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Canada

Draft Science Assessment of Plastic Pollution

Environment and Climate Change Canada
Health Canada

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List of abbreviations

Abbreviation	Meaning
µ-FTIR	Micro-Fourier transform infrared spectroscopy
AAB	Adopt-a-Beach™
AKT	Protein kinase B
ARG	Antibiotic resistant gene
ATP	Adenosine triphosphate
BALF	Bronchoalveolar lavage fluid
BW	Body weight
CaPSA	Canada's Plastics Science Agenda
CBD	Convention on Biological Diversity
CCME	Canadian Council of Ministers of the Environment
CMC	Carboxymethylcellulose
DDE	Dichlorodiphenyldichloroethylene
DNA	Deoxyribonucleic acid
DW	Dry weight
DWTP	Drinking water treatment plant
EC_{10}	10% effect concentration
EC_{50}	Median effect concentration
ECCC	Environment and Climate Change Canada
ECHA	European Chemicals Agency
EDS	Energy-dispersive X-ray spectroscopy
EFSA	European Food Safety Agency
ERK	Extracellular signal-regulated kinase
EU	European Union
FAO	Food and Agriculture Organization
FTIR	Fourier Transform Infrared Spectroscopy
GCMS	Gas chromatography mass spectrometry
GCSC	Great Canadian Shoreline Cleanup
GD	Gestational day
GESAMP	Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection
GI	Gastrointestinal
GM	Geometric mean
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	Glutathione
GST	Glutathione-S-transferase
HDPE	High-density polyethylene
HGT	Horizontal gene transfer
HOC	Hydrophobic organic compound
HPF	Hours post fertilization
IR	Infrared
IUCN	International Union for Conservation of Nature
LC_{50}	Median lethal concentration

LDH	Lactate dehydrogenase
LDPE	Low-density polyethylene
LoD	Limit of detection
LOEC	Lowest observed effect concentration
LOEL	Lowest observed effect level
LT_{50}	Median lethal time
MAPK	Mitogen-activated protein kinase
MEDITS	International bottom trawl survey in the Mediterranean
MEK	Mitogen-activated protein kinase kinase
MGE	Mobile genetic element
MMAD	Mass median aerodynamic diameter
MP	Microplastic
NOEC	No observed effect concentration
NOEL	No observed effect level
OECD	Organisation for Economic Co-operation and Development
PA	Polyamide
PAA	Poly(N-methyl acrylamide)
PAH	Polycyclic aromatic hydrocarbon
PAME	Protection of the Arctic Marine Environment
PAN	Polyacrylonitrile
PBDE	Polybrominated diphenyl ethers
PBT	Polybutylene terephthalate
PC	Polycarbonate
PCB	Polychlorinated biphenyl
PE	Polyethylene
PET	Polyethylene terephthalate
PLA	Polylactic acid
POP	Persistent organic pollutant
PP	Polypropylene
PS	Polystyrene
PU	Polyurethane
PUF	Polyurethane foam
PVC	Polyvinyl chloride
qPCR	Quantitative polymerase chain reaction
ROS	Reactive oxygen species
SAMP	Suspended atmospheric microplastic
SEM	Scanning electron microscopy
SOD	Superoxide dismutase
TEF	Toxic equivalency factor
UNEP	United Nations Environment Programme
UV	Ultra-violet
WHO	World Health Organization
WW	Wet weight
WWTS	Wastewater treatment system

Executive summary

Plastics are among the most universally used materials in modern society. Since the 1950s, the production and use of plastics has been increasing faster than that of any other material, mostly due to their durability and low cost. However, the improper management of plastic waste has led to plastics becoming ubiquitous in all major compartments of the environment. Plastic that is discarded, disposed of, or abandoned in the environment outside of a managed waste stream is considered plastic pollution. Plastic pollution has been detected on shorelines, and in surface waters, sediment, soil, groundwater, indoor and outdoor air, drinking water and food. In Canada, it is estimated that 1% of plastic waste enters the environment, representing 29 000 tonnes of plastic pollution in 2016. Since plastic degrades very slowly and is persistent in the environment, the amount of plastic pollution is anticipated to continue to increase over time. There are growing concerns that plastic pollution may adversely impact the health of the environment and humans.

The purpose of this report is to summarize the current state of the science regarding the potential impacts of plastic pollution on the environment and human health, as well as to guide future research and inform decision-making on plastic pollution in Canada. It provides a review of the available information on plastic pollution, including its sources, occurrence, and fate, as well as on the potential effects of plastics on the environment and human health. This report is not intended to quantify the risks of plastic pollution on the environment or human health, but rather to survey the existing state of science in order to guide future scientific and regulatory activities.

Plastics are often defined by their size, with macroplastics being larger than 5 mm and microplastics being less than or equal to 5 mm. Plastic waste can be released into the environment as complete materials (e.g., discarded single-use or short-lived products, such as plastic bags and straws), as large pieces of plastics (e.g., fragments of plastic products) or as microplastics (e.g., microfibres released from washing of clothes or microbeads released through wastewater). Microplastics can also be formed through the breakdown of larger plastic items in the environment.

While plastics can degrade, the rate at which they break down is very slow and can be affected by multiple factors, such as temperature and light. In water, the rate of degradation is temperature dependent, being slower in cold water. The lack of exposure to sunlight also slows down the degradation of plastics. While oxidation can promote the degradation of plastics in soil, the rate of degradation is still slow. Although biodegradable plastics and bioplastics are increasingly being used as alternatives to conventional plastics, they may not degrade more readily than conventional plastics once in the environment.

Plastic packaging is the biggest contributor of plastic waste in Canada, followed by the automotive, textile, and electrical and electronic equipment sectors. The release of synthetic microfibres from wastewater treatment systems (WWTS) is also anticipated to represent a significant source of microplastic pollution. In WWTS, microplastics removed from wastewater settle in sewage sludge and are then released to land through the application of biosolids. The presence of microplastics in outdoor

air is largely thought to be attributable to tire wear and tear, while microplastics in indoor air result from the shedding of fibres from clothing, furniture, carpeting and household goods.

It is clear that plastic pollution is found everywhere in the environment. In Canada, single-use plastics make up the bulk of plastic litter that is found in freshwater environments. The most common litter items collected on Canadian shorelines include bottle caps, plastic bags, plastic bottles, straws, and cigarette butts. Large numbers of microplastic particles are also found in fresh and marine surface waters. Globally, microfibres are the most abundant type of microplastics found in water. However, it is recognized that there is a lack of standardized, high-quality methods for sampling plastics, particularly for measuring and characterizing microplastics.

Microplastics are also found in sediment and soil. Through various mechanisms, such as the formation of biofilms—layers of microorganisms that form on a surface—microplastics in surface waters may eventually sink, leading to the accumulation of microplastics in the bottom sediment of both freshwater and marine environments. Soils are also expected to act as a major sink for plastic particles, as microplastics are likely to remain in soils for long periods due to factors such as vertical transport, which pulls particles down from the surface and thereby slows down their degradation. Microplastics may travel from soil into groundwater.

Air is also anticipated to be an important pathway for microplastic transport, and microplastics have been detected in both indoor and outdoor air. While there are no Canadian data available on the occurrence of microplastics in air, data from other parts of the world show that concentrations are higher in indoor air than in outdoor air. Indoors, microplastics are also found in settled house dust.

Current data on the occurrence of microplastics in food are limited, and most available information concerns microplastics found in seafood, specifically fish and shellfish from marine environments. In fish, microplastics have been found in both muscle tissue and the gastrointestinal tract, mostly as fragments and fibres. Microplastics have also been detected in mussels, clams, oysters, scallops and snails, and in a very small number of other foods, such as salt.

Internationally, a limited number of studies have investigated the presence of microplastics in tap and bottled water. Microplastics have been detected in up to 93% of bottled water samples from outside of Canada, with concentrations varying across bottle type (i.e., plastic, glass or cardboard) and intended use conditions (i.e., single-use versus multi-use bottles). In the case of tap water, some studies have detected microplastics while others have not. Drinking water treatment is anticipated to remove a large proportion of microplastic particles.

Plastic pollution has been shown to impact organisms and their habitats. Macroplastic pollution can cause physical harm to biota, often as a result of entanglement or ingestion. Entanglement can lead to suffocation, strangulation, or smothering, and a high frequency of reported entanglement occurrences has led to the direct harm or mortality of biota. Ingestion can lead to direct harm through physical damage; it can block airways or intestinal systems leading to suffocation or starvation. The observed effects of microplastics on biota are either primarily driven by physical effects or due to the presence of residual chemicals used to make the plastic or of other chemical pollutants from the environment that

may adsorb onto the plastic. Published studies on exposure to microplastics report conflicting observations of effects, even for the same endpoint in the same species. Conflicting information on ecotoxicological effects could be attributed to the difficulties that exist in testing the effects of microplastics in organisms and the lack of standardized test methods.

Humans may be exposed to microplastics via the ingestion of food, bottled water, and tap water, as well as through the inhalation of indoor and outdoor air. However, information on the human health effects of microplastics is limited. Some associations between exposures to high levels of microplastics and adverse health effects in laboratory animals and in humans have been reported, but the health effects cannot be linked to exposure in the general population. Occupational inhalation exposure studies show associations between work in microplastic-related industries and increased incidence of various respiratory symptoms and diseases. Conflicting observations have been made for cancers of the respiratory tract and digestive system.

Effects observed in animal studies are primarily associated with tissues related to where particles enter the body (e.g., effects on the digestive system after oral exposure and on the respiratory tract after inhalation). Effects following oral exposure include inflammation of the liver, oxidative stress, metabolic changes, and altered gut microbiota. Effects in the respiratory tract are likely related to the physical impact of microplastics as particulate matter and include oxidative stress, cytotoxicity, inflammation, and development of foreign body granulomas. In both ingestion and inhalation studies, movement of a small fraction of microplastic particles to lymphatic or systemic tissues has been observed. No dose-response relationship has been observed in mortality, survival time, behaviour, clinical observations, or tumour incidence from inhalation exposures.

In addition to physical impacts, there are concerns that plastics may serve as a means of transport for other chemicals. Since plastics can contain unbound monomers and chemical additives and can sorb persistent organic pollutants from the environment, it is possible that these substances may be transported to organisms or humans, where they may then be released. The extent of release is expected to depend on a variety of factors, such as the properties of the receiving environment, the plastic particle, and the bound chemical. The current literature suggests that, while the transport of chemicals via plastics is possible, the impact to biota is likely limited, and recent international reviews indicate that there is likely a low health concern for human exposure to chemicals from ingestion of microplastics from food or drinking water (EFSA 2016; FAO 2017; WHO 2019). However, further research would be required before a human health risk assessment on microplastics is possible. Many of the chemicals observed to be bound to plastic particles have been assessed by various programs at Environment and Climate Change Canada (ECCC) and Health Canada.

Plastics can also provide a habitat for microorganisms, including potential pathogens, through the formation of biofilms. There is currently no indication that microplastics-associated biofilms would impact human health. In addition, despite very limited data, it is anticipated that drinking water treatment would inactivate biofilm-associated microorganisms.

Given the increasing amounts of plastic pollution in the environment and the demonstrated ability of macroplastics to harm biota, it is anticipated that the frequency of occurrence of physical effects on individual environmental receptors will continue to increase if current trends continue.

There are a multitude of sources that contribute to plastic pollution. Under the precautionary principle, action is needed to reduce macroplastics and microplastics that end up in the environment.

In order to advance the understanding of the impacts of plastic pollution on the environment and human health, it is recommended that research be carried out in the following areas to address the key knowledge gaps identified in this report:

- Developing standardized methods for sampling, quantifying, characterizing, and evaluating the effects of macroplastics and microplastics;
- Furthering the understanding of human exposure to microplastics;
- Furthering the understanding of the ecotoxicological effects of microplastics;
- Furthering the understanding of the effects of microplastics on human health; and
- Expanding and developing consistent monitoring efforts to include poorly characterized environmental compartments such as soil.

1. Introduction

Plastics are part of the everyday lives of Canadians and populations around the world. Plastics are low cost, durable materials and can be used in a variety of applications (CCME 2018). For these reasons, global plastic production has been increasing over the past several decades at a rate faster than that of any other material (Geyer et al. 2017; CCME 2018). In Canada, total sales of plastic are estimated at $35 billion in 2017, with approximately 4 667 kt introduced to the Canadian market in 2016 (ECCC 2019a). Plastics are used in a variety of industrial sectors, and demand for plastic products continues to grow.

Poor management of plastics across their life cycle, as well as improper disposal, has resulted in large amounts of plastic waste entering the environment as plastic pollution (CCME 2018). In 2016, an estimated 9% of plastics were recycled, 86% were landfilled, 4% were incinerated for energy recovery, and 1% were released directly into the environment in Canada (ECCC 2019a). Of the 4 667 kt of plastics that entered the market in 2016, an estimated 3 268 kt were discarded as waste (ECCC 2019a). Of that amount, an estimated 29 kt were discarded outside of the normal waste stream (i.e., not landfilled, recycled or incinerated) in 2016, through direct release to the environment or through dumps or leaks (ECCC 2019a). As illustrated in

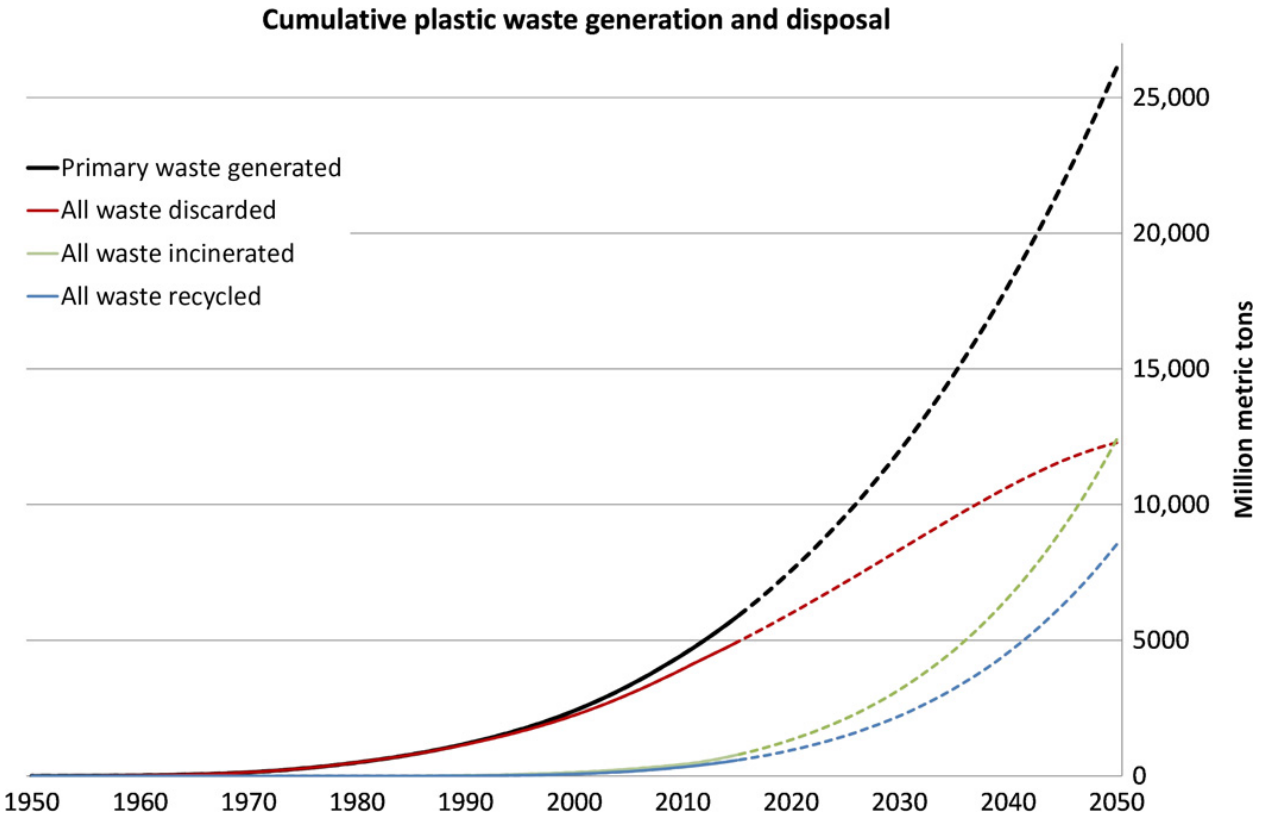

Figure 1, if plastic manufacturing continues at its current pace, the accumulation of plastics will continue to accelerate. It is estimated that by 2050 12 000 000 kt of plastic waste will have been discarded globally to landfills or the environment (Geyer et al. 2017).

With the growing public and scientific concern about the ubiquity of plastic pollution, there has been increasing global media attention on the potential impacts of plastic pollution on human health and the environment (CCME 2018; ECCC 2019c; SAPEA 2019). The Government of Canada has put forward Canada's Plastics Science Agenda (CaPSA), which aims to align current and future research investments across a range of disciplines (ECCC 2019c). The CaPSA framework identifies several key research priorities, including the detection of plastics in the environment, understanding and mitigating potential impacts on wildlife, human health and the environment, plastic design and alternatives, sustainable plastic production, and recycling and recovery.

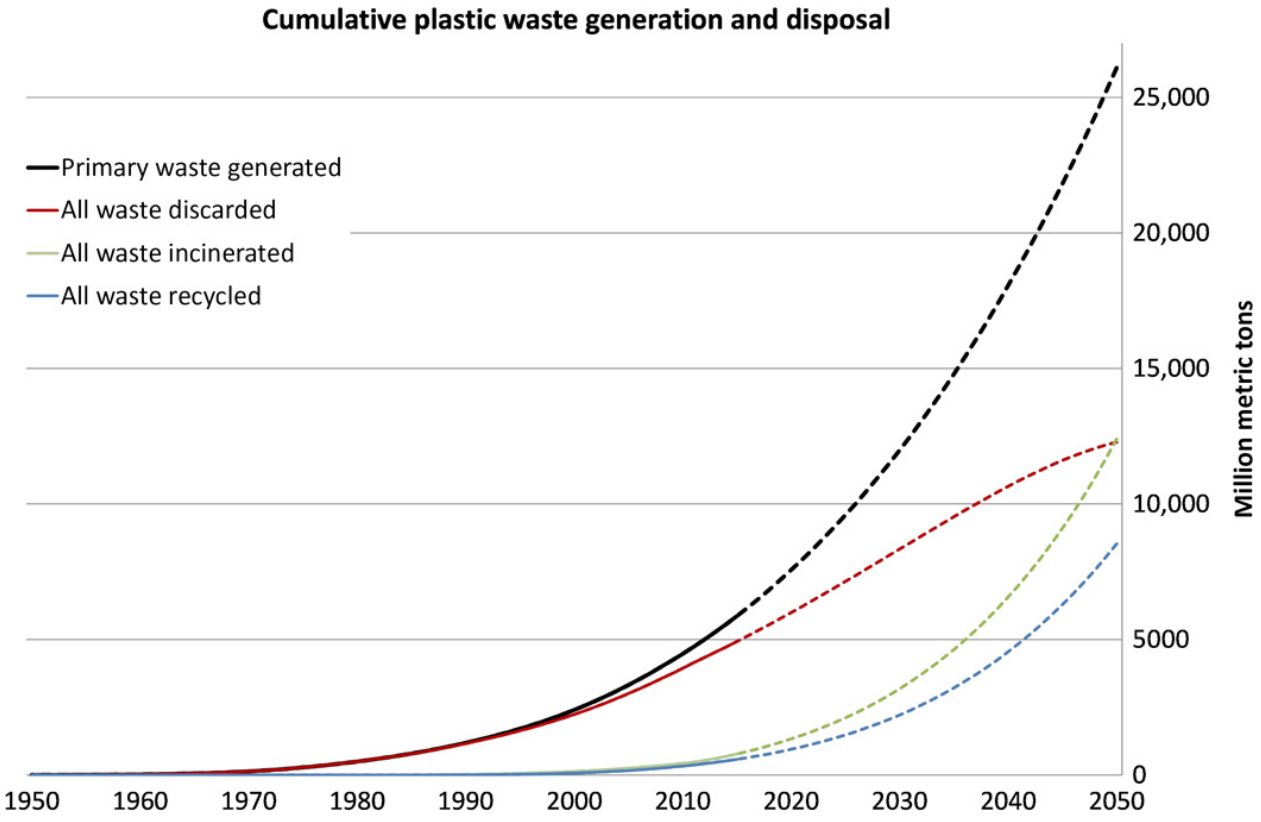

Figure 1: Global cumulative plastic waste generation and disposal. Solid lines show historical data from 1950 to 2015, and dashed lines show projections of historical trends to 2050 (reproduced with permission from Figure 3 of Geyer et al. 2017)

Long Form Description: This figure displays the global cumulative plastic waste generation and disposal. Increasing trends are shown for primary waste generated, all waste discarded, all waste incinerated and all waste recycled (reproduced with permission from Figure 3 of Geyer et al. 2017)

1.1 Scope

This report summarizes the current state of the science on the potential impacts of plastic pollution on the environment and human health and informs future research and decision-making on plastic pollution in Canada. For the purposes of this report, plastic pollution has been divided into two main types: macroplastics (plastics greater than 5 mm in size) and microplastics (plastics less than or equal to 5 mm in size) (see Section 1.2 for definitions). This report discusses the sources, occurrence, and fate of plastic pollution in the environment, as well as the potential impacts of plastics on human health and the environment. Information identified up to June 2019 was considered for inclusion in this draft science assessment, in addition to the 2019 WHO report on microplastics in drinking water and the 2019 report on microfibres commissioned by Ocean Wise.

This report is a review of the current state of the science on plastic pollution. It is not intended as a substitute for chemical risk assessment, and it is similar to the approach taken for the Science Summary on Microbeads (ECCC 2015). Typically, a chemical risk assessment is conducted to assess the potential for risk to the environment and human health associated with a substance. However, significant data gaps currently exist that preclude the ability to conduct a quantitative risk assessment, including a lack of standardized methods for monitoring microplastics and characterizing the environmental and human health effects of plastics, as well as inconsistencies in the reporting of occurrence and effects data in the scientific literature (Gouin et al. 2019). Indeed, risk assessment frameworks for evaluating the potential risks associated with plastics are currently under development. For example, see Gouin et al. (2019) for a discussion on the development of an environmental risk assessment framework for microplastics.

As the focus of this report is on plastic pollution, it is limited to a review of the occurrence of macroplastics and microplastics resulting from plastic waste entering the environment and does not examine non-environmental sources (e.g., via exposure from consumer products or self-care products). Moreover, it does not review the economics of waste management practices or evaluate the efficacy of waste management streams (e.g., recycling).

1.2 Definitions

Plastics are often categorized by their size. The term microplastic was originally used to differentiate between substances that could only be visualized through a microscope and larger macroplastics (ECCC 2015). However, there is no one definition of what constitutes a microplastic. For the purpose of this report, plastic particles less than or equal to 5 mm in size are defined as microplastics, while plastics greater than 5 mm are defined as macroplastics. Microplastics can be further defined as primary or secondary microplastics. Primary microplastics are intentionally produced plastic particles (such as pellets, powders, and beads) that are either intended for use as microplastics or as precursors for the production of plastic or plastic-containing products. Primary microplastics are widely used as abrasives in a variety of applications (UNEP 2016). Secondary microplastics are not produced intentionally, but are the result of the breakdown and fragmentation of larger plastic items (SAPEA 2019). Furthermore, microfibres are a specific type of microplastic defined as being fibrous in shape and less than or equal to 5 mm in length. Nanoplastics are considered to be a subset of microplastics. They are primary or

secondary microplastics that range from 1 to 100 nm in size in at least one dimension. Nanoplastics occur largely as a result of secondary sources of plastic pollution (i.e., the breakdown of larger plastics) (Rist and Hartmann 2018). This report will focus on plastics greater than 100 nm in size (i.e., microplastics and macroplastics).

For the purposes of this report, plastic waste that is released to the environment is defined as plastic pollution. In the scientific literature, plastic pollution has been referred to by a number of terms, such as plastic debris or plastic litter. This report will use the terms plastic pollution or plastic pollutants. Furthermore, in this report the term litter refers to any persistent, manufactured, or processed solid material discarded, disposed of, lost, or abandoned in the environment, including plastics, textiles, glass, metal, ceramics, and other persistent synthetic materials. This term will be used when the proportion of plastic pollution reported in the literature is unclear. In this report, plastic waste is considered to be plastics that enter the waste stream (e.g., landfilled, recycled or incinerated), whereas plastic pollution is considered to be plastic that is discarded, disposed of, or abandoned in the environment outside of a managed waste stream.

2. Composition, properties, and uses

All plastic materials are formed from long-chain polymers of very high molecular weight, often measured in the hundreds of thousands of kilodaltons (Sperling 2006). Synthetic polymers first appeared in the early 20th century, leading to the manufacture of plastic products such as Bakelite and nylon (commercial name for polyamides). Since then, polymer science has evolved, with a greater mechanistic understanding of the interrelationships between polymer structure, morphology, and physical and mechanical behaviour. This has resulted in the production of a myriad of plastic materials with varying physical and chemical properties.

Polymerization, the synthesis of polymers, can occur following one of two main processes: chain polymerization or stepwise polymerization. The process used to form polymers greatly influences their physical properties. Common chain polymer structures include polyethylene (PE), polypropylene (PP), polystyrene (PS), and polyvinyl chloride (PVC), whereas common stepwise polymers include nylons, polyethylene terephthalate (PET), polycarbonate (PC), and polyurethane (PU). These represent many of the most common forms of plastics typically found as environmental contaminants (Sperling 2006).

The physical properties of plastic, such as rigidity, flexibility, and elasticity, are influenced by the polymer's molecular weight distribution and organization of polymer chains (Sperling 2006; Verschoor 2015). Generally, high molecular-weight polymers with a complex organization that leads to strong covalent bonds between the polymers can result in the formation of a rigid plastic with a high melting point. In contrast, linear polymer organization with low molecular-weight distribution results in a more flexible plastic with a lower melting point. Combinations of different molecular weight distributions, different polymer chain organization, and/or blends of different types of polymers can produce a material that will be effective for its intended use (Sperling 2006).

Furthermore, many polymers are subject to additional processes aimed at enhancing efficacy with respect to an intended functionality. For instance, when heated, a linear polymer will flow, resulting in the formation of a thermoplastic (Sperling 2006). Thermoplastics are polymers commonly found in plastics that can be melted and reshaped into new objects. Commonly used thermoplastics include PVC, PE, PS, and PC (ECCC 2019b). To prevent flow upon heating, polymers can be cross-linked to produce a thermoset plastic (Sperling 2006). Thermoset plastics are polymers that are used for their resistance to mechanical forces, chemicals, wear, and heat, but they cannot be re-melted to form new objects. Examples include PU and unsaturated polyester polymers (ECCC 2019b).

Chemical additives can be added to polymers during production to alter the properties of plastics (Rochman et al. 2019). There are several categories of additives, including stabilizers and functional agents. Polymer stabilizers maintain the inherent properties of the material by protecting it against oxidative degradation. They include substances such as anti-oxidants, light stabilizers, metal deactivators, and ultra-violet absorbers. Functional agents can enhance the mechanical strength of a polymer or impart new characteristics. Examples of functional agents include flame-retardants, anti-static agents, lubricants, and plasticizers (ECCC 2019b). For instance, plasticizers can be added to soften a polymer by lowering its glass transition temperature or reducing the degree of crystallinity or melting point (Sperling 2006).

Polymer production can begin with either the use of recycled or recovered plastics or with natural resources (i.e., petroleum or plant-based starting material). These polymers are then used to manufacture plastic products (ECCC 2019b). Although many different types of plastic polymers are used in Canada, domestic plastic production is dominated by five polymer types. PE accounts for the majority of plastic production, with 3 700 kt produced in 2017, followed by PVC (210 kt), PET (166 kt), PU (122 kt) and polyamides (PA) (116 kt) (ECCC 2019b). Of the 4 800 kt of plastic polymers produced in Canada in 2016, 77% was exported. Further, there is a domestic demand of 3 800 kt, 71% of which is fulfilled through imports (ECCC 2019a).

The majority of plastic products in Canada are found in the packaging and construction sectors. Other major sectors include the automotive, electronic and electrical equipment, textiles, and agriculture sectors (ECCC 2019b). Examples of applications of various polymers are presented in Table 2-1.

Table 2-1: *Selected polymer applications*

Acronym	Name	Main application[a]
PP	Polypropylene	Rigid, semi-rigid and flexible packaging Automotive Houseware Electrical insulation
PE	Polyethylene	Rigid, semi-rigid and flexible packaging Agriculture film Houseware Electrical insulation Construction (pipes) Self-care products
PS	Polystyrene	Packaging (thermoformed containers) Foams
PMMA	Poly(methyl methacrylate)	Transparent applications in automotive and construction Medical Electronics
PC	Polycarbonate	Transparent applications in automotive and construction Medical Electronics
PLA	Polylactide – a specific type of polyester	Rigid, semi-rigid and flexible packaging
PET	Polyethylene terephthalate – a specific type of polyester	Rigid, semi-rigid and flexible packaging Textile synthetic fibres
PVC	Polyvinylchloride	Construction (pipes, profiles, flooring) Sheet and coated fabrics Electrical insulation
PTFE	Polytetrafluoroethylene	Anti-adhesive coatings Engineering parts

[a] Personal communication, email from Transportation and Manufacturing Division, National Research Council Canada, to the Ecological Assessment Division, Environment and Climate Change Canada, dated August 15, 2019; unreferenced

The packaging sector is the largest user of plastics in Canada, accounting for 33% of end-use plastics introduced to the market in 2016. Examples of plastic packaging products include plastic bags, water and soft drink bottles, as well as various packaging used for pharmaceuticals, toiletries, and cleaning compounds. PE is very commonly used in packaging, specifically for films and flexible packaging (ECCC 2019b). Two major types of PE are low-density polyethylene (LDPE) and high-density polyethylene (HDPE). LDPE is generally used in heavier duty films, such as high durability bags and protective sheeting, due to its toughness, flexibility, and relative transparency. HDPE possesses good chemical resistance and thus is widely used in thin-gauge carrier bags, chemical drums, toys, food wrapping material, and kitchenware. In addition to PE, other plastic polymers can also be seen in the packaging sector, such as PVC, PET and PP.

Construction is the second-largest end-use market for plastics in Canada, accounting for approximately 26% of all end-use plastics generated in 2016 (ECCC 2019a). Primary uses of plastics in the construction sector include plastic and foam building and construction materials, paints and coatings, profile shapes, and reconstituted wood and plywood. Plastics are broadly used in the construction of all types of buildings and are especially used in thermal insulation materials, as well as waterproofing and sealant materials. PVC is widely used in siding and window applications, floor and wall covering products, as well as pipe and pipe fittings. Clear PC sheets are used as a substitute for glass in greenhouses, transit shelters, and covered walkways due to its resistance to weathering. PU foam is used as insulation in commercial and residential properties (ECCC 2019b).

In an effort to improve fuel efficiency through weight reduction, the automotive sector has increased its use of plastics. While many different types of plastics are used in the sector, PU, PP, and PVC make up the vast majority of total plastics used in a vehicle. PU is used in cushioning applications such as seating, PP is used in automobile interiors, and PVC is used for faux leather. PC can be used to replace glass in cars, while foam, plastic, and fibre composites can be used in door panels, dashboards, and hoods (ECCC 2019b).

Other end-use sectors include the electronic and electrical equipment, textile, and agriculture sectors. Plastics are used in the electronics sector for computer and phone parts, as well as items such as electric wires and cables. The textile sector uses plastics for fibres in carpets, rugs, mats, and clothing. In the agriculture sector, plastics are used for fertilizer and pesticide packaging (e.g., agricultural films, mulches, and greenhouses) (Ekebafe et al. 2011; ECCC 2019b).

Given the variety of plastic materials that can be produced, the physical and chemical properties of plastic particles present in the environment will be complex (Rochman et al. 2019). With respect to shape and size, primary microplastics are intentionally engineered to be a particular size (e.g., virgin resin pellets used in plastic manufacturing processes) and will therefore likely show less variation than secondary microplastics. Secondary microplastics can have a range of shapes, including spheres and cylinders, but also fragments, fibres, and films (Kooi and Koelmans 2019). Secondary microplastics are also highly variable in size and density. Recognizing the inherent challenge associated with defining the physical properties of microplastic particles observed in the environment, Kooi and Koelmans (2019) suggest a method aimed at defining and characterizing the distributions of properties most commonly encountered. The approach proposed by Kooi and Koelmans (2019) may prove useful in developing tools for monitoring plastics in the environment, providing a greater mechanistic understanding of the environmental fate of microplastics, and allowing for easy comparison between studies.

Microplastics can exist as fibres, fragments, spheres, pellets, films, and foams, as shown in Figure 2. In general, certain shapes of microplastics originate from certain plastic products. For example, fibres are typically shed from fabrics, such as clothing and upholstery, whereas pellets are typically from industrial feedstock (Rochman et al. 2019).

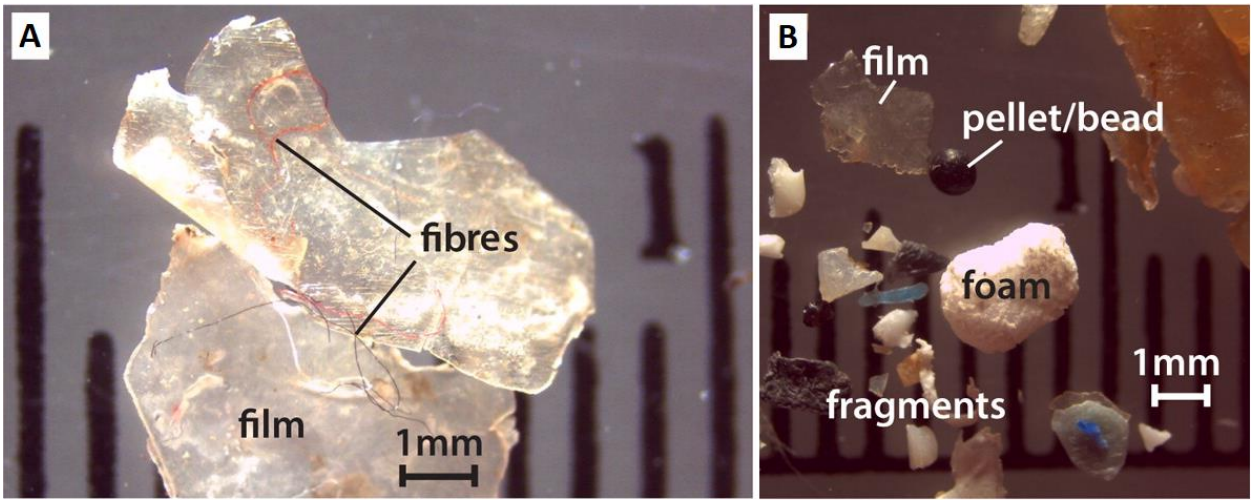

Figure 2: Microplastics found in the environment (reproduced and adapted with permission from Figure 2 of Baldwin et al. 2016)

Long Form Description: This figure shows microplastics found in the environment. Image A contains film and fibres. Image B contains film, pellet/bead, fragments, and foam. (Reproduced and adapted with permission from Figure 2 of Baldwin et al. (2016))

Density is a key property that influences the environmental fate of plastics (Rochman et al. 2019). Densities of plastic polymers such as PE, PS and PVC can range from 0.9 to 2.3 g/cm^3 (WHO 2019).

