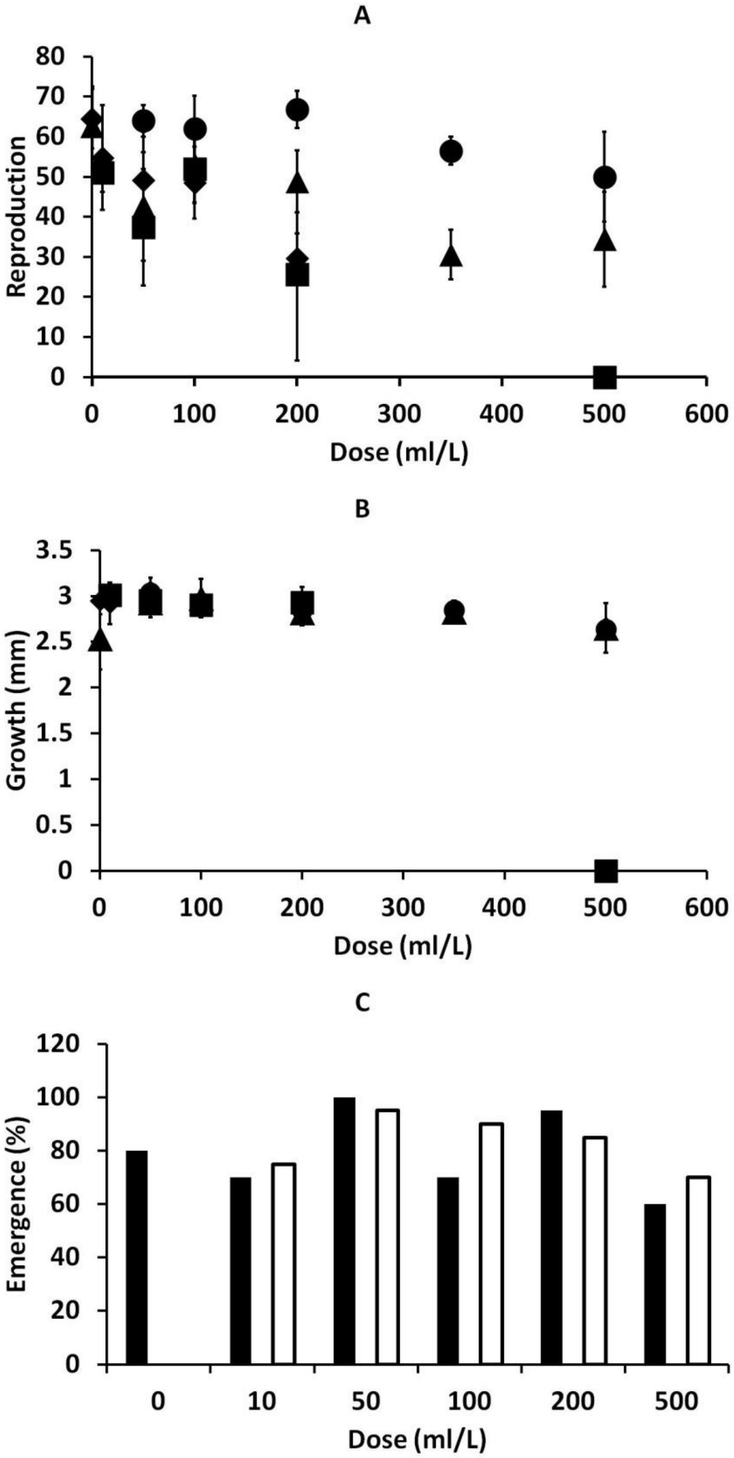


Figure 5.5 The chronic effects of NRL degradates on reproduction and growth to *D. magna*, and on emergence to *C. riparius*; where diamonds = absence and squares = presence of NRL degradates aged 112 days, triangles = absence and circles = presence of NRL degradates aged 56 days, and solid bars = absence and hollow bars = presence of NRL degradates aged 200 days.

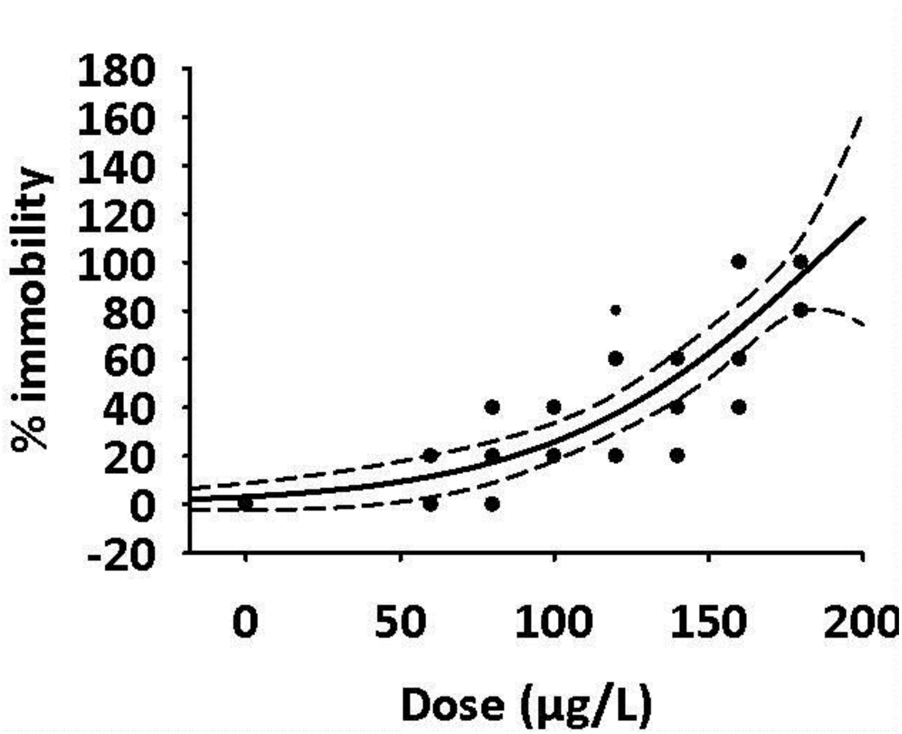


Figure 5.6 The acute toxicity of fluoranthene to the freshwater organism *D. magna*. The solid line represents the modelled response curve; the long dashed lines represent 95 % confidence intervals; and the short dashed line represents the effect concentration (EC_{50} = 136.3 µg/L).

