Volume 259

Pim de Voogt *Editor*

Reviews of Environmental Contamination and Toxicology

Reviews of Environmental Contamination and Toxicology

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Reviews of Environmental Contamination and Toxicology Volume 259

Editor
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Volume 259

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Foreword

International concern in scientific, industrial, and governmental communities over traces of xenobiotics in foods and in both abiotic and biotic environments has justified the present triumvirate of specialized publications in this field: comprehensive reviews, rapidly published research papers and progress reports, and archival documentations These three international publications are integrated and scheduled to provide the coherency essential for nonduplicative and current progress in a field as dynamic and complex as environmental contamination and toxicology. This series is reserved exclusively for the diversified literature on "toxic" chemicals in our food, our feeds, our homes, recreational and working surroundings, our domestic animals, our wildlife, and ourselves. Tremendous efforts worldwide have been mobilized to evaluate the nature, presence, magnitude, fate, and toxicology of the chemicals loosed upon the Earth. Among the sequelae of this broad new emphasis is an undeniable need for an articulated set of authoritative publications, where one can find the latest important world literature produced by these emerging areas of science together with documentation of pertinent ancillary legislation.

Research directors and legislative or administrative advisers do not have the time to scan the escalating number of technical publications that may contain articles important to current responsibility. Rather, these individuals need the background provided by detailed reviews and the assurance that the latest information is made available to them, all with minimal literature searching. Similarly, the scientist assigned or attracted to a new problem is required to glean all literature pertinent to the task, to publish new developments or important new experimental details quickly, to inform others of findings that might alter their own efforts, and eventually to publish all his/her supporting data and conclusions for archival purposes.

In the fields of environmental contamination and toxicology, the sum of these concerns and responsibilities is decisively addressed by the uniform, encompassing, and timely publication format of the Springer triumvirate:

Reviews of Environmental Contamination and Toxicology [Vol. 1 through 97 (1962–1986) as Residue Reviews] for detailed review articles concerned with any aspects of chemical contaminants, including pesticides, in the total environment with toxicological considerations and consequences.

Bulletin of Environmental Contamination and Toxicology (Vol. 1 in 1966) for rapid publication of short reports of significant advances and discoveries in the fields of air, soil, water, and food contamination and pollution as well as methodology and other disciplines concerned with the introduction, presence, and effects of toxicants in the total environment.

Archives of Environmental Contamination and Toxicology (Vol. 1 in 1973) for important complete articles emphasizing and describing original experimental or theoretical research work pertaining to the scientific aspects of chemical contaminants in the environment.

The individual editors of these three publications comprise the joint Coordinating Board of Editors with referral within the board of manuscripts submitted to one publication but deemed by major emphasis or length more suitable for one of the others.

Coordinating Board of Editors

Preface

The role of *Reviews* is to publish detailed scientific review articles on all aspects of environmental contamination and associated (eco)toxicological consequences. Such articles facilitate the often complex task of accessing and interpreting cogent scientific data within the confines of one or more closely related research fields.

In the 50+ years since *Reviews of Environmental Contamination and Toxicology* (formerly *Residue Reviews)* was first published, the number, scope, and complexity of environmental pollution incidents have grown unabated. During this entire period, the emphasis has been on publishing articles that address the presence and toxicity of environmental contaminants. New research is published each year on a myriad of environmental pollution issues facing people worldwide. This fact, and the routine discovery and reporting of emerging contaminants and new environmental contamination cases, creates an increasingly important function for *Reviews.* The staggering volume of scientific literature demands remedy by which data can be synthesized and made available to readers in an abridged form. *Reviews* addresses this need and provides detailed reviews worldwide to key scientists and science or policy administrators, whether employed by government, universities, nongovernmental organizations, or the private sector.

There is a panoply of environmental issues and concerns on which many scientists have focused their research in past years. The scope of this list is quite broad, encompassing environmental events globally that affect marine and terrestrial ecosystems; biotic and abiotic environments; impacts on plants, humans, and wildlife; and pollutants, both chemical and radioactive; as well as the ravages of environmental disease in virtually all environmental media (soil, water, air). New or enhanced safety and environmental concerns have emerged in the last decade to be added to incidents covered by the media, studied by scientists, and addressed by governmental and private institutions. Among these are events so striking that they are creating a paradigm shift. Two in particular are at the center of ever increasing media as well as scientific attention: bioterrorism and global warming. Unfortunately, these very worrisome issues are now superimposed on the already extensive list of ongoing environmental challenges.

The ultimate role of publishing scientific environmental research is to enhance understanding of the environment in ways that allow the public to be better informed or, in other words, to enable the public to have access to sufficient information. Because the public gets most of its information on science and technology from internet, TV news, and reports, the role for scientists as interpreters and brokers of scientific information to the public will grow rather than diminish. Environmentalism is an important global political force, resulting in the emergence of multinational consortia to control pollution and the evolution of the environmental ethic. Will the new politics of the twenty-first century involve a consortium of technologists and environmentalists, or a progressive confrontation? These matters are of genuine concern to governmental agencies and legislative bodies around the world.

For those who make the decisions about how our planet is managed, there is an ongoing need for continual surveillance and intelligent controls to avoid endangering the environment, public health, and wildlife. Ensuring safety-in-use of the many chemicals involved in our highly industrialized culture is a dynamic challenge, because the old, established materials are continually being displaced by newly developed molecules more acceptable to federal and state regulatory agencies, public health officials, and environmentalists. New legislation that will deal in an appropriate manner with this challenge is currently in the making or has been implemented recently, such as the REACH legislation in Europe. These regulations demand scientifically sound and documented dossiers on new chemicals.

Reviews publishes synoptic articles designed to treat the presence, fate, and, if possible, the safety of xenobiotics in any segment of the environment. These reviews can be either general or specific, but properly lie in the domains of analytical chemistry and its methodology, biochemistry, human and animal medicine, legislation, pharmacology, physiology, (eco)toxicology, and regulation. Certain affairs in food technology concerned specifically with pesticide and other food-additive problems may also be appropriate.

Because manuscripts are published in the order in which they are received in final form, it may seem that some important aspects have been neglected at times. However, these apparent omissions are recognized, and pertinent manuscripts are likely in preparation or planned. The field is so very large and the interests in it are so varied that the editor and the editorial board earnestly solicit authors and suggestions of underrepresented topics to make this international book series yet more useful and worthwhile.

Justification for the preparation of any review for this book series is that it deals with some aspect of the many real problems arising from the presence of anthropogenic chemicals in our surroundings. Thus, manuscripts may encompass case studies from any country. Additionally, chemical contamination in any manner of air, water, soil, or plant or animal life is within these objectives and their scope.

Manuscripts are often contributed by invitation. However, nominations for new topics or topics in areas that are rapidly advancing are welcome. Preliminary communication with the Editor-in-Chief is recommended before volunteered

review manuscripts are submitted. *Reviews* is registered in WebofScience™. Inclusion in the Science Citation Index serves to encourage scientists in academia to contribute to the series. The impact factor in recent years has increased from 2.5 in 2009 to 7.0 in 2017. The Editor-in-Chief and the Editorial Board strive for a further increase of the journal impact factor by actively inviting authors to submit manuscripts.

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Microplastics in the Food Chain: Food Safety and Environmental Aspects

József Lehel and Sadhbh Murphy

Contents

Abstract Plastic has been an incredibly useful and indispensable material in all aspects of human life. Without it many advances in medicine, technology or industry would not have been possible. However, its easy accessibility and low cost have led to global misuse. Basically, the production of the plastics from different chemical agents is very easy but unfortunately difficult to reuse or recycle, and it is thrown away as litter, incinerated or disposed of in landfill. Plastic once in the environment begins to degrade to very small sizes. Thus, many animals mistake them for food, so

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plastic enters a marine, terrestrial or freshwater food web. These microplastics although chemically inert have been shown to act as tiny "bio-sponges" for harmful chemicals found in the environment changing the nature of a plastic particle from chemically harmless to potentially toxic. It was believed that microparticles would simply pass through the gastrointestinal tract of animals and humans with no biological effect. However, studies have shown that they are sometimes taken up and distributed throughout the circulatory and lymphatic system and may be stored in the fatty tissues of different organisms. The result of the uptake of them showed potential carcinogenic effects, liver dysfunction and endocrine disruption. This review focuses on micro- and nanoplastics and their way entering marine and freshwater food webs, with particular attention to microplastic trophic transfer, their toxic side effects and influence to the human consumer in health and safety in the future.

Keywords Anthropogenic activity · Aquatic food chain · Environmental safety · Food safety · Freshwater fish · Marine fish

Abbreviations

ABS	Acrylonitrile butadiene styrene
BPA	Bisphenol A
FTIR	Fourier Transform Infrared Spectroscopy
GI	Gastrointestinal
HBDC	Hexabromocyclododecane
HDPE	High-density polyethylene
IPA	Isophthalic acid
LDPE	Low density polyethylene
NP	Nonylphenols
PAH	Polycyclic aromatic hydrocarbons
PCB	Polychlorinated biphenyls
PDBE	Polybrominated diphenyl ether
PE	Polyethylene
POP	Persistent organic pollutant
PP	Polypropylene
PPA	Polyphthalamide
PVC	Polyvinylchloride
SPI	Society of Plastic Industry
SUP	Single-use plastic
TPA	Terephthalic acid
UN	United Nations

1 Introduction

Plastic is intrinsic to modern life. Since the invention of Bakelite in 1930 plastic has lived up to its reputation as the "Material of a thousand uses" (Gilbert 2017). Human beings use plastic in different ways every single day, and it has propelled invention and advances in many industries including medicine, construction and engineering. The problem with plastics began with the development of a "throwaway culture" which has been feasible by the invention of "single-use plastics", most frequently used in the packaging of various products. This problem in countries that have underdeveloped waste disposal methods, to cope with the large volumes of plastic, has led to build-up of plastic materials in landfills, waste incinerators, or in the environment in the form of litter (Hayden et al. 2013; Shah et al. 2008). Plastic is favoured for its outstanding durability, but it is this trait which has led to problems associated with its degradation, especially when it reaches the environment.

It has been estimated that the amount of plastic entering the ocean yearly is eight million tonnes (Jambeck et al. 2015) and that plastic pieces floating around in the oceans water column could exceed 5 trillion (Eriksen et al. 2014). Plastic is accumulating in ecosystems at ever increasing rates. These plastic pieces have been found all around the world from deep ocean gyres to surface waters as well as in every terrestrial and freshwater habitat (Carbery et al. 2018; Rillig 2012).

Plastics are mostly made from petrochemical waste products of the fossil fuel industry, which are materials of high molecular mass usually derived from ethylene, propylene and styrene. During their manufacture and degradation greenhouse gases can be emitted such as ethylene, carbon dioxide and methane (Hayden et al. 2013; Soares et al. 2008). Various chemical additives can be added to the plastic during its manufacture depending on its potential/intended use. The top two additives used in plastic manufacture that were found in environmental plastic debris were (1) Phthalates such as Bisphenol A; (2) Flame retardants such as Nonylphenols (NP), Polybrominated diphenyl ethers (PDBEs) and hexabromocyclododecane (HBCD). The reason phthalates are added to plastic is that they increase the flexibility and durability (Oehlmann et al. 2009). The flame retardants are used in plastics as safety devices where the intention is to reduce the flammability of a product. These plastic additives may leach to the environment (Talsness et al. 2009). Plastic litter produced may become bioavailable to the organisms that reside there (Cheng et al. 2013). This is also how they become incorporated into marine, aquatic, or terrestrial food webs. Nonylphenols are mostly found in the effluent from wastewater treatment plants and have been found associated with many microplastics found as plastic debris (Mackintosh et al. 2004). These chemical additives have been linked to various health risks including endocrine disrupting activities, liver and kidney toxicity and teratogenicity. They can also leach into the environment in a similar way and are known as persistent organic pollutants (POPs). HBCD is often used in polystyrene products and has also been found in buoys used in fisheries and in marine debris, and has allegedly been linked to endocrine disruption and are also POPs (Al-Odaini et al. 2015; Yogui and Sericano 2009).

It is for this reason that it is important to produce, recycle, reuse and dispose of plastics in a way that is not wasteful or harmful to the environment to prevent unnecessary expenditure of chemical additives. In Germany there are waste processes in place that work very well whereas Ireland relies on shipping up to 95% of their plastic waste to other countries to be recycled, incinerated, or buried in landfill (O'Sullivan 2017; Patel et al. 2000).

Until 2017 China a significant amount of other countries' plastic and paper waste, but in December of that year, they declared that they would no longer be the world's dumping ground. So, countries have been faced with their own waste to deal with (O'Sullivan 2017).

The EU strategy on a circular economy in plastics includes a strong emphasis on improving the waste management of plastics, however, it is a complex process focusing to reducing waste, waste collection, sorting plastic types and improving recycling methods (European Commission 2018a). Certainly, the problems of wastes are intensified in that countries where there is no effective waste management system, and these countries can receive large amounts of plastic waste materials from the developed countries.

Most of the mismanaged plastic waste, and of the world's ocean plastics pollution has its origin in Asia. China produced the largest amount of plastic with about 60 million tonnes (MT) followed by the USA (38 MT), Germany (14.5 MT) and Brazil (12 MT) in 2018. Furthermore, highest quantity of the mismanaged plastic waste is also originated from China (28% of the global total waste) followed by Indonesia (10%), Philippines and Vietnam (6%), Thailand (3.2%), Egypt (3%), Nigeria (2.7%) and South Africa (2%). The amount of the mismanaged waste which can induce risks of ocean pollution is generally significantly lower in many countries of Europe and North America due to the modified and thus effective waste management, despite producing large quantities of plastic (Ritchie 2018).

Certainly, very large differences can be seen in the effectiveness of waste management across the world. In high-income countries (e.g. most of Europe, North America, Australia, New Zealand, Japan and South Korea), the waste management instillations and the infrastructure are very effective, because the discarded plastic wastes are stored in secure, closed landfills even if they are not recycled or incinerated. In many low-to-middle-income countries, the amounts of inadequately disposed waste can be high, thus there is a risk of pollution of rivers and oceans such as in many countries of South Asia and sub-Saharan Africa, where about 80–90% of plastic waste is stored and disposed inadequately (Ritchie 2018).

In Europe, the declaration for a ban on single-use plastic and the creation of a circular economy in 2019 were great steps forward on the road to tackling plastic waste production and disposal issues (European Commission 2019).

Plastic waste comes in many sizes such as macro-, meso-, microplastic, microfibres and nanoplastics. All sizes and types of plastic and their associated chemicals are making their way into the environment through legal and illegal dumping, littering and landfill. Macro- and mesoplastic cause obvious devastation to wildlife and nature through such processes as entanglement, as well as being an

Table 1 Type of microplastics (Batel et al. 2016)

Type of microplastics
Primary microplastic
It is often added to cosmetic products as exfoliant and then wash down the drain and into the freshwater rivers, lakes and the sea
Secondary microplastic
It is the result of larger meso- or macroplastics that have been broken down or degraded to smaller fragments by weathering through UV light and exposure to other physical or biological processes
Tertiary microplastic
They are plastic pellets that are the building blocks of plastic material

eye sore when found discarded or washed up in nature (Carbery et al. 2018; Hayden et al. 2013; Lusher et al. 2017).

Microplastics are divided into primary, secondary and tertiary materials (Batel et al. 2016) (Table 1).

If these tiny plastics make their way into ecosystems, they are often mistaken for a food source by a selection of invertebrates (within marine, freshwater and terrestrial ecosystems), as well as juvenile fish species and enter the food chain or causing damage to these creatures after direct and indirect ingestion (Rillig 2012).

Although chemically inert, plastic has shown to have the property of a "bio-sponge". This means that it is very conducive to the adherence of various chemicals both added to the plastic during production or taken up from the environment in which it has found itself in such as a polluted part of the ocean, freshwater rivers, lakes or the soil (Rochman et al. 2013b). This quality makes plastic potentially toxic if ingested due to the nature of the chemicals which have been found adsorbed to the surface of microplastics (Batel et al. 2016; Raza 2018).

Based on the simulation performed by Koelmans et al. (2017) most of the plastic (99.8%) entered the ocean is settled below the ocean surface layer with an annual additional 9.4 million tonnes settling. Due to the different types of plastics and the wide variety of chemical substances absorbed or adsorbed to them, their toxic effects and mechanisms of action are variable, and manifold resulted in widely differing responses in individuals and species with different biological characteristics (Koelmans et al. 2017).

Furthermore, the microplastics in the aquatic ecosystem can be taken up by the animals during the food web. The marine zooplankton can ingest relatively small amount of microplastics settled in the ocean surface layer (<0.07%), however, it can be enriched and concentrated in the food chain including mesopelagic fish, seabirds and other aquatic animals. Thus, large amount of microplastics can be removed by marine organisms via ingestion of plastic debris, however, they are again returned to the ocean surface layer after gut passage and egestion settled in faecal pellets. Due to it, the plastic debris can be sedimented to the ocean floor resulted in impacts to mesopelagic and benthic communities (Koelmans et al. 2017).

Microplastics have been aptly described as being ubiquitous in the environment; meaning that they have been found everywhere. This fact raises concerns regarding

potential microplastic incorporation into the human food chain. It has been proven through various studies that humans ingest plastic from an array of sources (Van Cauwenberghe et al. 2015). It is important to determine the main routes of ingestion and how they can be quantified and prevented, and to conduct toxicological studies to determine the concentrations in which they cause harm or are toxic to human consumers. Many studies have been done with these questions in mind. Most have been conducted under laboratory conditions and exposures have often been much higher than would be found naturally in the environment, however, they still provide an indication as to the problems microplastics may cause if they continue to build up in the environment or within organisms.

There have been already developed many projects on methods for quantifying plastic in the environment, although there is need for more standardization. So, it is difficult to grasp the scale of the problem that is why it is necessary to develop new methods for detecting plastics within food items and study bioindicator species to help us monitoring the plastics in the ecosystem and their effects. We must look at the trophic cascade to determine potential hazards that could be inflicted upon humans and animals within the complex food webs of various ecosystems (Batel et al. 2016; Carbery et al. 2018).

The problem is that plastic is not the only potential risk issue facing our environment. Climate change, over population, political unrest, habitat fragmentation and loss, forest fires, loss of biodiversity, collapse of fish stocks due to overfishing, invasive species, acidification of the oceans, and pollutants from other sources such as heavy metals also play their part in threatening global biodiversity and species worldwide, but plastic, too, contributes to the pressures facing in the natural world. The plastic problem is just additive to these pressing concerns and it is important to grasp the impact it may have in terms of food safety for human and animal consumers and on protecting the biodiversity of our wildlife habitats. It must be noted that whatever is damaging to the environment will be damaging to humans in some way.

"In isolation, microplastics might not be the single most toxic (lethal or sublethal) environmental contaminant. However, there are consistent past, present, and future trends of increasing a near-permanent plastic contamination of natural environments at a global scale" (Geyer et al. 2017).

This literature review is based on the most recent studies available about the trends in plastic production and human interaction with plastic, the routes in which plastic may enter the food chain and the potential toxic or harmful effects they may pose to invertebrate and vertebrate organisms as well as food safety and security issues regarding humans as the main consumer of interest.

2 Plastic Material

The word plastic was derived from the Greek word "Plastikos" which means "capable of being shaped or moulded". This aptly describes the ductile and malleable nature of the material we know as plastic. It is a material consisting of a wide range

of synthetic or semi-synthetic organic compounds which can be moulded into solid objects (Lusher et al. 2017).

"Plastic" is an umbrella term that refers to a very large family consisting of many different materials all with varying characteristics, properties and uses. Plastic can be utilized in many areas of life and this explains the ubiquitous nature of the product. Plastic polymers have innumerable applications from microplastics, food packaging, clothing, toys, medical implants, piping, plumbing, furniture, etc. (Lusher et al. 2017). The invention of plastic initially meant less reliance on natural materials such as wood, bone, tortoiseshell, horn, metal, glass and ceramics, which was a benefit to the environment. However, due to humans ever increasing reliance on plastic and its ability to find its way into the environment, among others plastic has proven quite the burden on the natural world, accumulating in terrestrial, marine and aquatic ecosystems (Andrady 2011; Machado de Souza et al. 2018).

Plastic is usually derived from either fossil fuel based or bio-based materials. Most plastics are not or only limited degradable, however, one part of them can be degradable if disposed of correctly, but plastic disposal most often follows three main routes: landfill, incineration, recycling, or littering (Hayden et al. 2013; Machado de Souza et al. 2018; Shah et al. 2008). From the aspect of environmental pollution plastic has become a focus since the fact that much of it finds its way into the environment through many routes. It was estimated that annual eight million tonnes of plastic waste enter the ocean then these plastics interact with almost 700 marine species (Andrady 2011; Gall and Thompson 2015). However, plastics can be incinerated without significant waste production (except for carbon dioxide production) in appropriate establishments. Basically, a well-designed incineration process can remove more polycyclic aromatic hydrocarbons, polychlorinated biphenyls and dioxins from the incoming air used in the installation than is emitted by the waste stream.

Plastic can be categorized according to size: macroplastics, mesoplastics, microplastics and nanoplastics, but there is a wide range of their sizes recommended by different articles. Plastics less than 5 mm in size or between 5 and 1,000 μm are regarded as microplastics (Smith et al. 2018; Van Cauwenberghe and Janssen 2014). Nanoplastics have not been settled a standard size definition, but generally they are below 0.1 μm (Boyle and Örmeci 2020; Lambert and Wagner 2016). Macro- (>25 mm) and mesoplastics (5–25 mm) typically make up the plastic litter that is visible to the naked eye; while microplastics and nanoplastics consisting of plastic we usually cannot see easily or at all (Smith et al. 2018). Macroplastics can cause problems such as entanglements, ingestion in larger animals, are an eyesore in the environment, etc., but micro- and nanoplastics can cause problems such as bioaccumulation and biomagnification within the food chain. If ingested, these plastics also pose a threat due to their potentially toxic effects when acting as a bio-sponge (Lusher et al. 2017).

It has been documented and will be discussed later how microplastics interact with or are ingested by many small invertebrates such as Daphnia, Mussels and Earthworm across a range of ecosystems with organisms being affected either at the

tissue or cellular level (Farrell and Nelson 2013; Lwanga et al. 2017; Setälä et al. 2014).

Sometimes plastic can have additives incorporated into their creation process for them to have a variety of uses. These additives have the potential to be harmful to the environment and cause also harm to body tissues in large quantities (Andrady 2011). These include: Ultraviolet stabilizers; Lubricants; Colourants; Flame retardants; Plasticizers; Anti-oxidants; Phthalates; BPA; Nonylphenol (Lusher et al. 2017; Tsuguchika et al. 2011; Yogui and Sericano 2009). Microplastics also play a role in transferring persistent organic pollutants adsorbed to their surfaces. In several studies microplastics were shown to have rather high amounts of harmful substances such as polycyclic aromatic hydrocarbons, polychlorinated biphenyls, dichlorodiphenyl-trichloro-ethanes, perfluoro-octane-sulfonate and perfluoro-octane-sulphonamide (Lusher et al. 2017). These substances are found as pollutants in the environment while also being attracted to and adsorbed by microplastics that are found in the same environment as the pollutant. The consequences of ingesting these particles have been studied in small invertebrates and fish and their detrimental effects have been noted under laboratory conditions. However, in a natural setting the ingestion of these chemical-laden microplastics may not have the same affect at least to people, who are exposed to relatively few of these (Bakir et al. 2014; Lusher et al. 2017). Microplastics also exist as microfibres from polyester and nylon clothing which, once washed, release tiny fibres which are washed down the drain and reach the same fate and consequence as microplastics (Vianello et al. 2018).

2.1 Top 5 Plastics Found in Waste

Global generation of most important types of the primary plastic wastes was as follows in 2015: 57 million tonnes (MT) for low-density polyethylene, 55 MT for polypropylene, 42 MT for polyphthalamide and 40 MT for high-density polypropylene followed by polyethylene terephthalate (32 MT), polystyrene (17 MT), polyurethanes (16 MT) and polyvinyl chloride (15 MT) (Geyer et al. 2017).

2.1.1 Low Density Polyethylene (LDPE)

LDPE has a Society of Plastic Industry (SPI) resin ID code 4. LDPE was developed in 1939 by an accidental leak of trace oxygen during an experiment to reproduce polyethylene. It is produced by the ICI process for producing ethylene and is a thermoplastic (Gilbert 2017). LDPE has a density range of 0.917–0.930 g/cm^3. It is a flexible but tough plastic that can undergo temperatures of up to 80°C (Lusher et al. 2017). When compared to High-Density Polyethylene it has roughly 2% more branching on its carbon atoms that have weaker intermolecular forces. This in turn translates to higher resilience but a lower tensile strength, it also has a lower density due to its molecules being less tightly packed and has also fewer crystalline

molecules due to the side branches. It produces methane and ethylene when exposed to solar radiation. This material is used for an array of products such as containers, six pack rings, juice and milk cartons, computer hardware and hard discs, playground slides, plastic hinges on shampoo or ketchup bottles, plastic wraps and corrosion resistant work surfaces (Tripathi 2002).

2.1.2 Polypropylene (PP)

PP has an SPI resin ID code 5 meaning it is recyclable. Polypropylene is also a thermoplastic polymer with many applications. It is produced from the monomer propylene using chain growth polymerization. PP is very similar to polyethylene with a density between 0.895 and 0.92 g/cm^3. It is a tough and flexible material especially when copolymerized with ethylene. It can be used as an engineering plastic. When it was discovered, it was produced in large amounts, competing with materials such as acrylonitrile butadiene styrene (ABS). It is a very economical plastic with good fatigue resistance, it has excellent resilience against many forms of stress such as impact and freezing, and it is also resistant to corrosion and chemical leaching. Polypropylene has many uses. It is most famous for its plastic living hinges; however, it can also be used in clothing, stationery, packaging, carpets, clear bags and piping. In areas where other plastics may melt propylene will not. Many medical devices are made from PP (Gilbert 2017; Malpass 2010).

2.1.3 Polyphthalamide (PPA)

Polyphthalamide (PPA) belongs to the polyamide (nylon) family and it is in fact a subset of thermoplastic synthetic resins characterized by 55% more moles of carboxylic acid portion of repeating units in the polymer chain comprised of a combination of terephthalic (TPA) isophthalic (IPA) acids. The backbone of this polymer made from aromatic acids means that this material has a very high melting point, chemical resistance and stiffness. This means that PPAs have a better chemical resistance, higher strength and stiffness even at higher temperature, they resist creep and fatigue, have good resistance to warping and have also good dimensional stability while not being sensitive to moisture absorption (Malpass 2010).

2.1.4 High-Density Polyethylene (HDPE)

HDPE stands for high-density polyethylene. It is an often-recycled plastic with an ISO resin code of 2. HDPE is a thermoplastic polymer produced from the monomer ethylene. It is mostly used for plastic bottles, packaging and piping as it has a high strength to density ratio.

The density of HDPE can range from 930 to 970 kg/m^3. HDPE has a slightly higher density than LDPE but has much less branches which means it has stronger

intermolecular forces and tensile strength than LDPE. It is a harder plastic and less transparent and can also undergo higher temperatures (120°C) for short periods of time. However, it cannot withstand an autoclave.

It has a wide range of applications some of which are: water pipes, wood plastic, plastic surgery skeletal and facial reconstruction, shampoo bottles, sewage mains, etc. (Nagar 2006).

2.1.5 Polyethylene (PE)

PE has an ISO resin code of 1. There are several kinds of polyethylene as described above. It is a thermoplastic although it can become thermoset if modified. PE has a low strength, hardness and rigidity but can be modelled into many shapes. It has a low melting point around 105°C, but melting temperatures can vary. It is very chemically stable and is not affected by strong acid or base or minor oxidizing agents. It is not readily degraded but some bacteria have been known to degrade this plastic, it can also become brittle when exposed to UV light. It is a very good insulator, and it has massive application opportunities in packaging, drink bottles, 3D printing, thin solar cells and cellotape (Nagar 2006).

ISO resin codes can help the consumer figure out whether a plastic is recyclable or not. However, there is considerable consumer confusion when it comes to what they indicate and also many plastic products are made of more than one plastic type meaning they are more difficult to recycle (Gilbert 2017).

3 Degradation of Plastic Polymers

Degradation of plastic is defined as reducing the molecular weight of the polymers within the plastic material (Andrady 2011). Plastic is well known for its durable and stable nature and these characteristics make the degradation process in the environment incredibly slow. This is way why plastics persist in nature when not disposed of correctly. Plastic polymers which make their way into the environment are exposed to many different types of weathering influences. There are five main methods by which plastic degrades, the name of the process refers to the cause and type of degradation. (Andrady 2011; Bellas et al. 2016; Gewart et al. 2015) (Table 2).

Due to their larger surface to volume ratio microplastics usually degrade faster than larger meso- or macroplastics. This is because their polymer surface is exposed and prone to breakdown by chemicals or enzymes. The result of degradation at the surface is for the inside to become exposed for degradation and results in the plastic becoming brittle and disintegrating into smaller particles or flakes (Hayden et al. 2013).

Most often this process begins with photodegradation due to exposure to UV light from the sun, which gives the initial energy required to incorporate oxygen into the polymers. Plastic polymers begin to degrade in an aerobic environment that will

Table 2 Main degradation methods (Andrady 2011; Bellas et al. 2016; Gewart et al. 2015)

Main degradation methods
1. Hydrolytic degradation – reacting with water
2. Exposure to heat or thermooxidative degradation – a slow process involving oxidative breakdown in a moderate temperature range
3. Thermal degradation – degradation involving high temperatures which are not normally present in the environment naturally
4. Photodegradation through UV light exposure
5. Biodegradation within microbial cells by cellular enzymes

inevitably lead to thermodegradation. Over time, the plastic polymers become more and more brittle and break into smaller particles as the polymer chain decreases in molecular weight. This process will then lead to biodegradation by microorganisms. These microbes convert the polymer chains into biomolecules or carbon dioxide. This process takes very long, up to 50 or more years to fully degrade, however, there is dispute as to whether these polymers ever fully degrade as some scientists believe they can persist in the environment or landfill sites infinitely. Low temperatures and oxygen availability such as conditions in the ocean or in river ways can greatly lengthen the degradation time of any plastic material (Andrady 2011; Hayden et al. 2013). This is why plastic can persist for long periods of time in landfill and in the ocean as there is less oxygen, and it is exposed to cold temperatures (Andrady 2011).

3.1 Biodegradable Plastic

The invention of "bio-plastic" has arisen alongside the increasing need for alternative materials to plastic with a shorter and more efficient degradation time. Three main types have emerged thus far, these include

- oxo-biodegradable plastic which contains polyolefin plastic, and this contains metal salts in small amounts that aid the degradation process.
- biodegradable plastic that can be broken down into water and carbon dioxide by microorganisms
- bio-based plastics which are made from biological and renewable sources, within them is a weaker polymer structure which leads more readily to degradation when compared to the plastics currently in use.

Many of these plastics are now available and labelled often as "compostable", however, they must first reach compost and little research has been done on their degradation time and effect on the environment (Lusher et al. 2017).

4 Plastic Waste Disposal

4.1 Burying in Landfill

Landfill is defined as the burying of waste on excavated land. This has got obvious negative connotations as it is using land that could otherwise be used in a more profitable way such as for forestry or agriculture. Burying plastic in landfill leads to very slow degradation as the environment lacks oxygen and plastic degrades better in an aerobic environment. This slow degradation means that the land is therefore not viable for many years (Andrady 2011; Hayden et al. 2013).

There is another problem with burying plastic in landfill in which some plastics can leach pollutants as they degrade (Zhang et al. 2004). These pollutants include and are not limited to volatile organic chemicals such as xylene, benzene, toluene, ethyl/trimethyl benzenes and bisphenol A (BPA), a compound used widely in many plastics and resins (Lusher et al. 2017; Urase et al. 2008; Xu et al. 2011). These compounds are a cause for concern if they are continuously being exposed to the environment through the dumping of large amounts of plastic in landfill, however, it is BPA that has been under the most scrutiny in recent years.

BPA has been linked to numerous health risks and some research has shown that BPA can leach into food, beverages and the soil from containers that are made with BPA. Exposure to BPA has become a special concern because of possible side effects on the brain and prostate gland of foetuses, infants and children, even being linked to adverse behaviour in children. BPA has also been listed as an endocrine disruptor (Lusher et al. 2017). Moreover, when it comes to landfill BPA can leach into the surrounding soil and it has been correlated to increased populations of sulphate reducing bacteria in soil which has led to a rise in production of hydrogen sulphide, this can have lethal consequences in high concentrations (Hayden et al. 2013; Tsuchida et al. 2011).

4.2 Incineration

Incineration is the burning of waste products. Many countries use this method to some degree. Two positive aspects when comparing to landfill are that there is much less space being used up in this process and in some cases the heat generated from burning the materials may be used for energy. On the other hand, many pollutants are released to the atmosphere through the process of burning (Zhang et al. 2004). These include polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), toxic carbon and oxygen based free radicals, smoke (particulate matter), PCFDs and particulate bound heavy metals. Greenhouse gases, ethylene, methane and CO_2 are also released in this process. In some cases, the negative effects of the combustion emissions can be controlled by various means; (1) activated carbon

addition, (2) flue gas cooling, (3) acid neutralization and (4) ammonia, addition to the combustion chamber and/or (5) filtration (Yassin et al. 2005).

Due to landfill and incineration having many negative environmental effects recycling was developed as a potential alternative (Astrup et al. 2009).

4.3 Recycling

Plastic waste is being produced globally at an even growing scale per year and this increases the pressure on landfill and incineration as disposal methods for the material. This magnifies the environmental drawbacks outlined above with both the space and time needed for landfill and the harmful pollutants produced by each method. Recycling is therefore being investigated as the most sustainable solution for the repurposing of the plastic produced each year. Unfortunately, at present only approximately 9% of single-use plastics are recycled annually. Not all plastic can be recycled to the same degree and so they must first be separated (Hayden et al. 2013; Tartakowski 2010).

Plastic materials have various melting points, so mixing the polymers of different plastics can affect the characteristics of the plastic. For example, if HDPE and PP are melted together, they will form a brittle and weak secondary plastic product (Sanchez-Soto et al. 2008). The key to successful recycling methods is the accurate separating of mixed plastics and the grouping of identical materials. There are various ways of separating plastics including; Tribo electric separation, X-ray fluorescence, Fourier transformed infrared technique, Froth flotation method, Magnetic density separation and Hyper spectral imaging technology (Kumar et al. 2015). Recycling can be divided into four main techniques, such as primary, secondary, tertiary and quaternary (Table 3). Each has pros and cons to its techniques, however, once recycled will forgo some of its properties with relation to tensile strength, dimensional accuracy and wear properties. Recycling can be divided into mechanical and chemical recycling. The first three types of recycling unfortunately do have their limitations because plastic materials can only undergo 2–3 recycling cycles before they become an unviable material, in which phase the last type of recycling is utilized (Kumar et al. 2015; Sadat-Shojai and Bakhshandeh 2011; Subramanian 2000).

5 EU Legislation Regarding Plastic Waste

In Europe alone 25 million tonnes of plastic waste is generated every year with less than 30% being collected for recycling. The ten most commonly found single-use plastic items together make up 86% of all single-use plastics and therefore roughly 43% of all marine litter found on European beaches by the latest count. This, together with discarded fishing gear, which accounts for 27% of plastic, together

Table 3 Types of recycling techniques (Kumar et al. 2015; Sadat-Shojai and Bakhshandeh 2011; Subramanian 2000)

Types of recycling techniques
Primary recycling
It is also known as re-extrusion or closed loop process, this can only be done with clean or semi-clean scrap material and is the recycling of a single type polymer which has properties of a virgin material. This method is popular and easy to use and results in a good quality product like its original. It is a type of mechanical recycling
Secondary recycling
It is also a mechanical type of recycling that it is also a popular choice and is application of choice for many manufacturers. This process usually produces fewer demanding products and its steps include cutting/shredding, contaminant separation, flakes separation by floating. After this the single polymer plastic is processed and milled together to make a granulated form, the washing and drying is performed to remove all the glue residue.
Tertiary recycling
The main types of it are chemical and thermal recycling. It is beneficial because it extracts the raw materials from which the plastic polymer was created from, such as the petroleum-based products for example. This means that it contributes towards energy sustainability because we are extracting the building blocks necessary to form other plastics. This is achieved through processes such as pyrolysis, cracking, gasification and chemolysis
Quaternary recycling
The waste material is processed to recover energy through incineration, this also leads to reduction of waste and the rest is sent to landfill. This is the best alternative when the plastic has been used to its limits. Although the environmental concerns outlined above are still applicable here

accounts for almost 70% of all discarded plastic products on beaches. European citizens are suspected to inhale and consume microplastic particles on a regular basis as they have been shown to be present in the food they eat, in the air they breathe and in the water they drink, these plastics may have detrimental effects on the health of humans, and it is time for the European Union to begin to try and solve the problem or at least get a handle on it (Geyer et al. 2017; Hayden et al. 2013; Lusher et al. 2017).

The reasons and objectives of the single-use plastic directive proposal created by the European Commission are very relevant to this literature review because they highlight the main issues concerned:

- the fact that plastic litter is building up in the environment especially in aquatic and marine ecosystems through blow off from land-based litter and direct legal and illegal dumping activities,
- the negative impact on biodiversity of wildlife,
- the potential hazards to human health which are an increasingly alarming cause for concern and cause for action on the behalf of heads of state (European Commission 2018b).

Plastics are integral materials in use all over the world. Many people and businesses depend on plastic daily, and life would be very challenging without it. However, due to their durable nature they persist for very long periods of time

in the environment and this has detrimental effects in nature and to human and animal health (Geyer et al. 2017; Hayden et al. 2013).

Another problem with plastic waste is that it often does not remain in one place, it can be moved by the elements in land and sea, through anthropogenic and animal activity and once in the ocean, it can move with the currents and effect neighbouring countries. Thus, a collaborative effort is required to tackle this problem effectively. Meetings such as the G7 and G20 and implementations such as the UN Sustainable Development Goals are important in facing this issue on a global scale and hopefully will strengthen their efforts and enhance success rates (European Commission 2018b).

5.1 Circular Economy Action Plan

In recent years, countries have begun to prioritize action on plastics in the Circular Economy Action Plan, which was adopted in December 2015 in order to help European countries and consumers use plastic in a more environmentally friendly and sustainable way. A circular economy is an economic system that is based on removing the repeated need for raw materials and resources and also cutting down or eliminating completely any waste produced. The backbone of this system is the four "R" principles; reduce, reuse, recycle and repair. Plastic products in the future must be designed to fit this model (European Commission 2018a, b, 2019).

Under the new strategy, the European Union will plan an urge on the following.

5.1.1 Make Recycling Profitable for Business

Packaging on plastic will be labelled more clearly to improve the ease of recycling and separating plastics when it comes to disposing of them. Across the EU, plastic waste will be sorted and separated according to a standardized system. This will add value to the plastic product and help set up a more competitive and resilient plastic industry. This will hopefully be achieved by new rules on plastic packaging.

5.1.2 Curb Plastic Waste

A reduction in plastic bag use has already been seen due to the 2015, Plastic bag directive tax levy. Currently the main area of focus is on single-use plastics and fishing gear. Promoting awareness of the problems single-use plastic and fishing gear can cause when it reaches the environment as well as restricting the use of single-use plastic will encourage the consumer to be more mindful of their use of these items. The Commission shall also take measures to reduce the use of microplastics in various products, as well as create more transparent labelling for biodegradable and compostable plastic.

5.1.3 Stop Littering at Sea

New rules will be introduced to make sure that any waste that is generated at sea must return to land to be managed and is not simply discarded at sea.

5.1.4 Drive Investment and Innovation

Guidance for European businesses and national authorities on how to reduce plastic waste will be provided by the commission. This will support innovation dedicated to developing more recyclable plastic products, making more efficient recycling processes, and removing hazardous substances as well as tracing any contaminants from recycled products.

5.1.5 Spur Change Across the World

While the EU will continue to work on its plastic management, they will also link up with other countries for support and inspiration on how to find global solutions and to develop international standards to the plastic strategy.

The "European Strategy for Plastics in a Circular Economy" was introduced in January 2018. This will hopefully change the way products are used, produced, recycled and designed within the EU. New research can be carried out with regard to how many times a certain type of plastic can be recycled or how it breaks down emitting less harmful contaminants into the environment. This will encourage various companies to be creative with their plastic use and design and can create opportunities to add value in the industry.

If this strategy is implemented correctly it could help Europe ascertain its "Sustainable Development Goals" and the climate commitments which were underlined in the 2016, Paris Agreement as well as the EU's own industrial policy objectives. This strategy could lead to a more sustainable production and consumption of plastic materials whilst also helping the EU reduce its marine, aquatic and terrestrial ecosystem litter, reduce greenhouse gas emissions and limit our dependence on fossil fuel.

On March 27th, 2019, the European Parliament published a press release displaying measures proposed by the Commission to reduce marine litter from the ten most often found single-use plastic items on European beaches, and abandoned fishing gear and oxo-degradable plastics.

This proposal, also tackling single-use plastic, is a giant step forward for Europe as it highlights the fact that there is a problem with our reliance on single-use plastic and linking it to an environmental issue. It is in keeping with the Circular Economy Plan objectives, because the very essence of "Single Use Plastic" is completely opposed to the nature of the plan in that it is not "circular" by any means. It uses a

Table 4 Different solutions to reduce the volume of non-reusable plastic wastes (European Commission 2018b, 2019)

Solutions/suggestions
• A complete ban on items which there are alternative items available in the market such as: Straws, cutlery, cotton buds, stirrers, sticks for balloons and many beverage containers that contain expanded polystyrene and all oxo-degradable plastic
• Reducing the consumption of any food or beverage containers and cups made of plastic
• "Extended Producer Responsibility schemes" which will cover the cost of litter cleans up will be applied to certain products like tobacco filters and fishing gear
• The introduction of new designs which attach lids to bottles so they are not separate anymore as well as incorporating up to 25% plastic in PET bottles from recycled sources

great deal of raw material and produces a lot of non-reusable waste (European Commission 2018b, 2019) (Table 4).

This Directive was based on the 2015, Plastic Bag Directive also known as the Plastic Bag Levy which was implemented by what is known as placing a tax on consumer behaviour that brought about rapid shift. It is predicted that these new measures shall have a positive effect on the economy and on the environment if implemented efficiently and correctly. We can cut down on our carbon dioxide emissions, avoid damage to the environment predicted to cost up to 22 billion Euros by 2030 and save consumers an estimated 6.5 billion Euros. After approval by the European Parliament, the Council of Ministers must finalize the formal adoption. Member states will then have 2 years to incorporate the legislation into their national law.

Below is the European strategy for plastic in a circular economy from the European Commission singling out the top 10 single-use plastic items (SUP) which have been found on Europe's beaches. These top 10 items make up 43% of total marine litter. Fishing gear represents 27% of the marine litter (Table 5).

6 Environmental Aspects

Microplastics are now known to be accumulating and persisting in the environment in various ecosystems, however, the true scale of environmental risk remains uncertain (Andrady 2011; Koelmans et al. 2017). Microplastics are a growing cause of concern for environmentalists and can get from the environment into an animal directly and indirectly. The direct way is realized through accidental consumption by non-discriminate feeding methods; the indirect way is through trophic transfer and consumption of contaminated animals in the trophic level described below (Nelms et al. 2018). Animals consume the microplastics mistaking them for their own food source, because the microplastics can be covered with prey or are similar in size, shape and appearance. Often the plastic can be covered in a biofilm which helps camouflage the plastic and confuse the consumer (Carbery et al. 2018; Naji et al. 2018). Microplastics are not only a concern because of the risks associated

Table 5 Type and origin of single-use plastic items (SUP) found on Europe's beaches (European Commission 2018a)

Type of plastic	Consumption reduction	Market restriction	Product design requirement	Marking requirements	Extended producer responsibility	Separate collection objective	Awareness raising measures
Food containers	X				X		X
Cups for beverages	X				X		X
Cotton bud sticks		X					
Cutlery, plates, stirrers, straws		X					
Stick for balloons		X					
Balloons				X	X		X
Packets and wrappers					X		X
Beverage containers, their caps and lids			X		X		X
Beverage bottles			X		X	X	X
Tobacco product filters					X		X
Wet wipes (sanitary)				X	X		X
Sanitary towels				X			X
Lightweight plastic carrier bags					X		X
Fishing gear					X		X

with consumption but also due to their ability to transfer pollutants adsorbed to their surfaces, however, whether the amount of these chemicals is enough to drastically interfere with animal or human's health is still relatively unknown (Bakir et al. 2014). In 2016 a report by the UN (United Nations) documented that over 800 animal species were affected by plastic through ingestion or entanglement (UNEP 2017). This figure has risen by 69% since a similar study was carried out in 1977, where plastics, mostly filaments were found in the stomach of 83% of 120 investigated decapod crustacean (*Nephrops norvegicus*) followed by tightly tangled balls of plastic strands in 62% of the animal tested (Murray and Cowie 2011).

Similarly, Kühn et al. (2015) noted that the number of the affected aquatic animals due to entanglement of ingestion of plastic debris was increased between 1997 and 2015 (from 267 species to 577 species) including, e.g. marine turtles (86 to 100%), marine mammals (43 to 66%) and seabirds (44 to 50%), but the variances between the number of the species investigated was lower.

Certainly, the increase in the number of cases has changed not only due to the increasing quantity of plastics released into the environment, but also with the development and use of newer and "more modern" and "finer" measurement methods, that are suitable for detection very small particles. The production of plastic was only two million tonnes (MT) in 1950 which was extremely increased till 2015 reaching 381 MT (Ritchie 2018). The extent of the contamination and the number of cases can be influenced by the examined area, among many others, e.g. the basin of the North Pacific Ocean is the most contaminated (Eriksen et al. 2014).

Furthermore, the size of the animals can determine the size of the ingested plastics (microplastic, nanoplastic, etc.). The plastic fibres can be consumed by filter-feeding aquatic organisms (mussels, oysters, etc.), however, the larger plastic objects (e.g. plastic films or food packaging materials, rope, hose, flowerpot, plastic sheeting) can be taken up by large fish species, whales and marine mammals or seabirds (de Stephanis et al. 2013).

In a food chain or ecological pyramid there are several levels of succession. These are known as "Trophic levels". The ingestion of plastic occurs within many different trophic levels (Boerger et al. 2010; Smith et al. 2018). Beginning at trophic level 1 with primary producers which are organisms that have the ability to carry out photosynthesis and therefore produce their own energy and food using sunlight. Following this is trophic level 2; this level is occupied by organisms which feed on the plants known as herbivores, the next trophic level 3 is occupied by an omnivorous or carnivorous predator and usually trophic level 4 or 5 is filled by the apex predator. Usually, the last trophic level is occupied by decomposers also known as "detritivores" (O'Callaghan 2013).

Typically, there are four to five trophic levels in any ecological system. When one organism consumes another, energy is transferred through the trophic levels, this is known as "Trophic level transfer efficiency". Energy will decrease as we move further up the levels. The goal of this review is to explore whether microplastics can be transferred through the trophic levels, and what the consequence of this interaction is (O'Callaghan 2013).

Studies have been carried out depicting the trophic transfer in both wild animals and animals subjected to microplastic exposure under laboratory conditions which have shown transfer of particles within food webs and throughout trophic levels (Farrell and Nelson 2013; Nelms et al. 2018; Setälä et al. 2014; Welden et al. 2018).

6.1 Terrestrial Ecosystem

Most of the current research has focused on marine ecosystems while the effects of microplastics have not been so well documented in the terrestrial systems, however, almost all the plastic present in the marine or freshwater ecosystems was first either created, used or discarded on land. This plastic would have faced various environmental influences that would affect its fate and effect on the terrestrial ecosystems and organisms within it. It is said that microplastics may accumulate in terrestrial and continental food webs in the same way as marine or freshwater systems, however, there is more research to be done in this area (Horton et al. 2017; Jambeck et al. 2015; Lebreton et al. 2017; Machado de Souza et al. 2018).

Where microplastics were found in the digestive tract of continental birds, they were often seen to be much smaller than their usual forage material, indicating accidental ingestion, or they arrived there through the route of trophic transfer (Gil-Delgado et al. 2017; Zhao et al. 2016).

One of the first quantitative assessments with regard to trophic transfer in terrestrial organisms showed the presence of microplastic in soil, earthworms and chicken faeces (Lwanga et al. 2017). There has been evidence to suggest that bioaccumulation of microplastics could be widespread in the terrestrial environment since it has been shown to accumulate in yeasts and filamentous fungi that suggests accumulation or magnification along the soil detrital food web (Machado de Souza et al. 2018; Schmid and Stoeger 2016).

Although the uptake of plastic is at a very low level with minimal toxicity at this stage, it is a concern that the continual and cumulative exposure to microplastics and these toxins could have in the long term resulted in increased toxicity, interaction or other unwanted effects. Indeed, in Lwanga et al. (2017) a growth reduction was observed in earthworms with 150 μm of microplastics in their food. This energy loss at the lower level of the trophic cascade affects the energy transfer to the higher trophic levels. This growth reduction could be associated with the poor nutritious quality of the microplastics, or the potentially toxic effects such as damage to the digestive histology and alterations in the gene expression (Rodriguez-Seijo et al. 2017). Furthermore, the plastics may be a vector for metal exposure in terrestrial invertebrates due to the affinity for zinc to adsorb to high-density polyethylene microplastics (Hodson et al. 2017).

An experiment exposing yeasts and filamentous fungi *Aspergillus* spp. to polystyrene nanobeads with a size of 50, 100 and 500 nm (for yeast) and 100 nm (for fungi), respectively, resulted in lethal toxicity and 100% mortality of yeasts and varying effects on the fungi based on their levels of hydrophobicity within their cells

(Miyazaki et al. 2015; Nomura et al. 2016). This shows the effect of microplastics at varying trophic levels and it is unknown as of yet what overall affect this will have on the terrestrial food web or chain, however it is known that pollution at any level, especially the subcellular level can incur negative effects later on (Machado de Souza et al. 2018).

Recently, nanoplastics are under closer scrutiny than the larger plastics, as these have the potential to adsorb an even larger amount of harmful and potentially toxic chemicals due to their small size and large surface area. If these particles become attached to the external surface of cells, they can influence several membrane processes involved in intracellular homeostasis in an organism exposed to them. Miyazaki et al. (2015) displayed how the nanoplastic exposure disrupted the electrostatic interaction between particles and cell walls, which in turn affected the membrane processes of cells. Carboxylate- and amine-modified ultraclean polystyrene particles with sizes of 24–110–190 nm were also shown to affect the lung responses in rabbits after exposure by causing peripheral thrombosis displayed after histopathological examination (Hamoir et al. 2003).

Linking these issues to humans as an apex terrestrial organism, the experiments were shown to report changes in gene expression, as well as inflammatory and biochemical responses after nanoplastics exposure (Forte et al. 2016; Galloway 2015). Forte et al. (2016) noted that the unmodified polystyrene nanoparticles with a size of 44 nm (1%) rapidly and more efficiently accumulated in the cytosol of gastric adenocarcinoma cells than that of 110 nm (1%) using energy dependent mechanism of internalization and a clathrin-mediated endocytosis pathway. Both size of nanoparticles modified cell viability, inflammatory gene expression and cell morphology, but the smaller particle produced strongly the up-regulation of interleukins (IL-6 and IL-8). The properties of nanoplastic adsorption are influenced by their surface, size, electric charge and hydrophobic properties (Schmid and Stoeger 2016).

It is vital that more research is done in the area of terrestrial organisms, trophic cascade and ecosystem interaction in order to display the potential risks involved with biodiversity and ecotoxicological effects of microplastic build-up in the food chain. This is important both to predict future negative impacts, to find ways to prevent them and to also develop government policies in order to protect the organisms and ultimately humans from potentially negative or toxic effects (Machado de Souza et al. 2018).

6.2 Marine Ecosystem

Microplastics are found throughout the ocean, from coastal areas to surface water to subtidal sediments, sea ice to the deepest gyres. Due to this distribution, they are available for ingestion to all marine animals, as illustrated in a number of studies and experiments (Carbery et al. 2018). There is evidence to suggest that microplastics, depending on their size, are transferred from animal to animal through the trophic

cascade within the marine ecosystem, including different fish species (e.g. herring [*Clupea harengus*], Atlantic mackerel [*Scomber scombrus*]), oysters (e.g. Pacific oyster [*Crassostrea gigas*]) and shellfish (e.g. blue mussel [*Mytilus edulis*]), crustaceans (e.g. brown shrimp [*Crangon crangon*], North Pacific krill [*Euphausia pacifica*], goose barnacles [*Lepas* spp.], Norway lobster [*Nephrops norvegicus*]) and Annelid worm (e.g. Northern lugworm [*Arenicola marina*]) (Carbery et al. 2018). This was a pivotal discovery as it shed new light on the potential bioaccumulation and biomagnification of these particles building up inside the marine food web. Microplastics have entered the food chain and if persistent enough, could in fact make their way to humans, with consequences yet unknown.

Microplastics are in the size range of plankton and grains of sand, meaning that they are often introduced readily into the food chain by the accidental ingestion by marine invertebrates consuming items of this size as their primary diet (Carbery et al. 2018). They are consumed by a variety of invertebrates including benthic species, selective and non-selective filter feeders, deposit feeders and detritivores (Browne et al. 2011; Van Cauwenberghe and Janssen 2014). Similarly, the nanoplastics may be harmful to the marine ecosystem and it is also very difficult to quantify. Positively charged nanoparticles have been shown to adsorb to the cellulose in algae, affecting its photosynthesizing abilities, as a primary producer this could have major knock-on effects in the marine food web (Battacharya et al. 2010).

One of the first studies which investigated the trophic level transfer of microplastics in marine animals was carried out in 2013 by Farrell and Nelson. The key to this investigation was determining whether microplastics did transfer from one organism to the next via ingestion, to quantify the microplastics transferred and to give a clue as to the persistence of the particles within the organism. This study was done on mussels and crabs and was the first to show "natural" trophic transfer of microplastics from one organism ingesting another. The blue mussels (*Mytilus edulis*), a very important food for many animals including humans, were exposed to 0.5 μm fluorescent polystyrene microspheres. The mussels were then fed to crabs (*Carcinus maenas*). The results showed that the crabs had a maximum amount of 0.4% microspheres that the mussels were exposed to initially. The important discovery was that the microspheres were not only found in the alimentary canal; in the stomach, hepatopancreas, but also in the ovary, gills and even into the haemolymph of the crab from the mussel.

Huntley and Boyd (1980) displayed that zooplankton, a typical marine trophic level 2 consumer, consumed microplastic spheres in place of phytoplankton. Researchers showed on Baltic Sea Zooplankton that microplastic spheres could also be passed from one trophic level to a higher one; mesozooplankton to macrozooplankton carried out by feeding fluorescent microspheres to grazing zooplankton (Desforges et al. 2015; Setälä et al. 2014). The focus of this experiment was to show direct ingestion of microspheres by many species of zooplankton found in the planktonic web and to verify microplastic introduction into the planktonic food web by then feeding the zooplankton to mysid shrimp and polychaete larvae species.

The ingested particles have the potential to pass through or block the gut, be absorbed, or accumulate in the digestive tract and therefore perhaps hinder digestion

or feeding. There is also the potential risk associated with chemicals adhered to the microplastics, indicated by Rochman et al. (2014) which revealed the potential for endocrine disruption in fish exposed to the chemicals present in the microplastics, which could have major repercussions for reproductive success and wildlife populations.

It was shown that the mysid shrimp and polychaete larvae ingested the microspheres both directly and indirectly by consuming the zooplankton that had been previously exposed. An important point was raised that since both of these species live in the "pelagic and benthic realm" it is possible for both animals to introduce microplastics into both food webs, causing potential harm to multiple species which prey on them (Setälä et al. 2014).

An experiment carried out on captive grey seals (*Halichoerus grypus)* and wild caught Atlantic mackerel (*Scomber scombrus*) indicated that roughly half of the scat samples and a third of the fish contained microplastic, with ethylene propylene being the most frequently found polymer in both. This was an important experiment because it indicated that microplastics could be transmitted indirectly to an apex predator and could outline how thorough eating fish humans could also be exposed to microplastics (Nelms et al. 2018).

Generally, the digestive tract of the aquatic organisms contains the largest amounts of microplastics and nanoplastics, however, it is normally removed from larger fish (e.g. tunas [*Tunnus* spp.], salmons [*Salmon* spp., *Oncorhynchus* spp., etc.], crustacean (e.g. *Nephrops* and *Homar* spp.) and other species before consumption resulted in limited risk to human consumers. In contrast, the most bivalves (e.g. blue mussel [*Mytilus edulis*], oysters [*Crassostrea* spp.], etc.), some echinoderms (brown sandfish [*Holothuria spinifera*], black teatfish [*H. nobilis*], Japanese sea cucumber [*A. japonicus*], giant California sea cucumber [*Parastichopus californicus*], etc.), and several small species of fish species (sprat spp. [*Sprattus sprattus*], sardine spp. [*Sardina pilchardus*], sardinellas (e.g. goldstripe sardinella [*Sardinella gibbosa*], round sardinella [*S. aurita*] are eaten whole leading to microplastic exposure (Carbery et al. 2018; Galloway 2015; Lusher et al. 2017).