Table 2-2 presents the densities of various plastic polymers. Polymers with a density greater than 1 are denser than water and are expected to sink, while those with a density less than 1 are expected to float. By analogy, the environmental fate and transport of macroplastics or microplastics released to the atmosphere are also likely to be influenced by their density. For example, denser microplastics are less likely to be readily dispersed by the wind (Rochman et al. 2019). The density of plastics and their buoyancy in water can also be influenced by the coating of plastics with microorganisms, algae, or plants (i.e., biofilms) (Woodall et al. 2014). Other factors, such as shape and size, can also govern the fate of plastics in the environment (Rochman et al. 2019).

Table 2-2: *Selected polymer densities*

Name	Density (g/cm^3)[a]
Polyethylene	0.965 – 0.971
Polypropylene	0.90 – 0.91
Polystyrene	1.04 – 1.10
Polyamides (nylon)	1.02 - 1.05
Acrylic	1.09 – 1.20
Polyvinylchloride	1.16 – 1.58
Poly methylacrylate	1.17 – 1.20
Polyurethane	1.20
Polyester	1.23 – 2.3
Polyethylene terephthalate – a specific type of polyester	1.37 – 1.45

[a] Hidalgo-Ruz et al. 2012

3. Sources of plastic waste and pollution

The sources of global plastic pollution are varied, and actual amount of plastic pollution is largely unknown (UNEP 2016). Important land-based sources of macroplastics to the marine environment include packaging, construction, household goods, and coastal tourism (UNEP 2016). It is estimated that 40% of all plastic production is used for packaging, a significant portion of which is used for the food and drink sector (UNEP 2016). Other land-based sources of microplastics around the world include cosmetics and personal care products, synthetic textiles and clothing, terrestrial transport (i.e., tire wear), and plastic producers and fabricators (i.e., accidental loss of resin pellets) (UNEP 2016; SAPEA 2019). With respect to sea-based sources of plastic pollution, the fisheries, aquaculture, and shipping sectors are major contributors (GESAMP 2016; UNEP 2016). Plastics in these sectors may be lost at sea by accident, abandonment, or deliberate disposal (UNEP 2016; SAPEA 2019). Macroplastics and microplastics from land- and sea-based sources can enter the ocean through various entry points (i.e., wastewater, rivers, coasts), depending on the region (UNEP 2016).

In Canada, the main industrial sectors contributing to the estimated 3 268 kt of plastic waste discarded 2016 are presented in Table 3-1. Plastic packaging is the single largest contributor of plastic waste, followed by the automotive, textile and electrical and electronic equipment sectors. In 2016, 33% of the plastics entering the Canadian marketplace was for use in packaging. However, due to the extremely short life cycle of plastics from packaging (i.e., most plastic packaging is single-use in nature) compared to plastics from other sectors, packaging accounts for 47% of the plastics discarded in that same year. Plastics generated from other industrial sectors, such as the automotive and construction sectors, have longer life cycles and therefore represent a smaller proportion of annual plastic waste as compared to packaging, which is typically discarded shortly after use (ECCC 2019a).

Table 3-1: *Main industrial sectors generating plastic waste in Canada in 2016 (ECCC 2019a)*

Sector	Proportion of total plastic waste
Packaging[a]	47%
Automotive	9%

Textiles	7%
Electrical and electronic equipment	7%
Construction	5%
White goods (e.g., large and small appliances)	4%
Agriculture	1%
Other[b]	19%

[a] Films (including plastic bags), bottles and other items for sectors including food and beverage, healthcare, consumer packaged goods, and cosmetics and personal care products, among countless other applications.
[b] Includes chemical products, toys, household furniture, etc. See ECCC (2019a) for a complete description.

Microfibres from laundering of textiles also represent a significant source to waterbodies. A report by Ocean Wise detailed the results of a study in which 38 different textile samples were tested for their shedding properties using a custom-designed washing machine test facility. The extent of microfibre shedding varied with the type of textile, with polyester, wool and cotton textiles releasing the largest amounts of microfibres. The report also estimates that the average Canadian household releases 533 million microfibres from laundry every year and that an estimated 878 tonnes of microfibres are released to water following wastewater treatment in Canada and the United States annually (Vassilenko et al. 2019).

Synthetic textiles and clothing are a large source of microplastic pollution (SAPEA 2019). Microfibres can be released from synthetic fabrics during wear and laundering, as well as from sources such as fishing gear (e.g., fishing nets) (ECCC 2019d). Carney Almroth et al. (2018) and De Falco et al. (2018) counted the number of microfibres released from different types of fabric under different laundering conditions. Both studies found that the use of a detergent increases the number of fibres released during washing. Powdered detergents, which often contain insoluble compounds that are able to create friction with the fabric, enable an even greater number of fibres to be released (De Falco et al. 2018). It has also been noted that powdered detergents have a higher pH compared to liquid detergents. While this is effective for soil removal, it can damage polyester fabrics by way of slow surface hydrolysis (Bishop 1995). Furthermore, fabric exposure to chemical detergents can cause the breakdown of synthetic fibres into smaller fibres (SAPEA 2019). The studies found that fleece garments and tightly knit fabrics released the greatest number of fibres during washing. It was found that on average, an adult-sized PET fleece garment releases an estimated 110 000 fibres during washing (Carney Almroth et al. 2018). A wash load of 5 kg of polyester garments was found to release 6 000 000 to 17 700 000 fibres, for an approximate weight of 0.43 to 1.27 g (De Falco et al. 2018).

Consumer products discarded to the environment or not properly managed may also represent a source of plastic pollution in the environment. While knowledge of the source of primary microplastics (i.e., the type and amount of microplastics intentionally used in consumer products) in Canada is limited, secondary microplastics may arise from the breakdown and fragmentation of macroplastics released to the environment. This may include consumer products such as toys, plastic gloves, appliances, electronics, mattress covers and flooring, as well as plastic materials used in packaging (Table 3-1).

3.1 Sources to water

Plastic pollution in the aquatic environment can arise from plastics released during land-based activities (e.g., through littering, inadequate waste management, landfill leachate, the use of plastics in agriculture, land application of biosolids, or direct release following abrasion or maintenance of plastic products (Boucher and Friot 2017; Alimi et al. 2018), from the deposition of airborne microplastics onto water (Hendrickson et al. 2018), or from water-based sources (e.g., fishing-related litter (Driedger et al. 2015)). Plastic pollution in water may also arise from the accidental release of raw plastic materials, such as spillage during transport (Driedger et al. 2015) and from releases from wastewater effluent (Murphy et al. 2016; Boucher and Friot 2017; Kay et al. 2018).

The Arctic Council's Protection of the Arctic Marine Environment (PAME) Working Group recently released the *Desktop Study on Marine Litter including Microplastics in the Arctic* as part of the first phase of a Marine Litter Project. The major sectors highlighted as sources of marine litter in the Arctic were fisheries, aquaculture, shipping, cruise tourism, and offshore resource exploration and exploitation. Approximately 640 kt of abandoned, lost or discarded fishing gear is estimated to be released to marine waters globally each year, accounting for 10% of all marine litter. In addition, releases from communities that are not connected to large waste management systems were flagged as sources of marine litter (PAME 2019).

3.1.1 Wastewater treatment

When wastewater containing plastics from domestic, commercial, and industrial sources passes through wastewater treatment systems[1] (WWTSs), most of the plastics are removed prior to discharge to the aquatic environment.

Based on a review of several published studies, Sun et al. (2019) reported significant reductions in microplastic concentrations when comparing influent and effluent in various WWTSs: concentrations ranged from 1 to 10 044 particles/L for influent and from to 0 to 447 particles/L for effluent. While large variations in microplastic concentrations can be observed between WWTSs (Sun et al. 2019), this may be due to differences in sample collection and analysis methods, as there are currently no standardized methods for the detection and quantification of microplastics in water. Other factors, such as catchment size, population served, wastewater source (residential, commercial, or industrial), and treatment technology, may also contribute to variations in influent and effluent concentrations and treatment efficiencies.

According to available data on the microplastic removal efficiencies of WWTSs, standard wastewater treatment systems using primary and secondary treatment processes can effectively remove most

[1] The term "wastewater treatment system" refers to a system that collects domestic, commercial and/or institutional household sewage and possibly industrial wastewater (following discharge to the sewer), typically for treatment and eventual discharge to the environment. Unless otherwise stated, the term wastewater treatment system makes no distinction of ownership or operator type (municipal, provincial, federal, indigenous, private, partnerships).

microplastics from the effluent before it is released to receiving waters (WHO 2019). Sun et al. (2019) estimated that 50% to 98% of microplastics can be removed during primary treatment, which involves skimming processes and settling stages, with larger particles being preferentially removed. Secondary treatment, which typically involves biological treatment to remove organic compounds, can increase microplastic removal to approximately 86% to 99.8% of microplastics (Sun et al. 2019; Raju et al. 2018). The addition of tertiary treatment can lead to the removal of 98% to 99.8% of microplastics, but removal efficiency is dependent on the type of treatment technology used (Sun et al. 2019). Advanced technologies such as rapid-sand filters, membrane bioreactors, and dissolved-air flotation can remove 95% to 99.9% of microplastics greater than 20 µm (Lares et al. 2018; Talvitie et al. 2017). Mintenig et al. (2019) observed complete removal of microplastics >500 µm and 95% of microplastics <500 µm using tertiary filtration.

Given the large volumes of effluent water leaving a WWTS, even a small fraction of microplastics remaining in the effluent water after treatment can translate into high absolute numbers of particles being released to the environment (Murphy et al. 2016). Effluent discharges have therefore been identified as an important pathway for the entry of microplastics into freshwater sources (Murphy et al. 2016).

It is estimated that a single WWTS discharges an average of 2 million microplastic particles per day (Sun et al. 2019). In a study conducted at a WWTS near Vancouver, it was estimated that 32 to 97 million microplastics per day are discharged in effluent (Gies et al. 2018), with fibres and fragments being the most abundant microplastic in the effluent. The study also estimated that of the 1.76 trillion microplastic particles that enter the WWTS each year, 1.28 trillion settle into primary sludge, 360 billion exit in secondary sludge, and 30 billion pass into the secondary treatment effluent and are released into the environment, corresponding to up to 99% removal of microplastics in the WWTS.

The most frequent polymers in WWTS influent and effluent are polyester, PE, PET and PA, with fibres accounting for approximately 52.7% of the microplastics found in wastewater, which is likely attributable to the large amount of fibres released during domestic laundering (Sun et al. 2019). A study conducted by the Swedish Environmental Research Institute found that microfibres were the predominant type of microplastics found in sewage sludge from WWTSs, which is consistent with observations in other studies (Magnusson and Norén 2014; Mahon et al. 2017; Li et al. 2018a).

3.2 Sources to soil

Microplastics can enter terrestrial environments through plastic products used in agriculture, such as plastic seed casings, ground covers, and crop mulch. Land application of biosolids, plastic pollution, and poorly managed landfills are also significant sources of releases to soil (Alimi et al. 2018).

The settling stages of the wastewater treatment process result in the production of sewage sludge that contains large amounts of microplastics (Mahon et al. 2017). It is estimated that 99% of microplastics are removed from the influent but are retained in sewage sludge (Magnusson and Norén 2014) and that the properties of microplastics, such as their hydrophobicity and surface charge, can affect their

accumulation in the solid phase (Murphy et al. 2016). However, the configuration of WWTSs differs, and thus removals from the influent vary from study to study (Novotna et al. 2019). Microplastics can therefore enter terrestrial environments through the application and use of sewage sludge as fertilizers for agriculture or landscaping purposes (Raju et al. 2018). In Europe and North America, around 50% of sewage sludge is recycled for use as fertilizer, and it is estimated that 44 to 300 kt of microplastics are added to farmlands in North America annually (Nizzetto et al. 2016).

3.3 Sources to air

Road traffic-related releases of particles from tire wear and tear may be an important source of microplastics to outdoor air (Kole et al. 2017; Prata 2018). Deposition and dispersion of these particles from the air may result in large accumulations of microplastics in water, and tire wear and tear is estimated to account for 5% to 10% of total microplastics in our oceans (Kole et al. 2017). Additional sources of microplastics in outdoor air are thought to include airplane tires, artificial turf, brake wear, thermoplastic road markings, waste incineration, construction, landfills, industrial emissions, and tumble dryer exhaust, although their relative contributions have not been well established (Dris et al. 2016; Magnusson et al. 2016; Kole et al. 2017; Prata 2018).

The primary source of microplastic particles in indoor air is thought to be the shedding of polymeric textile fibres from clothing, furniture, carpeting, and household goods due to wear and tear or abrasion (Sundt et al. 2014; Dris et al. 2016). For example, washing clothing made from synthetic materials has been shown to release microplastics into wastewater, and it is hypothesized that air- or tumble-drying these garments would also cause fragments to be transferred to indoor air, household dust or dryer lint (Wright and Kelly 2017; Prata 2018). Synthetic textile fibres have also been retrieved from a variety of surfaces, including outdoor surfaces, suggesting that clothing and other fabrics may be additional sources of microplastics in both outdoor and indoor air (Rauert et al. 2014; Dris et al. 2016; Prata 2018).

4. Environmental fate

This section reviews the available data on the fate of macroplastics and microplastics in three environmental compartments: water, soil, and air. It then discusses the persistence of plastics in the environment and the conditions under which they will break down (e.g., transition from macroplastics to microplastics). The fate of biodegradable plastics and bioplastics is also addressed.

The transport of plastic pollution often follows hydrological pathways (Windsor et al. 2019), with rivers being a key transport pathway (see Figure 3) (Alimi et al. 2018). From rivers, it is expected that the majority of plastic pollution will eventually be transported to the ocean. The mechanisms of transport are poorly understood, but are thought to be influenced by the shape, density, size, and surface condition (i.e., degree of weathering) of the plastic particle. It is also thought believed that the behaviour of macroplastics differs from microplastics since more energy would be required to transport

larger plastics through an ecosystem even if the same transport mechanism is used (Windsor et al. 2019).

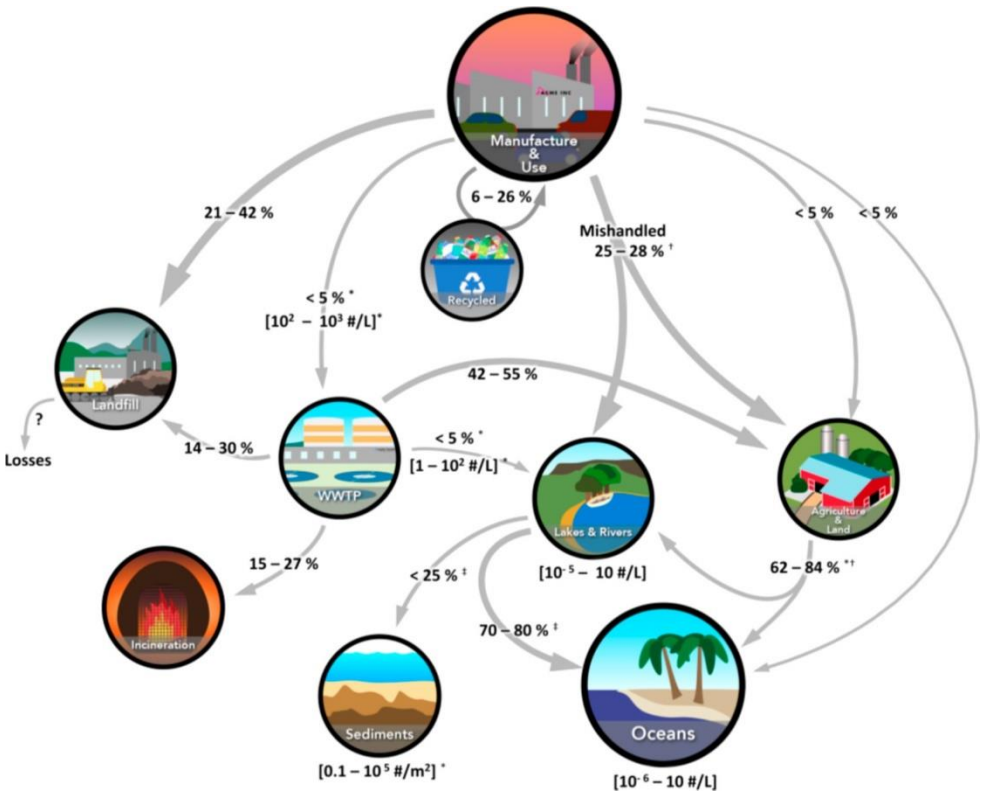

Figure 3: Estimated plastic loading and transport pathways in the environment (reproduced with permission from Alimi et al. 2018, © 2018 American Chemical Society).

Long Form Description: This figure shows the estimated plastic loading and transport pathways in the environment. Manufacture and use of plastic can result in plastics being landfilled, recycled, mishandled, or ending up in wastewater treatment plants. Mishandled plastics can end up on land, or in lakes and rivers, and subsequently in oceans and sediment. Plastics in wastewater treatment plants can be incinerated, end up in lakes and rivers or on land (reproduced with permission from Alimi et al. (2018). Copyright 2018 American Chemical Society.)

4.1 Degradation

Plastic degradation in the environment is slow and can be affected by multiple factors (Andrady 2015; Gewert et al. 2015). Plastics that are exposed to sunlight, oxidants, and physical stress over time will weather and degrade, although the extent of degradation depends on both the environment and the chemical composition of the plastic (Eubeler et al. 2010).

Owing to their chemical structure, common synthetic polymers are durable and can be resistant to degradation. However, there are multiple processes that can bring about the degradation of polymers. These include solar UV-induced photodegradation, thermo-oxidation, hydrolysis, and biodegradation

(i.e., degradation by microorganisms). The most common polymers in the environment, such as PE, PP, PS and PVC, possess a carbon backbone that is resistant to biodegradation. Therefore, in order for biodegradation of these polymers to occur, an abiotic degradation step is needed to first break them down into smaller, lower molecular weight fragments (Gewert et al. 2015; Ng et al. 2018). Given that plastic degradation occurs primarily through exposure to sunlight, degradation is most intensive in environments such as the sea surface and beaches (Andrady 2015).

The first visual effects of polymer degradation are changes in colour and cracking of the surface (Gewert et al. 2015). Surface cracking causes the inside of the plastic material to be further available for degradation, eventually leading to embrittlement and physical breakdown upon exposure to abrasive or mechanical forces, such as wind, waves, and physical impacts (Gewert et al. 2015; Ng et al. 2018; SAPEA 2019). Over time, fragmentation can result in plastics no longer being visible in the environment (Selke et al. 2015).

It is important to note that several degradation pathways may take place simultaneously since various factors initiate degradation. For that reason, degradation products may be more diverse than those expected for any specific pathway. In the marine environment, most plastics degrade first at the polymer surface that is exposed and available for chemical or enzymatic attack. Microplastics have higher surface-to-volume ratios than macroplastics and therefore degrade faster than macroplastics, but the process is still slow (Andrady 2015).

There are numerous gaps in research on plastic degradation. To estimate biodegradation, many studies examine factors such as weight loss, decrease in tensile strength, visual disappearance, or the growth of different microorganisms (Zumstein et al. 2019).

Commercial plastics often include additives that, when released, may degrade to form other chemicals. In addition, additives such as stabilizers may enhance resistance to degradation. A study by Selke et al. (2015) evaluated the effect of biodegradation-promoting additives on the biodegradation of PE and PET in compost, landfill, and soil environments. They found that none of the additives significantly increased biodegradation in any of the conditions, and there was no evidence that these additives promoted or enhanced biodegradation of PE or PET polymers (Selke et al. 2015).

4.1.1 Biodegradable plastics and bioplastics

Biodegradable plastics and bioplastics have been suggested as alternatives to reduce the environmental burden of conventional plastics. Biodegradable plastics can be derived from petroleum or bio-based resources, whereas bioplastics are defined as polymers derived from biomass.

Biodegradable plastics have chemical functionalities on their backbone that render them more susceptible to conventional degradation mechanisms (hydrolysis, ultraviolet light) or to decomposition by living organisms (Ng et al. 2018). These organisms, such as fungi or bacteria, decompose biodegradable plastics either aerobically or anaerobically. Some types of biodegradable plastics will not

mineralize[2] unless they are exposed to temperatures above 50°C for long periods of time, conditions that are found in industrial composting facilities but rarely in the natural environment (UNEP 2015).

Bioplastics do not possess any inherent superiority to petroleum-based plastics (Vert et al. 2012) and do not necessarily biodegrade more readily than conventional plastics, despite the common perception that they do (European Commission 2019). However, there can be advantages to sourcing plastic feedstock from renewable materials, such as helping with decarbonization efforts or providing demand for residual biomass that exists in integrated agriculture and forestry sectors. A full life cycle assessment would be required to demonstrate whether or not bioplastics are preferable to conventional plastics from an environmental perspective (Vert at al. 2012).

Oxo-plastics are formulated using conventional polymers, such as PE and PS, with the addition of heat and UV-activated additives to accelerate their fragmentation into very small pieces. While it is expected that accelerated fragmentation would also accelerate degradation, the degree and speed of fragmentation are dependent on conditions that change from day to day and by location. Therefore, there is no conclusive evidence that accelerating fragmentation will enable degradation (European Commission 2018).

In landfills, the majority of plastics will not have direct access to oxygen. Little to no biodegradation of oxo-plastics is expected in deeper landfill layers. In the marine environment, there is insufficient evidence to prove that oxo-degradable plastics will biodegrade in a reasonable timeframe (European Commission 2018). Furthermore, fragmenting oxo-plastics will result in increasing concentrations of microplastics that are less likely to be recovered in clean-up exercises. Fragmented plastics are not likely to fully biodegrade, resulting in a direct contribution to microplastic pollution (UNEP 2015). Increased fragmentation may, however, lead to fewer cases of animal entanglement (European Commission 2018).

Biodegradable and compostable plastics are typically labelled with reference to a certification that outlines the criteria needed to achieve biodegradation (e.g., biodegradability in industrial compost). Nazareth et al. (2019) studied the biodegradation of these types of plastics outside of their intended waste stream. The authors performed a biodegradation study in seawater for six plastic samples labelled as either biodegradable or compostable. Plastic samples were randomly selected from supermarkets, restaurants, and stores in Canada, the United States, and Brazil. After 180 days, four of six plastic samples showed no signs of chemical or morphological changes. Only one sample showed both chemical and morphological changes and was largely degraded after the test period. Prior to this experiment, Lambert and Wagner (2017) identified the need to differentiate degradation pathways under different conditions. For instance, polylactic acid (PLA) is biodegradable in industrial composting facilities, but does not biodegrade under natural conditions.

[2] Mineralization is the complete breakdown of a polymer as a result of abiotic and microbial activity into inorganic compounds (e.g., CO_2, H_2O, and methane) (UNEP 2015).

Overall, there is no significant evidence that biodegradable plastics will fully degrade in a natural environment, and there is no conclusive evidence as to the beneficial effect of biodegradable plastics, including oxo-plastics, on the environment (European Commission 2018).

4.2 Fate in water

The proportion of plastics present in surface waters and sediments varies depending on the biological (e.g., attachment of bacteria/algae), physicochemical (e.g., plastic density), and hydrodynamic conditions (e.g., mixing of the water column) (Alimi et al. 2018). Factors such as wind, surface water circulation, temperature and salinity influence the distribution of microplastics (Zbyszewski et al. 2014; Corcoran et al. 2015; Anderson et al. 2016).

In the aquatic environment, the rate of degradation of plastics is temperature-dependant, with degradation proceeding more slowly in cold water (Andrady 2015). Plastics found below the photic zone in the water column degrade very slowly, resulting in high persistence of plastics in the aphotic zone, particularly at the seafloor. In addition, biodegradation of plastics by microorganisms is negligible because of the slow kinetics of biodegradation at sea and the limited oxygen supply for these processes (Andrady 2015).

A study by Leonas and Gorden (1993) looked at disintegration rates of LDPE, PS, and a 2% ethylene-carbon monoxide polymer, as well as other blends in aqueous media. The results showed that while the ethylene-carbon monoxide polymer disintegrated[3] more rapidly than the other films evaluated, the aqueous environment significantly delayed, if not inhibited, the degradation of the other polymers.

Biber et al. (2019) studied the deterioration of different plastics in air and seawater. Macro-sized pieces of PE, PS, PET, and a commercial material marketed as biodegradable plastic were exposed to environmental conditions in air and water. All materials deteriorated more slowly in seawater than in air, likely due to reduced exposure to light and thus reduced photooxidation in seawater. The authors found that PS showed the most rapid deterioration and is likely to break down into microplastics faster than the other materials evaluated, but that all materials tested did deteriorate to microplastics. Given the requirements for breakdown, it is expected that plastic items likely remain in seawater and that the formation of microplastics would occur in areas where plastic pollution is exposed to oxygen and UV radiation, such as intertidal habitats and at the water surface.

4.2.1 Sediment

Plastics may remain in benthic systems of lakes and rivers or be transferred along an altitudinal gradient towards marine ecosystems. As plastics move from source to sink, they interact with the physical, chemical, and biological environments in ways that depend on the characteristics of the plastics (e.g., density) (Windsor et al. 2019).

[3] Disintegration is the breakdown of the polymer material as evidenced by the loss of physical and mechanical properties.

Besseling et al. (2019) found that microplastic concentrations on a volume basis are higher in sediments than in surface water. This can be explained by the settling of particles either as singular particles or in aggregated or fouled form. The authors also found that concentrations in beach sediments were higher than in subtidal sediments, which may be explained by the relatively low density of plastics compared to seawater, causing floating and suspended plastics to be washed ashore.

Sinking fecal matter from zooplankton that have ingested microplastics represents a mechanism by which floating plastics can be vertically transported away from surface waters and into deeper waters and the benthos, thus providing food for sediment-dwelling biota (Cole et al. 2016). Wieczorek et al. (2019) found that microplastics significantly altered the size, density, and sinking rates of zooplankton fecal pellets. In oceanic conditions, fecal pellets with reduced sinking velocities are more prone to consumption, fragmentation, and microbial degradation, resulting in their mineralization within the upper regions of the water column and therefore in reduced particulate organic matter export to deeper waters (Cole et al. 2016).

Fecal pellets containing microplastics that reside at the sea surface for a prolonged period are also more readily available for ingestion by other organisms, resulting in the trophic transfer of microplastics. Wieczorek et al. (2019) note that despite this, microplastics have been found in deep-sea sediments and benthic deep-sea organisms. Thus, an unknown proportion of microplastics are likely being transported to the seabed from fecal pellets where they become available to the benthos communities.

While sediment is largely expected to be a sink for macroplastics and microplastics (Eriksen et al. 2014; Woodall et al. 2014), there is significant mobilization and removal of microplastics in sediment during high flow events such as flooding (Hurley et al. 2018). Plastics in benthic sediments may be temporarily stored and remobilized by physical and biological processes. However, there is limited research on these mechanisms of plastic transport in aquatic systems (Windsor et al. 2019).

4.2.2 Impact of biofouling on aquatic distribution

Biofouling, also known as biofilm formation, is the coating of plastics with microorganisms, algae, or plants. This process can lead to a loss of buoyancy and thus promote the sinking of microplastics to the bottom of the water body (Weinstein et al. 2016; SAPEA 2019). It has been hypothesized that phytoplankton aggregates act as potential sinks for microplastics (Long et al. 2015). Kaiser et al. (2017) found that the sinking velocities of PS particles increased by 16% in estuarine water and 81% in marine water after a 6-week incubation period, which allowed for the particles to become coated with biofilms. The sinking of PE particles was not impacted by biofouling during 14 weeks of incubation in estuarine water, but in coastal water, their sinking velocity increased after 6 weeks. These results indicate that biofouling can enhance deposition of plastics to sediments and ocean beds (Kaiser et al. 2017). Further, Weinstein et al. (2016) indicated that biofilm formation on plastics decreases their UV transmittance, which could also inhibit the degradation of plastics in the environment.

4.3 Fate in soil

Although limited scientific information is available on the fate of plastics in the soil compartment, studies indicate that biodegradation can play a role in the fate of plastics in soil. Certain organisms, such as bacteria (Huerta Lwanga et al. 2018) or insect larvae (e.g., moths), can degrade plastics; however, this is not likely a relevant process in natural agroecosystems since they may not be naturally present in these environments (Ng et al. 2018). Alternatively, co-metabolism (i.e., the degradation of a compound in the presence of another compound used as a carbon source) is more likely to occur due to the abundance of carbon resources in soil (Ng et al. 2018).

The physicochemical state of plastics is also likely to be very dynamic in soil due to interactions with soil components, including organic matter (Ng et al. 2018). Interactions with certain pesticides can facilitate photodegradation or embrittlement of plastic particles (Schettini et al. 2014). As photo- and thermo-oxidative degradation pathways both rely on the combination of free radicals and oxygen, these processes only occur near or at the surface of soil (Ng et al. 2018).

In a study by Cosgrove et al. (2007), PU films were observed in soil at different organic carbon contents and different pH levels, and their fungal communities were compared. PU films appeared to be highly susceptible to biodegradation in soil and were degraded almost completely after 5 months (Cosgrove et al. 2007; Eubeler et al. 2010). In another study, biodegradation in compost was investigated for irradiated ethylene propylene copolymers, LDPE, and isotactic PP films (Eubeler et al. 2010). The results showed that degradation increased with increasing irradiation time; however, after 6 months of exposure, LDPE was still the slowest sample to be degraded as measured by weight loss (Eubeler et al. 2010). Ohtake et al. (1995) found no evidence of biodegradation for PS, PVC and urea formaldehyde resin that had been buried under soil for over 32 years. Another study found that a LDPE bottle buried in shallow soil under aerobic conditions for over 30 years underwent degradation on the surface, but the inner part was almost unchanged (Ohtake et al. 1996).

Following the release of microplastics to the terrestrial environment, particles can be transported to surface water bodies by wind and water erosion or dispersed through ingestion by organisms (Maaß et al. 2017; Hurley and Nizzetto 2018). There is also the potential for microplastics to leach into groundwater aquifers due to downward drainage from soils (Re 2018).

Soils are also expected to act as a major sink for plastic particles (Hurley and Nizzetto 2018). Microplastics are likely to be retained in soils for long periods of time due to factors such as vertical transport that draw the particles away from the surface, hindering degradation (Horton and Dixon 2017; Huerta Lwanga et al. 2017). Zubris and Richards (2005) studied fibres in soil as an indicator of the application of biosolids to land in the United States. The authors detected fibres in soil samples from field application sites up to 15 years after the application of sludge, and these data were corroborated with biosolids application records. Additionally, vertical transport of microplastics is possible via the movement of soil organisms (Huerta Lwanga et al. 2017; Maaß et al. 2017; Rillig et al. 2017) as well as agricultural processes (e.g., tilling), which can also cause physical damage to the particles (Ng et al. 2018).

4.4 Fate in air

Although research on the fate of microplastics in air is lacking, it is understood that air is likely an important pathway for the transport of microplastic particles (Dris et al. 2016).

When released into the atmosphere, microplastics can become suspended, or further transported, due to their light weight (Horton and Dixon 2017). Suspension and dispersion of particles in the air is dependent on factors such as the size, shape and density of a particle (lighter, less dense polymers can be carried more easily), wind conditions, and precipitation, which can facilitate deposition on land or water (Dris et al. 2016; Prata 2018).

Air currents and wind can transport particles long distances. Since air currents can be multidirectional, transport in air is less limited than transport in aquatic or terrestrial environments (Horton and Dixon 2017). For example, Allen et al. (2019) observed microplastic deposition in the French Pyrenees, a remote mountain catchment. Preliminary trajectory assessments showed that the microplastics had travelled up to 95 km from their source, indicating possible long-range transport. Microplastics have also been found in the Arctic Ocean in several studies. Lusher et al. (2015b) first reported the quantity of microplastics in surface and sub-surface Arctic polar waters. Subsequently, Bergmann et al. (2017) found large quantities of microplastics in Arctic deep-sea sediments, Kanhai et al. (2018) identified the abundance, distribution and composition of microplastics in sub-surface waters of the Arctic central basin, and Peeken et al. (2018) found microplastics in Arctic sea ice cores at five different locations and analyzed their content and composition. As with persistent organic pollutants, it is speculated that long-range atmospheric input of microplastics is one of several possible transport mechanisms (with others being prevailing currents and food-webs) to the remote Arctic Ocean (AMAP 2004; Kanhai et al. 2018; Peeken et al. 2018).

A number of studies have quantified microplastic presence in the atmosphere as well as in fallout (i.e., particles that settle on a filter during the sampling period). One study, for instance, observed atmospheric fallout of microplastics at a sampling site in a dense urban environment with a daily range of 2 to 355 particles/m^2 (Dris et al. 2016). A previous study by Dris et al. (2015) measured a total atmospheric fallout of 29 to 280 particles/m^2/day in the urban Greater Paris region. In Dongguan City, China, the concentrations of microplastics in atmospheric fallout samples collected from three sites over a period of 3 months were 31, 33, and 43 particles/m^2/day (Cai et al. 2017). Three different polymer types were identified in the microplastic samples (PE, PP, and PS), and fibres were the predominant shape of the microplastics sampled. In the metropolitan region of Hamburg, a median microplastic fallout concentration of 136.5 to 512.0 particles/m^2/day was found across six sampling sites over a 12-week sample collection period (Klein and Fischer 2019). Of the microplastics detected, 95% were fragments, with fibres making up the remaining 5%. During periods of higher rainfall, Dris et al. (2016) observed a higher number of fibres in atmospheric fallout; however, there were likely other temporal and mechanistic factors at play, which the authors did not identify.