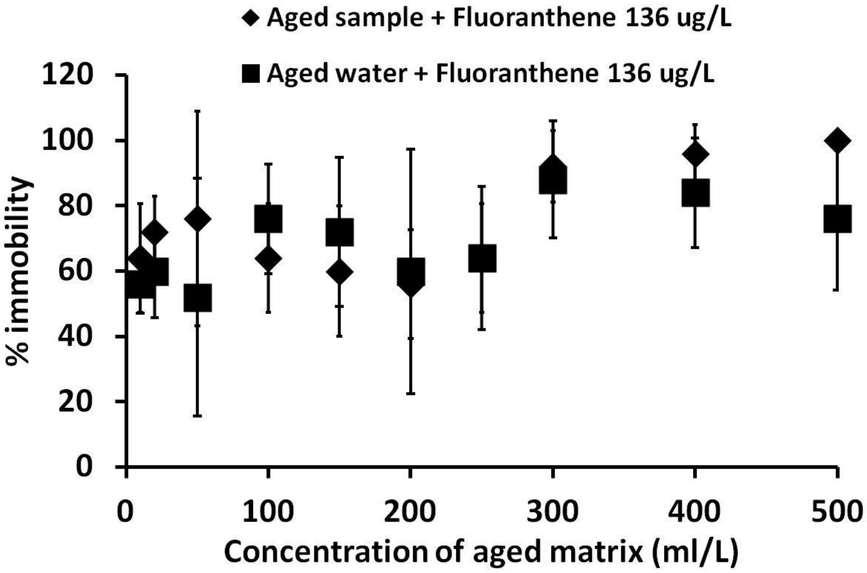


Figure 5.7 Immobility of *D. magna* in the NRL degradate mixture and aged water control samples (200 d), with the addition of fluoranthene at 136 µg/L.

5.3.4 Modelled concentration s of condoms to catchment surface waters

The annual mean concentrations of parent condom material for catchment surface waters were greatest for the Rivers Aire and Calder downstream from more highly populated locations of Leeds and Halifax (Fig. 5.7). The Nidd is also highlighted as having potentially sensitive stretches. The Rivers Derwent, Ouse, Swale, Ure and Wharfe have low predicted concentrations as the surrounding areas are predominately agricultural. When the model was run assuming 80 % screening efficiency by STPs, annual mean and median concentrations were 0.19 µg/L (± 0.3) and 0.06 µg/L (± 0.27), respectively; with predicted maximum concentration of 1.63 µg/L. The seasonal catchment concentrations describe a bell-shaped concentration curve over time with high peak concentrations in the summer months, when inputs to the river system are low.

5.3.5 Risk ratios and assessment

The PECs for the surface water modelling are clearly low, so in the worst case scenario, the annual maximum concentration was used (1.63 µg/L) to estimate risk ratios. The results of the ecotoxicity tests preclude the derivation of a genuine PNEC, so the chronic-based surface water PNEC was based on the concentration of NRL degradates in the samples tested (Table 5.1). The PEC of 1.63 µg/L is therefore five orders of magnitude lower than the highest concentrations of NRL degradates tested. Hence, the risk ratio for surface waters is below 1, so no significant risk is expected from NRL condom degradates.