Carbery et al. (2018) stated that although there had been many lab studies carried out which depict marine animals consuming microplastic and the biological effects this had on the animal in question, experiments had also shown evidence of trophic transfer of microplastics through these marine animals consuming each other, however, these had been conducted under experimental lab conditions and not mimicking actual levels available in the environment. Their paper outlines the importance for more research to be conducted from this aspect. Since plastic is present in many seafood items it did display that humans would be at risk from eating contaminated seafood products, adding to the fact that currently microplastic is not quantified or monitored within seafood being sold to humans it is very difficult to assess the actual risk posed to human health from the marine exposure route. This does raise concern considering medical studies conducted on humans and rats demonstrated movement of polystyrene and polyvinylchloride (PVC) particles from the gut cavity and circulatory system. The absorption of 100-nm size polystyrene particles was higher than that of 1-μm microplastics, however, the uptake of

poloxamer coated 60-nm polystyrene nanoparticles was reduced (1.5–3%) (Hussain et al. 2001).

However, nanoparticles are used to adsorb different drugs (peptides, proteins, oligonucleotides, antibacterials, antifungals, chemotherapeutics) on their surface (as nanoparticle encapsulated drug) inducing the absorption and/or translocation of the therapeutic medicines via the intestinal lymphatic system (e.g. Peyer's patches), and to increase their resistance against enzymatic degradation (Hussain et al. 2001).

Vethaak and Leslie (2016) also showed that the microplastic particles could cross the placenta and blood brain barrier. It is difficult to say whether humans are being also exposed to microplastics from meats and beverages as they can be present in other food or drink items from packaging or cling film wrap and they can even be inhaled through inhalation of tiny particles in the air.

It has been displayed that microplastics can be transferred through marine food webs, however, the effect this has on higher organisms and apex predators is still poorly understood (Carbery et al. 2018).

6.3 Freshwater Ecosystem

In comparison with marine ecosystems aquatic freshwater ecosystems have been less examined from the aspect of trophic transfer in food webs but there is new research beginning to emerge in the last few years. The threats in freshwater systems are the same as with terrestrial and marine and in fact recently microplastics have been found in lakes, rivers and estuaries all over the world. They are also thought to be an important contributor of terrestrial litter to the marine ecosystems. From the Danube it is estimated that over tonnes of plastic are deposited into the Black Sea annually (Free et al. 2014; Lechner et al. 2014). Microplastic ingestion by freshwater invertebrates has yet to be displayed outside of a lab experiment (Hurley et al. 2017).

Batel et al. (2016), conducted on *Artemia sp.*nauplii and zebrafish (*Danio rerio*) aimed to display how microplastics had the potential to transfer and accumulate along the artificial food chain in aquatic environments and to explore whether the harmful substances are transferred along with the plastics in an artificial food chain created under laboratory conditions. The results of the experiment showed that it is true that microplastics are transferred along with their associated chemicals through the various trophic levels. This experiment was important in displaying trophic transfer from an invertebrate to a vertebrate animal, however, the experiment displayed that the microplastic highlighted with fluorescent dye passed almost completely through the Zebra fish without much evidence for accumulation or absorption through the enterocytes or epithelial cells and also showed no evidence for severe disease to the zebrafish (Batel et al. 2016).

In Manchester, an experiment showed that *Tubifex tubifex* worms ingested microplastics and microfibres under lab conditions at varying concentrations. These worms were shown to ingest and tolerate very high concentrations of plastic, much higher than was shown in other freshwater or marine invertebrates. Therefore,

meaning they had the potential to pass on large quantities of plastic through the trophic levels. These worms are at the bottom of the food chain as it is a food source for many larger invertebrates such as leeches as well as small fish, salmon and trout which are a link to the human food chain (Hurley et al. 2017).

6.4 *Microplastics in Aquatic Organisms of Commercial Interest*

The main focus of this literature review is to explore how plastic enters the marine or freshwater ecosystems, how it can infiltrate food webs and what harm this may cause to humans and animals (Holman et al. 2013). Humans may be exposed to microplastics through various routes, however, a point of interest for this literature review is how microplastics may be taken up by the ingestion of fish and bivalves of commercial interest. Either fish farmed in fisheries, aquaculture centres or wild caught fish.

Fisheries and aquaculture centres have often used plastic in many forms such as ropes and netting, boat construction, boat maintenance, fish hold insulation, fish crates, seafood packaging and transportation, floats, fish crates and boxes, fish cages, pond lining, fish feeders, fish tanks.

Often netting and structures for catching fish are kept buoyant by different types of plastic buoys. Sometimes these structures break free or get lost in stormy weather conditions or when they become too old are simply discarded into the waterways such as oceans, lakes and rivers. Abandoned, lost, or otherwise discarded fishing gear (ALDFG) are said to be the most prominent form of plastic waste in the marine and freshwater environments, however, to date there are no definitive numbers for the quantity of ALDFG waste in these environments. These materials become marine litter and cause problems for animals that become entangled in the fishing gear, ropes and netting. This plastic also breaks down to smaller plastic particles (Lusher 2015).

There are many other sources of plastic in waterways, however, ALDFG has been shown to be a considerable contributor to marine and freshwater plastic waste.

It has been shown that animals from aquaculture centres are ingesting microplastics also (Cheung et al. 2018; Renzi et al. 2018). The most prone organisms to this are bivalves which have been cultivated in lagoons or estuaries contaminated with plastic (Lusher et al. 2017).

The potential for microplastics to interfere with the fishery and aquaculture industry is a cause for concern for humans both economically and with regard to the health of the consumer. Indeed, it is a threat to food hygiene and safety if we are marketing animals in the marine and aquaculture industry for human consumption which may be contaminated with harmful chemical containing microplastics or fibres. There is minimal information with regard to the impact of microplastics upon freshwater ecosystems which means it is difficult to accurately assess and

project the affect they will have upon aquaculture and freshwater fish species. There is the potential risk for food safety concerns and similarly for the revenues of fishery and aquaculture centres (Medrano et al. 2015).

Many species of commercial fish consumed by humans have been shown to have ingested microplastic. These species include but are not limited to Atlantic cod (*Gadus morhua*), European Pilchard (*Sardina pilchardus*), red mullet (*Mullus barbatus*), Atlantic horse mackerel (*Trachurus trachurus*), European Sea bass (*Dicentrarchus labrax*), bivalves (e.g. blue mussel [*Mytilus edulis*]and oysters [*Crassostrea gigas*]) and crustaceans (e.g. brown shrimp [*Crangon crangon*]) (Avio et al. 2015b; Bessa et al. 2018; Brate et al. 2016; Devriese et al. 2015; Güven et al. 2017; Lusher et al. 2013; Van Cauwenberghe and Janssen 2014).

Van Cauwenberghe and Janssen (2014) carried out research to explore the relationship between the ingestion of seafood and exposure to microplastic particles focusing on blue mussels (*Mytilus edulis*) and Pacific oysters (*Crassostrea gigas*) and displayed how they were a source of plastic exposure to humans but there are also other species and sources which pose a similar hazard. Their results are summarized, such as:

- European countries with high consumption of shellfish had average consumer levels of up to 11,000 microplastic particles per year. This was found in Belgium who had the highest per capital intake of microplastic particles in which the average intake was 72.1 g/day.
- European countries with very low consumption of shellfish had levels of approximately 1800 microplastic particles per year. This was countries like France and Ireland who had approx. 11.8 g/day consumption rates.

Initially, it was believed that commercial fish especially those that are farmed in managed centres and fisheries may not be exposed to the same amount of plastic as wild fish. However, a study carried out by Hanachi et al. (2019) in Germany showed through Fourier Transform Infrared Spectroscopy (FTIR) that microplastics were present in high quantities of fish meal being fed to fish farmed in aquaculture centres. This indicates that fish kept in this way may be at a higher risk of exposure to microplastics than their wild counterparts as they are sometimes being directly fed microplastics and have no other alternative non-contaminated food source. There was a positive relationship between the microplastics levels in fish meal and the plastic found to be ingested by common carp (*Cyprinus carpio*) which underlines the theory that fish meal created from marine sources may be a way in which plastics are introduced to cultured fish and thus the human food chain. During the experiment careful consideration was given to plastic contamination from other sources and so special measures were taken to ensure this did not happen. Upon examination, microplastics were discovered in the gastrointestinal (GI) tract and the gills; naturally, GI tracts contained the highest concentration of microplastics in comparison with the gills. The most common plastics found were identified as polystyrene and polypropylene. This study is very important in proving another way in which humans may be exposed to microplastics and how the plastics themselves are a human health and food safety risk (Hanachi et al. 2019).

There has also been evidence of fish and other farmed animals such as shrimps being fed meal made from other animals including other fish which contained plastic as above (GESAMP 2016). Food which has been sold for human consumption has been identified as containing microplastic, it has included fish and shellfish purchased in fish markets (Li et al. 2016; Neves et al. 2015).

When investigating Shrimp consumption, it was discovered that up to 175 microplastic particles were estimated to be consumed per person per year (Devriese et al. 2015). Where mussels are consumed by humans Vandersmeersch et al. (2015) discovered that the blue mussel (*Mytilus edulis*) and Mediterranean mussel (*Mytilus galloprovincialis*) from Denmark, France, Spain, Italy and The Netherlands all contained microplastic particles. In Belgium, a country that has very high shellfish consumption rates it was found that in every 10 g of mussels at least 3 to 5 microfibres were discovered (De Witte et al. 2014). In China, a study was conducted on microplastic presence in bivalves for commercial use and human consumption with interesting results. The study reported that per gram of bivalves there were 2–11 microplastic particles and figures varied from 4 to 57 items per individual bivalve (Li et al. 2016).

In the Persian Gulf, five shellfish species were found to have between 3.7 and 17.7 microplastic particles per individual (Naji et al. 2018).

In the Mediterranean microplastics were found in the stomach of important commercial fish and similarly in the liver and gastrointestinal tract of sardines and anchovies that are usually consumed whole (Avio et al. 2015b; Collard et al. 2017; Romeo et al. 2015). Commonly microplastics are found in the stomach and gastrointestinal tract and since this portion is removed in the seafood preparation process it is logical to expect that a consumers exposure to plastic particles is greatly reduced (Wright and Kelly 2017) and so fish that are consumed in its entirety such as sardines, sprats and other juvenile fish pose a more urgent threat, however, there is evidence to suggest that microplastic particles also migrate through to the muscles and other eviscerated parts of the fish. This was found in two species used for dried fish consumption (*Chelonia subviridis, Johnius belangerii)* that was found with much higher levels of microplastic particles in its viscera and gills. Proving that eviscerating in some cases does not remove the microplastic ingested by human consumers (Karami et al. 2017). Abassi et al. (2018) also noted microplastic in the muscle of another important commercial fish and crustacean. Although this is less common it does show that more research is needed to understand the transit of microplastic in commercially important fish worldwide and determine what risks to human food safety are involved.

A study conducted in Makassar, Indonesia and California, USA indicated that fish and shellfish being sold to the public for human consumption contained plastic. The study showed that in Indonesia 28% of individual fish and 55% of total fish sampled contained microplastic. Likewise, in the USA 25% of individual fish and 67% of totally fish sampled contained microplastic. In Indonesia, the debris found was seen to be mostly microplastic whereas in USA the debris was mostly microfibres. Debris was also found in 33% of shellfish sampled. This was a pioneering study indicating that fish being sold to the public were contaminated in

plastic. It was noted in the study that both Indonesia and the USA rank highly when it comes to poor management of anthropogenic waste (Rochman et al. 2014).

Microplastics were also noted to have contaminated 11 out of 25 most important species of fish which are part of the global marine fisheries, this raises a concern because as of yet not enough study has been done to show the interaction with humans and microplastics and it is an area that needs urgent attention from food safety authorities and the World Health Organization expert committee on food additives (Barboza et al. 2018; FAO 2017; Lusher et al. 2017).

Nowadays, the analytical methods for the detection and quantification of microplastics, nanoplastics and other sizes of plastics in the environment including water, sediments and ecosystem, in human tissues, blood and in foodstuffs should be developed and standardized, because the currently existed analytical methods including their type of methods, limit of detection, determination of different size, quality control, etc. are different and compared difficulty. Furthermore, most of the studies focus on "individual" effect caused by different size of plastics under laboratory circumstances (e.g. in mesocosm studies), and the population levels studies are missing focusing of microplastic uptake in farmed and wild aquatic animals (seafood), however, they may be influenced by multifactorial aspects including environmental and human activities.

7 Food Safety Aspects

7.1 Adverse Effects

The fragmented or degraded plastic particles such as micro- and nanoplastics and other types can destroy and perish different animal species including zoo- and phytoplankton, vertebrates and invertebrates which resulted in several unexpected, unwanted adverse effects (Sana et al. 2020). Adverse effects have been shown in various marine, freshwater and terrestrial organisms directly or indirectly caused by exposure to microplastics in laboratory conditions. These effects have included mortality, reduced feeding, body and metabolic rate, reduced allocation of energy for growth, decreased predatory performance, changes in behavioural responses and reduced swimming performance, decreased fertilization and larval abnormalities, neurotoxicity due to acetylcholinesterase inhibition and oxidative stress, intestinal damage and other several adverse effects. The details of these experiments are summarized in Table 6.

The micro- and nanoplastics have ability to absorb or adsorb and interact with other environmental and industrial pollutants (e.g. metals, pharmaceuticals, emitted other contaminants) in the environment during the food chain. Therefore, they can directly influence or modify on fate and toxicity of these substances or of each other to the environment and organisms including their toxic interaction in toxicokinetic and toxicodynamic properties (Sana et al. 2020).

Table 6 Adverse effects caused by microplastics in different organisms (marine, freshwater, terrestrial animal species) under laboratory conditions

Adverse effect	Animal species	Microplastic			Reference
		Type/name	Concentration	size	
Alterations of immunological responses, lysosomal compartment, peroxisomal proliferation, antioxidant system; neurotoxic effects; onset of genotoxicity; changes in gene expression profile	Mediterranean mussel (*Mytilus galloprovincialis*)	Polyethylene Polystyrene	0.5 μg/L, 5 μg/L, 50 μg/L	1,000–100 μm	Avio et al. (2015a)
Neurotoxicity; inhibition of acetylcholinesterase activity; increased lipid oxidation in brain and muscle; changed the activities of the energy-related enzymes	European seabass (*Dicentrarchus labrax*)	Fluorescence red polymer microspheres	0.26 mg/L, 0.69 mg/L	1–5 μm	Barboza et al. (2018)
Decreased food consumption; weight loss; energy depletion	Lugworm (*Arenicola marina*)	Polystyrene	1, 3, 10, 30, 100 g/L	–	Besseling et al. (2013)
Growth inhibition; increased mortality	Earthworms (*Eisenia fetida*)	Polystyrene	1%w/w, 2%w/w	58 μm	Cao et al. (2017)
Inhibition of acetylcholinesterase activity	Zebra danio (*Danio rerio*)	Polystyrene	1 mg/L	50 nm	Chen et al. (2017)
Liver inflammation; changes in metabolic profiles (energy and lipid metabolism); oxidative stress	Mouse (*Mus musculus*, ICR)	Polystyrene	–	5 μm, 20 μm	Deng et al. (2017)
Behavioural abnormalities: reduction of the predatory performance and efficiency, reduction of food intake	Common goby (*Pomatoschistus microps*)	Polyethylene	White: 1.2 g/cc density; black: 1.15 g/cc density; red: 0.98 g/cc density	420–500 μm	de Sá et al. (2015)
Negative effects on growth, reproduction	meta-analysis of studies performed on different species	Microplastics	–	–	Foley et al. (2018)

(continued)

Table 6 (continued)

Adverse effect	Animal species	Microplastic			Reference
		Type/name	Concentration	size	
Mortality	Daggerblade grass shrimp (*Palaemonetes pugio*)	Polyethylene, polypropylene, polystyrene	2000 particles/400 mL (=50,000 particles/L)	Sphere (30, 35, 59, 75, 83, 116, 165 μm); fragment (34, 93 μm), fibre (34, 93 μm)	Gray and Weinstein (2017)
Decreased growth rate	Earthworm (*Lumbricus terrestris*)	Polyethylene	–	<150 μm	Huerta-Lwanga et al. (2016)
Adverse effects on growth rate and fecundity	Copepod (*Paracyclopina nana*)	Polystyrene microbead	10 μg/mL	0.05 μm, 0.5 μm	Jeong et al. (2017)
Inflammation, oxidative stress and disrupted energy metabolism	Zebra danio (*Danio rerio*)	Polystyrene	–	5 μm, 70 nm	Lu et al. (2016)
Mortality; decreased fish predatory performance; inhibition of acetylcholinesterase activity	Common goby (*Pomatoschistus microps*)	Polyethylene	1.2 g/cc density	1–5 μm	Luis et al. (2015)
Lowest fertilization rate; developmental abnormalities on embryo	Sea urchin (*Paracentrotus lividus*)	Polystyrene Polyethylene	– –	6 μm 0–80 μm	Martínez-Gómez et al. (2017)
Altered behaviour and metabolism; depleted energy reserve	Crucian carps (*Carassius carassius*)	Polystyrene	130 mg/feed	24 nm, 27 nm	Mattsson et al. (2015)
Changes in morphology (developmental abnormalities)	*Hydra attenuata*	Polyethylene	0.01, 0.02, 0.04, 0.08 g/mL	<400 μm	Murphy and Quinn (2018)
Neurotoxicity; reduced acetylcholinesterase activity	Common goby (*Pomatoschistus microps*)	Polyethylene	18.4 μg/L, 84 μg/L	1–5 μm	Oliveira et al. (2013)
Toxic effects at tissue, cellular and molecular levels; histopathological damages and increased levels of antioxidant markers	Mussels (*Mytilus edulis*, *M. galloprovincialis*)	Polystyrene	32 μg/L	2 μm, 6 μm	Paul-Pont et al. (2016)

Pathological alterations of distal part of intestine	European seabass (*Dicentrarchus labrax*)	Polyvinyl chloride	0.1% (w/w) in feed pellet	<0.3 mm	Pedà et al. (2016)
Immobilization	*Daphnia magna*	Polyethylene	12.5–400 mg/L	1–100 μm	Rehse et al. (2016)
DNA damage; neurotoxicity; oxidative damage; genotoxicity	Clam (*Scrobicularia plana*)	Polystyrene	1 mg/L	20 μm	Ribeiro et al. (2017)
Liver toxicity, hepatic stress; changed endocrine function; gene expression	Japanese medaka (*Oryzias latipes*)	Low-density polyethylene	8 μg/L	3 mm (diameter)	Rochman et al. (2013b, 2014)
Decreased fecundity and negative impacts on subsequent generations	Pacific cupped oyster (*Crassostrea gigas*)	Polystyrene	23 μg/L	2 μm, 6 μm	Sussarellu et al. (2016)
Increased immune response	Blue mussel (*Mytilus edulis*)	High-density polyethylene	2.5 g/L	>0–80 μm	von Moos et al. (2012)
Reduction in feeding rate, body mass, metabolic rate	Norway lobster (*Nephrops norvegicus*)	Polypropylene	–	3–5 mm (length), 0.2 mm (diameter)	Welden and Cowie (2016)
Reduced feeding activity; longer gut residence times; inflammation; depleted energy reserves	Polychaete lungworm (*Arenicola marina*)	Polyvinylchloride polystyrene	1–5%	130 μm (mean diameter)	Wright et al. (2013)
Reduction of feed consumption and growth	Shore crab (*Carcinus maenas*)	Polypropylene microfibers	–	1–5 mm (length)	Watts et al. (2015)

Whether or not these effects will be seen in many humans is still a matter of discussion and yet to be proven (Cheng et al. 2013).

7.2 Potential Effects on Humans

Microplastics have been found in many foods such as beer, salt, honey, sugar, etc. although most studies have been carried out on seafood (Barboza et al. 2018). Microplastics have also been found in the faecal matter of human beings thus proving that we are being exposed to these particles and similarly that one of their intake routes is ingestion (Schwabl et al. 2019).

This review focuses on investigating the evidence available and determines the main risks to human health associated with the ingestion of microplastic particles. It has been shown in numerous studies that shellfish and fish of commercial interest are often contaminated with microplastic and it has been proven that this is a potentially reliable source of ingestion exposure to human consumers. It has also been shown in the research that these micro- and nanoplastics are laden with chemicals adsorbed to their surfaces and so humans are exposed to these toxicants as a result of ingestion (Barboza and Gimenez 2015; Barboza et al. 2018; Waring et al. 2018).

Depending on the shape, size, polymer type and additive of the microplastic particle consumed the fate may vary, the plastic may be passed through the gastro-intestinal tract unchanged or may be absorbed and distributed throughout the circulatory system (Lusher et al. 2017). From there it can enter cells and tissues and it is at this stage where there is a risk of potentially adverse effects. These effects may be changed, if the microplastic particle involved had previously been exposed to chemical contaminants or toxicants of any kind. As mentioned previously these contaminants were either added in the manufacture process or accumulated through exposure in the environment, these adverse effects can be passed from prey to predator and upwards through trophic levels (Avio et al. 2017; Chae and An 2017; Foley et al. 2018; Pedà et al. 2016; von Moos et al. 2012; Wright et al. 2013).

Polystyrene microplastics (23 μg/L; 2 μm, 6 μm) can reduce the larval progeny and development in Pacific supped oysters (*Crassostrea gigas*) after absorption (Sussarellu et al. 2016). Due to the uptake of microplastics (0.5 μm and 6 μm) delay of development and reduced fertility were noted in marine copepod (*Paracyclina nana*) (Jeong et al. 2017). Microplastics (0.5 μm) have been translocated into the tissues of blue mussels (*Mytilus edulis*) through the "treated" crab (*Carcinus maenas*), however, only 0.3% of the particles was detected in the haemolymph of the crab (Farrell and Nelson 2013). The larger particles, microplastics with a size of 3–5 mm have been observed in the tissues (Dos Santos and Jobling 1991), however, the smaller particles (50–500 μm) would not accumulate in the tissues of goldfish (*Carassius auratus*) (Grigorakis et al. 2017). However, gastric obstruction has been noted due to the larger plastic particles (Mazurais et al. 2015). Based on other scientific investigation, the smaller microplastics (<600 μm) can be detected in the liver of Flathead grey mullet (*Mugil cephalus*) (Avio et al.

2015b). The microplastics in the feed pellets containing 0.1% of polyvinyl chloride can induce alterations of the intestinal tract (Pedà et al. 2016). Microplastics ranging from 0.2 to 150 μm can be translocated into the lymphatic system in humans. This absorption is also detected in other mammals depending on the sizes, such as in dogs (size: 3–100 μm), in rabbits (size: 0.1–10 μm) and in rodents (size: 30–40 μm) (Walczak et al. 2015). Basically, the microplastics with smaller sizes (<20–150 μm) can penetrate through the mammalian intestines resulted in systemic exposure and clinical symptoms. However, the translocation of these particles is relatively low (<0.3%).

When humans consume food containing microplastics they may uptake and absorb them from their intestinal tracts through the microfold cells (M-cells), Peyer's patches and other lymphatic tissue in the intestines. It is through this action that microplastic can be absorbed into the lymphatic system, however, this depends on particle size. This action has been displayed in many animal models, rabbits, rodents and dogs as well as humans (Hussain et al. 2001). There is mounting evidence and literature to suggest that plastic associated toxic substances can be transferred to exposed wildlife and is a threat to human health through the route of consumption (Van Cauwenberghe and Janssen 2014).

Basically, human consumers can be exposed to microplastics by the food, especially seafood which can contain high ratio of microplastic pollutants, but they may be found in other food items such as beer (Liebezeit and Liebezeit 2014) and honey (DR 2015; Liebezeit and Liebezeit 2013), but its origin is questionable (they may be contaminated during the manufacture due to plastic filter or the natural raw materials may be contaminated). However, findings available for composition, size, form, or concentration of microplastics in the food are poorly researched and quantified (BfR 2015; Van Cauwenberghe and Janssen 2014). Generally, the gastrointestinal tract of aquatic organisms contains the largest quantities of microplastics, however, the digestive part is generally removed before consumption, except, e.g. for most bivalves, some edible echinoderms, and different smaller species of fish (e.g. sprats, sardines, etc.) that are eaten whole. Although only the smaller microplastics with a size of 150 μm may translocate across the mammalian intestinal epithelium resulted in systemic exposure and possible clinical signs. However, the uptake of these smaller particles is relatively limited ($\leq$0.3%), but the penetration of the smallest particles ($\leq$20 μm) is better into organs inducing possible systemic signs (Lusher et al. 2017).

Microplastic particles (polyethylene, polypropylene) with a size of more than 1 μm in the cosmetic products can induce skin damage in humans (BfR 2015).

The ingestion of microplastics results in chromosomal aberration leading to infertility, obesity and cancer (GESAMP 2015).

Although at present there is no data accurately describing the toxicity of translocated microplastics in humans, it is known that these particles adsorb luminal molecules and so have ability to translocate them to mucosal cells (Powell et al. 2010). It is possible that the particles ingested could incite pro-inflammatory and immune stimulatory effects in the gut due to the agents adsorbed to their surfaces. These microplastic particles can then influence effect on the circulatory system, the

immune system, the lymphatic system and cell health (EFSA 2016). These processes would occur due to the fact predicted by Wright and Kelly (2017) that microplastics may cause necrosis to and compromise immune cells, may cause inflammation in tissue and can cause cellular proliferation.

There is much literature reporting on the organic pollutants and toxicants present in or on plastic found in the marine, terrestrial and freshwater ecosystems (Endo et al. 2005; Mato et al. 2001). Researchers described indirect evidence of uptake of contaminants that had been absorbed into the tissues of sea birds and transfer of plastic to seabirds' tissues (Tanaka et al. 2013; Teuten et al. 2009). It is logical to assume the toxicity expected from these chemicals and additives, however, the actual effects on human remain to be investigated fully, the monomers that leach from plastic have potential to cause acute and chronic processes including oncogenic and neurologic effects in the human consumer that is continuously exposed to microplastic particles (ATSDR 2015). Ingested microplastics have been discovered in the adipose tissue of sea birds and in some lugworms and fish an accumulation of PBDEs was found in their tissues (Browne et al. 2013; Rochman et al. 2013a; Tanaka et al. 2013). As we gather more research on the effects of wildlife exposed to and ingesting microplastic we are beginning to see the potential fate for the microplastic and human relationship and how the human consumer may be affected by the ingestion of these toxicant and chemical-laden microplastic particles (Van Cauwenberghe and Janssen 2014).

Nanoplastics due to their size and hydrophobic properties mean they can potentially pass through the blood brain barrier, placenta, gastrointestinal tract and lungs which offer sites where damage could be caused. Nanoplastics have a large surface area to volume ratio and so this makes them very chemically reactive, if they accumulate enough and have a large concentration of contaminants, they have been shown to have various effects after chronic exposure; in vitro in the lungs, liver and brain cells (GESAMP 2016). Ingestion of nanoplastics has been linked to various effects, like to what is seen in microplastic exposure studies such as: oxidative stress, influence on nutrient absorption, gut microflora alterations, inflammatory responses, reproduction, cardiopulmonary responses, alterations of endogenous metabolites, genotoxicity (EFSA 2016).

The effect of ingesting micro- or nanoplastics could be caused by either the plastic itself or the associated absorbed toxins, although according to some research the amount at which these particles are consumed through seafoods appears negligible when we consider the amount absorbed from the gastrointestinal tract and into the tissues. An example provided showed that even if a human consumed a portion of mussels weighing 225 g that the amount of exposure would be roughly 7 μg of plastic meaning that the exposure to PBTs or additives would be less than 0.1% of the dietary exposure to these compounds. This would indicate that it is unlikely to cause harm to the health of a human being (Lusher et al. 2017). This does not consider into account developing countries where rivers are badly polluted with plastic and environmental contaminants where many people rely on fish and seafood as their main source of protein. These people would be at a greater risk to the effects of toxic microplastic particles (McCormick et al. 2014). Although BPA has been

found to have potentially harmful characteristics, it is still used as a food packaging material additive because it has been registered as "safe" by the European Union, since the European Food Safety Authority have said that it poses no threat or health risk to human consumers at the current exposure levels (EFSA 2015).

Humans are exposed through the food, water and air (Vethaak and Leslie 2016). As stated above there have been a few studies that have given insight to the possible risks involved. A study conducted on mouse and human models showed that plastic particles caused lung and gut damage and that nanoparticles could indeed penetrate through the special barriers including the blood brain barrier and the human placenta (Vethaak and Leslie 2016). Basically, the smaller plastic particles can pass more easily through the different membranes than the larger ones, e.g. polystyrene particles with a size of 50–100 nm more readily penetrate through the Peyer's patches and the villi of intestine than the larger solid plastics over 300 nm. The uptake of polystyrene microplastic through the intestines was higher and the presence of the food further increases the absorption due to the delayed transit time of the gastrointestinal tract. However, other properties (e.g. combination of size, surface charge, hydrophilicity) can influence the uptake of them. It was experienced when the absorption of small size (2.5 nm) of polylysine dendrimers was lower than that of larger polystyrene particles between 100 nm and 3 μm. Some of the problems included cell damage, inflammation and energy impairment functions (GESAMP 2015).

Previously it has been stated how microplastic acts as a bio-sponge attracting chemicals to adhere to its surface. Such chemicals include BPA that has been shown to behave as an endocrine disruptor. However, whether the rate and concentration at which humans are exposed to these chemicals are enough to cause damage are yet to be determined. Nanoparticles have been shown to interfere with cell signalling and uptake processes which could have an impact on the pharmacokinetic properties of various pharmaceutical drug and toxin interactions (GESAMP 2015).

When microplastics act as a bio-sponge to human pathogens and parasites, the studies show that harmful bacteria such as *E. coli*, *Bacillus cereus* and *Stenotrophomonas maltophilia* have been found in higher concentrations on the microplastic substrate off the Belgian coast (McCormick et al. 2014). This would become a bigger issue in countries with poor sanitation and very high populations where wastewater and drinking or bathing water may be in contact. Larger plastic debris is also capable of creating habitats for parasite bearing freshwater snails and so helping their populations increase and spread disease (McCormick et al. 2014). The microplastics can also serve as a "transport molecule" for microbes, e.g. for pathogenic bacteria or even viruses resulted in increase in the occurrence of pathogenic or even non-indigenous species, however, the exact mechanisms are still not known. Thus, the possibility of different sizes of plastics as carrier molecule for microorganisms must be further studied and evaluated (Lassen et al. 2015; GESAMP 2015).

Another less direct way that microplastics can affect the aquatic/marine ecosystem is through their effect on juvenile fish species. A study carried out on how young fish, crab and shrimp species can sometimes mistakenly feed on microplastics that

Table 7 Juvenile fish at risk of microplastic ingestion (Parker 2019)

Juvenile fish and other aquatic organisms at risk of microplastic ingestion	
Common name	Scientific name
Hound needlefish	*Tylosurus crocodilus*
Sergeant major damselfish	*Abudefduf saxatilis*
Amberjack	*Seriola lalandi*
Chub	*Squalius cephalus*
Triggerfish spp.	*Balistidae*
Sailfin flying fish	*Parexocoetus brachypterus*
Flying fish spp.	*Exocoetidae*
Man-of-war fish	*Physalia physalis*
Bigwing halfbeak	*Oxyporhamphus micropterus*
Mahi-mahi	*Coryphaena hippurus*
Tropical halfbeak	*Hyporhamphus affinis*
Flat needlefish	*Ablennes hians*
Large-scaled lanternfish	*Neoscopelus macrolepidotus*
Decapod shrimp larvae	e.g. *Crangon crangon*
Purple pelagic snail	*Janthina janthina*
Blue shrimp	*Neocaridina* sp.
Crab larvae, megalops stage	e.g. *Liocarcinus vernalis*
Pelagic snail	e.g. *Cavolinia gibbosa*
Blue copepod	e.g. *Pontella valida*, *Acartia erythraea*
Medusa (jellyfish)	e.g. *Chrysaora fuscescens*
Polychaete worm	e.g. *Spirobranchus giganteus*
Blue button hydroid	*Porpita porpita*
Pelagic sea slug	*Glaucus atlanticus*
Flatworm	e.g. *Pseudobiceros bedfordi*
Comb jelly	e.g. *Mnemiopsis leidyi*
Peanut worm	e.g. *Sipunculus nudus*

can lead to malnutrition due to false satiation as well as causing blockages, damage to their enterocytes and death (Tables 7 and 8). When young fish do not have access to proper nutrition it will lead to inadequate weights of older, mature fish. With marine and freshwater habitats already under stress from overfishing, pollution, ocean acidification, etc. it is unhelpful to have another stressor which could affect the delicate juvenile fish and perhaps prevent them from reaching adulthood. Most of the plastic found in nets was very small, degraded fragments which are very difficult to identify (Parker 2019).

The amount of micro and nanoplastics present in the environment is going to increase with time following global trends in plastic production if nothing is done to manage their introduction (Geyer et al. 2017). A ban has been placed on microplastics being used as primary additives into cosmetic and cleaning products but there are still secondary and tertiary microplastics to be concerned about. Human and animal exposure to these microplastics will increase alongside this and it is very

Table 8 The most frequent type of microplastic particles from various primary and secondary sources (Parker 2019)

Type of microplastic particles
Polypropylene or polyethylene fragment
Preproduction pellet, polypropylene or polyethylene
Braided line from fishing or cargo net
Marker-pen cap
Monofilament fishing line, nylon
Tube for spacing oysters on oyster farm
Flexible low-density polyethylene
Possible latex balloon
Packaging sheet, probably polyethylene food wrapper
Expanded polystyrene, probably from a take-out container
Soda bottle cap, high-density polyethylene

important that studies are conducted in a practical and realistic manner in order to accurately discern the risks to human health and safety due to this constant exposure to potentially harmful and toxic particles. It is known that fish provide an excellent source of lean protein packed with nutrients and have many health benefits. However, evidence is emerging that indicates fish are accumulating contaminants from the surrounding environment which now indicates the fact that the fish products could be harmful to human health and safety which thus diminishes and contradicts the health benefits in store when consuming seafood (Lusher et al. 2017).

If this problem has negative knock-on effects as are predicted it could have a major impact on humans especially those who rely on fish for their livelihoods or as their main food source.

8 Conclusions

The main aim of this review was to research the literature and explore the true scope of knowledge available on plastic waste and especially micro and nanoplastics and how they interact with humans and animals. Particular attention was paid to fish in aquaculture centres and commercial fish and seafood to explore whether humans are at a greater or lesser risk from consuming plastic when ingesting these fish. Initially it was thought that fish in aquaculture centres would not be exposed to microplastic because of the nature of their upbringing, however there is evidence that these fish are being directly fed large quantities of marine based feed that is laden with microplastic. It suggests that their exposure could be higher when compared to a natural wild counterpart. From several studies it is evident that humans ingest microplastics. The main scope of interest to date has been seafood and food of marine source, and this is where most of the research has been done. However, we can ingest plastic from numerous other sources. More evidence is emerging outlining the situation in terrestrial and freshwater ecosystems, but marine habitats have received more attention. It has been shown how organisms consume

Table 9 Relevant knowledge gaps

Relevant knowledge gaps
• Develop a standardized, reliable quantifying method for microplastics and nanoplastics
• Identify bioindicator species
• Develop realistic schemes and policies for waste collection, dumping and recycling, reinvent labelling on plastic packaging items depicting clear recycling and disposal instructions
• Improve consumer awareness and encourage zero waste initiatives globally
• Identify more clearly how humans are affected by microplastics and their associated contaminants through toxicological studies and experiments
• Improve screening of animal feed especially meals fed to fish of commercial interest kept in fisheries and aquaculture centres
• Develop methods to clean up the environment especially the ocean and create policies to stop dumping and littering of plastic in the ocean, rivers, forests, etc.
• Identify clear methods to assess the damage caused by microplastics when they encounter with a biological organism
• Identify the risks involved with juvenile fish ingesting plastics and how this may affect fish stocks, especially in areas where fish are the main source of food or livelihood to a community

microplastic particles and that these particles can be transferred through trophic levels and accumulated in small amounts in higher organisms through indirect means, although the majority will remain in the gastrointestinal tract. Nanoplastics are shown to be more hazardous because of their nature to be absorbed and therefore the difficulty of properly assessing where they end up, and what are their effects in the human body, and they are difficult to quantify. They can also absorb larger amounts of harmful chemical contaminants based on their larger surface area to volume ratio. When it comes to the toxicity of these particles, it is likely to be related to dose, size and associated chemicals which have adsorbed to the surface owing to the bio-sponge properties. Although most microplastics appear to build up in the GI tract, there is research showing that they can also build up in the adipose tissue of various organisms. Although chemical additives found adsorbed to microplastics include endocrine disruptors such as BPA which are harmful to humans, it has been discussed that the amounts they are available may be negligible and cause no harm when ingested and according to UNEP (2017) they are of no concern to human health and safety. However, more clarification is needed on this issue to determine the real risks at hand. The current knowledge depicts how global understanding is limited based on the sources, bioavailability, exposure, fate and toxicity of microplastic particles and their associated contaminates, however the gaps are being slowly filled as research in this area is growing. Simultaneously, the amount of plastic in production and in use is also growing, a considerable part of which is likely to end up as litter. We must get a handle on this problem before it becomes too heavy a burden. Upon completing the research for this literature review the areas that need further study regarding this topic are summarized in Table 9.

There is undeniable evidence that the production of plastic and plastic waste has surpassed necessity and the methods through which these processes are managed globally need redesigning and improving in order to combat the problem at hand.

Humans and animals from detritivores to apex predators are ingesting plastic. The cumulative knock-on effects of this interaction may have detrimental effects upon humans and animals alike. It is vital that more research is done to discover the potential risk to human health and safety, especially regarding ingestion from various sources as the main route of interest. The pinnacle note of interest from this review is that there is simply not enough research available on the relationship between microplastic humans are consuming and the potential toxicity and harm they may or may not cause upon ingestion. It should be prioritized as an area of concern for the global food safety authorities to clarify this issue.

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Conflicts of Interest Herewith the authors declare that no actual or potential conflict of interest occurred including any financial, personal or other relationships with other people or organizations within 3 years of beginning the submitted work that could inappropriately influence, or be perceived to influence, our work.

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Toxicity of Graphene: An Update

Thiyagarajan Devasena, Arul Prakash Francis, and Sundara Ramaprabhu

Contents

Abstract Graphene possesses wider biomedical applications including drug delivery, photothermal ablation of tumors, biosensors, and also in the disease diagnosis. The accidental or intentional exposure of the environment including plants, ecosystem, and humans toward graphene is gradually increasing. Therefore, graphene toxicity becomes a critical issue to be addressed despite their diverse applications in multiple fields. In this situation, the scientific community as well as the general public must get awareness about the toxicity of graphene. This article, therefore, reviews the investigations on graphene toxicity. This review reveals the toxicity of graphene in vitro, in vivo models along with the environmental toxicity. The advantages of graphene toxicity in bacterial cells and cancer cells were also reviewed.

Keywords Ecosystem · Environmental · Graphene · In vitro · In vivo · Toxicity

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1 Introduction

Graphene is a two-dimensional nanomaterial with Sp^2-bonded carbon atoms. They possess wider applications owing to their unique optical and electrical properties. For example, it is widely used in intracellular delivery of anticancer drugs, photothermal ablation of tumors, sensing biomolecules, and in disease diagnosis (Novoselov et al. 2004; Liu et al. 2008, 2017; Sun et al. 2008; Yang et al. 2008; Zhang et al. 2010a; Mohammadi Gazestani et al. 2018). The literature review indicates that the electrical, therapeutic, and diagnostic applications of graphene may be a boon in future nanotechnology. As a result, the accidental or intentional exposure of the society including plants, ecosystem, and humans toward graphene is gradually increasing. Therefore, graphene toxicity becomes a critical issue to be addressed before their further applications in multiple fields. In this situation the scientific community as well as the general public must get awareness about the toxicity of graphene. Also, the risk to benefit ratio needs to be accurately evaluated before any medical application. This article, therefore, reviews the investigations on graphene toxicity. Studies on the in vitro toxicity, in vivo toxicity, and environmental toxicity of graphene were updated. The benefits of graphene toxicity in bacterial cells and cancer cells were also reviewed. Physiochemical properties and biological interactions are two major parameters influencing the toxicity of graphene. Hence these parameters were reviewed before entering into the in vitro and the in vivo toxicity of graphene. Our review reveals the high risk to benefit ratio of graphene, which should be seriously considered before applying for biomedical use.

2 Physiochemical Properties Determine the Fate and the Toxicity of Graphene

The toxicity of graphene depends on its environmental fate which in turn is determined by its interdependent physiochemical properties:

- Size
- Shape
- Edges, holes, and corrugation
- Nanohole
- π-π stacking
- Surface charge,
- Conductivity
- Hydrophilicity: hydrophobicity
- Surface functionalization

Size is the paramount factor that determines the biological response to graphene and its derivatives. A significant cytotoxicity of HeLa cells was observed with GO of particle size around 200 nm, whereas GO with smaller size shows higher viability,

suggesting that the cell membrane damage easily occurs with larger sized GO (Zhang et al. 2013). Larger nano GO flakes were reported to reduce the viability of HeLa cells and macrophages in a concentration dependent manner as compared to smaller flakes (Mendes et al. 2015). In addition, the viability reduction correlates with the time and the concentration of the GO nanoparticles to which the cells are exposed. In in vivo studies the size and mass are related to the exposure dose of nanomaterials. An increase in size decreases the circulatory time and enhances the translocation into the tissue. Smaller size with a higher edge to center ratio results in the generation of reactive oxygen species (ROS). However, in the biological medium, the graphene particles tend to aggregate making the size dependence of subcellular localization, tissue distribution, bio persistence, toxicity, and pharmacokinetics and clearance difficult to investigate (Yang et al. 2012; Sharifi et al. 2012). Therefore, the size dependence of in vivo toxicity is still obscure and there are only a few in vivo studies on the toxicity of graphene and its derivatives.

The diverse shapes of graphene-like symmetric hexagon, asymmetric hexagon, rectangle, rhombohedrum, regular triangle, ribbon, and Ω shapes diversify the electronic properties and hyperpolarizability or spinning multiplicity. As a result, the membrane-warping process is affected during cellular uptake processes like endocytosis or phagocytosis. GO nanoribbons were described as more cytotoxic response when compared to GO nanoplatelets. High aspect ratio of GO nanoribbons was reasoned for its cytotoxicity property (Khim Chng et al. 2014). Though graphene platelets were not entered into the cells, they possess a strong tendency to localize close to the cells and induce apoptosis due to the loss of membrane integrity (Jaworski et al. 2013). As with size dependence, the shape dependence of toxicity is also unexplored.

The swinging bonds at the edges of the graphene (zig-zag or armchair conformations) are active moieties which interact with the atoms or molecules constituting the cell membrane and the surface of the microbes resulting in damage to the cells (Liu et al. 2011).

Nanoholes in the carbon plane being generated by various factors like the removal of carbon atoms, reduction of graphene oxide (GO) influences the aqueous solubility in the biological media, thus affecting the toxicity (Shi et al. 2021).

According to Meyer et al., graphene possesses nano corrugations (i.e., they are not flat) which renders them more flexible with liquid-like topography thus increasing the probability of adhesion to the cell membrane (Meyer et al. 2007; Koenig et al. 2011).

Graphene and its derivatives established non-specific binding of organic biomolecules (nucleic acids, proteins/enzymes, and aromatic molecules) via its π-stacking interactions or ionic interactions leading to biological responses. However, functionalization of the surface of graphene or GO may reduce the non-specific binding (Park et al. 2010; Balapanuru et al. 2010; Wu et al. 2011; Kim et al. 2011; Ruiz et al. 2011).

Being a conductor of electrons, graphene functions as an electronic conduit between cells and manipulates the signal transfer and ionic channel functions of the cells (Geim 2009; Mohanty et al. 2011; Kotchey et al. 2011).

GO and rGO vary in their wettability due to variation in their surface oxidation state. GO exhibits distinct hydrophilicity which is responsible for rapid intracellular uptake. Relatively, rGO with good degree of hydrophobicity shows strong adsorption and aggregation on the cell surface. But both are capable of interacting with proteins and lipids and disrupt the cellular integrity (Ou et al. 2016). Perhaps, as a consequence of cell surface disruption the rGO will be internalized as GO. Thus, both are equally involved in inducing cellular toxicity but with different mode of uptake owing to different wettability (Chatterjee et al. 2014). In general, graphene family nanomaterials (including pristine graphene, GO, and rGO) were reported to establish strong hydrophobic interactions with the cell membrane causing morphological extension of F-actin leading to filopodial and cytoskeletal dysfunction (Burton and Jauniaux 2011).

Graphene produces a biological response on interaction with vital biomolecules. Graphene comes in different commercial forms such as graphene oxide (GO), reduced graphene oxide (rGO) graphene powder, solution or paste; graphene nanoplatelets and functionalized grapheme with wider applications. We have generalized the term graphene in this review title as the toxicity of all the forms is discussed here. The biological response toward graphene and its derivatives is due to their interaction with vital biomolecules like DNA, protein. Interaction between DNA and graphene derivatives depends on the strand structure and the nitrogenous bases. DNA oligomers with <15 base pairs are capable of self-assembly on the graphene forming DNA nanoforests (Zhao 2011). Graphene can easily bind to a single-stranded DNA but not to double-stranded DNA while GO can bind to both (Lei et al. 2011). Among the nitrogenous bases, guanine has the highest binding strength toward graphene (Antony and Grimme 2008; Varghese et al. 2009). Nevertheless, A-T base pairs have a higher probability of interaction with graphene as compared to G-C base pairs (Zhao 2011). The interaction between the DNA and the graphene is mediated by hydrogen bonding and π-π stacking. Graphene can intercalate more efficiently at the major groove of the DNA leading to scission and strand break (Ren et al. 2010).

Under neutral pH, graphene interacts with the building blocks of proteins via Van der Waal's force and the protein molecules localize to a greater extent in the edges of the graphene. However, hydrogen bonding dominates in GO-amino acid conjugate. Pristine graphene owing to large water contact angle damages the hydrogen bonds of the proteins by dispersion and hydrophobic interaction (Kim et al. 2011). A recent molecular dynamics study by Puigpelat et al. revealed the adsorption of graphene flakes to hydrophobic regions of lipid bilayers and suggested that the graphene nanoflakes display a diffusive dynamic in the membrane plane. As lipid bilayer is protective in all cellular organelles including nucleus, it could be suggested that the graphene and its derivatives have an impact on normal cellular architecture and metabolism (Puigpelat et al. 2019). Thus, we infer that graphene and its derivatives may execute its biological effect by interacting with vital biomolecules such as DNA protein and lipids.

3 In Vitro Toxicity

Several studies have been done in cell lines to explore the toxicity of graphene (Hu et al. 2010; Ryoo et al. 2010). Studies on PC12 cell lines have revealed the potential of graphene to induce caspase 3, to enhance the release of the cytosolic enzyme, lactate dehydrogenase and to elicit the generation of reactive oxygen species (Zhang et al. 2010b). Caspase 3 is a marker of apoptosis and its activation is believed to induce programmed cell death (Francis et al. 2014). Lactate dehydrogenase is a major cytosolic enzyme, whose burst release indicates the cell membrane damage. Reactive oxygen species are highly reactive unstable molecules capable of damaging cellular biomolecules like DNA, proteins, enzymes (Rajasekar and Devasena 2015) and membrane lipids (Suganya and Devasena 2015). Therefore, studies on the graphene- treated PC12 cell lines indicates diverse cellular changes like: (i) apoptosis (ii) membrane damage (iii) enzyme imbalance (iv) oxidative stress (v) bimolecular damage, and (vi) lipid peroxidation. These events finally influence the morphology and functions of the cells. Possible cellular aberrations induced by graphene are schematized in Fig. 1. As PC12 cell lines are used to derive information about the brain-related diseases and disorders, studies by Zhang et al. assume much significance and can be used to explore the details about the neurotoxicity of graphene (Zhang et al. 2010b). Kang et al. revealed the toxic effects of graphene oxide (GO) and reduced graphene oxide (rGO) materials on PC12 cells. They reported that the GO and rGO exposure significantly altered the phosphorylation levels of ERK signaling in PC12 cells (Kang et al. 2017).

Fibroblasts are the cells of connective tissue that produce the precursors of extracellular matrix components and help to maintain the structural integrity of the connective tissues. Liao et al. have determined the toxicity of compacted graphene

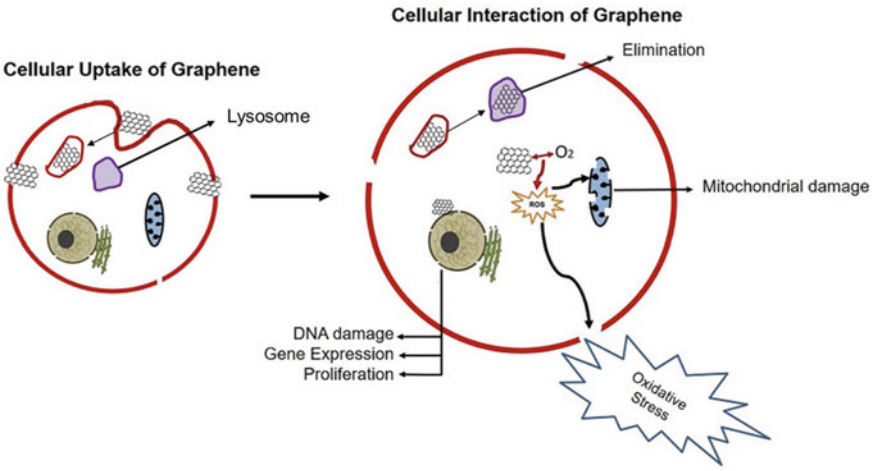

Fig. 1 Cellular uptake of graphene and its interaction

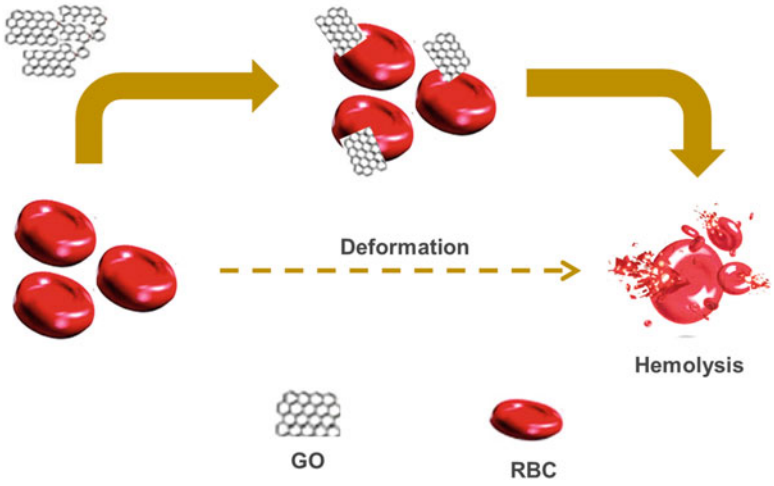

Fig. 2 Hemolytic effect of GO

sheets in skin fibroblast cells by using the trypan blue exclusion and reactive oxygen species assay (Liao et al. 2011). This study demonstrates that densely packed graphene is more toxic than sparsely packed ones.

Red blood cells (RBCs) are an ideal and reliable model to study the toxicity of the material (Francis et al. 2015). Liao et al. have determined the toxicity of GO using RBCs. GO affects the RBC membrane integrity and induces hemolysis leading to efflux of hemoglobin (Fig. 2) (Liao et al. 2011). The rate of hemolysis increased with decreased GO size. RBC toxicity depends on the size, particulate state, and oxygen content or surface charge of graphene. A previous report suggested that the different forms of graphene treated with chicken embryo RBC cause damage to the structure of RBC in a dose-dependent manner (Jaworski et al. 2017). Singh and co-workers have investigated the influence of graphene oxide and reduced graphene oxide on ultrastructural details of platelets. They revealed strong aggregation in treated platelets thus alarming the feasibility of arterial thrombotic events like ischemic heart disease and stroke (Singh et al. 2012).

GO was reported to induce lung toxicity in a dose-dependent manner. 50 μg mL^{-1} of GO induced obvious toxicity and 20 μg mL^{-1} of GO was found to be non-toxic. GO nanoparticles can internalize into the fibroblast population of lungs leading to (1) decreased cell adhesion, (2) cell floating, and (3) apoptosis. As these features are characteristic of cell death, GO was considered as toxic to the lungs at a dose >20 μg mL^{-1} (Wang et al. 2011). As the GO predominantly accumulates in the lungs, there is a greater possibility for the inhibition of cell adhesion and consequent floating and apoptosis. Therefore, the dose of exposure of graphene should be extrapolated to humans to assess the toxicity risk. Studies on lung epithelial cells show that GO (50 μg mL^{-1}) produce a slight loss of cell viability due to oxidative stress (Chang et al. 2011). Studies on A549 cells disclose the cytotoxic ability of

hydrazine-treated rGO (Hu et al. 2010). Comparing this study with that of Wang et al., it is interesting to note that the same dose of GO is highly toxic to lung fibroblasts but insignificantly toxic to lung epithelial cells. Thus, we could infer that the degree of GO toxicity varies with the cell type. Wang et al. compared the toxic effects of rGO-nanoscale zerovalent iron (rGO-nZVI) along with GO and rGO against human bronchial epithelial cells (BEAS-2B). The results revealed that the toxic behavior of rGO-nZVI nanohybrids was found to be less than that of rGO/GO (Wang et al. 2018).

Graphene nanoplatelets with 1-10 layers were reported to jeopardize the membrane integrity and phagocytic capacity of macrophages at a dose of 5 μg cm^{-2}. This was thought to be mediated via reactive oxygen species generation (Schinwald et al. 2012). We suggest that the graphene nanoplatelets may lead to immunological malfunction due to phagocytosis inhibition. Therefore, this issue should be seriously addressed.

Li et al. have disclosed the intracellular translocation of graphene sheets using confocal fluorescence imaging and electron microscopic imaging (Li et al. 2013). They revealed that the sharp edges at the corners of the graphene sheets namely asperities pierce through the membrane and infiltrates in the cytoplasm and induce toxicity in human lung epithelial cells, human keratinocytes, and murine macrophages. The membrane piercing may impair cellular integrity and function. Besides, cellular uptake and infiltration in the cytoplasm may damage vital biomolecules including DNA, proteins, and enzymes, thus leading to cell death. Thus, further studies on graphene-induced changes in cellular metabolism are very essential in the present scenario.

Das et al. have reported that GO induces cellular toxicity by altering the level of mRNAs of heme oxygenase 2 and thioredoxin reductase (Das et al. 2013). GO affects the mitochondrial membrane potential and membrane integrity leading to cell death. In HepG2 cell lines, GO was reported to cause increased accumulation of calcium, augmented auto-phagosomes, increased oxidative stress associated with mitochondrial DNA and enzyme damage finally leading to structural and functional impairment in the mitochondria (Lammel et al. 2013). Studies on murine peritoneal macrophages model reveal that GO accumulates in the lysosomes leading to lysosomal membrane destabilization (Wan et al. 2013). Studies on the in vitro toxicity of graphene on various experimental models are summarized in Table 1.

Altogether, studies on graphene-induced in vitro toxicity reveal the following:

1. The toxicity is determined by the extent of GO aggregation.
2. The mode of interaction of graphene with the cells also determines the toxicity.
3. Densely packed graphene sheets are more toxic.
4. Toxicity depends on the size, particulate state, oxygen content, or surface charge of graphene.
5. Greater than 20 μg mL^{-1} is the pulmonary toxic dose of GO.
6. The same dose of GO is more toxic to fibroblasts than the epithelial cells of the lungs. i.e., GO toxicity is different for different cell types.