In general, atmospheric concentrations of microplastics are likely to be correlated with population density, as human activities strongly influence the environmental release of microplastics. The fate and

transport will depend on prevailing meteorological conditions, with long-range transport from urban source regions to remote locations highly probable.

5. Occurrence

This section reviews the available data on the occurrence of macroplastics and microplastics in the water, soil and air compartments, as well as in other matrices through which humans may be exposed to microplastics of environmental origin (namely food and drinking water). Occurrence in biota, with the exception of occurrence in food, is covered in Section 6. Where possible, Canadian occurrence data are presented. However, since Canadian occurrence data are often lacking, data from other areas around the world are also presented in many instances.

The absence of standardized methods and analytical techniques poses a significant challenge to quantifying microplastics in the environment. As a result, it is not possible to quantitatively characterize environmental or human exposure levels at this time. In light of these limitations and due to the small number of studies available, this report does not undertake a quantitative exposure assessment.

5.1 Environmental occurrence

5.1.1 Occurrence in the aquatic environment

Plastic pollution in the aquatic environment is summarized below with a focus on four compartments: shorelines, surface waters, benthic zone (i.e., the bottom of a water body) and groundwater. As there are no standardized procedures for quantifying microplastics in the environment, the focus was on studies that, where possible, applied practices such as the use of controls, use of appropriate and clean glassware, and application of contamination avoidance measures. For occurrence of microplastics on shorelines and in surface water, preference was given to studies in which microplastics were identified with an analytical method such as Fourier transform infrared spectroscopy (FTIR), Raman spectroscopy, or pyrolysis gas chromatography mass spectrometry (GCMS). If any studies included in this report deviated from these criteria, it is explicitly mentioned in the text.

Shoreline

In an effort to remove litter from Canada's shorelines, 21 300 cleanups have been organized by the Great Canadian Shoreline Cleanup (GCSC) across the country since 1994 (GCSC 2018a). Of the top 10 most common litter types collected during the 2018 Shoreline Cleanup, seven were either plastics or items containing plastic. Plastic items included cigarette butts, tiny plastics or foam, bottle caps, plastic bags, plastic bottles, straws, and food wrappers (GCSC 2018b). A total of 0.1 kt of litter was removed from Canadian shorelines in 2018 (GCSC 2018b). Figure 4 illustrates the contribution of plastics to shoreline litter collected during historical beach cleanup surveys of the Great Lakes.

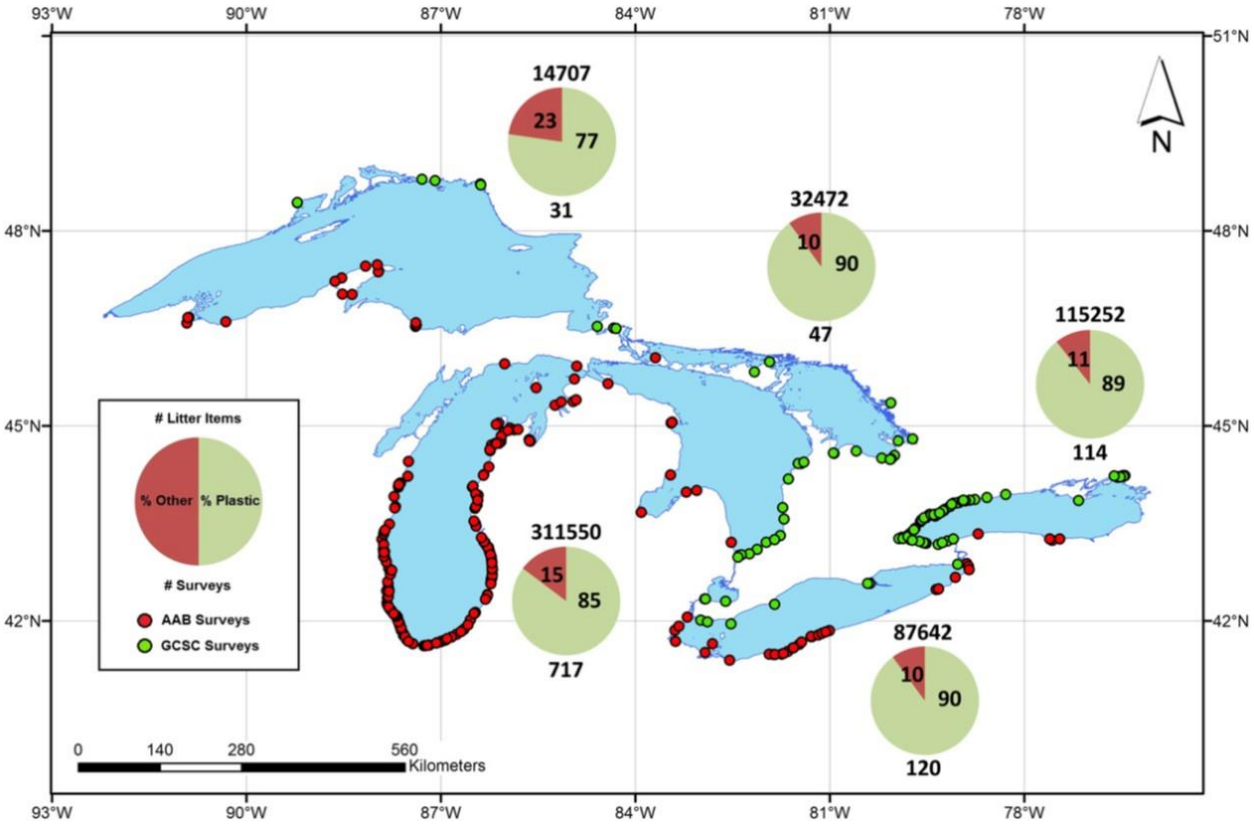

Figure 4: Great Lakes beach cleanup surveys (reproduced with permission from Figure 2 of Driedger et al. 2015)

Long Form Description: Great Lakes beach cleanup surveys. The figure shows the locations of beach cleanup surveys, the number of surveys conducted, and the percentages of anthropogenic litter comprised of plastic for each of the Great Lakes. Lake Ontario: 115252 litter items collected from 114 surveys (89% plastic); Lake Erie: 87642 litter items collected from 120 surveys (90% plastic); Lake Huron: 31472 litter items collected from 47 surveys (90% plastic); Lake Michigan: 344550 litter items collected from 717 surveys (85% plastic); Lake Superior: 14707 litter items collected from 31 surveys (77% plastic). The data used in the figure were collected by Adopt-a-Beach™ (AAB) and Great Canadian Shoreline Cleanup (GCSC) volunteers in 2012. (Reproduced with permission from Figure 2 of Driedger et al. 2015)It is worth noting that brittle plastic materials may break into smaller fragments during analysis, and broken pieces may be quantified as microplastics rather than macroplastics, thus affecting counts (Esiukova 2017). On the other hand, other methods of plastic quantification may underestimate the amount of microplastics, such as surveys on rocky shorelines. McWilliams et al. (2018) highlighted the need to further develop protocols and techniques to sample microplastics on rocky shores where marine litter may be caught between rocks and crevasses, thus increasing their lifetime on the shore. In addition, waves may grind macroplastics against rocky shores, accelerating their breakdown into microplastics. The authors conducted an accumulation survey of Fogo Island beach in Newfoundland and found that 82% of marine litter collected from the rocky beach surface consisted of plastics, with 67% of litter being smaller than 1 cm^3.

McWilliams et al. (2018) also conducted a standing stock survey to assess the abundance of plastics at different depths of the beach on Fogo Island. This was done by first picking visible particles from the top layer of shoreline, followed by shovelling a 5 cm layer into a tray. Stratified sampling was performed to a depth of 20 cm. Across all layers, glass comprised 75.7% of litter, and plastics comprised 17.9%. More than 82% of the plastic particles sampled were macroplastics. Plastic particles were found throughout the different depths sampled, with the vast majority of smaller items found below the surface. Plastic particles below the surface were found to be smaller and more abundant than particles on the surface. The surface accumulation survey in conjunction with the standing stock survey provides insight into how many particles would be missed by a survey that only considers particles on the surface.

Proximity to industrial sources may be associated with higher concentrations of plastics (Zbyszewski et al. 2014; Zbyszewski and Corcoran 2011; Ballent et al. 2016; Driedger et al. 2015). Zbyszewski et al. (2014) collected samples along the shorelines of Lake Erie, Lake Huron and Lake St. Clair. Macroplastics and microplastics were found along all shorelines. Additionally, Zbyszewski and Corcoran (2011) found that along the shoreline of Lake Huron, pellets represented the majority of the plastic collected. Most of the collected industrial pellets were PE and PP that are similar to those produced by petrochemical companies. It should be noted that more than 94% of the total plastic pellets were found at one beach in Sarnia (Zbyszewski and Corcoran 2011).

Munier and Bendell (2018) visually identified and collected plastic pollution on the beach surface of Burrard Inlet in British Columbia. Of the 150 items collected, 144 were plastics, which were divided into 7 major user groups: bags, car/bike parts, everyday items, food associated, packaging, functional use, and children's toys. The majority of the plastics were wastes related to food consumption, such as cups, straws and forks, and packaging.

Corcoran et al. (2015) collected 6172 plastic pieces from Humber Bay Park West beach on the northwest shoreline of Lake Ontario. Excluding polystyrene, which was only quantified by mass due to the large quantity collected, the plastics concentration was 21.8 items/m^2, with industrial pellets being the most common type of plastic, followed by fragments. The majority of pellets and fragments had accumulated within organic matter along the strandline. Ballent et al. (2016) also examined the presence of microplastics in beach sediment along the shorelines of Lake Ontario. Unlike Corcoran et al. (2015), fragments were the predominant type of microplastic detected in beach sediment, followed by fibres. Beach sediment contained an average of 140 particles/kg dw.

In Lake Erie, Dean et al. (2018) collected 12 sediment samples from six beaches at the foreshore (between low- and high-water marks) and backshore (high-water mark to inland limit of beach). All samples contained microplastics, with a range of 50 to 146 particles/kg. Most of the backshore samples contained higher concentrations of microplastics than the foreshore samples. The dominant microplastic type was fibres, followed by fragments. It should be noted that although precautions were taken against the contamination of samples from microplastics during processing, some samples were stored or sampled in PET jars or PVC liners, and sometimes new and unopened plastic containers were used in the field without prior rinsing (Dean et al. 2018).

Single-use plastics are one of the most common types of macroplastics found on shorelines internationally. In Canada, 17% of collected shoreline litter consisted of plastic single-use food and beverage items (GCSC 2018b). Similarly, Earthwatch Europe (2018) found that single-use plastics are a significant category of plastic waste in European freshwater environments. The top 10 most prevalent macroplastics in European freshwater environments were plastic bottles (14% of identifiable plastic pollutants), food wrappers (12%), cigarette butts (9%), food takeout containers (6%), cotton bud sticks (5%), cups (4%), sanitary items (3%), smoking-related packaging (2%), plastic straws, stirrers and cutlery (1%), and plastic bags (1%) (Earthwatch Europe 2018). Cigarette butts rank high on both European and Canadian litter lists, with cigarette butts topping Canada's 2018 Shoreline Cleanup for the highest abundance of litter and smoking-related litter making up 42.1% of the types of litter collected. In the northeast Atlantic, marine litter ranging from 2 to 30 cm was collected on beaches in the Azores, and plastic items accounted for 93% (26 321 items) of all litter. The collected litter consisted of 15.1% single-use items, 7.9% fishing-related items, and 71% fragments (Pieper et al. 2015). In the southern Caribbean, 42 585 litter items greater than 25 mm were collected at 10 locations on sandy beaches in Aruba. Of the litter collected, 89% (38 007 items) consisted of plastics. The collected litter was composed of 51% single-use plastics, of which 18% was bottle or container caps, 9% straws and 7% cigarettes. Additionally, 5% of all litter collected was fishing-related and 40% consisted of fragments of undiscernible origin (de Scisciolo et al. 2016).

In Canada, fishing-related litter made up only 1% of the litter collected in the 2018 Canadian Shoreline Cleanup (GCSC 2018b), whereas international levels show higher abundances in the water. Additionally, plastic items related to fishing activity make up a significant amount of plastic waste found on shorelines globally (Browne et al. 2010; Chen et al. 2019; PAME 2019). Fishing-related litter is especially significant in the Arctic, where most of the marine litter analyzed in the northern parts of Norway, the Barents Sea region, and the Arctic originate from fishing-related activities (Hallanger and Gabrielsen 2018). Fishing-related litter also accounted for 48% to 100% of the mass of litter on the beaches of Svalbard, Norway (PAME 2019). An average of 1040 plastic items/km were collected in Iceland, corresponding to an average of 104 kg/km that mostly originated from Icelandic fisheries (Kienitz 2013).

Plastics have been reported on shorelines around the world. Microplastics have been found on every Californian beach sampled by Horn et al. (2019), and fibres accounted for 95% of the microplastic items. Macroplastics have been found on beaches surveyed in Polynesia (Connors 2017) and on shorelines in East China (Chen et al. 2019). In China, microplastics were collected on beaches adjacent to the Bohai and Yellow Seas, where flakes were the most abundant type of plastic (Zhou et al. 2018). On surveyed South African beaches, industrial pellets were the most abundant type of plastic (Ryan et al. 2018). Typically, pellets enter the environment via accidental spills on land or at sea, and weather conditions play a factor in industrial pellet accumulation, as well as the presence of beached organic materials (e.g., woods, weeds) in which they may become entrapped (Corcoran et al. 2015; Ryan et al. 2018). Microplastics have also been collected on beaches along the southeast coast of India (Karthik et al. 2018). Finally, both macroplastics and microplastics are widely found in the Arctic, despite its distance from industrialized and highly populated areas (PAME 2019). Refer to Section A-1 of Appendix A for further data on the occurrence plastics on shorelines.

Surface water

Several studies have looked at microplastic occurrence in Canadian freshwater bodies, with a focus on the Great Lakes. According to Driedger et al. (2015), areas with greater human and industrial activity in the Great Lakes region are generally associated with a higher abundance of plastic pollution in the adjacent Great Lakes basins.

In the Government of Canada's 2015 Science Summary on Microbeads (ECCC 2015), several publications reporting on the presence of microplastics in surface water were summarized. Building on this, a review of additional current literature on Canadian occurrence of microplastics in surface water is provided below.

Higher concentrations of plastics can be found near harbours or recreational areas and shipping routes (UNEP 2016). Hendrickson et al. (2018) studied microplastic occurrence in the surface waters of western Lake Superior. Sample sites were selected to include environments suspected to differ in microplastic distribution based on their proximity to presumed sources of microplastic pollution, such as WWTSs, urban shorelines and river outflows. On average, the estuary and harbour regions had the greatest abundance of microplastics, followed by open water sites and then nearshore sites. The average abundance for all sites was 37 000 particles/km^2. Fibres were the most abundant type of particle, followed by fragments, films, beads, and foams.

Anderson et al. (2017) found microplastics in all surface water samples collected from Lake Winnipeg at densities ranging from 66 788 to 293 161 particles/km^2. Microplastic densities in Lake Winnipeg were significantly higher than those reported for Lake Superior and Lake Huron, but were comparable to those of Lake Erie. There were no significant differences between nearshore and offshore sites. Fibres were the most common plastic type, whereas films and foams were the least common. In general, microfibres are one of the most common types of microplastic found in the aquatic environment. However, the distribution of microplastic type may also depend on the sample location as well as the method of quantification.

Globally, plastics have been reported in fresh and marine surface waters, and extensive research has been done in marine surface waters. Macroplastics have been found in the Adriatic Sea, where plastic bags constitute nearly one-third of floating macroplastics (Zeri et al. 2018). Foamed polystyrene items were the most frequently observed macroplastics surveyed in the South Pacific, North Pacific, South Atlantic, and Indian Oceans as well as around Australia (Eriksen et al. 2014). As plastic spreads throughout the world's oceans, it accumulates in subtropical gyres, such as the North Atlantic Subtropical Gyre and the North Pacific Subtropical Gyre, which is commonly referred to as the Great Pacific Garbage Patch (Eriksen et al. 2014; Poulain et al. 2019). Microplastics have also been found in Lake Michigan in the United States (Mason et al. 2016), in the Mediterranean Sea (de Haan et al. 2019) and in the Northwestern Pacific Ocean (Pan et al. 2019). Finally, macroplastics and microplastics have been found in Arctic surface water and in sea ice, and the majority of microplastics were fibres (Obbard et al. 2014; Lusher et al. 2015b; Peeken et al. 2018). Refer to Section A-2 of Appendix A for further data on plastic occurrence in surface waters.

Benthic zone

Microplastics have been reported in the benthic zone of Canadian waters. For example, in a study on the abundance and distribution of microplastics in surface sediments in Baynes Sound and Lambert Channel in British Columbia (Kazmiruk et al. 2018), microplastics were found to be abundant in the sediment samples from all sampling locations. Microbeads were the most common type of microplastic found, with a maximum of 25 368 beads/kg sediment sampled at one site, followed by fibres and fragments.

Ballent et al. (2016) quantified microplastics in Lake Ontario in nearshore, tributary, and beach sediment. Fragments and fibres were the dominant type of microplastic in the size range of less than 2 mm, and fragments and industrial pellets were the primary type of microplastic in the greater than 2 mm size range. Fibres were most abundant in nearshore samples, and pellets were present in all depositional environments, but not in sediment traps. Polyethylene was the most common type of polymer in the samples analyzed. The mean microplastic abundance was 760 particles/kg sediment. The highest abundances of microplastics were found in nearshore sediments, with 980 particles/kg dw, followed by tributary and beach sediments. Lake-bottom samples were also collected from Lake Ontario, with a total of 35 pieces being found in the two sample cores (Corcoran et al. 2015). No plastics were found in samples collected from depths greater than 8 cm.

Dean et al. (2018) examined microplastic occurrence in nearshore and tributary sediment along the shoreline of Lake Erie. Benthic sediment was sampled from Lake Erie nearshore locations, from the mouth of the Grand River, and from the Detroit River. Sediment samples were also collected from two northwestern Lake Erie tributaries and two northeastern tributaries. The concentration of microplastic particles in nearshore samples ranged from 0 to 391 particles/kg sediment, and fibres were the primary type of microplastic, followed by fragments. Tributary samples ranged from 10 to 462 particles/kg sediment with fragments dominating the samples, followed by fibres. A tributary sample from the Welland Canal, which is exposed to high shipping traffic and a sizable population, contained the largest concentration of microplastics. The passive sediment trap sample contained no microplastics, whereas the grab sample from the same location contained 390 particles/kg sediment (Dean et al. 2018).

Globally, plastics have been reported in marine sediment, where they are typically dominated by microplastics. Macroplastics have been found in sediment in Argentina and the United Kingdom, and the dominant type of plastic was packaging and wrappers (Browne et al. 2010; Blettler et al. 2017). Macroplastics and microplastics have also been found in sediment in Italy, with fibres being the most abundant type of microplastics (Fastelli et al. 2016). Similarly, fibres were the predominant type of microplastic found in Croatia and in the Arctic (Sundet et al. 2016; Blašković et al. 2017; Renzi et al. 2019). Microplastics found in river sediment in Shanghai consisted primarily of spheres, and the most dominant polymer was polypropylene, similar to the situation in Hungary and on Rameswaram Coral Island, along the southeast coast of India (Peng et al. 2018; Vidyasakar et al. 2018; Bordós et al. 2019). Plastics have been collected from the Spanish Mediterranean seafloor (García-Rivera et al. 2018), the Arctic seafloor (PAME 2019), and the Pacific Ocean's Mariana Trench (Morelle 2019). Refer to Section A-3 of Appendix A for further data on plastic occurrence in the benthic zone.

Groundwater

Groundwater is likely less vulnerable to microplastic pollution than surface waters (WHO 2019), although it has recently been hypothesized that microplastics from soils may be transported to and within aquifer systems (Re 2019). Currently, there is very little empirical data on the occurrence of microplastics in groundwater.

Mintenig et al. (2019) investigated the presence of microplastics in drinking water derived from groundwater sources in the northwest region of Germany. Groundwater (i.e., raw water) was supplied from wells at least 30 m in depth and microplastics over 20 µm were detected. Despite the use of very large volumes of water (1 000 L), very low microplastic concentrations were observed in groundwater, ranging from 0 to 0.007 particles/L, with a mean of 0.0007 particles/L. All identified microplastics were found to be small fragments between the sizes of 50 and 150 µm, with the predominant polymer types being polyester, PVC, PE, PA and epoxy resin.

A South African scoping study surveyed microplastics in groundwater from four boreholes in Potchefstroom (North West), South Africa (Bouwman et al. 2018). The mean microplastics concentration reported was 0.167 particles/L. From the microplastics identified, many of the fragments were in the lower size class range below 600 µm.

Panno et al. (2019) studied the occurrence of microplastics in springs and wells (<65 m) from two karst aquifers in the U.S. state of Illinois. Previous studies on the groundwater chemistry in these areas reported data suggesting input from septic effluent. The authors reported the presence of microplastics in 16 of the 17 water samples collected, with a median concentration of 6.4 particles/L and a maximum of 15.2 particles/L.

5.1.2 Occurrence in soil

The occurrence of plastics in soil is not as well studied as it is in water and sediment. Soil is an important environmental compartment in which to quantify microplastics as they may enter soils via plastic mulching or application of biosolids, among other sources. Given the lack of research on microplastic occurrence in soil, the criteria for selecting studies for this report were less stringent than for occurrence in surface waters.

In Germany, agricultural farmland was found to have 206 macroplastic pieces per hectare. The mean concentration of microplastics sized 1 to 5 mm was 0.34 particles/kg dry weight (dw) of soil (ranging from 0 to 1.25 particles/kg dw). The most common type of polymer for both macroplastics and microplastics was polyethylene (67.9% and 62.50%, respectively) (Piehl et al. 2018).

Liu et al. (2018) found plastics in farmland soil around the suburbs of Shanghai, China. Macroplastic particles sized 5 mm to 2 cm were found at a concentration of 6.75 items/kg in shallow soil (0 to 3 cm) and 3.25 items/kg in deep soils (3 to 6 cm). Microplastic concentrations were 78.0 items/kg in shallow soil and 62.5 items/kg in deep soil. In general, Liu et al. (2018) found that topsoil contained higher concentrations of larger sizes of plastic particles. Fibres, fragments and films were the most common

types of plastics and the majority of all plastics collected were PP and PE. Zhang and Liu (2018) also explored microplastic occurrence in arable land in southwestern China. The study area consisted of two cropped areas at the upstream and estuary of the Chai River, as well as a buffer zone, which was converted from cropland in 2009 to host indigenous trees. Plastic particles were found in all samples, ranging from 7 100 to 42 960 particles/kg, much higher than the concentrations measured by Liu et al. (2018). In addition, most microplastics were less than 1 mm in size and the dominant type of microplastic was fibres, constituting an average of 92% of samples. During wastewater treatment processes, microplastics can settle in sewage sludge, which can then be transferred to agricultural soils and used as fertilizer (Corradini et al. 2019). Corradini et al. (2019) sampled 30 agricultural fields in Chile with similar soil chemical and physical characteristics, but with different sludge application records over the past 10 years. The authors found high concentrations of microplastics in the soil and reported that microplastics accumulate in the soils with successive sludge applications. Scheurer and Bigalke (2018) found microplastics at concentrations up to 55.5 mg/kg (593 particles/kg) in floodplain soil samples in Switzerland, with a mean concentration of 5 mg/kg. Macroplastics sized 5 mm to 2.5 cm were also found but in much lower concentrations.

5.1.3 Occurrence in air

Indoor air

Limited data are available on exposure to microplastics in the indoor environment. Only two studies were identified in which indoor air was sampled, and three studies were identified in which “fallout” from indoor air or settled dust (i.e., particles sampled from surfaces or vacuum cleaner bags) was collected. Generally, particles were examined and counted microscopically and characterized by size, shape, and composition. However, collection and analysis techniques varied, and therefore comparison between studies is not possible.

In the indoor environment, microplastics are more likely to occur in settled dust than in air, as they have a higher density than air (Henry et al. 2019). This route of exposure is particularly relevant to toddlers and young children, given behaviours such as crawling and hand-to-mouth activity. However, no data have been identified on partitioning of microplastics in indoor environments, and inhalation is therefore also considered a potential exposure route.

Dris et al. (2017) looked at fibres in indoor air, indoor fallout, and settled dust in two apartments and an office in urban Paris. They found that approximately 33% of the fibres were synthetic, including PA, PP and PE. The authors reported air concentrations of 1 to 60 fibres per m^3 (median 5.4 fibres/m^3), and dust concentrations of 190 to 670 fibres per mg. The method was limited to fibres greater than 50 µm in length. However, there was an inverse relationship between the number of fibres and their size, suggesting that smaller fibres could be present in larger numbers. Fibre concentrations in indoor air were significantly higher than in outdoor air.

Vianello et al. (2019) sampled indoor air in three apartments in Denmark and found that microplastics comprised 4% of the particles identified. The average number of microplastic particles in the samples

was 9.3 per m^3. Most (81%) of the microplastics were polyester; other polymers identified included PE, PP, and polyacrylonitrile (PAN). Both fibre and fragment shapes were observed, and the size limit for detection was 11 µm. Like Dris et al. (2017), Vianello et al. (2019) reported an inverse relationship between sample microplastic concentration and median of the size distribution.

Dust was examined from 39 homes in different locations across China (Liu et al. 2019a). PET was identified in all samples, and PC was found in 74% of samples. The method used could detect particles in the range of 50 to 2 000 µm; most microplastic particles were fibrous in shape. Synthetic polymers accounted for approximately 40% of the fibres collected, including polyester, PU, PA, PE, PP, and PAN. A concentration of 17 to 620 fibres per mg of dust was reported. The study authors also reported a concentration of PET in dust by mass (median of 27 µg per mg) and a concentration of PC in dust by mass (median of 0.005 µg per mg).

An earlier study (Schneider et al. 1996) looked at personal exposure to fibres at some European sites, using personal sampling pumps to collect airborne dust. The composition of fibres was not determined, but synthetic organic fibres may have included PE, PP, poly(vinyl alcohol), polyester, PA, and polytetrafluoroethylene.

Cox et al. (2019) did a crude estimate of inhalation exposure to microplastics using the air concentrations of fibres from Dris et al. (2017) and Tunahan Kaya et al. (2018) (see outdoor air exposure section), assuming 33% of the fibres and particles were actually microplastics (Dris et al. 2017). Similarly, Prata (2018) used the data from Gasperi et al. (2015) to estimate the number of airborne microplastics that could enter the human lung each day. However, as discussed above, no quantitative estimate of exposure to microplastics from indoor air and dust was conducted for this assessment due to the limited number of studies available, the very small sample sizes, and the varying techniques and criteria applied for sample collection and particle characterization.

Outdoor air

Only a few studies have investigated microplastics in outdoor air samples. The monitoring methods employ sampling techniques in which predetermined volumes of air are passed through filters onto which particles are collected. Confirmation of microplastic particles among other particles is then completed using traditional methods. There are no Canadian data available, but limited studies were conducted in Europe, Asia and the Middle East.

Dris et al. (2017) measured total fibre concentrations, including microplastic fibres, approximately 3 metres from the roof surface of an office building located roughly 10 km from the centre of Paris (four times throughout the year to account for seasonal variations). The concentration of fibres measured outdoors ranged between 0.3 and 1.5 fibres/m^3 (median of 0.9 fibres/m^3) and was significantly less than concentrations measured indoors within the office and at two residential sites in the same region. One sample collected on a rainy day in winter contained five times more fibres, suggesting that the rain caused fallout of the fibres. The methodology used in this study has a lower observation limit of 50 µm. However, the results revealed a more elevated number of particles in the smaller size fraction, suggesting that microplastics smaller than 50 µm could be present in greater numbers.

The concentration of suspended atmospheric microplastics (SAMPs) measured in Shanghai ranged from 0 to 4.18 SAMPs/m^3 (mean of 1.42 SAMPs/m^3) (Liu et al. 2019b). Microplastic fibres comprised 67% of the SAMPs, followed by fragments and granules (30% and 3%, respectively). The size and concentration of microplastics was shown to vary with altitude. The concentration of SAMPs was highest closer to the ground (1.7 metres), and lower at higher altitudes (33 and 80 metres). Larger sized particles (>5 000 µm) were also detected near ground level and not at higher altitudes. SAMPs were shown to represent 54% of the total particles collected and were comprised mostly of PET, PE, polyester and PAN. Poly(N-methyl acrylamide) (PAA) was the predominant SAMP at the highest altitude. It should be noted that rayon was included in the definition of SAMPs. Because this material is synthesized using cellulose, it is not always grouped with microplastics. This study estimates that the average adult in Shanghai inhales 21 microplastic particles per day.

The limited data on microplastics in outdoor air, measured in France and China, collectively identify an exposure level of approximately 1 microplastic particle per m^3 of air. The primary exposure form is through microplastic fibres. However, there is significant uncertainty with regards to exposure to smaller microplastic particles, particularly those below 50 µm. In outdoor air, it is anticipated that most human inhalation exposures would occur near ground level and that concentrations would depend on many factors, including geographical proximity to outdoor microplastic sources, wind, temperature and precipitation (Prata 2018). Since people spend less time in outdoor or transit environments, they would be exposed to fewer microplastics outdoors than indoors.

5.2 Occurrence in food and drinking water

5.2.1 Occurrence in food

Current knowledge of the occurrence of microplastics in food is limited. The point sources of confirmed microplastics in food are currently unknown, although microplastics likely enter the food chain through plastic waste breaking down in environmental matrices, such as water and air. For example, animal species consumed by humans may ingest microplastics from aquatic environments or become exposed via trophic transfer of microplastics from prey to predator (EFSA 2016; Toussaint et al. 2019). It is also possible for ambient microplastics in the air to settle on food items (Catarino et al. 2018; Li et al. 2018a). In a number of microplastics occurrence studies, contamination of laboratory control blanks and test samples by background and/or ambient air microplastics was reported as a methodological challenge (Mathalon and Hill 2014; Lachenmeier et al. 2015).

Food manufacturing, processing, and handling, as well as food packaging materials, have also been suggested as potential point sources of microplastics in food (Karami et al. 2018; Oßmann et al. 2018; Schymanski et al. 2018). However, to date, there is no conclusive scientific evidence that food packaging materials, when used as intended (i.e., under normal conditions of use), are a source of microplastics in food or bottled water. Further studies are needed to determine whether food manufacturing, processing and/or handling, as well as food packaging materials, may contribute to microplastic concentrations in food.

The majority of available data on findings of microplastics in foods pertain to analyses conducted internationally and, unless otherwise stated, are not Canadian specific data. Most studies have focused on investigating microplastic content in seafood, specifically fish and shellfish harvested from non-Canadian marine environments (EFSA 2016; FAO 2017; Lusher et al. 2017; Barboza et al. 2018; Toussaint et al. 2019).

The available data for other animal species that may be consumed as part of the diet of Indigenous Peoples is summarized in Section 6. It is noted that the available research is limited to identifying macroplastics and microplastics from the perspective of animal health. It is not anticipated that consumption of gastrointestinal (GI) tracts, which would likely have the greatest amount of microplastics for marine mammals, fish and seabirds, is a major source of country food consumption. For example, according to the Inuit Health Survey, the most commonly consumed country foods include the flesh and organs of various mammals, birds and fish, but the survey does not indicate that the GI tract is usually consumed. There is a need for research to quantify the presence of microplastics in the animal tissues and organs that are typically consumed.

While some peer-reviewed studies report the presence of microplastics in certain foods, the particles were not confirmed as plastic, as the methodology employed relied on visual inspection or crude staining (Mathalon and Hill 2014; Desforges et al. 2015; Lachenmeiser et al. 2015; Liebezeit and Liebezeit 2013, 2014, 2015; Rochman et al. 2015; Wójcik-Fudalewska et al. 2016; Karlsson et al. 2017; Kosuth et al. 2018; Renzi et al. 2018). Given the lack of certainty that the particles reported in these studies are, in fact, microplastics, these results are not considered further in this report.

The available data on the occurrence of microplastics in food, including bottled water, are summarized below, with further details available in Appendix B.

Fish and shellfish

The presence of microplastics in the GI tract of over 150 fish species is well-documented, with microplastic content ranging in number from 0 to 20 microplastics per fish and ranging in size from 130 µm to 5 mm (Lusher et al. 2013; Campbell et al. 2017; EFSA 2016; FAO 2017; Barboza et al. 2018; Hantoro et al. 2019; Liboiron et al. 2018, 2019; Slootmaekers et al. 2019; Toussaint et al. 2019). There is significantly less information available on microplastic occurrence in fish muscle, which is the tissue of bony fish that is typically consumed (Karami et al. 2017a; Abbasi et al. 2018; Akhbarizadeh et al. 2018). The existing information indicates that microplastic concentrations in muscle tissue are lower than what has been reported in the GI tract of bony fish. The majority of dried fish muscle tissue samples purchased from local markets in Malaysia did not contain any microplastics, with concentrations ranging from 0 to 3 microplastics per fish (Karami et al. 2017a). Conversely, microplastics were detected in all analyzed fresh fish samples from the Persian Gulf, at concentrations ranging from 3.1 to 4.6 microplastics per fish (Abbasi et al. 2018) or 0.57 to 1.85 microplastics per gram of fish muscle tissue (Akhbarizadeh et al. 2018). Most microplastics were larger than 100 µm, with fragments and fibres being the predominant particle shapes in fish muscle tissue (Abbasi et al. 2018; Akhbarizadeh et al. 2018).

Microplastics have been detected in a number of edible species of molluscs, including mussels, clams, oysters, scallops, and snails (Barboza et al. 2018; Toussaint et al. 2019). The most commonly investigated species of molluscs is the blue mussel, which was found to contain 0 to 10 microplastics per individual mussel or 0.2 to 2.9 microplastics per gram of meat (De Witte et al. 2014; Van Cauwenberghe et al. 2015; Catarino et al. 2018; Li et al. 2015, 2018a; Toussaint et al. 2019; Van Cauwenberghe and Janssen 2014). Similar concentrations of microplastics have been reported in clams, oysters, scallops, and snails (Van Cauwenberghe and Janssen 2014; Li et al. 2015; Naji et al. 2018; Su et al. 2018; Hantoro et al. 2019). Fibres and fragments were the most commonly detected shape, ranging in size from 5 µm to up to 4.7 mm (EFSA 2016; FAO 2017; Catarino et al. 2018; Li et al. 2018a; Naji et al. 2018; Su et al. 2018). The concentration of microplastics detected in mussels varies; with higher concentrations of microplastics observed in the tissue of mussels harvested from waters with higher environmental concentrations of microplastics (EFSA 2016; Li et al. 2016b; FAO 2017; Hantoro et al. 2019).