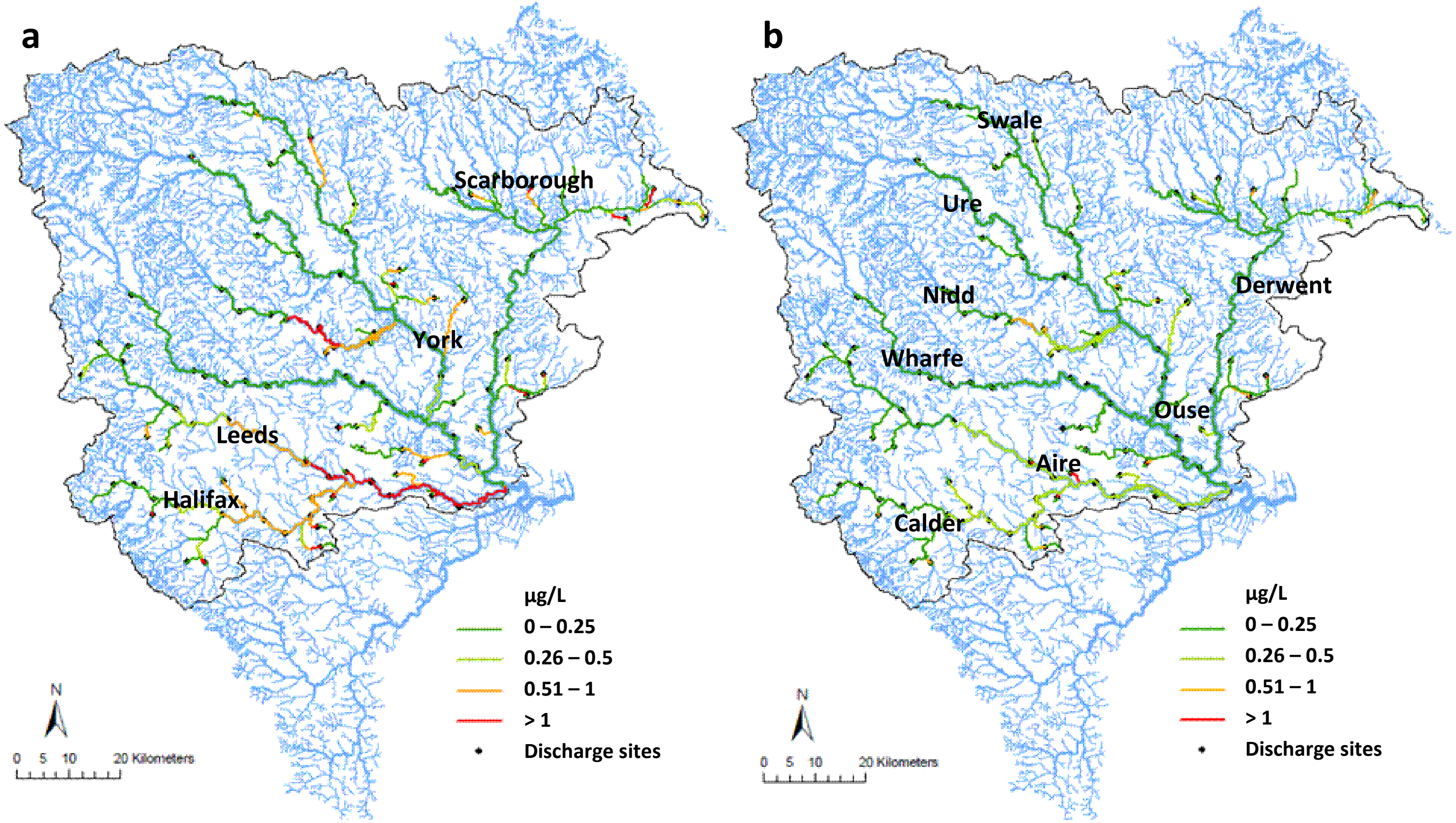


Figure 5.8 The annual mean surface water PECs of parent condom material in the Ouse and Derwent catchment. Plot (a) corresponds to 50 % screening efficiency and identifies the major urban centres, and plot (b) corresponds to 80 % screening efficiency and identifies the major rivers.

5.4 Discussion

The sources from which PBMs enter the environment are becoming better understood, and the importance of each is known to differ between geographical locations, depending on infrastructure (Chapter 2). However, the environmental releases of PBMs are difficult to quantify, because inadvertent littering is problematic to monitor, and in general people do not admit to littering or dumping of unwanted waste. This is the first study to quantify the environmental release of a case-study PBM, and to then combine this with long-term effect data for naturally weathered degradates. Condoms are an interesting case study material as they are a personal care product with a complex chemical makeup and a high market turnover. To facilitate their study, a survey was designed to investigate disposal habits. Condom use with subsequent exposure to WWTPs was estimated based on a questionnaire survey. Factors that may influence condom usage are the frequency of intercourse and the probability of a condom being used, and disposal habits are influenced by a range of socio-demographic factors, such as age, and education (Kao and Manczak, 2010; Meekers and van Rossem, 2005).

The major rivers in the catchment system have a number of sewage treatment facilities and combined sewage overflows distributed along them and will receive wastewater discharges from both domestic and industrial effluents. The occurrence of SRD and residues of such materials in river systems can vary and depends on population demographics, individuals’ flushing habits and the types of items flushed. The intensity and type of flow in the receiving watercourse will determine the environmental concentrations. When trying to quantify the amount of condom material that may enter the environment via overflow events, the variability of spill events should be taken into account.

The nature of NRL degradates was shown to be made up of a complex mixture of substances (Chapter 3 and 4), and individual components could not be tested. Therefore, it was decided to test the direct toxicity of the NRL mixture. None of the samples generated were toxic even though zinc concentrations (Fig. 5.4) are within the range (0.05 mg L^{-1} to 2.5 mg L^{-1}) reported as causing long-term effects on microbenthic communities (Niederlehner and Cairns, 1993; Paulsson et al., 2000; Loez et al., 1995). However, zinc toxicity is generally associated with the presence of free zinc and the zinc measured is more likely associated with zinc based accelerators and activator compounds. The presence of humic acids in the degradation mixture may also help to mitigate both acute and chronic toxicity. Humic acid is thought to zinc toxicity by decreasing the amount of free metal ions though zinc–humic acid complexes, these complexes have high molecular weight, are relatively stable with regard to metal-exchange reactions, and consequently the metals were less bioavailable (Koukal et al., 2003; Paulauskis and Winner, 1988).

These results largely agree with those of Lither *et al*., (2009) who conducted an extensive acute screening of leachates from 32 new polymer-based consumer products to *D. magna*, and found 48 h EC_{50} of leachates ranged from an equivalent of 5-80 g of material/L. The additional concern was the chronic exposures of organisms to the physical component of the NRL degradate mixture (< 1.6 µm). Studies using engineered nano particles (ENPs), such as nano-TiO_2 (Zhu *et al*., 2010), quantum dots (Lewinski *et al*., 2010), nano-Ag (Zhao and Wang, 2010), and carbon nano-tubes (Petersen *et al*., 2009), have shown uptake by *D. magna*. In this case, uptake of the physical component was not directly measured, but the chronic *D. magna* and *C. riparius* tests did not show any effects. The exposure of the test organisms to the complex NRL degradate mixture was more reflective of realistic environmental exposure scenarios, and the impacts take into account the interacting effects of both physical and chemical degradates. Future studies may want to monitor effects towards fish species, and investigate developmental abnormalities and subsequent multigenerational effects that can occur when reproduction and growth in the first generation are not affected.

To examine some of the broader implications of PBM degradates, fluoranthene was added to assess any indirect effects. The concentrations of POPs in aquatic systems are typically low, but the risk posed by microscopic particles concentrating high levels of POPs could be significant (Andrady, 2011). Fluoranthene was chosen as a test substance as this has previously been shown to sorb to plastic fragments collected from the North Pacific Central Gyre and the Japanese coast (Teuten *et al*., 2009). It was thought that its relatively high Log K_{ow} of 5.12 would allow the compound to adsorb to the micro and nano NRL particles in the degradate mixture. This could then give two possible outcomes that would differentiate the NRL degradate samples from those where NRL degradates were absent, i) a case where the compound is sorbed to the particles so is unavailable to exert an effect or, ii) a case where the compound is sorbed to the NRL particles and exerts an effect through ingestion. It has already been shown that nano-TiO_2 has the potential to facilitate transport of cadmium into the gut of *D. magna* (Hartmann *et al.*, 2012). If POPs were to be associated with polymer nano-sized particles, because of their small size and increased surface area, this may facilitate the uptake of POP into organisms and cells. However, no such relationships were observed. It is thought that in this case, the fluoranthene is mainly present in the water phase for both treatment groups, because the mass of particles in the degraded latex sample is very low. Further investigation into the indirect effects of micro and nano polymer particles should focus on uptake and depuration of POPs in the presence and absence of polymer particles.