Table 1 In vitro toxicity studies of graphene

Sl. no.	Model used	Toxicological changes	Reference
1.	Raji, HCT-116, OVCAR-3, U87MG, MDA-MB-435 and MCF-7 cell lines	Negligible toxicity of PEGylated graphene oxide	Liu et al. (2008); Sun et al. (2008)
2.	A549 cells	Cytotoxic cell death	Hu et al. (2010)
3.	PC 12 cell lines	Activation of caspase Enzyme imbalance Oxidative stress	Zhang et al. (2010b)
4.	Mouse pheochromocytoma cells, human oligodendroglia cells, and human fetal osteoblasts	Biocompatible	Agarwal et al. (2010)
5.	Mouse neuronal cells were grown on graphene film	Improved cell multiplication and neurite growth	Li et al. (2011)
6.	Human lung epithelial cells	Slight loss of cell viability due to oxidative stress	Chang et al. (2011)
7.	Skin fibroblast cells	Generation of reactive oxygen species	Liao et al. (2011)
8.	Human lung fibroblast cells	Reduced cell adhesion Increased cell floating Apoptosis	Wang et al. (2011)
9.	Mammalian colorectal adenocarcinoma HT-29 cells	Promoted adhesion and growth	Ruiz et al. (2011)
10.	Gram-positive and Gram-negative bacterial cells	Membrane damage owing to reactive oxygen species-independent oxidative stress	Akhavan and Ghaderi (2010); Liu et al. (2011)
11.	Macrophages	Loss of membrane integrity and phagocytosis capacity	Schinwald et al. (2012)
12.	Coarse-grained simulations Atom simulations	Cell membrane penetration by asperities	Li et al. (2013)
13.	Human lung epithelial cell line Human keratinocytes Murine macrophages	Cell membrane penetration by asperities	Li et al. (2013)
14.	Human umbilical vein endothelial (HUVEC) cells	Augmentation in the level of heme oxygenase1 and thioredoxin reductase mRNA	Das et al. (2013)
15.	Liver cell line HepG2	Mitochondrial dysfunction	Lammel et al. (2013)
16.	Murine peritoneal macrophages	Lysosomal membrane destabilization and degradation	Wan et al. (2013)
17.	Glioblastoma cells	Induction of apoptosis	Jaworski et al. (2013)
18.	HepG2	Loss of membrane integrity Internalization into cytoplasm Induction of oxidative stress with elevated reactive oxygen species	Lammel et al. (2013)

(continued)

Table 1 (continued)

Sl. no.	Model used	Toxicological changes	Reference
19.	Chicken embryo	Alteration in the brain ultrastructure	Sawosz et al. (2014)
20.	Drosophila melanogaster larvae	Genotoxicity of graphene composite Upregulation of heat shock protein hsp70	Siddique et al. (2014)
21.	Red blood cells (RBCs)	Hemolysis	Liao et al. (2011); Jaworski et al. (2017)
22.	*Caenorhabditis elegans*	Morphological abnormalities in the pharynx and the intestine	Rive et al. (2019)

7. Graphene nanoplatelets jeopardize phagocytosis which may lead to immunological malfunction.
8. Graphene's asperities pierce the biological membranes and make way into the cells.
9. Pristine graphene alters the brain's ultrastructure and DNA metabolism.
10. GO induces toxicity by altering the mRNA levels of enzymes such as heme oxygenase 1 and thioredoxin reductase (it influences on of the biomolecule mRNA).
11. GO induces mitochondrial dysfunction and degradation via altering the membrane potential and eliciting ROS generation.
12. GO induces lysosomal destabilization.

4 In Vivo Toxicity

In vivo toxicology studies help in terms of identifying adverse effects, providing mechanistic data, establishing dose-response relationships, and aiding the process of establishing standards (Krewski et al. 2010; Parasuraman 2011). These studies also play an important role in hazard identification and prevention of human disease. Besides, these investigations also concern the relationships between the metabolic handling of the chemical and its interactions with target molecules (mechanism of action), identification of methods for biological monitoring of exposure and early health effects, and identification of preexisting pathologic states that may increase susceptibility to the chemical. Overall, toxicological investigations using animals often serve to establish a tentative acceptable exposure level. In general, animal studies are conducted in two species, one rodent (e.g., rat, mouse) and one non-rodent (e.g., dog, nonhuman primate). In addition, for special studies (e.g., vaccine studies) other species (e.g., rabbits, ferrets, hamsters, minipigs) are also used.

In in vivo systems the interactions of the nanostructures with biological components, such as proteins and cells could lead to unique biodistribution, clearance, immune response, and metabolism (Fischer and Chan 2007). An understanding of the relationship between the physical and chemical properties of the nanostructure and its in vivo behavior would provide a basis for assessing toxic response. Orally ingested nanoparticles can cross the small intestine by per-sorption and further can be distributed into the blood, brain, lung, heart, kidney, spleen, liver, intestine, and stomach (Hillyer and Albrecht 2001; Zhang et al. 2015; Fadeel et al. 2018). Moreover, the extent of uptake of insoluble particles through the digestive tract and their pathway is known to be size-dependent (Hodges et al. 1995; Donaldson et al. 2000).

Previous studies reported that inhalation of pristine graphene and GO produce severe pulmonary distress with excessive inflammation (Duch et al. 2011). However, surface-functionalized graphene with better hydrophilicity and stability was less toxic (Singh et al. 2011; Yang et al. 2012). Subsequent investigation on the distribution of graphene confirmed its penetration into the tissues of the heart, spleen, kidney, bone marrow, and liver. In addition, GO administration produces dose-dependent pulmonary toxicity, granulomatous lesions, pulmonary edema fibrosis, and inflammatory cell infiltration (Zhang et al. 2011; Duch et al. 2011). Wang et al. have investigated the toxicity of as-prepared GO in mice (Wang et al. 2011). Intravenously-administered GO was found to induce dose-dependent pulmonary toxicity with predominant accumulation in the lungs. The maximal toxic dose was reported to be 10 mg/kg. Thus, it could be suggested that the lung is the first destination for the intravenously navigated GO. The permissible exposure limit of carbon materials such as graphite was reported as 15 mg/m^3 by Occupational Safety and Health Administration and 5 mg/m^3 by National Institute for Occupational Safety and Health, USA. Graphene exposure in rats shows the small aggregates of graphene alveolar macrophages in the lungs of all treated animals. Most of these macrophages were observed in the lumen of alveoli, few occurred in the alveolar wall, the alveolar ducts, and in terminal bronchioles. Micro-granulomas were characterized by small particle-loaded aggregates of macrophages that were found to be connected to the alveolar septum in a dose-dependent manner. 28-day repeated nose-only graphene inhalation study in Sprague-Dawley rats show low toxicity, while the inhaled graphene was uptaken by macrophages and also translocated to lung lymph nodes (Kim et al. 2016).

Experiments in chicken embryos treated with pristine graphene revealed altered ultrastructure of the brain and impairment in the DNA synthesis in the brain cells (Sawosz et al. 2014). GO at a dose of 4 mg/mL when administered to mice was found to accumulate in the reticuloendothelial system (RES) including liver and spleen causing mortality associated with impairment in the renal clearance. In addition, GO creates granuloma in the kidney, lung liver, and spleen of mice (Wang et al. 2011). Investigation on the effect of graphene-zinc oxide nanocomposite in larvae of transgenic Drosophila melanogaster confirms the cytotoxicity (as revealed by hsp70 expression) as well as genotoxic damage (as revealed by comet assay) in the midgut cells of the larvae. This study discloses the

internalization of graphene into the nucleus (Siddique et al. 2014). The women population is relatively more affected by toxicity because of the susceptibility of the female reproductive system and fetal development (Sun et al. 2013).

Records on pulmonary toxicity of graphene are inadequate. Duch et al. have intratracheally instilled aggregated and nanoscale graphene and observed the inflammation and toxicity indexed by increased cell counts, and interleukin (IL)-6 concentrations in the lungs (Duch et al. 2011). Schinwald et al. have investigated the pulmonary toxicity of graphene after pharyngeal aspiration or intrapleural injection in mice (Schinwald et al. 2012). Analysis of bronchoalveolar lavage fluid and pleural lavage fluid revealed the increased polymorphonuclear neutrophils and higher levels of pro-inflammatory cytokines (monocyte chemoattractant protein (MCP)-1, macrophage inflammatory protein (MIP)-1R, MIP-1, IL-1β). Ma Hock et al. studied the pulmonary toxicity of graphene after head-nose exposure in male Wistar rats. 10 mg/m^3 of graphene caused inflammatory response and microgranuloma in the lungs (Ma-Hock et al. 2013). Qu et al. investigated the effect of intraperitoneally injected GO on BALB/C male mice and reported that GO induces toxicity to erythroid cells and induces apoptosis (Qu et al. 2013). This may lead to an imbalance in the erythropoiesis. GO and carboxylated GO at a dose of >4 μg/mL were able to induce plasma membrane damage, internalization into the cytoplasm and elicit oxidative stress in liver cell lines (HepG2). On the other hand, few studies reported that the carbon nanoparticles possess negligible toxicity in animal model followed by prolonged exposure. Intraperitoneally injected carbon nanoparticles like diamond, graphite, and graphene oxide in rat were reported that the particles did not affect health and growth of rats even it was retained in the body as agglomerates (Kurantowicz et al. 2015b; Strojny et al. 2015).

Toxicological effect of graphene-based materials can be explored based on their effect in vital organs like lung (inhalation toxicity), brain (neurotoxicity), gonads (reproductive toxicity). The inhalation toxicity of graphene is in the order graphene layers < graphite < rGO < GO. The inflammatory effect is more pronounced by graphene derivative with negative surface charge (Ema et al. 2017). All types of graphene-based materials mentioned above were reported to slowly accumulate in the central nervous system and show long-time persistence which accounts for chronic neurotoxicity (Baldrighi et al. 2016). GO affects sperm motility and sperm DNA function in male mice (Nirmal et al. 2017). In female mice, rGO caused fetal loss during later stage of pregnancy (Xu et al. 2015).

On the whole, studies on graphene-induced in vivo toxicity reveal the following:

1. The toxicity of GO depends on the biocompatible surface functions.
2. Functionalized and unfunctionalized GO have different bioaccumulation on similar portal entry.
3. Functionalized GO has a higher probability of excretion from the animal system.
4. Graphene at a dose of 10 mg/m^3 induces pulmonary toxicity by altering the cytokine levels and cell counts in the lung fluid in the mammalian model.
5. GO accumulates in the RES and kidney and induces impairment in renal clearance and mortality.

6. Graphene composites are internalized into the nucleus leading to genotoxicity and upregulation of hsp70.
7. GO induces granuloma in the liver, kidney, lung and spleen of mice.
8. Intra-abdominally injected GO did not induce reproductive toxicity in male mice.
9. Intratracheally instilled aggregated and nanoscale graphene leads to pulmonary inflammation.
10. Graphene exposure in mice through pharyngeal aspiration or intrapleural injection leads to lung inflammation.
11. Exposure of graphene in male Wistar rats by head-nose only inhalation develops microgranuloma in lungs.
12. Intraperitoneally-injected GO on BALB/C male mice induces toxicity to erythroid cells.

Studies on the in vivo toxicity of graphene on various experimental models are summarized in Table 2.

5 Simulation Studies on Graphene Toxicity

The interaction between the graphene and the biological material should essentially be studied for understanding its cellular uptake, toxicity, and toxicity prevention methods. Li et al. have investigated the interaction between graphene sheets and cell membranes (Li et al. 2013). In addition to in vitro cell line studies, they have also used model lipid bilayers by combining coarse-grained molecular dynamics (MD), all-atom MD, analytical modeling. They have suggested that the corners of graphene sheets, asperities that prevail in the asymmetrical boundaries of the graphene sheets pierce and penetrate along the membrane. By this mechanism, even multilayer sheets may make a way into the cell. This simulation study correlates with the report, ideally done by the same team on graphene sheet entry into human keratinocytes, human lung epithelial cells, and murine macrophages. Molecular dynamics study suggests that 2D nanomaterials such as graphene and graphene oxide flakes provide a better surface for anchoring protein residues (Xiaoli et al. 2020). In this context, Jo et al. have reported the cytotoxicity of graphene nanosheets to the blood coagulation protein using simulation studies (Jo et al. 2017). Overall, according to simulation studies, the 2-dimensional nature of graphene in any form is responsible for binding to the major biomolecules such as lipids and proteins which may induce toxicity.

Table 2 In vivo toxicity studies of graphene

Sl. No.	Model used	Particle used	Route of administration	Dosage	Toxicological changes	Reference
1	C57BL/6 mice	Pristine graphene or GO	Intratracheal	50 μg/mouse	Inflammation, increased cell counts, increased interleukin (IL)-6 concentrations	Duch et al. (2011)
2	Mice	GO, rGO	Intravenous	250 μg/kg	Platelet-rich thrombi occluding lung vessels	Singh et al. (2011)
3	Mice	GO	Intravenous	1 mg kg − 1	Pulmonary toxicity	Zhang et al. (2011)
4	Mice	GO	Intravenous	0.1, 0.25, 0.4 mg/mouse	Granuloma formation in the kidney, liver, lungs, and spleen	Wang et al. (2011)
5	Mice	GO	Intravenous	0.1, 0.25, 0.4 mg/mouse	Accumulation in the RES Impairment in the renal clearance	Wang et al. (2011)
6	Balb/c mice	Pegylated GO	Intravenous	4 mg/kg	No noticeable abnormality was observed in liver, spleen, kidney	Yang et al. (2012)
7	Mice	Graphene platelets	Pharyngeal aspiration	50 μg	Expression of pro-inflammatory cytokines (MIP-1α, MCP-1, MIP-2, IL-8, and IL-1β) in the bronchoalveolar lavage and pleural lavage were increased with the exposure of graphene platelets	Schinwald et al. (2012)
8	Wistar rats.	Graphene	Head-nose exposure	10 mg/m^3	Inflammatory response and microgranuloma in the lungs	Ma-Hock et al. (2013)
9	BALB/C male mice	GO	Intraperitoneal	10 mg/kg	Toxicity to erythroid cells	Qu et al. (2013)
10	Male mice	GO	Intravenous	25 mg/kg	Not toxic to reproductive system	Liang et al. (2015)
11	Sprague-Dawley rats	Graphene	Nose-only inhalation	0.12, 0.47, and 1.88 mg/m^3	Pulmonary toxicity	Kim et al. (2016)
12	Rats	Diamond, graphite, GO	Intraperitoneal	4 mg /kg	No toxic effects on blood and no effects on growth small aggregates of nanoparticles were observed in liver and spleen	Kurantowicz et al. (2015b); Strojny et al. (2015)

6 Environmental Toxicity

Graphene and related materials are released into the environment from the large-scale manufacturing source, leaching out from enriched products, accidental spills during industrial production, and improper disposal of the wastes containing graphene-based consumer products (Jastrzębska and Olszyna 2015). The potential exposure and environmental pathways involved in graphene toxicity are air, soil, and the food web in which relevant organisms at different levels of the ecological chain such as bacteria, algae, plants, invertebrates, and vertebrates are affected. The pathways of environmental exposure to graphene are schematized in Fig. 3. Jastrzebska et al. have summarized the effects of graphene family materials on soil and water and reviewed the preliminary impact assessment and potential pathways of distribution of graphene-based materials in the environment (Jastrzębska and Olszyna 2015). Graphene family nanoparticles are transmitted through aquatic environment food chains, however, the detailed pathway through the food chain and the bio-interfacial interactions involved are still unclear (Ma and Lin 2013; Zhao et al. 2014). The Graphene Flagship Project (One of the European Commission's Future and Emerging Technology (FET) Flagship Projects) that was launched in

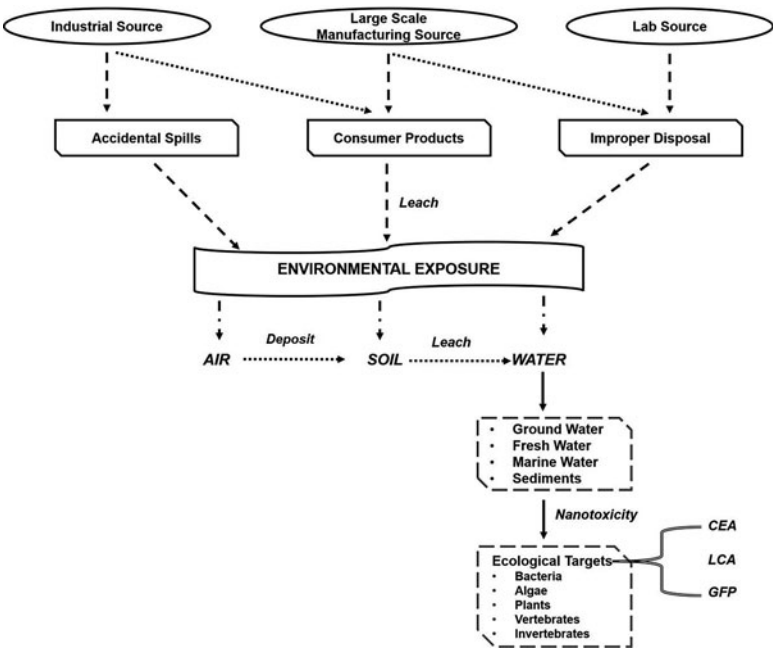

Fig. 3 The flow pathways of environmental exposure to graphene. *CEA* Comprehensive Environmental Assessment tool, *LCA* Life Cycle Assessment tool, *GFP* European Commission's Graphene Flagship Project

2013 is executing long-term, multidisciplinary research through a network of academic and industrial research teams of more than 20 countries in the world. The mission of this flagship is to address to explore the ecotoxicity of graphene by analyzing bacteria, photoautotrophs, invertebrates, and vertebrates in a variety of ecosystems (Fadeel et al. 2018). Aquatic bacteria and algae residing at the bottom of the aquatic food chain are the first victims. These are consumed by invertebrates making the later the next level recipients of toxicity. Fishes are the secondary consumers that feed on crustaceans invertebrates priming the process of biomagnification (Peralta-Videa et al. 2011).

As GO is widely used in various fields such as biology, chemistry, medicine, exploration, and environmental protection (Dreyer et al. 2010; Novoselov et al. 2012), there is a higher probability for release into the environment. Hence its ecological risk should be explored. Plants being the primary producers in ecosystems are easily vulnerable to toxicity induced by nanoparticles including GO. Nevertheless, very few investigations have been carried out on the phytotoxicity of GO. In addition, plants are extensively exposed to arsenic in agriculture. In these circumstances, Bartlem et al. have revealed that GO amplified the arsenic-induced toxicity in plants (Bartlem et al. 2015). GO in combination with arsenic led to a fall in the biomass and root count and an increase in oxidative stress. Metabolism of fatty acids, carbohydrates and amino acids were altered in GO + arsenic- treated plants. Begum et al. revealed that graphene inhibited the plant growth and biomass in by inducing phytotoxicity via oxidative stress and necrosis (Begum et al. 2011). Water is an important environmental component vulnerable to graphene family nanomaterials. Graphene, GO, and rGO were reported to be toxic to aquatic organisms like bacteria, fungi, plants, fishes, and vertebrates which is due to cell membrane damage (Zhao et al. 2014). These studies clearly emphasize the threat of GO to the environment.

Several studies have focused on the toxicity of graphene materials using Escherichia coli. Graphene-based materials establish direct physical contact with the bacteria and affect the membrane integrity, metabolic process, and morphological architecture (Akhavan and Ghaderi 2010; Tu et al. 2013; Efremova et al. 2015). The carbon radical density of hydrated graphene oxide is the principal factor involved in degrading cellular viability (Li et al. 2016). Among photoautotrophs like cyanobacteria and seed plants the internalization and hence the toxicity depends on different stages of growth and age of the plants. Fully grown tissues hinder the entry of graphene materials due to their relatively thicker cell wall barrier as compared to young tissues (Navarro et al. 2008; Tang et al. 2015). The effect of graphene on A. thaliana (a model plant highly suitable for nanotoxicity studies) shows endocytosis-mediated entry and reactive oxygen species-induced nuclear fragmentation, membrane damage, and mitochondrial dysfunction (Begum and Fugetsu 2013).

In microalgae, one of the important primary producers, graphene oxide interacts with the cell surface induces toxicity, oxidative stress, reducing the chlorophyll content or sequestration of nutrients (Du et al. 2016; Zhao et al. 2017). In terrestrial invertebrates, graphene oxide was reported to generate hydroxyl radical leading to

the formation of oxidizing cytochrome C intermediates and also cause germ cell apoptosis (Zhang et al. 2012; Jung et al. 2015; Zhao et al. 2016). Aquatic invertebrates are capable of accumulating graphene oxide after exposure which is capable of transferring to neonates accompanied by a decrease in feeding rate and mobility rate (Guo et al. 2013; Souza et al. 2018). Studies on the toxicity of graphene oxide using the vertebrate model show physical blockage of the gills and digestive tract impairing the nutrient absorption and causing anoxia (Liu et al. 2014).

Thus, graphene toxicity is mediated due to its potential exposure and environmental pathways through air, soil, and the food web. As a result, beneficial bacteria, microalgae, and aquatic organisms and other life forms at different levels of the ecological chain including plants, invertebrates, and vertebrates are affected. In this situation, special environmental impact assessment tools such as the Comprehensive Environmental Assessment (CEA) extended to Life Cycle Assessment (LCA) to identify risks associated with the product are of utter importance in addressing this issue. These tools execute hazard identification (of graphene in a specific product as a function of both exposure potential and toxicity), dose-response assessment, exposure assessment, and risk characterization ecosystems. Considering the ecotoxicity of graphene, guidelines have been suggested which recommends the use of individual graphene sheets for easy dispersibility and minimal clumping; and the use of surface-engineered graphene material to enhance the biodegradability (Bussy et al. 2013).

7 Toxicity Is Beneficial in the Context of Cancer and Microbiology!

In few cases, graphene toxicity can be taken as an advantage. Graphene platelets have an apoptotic effect which might be useful for cancer therapy. Toxicity of GO to the skin has proved to be beneficial in killing the skin cancer cells via photothermal therapy. The topical application of GO nanoparticles leads to their accumulation in skin cancer cells. Subsequent irradiation with near infrared light generates heat results in the local killing of tumor cells with little side effects as opposed to intravenous administration (Jung et al. 2014). GO supports molecular imaging and pH-sensing due to its pH-dependent fluorescence emission in the visible/near-infrared region (Shamsipur et al. 2019). In addition its water soluble property creates a platform for functionalization and thus acts as a multiple therapeutics (Deb et al. 2018). Previous study demonstrated the potential use of GO as non-invasive optical sensor. The results showed a decreased green/red (550/630 nm) fluorescence intensity ratios toward HeLa and MCF-7 cancer cells in comparison with HEK-293 healthy cells suggesting the use of GO as biological imaging and cancer sensing agent (Campbell et al. 2019). Doxorubicin (DOX) loaded folic acid conjugated chitosan/amine functionalized GO shows a high loading capacity with higher drug release rate at pH 5.3. Moreover, the results display an effective intracellular uptake

of the drug into HeLa cells through FA receptors suggesting functionalized GO as a suitable drug carrier for anticancer drug delivery (Anirudhan et al. 2020). Fluorouracil loaded GO based nanosheets functionalized with GE11 (ligand for epidermal growth factor receptor) show 90% tumor inhibition in colorectal cancer (CRC) bearing mouse model (Qiu et al. 2020). Wierzbicki et al. 2018 demonstrated the use of graphite nanoparticles and graphene oxide nanoplatelets in glioma angiogenesis and its microenvironment. The results revealed a significant decrease in the angiogenic potential of wild-type p53 glioma cell line treated with graphite nanoparticles and graphene oxide nanoplatelets (Wierzbicki et al. 2018). Graphene and rGO were reported for its anticancer properties in glioblastoma multiforme cells. Interestingly, rGO showed the highest level of apoptosis compared to graphene. The efficacy of rGO was associated with the presence of oxygen-containing functional groups (Szczepaniak et al. 2018). Pristine graphene platelets induced dose-dependent cytotoxicity in glioma cells through mitochondrial membrane potential depletion and ROS overproduction. Moreover reduction in mass and volume of tumors were reported with the in vivo model (Jaworski et al. 2019). GO was proved to down-regulate the mRNA expression of mitochondrial oxidative phosphorylation (OXPHOS) nuclear genes in glioblastoma cell line more efficiently than rGO and pristine graphene (Szmidt et al. 2019). Aminated GO particles exhibit higher cytotoxic potential through the ROS induction, subsequent DNA damage, and apoptosis than pristine GO was suggested for the treatment of colon cancer (Krasteva et al. 2019).

The toxicity of graphene may be advantageous in the context of microbiology. The bacterial toxicity of graphene nanosheets makes them an excellent antibacterial agent. GO nanowalls were reported to be toxic to the gram-positive Staphylococcus aureus probably due to direct interaction with the outer surface of the bacterial cell. GO reduced with hydrazine was found to be even more toxic than unreduced GO, due to better charge transfer between the organism and the edges of the nanowalls (Orecchioni et al. 2017). Reduced graphene nanowalls and graphene nanowalls were found to induce membrane damage and significant loss of viability in *E. coli* and *S. aureus* cells (Akhavan and Ghaderi 2010). Studies on E coli reveal that nanosheets of GO and rGO damage the cell membrane and impair cellular metabolism (measured using ATP assay) and cell viability (measured by spread plate method) (Hu et al. 2010). Kurantowicz et al. reported the antimicrobial property of pristine graphene, GO, and rGO against *Listeria monocytogenes* and *Salmonella enterica*. The complete inhibition of both pathogens was observed with all type of graphenes at high concentration. While at lower concentration, similar effects were only observed with GO (Kurantowicz et al. 2015a). Liu et al. rated the bacterial toxicity of the graphene and its derivatives against the E coli in the following order:

Graphene oxide dispersion > reduced graphene oxide > graphite > graphite oxide.

The proposed mechanism was the deposition of bacterial cells on the graphene and its relatives followed by reactive oxygen species-independent oxidative stress (Liu et al. 2011). GO exposure in *S. aureus* and *E. coli* induces the degradation of bacterial intracellular components through oxidative stress. In addition, the cellular

membrane was easily pierced by extremely sharp edges of GO and thereby reducing the cell viability due to the loss of intracellular molecules. However, the cell membrane of S. *aureus* was more susceptible to GO in comparison with *E. coli* (Farid et al. 2018). Treatment with higher concentrations of GO resulted in the bacterial cell (*S. aureus* and *E. coli*) death (Valentini et al. 2019). Silver nanoparticles (Ag-NPs) decorated GO nanocomposite was reported as novel multifunctional antibacterial and antifungal material. The results revealed that the nanocomposite possesses high antimicrobial potential against bacteria and yeast cells in comparison with that of Ag-NPs and GO (Jaworski et al. 2018).

Morphology of graphene and its derivatives gained a major part in tissue engineering. N-acetyl cysteine-loaded GO hybrid membrane was reported to promote fibroblast migration and proliferation on account of its better mechanical property and stronger water retention capacity. Moreover, the hybrid membrane showed complete healing in the rat wound model and its anti-scar effect was evidenced by the decreased mRNA expression of profibrotic and overexpression of anti-fibrotic factors (Li et al. 2019). Nanoscale graphene with crumpled morphologies promotes the differentiation of C2C12 mouse myoblast cells into myotubes more efficiently than flat graphene supports its value in tissue engineering (Kim et al. 2019). GO nanofilm supplemented with L-Glu showed an improved myogenic potential of myocytes that involved in muscle formation (Zielińska-Górska et al. 2020).

Hence, the toxicity of graphene and its derivatives may be exploited for killing cancer cells of skin (GO), breast (GO), glioma (graphite nanoparticles), and colon (aminated GO) as well for killing bacterial cells (pristine graphene, GO, and rGO). This graphene and its derivatives may evolve as effective anticancer and antibacterial product.

8 How to Reduce the Toxicity?

Studies indicate that functionalization of graphene with biocompatible material and using of graphene in the form of the film may reduce the toxicity to a certain extent. Coating of GO with a biocompatible material such as polyethylene glycol has shown negligible toxicity in cell lines. This result was confirmed by repeating the experiments in different cell lines. GO was found to be significantly less toxic to Raji, HCT-116, OVCAR-3, U87MG, MDA-MB-435, and MCF-7 cell lines even at high concentrations up to 100 mg/L (Liu et al. 2008; Sun et al. 2008). Intensive investigations by Yang et al. and others in mice model have revealed the non-toxic nature of PEG-functionalized GO of 5–10 nm size, after intravenous administration. Parallelly, the GO nanoparticles were found to be predominantly accumulated in reticuloendothelial system (liver and spleen) instead of lungs without producing toxicity at the tested dose of 20 mg/kg (Yang et al. 2008, 2010, 2012; Zhou et al. 2009; Zhang et al. 2010a). These findings reveal that the functionalized and unfunctionalized GO has the different fate of accumulation and different level of toxicity though they have similar portal entry. Thus, we could suggest that

functionalized GO is more biocompatible and less toxic than the naked GO. Also, the functionalized GO has more probability for excretion. Apart from the functional moieties, the dimension of the graphene plays a role in toxicity induction. This was evident from the investigations on the toxicity of graphene film. Mouse pheochromocytoma cells, human oligodendroglia cells, and human fetal osteoblasts were found to be compatible with GO film (Agarwal et al. 2010). Interestingly, Ruiz et al. have reported that graphene oxide film promoted the adhesion, growth and maintained the normal morphology of mammalian colorectal adenocarcinoma HT-29 cells (Ruiz et al. 2011). An investigation by Li et al. has demonstrated the significant biocompatibility, neurite growth, and multiplication of mouse neuronal cells grown on graphene film (Li et al. 2011). Go functionalized with polyvinylpyrrolidone possesses good biocompatibility immune-enhancement and immune-adjuvant in human-derived immune cells such as dendritic cells, T lymphocytes, and macrophages, thus having the potential to emerge as an immune-adjuvant candidate (Zhi et al. 2013). GO was reported for non-cell-specific cytokine production throughout cell populations, on the other hand, amino-functionalization of GO changes the effect on human immune cells: and produce a more specific, polarized Th1 response (Orecchioni et al. 2017). Amino functionalized GO treatment in C. elegans did not cause serious detrimental effects, i.e. lower innate immune response and not affecting pmk-1. Interestingly, an extended lifespan was observed with amino-functionalized GO treated animals. While GO exposure significantly decreased animal size and induced morphological abnormalities in the pharynx and the intestine (Rive et al. 2019).

9 Conclusion

This review reveals the toxicity of graphene and its relatives in cell lines, animals, plants, and microbes. Simulation studies also revealed the graphene toxicity. Though there are massive numbers of studies on the in vitro and in vivo toxicity of graphene, the influence of size, surface function, and route of administration on the toxicity, reticuloendothelial uptake profile, and excretion requires further study. Graphene and its family members were also reported to be less toxic if properly functionalized. Therefore, identification of ideal biocompatible surface functional moieties and treatment regime for graphene toxicity also warrants intensive studies as it is very essential for the society. These studies will open avenues for preclinical single and repeat dose safety studies.

Future Prospects

As per the available literature, the toxicity of graphene-based materials was reported in microorganisms, plants, aquatic and terrestrial inhabitants of the ecosystem. Adverse effects of graphene in vitro and in vivo in the mammalian system have also been explored. Graphene is capable of accumulating in different vital organs and the toxicity is determined by the surface functionalization and the resulting

change in dispersibility and biodegradability. However, the development of standardized protocols or certified reference materials for the ecological toxicity assessment of different samples and the review thereof is still in infancy and is evidently a challenge to be addressed in the future. Lack of unanimity in the quantifiable toxicity markers such as LD50, LC50, and MIC, germ cell apoptosis, and impairment of germination is a demerit in ecotoxicology. Hence, misinterpretations of inhibitory markers should be avoided and the issue should be addressed with extra care. Chronic effects of graphene should be evaluated on different organs of the experimental model with an emphasis on bioaccumulation and biodegradation. Another important concern is the environmental concentrations of anthropogenic graphene which is very much essential for toxicological studies.

Conflict of Interest The authors declare that they have no conflict of interest.

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Multi-Level Gene Expression in Response to Environmental Stress in Aquatic Invertebrate Chironomids: Potential Applications in Water Quality Monitoring

Kiyun Park and Ihn-Sil Kwak

Contents

Abstract In freshwater ecosystems, aquatic invertebrates are influenced continuously by both physical stress and xenobiotics. Chironomids (Diptera; Chironomidae), or non-biting midges, are the most diverse and abundant invertebrates in freshwater habitats. They are a fundamental link in food chains of aquatic ecosystems. Chironomid larvae tolerate stress factors in their environments via various physiological processes. At the molecular level, environmental pollutants induce multi-level gene responses in *Chironomus* that regulate cellular protection through the activation of defense processes. This paper reviews literature on the transcriptional responses of biomarker genes to environmental stress in chironomids at the molecular level, in studies conducted from 1991 to 2020 (120 selected literatures of 374 results with the keywords "*Chironomus* and gene expression" by PubMed search tool). According to these studies, transcriptional responses in

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chironomids vary depending on the type of stress factor and defensive responses associated with antioxidant activity, the endocrine system, detoxification, homeostasis and stress response, energy metabolism, ribosomal machinery, apoptosis, DNA repair, and epigenetics. These data could provide a comprehensive overview of how *Chironomus* species respond to pollutants in aquatic environments. Furthermore, the transcriptomic data could facilitate the development of genetic tools for water quality and environmental monitoring based on resident chironomid species.

Keywords Aquatic monitoring · Chironomidae · Environmental stressor · Gene expression · Transcriptome

1 Introduction

Freshwater ecosystems are continuously exposed to numerous pollutants generated by anthropogenic activities, in addition to fluctuating or shifting environmental conditions. Over time, the aquatic invertebrates inhabiting freshwater become vulnerable to physical stress (e.g., temperature) and xenobiotics. The stress factors could elicit gene responses to regulate defense mechanisms that could facilitate survival and tolerance under stressful environments in aquatic ecosystems. The gene responses could be employed as pollution biomarkers for monitoring the quality and health of complex environments (Mantilla et al. 2018).

Molecular technologies have been utilized in ecotoxicology since the early 1990s, leading to the emergence of a research field referred to as ecotoxicogenomics (Neumann and Galvez 2002). At the molecular level, exposure of organisms to stress could trigger a cascade of effects and stimulate the transcriptional expression of genes participating in cellular homeostasis. If the stress factor persists or its levels increase gradually, it could lead to cellular damage and breakdown of the comprehensive defense systems associated with antioxidant mechanisms, endocrine processes, detoxification, homeostasis and stress response, immune processes, energy metabolism, ribosomal machinery, apoptosis, DNA repair, and epigenetics (Fedorenkova et al. 2010; Mantilla et al. 2018). The detection of transcriptional profiles has been proposed as an approach that could provide insights into, and facilitate risk assessment of, actual and potential damage caused by environmental stress. Such tools could be employed as early and sensitive warning systems of environmental contaminants or change before the impacts are discernible at the population level in biological communities (Clements 2000; Steinberg et al. 2008; Fedorenkova et al. 2010; Lauritano et al. 2012; Marinkovic et al. 2012; Mantilla et al. 2018). Here, we focus on transcriptional responses of genes that mediate defense mechanisms in aquatic invertebrates and classify the types of stressors involved into physical factors and chemical compounds, based on the results of studies that have been conducted on aquatic chironomids from 1991 to 2020.

2 Chironomids as Bioindicator Species in Aquatic Ecosystem

Chironomids (Diptera; Chironomidae), known as non-biting midges, are the most abundant benthic macroinvertebrates in freshwater habitats (Armitage et al. 1995). In aquatic ecosystems, they act as deposit-feeders, feeding on deposited organic matter, and are an essential food resource for a wide range of animals (Armitage et al. 1995; Allgeier et al. 2019). The chironomid life cycle involves the metamorphosis of four developmental stages (Fig. 1). Chironomidae develop from an egg, passing through a series of four metamorphoses (instars), a pupa stage, and final emergence as adults. The reproductive cycle begins when the chironomid lays egg masses, comprising a gelatinous matrix (Fig. 1a), on a hard substrate at the water's edge. After hatching, larval instars undergo developmental molts in four stages (Fig. 1b),

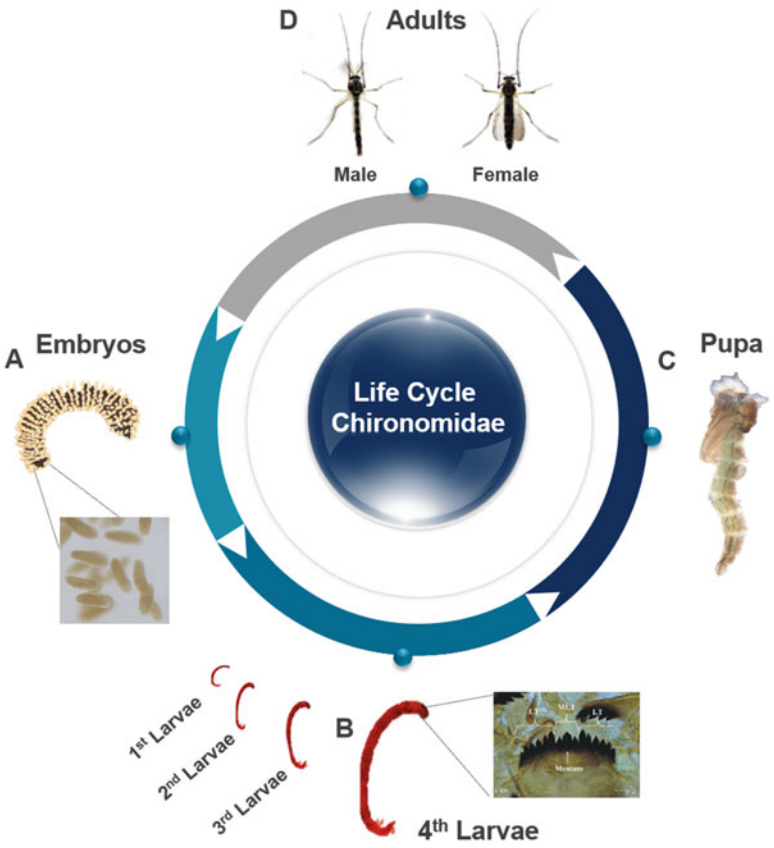

Fig. 1 Different developmental stages of Chironomidae. (**a**) An egg mass. Each egg mass contains hundreds of eggs surrounded by a gelatinous matrix. (**b**) The larvae (red-colored because the hemolymph contains hemoglobin) develop four molt stages before transforming into a pupa. (**c**) Pupal stage. (**d**) Adult male and female

distinguishable based on head diameter and body length (Failla et al. 2015). The larval metamorphoses, from planktonic first instar to benthic fourth instar, occur in the aquatic phase (Laviad and Halpern 2016). These larval bodies display a characteristic red color because of the presence of hemoglobin (Fig. 1b). After a pupation lasting 16–18 days (Fig. 1c), adults emerge at the terrestrial phases (Khosrovyan and Kahru 2020). Chironomid species develop entirely in an interphase of sediment and water, except during the adult stage in which they transition to the air (Fig. 1d) (Halpern and Senderovich 2015; Laviad and Halpern 2016). The entire life cycle is completed in approximately 1 month (Armitage et al. 1995); therefore, chironomids must reproduce rapidly and in high numbers.

Chironomid larvae are macroinvertebrates inhabiting benthic environments and are key components of the benthic communities of freshwater ecosystems (Pinder 1986). They are a major link between producers and secondary consumers in food chains of aquatic environments (Mantilla et al. 2018). The number of species in the Chironomidae family is estimated to be approximately 15,000–20,000 (Ali 1995). They can survive in aquatic environments with limited nutrient resources and are found in almost all types of aquatic habitats, although they mainly inhabit lotic and lentic environments (Epler 2001). In addition, they can put up with extreme environmental fluctuations in organic pollution, heavy metal loads, pH, temperature, salinity, depth, and flow rate (Armitage et al. 1995; Park and Kwak 2014; Muñiz-González and Martínez-Guitarte 2020a), thereby dominating polluted environments (Richardson and Kiffney 2000; Arambourou et al. 2019; Im et al. 2019; Muñiz-González and Martínez-Guitarte 2020b).

Members of the chironomid family are widely used in ecotoxicological research to investigate the potential impacts of environmental change because of their short life cycles, widespread distribution, and ability to be cultured easily, in addition to their status as model organisms in standardized tests (OECD 2004a, b, 2010, 2011). Numerous studies have been conducted to evaluate their responses to toxicity based on biological endpoints such as survival, growth, emergence, sex ratio, mouthpart deformities, and developmental parameters (Nieto et al. 2017; Park and Kwak 2018; Arimoro et al. 2018; Im et al. 2019). The genotoxic effects of exposure to pollutants have also been observed directly in the giant polytene chromosomes from the salivary gland cells of chironomids (Michailova et al. 2003, 2006; Planelló et al. 2007). In addition, over the last two decades, the differential expression of numerous genes has been reported in chironomid species following exposure to various contaminants (Mantilla et al. 2018). Such studies suggest that chironomid species are appropriate model organisms for use in the evaluation of potential toxicity at the molecular level.

3 Molecular Genetic Research on Environmental Stress in Chironomids

Aquatic organisms are frequently exposed to fluctuations in temperature, mainly increases, due to climate change derived from anthropogenic effects. In the early stages of research, molecular studies on chironomids investigated the molecular

mechanisms underlying heat stress responses. Heat-shock induced proteins were first reported in *Chironomus tentans* salivary glands by Vincent and Tanguay (1979), and heat shock proteins (*HSPs*) were first characterized in *C. tentans* salivary glands by Tanguay and Vincent (1981). *HSP* genes control the stress response and are developmentally regulated, which is important for insects to survive and adapt to their environments (Zhao and Jones 2012). A 70-kDa heat shock cognate (*HSC70*) gene was cloned in *C. tentans* and *Chironomus yoshimatsui*, and the responses of heat shock protein 70 (*HSP70*) following exposure to Cu were reported in *C. tentans* using the western blotting technique by Karouna-Renier et al. (2003) and Karouna-Renier and Zehr (2003). In addition, the northern blotting technique facilitated studies on gene expression in response to external stimuli in chironomids prior to the year 2006 (Govinda et al. 2000; Yoshimi et al. 2002). The earliest studies quantifying gene expression patterns using reverse transcription-quantitative polymerase chain reaction (RT-PCR) in chironomids are those of Lee et al. (2006), who investigated the expression of *HSP* and hemoglobin genes (*Hbs*) in *C. tentans* in response to various environmental pollutants. Furthermore, Martínez-Guitarte et al. (2007) investigated the expression patterns of ribosomal proteins L11 (*RPL11*) and L13 (*RPL13*) in *Chironomus riparius* under conditions of heat shock or Cd exposure, and Park and Kwak (2008a) evaluated *HSPs* expression in *C. riparius* in response to exposure to di(2-ethylhexyl) phthalate (DEHP).

One study by Xiuwei et al. (2009) evaluated the expression levels of 11 glutathione S-transferases (*GSTs*) genes after exposure to an herbicide in *C. tentans,* while another measured those of various biomarker genes (*HSPs, CYPs, GSTs,* and *Hbs*) in response to exposure to a veterinary antibiotic in *C. riparius* (Park et al. 2009). Although research has been conducted on chironomids since 1991, since 2009 gene response research has been emphasized by global research teams. Numerous studies have investigated the gene response against xenobiotics in chironomids, as well as physical stimuli, such as temperature. As illustrated in Fig. 2, in chironomids, gene responses to heavy metals and endocrine disruptor chemicals (EDCs) have been those which are predominantly studied. The other highly studied xenobiotics are ultra-violet (UV) filters, insecticides/pesticides, antibiotics, herbicides, and nanoparticles (5–11%). Other investigated chemicals have been fungicides, carcinogenic substances, biocides, synthetic estrogen, as well as the combined exposures to these agents (Fig. 2a). In addition, published articles have reported the gene expression responses of chironomids to EDCs, such as nonylphenol (NP; 31%), DEHP (25%), bisphenol-A (BPA; 20%), benzyl butyl phthalate (BBP; 9%), pentachlorophenol (PCP; 4%), phenol (4%), diethyl phthalate (DEP; 2%), bisphenol S (BPS; 2%), and bisphenol P (BPP; 2%) (Fig. 2b). Furthermore, the effects of heavy metals, such as Cd (69%), Cu (20%), Pb (5%), Zn (3%), and Cr (3%), on gene expression have been studied (Fig. 2c).

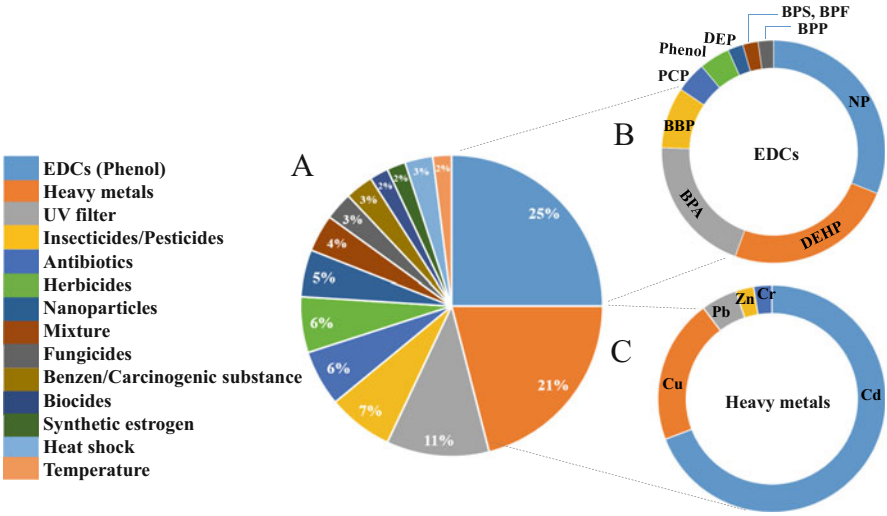

Fig. 2 The proportions of molecular studies on physical and chemical stress factors in Chironomids. (**a**) Total percentage of physical and chemical stressor types. (**b**) The proportions of studies exploring the effects of various types of EDCs. (**c**) The proportions of studies exploring the effects of heavy metals

3.1 Gene Responses to Physical Stressors in Chironomids

3.1.1 Heat Shock

Warming climate and environmental pollution confer the greatest influence on biogeochemical processes in global ecosystems. Temperature is a key environmental factor that may impact the life cycles of *Chironomus* individuals (Park and Kwak 2014). Molecular research in chironomids began with the investigation of the responses of *HSP* genes to heat shock (Vincent and Tanguay 1979; Tanguay and Vincent 1981). Gene responses to heat shock have been reported in three species in the family Chironomidae (Table 1). The responses of *HSP* genes to heat shock have been studied; the upregulation of *HSP70* and small *HSPs* (*HSP17, HSP23, HSP24,* and *HSP27*) was observed, along with the downregulation of small *HSPs* (*HSP21* and *HSP22*) (Table 1). The *HSPs*, considered stress proteins and extrinsic chaperones, function as helper molecules for all protein and lipid metabolic activities, as determined by studies that have recognized upregulation in response to external changes as well as heat stress (Roberts et al. 2010; Martín-Folgar et al. 2015). However, the expression patterns of ribosomal proteins indicated downregulation or non-significant responses to heat shock (Martínez-Guitarte et al. 2007).

Table 1 Gene expression profiles in response to heat shock, temperature, and UV filters in chironomids, reported in studies from 1991 to 2020. Full names of the analyzed genes are listed in Supplementary Table 1

Stressor		Species (Chironomidae)	Gene	Response	Reference
Heat shock	35, 39°C	*Chironomus riparius*	*HSP17, HSP21, HSP22, HSP23, HSP24, HSP34, HSP27, HSP70*	Upregulation (*HSP17, HSP23, HSP24, HSP27, HSP70*) Downregulation (*HSP21, HSP22*)	Martín-Folgar et al. (2015)
	35°C	*Chironomus riparius*	*HSP27*	Upregulation (heat shock) Downregulation (cold shock)	Martínez-Paz et al. (2014)
	35°C	*Chironomus riparius*	*HSP70*	Upregulation	Morales et al. (2011)
	37°C	*Chironomus yoshimatsui*	*HSP70*	Upregulation	Yoshimi et al. (2009)
	35°C	*Chironomus dilutus*	*HSP70*	Upregulation	Karouna-Renier and Rao (2009)
	35°C	*Chironomus riparius*	*RPL11, RPL13*	No significant change	Martínez-Guitarte et al. (2007)
Temperature	18.5, 23°C	*Chironomus riparius*	*EcR, InR, Met, CYP4d2, CYP6b7, GSTd6, GSTo1, MRP-1, HSP22, HSP27, HSP70, HYOU, Gp93*	Upregulation (*HSP22*) Downregulation (*CYP6b7, GSTd6, GSTo1*)	Muñiz-González and Martínez-Guitarte (2020a)
	10, 20, 30°C	*Chironomus riparius*	*EcR, USP, ERR, CAT, Px, SOD, GPx*	Upregulation	Park and Kwak (2014)

(continued)

Table 1 (continued)

Stressor		Species (Chironomidae)	Gene	Response	Reference
	26, 32°C	*Pseudodiamesa branickii*	*HSP70*	Upregulation	Bernabò et al. (2011)
	15, 20, 25, 30°C	*Belgica Antarctica*	*HSP70, HSP90, small HSP*	Upregulation	Rinehart et al. (2006)
UV filter	Benzophenone-3 (BP3) (organic compound used as common ingredient in sunscreen)	*Chironomus riparius*	*CYP12A2, CYP6B7, CYP9F2, CYP4D2, MRP1, GSTd3, GSTd6, GSTe1, GSTo1, GSTt1*	Upregulation (*MRP1, GSTd3, GSTe1, GSTo1*)	Martínez-Guitarte (2018)
		Chironomus riparius	*EcR, HSP70*	Upregulation	Ozáez et al. (2016b)
		Chironomus riparius	*EcR, USP, ERR*	Upregulation (*EcR*)	Ozáez et al. (2013)
	4-methylbenzylidene camphor (4MBC) (organic camphor derivative used as a UVB filter in sunscreen and cosmetic products)	*Chironomus riparius*	*CYP12A2, CYP6B7, CYP9F2, CYP4D2, MRP1, GSTd3, GSTd6, GSTe1, GSTo1, GSTt1*	Upregulation (*MRP1, GSTd3, GSTe1, GSTo1*)	Martínez-Guitarte (2018)
		Chironomus riparius	*EcR, HSP70*	Upregulation	Ozáez et al. (2016a, b)
		Chironomus riparius	*EcR, USP, ERR*	Upregulation (*EcR*)	Ozáez et al. (2013)
	Octyl-p-methoxycinnamate (OMC) (organic compound used as an ingredient in sunscreens and lip balms)	*Chironomus riparius*	*EcR, HSP70*	Upregulation	Ozáez et al. (2016a, b)
		Chironomus riparius	*EcR, USP, ERR*	Upregulation (*EcR*)	Ozáez et al. (2013)
	2-ethylhexyl-4-methoxycinnamate (EHMC) (octinoxate is an oily UV absorber used in sunscreens)	*Chironomus riparius*	*EcR, HSP70*	Upregulation	Ozáez et al. (2016a)

	4-hydroxybenzophenone (4HB) (organic compound used as ingredient in sunscreen)	*Chironomus riparius*	*EcR, HSP70*	Upregulation	Ozáez et al. (2016a)
		Chironomus riparius	*EcR, USP, ERR*	Upregulation (*EcR*)	Ozáez et al. (2013)
	Octocrylene (OC) (organic compound used as synthetic UV absorber of sunscreens)	*Chironomus riparius*	*EcR, E93, Kr-h1, Dronc, Dis, CYP18a1, Met, JHAMT, InR, MAPR, CYP4d2, CYP6b7, CYP9f2, CYP12a2, GSTd3, GSTe1, GSTo1, GSTt1, MRP1, ABCB6, HSP70, HSC70–5, HYOU1, HSP40, HSP90, Gp93, HSP60, HSP10, HSP17, HSP21, HSP22, HSP23, HSP24, HSP27, DECAY, XRCC1, NLK, ATM, Def, prePO*	Upregulation (*MAPR, GSTt1, HSP70*)	Muñiz-González and Martínez-Guitarte (2020b)
		Chironomus riparius	*HSP70, RPL13, GSTd6, GSTe1, GSTo1, GSTt1, JHAMT, HYOU1, Efl, Defensin, PPO1, Kr-h1, CYP18A1, MAPR, CYP12A2, CYP6B7, CYP9F2, CYP4D2*	Downregulation (*HYOU1*) No significant changes	Muñiz-González and Martínez-Guitarte (2018)
		Chironomus riparius	*EcR, HSP70*	Upregulation	Ozáez et al. (2016a)
		Chironomus riparius	*EcR, USP, ERR*	Upregulation (*EcR*)	Ozáez et al. (2013)
	2-ethylhexyl 4-(dimethylamino) benzoate (OD-PABA) (organic compound related to the water-soluble compound PABA)	*Chironomus riparius*	*EcR, E93, Kr-h1, DRONC, Dis, CYP18a1, Met, JHAMT, InR, MAPR, CYP4d2, CYP6b7, CYP9f2, CYP12a2, GSTd3, GSTe1, GSTo1, GSTt1, MRP1, ABCB6, HSP70, HSC70-5, HYOU1, HSP40, HSP90, Gp93, HSP60, HSP10, HSP17, HSP21, HSP22, HSP23, HSP24, HSP27, DECAY, XRCC1, NLK, ATM, Def, prePO*	Upregulation (*GSTt1, HSP70*)	Muñiz-González and Martínez-Guitarte (2020b)

(continued)

Table 1 (continued)

Stressor		Species (Chironomidae)	Gene	Response	Reference
		Chironomus riparius	*HSP70, RPL13, GSTd6, GSTe1, GSTo1, GSTt1, JHAMT, HYOU1, Efl, Defensin, PPO1, Kr-h1, CYP18A1, MAPR, CYP12A2, CYP6B7, CYP9F2, CYP4D2*	Downregulation (*HYOU1*) No significant changes	Muñiz-González and Martínez-Guitarte (2018)
		Chironomus riparius	*EcR, HSP70*	Upregulation	Ozáez et al. (2016a)
		Chironomus riparius	*EcR, USP, ERR*	Upregulation (*EcR*)	Ozáez et al. (2013)
	Gamma radiation	*Chironomus ramosus*	*HSP70*	Upregulation	Datkhile et al. (2011)

3.1.2 Temperature

Temperature is important in invertebrate organisms, such as *Chironomus,* since they are poikilothermic (Everatt et al. 2015; Wojda 2017). Recently, a study reported that *C. riparius* may adapt rapidly to temperature changes via genome-wide selection (Pfenninger and Foucault 2020). Increasing temperature induced upregulation of *HSP70* gene in *Pseudodiamesa branickii* and *Belgica antarctica* (Table 1). Temperature changes over the course of development alter the expression of hormone-related genes (Ecdysone receptor, (*EcR*), Ultraspiracle *(USP*), and Estrogen related receptor (*ERR*)) and antioxidant enzymes (Catalase (*CAT*), Peroxidase (*Px*), Superoxide dismutase (*SOD*), and Glutathione peroxidase (*GPx*)) (Park and Kwak 2014). Muñiz-González and Martínez-Guitarte (2020a) reported the downregulation of *CYP6B7*, GST omega 1 (*GSTo1*), and GST delta 6 (*GSTd6*) genes and the upregulation of *HSP22* as well as the increased activities of phenoloxidase (*PO*) and acetylcholinesterases (*AchE*) enzymes in *C. riparius* exposed to a temperature of 23°C.

3.2 Gene Responses to Chemical Stressors in Chironomids

3.2.1 UV Filters

UV filters are compounds used to prevent damage to skin from UV radiation (Ozáez et al. 2016a). It is an emerging contaminant that is ubiquitous in aquatic systems due to its widespread use in personal care and industrial products, including plastics, cosmetics, paints, and textiles (Ozáez et al. 2016b). Since it was reported that *HSP70* expression was induced by gamma radiation in *C. ramosus*, gene responses have been observed in *C. riparius*, in response to seven types of UV filters, including benzophenone-3 (BP3), 4-methylbenzylidene camphor (4MBC), octyl-p-methoxycinnamate (OMC), 2-ethylhexyl-4-methoxycinnamate (EHMC), 4-hydroxybenzophenone (4HB), octocrylene (OC), and 2-ethylhexyl 4-(dimethylamino) benzoate (OD-PABA) (Table 1). It has been suggested that UV filters cause endocrine disruptions due to the alteration of genes related to the endocrine system, specifically, hormone 20 hydroxyecdysone or the juvenile hormone, thereby modulating the expression of these hormones and modifying their metabolism (Table 1). BP3 or 4MBC exposure has also been shown to disrupt the expression of genes related to detoxification responses, including phase II members, such as *GSTs* (GST delta 3 (*GSTd3*), GST epsilon 1 (*GSTe1*), and *GSTo1*), as well as phase III members, such as multidrug resistance protein 1 (*MRP1*) (Martínez-Guitarte 2018). *HSP70* expression increased in *C. riparius* when exposed to all UV filters, while the expression of hypoxia upregulated 1 (*HYOU1*) gene of the *HSP70* family decreased after exposure to OD-PABA (Table 1).