Occurrence data on the presence of microplastics in crustaceans is extremely limited. The average microplastic content in green tiger prawns sampled from the Persian Gulf was 7.8 microplastics per individual (muscle tissue and exoskeleton combined), with filamentous fragments measuring 100 to 250 µm identified as the most abundant type of microplastic (Abbasi et al. 2018). Conversely, microplastics were observed in the digestive tract, head, and gills of whole brown shrimp, but not in the abdominal muscle tissue of peeled brown shrimp, sampled from the Clyde Sea (Devriese et al. 2015). Microplastics have also been found in the guts of lobsters at concentrations of up to 0.80 mg per individual, with fibres being the most frequently observed shape (Murray and Cowie 2011; Welden and Cowie 2016).

Other foods

The occurrence of microplastics has also been reported in a very small number of other foods, including honey, sugar, beer, and salt (EFSA 2016; Peixoto et al. 2019; Toussaint et al. 2019). One study reported that the majority of fibres in honey samples were naturally occurring cellulose fibres, with only a small portion of fibres confirmed to be PET by spectroscopy, but the number of PET fibres was not reported (Mühlschlegel et al. 2017). The remaining honey studies and all sugar and beer studies used a non-specific staining method to identify particles in the food items and thus, none of these particles could be confirmed as plastic (Liebezeit and Liebezeit 2013, 2014, 2015; Lachenmeier et al. 2015; Kosuth et al. 2018).

A recent review of microplastics in salt reported that their presence in commercial salts was common, although microplastic concentrations varied considerably depending on the origin and type of salt (Peixoto et al. 2019). Sea salts contained the highest concentrations of microplastics, ranging from 0 to 19 800 microplastics per kg of salt (Yang et al. 2015; Iñiguez et al. 2017; Karami et al. 2017b; Gündoğdu 2018; Kim et al. 2018; Renzi and Blašković 2018; Seth and Shriwastav 2018). Concentrations in lake and rock/well salts were much lower, ranging from 0 to 800 microplastics per kg of salt and 0 to 204 microplastics per kg of salt, respectively (Yang et al. 2015; Iñiguez et al. 2017; Karami et al. 2017b;

Gündoğdu 2018; Kim et al. 2018). In most studies of salt, microplastics <500 µm accounted for the largest proportion of detected microplastics, with fragments and fibres being the most abundant microplastic shape, regardless of salt type (Yang et al. 2015; Iñiguez et al. 2017; Karami et al. 2017b; Gündoğdu 2018; Kim et al. 2018; Renzi and Blašković 2018; Seth and Shriwastav 2018).

Bottled water

A few studies have evaluated the occurrence of microplastics in bottled water (Wiesheu et al. 2016; Kosuth et al. 2018; Mason et al. 2018; Oßmann et al. 2018; Schymanski et al. 2018; Szeto et al. 2018; Zuccarello et al. 2019). In one study, microplastics were detected in 93% of bottled water samples purchased from 19 locations in nine countries outside of Canada, with an average concentration of 10.4 microplastics ≥100 µm/L (Mason et al. 2018). Similar results were observed in an unpublished Canadian bottled water study (Szeto et al. 2018). In both studies, the number of particles in the 6.5 to 100 µm size range were reported. However, spectroscopic analyses were not performed at this size range, and thus the particles could not be confirmed as plastic (Mason et al. 2018; Szeto et al. 2018).

Microplastic concentrations are reported to vary across bottle type (i.e., plastic, glass or cardboard) and intended use conditions (i.e., single-use versus multi-use bottles) (Oßmann et al. 2018; Schymanski et al. 2018). The highest concentrations of microplastics were reported in water from older multi-use plastic bottles, followed by glass bottles, newer multi-use plastic bottles, single-use plastic bottles, and cardboard boxes (Oßmann et al. 2018; Schymanski et al. 2018). Approximately 78% to 98% of the microplastics detected in bottled water samples were between 1 and 5 µm, with less than 7% of microplastics >10 µm (Oßmann et al. 2018). The point source of microplastics in bottled water is still unknown, and the variation in the reported microplastic concentrations does not seem to correlate with bottle type alone. This suggests that the origin of reported findings of some microplastics in bottled water may be environmental (i.e., from the source water and air as a result of secondary microplastics forming in the environment).

5.2.2 Occurrence in drinking water

A limited number of studies have measured microplastics in tap water, and even fewer are considered reliable due to concerns with quality assurance measures (WHO 2019). Average microplastic particle concentrations in tap water have been reported to range from 0.0007 to 628 particles/L (WHO 2019), and microplastics as small as 1 µm in size have been measured in drinking water (Pivokonsky et al. 2018). Due to the limitations of existing detection techniques, no information is available on the occurrence of particles below 1 µm in size. The most predominant polymer types detected were PET and PP in the form of fibres and fragments (WHO 2019).

In a WHO-commissioned review, Koelmans et al. (2019) reviewed 50 studies on microplastics in tap water, bottled water and freshwater. The majority of the studies were missing at least one of nine critical aspects of quality assurance (Koelmans et al. 2019). Specifically, the authors noted uncertainties with the concentrations reported in many of these studies and concluded that any information presented on the presence of microplastics in water must be interpreted with this knowledge. Relevant

studies on microplastics in tap water and freshwater are summarized below. See section 5.2.1 for a review of relevant bottled water studies.

Pivokonsky et al. (2018) examined raw surface water and treated drinking water for microplastics from three drinking water treatment plants (DWTPs) in urban areas of the Czech Republic. Drinking water samples were analyzed by scanning electron microscopy (SEM), FTIR and Raman spectroscopy. The results from this quantitative analysis indicated average concentrations of 338, 443 and 628 particles/L for drinking water at each of the respective DWTPs, with microplastics smaller than 10 µm accounting for up to 95% of particles retained. Although 12 different materials were identified, PET and PP were found to be the prevailing microplastics in treated water collected at two of the DWTPs, while PP and PE were most abundant in treated water collected at the third DWTP. Some of the limitations that may affect the overall quality and reliability of this dataset include the use of small sampling volumes and failure to take sufficient measures to control background contamination (i.e., wiping down surfaces and working under clean air conditions) (Koelmans et al. 2019).

Strand et al. (2018) did not find significant concentrations of microplastics in tap water sampled from 17 different locations across Denmark sourced by groundwater. Samples were visually examined by stereomicroscopy for all particles >100 µm displaying microplastic-like characteristics. Only a single sample concentration was reported above the level of detection (LoD) of 0.58 particles/L, at 0.6 particles/L. Chemical analysis by FTIR revealed that of the particles exhibiting microplastic properties, only 3% were confirmed to be microplastics, with the remainder identified as cellulose-like material (76%), as having poor spectra (10%), as having an unknown spectra (7%), or as protein-like material (4%). Polymer types were reported as PP, PS and PET. Given the very low level of each type of plastic polymer identified in the tap water samples, the authors caution against drawing conclusions on the origin of the plastic contamination. Additional tap water samples were collected to investigate the occurrence of smaller microplastics 10 to 100 µm, and chemical analyses were performed by FTIR. Only a single concentration of 0.8 particles/L was reported above the LoD of 0.3 particles/L, in the form of fragments comprised of PP, PET, acrylonitrile butadiene and PU. Despite the small sample volumes used in this study, the data presented was found to be among the most reliable studies on the occurrence of microplastics in drinking water (WHO 2019).

In a study on tap water derived from the purification of groundwater in northwestern Germany, Mintenig et al. (2019) investigated the abundance of microplastics at different locations within the drinking water supply chain. Particles were characterized using FTIR imaging, and microplastics down to a size of 20 µm were identified. Results indicated a low level of microplastic contamination of tap water derived from groundwater, with concentrations in both raw and drinking water ranging from 0 to 7 x 10^3 particles/L and a reported mean of 0.7 x 10^{-3} particles/L. Microplastic particles identified were small fragments between 50 and 150 µm in size, with the predominant polymer types identified being polyester, PVC, PE, PA and epoxy resin. Although this study lacks some aspects of quality assurance, such as the use of clean air conditions and absence of positive controls (Koelmans et al. 2019), when assessed on key quality control criteria, it was found to score the highest of all tap water studies by the WHO (WHO 2019).

Two studies (Uhl et al. 2018; Kosuth et al. 2018) were identified but not considered reliable due to uncertainty about whether the methods used could accurately identify particles as plastic. In one study, no particles were observed in treated or distributed water in 24 DWTPs in Norway (Uhl et al. 2018). In another study, Kosuth et al. (2018) evaluated synthetic particles in tap water from 14 countries across five continents and found particles in 81% of samples, with the most abundant type being fibres. Concentrations ranged from 0 to 61 particles/L, with an overall mean of 5.45 particles/L.

Finally, the possibility exists that microplastic contamination could occur at some point in the water supply chain as a result of abrasion of water pipes containing plastic materials, or from membrane filters made of polymers (Novotna et al. 2019). Further research is required to investigate this possibility.

5.2.3 Drinking water treatment

DWTPs provide a barrier against the introduction of waterborne microplastics in drinking water. The current literature, while limited, shows that drinking-water treatment can be effective at removing microplastics. However, given the lack of standardized methods for quantifying microplastics in water, further research is required in this area (Novotna et al. 2019).

Drinking water treatment typically occurs via clarification or membrane processes. Clarification processes are the most commonly used methods for removing particles from drinking water and involve techniques such as coagulation, flocculation, flotation, and/or filtration (Novotna et al. 2019). Membrane processes involve the use of diffusion membranes (e.g., reverse osmosis) or porous membranes (e.g., microfiltration, ultrafiltration). Diffusion membranes allow only dissolved substances (such as ions and specific dissolved substances) to pass through, whereas porous membranes allow only particles of a certain size to pass (Crittenden et al. 2012). As most observed microplastics are above the membrane size thresholds for porous membranes (i.e., 0.1, 0.01 and 0.001 µm for micro-, ultra- and nano-filtration respectively), porous membranes have the potential to be very effective at removing microplastics (Crittenden et al. 2012). For example, a laboratory study by Ma et al. (2018) found complete rejection of PE microplastics by an ultrafiltration membrane. The type of drinking water treatment process may affect the efficiency of DWTPs in removing microplastics. However, further research is required to inform drinking water treatment optimization for microplastics. Pivokonsky et al. (2018) observed microplastic removal rates of between 70% and 82% for three DWTPs employing conventional coagulation, clarification, and filtration. In a study using groundwater, Mintenig et al. (2019) found no significant difference between source water and treated water, although microplastic concentrations were very low in both source and treated water, varying from 0 to 0.007 particles/L (Mintenig et al. 2019).

Microplastic properties (e.g., size, shape, and surface properties), as well as water properties (e.g., pH and organic matter content) may also impact the efficiency of microplastic removal during different treatment processes. As microplastics are hydrophobic, adsorption of organic materials to the particles can occur, which can prevent their aggregation and thus make separation more difficult (Napper et al. 2015; Koelmans et al. 2016). Hydraulic forces can also break down large aggregates or particles

themselves, creating smaller particles that may not be removed as easily during the clarification process (Jarvis et al. 2005). Ma et al. (2018) found that while pH and turbidity of the water had little effect on the microplastics removal efficiency, the microplastics themselves can actually influence the turbidity of water at sufficient concentrations.

6. Impacts on environmental health

This section reviews data on the effects of both macroplastics and microplastics on environmental receptors. Each subsection begins with discussion of occurrence in biota, followed by an overview of their effects. There are no standardized methods for testing the effects of microplastics. For the purposes of this report, the following criteria were used to select the studies: the study reported details of the analytical techniques, the study reported the type of plastic used (i.e., polymer, size, shape, virgin vs. aged), and the study monitored and reported measured concentrations that were similar to the nominal (i.e., theoretical) concentrations. Although it was found that preservatives or surfactants on plastics may be the cause of acute toxicity to organisms rather than the plastic particle itself, the washing of test particles is not currently standard practice, and this was not considered in the above criteria. Furthermore, studies were selected so as to cover a variety of organism types and effects.

6.1 Macroplastic

Plastic pollution can have various effects on organisms and their habitats, depending on the size and type of plastic, and the level of biological organization (Werner et al. 2016). In 2016, the Secretariat of the Convention on Biological Diversity (CBD) reported that a total of 817 marine species had been affected in some way by marine litter (CBD 2016), up 23% from the same assessment performed four years earlier. It also found that over 80% of this marine litter was plastic (CBD 2012, 2016). A literature review by Gall and Thompson (2015) of 340 publications involving 693 species found that plastic pollution accounted for 92% of the reported interactions between pollution and individuals globally.

Rochman et al. (2016) conducted an extensive literature review of primary publications (283 papers) on marine litter (including macro- and micro-sized plastic pollution) published through to 2013. The authors compiled the perceived and demonstrated effects of litter and sorted them by levels of biological organization: suborganism, organism, population and assemblage. Micro-sized litter (defined as <1 mm in this study) accounted for 71% of the demonstrated impacts, while macro-sized litter (defined as >1 mm in this study) accounted for 29%. A further breakdown of these effects by level of biological organization shows that of the demonstrated impacts from macro-sized litter, the majority were classified as suborganismal, with the most common effects being seen in tissues (e.g., inflammation or lacerations) and organ systems (e.g., poor functioning). Of the demonstrated impacts at the suborganismal level, 78% were due to micro-sized litter, 74% of which were caused solely by plastics. Other demonstrated effects include effects on cells (e.g., necrosis, viability), in organs (e.g., change in size, lesions) and macromolecules (e.g., protein, DNA damage). All of these demonstrated impacts of

macro-sized litter were found to be from plastic pollution. The remaining demonstrated effects were divided between the organismal level and the ecological level. At the organismal level, the main effect observed was death to an individual, whereas at the ecological level, the main effect was on assemblages (i.e., change in abundance or diversity of biota). The most common items reported to cause an effect were lost and abandoned fishing gear or other plastic items, such as rope, bags, straws and degraded fragments.

The adverse effects of macroplastic pollution include entanglement, ingestion, and impacts on habitat integrity (Gall and Thompson 2015; Rochman et al. 2016; Werner et al. 2016).

6.1.1 Entanglement

Entanglement from macroplastics can occur from ropes, nets, cable ties, plastic bags, packaging bands and rings (such as for cans in bulk), and other string-like items (Werner et al. 2016). Observations of entanglement are reported more frequently than other impact pathways, likely due to its very visible nature (Werner et al. 2016). For example, Gall and Thompson (2015) found reported occurrences of entanglement for 30 896 individuals from 243 species. Of these reported cases, 79% were linked to direct harm or mortality, and the majority of these incidents involved plastic rope and netting. As well, Rochman et al. (2016) found that 29% of demonstrated impacts at the organismal level were caused by entanglement. The species most commonly impacted by entanglement events were marine invertebrates (75 species), seabirds (49 species), fish (27 species), and marine mammals (10 species).

Entanglement in the marine environment is often due to “ghost fishing,” which occurs when lost, abandoned, or discarded fishing gear continues to catch fish in the ocean or on the seafloor (Hallanger and Gabrielsen 2018; PAME 2019). In the Arctic, old fishing-related products were found entangled with dead seabirds, dead and living Svalbard reindeers (*Rangifer tarandus platyrhynchus*), and seals (Hallanger and Gabrielsen 2018). In addition, Page et al. (2004) found the entanglement rates of Australian sea lions (*Neophoca cinerea*) and New Zealand fur seals (*Arctocephalus forsteri*) to be 1.3% and 0.9%, respectively, in 2002. These are some of the highest reported entanglement rates for all seal species. The authors estimated that 1478 seals die from entanglement events each year in Australia. In one incident in 2018, at least five seals were entangled in a “ghost net” and drowned in the Fraser River in British Columbia (Rasmussen 2018). In addition, a review of global data by Ryan (2018) reported that a total of 265 bird species were reported to be entangled in discarded plastics or other synthetic materials. Fishing gear was determined to be the cause of entanglement in 83% of species.

Votier et al. (2011) examined the use of macroplastics as nesting material by northern gannets (*Morus bassanus*) in Grassholm, Wales and assessed the associated entanglement events. Nests contained an average of 469.9 g dw of plastic and the preferred material used was synthetic rope. The authors estimate that 65.6 birds were entangled on an annual basis, with the majority being full-grown nestlings.

Large plastics such as bags, sheets, and films can also cover plants, sponges, and corals, affecting gas exchange and their photosynthetic capacities (Werner et al. 2016). This phenomenon, known as “smothering,” can lead to mortality of affected vegetation (Kühn et al. 2015). Rochman et al. (2016)

found that 8% of deaths at the organismal level were due to smothering when examining demonstrated effects. Smothering by plastic pollution can also lead to sublethal effects in these organisms. To study the effects of smothering on cold-water corals (*Lophelia pertusa*), Chapron et al. (2018) used 10 x 10 cm pieces of LDPE to represent fragments of plastic bags, which have been seen covering polyps in the field. They observed a decrease in growth rates from 3.59 mm/year in control aquaria conditions to 2.51 mm/year in the test group exposed to macroplastics. Although these findings are not statistically significant, the plastics may have acted as physical barriers to feeding, leading to impaired energy acquisition and slower growth rate. In addition, activity was 11% lower in coral exposed to macroplastics in comparison to control conditions after 7 days. However, activity was enhanced after 20 days, which the authors hypothesized to be a compensatory physiological response to enhance capture efficiency or a mechanism to cope with long-term low oxygen supply (Chapron et al. 2018). Macroplastic exposure also led to a noticeable decrease in feeding rates throughout the duration of the experiments.

Similarly, Qi et al. (2018) found that exposing soil to plastic films (1% w/w) had weak effects on the growth of wheat (*Triticum aestivum*). Plastic mulch films, comprised of 37.1% Pullulan (a polysaccharide), 44.6% PET and 18.3% polybutylene terephthalate (PBT), had stronger negative effects on wheat growth compared to the PE mulch. The authors note that this might be related to the presence of PET and PBT in the biodegradable mulch, which have been shown in previous studies to have stronger negative effects on soil-plant systems than LDPE (Qi et al. 2018). However, exposure to both types of films inhibited wheat growth with respect to plant height at day 40 and shoot biomass at 2 months. The plants in both biodegradable plastic mulch treatments also displayed fewer leaves, decreased leaf surface areas, and thinner stems.

6.1.2 Ingestion

Ingestion of plastics is another pathway that can lead to potential adverse effects. Ingestion of plastic can be intentional (e.g., where an organism eats the plastic, mistaking it for food), or unintentional (e.g., where predators feed on prey that have ingested plastics). Filter-feeding or detritus-feeding species are especially prone to unintentional plastic ingestion (Werner et al. 2016).

In the Mediterranean Sea, PE macroplastics were found in the gastrovascular cavity of 2 of 20 sampled jellyfish (*Pelagia noctiluca*) (Macali et al. 2018). Bernardini et al. (2018) also sampled 139 blue sharks (*Prionace glauca*) from the Mediterranean Sea. Blue sharks in the Mediterranean basin are categorized as a "Critically Endangered" species by the International Union for Conservation of Nature. Of the 95 adult blue sharks that were examined and had full stomachs, 24 contained plastic pollutants. Juveniles were also found to have a greater frequency of ingested plastics. In addition, macroplastics accounted for more than 70% of all plastic pieces. The majority of ingested plastic items were sheet-like (72.38%), followed by fragments (18.10%) and threadlike plastic items (5.71%), with the most common polymer found being PE.

The GI tracts of Atlantic cod (*Gadus morhua*), European flounder (*Platichthys flesus*), common dab (*Limanda limanda*), Atlantic herring (*Clupea harengus*), and Atlantic mackerel (*Scomber scombrus*) caught from the North Sea and Baltic Sea were sampled for plastics by Rummel et al. (2016). Of the 290

investigated fish, 16 contained plastics (approximately 74% microplastics and 26% macroplastics). Macroplastics and microplastics were found in the GI tracts of 47.7% of the coastal fish and 2.4% of the offshore fish collected from Scottish marine waters by Murphy et al. (2017), or 29.7% (n=63) of all fish sampled. The mean number of plastic pieces found per fish was 1.8, with polyamide being the most common polymer. Choy and Drazen (2013) also found plastics in the stomachs of 7 different species of pelagic fish from the central North Pacific Subtropical Gyre, many of which were macroplastics.

According to the Canadian Sea Turtle Network, almost 40% of leatherback turtles (*Dermochelys coriacea*) have been found to have plastics in their stomachs (Campbell 2018). Schuyler et al. (2014) conducted a global analysis of plastic ingestion in various sea turtle species and found that the most commonly ingested anthropogenic pollutants were plastics. Plot and Georges (2010) reported a field observation of an adult leatherback turtle that expulsed 2.6 kg of plastic pollutants, consisting primarily of plastic bags and plastic fragments. Plastics have also been found in green sea turtles (*Chelonia mydas*) (Özdilek et al. 2006; Stamper et al. 2009).

Lusher et al. (2015a) studied two adult and one juvenile True's beaked whales (*Mesoplodon mirus*) that were found stranded on the coast of Ireland. Analysis of the contents of their stomachs and intestines revealed that both adults appeared to have ingested macroplastics, but it could not be determined whether the whales died as a direct consequence of plastic ingestion (Lusher et al. 2015a). Marine litter was also found in the stomachs and intestines of 26 out of the 175 (approximately 15%) dead Magellanic penguins (*Spheniscus magellanicus*) collected from the Brazilian coastal zone, roughly 58% of which was plastics (Brandão et al. 2011).

Several news articles have also reported occurrences of marine wildlife washing up on shore with plastics in their stomachs. For example, in Indonesia, a dead sperm whale washed ashore with nearly 6 kg of plastic waste in its stomach, including 115 plastic cups and 25 plastic bags (Times Colonist 2018). Examples of microplastics occurrence in a variety of other wildlife species, such as rough-toothed dolphin (Alesali and Lear 2019), sperm whale (Magra 2019), pilot whale (Zachos 2018), and harp seal (McKenzie 2018), have also been reported.

Gall and Thompson (2015) reported occurrences of marine litter ingestion for 13 110 individuals of 208 species, and Kühn et al. (2015) reported that the number of species known to ingest plastics increased by approximately 87% from 1997 to 2015 (177 to 331 species) and that marine litter ingestion has been recorded in 50.4% of marine mammal species, 40.4% of seabird species, and 100% of turtle species. However, cases of plastic ingestion leading to direct harm or death is less frequent in comparison to entanglement. Gall and Thompson (2015) found that only 4% of reported cases of ingestion resulted in direct harm or death. In contrast, Rochman et al. (2016) found that 63% of deaths were due to ingestion of marine litter. Specifically, demonstrated impacts from ingestion were observed in marine mammals (2 species), sea turtles (1 species), seabirds (1 species), and marine invertebrates (2 species).

Ingestion of plastics by organisms has been shown to have consequences from several pathways. Current literature shows that the most clear adverse effects from plastic ingestion is the blockage of intestinal systems, preventing feeding and thus leading to possible starvation. For example, a common

dolphinfish (*Coryphaena hippurus*) caught in the Western Equatorial Atlantic had a large plastic bowl measuring 99.57 cm^2 in its stomach (Menezes et al. 2019). Researchers suggested that the bowl was likely blocking its digestive tract, leading to starvation. A study by Pierce et al. (2004) reported plastic ingestion by a male northern gannet (*Morus bassanus*) and a female greater shearwater (*Puffinus gravis*). Both birds had blockages of the pylorus, which prevented feeding, leading to starvation and death. Ulcerations near the pylorus were also seen in the northern gannet, which matched up exactly with the shape of the bottle cap found in its esophagus that was thought to have been dislodged from the gizzard.

Ingested plastics can also damage organs and intestinal systems. Brandão et al. (2011) observed a dead Magellanic penguin (*Spheniscus magellanicus*) whose stomach had been perforated by a plastic straw. Jacobsen et al. (2010) studied two sperm whales (*Physeter macrocephalus*) post-mortem, both of which had netting, fishing line, and plastic pollutants such as bags in their stomachs. The cause of death in both whales was suspected to be gastric impaction, as one whale had a ruptured stomach and the other was emaciated. Stamper et al. (2009) observed an emaciated green sea turtle (*Chelonia mydas*) floating off the coast of a Florida beach. The turtle displayed signs of cachexia, lethargy, increased buoyancy, obstipation, and anorexia. Radiographs confirmed the presence of plastics in the GI tract, hindering regular function. After the removal of 74 foreign objects (including latex balloons, string, nylon rope, and soft and hard plastics) via enemas, the turtle showed improvements in its health, appetite, and behaviour. The authors note that this demonstrates a cause-and-effect relationship between plastic ingestion and morbidity in organisms (Stamper et al. 2009).

6.1.3 Habitat integrity and rafting (organism transport)

The presence of plastic pollution in water bodies can also pose potential problems for ecosystem function, biodiversity, and habitat integrity (Werner et al. 2016). An increasing amount of plastic pollution in surface waters has the potential to act as a stressor on ecosystem dynamics and habitat integrity (CBD 2012). Plastics can be effective transport mediums due to their potential for surface adhesion and to the low density of certain types of plastic and can potentially accentuate transport of organisms or other organic matter, a phenomenon known as “rafting” (Werner et al. 2016). This process can also occur with naturally occurring material such as wood, but the increasing prevalence of plastic pollution in surface waters increases the likelihood for organisms to be transported, which can pose a threat to the receiving environment. Gall and Thompson (2015) identified 34 reports of organisms rafting on marine litter, including packaging, fragments, and intact items (plastic or otherwise). Of the 259 total species described in these reports, 6 were listed as being invasive (i.e., non-native). However, the authors note that this is likely an underrepresentation (CBD 2012; Gall and Thompson 2015). The transport of non-native species is a particular concern, as they have the potential to negatively impact the structure of other well-established ecosystems by becoming predators to native species and/or outcompeting them for resources, leading to a loss of biodiversity (Werner et al. 2016). Non-native species could also transport diseases to which native species have not previously been exposed and could alter the genetic diversity in the ecosystem, which can have repercussions for generations to

come. Furthermore, plastic pollutants can also act as an artificial habitat for the colonization and growth of microorganisms that can affect species assemblage (Werner et al. 2016).

Katsanevakis et al. (2007) studied the impacts of marine litter on the abundance and community structure of epibenthic megafauna in the Aegean Sea. They demonstrated that an increase in marine litter caused a marked and gradual increase in both the total abundance and number of species, changing the structure of the megafaunal community. This was attributed to the fact that the litter was able to provide refuge for mobile species and to act as a colonization site for hard-substratum sessile species. This change in dynamics can have significant long-term effects on the ecosystem, such as altered predator-prey dynamics.

6.2 Microplastic

6.2.1 Uptake, ingestion, and egestion

Microplastics have been found in many species, including invertebrates, fish, turtles, mammals, and birds. Given the lack of standardized methods for quantifying occurrence in biota as well as the limited data on occurrence in Canadian species, criteria for selecting reliable studies (e.g., studies that used an analytical method to identify microplastics) were identified but many studies did not meet these standards. Moving forward, it is recommended that a standardized method for quantifying microplastics in biota be developed.

A review by Provencher et al. (2017) showed that the literature on global macroplastic and microplastic ingestion in marine vertebrates is dominated by seabirds and that there is an increasing number of reports in fish, turtle and mammals each year. Fibres and fragments are the most common microplastic types found in organisms (Burns and Boxall 2018). For example, Beer et al. (2018) visually identified microplastics in 20% of the 814 fish they studied in the Baltic Sea, with 93% of these being fibres. Collicutt et al. (2019) determined by light microscopy that over 90% of the microplastics they found in juvenile Chinook salmon (*Oncorhynchus tsawytscha*) were fibrous in nature.

As in the case of macroplastics, several factors can affect the intake and ingestion of microplastics by organisms. In laboratory studies, Scherer et al. (2017) demonstrated that co-exposure of microplastics with algae significantly decreased ingestion of microplastics by *Daphnia magna*. Weber et al. (2018) demonstrated that exposure concentration and age of the freshwater amphipod *Gammarus pulex* affected its microplastic body burden. Feeding selectivity of biota is also thought to be a driving factor for microplastic ingestion: nonselective filter feeders are more prone to direct microplastic uptake, whereas more specialized feeders will uptake microplastics indirectly through ingestion by their prey (Scherer et al. 2018). Uptake of microplastics via prey ingestion is discussed further below. Select reported ingestion events are outlined below, with Canadian and global examples.

Liboiron et al. (2019) studied the GI tracts of three fish species commonly used for human consumption on the island of Newfoundland: Atlantic cod (*Gadus morhua*), Atlantic salmon (*Salmo salar*) and capelin (*Mallotus villosus*). The frequency of occurrence of macroplastic and microplastic ingestion by Atlantic salmon and capelin was 0% for specimens collected between 2015 and 2016 (a total of 419 fish). In

Atlantic cod examined during the same period, the frequency of occurrence of plastic ingestion was 1.68%. These results are consistent with a previous study by Liboiron et al. (2018), in which 134 silver hake (*Merluccius bilinearis*) from the south coast of Newfoundland were studied and found to have a 0% frequency of occurrence of plastic ingestion.

In a study of microplastics in juvenile Chinook salmon on the east coast of Vancouver Island, Collicutt et al. (2019) found that 59% of the specimens examined contained at least one plastic particle, with an average of 1.15 microplastic pieces per individual. It should be noted that plastic identification was not confirmed using an analytical method other than visual identification using light microscopy.

In a study of microplastics in fish from a prairie creek downstream of a WWTS in Regina, Saskatchewan (Campbell et al. 2017), five species of fish were collected: fathead minnow (Pimephales promelas), northern pike (Esox lucius), white sucker (Catostomus commersoni), emerald shiner (Notropis atherinoides), and five-spine stickleback (Eucalia inconstans). Of the 181 fish sampled, 73.5% had between 1 and 20 microplastics in their GI tracts, ranging from 83.3% of the northern pike, an apex predator, to 50.0% of the fathead minnows. The number of microplastics varied significantly between the five species sampled. This inter-species variation is suspected to be attributable to differences in feeding habits. The northern pike, an apex predator, had the highest proportion of sampled fish with microplastics present in their GI tracts at 83.3%, while the fathead minnow had the lowest, at 50.0%. The authors acknowledge that characterization of plastics using spectroscopic identification methods was not performed in this study. However, a hot needle was used to test whether the suspected plastic particles melted, to confirm that the particle was plastic (Campbell et al. 2017). It should be noted that some types of plastic will not melt under these conditions (i.e., thermosets).

O'Hara et al. (2019) conducted a study on the seasonal variability of exposure of Cassin's auklets (*Ptychoramphus aleuticus*) to microplastic pollution. Following a series of storm events, 707 carcasses were found on the beaches of Vancouver Island and Haida Gwaii in British Columbia. A total of 85 carcasses were collected for examination, and plastics were found in 40% of the birds. Macroplastic and microplastic pieces in the stomachs of the birds were visually identified and separated. The average number of plastic pieces ingested per bird was 1.6, with an average mass of 0.0085 g, and one outlier ingested 61 pieces of plastic. Furthermore, ingested plastics were predominantly microplastics (86.6%). There was no significant difference between the number of pieces ingested by age, sex, or health condition of the bird (O'Hara et al. 2019). Similarly, Poon et al. (2017) studied plastic ingestion by the northern fulmar (*Fulmarus glacialis*) in the Canadian high Arctic. None of the northern fulmars sampled in 2013 contained more than 0.1 g of plastics. Provencher et al. (2018c) demonstrated that northern fulmars excrete microplastics via their guano and found that the number of pieces of plastic in the gut was positively related to the number of microplastics in the guano.

Plastics have been identified in organisms from several regions of the world. Representative studies are presented below to demonstrate that microplastic ingestion by biota occurs globally. An exhaustive review has been conducted by Provencher et al. (2018a).

Microplastics have been found in gudgeons (*Gobio gobio*) in Flemish rivers in Belgium (Slootmaekers et al. 2019). Gudgeons from 15 rivers at 17 locations were sampled to study the occurrence of microplastics in their intestines. Microplastic contamination was found in four of the rivers studied. Of the 78 fish examined, 9% contained microplastic particles in their intestines, and only 1 fish had ingested more than 1 particle. A total of 16 suspected plastic particles were extracted from all sampled fish; however, only 8 particles were identified to be plastic following μ-FTIR analysis. Overall, 7 different polymers were identified: ethylene-vinyl acetate copolymer, PP, PET, PVC, cellophane, polyvinyl acetate and PA (Slootmaekers et al. 2019).

In the heavily industrialized city of Tuticorin, India, Kumar et al. (2018) investigated the occurrence of microplastics in Indian mackerel (*Rastrilliger kanagurta*) and honeycomb grouper (*Epinephalus merra*) on the southeastern coast. Of the 40 fish sampled, 12 had plastic particles in their intestines. FTIR analysis revealed that the particles were PE and PP. Fibres constituted 80% of particles, whereas fragments constituted the remaining 20%.

While the ingestion of microplastics has been widely demonstrated, egestion has also been shown to be possible in some organisms. For example, Grigorakis et al. (2017) found that goldfish (*Carassius auratus*) have efficient gut clearance of microbeads and microfibres: the time required for 90% clearance was 33.4 hours. Mazurais et al. (2015) found complete egestion of PE microbeads from European seabass (*Dicentrarchus labrax*) larvae after 48 hours. In invertebrates, significant microplastic egestion was seen in studies by Chua et al. (2014), Blarer and Burkhardt-Holm (2016), Frydkjær et al. (2017), and Hämer et al. (2014). In *Hyalella azteca*, an amphipod crustacean, microplastic fibres were found to be more slowly egested than microbeads during acute exposure; however, both were able to be completely egested (Au et al. 2015).

6.2.2 Ecotoxicological effects

Despite the ability of some organisms to egest plastic particles, microplastics have been shown in the current literature to have adverse effects on organisms. In their respective literature reviews, Rochman et al. (2016) and the Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP) (2016) reported that, in the laboratory, the effects from micro-sized litter (consisting primarily of plastic) were overwhelmingly seen at the suborganismal level. The predominant observed effects at this level were in macromolecules, cells, and tissues and can include inflammation and changes in gene expression (Rochman et al. 2016; GESAMP 2016). The remaining demonstrated effects were at the organismal level, primarily due to individual deaths (Rochman et al. 2016). In addition, Foley et al. (2018) conducted a meta-analysis of 43 papers published before October 2016 and observed that while effects from microplastic exposure were highly variable across taxa, the most consistently reported effect across both marine and freshwater taxa was a reduction in the consumption of natural prey. It should be noted, however, that effect studies do not have standards for testing particle size or concentration (Burns and Boxall 2018). Currently, concentrations of microplastics used in effect studies are much higher than those measured in the environment. Furthermore, effects studies focus on particle sizes much smaller than those currently sampled for in the environment (SAPEA 2019). Particle concentration can also influence toxicity, as higher concentrations are expected to overwhelm biological

clearance mechanisms and cause responses that are not otherwise observed at lower doses (WHO 2019).