5.5 Conclusion

The present study attempted to quantify the emission of a case study polymer from the domestic household via the sewage waste stream. This information was then used to predict potential environmental concentrations of condom material at a catchment scale. To put the predicted concentrations into context, the effects of NRL degradates to two freshwater organisms, were investigated. The NRL degradates samples used, as far as possible, were degraded under environmentally relevant conditions, and produced a complex mixture of substances. The results were conclusive in that no detrimental effect as a consequence of exposure to NRL degradates was observed. As the concentrations used were orders of magnitude higher than the modelled surface water concentrations, it can be assumed that NRL degradates pose limited environmental risk. It is recommend that future tests are conducted by widening the range of PBMs used by identifying those that have a large production volume, high content of additives, and high potential for additives to have an adverse effect. The wider implications of the environmental occurrence of PBM particles needs future investigation in terms of which polymers adsorb POPs, by using polymer particles of varying sizes and types. This is important because POPs have the potential to display different sorption behaviours to different polymer types. Sub-lethal concentrations of POPs and particle mixtures should also be investigated to quantify any long-term effects.

Chapter 6

General Discussion

6.1 Aim of thesis

The first documentation of the environmental occurrence of PBMs was in the early 1970s (Carpenter et al., 1972; Colton et al., 1974). Since then, human activities have resulted in large volumes of PBMs entering the environment (reviewed in Chapter 2). In recent years, the environmental occurrence of PBMs and their associated chemical additives in the aquatic environment have been recognized as an emerging worldwide problem, and their impacts are now a subject of wider scientific and social interest (Hammer *et al.*, 2012; Thompson *et al.*, 2009). In the present study, the degradation of a case study PBM in aquatic systems was investigated (chapters 3 and 4), and the subsequent ecological effects of formed degradation products to freshwater organisms were explored (chapter 5). The case study material was a mixture of *cis*,1-4 polyisoprene (Fig. 4.1) and a number of additive compounds used to manufacture NRL condoms.

6.2 Main findings

Outdoor microcosm studies were established to investigate the degradation of NRL under a range of environmental variables. This experimental design was chosen to expose the NRL samples to natural cycles of sunlight and temperature. The variables investigated were seasonal variations in weather conditions, difference in media pH, sample movement, material thickness, and the presence or absence of light.

6.2.1 Effects of the investigated variables

The driving variable was shown to be the presence of sunlight, because when light was completely excluded, weight loss was negligible over the study duration. The importance of sunlight was further highlighted by the investigation into how seasonal variations in weather conditions impacted weight loss. When exposure was initiated under winter conditions, the weight loss data indicated a lag-phase until sunlight intensity reached a point in the spring where decomposition of the NRL was induced. Previous studies have also shown increased outdoor degradation during summer conditions (Al-Salem, 2009). A number of approaches have been developed to model degradation kinetics, mainly for pesticides. Here, the FOCUS (Forum for the Co-ordination of pesticide fate models and their Use) models were adapted and fitted to the experimental data. This process showed that biphasic degradation patterns best describe the measured data as periods of elevated sunlight intensity correspond to increased weight loss and periods of declining sunlight intensity correspond to decreased weight loss.

The pH of the degrading solutions was shown to affect the degradation half-lives for the latex film used. The calculated DT_{50} shows that the NRL degraded faster in the lower pH treatment than in the freshwater (pH 7.9) or salt water solution. Generally, degradation rate of PBMs is considered to be much slower in marine environments because of lower surrounding water temperatures (Watters *et al.*, 2010). Movement is an environmental variable that needs to be considered as receiving environments are generally not static. The inclusion of movement was found to slow the degradation rate (DT_{50} 220 d) is terms of the calculated DT_{50}, when compared to the freshwater treatment of the same pH (DT_{50} 158 d). Possible explanations for this are that the movement applied in this study design was enough to cause disintegrated segments to coagulate, forming a thicker material. The thickness of the material was shown to greatly influence weight loss.

6.2.2 The formation of degradation products

Exposure to sunlight was show to initiate changes in the chemical functionality of the latex film. These changes included the chain scission of molecular bonds that is often random, making degradation pathways difficult to quantify. However, bond cleavages are most likely to occur first at those bonds with lower strength (Rooj *et al.*, 2011). This process is termed ‘depolymerisation’ and is generally thought to start at polymer end chains until fragmentation of the material occurs; opening up new areas for further degradation. Chain stripping is another degradation mechanism were side groups attached to the polymer chain are cleaved (Wilkes, 2005). These are often of a lower molecular mass so are easily volatile (Wilkes, 2005), suggesting the atmosphere acts as a sink for PBM degradation products. Cross-linking reactions are also known to occur were new bonds between polymer chains are created (Collin *et al.*, 2012; Wilkes, 2005).

A key finding in this work was the characterisation of particles in the nano-range formed during the degradation process. The lower concentrations observed in the marine water treatment, when compared to the freshwater treatment is most likely the result of differences in water density. Salt water is denser than freshwater; and so any particles disintegrating from the parent latex will float and agglomerate into larger clusters, or come into contact with larger floating latex particles and from aggregates. No differences in NP formation between the NRL film and thicker NRL material were observed, even though weight loss for the thicker material was minimal. The particles measured in the thicker material treatment indicate surface erosion of the polymer (Fig. 6.1) (Klemuck, 1990; Tushii *et al.*, 1997).

The decomposition of the NRL material initiated the release of zinc compounds for the latex into solutions, and the formation of a dissolved component of unknown substances. There are many challenges associated with the characterisation of unknown mixtures, especially those that are potentially undergoing continuous transformations. The major sample preparation steps used included sample pre-treatment (filtering), clean-up (SPE and liquid-liquid extraction), and concentration (N flow) (chapter 4). Owing to the potential diversity of physic-chemical properties degradation products may exhibit, analytical challenges and limitations can limit multiclass compound analysis (Comerton *et al.*, 2009). The approach used in this work was to combine LC-MS and GC-MS techniques as this widens the range of compound properties that can be measured. The predicted formulas generated by LC-TOF-MS, for the different pH type used, were similar indicating that a common degradation pathway potentially exists.