3.2.2 Heavy Metals

Heavy metals are contaminants with a growing presence in aquatic environments due to increased industrial development and human activity (Martín-Folgar and Martínez-Guitarte 2019). Freshwater invertebrates are frequently exposed to heavy metal contamination. Heavy metals represent one of the most studied chemical stress factors with regard to their impacts on gene expression in chironomids (Fig. 2). Cd, the most extensively studied heavy metal in chironomids (Fig. 2), is ubiquitous and highly toxic, and is of ecotoxicological relevance for aquatic invertebrates because of its natural occurrence in the environment and its impact as an industrial pollutant with carcinogenic potentials (Martín-Folgar and Martínez-Guitarte 2017. Cd exposure has been reported to upregulate the expression of antioxidant genes (Total glutathione (*GSHt*), *CAT*, and GST sigma 4 (*GSTs4*)), detoxification-related genes (Phospholipid hydroperoxide glutathione peroxidase (*PHGPx*), *GSTd3*, *GSTe1*, and Carboxylesterase (*CarE*)), and GSH biosynthesis genes (γ-glutamylcysteine synthetase (*GCS*) and glutathione synthetase (*GS*)) in *C. riparius* (Table 2). In addition, genes related to apoptosis and stress response (Death regulator Nedd2-like caspase (*DRONC*), Glycoprotein 93 (*Gp93*), StAR-related lipid transfer domain-containing protein (*START1*)), digestive system (Serine-type endopeptidase (*SP*)), and hormonal processes (*EcR*) have been reported to be upregulated in *C. riparius* (Table 2). Furthermore, Martín-Folgar and Martínez-Guitarte (2017) observed the upregulation of the small *HSP23, HSP24, HSP27,* and *HSP34,* while the expression of *HSP17* and *HSP21* was downregulated in *C. riparius* following exposure to Cd. Cu exposure has also been reported to increase the levels of expression of numerous genes (*DRONC, Gp93, CYP4G, HSP70, HSC70,* and *HSP10*), with these findings being consistent with the results reported for Cd toxicity. *HSP70* expression was upregulated in *Chironomus* following exposure to Cd and Cu, with the levels of expression being influenced by the concentrations of the heavy metals.

In *Chironomus tepperi*, Cd or Cu exposure induced upregulation of *GCS* and *GS* gene expression, and downregulation of the S-Adenosyl-L-homocysteine (*SAH*) and Adenosylmethionine (*SAM*) genes (Jeppe et al. 2014). However, the genes related to cysteine metabolism (Cystathionine-β-synthase (*CβS*)) exhibited contradictory responses to Cd (upregulation) and Cu (downregulation) toxicity in *C. tepperi*. Cystathionine-γ-lyase (*CγL*) gene expression increased only to Cu exposure, not that of Cd (Table 2). Cysteine is a precursor of GSH that is involved in antioxidant defense pathways. In addition, cysteine metabolism participates in detoxification processes under environmental stress conditions (Jeppe et al. 2014). In addition, metallothionein (*MT or Mtn*) gene expression is induced by Cd or Zn exposure. Zn toxicity increased expression of *SAH* mRNA in *C. tepperi*. Zheng et al. (2018) reported the upregulation of metabolism-related glycolytic enzyme (Enolase 1 (*Eno1*)) to Cd toxicity in *Propsilocerus akamusi*. Reduced Balbiani ring 2 (*BR2*) activity and no significant change in *BR1* were observed in *Chironomus ninevah* larvae in response to Cu toxicity (Aziz et al. 1991). Furthermore, exposure to Pb or Cr induced the upregulation or downregulation of *HSP70* and *Hbs* in the chironomids.

Table 2 Gene expression profiles in response to heavy metals and nanoparticles in chironomids, reported in studies from 1991 to 2020. Full names of the analyzed genes are listed in Supplementary Table 1

Stressor		Species (Chironomidae)	Gene	Response	Reference
Heavy metal	Cd	*Chironomus plumosus*	*HSP70, SP*	Upregulation	Park and Kwak (2020)
		Chironomus riparius	*DRONC, IAP1, PO1, Defensin, Gp93, Ctr1*	Upregulation (*DRONC, Gp93*)	Martín-Folgar and Martínez-Guitarte (2019)
		Propsilocerus akamusi	*Eno1*	Upregulation	Zheng et al. (2018)
		Propsilocerus akamusi	*PaHbV, PaHbVII*	Tissue-dependent up- or downregulation	Zheng et al. (2017)
		Chironomus riparius	*P38 MAPK*	Up- or downregulation depending on the concentration and exposure	Park and Choi (2017)
		Chironomus riparius (wild population)	*GSH_t, CAT, GSTs, LPO*	Upregulation (*GSH_t*)	Pedrosa et al. (2017)
		Chironomus riparius	*HSP17, HSP21, HSP22, HSP23, HSP24, HSP27, HSP34*	Upregulation (*HSP23, HSP24, HSP27, HSP34*) Downregulation (*HSP17, HSP21*)	Martín-Folgar and Martínez-Guitarte (2017)
		Chironomus riparius	*GST, CarE, CAT CYP450, HSP70*	Upregulation	Chen et al. (2016)
		Chironomus tepperi	*SAH, SAM, CβS, CγL, GCS, GS, GSTd1, Mtn*	Upregulation (*CβS, GCS, GS, Mtn*) Downregulation (*SAH, SAM*)	Jeppe et al. (2014)

(continued)

Table 2 (continued)

Stressor		Species (Chironomidae)	Gene	Response	Reference
		Chironomus riparius	*HSP27*	Upregulation	Martínez-Paz et al. (2014)
		Chironomus riparius	*CYP9AT2*	Upregulation	Nair et al. (2013)
		Chironomus riparius	*GCS, GS*	Upregulation	Nair et al. (2013)
		Chironomus riparius	*Cu-ZnSOD, MnSOD*	Up- or downregulation	Park and Kwak (2012)
		Chironomus riparius	*PHGPx*	Upregulation	Nair et al. (2012)
		Chironomus riparius	*START1*	Upregulation	Nair and Choi (2012)
		Chironomus riparius	*TrxR1*	Downregulation	Nair and Choi (2012)
		Chironomus riparius	*CAT, GST d3, GSTs4, GSTe1*	Up- or downregulation	Nair et al. (2011)
		Chironomus riparius	*RPL15*	Downregulation	Nair and Choi (2011)
		Chironomus riparius	*GSTd1, GSTd2, GSTd3, GSTe1, GSTs1, GSTs2, GSTs3, GSTs4, GSTt1, GSTz1, GSTo1, GSTu1, GSTu2*	Upregulation (*GSTd3, GSTs4, GSTe1*)	Nair and Choi (2011)
		Chironomus riparius	*HSP70*	Upregulation	Morales et al. (2011)
		Chironomus riparius	*HSP70, HSC70, HSP90, HSP40, EcR, USP*	Upregulation (*HSP70, EcR*)	Planelló et al. (2010)

		Chironomus yoshimatsui	*HSP70*	Upregulation	Yoshimi et al. (2009)
		Chironomus riparius	*RPL11, RPL13*	No significant change	Martínez-Guitarte et al. (2007)
		Chironomus riparius	*45S rRNA, 32S rRNA, 28S rRNA*	Downregulation (*45S rRNA, 32S rRNA*) No significant change (*28S rRNA*)	Planelló et al. (2007)
		Chironomus tentans	*HSP70, HSC70, HbA, HbB*	Up- or downregulation	Lee et al. (2006)
		Chironomus tentans	*CTTUB1*	Altered regulation	Mattingly et al. (2001)
		Chironomus riparius	*HSP70, Rp*	Upregulation (*HSP70*) Downregulation (*Rp*)	Govinda et al. (2000)
	Cu	*Chironomus riparius*	*DRONC, IAP1, PO1, Defensin, Gp93, Ctr1*	Upregulation (*DRONC, Gp93*)	Martín-Folgar and Martínez-Guitarte (2019)
		Chironomus riparius (Wild population)	*CYP4G, HSP70, HSC70, HSP10,*	Upregulation	Bernabò et al. (2017)
		Chironomus riparius	*HSP70, HSC70, HSP40, HSP10, CYP450*	Up- or downregulation	Lencioni et al. (2016)
		Chironomus tepperi	*SAH, SAM, CβS, CγL, GCS, GS, GSTd1, Mtn*	Upregulation (*CγL, GCS, GS, GSTd1*) Downregulation (*SAH, SAM, CβS*)	Jeppe et al. (2014)
		Chironomus yoshimatsui	*HSP70*	Upregulation	Yoshimi et al. (2009)

(continued)

Table 2 (continued)

Stressor		Species (Chironomidae)	Gene	Response	Reference
		Chironomus dilutus	*HSP70*	Upregulation	Karouna-Renier and Rao (2009)
		Chironomus ninevah	*BR1, BR2 activity*	Downregulation No significant change	Aziz et al. (1991)
	Pb	*Chironomus riparius*	*HbA, HbB, HbC, HbD, HbE*	Up- or downregulation	Ha and Choi (2008)
		Chironomus tentans	*HSP70, HSC70, HbA, HbB*	Up- or downregulation	Lee et al. (2006)
	Zn	*Chironomus tepperi*	*SAH, SAM, CβS, CγL, GCS, GS, MT*	Upregulation (*SAH, MT*)	Long et al. (2015)
	Cr	*Chironomus tentans*	*HSP70, HSC70, HbA, HbB*	Up- or downregulation	Lee et al. (2006)
Nanoparticle	Silver nanoparticle (AgNPs) (antimicrobial agents that have a wide spectrum of action, including against pathogenic bacteria)	*Chironomus riparius*	*P38 MAPK*	Up- or downregulation depending on the concentration and exposure	Park and Choi (2017)
		Chironomus riparius	*CuZnSOD, MnSOD, CAT, GSTe1, HO-1, TF, START-1, HSP70*	Up- or downregulation	Park et al. (2015)
		Chironomus riparius	*CuZnSOD, MnSOD, CAT, PHGPx1, TrxR1, GSTd3, GSTs4, GSTe1*	Upregulation (*CuZnSOD, PHGPx1, CAT, TrxR1, GSTd3, GSTs4, GSTe1*) Downregulation (*MnSOD*)	Nair et al. (2013)
		Chironomus riparius	*EcR*	Up- or downregulation	Nair and Choi (2012)
		Chironomus riparius	*RPL15*	Downregulation	Nair and Choi (2011)

		Chironomus riparius	*GSTd1, GSTd2, GSTd3, GSTe1, GSTs1, GSTs2, GSTs3, GSTs4, GSTt1, GSTz1, GSTo1, GSTu1, GSTu2*	Upregulation (*GSTd3, GSTs4, GSTe1*)	Nair and Choi (2011)
		Chironomus riparius	*RPL15, GnRH1, BR2.2*	Upregulation (*GnRH1, BR2.2*) Downregulation (*RPL15*)	Nair et al. (2011)
	Zinc oxide nanoparticles (ZnONPs) (inorganic metal oxide used in an increasing number of industrial products)	*Chironomus riparius*	*CuZnSOD, MnSOD, CAT, PHGPx, TrxR1, GSTd3, GSTe1, GSTs4, CYP9AT2, HSP70*	Upregulation	Nair and Chung (2015)
	Multi-walled carbon nanotubes (MWCNTs) (one of the most advantageous nanofillers in the production of polymer composites)	*Chironomus riparius*	*XRCC1, ATM, HSP27, HSP70, Caspase DECAY, Actin*	Downregulation (*ATM, HSP27, HSP70*) Upregulation (*Caspase, DECAY*)	Martínez-Paz et al. (2019)

3.2.3 Nanoparticles

Toxicity of nanoparticles (silver nanoparticles (AgNPs), zinc oxide nanoparticles (ZnONPs), and multi-walled carbon nanotubes (MWCNTs)) has been reported only in *C. riparius* larvae (Table 2). AgNPs are used extensively in various commercial products, including deodorants, shampoos, detergents, washing machines, and medical products due to their antibacterial properties (Nair et al. 2013); they are ultimately released into aquatic environments. Nair et al. (2011) observed the upregulation of gonadotropin-releasing hormone-related genes and BR2 following exposure to AgNPs, leading to the upregulation of the expression of antioxidation and detoxification-related genes (*CuZnSOD, PHGPx1, CAT*, Thioredoxin reductase 1 (*TrxR1*), *GSTd3, GSTs4*, and *GSTe1*) (Table 2). The antioxidant defense systems of chironomids protect them against the toxic effects of reactive oxygen species (ROS) in nanoparticles, and they maintain cellular homeostasis by eliminating ROS. Similar to the results observed for AgNPs, ZnONPs induced the expression of genes related to detoxification and oxidative stress, as well as *HSP70* (Nair and Chung 2015). Martínez-Paz et al. (2019) suggested genotoxic effects following exposure to MWCNTs in *C. riparius*, due to the upregulation of *Caspase* and Death executioner caspase related to Apopain/Yama (*DECAY*), and the downregulation of DNA damage-related genes (Ataxia telangiectasia mutated (*ATM*)).

3.2.4 Endocrine Disruptor Chemicals (EDCs)

EDCs mimic the action of endogenous estrogen hormones, and in turn, interfere with the endocrine signal pathways of organisms (Park and Kwak 2010). Gene response studies have reported the effects of various EDCs (Di(2-ethylhexyl) phthalate (DEHP), Diethyl phthalate (DEP), Butyl benzyl phthalate (BBP), Bisphenol-A (BPA), Nonylphenol (NP), Bisphenol P (BPP), Bisphenol S (BPS), Bisphenol F (BPF), Pentachlorophenol (PCP), and phenol) in five chironomid species, although the gene expression profiles have been studied mainly in *C. riparius* (Table 3). Phthalates such as DEHP, DEP, and BBP, which are industrial additives, are extensively used in the manufacture of plastic products as plasticizers (Park and Kwak 2009a). The potential toxicity of DEHP as an EDC in chironomids was confirmed by the upregulation of hormone receptor genes (*EcR, USP,* and *ERR*) in a study by Park and Kwak (2010, 2014). In addition, exposure to DEHP triggers oxidative stress (alteration of the levels of *CAT, Px, SOD,* and *GPx*), causing metabolic disruption by affecting the expression of calcium-binding proteins (*Calponin*), metabolizing enzymes (alcohol dehydrogenase (*ADH*)), digestive enzymes (*SP*), ribosomal proteins (*RpS3*, *RpS6, RPL4,* and *RPL13*), and chaperone proteins (*HSP70, HSC70, HSP40*, and *HSP90*) (Table 3). Furthermore, DEP toxicity increases the activities of antioxidant genes (*SOD, CAT,* and *GPx*) as well as those of the synaptic neurotransmitter enzyme *AchE* in *C. circumdatus*. Moreover, *HSP70* and *HSP27* are upregulated and *HSP40* and *GST* are downregulated in *C. riparius*

Table 3 Gene expression profiles in response to endocrine disruptor chemicals and phenols in chironomids, reported in studies from 1991 to 2020. Full names of the analyzed genes are listed in Supplementary Table 1

Stressor		Species (Chironomidae)	Gene	Response	Reference
EDCs & Phenol	Di(2-ethylhexyl) phthalate (DEHP) (manufactured chemical that is commonly added to plastics to make them flexible)	*Chironomus riparius*	*RPL4, RPL13, HSC70, HSP70, HSP40, HSP27, EcR, GAPDH, CYP4G, GST*	Downregulation	Herrero et al. (2017)
		Chironomus riparius	*EcR, USP, ERR, CAT, Px, SOD, GPx*	Upregulation	Park and Kwak (2014)
		Chironomus riparius	*RpS3, RpS6*	Upregulation (*RpS3*)	Park and Kwak (2012)
		Chironomus riparius	*HSP70, HSC70, ITS2, EcR, USP*	Up- or downregulation	Planelló et al. (2011)
		Chironomus riparius	*HSP70*	Upregulation	Morales et al. (2011)
		Chironomus riparius	*ERR*	Upregulation	Park and Kwak (2010)
		Chironomus riparius	*ADH*	Upregulation	Park and Kwak (2009a)
		Chironomus riparius	*Calponin*	Downregulation	Park and Kwak (2009b)
		Chironomus riparius	*HSP40, HSP90*	Upregulation	Park and Kwak (2008a)
		Chironomus riparius	*SP*	Downregulation	Park and Kwak (2008b)
		Chironomus tentans	*HSP70, HSC70, HbA, HbB*	Up- or downregulation	Lee et al. (2006)

(continued)

Table 3 (continued)

Stressor		Species (Chironomidae)	Gene	Response	Reference
	Diethyl phthalate (DEP) (a member of the phthalic acid ester class of plasticizers)	*Chironomus circumdatus*	*SOD, CAT, GPx, AchE, GR, HSP70*	Upregulation (*SOD, CAT, GPx, AchE, HSP70*) Downregulation (*GR*) Up- or downregulation (*EcR*)	Shaha and Pandit (2020)
	Butyl benzyl phthalate (BBP) (man-made phthalate ester that is mostly used in vinyl tile as a plasticizer)	*Chironomus riparius*	*ITS2, RPL4, RPL11, RPL13*	Downregulation	Herrero et al. (2016)
		Chironomus riparius	*HSC70, HSP70, HSP40, HSP27, HSP10, EcR, ERR, CYP4G, GPx, GST*	Upregulation (*HSP70, HSP27*) Downregulation (*HSP40, GST*)	Herrero et al. (2015)
		Chironomus riparius	*HSP70, HSC70, ITS2, EcR, USP*	Up- or downregulation	Planelló et al. (2011)
		Chironomus riparius	*HSP70*	Upregulation	Morales et al. (2011)
	Bisphenol-A (BPA) (organic synthetic compound used as a structural component in polycarbonate beverage bottles)	*Chironomus riparius*	*EcR, E74, CYP18a1, Shadow, DRONC, MRP1*	Upregulation (*Shadow, MRP1*)	Morales et al. (2020)
		Chironomus riparius	*HSP27*	Upregulation	Martínez-Paz et al. (2014)
		Chironomus riparius	*CYP9AT2*	Downregulation	Nair et al. (2013)
		Chironomus riparius	*RpS3, RpS6*	Upregulation (*RpS3*)	Park and Kwak (2012)
		Chironomus riparius	*CYP4G*	Downregulation	Martínez-Paz et al. (2012)
		Chironomus riparius	*HSP70*	Upregulation	Morales et al. (2011)
		Chironomus riparius	*ERR*	Upregulation	Park and Kwak (2010)

		Chironomus riparius	*HbA, HbB, HbC, HbD, HbE*	Up- or downregulation	Ha and Choi (2008)
		Chironomus riparius	*HSP70, EcR*	Upregulation	Planelló et al. (2008)
		Chironomus tentans	*HSP70, HSC70, HbA, HbB*	Up- or downregulation	Lee et al. (2006)
	Nonylphenol (NP) (estrogenic endocrine active chemical that is present in detergents)	*Chironomus riparius*	*P38 MAPK*	Up- or downregulation depending on the concentration and exposure	Park and Choi (2017)
		Chironomus riparius	*HSP27*	No significant change	Martínez-Paz et al. (2014)
		Chironomus riparius	*CYP9AT2*	Downregulation	Nair et al. (2013)
		Chironomus riparius	*GCS, GS*	Upregulation	Nair et al. (2013)
		Chironomus riparius	*RpS3, RpS6*	Upregulation (*RpS3*)	Park and Kwak (2012)
		Chironomus riparius	*EcR*	Upregulation	Nair and Choi (2012)
		Chironomus riparius	*START1*	Downregulation	Nair and Choi (2012)
		Chironomus riparius	*CYP4G*	Downregulation	Martínez-Paz et al. (2012)
		Chironomus riparius	*CAT, GSTd3, GSTs4, GSTe1*	Up- or downregulation	Nair et al. (2011)
		Chironomus riparius	*HSP70*	Upregulation	Morales et al. (2011)
		Chironomus riparius	*ERR*	Upregulation	Park and Kwak (2010)
		Chironomus riparius	*HbA, HbB, HbC, HbD, HbE*	Up- or downregulation	Ha and Choi (2008)

(continued)

Table 3 (continued)

Stressor		Species (Chironomidae)	Gene	Response	Reference
		Chironomus riparius	*HSP70*	Upregulation	Lee et al. (2006)
		Chironomus tentans	*HSP70, HSC70, HbA, HbB*	Up- or downregulation	Lee et al. (2006)
	Bisphenol P (BPP) (BPA analog)	*Chironomus tentans*	*EcR, E74*	Upregulation	Wang et al. (2019)
	Bisphenol S (BPS) (chemical cousins of BPA that are commonly found in plastics, food and beverage can linings, and other consumer products)	*Chironomus riparius*	*EcR, E74, CYP18a1, Shadow, DRONC, MRP1*	Upregulation (*EcR, E74, Shadow*)	Morales et al. (2020)
		Chironomus riparius	*EcR, ERR, E74, Vtg, CYP18A1, HSP70, HSP40, CYP4G, GPx, GSTd3, ITS2, RPL4, RPL13*	Upregulation (*EcR, ERR, E74, CYP18A1, HSP70, HSP40, CYP4G, GPx, GSTd3*)	Herrero et al. (2018)
	Bisphenol F (BPF) (small aromatic organic compound used as plastics and epoxy resins)	*Chironomus riparius*	*EcR, E74, CYP18A1, Shadow, DRONC, MRP1*	Upregulation (*E74, Shadow, CYP18A1, DRONC*)	Morales et al. (2020)
	Pentachlorophenol (PCP) (organochlorine compound used as a pesticide and a disinfectant)	*Chironomus riparius*	*EcR, E74, ERR, HSP70, CYP4G, HSP27, USP, GST*	Upregulation (*EcR, E74, ERR, HSP70, CYP4G*) Downregulation (*HSP27*) No significant change (*USP, GST*)	Morales et al. (2014)
		Chironomus riparius	*HSP70*	Upregulation	Morales et al. (2011)
	Phenol (organic compounds characterized by a hydroxyl group attached to a carbon atom that is part of an aromatic ring)	*Chironomus kiiensis*	*CYP6EV11*	Upregulation	Zhang et al. (2018)
		Chironomus kiinensis	*CYP6EW3, CYP6EV9, CYP6FV1, CYP6FV2*	Up- or downregulation	Cao et al. (2016)

following exposure to BBP. Herrero et al. (2016) observed a reduction in the expression of rDNA activity-related genes (*ITS2*) and ribosomal proteins (*RPL4, RPL11,* and *RPL13*) associated with protein synthesis and homeostasis in response to BBP toxicity (Table 3).

Phenols are one of the major organic pollutants and are frequently detected in aquatic environments because of their relatively high-water solubility, chemical stability, and environmental mobility (Cao et al. 2013; Zhang et al. 2018). Some types of phenols have been suggested to act as EDCs because they induce the expression of hormone-related genes (*EcR*) in *C. riparius* (Planelló et al. 2008; Park and Kwak 2010). BPA and its analogues (BPS and BPF) modified the expression of genes involved in the endocrine pathway (*EcR*, Ecdysone-induced protein 74 (*E74*), and *shadow*) in *C. riparius* (Morales et al. 2020). Upregulation of *HSP70* and *HSP27* was detected in *C. riparius* after exposure to several doses of BPA (Planelló et al. 2008; Morales et al. 2011; Martínez-Paz et al. 2014). Ribosomal proteins (*RpS3*) are also upregulated in response to BPA toxicity (Park and Kwak 2012; Nair et al. 2013).

However, BPA toxicity inhibited the phase I detoxification response in *C. riparius* through the downregulation of cytochrome enzyme activity (*CYP9AT2* and *CYP4G*), while exposure to NP induced the transcriptional expression of GSH biosynthesis genes (*GCS, GS*), a ribosomal protein gene (*RpS3*), a stress gene (*HSP70*), as well as a hormone-related gene (*EcR*) (Table 3). The reduced expression levels of the phase I detoxification-related genes (*CYP9AT2, CYP4G*) and StAR-related lipid transfer domain-containing protein (*START1*) were also observed in *C. riparius* following exposure to NP. Herrero et al. (2018) reported BPS toxicity based on the response of genes such as *EcR, ERR, E74, Vtg,* and *CYP18A1*, which are related to signaling and degradation ecdysone pathways. In addition, BPS exposure induced modified expression of the genes associated with stress responses (*HSP70, HSP40*), phase I (*CYP4G*) and phase II (*GSTd3*) of the detoxification response, and antioxidation (*GPx*), all of which are vital for survival and adaptive regulation. As displayed in Table 3, there was enhanced upregulation of hormone-related genes (*EcR, ERR*, and *E74*) following exposure to BPP and PCP, suggesting that they are EDCs in chironomids (Morales et al. 2014; Wang et al. 2019). In addition, exposure to PCP induced the upregulation of *HSP70* and *CYP4G* and the downregulation of *HSP27*. The oxidative metabolism responses following exposure to phenol observed in *Chironomus kiiensis* and *Chironomus kiinensis* larvae were altered expression profiles of five cytochrome P450 enzymes (Table 3).

3.2.5 Insecticides

Insecticides are substances used to control insects in agriculture, industry, and medicine. In molecular research, the effects of four types of insecticides in four chironomid species have been investigated (Table 4). Chlorpyrifos, an organophosphate insecticide, induced phase I detoxification responses through the activation of cytochrome P450 enzymes (*CYP6EV1, CYP4DG2, CYP4DG1, CYP6EX3,*

Table 4 Gene expression profiles in response to insecticides, antibiotics, herbicides, fungicides, biocides, carcinogenic substances, and synthetic estrogen in chironomids, reported in studies from 1991 to 2020. Full names of the analyzed genes are listed in Supplementary Table 1

Stressor		Species (Chironomidae)	Gene	Response	Reference
Insecticides	Chlorpyrifos (organophosphorus insecticide)	*Chironomus tentans*	*CYP6EV1, CYP4DG2, CYP4DG1, CYP6EX3, CYP6EV3*	Upregulation	Tang et al. (2018)
		Chironomus riparius	*CYP9AT2*	Upregulation	Nair et al. (2013)
		Chironomus riparius	*Cu-ZnSOD and MnSOD*	Upregulation (*MnSOD*-24 h) Downregulation (*Cu-ZnSOD*)	Park and Kwak (2012)
		Chironomus tentans	*HSP70, HSC70, HbA, HbB*	Upregulation (*HSP70, HSC70*) Downregulation (*HbA, HbB*)	Lee et al. (2006)
	Bifenthrin (pyrethroids insecticide)	*Chironomus tepperi*	*SAM, Mtn, SAH, CβS, GCS*	Downregulation Upregulation (*SAM*)	Jeppe et al. (2017)
	Azadirachtin (neem oil, bioinsecticide)	*Chironomus riparius* (wild population)	*HSP70, HSC70, HSP40, HsP10, CYP450*	Upregulation	Lencioni et al. (2016)
	Etofenprox (pyrethroids insecticide)	*Chironomus yoshimatsui*	*HSP70*	Upregulation	Yoshimi et al. (2002, 2009)
	Fenitrothion (organophosphorus insecticide)	*Chironomus tentans*	*HSP70, HSC70, HbA, HbB*	Downregulation	Lee et al. (2006)
		Chironomus yoshimatsui	*HSP70*	Upregulation	Yoshimi et al. (2002, 2009)
	Endosulfan (organochlorine insecticide)	*Chironomus tentans*	*HSP70, HSC70, HbA, HbB*	Upregulation (*HSP70, HSC70*) Downregulation (*HbA, HbB*)	Lee et al. (2006)

Antibiotics	Sulfadiazine (SDZ) (sulfonamide antibacterial antibiotic)	*Chironomus riparius*	*HSP70, HSP27, EcR, E74*	Upregulation	Xie et al. (2019a)
	Tetracycline (TC) (broad-spectrum polyketide antibiotic)	*Chironomus riparius*	*SOD, GST, HSP70, HSP27, EcR, E74*	Upregulation (*HSP70, HSP27, EcR, E74*) Downregulation (*SOD, GST*)	Xie et al. (2019b)
	Sulfathiazole (STZ) (short-acting sulfonamide antibiotic)	*Chironomus riparius*	*HSP70, HSP40, HSP90, HSP27, ERR, EcR, USP, E74, CAT, SOD, GST, Px, GPx*	Upregulation (*HSP70, HSP40, HSP90, ERR, EcR, USP, E74, CAT, SOD, GST*) Downregulation (*HSP27, Px, GPx*)	Park and Kwak (2018)
		Chironomus riparius	*RpS3, RpS6*	Upregulation (*RpS3*)	Park and Kwak (2012)
	Fenbendazole (FBZ)	*Chironomus riparius*	*RpS3, RpS6*	Upregulation (*RpS3*)	Park and Kwak (2012)
	(broad-spectrum benzimidazole anthelmintic)	*Chironomus riparius*	*HSP70, HSP40, HSP90, CYP450, GST, HbA, HbB*	Upregulation (*HSP70, HSP40, HSP90, CYP450, GST, HbA*)	Park et al. (2009)
	Lincomycin (LCM) (lincosamide antibiotic)	*Chironomus riparius*	*RpS3, RpS6*	Upregulation (*RpS3*)	Park and Kwak (2012)
	Actinomycin D (DACT) (anti-neoplastic antibiotic)	*Chironomus riparius*	*HSP70, Rp*	Upregulation (*HSP70*) Downregulation (*Rp*)	Govinda et al. (2000)
	Triclosan (TCS) (broad-spectrum antimicrobial used as an antiseptic)	*Chironomus riparius*	*CYP4D2, CYP6B7, CYP9F2, CYP12A2, GSTd3, GSTd6, GSTe1, GSTo1, GSTt1*	Upregulation (*CYP12A2, GSTd3, GSTe1, GSTo1, GSTt1*) Downregulation (*GSTd6*)	Martínez-Paz (2018)
		Chironomus riparius	*EcR, USP, ERR, E74, HSP70*	Upregulation	Martínez-Paz et al. (2017)
		Chironomus riparius	*HSP27*	Upregulation	Martínez-Paz et al. (2014)

(continued)

Table 4 (continued)

Stressor		Species (Chironomidae)	Gene	Response	Reference
Herbicides	Atrazine (triazine herbicide)	*Chironomus tentans*	*CYP6EV1, CYP4DG2, CYP4DG1, CYP6EX3, CYP6EV3*	Upregulation	Tang et al. (2018)
		Chironomus tentans	*CteHb-II, CteHb-III*	Downregulation	Anderson et al. (2008)
		Chironomus tentans	*CYP450 family 4*	Upregulation	Londoño et al. (2004)
	Paraquat (PQ) (chemical herbicide or weed killer)	*Chironomus riparius*	*Cu-ZnSOD, MnSOD*	Upregulation	Park and Kwak (2012)
		Chironomus riparius	*TrxR1*	Upregulation	Nair and Choi (2012)
		Chironomus riparius	*CAT, GSTd3, GSTs4, GSTe1*	Up- or downregulation	Nair et al. (2011)
		Chironomus tentans	*HSP70, HSC70, HbA, HbB*	Upregulation (*HSP70, HSC70*) Downregulation (*HbA, HbB*)	Lee et al. (2006)
	2,4-Dichlorophenoxyacetic acid (2,4-D) (chlorophenoxy herbicide)	*Chironomus riparius*	*HSP70, HSP40, HSP90, GST*	Upregulation	Park et al. (2010)
	Alachlor (chloroacetanilide herbicide)	*Chironomus tentans*	*GSTd1, GSTu2, GSTu4, GSTs1, GSTs2, GSTs3, GSTs4, GSTo1*	Upregulation (*GSTd1, GSTs2, GSTs3*)	Li et al. (2009)

Fungicide	Azoxystrobin (methoxyacrylate analogue and a strobilurin fungicide)	*Chironomus dilutus*	*RAP1A, PCKA, AMPK, PPP2R5, ACC1, FASN, ATP2A, CALM, CAMK2, ORAI 1, PRKCA, ATR, CHK1, GADD45, Cyto c, CASP7, TRAF4, EGFR, MAP3K5, RPS6KA, HSP90a, HSP90b, PTPN11, NFKB1, Cyt b, ATPEF1A, ATPEVLA, COX 11, AKT, GNAI, GNAS, PKA, MAP2K1, ACOX1, RAC 1, GSK3B, PI3K, FAK, INSR*	Upregulation (*RAP1A, PCKA, AMPK, PPP2R5, ACC1, FASN, ATP2A, CALM, CAMK2, ORAI 1, PRKCA, ATR, CHK1, GADD45, Cyto c, CASP7, TRAF4, EGFR, MAP3K5, RPS6KA, HSP90a, HSP90b, PTPN11, NFKB1*) Downregulation (*Cyt b, ATPEF1A, ATPEVLA, COX 11, AKT*) Up- or downregulation (*GNAI, GNAS, PKA, MAP2K1, ACOX1, RAC 1, GSK3B, PI3K, FAK, INSR*)	Wei et al. (2020)
	Vinclozolin (Vz) (dicarboximide fungicide)	*Chironomus riparius*	*ATM, NLK, XRCC1, DECAY*	Up- or downregulation (*ATM, NLK, XRCC1*)	Aquilino et al. (2018)
		Chironomus riparius	*EcR, E74, Kr-h1, Disembodied, CYP18A1, FOXO, MAPR, HSP70, HSP24, Gp93, CYP4G, GSTd3*	Upregulation (*EcR, E74, Kr-h1, HSP70, HSP24, Gp93, CYP4G, GSTd3*) No significant change (*CYP18A1, FOXO, MAPR, disembodied*)	Aquilino et al. (2016)
Biocide	Tributyltin (TBT) (organotin compound)	*Chironomus riparius*	*HSP27*	No significant change	Martínez-Paz et al. (2014)
		Chironomus riparius	*EcR, USP, ERR, E74, Vtg, HSP70*	Upregulation (*EcR, USP, ERR, E74*) Downregulation (*HSP70*) No significant change (*Vtg*)	Morales et al. (2013)
		Chironomus riparius	*CYP4G*	Upregulation	Martínez-Paz et al. (2012)
		Chironomus riparius	*HSP70*	No significant change	Morales et al. (2011)

(continued)

Table 4 (continued)

Stressor		Species (Chironomidae)	Gene	Response	Reference
Benzene/ carcinogenic substance	Benzo[a]pyrene (B[a]P) (polycyclic aromatic hydrocarbon)	*Chironomus riparius*	*CYP9AT2*	Upregulation	Nair et al. (2013)
		Chironomus riparius	*Cu-ZnSOD, MnSOD*	Up- or downregulation	Park and Kwak (2012)
		Chironomus riparius	*HbA, HbB, HbC, HbD, HbE*	Up- or downregulation	Ha and Choi (2008)
		Chironomus tentans	*HSP70, HSC70, HbA, HbB*	Upregulation (*HSP70, HSC70*) Downregulation (*HbA, HbB*)	Lee et al. (2006)
	Octachlorostyrene (OCS) (by-product of normal industrial chemical processes)	*Chironomus tentans*	*CAT, Px, MDA, HSC70, HSP70, HbA, HbB, HbC, HbD, HbE*	Upregulation	Lee and Choi (2009)
	Carbon tetrachloride (CCl_4) (ozone-depleting substance)	*Chironomus tentans*	*HSP70, HSC70, HbA, HbB*	Upregulation (*HSP70, HbA, HbB*) Downregulation (*HSC70*)	Lee et al. (2006)
Synthetic estrogen	Ethinylestradiol (EE) (synthetic estrogen)	*Chironomus riparius*	*CYP9AT2*	Downregulation	Nair et al. (2013)
		Chironomus riparius	*HSP70*	No significant change	Morales et al. (2011)
		Chironomus tentans	*HSP70, HSC70, HbA, HbB*	Upregulation (*HSP70, HSC70*) Downregulation (*HbA, HbB*)	Lee et al. (2006)

CYP6EV3, and *CYP9AT2*) in *C. riparius* and *C. tentans*. According to Jeppe et al. (2017), exposure to bifenthrin altered cysteine metabolism via the upregulation of *SAM* and the downregulation of *CβS* and *GCS* in *C. tepperi*. In addition, *HSP70* expression is induced in *C. yoshimatsui* following exposure to etofenprox or fenitrothion (Table 4). Lencioni et al. (2016) reported upregulated expression of *HSPs* (*HSP70, HSC70, HSP40*, and *HSP10*) depending on the exposure doses of azadirachtin in wild *C. riparius* populations. In Lee et al. (2006), *endosulfan*, an off-patent organochlorine insecticide, upregulated the expression of *HSPs* (*HSP70* and *HSC70*) and downregulated the expression of *Hbs* (*HbA* and *HbB*).

3.2.6 Antibiotics

The effects of three veterinary antibiotics (sulfathiazole (STZ), fenbendazole (FBZ), and lincomycin (LCM)) and four antibiotic compounds (sulfadiazine (SDZ), tetracycline (TC), actinomycin D (DACT), and triclosan (TCS)) on the antioxidant system, stress response, and protein synthesis were studied in *C. riparius* larvae (Table 4). *SDZ*, a sulfonamide antibacterial agent, was associated with induction of *HSP* genes (*HSP70* and *HSP27*) and hormone signals (*EcR* and *E74*). TC exposure also led to the upregulation of these genes (*HSP70, HSP27, EcR*, and *E74*) and downregulation of *SOD* and *GST* in *C. riparius*. Meanwhile, exposure to STZ provoked the upregulation of endocrine-related genes (*EcR, USP,* and *E74*), stress response genes (*HSP70, HSP90,* and *HSP40*), antioxidant enzyme *CAT* and *SOD*, and the phase II enzyme *GST* associated with the detoxification response (Park and Kwak 2018). However, *HSP27* and antioxidant genes, such as *Px* and *GPx,* were downregulated in *C. riparius* exposed to STZ. Park et al. (2009) observed that FBZ exposure induced the transcriptional expression of *HSPs* (*HSP70, HSP90,* and *HSP40*), detoxification enzymes (*CYP450* and *GST*), and *Hbs* (*HbA*). Moreover, *RpS3*, a ribosomal protein, showed high expression following exposure to the veterinary antibiotic, LCM, as well as STZ and FBZ. Increased *HSP70* expression was also observed in *C. riparius* following exposure to DACT by Govinda et al. (2000). TCS is an antimicrobial agent used in a diverse range of products, from personal care to consumer-based (Martínez-Paz 2018). In detoxification processes, varying cytochrome P450 enzyme expression patterns (for instance, upregulation of *CYP12A2* during phase I detoxification, or upregulation of *GSTd3, GSTe1, GSTo1,* and *GSTt1* and downregulation of *GSTd6* during phase II detoxification) were observed following exposure to TCS (Table 4). The transcriptional expression of *HSPs* (*HSP70* and *HSP27*) and hormone-related genes (*EcR, ERR,* and *E74*) also increased in *C. riparius* following exposure to TCS.

3.2.7 Herbicides

Atrazine, a triazine herbicide which is used in agricultural and residential settings, has been reported to induce the upregulation of phase I detoxification enzymes

(*CYP6EV1, CYP4DG2, CYP4DG1, CYP6EX3, CYP6EV3,* and *CYP450 family 4*) and the downregulation of *Hbs* (*CteHb-II, CteHb-III*) in *C. tentans*. Paraquat (PQ) exposure also induced the upregulation of a detoxification gene (*TrxR1*), antioxidant enzymes (*Cu-ZnSOD* and *MnSOD*), and stress-related genes (*HSP70* and *HSC70*) (Table 4). Concurrently, Park et al. (2010) reported that 2,4-Dichlorophenoxyacetic acid (2,4-D) toxicity induced the expression of genes related to cellular homeostasis (*HSP70, HSP90*, and *HSP40*) and detoxification metabolism (*GST*) in *C. riparius*. Li et al. (2009) also observed induced expression of phase II detoxification enzymes (*GSTd1, GSTs2,* and *GSTs3*) in *C. tentans* exposed to alachlor (Table 4).

3.2.8 Fungicides and Biocides

Azoxystrobin, a strobilurin fungicide, is a typical mitochondrial complex III inhibitor; as such, it induces mitochondrial dysfunction as a mitotoxicant (Wei et al. 2020). Recent data using RNA sequencing and real-time RT-PCR reported that exposure to azoxystrobin led to the upregulation of AMPK signaling genes (phosphoenolpyruvate carboxykinase (GTP) (*PCKA*), 50-AMP-activated protein kinase, regulatory gamma subunit (*AMPK*), threonine-protein phosphatase 2A regulatory subunit B′ (*PPP2R5*), Acetyl-CoA carboxylase/biotin carboxylase 1 (*ACC1*), and fatty acid synthase, animal type (*FASN*)), DNA damage-related genes (serine/threonine-protein kinase (*ATR*), serine/threonine-protein kinase (*CHK1*), and growth arrest and DNA-damage-inducible protein (*GADD45*)), apoptosis pathway-related genes (Cytochrome c (*Cyto c*), Caspase 7, TNF receptor-associated factor 4 (*TRAF4*), epidermal growth factor receptor (*EGFR*), Mitogen-activated protein kinase kinase 5 (*MAP3K5*), and ribosomal protein S6 kinase alpha-1/2/3/6 (*RPS6KA*)), and immune pathway-related genes (molecular chaperone HtpG (*HSP90a*), heat-shock protein 90 kDa beta (*HSP90b*), tyrosine protein phosphatase non-receptor type 11 (*PTPN11*), signal transducer and activator of transcription 5B (*STAT5B*), and nuclear factor NF-kappa-B subunit (*NFKB1*)) in *Chironomus dilutus* (Wei et al. 2020) (Table 4). However, azoxystrobin exposure decreased the expression of mitochondria-related pathway genes (Cytochrome b (*Cyt b*), F-type Hþ--transporting ATPase subunit A (*ATPEFlA*), V-type Hþ-transporting ATPase subunit A (*ATPEVlA*), and cytochrome c oxidase assembly protein subunit 11 (*COX 11*)). Vinclozolin (Vz), a fungicide applied in agriculture, is a contaminant that produces antiandrogenic effects in reproduction (Aquilino et al. 2016, 2018). Exposure to low concentrations of Vz activated the cellular DNA repair process through the induced expression of the *ATM*, Nemo-like kinase (*NLK*), and X-Ray Repair Cross Complementing 1 (*XRCC1*) genes in *C. riparius*. However, such repair mechanisms in response to Vz toxicity were inhibited at high Vz concentrations (Aquilino et al. 2018). In addition, exposure to Vz induced the expression of ecdysone response genes (*EcR, E74,* and Krüppel Homolog 1 (*Kr-h1*)), cellular stress-related genes (*HSP70, HSP24,* and *Gp93*), and phase I and phase II detoxification genes (*CYP4G* and *GSTd3*) in *C. riparius* (Table 4). Tributyltin (TBT), a

biocide, is associated with adverse effects in the course of development and reproduction in chironomid larvae in aquatic ecosystems (Morales et al. 2013). It induces endocrine-disrupting activities, as demonstrated by the upregulation of hormone-related genes (*EcR, USP, ERR*, and *E74*), while *HSP70* expression seems to be downregulated or altered non-significantly in *C. riparius* following exposure to TBT (Table 4).

3.2.9 Carcinogenic Substances and Synthetic Estrogen

B[*a*]P, a polycyclic aromatic hydrocarbon, is ubiquitous in tobacco smoke, coal tar, and various foods, especially, grilled meats (Ha and Choi 2008). Nair et al. (2013) reported the activation of the phase I detoxification enzyme, *CYP9AT2,* in response to B[*a*]P exposure. However, there are simultaneous patterns of up- or downregulation of antioxidant, cellular stress-related, and hemoglobin genes depending on the exposure time and concentrations of B[*a*]P (Table 4). Lee and Choi (2009) observed transcriptional responses of stress response genes (*HSC70* and *HSP70*) and *Hbs* (*HbA* and *HbB*) in *C. tentans* exposed to octachlorostyrene (OCS), a persistent and bioaccumulative toxicant. In addition, exposure to carbon tetrachloride led to the upregulation of *HSP70, HbA*, and *HbB,* and the downregulation of *HSC70* in *C. tentans*. Furthermore, *HSP70* and *HSC70* expression was induced by exposure to ethinylestradiol (EE), while the activities of *Hbs* (*HbA, HbB*) and *CYP9AT2* were downregulated in *C. riparius* following exposure to EE (Table 4).

3.2.10 Mixture and Field Exposure

Chemical pollutants and external stress factors simultaneously occur in aquatic environments, and the toxic effects of such factors lead to the formation of diverse phenotypes in organisms (Chen et al. 2016). In chironomids, studies investigating the ecotoxicological responses to combined exposures that simulate actual environmental conditions have been increasingly performed (Im et al. 2019). Diclofenac, a non-steroidal anti-inflammatory drug, is a class of generally prescribed pharmaceuticals for both humans and domestic livestock (Xie et al. 2020). Combined exposures of diclofenac and Cd induce oxidative damage (*CuZnSOD, MnSOD,* and *CAT*) and disrupt phase I (*CYP4G* and *CYP9AT2*) and phase II (*GSTd3, GSTe1,* and *GSTs4*) detoxification processes in *C. riparius* larvae (Table 5). Muñiz-González and Martínez-Guitarte (2020b) reported the upregulation of endocrine system-related genes (Juvenile hormone acid methyltransferase (*JHAMT*), Mitochondrial ATP production rates (*MAPR*)), a phase II detoxification gene (*GSTt1*), and a stress gene (*HSP70*) after exposure to mixtures of BPA, OC, and OD-PABA in *C. riparius*. In *C. dilutus* larvae, a mixture of pyrethroids and Cd induced the activity and expression of defense metabolism-related enzymes (*GST, CarE, CAT,* and *CYP450*) and *HSP70* (Table 5). Combined exposure to different temperatures (18.5 or 23°C) and the UV filter BP3 increased the transcriptional expression of

Table 5 Gene expression profiles in chironomids in response to mixed and field sample exposures reported in studies from 1991 to 2020. Full names of the analyzed genes are listed in Supplementary Table 1

Stressor		Species (Chironomidae)	Gene	Response	Reference
Mixture and field sample exposures	Temperature (18.5 or 23°C) and UV benzophenone-3 (BP3)	*Chironomus riparius*	*EcR, InR, Met, CYP4D2, CYP6B7, GSTd6, GSTo1, MRP-1, HSP22, HSP27, HSP70, HYOU, Gp93, GST, PO, AChE*	Upregulation (*MRP-1, EcR, HSP27*) Downregulation (*CYP6B7*) Up- or downregulation (*GSTd6, GSTo1, HSP22, HSP70*)	Muñiz-González and Martínez-Guitarte (2020a)
	Diclofenac and Cd	*Chironomus riparius*	*CuZnSOD, MnSOD, CAT, GSTd3, GSTe1, GSTs4, CYP4G, CYP9AT2*	Upregulation (single exposure) Downregulation (combined exposure)	Xie et al. (2020)
	BPA, OC, and OD-PABA	*Chironomus riparius*	*EcR, E93, Kr-h1, Dronc, Dis, CYP18a1, Met, JHAMT, InR, MAPR, CYP4d2, CYP6b7, CYP9f2, CYP12a2, GSTd3, GSTe1, GSTo1, GSTt1, MRP1, ABCB6, HSP70, HSC70–5, HYOU1, HSP40, HSP90, Gp93, HSP60, HSP10, HSP17, HSP21, HSP22, HSP23, HSP24, HSP27, DECAY, XRCC1, NLK, ATM, Def, prePO*	Upregulation (*JHAMT, MAPR, GSTt1, HSP70*)	Muñiz-González and Martínez-Guitarte (2020b)
	Pyrethroids and Cd mixture	*Chironomus dilutus*	*GST, CarE, CAT CYP450, HSP70*	Upregulation	Chen et al. (2016)
	Reclaimed water and its fortification with carbamazepine and triclosan	*Chironomus riparius*	*EcR, ERR, E75, Vtg, HSP70, HSC70, HSP24, HSP10, Gp93, GSTd3, GPx, CYP4G*	Upregulation (*EcR, Vtg, HSP70, HSC70, HSP24, HSP10, Gp93, GSTd3, CYP4G*)	Planelló et al. (2020)
	Heavy metal-contaminated sediments	*Chironomus riparius*	*EcR, InR, Vtg, GST, GPx, SOD, CAT, TrxR1, CYP4G, FeH, FeL, HbA, HbB, GAPDH, HSP70, HSP40, HSP10, Gp93, NLK, XRCC1, ATM, DECAY*	Upregulation (*FeL, HbB, Gp93*) Downregulation (*EcR, InR, GST, SOD*)	Arambourou et al. (2020)

	Metal-contaminated field sediments (MCFS)	*Chironomus riparius*	*SAM, SAH, DNMT, GPx, GSTd3, GSTs4, GSTe1, CAT, HO, Cu-ZnSOD, MnSOD*	Downregulation (*SAM, SAH, DNMT, GSTd3, GSTs4, GSTe1, HO, ZnSOD, MnSOD*), Upregulation (*GPx*), No significant change (*CAT*)	Im et al. (2019)
	Field-collected contaminated sediments	*Chironomus riparius*	*EcR, Vtg, InR, HSP40, HSP70, HSP10, Gp93, CAT, CYP4G, GST, GPx, SOD*	Upregulation	Arambourou et al. (2019)
	Oil sands process-affected water (OSPW)	*Chironomus dilutus*	*GST, CAT, GPx, USP, ESR, ERR*	Up- or downregulation	Wiseman et al. (2013)
	Complex mixtures of environmental contaminants in their natural habitats	*Chironomus riparius* (natural population)	*HSP70, HSC70, GST, EcR, P450*	Up- or downregulation (*HSP70, GST*)	Planelló et al. (2015)
	Anhydrobiosis	*Polypedilum vanderplanki*	*HSP90, HSP70, HSC70, HSP60, HSP20, p23, Hsf1*	Upregulation	Cornette et al. (2010)

the detoxification enzyme (*MRP-1*), an endocrine-related gene (*EcR*), and a stress gene (*HSP27*) in *C. riparius*.

Wiseman et al. (2013) suggested that the toxicity of oil sands results in water induced oxidative stress and the disruption of endocrine processes in *C. dilutus* larvae through the altered expression profiles of *GST, CAT, GPx, USP,* ecdysteroid receptor (*ESR*), and *ERR* (Table 5). Planelló et al. (2015) investigated the transcriptional responses and metabolic activity in natural populations of *C. riparius* larvae sampled in three rivers in Spain with different levels of pollution. The levels of *HSP70* and *GST*, applied as biomarkers, exhibited different responses to different levels of pollution. Field-collected sediments with different levels of PAHs, phthalates, and pesticides altered the molecular levels of endocrine-, defense-, and biotransformation-related genes in the course of development of *C. riparius* larvae (Arambourou et al. 2019). In addition, the expression levels of a biotransformation-related gene (*FeL*), an oxygen transport gene (*HbB*), and a stress gene (*Gp93*) were upregulated in *C. riparius* following exposure to metal-contaminated sediments collected from a field environment (Arambourou et al. 2020). In addition, Im et al. (2019) reported that the expression of DNA damage-, oxidative stress-, and development-related genes was altered in early generations of *C. riparius* raised in metal-contaminated field sediments. Recently, Planelló et al. (2020) observed variation in the expression of cellular stress-related genes (*HSP70, HSC70, HSP24*, *HSP10,* and *Gp93*), defense metabolism-related genes (*GSTd3, GPx,* and *CYP4G*), and endocrine-related genes (*EcR, ERR, E75,* and *Vtg*) in *C. riparius* after exposure to reclaimed water and its fortification with carbamazepine and TCS (Table 5). Conversely, in a cryptobiotic midge, *Polypedilum vanderplanki*, anhydrobiosis associated with the induction of *HSP90, HSP70, HSC70, HSP60, HSP20,* desiccation-inducible small *HSP* (Protein 23 (*p23*)) and heat shock factor 1 (*Hsf1*), was revealed by anhydrobiosis-related expressed sequence tag database analysis (Cornette et al. 2010).

4 Discussion

The transcriptional responses of differentially expressed genes reflect the broad defense mechanisms in chironomid larvae occurring in response to shifting environmental conditions in natural aquatic ecosystems. One of the primary goals of aquatic ecotoxicology research is to identify the specific pathways of stress and protective responses. Over the past three decades, gene responses in chironomids have been explored under various molecular signaling categories, including stress, hormone signals, antioxidation, detoxification, ribosomal process, apoptosis, metabolism, hemoglobin, digestive system, DNA damage, and DNA methylation (Fig. 3).

Out of the multiple genes involved in molecular defense responses in chironomids, the most extensively studied genes are stress-related genes such as *HSPs*. *HSPs* are critical for the maintenance of protein homeostasis and cell survival. Primarily, *HSP70* has been studied by exposing chironomids to various stress

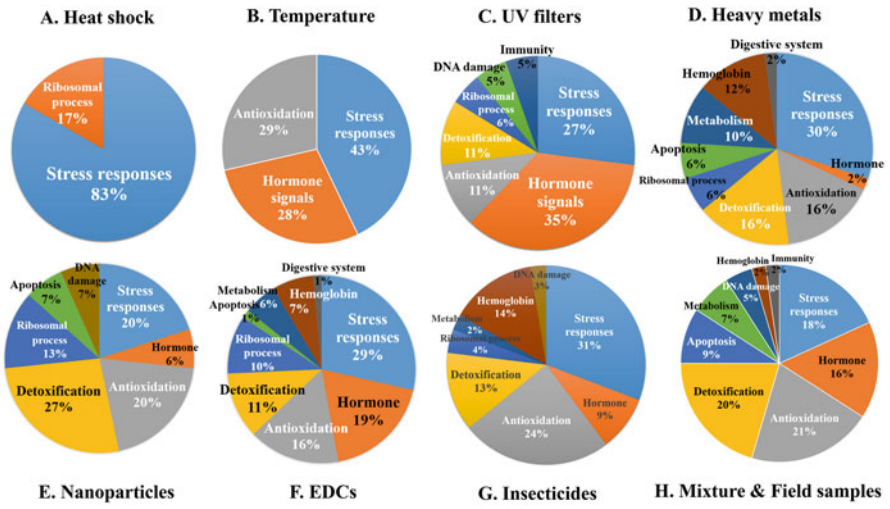

Fig. 3 Proportions of studies on gene expression responses to seven classes of stress factors including heat shock (**a**), temperature (**b**), UV filters (**c**), heavy metals (**d**), nanoparticles (**e**), EDCs (**f**), insecticides (**g**), and mixed and field samples (**h**)

factors, including high temperatures and xenobiotics, as well as mixtures or field sample exposures. Its expression seems to be activated under the influence of such stress factors. Therefore, responses of *HSP70* to stress in cells, determined via expression, are potential indicators of pollution in environments (Morales et al. 2011; Planelló et al. 2020). In addition, the expression of stress-related genes following exposure to heavy metals, EDCs, and insecticides, as well as heat shock and changing temperature, has been studied extensively in chironomids. Among the different classes of highly conserved *HSPs*, the responses of low-molecular-weight *HSPs* following exposure to environmental stress factors have also been studied. Notably, the upregulation of *HSP27* has been observed in chironomids exposed to Cd, BBP, BPA, SDZ, TC, and TCS, as well as heat shock (Martínez-Paz et al. 2014; Herrero et al. 2015; Martín-Folgar et al. 2015; Martín-Folgar and Martínez-Guitarte 2017; Xie et al. 2019a, b). *HSP27*, a molecular chaperone, plays a cytoprotective role through its antioxidant activity and can ameliorate the toxic effects of misfolded proteins during cell stress. It can directly inhibit apoptotic pathways and promotes neuronal survival (Read and Gorman 2009). However, the expression of *HSP27* is downregulated following exposure to nanoparticles (MWCNTs), EDCs (DEHP, PCP), and antibiotics (STZ), while no significant expression was induced in response to NP and TBT toxicity (Morales et al. 2014; Herrero et al. 2017; Martínez-Paz et al. 2019). The response of *HSP27* varies depending on the stress factor and type of exposure. Meanwhile, *HYOU1* was downregulated following exposure to UV filters (OC, OD-PABA) (Muñiz-González and Martínez-Guitarte 2018), and *Gp93* was upregulated in response to exposure to Cu (Martín-Folgar and Martínez-Guitarte 2019).

With regard to the effects of UV filters or EDCs, numerous studies have been reported that have determined that they influence the ecdysone pathway as well as the associated hormone signals, especially, *EcR*, in chironomids. The ecdysones, including *EcR*, are considered major regulators of growth, development, and metamorphosis in aquatic invertebrates (Herrero et al. 2018; Park and Kwak 2018). Activation of hormone-related genes provides evidence of disturbance in the endocrine system. Due to the upregulation of *EcR* following exposure to UV filters, it has been suggested that UV filters (BP3, 4MBC, OMC, EHMC, 4HB, OC, and OD-PABA) are chemicals that disrupt the endocrine system. Some types of phthalates and phenols are also EDCs that disturb endocrine hormone signals. Exposure to the fungicide Vz altered the transcriptional activity of an ecdysone signal pathway gene, *Kr-h1*, by increasing its mRNA expression levels. *EcR* is expressed in response to exposure to EDCs, while the expression profiles of other hormone-related genes, such as *USP* and *ERR*, depend on the characteristics of the stress factors (concentrations and period of exposure).