In the Government of Canada's 2015 science summary on microbeads (ECCC 2015), 130 publications on the fate and effects of microplastics were examined and reviewed. Several key studies were summarized in the assessment report. The report noted a scarcity of information on long-term and multigenerational effects of microbeads; however, short-term and direct effects are well described. Physical effects were identified as the primary driver for effects to organisms. Some examples of effects in organisms from microbead exposure that have been described in literature include: decreased survival and fecundity (Lee et al. 2013), decreased reproduction from impedance of feeding behaviour (Cole et al. 2015), liver stress (Rochman et al. 2013), altered gene expression (Rochman et al. 2014), and possible genotoxicity in the form of DNA damage (Avio et al. 2015). Au et al. (2015) found that acute exposure to microfibres produced greater toxicity (due to physical effects) to *Hyalella azteca* than spherical beads, with 10-day median lethal concentration (LC_{50}) values of 71.43 microfibres/mL and 4.64×10^4 microbeads/mL, respectively. Hämer et al. (2014) observed no impact on survival, growth, and intermolt duration in isopods (*Idotea emarginata*) following chronic exposure to microplastic particles of multiple forms. More detailed summaries of these studies can be found in ECCC (2015).

In its proposal for a restriction on intentionally added microplastics, the European Chemicals Agency (ECHA) reviewed and summarized 25 influential scientific papers on the ecotoxicological effects of microplastics (ECHA 2019). The papers include data that overlap with those from studies cited in ECCC (2015). Experimental data cited by the ECHA in its proposal that were not discussed in ECCC (2015) are summarized briefly below. For more detailed summaries of these studies, please refer to ECHA (2019).

- Earthworms (*Lumbricus terrestris*) showed a decrease in growth rate with exposure to high concentrations of LDPE particles (<150 µm; 28, 45, 60% dw), but reproduction was not affected (Huerta Lwanga et al. 2016).
- Zebrafish (*Danio rerio*) exposed to PS microspheres (5 µm) exhibited inflammation, lipid accumulation in liver, oxidative stress, and altered metabolomics profiles (Lu et al. 2016).
- European sea bass (*Dicentrarchus labrax*) feeding on PVC pellets (<0.3 mm; 1.4% bw) had significant structural damage to the intestine (Pedà et al. 2016).
- *Daphnia magna* that ingested PE particles (1 µm; 12.5 to 200 mg/L) experienced immobilization that increased with concentration and time following 96-hour exposure (Rehse et al. 2016).
- Pacific oysters (*Crassostrea gigas*) exposed to PS spheres (2 and 6 µm; 0.023 mg/L) had significant reductions in oocyte number, oocyte diameter, sperm velocity, and larval development of offspring following 2-month exposure (Sussarellu et al. 2016).
- Mussels (*Mytilus edulis*) and lugworms (*Arenicola marina*) exposed to PS microspheres (10 µm, 30 µm, 90 µm; 110 particles/mL seawater for mussels, 110 particles/g for lugworms) showed increased metabolism, but no adverse effects on energy allocation (Van Cauwenberghe et al. 2015).
- Common shore crabs (*Carcinus maenas*) feeding on PP microfibres (1 to 5 mm in length; 1% plastic) showed a decrease in food consumption rates over time and a drastic reduction in energy available for growth, with minimal lasting consequences (Watts et al. 2015).

- Marine worms (*Arenicola marina*) had reduced feeding activity and reduced available energy reserves from ingestion of unplasticized PVC treatments (130 µm mean diameter; 5% by weight) (Wright et al. 2013).

A review of additional current literature on ecotoxicological effects of microplastics is provided below for each environmental compartment of interest. Relevant studies are outlined in the sections below, with more detailed summaries provided in Appendix C, including information on the size, concentration and polymer type of the particles. Due to physicochemical similarities, information on primary microplastics was used as surrogate information where information on secondary microplastics was not readily available.

Water

The aquatic environment, and in particular marine organisms, has been the focus of much of the ecotoxicological research on plastics (SAPEA 2019). In freshwater studies, invertebrates have been the focus of research on sensitivity to microplastic exposure (Adam et al. 2019).

Studies on the effects of plastics on organisms in both freshwater and marine environments are presented below, by level of biological organization.

Vertebrates

Yin et al. (2018) exposed the fish species *Sebastes schlegelii* to PS spheres and observed a reduction in foraging time and swimming speed, an increase in shoaling behaviour, and a feeding time of almost twice that of the control. Goldfish (*Carassius auratus*) exposed to ethylene vinyl acetate fibres, PS fragments, and polyethylene acrylate pellets also exhibited sublethal effects such as weight loss, histological changes to the GI tract and intestines, inflammation of the liver, and physical damage to the jaw, including incisions from chewing fragmented particles, but no mortality (Jabeen et al. 2018).

Similarly, in zebrafish (*Danio rerio*) exposed to PA, PE, PP, and PVC microplastics, no significant difference in lethality was observed; however, microplastics caused intestinal damage such as cracking of villi and splitting of enterocytes (Lei et al. 2018b). A study by Qiao et al. (2019) had reported similar findings when using PS microplastics, such that zebrafish exposed to virgin PS beads had significant intestinal damage, inflammation, oxidative stress, and altered gut microbiomes.

At the molecular level, Qiang and Cheng (2019) found that exposure to PS microplastics induced upregulated expression of inflammation and oxidative stress-regulated genes in zebrafish larvae. *S. schlegelii* showed a significant reduction in crude protein and lipid contents and had black bile in their gallbladders, indicating gastrointestinal function disorder resulting from accumulation of PS spheres in their intestinal tract (Yin et al. 2018).

Conversely, several current studies report no significant effects on vertebrates for any of the endpoints measured. De Felice et al. (2018) exposed tadpoles of African clawed frog (*Xenopus laevis*) to PS microplastics and found no significant effects on mortality, body growth, or swimming activity during their early life stages, despite observing microplastics in the digestive tracts of all exposed tadpoles.

Further, Ašmonaitė et al. (2018) observed no significant histological effects or inflammatory responses in rainbow trout (*Oncorhynchus mykiss*) exposed to PS microplastics, and Jacob et al. (2019) observed no effects on foraging or predation avoidance in coral-reef fish (*Acanthurus triostegus)* exposed to PS microbeads. Dietary exposure to PVC, PA, PE, and PS microplastics also did not affect stress responses, growth rate, or induce pathology changes in seabream (*Sparus aurata*) (Jovanović et al. 2018).

Invertebrates

In cladocerans (*Daphnia* spp.), increased microplastic concentrations led to increased mortality (Aljaibachi and Callaghan 2018; Martins and Guilhermino 2018; Pacheco et al. 2018). However, Jaikumar et al. (2018) suggest that mortality might also be temperature-dependent. Martins and Guilhermino (2018) further observed that microplastic exposure could have transgenerational effects in *D. magna*. Females descending from groups exposed to microspheres showed reduced growth, reproduction, and population growth rates up to the F_3 generation, indicating that complete recovery from chronic exposure may take several generations for this species. Tang et al. (2019) found no mortality in *Daphnia* exposed to PS particles, but observed a reduction in body growth rate and increased transcription of arginine kinase and permease (enzymes involved in oxidative defence and energy production).

Freshwater crabs (*Eriocheir sinensis*) exposed to PS microspheres similarly showed a decrease in weight gain, reduced hepatosomatic index, and several biochemical effects, such as an increase in transcription of genes involved in the oxidative stress response and anti-inflammation pathways (Yu et al. 2018).

Similarly, Jeong et al. (2017) found that exposing the marine copepod *Paracyclopina nana* to PS microbeads increased antioxidant enzyme activity in a size-dependent manner. A previous study by Jeong et al. (2016) found similar results when studying the monogonont rotifer *Brachionus koreanus*: several antioxidant enzymes showed increased activity in rotifers exposed to PS microbeads, indicating a defence mechanism against oxidative stress.

Beiras et al. (2018) studied rotifers as well as the crustacean *Tigriopus fulvus* and determined lowest observed effect concentrations (LOECs) of 0.01 mg/L for rotifer immobility and 1.0 mg/L for rotifer and crustacean mortality, using PE particles.

For the freshwater invertebrate *Gammarus pulex,* Weber et al. (2018) found no significant effects on juvenile survival, development (molting), metabolism, or feeding activity following chronic exposure to PET. Another study by Redondo-Hasselerharm et al. (2018) exposed *G. pulex* to PS microplastics. While the survival of *G. pulex* was not affected, the organisms experienced a significant reduction in growth, with a 28-day EC_{10} (10% effect concentration) of 1.07% plastic weight in sediment dw.

Studies have also been conducted on coral species. Chapron et al. (2018) found that marine corals (*Lophelia pertusa*) exposed to LDPE microbeads had significantly lower prey capture rates and reduced skeletal growth rates and calcification compared to the controls. Hankins et al. (2018) found no significant effects on calcification in either the large polyp coral *Montastraea cavernosa* or the small polyp coral *Orbicella faveolata* despite active ingestion of PE microbeads.

Microplastic exposure has also been studied at early developmental stages for invertebrates. Lo and Chan (2018) found that larval and juvenile sea snails (*Crepidula onyx*) were not affected by exposure to environmentally-relevant concentrations of PS particles. At higher concentrations, the larvae grew slower and settled at a smaller size compared to control conditions. In addition, individuals exposed to microplastics only in their larval stages displayed slower growth rates even after the removal of the microparticles, indicating a possible legacy effect (Lo and Chan 2018). Similarly, blue mussel (*Mytilus edulis*) larvae with PS bead exposure experienced no changes in growth rate; however, there was an increase in the amount of abnormally developed larva (Rist et al. 2019). Beiras et al. (2018) found no significant effect on mussel embryonic development under static conditions from virgin PE microplastics.

Primary producers

Green algae (*Chlorella pyrenoidosa*) exposed to PS beads displayed inhibited growth rates that corresponded to increasing plastic concentration (Mao et al. 2018). Reduced photosynthetic activity and damaged cell membranes were also evident; however, a recovery of algal biomass and photosynthetic activity was seen during the later phases of growth, which may be linked to detoxification mechanisms. Additionally, Gambardella et al. (2018) found that green microalga (*Dunaliella tertiolecta*) exposed to PS microbeads experienced a dose-dependent inhibition of growth: inhibition reached 40% at the highest concentration.

Current studies also exist that show an absence of significant effects on primary producers for endpoints tested. Sjollema et al. (2016) exposed both freshwater and marine microalgal species to uncharged virgin PS microbeads and negatively-charged beads and found an absence of significant effects on photosynthesis from exposure to all treatments. Further, Garrido et al. (2019) found no effect on the daily growth rate of the microalgae *Isochrysis galbana* exposed to PE particles at any of the tested concentrations.

Soil

Experimental studies involving biota in the soil compartment are limited, but the studies that do exist show that microplastic exposure can negatively impact organism health and behaviour.

Ju et al. (2019) showed that exposing soil springtails (*Folsomia candida*) to PE microplastics for 28 days led to an increase in avoidance behaviours and an inhibition of reproduction rate by up to 70.2% at the highest exposure concentration. Additionally, the exposed springtails had significantly decreased bacterial diversity in their guts. Similarly, Kim and An (2019) found that microplastic infiltration into soil system bio-pores caused movement inhibition in the invertebrate *Lobella sokamensis*.

PS microplastics also caused toxicity to the soil invertebrate *Caenorhabditis elegans* following a 3-day exposure period (Lei et al. 2018a). Nematodes exposed to 1.0 µm PS particles had lower survival rates, shorter average lifespans, decreased average body lengths, and significant damage to GABAergic neurons in comparison to the other microplastic sizes tested.

Sediment

Although the sediment compartment has also been less studied than the water compartment, the current literature indicates that microplastics may have adverse effects on sediment-dwelling organisms.

Ziajahromi et al. (2018) exposed sediment-dwelling midge (*Chironomus tepperi*) larvae to four different size ranges of virgin PE microplastics to assess development. They concluded that midge survival was size-dependent; organisms exposed to microplastics that were similar in size to their normal food sources (10 to 27 µm) had a survival rate of 57% compared to 92% in the negative control group, as well as significantly smaller body sizes and head capsule lengths. Further, Leung and Chan (2018) found that PS microplastics significantly increased mortality and decreased body part regeneration in polychaetes (*Perinereis aibuhitensis*) after a 4-week exposure period in a size-dependent manner. In addition, sediment-dwelling bivalves (*Ennucula tenuis, Abra nitida*) exposed to fragmented PE microplastics in three size classes displayed a dose-dependent decrease in energy reserves; however, no significant mortality was observed (Bour et al. 2018). The exposed *E. tenuis* also had significantly lower lipid content for only one condition, while lower protein content was observed in *A. nitida* from exposure to the largest particles at all concentrations.

Nematodes (*Caenorhabditis elegans*) exposed to PA, PE, PP, and PVC microplastics had decreased survival rates, body length, and reproduction, as well as reduced calcium levels and increased expression of enzymes, indicating oxidative stress and intestinal damage (Lei et al. 2018b).

In contrast to the above-summarized research, the current literature also contains studies that show an absence of adverse effects on organisms exposed to microplastics in sediment. Redondo-Hasselerharm et al. (2018) observed no significant effects on survival or growth of the freshwater benthic macroinvertebrates *Hyalella azteca, Asellus aquaticus, Sphaerium corneum,* and *Tubifex* spp. from exposure to PS microplastics. Further, they observed no effects on the reproduction of the freshwater worm *Lumbriculus variegatus*.

6.2.3 Trophic transfer

There is limited information on the ability of microplastics to travel through different trophic levels, as seen in a food chain. Very few studies have looked at trophic transfer, and even fewer have studied the importance of bioconcentration, biomagnification, and bioaccumulation (Provencher et al. 2018a). Hammer et al. (2016) is one of the few studies that demonstrate vertical transfer of plastic particles within a food web. In that study, plastics found in the guts of great skuas (*Stercorarius skua*) from the Faroe Islands corresponded to the plastic contents of their prey (surface-feeding seabirds), implying indirect consumption.

Additionally, Cuthbert et al. (2019) demonstrated transference of microplastics in predatory midge larvae (*Chaoborus flavicans*) that consumed mosquito (*Culex pipiens)* larvae exposed to 2 µm PS microplastics. They found that the amount of microplastics transferred correlated with feeding rates towards mosquito larvae.

To study transfer along a natural food chain, Batel et al. (2016) exposed nauplii of the brine shrimp *Artemia* to microplastics ranging from 1 to 5 µm or from 10 to 20 µm, then fed the nauplii to zebrafish (*Danio rerio*). They observed that while the zebrafish were able to uptake the microplastic particles, no significant accumulation or further retention was observed within their intestinal tract, and no transfer to other organs was observed. Similarly, Welden et al. (2018) found by examination of stomach contents that trophic transfer of microplastics occurred between sand eels (*Ammodytes tobianus*) and their predator, plaice (*Pleuronectes platessa*) from the Celtic Sea. However, the microplastics were egested in the plaice.

Some studies suggest that unintentional ingestion, rather than trophic transfer, is the primary means by which microplastics are ingested. Chagnon et al. (2018) found no accumulation of microplastics in stomachs of yellowfin tuna (*Thunnus albacares*), a large predatory fish from Easter Island, despite plastics being found in the guts of its prey. Hipfner et al. (2018) also concluded that two fish species from the northeastern Pacific Ocean, the Pacific sand lance (*Ammodytes personatus*) and the Pacific herring (*Clupea pallasii*), do not act as significant conduits for the vertical transfer of microfibres to marine piscivores along the coast of British Columbia.

6.2.4 Translocation

While mechanisms of translocation from an organism's gut to other parts of its body are not well studied to date, the current literature has shown that translocation is usually size-dependent. For example, Lu et al. (2016) found that particles <5 µm can translocate to fish liver from the gut, while 20 µm particles cannot (Jovanović 2017). Smaller particles have the potential to more easily enter the circulatory system, but can also be egested more easily than larger microplastic particles (Jovanović 2017; Burns and Boxall 2018).

Current studies show that translocation occurs in some organisms and organs, while other studies contradict these findings. For example, translocation of 0.5 µm PS spheres to the haemolymph, gills, and ovary was observed in crabs (*Carcinus maenas*) (Farrell and Nelson 2013). In zebrafish (*Danio rerio*), Lu et al. (2016) found 5 µm PS particles in the gills, liver, and gut, while 20 µm particles were only found in the gills and gut. In bivalves, tissue translocation of 3.0 or 9.6 µm PS spheres from the digestive tract to the circulatory system was seen in mussels (*Mytilus edulis*) by Browne et al. (2008). However, a study by Sussarellu et al. (2016) using Pacific oysters (*Crassostrea gigas*) showed no evidence of PS sphere (2 and 6 µm) translocation. Limited information in fish also shows very small amounts of microplastics in fish muscle (Karami et al. 2017a; Abbasi et al. 2018; Akhbarizadeh et al. 2018).

The conflicting results observed in these studies may be attributable to species-specific differences and/or false positive results that may occur as a result of leaching of fluorescent dye, which is often used to track particle ingestion. Schür et al. (2019) tested this theory and found that fluorescent droplets did not always co-localize with the plastic PS beads ingested by *Daphnia magna*. Using confocal laser scanning microscopy, 1 µm beads did not co-localize with the fluorescent dye in the gut and there was a rapid loss of fluorescence upon investigation. Fluorescence was also observed in lipid droplets outside of the digestive tract, but plastic particles were not detected in these same lipid droplets. Therefore, given

that false positives may occur in uptake studies that did not take precautions to avoid potential artifacts by ensuring the stability of dyes, controlling for dye leaching (e.g., by pre-washing the particles), or using microscopic imaging to confirm plastic presence, results should be interpreted with caution.

7. Impacts on human health

7.1 Macroplastic

Human exposure to macroplastic pollution is not anticipated to occur, and the effects of macroplastics on human health are therefore not considered in this report.

7.2 Microplastic

Humans may be exposed to microplastics through the ingestion of food and drinking water (see Section 5.2) and the inhalation of indoor and outdoor air (see Section 5.1.3). The toxicity of microplastics via the ingestion and inhalation routes of exposure is reviewed below. Where possible, inferences are made from epidemiological studies on microplastics in humans and experimental studies on microplastics in animal models. A comprehensive review of in vitro studies on microplastics was not conducted as their relevance to human health is unclear. The effects of biofilms on human health are also discussed.

Upon ingestion or inhalation, microplastics may exert effects due either to their physical presence in the gut or lung or to the chemical composition of the plastic polymers themselves or their monomers, additives or sorbed substances. The World Health Organization (WHO) recently carried out an assessment of human exposure to microplastics in drinking water using conservative worst-case estimates of the levels of additives and sorbed chemicals on microplastics (WHO 2019). The Food and Agriculture Organization of the United Nations (FAO) and the European Food Safety Authority (EFSA) conducted a similar assessment of exposure to microplastics in seafood (EFSA 2016; FAO 2017). These evaluations concluded that exposure to microplastics and/or chemicals associated with microplastics are considered to be a low concern to human health (EFSA 2016; FAO 2017; WHO 2019). The reader is referred to those reports for further information on the exposure and risk assessments conducted therein.

7.2.1 Effects from oral exposure

Physicochemical properties affecting uptake and toxicity

Following ingestion, microplastic uptake and translocation are strongly dependent on the physicochemical properties of the ingested particles (FAO 2017; Wright and Kelly 2017; WHO 2019). Particle size is an important determinant of absorption through the intestinal epithelium. Smaller particles have larger surface-area-to-volume ratios, which can increase their ability to translocate to internal organs and increase bioreactivity (WHO 2019). A higher surface-area-to-volume ratio may also increase the sorption capacity of microplastics for environmental contaminants. Smaller particles may

also be more susceptible to fragmentation, and while degradation of microplastics to smaller polymers has been demonstrated for Antarctic krill, it is uncertain whether this occurs within the human GI tract (WHO 2019). Particle concentration can also influence toxicity, as higher concentrations are expected to overwhelm biological clearance mechanisms and cause responses that are otherwise not observed at lower doses (WHO 2019). At present, it is unclear how other properties, such as shape and surface chemistry, may affect the uptake, retention, and/or toxicity of ingested microplastics (Stock et al. 2019; WHO 2019).

Toxicokinetics

There are limited data regarding the fate of orally ingested microplastics in mammalian species. After oral ingestion, microplastics may accumulate in the gastrointestinal tract, translocate from the gastrointestinal tract into organs or tissues, or be excreted (Carr et al. 2012; Galloway 2015; Duis and Coors 2016).

Several mechanisms of uptake have been proposed for microplastics, including endocytosis via microfold cells (M cells) of the intestinal Peyer's patches and paracellular persorption (see EFSA 2016, FAO 2017, and Wright and Kelly 2017 for an extensive review of the toxicokinetics of microplastics). Based on limited data, it is expected that the largest fraction of orally ingested microplastics (>90%) will be excreted in the feces (EFSA 2016; FAO 2017). Microplastics greater than 150 µm are also expected to remain confined to the gut lumen and be excreted (EFSA 2016; FAO 2017; WHO 2019). Various types of microparticles have been shown to translocate across the mammalian GI tract into the lymphatic system at sizes ranging from 0.1 to 150 µm (Hussain et al. 2001; EFSA 2016; FAO 2017). For example, in one study, PVC microplastics (5 to 110 µm) were detected in the portal veins of dogs (Volkheimer 1975). Given these findings, it is expected that microplastics ≤150 µm will end up in the lymph nodes and may result in systemic exposure, although absorption is expected to be low (≤ 0.3%; EFSA 2016; FAO 2017). Only very small microplastics (<1.5 µm) are expected to enter into capillaries and penetrate deeply into tissues (Yoo et al. 2011; EFSA 2016). This is consistent with a recent 28-day study in which mice were administered high concentrations of a mixture of PS microplastics of various sizes by oral gavage 3 times per week (Stock et al. 2019). Only a few microplastics were detected in the intestinal walls (no quantitative analysis completed), representing a very low uptake by the GI tissue, and no microplastics were found in the liver, spleen or kidney. Conversely, another study reported significant translocation of 5 µm and 20 µm PS microplastics to the liver and kidney in mice (Deng et al. 2017), although these data are of questionable quality due to notable limitations in study design, data reporting, and biological plausibility of results (Tang 2017; Böhmert et al. 2019; Braeuning 2019). Based on a single human ex vivo placental perfusion model, fluorescently-labelled polystyrene beads <240 nm may be taken up by the placenta (Wick et al. 2010).

Studies in humans

No epidemiological or controlled dose studies that evaluated the effects of orally ingested microplastics were identified in humans.

Studies in experimental animals

A small number of animal studies have evaluated the potential adverse effects of orally ingested microplastics (Merski et al. 2008; Mahler et al. 2012; Deng et al. 2017, 2018; Lu et al. 2018; Rafiee et al. 2018; Jin et al. 2019; Stock et al. 2019). Studies were limited to a few types of virgin microplastics and tested either unknown or high concentrations of microplastics that were not necessarily reflective of anticipated human exposure. Test concentrations in toxicity studies are orders of magnitude higher than would be anticipated for humans. Therefore, it was not possible to adequately evaluate the health risk of orally ingested microplastics with the currently available animal data (EFSA 2016; FAO 2017; Wright and Kelly 2017; WHO 2019). The WHO conducted the most recent review of the toxicological data on microplastics ingestion. Consistent with previous reviews by the EFSA (2016) and FAO (2017), the WHO concluded that there were insufficient data to allow for a robust evaluation of the potential human health risks of ingested microplastics, although there was no information to suggest it represented a potential human health concern (WHO 2019). Relevant toxicological studies are briefly summarized below, with more detailed descriptions, including test concentrations, provided in Table D-1 in Appendix D.

In a 90-day study that was compliant with test methods from the Organisation for Economic Co-operation and Development (OECD), rats fed a daily diet that contained up to 5% milled PE and PET fabric exhibited no treatment-related adverse effects on blood parameters, organ weights, or histopathology (Merski et al. 2008). Based on the absence of observed toxicity, the highest test dose was considered to be the no observed effect level (NOEL), equivalent to approximately 2 500 mg/kg body weight (bw)/day (WHO 2019). Fibre concentrations were not reported.

Other studies have reported adverse health effects in mice following the administration of very high oral doses of microplastics, several orders of magnitude above expected microplastic concentrations in food and drinking water (Deng et al. 2017, 2018; Lu et al. 2018; Jin et al. 2019). These studies have been extensively criticized for their lack of reliability and relevance (Böhmert et al. 2019; Braeuning 2019; Tang 2017; WHO 2019; Stock et al. 2019). Exposure to high concentrations of PS microplastics in drinking water was associated with alterations in lipid metabolism, gut microbiota composition, amino acid and bile acid metabolism, mucus secretion, and reduced intestinal barrier function in mice (Jin et al. 2019; Lu et al. 2018). Inflammation and lipid droplets were reported in the livers of mice administered high concentrations of PS microplastics by gavage (Deng et al. 2017), but the presence of these effects cannot be determined due to poor quality histological images (Braeuning 2019). Deng et al. (2017) also reported changes in metabolic profiles suggestive of disturbances in energy and lipid metabolism, oxidative stress, and neurotoxic responses. However, the relevance of these metabolic endpoints in assessing the potential human health effects of microplastics is difficult to interpret (Tang 2017; Braeuning 2019; WHO 2019).

More recently, a 28-day mouse study evaluated the potential adverse effects of a mixture of various sizes of PS microplastics (1, 4 and 10 µm) administered via oral gavage 3 times per week using male heme oxygenase-1 reporter mice, a transgenic mouse model used to evaluate oxidative stress and inflammatory responses (Stock et al. 2019). In contrast to previous studies (Deng et al. 2017, 2018), the

authors reported no evidence of oxidative stress or inflammation. While very high microplastic doses were selected for purposes of consistency with other rodent oral toxicity studies, the selected treatment scheme involved dosing the animals 3 times per week, which was intended to be more representative of a realistic human exposure scenario. However, given the high level of uncertainty surrounding human exposure to microplastics, it is unclear whether this experimental dosing regime (i.e., 3 times per week) was in fact more representative of human exposure than daily dosing regimes.

7.2.2 Effects from inhalation

There may be hazards associated with the inhalation of microplastic particles due to their physical presence in the lung that are independent of chemical-related hazards. The scientific literature demonstrating the specific effects of microplastics on the lung is emerging, but their potential to cause effects in the respiratory tract or to translocate to other tissues remains uncertain. Still, inferences can be made from concepts of particle toxicology. Overall, toxicity related to the physical hazard of particles can include oxidative stress, cytotoxicity, inflammation, translocation to other tissues and, in some exceptionally elevated exposure circumstances, particle overload (elevated alveolar burden of particles that can impair clearance) (Prata 2018). Poorly soluble particles that are not inherently toxic, such as carbon black and TiO_2, have been shown to cause inflammation and tumours in rodents, albeit at very elevated levels of exposure (Borm and Driscoll 2019). Inhalation of fine particles is also associated with adverse respiratory and cardiovascular effects, although it is not possible to draw any conclusions regarding particle-driven effects of microplastics exposure at this time.

Potential toxicity of particles will largely depend on particle size and shape, which will influence their deposition in the respiratory tract, their interaction with biological matrices, their potential to translocate, and the efficiency of particle clearance mechanisms. In general, inhalable particles larger than 10 µm in aerodynamic equivalent size will deposit mostly in the extrathoracic region, whereas particles below 10 µm can reach the tracheobronchial regions of the lung (US EPA 2009). It is expected that the majority of these particles will be removed from the airways by means of mucociliary clearance (i.e., trapping of the particles in mucus and coughing), though such clearance can result in ingestion of the particles and subsequent gastrointestinal exposure (Gasperi et al. 2018). In theory, small particles below 2.5 µm in size can reach the alveolar region of the lung. These particles are removed through phagocytosis by alveolar macrophages, although there is some conflicting evidence demonstrating that very small particles in the nano-size range can evade alveolar clearance mechanisms and potentially accumulate in the lung, eventually reaching the interstitium (Li et al. 2016a).

In the case of fibres, deposition patterns are more difficult to predict. Given their length, most microplastic fibres are expected to be deposited either in the extrathoracic region or in the upper airways and removed via mucociliary clearance (Gasperi et al. 2018). In general, longer plastic fibres, although flexible, are more likely to be associated with evasion of clearance mechanisms (Prata 2018). The area in which deposition occurs and residency time in the lung will greatly influence physical hazards associated with microfibres. Although there are insufficient data that are specific to microplastics, the observation of plastic microfibres in lung tissue biopsies of workers from a synthetic textile industry, as well as in healthy and neoplastic

lung tissues from lung cancer patients, substantiates the plausibility of pulmonary microplastic retention through inhalation (Pauly et al. 1998; Wright and Kelly 2017).

There are few studies that evaluate microplastic particle translocation from the lung following inhalation. It is possible that microplastics can translocate from the lung to systemic circulation or to the lymphatic system, potentially reaching other tissues. One study has examined translocation following intratracheal instillation in pregnant rats (equivalent to 2.4×10^{13} particles) and revealed systemic translocation to placenta, whole pup, fetal liver, heart and spleen (Fournier et al. 2018). Rats intratracheally instilled with radiolabelled PS particles of 56.4 and 202 nm in size exhibited only a small fraction (<2.5%) of particle translocation into systemic circulation in healthy rats, which increased to 4.7% for the smaller particles in the presence of lipopolysaccharide-induced lung inflammation (Chen et al. 2006). The likelihood of translocation is expected to increase with decreasing particle size and increased residency time as well as in individuals with compromised lung function and during events of inflammation (i.e., due to increased cellular permeability) (Galloway 2015). The alveolar region of the lung is a site of potential concern, in part because smaller particles can penetrate this region of the lung (and because they are, by nature, more reactive due to their high surface area), and in part because of the exchanges with systemic circulation that occur at this site. In the upper airway, particles may diffuse through mucus and reach underlying epithelium, where translocation may occur. However, diffusion through mucus is not expected to occur for insoluble particles such as microplastics. It should be noted that, in rats, ultrafine particles have been shown to reach brain tissue via translocation from the nasal cavity through the olfactory nerve (Oberdörster et al. 2004).

There is a paucity of information on the physical hazards related to inhalation of microplastics. Future studies should focus on confirming and exploring the toxicological mechanisms of the physical hazards associated with microplastics, including their effects on the lung and cardiovascular system and their capacity to translocate to extra-pulmonary tissues.

Studies in humans

In the only controlled dosing studies of microplastics in humans, participants were exposed to printer toner, which was not considered relevant for this evaluation. Epidemiology studies of microplastics in indoor or ambient air could not be found for the general population. Health effects studies of microplastics are limited to several occupational epidemiology studies and a lung biopsy study; these studies are summarized below.

Two reviews (Wright and Kelly 2017; Prata 2018) summarized the outcomes of occupational epidemiology studies in individuals who worked with synthetic textiles, nylon flock, and PVC. The studies identified associations between work in these industries and increases in adverse respiratory effects, including airway lesions and fibrosis, decreased pulmonary function, wheezing, dyspnea, inspiratory crackles, chronic cough, chronic mucous production, eye and throat irritation, increased bronchial responsiveness, bronchitis, bronchiolitis, emphysema, asthma, pneumoconiosis, interstitial lung disease, and acute respiratory failure (Wright and Kelly 2017; Prata 2018). Several studies also found associations between work in these industries and cancers of the digestive system and respiratory tract, but not all

studies investigating these effects identified the association. Despite the associations between exposures to plastic particulates or fibres and adverse health effects, no firm conclusions on human health effects can be made owing to confounding variables such as co-exposures with other workplace hazards that could contribute to respiratory effects.

A third review discussed the epidemiological evidence of health effects in women working in plastics manufacturing and processing industries, but did not specifically address microplastics (DeMatteo et al. 2012). Epidemiology studies identified associations between work in plastics industries and breast cancer, spontaneous abortion, and infertility. As exposures to microplastics were not specifically discussed in these studies, it is unclear whether associations with these health effects are related to inhalation of plastic particulates and fibres or exposure to other substances used in the production of plastic.

Epidemiology studies have been developed for other occupations with exposure to microplastics. However, most studies limited exposure categorization to occupation, and therefore adverse outcomes from exposure to microplastics were not specifically investigated. A small subset of the epidemiology studies included analyses specifically related to exposure to plastic particulates or fibres; only these studies are discussed here. No increases in lung or respiratory tract cancer were associated with exposure to PU dust in polyurethane foam (PUF) workers (Sorahan and Pope 1993; Mikoczy et al. 2004; Pinkerton et al. 2016). In pattern and model makers, an increase in lymphocytopenia was significantly associated with exposure to plastic dusts, but no exposure–response relationship was observed (Demers et al. 1994).

The relevance of occupational data on airborne microplastics to the general population is unknown, as extrapolation from high-dose occupational exposures to lower doses, as would be expected for the general population, is difficult in the absence of health effect data at lower concentrations. A further limitation of the dataset is that most studies did not investigate the impact of dose–response on the health outcomes. Additionally, workers in the studies might have had co-exposures to other chemicals associated with adverse health effects, such as monomers, catalysts, additives, and other compounds used in the workplace.

Studies in experimental animals

Studies of inhaled microplastics were identified for rats (Laskin et al. 1972; Thyssen et al. 1978; Hesterberg et al. 1992; Warheit et al. 2003; Ma-Hock et al. 2012), hamsters (Laskin et al. 1972), and guinea pigs (Pimentel et al. 1975). The microplastic constituents in the studies included PP fibres (Hesterberg et al. 1992), PU particulate (Laskin et al. 1972; Thyssen et al. 1978), nylon fibres or particulate (Pimentel et al. 1975; Warheit et al. 2003), PAN particulate (Pimentel et al. 1975), and acrylic ester copolymer (Ma-Hock et al. 2012). Exposure duration varied, with one longer-duration study of 325 days, two studies of subchronic duration (12 to 13 weeks), and three studies of subacute duration (5 to 30 exposure days). Detailed descriptions of these studies, including test concentrations and results, are presented in Table D-2 in Appendix D.