The GC-MS data represented that of an unresolved complex mixture. A method of visualising this type of data is to apply PCA. The purpose of the pre-processing methods applied is to produce a better dataset without any relevant information loss (Christensen *et al.*, 2005). PCA is a good solution to reduce the number of variables in a dataset. Sample visualisation using PCA analysis showed separation of control samples from the degraded NRL samples, and when light was excluded, samples containing the latex film grouped themselves with the controls. This suggests minimal leaching of additives compounds and indicates that the additives used in the manufacturing processes are tightly bound to the polymer matrix. The PCA also highlights that a continuing process of transformations are occurring, as the spread of the samples in principal component space indicates the formation and subsequent degradation of unresolved mixture.

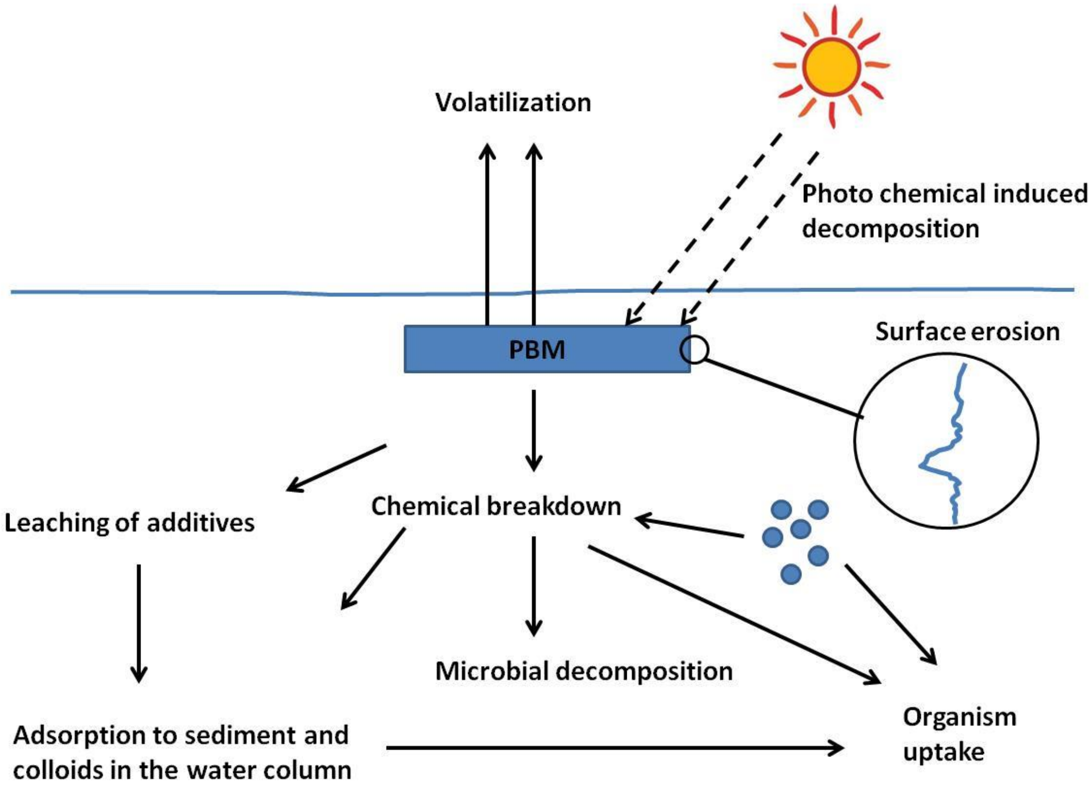


Figure 6.1 Processes acting on polymer-based materials in the environment

6.2.3 Ecological effects of NRL degradation products

This study aimed to investigate the ecological effects of NRL degradates to freshwater organisms. It was hypothesized that aquatic organisms would experience reduced viability, in terms of reproduction, growth and emergence, as a result of exposure to the combination of microscopic particles and chemical NRL degradates. Based on the results generated in this work, NRL degradates were shown to be no more toxic to the organisms tested than water aged for the same period of time.

6.3 Implications of the main findings

The results from Chapters 3 and 4 demonstrated that the degradation of NRL involves the disintegration of the material into increasingly smaller polymer fragments, including microscopic and nano-sized particles; chemical transformation of the parent PBM and polymer fragments; degradation of the PBM and polymer fragments into non-polymer organic molecules; and transformation/degradation of these non-polymer molecules into other compounds. For different PBMs, the types of degradates formed will depend on the degradation mechanism, which will be influenced by polymer and additive types used, and environmental factors. Examples of substances released from various PBMs are phthalates from plasticized PVC (Rijk and Ehlert, 2001; Tonning *et al*., 2010), flame retardants from television housings (Kim *et al*., 2006); bisphenol-A from PC food contact materials (Brede *et al*., 2003; Geens *et al*., 2010), lead from unplasticized PVC pipes (Al-Malack, 2001), and nonly-phenol from food contact materials (Fernandes *et al*., 2008). Chlorine (PVC), nitrogen (nylons, PURs, and polyacrylonitrile), and fluorine containing compounds (PTFE and polyvinyidene fluoride) can also be released during degradation processes (Folarin and Sadiku, 2011). Alongside these, many volatile organic compounds are also known to be released during a PBMs lifecycle (Lundgren *et al*., 1999).

The diverse nature of the NRL degradates formed may have important implications for their persistent, bioaccumulation and toxicity assessments. Microscopic polymer particles have been recovered from beach sands and estuary sediments, and may persist within environmental systems for long-time periods. The accumulation of micro and nano PBM particles into organisms can occur directly via ingestion while feeding, or uptake via respiratory surfaces (gills). The bioaccumulation of chemical degradates can occur directly from aqueous exposure, by both adsorption to external surfaces and uptake via respiratory surfaces (gills). The extent of transfer through the food chain, for both the microscopic particles and chemical NRL degradates, from prey to predators will be dependent on, i) the feeding behaviour of the prey (sediment ingestors, filter feeders) and that of the predator, as predators can feed on their prey whole or consume only internal tissues (Wang and Wang, 2006); and ii) the chemical properties and particles size.

Once in the environment PBMs are known to provide a surface for sorbing and concentrating persistent organic pollutants (POPs) (Endo *et al*., 2005; Ogata *et al*., 2009; Teuten *et al*., 2009). Fragmented polymer particles can be ingested by filter feeding and non-selective feeding organisms, providing a potential pathway for uptake of POPs into organisms (Ryan *et al*., 1998). A preliminary investigation made in Chapter 5 reported the interaction of fluoranthene with the generated NRL degradates. When POPs adsorb to polymer particles, the uptake of POPs into organisms and cells may be facilitated, due to their small size and increased surface area. However, this has yet to be fully explored. An understanding of the ecological effects of microscopic polymer particles and their interaction with other environmental pollutants is therefore essential to understanding the risks of PBMs to environmental systems. A more complete assessment of the toxicity and environmental risks surrounding the interactions of PBMs and persistent organic pollutants is required with a wider methodological approach covering different POPs and different polymer types of different size ranges.