Antioxidation and detoxification activities are the second line of defense in the protection of cellular homeostasis against stress. Oxidative stress induced by environmental pollutants activates antioxidant defense enzymes, including *CAT, SOD, LPO,* and *CYP450* isoforms of the phase I detoxification process. Particularly, *CYP9AT2* is upregulated in response to exposure to Cd, ZnONPs, chlorpyrifos, or B[a]P, whereas it is downregulated in response to exposure to BPA, NP, or EE (Nair et al. 2013; Nair and Chung 2015). In *C. tentans* larvae, exposure to chlorpyrifos or atrazine induces the activation of *CYP450* isoforms, including *CYP6EV1, CYP4DG2, CYP4DG1, CYP6EX3,* and *CYP6EV3* (Tang et al. 2018). Moreover, *CYP12A2, CYP6EV11,* and *CYP18A1* are upregulated in response to TCS, phenol, and BPS toxicity, respectively (Martínez-Paz 2018; Zhang et al. 2018; Herrero et al. 2018). The upregulation of *CAT* and *LPO* reflects ROS scavenger activities under conditions of toxicity in cellular environments. The activity of *SODs*, including *CuZnSOD* and *MnSOD*, exhibited either up- or downregulation following exposure to stress. Detoxification enzymes, such as *TrxR1, PHGPx, CarE,* and *GST* isoforms, have been studied following exposure to UV filters (BP3, 4MBC), heavy metals (Cd, Cu), nanoparticles (AgNPs, ZnONPs), BPS, STZ, alachlor, TCS, and Vz in chironomids. *TrxR1* expression was upregulated following exposure to nanoparticles (Nair et al. 2013) and downregulated following exposure to Cd (Nair and Choi 2012). The response of *GST* isoforms varies according to the species and stressor types. Exposure to pollutants such as Cd, AgNPs, ZnONPs, BPS, Vz, and TCS, is associated with the upregulation of *GST* enzymes, including *GSTd3, GSTs4, GSTe1, GSTo1,* and *GSTt1*, in *C. riparius* (Nair and Choi 2011; Nair and Chung 2015; Aquilino et al. 2016; Herrero et al. 2018; Martínez-Paz 2018). Particularly, *GSTd3* is a potential indicator that could be used to evaluate the toxic effects of exposure to pollutants. The upregulation of *GSTs* was also observed suggesting alteration of *GSTd1* expression following exposure to Cu in *C. tepperi* (Jeppe et al. 2014), and the *GSTd1, GSTs2,* and *GSTs3* expression profiles following exposure to alachlor in *C. tentans* (Li et al. 2009). Increased expression levels of *MRP1* and *PHGPx* are also associated with detoxification processes that respond to exposure to

UV filters (BP3 and 4MBC in the case of *MRP1*, and AgNPs and ZnONPs in the case of *PHGPx*) (Nair et al. 2013; Nair and Chung 2015; Martínez-Guitarte 2018). *CarE*, a crucial class of detoxification enzymes, is also upregulated in response to exposure to Cd. However, no studies have been conducted on *CarE* responses following exposure to insecticides in chironomids, although it is one of the enzymes involved in insecticide resistance (Chen et al. 2016).

Cysteine metabolism genes, such as *CβS, CγL, GCS,* and *GS*, were upregulated following exposure to Cu in *C. tepperi* (Jeppe et al. 2014). The expression of *Eno1*, a glycolytic enzyme, is induced significantly by Cd toxicity (Zheng et al. 2018). In addition, exposure to DEHP upregulated the transcriptional expression of metabolic genes such as *ADH* and *Calponin* (Park and Kwak 2009a, b). Still, few genes involved in apoptosis have been analyzed in chironomids. *DRONC*, which encodes an initiator caspase, is critical for caspase-dependent cell death. Exposure to Cd or Cu induced the upregulation of *DRONC* (Martín-Folgar and Martínez-Guitarte 2019). However, *p38MAPK* expression was up- or downregulated depending on the concentrations and period of exposure to heavy metals or nanoparticles. *p38MAPK* regulates apoptosis and the release of cytokines by macrophages (Park and Choi 2017). In addition, *ATM*, which is involved in DNA repair, was downregulated by MWCNTs (Martínez-Paz et al. 2019). The expression of a digestive endopeptidase enzyme, *SP*, is upregulated by Cd exposure (Park and Kwak 2020) or downregulated by DEHP exposure (Park and Kwak 2008b), while the expression of ribosomal proteins was mostly downregulated in response to exposure to various pollutants (Park and Kwak 2012). The responses of hemoglobin genes are potential indicators for monitoring changes in oxygen transport systems of *Chironomus* (Zheng et al. 2017). Altered *Hbs* expression trends were observed in response to exposure to heavy metals, EDCs, or insecticides (Lee et al. 2006; Planelló et al. 2007). However, *Hbs* expression profiles varied depending on the types of stress factors.

To closely monitor the aquatic environmental status, molecular responses of multi-level genes have been studied for their response to toxicity in mixed or field exposure. Recent studies by Arambourou et al. (2019, 2020) revealed that exposure to field-collected sediments with heavy metal contamination (Cd, Pb, and Zn) altered lipidomic and transcriptional profiles, as well as the measured biological parameters in *C. riparius* over the course of its life cycle. *EcR, GST, SOD*, and *InR* genes were downregulated in larvae exposed to field-collected sediments with high metal concentrations (Arambourou et al. 2019). In addition, epigenetic response genes, such as *SAM, SAH,* and DNA methyltransferase (*DNMT*), were downregulated under conditions of heavy metal toxicity in contaminated field sediments (Im et al. 2019). Transcriptional expression of *FeL, HbB,* and *Gp93* was upregulated depending on the concentration gradients of heavy metal contaminants in sediments (Arambourou et al. 2020). Furthermore, Planelló et al. (2020) reported that cellular stress-related genes (*HSP70, HSC70, HSP24,* and *Gp93*), endocrine-related genes (*EcR* and *Vtg*), and detoxification-related genes (*GSTd3* and *CYP4G*) were upregulated significantly in *C. riparius* exposed to reclaimed water fortified with a binary mixture of carbamazepine and TCS. In addition, it has recently been reported that Cd alters

developmental features and induces the upregulation of *SP* transcripts in wild *C. plumosus* populations (Park and Kwak 2020).

Integrated and multi-level gene responses to environmental pollutants are more rapidly accessible through transcriptomic approaches, such as RNA sequencing using next-generation sequencing (NGS), which has recently been employed for ecotoxicological assessments in chironomids (Zhang et al. 2020; Wei et al. 2020). To identify the mechanisms underlying the toxicity of various pollutants ($CdCl_2$, NP, and TCS) and molecular biomarkers, transcriptomes of 31,132 unigenes were identified in *C. dilutes* larvae, and life stage-specific gene sets and chemical-specific gene expression responses were characterized (Zhang et al. 2020). Wei et al. (2020) reported the major pathways of neonicotinoid toxicity in *C. dilutes* by analyzing the toxicogenomic profiles. Such techniques could facilitate the simultaneous screening of numerous genes and transcriptomic profiling of various cellular and molecular signal patterns against toxicity with regard to different and complex pollutants in chironomids.

Chironomids are aquatic invertebrates that reflect toxic alterations in water and sediments in aquatic environments based on their life-cycle characteristics, larval characteristics in aquatic sediments, and embryonic and pupal development in water (Fig. 1). The results of such ecotoxico-transcriptomic studies on responses to environmental pollutants could offer insights into the identification and characterization of gene profiles useful for monitoring the health status of aquatic environments using resident organisms, such as chironomids.

5 Conclusion

- From 1991 to 2020, multi-level gene expression studies in chironomids have been reported regarding 12 molecular signaling categories, including stress, hormone signals, antioxidation, detoxification, ribosomal process, apoptosis, metabolism, oxygen transport capacity of hemoglobin, the digestive system, DNA damage, immune defense, and DNA methylation.
- Gene expression profiles in chironomids respond to various environmental factors, including both physical stimuli, such as heat shock or temperature changes and chemical exposure (e.g., UV filters, heavy metals, nanoparticles, EDCs, insecticides, antibiotics, herbicides, fungicides, and other toxicants).
- Although the transcriptional responses of genes in chironomids to the exposure of various heavy metals and EDCs have been investigated, the most studied have been those responses to Cd, NP, and DEHP.
- The transcriptional responses of multi-level genes to toxic pollutants enhance our understanding of the ecological and toxicological relevance of the survival and adaptation of chironomids following environmental changes, and the identification and development of genetic tools for aquatic environment monitoring based on resident species.

- Aquatic invertebrate chironomids are bioindicator species that could be applied in the monitoring of aquatic environments considering their life cycles, which include a planktonic habit in the first-instar larvae and benthic habit in the developed larvae.
- With regard to water quality monitoring applications, transcriptomic data from chironomids could supplement physicochemical data in assessing aquatic environments.
- Recent transcriptomic studies have attempted to characterize the mechanisms underlying the toxicity of various pollutants and elucidate the development of specific molecular biomarkers based on unigenes in chironomid larvae, particularly in field or natural populations.

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Role of Structural Morphology of Commodity Polymers in Microplastics and Nanoplastics Formation: Fragmentation, Effects and Associated Toxicity in the Aquatic Environment

Cassandra Johannessen and Shegufa Shetranjiwalla

Contents

Abstract With the continued growth in plastic production, its ubiquitous use and insufficient waste management and disposal, the increased levels of plastics in the environment have led to growing ecological concerns. The breakdown of these plastic macromolecules to smaller micro and nanosized particles and their detection in the aerial, aquatic, marine and terrestrial environments has been reviewed extensively, especially for thermoplastics. However, the formation of micro and nanoplastics has typically been explained as a physical abrasion process, largely overlooking the underlying chemical structure-morphology correlations to the

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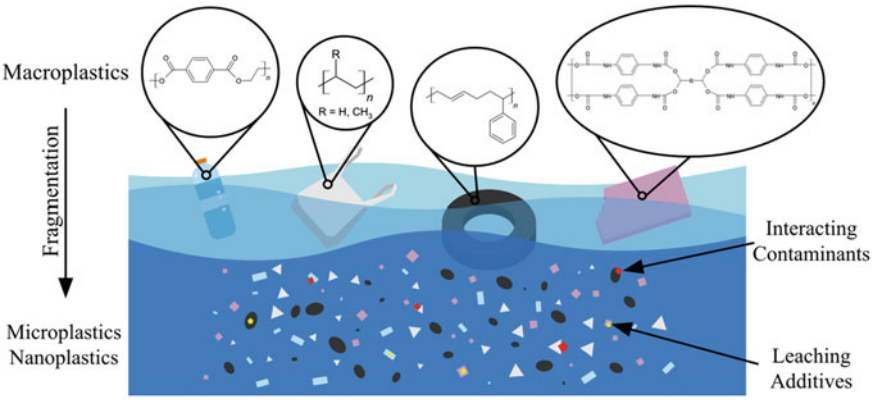
degradation mechanisms of the plastics. This is particularly true for the common commodity thermosets. This review focuses on the degradation pathways for the most widely produced commodity thermoplastics and thermosets into microplastics (MP)s and nanoplastics (NP)s, as well as their behaviour and associated toxicity. Special emphasis is placed on NPs, which are associated with greater risks for toxicity compared to MPs, due to their higher surface area to volume ratios. This review also assesses the current state of standardized detection and quantification methods as well as comprehensive regulations for these fragments in the aquatic environment.

Graphical Abstract



Keywords Aquatic · Commodity plastics · Degradation mechanisms · Microplastics and nanoplastics · Thermoplastics · Thermosets

Abbreviations

Đ	Dispersity
FTIR	Fourier-Transform Infrared Spectroscopy
HDPE	High density polyethylene
LDPE	Low density polyethylene
MPs	Microplastics
MW	Molecular weight
NPs	Nanoplastics
PAHs	Polycyclic aromatic hydrocarbons
PBDE	Polybrominated diphenyl ethers
PCBs	Polychlorinated biphenyls
PCPs	Personal care products

PE	Polyethylene
PET	Polyethylene terephthalate
PNEC	Predicted no effect concentration
PP	Polypropylene
PS	Polystyrene
PU	Polyurethane
PU-ET	Polyether-based polyurethane
PVC	Polyvinylchloride
SBR	Styrene-butadiene rubber
TWP	Tire and roadway particles
WWTPs	Wastewater treatment plants

Highlights

- The structure-morphology-properties of commodity polymers that influence formation of MPs/NPs are discussed.
- The contribution of thermosets to MPs/NPs pollution is considered.
- Degradation mechanisms that induce fragmentation into MPs/NPs are discussed.
- Unique polymer specific toxicity studies for MPs/NPs are reviewed.

1 Introduction

There is consensus that the inertness and the robust chemical nature of plastics that results in long residence times in the environment and resistance to degradation has led to negative environmental impacts (Andrady 2003; Galloway et al. 2017; Avio et al. 2017). Since their genesis, it is estimated that 8,300 million metric tonnes of virgin plastics has been produced, of which 6,300 million metric tonnes has become waste (Geyer et al. 2017). Significantly, 79% of this waste has been deposited into landfills or entered the natural environment, and it is estimated that 4.8–12.7 million metric tonnes of plastics enters the oceans every year (Jambeck et al. 2015; Geyer et al. 2017). The majority of these plastics do not biodegrade but fragment into microplastics (MPs) and nanoplastics (NPs), and the environmental impacts of MPs/NPs in marine and freshwater environments have been extensively studied (Koelmans et al. 2015; Ivleva et al. 2017; Chae and An 2017; Alimi et al. 2018). However, the mechanisms of degradation that generate these small particles have been largely overlooked. The degradation mechanisms are a function of the structure, morphology and properties of the polymers that make up the synthetic plastic commodities. These "commodity plastics" include polymer resins and synthetic fibres that may include additives that enhance and tune the polymer properties. The most-produced commodity plastics include polyethylene (PE), polypropylene (PP) and polyvinylchloride (PVC), followed by the polyester polyethylene terephthalate (PET), then polyurethane (PU) and polystyrene (PS). These materials are

widely used in single-use packaging applications (Geyer et al. 2017). These thermoplastics are typically implicated in the formation of MPs and NPs, but the contributions of thermosetting plastics such as PU foams, epoxy resins, synthetic rubbers and cross-linked PS have largely been overlooked, even though they constitute 12–20% of the world's total plastic consumption (Biron 2018). This review presents the structure-morphology-property characteristics of commodity thermoplastics and thermosets and correlates these to the degradation pathways that can produce MPs and NPs. Also reviewed are the fate, toxicity, detection and regulations for the commodity plastics responsible for generating MPs/NPs, with an emphasis on the structure-morphology-properties that elicit their impacts in the aquatic environment.

1.1 Plastics, Microplastics and Nanoplastics

Polymers are macromolecules composed of repeating subunits that may occur naturally, like wood (repeat units of monosaccharide glucose, -$C_6H_{12}O_6$-), but are also synthesized industrially to produce commodity plastics. For instance, PP and PE, with repeat units of -$CH_2CH(CH_3)$- and -CH_2- respectively, are the most widely produced commodity plastics (Geyer et al. 2017). Linear and networked polymers are generally classified as thermoplastics and thermosets, respectively, resulting from the monomer functionality during polymerization or cross-linking (Fig. 1). Thermoplastics have high impact-resistance, can be reprocessed, and are therefore recyclable. Thermosets, by contrast, cannot be reshaped due to their cross-linked structure and are heat-resistant, making them suitable for high performance applications (Cowie and Arrighi 2007; Carraher 2013). Thermoplastics and thermosets constitute the major commodity plastics used for various applications in industry, such as resins, fibres, coatings, structural building materials and elastomers. Notably, elastomers are frequently grouped with thermosets rather than thermoplastics. These hard cross-linked materials, often referred to as thermosetting rubbers, have applications in the productions of tires, drive belts and biomedical devices (Mark 2017). Additives are chemical species that constitute a major component of commodity plastics and are added during the manufacturing process to enhance polymer properties or as flame retardants. Figure 1 shows the molecular architecture and structural morphology of representative commodity thermoplastics and thermosets, as well as the structures of commonly used additives.

Macroplastics (>25 mm) and mesoplastics (5–25 mm) are thermoplastic or thermosetting polymers that are readily visible to the naked eye, such as water bottles and water bottle lids (Alimi et al. 2018). These polymers often contain fractures, pits and grooves that are ideal loci for both physical and chemical degradation processes to act on. The polymer is aged by a combination of environmental influences such as mechanical degradation initiated by friction and abrasive forces, physical degradation from repeated freezing/thawing or wetting/drying, UV light initiated photodegradation and hydrolytic/oxidative chemical degradation, all

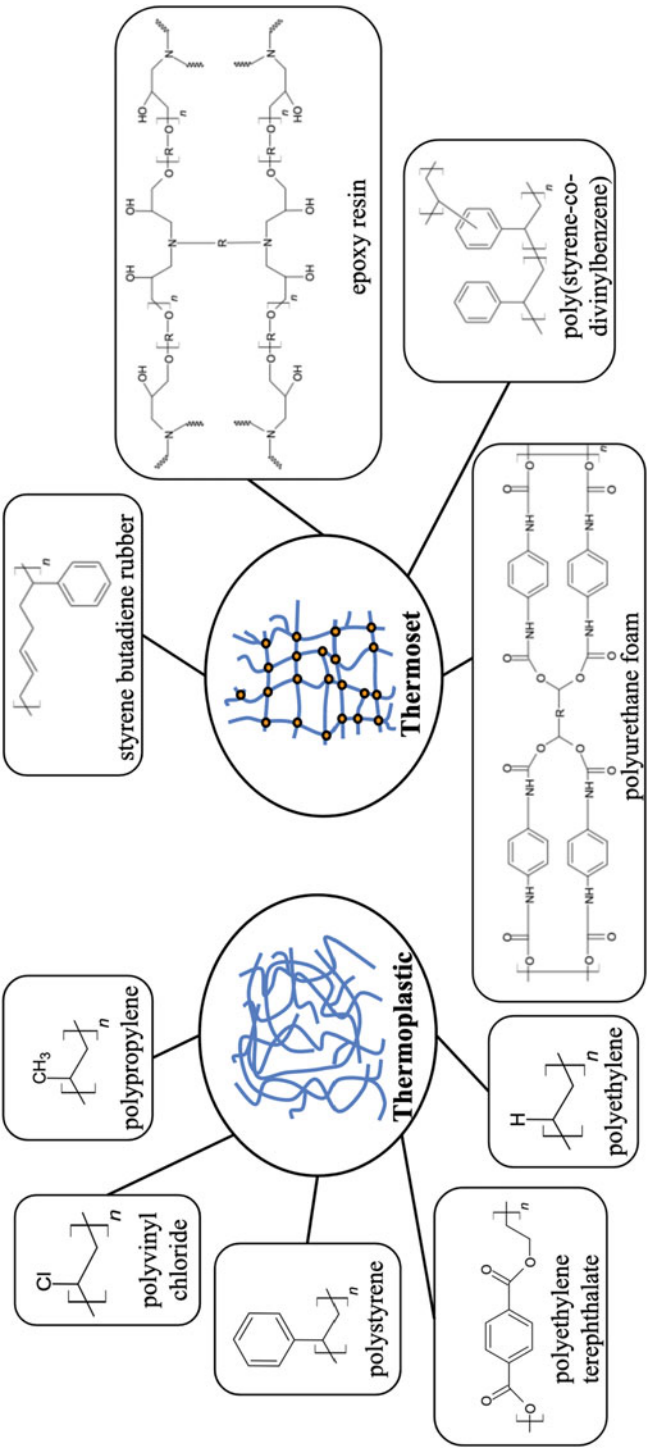


Fig. 1 Molecular architecture and structural morphology of representative commodity thermoplastics and thermosets including the structures of commonly used additives

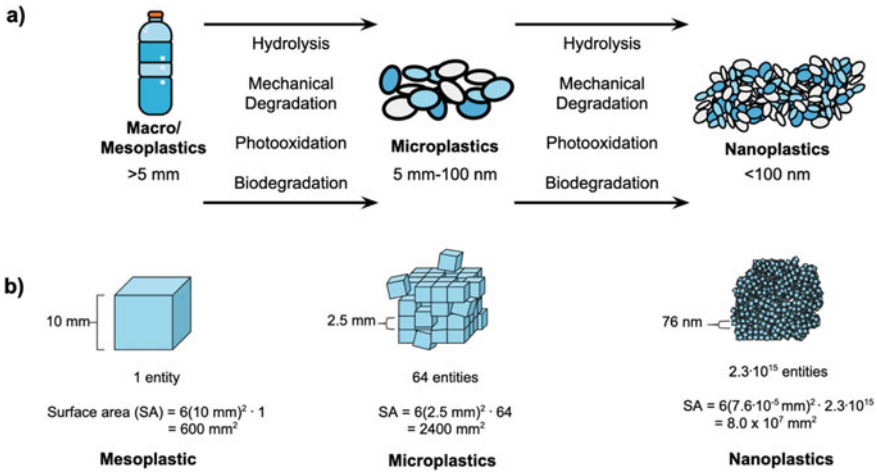


Fig. 2 (**a**) Routes of formation for secondary MPs and NPs (**b**) Relative surface area increase of a 10 mm cube (surrogate for a mesoplastic) upon fragmentation into MPs and NPs. SA = Surface Area

leading to deterioration of polymer properties and embrittlement. In case of high fluctuation in temperature, the polymer may undergo thermal degradation as well (Klein et al. 2018). These processes result in the production of smaller macro and mesoplastics, or even smaller plastic fragments such as MPs (5 mm to 100 nm) and NPs (<100 nm) (Alimi et al. 2018). The minute size of MPs and NPs poses a particular challenge in the environment for detection, collection, recycling or disposal, resulting in MP/NP counts as high as 10^4 per m^3 in coastal environments (Andrady 2017).

MPs and NPs are broadly classified as primary or secondary products, based on their origin. Primary MPs/NPs are intentionally produced for specific applications, such as for air- and sand-blasting, in paints and adhesives, and as microbeads in personal care products (PCPs) (Cole et al. 2011; Koelmans et al. 2015; Andrady 2017). The production, importation and sale of the latter are now prohibited in Canada and the USA (U.S Food and Drug Administration 2017; Government of Canada 2017). Secondary MPs/NPs (also known as "daughter" MPs/NPs) are derived from larger polymer plastics that are degraded through environment exposure. Relevant degradation pathways for the formation of secondary MPs/NPs include abiotic and, to a lesser extent, biotic degradation (Fig. 2a) (Andrady 2011). MPs/NPs have been shown to have negative biological effects on aquatic organisms (Galloway et al. 2017; Chae and An 2017; Nelms et al. 2018; Franzellitti et al. 2019). When ingested by filter feeding aquatic organisms, which is facilitated by their small size, the plastics may leach harmful chemical additives (Rochman et al. 2013). In addition, MPs have demonstrated the ability to sorb contaminants from the environment (Cole et al. 2011). For example, persistent organic pollutants such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) have been

demonstrated to sorb onto MPs, creating risks for toxicity to organisms that may ingest these plastics (Rochman et al. 2013; Alimi et al. 2018; Tan et al. 2019).

This also explains why NPs are an environmental concern from a toxicological standpoint. NPs are, by definition, much smaller in size than MPs, and therefore have greater surface area-to-volume ratios than both bulk plastics and MPs. To provide context, if a 10 mm mesoplastic were to break down into 76 nm cubical nanoparticles, the relative surface area could increase by five orders of magnitude (Fig. 2b). An increase in surface area may result in increased absorption of toxic hydrophobic organic pollutants that are later released into the aquatic environment and organisms (Velzeboer et al. 2014; Liu et al. 2016; Rios Mendoza et al. 2018; Liu et al. 2018; Jeong et al. 2018; Stapleton 2019). Interestingly, NPs have been shown to have joint toxicity relationships with some compounds, such as PAHs, that MPs do not (Ma et al. 2016). In addition, these particulates have shown the capacity to disrupt cell membranes and interfere with cellular functions, which is facilitated by their small size (Shen et al. 2019; Hollóczki and Gehrke 2020). NPs also present a greater issue in terms of detection and quantification, in comparison with MPs, due to their small size (Rios Mendoza et al. 2018; Alimi et al. 2018).

Formation of secondary MPs/NPs has primarily been explained by physical abrasion, overlooking the degradation mechanisms associated with the polymeric macro and microstructure and morphology. The present review aims to explain the formation of MPs and NPs from the perspective of the polymeric structure and morphology, and the potential for depolymerization of the original plastic products. The purpose of this review is to develop a more complete understanding of this process as it pertains to contamination of the aquatic environment.

2 Sources and Behaviour

The discharge of PCPs into domestic waters is a significant source of primary MPs/NPs found in the aquatic environment (van Wezel et al. 2016; Alimi et al. 2018). Microbeads, a type of primary MP used in cosmetics, are typically composed of thermoplastic polymers such as PE, PP and PS (Duis and Coors 2016; Lei et al. 2017; Praveena et al. 2018). The presence of PE MPs and their impact on the aquatic environment have been reported in numerous studies and this has provided evidence to support prohibition of these products in many regulatory jurisdictions worldwide (Rochman et al. 2015; Kalčíková et al. 2017; Hernandez et al. 2017). These plastic particulates have been frequently detected in sewage sludge, as well as outgoing effluents from wastewater treatment plants (WWTPs) (Murphy et al. 2016; van Wezel et al. 2016; Mason et al. 2016; Sun et al. 2019). WWTPs are considered to be a barrier for the discharges of MPs into the aquatic environment, but the reported removal efficiencies of these plastics vary significantly, depending on the wastewater treatment process. For example, one study showed that a membrane bioreactor

used to treat primary effluent showed an MP removal efficiency of 99.9%, whereas a disc filter system used to treat secondary effluent showed removals of 40–98.5% (Talvitie et al. 2017). Despite these removal processes, WWTPs are considered a significant source of MP pollution due to the large volume of water that a plant processes and discharges into the aquatic environment (Murphy et al. 2016; Edo et al. 2020). Furthermore, treatment processes may not efficiently remove NPs as the sizes removed in highest proportions are between 25 μm and 300 μm, indicating that the current treatment processes have greater specificity for larger sized MPs (Estahbanati and Fahrenfeld 2016; Talvitie et al. 2017; Sun et al. 2019; Edo et al. 2020). Besides WWTPs, other routes for the entry of MPs/NPs into aquatic ecosystems are spillage of raw plastic pellet material, as well as improper disposal of industrial abrasives (Jiang 2018; Alimi et al. 2018). In addition, tire and road wear have recently received attention for their contribution to MP pollution and are regarded as one of the most important sources of MPs in the environment (Jan Kole et al. 2017; Sommer et al. 2018; Wagner et al. 2018; Leads and Weinstein 2019; An et al. 2020). Tire and roadway particles (TWP) are formed by mechanical abrasion due to the high friction that occurs between vehicle tires and roadways (Jan Kole et al. 2017; Knight et al. 2020). The resultant particles are largely composed of thermosetting synthetic polymers such as styrene-butadiene rubber (SBR) (Leads and Weinstein 2019; An et al. 2020; Knight et al. 2020). It is estimated that the per capita emission of MPs from tire wear ranges from 0.23 to 4.7 kg per year, of which 5–10% is predicted to enter oceans through road runoff (Jan Kole et al. 2017). These particles have unique sorption properties compared to other types of MPs, and their interactions with other contaminants are poorly understood, justifying future research (Hüffer et al. 2019).

Secondary MPs and NPs arise from fragmentation of polymer chains exposed to the environment. In addition to degradation of the neat polymer, the presence of low-molecular weight additives in commodity plastics are of growing concern. A recent study estimated that 190 t of 20 chemical additives entered the oceans in 2015 through only seven common plastic debris items (bottles, bottle caps, expanded PS containers, cutlery, grocery bags, food wrappers and straws) (De Frond et al. 2019). These additives have been shown to be a major component of derived secondary MPs or NPs, are often toxic, and can leach into aquatic environments, endangering organisms and the ecosystem (Hermabessiere et al. 2017; De Frond et al. 2019; Shen et al. 2019; Capolupo et al. 2020). Bis(2-ethylhexyl) phthalate, used as a plasticizer in PVC (Rijk and Ehlert 2001) and decabromodiphenyl ether, used as a flame retardant in PS resins and upholstery fabrics (Kim et al. 2006), are just two of the additives that are implicated in migration causing toxicity. Their molecular structures (Fig. 1) exhibit aromatic rings substituted with long alkyl chains or polyhalogens that promote persistence in the environment (Boethling et al. 2007).

2.1 Properties of Commodity Polymers

The most widely produced commodity plastics are polymerized through addition or step-growth reactions. Polyolefin thermoplastics such as PE, PP, PVC and PS are produced from addition polymerization using radical, anionic or cationic initiators. Addition polymerization is also used to produce commodity thermoset polymers such as cross-linked PS. Polyester PET, polycarbonates, polyamides or nylons and thermoplastic PU polymers are synthesized by step-growth polymerization where the bifunctional monomers condense, producing a by-product such as water. PU foams and epoxy resins are examples of products that are step-growth thermosets. The mass and distribution of the numerous polymer chains of different sizes formed during polymerization are characterized by the average molecular weight (MW) and dispersity (Đ), respectively (Carraher 2013). These parameters are important descriptors of the structural morphology and the physical properties of a polymer and may be key to predicting the mechanisms for formation of MPs and NPs.

The bulk physical properties of a polymer are directly impacted by the morphologies that are associated with the intermolecular interactions of the chains. When chains fold in parallel and align with one another in a packed configuration, crystal-like domains are formed. These crystalline regions impart rigidity and strength to the plastic. Crystalline domains are embedded in the major disorderly segments called amorphous regions of the polymer that impart flexibility.

Typically, commodity plastics are semi-crystalline, with varying degrees of crystallinity (Carraher 2013; Andrady 2015). Based on the desired application, the crystalline morphology can be "tuned" in commodity polymers through versatile molecular design (i.e. tacticity and stereochemistry) that alter the MW, branching and thermal processing. Higher proportions of crystalline domains shield the more susceptible amorphous fraction from fragmentation due to reduced accessibility for oxidative degradation, embrittlement from ductile deformation and weathering.

The degree of crystallinity can influence the fate of MPs and NPs in the aquatic environment. Generally, higher crystallinity in PE-like polymers increases the density, which influences the buoyancy and determines the location of plastic fragments in the water column (Andrady 2017). For example, high crystallinity PE (80–90%) is more dense than low-crystallinity PE (30–50%). Nevertheless, MPs/NPs composed of PE will tend to initially remain in the upper water column where they could be accumulated by planktonic filter feeders. By contrast, MPs/NPs made from more dense polymers, such as PET, will sink into the sediments and could be accumulated by deposit feeders (Wright et al. 2013). Fouling and aggregation of other suspended particles, promoted by the high surface area of MPs/NPs, will eventually lead to them sinking in the water column and their deposition in sediments (Kaiser et al. 2017; Besseling et al. 2019). Table 1 summarizes the correlation between the properties and the composition of commodity plastics, their fragmentation patterns and the implications for the fate and toxicity of MPs/NPs in the aquatic environment.

Table 1 Properties and composition of commodity polymers and the implications for the formation of microplastics (MPs) and nanoplastics (NPs)

Property of Polymer	Implications for MP/NP derivatives	Reference
Molecular weight and dispersity	• High MW polymers exhibit larger cumulative intermolecular forces, resulting in resistance to fragmentation • Low MW polymers are brittle and have a high surface area, which promotes degradation and accelerates fragmentation. • High Đ correlates to high proportion of low MW chains, promoting degradation and increasing rate of fragmentation • High MW and Đ ~ 1 mitigate degradation by microbial attack	Arutchelvi et al. (2008), Carraher (2013), Gewert et al. (2015), Andrady (2017)
Morphology	• Degree of crystallinity impacts the rate of fragmentation, and therefore the rate of MP/NP formation • Initial degradation and crack propagation typically occur selectively in the amorphous regions of the polymer • High crystallinity mitigates oxidative degradation by abiotic or biotic mechanisms • Amorphous polymers readily adsorb organic pollutants, with potential adjunct toxicity effects	Daglen and Tyler (2010), Carraher (2013), Hartmann et al. (2017), Andrady (2017), A. Glaser (2019)
Density	• Polymers with greater density tend to sink in the water column • Low density polymers float, then interact with dissolved organic matter and suspended particles before eventually descending in the water column due to fouling and aggregation • High packing density reduces susceptibility to microbial attack and biodegradation	Arutchelvi et al. (2008), Kowalski et al. (2016), Andrady (2017), Kaiser et al. (2017)
Composition	• Heteroatoms (O, N, Cl) in the polymer backbone resist thermal degradation compared to hydrocarbon polymers • Heteroatoms in polymers are susceptible to hydrolytic cleavage in the aquatic environment, • Heteroatoms stimulate biotic deterioration • Hydrophobic polymers are not biodegradable without impurities • Structural complexity, through the addition of functional groups associated with the main chain inhibits biodeterioration but may enhance the rate of UV degradation through the stabilization of radicals • Presence of chromophores in the polymer promotes MP/NP formation through UV-induced pathways • High degree of cross-linking, such as in thermosetting plastics, mitigates the formation of fragments through thermal and chemical degradation pathways	Göpferich (1996), Arutchelvi et al. (2008), Gewert et al. (2015), Liu et al. (2016)

(continued)

Table 1 (continued)

Property of Polymer	Implications for MP/NP derivatives	Reference
	• Conjugation, such as in aromatic rings, permits increased interfacial interactions with organic aromatic pollutants such as polyaromatic hydrocarbons through π-π interactions, enhancing adsorption and increasing the associated potential risk for toxicity	
Additives/ Stabilizers	• Presence of antioxidants and stabilizers decreases susceptibility to degradation • Presence of biodegradable copolymers or fillers such as starch blends and pro-oxidants enhances degradation through abiotic and biotic degradation pathways • Leaching of additives/stabilizers from MPs/NPs may increase associated toxicity	Shah et al. (2008), Muthukumar et al. (2010), Hermabessiere et al. (2017)

2.2 Mechanical Properties

The mechanical properties of commodity polymers are a key indicator of their performance (Nielsen and Landel 1993). The performance of commodity polymers is determined by their molecular parameters such as structure, composition, molecular weight, dispersity and morphology (Table 1). When the polymer degrades, it causes a deterioration of these molecular parameters resulting in the corresponding loss of mechanical properties.

A polymer degrades in three stages with varying rates of deterioration of the molecular weight and crystallinity (Albertsson and Karlsson 1988; Shetranjiwalla et al. 2017). During the first stage, material changes are rapid, causing a swift change in mechanical properties. However, in the second stage the changes in mechanical properties are asynchronous with the extensive loss in molecular weight. This is attributed to *chemicrystallization* where the cleaved polymer chains gain mobility and realign to show an initial increase in crystallinity. Due to this phenomenon, the smaller molecular fragments do not diffuse out of the matrix and polymer chains do not reach a molecular weight below a critical value, resulting in relatively slower changes in mechanical properties. The third stage is characterized by the rapid extraction of the oligomers from the eroded matrix causing extensive mass loss and embrittlement. Subsequently, the mechanical strength of the plastic decreases while the rate of embrittlement increases (Hawkins 1984; Göpferich 1996). Also, residual internal stresses caused during the processing of commodity polymers impact the mechanical properties during degradation as these produce nucleation sites that allow for pore and crack formation (Suresh et al. 2011). In the aquatic environment, water penetration disrupts molecular bonding and alters the

morphology of commodity thermoplastic and thermoset polymers by promoting migration of fragmented species. The water acts as a medium for migration of plasticizers out of the degrading matrix, just as it does for the fragmented species (Saharudin et al. 2016; Wei et al. 2019). The plasticizers are usually additives that are used to improve mechanical properties such as flexibility. Water also acts as a plasticizer (Houchin and Topp 2009) and can form hydrogen bonds with the heteroatom containing polymers, altering the carbonyl index and the morphology (Shetranjiwalla et al. 2017). Changes in the tensile strength, elongation at break, formation of surface cracks and pores, and changes of the carbonyl index are key indicators that the mechanical properties of a polymer are failing due to degradation (Naddeo et al. 2004; Chamas et al. 2020).

3 Fragmentation Pathways

Secondary MPs/NPs are produced through both biotic and abiotic degradation processes. The physicochemical properties of the polymer before it biodegrades or fragments are key to understanding the ease of formation and subsequent behaviour of these small plastic by-products (Andrady 2017; Alimi et al. 2018). Information on the mechanisms of degradation of polymers into MPs/NPs in the aquatic environment is limited, and therefore, these processes must be extrapolated from the known routes of polymer degradation. In this review, degradation is not taken to mean complete mineralization, but rather the alteration of polymer structure into smaller entities by either biotic or abiotic processes.

As summarized in Table 1, polymers degrade into MPs/NPs in the environment through chemical, biological and physicomechanical processes and the chemical composition of the polymer is key in identifying the dominant degradation pathways. For example, exocyclic double bonds in a polymer backbone react actively through photooxidation, increasing degradation. Side chain functional groups may contribute to degradation by rendering alpha carbons vulnerable to photooxidation. Heteroatom containing polymers may stimulate hydrolytic degradation but resist thermal degradation (Nayak et al. 2017). It is also known to some extent that photochemical degradation of conjugated polymers such as conductive polythiophenes occurs when molecular oxygen destroys the pi conjugation, affecting either the allylic hydrogen or side chains (Koch et al. 2009).

Degradation of polymer plastics commences at the exterior surface and continues inwards to the bulk of the plastic (Gewert et al. 2015). Fragmentation occurs through the process of crack propagation, which readily occurs in amorphous regions that are subjected to ductile deformation (Andrady 2017). Oxidative degradation, a process catalysed by solar radiation, acts primarily on the amorphous regions of plastics due to their high oxygen permeability. Presumably, this only occurs in the upper part of the water column where there is high solar radiation. During degradation, oxygen-dense functional groups are formed in the amorphous regions and the average MW of the polymer decreases (Table 1). However, high densities of embedded crystalline

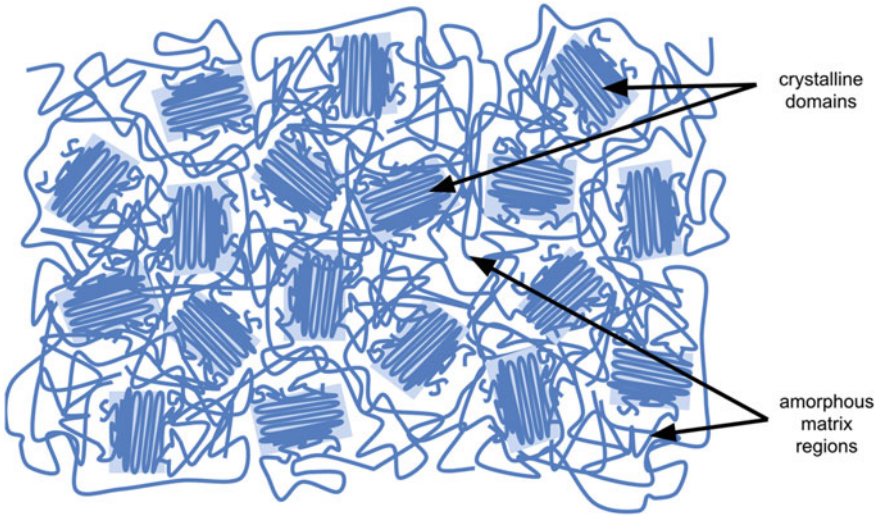
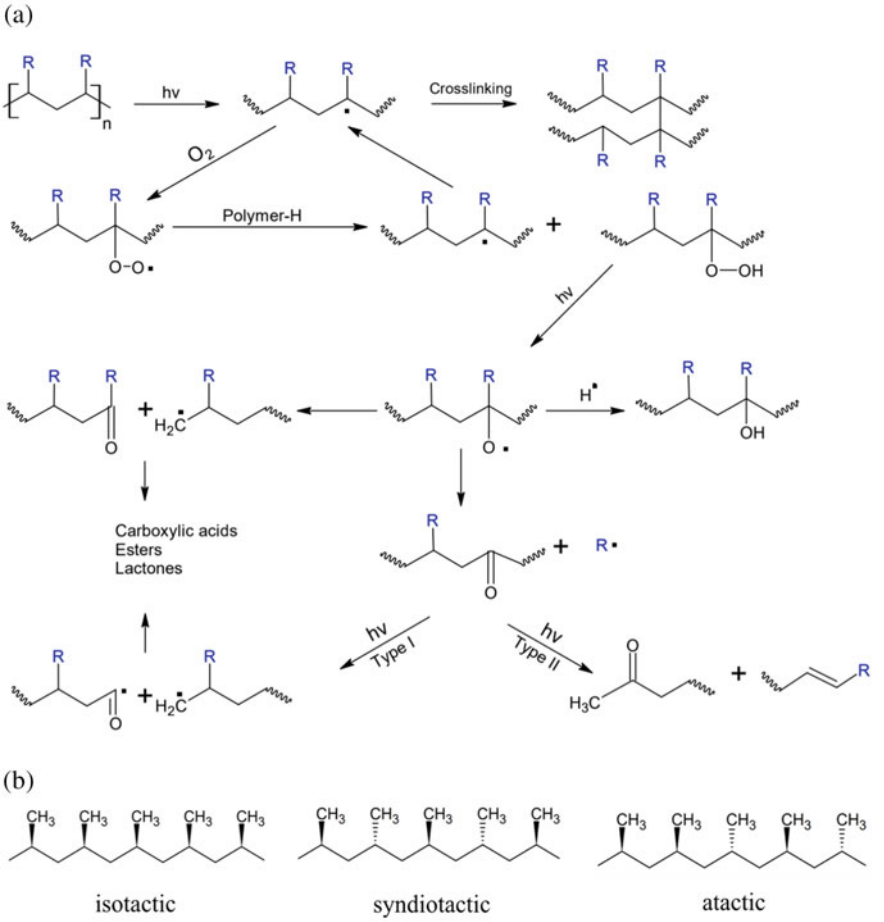


Fig. 3 Crystalline domains embedded in an amorphous matrix for semi-crystalline polymers

regions inhibit access to amorphous regions and mitigate degradation (Andrady 2017). Therefore, the degree of crystallinity is an important morphological factor in determining the rate of fragmentation. Semi-crystalline commodity plastics such as PE, PP and PET are composed of crystalline regions that are encased in the amorphous matrix (Fig. 3). These plastics therefore are subject to high rates of oxidative degradation and are commonly found as MPs (Cooper and Corcoran 2010; Ojeda et al. 2011; Andrady 2011, 2017; Song et al. 2017).

Abiotic photodegradation processes are typically initiated by UV light, reacting at chromophore sites within the polymer. Chromophores that absorb light at various frequencies are often present, even in polyolefins, due to structural impurities such as double bonds in residual monomers or additives. Degradation products typically contain oxidized molecules such as esters, ketones, alcohols, carboxylic acids and lactones (Ojeda et al. 2011; Yousif and Haddad 2013; Gardette et al. 2013; Gewert et al. 2015). Scheme 1a represents the typical degradation pathway for the photo-oxidation of polyolefinic polymers. Scheme 1b shows, using the example of PP, the stereoisomers formed in polyolefins by the variation in stereochemistry of repeating sidechains that impact the rate of degradation.

Biotic processes of degradation (i.e. biodegradation) of polymers are an important but less considered mechanisms for the formation of MPs/NPs following initial fragmentation of the bulk plastic (Shah et al. 2008). The ease of biodegradability also depends on the chemical composition, degree of crystallinity, the hydrophilicity, size profile, bond types, presence of additives/blends and surface area of the polymer (Zheng et al. 2005; Arutchelvi et al. 2008). However, the most common plastics in the aquatic environment (i.e. polyolefins) are not susceptible to microbial attack due to their hydrophobic backbones, large packing density and high MWs



Scheme 1 (**a**) Typical photooxidation pathway for polyolefin commodity polymers. Type I and Type II refer to Norrish Type I and Type II photochemical cleavage of carbonyls, respectively. R = H, CH_3, Ph (**b**) Stereoisomers of polypropylene, typical of polyolefinic polymers, formed by the variation in stereochemistry of repeating sidechains

(Arutchelvi et al. 2008). However, biodegradation is enhanced by the presence of structural impurities incorporated into the polymer either during manufacturing stages or during earlier stages of degradation.

Importantly, plastics transform as they degrade in the environment, undergoing cross-linking or chain scission reactions that alter their chemical structure and composition. These structural modifications are especially prevalent at the point of fragmentation and not only dictate the composition of secondary MPs/NPs but also influence detection by spectroscopic techniques. The spectral characteristics of secondary MPs/NPs are often very different from the neat polymer (Lenz et al. 2015), often preventing identification from available spectral databases.

4 Common Commercial Plastics and Their MP/NP Derivatives

The identity and concentrations of MPs/NPs that have been detected in the aquatic environment have been recently summarized (Wan et al. 2018; Erni-Cassola et al. 2019; Li et al. 2020). The most commonly found MPs are fragments of commodity thermoplastics that include PE, PP, PS, PVC and PET (Andrady 2017). In addition to the thermoplastic polymers, thermoset polymeric resins such as SBR used in tires are estimated to contribute 10% of the MP emissions (Jan Kole et al. 2017). Epoxy and PU foams, although currently less frequently detected as MPs and NPs, must also be considered due to their significant production volumes. Table 2 summarizes the most prominent degradation pathways for each aforementioned polymer.

Table 2 Summary of prominent degradation pathways for commodity polymers

Commodity polymer	Type	Degradation process	Degradation products
Polyethylene (PE) and Polypropylene (PP)	Thermoplastic	Photooxidative degradation	Products rich in hydroxy, ketone, carboxyl and ester functional groups
Polystyrene (PS)		Photooxidative degradation	Peroxy-radicals, carbonyl containing compounds, aliphatic ketone, styrene monomers and oligomers
Polyvinyl chloride (PVC)		Mechanical degradation and UV-induced degradation	Unsaturated polymers and hydrochloric acid
Polyethylene terephthalate (PET)		(a) Photooxidative degradation and (b) hydrolytic degradation	(a) Carboxylic acid and vinyl end groups (b) carboxylic acid and hydroxyl-ester end groups
Polyurethane forms synthesized from polyether (PU-ET) or polyester (PU-ES) polyols	Thermoset	PU-ET: photooxidation PU-ES: hydrolysis	PU-ET: Carboxylic acids, formates, lactones, ethene, glycol derivatives and benzoic acids (if aromatic) PU-ES: alcohol and carboxyl products
Epoxy resin		Photooxidative degradation	Amide containing by-products that can further degrade into propenal
Poly(styrene-co-divinylbenzene)		Photooxidative degradation	Peroxy-radicals, carbonyl containing compounds, aliphatic ketone, styrene monomers and oligomers (hypothesized)
Styrene-butadiene rubber (SBR)		Physical ablation and thermal oxidative degradation	Anhydrides, peresters, carboxyl acids, ethers and alcohols

4.1 Thermoplastics

Single-use packaging from plastic bags, bottles from food and beverage, healthcare applications, packaged consumer goods, cosmetics and PCPs are made from commodity thermoplastics. After first use, these materials lose 95% of their material value (an estimated loss of 80–120 billion dollars annually) and contribute to secondary MP/NP pollution (Ellen MacArthur Foundation, Mckinsey, and Company 2016). Selected properties that determine their fate in the aquatic environment for these thermoplastics are provided in Online Resource 1 (Table S1). In the following section, the structural properties that define the environmental fate of the most common thermoplastics are discussed in detail.

4.1.1 Polyethylene

PE is the most widely used plastic in the world with a combined global production volume for low and high density PE of 116 million metric tonnes per year (Geyer et al. 2017). PE has a density that can range from 0.91 to 0.97 g cm^{-3}, allowing it to float on the water surface, although the accumulation of other debris can increase the density so that it eventually sinks to the sediment (Fazey and Ryan 2016). PE has been widely detected as MPs (Wan et al. 2018; Erni-Cassola et al. 2019; Li et al. 2020). For example, 70% of MPs detected in sediments from the Brisbane River in Australia, where maximum MP concentrations were 129.20 mg $kg^{-1,}$ were identified as originating from PE (He et al. 2020).

PE is the simplest polymeric thermoplastic with the general formula of $(C_2H_4)_n$, containing a saturated carbon backbone. There are a multitude of commonly produced grades of PE, each exhibiting different properties. Low density PE (LDPE) has a low to moderate crystallinity, whereas high density PE (HDPE) has a high crystallinity (Table S1). Consequently, the rate of oxidative degradation of LDPE is higher than that of HDPE due to the higher oxygen permeability in the amorphous regions of the plastic. Mechanical degradation is also elevated in LDPE compared to HDPE, due to the higher ability for crack propagation in the amorphous regions. Also, due to the variations in MW, degree of cross-linking, additives and tensile strength, the specific degree of weathering varies with the grade of PE. Although the physical disintegration of polymer properties due to environmental influences is considered a long-term process, secondary MPs are continually being produced. MPs derived from PE (among other plastic types such as PP) were observed to be produced from the commodity polymer during every tidal cycle in salt marshes (Weinstein et al. 2016). However, complete degradation of a polymer takes several years (Arutchelvi et al. 2008) and is slower in the aquatic environment than in air, which is attributed to lower exposure to sunlight, and lower temperatures and oxygen levels (Weinstein et al. 2016).

The degradation of PE proceeds through the photooxidative degradation of chromophores catalysed by UV light in the presence of structural impurities,

resulting in the generation of free radicals (Scheme 1) (Gijsman et al. 1999; ter Halle et al. 2017). During propagation, peroxy-radicals are formed with molecular oxygen producing carbonyl degradation products. Aldehyde and ketone products undergo Norrish type I and II reactions, resulting in fragments that include aliphatic carboxylic acids, esters, ketones, alcohols, lactones, vinyls and various oligomers (Gardette et al. 2013; Gewert et al. 2015). Degradation through UV radiation ultimately reduces the overall MW of the polymer and renders it brittle and susceptible to accelerated fragmentation (ter Halle et al. 2017). The fragmentation pathway for the formation of low MW chains include the insertion of oxidized species on the surface that leads to increased hydrophilicity, which promotes biodegradation processes that would not normally occur in a hydrophobic commodity polymer (Arutchelvi et al. 2008). Degradation of PE by microorganisms originates at a terminal methyl group and results in the formation of hydroxy, ketone, carboxyl and ester functional groups as well as water and carbon dioxide as by-products (Arutchelvi et al. 2008; Gewert et al. 2015). Multicellular organisms can also contribute to biodegradation as a recent study reported that Antarctic krill (i.e. euphausiid shrimp) can biodegrade PE-derived MPs into smaller NPs that are capable of crossing biological barriers and can be readily consumed by aquatic organisms (Dawson et al. 2018).

4.1.2 Polypropylene

PP is the second most-produced plastic with a production of 68 million metric tonnes per year (Geyer et al. 2017). PP is used for packaging and labelling of goods. With a density of 0.90 g cm^{-3}, PP will initially float in the aquatic environment until it sinks due to fouling. MPs derived from PP have been frequently detected in the aquatic environment (Wan et al. 2018; Erni-Cassola et al. 2019; Li et al. 2020). Degradation of PP proceeds primarily through chain scission resulting in a lower MW distribution (Canevarolo 2000). PP is a branched thermoplastic with a methyl group attached to alternate carbon stereocenter in the repeat unit, generating three stereoisomers: isotactic PP, syndiotactic PP and atactic PP (Scheme 1b). The specific rate of degradation has been shown to vary with tacticity. Studies of thermo-oxidative degradation of molten state PP show that isotactic PP degrades at a much faster rate compared to the other stereoisomers following a bimolecular chain initiation in contrast to the unimolecular chain initiation in atactic PP. Isotactic PP is constituted mainly of meso dyads that represent two adjacent carbons with methyl groups on the same side whereas atactic PP exhibits a mainly racemic configuration that mitigates thermo-oxidative degradation (Hatanaka et al. 1999a). Oxidative degradation has also been shown to proceed more readily in the isotactic form than in the syndiotactic form as the activation energy required for the abstraction of the tertiary hydrogen is considerably higher for the latter stereoisomer (Hatanaka et al. 1999b). In addition, it has been demonstrated that long isotactic sequences in PP promote the propagation of oxidative degradation (Chammingkwan et al. 2017). Interestingly, oxidative

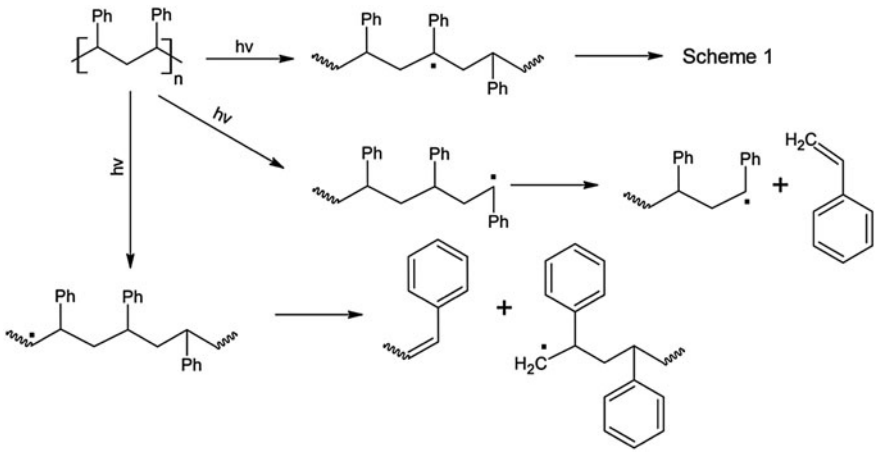
degradation in isotactic PP was shown to be independent of MW and the presence of other additives such as catalyst residues (Hatanaka et al. 1999a).

The UV-induced degradation of PP is similar to that of PE (Gewert et al. 2015). PP also requires the presence of chromophores through the presence of additives or structural impurities in order to initiate the UV degradation process (Gijsman et al. 1999). Propagation occurs via the abstraction of the tertiary hydrogens, resulting in tertiary peroxy-radicals with molecular oxygen (Scheme 1a). These peroxides decompose into alkoxy and hydroxyl radicals, continuing propagation to form ketones, alcohols, esters and carboxylic acids as possible oxidation products (Rjeb et al. 2000). Photooxidative degradation of PP is a relatively faster process compared to the photooxidative degradation of PE (Carlsson and Wiles 1976; Ojeda et al. 2011). This is attributed to the lower chemical resistance of PP arising from the stabilization of the produced radicals at the tertiary carbon compared to the thermodynamically less favoured stabilization at the secondary carbon of PE. Also, the branched methyl group in PP impedes chain stacking resulting in a less crystalline morphology. The presence of plastic additives such as antioxidants and UV stabilizers decreases the rate of abiotic degradation of the polymer. However, some additives such as starch blends and pro-oxidants promote degradation in polymer blends through abiotic and biotic pathways compared to pure PP (Zheng et al. 2005; Muthukumar et al. 2010). Like PE, fragmentation of PP leads to the insertion of hydrophilic groups on the surface of the polymer (Scheme 1a), allowing for biodegradation by microorganisms. Biodegradation of PP results in the formation of ester and hydrogen peroxides as well as hydrocarbons and plasticizers (Cacciari et al. 1993). PP is more resistant to microbial attack than PE due to the increased structural complexity attributed to the methyl group in the beta position instead of a hydrogen (Sudhakar et al. 2007; Gewert et al. 2015).

4.1.3 Polystyrene

Commodity PS is produced in volumes of 25 million metric tonnes per year (Geyer et al. 2017) and is commonly used in the production of moulds, cutlery and as packaging material. In the aquatic environment, PS is expected to sink due to its typical density of 1.04 g cm^{-3}. However, the expanded form of PS, which includes air trapped in the polymer matrix during production may float (Andrady 2015). MPs derived from PS have been commonly detected in the aquatic environment (Wan et al. 2018; Erni-Cassola et al. 2019; Li et al. 2020). Weathering simulations have established that there is significant production of NPs from bulk PS over time (Lambert and Wagner 2016).

PS generates tactic stereoisomers due to the alternately repeating phenyl group. Atactic PS that is highly amorphous is the most common form of commodity PS. The steric hindrance caused by the large phenyl group inhibits formation of the crystalline and semi-crystalline syndiotactic and isotactic forms of PS, respectively. Degradation of PS proceeds through both chain scission and chain branching, with end chain scission being the predominant mechanism (Singh and Sharma 2008;



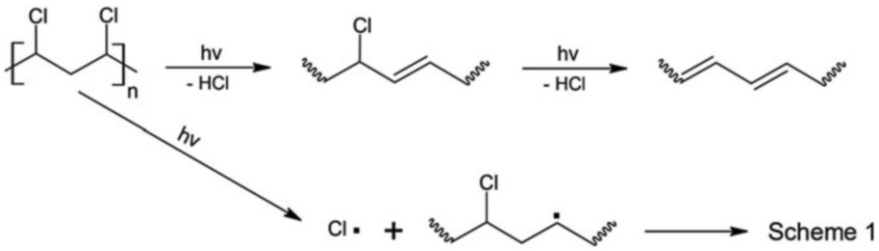
Scheme 2 Simplified scheme for the photooxidation of PS

Gewert et al. 2015). UV-induced degradation leads to the production of radicals through excitation in the phenyl ring, resulting in cleavage of the benzylic C–H bond (Gewert et al. 2015). The benzylic radical can move along the PS chain, triggering differing degradation processes (Scheme 2). For example, chain scission can occur through macroradical disproportionation and the styrene monomer can be formed through the formation of a terminal radical. If two radicals are close to each other, cross-linking may occur as depicted in Scheme 1a. In addition, the PS radical can react with oxygen to produce peroxy-radicals, which undergo chain scission to produce carbonyl containing compounds (Scheme 1a) (Yousif and Haddad 2013). Aliphatic ketone groups may be produced through the photolysis of the peroxides; however, the styrene monomer is the most common volatile degradation product with many oligomers of styrene present (Yousif and Haddad 2013; Gewert et al. 2015). PS is considered largely resistant to biotic degradation attributed to its high MW and structural complexity (Gewert et al. 2015; Ho et al. 2018).