Effects observed in inhalation studies tended to be consistent and independent of duration, type of plastic, and species. Observations consistent with foreign body reactions were common in the studies. This included an increase in activity or number of inflammatory cells, which contained fibres or particles (primarily in lung tissues and bronchoalveolar lavage fluid [BALF], but also in the lymphatic system) and which were often accompanied by granulomas. In areas of lungs associated with particle deposition, hyperplasia, emphysema, and edema were observed. Studies that euthanized animals at various timepoints post-exposure tended to indicate a reversibility of effects, which might suggest that the effects are adaptive rather than adverse responses. No dose-related effects were observed in mortality, survival time, behaviour, clinical observations, tumour incidence, or fibrosis. LOECs adjusted to reflect intermittent exposure ranged from 0.48 to 2.3 mg/m^3. One exception was for the shortest duration study, in which no treatment-related changes in BALF or histology were observed up to the adjusted no observed effect concentration (NOEC) of 2.7 mg/m^3 in rats exposed for 5 days and followed up to 24 days post-exposure (Ma-Hock et al. 2012). However, most studies were not performed according to OECD test guideline methods. Moreover, the human relevance of these animal studies is unclear, as exposures in the studies are much higher than would be expected in humans under typical exposure scenarios.

The inhalation studies are also supported by observations in intratracheal instillation studies in rats. Exposures in the studies were to PVC particulate (Agarwal et al. 1978; Pigott and Ishmael 1979; Xu et al. 2004), nylon fibres or particulates (Porter et al. 1999), PS particulate (Brown et al. 2001; Fournier et al. 2018), or PU particulate (Stemmer et al. 1975). Most of the studies incorporated only one exposure level, and contained a single intratracheal instillation, except for one group in Fournier et al. 2018 (every second day). The rats in the various studies were followed from 1 day to 24 months post-instillation. In general, the foreign body reactions observed in inhalation studies were also observed in the intratracheal studies. One study demonstrated that effects from washed PVC particulates were equal to or greater than those from unwashed PVC particulates, suggesting that adverse effects were from the plastic particulate itself rather than from adsorbed additives (Xu et al. 2004). Additional pulmonary effect observations are outlined in Table D-2 in Appendix D. A developmental study also observed an increase in fetal reabsorption sites and evidence of particle translocation from the lungs (placenta, whole pup, fetal liver and heart, and maternal heart and spleen) (Fournier et al. 2018). Although results from the intratracheal studies corroborate effects observed in the inhalation studies, the route of exposure is of lesser quantitative relevance because it does not accurately represent deposition patterns and dosing that would be observed from inhalation.

A review of the toxicology of *p*-aramid (an aromatic polyamide commonly known as Kevlar) fibrils was also identified (Donaldson 2009). Studies of rat lungs identified effects at high exposure levels, such as inflammation, increased cell proliferation, fibrosis, and development of cystic keratinizing squamous cell carcinoma (a tumour stated to be of questionable relevance to humans due to an absence of a human homologue).

7.2.3 Effects of biofilms

Microplastics provide a unique and extensive surface for microorganisms to attach to and colonize in water environments, forming biofilms (Zettler et al. 2013; De Tender et al. 2015; McCormick et al. 2016; Oberbeckmann et al. 2018; Kettner 2018; Arias-Andres et al. 2018, 2019). However, very few studies have analyzed microplastics-associated biofilms.

Biofilms consist of accumulations of microorganisms, typically encased in a self-secreted matrix of extracellular polymeric substances, containing both organic and inorganic matter (Liu et al. 2016; Prest et al. 2016; WRF 2017). The structure of the extracellular polymeric substances provides protection from stressors (e.g., predators, disinfectants), and aids in uptake and utilization of nutrients (Flemming and Wingender 2010; Prest et al. 2016). Biofilms are ubiquitous in the environment (Hall-Stoodley et al. 2004; Yadav 2017) and in drinking water distribution systems (Liu et al. 2016; Prest et al. 2016; WRF 2017), where they provide a habitat for the survival and growth of microorganisms, including potential pathogens (US EPA 2002; Batté et al. 2003; Berry et al. 2006; Liu et al. 2016).

The higher surface-to-volume ratio of microplastics facilitates the absorption of organic matter, which serves as nutrients for microorganisms, thereby promoting biofilm formation. The transport of microplastics over long distances and through the water column (Peng et al. 2017) affords opportunities for attachment of microbial "hitchhikers" and biofilm formation (Kirstein et al. 2016; Zalasiewicz et al. 2016; Keswani et al. 2016). These plastic-associated biofilm communities are sometimes referred to as "plastispheres" (Zettler et al. 2013) and tend to differ from microorganisms in surrounding water or on natural aggregates/particles (Zettler et al. 2013; Hoellein et al. 2014; McCormick et al. 2016; Oberbeckmann et al. 2016; Kettner et al. 2017; Arias-Andres et al. 2018, 2019). Gene sequencing studies have demonstrated that microbial communities on microplastics are less diverse than those on non-plastic substrates (Zettler et al. 2013; Harrison et al. 2014; McCormick et al. 2014, 2016; Ogonowski et al. 2018a), suggesting that microplastics may select for specific microbial colonizers. In other words, the physicochemical properties of microplastics influence the composition and structure of the associated biofilm community (Bhardwaj et al. 2012; Zettler et al. 2013; Harrison et al. 2014; McCormick et al. 2014, 2016). It is unclear what impact this has, but some have hypothesized that it may result in reduced competition and predation, leading to the emergence of potential pathogens (Amalfitano et al. 2014; Keswani et al. 2016; Andrady 2017). Other factors, including environmental conditions (e.g., salinity, temperature), can also influence biofilm formation on microplastics (Harrison et al. 2018; Oberbeckmann et al. 2018; WHO 2019). In addition, microorganism features, such as the hydrophobicity of their cell walls and cell surface charge, can impact attachment to microplastics (Rummel et al. 2017).

Biofilm constituents commonly found on microplastics include various non-pathogenic microorganisms, comprising species of *Pseudomonas*, *Arcobacter*, *Erythrobacter*, *Streptococcus*, *Staphylococcus*, *Aspergillus*, *Penicillium* and *Phanerochaete* (Bhardwaj et al. 2012; McCormick et al. 2014). Pathogenic bacterial sequences, primarily those of *Vibrio*, have been detected in microplastic-associated biofilms (Zettler et al. 2013; De Tender et al. 2015; Kirstein et al. 2016). However, aside from one study (Kirstein et al. 2016), species identification was not possible, and it is therefore unknown whether the organisms

were of human health concern. In the study by Kirstein et al. (2016), *Vibrio* spp. of potential human health significance were identified, namely *V. parahaemolyticus*, *V. fluvialis*, and *V. alginolyticus*.

The increased cell density and proximity, improved nutrient availability, and protection afforded by an extracellular polymeric substances matrix make biofilms an ideal environment for interactions between microorganisms, including those on microplastics. Among these interactions is conjugation, the transfer of genetic material through direct cell-to-cell contact (Cook et al. 2011; Stalder and Top 2016). Conjugation is a method of horizontal gene transfer (HGT), the primary mechanism for spread of antibiotic resistance, whereby a mobile genetic element (MGE), such as a plasmid, containing antibiotic resistance genes (ARGs) is transferred from a donor to a recipient cell (Von Wintersdorff et al. 2016). A few studies have shown that ARGs are more frequently transferred between microplastic-associated biofilm members than free-living bacteria or biofilms associated with natural aggregates (Arias-Andres et al. 2018; Eckert et al. 2018a,b; Imran et al. 2019; Laganà et al. 2019). Transfer also occurred between a broader (i.e., more distantly related) group of microorganisms on the microplastics than in the natural environment. These findings suggest that microplastic-associated biofilms provide a favourable environment (i.e., "hot spot") for HGT events and may select for antibiotic resistant microorganisms and ARGs, which may then be transported to different habitats. Transfer of ARGs via microplastics has been observed between wastewaters and the aquatic environment (Eckert et al. 2018a,b). Transfer events on microplastics may be further amplified through exposure to metals, as metal resistance genes are present on the same plasmid as antibiotic resistance genes (Baker-Austin et al. 2006; Wright et al. 2006; Seiler and Berendonk 2012; Zhang et al. 2018; Imran et al. 2019).

Although research in this area is very limited, studies suggest that plastic-associated biofilms in water may harbour potential human pathogens and ARGs. Given that microplastics can travel long distances (see Section 4, WHO 2019), there is a possibility that these organisms and/or ARGs may be dispersed across waters and enter drinking water sources. Despite this, there is no indication of how prevalent these organisms are or of how long they persist and/or remain infectious while in a plastisphere. Moreover, conventional drinking water treatment is expected to significantly reduce microplastics and inactivate associated biofilm organisms (see Section 4.1.3, WHO 2019). Thus, there is currently no evidence to suggest that microplastic-associated biofilms in drinking water pose a risk to human health.

Microorganisms might also adhere to the surface of airborne microplastics, but data are limited. Microorganisms have been measured in airborne particulates (Noble et al. 1963; Brodie et al. 2007), although no data exist specifically for plastic particulates. Adherence and growth of microorganisms on airborne microplastics might be limited because they could be dependent on the contact of microorganisms and microplastics in the environment. If contact does occur, however, the plastic particulates might protect and shield adhered microorganisms (Prata 2018). While no data could be found on the characterization of microbial communities potentially colonizing airborne microplastics, lung infections could theoretically occur if pathogenic species were adhered to microplastics and inhaled (Prata 2018).

8. Transport of chemicals

In addition to the physical hazards presented by plastic particles themselves, it is possible that effects could occur as a result of exposure to residual monomers, chemical additives, and sorbed environmental contaminants (e.g., persistent organic pollutants (POPs) and metals) that may leach from microplastic particles (Munier and Bendell 2018; SAPEA 2019). Although there is potential for environmental or human exposure to these compounds, these chemicals are considered to be under the purvue of various programs at Environment and Climate Change Canada and Health Canada and are not discussed further in this report.

Any effects observed from the transport of chemicals are highly context dependent. For example, the type of plastic and the physicochemical properties of the sorbed chemical are known to have an effect on sorption ability. In general, PE shows a greater ability to sorb contaminants, while PET and PVC have a lower sorption capacity (Alimi et al. 2018). Plastics with high surface-area-to-volume ratios (i.e., small, elongated, or have an irregular shape) tend to have higher sorption capacities (Rochman 2015). For instance, PVC was shown to have significantly greater absorption of copper than PS, which could be due to its higher surface area and polarity (Brennecke et al. 2016; Munier and Bendell 2018). Sorption can also be affected by factors such as age, shape and porosity of the particle, salinity and pH of the environment (increased salinity and particle age tend to increase sorption, and alkaline environments favour sorption of cations), and the concentration of metals and other contaminants in the surrounding waters (Munier and Bendell 2018; Guo and Wang 2019). Di and Wang (2018) sampled surface waters and sediments from China's Three Gorges Reservoir and found that several contaminants were adsorbed to the surface of the recovered microplastics, including organic solvents and pharmaceutical intermediates.

The properties of the receiving environment can also affect contaminant transfer. Mohamed Nor and Koelmans (2019) found that the transfer of polychlorinated biphenyls (PCBs) from microplastics in simulated gut fluid is biphasic and fully reversible. More specifically, the effect of microplastics in the gut depends on the contents of the gut system. Ingested plastics acted as a source of hydrophobic organic compounds (HOCs) in clean gut systems, whereas in contaminated gut systems, clean microplastics rapidly extracted PCBs from food or other organic matter (Mohamed Nor and Koelmans 2019). The authors concluded that chemical contamination and cleaning can occur simultaneously when microplastics are ingested.

Although many of the compounds associated with plastic have short biological half-lives and are not persistent, plastic particles within the body could present a long-term source of exposure to the chemicals (Engler 2012). While recent reviews indicate that there is a low health concern for human exposure to chemicals from ingestion of microplastics from food or drinking water (EFSA 2016; FAO 2017; WHO 2019), further research would be required before a human health risk assessment on microplastics is possible. No data could be found on the transfer of these compounds in the human respiratory or GI tract.

Sorbed chemicals

Provencher et al. (2018b) found no significant correlations between concentrations of various PCB congeners in northern fulmars (*Fulmarus glacialis*) and the amount of ingested plastics when using a toxic equivalency factor (TEF) approach. They found that plastics did not contribute to the PCB concentrations in the birds and that the PCB congener profile between ingested plastics and the liver differed (Provencher et al. 2018b). This could be the result of the ability of northern fulmars to metabolize or bio-transform contaminants such as PCBs (Letcher et al. 2010; Provencher et al. 2018b). In a study using goldfish (*Carassius auratus*), Grigorakis and Drouillard (2018) observed lower dietary assimilation efficiencies (13.4%) for PCBs sorbed to microplastics compared to efficiencies (51.6%) for PCBs associated with food. The authors concluded that the lower bioavailability of PCBs associated with microplastics indicates that microplastic presence is unlikely to increase PCB bioaccumulation in fish. In a study by Devriese et al. (2017), Norway lobsters (*Nephrops norvegicus*) exposed to PCB-loaded PE or PS microplastics showed no significant bioaccumulation of the chemicals, with uptake of the PCBs being limited. Furthermore, Gerdes et al. (2019) found a positive correlation between the elimination rate of PCBs in *Daphnia magna* and the presence of microplastics. More specifically, the presence of microplastics together with PCBs was able to increase the elimination rate of high-molecular-weight PCB congeners in *D. magna* fourfold.

Diepens and Koelmans (2018) introduced a theoretical model simulating transfer of microplastics and HOCs in aquatic Arctic food webs. Simulated scenarios showed that PCBs biomagnify to a lesser extent with higher levels of microplastic ingestion, which supports the evidence previously described. Conversely, the same model also indicated that polycyclic aromatic hydrocarbons (PAHs) biomagnify more with elevated levels of microplastic ingestion. Under different conditions, Magara et al. (2018) found that the uptake and accumulation of fluoranthene (a PAH) in blue mussels *(Mytilus edulis*) were not affected by incubation with microplastics and that incubation with microplastics reduced the bioavailability of fluoranthene. In a study modelling the transfer of POPs from PVC and PE to benthic invertebrates, fish, and seabirds, Bakir et al. (2016) found that food and water were the main pathways of exposure for all organisms, and input from microplastic particles was negligible.

Tanaka et al. (2013) studied the occurrence of polybrominated diphenyl ethers (PBDEs) in tissues of short-tailed shearwater (*Puffinus tenuirostris*) seabirds, in their natural prey, and in plastics in the stomach of the seabirds. In 3 of the 12 short-tailed shearwaters examined, they detected higher-brominated congeners of PBDEs that were not present in their prey, i.e., lanternfish and squid, which were also sampled from the same area as the seabirds. However, they did detect these PBDEs on the plastics found in the stomachs of the 3 birds, which suggests that plastic-derived chemicals were transferred from the ingested plastic to the seabird tissue.

Hydrophobic POPs of potential human health concern (such as PCBs, PAHs, and organochlorine pesticides) can readily sorb to plastics. For that reason, plastic compounds such as PE and PU are used as passive samplers in environmental monitoring (WHO 2019).

Studies on microplastic-associated sorbed pollutants in drinking water could not be identified, but increased POPs in microplastics have been measured in marine environments and shorelines near urban environments (Wang et al. 2017; Pellet Watch 2019).

Limited data exist on the sorption of chemicals to microplastic particulates in outdoor air, indoor air, or indoor dust. Adsorption of organic pollutants in air to plastic particulates could theoretically occur, but would be dependent on the duration of microplastic suspension in air (Prata 2018). One study reported that no significant adsorption of PCBs, dichlorodiphenyldichloroethylene (DDE) or nonylphenol occurred on virgin PP pellets released in the atmosphere for 6 days (Mato et al. 2001). Therefore, the contribution of microplastics to inhalation of sorbed chemicals is unknown but potentially limited, although it is anticipated to be dependent on environment (e.g., urban versus rural environments, proximity to point sources). Overall, current research shows that while microplastics are able to transport POPs, the evidence suggests that the impact of this exposure pathway is minimal (Burns and Boxall 2018).

Monomers

Plastics are manufactured through the polymerization of monomers, which vary in toxicity. Some of the more hazardous compounds include acrylonitrile, acrylamide, 1,3-butadiene, ethylene oxide, and vinyl chloride (Lithner et al. 2011). Depending on the polymerization process, up to 4% of the plastic material can be composed of residual monomers from incomplete polymerization (Araújo et al. 2002; Lithner et al. 2011). Plastics can also be degraded (through biological processes and weathering) into monomers and oligomers, but few data exist on the contribution of these processes to human exposures to monomers (WHO 2019).

Additives

As discussed in Section 2, plastic additives can include polymer stabilizers, flame retardants, lubricants, plasticizers, and colourants. Compounds with potential human health effects that are additives of plastics include phthalates, polybrominated diphenyl ethers (PBDEs), lead, and cadmium (WHO 2019), among others. Plastic additives are mostly not co-polymerized, resulting in increased likelihood of being leached into the environment (Wright and Kelly 2017; Hahladakis et al. 2018). Molecular weight of additives and age of plastics are factors that can influence the rate of migration of additives from plastics to the surrounding environment (Hansen et al. 2013; Suhrhoff and Scholz-Böttcher 2016; Jahnke et al. 2017). Limited data exist on the contribution of microplastics to concentrations of plastic additive compounds in the environment, but there is evidence of potential migration pathways for the compounds in sources of human relevance, such as food (Helmroth et al. 2002; Muncke 2011), water (WHO 2019) and indoor dust (Rauert et al. 2014).

9. Knowledge gaps and considerations for future research

Several knowledge gaps were identified during the writing of this report and are outlined below with the objective of encouraging further research. Addressing these knowledge gaps will contribute to the

understanding of the environmental and human health risks of plastic pollution and will inform science-based policy and regulatory decisions related to plastic pollution.

9.1 Occurrence

While the approach to observing macroplastics is relatively obvious given their size, there is a general lack of consistency and reliability in the methods used to sample and quantify microplastics in the environment and other media (e.g., drinking water and food). Many studies rely only on visual identification to determine if a particle is plastic. This can lead to a high false positive rate (especially at sizes smaller than 1 mm) and does not allow for proper characterization of plastics. For instance, when fibres visually identified as microplastics from the GI tracts of eelpout (*Zoarces viviparus*) were analyzed with µATR-FTIR by Wesch et al. (2016), none of the fibres were determined to be of synthetic origin. Given these findings, the authors question whether visual identification alone is sufficient to determine if microfibres are microplastics and call for standardized approaches for identifying and monitoring microplastics. Non-specific fluorescence staining methods have been suggested as a potential rapid-screening approach for detecting and quantifying microplastics in various media (Erni-Cassola et al. 2017; Maes et al. 2017; Prata et al. 2019). However, a major drawback of these staining methods is the possible introduction of false positives through the staining of biological organisms, such as marine algae or organic matter.

Spectroscopy techniques, such as FTIR, Raman spectroscopy and pyrolysis GCMS, are currently the preferred methods for plastic characterization and are often used following separation of plastics from sample media and visual identification using a microscope. Although they increase the accuracy of the identification of microplastics, spectroscopic analyses have limitations that can lead to the underestimation of microplastics in samples. With Raman spectroscopy, the generation of fluorescence can overpower the Raman spectrum produced, which can hinder the identification of potential plastics (Rezania et al. 2018). Furthermore, the signal can be heavily influenced by dyes, as well as by microbiological, organic and inorganic substances (Nguyen et al. 2019). With infrared (IR) spectroscopy, black or dark particles are not detected because they have a high absorption rate (Rezania et al. 2018), and particles below 20 µm may not yield enough absorbance interpretable spectra (Li et al. 2018b). Pyrolysis GCMS lacks reproducibility, as results are highly dependent on sample preparation and pyrolysis type. Thermal desorption GCMS is best used for samples of high mass (up to 100 mg) but lacks the sensitivity of pyrolysis GCMS (Nguyen et al. 2019). Microplastic counts can also be overestimated. Using SEM with energy dispersive X-ray spectroscopy (EDS), Anderson et al. (2017) found that, on average, 23% of the particles that were visually identified as plastics were not plastic. Burns and Boxall (2018) highlight that the error rate for identifying particles as plastic ranges from 33% to 70%. While analytical methods may help to confirm the synthetic nature of microplastics sampled in the environment, the inconsistencies in sampling methods (e.g., size of subsamples and sampling strategies) can limit the comparability of such analyses.

Studies investigating the occurrence of plastics in the environment and other media often use different units to report plastic abundance (e.g., plastics per area vs. plastics per unit volume), thereby limiting comparisons between studies and generalizability of results. Standardized reporting metrics are

required to ensure reporting consistency and study comparability (Burns and Boxall 2018). Another major gap in the analytical process is that there are no inter-laboratory studies, which are useful for method validation. Furthermore, due to variability and difficulty in quantifying microplastics, large standard deviations have been reported for the occurrence of microplastics in the environment and, in some instances, the standard deviation value exceeds the reported measurement.

In water, microplastics are sampled at size ranges that are compatible with available sampling apparatus (e.g., trawl nets, which have a mesh size of 300 to 350 µm). This means that microplastics smaller than 300 µm can often go undetected. This is an issue for microfibres in particular given their narrow size (Covernton et al. 2019). Sampling methods therefore need to be developed to support the characterization of the smaller size fractions of plastics in the environment. Further, sampling depths vary across studies and are not standardized (i.e., trawl nets would be biased to less dense plastics that are present near the surface of surface waters, and studies conducted at a greater depth would be biased against denser plastics).

A limited number of published studies report on environmental monitoring and effects in freshwater environments, and few data are published on the terrestrial environment (Burns and Boxall 2018; Provencher et al. 2018a). There is a need to expand work to include monitoring studies to other ecosystems, particularly terrestrial ecosystems.

In terrestrial matrices, studies of microplastic occurrence are scarce, possibly due to difficulty in translating research ideas in a marine context to a terrestrial context (Rillig 2012; da Costa et al. 2019). For example, there are no parallels for the accumulation of microplastics along shorelines in a terrestrial setting. In addition, it is often more difficult to isolate and characterize microplastics from a soil matrix; soil can contain varying levels of organic matter, which can distort signals and present problems when using FTIR and Raman spectroscopy for plastic characterization (Bläsing and Amelung 2018). Furthermore, there is a lack of standardized protocols for soil sampling and analysis in various soil types (da Costa et al. 2019). It has been suggested that a standard step-by-step approach be employed for terrestrial samples, involving removal of adherent fragments, mineral phase, and organic matter, followed by microplastic identification and quantification (da Costa et al. 2019).

There is also a lack of appropriate quantitative data for microplastic presence in drinking water and in water discharged after wastewater treatment, and limited information is available on the fate of microplastics during the wastewater treatment process, including particle breakdown, particle composition, removal efficiency, and subsequent release of these microplastics to other environmental compartments.

Occurrence data for microplastics in food is also scarce, with little to no Canadian-specific data. Data that do exist are focused on wild marine fish and shellfish, with limited occurrence data for freshwater and farmed species or other foods. In addition, occurrence data are needed for the tissues and organs of animals that are consumed by humans. Data are lacking on the potential effects of cooking or food processing (e.g., fresh versus frozen food) on microplastic concentrations, the impact of the food matrix on microplastic bioavailability (e.g., water-based versus solid/dry foods), and the potential point

source(s) of exposure to microplastics in food. Further studies are needed to determine whether food manufacturing, processing and/or handling as well as food packaging materials may contribute to microplastic concentrations in food.

There are currently no validated or recognized methods for the collection or analysis of microplastic samples in air, and little information is available on the partitioning of microplastic particles between air and dust. In order to accurately assess microplastic exposure from air, there is a need to develop and validate accurate, precise and reproducible methods for the sampling, extraction, characterization, and quantification of airborne microplastics and microplastics in settled dust and air, including robust quality assurance and control protocols. As Canadians spend approximately 90% of their time indoors, data on both indoor and outdoor microplastic exposures are needed to determine personal exposures, to understand their sources, pathways, fate, and distribution, and to identify and prioritize specific microplastic categories or mixtures for future research. There is also a need to explore the relationship between airborne microplastic particles and particulate matter. For example, knowing what proportion of particulate matter is composed of plastic polymers and knowing whether airborne plastic particles behave similarly to other airborne particulates would be useful in determining whether inferences can be made from the wealth of knowledge that exists on particulate matter.

Several researchers have identified the need for standardized protocols and stricter quality assurance in literature to ensure the availability of more high quality occurrence and exposure data in all media (Burns and Boxall 2018; Hermsen et al. 2018; Gouin et al. 2019; Koelmans et al. 2019). For sampling methods, this would include collection media, equipment, and handling procedures, as well as laboratory analysis practices. Due to the ubiquity of plastics, additional care must be taken throughout the entire process, from sample collection to laboratory analysis, to prevent sample contamination.

The importance of protocol development can be demonstrated by Provencher et al. (2017, 2019), who developed the only standardized protocols for monitoring and studying ingested plastics in seabirds. They include standardized field and lab techniques, as well as reporting guidelines for data (Provencher et al. 2017, 2019). The use of these standardized techniques by the international seabird community has led to spatial and temporal tracking of trends in plastics in the marine environment.

It has been recommended that a standardized quality criteria be developed that can be used to evaluate the appropriateness of studies on microplastic occurrence and effects. Hermsen et al. (2018) proposed several areas that should be evaluated when scoring the quality of microplastic ingestion studies: sampling method and strategy, sample size, sample processing and storage, laboratory preparation, clean air conditions, negative and positive controls, target component, sample treatment, and polymer identification. When reviewing current studies on microplastic ingestion by biota, they identified negative controls, polymer identification, laboratory preparation, and sample treatment as areas that were particularly lacking in quality and available information. Koelmans et al. (2019) evaluated 50 microplastic studies in freshwater surface water and drinking water using the same method identified by Hermsen et al. 2018. Only 4 studies scored positively on all proposed quality criteria; 92% of the reviewed studies were not considered complete or reliable on at least one criterion. It should be noted that Hermsen et al. (2018) and Koelmans et al. (2019) acknowledge that their criteria are not an

absolute judgement of the value of studies since not all aspects of studies could be captured in their scoring system. Moving forward, the use of standardized quality criteria will ensure that only data of acceptable quality are being used to inform scientists and policy makers and that the data are both reproducible and directly comparable.

There is also a paucity of data on the common or important sources of microplastics in the environment and other media, such that identifying source contributions of microplastics is difficult. There is a need to develop libraries that can be used to link samples to their sources using their chemical composition (polymer and additive chemicals) and other physical properties. Furthermore, establishing a taxonomy of microplastics based on morphology may also be informative in determining sources (Helm 2017).

Lastly, data on the occurrence and effects of nanoplastics is still emerging and is poorly understood. It is unclear whether and how nanoplastics may form in the environment (e.g., whether they are formed by processes such as the weathering of macroplastics or microplastics). There is a lack of appropriate analytical methodologies for nanoscale materials in all media, making accurate measurements of environmental occurrence and behaviour of nanoplastics difficult to evaluate (SAPEA 2019). As nanoplastics are inherently more difficult to test and measure, the importance of plastics fragmenting to the nano scale remains unclear at this time (Koelmans et al. 2015).

9.2 Environmental effects

The size ranges and concentrations of microplastics used in ecotoxicological research do not reflect the concentrations or sizes of microplastics collected in the environment using current sampling techniques. Microplastic effects studies are performed using either concentrations that are much higher than those currently reported in the environment or very small microplastics for which limited occurrence data exists (SAPEA 2019). Researchers studying effects should use plastics of similar size, shape, and composition to those found in the environment. Additionally, there is a need to further investigate the relationship between microplastics and natural particles that exist in the environment that induce similar effects in biota. Currently, experimental designs do not differentiate plastic-specific effects from those caused by other particles, such as clay or cellulose (Ogonowski et al. 2018b). Furthermore, effects studies are largely conducted with PS microplastic spheres, which are not representative of plastics found in the environment. More frequently detected microplastics (i.e., PP, polyester, and PA, among others) are underrepresented in effect studies (SAPEA 2019).

There is therefore a need to develop standard methods for testing the potential for adverse effects associated with exposure to plastic. For example, there is a need to evaluate the relationship between properties of plastic (e.g., particle size, shape and particle number) and toxicity. There is a corresponding lack of consistency in reporting test concentrations in studies; some studies report weight/volume, while others report particle number/volume

Pristine microplastics will likely contain additives such as catalysts. Consequently, it is possible that the resulting effects observed when testing these microplastics could be due to the catalyst and not solely to the polymer. For example, Pikuda et al. (2019) found that the acute toxicity to *D. magna* was

associated with sodium azide, a surfactant, and not the plastic PS particles themselves. When the sodium azide was removed from the plastics, the PS particles no longer caused mortality. Thus, plastic particles used to test effects in organisms should be washed to remove any additives that may cause effects that can be confused with effects caused by the particles themselves. Currently, this is not standard practice, and this was not considered when selecting studies for this report.

Burns and Boxall (2018) suggested that research in this field should move to the use of environmental degradation studies. Follow-up ecotoxicological studies should then be conducted using the resulting materials identified in the degradation studies. There is a need to develop certified standard reference materials that are environmentally relevant and meet the needs for risk assessment. This would help to characterize the effects of environmentally relevant plastics. Experiments that consider chronic effects (including effects of long-term retention within organisms) using consistent endpoints should also be completed. Provencher et al. (2018a) highlighted a need for studies that examine plastic transfer between predator and prey, as well as the biomagnification, bioaccumulation, and bioconcentration of these transferred plastics. Further research is needed on the mechanisms of absorption, distribution, metabolism, and excretion of microplastics and on the feasibility of a read-across approach from particle translocation studies. There is also a need to develop a better understanding of the sub-lethal, interactive and cumulative effects of plastics with other factors. For example, although a recent study has shown that there may be sub-lethal effects related to plastic ingestion on the blood chemistry of flesh-footed shearwaters in the southern hemisphere, the authors are unable to make definitive links at this time (Lavers et al. 2019). Further, while studies such as those by Lavers and Bond (2016) on ingested plastics as a route for the transport of trace metals have indicated that concentrations of certain metals were positively related to plastic mass, generalizations about the transfer of trace elements from ingested plastics are not yet possible as the mechanisms underlying this process are unknown. In addition, some studies on microplastic ingestion have only examined a portion of an organism's digestive tract, which may lead to an underestimation of ingestion rates, since other components of the GI tract may also contain microplastic particles. To accurately estimate all ingested microplastic, it is recommended that the entire GI tract, from esophagus to vent, of fish and the entire body for smaller species (i.e., bivalves) be examined (Hermsen et al. 2018).

There is a lack of research on microplastics in soil, and further study is needed to fully understand the interactive effects that plastic pollution will have on soil fauna and potential uptake into food crops. Finally, while some sources and occurrences of microfibres have been identified, further work is needed to fully understand their distribution and fate in the environment, as well as the effects this type of plastic pollutant presents.

Recent research has begun to explore links between plastic pollution and climate change. For example, Royer et al. (2018) showed that commonly used plastics produce greenhouse gases when exposed to ambient solar radiation, and virgin plastics had higher emissions of hydrocarbon gases than environmentally aged plastic pellets. This suggests that plastic pollution may be contributing to climate change. There is also evidence to suggest that climate change could contribute to increased wildlife exposure to plastic pollution. For example, Drever et al. (2018) reported that, under unusually warm ocean temperatures, red phalaropes (*Phalaropus fulicarius*) were found feeding closer to shore. The

authors indicated that distribution shifts of the birds closer to shore resulted in increased exposure to plastic pollution.

In addition to the uncertainties inherent in microplastic toxicity testing described above, there is a need to conduct toxicity tests on nanoplastics; however, these may be also confounded by the suspension matrix used (Pikuda et al. 2019). Toxicity results for studies using commercially formulated nanoparticles, which are likely to contain preservatives, antimicrobials, or surfactants, must therefore be carefully considered (Pikuda et al. 2019).

9.3 Human health effects

In order to better understand the potential human health effects of microplastics resulting from both oral and inhalation exposures, an improved understanding of the extent and nature of human exposure and potential toxicological hazards is required.

With respect to the potential human health impacts of microplastic ingestion (e.g., from drinking water and/or food) and inhalation (e.g., from indoor and ambient air), more research is needed on the uptake and fate of microplastics in the GI and respiratory tracts and on the bioavailability of chemical substances associated with microplastics. In addition, from an inhalation perspective, there is a need to better characterize microplastics exposure for particles of aerodynamic diameter in the micron scale (<1 mm), with a focus on inhalable particles (<10 μm) and especially respirable particles (<2.5 μm) that can penetrate deep into the lungs. There is also a need to understand the physical characteristics of microplastics (e.g., length, diameter, polymer type, surface chemistry) that may determine their bioavailability, tissue distribution, and potential relevance to human health.

Toxicological research using appropriate cell models and experimental animals that would be needed to identify target tissues and threshold dose and to inform hazards for human health risk assessment is generally lacking. More research is also required to improve the understanding of whether the characteristics (e.g., size, shape, composition) of microplastics influence their potential adverse effects. In addition, as information emerges regarding the health-relevant properties of microplastics, standardized reporting metrics are needed to ensure that those features are adequately characterized in scientific reports.

There is also a need to understand the extent to which microplastics may act as a vector for transporting other chemicals (e.g., chemicals additives, adsorbed environmental contaminants) and to determine whether they have an impact on human health. While recent reviews indicate that there is a low health concern for human exposure to chemicals from ingestion of microplastics from food or drinking water (EFSA 2016; FAO 2017; WHO 2019), further research would be required before a human health risk assessment on microplastics is possible. Further research investigating the toxicity of nanoplastics is also required, as described above.

Lastly, there is also a need for improved characterization of microplastic-associated biofilms in drinking water, drinking water sources and air. Gaining increased knowledge in such areas as the factors shaping

biofilm composition, the taxonomy of biofilm communities, and biofilm activity and interactions (e.g., transfer of ARGs) would contribute to the understanding of the importance of biofilms on human health.

10. Findings

Plastic pollution, in the form of macroplastics and microplastics, is ubiquitous in the environment. It is estimated that, in 2016, 1% of all plastic waste in Canada, or 29 kt, was discharged to the environment as litter. Since plastics degrade very slowly and are persistent in the environment, the frequency of occurrence of plastic pollution in the environment is expected to increase.

Macroplastics have been demonstrated to cause physical harm to environmental receptors on an individual level and to have the potential to adversely affect habitat integrity. Organisms have been shown to ingest macroplastics and to become entangled in macroplastics, which can result in direct harm and in many cases, mortality.

The evidence for potential effects of microplastic pollution on environmental receptors is less clear and sometimes contradictory, and further research is required. For example, although there are reports indicating that exposure of environmental receptors to microplastics can lead to mortality, developmental and reproductive effects, effects on feeding and energy production, and biochemical or molecular-level effects, a similar number of reports have found no effects.