6.4 Suggested methods for assessing the environmental risks of PBMs

This work has focused on a case study polymer, but assessing the environmental risk of PBMs remains an open question. PBMs are composed of polymers that determine the polymer type and additives that enable processing and give the material its desired properties. From a toxicity assessment point of view PBMs are considered as inert and of low environmental risk. However, several monomers and additives used in their production are identified as hazardous (Table 6.1).

Presently, only some polymer-based consumer products have restrictions on their chemical makeup, for example food contact materials (European Commission, 2002). However, there is the potential for the release of chemicals from PBMs to the environment at all lifecycle stages. For example, low molecular mass additives that are weakly bound or not bound, unpolymerised residual monomers, and degradates formed during exposure to weathering processes. Here, an extensive degradation study was undertaken, but this may not be appropriate for all new PBMs because of the time scale involved. However, BFRs, BPA and phthalates used in PBMs are widely occurring environmental pollutants (Table 2.6). Therefore, a more complete assessment of the environmental risks of PBMs that includes a wide methodological approach to covered emissions and ecotoxic effects is needed.

Table 6.1. Polymer materials identified as containing hazardous compounds (Rossi and Lent, 2006).

Polymer type	**Identified hazardous compounds**
PVC	Formation of dioxins during manufacturing and waste incineration. Additives include phthalate plasticizers which are suspected endocrine disrupting compounds. Older PVC formulations used lead and organotins to act as stabilisers, lead is a neuro-toxicant and organo-tins are known to impact sexual development.
ABS	A copolymer of acrylonitrile, butadiene-1,3 and styrene, all of which are classified as probable carcinogenic by the International Agency for Reseasrch on Cancer (IARC).
EVA	Made from vinyl acetate feedstock which an IARC possible carcinogen.
PC	Manufactured from BPA which as suspected endocrine disrupting compound.
PS	The monomer styrene is a suspected carcinogen.
PUR	Manufactured from formaldehyde an IARC known carcinogen and a variety of other chemicals that are classified as hazardous.
PET	Manufactured from ethylene glycol a possible developmental toxicant.

The environmental risk assessment of PBMs could be addressed through a more controlled assessment. This should involve leaching tests using extraction solvents to enhance chemical migration from the PBM to a surrounding contact media, combined with emission tests for volatile compounds following Bradley *et al*., (2007) and Lundgren *et al*., (1999). These tests should be supported by a robust analytical methodology that assess a wide range of compound properties, such as volatile, semi-volatile, non-volatile substances, and where appropriate the analysis of metals. This type of analytical strategy would allow for the identification of compounds in the generated leachate through the use of LC-TOF-MS and GC-MS that generate accurate mass and predicted formulae. The substances identified in the generated leachate can then be assessed for their persistence and bioconcentration potential using QSAR approaches, such as the EPI Suite. Persistence can be assessed using the BIOWIN model to evaluate biodegradation potential and bioconcentraion can be assessed using the BCFBAF model. The toxicity screening of the generated leachate mixture should then take a direct testing approach. This approach would use a dilution series to assess acute effects following Lithner *et al*., (2009); where acute effects at low concentrations are measured chronic effects should then be tested. This direct toxicity testing approach also has the advantage of assessing multiple substances at once, many of which may only be present at lower concentrations. This type of approach has the potential to differentiate between toxic and non-toxic polymer-based products. Therefore, providing against the unintended spread of chemicals into the environment.

6.5 Conclusions

The environmental occurrence, degradation, and effects of PBMs are issues gaining a wide social and scientific audience. NRL condoms represent an interesting case study material as they are a complex mixture of their representative polymer (polyisoprene), and a variety of additive compounds. Outdoor microcosm experiments were initiated to mimic natural conditions as far as possible. The results demonstrated that environmental degradation processes produce a complex mixture of microscopic particles, dissolved and volatile substances. The ecotoxicity of the degradates mixture was shown to have no toxic effects to the organisms tested. The types of PBM degradation products produced and their subsequent effects will depend on both the polymer and additive types used to manufacture a particular product. When thinking about the correct disposal methods for condoms, environmental education through a company’s advertising, combined with correct product labelling could help to prevent littering and flushing. Finally, from a wider perspective, the environmental risks of PBMs could be reduced by developing risk assessment methods that limit the spread of hazardous chemical to the environment.

6.6 Recommendations

Recommendations specific to this thesis:

1. To further understand the drivers that initiate changes in chemical functionality in the aquatic environment, an experiment where hydrolysis is specifically addressed would provide a better understanding of the degradation pathway.
2. As part of this work a degradation experiment using natural sediment sourced from a local stream was undertaken, however, the results from this study were inconclusive. Studies should be performed to understand to what extent

a realistic microbial community, and not a concentrated laboratory culture, utilise these dissolved substances and microscopic particles as a food source. This would result in a better understanding of the ultimate fate of NRL in aquatic systems.

3. Results from the outdoor degradation experiments show that as the NRL degrades in the aqueous medium a complex mixture of dissolved substances is formed. The PCA analysis showed that when movement was included as a variable, a more complex mixture of substances was produced. Studies performed using continuous movement would provide a more realistic scenario than static systems. This would result in an improved understanding of the degradation pathways.
4. A number of studies have investigated the uptake of microscopic polymer particles in a range of marine organisms. Further work is now required to better understand the long-term effects of polymer particle uptake and to what extent these particles stay in the organism or pass through them.
5. A number of studies highlight the ingestion of microscopic polymer particles as presenting a potential uptake pathway for POPs into non-selective feeding organisms. Studies that investigate the sorption behaviour of a range of POPs to a range of polymer particles of various types and sizes should be performed to better understand these interactions. This should then be followed by uptake and depuration studies for specific POPs and particle mixtures, in the presence and absence of polymer particles.