4.1.4 Polyvinyl Chloride

PVC is widely produced in volumes of 38 million metric tonnes per year (Geyer et al. 2017). It is used in packaging, as bank cards, and as tiles and pipes in construction. PVC is negatively buoyant (1.16 to 1.58 g cm^{-3}), therefore is expected to sink in the aquatic environment. MPs derived from PVC have been widely detected in aquatic environments, including in Canada, India, Singapore and the Southern Ocean (Mohamed Nor and Obbard 2014; Ballent et al. 2016; Isobe et al. 2017; Sruthy and Ramasamy 2017).

PVC also generates stereoisomers and is a highly amorphous polymer, making it sensitive to mechanical degradation through crack propagation (Yassin and Sabaa 1990). Due to its halogenated nature, PVC is also highly sensitive to UV radiation;



Scheme 3 Simplified scheme for the photooxidation of PVC

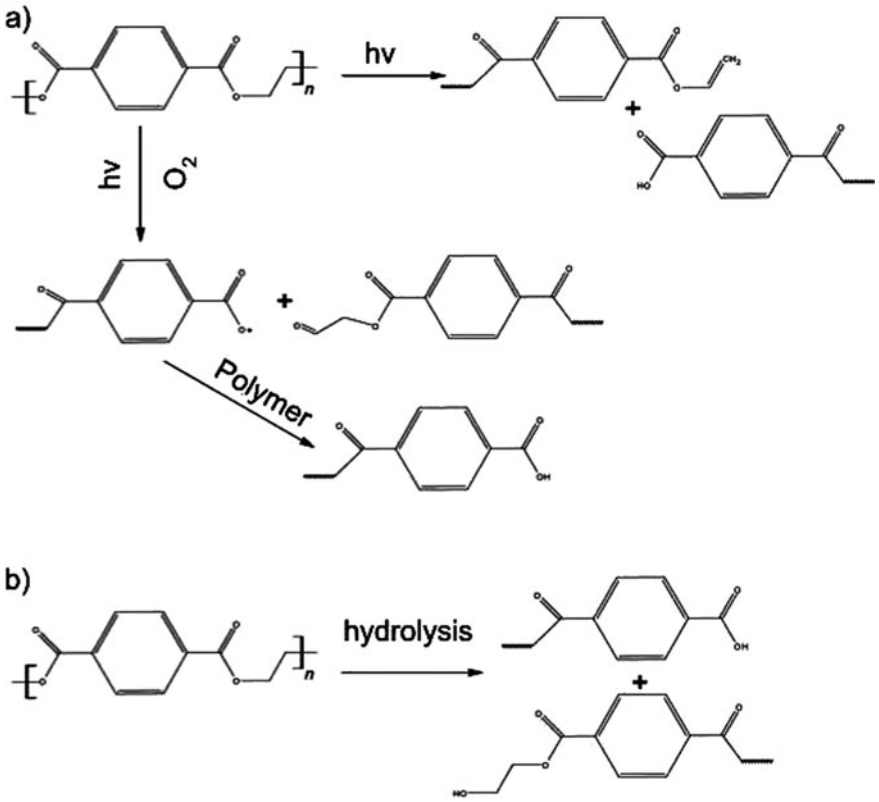
resulting in rapid deterioration in a matter of weeks (Wypych 2015). Chromophoric impurities initiate UV degradation. Often, carbonyls, dienes, trienes and longer conjugation products are generated during thermal processing and long-term storage of PVC, greatly increasing the polymer's susceptibility to UV degradation (Wypych 2015). Upon initiation by photons, a radical can form on the unsubstituted carbon, resulting in dechlorination. This produces an unsaturated polymer and hydrochloric acid as a by-product (Scheme 3) (Winkler 1959; Gewert et al. 2015; Andrady 2017). The allylic bond can also be cleaved, forming a radical in the α-position that propagates photooxidation (Scheme 1a) (Yousif and Hasan 2015). Acid-induced degradation of the polymer also increases deterioration.

PVC has limited biodegradation potential and it is proposed that the dechlorination of PVC must precede biodegradation (Gewert et al. 2015; Glaser 2019).

4.1.5 Polyethylene Terephthalate

PET has a global production of 33 million metric tonnes per year (Geyer et al. 2017). It is commonly used as bottles, clothing and for manufacturing purposes. With a density of 1.29 to 1.40 g cm^{-3}, this plastic tends to sink in the water column and is one of the most reported MPs in the freshwater environment, after PE, PP and PS (Li et al. 2020). PET has a general formula of $(C_{10}H_8O_4)_n$ and is semi-crystalline showing susceptibility to fragmentation through crack propagation, but due to the presence of heteroatoms, PET shows increased thermal stability (Gewert et al. 2015). PET can undergo rapid photooxidation via radical reactions detailed previously with the hydroperoxide formation, resulting in chain scission (Venkatachalam et al. 2012; Gewert et al. 2015). The ester bond may also be cleaved, resulting in carboxylic acid and vinyl end groups (Scheme 4a) (Gewert et al. 2015).

In the aquatic environment, PET may also undergo hydrolytic degradation due to the presence of the hydrolytically susceptible ester groups forming compounds with a carboxylic acid end group and an hydroxyl-ester end group (Scheme 4b) (Awaja and Pavel 2005; Gewert et al. 2015). Hydrolysis of PET takes place in the amorphous regions of the polymer through chain scission (Arhant et al. 2019). The rate of hydrolysis and subsequent MP/NP formation will depend on the MW, Đ and percent crystallinity of the polymer.



Scheme 4 Degradation pathways for PET by: (**a**) photooxidation and (**b**) hydrolysis

PET is not likely to undergo biodegradation due to its complex cumulative intermolecular dipole–dipole, π-π and van der Waals interactions that prevent access for microbial attack.

4.2 Thermosets

Thermosets are cross-linked polymers (Fig. 1) that are produced on an industrial scale and exhibit diverse structural features corresponding to their versatile chemistry (Biron 2018). Thermosets degrade in the environment, often by different mechanisms than that of thermoplastics, due to their inherent heat and chemical resistances. Degradation in high-demand thermosets such as PUs, epoxy resins, poly(styrene-co-divinylbenzene) and SBR is largely dependent on the physical or chemical nature and extent of cross-linking that determines the crystallinity and other physical properties of the polymers. The extent of cross-linking may be tuned

by varying the monomer, catalysts, cross-linking agent, curing time and polymerization method leading to a range of properties that are industry specific.

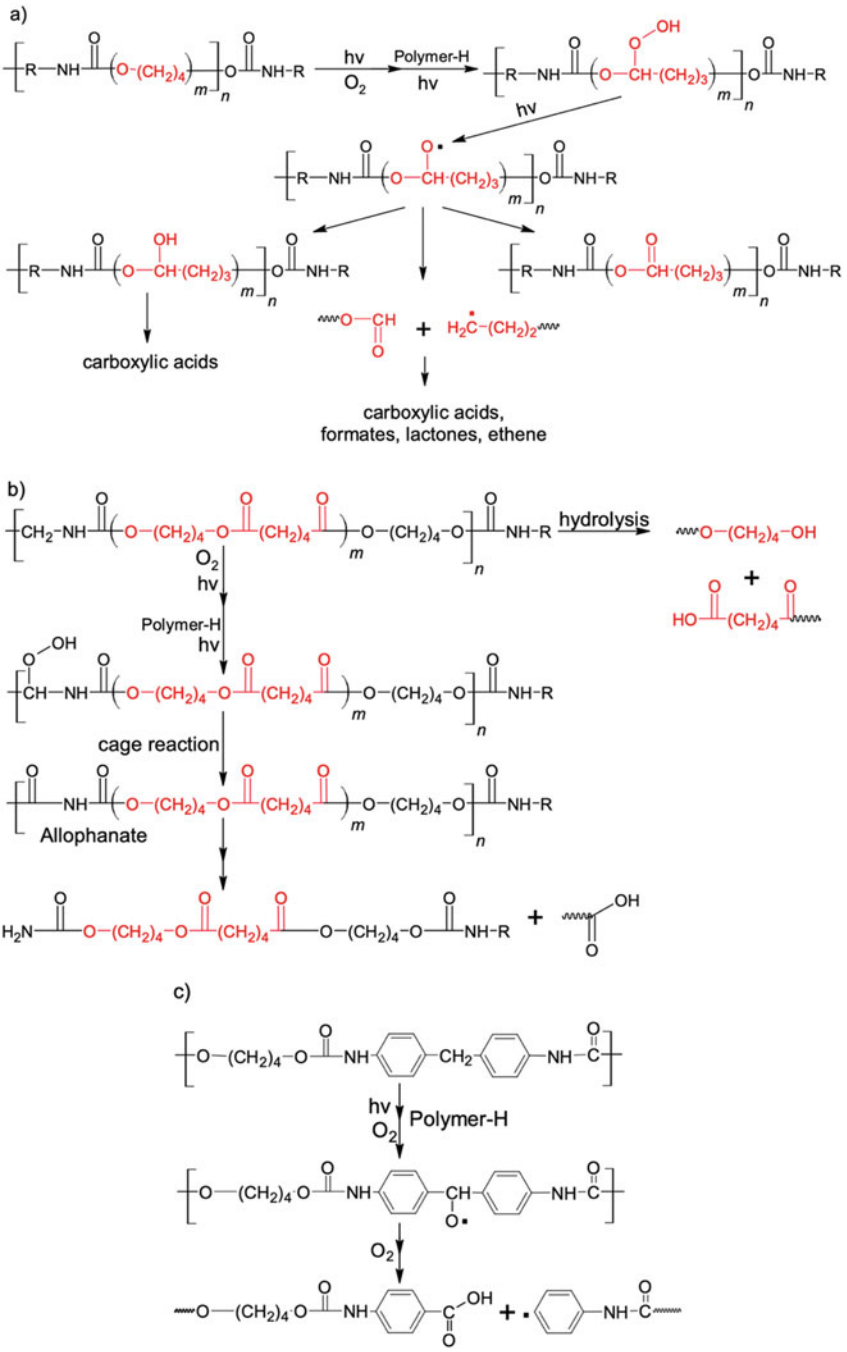
Thermosets are characterized by their thermal stability and mechanical strength. Typical thermal stability and mechanical strength data for epoxy resins, which are the most investigated thermosets, are provided in Online Resource 1 (Table S2). Here, epoxy resins serve to act as representative materials to illustrate the impact of monomer type, cross-link density and other important structural parameters on physical and mechanical properties. Unlike thermoplastics, which decrease in mechanical strength with increasing temperature and demonstrate viscous flow, cross-linked thermosets retain modulus at approximately 10^6 Pa due to the loss in chain mobility. The geometrically restricted network in thermosets results in brittle materials that are susceptible to crack development that act as stress concentrators that eventually propagate to form MPs/NPs. Toughening agents and nanofiller reinforcement such as nanofibres, micro-sized liquid rubber or thermoplastic particles institute further components in thermosets prone to eventual MP/NP creation (Garg and Mai 1988; Zeng et al. 2012).

4.2.1 Polyurethane

PU plastics have a global production of 27 million metric tonnes per year (Geyer et al. 2017) and are synthesized from polyols and diisocyanates forming urethane linkages in their backbone (Fig. 1). PU can be either thermosets or thermoplastics. The most commonly produced PU plastics are thermoset foams, which correspond to at least 50% of global PU consumption (Gama et al. 2018). PU foams are typically synthesized from polyether (PU-ET) or polyester (PU-ES) polyols. The PU-ET forms are widely used for their cost efficiency. In one study, PU-based MPs were the third most detected particles from sediments of Lake Ontario, Canada (Ballent et al. 2016).

Structural pores in PU foams allow for easy access to oxygen, UV radiation and water into the material, driving degradation at reactive sites (Shashoua 2008). However, due to their innate structural differences, PU-ET and PU-ES vary in terms of their susceptibility to different degradation pathways. The dominant abiotic pathway of degradation for PU-ET is photooxidation, whereas for PU-ES it is hydrolysis (Lattuati-Derieux et al. 2011; Le Gac et al. 2013). Degradation results in chain scissoring and crumbling of the foams (Shashoua 2008).

Photooxidation of aliphatic PU-ET commences at the α-methylene position with respect to the oxygen, forming hydroperoxides through radical reactions typical of hydrocarbons (Scheme 5a), producing carboxylic acids, formates, lactones and ethene (Wilhelm and Gardette 1998). The formation of glycol derivatives has also been reported (Lattuati-Derieux et al. 2011). After the oxidation of the polyether portion, the methylene group α to the NH may oxidize to give carboxylic acids and primary urethane (Wilhelm and Gardette 1998). Oxidation at this position has also been observed in PU-ES (Scheme 5b) (Wilhelm and Gardette 1997). PU-ES is extremely labile to degradation in aquatic environments through hydrolysis



Scheme 5 Degradation pathways for aliphatic and aromatic PUs: (**a**) Simplified photooxidation in aliphatic PU-ET; (**b**) Photooxidation and hydrolysis in aliphatic PU-ES; (**c**) Photooxidation in aromatic PU-ET

(Le Gac et al. 2013), attributed to the hydrolytic cleavage of the ester bond, forming alcohol and carboxyl products (Scheme 5b) (Lattuati-Derieux et al. 2011). The carboxylic acid products auto-catalyse and accelerate depolymerization (Gewert et al. 2015). This process is fast even at lower temperatures. Additionally, the hydrolysis of the urethane bonds has also been observed (Shetranjiwalla et al. 2017). In aromatic PU, oxidation of the urethane segments has been observed through the formation of a radical on the central methylene carbon between the aromatic groups, ultimately producing end groups that resemble benzoic acid (Scheme 5c) (Gardette et al. 1999). Mechanisms shown in Scheme 5 illustrating linear PUs are also applicable to thermoset PUs composed similarly of ether, ester and carbamate functional groups. Of note, however, is that the degradation at these functional groups is significantly reduced in thermoset PUs due to the limited accessibility for photooxidation and hydrolysis in the highly cross-linked structure.

PUs also biodegrade into MPs/NPs (Howard 2002), but this largely depends on the type of polyol used. PU-ES have shown greater susceptibility to biodegradation than PU-ET due to the sterically encumbered ester bonds that reduce packing density, resulting in significant amorphous regions susceptible to microbial attack compared to the more crystalline regions of PU-ET (Nakajima-Kambe et al. 1999). The mechanism of microbial degradation for PU-ES is thought to be through the hydrolysis of ester bonds by esterase enzymes, which is limited to the surface of the foam due to the inability of enzymatic fusing into the bulk polymer (Howard 2002; Gewert et al. 2015).

4.2.2 Epoxy Resins

Epoxy resins are produced by epoxide ring-opening polymerization reactions typically with nucleophilic amines (Fig. 1). They have application in electrical insulators, paints, structural adhesives, and as primary MPs and potentially NPs in personal care products (Amec Foster Wheeler Environment and Infrastructure UK Limited 2017). Epoxy resins are cross-linked thermosets with high adhesive and mechanical strength. The global demand for these resins is approximately 3.2 million metric tonnes per year (Expert Market Research 2020); smaller in magnitude than other commodity plastics, however, epoxy-derived MPs have been previously detected (Xu et al. 2020). Notably, epoxy resin MPs have been detected in drinking water, which may be linked to its use as a corrosion prevention coating in drinking water treatment plants (Mintenig et al. 2019; Shruti et al. 2020).

The elucidation of degradation routes for epoxy resins is challenged by the high versatility of the structures in use. However, presence of heteroatoms (O, N) generally makes epoxy resins sensitive to photooxidation, although their specific mechanism of degradation depends on the exact chemical structure and curing agents used. In fact, the type of curing agent used in the manufacturing process of epoxy resin impacts its network density (Table S2) affecting degradability. Resins cured with anhydride agents prove more resistant to photo and thermal oxidation than those cured with amine agents (Delor-Jestin et al. 2006). However, anhydride-

cured epoxies have undergone hydrolysis due to the residual anhydride groups present in the polymer matrix (El Yagoubi et al. 2015). For amine-crosslinked epoxies, photoinitiation occurs in the phenoxy portion of the resin and propagation depends on the concentration of amines and the electron density at the nitrogen atom. In the case of diglycidyl ether of bisphenol A cured with diamine-terminated poly (propylene glycol), photodegradation was shown to proceed through chain scission primarily due to the oxidation of the diglycidyl ether of bisphenol A and amine cross-links (Mailhot et al. 2005). The environmental degradation of nitrogen-containing aromatic epoxy resins occurs through hydrolysis and photooxidation reactions which commence on the surface of the resin form amide containing by-products that could further degrade into propenal (Luoma and Rowland 1986). Epoxy resins are considered largely resistant to biodegradation (Wagner et al. 1996) which is attributed to its highly cross-linked nature.

4.2.3 Poly(Styrene-Co-Divinylbenzene)

Poly(styrene-co-divinylbenzene) is synthesized by copolymerizing styrene with divinylbenzene to form a cross-linked material used industrially as ion-exchange resins for water softening and purification techniques (Sidwell and Willoughby 2006). It has a density of 1.0–1.3 g cm^{-3} in its water-swollen state (Mani et al. 2019).

Poly(styrene-co-divinylbenzene) is synthesized as microspheres and is a possible source of primary MPs in the aquatic environment. From samples analysed from the Rhine river, 69% of the micro-sized spherules were identified to be cross-linked PS-divinylbenzene (Mani et al. 2019). Due to the frequent detection of cross-linked PS-divinylbenzene in the Rhine River in Germany, it is expected that other areas in which PS has been identified also have cross-linked PS-divinylbenzene present in significant proportions, urging further investigation into the contribution of this thermoset polymer to MP/NP pollution. It is also worth considering that PS-divinylbenzene may degrade in the environment and lose its characteristic spectral features, rendering it more similar to pure PS spectroscopically, resulting in misidentification of thermoset PS-divinylbenzene as thermoplastic PS (Mani et al. 2019). The abiotic degradation for this polymer is potentially linked to the photo-oxidation of residual monomer or structural impurities as seen in PS and compounded by fouling-originated biodegradation.

4.2.4 Styrene-Butadiene Rubber

Styrene-butadiene rubber (SBR) is a synthetic thermoset elastomer with global production estimated at 7.25 million metric tonnes per year (Il'in and Rezova 2015). SBR has been commonly used as a filler in chewing gum, of which the primary MP content is estimated to be 2.4% by weight (Verschoor et al. 2014). SBR is also cross-linked and vulcanized by various additives for tires production (Siegfried et al. 2017; Eisentraut et al. 2018). The density of SBR is

0.94–1.04 g cm^{-3}, so it is expected to be initially located in the upper water column. Consequent to its widespread use, SBR has been detected as MPs (Eisentraut et al. 2018; Scheurer and Bigalke 2018; Nelms et al. 2018).

SBR degrades primarily through a physical abrasion and thermal oxidative degradation processes in which many oxygenated chemicals are produced as by-products (Guo et al. 2014; Jan Kole et al. 2017; Siegfried et al. 2017; Eisentraut et al. 2018). The shear stress and heat created from the friction of tires on the roads is capable of releasing MPs that have a direct route of entry into the aquatic environment via road runoff (Jan Kole et al. 2017). As a result, the specific concentrations of SBR that enter the aquatic environment depend heavily on the proximal amount and frequency of road run-off events and the density of vehicular traffic (Wagner et al. 2018). The magnitude of research into thermoset MPs that derive from tire wear will surely increase in the near future.

5 Toxicity of Commodity Polymer MPs and NPs

The toxicity of all MPs/NPs depends on their identity and concentration, their shape and size distribution, the presence of any co-contaminants, the characteristics of the exposed organism (e.g. species, life stage) and the testing methods (e.g. acute, chronic). Select determining factors of plastic particle toxicity have been recently summarized by Kögel et al. (2020). It has recently been reviewed that many toxicology studies surrounding MPs and NPs lack quality assurance and quality control (De Ruijter et al. 2020). Furthermore, toxicity assessments for MPs/NPs have been criticized for being conducted at concentrations above those expected in the aquatic environment. In fact, researchers have suggested that the environmental levels of MPs and NPs are not suspected to cause adverse effects except at hotspot locations (Burns and Boxall 2018; Everaert et al. 2018). This prompts the need for toxicology studies at environmentally relevant concentrations in order to draw meaningful conclusions about the true hazards of MPs/NPs. In addition, there is a severe lack of toxicity assessment standardization, with many tests being incomparable in terms of the size, shape and type of the plastic tested. In response to these shortcomings, Koelmans et al. (2020) recently proposed a method of recalibrating species sensitivity distributions (SSD) to correct for incompatible toxicity data. SSDs are cumulative probabilities distributions that incorporate toxicity data from multiple diverse species in order to predict the percentage of species that are likely to be affected by a range of containment concentrations. By recalibrating previously developed SSDs to correct for the variation between MP used in effect studies compared to those found in nature, Koelmans et al. (2020) found 28% of the studied freshwater location (lower limit of the 95% confidence interval) have MP concentrations that would result in exposure risk. The utilization of harmonizing toolkits such as this would increase the reliability of MP and NP risk assessments.

Currently, there are much fewer studies on the toxicology of NPs relative to MPs, which can potentially be attributed to the technical challenges of detecting NPs.

Additionally, many commodity plastics remain understudied. For example, thermosets are severely underrepresented in toxicology studies. Where these studies have been conducted, they largely focus on the toxicity of the leachates from these particulates and not on the thermosetting plastics themselves. Selected toxicological studies pertaining to the MPs/NPs derived from both commodity thermoplastics and thermosets are described below.

5.1 Thermoplastics

5.1.1 Polyethylene

The toxicity of PE-based MPs has been extensively investigated in the aquatic environment. MPs derived from PE have been shown to cause a strong inflammatory response to *Mytilus edulis* (i.e. blue mussels) (Von Moos et al. 2012), as well as immobilization of *Daphnia magna* (i.e. water fleas) (Rehse et al. 2016). Zebrafish (*Danio rerio*) fed with 2 mg l^{-1} of MPs (10–600 μm) derived from PE demonstrated abnormal behaviours such as seizures, and the MPs accumulated in the gill and intestine (Mak et al. 2019). PE-derived MPs (~38 μm) also caused reduced larval survival rates in zebrafish (Malafaia et al. 2020). Virgin PE fragments have been shown to cause stress to fish, however PE fragments containing chemical pollutants sorbed from the marine environment have shown a heightened tendency for toxicity (Rochman et al. 2013). A recent study indicated that NPs generated from PE debris from the North Atlantic gyre produced greater inhibition of algal growth than NPs produced from industrial PE pellets (Baudrimont et al. 2019). Optimistically, not all studies suggest that MPs derived from PE have a significant toxicological effect on aquatic organisms. For example, ingestion of MPs derived from PE did not cause acute toxicity to zooplankton (Beiras et al. 2018). Furthermore, the predicted no effect concentration (i.e. PNEC) for PE-based MPs for toxicity to the freshwater polyp, *Hydra attenuate* was six orders of magnitude above concentrations expected in the aquatic environment (Murphy and Quinn 2018), such as the 2.46 particles per cubic metre detected in the northeast Atlantic (Lusher et al. 2014).

5.1.2 Polypropylene

Studies on the toxicity of PP-derived MP/NP to aquatic organisms are relatively few and do not reflect the worldwide growth in production of PP (PlasticsEurope 2019; Kögel et al. 2020). Nevertheless, MPs from PP have been detected in aquatic regions near coral reefs, where the polyps of scleractinian (stony) corals have ingested these plastic particles (Hall et al. 2015; Reichert et al. 2018). Exposure to PP-based MP fibres has also been shown to cause reduced body mass of Norway lobsters, among other effects on body condition (Welden and Cowie 2016). Leachates from a semi-crystalline PP DVD case subjected to UV-irradiation showed a significant increase

in toxicity to harpacticoid copepods (Bejgarn et al. 2015). Leachates from PP MPs have also been shown to impact the development of embryos of brown mussels (*Perna perna*) (Gandara et al. 2016). Interestingly, the freshwater amphipod *Hyalella azteca* showed greater toxicity from PP-based MP fibres originating from fragmentation of fishing lines, ropes and clothing than it did to PE-based MP particles, which was attributed to the longer residence times of the fibres in the gut (Au et al. 2015). Studies of PP-derived NPs should be a research focus in the future.

5.1.3 Polystyrene

The derivatives PS may be the most studied secondary MP/NP, which is inconsistent with its lower production relative to PE and PP (Kögel et al. 2020). Studies on PS-derived MPs (5 μm and 50 μm) on zebrafish at concentrations at 100 and 1,000 μg L^{-1} demonstrated that these MPs had an impact on the metabolic profiles of these fish and induced microbiota dysbiosis and inflammation in the gut (Jin et al. 2018; Wan et al. 2019). PS-based MPs also accumulated in the gills and intestines of marine Korean rockfish (*Sebastes schlegelii*), reducing growth, energy reserves and nutrient quality for this organism exposed at a concentration of 1×10^6 particles per litre (Yin et al. 2018). Filter feeding animals have been shown to accumulate PS-based MPs, even at low concentrations (Messinetti et al. 2018). In studies with juvenile tunicate (i.e. ascidian) filter feeders and early life stages of sea urchins (i.e. plutei), the larval development of these organisms was impacted by exposure to 10 μm particles at concentrations between 0.125 and 25 μg mL^{-1}. Scallops (*Pecten maximus*) exposed to PS-derived NPs at environmentally relevant concentrations showed rapid accumulation of particles at a rate that was higher for particles of 24 nm in size relative to larger particles of 250 nm. Radiographs illustrated that smaller NPs were distributed throughout the whole body of the scallops, diffusing through tissues, whereas the larger particles were primarily confined to the intestine (Al-Sid-Cheikh et al. 2018).

5.1.4 Polyvinyl Chloride

Although PVC is widely produced, there have been relatively few studies of the toxicity of MPs/NPs derived from this class of polymer. PVC-based MPs have been shown to induce stress in *Sparus aurata* (gilt-head bream), but did not significantly impact the immune system activity (Espinosa et al. 2017, 2018). Virgin MPs did not show significant effects on the white blood cells of *Sparus aurata* or *Dicentrarchus labrax* (European sea bass), although PVC did induce more changes than PE (Espinosa et al. 2018), indicating that the types of polymer is a factor in toxicity. However, ingestion of PVC-based MPs has been shown to impact the intestinal tissue of *Dicentrarchus labrax* (Pedà et al. 2016). *Perna viridis* (Asian green mussel)

exposed to various concentrations (21.6, 216, 2,160 mg L^{-1}) of MPs (1–50 μm) derived from PVCs contaminated with the PAH compound fluoranthene showed a concentration dependent decline in filtration, respiration rates and survival (Rist et al. 2016). Moreover, when fry of *B. gonionotus* (Silver barb) were exposed to virgin PVC fragments (90% of MPs were below 310 μm in size) at concentrations of 0.5 and 1.0 mg l^{-1}, these fish experienced thickening of the mucosal epithelium and increased levels of whole body trypsin and chymotrypsin (Romano et al. 2018). Aged PVC-based MPs have shown a greater toxicological impact than virgin MPs on freshwater algae (*Chlamydomonas reinhardtii*) (Wang et al. 2020). The leachates from PVC have been of specific interest for toxicological purposes, and their effects have been investigated for some aquatic organisms. Leachates from these MPs have been shown to have toxic effects on *Amphibalanus amphitrite* (striped barnacle), *Nitocra spinipes* (harpacticoid copepod) and *Daphnia magna* (Gandara et al. 2016). The phthalates used in PVC have also been studied for their endocrine effects on aquatic organisms and have been shown to impact the development and reproduction of aquatic species (Mathieu-Denoncourt et al. 2015).

5.1.5 Polyethylene Terephthalate

The number of toxicity studies for PET MPs and NPs does not reflect the high production volume of this plastic material that is used in packaging materials. To the best of our knowledge, the first study in the peer-reviewed literature on the effects of PET-based MPs was published in 2016 on the effects of these MPs to *Daphnia magna* at concentrations ranging from 12.5 to 100 mg l^{-1} (Jemec et al. 2016). Results indicated that the organism was capable of ingesting very long PET microfibers (up to 1,400 μm) resulting in increased mortality, although the results varied between different replicates of the experiment which is suspected to be a consequence of the variable sedimentation rates of the utilized MPs. Another recent investigation of the toxicity to the freshwater amphipod, *Gammarus pulex,* found that concentrations ranging from 0.8 to 4,000 particles per mL of PET-based MPs with dimensions of 10–150 μm did not negatively impact the survival or developmental cycle, although the organism readily consumed and accumulated the particles (Weber et al. 2018). One factor limiting toxicological investigations of PET-based NPs in the aquatic environment is the difficulty in acquiring these NPs for research. This problem could be solved by a novel production method for PET nanoparticles which involves dissolving PET debris in a concentrated solution of trifluoroacetic acid and reprecipitating the particles with a diluted trifluoroacetic acid solution (Rodríguez-Hernández et al. 2019).

5.2 *Thermosets*

5.2.1 Polyurethanes

Although MPs from aquatic environments are increasingly being identified as derived from PU, the toxicity of these plastics and of PU-based NPs has not been adequately investigated. However, leachates from PU-based MPs have been shown to cause mortality in *Daphnia magna* and *Nitocra spinipes* (water copepod) (Lithner et al. 2009; Bejgarn et al. 2015). Future research is imperative to investigate the toxicity of PU foams on aquatic organisms based on the size of their degradation products (i.e. MPs vs. NPs) and their composition.

5.2.2 Epoxy Resins

Epoxy resins are a versatile class of chemicals, however, to the best of our knowledge, there has only been one study published in the peer-reviewed literature that has reported the toxicology of epoxy-derived MPs, and this study showed that leachate from epoxy caused toxic effects to *Daphnia magna* (Lithner et al. 2012). Bisphenol A, a common monomer used as a plasticizer in many epoxy resins has shown to be toxic to aquatic life and is a known endocrine disrupting compound (Kang et al. 2007; Huang et al. 2012). The toxicological impacts of epoxy resin MPs/NPs in the aquatic environment should be a research priority.

5.2.3 Poly(Styrene-Co-Divinylbenzene)

Toxicity data on the primary MPs of poly(styrene-co-divinylbenzene) is non-existent in the peer-reviewed literature, to the best of our knowledge. However, aquatic organisms such as sea urchin, sea star, sand dollar, brittle star and sea cucumber larvae have been shown to ingest cross-linked PS-divinylbenzene MPs (10–20 μm) (Wright et al. 2013), which underlines the importance of related studies.

5.2.4 Styrene-Butadiene Rubber

There are no studies in the peer-reviewed literature on the toxicity of MPs/NPs derived from SBR to organisms in the aquatic environment. However, recently reported effects of tire wear particles, suspected to contain mainly SBR, on biota in the aquatic environment indicate their potential for toxicity (Wagner et al. 2018). Future work is required that builds on this study to assess the impact of SBR-derived MPs/NPs on aquatic organisms. This should be of great interest due to the knowledge that tire wear particulates are widespread in the environment (Jan Kole et al. 2017). Furthermore, it is now understood that additives readily migrate from

polymeric tire material and enter the aquatic environment through road runoff (Johannessen et al. 2021a, b). A transformation product (6PPD-quinone) of the tire additive 6PPD has been implicated in causing mass coho salmon mortality and has been since detected in urban watersheds (Tian et al. 2021; Johannessen et al. 2021c). This recent research highlights the need for understanding of the tire additive leaching process from SBR MPs and NPs.

5.2.5 Interactions with Other Contaminants

MPs and NPs have been demonstrated to act as both sources and sinks for environmental contaminants (Wan et al. 2018; Alimi et al. 2018). For example, MPs can act as vectors for metal contaminants in the aquatic environment (Davranche et al. 2019). Copper and zinc readily leach from virgin PS and aged PVC-derived MPs into water (Brennecke et al. 2016). It is of note that the adsorption of the heavy metals was greater in the aged PVC, attributed to the greater polarity associated with the structure of PVC compared to PS and its higher surface area. Despite similar particle sizes, the irregular and rectangular shape of aged-PVC particles also allowed for more adsorption compared to the spherical virgin PS beads. The ability for MPs/NPs to act as vectors for heavy metals is concerning due to associated toxicity. Recently, it was demonstrated that the combination of MPs (largely PE) extracted from popular facial scrubs and cadmium had an integrated toxic effects on common carp (*Cyprinus carpio*) (Banaee et al. 2019). Another recent study also confirmed the synergistic toxicity of metal-polymer MP, where PS-derived NPs in combination with gold ions caused heightened responses in zebrafish embryos compared to pure PS (Lee et al. 2019). In this study, the LC50 of the gold ion dropped from 1.88 μg mL^{-1} to 1.25 μg mL^{-1} when embryos were exposed concurrently with PS. This study also indicated that smaller (50 nm) PS-based NPs resulted in a higher toxicity in association with gold ions than larger (200 and 500 nm) PS-based NPs did. These results illustrate that further investigations are needed with a larger range of heavy metal contaminants, polymer particle size ranges, and plastic compositions for a better understanding of the role of MPs/NPs as vectors for heavy metals.

MPs and NPs have been shown to sorb many organic contaminants (Liu et al. 2016; Alimi et al. 2018; Ferreira et al. 2019). The equilibrium distribution constants for many aquatically available priority organic pollutants favour the adsorption into the plastic by partitioning (Andrady 2011). Polymers with larger amorphous regions are more susceptible to interactions with these organic pollutants than crystalline regions, as the former are more permeable to these compounds. Some MPs/NPs (specifically PS-derived) have been shown to bind very effectively with other hydrophobic compounds such as PAHs and PCBs, which are ubiquitous in many aquatic environments (Velzeboer et al. 2014; Liu et al. 2016). PS-based MPs/NPs have also shown a potential for the sorption of aromatic organic pollutants, stimulated by strong π-π interactions between both compounds (Liu et al. 2016). The interchain steric hindrance due to the bulky phenyl groups account for its amorphous nature and likely increased sorption capacity (Pascall et al. 2005).

Studies have shown that PP-based MPs are also capable of sorbing PCBs in the aquatic environment, and this sorption capacity was shown to be size dependent. The capacity for sorption of PCB congener 77 by PP increased with decreasing particle size and temperature, and PP particles between 0.425–0.85 mm showed a maximum predicted sorption capacity of 350 μg g^{-1} for PCB 77 in simulated seawater (Zhan et al. 2016). PP-based MPs have been shown to have sorption capacity for polybrominated diphenyl ethers (PBDE) that were historically used as flame retardants in commodity plastics (Fig. 1). PP-based MPs showed higher sorption of the PBDE than PE-based MPs, commensurate with the higher surface area (Xu et al. 2019). The capacity of pharmaceuticals and synthetic musks for sorption to PP-based MPs has also been recently investigated and has been demonstrated to increase with decreasing particle size (Zhang et al. 2018; Zhang et al. 2019). Due to the finding that sorption increases with decreasing particle size, it is likely that PP-based NPs will show increased sorption with aquatic contaminants compared to MPs, requiring future studies to confirm this. The size effect of sorption was also investigated for sorption of the PAH, phenanthrene, in PS-based MPs/NPs and the study results indicated that sorption of the organic contaminants increased with decreasing size until a point (~50 nm) after which sorption declined due to aggregation of the NPs (Rios Mendoza et al. 2018).

The polarity of organic contaminants also significantly influences the sorption of contaminants by NPs and therefore potentially, the toxicity. A recent study for PS-based NPs demonstrated that low-polarity contaminants showed significantly enhanced transport facilitated by PS-based NPs relative to polar compounds (Liu et al. 2018). This is explained by the adsorption of nonpolar compounds in the inner matrix of the NPs, which is the hydrocarbon-based amorphous regions of polymer, which enable encapsulation of the contaminants compared to the surface adsorption favoured by polar compounds. A comprehensive comparison of the acute synergistic toxicity of phenanthrene and PS particles of various sizes in *Daphnia magna* clearly showed that larger particles (MPs) were much less toxic than smaller particles (NPs) (Ma et al. 2016). NPs have been observed to pass through the blood-brain barrier, enter cells via endocytosis, and penetrate fish egg chorion (Liu et al. 2016; Lee et al. 2019). These results confirm the need for research on the impacts of NP fragments to aquatic organisms.

6 Detection

The development of methods for the extraction, identification and quantitative of MPs/NPs has proven to be challenging (Koelmans et al. 2015; Andrady 2017). Cost-effective and accurate analytical methods and a unified method of reporting concentrations are crucial for understanding the fate and effects of MPs/NPs in the aquatic environment. Methods have yet to be validated or standardized and are therefore largely in the developmental and experimental stages (Toussaint et al. 2019). There is evidence that current methods underestimate the concentration of MPs in the

aquatic environment by ignoring particles below 300 μm for quantitative evaluations (Conkle et al. 2018).

To date, MPs have largely been assessed by visual examination, although this method is highly critiqued (Renner et al. 2018). The detection and quantification of MPs has been recently reviewed (Renner et al. 2018; Barbosa et al. 2020). Optical spectroscopy, specifically Fourier-Transform Infrared Spectroscopy (FTIR) and Raman spectroscopy, is a frequently used analytical technique for identification and quantification of MPs (Käppler et al. 2016; Kniggendorf et al. 2019; Toussaint et al. 2019). Typically, both techniques have been required to generate a complete profile of MPs in environmental samples (Käppler et al. 2016). However, limitations in spectroscopic validation of MPs may arise from the changes that MPs undergo during degradation when compared to a certified reference material (Lenz et al. 2015).

Numerous studies have confirmed that NPs pose a particular challenge for detection (Peiponen et al. 2019; Ferreira et al. 2019). Where techniques such as FTIR and Raman spectroscopy have proven to be successful for identifying and quantifying MPs, these methods do not possess the resolution required for detecting NPs in environmental samples. The progress and complications surrounding the analysis of environmental NPs have recently been reviewed (Cai et al. 2021). The methods for the detection and quantification of these plastic particles are poised to become more robust and standardized over the coming years.

7 Regulations and International Initiatives

To combat the growing issue of MP and NP pollution, some regulatory jurisdictions have implemented bans on products containing primary MPs. However, comprehensive legislation does not currently exist for other forms of MPs/NPs (Rochman et al. 2019). Regulations controlling the release of small plastic contaminants into the environment have largely been focused on microbeads. In 2018, Canada prohibited creation and distribution of toiletry products containing microbeads through the Microbeads in Toiletries Regulations, extending the prohibition to the sale of natural health products and non-prescription drugs in 2019 (Government of Canada 2017). The USA also adopted similar legislation pertaining to microbeads in toiletry products under The Microbead-Free Waters Act (U.S Food and Drug Administration 2017). The European Union called for a ban on intentionally added MPs in PCPs, cosmetics, detergents and cleaning products by 2020 (European Parliament 2018).

However, reducing the levels of MPs/NPs in the aquatic environment will be largely dependent on regulating the macroplastic waste that is the source of the MPs/NPs. Therefore, legislation must not only target primary MPs/NPs, but also large plastic products such as single-use plastics. A comprehensive review on the regulation of plastic pollution from a circular economy perspective was recently published by Syberg et al. (2021). In 2018, the European Union banned single-use plastics such as plates and cutlery (European Parliament 2018). Some states in the

USA have implemented plastic bag bans, such as California, Hawaii and New York. Canada seeks to ban many single-use plastics (such as some plastic bags, cotton swabs, plates and cutlery) by 2022. Intergovernmental and international organizations also have a role in promoting controls over the release of plastic waste. Goal 14 of the United Nations' Sustainable Development Goals sets targets for conserving and sustaining the aquatic environment. This goal focuses on reducing all forms of marine pollution, including plastic pollution.

In February of 2019, a meeting of the Chief Science Advisors to the G7 Nations was held to discuss MP pollution (Office of the Chief Science Advisor 2019). Following the recommendations from the Science Advice for Policy by European Academies (SAPEA) organization in 2019, the committee recognized the need for comprehensive scientific data and standardized analytical and toxicology methods to inform the development of policy (Science Advice for Policy by European Academies 2019). This confirms the need for further studies and reviews on the topic of MP/NP pollution. Notably, a three-year investigation was initiated in Canada to determine the biological impacts of MPs/NPs in the aquatic environment (Fisheries and Oceans Canada 2018). This study will utilize both *in vitro* and *in vivo* methods to determine the toxic effects of MPs/NPs and the potential adjunct toxicity of chemical contaminants associated with MPs/NPs.

8 Conclusions

The behaviour and fate of MPs and NPs in the aquatic environment depends on the chemical structure, composition, surface area and origin of the polymer from which they are derived. MPs/NPs that are derived from not only commodity thermoplastics such as PE and PS, but also from thermosetting products such as epoxy resins and SBR have been detected in the aquatic environment. The contribution of thermosetting plastics to MP/NP pollution has been largely overlooked, including assessments of the toxicity of these products.

In this review, various properties of commodity thermoplastic and thermosetting polymers such as chemical composition, tacticity, morphology, MW, Đ, density, presence of additives and their fragmentation pathways were discussed. The implications for pollution and associated toxicity in the aquatic environment were described in detail. Importantly, future research priorities related to MPs/NPs derived from thermoset polymers were highlighted. The impact that structural modification of MPs derived from commodity thermoplastics and thermosets has on the fate, toxicity and detection of these products was reviewed comprehensively. Current gaps in methods to detect MPs/NPs were identified providing direction for regulation of plastic pollution. Trends in contaminant sorption related to the chemical structure and particle size of the plastic particulates were also described.

The structure of commodity thermoplastics and thermoset polymers is key to understanding the mechanisms for fragmentation of the polymers into MPs/NPs and their consequent behaviour and fate in the aquatic environment. Studies with

environmentally relevant concentrations using validated analytical techniques that can detect, characterize and quantify these small particles are necessary to assess the true environmental impacts of MPs and NPs.

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Authors' Contributions

Cassandra Johannessen: Writing, editing, data curation, literature research, visualization, chemical structures/schemes/figures.

Dr. Shegufa Shetranjiwalla: Conceptualization, writing, editing, literature research, resources, supervision.

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Prioritization of Pesticides for Assessment of Risk to Aquatic Ecosystems in Canada and Identification of Knowledge Gaps

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Contents

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Abstract Pesticides can enter aquatic environments via direct application, via overspray or drift during application, or by runoff or leaching from fields during rain events, where they can have unintended effects on non-target aquatic biota. As such, Fisheries and Oceans Canada identified a need to prioritize current-use pesticides based on potential risks towards fish, their prey species, and habitats in Canada. A literature review was conducted to: (1) Identify current-use pesticides of concern for Canadian marine and freshwater environments based on use and environmental presence in Canada, (2) Outline current knowledge on the biological effects of the pesticides of concern, and (3) Identify general data gaps specific to biological effects of pesticides on aquatic species. Prioritization was based upon recent sales data, measured concentrations in Canadian aquatic environments between 2000 and 2020, and inherent toxicity as represented by aquatic guideline values. Prioritization identified 55 pesticides for further research nationally. Based on rank, a sub-group of seven were chosen as the top-priority pesticides, including three herbicides (atrazine, diquat, and S-metolachlor), three insecticides (chlorpyrifos, clothianidin, and permethrin), and one fungicide (chlorothalonil). A number of knowledge gaps became apparent through this process, including gaps in our understanding of sub-lethal toxicity, environmental fate, species sensitivity distributions, and/or surface water concentrations for each of the active ingredients reviewed. More generally, we identified a need for more baseline fish and fish habitat data, ongoing environmental monitoring, development of marine and sediment-toxicity benchmarks, improved study design including sufficiently low method detection limits, and collaboration around accessible data reporting and management.

Keywords ECOTOX · Fish · Fungicide · Herbicide · Insecticide · Primary producers

1 Introduction

The very properties of pesticides that make them effective for their registered uses (i.e., those imparting biological activity towards targets) can also pose issues for the wider environment (Johnson et al. 2020). Pesticides can enter aquatic environments via direct application (e.g., for controlling aquatic plants), via overspray or drift during application, or by runoff or leaching from fields during rain events (Breckels and Kilgour 2018; Bartlett et al. 2016; Struger et al. 2016). Registrations for direct use in water tend to be fairly limited, aside from pesticides used specifically for aquaculture, and labels typically instruct the use of buffer zones or other approaches to prevent the entry of pesticides via spray drift, so runoff and leaching poses arguably the biggest challenge for controlling the unintended entry of current-use pesticides into aquatic environments. This is consistent with reported detection rates and concentrations of pesticides that are strongly correlated to season (i.e., application timing), precipitation (i.e., driven by runoff events), pesticide use patterns, and

land use (Fairbairn et al. 2016; Metcalfe et al. 2016; Harris et al. 2008; Baldwin et al. 2016; Rosic et al. 2020; Sanford and Prosser 2020).

Long-term bivalve (Alvarez et al. 2014) and land-locked Arctic char (*Salvelinus alpinus;* Cabrerizo et al. 2018) monitoring studies suggest that concentrations of banned or restricted organic contaminants (e.g., PCBs [polychlorinated biphenyls], organochlorine pesticides) in aquatic environments have decreased over time, resulting in a shift in research focus towards contaminants of emerging concern (CECs), including current-use pesticides. Newer pesticide chemistries tend to be less persistent and bioaccumulative than previous generations of contaminants, as well as less likely to partition into food web-associated lipids (Harris et al. 2008; Alvarez et al. 2014; Daughton and Ternes 1999). For example, in coho salmon (*Oncorhynchus kisutch*) habitat in British Columbia, current-use pesticides were the most prominent active ingredients detected in water and air samples, while sediment and biota samples contained both current-use and legacy pesticides (Harris et al. 2008).

Health Canada's Pest Management Regulatory Agency (PMRA) is responsible for the registration of pesticides in Canada, including the evaluation of potential for human or environmental risks. In order to make (re-)evaluations of the economic benefits and potential environmental and human health risks of particular pesticide ingredients, PMRA requires data pertaining to chemical fate and movement in the environment, toxicity towards non-target receptors, and where available, measured concentrations from environmental media. Although pesticide use data are not collected in Canada, under the *Pest Control Products Act* (S.C. 2002, c. 28; Government of Canada 2002), registrants are required to report annual sales to PMRA (Health Canada 2017, 2020). As such, extrapolations to pesticide use can be made based on the assumptions that all purchased products will be applied in the region in which they were purchased and will be applied within the year of purchase (Government of British Columbia 2015; ECCC 2011).

Over 132 million kilograms of active ingredient (kg a.i.) was sold in Canada in 2017 and over 121 million kilograms in 2018 (the most recent year for which complete data were available at the time of review), comprising >7,400 registered products (Health Canada 2017, 2020) and 658 active ingredients (PMRA 2019a). While sales declined in 2018 relative to the previous year, there was an increasing trend in sales over the preceding 5-year period (Health Canada 2017, 2020). Pesticide use has generally increased over the past 35 years, and this has been attributed to shifts from livestock to food cropping, as well as adoption of no-till or reduced tillage practices, which can increase the need for pesticide use and consequently increase the potential for runoff (Agriculture and Agri-Food Canada 2020; Malaj et al. 2020). The agricultural sector is the greatest user of pesticides (Sheedy et al. 2019; Health Canada 2017) and a relatively small number of active ingredients constitute the majority of pesticides purchased and applied in Canada (Health Canada 2017, 2020; Table S1), though dominant active ingredients can vary by geographical region (Table 1), posing a challenge for determining priorities on a national scale.

As noted by Fairbrother et al. (2019), the identification of research priorities is crucial for government organizations in allocating finite resources in the face of

Table 1 National and provincial pesticide sales for the top ten pesticide active ingredients in each jurisdiction based on the most recently published reports. Note: Manitoba data reflect average use between 1996 and 2006, and Ontario data are use data as opposed to sales

Canada (2017)[a]		Canada (2018)[b]		British Columbia (2015)[c]		Alberta (2013)[d]		Manitoba (1996–2006)[e]		Ontario (2013–2014)[f]		PEI (2014)[g]	
Active ingredient	Kg ai	Active ingredient	Kg ai	Active ingredient	Kg ai	Active ingredient	Kg ai	Active ingredient	Kg ai	Active ingredient	Kg ai	Active ingredient	Kg ai
Glyphosate	>50,000,000	Glyphosate	>25,000,000	Mineral oil	262,513	Glyphosate	8,667,959	Glyphosate	832,651	Glyphosate	2,909,184	Mancozeb	303,957
Available chlorine (as sodium hypochlorite)	>10,000,000	Available chlorine (as sodium hypochlorite)	>10,000,000	Hydrogen peroxide	257,332	MCPA	920,011	MCPA	478,520	Metolachlor	768,804	Chlorothalonil	140,491
Creosote	>5,000,000	Creosote	>5,000,000	Glyphosate	250,505	Glufosinate	694,347	Bromoxynil	191,016	Atrazine	297,603	Mineral oil	140,961
Surfactant blend	>1,000,000	Prothioconazole	>1,000,000	Sulphur	57,875	2,4-D	565,726	Ethalfluralin	169,077	Mancozeb	256,042	Mono and di-potassium phosphite	124,733
Glufosinate ammonium	>500,000	Glufosinate ammonium	>1,000,000	*Bacillus thuringiensis*	48,657	Triallate	367,417	2,4-D	164,929	Chlorothalonil	99,286	Glyphosate	57,696
Borates	>500,000	Bromoxynil	>1,000,000	Diazinon	42,651	Bromoxynil	315,621	Glufosinate ammonium	141,507	Metribuzin	90,922	Metiram	39,535
2,4-D	>500,000	MCPA	>1,000,000	Mineral spirits	40,180	Surfactant blend	299,028	Trifluralin	116,457	Captan	88,851	Phorate	27,557
Mineral oil	>500,000	Surfactant blend	>1,000,000	Chlorothalonil	40,051	Petroleum hydrocarbon blend	215,139	Sethoxydim	96,530	MCPA/MCPB	87,431	Linuron	27,528
Available chlorine (as trichloro-s-triazinetrione)	>500,000	Borates	>1,000,000	Carbon dioxide gas	35,139	Fluroxypyr	156,866	Dichlorprop	91,632	Dimethenamid-P	62,618	Diquat	22,409
Mancozeb	>500,000	2,4-D	>1,000,000	Clodinafop-propargyl	27,687	Methylated canola oil	134,649	Imazamethabenz	68,784	Bromoxynil	60,330	MCPA	18,361
Total herbicides	77,765,728	Total herbicides	66,232,905	Total herbicides	1,175,251	Total herbicides	13,200,340	Total Herbicides	n/a	Total herbicides	4,564,800	Total herbicides	161,568

Total insecticides	4,932,766	Total insecticides	3,836,995	Total insecticides	1,177,716	Total insecticides	200,572	Total insecticides	n/a	Total insecticides	57,500	Total insecticides	190,713
Total fungicides	9,928,052	Total fungicides	13,724, 886	Total fungicides	507,373	Total fungicides	807,883	Total fungicides	n/a	Total fungicides	774,600	Total fungicides	647,644
Total antimicrobials	34,864,449	Total antimicrobials	34,822,207			Total surfactants and adjuvants	1,010,265						
Total vertebrate control	163,405	Total vertebrate control	156,629										
Total other	5,958,314	Total other	3,980,511										

References: [a]Health Canada (2017), [b]Health Canada (2020), [c]Government of British Columbia (2015), [d]Government of Alberta (2015), [e]Wilson (2012), [f]Farm and Food Care Ontario (2015), [g]PEI Environment, Water, and Climate Change (2015)

infinite research questions. With the increasing number of active ingredients registered for use in Canada (PMRA 2017), there is a considerable challenge to balance deepening our understanding of compounds deemed to be of greatest concern with expanding the research to cover a greater proportion of compounds (Johnson et al. 2020).

The specific objectives of this literature review were as follows:

1. To identify those current-use pesticides of potential concern for Canadian marine and freshwater environments;
2. To outline current knowledge on the biological effects with a focus on apical endpoints (i.e., those related to survival, growth, and reproduction), but also with consideration of other sub-lethal effects of the identified pesticides of top concern on aquatic organisms;
3. To identify and review any important data gaps specific to biological effects on aquatic species that became evident during the detailed review of the literature.

Where possible, focus was placed on species that are part of, or contribute to, fisheries, including Indigenous fisheries. Fish are defined broadly under Canada's Fisheries Act (R.S.C., 1985, c, F-14; Government of Canada 1985) including not only fish, but also marine mammals, shellfish, and crustaceans. These species include those accessed as part of fisheries, but also many organisms that act as prey and contribute to habitat structure. Outcomes of this exercise were intended to inform policymakers on current concerns regarding pesticides and Canadian fisheries, and to guide considerations for future research priorities.

2 Methods

2.1 Scope of the Review

The aim of this literature review was to focus on current-use pesticides with the greatest potential to enter aquatic environments via terrestrial runoff and leaching, intentional or incidental overspray, and long-range transport to remote regions (e.g., the Arctic). An emphasis was made on published literature because, although part of the pesticide registration process in most jurisdictions includes submission of data on the fate and effect of pesticides in the environment by the registrant to government regulators, these data are often not publicly available. For all literature searches, emphasis was placed on recent research, particularly peer-reviewed works published since 2016. Active pesticide ingredients detected in the environment but not currently registered for use in Canada by the PMRA (e.g., carbofuran, bendiocarb) were excluded because these are no longer "current-use" and concentrations are expected to continue to decrease over time. Products used as pesticides for which environmental concentrations of their components could not be solely attributed to a pesticide-related application were excluded from this review, including antimicrobials (e.g., available chlorine, hydrogen peroxide), those lacking an explicitly-defined or consistent chemical structure (e.g., surfactant blend, mineral

spirits, mineral oil, creosote, petroleum hydrocarbon blend), and sulphur, carbon dioxide gas, and borates. Drugs used in aquaculture, which are sometimes categorized as pesticides, were additionally deemed out of scope and excluded from the review.

2.2 *Identification of Pesticides of Top Concern for Aquatic Biota*

The first step in determining current-use pesticide active ingredients of top concern for Canadian waters was to construct a long list of candidates based on (1) quantities used and (2) environmental presence of analytes (pesticide active ingredients, their degradates, and/or metabolites). To determine the quantity of use by volume, recent sales numbers were obtained as a proxy, based on the assumption that all products purchased were applied in the same year. Specifically, the top ten active ingredients sold nationally and for each province were determined where sales data were made voluntarily available (see Table 1). To determine environmental concentrations of analytes, a detailed literature search was conducted in February 2020 using various sources including peer-reviewed journal articles, government reports, university theses, and publicly-available databases. Data on concentrations of pesticides in the environment reported in studies were compiled (mean, median, maxima, as available) since availability of raw data tended to be limited and reporting varied among sources (e.g., detection limits, raw data versus summary statistics). Analytes occurring at "high concentrations" in the environment were defined as those within the top ~10% of measured means and/or maximum concentrations of all analytes in the collected dataset. Data were considered from 2000 to the present to account for the cyclical nature of monitoring programs and large number of Canadian water bodies.

The next step in the process was to reduce the long list of candidates and identify those of top priority using a series of criteria: (1) volume of sales in Canada in 2017 or 2018 (as reported by Health Canada 2017, 2020; score based on the greater of the 2 years); (2) mean environmental detection rate, based on available data; (3) mean, 95th percentile, and maximum reported concentration in Canadian surface water between 2000 and present (each as a separate criterion); (4) most conservative Canadian Water Quality Guideline for the Protection of Aquatic Life (CWQG-PAL) (or USEPA guideline, if no CWQG-PAL available); (5) whether the 95th percentile of measured concentrations exceeds the guideline value; (6) ratio of the 95th percentile concentration to the guideline threshold (representative of a hazard ratio), and (7) the registration status in the European Union (according to European Commission 2016). The EU was used as a representative international jurisdiction as their review process tends to be one of the most stringent globally, and represents a large number of member nations (Handford et al. 2015; Bozzini 2017). The USA was not used as there is typically synergy with Canadian regulations. Each active ingredient was then assigned a graded score between 0 and 3 for each of 9 criteria (Table 2). The scores for the guideline value and whether the guideline was exceeded

Table 2 Criteria and scoring matrix for ranking relative priority of pesticide active ingredients, including sales, detection frequency, measured concentrations, inherent toxicity (as indicated by guideline values), and regulatory status in other jurisdictions (i.e., European Union)

Scoring rubric	2017/2018 sales (kg a.i.)	Mean study detection rate (where reported)	Most conservative guideline (µg/L) (weighted x2)	Mean concentration (µg/L)	95th percentile reported concentrations (µg/L)	Max reported concentration (µg/L)	95th percentile exceeded guideline (weighted x2)	Ratio of 95th: guideline	EU Status
3 points	>500,000	≥50%	<1 µg/L	≥1.0 µg/L	≥100 µg/L	>100 µg/L	Yes	>10/ Not monitored	Not approved
2 points	>100,000	<50%/Not monitored	<10 µg/L or none	<1.0 µg/L	<100 µg/L/Not monitored	<100 µg/L/Not monitored	Not monitored	>5	N/A
1 point	>50,000	<10%	<100 µg/L	<0.5 µg/L	<10 µg/L	<10 µg/L	No, but few data	>1	Not approved in EU, but in many European countries
0 points	<50,000	<5%	>100 µg/L	<0.1 µg/L	<1 µg/L	<1 µg/L	No	<1	Approved

were doubled to avoid decisions made with an over-reliance on measured concentrations. As such, a total score of 33 points was possible. A balanced degree of conservatism was imparted by assigning scores of 2 points for situations where data were limited (e.g., compounds not typically monitored or where samples have only been collected in a small number of locations or sampling events). As a final screening step to select the most important top-priority pesticides for detailed review, those active ingredients receiving 23 points or more (averaging over 2/3 on each criterion) were retained as the final list. A state of the science review on biological effects in aquatic biota was conducted for these top-scoring active ingredients, including an assessment of the availability of aquatic toxicity data.