The current literature on the human health effects of microplastics is limited. Potential exposure pathways include air, water and food. While some occupational epidemiology and experimental animal studies show the potential for effects at high exposure concentrations, they are of questionable reliability and relevance, and further research on the potential for microplastics to impact human health is required.

Given the increasing amounts of plastic pollution in the environment and the demonstrated ability of macroplastics to harm environmental receptors, it is anticipated that the frequency of occurrence of physical effects on individual environmental receptors will continue to increase if current trends continue.

As discussed in this report, there are a multitude of sources (see Section 3) that contribute to plastic pollution. In keeping with the precautionary principle, action is needed to reduce macroplastics and microplastics that end up in the environment.

In order to advance the understanding of the impacts of plastic pollution on the environment and human health, it is recommended that research be conducted to address key knowledge gaps identified in this report. This includes studies to improve the understanding of both exposure to and potential toxicity of plastics. More specifically, research is recommended in the following areas:

- Developing standardized methods for sampling, quantifying, characterizing and evaluating the effects of macroplastics and microplastics;
- Furthering understanding of human exposure to microplastics;

- Furthering understanding of the ecotoxicological effects of microplastics;
- Furthering understanding of the effects of microplastics on human health; and
- Expanding and developing consistent monitoring efforts to include lesser characterized environmental compartments such as soil.

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Appendix A: Additional occurrence of plastics in the global environment

A-1. Shoreline

Plastic pollution has been detected on shorelines around the world. For example, one study found macroplastics on every beach surveyed on an island in French Polynesia, where plastics accounted for 20% to 100% of all litter items (Connors 2017).

Chen et al. (2019) collected marine litter around a tourist city in East China and found that plastic pollutants made up the majority of all floating, benthic, and beached litter. Grocery bags were the most commonly found litter item in all three areas. On shorelines, foams from fishing and aquaculture were found at similar concentrations as grocery bags. The average density of floating microplastics was 36 456 items/km^2.

In a study by Horn et al. (2019), microplastics were found on all 51 Californian beaches sampled. The average microplastic count was 11.8 items per 100 mL of sediment. Fibres accounted for 95% of the microplastic items. The polymers identified were PP, isotactic PP, atactic PP, polyacrylate, PE, and polyester.

Ryan et al. (2018) collected litter items sized 2 to 25 mm at South African beaches and reported that plastics comprised 99% of all litter items by number, and that industrial pellets (which form the feedstock of the plastics industry) were the most abundant type of plastic. Typically, pellets enter the environment via accidental spills on land or at sea. Corcoran et al. (2015) found that weather conditions are a factor in industrial pellet accumulation, as is the presence of beached organic material in which they may become entrapped.

Zhou et al. (2018) studied the occurrence of microplastics on beaches adjacent to China's Bohai Sea and Yellow Sea. Microplastics were both visually identified and analyzed using FTIR, which determined that PE and PP made up the majority of samples. Flakes were the most abundant microplastic, followed by foams, fragments, fibres, pellets, films, and sponges. The abundance of microplastics between sampling sites varied significantly, ranging from 1.3 to 14 712.5 particles/kg dry weight (dw), with an overall average of 740 particles/kg. Similarly, Karthik et al. (2018) studied the occurrence of microplastics on beaches along the southeast coast of India. Microplastic particle concentration along the coast ranged from 2 to 178 particles/m^2, with a mean of 46.6 particles/m^2. FTIR analysis identified PE, PP, and PS as the main components of identified plastics.

Plastics, both macro and micro, are widely found in the Arctic, despite its distance from industrialized and highly populated areas. Plastics have been found in all abiotic environments of the European Arctic, and monitoring of beach litter in the Atlantic Arctic in 2017 revealed that the amount beach litter varied from a mean of 1 475 items per 100 m in the spring to 195 items per 100 m in the summer months (PAME 2019).

A-2. Surface water

Plastic waste is found in fresh and marine surface waters worldwide, and extensive research has been done on the occurrence of microplastics in marine surface waters. A brief summary of selected papers is presented below.

In the United States, Mason et al. (2016) collected surface water samples from Lake Michigan and found an abundance of microplastic particles ranging from about 1 400 to 100 000 particles/km^2 (mean of 17 267 particles/km^2), with 59% of the particles in the size range of 0.355 to 0.999 mm. Microplastic abundances were fairly evenly distributed across the lake surface despite a seasonal gyre that developed in the southern end of the lake. Fragments dominated, followed by fibres and line, and the most common type of microplastics was PE, followed by PP. A study by Wang et al. (2018) investigated microplastics in freshwater in China. Concentrations in Dongting Lake and Hong Lake ranged from 900 to 4 659 particles/m^3, and the concentrations were much higher in the outlet channel between Dongting Lake and the Yangtze River, an area with heavy shipping traffic. The microplastics were mainly PE and PP, and the majority were fibres. Additionally, more than 65% of all microplastics were smaller than 2 mm (Wang et al. 2018).

Surface water samples were collected along the Rhine River in Europe, and microplastics were found in all samples with an average concentration of 892 777 particles/km^2. A peak concentration of 3.9 million particles/km^2 was measured in a single sample collected at Rees, in Germany, supporting the finding that higher microplastic concentrations are found near densely populated areas. Most of the microplastics recovered were spheres, followed by fragments (Mani et al. 2015).

Macroplastics have been observed floating on the Arctic sea surface, and microplastics have been found in Arctic Ocean surface waters and in the water column. Of the microplastics observed in surface and subsurface waters (to a depth of 6 m), 95% were fibres (Hallanger and Gabrielsen 2018). Plastics may also become entrapped in sea ice, and microplastics levels ranging from 38 to 234 particles/m^3 of ice have been measured (Obbard et al. 2014). More recently, Peeken et al. (2018) measured microplastic abundances in Arctic sea ice ranging from 1.1×10^6 particles/m^3 to 1.2×10^7 particles/m^3, with highly variable concentrations. Most of these microplastics were smaller than 50 µm in size. Of the 17 polymers identified, PE was the most common, with a mean of 48%.

In the Adriatic Sea, Zeri et al. (2018) found significantly higher macroplastic abundance in offshore waters (>4 km) than in inshore waters, but higher abundances of microplastics in nearshore waters (≤4 km) than in offshore waters. The authors collected 22 245 particles of floating microplastics from surface waters, and visually identified 658 floating macroplastics, which accounted for 91.4% of litter items recorded. They found that 29% of the macroplastics was plastic bags, 22% was plastic pieces, 15% was sheets, 13% was fish boxes of expanded PS, 8.8% was cover/packaging, 4.3% was PS pieces, and 1.4% was plastic bottles.

Floating litter collected in Vietnam consisted of a mean of 26% plastics by weight. Of the total plastic mass, 37% was plastic bags, 14% plastic packaging (single-use food containers), and 48% was other plastics, such as plastic bottles, food wrappers, cups, and cutlery (Lahens et al. 2018).

The occurrence and aggregation potential of microplastics in the Mediterranean Sea has been reported by several researchers. For example, de Haan et al. (2019) collected surface water samples using 335 µm mesh nets, which yielded 2 489 plastic particles. Microplastics made up 94.6% of plastic abundance and 55% of plastics by weight, averaging 0.10 items/m^2. The three most abundant polymers were LDPE and HDPE (54.5%), PP (16.5%) and PS (9.7%) (de Haan et al. 2019).

Bordós et al. (2019) examined microplastic occurrence in Hungary. Given the use of a 2 mm pre-filter during sampling, microplastics between 2 mm and 5 mm were not sampled. Suspected plastics particles were visually identified and analyzed under a FTIR microscope and six polymer types were identified: PE, PP, PS, polytetrafluoroethylene, polyacrylate, and polyester. Of the 13 water samples taken, 12 contained microplastics ranging from 3.52 to 32.1 particles/m^3 with an average of 13.8 particles/m^3. All water entering sampling locations (i.e., influents) had higher microplastic concentrations than the water leaving that sampling location (i.e., effluents).

Pan et al. (2019) reported microplastics in surface waters across the Northwestern Pacific Ocean. The concentration of particles collected from 18 stations varied significantly, ranging from 6.4 x 10^2 to 4.2 x 10^4 items/km^2. Microplastics were analyzed by Micro-Raman spectroscopy, yielding a distribution of 57.8% PE, 36.0% PP, and 3.4% nylon.

Poulain et al. (2019) investigated the concentration of microplastics in the North Atlantic Subtropical Gyre. Microplastics were categorized as small microplastics (SMPs, 0.025 to 1 mm) and large microplastics (LPMs, 1 to 5 mm). SMPs were collected by a 25 µm mesh net, and LMPs by a 300 µm mesh net. The authors accounted for the decreased buoyancy of SMPs compared to LMPs and applied a correction factor for the increased susceptibility of microplastics to wind-driven vertical transport. The concentrations of LMPs and SMPs corrected for vertical transport are 50 to 1000 g/km^2 and 5 to 14 000 g/km^2, respectively.

Eriksen et al. (2014) conducted 680 net tows of global surface water and found plastics in 92.3% of the tows. Visual surveys in the South Pacific, North Pacific, South Atlantic, Indian Ocean, and water around Australia also indicated that foamed PS items were the most frequently observed macroplastics. The authors estimated that there are 5.25 trillion particles of plastic floating at sea, totalling to 268 940 tonnes. Their results indicate that plastic pollution has spread throughout the world's oceans and that plastics accumulate in subtropical gyres. There is an area with accumulation of buoyant plastics in the North Pacific Subtropical Gyre that is commonly referred to as the Great Pacific Garbage Patch (Eriksen et al. 2014). Lebreton et al. (2018) predicted that a 1.6 million km^2 zone of the Great Pacific Garbage Patch contains 1.8 trillion pieces of plastics and weighs 79 000 tonnes. The average plastic mass concentration measured inside the Great Pacific Garbage Patch has shown exponential increase over the last decades, from 0.4 kg/km^2 (n = 20) in the 1970s to 1.23 kg/km^2 (n = 288) in 2015 (Lebreton et al. 2018).

A-3. Benthic zone

Plastic waste has also been detected in marine sediments around the world and is typically dominated by microplastics. Dai et al. (2018) reported the occurrence of microplastics in the surface water, water column and sediment of the Bohai Sea of the Pacific Ocean. Microplastics were detected in all 20 surface water samples, ranging from 0.4 to 5.2 particles/L, with an average of 2.2 particles/L. The average concentration of microplastics in the water column ranged from 1.6 to 6.9 particles/L. There was no clear trend in microplastic accumulation at any specific depth along the water column, and the abundance in sediments was inconsistent with the water column. The surface sediment concentration ranged from 31.1 to 256.3 particles/kg. Fibres dominated the type of microplastics found in both water and sediment, followed by fragments. μ-FTIR analysis identified that the polymer with the highest density in surface waters was PS, whereas PET and PVC were found at highest densities in deeper water.

In Argentina, an average of 25 macroplastic items/m^2 and 704 microplastic fragments/m^2 were collected from sediment. The macroplastic pollutants were categorized into 24 types, and the most dominant types were food wrappers (PP and PS), bags (HDPE and LDPE), bottles (PET), and disposable Styrofoam food containers (PS) (Blettler et al. 2017). In a study in the United Kingdom, the main types of macroplastic waste found in sediment were packaging, fishing and shipping waste (Browne et al. 2010). Macroplastics and microplastics were found in sediments from a marine protected area in Italy, ranging from a mean of 11.9 to 46.4 pieces and 151.0 to 678.7 pieces per kg dw of sediment, respectively. Greater than 85% of the microplastics were fibres (Fastelli et al. 2016). Bordós et al. (2019) sampled sediment in Hungary and found that 9 of the 12 sediment samples contained microplastics ranging from 0.46 to 1.62 particles/kg, with an average of 0.81 particles/kg. The most dominant polymer was PP. Marine litter in Croatia ranged from 3.4 items/kg dw to 528 items/kg dw, with macroplastics making up 1.3% to 11.3% of samples. Like in Italy, fibres were the most abundant type of microplastic found in Croatia, ranging from 39.9% to 90.1% of the total number of plastic items (Renzi et al. 2019; Blašković et al. 2017). Blašković et al. (2017) found no correlation between the extent and pattern of plastic contamination and sediment grain size or sampling depth. In Svalbard in the Arctic, fibres were once again found to be the dominant microplastic in sediment at depths of 40 to 79 m, where they were sampled at a density of 9.2 fibres/kg (Sundet et al. 2016).

Vidyasakar et al. (2018) conducted the first study on the distribution and characteristics of plastic pollutants in marine sediment on Rameswaram Island, along the southeast coast of India. PP was the most abundant polymer type, followed by PE, PS, nylon, and PVC. Irregularly shaped plastics were most plentiful at 69.2%, followed by fibres at 17.9% and pellet-shaped plastics at 12.9%.

Microplastics have been found in large quantities in river sediment in Shanghai (Peng et al. 2018), at concentrations ranging from 5.3 particles/100 g dw to 160 particles/100 g dw. The average concentration across all sites was 80.2 particles/100 g dw. Residential areas showed the highest level of microplastic concentration, followed by parks, rural areas, and tourist areas. Spheres constituted the majority of microplastics at 88.98%, followed by fibres (7.55%) and fragments (3.47%). The two most dominant polymer types identified by μ-FTIR were PP and polyesters (Peng et al. 2018).

García-Rivera et al. (2018) derived data from the MEDITS (International Bottom Trawl Survey in the Mediterranean) program surveys and found that, over 11 years, 2197.8 kg of marine litter was collected from the Spanish Mediterranean seafloor (collected 5 stratum levels at depths from 0 to 800 m) and was comprised of 29.3% plastics by weight. They reported that the amount of marine litter generally remained stable over the survey period. Deep sea litter in the Arctic reportedly increased from 346 items/km^2 in 2004 to 8082 items/km^2 in 2014, with plastics accounting for 47% of litter (PAME 2019). Recently, an American explorer found a plastic bag and candy wrappers on the seafloor of the Pacific Ocean's Mariana Trench at a depth of nearly 11 km, the deepest submarine dive to date (Morelle 2019).

Appendix B: Additional information on occurrence of microplastics in food

Table B-1: Summary of the occurrence data for microplastics (MPs) in food

Food Item	Concentration	Size (µm)	Shape	Reference(s)
Fish	0 to 20 MPs/fish (gastrointestinal tract)	130 to 5 000	Predominantly fragments and fibres	Barboza et al. 2018; Campbell et al. 2017; EFSA 2016; FAO 2017; Hantoro et al. 2019; Liboiron et al. 2018, 2019; Lusher et al. 2013; Slootmaekers et al. 2019; Toussaint et al. 2019
Fish	0 to 4.6 MPs/fish (muscle); 0.57 to 1.85 MPs/g fish (muscle)	100 to 5 000 (fibres) 100 to 500 (fragments)	Predominantly fragments and fibres	Abbasi et al. 2018; Akhbarizadeh et al. 2018; Karami et al. 2017a
Molluscs	0 to 10 MPs/individual mussel;[a] 0.2 to 2.9 MPs/g mussel	5 to 4 700	Predominantly fragments and fibres	Catarino et al. 2018; De Witte et al. 2014; Li et al. 2015, 2018a; Naji et al. 2018; Patterson et al. 2019; Su et al. 2018; Toussaint et al. 2019; Van Cauwenberghe and Janssen 2014; Van Cauwenberghe et al. 2015
Crustaceans	1.23 MPs/individual whole shrimp;[b] 0.68 MPs/g whole shrimp wet weight	200 to 1 000	Predominantly fibres	Devriese et al. 2015
Crustaceans	7.8 MPs/individual prawn (muscle tissue and exoskeleton)	100 to 250	Predominantly filamentous fragments	Abbasi et al. 2018
Crustaceans	0.80 mg of MPs/individual lobster (gastrointestinal tract)	Not reported	Predominantly fibres	Murray and Cowie 2011; Welden and Cowie 2016
Salt[c]	0 to 19 800 MPs/kg sea salt	4 to 5 000	Fragments and fibres were most abundant shape for all salt types	Gündoğdu 2018; Iñiguez et al. 2017; Karami et al. 2017b; Kim et al. 2018; Lee et al. 2019; Peixoto et al. 2019; Renzi and Blašković 2018; Seth and Shriwastav 2018; Yang et al. 2015

[a] Mussels are the most frequently investigated species of mollusc. Similar concentrations of microplastics have been reported in clams, oysters, scallops, and snails.

[b] Microplastics were only observed in the digestive tract, head, and gills of the whole shrimp and not the abdominal muscle tissue of peeled shrimp.

[c] Microplastic concentrations in salt varied considerably depending on the origin and type of salt.

Table B-2: Summary of the occurrence data for microplastics in bottled water

Reference	Type of Bottle	Concentration (microplastics/L)	Size (µm)	Shape	Location
Mason et al. 2018	Plastic (not specified if single or multi-use)	10.4	>100	Predominantly fragments	Multiple locations worldwide (Brazil, China, France, Germany, India, Indonesia, Italy, Lebanon, Mexico, United Kingdom, United States of America)
Oßmann et al. 2018	Single-use PET plastic	2 649 ± 2 857	≥1	Not reported	Germany
	Multi-use PET plastic (newer bottles)	2 689 ± 4 371			
	Multi-use PET plastic (older bottles)	8 339 ± 7 043			
	Glass	6 292 ± 10 521			
Schymanski et al. 2018	Single-use PET plastic	14 ± 14	≥5	Not reported	Germany
	Multi-use PET plastic	118 ± 88			
	Cardboard	11 ± 8			
	Glass	50 ± 52			
Szeto et al. 2018	Plastic (not specified if single or multi-use)	10.2	>100	Not reported	Canada

Appendix C: Additional information on ecotoxicological studies

Table C-1: Aquatic: freshwater

Organism and Duration of Exposure	Microplastic Type and Concentration	Summary of Effects	Source
Daphnia magna (Water flea) For uptake experiments, exposure was 15, 30, 60, 120 and 240 minutes For depuration experiments, exposure was 1 hour For differential food regime experiments, exposure was 1 hour For chronic toxicity tests, exposure was 21 days	Yellow-green fluorescent, carboxylate-modified PS (2 µm) were used for uptake and depuration experiments Non-fluorescent PS microplastics (2 µm) were used for toxicity tests For uptake and depuration experiments, microplastic concentration was 1.46×10^{2} mg/L and algae concentration was 1.00×10^{-1} mg/L For differential food regime experiments, microplastic concentrations were 6.93×10^{-4}, 1.39×10^{-3}, 2.77×10^{-3}, 5.54×10^{-3}, 8.31×10^{-3}, and 1.11×10^{-2} mg/L; algae concentrations were 5.00×10^{-2}, 1.00×10^{-1}, 2.00×10^{-1}, 4.00×10^{-1}, 6.00×10^{-1}, 8.00×10^{-1} mg/L Control groups for uptake, depuration, and differential food regime experiments were not exposed to algae For chronic toxicity tests, microplastic concentrations were 1.39	Uptake and depuration tests of microplastics indicate that *D. magna* fed both microplastics and algae consumed a significantly lower amount of microplastics than *D. magna* that only ate microplastics. Using differential food regime experiments, it was also found that this effect could also be seen when using low concentrations of algae and that increasing algal concentrations led to decreasing microplastic uptakes. In adult *D. magna*, mortality was seen in all treatment groups compared to the control following 7 days of exposure. When using a low algal concentration (1.00×10^{-1} mg/L) with a relatively higher microplastic concentration (1.11×10^{-2} mg/L), the LT_{50} was 10.09 ± 0.70%, which is slightly lower than the control at the same algal concentration. No impact on reproduction was seen. In neonate *D. magna*, mortality in those fed a low algal concentration (1.00×10^{1} mg/L) and microplastics was significantly higher than neonates fed only algae. No effect on mortality was found for a high algal concentration (8.00×10^{-1} mg/L) and microplastic uptake. There were no significant differences in reproduction between identical food regimes with and without microplastics.	Aljaibachi and Callaghan 2018

	× 10^{-3} mg/L (low) and 1.11 × 10^{-2} mg/L (high); algae concentrations were 1.00 × 10^{-1} mg/L (low) and 8.00 × 10^{-1} mg/L Control group for chronic toxicity tests was not exposed to microplastic		
Xenopus laevis (African clawed frog) tadpoles Developmental stages 36 to 46	Blue PS microplastics (2.75 ± 0.09 µm) at 0.125 µg/mL, 1.25 µg/mL, and 12.5 µg/mL (nominal) Control group was not exposed to microplastic	Microplastics were found in the tadpoles' digestive tract from each tested concentration; however, SEM analyses suggest no mechanical damage in the epithelium walls as a result. Microplastics were not found in the gills. No significant effects on mortality, body growth, or swimming activity (swimming speed or distance moved) during early life stages were seen.	De Felice et al. 2018
Carassius auratus (Goldfish) 6 weeks	Ethylene vinyl acetate fibres (0.7–5.0 mm), PS fragments (2.5–3.0 mm), and polyethylene acrylate pellets (4.9–5.0 mm) Fish were fed concentrations of 1.36%, 1.94%, and 3.81% (g (food+microplastics)/g ww[b] fish) for the fibres, fragments, and pellets, respectively Control group was given food pellets that contained no microplastic	Various sublethal effects, but no mortality, were observed. Fish exposed to plastic fibres, fragments, and pellets showed significant weight loss compared to the control group. Fragments and pellets were chewed and expelled by fish. The highest occurrence of changes in the upper (27.0%) and lower (30.4%) jaws were seen in the fragment group, followed by the fish exposed to pellets. Damage to the buccal cavity was seen in 80.0% of fish that chewed plastic fragments. This damage ranged from slight exfoliation to deep incisions. In addition, 13.1% of fish exposed to fragments showed sinusoid dilation in their livers.	Jabeen et al. 2018

		Fibres were found in the gills, gastrointestinal (GI) tract, and feces, but were not likely to accumulate in the GI tract. The frequency of pronounced changes in the upper jaw was the highest in fish exposed to fibres. Additionally, this group showed pronounced and severe damage in their livers. The highest organ index values for the upper jaw, liver, and intestines of fish were also seen in those exposed to fibres. The distal intestine displayed more pronounced and severe changes in comparison to the proximal intestine, which could also be a result of fibre ingestion.	
Daphnia magna *Daphnia pulex* *Ceriodaphnia dubia* (Water fleas) 96 hours	Green fluorescent plastic microspheres (1–5 µm) were used as primary microplastic models Irregularly-shaped PE microplastics (approx. 1–10 µm) were used as secondary microplastic models Concentrations were 10^3, 10^4, 10^5, 10^6, 10^7 particles/mL Control group was not exposed to microplastic	Using no-effect concentration estimates and three different temperatures (18°C, 22°C, 26°C), the sensitivity of *D. magna* and *D. pulex* to primary and secondary microplastics was found to drastically increase with temperature. This effect was not seen in *C. dubia*. At the lowest tested temperature (18°C), *C. dubia* was the most sensitive species. At the highest temperature (26°C), *D. magna* and *D. pulex* were more sensitive. Primary microplastics were found to be more toxic than secondary microplastics in *C. dubia*. For all species, survival was time-dependent as seen in LC_{50} estimates compared at 48 hours and 96 hours. In *D. magna*, for example, the 48-hour LC_{50} was 32.0 particles/mL, whereas the 96-hour LC_{50} was 18.0 particles/mL at 18°C.	Jaikumar et al. 2018
Danio rerio (Zebrafish)	Virgin PA, PE, PP, and PVC particles (mean diameter of about 70 µm)	In *D. rerio*, there were no significant differences in lethality following 0.001–10.0 mg/L microplastic	Lei et al. 2018b

10 days	Two kinds of PS (nominal sizes of 1.0 µm and 5.0 µm) particles were used: virgin PS for the toxicity test and red-fluorescently-labelled PS to examine microplastic distribution in *C. elegans* For *D. rerio*, concentrations of 0.001 mg/L, 0.01 mg/L, 0.1 mg/L, 1.0 mg/L and 10.0 mg/L were used For *D. rerio*, dechlorinated tap water was used for the control group	exposure. In the surviving fish, PA, PE, PP, and PVC particles caused intestinal damage (including cracking of villi and splitting of enterocytes) in 73.3% to 86.7% individuals.	
Chlorella pyrenoidosa (Green algae) 30 days (comprised of three growth periods: lag phase, logarithmic phase, and stationary phase)	PS microbeads (1.0 µm) at 10 mg/L, 50 mg/L, and 100 mg/L in algal cultures Control group was pre-cultured *C. pyrenoidosa* in the logarithmic growth phase added into BG-11 medium without microplastic	1.0 µm PS caused a dose-dependent decrease in *C. pyrenoidosa* growth from the lag to early logarithmic phases (day 0 to 22). At 10, 50 and 100 mg/L PS, there was a growth inhibition ratio of 20.9%, 28.4% and 38.1%, respectively. From the lag to early logarithmic phases, microplastics (100 mg/L) had a negative effect on photosynthesis. However, the end of the stationary phase onwards showed a stimulation of photosynthesis that was also dose-dependent. In the presence of microplastics, distorted thylakoids and cell wall thickening were also observed. Following 25 days of exposure, cell morphology mostly recovered.	Mao et al. 2018
Daphnia magna (Water flea) 21 day exposure	Red fluorescent microspheres (1–5 µm) at 0.1 mg/L	Chronic exposure of *D. magna* to microplastics caused parental mortality (10% to 100%) and a significant decrease in growth, reproduction (total offspring and	Martins and Guilhermino 2018

4 sequential generations	Control group was exposed to a clean test medium	mobile juveniles), and population growth rate. In two treatment groups, microplastic-exposed populations were extinct in the F_1 (2^{nd}) generation. Juveniles produced by microplastic-exposed females were immobile. Some recovery was visible in the F_1 population, such as an increase in production of mobile juveniles and earlier first brood release. However, females descending from the exposed population in F_0 (called the recovery model population) still experienced a significant reduction in growth, reproduction, and population growth rate up to the F_3 generation, in comparison to controls. These findings demonstrate that full recovery from developmental and reproductive effects may take several generations.	
Daphnia magna (Water flea) 14 and 21 days	Fluorescent red microspheres (1–5 µm) at 0.02 mg/L and 0.2 mg/L (nominal) Control group was exposed to hard water without microplastic	When exposing *D. magna* for 14 days to microplastics, there was a significant reduction in the number of total offspring and a higher frequency of immobile juveniles. No effects on parental female mortality were seen. When exposing *D. magna* for 21 days to microplastics, there was a dose-dependent effect on mortality. At 0.02 mg/L, microplastics induced 10% of mortality. However, at 0.2 mg/L, microplastics induced 30% of mortality. There were no significant effects on growth. In the 21-day exposure treatment, microplastics also reduced the reproductive fitness of *D. magna*. Exposure increased the time of first	Pacheco et al. 2018

		brood release (49%) and reduced the total number of broods released by 71%. Similar to the 14-day treatment, there was also a decrease in the number of offspring and induction of immobile juveniles. There was no effect on the number of aborted eggs in both exposure regimes.	
Danio rerio (Zebrafish) For distribution experiments, exposure times were 20 hours (4–24 hpf[c]) and 92 hours (4–96 hpf) For uptake and qPCR[d] experiments, exposure was 92 hours (4–96 hpf) For developmental effects experiments, exposure was 68 hours (4–72 hpf) For free swimming and light-to-dark experiments, exposure was 116 hours (4–120 hpf)	Green fluorescent PS microplastics (1 µm) For distribution, developmental effects, free swimming, light-to-dark, and qPCR analysis, concentrations used were 100 µg/L and 1 000 µg/L For uptake experiments, concentrations used were 10, 100 and 1 000 µg/L Control group was exposed to embryo medium without microplastic	Microplastics were found to adhere to the embryo chorion and its distribution increased with increasing PS concentration. Microplastic uptake also increased with increasing exposure concentrations. Hatching rate was slightly reduced with exposure; however, this result was not significant. Development speed (in terms of body length and yolk sac area) of larvae was also not impacted significantly from 4–72 hpf. Larvae did not display any obvious malformations. In the free swimming test, exposure to 1 000 µg/L microplastics led to a significant decrease in both swimming distance and larvae speed in dark conditions by 3.2% and 3.5%, respectively. Using an alternating light-to-dark photoperiod stimulation, a significant reduction in swimming competence was also seen in dark conditions. At 1 000 µg/L exposure, total swimming distance was reduced by 2.6% and swimming speed was 2.8% lower in comparison to the control. No significant differences were found when exposed to light conditions. In the 1 000 µg/L exposure group, *il1b* and *cat* expression were upregulated to 165% and 121%,	Qiang and Cheng 2019

		respectively. No significant changes were seen in *sod* expression.	
Danio rerio (Zebrafish) 21 days	PS microbeads (5 µm) at 50 µg/L and 500 µg/L Control group was exposed to culture water without microplastic	Significant intestinal damage was observed in 78% and 86% of the histological sections sampled for the 50 µg/L and 500 µg/L treatment groups, respectively. Microplastic exposure was found to induce intestinal oxidative stress and increased permeability. In addition, there was significant alterations in the intestinal metabolic profiles and gut microbiome.	Qiao et al. 2019
Daphnia magna (Water flea) 10 days	Uncoated PS particles (1.25 µm) at 2 mg/L, 4 mg/L, and 8 mg/L Control group was not exposed to microplastic	No mortality occurred in all treatments. Reduction in body growth rate, an indicator of population fitness, was also seen with microplastic exposure. Following PS exposure, transcript level of TRxR in *D. magna* (vital in mediating oxidative defence) significantly increased (2.5–5-fold) with PS concentrations of 2 and 4 mg/L. Transcript level declined at 8 mg/L, but was still significantly higher in comparison to the control group. Arginine kinase (vital in cellular energy production and ATP buffering) transcript level was significantly elevated in the presence of PS (approx. 5-fold at 2 mg/L). Transcript level of permease (facilitates removal of cytotoxic compounds from cells) increased 1.4–1.8 fold when exposed to 2 and 4 mg/L PS. Exposure to 8 mg/L lowered transcription compared to the control.	Tang et al. 2019
Gammarus pulex (Amphipod)	Irregular particles (10–150 µm) were prepared from green fluorescent	In the uptake experiment, no mortality was found. In addition, body burden was found to be dependent on dose and age. Body	Weber et al. 2018

In the uptake study, exposure was 24 hours In the chronic exposure study, exposure was 48 days	soft drink bottles made from PET In the uptake study, concentrations used were 0.8 particles/mL, 40 particles/mL, and 4000 plastics/mL In the chronic exposure study, concentration used were 0.8 particles/mL, 7 particles/mL, 40 particles/mL, 400 particles/mL and 4000 particles/mL Negative control group was exposed only to ISO medium; solvent control group was exposed to ISO medium with 10% cetyl alcohol	burden was significantly higher in juveniles in comparison to adults for the 0.8 particles/mL and 4000 particles/mL treatments. No significant difference was seen at 40 particles/mL. Furthermore, a higher dosage of microplastics was associated with a significantly higher body burden in both juveniles and adults. In the chronic exposure study, no significant effects were seen on feeding activity, energy reserves and molt periods. Mortality rates also did not vary in juveniles; however, mortality was significantly increased in adults for the 7 particles/mL and 400 particles/mL treatments compared to the control.	
Eriocheir sinensis (Chinese mitten crab) For uptake experiments, exposure was 7 days For toxicity tests, exposure was 21 days	Two kinds of PS microspheres (5 µm) were used: fluorescent microspheres for uptake and accumulation experiments, and virgin microspheres for toxicity tests For uptake experiments, a concentration of 40 000 µg/L was used For toxicity tests, nominal concentrations were 40 µg/L (5.4×10^2 particles/mL), 400 µg/L (5.4×10^3 particles/mL), 4 000 µg/L (5.4×10^4 particles/mL) and 40 000 µg/L (5.4×10^5 particles/mL)	No significant differences in survival were seen with microplastic exposure. Weight gain, specific growth rate, and hepatosomatic index generally decreased with increasing microplastic concentration, with the exception of specific growth rate in the 40 µg/L group. In the uptake experiments, microplastics (40 000 µg/L) accumulated in the gills, liver and guts of *E. sinensis*. Acetylcholinesterase, alanine aminotransferase, and catalase activities in all treatment groups were significantly lower than seen in the control. The activities of superoxide dismutase, aspartate transaminase, GSH[e], and GPx[f] increased in crabs exposed to 40 and/or 400 µg/L	Yu et al. 2018

	Control group was not exposed to microplastic	microplastics. However, there was a general decrease in activity with high exposure (4 000 and 40 000 µg/L). Genes encoding the antioxidants SOD[g], catalase, GPx, and GST[h] in the liver initially increased and then decreased in expression following exposure. Further, there was an increased expression of the gene encoding p38 in the MAPK[i] signaling pathway with treatment of 4 000 µg/L and 40 000 µg/L microplastics, but significant reductions in the expression of ERK[j], AKT[k], and MEK[l]. No significant differences in transcription were found with the gene encoding c-Jun N-terminal kinase. These results show that microplastic exposure can induce oxidative stress the liver of *E. sinensis*.	