More general recommendations:

1. A number of studies now suggest that polymer particles used in facial scrubs and synthetic fibres from clothing will pass though the WWTPs. Further work is now needed to better understand the fate of these particles and fibres in WWTPs. Results could inform as to whether exposure of the terrestrial environment is of concern through land application of treated sludge.
2. The terrestrial environment is also a sink of polymer debris. Outdoor microcosm studies could also be used to investigate the fate of polymers in a range of soil types.
3. Following on from the two points made above, the uptake of PBM particles into soil organisms and their long-term effects should also be investigated.

4. This work identified the atmosphere as a receiving environment for volatile degradation products. The diffuse release of volatile compounds from polymer-based products, during all lifecycle stages, needs to receive more attention. This will help to reduce the risks from chemicals used in the production of PBMs, by identifying compounds for substitution.

Appendix 1

Table A.1 pH data for all treatments

August 2010 treatment				
Time (days)	**Control**	**Rep a**	**Rep b**	**Rep c**
0		6.47	6.35	6.29
10	5.75	4.82	4.53	4.56
20	6.15	4.08	4.14	4.02
35	6.24	4.22	4.79	4.49
50	6.37	4.82	4.59	4.92
90	5.22	4.52	4.05	4.62
120	5.85	4.51	4.44	4.73
250		4.48	4.44	4.87

November 2010 treatment				
Time (days)	**Control**	**Rep a**	**Rep b**	**Rep c**
0		7.13	6.89	7.09
30	8.23	7.69	7.58	7.39
60	5.57	6.28	6.31	6.2
90	6.03	4.86	6.2	5.82
120	6.11	5.42	4.75	5.99
150	6.43	6.85	6.78	4.45
200	6.39	4.55	4.82	4.65

Freshwater treatment				
Time (days)	**Control**	**Rep a**	**Rep b**	**Rep c**
0				
1	7.58	7.47	7.69	7.75
3	7.70	7.65	7.68	7.79
7	7.81	7.67	7.61	7.78
14	7.93	7.58	7.72	7.65
28	8.22	8.49	8.52	8.31
56	8.01	7.89	7.83	7.87
112	8.27	8.00	7.94	8.04
200	7.18	7.33	7.34	7.38

Simulation of movement treatment				
Time (days)	**Control**	**Rep a**	**Rep b**	**Rep c**
1	7.83	7.84	7.83	7.86
3	7.89	7.83	7.84	7.82
7	7.93	7.85	7.79	7.77
14	8.05	7.82	7.82	7.86
28	8.10	8.15	8.01	8.26
56	7.99	8.10	8.01	8.07
112	8.02	8.01	8.05	7.93
200	8.11	8.56	8.16	8.24

Table A.1: pH data for all treatments (continued)

Exclusion of light treatment				
Time (days)	**Control**	**Rep a**	**Rep b**	**Rep c**
1	7.65	7.78	7.88	8.00
3	7.64	7.72	7.74	7.81
7	7.66	7.68	7.69	7.71
14	7.98	7.98	7.89	7.87
28	8.37	8.09	8.14	8.21
56	8.23	8.88	9.08	8.87
112	8.07	8.09	8.13	8.07
200	7.86	7.88	7.76	8.05

Thicker material treatment				
Time (days)	**Control**	**Rep a**	**Rep b**	**Rep c**
1	7.77	7.80	7.81	7.83
3	7.76	7.79	7.82	7.85
7	7.87	7.87	8	7.97
14	8.17	8.22	8.24	8.23
28	8.28	8.14	8.12	8.14
56	8.12	8.13	8.24	8.16
112	8.11	7.96	7.97	8.02
200	7.99	8.16	8.22	8.24

Lower pH treatment				
Time (days)	**Control**	**Rep a**	**Rep b**	**Rep c**
1	5.61	5.41	5.49	5.65
3	5.69	5.65	5.82	5.55
7	5.43	5.71	5.63	5.83
14	5.70	5.98	5.63	5.6
28	5.63	5.65	5.58	5.71
56	5.59	5.27	5.61	5.64
112	5.90	5.44	5.14	5.81
200	5.81	5.51	5.16	5.48

Marine water treatment				
Time (days)	**Control**	**Rep a**	**Rep b**	**Rep c**
1	8.28	8.32	8.32	8.33
3	8.23	8.24	8.19	8.22
7	8.28	8.16	8.24	8.16
14	8.25	8.11	8.06	8.01
28	8.32	8.06	8.11	8.09
56	8.44	8.23	8.29	8.23
112	8.29	8.15	8.09	8.12
200	8.08	7.90	7.86	8.06

Table A.2 Optimised parameters derived for models used in modelling the weight loss of latex under different environmental conditions.

	August 2010	November 2010	Freshwater	Lower pH	With movement	Marine water
Parameters						
M_o	100	100	100	100	100	100
M_1			64	73.763	60	63
M_2			44.329	35	46.184	45.763
k_1	0.018	0.0005	0.008	0.005	0.02	0.006
k_2	0.0035	0.016	0.015	0.018	0.015	0.035
k_3			0.0002	0.005	0.00001	0.0002
t_b	44	105	10	14	3	14

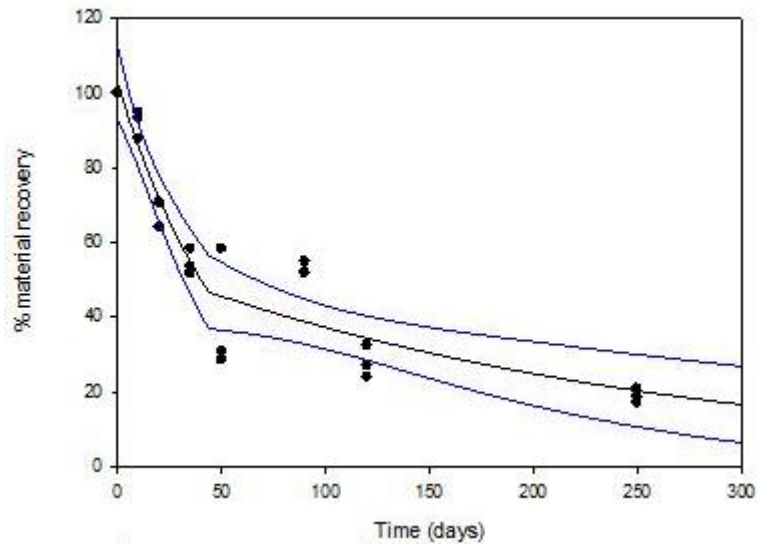
Appendix 2

Alternative modelling approach

This study used the FOCUS degradation models (page 80) to describe the measured weight loss data. This approach has a number of limitations that include (i) the parameters used to define the model are not calibrated and (ii) the models are over parameterised meaning the model may provide an inappropriate fit for the measured data.