To gather supporting data for this scoring process and state of the science review, peer-reviewed literature key word and Boolean searches were made using the Web of Science and the following keywords: "pesticide*", "herbicide*", "insecticide*", "fungicide*", "aquatic", "freshwater", "marine", "ecosystem", "Arctic", "remote", "Canada", "fish", "invertebrate", "algae", "toxic*", "effect*", and "emerg*". From the search results, abstracts were reviewed as a screening step to deem the article of potential relevance or not based on the objectives of determining environmental presence and effects in aquatic biota. Articles were then read in full, and reference lists reviewed for further useful publications. Additional searches were also made on Government of Canada websites (PMRA, Department of Fisheries and Oceans – DFO, Northern Contaminants Program – NCP, Environment and Climate Change Canada – ECCC) and provincial/territorial government websites. Data were queried from the ECCC National Long-term Water Quality Monitoring database (Government of Canada 2016) to identify pesticides that have been measured/detected by federal programs and those that are not currently monitored in Canadian waters.

To evaluate the current availability of aquatic toxicity data for the final list of top-priority pesticide active ingredients for detailed review, the United States Environmental Protection Agency ECOTOX database (USEPA 2020a) was queried for all effects, all endpoints, for algae, crustaceans, molluscs, insects, other invertebrates, and fish, for all aquatic-only exposures. For quality assurance, data were retained only for studies where concentrations of active ingredient had been measured, as recommended by Hanson et al. (2019a) and Van Der Kraak et al. (2014) in their evaluations of strength and relevance of atrazine studies. Further searches of USEPA and PMRA regulatory documents plus within Web of Science and Google Scholar were performed for each active ingredient in an effort to find any studies that had not been entered into ECOTOX as of March 31, 2020.

The intention of this evaluation approach was to cast a wide net of active ingredients and identify those that are relatively likely to be found in Canadian surface waters (and above guideline values), those that are inherently toxic to aquatic organisms, and those that have been identified in other jurisdictions as posing a potential risk. This approach was consistent with previous work conducted to identify priority pesticides in other countries (Australia: Rosic et al. 2020; UK: Johnson et al. 2020).

It is important to recognize that the prioritization process employed in this study was simple and conservative, as the objective was to prioritize, from a relatively long

list, the pesticides of potential concern for Canadian marine and freshwater environments. This simplistic and conservative approach was necessitated by having limited access to all of the raw data on exposure and effect that have been generated for each pesticide. It is also important to recognize the limitations in each of the criterion used in this prioritization exercise. The quantity of an active ingredient sold in a particular year does not directly relate to the quantity entering aquatic ecosystems and the toxicity of the active ingredient. The criterion related to the measurement of the active ingredient in aquatic ecosystems does bias the prioritization towards active ingredients that are included in monitoring efforts and are appreciably soluble in water. The use of the maximum reported concentration in the environment can give weight to measurements that could be considered outliers. For this reason, the mean and 95th percentile of measured concentrations were also included as criteria. The use of detection of frequency is also problematic because the significance of this criterion is dependent on the temporal and spatial extent of sampling. It is also important to recognize that relatively high concentrations measured in aquatic ecosystems do not justify an active ingredient being considered a priority. Those measured concentrations need to be related to a threshold of toxicity. For this reason, the criterion of the ratio of 95th centile of measured concentrations to the most conservative water quality guideline was included. This criterion relates the level of exposure to the potential level of effect. Consequently, this criterion was also given twice as much weight as the other criteria (Table 2). While each criterion has limitations when used in isolation, the combination of the criteria in priority setting should limit the influence of the bias present in any one criterion (Egeghy et al. 2011; Salvito et al. 2002).

3 Pesticides in the Canadian Aquatic Environment

3.1 National Results and Regional Contexts

In the course of the literature review, increased use of pesticides generally indicated that pressures on water quality would also be expected to increase. The ECCC Water Quality in Canadian Rivers sustainability indicator concluded that water quality was generally lower in areas with high populations and agriculture or forestry pressures (ECCC 2020). A related indicator, the Indicator of the Risk of Water Contamination by Pesticides, declined from 2006 to 2011 as a result of increased pesticide use, indicating increased risk of contamination (Agriculture and Agri-Food Canada 2020). Based on a recent risk assessment for pesticide use in Ontario, adoption of newer chemical formulations has generally resulted in growers applying greater amounts of less hazardous active ingredients because they are also less potent towards targets (Van Eerd 2016). Likewise, pesticide use intensity (kg applied per area of cropland) increased in Alberta from 0.76 kg/ha in 1988 to 1.33 kg/ha in 2013 (Government of Alberta 2015).

The most recent Government of Quebec pesticide sales report suggested that of all pesticide types, herbicides pose the greatest current risk to the environment in that province, consistent with herbicides comprising 69% of active ingredients purchased for agricultural use (Government of Québec 2011, 2017). The five active ingredients deemed to pose the greatest environmental risks in Quebec were atrazine, chlorpyrifos, S-metolachlor, imazethapyr, and chlorimuron-ethyl based on the Quebec pesticide risk indicator, which integrates data related to fate, behaviour, toxicity, and usage patterns (Government of Québec 2017). It was noted in the Quebec Pesticide Strategy 2015–2018 that atrazine and chlorpyrifos accounted for <5% of sales but 20% of environmental risk indicators for the province (Government of Québec 2015).

In Ontario and Quebec, S-metolachlor has been identified as one of the pesticides of greatest risk to environmental receptors in those regions (Van Eerd 2016; Government of Québec 2017; Corsi et al. 2019). The risk assessment by Van Eerd (2016) also identified dimethenamid-P, chlorothalonil, and metribuzin as top active ingredients of potential concern for the environment in Ontario based on Environmental Impact Quotients (EIQs) calculated using the method developed by Kovach et al. (1992). This approach integrates toxicity data for human health, fish, birds, bees, and arthropods, as well as environmental fate data to determine the EIQs, which are then multiplied by application rate (kg a.i.) to determine risk (Van Eerd 2016). Chlorothalonil is applied in large volumes across Canada (>500,000 kg a.i. in 2017 and 2020; Health Canada 2017, 2020) and in Ontario (fifth largest volume pesticide applied in 2013–2014; Farm and Food Care Ontario 2015), and has relatively low toxicity benchmarks for sensitive non-target organisms (Van Eerd 2016). In national surface water monitoring, chlorothalonil was among the top five active ingredients most likely to exceed CCME guideline values, particularly in British Columbia and the Atlantic region (ECCC 2011).

The intensity of pesticide use in farming on Prince Edward Island (PEI) and in the Lower Fraser Valley, British Columbia, is high due to the climates and type of crops grown and as a result, most local water bodies are susceptible to contamination by pesticides (ECCC 2011). Specifically, ECCC (2011) noted that approximately 20% of the land area in PEI is involved in potato production (relatively high pesticide-intensity required compared to other crops under local conditions), and over 40% of pesticides purchased in British Columbia are applied in the Lower Fraser Valley. Likewise, greater than two million kg a.i, were sold in 2013 in the North Saskatchewan River, Battle River, and Red Deer River basins in Alberta, with between 1.5 and two million kg a.i. sold in both the Oldman River and Peace River basins (Government of Alberta 2015). In a survey of southern Alberta watersheds, the most frequently detected pesticides were those that had the highest sales, the greatest solubility in water, and relative stability in the environment; these were namely auxin mimics (2,4-D, dicamba, MCPA, mecoprop, fluroxypyr, and clopyralid; Sheedy et al. 2019). As such, monitoring of fish-bearing habitats for the most commonly purchased pesticides (by sales volume) would be prudent in those regions, especially if species at risk are present.

3.2 Top-Priority Active Ingredients

As noted by Malaj et al. (2019) and Anderson et al. (2015), the lack of centralized tracking of pesticide use and presence in the aquatic environment in Canada presents a challenge in assessing their potential environmental risks. Concentration data were obtained from government databases, provincial and national reports, and peer-reviewed papers for pesticides measured in Canadian waters from 2000 to the present. The details for each of the references used including which pesticides were measured, the sampling location, and in what type of water body were tabulated (Table S2). Contemporary sales information was not available for several provinces, but recent annual reports (within the past 5 years) were obtained for Ontario, Quebec, Prince Edward Island, Alberta, and British Columbia, and average use data were located for Manitoba between 1996 and 2006. The active ingredients constituting the greatest sales by volume for each province are shown in Table 1, along with the national sales data (Health Canada 2017, 2020). Of the top ten active ingredients sold in Canada annually between 2013 and 2018 (i.e., previous three sales reports), six have remained consistent – glyphosate, available chlorine (as sodium hypochlorite), creosote, 2,4-D, surfactant blend, and glufosinate ammonium (Health Canada 2017, 2020; Table 1, Table S1). Several of these were not considered further in the prioritization process as they contained multiple components whose presence in the aquatic environment may not be confidently attributed to pesticide application (i.e., mineral oil, surfactant blend, creosote, and available chlorine).

The environmental concentrations representing the top ~10% of pesticides in Canadian waters were identified from water quality monitoring data as $\geq$0.5 μg/L or 1.0 μg/L for mean and maximum concentrations, respectively. Using the inclusion data described in Sects. 2.1 and 2.2 (i.e., sales data and measured concentrations), a total of 55 pesticide active ingredients were screened into the initial long list for further consideration as potential priorities for Canadian fisheries. Of these, 8 achieved a score of 23 or greater, based on some combination of large sales volumes, frequent detection, low toxicity thresholds, measured concentrations exceeding the most sensitive guideline, and registration status in the EU. These formed a final short list of top-priority pesticides for aquatic environments in Canada. Diazinon was removed from the final list by professional judgement because sales declined sharply from 2017 to 2018 (<50,000 kg a.i. and <5,000 kg a.i., respectively) in response to recent registration reassessment (Health Canada 2017, 2020). Several previous label uses of diazinon were phased out between 2013 and 2016 (PMRA 2013), and reduced concentrations in water would be expected as a result, as was observed in the U.S. following additional label restrictions (USDA 2018 and references therein).

As such, the final list of top-priority active ingredients included a total of seven pesticides: three herbicides (atrazine, diquat, and S-metolachlor), three insecticides (chlorpyrifos, clothianidin, and permethrin), and one fungicide (chlorothalonil). These active ingredients will be reviewed in more detail in the sections below.

Ranked lower than the final list of top-priority active ingredients, were two tiers of active ingredients with scores slightly below the cut-off of 23. Carbaryl, imidacloprid, malathion, mancozeb, metiram, prothioconazole, thiamethoxam, trifluralin, and 2,4-D received scores between 20 and 22 (Table 3). Clodinafop-propargyl, deltamethrin, metribuzin, pyraclostrobin, and sethoxydim received scores between 18 and 19 (Table 3). These active ingredients are worth mentioning as a number of them (e.g., 2,4-D, mancozeb, prothioconazole) are widely used in Canada (Table 1) or are closely related to active ingredients in the top-priority list. For example, thiamethoxam was not in the top-priority list with a score of 22 but it is metabolized into clothianidin (Nauen et al. 2003), which did make the top-priority list. Consequently, consideration should also be given to the risk that these active ingredients with relatively high priority scores could pose to aquatic ecosystems.

3.3 Review of Top-Priority Active Ingredients

Overall, the seven top-priority active ingredients from the current review ranged from practically non-toxic (LC50 >100,000 μg/L) to very highly toxic (LC50 <100 μg/L) towards fish, aquatic invertebrates, and aquatic primary producers under acute exposure scenarios (categories determined according to USEPA 2017a). In terms of data availability on ECOTOX or in the published literature, datasets were typically more complete for freshwater than marine species, though there was considerable variation between active ingredients (Table S3). Generally, those compounds ranking highest in priority had broader data coverage. For example, atrazine had at least one toxicity endpoint from a study with measured exposure concentrations for nearly all acute, chronic, growth/development, and reproduction/population abundance study classes outlined in Table S3. Acute and chronic survival data were available for most active ingredients, but data were sparser for other apical endpoints (i.e., growth, development, and reproduction). Molluscs generally represented the least-studied class of organism, while fish, primary producer, and crustacean datasets were complete for all seven active ingredients for acute exposures. Whiteside et al. (2008) similarly noted the greater availability of fish and crustacean data for agricultural active ingredients registered in Canada compared to other taxa. For some active ingredients, PMRA and/or USEPA explicitly noted that additional data were required to support regulatory risk assessment (Table S3).

3.3.1 Herbicides

Atrazine

Atrazine is a broad-spectrum herbicide commonly used to control weeds, particularly in corn crops, as well as sorghum, sugarcane, and fallow, and for non-agricultural applications such as sod and Christmas tree farms (USEPA 2016).

Table 3 Outcome of the ranking exercise to determine relative priority of pesticide active ingredients, including sales, detection frequency, measured concentrations, inherent toxicity (as indicated by guideline values), and regulatory status in other jurisdictions (i.e., European Union). The list of 55 candidate compounds was derived based on relative use volume and/or measured concentrations in Canadian surface waters between 2000 and 2020. Active ingredients are listed in order from highest score to least based on an assigned score between 0 (white) and 3 (darkest shade) for each criterion (details provided in the notes and scoring rubric below). Active ingredients scoring of 23 or greater are indicated in green shading and were selected for detailed review ($n = 7$)

Active ingredient	Type	2017 sales (kg a.i.)	2018 sales (kg a.i.)	Mean study detection rate (where reported)	Most conservative guideline (µg/L) (x2)[c]	Mean concentration (µg/L)	95th concentration (µg/L)	Max reported concentration (µg/L)	95th exceeds guideline (x2)[c]	Ratio of 95th: guideline	EU status	Score (/33)	Reviewed
Atrazine	Herbicide	>500,000	>500,000	65%	1.8	2.0	9.7	62.0	Yes	5.4	Not approved	27	✓
Chlorothalonil	Fungicide	>1,000,000	>500,000	19%	0.18	0.56	3.7	7.83	Yes	20.4	Not approved	27	✓
Diazinon[b]	Insecticide	<50,000	>1,000	10%	0.08	1.3	8.3	12.5	Yes	104	Not approved	26	
Clothianidin	Insecticide	>100,000	>25,000	80%	0.05	0.16	0.51	3.1	Yes	10.3	Not approved	25	✓
Chlorpyrifos	Insecticide	>100,000	>100,000	28%	0.002	0.21	0.60	4.00	Yes	301	Not approved	24	✓
Diquat	Herbicide	>500,000	>500,000	Not monitored	0.75	Not monitored	Not monitored	Not monitored	Not monitored	Not monitored	Not approved in EU, but approved in many European countries	24	✓
Permethrin	Insecticide	>50,000	>25,000	LOD>guideline[d]	0.004	0.14	0.83	1.1	Yes	208	Not approved	24	✓
S-metolachlor	Herbicide	>500,000	>1,000,000	61%	7.8	2.1	9.9	41	Yes	1.3	Approved in S-metolachlor form; metolachlor not approved	24	✓
Carbaryl	Insecticide	<50,000	>25,000	12%	0.2	0.45	1.8	2.90	Yes	8.95	Not approved	22	
Imidacloprid	Insecticide	<50,000	>100,000	37%	0.23	0.38	2.2	10.4	Yes	9.7	Approved	22	
Thiamethoxam	Insecticide	>100,000	>100,000	72%	0.74	0.32	1.4	4.5	Yes	1.9	Not approved in EU, but approved in many European countries	22	
2,4-D	Herbicide	>500,000	>1,000,000	53%	4	0.81	4.2	8.2	Yes	1.1	Approved	21	
Mancozeb	Fungicide	>1,000,000	>1,000,000	Not monitored	1.35	Not monitored	Not monitored	Not monitored	Not monitored	Not monitored	Approved	21	
Prothioconazole	Fungicide	>500,000	>1,000,000	Not monitored	None	Not monitored	Not monitored	Not monitored	Not monitored	Not monitored	Approved	21	
Trifluralin	Herbicide	>500,000	>100,000	8%	0.2	0.07	0.24	1.2	Yes	1.2	Not approved	21	
Malathion	Insecticide	>100,000	>25,000	2%	0.049	0.44	1.4	5.5	Yes	28	Approved	20	
Metiram	Fungicide	>100,000	>100,000	Not monitored	1	Not monitored	Not monitored	Not monitored	Not monitored	Not monitored	Approved	20	
Deltamethrin[a]	Insecticide	<50,000	>25,000	LOD>guideline[d]	0.0004	0.10	0.10	0.10	Yes	250	Approved	19	
Metribuzin	Herbicide	>100,000	>100,000	15%	1	0.50	2.9	7.1	Yes	2.9	Approved	19	
Pyraclostrobin	Fungicide	>100,000	>100,000	52%	1.5	0.38	1.79	2.31	Yes	1.2	Approved	19	
Clodinafop-propargyl	Herbicide	>100,000	>100,000	Not monitored	14	Not monitored	Not monitored	Not monitored	Not monitored	Not monitored	Approved	18	
Sethoxydim	Herbicide	>50,000	>100,000	Not monitored	210	Not monitored	Not monitored	Not monitored	Not monitored	Not monitored	Not approved	18	
Dimethenamid-P	Herbicide	>100,000	>100,000	32%	5.6	7.0	4.8	130	No	0.86	Approved in dimethenamid-P form; dimethenamid not approved	16	
Phorate	Insecticide	<50,000	>10,000	3%	0.03	0.02	0.06	0.06	Yes	2.0	Not approved	16	
Bentazon	Herbicide	>500,000	>500,000	60%	510	5.3	16.9	34.0	No, but few sampling locations	0.03	Approved	15	
Dicamba	Herbicide	>100,000	>100,000	58%	10	3.3	5.0	105	No	0.50	Approved	14	
Diuron	Herbicide	<50,000	>5,000	1%	1.6	0.45	2.9	2.86	Yes	1.8	Approved	14	
Glyphosate	Herbicide	>50,000,000	>25,000,000	75%	800	7.30	22.5	140	No	0.03	Approved	14	

Imazethapyr	Herbicide	>100,000	>100,000	59%	8.1	0.26	0.96	1.9	No	0.12	Not approved	**14**	
EPTC	Herbicide	<50,000	>25,000	6%	39	2.31	15.9	69	No	0.41	Not approved	**13**	
Linuron	Herbicide	>100,000	>100,000	7%	7	0.32	1.3	1.7	No	0.18	Not approved	**13**	
MCPA	Herbicide	>1,000,000	>1,000,000	34%	2.6	0.51	2.5	4.9	No	0.94	Approved	**13**	
Mecoprop	Herbicide	>100,000	>100,000	42%	13	3.0	3.0	103	No	0.23	Approved	**13**	
Dimethoate	Insecticide	>100,000	>25,000	6%	6.2	0.78	4.1	8.50	No	0.66	Not approved in EU, but approved in many European countries	**12**	
Ethalfluralin	Herbicide	>500,000	>500,000	0%	0.4	0.01	0.02	0.02	No	<1	Not approved	**12**	
N-N-diethyl-meta-toluamide (DEET)	Insecticide	>100,000	>100,000	60%[d]	37500	1.5	4.5	5.1	No, but few data	0.0001	Approved	**12**	
Boscalid	Fungicide	>100,000	>100,000	33%[d]	116	1.4	9.7	14.2	No	0.08	Approved	**11**	
Flumetsulam	Herbicide	<50,000	>1,000	30%	3.1	0.18	0.64	1.14	No	0.21	Not approved	**11**	
Bromoxynil	Herbicide	>1,000,000	>1,000,000	16%	5	0.15	0.69	0.95	No	0.14	Approved	**10**	
Simazine	Herbicide	<50,000	>10,000	17%	10	0.35	2.8	3.7	No	0.28	Not approved	**10**	
Captan	Fungicide	>100,000	>100,000	5%[d]	1.3	0.03	0.06	0.06	No, but few data	0.05	Approved	**9**	
Glufosinate ammonium	Herbicide	>1,000,000	>1,000,000	2%	72	0.30	1.5	5.60	No	0.02	Not approved in EU, but approved in many European countries	**9**	
Metalaxyl	Fungicide	>50000	>100,000	27%	374.0	0.51	1.3	10.3	No	0.003	Approved	**9**	
Fluroxypyr	Herbicide	>500,000	>500,000	11%[d]	7150	0.05	0.06	0.06	No, but few data	0.00001	Approved	**8**	
Triallate	Herbicide	>100,000	>100,000	1%	0.24	0.01	0.02	0.05	No	0.10	Approved	**8**	
Clopyralid	Herbicide	<50,000	>100,000	25%	6900	0.22	1.1	2.3	No	0.0002	Approved	**7**	
Tebuconazole	Fungicide	>100,000	>1,000,000	29%	11	0.04	0.27	0.31	No	0.02	Approved	**7**	
Flonicamid	Insecticide	<50,000	>500	23%	3000	0.56	3.2	5.9	No	0.001	Approved	**6**	
MCPB	Herbicide	<50,000	>5,000	2%	7.3	0.20	0.77	3.1	No	0.10	Approved	**6**	
Clomazone	Herbicide	>50,000	>25,000	4%[d]	167	0.15	0.51	0.51	No, but few data	0.003	Approved	**5**	
Myclobutanil	Fungicide	<50,000	<500	7%	11	0.39	0.29	7.5	No	0.03	Approved	**5**	
Dichloroprop (2,4-DP)	Herbicide	<50,000	>10,000	13%	40	0.05	0.11	0.81	No	0.00	Approved	**4**	
Imazamethabenz	Herbicide	<50,000	>25,000	6%	5000	0.01	0.02	0.13	No	0.00	Not approved	**4**	
Picloram	Herbicide	<50,000	>10,000	2%	29	0.14	0.34	1.55	No	0.01	Approved	**4**	
Nicosulfuron	Herbicide	<50,000	<500	36%	43000	0.15	0.54	0.56	No	0.00001	Approved	**3**	

Notes: Sales scores were based on the greater sales volume of 2017 or 2018 (Health Canada 2017; Health Canada 2020)

[a]Deltamethrin was included in the long list by professional judgement based on the very low CCME value, which was exceeded in monitoring data, despite not meeting the criteria for top compound and/or mean concentration $\geq$0.5 µg/L and/or maximum concentration $\geq$1.0 µg/L

[b]Diazinon was removed from the top-priority list by professional judgement based on phasing out of uses by PMRA in 2013 and 2016 (PMRA 2013), and sales <5,000 kg a.i. in 2018 reflecting those usage changes

[c]A double weighting was applied to two criteria to avoid excessive reliance on measured environmental concentrations, particularly in cases where data were not available, but rather to consider inherent toxicity and the context of the guideline as more than merely presence is required to indicate a potential issue

[d]Denotes a compound for which few data were available for comparison to a specific criterion or for which sampling had a very limited geographic coverage. As such, these scores were bumped up to the next level to impart conservatism. e.g., mean detection rate for boscalid was based on data from only two provinces, so the score of 2 for 33% was upgraded to a 3 to account for uncertainty

Atrazine acts by binding to the plant-specific plastoquinone-binding protein in photosystem II, resulting in oxidative damage and cell plant death via starvation (Zhu et al. 2009). It is among the most well-studied current-use pesticides, with nearly 2000 records in the ECOTOX database for studies with analytical confirmation (USEPA 2020a), and has recently been reviewed by de Albuquerque et al. (2020).

Presence in the Aquatic Environment

Atrazine is frequently detected in Canadian freshwater samples (Table 3), particularly in Ontario and Quebec where it is one of the most commonly purchased pesticides (Table 1). In a 2010–2013 study in Great Lakes tributaries, atrazine was the pesticide most commonly measured above water quality guidelines, with overall detection rates of 30% and maximum concentration of 40 μg/L (Baldwin et al. 2016). In another study, atrazine was detected in all monitored sites in watersheds and receiving waters of Lake Ontario (Metcalfe et al. 2016), as well as frequently in studies in the Niagara Region (Bartlett et al. 2016), and in Quebec rivers (Giroux 2010, 2015; Giroux and Pelletier 2012) and rural drinking water (Husk et al. 2019). Concentrations of atrazine up to 0.52 μg/L were reported in the lower Red River, Manitoba, in 2014–2015, with a general increase in measured mean, median, and maximum concentrations compared to concentrations measured in a 1993–1995 study (Challis et al. 2018). Desethylatrazine, a metabolite of atrazine, was one of the most frequently detected pesticide analytes in a surface water study along the St. Lawrence River and its tributaries (Montier-León et al. 2019).

Toxicity Towards Aquatic Organisms

Atrazine is classified as moderately toxic towards fish, highly toxic towards freshwater aquatic invertebrates, and very highly toxic towards marine aquatic invertebrates based on acute toxicity data deemed appropriate for risk assessment (USEPA 2016, 2017a). Recent weight-of-evidence reviews have been conducted with regard to effects in fish, amphibians, and reptiles (Van Der Kraak et al. 2014; Hanson et al. 2019a), aquatic plant communities (Moore et al. 2017), and periphyton, phytoplankton, and macrophytes (Hanson et al. 2019b). It was noted by Hanson et al. (2019b) that there were insufficient marine studies on primary producers of sufficient quality for risk assessment, so there might yet be gaps in our understanding of atrazine in the environment, albeit fewer than some other less-studied pesticides. An extensive discussion of toxicity data available from the open literature is provided by USEPA (2016) as part of its recent ecological risk assessment of atrazine, with evaluation of study quality.

The exercise by Moore et al. (2017) compared four methods for establishing a protective level of concern (LOC) for aquatic plant communities against which USEPA could compare monitoring data as part of the risk assessment process. Based on mesocosm, microcosm, and individual species data for 26 primary producers, 60-day LOCs ranged from 19.6 to 26 μg/L. Using a weight-of-evidence

approach, the authors concluded that the most statistically reliable method resulted in a weighted LOC of 23.6 µg/L. Below this concentration, atrazine would not be expected to cause significant adverse effects in aquatic plant assemblages (Moore et al. 2017). Two high quality studies on freshwater primary producers were evaluated by Hanson et al. (2019b) that were not captured in the Moore et al. (2017) weight-of-evidence. Knežević et al. (2016) reported 7–12-days EC50 concentrations ranging from 100.9 to >1,280 µg/L for frond weight and number in the duckweed (*Lemna minor*) and Baxter et al. (2016) reported 96-h EC50s of 87.6 µg/L and 41.9 µg/L for phytoplankton growth and photosystem II yield, respectively. The reported EC50 values were >23.6 µg/L, consistent with the conclusions from Moore et al. (2017).

The risk assessment endpoints used for freshwater invertebrates by USEPA (2016) were an acute LC50 of 720 µg a.i./L for the midge *Chironomus dilutus* (formerly *Chironomus tentans*) and a chronic lowest observed adverse effect concentration (LOAEC) of 140 µg a.i./L for second generation growth in the shrimp, *Gammarus fasciatus*. For estuarine and marine invertebrates, the most sensitive endpoints were an acute LC50 of 48 µg a.i./L and a chronic no observable effect concentration (NOAEC) of 3.8 µg a.i./L for opossum shrimp (*Neonmysis integer*; USEPA 2016). More recent studies in aquatic invertebrates (crustaceans, insects, and molluscs) reported by de Albuquerque et al. (2020) and Brain et al. (2021) generally indicate biochemical effects (e.g., antioxidant biomarker activity, DNA damage) could occur at environmentally relevant concentrations (<100 µg/L), with changes to growth, reproduction, or community endpoints at concentrations greater than would be expected under typical atrazine use.

With respect to fish, acute toxicity is low and studies selected for the USEPA Ecological Risk Assessment reported LC50 (concentration resulting in 50% mortality) values of 5,300 µg a.i./L for rainbow trout (*Oncorhynchus mykiss)* and 2,000 µg a.i./L for sheepshead minnow (*Cyprinodon variegatus*) (USEPA 2016). Based on over 1,290 data points, Van Der Kraak et al. (2014) concluded that at environmentally relevant concentrations (defined as 100 µg/L or less), atrazine and its metabolites can cause significant changes in gene expression, biochemical endpoints (e.g., induction of detoxification enzymes), or concentrations of hormones in fish, amphibians, and reptiles, but that these did not translate into adverse outcomes at higher levels of biological organization including those that might impact population stability (i.e., mortality, fecundity) and community-level effects. Additional weight-of-evidence evaluation from Hanson et al. (2019a) supported these conclusions for fish, amphibians, and reptiles. Likewise, studies with fathead minnow (*Pimephales promelas*) and Japanese medaka (*Oryzias latipes*) reported no adverse effects on reproduction at concentrations up to 105 µg/L and 244 µg/L, respectively (Brain et al. 2018), and a life-cycle assessment with fathead minnow found no significant effects on growth, survival, or reproduction at concentrations up to 150 µg/L (Dionne et al. 2021).

The high solubility and low octanol-water partitioning coefficient (log K_{ow} of 2.70, Table 4) of atrazine suggest a low potential for bioaccumulation. Studies in

Table 4 Physical and chemical properties of current-use pesticides deemed to be of top tier concern ($n = 7$) for Canadian aquatic environments and fisheries based on the scoring criteria outlined in Table 2

Pesticide	Group[a]	Mechanism/ mode of action	Crops/uses	Log K_{ow}	Log K_{oc}	Degradates, metabolites	Solubility	Half-life in water (days)	References
Herbicides									
Atrazine	Triazines, Tetrazines	PSII inhibitor	Corn, sorghum, fallow, turf/control broadleaf and grassy weeds	2.70	1.88	DIA, DEA, DACT/DDA, OIET, OIAT, OEAT	33 mg/L	38–155	USEPA (2016)
Diquat	Quaternary ammoniums	Formation of peroxide and free radicals	General weed control, aquatic plant control, dessication of seed, fruit, vegetable, and ornamental crops	−4.6	2.84–6.90	None	700,000 mg/L	74	USEPA (2015a); Roede and Miller (2014)
S-Metolachlor	Anilides/ anilines	Inhibition of cell division	Corn, soybeans, ornamental crops/pre-plant, pre-emergence, or early post-plant control of grasses and broadleaf weeds	3.05	1.33–2.57	CGA-354743, CGA-51202, CGA-40172, CGA-41507, CGA-351916	530 mg/L	33–150	USEPA (2019b)
Insecticides									
Chlorpyrifos	Thiophosphates	AChE inhibitor	Grains, corn, fruit and vegetable crops, ornamentals/control insects and mites	5.0	1.61–4.72	Chlorpyrifos-oxon, TCP	0.73 mg/L	30–50	CCME (2008); Giesy and Solomon (2014)
Clothianidin	Guanidines	Nicotinic ACh receptor binding	Corn, cereal crops/control of piercing sucking pests, coleopteran pests, others	0.7–1.12	1.78–2.54	MNG, TMG, TZNG, TZMU	327 mg/L	10–158	PMRA (2018a); USEPA (2017a, 2020b)

Permethrin	Pyrethroids	Nerve axon Na+ channel modulation	Control insects on agricultural crops, public health mosquito control	6.1	4.45–5.69	trans-DCVA, 3-phenoxybenzoic acid, phenoxybenzyl alcohol, phenoxybenzoic aldehyde, cis-DCVA	0.006–0.2 mg/L	38–175	USEPA (2007); PMRA (2017)
Fungicides									
Chlorothalonil	Benzonitriles	Unknown; inhibits spore formation by binding with glutathione	Food crops, feed crops, greenhouse crops, industrial preservative/ fungicide, antimicrobial	2.88–3.8	3.05–4.08	SDS-3701, SDS-46851, SDS-47523, R417888, R471811, R419492, SDS-67042, SDS-66382, others	0.8–0.96 mg/L	<1–21.5	USEPA (2012); PMRA (2011)

[a]Group information derived from Health Canada (2017)

bluegill sunfish (*Lepomis macrochirus*) have reported maximum bioconcentration factors of 7.7–15, and >70% depuration after 21 days (USEPA 2016).

The current CWQG-PAL for atrazine in freshwater is 1.8 μg/L; no marine guideline has been recommended (CCME 1999a). It was derived based on the most sensitive plant-based maximum allowable toxicant concentration (MATC) value of 17.6 μg/L divided by a safety factor of 10 (CCME 1999a). An aquatic plant community Concentration Equivalent Level of Concern was established by USEPA to be 3.4 μg a.i./L. Above this level, changes in productivity, structure, and/or function of aquatic plant communities could be expected (USEPA 2016).

Exposure Risks for Aquatic Organisms

Over 500,000 kg a.i. of atrazine was purchased in each of 2017 and 2018 in Canada (Health Canada 2017, 2020). Corsi et al. (2019) concluded that atrazine was among the priority chemicals of ecological concern for the Great Lakes region. Atrazine is both persistent and mobile in the aquatic environment, so runoff and leaching into surface waters is predicted (USEPA 2016), and does occur, as evidenced by ubiquitous detection of atrazine in the environment. As noted, concentrations have been reported above this guideline, suggesting potential risks to aquatic organisms, if concentrations reach toxicological thresholds for sensitive species of primary producers, invertebrates or fish.

Few data were available in the ECOTOX database for aquatic insects or invertebrates (USEPA 2020a), but data available for molluscs and crustaceans indicate these taxa might be less sensitive to atrazine than fish, macrophytes, or algae (Fig. 1). Using the 23.6 μg/L aquatic plant LOC from Moore et al. (2017) as a protective threshold for effects, concentrations have very rarely been reported to exceed this concentration; the only exceedances in the collected data set were in Baldwin et al. (2016) in tributaries of the Great Lakes (40.2 μg/L – sampling location actually in the USA) and in a Quebec river studied by Giroux (2010; mean concentration of 62.0 μg/L). As such, atrazine has reached concentrations in Canadian waters that could have effects on algae and aquatic plants, but monitoring data indicate this would be expected very infrequently. Continued monitoring of atrazine in aquatic environments is recommended, particularly in regions of common use, but ideally should also be performed within the context of monitoring for changes in primary producer communities.

Diquat

Diquat is most commonly applied as diquat dibromide or in formulation with paraquat. It can be used for general weed control on non-cropped land, as well as for pre- or post-harvest desiccation of alfalfa, cotton, flax, and other fruit, vegetable, or ornamental crops (Roede and Miller 2014; USEPA 2015a). Diquat exerts its toxicity on plants by inhibiting photosynthesis via repeated sequestration of electrons from photosystem I and generation of peroxide and free radical by-products

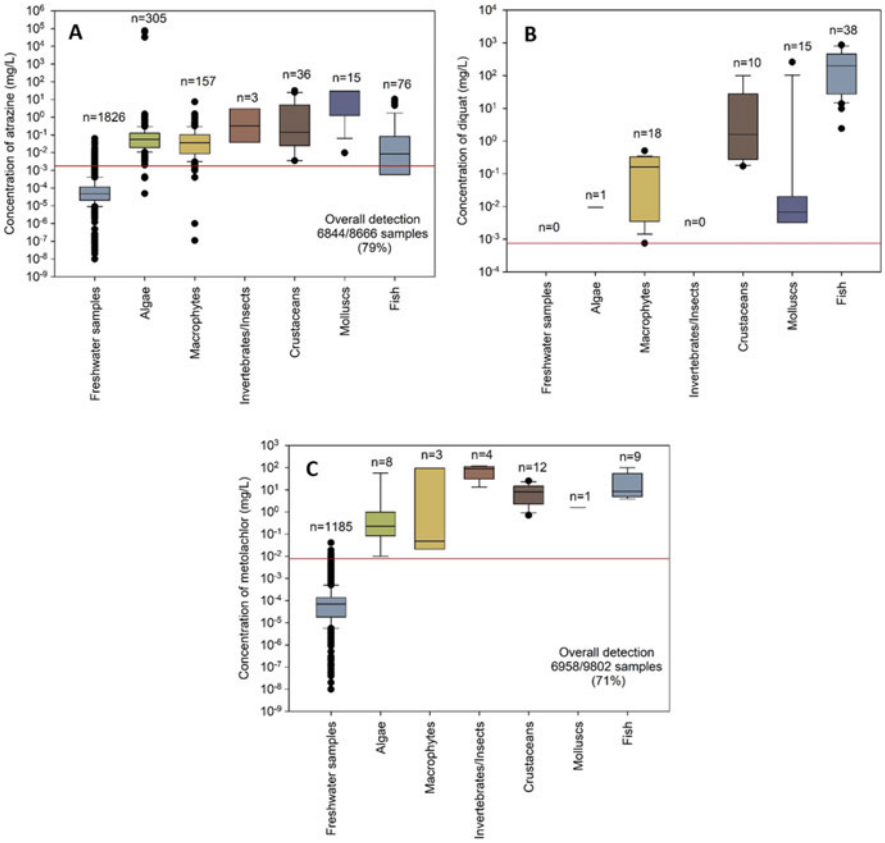

Fig. 1 Comparison of detectable concentrations of herbicides ((**a**)-atrazine, (**b**)-diquat, and (**c**)-metolachlor) measured in Canadian freshwater samples with effective concentrations (LCXX, ECXX, LOEL, LOEC values, where XX can be any number, e.g., LC10, EC50) reported in the ECOTOX database for aquatic toxicity tests using algae, invertebrates/insects, fish, molluscs, and crustaceans. Horizontal lines within each box indicate the 25th, 50th, and 75th percentiles of measurements reported, while the tenth and 90th percentiles are indicated by the whiskers (note: concentrations <LOD are not included, and values reflect data available in raw and summary form. The *n*-value reflects the number of measured concentrations >LOD or the number of toxicity data records). The red line represents the Canadian Water Quality Guideline for the Protection of Aquatic Life (CWQG-PAL; atrazine, S-metolachlor) or USEPA OPP Aquatic Life Benchmark for those active ingredients without a CWQG-PAL (diquat). The overall detection is the per cent of samples in which the herbicide was detected. The difference between *n* and the total number of samples used in the calculation of the overall detection is due to studies reporting a summary statistic of concentration, e.g., mean, the frequency of detection, and the total number of samples collected without providing the raw data

(Homer et al. 1960). Diquat is also registered in Canada for direct application to water for the control of aquatic plants, particularly free-floating weeds (Breckels and Kilgour 2018).

Presence in the Aquatic Environment

With spray application, diquat is anticipated to enter the aquatic environment directly (in the case of aquatic applications), via spray drift, or by runoff. Diquat degrades rapidly in water, with a half-life of <48 h (Roede and Miller 2014). However, it binds very tightly to soil and sediment particles, making it biologically unavailable, but potentially extending the persistence in aquatic and terrestrial systems (Roede and Miller 2014). In the preliminary ecological risk assessment for diquat dibromide performed by USEPA (2015a), it was noted that surface water monitoring data for diquat in the USA was very limited and likely did not capture higher-level exposure scenarios. Data also did not represent aquatic applications (USEPA 2015a). This situation was also observed in Canada in this review, whereby monitoring data were not found in the government databases accessed nor peer-reviewed literature, which has also been noted by others (e.g., Sesin et al. 2018).

Toxicity Towards Aquatic Organisms

As diquat is used to control aquatic plants, it is unsurprising that diquat was highly toxic towards model species of aquatic plants and algae. In a 14-day test with the duckweed *L. gibba*, the EC50 for frond number was 0.0047 mg/L, while in a 120-h exposure with the freshwater diatom *Navicula pelliculosa*, the EC50 value for reduced yield was 0.00070 mg/L. A 96-h LC50 value of 0.01 mg/L was reported for the *L. minor* duckweed species, by Garlich et al. (2016). A 42-day outdoor mesocosm study was conducted with native and non-native macrophytes collected in Ontario at concentrations corresponding to 0.4 to 100% of recommended label rate for managing nuisance macrophytes. In both the mesocosms and in 14-days single species greenhouse tests, almost 100% mortality was observed in all test species of aquatic macrophytes (*Elodea canadensis* Michx., *Myriophyllum spicatum* L., *Ceratophyllum demersum* L.,) and flowering plants (*Hydrocharis morsus-ranae* L.) at 0.074 mg/L (6% of label rate; Sesin et al. 2018).

Both freshwater and estuarine/marine invertebrates are highly sensitive to diquat. For example, the reported 96-h EC50 value for the model invertebrate amphipod, *Hyalella azteca* was 0.09 mg/L. In a chronic test (168-days) with the freshwater snail species *Lymnaea stagnalis,* development was delayed and food consumption reduced at a concentration of 0.0032 mg/L in formulation (USEPA 2015a). The reported 21-day LOAEC for survival of *D. magna* was 0.057 mg/L. Similar toxicity of diquat was also observed in the marine mysid shrimp (*Americamysis bahia*), with reported 96-h LC50 and 31-day LOAEC (female dry weight) values of 0.42 mg/L and 0.104 mg/L, respectively (USEPA 2015a). Given that diquat binds tightly to sediment, potential toxicity of diquat towards benthic invertebrates was investigated using the amphipods *H. azteca* and *Leptocheirus plumulosus.* The 42-day LOAEC

for reproduction of *H. azteca* was 23 mg/kg of sediment, while no effects were observed in *L. plumulosus* after a 10-day exposure to concentrations of diquat up to 110 mg/kg (USEPA 2015a).

Acute exposure to diquat can result in slight to high toxicity in freshwater and marine fish species (Fig. 1, USEPA 2017a). For freshwater fish, a 96-h LC50 value of 0.75 mg/L was reported for walleye (*Stizostedion vitreum*) and growth of fathead minnow was reduced in early-life stages at concentrations ≥0.316 mg/L (34-days LOEAC; USEPA 2015a). In the marine sheepshead minnow, these same endpoint effects thresholds were considerably higher, with values of 51.1 and 7.7 mg/L, respectively (USEPA 2015a). A study with juvenile rainbow trout reported a continuous exposure 96-h LC50 of 9.8 mg/L (McCuaig et al. 2020). Under pulsed conditions, embryos/alevin had decreased survival and changes in body morphometrics (decreased length and weight) following two 24-h pulses exposures at 9.3 mg/L, while juveniles were not significantly affected at this concentration (McCuaig et al. 2020). The toxicity of diquat towards fish is likely attributable to the habitat-level depletion of oxygen levels in aquatic systems following decomposition of targeted aquatic plants (Roede and Miller 2014; USEPA 2015a).

Diquat is not expected to bioaccumulate, as evidenced by reported BCF values ranging from 0.7 to 2.5, strong binding to sediment and soil particles, and a log K_{ow} value of −4.6 (Breckels and Kilgour 2018 and references therein, USEPA 2015a). No freshwater or marine CWQG-PAL has been recommended (CCME n.d.), but the lowest USEPA Aquatic Life Benchmark is 0.75 µg/L, based on vascular plant toxicity (USEPA 2019a).

Exposure Risks for Aquatic Organisms

Diquat was among the top ten herbicides sold in Canada in 2017, and had a sales volume of >500,000 kg a.i. in both 2017 and 2020 (Health Canada 2017, 2020). Toxicity incidents in aquatic organisms have been reported in the USA as a result of exposure to diquat, and the USEPA reported potential risks for fish, aquatic invertebrates, and/or aquatic plants for both terrestrial and aquatic use patterns (USEPA 2015a). Notably, non-target aquatic vascular and non-vascular plants were deemed at risk of experiencing toxic effects as a result of nearly all uses (USEPA 2015a).

It was highlighted by Breckels and Kilgour (2018) that despite the use of diquat in aquatic applications for 20 years in Canada, few studies had been conducted under field conditions to assess the risks to Canadian aquatic organisms. However, available field studies summarized by Breckels and Kilgour (2018) suggested that direct applications of diquat showed little if any effect on aquatic invertebrates, fish, and amphibians, even at concentrations initially exceeding LC50 or EC50 values reported from laboratory studies.

The conservative nature of the scoring system used for this review was such that by having few measured data available for diquat in Canadian systems, it was deemed one of the active ingredients of greatest interest or concern. Increased monitoring data would help to fill this knowledge gap and provide a clearer picture of the true risks posed by diquat under current-use patterns.

S-Metolachlor

S-metolachlor is applied pre-plant, pre-emergence, or early post-plant control of grasses and broadleaf weeds in crops such as corn, soybean, and ornamental crops. S-metolachlor is the enantiomerically-enriched form of metolachlor (88% S-metolachlor, 12% R-metolachlor) and has a separate registration, as well as greater potency. Metolachlor disrupts plant cell elongation and division by inhibiting enzymes involved in the production of long-chain fatty acid and the growth hormone gibberellin (Rose et al. 2016). Toxicological and environmental measurements are fluid between the two forms and data are generally bridged for risk assessment purposes (USEPA 2019b).

Presence in the Aquatic Environment

Metolachlor is highly water soluble and moderately mobile in the environment. It is primarily degraded by aerobic metabolism with half-lives of 14.6 to 231 days in soil and 33 to 54.5 days in water (USEPA 2019b). The physical and chemical properties of S-metolachlor (e.g., log K_{ow} of 3.05) suggest potential movement into benthic sediments; however, concentrations are expected to remain considerably lower than those measured in the water column (USEPA 2019b; Elias 2016).

Metolachlor has been detected frequently in water samples collected in Canadian waters (up to 100% of samples in some cases), particularly those from Ontario and Quebec (ECCC 2011; Bartlett et al. 2016; Larue 2019). The maximum reported concentration of 41 µg/L (Giroux 2010) was exceptional, but concentrations between 5 and 10 µg/L were reported by several studies (Fig. 1). Fewer data are available for sediments; for example, from Great Lakes tributaries (maximum of 12.8 µg/kg, 3% detection rate – Elliot et al. 2017) and Nathan Creek, British Columbia (mean of 35 µg/kg – Harris et al. 2008).

Toxicity Towards Aquatic Organisms

S-metolachlor was classified as moderately toxic towards fish and aquatic invertebrates under acute exposure conditions (USEPA 2017a, 2019b). The most sensitive NOAEC values used for the ecological risk assessment by USEPA were as follows: freshwater fish – 30 µg/L, marine fish – 1,000 µg/L, freshwater invertebrates – 3,200 µg/L, marine invertebrates – 130 µg/L, aquatic vascular plants – 14 µg/L (duckweed, *L. gibba*), and aquatic non-vascular plants – 8 µg/L (green algae) (USPEA 2019b).

As indicated by the risk assessment values from USEPA (2019b), aquatic plants and primary producers can be susceptible to S-metolachlor, but toxicity thresholds vary. For green algae, chlorophyll concentration, growth, and cell morphology endpoints of *Chlorella pyrenoidosa* had a 96-h LC50 value of 68 µg/L (Liu and Xiong 2009). The microalga *Parachlorella kessleri* had a reported 72-h EC50 value of 1,090 µg/L, but sub-lethal effects, including decreased growth, changes in cellular antioxidant activity, and decreased pigment concentrations, were observed following

exposure to S-metolachlor at concentrations ≤200 μg/L (Maronić et al. 2018). In the green algae, *Scenedesmus obliquus,* 100 μg/L induced generation of reactive oxygen species and increased cell membrane permeability after 96 h, while significant changes in chlorophyll-*a* and -*b* were reported at 50 μg/L (Liu et al. 2016). A comparison of sensitivities among three marine microalgae (chlorophyte *Tetraselmis suecica*, diatom *Ditylum brightwellii*, and dinoflagellate *Prorocentrum minimum*) reported 72-h EC50 values of 21,300, 423, and 70 μg/L, respectively, with significant reductions in cell counts and chlorophyll-*a* production (Ebenezer and Ki 2013). Similar responses were observed for the freshwater alga *Raphidocelis subcapitata* and marine alga *Dunaliella tertiolecta*, with reported EC50 values for growth of 118 μg/L and 11,300 μg/L, respectively, after 72–96 h (Machado and Soares 2019). Literature EC50 values cited by Machado and Soares (2019) for *R. subcapitata* ranged from 44.3 μg/L for chlorophyll concentration to 5,510 μg/L for growth rate. A 10% inhibition of growth (EC10) was observed at 45 μg/L for *R. subcapitata* and at 5,620 μg/L for *D. tertiolecta (*Machado and Soares 2019), demonstrating the wide range of sensitivities across primary producers.

A wide range of effect concentrations for invertebrates have been reported in the literature, some examples of which are described below. Exposure to 100 μg/L of metolachlor induced an eight-fold reduction in egestion rates of the aquatic gastropod *Physa acuta*, but had no significant effects on another species, *Helisoma anceps* (Elias and Bernot 2017). The amphipods *Gammarus* cf. *orinos* and *G. pulex* exhibited similar sensitivity to S-metolachlor as the isopod *Asellus aquaticus,* with reported 96-h EC50 values of 8,470–11,780 μg/L (Maazouzi et al. 2016). Metolachlor concentrations up to 100 μg/L caused immobilization of up to 10% of chironomids (*Chironomus tentans*) in a 72-h assay; at 1000 μg/L, 58% of test organisms were immobilized and AChE activities were significantly reduced (Jin-Clark et al. 2008). Chronic bioassays with the water flea *Daphnia longispina* revealed greater toxicity of S-metolachlor in formulation (Primextra® GOLD) compared to the technical product, with 21-day reproduction EC50 values of 4,100 μg/L and 8,240 μg/L, respectively (Neves et al. 2015).

Juvenile marbled crayfish (*Procambarus virginalis*) exhibited slower growth, increased mortality, behavioural excitation, and delayed ontogenetic development with chronic exposure (45 days) to concentrations of 1.1 μg/L. Long-term exposure to 11 and 110 μg/L of S-metolachlor caused significant changes in levels of oxidative stress biomarkers and antioxidant enzymes, with histopathological changes in the hepatopancreatic tissue observed only at the highest exposure concentration (110 μg/L) (Velisek et al. 2019). Chronic toxicity of metolachlor OA (a major metabolite of S-metolachlor and metolachlor) was also evaluated in juvenile marbled crayfish. Exposure to 4.2 μg/L for 45 days resulted in significantly reduced growth and antioxidant enzymatic activity. At 42 and 420 μg/L, changes in hepatopancreas and gill histomorphology were observed, but there were no changes in behavioural endpoints (Velisek et al. 2018). In the benthic clam *Scrobicularia plana,* acute (96 h) exposure to relatively high concentrations of S-metolachlor (2,048–46,410 μg/L) resulted in changes to fatty acid composition, and increased glucose and decreased glycogen in tissues, indicating stress response (Gutiérrez et al. 2019a). The LC10

concentrations of S-metolachlor for mortality in this clam species were previously determined to be 16,285 µg/L for smaller individuals and 30,065 µg/L for larger individuals (Gutiérrez et al. 2019b).

Among acute toxicity studies for fish species, reported 96-h LC50 values have included 10,000 µg/L in bluegill, 45,210 µg/L in zebrafish (*Danio rerio*), 8,850 in sheepshead minnow, 4,900 in channel catfish (*Ictalurus punctatus*), and 8,600 µg/L in guppy, and 3,900 µg/L in rainbow trout (Quintaneiro et al. 2017; CCME 1999b; Munn et al. 2006). Short-term exposure to 29,000 µg/L of metolachlor induced significant malformations in early-life stage zebrafish, with biochemical changes at 500 µg/L and higher (Quintaneiro et al. 2017). The CCME guideline of 7.8 µg/L was derived based on the lowest reproduction endpoint for fathead minnow (780 µg/L) with a safety factor of 0.01 to account for limited chemical fate and chronic toxicity data (CCME 1999b). However, more conservative aquatic quality indices of 1.62 µg/L for acute exposure and 0.162 µg/L for chronic exposure were recommended by Tsaboula et al. (2019).

The log K_{ow} value (3.05) suggests potential for bioaccumulation, but a bioconcentration study submitted for the USEPA risk assessment concluded that this potential is small (USEPA 2019b).

Exposure Risks for Aquatic Organisms

Sales of S-metolachlor exceeded 500,000 kg a.i. in 2017 and 100,000 kg a.i. in 2020 (Health Canada 2017, 2020), thus large quantities of this compound are being applied, and concentrations in the environment have been measured (though infrequently reported in the literature) above the CCME guideline and above toxicity endpoints for sensitive aquatic species (Fig. 1). In both Ontario and Quebec, S-metolachlor has been identified as one of the pesticides of greatest risk to environmental receptors in those regions based on measured concentrations, ecotoxicity data, and risk indicators such as hazard quotients (Van Eerd 2016; Government of Québec 2017; Corsi et al. 2019). S-metolachlor is currently under evaluation by PMRA with anticipated public consultation activities to begin late 2020 (PMRA 2019b).

In the recent risk assessment by USEPA (2019b), potential risks were identified for freshwater fish under chronic exposure and for water column invertebrates, though these were considered relatively low (risk quotients of 0.22–3.70 and 0.13–1.05, respectively). Likewise, benthic invertebrate risk quotients exceeded the level of concern. True risks were presumed to be low; however, lack of measured sediment concentrations resulted in uncertainty in the assessment (USEPA 2019b). Risks to aquatic plants as a result of runoff were identified under all use scenarios (USEPA 2019b).

Given the sensitivity reported in non-vascular plant studies (EC50 of 8.0 µg/L and NOAEC of 1.5 µg/L), the Canadian guideline value of 7.8 µg/L might not be protective of these species. Generally, fish would be expected to be more tolerant of the typical S-metolachlor concentrations anticipated in Canadian waters, and

effects on fisheries would be more likely via indirect habitat or food web-mediated effects resulting from damage to sensitive aquatic plants.

3.3.2 Insecticides

Chlorpyrifos

Chlorpyrifos is used agriculturally for insect control in a variety of field and greenhouse crops, as well as for control of mosquito larvae in standing water and management of destructive forestry pests (PMRA 2019c). Chlorpyrifos inhibits acetylcholine (AChE) breakdown by binding to cholinesterase in axon synapses (Giesy and Solomon 2014). Like atrazine, chlorpyrifos is one of the most studied pesticide active ingredients, with over 2000 records in the ECOTOX database (USEPA 2020a) and several reviews or risk assessments available in the published literature (e.g., Giesy and Solomon 2014; Giddings et al. 2014; Alvarez et al. 2019; Giesy et al. 2014; Juberg et al. 2013). As such, only a brief review of the state of the science with respect to aquatic biota will be provided below.

Presence in the Aquatic Environment

Chlorpyrifos has moderate environmental persistence and may dissipate via photolysis, hydrolysis, microbial degradation, and/or volatilization (CCME 2008; Giesy and Solomon 2014). Its major metabolite, chlorpyrifos-oxon, is toxicologically active but does not persist in the environment and is not found in surface waters (Giesy and Solomon 2014). Immediately following application, volatilization is the dominant process, but within days of application, chlorpyrifos will be strongly bound to soil (Giesy and Solomon 2014). Half-lives in soil can vary considerably, depending on soil properties and microbial activity, with reported values ranging from <1 week to >24 weeks (CCME 2008). Under field conditions, chlorpyrifos does not persist in the water column, but tends to be bound to sediment. Reported half-lives in water range from <1 to 50 days, and in sediment from 1 to 34 days (CCME 2008, Giesy and Solomon 2014).

As is true for many pesticides, chlorpyrifos presence in flowing waters tends to occur in pulses, with peak concentrations persisting only for a limited period (estimated as 2 days for chlorpyrifos) and concentrations declining thereafter (Giesy and Solomon 2014), so exposure duration and recovery are important considerations for assessment of risk.

Chlorpyrifos has been detected in water bodies across Canada since 2000, including remote lakes in Ontario and Arctic seawater (Hoferkamp et al. 2010; Kurt-Karakus et al. 2011). Reported concentrations have surpassed both acute and chronic CCME guideline values – 0.02 and 0.002 μg/L, respectively – reaching up to 4 μg/L in Quebec rivers (as reported by Giroux 2010; Fig. 2).

Toxicity Towards Aquatic Organisms

As noted in the risk assessment by Giddings et al. (2014), and observed in the ECOTOX database results (USEPA 2020a, Table S3), data on toxicity of chlorpyrifos towards aquatic plants are limited, but this is consistent with the lack of AChE receptors in plants and tolerance reflected in reported marine algae tests (EC50s of 138–769 μg/L). The 72-h EC50 values for growth of two freshwater microalgae, *Chlorella pyrenoidosa* and *Merismopedia* sp., were even greater, at 11,460 μg/L and 25,800 μg/L, respectively (Chen et al. 2016). Changes in chlorophyll-*a* concentrations were observed with 8-day exposure to concentrations ≥2,400 μg/L, as well as concentration-dependent growth inhibition at concentrations up to 38,400 μg/L (Chen et al. 2016).

In the risk assessment conducted by Giddings et al. (2014), acute toxicity data were included from 23 crustacean species, with LC50 values ranging from 0.035 to 457 μg/L, and an HC5 value (the concentration at which 5% of species are expected to exhibit effects) of 0.034 μg/L. Aquatic insects were similarly sensitive, with LC50 values for 17 species ranging from 0.05 to >300 μg/L and an HC5 of 0.087 μg/L (Giddings et al. 2014). Some insects also exhibit sub-lethal sensitivity at very low concentrations of chlorpyrifos. For example, the swimming behaviour of the Alpine chironomid *(Diamesa zernyi*) was significantly affected after 72-h exposure to chlorpyrifos at 0.11 μg/L (Di Nica et al. 2019). Fish were less sensitive as a group (Fig. 2), with LC50 values of 0.53 to >806 μg/L for 25 assessed species and an HC5 of 0.812 μg/L. Aquatic molluscs were deemed to be relatively insensitive, with LC/EC50 values of 154 to >806 μg/L (Giddings et al. 2014).