[a] Median lethal time
[b] Wet weight
[c] Hours post fertilization
[d] Quantitative polymerase chain reaction
[e] Glutathione
[f] Glutathione peroxidase
[g] Superoxide dismutase
[h] Glutathione-S-transferase
[l] Mitogen-activated protein kinase
[j] Extracellular signal-regulated kinase
[k] Protein kinase B
[l] Mitogen-activated protein kinase (MAPK) kinase

Table C-2: Aquatic: marine

Organism and Duration of Exposure	Microplastic Type and Concentration	Summary of Effects	Source
Oncorhynchus mykiss (Rainbow trout) 4 weeks	Colourless PS particles (100–400 µm) at approx. 500– 700 particles/day/fish Control group was not exposed to microplastic	Using histological analysis, no significant effects were seen on the abundance of mucus-secreting goblet cells in the proximal and distal segments of the trout intestine. In addition, there were no adverse changes in tissue morphology, paracellular permeability, and intestinal transporting functions (^{3}H-lysine transport, ion transport capacity, and net ion flow) in the intestines following exposure. PS microplastics did not induce pro-inflammatory or anti-inflammatory responses in the distal and proximal segments of the intestines.	Ašmonaitė et al. 2018
Brachionus plicatilis (Rotifer) 48 hours *Tigriopus fulvus* (Crustacean) 48 hours *Acartia clausi* (Marine copepod) 48 hours *Mytilus galloprovincialis* (Mussel) 48 hours	Non fluorescent LDPE microplastics (1–500 µm) Fluorescent green and red PE microplastics were used to examine particle ingestion in rotifers, copepod, and mussel larvae (nominal size of 1–5 µm) Virgin microplastic loads tested varied with each organism and consisted of 0, 0.01, 0.1, 1, 3, 10, 20, 30, 50, 100 mg/L Control group was exposed to 0.22 µm-filtered seawater without microplastic	Virgin microplastics had no significant effect on mussel embryonic development at any concentration under static conditions or in a rotary wheel. However, orbital shaking at 200 rpm significantly reduced the percentage of D-veliger larvae following exposure. Virgin microplastics did not cause any significant effect at any concentrations below 30 mg/L in any of the species tested. Exceptions of this were for the 1–4 µm particles, which produced a LOEC of 0.01 mg/L for *B. plicatilis* immobility, LOEC of 1 mg/L for *B. plicatilis* mortality (LC_{50} >10 mg/L), and a LOEC of 1 mg/L for *T. fulvus*	Beiras et al. 2018

Paracentrotus lividus (Sea urchin) 48 hours *Oryzias melastigma* (Fish) 1–13 days post fertilization		mortality (LC_{50} = about 1.82 mg/L).	
Lophelia pertusa (Cold-water coral) For capture rate and polyp activity experiments, exposure was 7, 20, or 47 days For coral growth rate experiments, exposure was 69 days	LDPE microbeads (500 µm) at 350 beads/L Control group was not exposed to microplastic; control measurements were done in flumes containing no corals to quantify zooplankton sedimentation for the prey capture rate experiment	The capture rates of corals were significantly lower than in the controls at 7 and 20 days after microplastic exposure. After 47 days however, they were not significantly different from the controls, indicating a possible behavioural compensatory response over time. Although microplastics did not impact polyp behaviour, coral exposed to microplastics also had a significantly lower skeletal growth rate in comparison to the control and *in situ* experimental conditions. Calcification was also reduced.	Chapron et al. 2018
Isochrysis galbana, clone T-ISO (Microalgae) 72 hours	PE micronized powder (1.4–42 µm; average particle size of 3.29 µm) at 0.5 mg/L, 1 mg/L, 10 mg/L and 25 mg/L Control group was microalgae with surfactant at its highest concentration	Daily growth rate was not affected by microplastic exposure for all test concentrations. A lower percentage of cellular inhibition was seen when chlorpyrifos were sorbed to microplastics, indicating that it could modulate its toxicity in *I. galbana*.	Garrido et al. 2019
Montastraea cavernosa (Large polyp coral) *Orbicella faveolata* (Small polyp coral)	Experiment 1 (Effects of microbeads on calcification): Fluorescent, PE microbeads (size ranges of 90–106 µm, 425–	In experiment 1, no significant differences on calcification were seen between the control and the exposed group treated with microplastics.	Hankins et al. 2018

2 days	500 µm, and 850–1 000 µm). Experiment 2 (Determination of ingestion size ranges and retention): Uncured, PE microbeads (size ranges of 212–250 µm, 425–500 µm, 850–1000 µm, 1.7–2.0 mm, and 2.4–2.8 mm). Polyps were fed 3 microbeads from each size class. Experiment 3 (Comparing microbeads and microfibres): Uncured, fluorescent, PE microbeads (425–500 µm) and uncured, fluorescent polyester microfibres (3–5 mm long). Polyps were fed 3 plastics of each type. Control groups were not exposed to microbeads; for experiment 2, control group was given food that contained no microbeads	In experiment 2, it was determined that *M. cavernosa* and *O. faveolata* ingested 425–500 µm, 850–1 000 µm, 1.7–2.0 mm, and 2.4–2.8 mm microbeads offered. However, a 212–250 µm size class did not elicit a feeding response in either species. No significant differences in egestion were evident in any size classes. In experiment 3, *M. cavernosa* egested 100% of the microbeads and microfibres. *O. faveolata* egested means of 80.0% ± 23.3 and 76.7% ± 35.3 for microbeads and microfibres, respectively. There was no significant difference in ingestion between microbeads and microfibres.	
Acanthurus triostegus (Convict surgeonfish) 3, 5 and 8 days	PS microbeads (90 µm) at 5 particles/mL (nominal) Control group was exposed to seawater without microplastic	Exposure to microbeads for 3, 5 and 8 days did not alter the foraging activity (measured as number of bites) in *A. triostegus*. The survival of post-larvae to predation was also not significantly affected, compared to the control.	Jacob et al. 2019
Brachionus koreanus (Monogonont rotifer) For toxicity tests, exposure was 12 days	Non-functionalized PS microbeads (0.5 µm and 6 µm) For toxicity tests, concentrations used were 0.1 µg/mL,	Toxicity of beads was size- and concentration-dependent. In the 6 µm treatment group, *B. koreanus* had slightly irregular growth, and no significant changes in fecundity and life span.	Jeong et al. 2016

For ingestion, egestion, ROS[a] levels, MAPK activation, and antioxidant enzyme experiments, exposure was 24 hours	1 µg/mL, 10 µg/mL, and 20 µg/mL For ingestion, egestion, ROS levels, MAPK activation, and antioxidant enzyme experiments, the concentration used was 10 µg/mL Control group was not exposed to microplastic	Microbeads were ingested by the rotifers at both sizes. The authors hypothesize that 0.5 µm microplastics have longer retention times that correlate to more negative effects. Increased enzymatic activities of GPx, GR[b], GST, and SOD were seen for the 0.5 µm beads. Exposure to 6 µm microplastics had levels similar to that of control conditions. The level of total GSH content was not significantly different for any exposure concentration.	
Paracyclopina nana (Marine copepod) 24 hours	Non-functionalized PS microbeads (0.5 µm and 6 µm) For toxicity tests and ROS levels experiments, concentrations used were 0.1 µg/mL, 1 µg/mL, 10 µg/mL, and 20 µg/mL For ingestion, egestion, western blot, and antioxidant enzyme experiments, the concentration used was 10 µg/mL Control group was not exposed to microplastic	Microbeads of both sizes were ingested but egestion was size-dependent; fluorescence was present for the 0.5 µm microbeads 24 hours after exposure, but not in the 6 µm group. *P. nana* exposed to 0.5 µm microbeads showed delayed molting. No observable effects were seen with 6 µm microbeads. ROS levels were increased in the 0.5 µm group compared to the control, however not significantly. In addition, the antioxidant enzymes GPx, GR, GST, and SOD had higher activity in the 0.5 µm group.	Jeong et al. 2017
Sparus aurata (Gilt-head seabream) 45 days	6 microplastics were used: PVC (high molecular weight; 75.6 ± 15.3 µm), PA (111.7 ± 32.2 µm), PE (ultra-high molecular weight; 23.4 ± 7.6 µm), PS (51.0 ± 36.3 µm), PE (average molecular weight medium density; 54.5 ±	Total biomass of the fish per tank was not affected by microplastic exposure. Levels of glucose, aspartate transaminase, alanine transaminase, lactate dehydrogenase, and gamma-glutamyl transferase did not differ significantly from control	Jovanović et al. 2018

	21.3 µm), PVC (low molecular weight; 87.6 ± 16.8 µm) Concentration used was 0.1 g/kg body weight/day Control group was given food that contained no microplastic	conditions following exposure, indicating a lack of stress. The retention of virgin microplastics in the *S. aurata* GI tract was low. However, 5.3% of all livers examined contained at least one plastic particle following 24 hours. In addition, there was no significant difference in overall histopathology between the different treatment groups.	
Crepidula onyx (Slipper limpet) 95 days post hatching	PS microplastics (2–5 µm) In the first trial, microplastic concentrations were 30% (low plastic ratio) and 70% (high plastic ratio) of algal concentration used. Final microplastic concentrations were 6 x 10^4 particles/mL and 1.4 x 10^5 particles/mL for the low and high plastic ratio treatments, respectively. In the second trial, an additional treatment of 10 particles/mL was added Control group was fed algae	Exposure to 10 particles/L microplastics had no significant effect on growth rate and settling rate in larval *C. onyx*. No significant difference was seen for juveniles at this concentration. Larval survival was not affected by microplastic addition at a high plastic ratio. In trial 1, adding microplastics appeared to negatively affect growth rates in larvae. Growth rate was reduced when using low plastic ratio and high plastic ratio treatments in comparison to the control. However, there was a trend that settling rate increased in larvae exposed to microplastic. Settling occurred earlier at a smaller size in this group as a result of their reduced growth rate. Microplastic exposure did not have an effect on survival rates or penis development in juveniles; however, there was a negative effect on growth rate. The microplastic group had a 25% slower growth rate in comparison to the control group.	Lo and Chan 2018

		C. onyx exposed to only microplastics during their larval stage continued to display slower growth rates than the control, even if microplastics were not present in their environment for 65 days. This finding indicates a legacy effect of microplastic exposure.	
Mytilus edulis (Blue mussel) For ingestion and egestion tests, exposure time was 4 hours For the larval growth tests, exposure time was 15 days	Fluorescent PS beads (2 µm) For ingestion and egestion tests, PS concentrations used were 0.70 mg/L, 1.05 mg/L, and 1.40 mg/L (based on a plastic-to-algae ratio) For the larval growth tests, concentrations used were 0.42 µg/L, 28.2 µg/L and 282 µg/L Control group was not exposed to microplastic; for ingestion and egestion tests, control group was only exposed to algae	The body burden (mass of microplastics per individual) was found to be 4.9 ng/larvae, 3.4 ng/larvae, and 3.1 ng/larvae for the 2 µm beads for bead concentrations of 1.40 mg/L, 1.05 mg/L, and 0.70 mg/L, respectively. No significant effect on larval growth rate was seen, but exposure to beads led to an increase in abnormally developed larva. Malformations were more frequent with increasing concentrations and exposure times. From day 11 on, 40% to 60% of all larvae showed signs of abnormal development.	Rist et al. 2019
Thalassiorira pseudonana (Marine diatom algae) *Dunaliella tertiolecta* (Marine flagellate algae) *Chorella vulgaris* (Green microalgae) 72 hours	Uncharged PS microbeads (0.5 µm and 6.0 µm) were used on *D. tertiolecta* Negatively charged carboxylated PS microbeads (0.5 µm) were used on all three test species Concentrations used were 25 mg/L and 250 mg/L (nominal) Control group was not exposed to microplastic	Using pulse amplitude modulation fluorometry, uncharged and negatively charged beads displayed no significant effect on photosynthetic efficiency in all three test species. A small decrease (11%) in *D. tertiolecta* growth was observed with exposure to uncharged 0.5 µm beads along with a 13% inhibition of growth rate. Effects were less than 10% for the 6 µm beads.	Sjollema et al. 2016

Sebastes schlegelii (Jacopever) 14 days	Green fluorescent PS microbeads (15 µm) at 1×10^6 microplastics/L Control group was not exposed to microplastic	Microplastics were found in the gills and intestines following 14-day exposure and 7-day depuration. No translocation to the liver was seen, however. 14-day exposure to microplastics caused feeding time to significantly increase (by approximately 2-fold). Foraging time was rapidly reduced and shoaling behaviour (staying in close proximity from one another) was shown through a reduction in mean distance between fish. In addition, mean swimming speed was reduced and fish used a significantly smaller volume of their tank when foraging in comparison to control fish. Histopathological changes in the liver (hyperaemia), gallbladder (bile turned black in colour), and intestines (altered morphology) of fish were seen following 14 day exposure to microplastics. After 14-day exposure and 7-day depuration, no mortalities were observed; however, there was a significant reduction in growth and energy reserves. Weight gain rate decreased from 8.92 ± 0.98% in controls to 3.09 ± 0.32% in the microplastic-exposed group.	Yin et al. 2018

[a] Reactive oxygen species
[b] Glutathione reductase

Table C-3: Soil

Organism and Duration of Exposure	Microplastic Type and Concentration	Summary of Effects	Source
Folsomia Candida (Soil springtail) 28 days	PE beads (<500 µm; size distribution of 32% with <50 µm, 25% between 50 and 200 µm, and 43% between 200 and 500 µm) Concentrations used were 0.005%, 0.02%, 0.1%, 0.5%, 1% microplastics w/w in dry soil Control group was exposed to soil without microplastic	Average survival rates were higher than 80% in all three conditions. Springtails displayed significant avoidance behaviours at 0.5% and 1% (microplastics w/w in dry soil) that appeared to be concentration-dependent. The avoidance rates were 59% and 69%, respectively. Reproduction rate decreased with increasing microplastic concentrations. At the highest tested concentration of 1% microplastics, the reproduction rate was reduced by 70.2%. The EC_{50} was 0.29% microplastics w/w in dry soil. At concentrations of 0.5% dw soil, MPs significantly altered the microbial community (and decreased bacterial diversity in the springtail gut). Alphaproteobacteria and *Wolbachia* were significantly less prevalent when exposed to microplastics. However, Bradyrhizobiaceae and *Ensifer* were significantly increased in the exposed group.	Ju et al. 2019
Lobella sokamensis (Soil springtail) 3 minutes	Plastic microbeads (average diameters of 0.50 ± 0.01 µm, 29 ± 4 µm, and 248 ± 14 µm) Plastic fragments (average diameters of 44 ± 39 µm,	The influx of microplastic particles in soil disrupted the movement of *L. sokamensis*. The springtails moved to avoid becoming trapped, and this behaviour created bio-pores in the soil system. The influx of plastic particles into these cavities subsequently immobilized the springtails	Kim and An 2019

	282 ± 131 µm, and 676 ± 479 µm) Concentrations in soil were 4 and 8 mg/kg for the roughly 0.50 µm microbeads and for the remaining microplastic sizes, the concentration used was 1 000 mg/kg Concentrations in solution were 10 mg/L and 20 mg/L for the roughly 0.50 µm microbeads Control group was exposed to soil without microplastic and deionized water	within. Using a movement index to quantify springtail behaviour, it was found that movement was significantly different in all size groups in comparison to the control. Specifically in the roughly 0.50 µm microbead solution at 8 mg/kg, movement decreased significantly compared to the other treatment groups.	
Caenorhabditis elegans (Nematodes) 3 days	PS microplastics (0.5 µm, 1.0 µm, 2.0 µm, and 5.0 µm) at 1.0 mg/L Control group was exposed to suspension solution without microplastic	PS microplastics displayed size-dependent effects on lethality. Survival rates were reduced in all treatment groups. The 1.0 µm group had the lowest mean reduction in survival of 32.27%. In addition, the 1.0 µm group also had significant decreases in body length and average lifespan. Microplastic exposure resulted in an increase in number of head thrashes and body bends in the 0.5 µm group but decreases in locomotion for the other treatment groups. However, exposure to 2.0 µm PS led to significant increases in mean crawling speed. Exposure to microplastics led to damage in cholinergic neurons (i.e., broken ciliated dendrites) in all treatment groups, indicating a downregulation of *unc-17*	Lei et al. 2018a

		(encodes acetylcholine in cholinergic neurons). Damage to GABAergic neurons was also seen in the 1.0 µm group. PS microplastics upregulated the expression of *gst-4* (encodes glutathione S-transferase-4, a key enzyme involved in oxidative stress).	

Table C-4: Sediment

Organism and Duration of Exposure	Microplastic Type and Concentration	Summary of Effects	Source
Ennucula tenuis (Bivalve) *Abra nitida* (Saltwater clam) 4 weeks	PE fragments (size ranges of 4–6 µm, 20–25 µm, 125–500 µm) at 1 mg/kg, 10 mg/kg, and 25 mg/kg dry sediment A low background contamination with perfluorooctane sulfonate was found in microplastics Control group was exposed to clean sediment	No significant changes in mortality, condition index, or burrowing behaviour were seen between treatments in both species. In *E. tenuis*, there were no significant changes in protein and carbohydrate content. However, there was a significant reduction in lipid content (64%) for individuals exposed to 20–25 µm at 10 mg/kg. In addition, a dose-dependent decrease in total energy was evident in all size groups. In *A. nitida*, there was a significant decrease in protein content from individuals exposed to 125–500 µm PE. Apparent, but not significant, changes in lipid content, carbohydrate content, and total energy were seen.	Bour et al. 2018
Perinereis aibuhitensis (Clamworm)	PS microspheres (size ranges of 8–12 µm and 32–38 µm) at 100 beads/mL and	The presence of microplastics increased mortality in *P. aibuhitensis*, with 8–12 µm microbeads having a	Leung and Chan 2018

4 weeks	1 000 beads/mL (nominal) Control group was exposed to 0.45 µm-filtered seawater without microplastic	significantly higher effect than the other treatments. For example, exposure to 8–12 µm microspheres at 100 beads/mL led to an average survival of 38% compared to over 80% in the control. Segment regeneration was size-dependent, with the slowest rate being observed in worms exposed to 8–12 µm (smaller size) microspheres at 1 000 beads/mL. Regeneration was 8.3 ± 1.4% for this group, compared to 20.7 ± 2.5% in the control group. In addition, worms exposed to a lower concentration of microplastics displayed a higher percent of segment regenerated.	
Hyalella azteca (Amphipod) *Asellus aquaticus* (Isopod) *Sphaerium corneum* (Bivalve) *Lumbriculus variegatus* (Worm) *Tubifex* spp. (Worm) 28 days	Irregular PS fragments (20–500 µm) mixed with sediment at 0.1%, 1%, 5%, 10%, 20%, 30% and 40% sediment dw Control group was exposed to sediment without microplastic	In *H. azteca*, *A. aquaticus*, *S. corneum*, and *Tubifex* spp., microplastics had no significant effect on mortality at all test concentrations. In *Lumbriculus variegatus*, no effects were found on reproduction (measured as reproduction factor). No differences in growth were seen in *A. aquaticus*, *S. corneum*, *H. azteca*, *L. variegatus*, and *Tubifex* spp. In *H. azteca*, there were no differences in feeding activity at all concentrations. In *L. variegatus and Tubifex* spp., microplastic exposure had no negative effect on egestion. No microplastics were found in the body and fecal pellets of *H. azteca*.	Redondo-Hasselerharm et al. 2018

Chironomus tepperi (Sediment dwelling midge) 5 day growth assay and 10 day emergence assay	Blue/white PE microplastics (size ranges of 1–4 µm, 10–27 µm, 43–54 µm, 100–126 µm) at 500 particles/kg sediment Control group was exposed to unspiked sediment; additional control assays using moderately hard water with and without Tween-20 (surfactant) were also conducted to ensure that larvae were appropriately sensitive and Tween-20 did not affect results	Using a 5-day growth assay, survival rates of midges exposed to microplastics were size-dependent, and the effects were found to be more pronounced with smaller particle sizes. Survival rate was the lowest in the 10–27 µm (57% survival) treatment group in comparison to the control (92% survival). Exposure to the highest tested concentration did not have any significant effect on survival. A size-dependent effect was also seen in larvae growth, where exposure to smaller microplastics led to significant decreases in body length. Exposure to 10–27 µm also led to the smallest body length (7.6 ± 2.4 mm) compared to the control (12.9 ± 3.1 mm). No significant changes were seen for the 100–126 µm group. The length of larvae head capsule was not affected by exposure to any treatment, with the exception of 10–27 µm, which had a significant reduction in mean head capsule length. SEM imaging also revealed reductions in the size of the head capsule and mouth for this group. It is hypothesized that the 10–27 µm particles had the greatest effects since they are the ideal size for consumption and to avoid egestion. Using a 10-day emergence assay, it was found that exposure to microplastics negatively affected the total	Ziajahromi et al. 2018

		number of emerged adults. There was a significant reduction in emergence rate for all microplastic size ranges. For the 10–27 µm group, the emergence rate was only 17.5%, compared to 92% in the control.	
Caenorhabditis elegans (Nematode) 2 days	For *C. elegans*, concentrations of 0.5 mg/m^2, 1.0 mg/m^2, 5.0 mg/m^2 and 10.0 mg/m^2 were used For *C. elegans*, nematode growth medium agar seeded with *Escherichia coli* OP50 was used for the control group	In *C. elegans*, PA, PE, PP, and PVC microplastics had significant effects on their survival, with the exception of PVC at 0.5 mg/m^2. PS particles displayed a significant size-dependent effect on lethality, with the 1.0 µm particles causing strong lethality and the 5.0 µm particles causing moderate lethality. In addition, exposure to 5.0 mg/m^2 microplastics led to reductions in average body length and reproduction (embryo number and brood size). Microplastic exposure also led to decreased intestinal calcium levels and increased *gst-4* expression. In *C. elegans*, 1.0 µm PS particles showed the highest toxicity, highest accumulation in the intestines, lowest Ca^{2+} level in the intestine, and greatest expression of *gst-4* of the different sizes tested.	Lei et al. 2018b
Gammarus pulex (Amphipod) 28 days	Irregular PS fragments (20–500 µm) mixed with sediment at 0.1%, 1%, 5%, 10%, 20%, 30% and 40% sediment dw[a] Control group was exposed to sediment without microplastic	In *G. pulex*, microplastics had no significant effect on mortality at all test concentrations. *G. pulex* had a significant reduction in growth following exposure to high microplastic concentrations (10–40%) compared to controls. The EC_{50}[b] value was determined to be 3.57% sediment dw (±3.22) and the EC_{10}[c] value was 1.07%.	Redondo-Hasselerharm et al. 2018

		There were no differences in feeding activity at all concentrations. In addition, *G. pulex* had microplastics present in the body and fecal pellets at all concentrations following a 24-hour depuration time. Uptake by *G. pulex* was found to be proportional to the concentration of microplastic in the sediment.	

[a] Dry weight
[b] Median effective concentration
[c] 10% effect concentration

Appendix D: Additional information on toxicological studies

Table D-1: Ingestion toxicity studies

Species, route and exposure duration	Microplastic tested	Concentration	Summary of Effects	Source
Rats Dietary 90 days (7 d/week)	Nonwoven, spunbond polymer fabric made of PE and PET (milled to fine powder) Particle sizes and counts were not reported, although based on typical diameter range of spunbond fibres, particles were likely in the range of 1 to 50 µm (Welle et al. 2018)	Test diet was prepared by mixing ground test material in basal diet at target levels of 0%, 0.5%, 2.5% or 5%	No toxicologically relevant treatment related effects were observed in any of end points evaluated in the feeding study i.e., no treatment-related adverse effects on blood parameters, organ weights or histopathology of the liver NOEL[a] not identified by authors but can be considered the highest dose, which is equal to 2 500 mg/kg bw/day (assuming 5% food factor for rats) (WHO 2019)	Merski et al. 2008
Mice Oral gavage 28 days (7 d/week)	Fluorescent PS 5 µm and 20 µm in diameter	1.46 x 10^6 items of 5 µm particles at 0.1 mg/day 2.27 x 10^4 items of 20 µm particles at 0.1 mg/day	PS accumulation in the liver, kidney and gut of exposed mice for both 5 µm and 20 µm particle sizes (Translocation to the liver and kidney reportedly occurred and particles could be detected 1 week after cessation of exposure)	Deng et al. 2017
Mice Oral gavage	Virgin PS 5 µm and 20 µm in diameter	1 × 10^5 items of 5 µm particles at 0.01 mg/day	Inflammation and lipid droplets were observed in the livers of treated mice at highest dose	Deng et al. 2017

28 days (7 d/week)		2×10^3 items of 20 µm particles at 0.01 mg/day 1×10^6 items of 5 µm particles at 0.1 mg/day 2×10^4 items of 20 µm particles at 0.1 mg/day 5×10^6 items of 5 µm particles at 0.5 mg/day 1×10^5 items for 20 µm at 0.5 mg/day	Incidence or severity data not reported Energy metabolism: Both sizes of PS induced a decrease in ATP level and significant decrease in LDH[b] activity in a dose-dependent matter Lipid metabolism: Decreases in all treatments for the levels of total cholesterol and triglycerides Biomarkers of oxidative stress: Increased GPx activity (more so in 5 µm group) and SOD Decrease in catalase activity in almost all the treatment groups Potential for neurotoxicity: Decreased acetylcholinesterase activity in liver after exposure to two sizes of PS microplastics, but more so in 5 µm group	
Mice Oral gavage 28 days (3 times /week)	PS 1 µm, 4 µm, 10 m in diameter	Mixture of 1 µm (4.55×10^7 particles), 4 µm (4.55×10^7 particles), and 10 µm (1.49×10^6 particles) PS in CMC[c] at a volume of 10 mL/kg/bw	No evidence of occurrence of inflammation and/or oxidative stress following exposure of mice to PS microparticles Little presence of particles in cells of the jejunum and duodenum. No particles were found in other organs (liver, spleen, kidney)	Stock et al. 2019

Mice Drinking water 6 weeks (continuous exposure)	Virgin and fluorescent PS 5 µm in diameter	1.456×10^6 particles/L of 5 µm particles at 100 µg/L 1.456×10^7 particles/L of 5 µm particles at 1 000 µg/L	Accumulation of 5 µm PS in gut with 1 000 µg/L exposure Gut microbiota dysbiosis (change in the composition of the gut microbiota in the cecal contents of the mice) at both doses Intestinal barrier dysfunction Alterations in amino acid and bile acid metabolism with 1 000 µg/L exposure	Jin et al. 2019
Mice Drinking water 5 weeks (continuous exposure)	PS 0.5 µm and 50 µm in diameter	1.456×10^{10} particles/L of 0.5 µm at 100 µg/L in drinking water 1.456×10^{10} particles/L of 0.5 µm at 1 000 µg/L in drinking water 1.456×10^4 particles/L of 50 µm at 100 µg/L in drinking water 1.456×10^4 particles/L of 50 µm at 1 000 µg/L in drinking water	Altered hepatic lipid metabolism Altered gut microbiota composition	Lu et al. 2018
Mice Drinking water 90 days (continuous exposure)	PE and organo-phosphorus flame retardants (OPFRs) (TCEP and TDCPP) or PS and OPFRs	2 000 µg/L PS (3.7×10^8 items/L) and 10 µg/L OPFRs 2 000 µg/L PS (3.7×10^8 items/L) and 100 µg/L OPFRs 2 000 µg/L PE (3.7×10^8 items/L) and 10 µg/L OPFRs	Increased oxidative stress, increased neurotoxicity, enhanced disruption of amino acid metabolism and energy metabolism from co-exposure. No microplastic-only control group; it is unclear what component of the treatment contributed to the effects	Deng et al. 2018

		2 000 µg/L PE (3.7 x 10^8 items/L) and 100 µg/L OPFRs		

[a] No observed effect level
[b] Lactate dehydrogenase
[c] Carboxymethylcellulose

Table D-2: Inhalation toxicity studies

Species, route and exposure duration	Microplastic tested	Concentration	Summary of Effects	Source
Rats Nose-only inhalation 90 days (6 h/d, 5 d/wk)	PP fibres (GM[a] diameter of 1.2 µm and length of 11.6 to 14.7 µm)	13.0, 28.1, or 59.6 mg/m^3 (12.1, 20, or 48.1 fibres/cm^3)	Dose-related increase in incidence and severity of fibre-containing macrophages and microgranulomas, with bronchiolization at high concentration. Reversible at two lower concentrations. LOEC[b] = 13 mg/m^3 $LOEC_{adj}$[c] = 2.3 mg/m^3	Hesterberg et al. 1992
Rats Inhalation in chamber air 12 weeks (6 h/d, 5 d/wk)	Freshly generated PUF particulates (94% <5 µm and 83% <3 µm)	8.65 mg/m^3	No effect on body weight, survival time, behaviour, or tumour incidence. Intra-alveolar granulomas and peribronchial and perivascular lymphocyte infiltration. LOEC = 8.65 mg/m^3 $LOEC_{adj}$ = 1.54 mg/m^3	Thyssen et al. 1978
Rats Inhalation in chamber air 30 exposure days (6 h/d, 5 d/wk)	Freshly generated PUF particulates (median diameter 0.7 µm)	3.6, 20.5 mg/m^3	No effect on mortality or weight. Hemorrhage congestion edema at high concentration. No increase in pneumonitis or lymphocytic infiltration. Dose-related increase in tracheal	Laskin et al. 1972

			hyperplasia. Increase in tracheal squamous metaplasia only at low concentration. Increase in bronchio-alveolar changes (centrilobular emphysema and macrophages) only at high concentration. No increase in bronchial hyperplasia or squamous metaplasia. Lung and lymph macrophages contained particles. Squamous cell carcinoma observed in 1 rat in each treatment group. LOEC = 3.6 mg/m^3 $LOEC_{adj}$ = 0.64 mg/m^3	
Hamsters Inhalation in chamber air 30 exposure days (6 h/d, 5 d/wk)	Freshly generated PUF particulates (median diameter 0.7 µm)	3.6, 20.5 mg/m^3	No increase in mortality. Weight loss only at low concentration. Hemorrhage congestion edema at high concentration. No increase in pneumonitis or lymphocytic infiltration. Histological changes limited to bronchial hyperplasia. LOEC = 3.6 mg/m^3 $LOEC_{adj}$ = 0.64 mg/m^3	Laskin et al. 1972
Rats Nose-only inhalation 4 weeks (20 exposure days), 6 h/d, 5 d/wk	Uncoated nylon fibre-shaped particulates (mean length and diameter of 9.8 and 1.6 µm, respectively)	4.0, 15, 57 fibres/cm^3 (0.6, 2.7, 19.6 mg/m^3)	No effect on body weight, lung weight, or clinical observations. Reversible increase in total cell counts in BALF in 57 fibres/cm^3 group (with an	Warheit et al. 2003

			increase in neutrophil fraction). Absence of evidence of pulmonary inflammation, biomarkers of lung injury, and cell proliferation. Nylon particulates contained in BALF and nasal lymphoid macrophages; higher and more persistent at high concentration. No impact on phagocytic abilities of macrophages. No significant changes in cell proliferation rates. NOEC[d] = 15 fibres/cm^3 (2.7 mg/m^3) $NOEC_{adj}$[e] = 2.7 fibres/cm^3 (0.48 mg/m^3)	
Guinea pigs Inhalation in chamber air 325 days	Nylon and Orlon (PAN) particulates (dimensions not stated)	2 g pulverized 3 times/d	Nodular subpleural foci within areas of emphysema in interalveolar septa. Foci consisted of edema, reticular fibres, and granulomas containing histiocytes and fibroblasts. Lesions contained inhaled particles. LOEC = 6 g/day	Pimentel et al. 1975
Rats Nose-only inhalation 5 days (6 h/day)	Acrylic ester copolymer, with and without a nanoparticle fraction (MMAD[f] of 1.2 µm and median diameter of 0.4 µm for both test	3.4 and 10.6 mg/m^3 for both test compounds	No treatment-related effect on body weight, clinical observations, hematological parameters, BALF parameters (total	Ma-Hock et al. 2012

	compounds, but size distribution varied in the two aerosol types)		and differential cell counts or biochemical indicators of lung injury) or lung and lymph node histology. NOEC = 10.6 mg/m^3 $NOEC_{adj}$ = 2.7 mg/m^3	
Rats Intratracheal Single instillation	PVC particulates (<5 µm)	25 mg suspended in 1 mL saline	No effect on mortality. Reversible increase in activity of lung succinic dehydrogenase and adenosine triphosphatase and lysosomal enzymes. Vascular and inflammatory changes, hyperplasia, interstitial fibrosis, and granulomas in areas of lungs corresponding to particulate deposition; effects were reversible as particulate was cleared. LOEL[g] = 25 mg	Agarwal et al. 1978
Rats Intratracheal Single instillation	PVC particulates as suspension or emulsion (various groups with mass median diameters ranging from 13 to 130 µm); one group exposed to a copolymer with vinyl acetate	2 mg in 0.2 mL saline	Small foci of granular material with mild inflammation, in alveoli and alveolar ducts. No fibrosis; no lymphatic changes. LOEL = 2 mg	Pigott and Ishmael 1979
Rats Intratracheal Single instillation	PVC particles as produced or washed (to remove adsorbed additives); median size of about 2 µm	10 or 50 mg/kg	No effect on body weight. BALF: elevated LDH, total protein level, total cell count, and neutrophils in 50 mg/kg groups at 2 and 7 days after instillation, but	Xu et al. 2004

			decreased to control levels at later timepoints; most effects persisted longer in washed PVC than non-washed. Histology: High-dose PVC groups had thickened aveolar walls accompanied by clusters of inflammatory cells and particles at 2 days post-instillation, with increased inflammation at 7 days in the washed PVC. Foci on lung surface at 28 days (less obvious at 90 days) and increase in macrophages (with no fibrosis) at 90 days.	
Rats Intratracheal Single instillation	Milled nylon tow (i.e., uncut nylon strands) (average width and length of 2 µm and 14 µm, respectively)	10 mg/kg bw of particulates in saline	Significant increase in breathing rate. Suppurative pneumonia around bronchioles; histiocytic inflammation in alveoli near fibres; no fibrosis. Significant increase in polymorphonuclear leukocyte cell count in BALF. Significant increase in chemiluminescence but not cell count for alveolar macrophage. Significant increase in albumin (indicator of blood-gas barrier	Porter et al. 1999

			deterioration) and matrix metalloprotease activity (indicator of inflammation). LOEL = 10 mg/kg	
Rats Intratracheal Single instillation	PS microspheres (64, 202, or 535 nm)	1 mg in 0.5 mL saline	BALF: Significant increase in total cells for 64 and 202 nm particles. Increase in protein in 64 and 535 nm particles, and increase in LDH activity (suggestive of cell death) in 64 nm particles. LOEL = 1 mg	Brown et al. 2001
Rats Intratracheal Single instillation	PU particles from aged (PUF I) or freshly-prepared (PUF II) foam (aerodynamic diameter of ≤10 µm for 93.5% of particles and ≤5 µm for 52% of particles)	20 mg/mL in saline	Early lymphocytic infiltration and macrophage activity in lungs, later accompanied by alveolar wall thickening, epithelization, and fibrosis, which at 18 and 24 months progressed to scarring and perifocal emphysema. Hyperplasia in bronchial epithelium and benign intrabronchial adenomas from PUF II.	Stemmer et al. 1975
Pregnant rats Intratracheal Repeat dose: instillation every other day, GD[h] 5 to 19	20 nm PS	2 974 µg total (equivalent to 952 µg/dose); 2.4 × 10^{13} particles. In 300 µL saline	Significant increase in reabsorption sites in exposed rats (both acute and repeat). Evidence of particle translocation from lung: repeat study – placenta, whole pup, and fetal liver; acute study – maternal heart, spleen,	Fournier et al. 2018 (abstract only; no full-text)

Acute: single instillation on GD 19			placenta, fetal heart, fetal liver, and whole pup.	

[a] Geometric mean
[b] Lowest observed effect concentration
[c] Lowest observed effect concentration, adjusted for continuous exposure
[d] No observed effect concentration
[e] No observed effect concentration, adjusted for continuous exposure
[f] Mass median aerodynamic diameter
[g] Lowest observed effect level
[h] Gestational day