An alternative modelling approach using SigmaPlot version 12.3 is now demonstrated for the Hockey-Stick model using two examples (a) the August 2010 treatment, and (b) the freshwater treatment. This software package uses confidence bands to represent the uncertainty in the estimated curve, and gives standard error values for the model parameters as described in brackets in the figures below. The calculated DT50 is then 39.9 days as compared to 38.51 days.

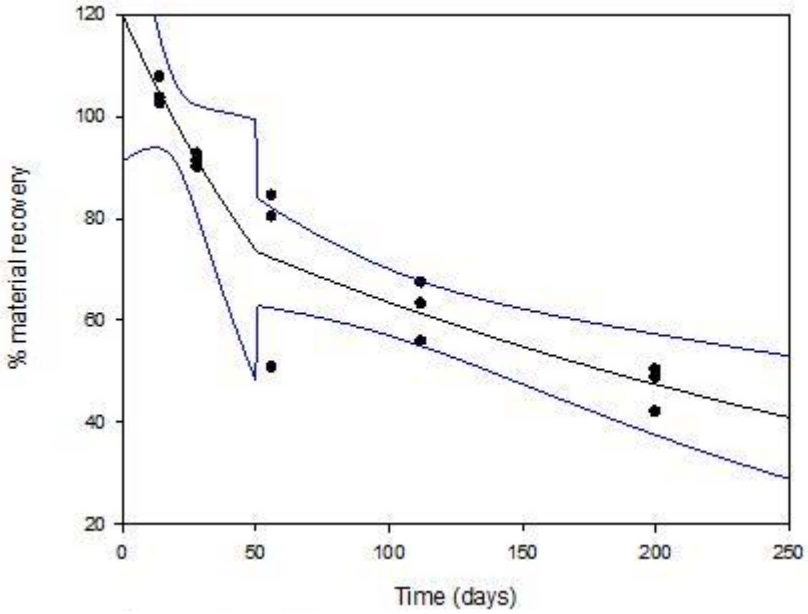
Example A – August 2010 Treatment



	August 2010 original parameter	SigmaPlot parameters
Parameters		
M_o	100	102.3 (4.655)
k_1	0.018	0.018 (0.0029)
k_2	0.0035	0.004 (0.0014)
t_b	44	43.832 (10.241)

Example B – Freshwater treatment

The second set of experiments that were started in 2011, because of the closer sampling time used an initial increase in weight was measured. This increase in weight was originally included in the modelling procedure. However, this leads to over parameterisation of the model and therefore it cannot be calibrated. An alternative approach is to apply the hockey stick model at the point where the weight increase is at its highest, in this case at day 14. The calculated DT_{50} is then at 181 days as compared to 158 days



	SigmaPlot parameters
Parameters	
M_o	119 (13.024)
k_1	0.0097 (0.0051)
k_2	0.0029 (0.0009)
t_b	50.488 (25.5)

Glossary

Acrylonitrile butadiene rubber	NBR
Assessment factor	AF
Atomic absorption spectrometry	AAS
Attenuated Total Reflection	ATR
Bisphenol A	BPA
Brominated flame retardants	BFRs
Butyl Benzyl Phthalate	BBP
Continuous plankton recorder	CPR
Degradation products	DPs
Department for the Environment, Food and Rural Affairs	DEFRA
Di ethyl phthalate	DEP
Di methyl phthalate	DMP
di-(2-ethylhexyl) phthalate	DEHP
Dichlorodiphenyldichloroethylene	DDE
Dichlorodiphenyltrichloroethane	DDT
Dichloromethane	DCM
di-isodecyl phthalate	DIDP
di-isononyl phthalate	DINP
Di-n-butyl phthalate	DBP
Dioctyl phthalate	DOP
Dissolved organic carbon	DOC
Ethylhexyl phthalate	MEHP
European Chemicals Agency	ECHA
Food and Environment Research Agency	Fera
Fourier Transform Infra-Red spectroscopy	FTIR
Gas chromatography – mass spectrometry	GC-MS
Hexabromocyclododecane	HBCD
High-density polyethylene	HDPE
Linear low density polyethylene	LLDPE
Liquid chromatography-time-of-flight mass spectrometry	LC-TOF-MS
Low density polyethylene	LDPE

Nanoparticle tracking analysis	NTA
Nanoparticles	NPs
National Institute of Standards and Technology	NIST
Natural rubber latex	NRL
No-observed effect concentrations	NOEC
Persistent organic pollutants	POPs
Poltetrafluoroethylene	PTFE
Polyaromatic hydrocarbons	PAHs
Polybrominated diphenyl ethers	PBDE
Polycaprolactone	PCL
Polycarbonate	PC
Polychlorinated biphenyls	PCB
Polyethylene	PE
Polyethylene terephtalate	PET
Polyhydroxyalkanoates	PHA
Polyhydroxybutyrate	PHB
Polylactides	PLA
Polymer-based materials	PBM
Polymethylpentane	PMP
Polyphenylene sulphide	PPS
Polypropylene	PP
Polystyrene	PS
Polyurethane	PUR
Polyvinyl chloride	PVC
Poor quality match	PQM
Predicted environmental concentration	PEC
Predicted no-effect concentrations	PNEC
Principal component analysis	PCA
Registration, Evaluation, Authorisation and Restriction of Chemicals	REACH
Root mean square error	RMSE
Screen capture ratios	SCR
Sewage related debis	SRD

Solid phase extraction	SPE
Styrene-butadiene	SBR
Sum of square residuals	SSRes
Terra-brominated bisphenol A	TBBPA
unplasticised PVC	uPVC
Unresolved complex mixture	UCM
Waste Water Treatment Plant	WWTP
Zinc diethyl dithiocarbamate	ZDEC
Zinc mercaptobenzothiazole	ZMBT

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