A more recent risk assessment by Alvarez et al. (2019) included chronic toxicity data for aquatic species ranging from 0.21 μg/L (AChE inhibition) to 171,000 μg/L (immobility) and noted that shrimp, cladoceran, and amphipod species were generally most sensitive to chlorpyrifos. Acute and chronic HC5 values for all taxa were calculated as 0.064 and 0.007 μg/L (Alvarez et al. 2019), indicating that the CCME chronic benchmark (0.002 μg/L) may not be protective of the most sensitive members of arthropod taxa. More conservative aquatic quality objectives were also recommended by Tsaboula et al. (2019) – 0.01 μg/L for acute exposure and 0.001 μg/L for chronic exposure.

Zooplankton assemblages in mesocosm studies were significantly shifted as a result of exposure 0.17–2.3 μg/L of chlorpyrifos (Pereira et al. 2017; Xiao et al. 2017). Recovery to control conditions was not observed over 56 days, despite rapid disappearance of chlorpyrifos from the system (Xiao et al. 2017). The growth, longevity, and reproduction of the rotifer *Brachionus koreanus* were not significantly affected by 10-day exposure to 10 μg/L, but at a concentration of ≥100 μg/L, growth was reduced, lifespan was shortened, and fewer offspring were produced (Kim et al. 2016).

Inhibition and recovery of AChE activity (whole body) was observed in post-larval American lobster (*Homarus americanus*) following exposure to 0.5 μg/L for 48-h and recovery for 9–15 days. A concentration of 0.82 μg/L caused sub-lethal effects on lobster growth, including decreased growth rate, decreased moult

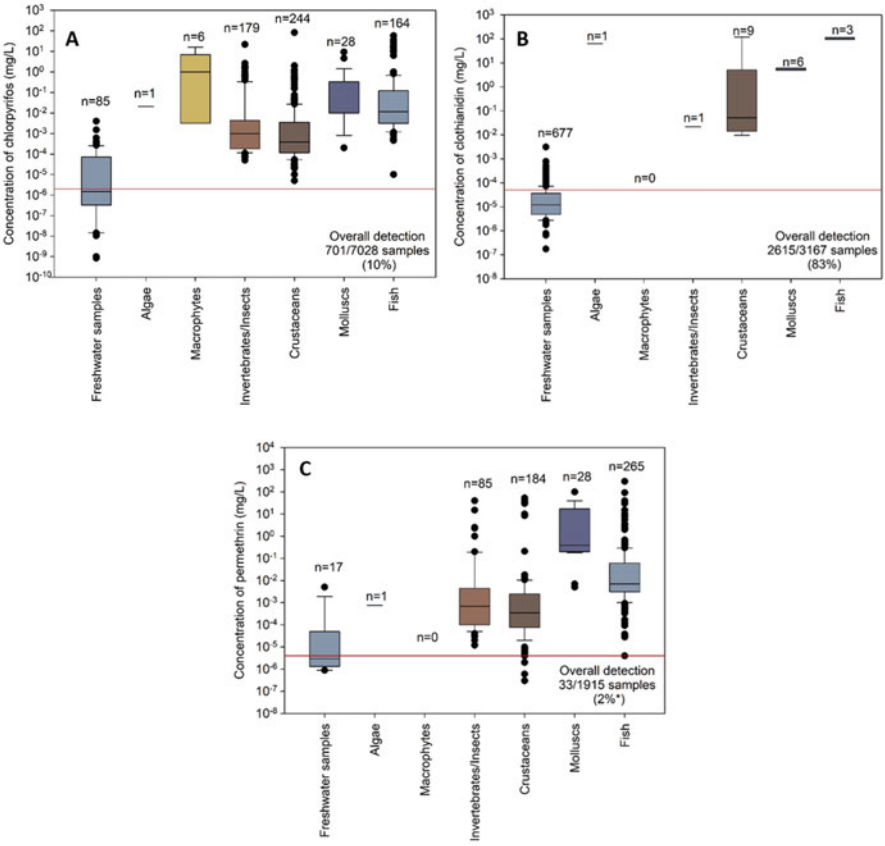

Fig. 2 Comparison of detectable concentrations of insecticides ((**a**)-chlorpyrifos, (**b**)-clothianidin, and (**c**)-permethrin) measured in Canadian freshwater samples with effective concentrations (LCXX, ECXX, LOEL, LOEC values, where XX can be any number, e.g., LC10, EC50) reported in the ECOTOX database for aquatic toxicity tests using algae, invertebrates/insects, fish, molluscs, and crustaceans. Horizontal lines within each box indicate the 25th, 50th, and 75th percentiles of measurements reported, while the tenth and 90th percentiles are indicated by the whiskers (note: concentrations <LOD are not included, and values reflect data available in raw and summary form. The *n*-value reflects the number of measured concentrations >LOD or the number of toxicity data records). The red line represents the Canadian Water Quality Guideline for the Protection of Aquatic Life (CWQG-PAL; chlorpyrifos and permethrin) or USEPA OPP Aquatic Life Benchmark for those active ingredients without a CWQG-PAL (clothianidin). The overall detection is the per cent of samples in which the insecticides were detected. The difference between *n* and the total number of samples used in the calculation of the overall detection is due to studies reporting a summary statistic of concentration, e.g., mean, the frequency of detection, and the total number of samples collected without providing the raw data

increment, and increased intermoult period, while the 48-h IC50 for normal movement was 0.66 µg/L (Taylor et al. 2019). At sub-lethal concentrations ranging from 0.03 to 100 µg/L, acute chlorpyrifos exposure induced changes in protein content of tissues and enzymatic activity in digestive glands and gills of the mussel *Mytilus galloprovincialis* (Kovačić and Medić 2016). In another mussel species (*Villosa iris*), mean viability of glochidia was not significantly different from the control at a concentration of 360 µg/L following a 48-h exposure period. This species is listed as of "special concern" in Canada, but it was suggested that chlorpyrifos would pose a minimal risk for survival and viability of its glochidia (Salerno et al. 2018).

Short-term (36- to 96-h) LC50 values for fish species used in the development of Canadian guidelines ranged from 1.3 to 280 µg/L ($n = 12$), and those for invertebrates ranged from 0.04 to 10 µg/L ($n = 9$; CCME 2008). Using a species sensitivity distribution approach, a guideline of 0.02 µg/L was established, reflecting the toxic nature of chlorpyrifos towards aquatic organisms. Long-term guidelines for freshwater and marine exposure are both set at 0.002 µg/L, based on a 96-h LC50 of 0.04 µg/L for *Hyalella azteca* and a safety factor of 20 (CCME 2008).

Sub-lethal studies in fish have often examined AChE activity (as this is the pathway targeted by chlorpyrifos), as well as growth, histological, developmental, and behavioural endpoints. Swimming behaviour in Japanese medaka (0, 20, and 40-days post-hatch) was significantly impacted by chlorpyrifos at concentrations $\geq$12.5 µg/L (Sastre et al. 2018). The liver somatic index of fingerling African sharptooth catfish (*Clarias gariepinus*) was significantly increased by exposure to 12.8 µg/L, and exposure to 400 µg/L caused erratic swimming, hyperactivity, and lack of startle response (Kanu et al. 2019). A study in adult zebrafish reported structural damage (vacuolization) in gonads after 96-h exposure to chlorpyrifos at 200 µg/L (Manjunatha and Philip 2016). Concentrations $\geq$150 µg/L caused histopathological changes in both testes and ovaries of banded gourami (*Trichogaster fasciata*) over a 60-day exposure period (Sumon et al. 2019). In another reproductive study, significant decreases in Coruh trout (*Salmo coruhensis)* spermatozoa motility rate and duration as a result of in vitro exposure to 5 µg/L or more of chlorpyrifos (Kutluyer et al. 2019). Long-term (30-day) exposure to 5 µg/L caused severe behavioural changes in spotted snakehead (*Channa punctatus*), as well as pathological lesions in gill tissue, and structural changes in hepatic and intestinal tissues (Stalin et al. 2019).

Changes to histological endpoints have been identified in fish gill, eye, and brain tissues in a number of species. Histopathological changes and loss of structural integrity were observed in gill tissues of common carp following a 45-day study with 14.5 µg/L chlorpyrifos, likely caused via oxidative stress and cell apoptosis (Jiao et al. 2019). Similar histopathological changes were observed in fingerling barramundi (*Lates calcifer*) following chronic (30-day) exposure to concentrations of chlorpyrifos as low as 0.04 µg/L. Specifically, the intercellular space in the photoreceptor of the fish retina increased at 0.04 µg/L, and exposure to 0.09 µg/L also induced changes to the primary and secondary lamellae of the gill (Marigoudar et al. 2018a). A concentration of 0.09 µg/L was also reported by Marigoudar et al. (2018b) as causing hyperplasia of secondary lamellae in fingerling flathead grey mullet

(*Mugil cephalus)*, while in milkfish (*Chanos chanos)*, gill histopathology was observed at 0.32 μg/L. Qiu et al. (2017) similarly observed changes in fish eyes as a result of chlorpyrifos exposure, reporting increased AChE activity in eyes and consequent persistent startle response in Japanese medaka following a 4-day exposure to 24 μg chlorpyrifos/L. In addition, transient hyperactivity and increased brain AChE activity were observed, but did not persist past the 21-day recovery period (Qiu et al. 2017). Long-term (90-days) exposure to 12 μg/L caused anaemia and reduced growth in freshwater Nile tilapia (*Oreochromis niloticus*; Majumder and Kaviraj 2019).

Although the high log K_{ow} that is generally cited for chlorpyrifos suggests the potential for bioaccumulation (Table 4), a weight-of-evidence review by Giesy et al. (2014) concluded that chlorpyrifos did not meet the criteria to be classified as "bioaccumulative" as per European EC Regulation No. 1107/2009 classifications, only "toxic". Likewise, another weight-of-evidence review concluded that chlorpyrifos does not demonstrate potential for interaction with oestrogen, androgen, or thyroid pathways at concentrations less than those causing effects via cholinesterase inhibition, so additional endocrine testing is not warranted (Juberg et al. 2013). Generally, the only toxicity data gaps identified by Giesy and Solomon (2014) in terms of risk assessment were related to terrestrial pollinators and not aquatic organisms.

Exposure Risks for Aquatic Organisms

Chlorpyrifos was among the top 20 chemicals (not just pesticides) of concern in a UK review, based on the overlap of measured river concentrations and aquatic toxicological effects thresholds (Johnson et al. 2017). Mesocosm studies reviewed in Giddings et al. (2014) generally support the conclusion that concentrations of chlorpyrifos <0.1 μg/L would not be expected to cause significant effects to aquatic communities; however, concentrations above this threshold have been measured in Canadian surface waters (Fig. 2).

Chlorpyrifos recently underwent a re-evaluation review by the PMRA and risks to aquatic biota were found to be unacceptable for most uses. As such, continued registration will only be for a limited number of uses, including treatment of temporary standing water for mosquito larvae and use in greenhouse ornamentals (PMRA 2019c). It is expected that these changes will result in decreased entry of chlorpyrifos into aquatic environments, and thus, reduced risk to fish and fish habitat. Continued monitoring of water concentrations would be advisable for tracking outcomes of the proposed registration changes.

Clothianidin

Clothianidin is both an active ingredient in its own right and a degradate of thiamethoxam (Anderson et al. 2015); as such, some information for thiamethoxam has also been included in this review. Clothianidin and thiamethoxam are

neonicotinoid insecticides used for the control of piercing sucking pests, coleopteran pests, and other pests in corn and cereal crops (PMRA 2018a; USEPA 2017b, c, 2020b). Clothianidin, like other neonicotinoid insecticides, inhibits the insect nicotinic acetylcholine receptor (Anderson et al. 2015 and references therein). In August 2018, the PMRA proposed a phase out of all outdoor agricultural applications of clothianidin and thiamethoxam (PMRA 2018a). A recent Special Review of these two active ingredients was undertaken by PMRA with particular focus on potential effects on aquatic invertebrates (PMRA 2019b). The decision outcome released in March 2021 resulted in cancellation of certain uses found to pose an unacceptable risk, as well as reduced application rates for acceptable uses and new or revised spray buffers (PMRA 2021a, b).

Presence in the Aquatic Environment

Both clothianidin and thiamethoxam are water soluble, allowing them to be readily transported systemically in plants, but also causing potential for leaching and runoff into surface water systems (Anderson et al. 2015 and references therein). Neonicotinoids have also been shown to persist through water treatment systems without being removed (Klarich et al. 2017); however, half-lives in aquatic environments are typically on the order of hours to weeks (Anderson et al. 2015; USEPA 2017b, c; PMRA 2018a). Reported soil half-lives for clothianidin range from 34 to >5,000 days, and from 46 to 464 days for thiamethoxam, suggesting potential for persistence and availability for movement into aquatic environments (USEPA 2017b, c).

Surface water sampling of neonicotinoids in Canada and elsewhere has expanded since 2010 to include clothianidin, thiamethoxam, and others, as neonicotinoids became of greater environmental and public interest. Maximal concentrations of clothianidin and thiamethoxam up to approximately 3–5 μg/L have been reported in Canadian surface waters, typically in agricultural areas (Giroux 2019, Main et al. 2016, PMRA 2018a, Fig. 2). In the PMRA proposed special review decision, a lack of Canadian estuarine or marine monitoring data was noted (PMRA 2018a), representing a gap for risk assessment.

Toxicity Towards Aquatic Organisms

Generally, clothianidin is more toxic towards aquatic organisms than its parent compound thiamethoxam (USEPA 2017c). Algae and macrophytes did not demonstrate sensitivity towards either clothianidin or thiamethoxam (Anderson et al. 2015 and references therein), with reported chronic NOAEC values of 6,350 and 12,000 μg/L, respectively, for the saltwater diatom *S. costatum,* and 520 and 22,000 μg/L, respectively, for duckweed (*L. gibba*) (USEPA 2017b, c). Further studies with primary producers reported acute toxicity thresholds (NOEC, ECx) ranging from 47,000 to >100,000 μg/L (Finnegan et al. 2017).

Broadly, aquatic invertebrate species are very sensitive to clothianidin, based on available data for amphipods, molluscs, dipteran insects, and cladocerans (USEPA

2017b; PMRA 2018a). However, data from the ECOTOX database (USEPA 2020a, Fig. 2) and literature suggest molluscs are slightly more tolerant than other invertebrate taxa. For example, Prosser et al. (2016) reported 48-h LC10 (mean viability) values of >478 and >691 μg/L for clothianidin and thiamethoxam, respectively, for wavy-rayed lampmussel (*Lampsilis fasciola)* glochidia. For juvenile rams-horn snails (*Planorbella pilsbryi*), clothianidin induced a 50% reduction in growth and biomass at 122.0 and 33.2 μg/L, respectively. Thiamethoxam reduced snail growth and biomass by 50% at concentrations of 52.1 and 51.3 μg/L, respectively (Prosser et al. 2016). Glochidia of another mussel species, *V. iris*, did not experience reductions in viability after 24 or 48-h exposure to 13,800 μg/L of clothianidin or 17,400 μg/L of thiamethoxam (Salerno et al. 2018).

As expected, based on the mode of action and insecticidal properties of neonicotinoids, aquatic insects are most susceptible to acute toxicity from exposure to neonicotinoids (Anderson et al. 2015; Sànchez-Bayo et al. 2016). The most sensitive effects values for chronic clothianidin exposures deemed acceptable for risk assessment by USEPA ranged from 0.020 μg/L (*Chironomus dilutus*, 40-days emergence) to 120 μg/L (*D. magna*, 21-days reproductive NOEC; USEPA 2017b). In a mesocosm study with eight species of aquatic invertebrates, the reported 48-h LC50 values ranged from 2 μg/L for diving beetle (*Graphoderus fascicollis)* to 1,245 μg/L for damselfly (*Lestes unguiculatus*) (Miles et al. 2017). Interestingly, Shahid et al. (2018) demonstrated adaptation of the amphipod, *G. pulex,* such that individuals from pesticide-exposed populations had a mean clothianidin EC50 of 218 μg/L compared to 81 μg/L for non-exposed populations. Data from exposure of cladocerans to formulated clothianidin suggest that components of the formulations can contribute additive toxicity with respect to the technical ingredient (PMRA 2018a; Takács et al. 2017). Two sediment studies of quality suitable for risk assessment were identified, with a most sensitive endpoint of 1.1 μg/L in pore water (10-days NOEC, dry weight of *C. dilutus*; PMRA 2018a). Notably, sub-lethal effects have been reported for aquatic invertebrates at concentrations of clothianidin well below immobilization/mortality endpoints, with effects including reduced reproduction, growth, and emergence, as well as changes to population sex ratios (USEPA 2017b).

While toxicity endpoint values were slightly higher for thiamethoxam than for clothianidin, some aquatic invertebrates were still very sensitive to exposure. For example, the acute 48-h EC50 (mobility) and chronic NOAEC (larval survival) values reported for *Chironomus riparius* midges were 35 μg/L and 0.74 μg/L, respectively (USEPA 2017c). Pickford et al. (2018) reported a thiamethoxam NOEC of 0.3 μg/L for a 35-day outdoor mesocosm exposure with mayflies (*Cloeon dipterum*). A similar 28-day LOEC for larval growth and emergence rate (1.6 μg/L) was reported for *C. xanthu*s midges based on a laboratory partial life-cycle test (Ferreria-Junior et al. 2018). A 30-day NOEC (emergence) of 10 μg/L was also reported for *C. riparius*, confirming its relative sensitivity (Finnegan et al. 2017). Cavallaro et al. (2017) reported a 40-day EC50 (emergence) value for *C. dilutus* of 4.13 μg thiamethoxam/L compared to 0.28 μg clothianidin/L. The 96-h behavioural EC50 values for mayfly *Hexagenia* spp. were 630 μg/L for thiamethoxam and

24 µg/L for clothianidin in a water-only test conducted by Bartlett et al. (2018), which were well below the reported LC50 values of >10,000 and 2000 µg/L, respectively. Five crustacean species exposed to thiamethoxam in 48-h assays had EC50 values ranging from 84 to 3,000 µg/L (Finnegan et al. 2017). For nymphs of another species of mayfly (*Deleatidium* spp.), the IC50 for immobility and EC50 for impairment were both >4 µg/L following 28-day thiamethoxam exposure. In contrast, the median concentrations of clothianidin causing immobility and impairment were 1.24 and 1.02 µg/L, respectively, on Day 28 (Macaulay et al. 2019). Concentrations of thiamethoxam and clothianidin ≤4 µg/L also had transient effects on moulting propensity over the 28-day exposure duration (Macaulay et al. 2019). The mysid shrimp (*Mysidopsis bahia*) was less sensitive to thiamethoxam, with 96-h LC50 and chronic NOAEC values of 6,900 µg/L and 1,100 µg/L, respectively (USEPA 2017c). In the context of shrimp aquaculture, Butcherine et al. (2019) suggested that more acute and chronic data were needed to characterize effects of neonicotinoids on different developmental stages of shrimp and more broadly, sub-lethal responses (e.g., biochemical) of commercially harvested crustaceans.

Based on the available acute toxicity data, PMRA calculated an HC5 for clothianidin of 1.5 µg/L for all invertebrate taxa (PMRA 2018a). This is consistent with acute HC5 values reported by Raby et al. (2018a) – 0.14 µg/L for immobilization and 4.13 µg/L for EC50 and LC50 endpoints for clothianidin, and 6.09 µg/L and 12.29 µg/L, respectively, for thiamethoxam. Basley and Goulson (2018) also reported reduced colonization of microcosms by invertebrate populations when treated with clothianidin or thiamethoxam at up to 15 µg/L. The chronic PMRA reference value for clothianidin is 0.0015 µg/L based on the HC5 approach (PMRA 2018a), reflecting the highly toxic nature of this active ingredient towards sensitive invertebrates. In prairie wetlands, a mean total neonicotinoid concentration of 0.131 µg/L resulted in lower overall emergence and a shift towards more disturbance-tolerant insect species (Cavallaro et al. 2019). From a 56-day mesocosm study, a time-weighted average concentration of 0.281 µg/L was considered a reasonable NOEC for community-level effects following a single application of clothianidin (PMRA 2018a).

Clothianidin and thiamethoxam are practically non-toxic towards fish on an acute exposure basis (USEPA 2017a), with reported 96-h LC50 values of >91,400 µg/L to 117,000 µg/L for clothianidin (USEPA 2017b; Anderson et al. 2015) and ≥ 80,000 µg/L for thiamethoxam (Anderson et al. 2015; USEPA 2017c; Finnegan et al. 2017). Whiteside et al. (2008) estimated the HC5 values for fish species at 10,500 and 10,900 µg/L, respectively, for clothianidin and thiamethoxam. However, Baldissera et al. (2018) reported oxidative stress and disruption of gill biochemistry following 96-h exposure of silver catfish (*Rhamdia quelen)* to 3.75 µg/L of thiamethoxam, and exposure to 0.15 µg clothianidin/L significantly increased whole body 17β-estradiol in swim-up sockeye salmon (*Oncorhynchus nerka*) fry (Marlatt et al. 2019).

Clothianidin and thiamethoxam have very low octanol-water coefficients (low K_{ow} values of 1.12 and − 0.13, respectively; Table 4) and are not expected to bioaccumulate (USEPA 2017b, c; PMRA 2018a, b, c).

Exposure Risks for Aquatic Organisms

By 2010, neonicotinoid constituted 27% of insecticides used globally (Casida and Durkin 2013), but the European Union instituted a ban on nearly all uses of imidacloprid, thiamethoxam, and clothianidin as of 2018 due to potential risks to honeybees and other pollinators (Jactel et al. 2019). Thiamethoxam is also on the 2019 European Union watch list and is one of the most frequently detected pesticides in surface water, groundwater, and wastewater treatment plant influent sampling data collected from 21 countries (Pietrzak et al. 2019). The proposed interim decision from the USEPA includes application rate reductions, cancelling certain uses of clothianidin, restricting certain uses of thiamethoxam, and label changes in an effect to mitigate the potential risks to aquatic invertebrates and terrestrial pollinators (USEPA 2020b), consistent with the outcome of the Special Review in Canada (PMRA 2021a, b).

In the literature review by Anderson et al. (2015), it was noted that the interim water quality guideline of 0.23 μg/L for imidacloprid (and used as a surrogate for clothianidin and thiamethoxam) would likely not be protective of the most sensitive aquatic invertebrates. The USEPA benchmarks are 0.05 μg/L of clothianidin and 0.74 μg/L of thiamethoxam, each of which was surpassed by its respective active ingredient in water samples from Canadian waters (Fig. 2,). However, Finnegan et al. (2017) calculated 5% hazard concentrations for freshwater invertebrates based on acute toxicity data and found the likelihood of thiamethoxam exceeding this level in North American waters to be <1%. Pickford et al. (2018) also concluded that mayflies and similarly sensitive aquatic insects would be unlikely to experience effects of thiamethoxam exposure, based on the 95th percentile of reported concentrations in surface waters (0.054 μg/L) falling below the 35-day NOEC of 0.3 μg/L. Likewise, Raby et al. (2018b) compared measured concentrations of neonicotinoids from Ontario waters with species-specific EC10 values and concluded clothianidin and thiamethoxam posed little to no hazard. Given the low demonstrated toxicity of clothianidin and thiamethoxam towards fish, direct effects would not be expected, but indirect food web-mediated effects are possible. Monitoring efforts should continue as the Special Review Decision label changes and new spray buffer zones are put into practice to evaluate the effectiveness of the proposed mitigations in preventing unintended consequences of clothianidin and thiamethoxam use in aquatic invertebrates.

Permethrin

Permethrin is a broad-spectrum synthetic pyrethroid insecticide used to control insect pests in a variety of agricultural crops (e.g., legumes, tobacco, grains, oilseeds), as well as for public health applications (e.g., mosquito, bedbug, and/or flea control) (PMRA 2017; USEPA 2007, 2020c). Permethrin acts by disrupting sodium channel proteins in neural cells, which alters membrane polarization (USEPA 2007).

Presence in the Aquatic Environment

Permethrin is slightly to moderately persistent, degrading slowly from the environment with aquatic half-lives ranging from 38 to 175 days (USEPA 2007; PMRA 2017). It is expected to reach the aquatic environment via spray drift or runoff, after which it adsorbs strongly to soils, sediments, and suspended solids. While this binding reduces bioavailability, there is potential for increasing concentrations of permethrin in sediments and consequent risks for benthic communities (USEPA 2007).

Relatively few monitoring data were located for permethrin (which was also noted by PMRA 2017), but for those samples collected and analysed, detection rates were typically quite low ($\leq$2%). However, many studies had a reported limit of detection (LOD) that was greater than the CWQG-PAL value of 0.004 μg/L (CCME n.d.), suggesting that concentrations present in water could exceed guideline values without being detected. The maximum reported concentrations were 1.1 μg/L, measured in Quebec rivers by Giroux (2019), and 5.04 μg/L in a sample from New Brunswick (reported by PMRA 2017) (Fig. 2). PMRA (2017) also suggested that surface water monitoring programs might be missing peak concentrations due to the location and timing of sampling.

Toxicity Towards Aquatic Organisms

Permethrin toxicity data for freshwater vascular aquatic plants were not available in the risk assessments performed by USEPA (2007) or PMRA (2017), but data were available for several algal species. Acute LC50 values ranged from 12.5 μg/L (72-h, growth inhibition, *Chlamydomonas reinhardtii)* to >100 μg/L (12-days growth inhibition and biomass reduction, *Chlorella pyrenoidsa* and *Scenedesmus quadricaudata*; PMRA 2017, Stratton and Corke 1982). For the marine alga *Dunaliella tertiolecta*, EC50 values for growth inhibition ranged from 68 to 124 μg/L. These endpoints are considerably greater than endpoints for fish or invertebrates, consistent with the assumption made by USEPA (2007) that algae and macrophytes would be less susceptible to permethrin based on its mode of action (i.e., nervous system disruption).

Permethrin can be very toxic towards other aquatic organisms, as demonstrated by its low freshwater CWQG-PAL (0.004 μg/L; CCME n.d.). As of late 2015, the USEPA had received a total of 27 reports of fish kill incidents associated with permethrin since its registration, most of which occurred prior to label changes instituting a requirement for vegetative filter strips bordering areas of application to reduce runoff (PMRA 2017). Generally, fish and invertebrates are less sensitive to transformation products of permethrin than the parent compound, so risk assessment focuses on permethrin (PMRA 2017).

For the mayfly *Hexagenia bilineata,* a 48-h EC50 of 0.1 μg/L was reported, and in a life-cycle test with *D. magna,* the NOAEC and LOAEC for reproduction and growth were 0.0047 μg/L and 0.084 μg/L, respectively (USEPA 2007, PMRA 2017). Permethrin was also very highly toxic towards the marine mysid shrimp

(*A. bahia*) resulting in a reported 96-h LC50 value of 0.019 μg/L and 30-day life-cycle LOAEC for reduced survival at 0.024 μg/L (USEPA 2007); while the LOAEC value would be expected to be lower than the 96-h LC50, these data remain among the few calculated for a marine invertebrate and thus are worth reporting for comparison. Aquatic invertebrate HC5 values were calculated using data for freshwater ($n = 25$ acute endpoints) and estuarine/marine ($n = 11$ acute endpoints) invertebrates; these were 0.019 μg/L and 0.002 μg/L, respectively (PMRA 2017). In the benthic invertebrates *C. dilutus* and *H. azteca,* reported 10-day LC50 values for permethrin in sediments were 24.5 $\mu g/g_{oc}$ (Maul et al. 2008) and 4.88 $\mu g/g_{oc}$ (Amweg et al. 2005), respectively. However, given the tendency for permethrin to sorb strongly to sediment, toxicity testing and monitoring of sediments for permethrin remain a relative knowledge gap specific to this active ingredient.

Beyond survival, exposure to permethrin caused changes in other endpoints, particularly growth, in benthic invertebrates. The EC50 for chironomid immobilization was 11.5 $\mu g/g_{oc}$ and IC50 values for significant reductions in ash-free dry mass and instantaneous growth rate were 27.4 and 27.2 $\mu g/g_{oc}$, respectively (Maul et al. 2008). Growth of *H. azteca* was significantly inhibited following 10-day exposure to concentrations ranging from 0.68 to 5.3 $\mu g/g_{oc}$ (Amweg et al. 2005).

An in-situ exposure was conducted in a Wyoming stream to investigate effects on non-target invertebrates of permethrin application via typical municipal fogging for mosquito control. Measured concentrations in the stream were <0.25 μg/L (<LOD), but resulted in a significant increase in drifting aquatic invertebrates and decrease in benthic invertebrate biomass downstream of the application site (Wurzel et al. 2020). The authors noted that a high number of taxa were included in the drifting biomass, so it was not only the traditionally "sensitive" species that were affected, but also the community assemblage (Wurzel et al. 2020).

Acute toxicity data are available for a number of fish species, including greenback cut-throat trout (*Oncorhynchus clarkistomias)*, white sucker (*Catostomus commersonii*), largemouth bass, and rainbow trout (PMRA 2017). Among the most sensitive endpoints used for risk assessment were the reported 96-h LC50 for bluegill sunfish was 0.79 μg/L and the marine Atlantic silverside (*Menidia menidia*) value of 2.2 μg/L (USEPA 2007). Reduced survival was observed in a fathead minnow full life-cycle test at an LOEC of 0.41 μg/L and in a 28-day early-life-stage test with sheepshead minnow at an LOEC of 10 μg/L (USEPA 2007, PMRA 2017). Sufficient data were available to derive HC5 values for freshwater ($n = 30$ acute endpoints) and estuarine/marine fish ($n = 10$ acute endpoints); these were 1.2 μg/L and 2.38 μg/L, respectively (PMRA 2017).

The octanol-water partition coefficient for permethrin is relatively high (log K_{ow} of 6.1), suggesting that permethrin would bioaccumulate in aquatic organisms (USEPA 2007, PMRA 2017). Field- and lab-derived bioaccumulation or bioconcentration factors range from 114 to 2,714 and 30 to 1,100, respectively. Additionally, there is evidence of both bioaccumulation in benthic invertebrates and biomagnification in marine wildlife (PMRA 2017). The current marine CWQG-PAL is 0.001 μg/L (CCME n.d.), which is consistent with the calculated HC5 values, but no North American sediment benchmarks are currently available.

Exposure Risks for Aquatic Organisms

Permethrin is among the most commonly applied pyrethroids currently used in Canada, with sales quantities <100,000 kg a.i. ((Health Canada 2017, 2020). Permethrin was deemed among the top five pesticides posing risks to the aquatic environment in the UK, based on measured concentrations and toxicity profiles (Johnson et al. 2017). It the recent re-evaluation of permethrin, PMRA (2017) concluded that concentrations in Canadian waters did occur at levels that could pose risks to invertebrates, fish, and amphibians (shown in Fig. 2), though infrequently. As such, spray buffer zones and 10 m vegetative filter strips were proposed as new mandatory requirements to protect aquatic environments (PMRA 2017), which would be expected to reduce concentrations in Canadian aquatic environments.

However, there is still a paucity of water and sediment monitoring data with appropriate detection limits for permethrin. In addition, the HC5 values calculated by PMRA for aquatic invertebrates (0.019 and 0.002 μg/L for freshwater and estuarine/marine, respectively) suggest that the current CCME marine guideline of 0.001 μg/L might not be protective of the most sensitive ~5% of non-target invertebrate species. As concluded by USEPA (2020c), the primary risks of permethrin to the aquatic environment are for aquatic invertebrates, but under certain use patterns, fish could also be affected, particularly in the context of cumulative risks from pyrethroid and pyrethrin insecticides as a class with a common mode of action. The data in Fig. 2 also demonstrate that reported effects endpoints for fish could be surpassed in Canadian waters, and effects to fish could occur both directly and via indirect effects on food. As discussed in further detail in Sect. 4.4, monitoring of permethrin in the Canadian aquatic environment requires additional considerations and methodological improvements.

3.3.3 Fungicides

Chlorothalonil

Chlorothalonil is used as both a contact fungicide for a wide range of agricultural crops (including stone fruits, highbush blueberries, potatoes) and on turf, and as a material preservative in paint (PMRA 2018b). Chlorothalonil acts by deactivating the antioxidant co-enzyme glutathione through chemical reduction which inhibits spore formation in fungi (USEPA 2012).

Presence in the Aquatic Environment

Generally, environmental fate data suggest that chlorothalonil is rapidly transformed under both aerobic and anaerobic water/sediment systems (USEPA 2012). Chlorothalonil readily degrades in the aquatic environment, with reported half-lives ranging from 0.18 to 8.8 days but there is potential for adsorption to sediment

or suspended materials (ECCC 2011). Soil half-lives (estimated between 33 and 81 days) suggest that chlorothalonil could remain available for runoff for weeks to months after application (USEPA 2012). However, the fate dataset was found to be of insufficient quality for risk assessment for many of the environmental transformation pathways and additional studies are needed (USEPA 2012).

In national surface water monitoring, chlorothalonil was among the top 5 active ingredients most likely to exceed CCME guideline values, particularly in British Columbia and the Atlantic region (ECCC 2011). It has also been detected in Arctic lakes at concentrations up to 2.8 ng/L (Hoferkamp et al. 2010).

Toxicity Towards Aquatic Organisms

Few data are available for aquatic plants, but acute toxicity EC50 values for freshwater diatoms and vascular plants (duckweed, *L. gibba*) submitted to UESPA for registration review were 12 and 640 µg/L, respectively, while NOAEC values were 3.9 and 290 µg/L (USEPA 2012). Chlorothalonil was classified as acutely very highly toxic towards fish and invertebrates in a USEPA ecological risk assessment (USEPA 2012, 2017a). A weight-of-evidence Tier 1 screening concluded that chlorothalonil does not exert toxicity via interactions with oestrogen, androgen, or thyroid pathways in fish or mammals (USEPA 2015b).

Overall, for invertebrates, crustaceans, and molluscs were sensitive to chlorothalonil at concentrations ranging from 1.8 µg/L to >10,000 µg/L (CCME 1999c). Acute (48-h EC50) and chronic (21-day NOAEC) toxicity endpoints for *D. magna* were 54 and 0.6 µg/L, respectively. The shell deposition of Eastern oyster (*C. virginica*) was affected at even lower exposure thresholds, with a reported 96-h EC50 of 3.6 µg/L (USEPA 2012). A laboratory study of field-collected soft-shell clams (*Mya arenaria*) reported a chronic LC50 of >100 µg/L, and no significant induction of haemic neoplasia at this concentration after 35 days (Pariseau et al. 2009). As reported for chlorpyrifos and phorate, exposure of molluscs to chlorothalonil resulted in significant inhibition of AChE activity. A LOEC of 10 µg/L was reported for gill AChE activity inhibition in two marine species, Pacific oyster (*Magallana gigas*) and bay mussel *M. edulis* (Haque et al. 2019). Exposure to 100 µg/L of chlorothalonil induced an eight-fold reduction in egestion rates in the aquatic gastropod *P. acuta*, but had no significant effects on another freshwater snail species (Elias and Bernot 2017). Likewise, 100 µg/L significantly induced AChE activity in the estuarine polychaete *Laeonereis acuta*, as well as increased lipid peroxidation (da Silva Barreto et al. 2018).

Acute and chronic toxicity data were available for a number of fish species. Reported literature values for acute toxicity (96 h-LC50) in rainbow trout ranged from 10.5 to 195 µg/L, and chronic effects on survival and behaviour occurred at concentrations above 2.3 µg/L (CCME 1999c). A freshwater NOEC of 1.3 µg/L was reported from a study with fathead minnow (USEPA 2012). Consistent with this, a more recently published standard full life-cycle study conducted with fathead minnow reported a reproductive NOEC of 1.4 µg/L (Hamer et al. 2019). In addition, pulsed exposures (up to 3 pulses, 6 h to 11 days in duration) at concentrations up to

15.5 µg/L did not result in significant effects on fish fecundity (Hamer et al. 2019). A recent study with zebrafish embryos reported 21.9% mortality after 96-h exposure to 50 µg/L chlorothalonil and 57.3% mortality for the same concentration of 4-hydroxychlorothalonil, suggesting that the metabolite is more acutely toxic to fish than the parent (Zhang et al. 2016). For the marine three-spine stickleback (*Gasterosteus aculeatus*), chlorothalonil 96-h LC50 concentrations ranged from 27 to 4,700 µg/L (CCME 1999c). However, USEPA deemed acute and chronic toxicity of marine fish a data gap for which there were not acceptable studies available (USEPA 2012). Since the 2012 assessment, a study of early-life stage Pacific sockeye salmon (*Oncorhynchus nerka*) was conducted to investigate effects of chlorothalonil on development timing and success. Exposure to 5 µg/L reduced survival to hatch and increased incidence of finfold deformities and delayed hatch (Du Gas et al. 2017). Treatments of both 0.5 and 5 µg/L resulted in premature emergence (Du Gas et al. 2017). Sperm motility in estuarine guppy *(Poecilia vivipara*) was similarly sensitive, with significant effects observed after 96-h exposure to 1 or 10 µg/L of chlorothalonil (Chaves et al. 2020).

The CCME guidelines for protection of aquatic life are 0.18 µg/L in freshwater and 0.36 µg/L in marine environments for chlorothalonil total (including its 4-hydroxy transformation product) (CCME 1999c, Fig. 3). These were derived from the most sensitive chronic endpoints, a 22-day LOEC of 1.8 µg/L in *D. magna*, and 96-h EC50 of 7.3 µg/L in Eastern oyster (*C. virginica*), with safety factors of 0.1 and 0.05 applied (CCME 1999c).

The reported range of octanol-water coefficients for chlorothalonil (log K_{ow} of 2.88 to 3.8, Table 4) suggests some potential for bioaccumulation (PMRA 2011). Bioconcentration studies in fish and oysters reported bioconcentration factors (BCF) of 9 to 5,812 and 2,600, respectively, suggesting that these organisms can adsorb chlorothalonil into their tissues to some extent (USEPA 2012; PMRA 2011). However, depuration from fish tissues was fairly rapid (31–35% in the first day) following cessation of exposure (USEPA 2012) and parent chlorothalonil is not expected to bioconcentrate appreciably (PMRA 2011).

Exposure Risks for Aquatic Organisms

Sales in Canada in 2017 exceeded 1,000,000 kg of chlorothalonil and 500,000 kg in 2020 (Health Canada 2017, 2020), and concentrations of chlorothalonil have been measured in Canadian water bodies above the CCME freshwater guideline, as well as above effects concentrations reported in sensitive taxa (Fig. 3). Canadian incident reports collected by PMRA provide evidence of heavy rainfall events resulting in significant runoff of chlorothalonil and concentrations reaching levels of concern for fish (PMRA 2018b). In 2016, there were three environmental pesticide incidents reported to PMRA, including one major incident, that were attributed to runoff of chlorothalonil that resulted in fish mortality (PMRA 2016). It was found that two of these incidents related to runoff from Prince Edward Island potato fields following application of chlorothalonil according to label directions (PMRA 2018b). In response, PMRA initiated a Special Review regarding the environmental fate and

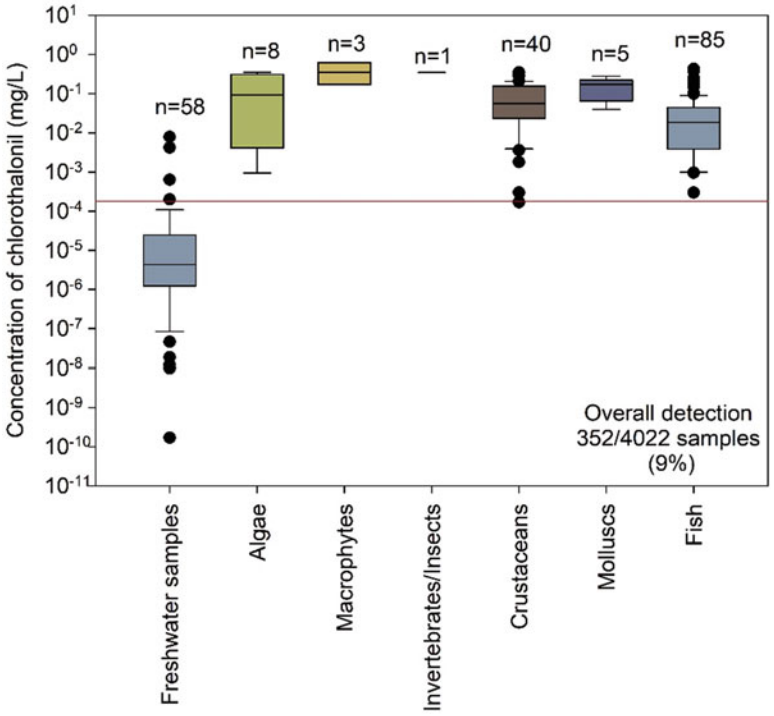

Fig. 3 Comparison of detectable concentrations of the fungicide chlorothalonil measured in Canadian freshwater samples with effective concentrations (LCXX, ECXX, LOEL, LOEC values, where XX can be any number, e.g., LC10, EC50) reported in the ECOTOX database for aquatic toxicity tests using algae, invertebrates/insects, fish, molluscs, and crustaceans. Horizontal lines within each box indicate the 25th, 50th, and 75th percentiles of measurements reported, while the tenth and 90th percentiles are indicated by the whiskers (note: concentrations <LOD are not included, and values reflect data available in raw and summary form. The *n*-value reflects the number of measured concentrations >LOD or the number of toxicity data records). The red line represents the Canadian Water Quality Guideline for the Protection of Aquatic Life (CWQG-PAL). The overall detection is the per cent of samples in which chlorothalonil was detected. The difference between *n* and the total number of samples used in the calculation of the overall detection is due to studies reporting a summary statistic of concentration, e.g., mean, the frequency of detection, and the total number of samples collected without providing the raw data

ecotoxicology of chlorothalonil with regard to agricultural and turf uses (PMRA Re-evaluation Decision RVD2018-11). A separate review of the use in paint coatings is currently underway (REV2018-02).

The risk assessment by PMRA found that there could be risks of chlorothalonil to aquatic organisms, particularly fish (PMRA 2018b). As a result, label and registration risk mitigations have been updated, including reducing the number of allowable applications per year for potatoes (from 12 to 3), in an effort to reduce risks to aquatic organisms (PMRA 2018b). Continued collection of monitoring data will

support PMRA in future assessments of the success of this approach in preventing adverse effects to receptors in Canadian aquatic habitats.

4 General Knowledge Gaps and Recommendations

For well-studied pesticides such as atrazine and chlorpyrifos, continued monitoring of both concentrations in the receiving environment and responses of communities there is required, not necessarily more research to fill data gaps. For the other active ingredients reviewed in this exercise, additional measured concentrations in water and/or sediment are needed to provide coverage across the broad expanse of receiving waters in Canada. In addition, a number of broader issues came to light that would not necessarily be specific to Canada alone or any one active ingredient. However, given the specific geographical, regulatory, and biophysical context of this country, addressing these higher-level knowledge gaps would improve our confidence in pesticide risk assessment and future refinement of the presented prioritization.

4.1 Baseline Fish and Fish Habitat Data

Monitoring of aquatic communities for abundance, diversity, and community-level variability is needed to establish baseline conditions for Canadian fisheries and place environmental chemistry and ecotoxicology data in context (Johnson et al. 2020; Johnson and Sumpter 2016). Without a strong understanding of typical conditions and natural fluctuations that can be expected within populations of fish, invertebrates, zooplankton, phytoplankton, and aquatic plants, it is not possible to detect changes as a result of pesticide exposure. This is particularly relevant in regions where agricultural pressures are strong, and thus, presence of pesticides is more likely, and where those pressures coincide with sensitive life stages (e.g., hatching and/or populations). Toxicity assays seek to, under controlled conditions, predict the concentrations of a pesticide that could induce significant changes to non-target receptors; however, these are not representative of real-world conditions. As such, ground-truthing of toxicity endpoints with field monitoring of water (and sediment, as relevant) is an important piece. While some studies have correlated measured concentrations of pesticides with observed changes in non-target aquatic invertebrate populations (e.g., Bartlett et al. 2016; Bashnin et al. 2019), this remains an important data gap for managing risks to fish, as noted previously by others (e.g., Scholz et al. 2012).

4.2 Pesticide Monitoring Data

Monitoring of surface waters for pesticides in Canada by provincial and federal agencies is a challenging undertaking requiring intentionality to address the existing gaps. For example, there is limited "on the ground" presence in the provinces of Manitoba or Saskatchewan of the federal department of Environment and Climate Change. For context, these are prairie regions with extensive agricultural areas, and Manitoba receives waters from across the Canadian and U.S. prairies/Midwest into some of the world's largest lakes (Environment Canada and Manitoba Water Stewardship 2011). While federal monitoring is limited, there is a strong ENGO-run community-based water monitoring network for Lake Winnipeg that could help to bridge the gap (Lake Winnipeg Data Stream 2021). To do so effectively, we propose that there needs to be clear communication, data-sharing understandings, and "apples to apples" sampling and reporting so that larger data products can be developed smoothly.

In another example, in the province of Ontario, the Ontario Ministry of the Environment, Conservation, and Parks is doing their best with the resources they have, but their monitoring program consists of taking 4–10 water grab samples per year from 18 to 20 sites across Ontario. The federal government has conducted pesticide monitoring in surface waters through the National Water Quality Pesticides Surveillance Program (Government of Canada 2016). However, the number of sites monitored across the country, the frequency of sampling at each site in a year, and the availability of the data is not clear (Government of Canada 2016). In contrast, the German Federal Environment Agency (Umweltbundesamt) conducts surveillance and operational monitoring (Arle et al. 2016). Surveillance monitoring assesses long-term changes in water quality over a relatively large scale, i.e., within a river catchment or sub-catchment (up to 2,500 km^2). Germany's surveillance monitoring includes more than 500 monitoring stations located in major rivers or major tributaries across the country. These sites are sampled 4–13 times in a year every 6 years (Arle et al. 2016). Operational monitoring involves more intensive sampling of water bodies that may be at greater risk of exceeding water quality guidelines. In Germany, the operational monitoring programs involve 10,000 stations along river and streams that are sampled 4–13 times in a year every 3 years (Arle et al. 2016).

The shortcomings of pesticide monitoring in Canada can be illustrated in Health Canada's PMRA listing monitoring data as a source of uncertainty in their recent risk assessment of the two neonicotinoid insecticides thiamethoxam and clothianidin to aquatic invertebrates (PMRA 2018a, c). In order to understand the risk that pesticides could pose to Canadian fisheries and aquatic ecosystems, comprehensive open-access data on exposure of Canadian aquatic ecosystems to pesticides is needed. A centrally managed, bilingual (reflective of the English and French official languages of operations in federal and provincial governments and academic institutions in Canada), up-to-date repository for pesticide data would help to integrate results from different sampling programs and allow the cumulative data to be used by all interested parties.

Canada would benefit from a collaborative and sustained monitoring program that incorporates knowledge of land use, agronomic practices, seasonal variation, pesticide fate, and sensitivity of aquatic species or communities to decide when and where to sample a representative variety of receiving water bodies. There is also a need for such programs to consider monitoring surface waters following heavy precipitation, irrigation, and/or snowmelt events, as these events have been shown to increase the probability of pesticide movement from agriculture areas into surface water (Waite et al. 1992; Guo et al. 2007; Davis et al. 2013).

4.3 Marine and Sediment Benchmarks

For many of the pesticide analytes of interest, Canadian benchmarks (freshwater and/or marine) do not yet exist to help researchers and policy makers put measured environmental concentrations into context (PMRA 2019c; Metcalfe et al. 2019; Johnson et al. 2020). In the present review, freshwater CWQG-PALs were only found for five of the seven important priority active ingredients; of these, only two (atrazine and chlorothalonil) also had a marine CWQG-PAL, and none had sediment guidelines (CCME n.d.). There are locations in Canada where coastal agriculture is established, and pesticide concentrations in these areas may be important for marine species.

Johnson et al. (2020) posited that there are gaps in terms of chronic toxicity, persistence, and bioconcentration for most registered chemicals in Europe and North America, which is consistent with the findings of the ECOTOX database review and regulatory findings for many of the active ingredients highlighted by the current exercise. This is particularly true for marine waters and for sediment, although only some current-use pesticides will be expected to partition into sediments, and only some compounds have been analysed for in sediments. In an attempt to address this gap, Nowell et al. (2016) recently developed proposed sediment-toxicity benchmarks for 129 current-use pesticides using the model amphipod *H. azteca* and insect *C. dilutus* as benthic invertebrate models. This work should continue, as appropriate for the specific physicochemical properties and use patterns for individual active ingredients.

4.4 Mixture Toxicity

Pesticides are frequently detected as mixtures in surface waters and sediments (e.g., ECCC 2011; Harris et al. 2008; Metcalfe et al. 2016, 2019), indicating that aquatic biota can be exposed to more than one active ingredient simultaneously, as well as other environmental contaminants. For example, Baldwin et al. (2016) collected water samples ($n = 709$) from 57 tributaries of the Great Lakes between 2010 and 2013 for analysis of organic contaminants. At 35% of sites and in 34% of samples,

there were ten or more compounds detected within a single sample, typically a combination of PAHs, flame retardants, caffeine, detergents, and/or pesticides. Atrazine was the most frequently detected pesticide and exceeded aquatic toxicity benchmark values at some sites, as did dichlorvos and carbaryl (Baldwin et al. 2016). Similarly, sampling in the Niagara Peninsula in 2004 to 2006 revealed frequent presence of atrazine, metolachlor, simazine, 2,4-D, mecoprop, dicamba, and clopyralid (Bartlett et al. 2016). In corn and soybean-dominated regions of Quebec, glyphosate, nicosulfuron, imazethapyr, bentazon, and dicamba were detected in over 60% of river water samples, while atrazine and metolachlor were present in nearly all samples collected (Giroux 2010).

Further compounding this issue, it is also common for pesticides to be applied as mixtures; for example, MCPA is typically applied in combination with other chemicals such as 2,4-D (USEPA 2014). Neonicotinoids are also often detected in mixtures due to widespread use and the degradation of thiamethoxam to clothianidin (Maloney et al. 2018). Concentrations of pesticides can also be very seasonally driven (i.e., by precipitation patterns and application schedule, Baldwin et al. 2016, Giroux 2015, 2019), so timing of sampling in the context of mixtures is important. Additionally, samples have also often indicated the presence of other stressors, including excess nutrients, metals, and/or pharmaceuticals and personal care products (Bartlett et al. 2016) and these multiple stressors can have additive, synergistic or even antagonistic effects on aquatic biota (Liess et al. 2019).

Several field and laboratory studies have examined potential effects of mixtures on aquatic non-target organisms. For invertebrates, for example, using *C. dilutus*, Maloney et al. (2018) demonstrated weak synergism of neonicotinoid mixtures and deviation from the concentration additive reference model. Chlorpyrifos (0.17 μg/L) and terbuthylazine (8.5 μg/L) had no effects on feeding rates of the planktonic crustacean *D. magna* during individual 28-day exposures, but when applied as a mixture, feeding rates were reduced by over 50% compared to controls (Pereira et al. 2017). In-situ caging studies with the amphipod *H. azteca* revealed effects of pesticide mixtures on survival and AChE activity. Organophosphate insecticides were deemed to be the likely drivers of toxicity, though excess nutrients and metals may have also acted in conjunction since these exceeded guideline values at some sites (Bartlett et al. 2016). At a community level, the Albemarle-Pimlico Estuarine System in the USA experienced substantial losses in submerged aquatic vegetation community which could be attributed to herbicide mixtures of atrazine, alachlor, and metolachlor (Powell et al. 2017).

While Canadian guidelines for the protection of aquatic organisms are available for some of these compounds, there remains a monumental challenge to assess the potential toxicity of mixtures, which can include pesticides, and approaches are needed to consider total pesticide burden (Metcalfe et al. 2019; Cruzeiro et al. 2017; Bopp et al. 2019; Kienzler et al. 2016). Mixture toxicity and risk assessment of multiple stressors or toxicants was universally highlighted as a top research priority at workshops held with scientists in North America (Fairbrother et al. 2019), Europe (Van den Brink et al. 2018), and Latin America (Furley et al. 2018), and represents a substantial gap in our understanding of the potential effects of current-use pesticides

on Canadian aquatic biota. One proposed response has been to determine those compounds that pose the greatest risk (or "drivers of toxicity") and test mixtures for those that would reasonably be expected to co-occur (Johnson et al. 2017; Van den Brink et al. 2018). A number of initiatives and studies have been undertaken to address chemical mixtures and their assessments in the environment (Bopp et al. 2018; PMRA 2019a, Verbruggen and Van den Brink 2010, Maazouzi et al. 2016) but further work is needed to identify mechanistically how combinations of active ingredients could have synergistic effects and under what specific field conditions. It should be noted here that all 55 pesticides that were screened into the present long list of current-use compounds, and particularly the 29 top-priority ones, may be important components of mixtures based on their use volumes and presence in the environment.

4.5 Study Design and Analytical Methods for Compounds with Low Benchmarks

Pesticides can be both present in the environment and biologically active at very low concentrations, necessitating sensitive analytical methods as part of the monitoring and risk assessment of these compounds. In the 2018–2019 annual report from PMRA, high limits of detection were among the key challenges identified (PMRA 2019a). The need for sensitive and reliable analytical chemistry methods to support contaminants of emerging concern (including pesticides and their metabolites and degradation products) was also one of the top priorities identified by Fairbrother et al. (2019) and Furley et al. (2018) in global surveys of environmental scientists.

Permethrin is one active ingredient that has posed a challenge for water quality monitoring programs and regulatory risk assessment as a result of inadequate method detection limits. In the recent re-evaluation review performed by PMRA, it was stated that, "*available Canadian water monitoring data are not robust enough to fully characterize the risks to aquatic invertebrates because 2405 of 2600 samples (93%) of the samples collected and analyzed for permethrin had limits of detection that were higher than the toxicity endpoint for aquatic invertebrates (HC5 = 0.019 µg/L). The analytical methods were not sensitive enough to capture detections of permethrin in water that could potential be a concern to aquatic invertebrates*" (PMRA 2017). Without scientifically rigorous and defensible monitoring data, true risks for environmental receptors for permethrin (and other active ingredients) cannot be determined.

Giroux (2015) noted that chlorpyrifos and diazinon presented particular challenges for achieving appropriate method detection limits due to their relatively low guideline values and potential for toxicity at fractions of a microgram per litre. For example, in Baldwin et al. (2016), the method detection limits reported for chlorpyrifos and diazinon were both 0.16 µg/L and the lab reporting limits were up to 0.32 µg/L, while the EPA Aquatic Life Benchmarks (maximum concentration) for

these compounds are 0.083 μg/L and 0.17 μg/L, respectively (USEPA 2019a), and the short-term CCME water quality guideline for chlorpyrifos is 0.02 μg/L (CCME 2008). With potential for acute toxicity of chlorpyrifos towards invertebrates at concentrations as low as 0.05 μg/L (Giddings et al. 2014), analytical methodologies need to be appropriate and sufficient to support these compounds with very low concentrations and correspondingly low toxicity endpoint concentrations.

There is a need to consider which target compounds will be analysed for and how, when sampling will take place, and the way in which samples will be collected, as these can all affect aquatic sampling results and the broader interpretation of potential risks for non-target aquatic organisms (Metcalfe et al. 2016, 2019). Grab samples typically have relatively small volumes of water, presenting a challenge to detect very low concentrations of target analytes. By comparing calculated concentrations of CECs obtained using three different passive sampling devices, Alvarez et al. (2014) concluded that a combination of samplers would provide the most useful characterization of contaminants in aquatic environments. However, the authors also noted that the use of biota for CEC monitoring (i.e., body burdens) would not be particularly informative, given the hydrophilic nature of many of these compounds (Alvarez et al. 2014).

Timing and location of sampling are important considerations for study design. For example, limited monitoring data were available for phorate from federal monitoring programs, and most results were below the limit of detection (ECCC 2011; Government of Canada 2016). However, data were absent for the Atlantic region, despite phorate being among the top pesticides sold in P.E.I. (PEI EWCC 2015; Lichtenberger 2017). While it is sold at relatively low volumes, a science-based benchmark was proposed by ECCC (2011) for phorate at 0.03 μg/L, reflecting the relatively high toxicity towards aquatic organisms. A PMRA re-evaluation review is scheduled to begin in 2020–2021, for which monitoring data for evaluating exposure risks under current-use patterns and label conditions, as well as toxicity data to fill any gaps, will be necessary.

Several studies have noted that greater concentrations of pesticides were measured in tributaries and wetlands compared to mainstem waterways (e.g., Sheedy et al. 2019; Montier-León et al. 2019), necessitating consideration of where samples should be collected from within a system. Also, it can be necessary to consider inputs from the USA or other provinces. For example, the Lake Winnipeg watershed integrates inputs from agriculturally intensive regions of the USA and Canada. Challis et al. (2018) reported that the USA seemed to be a major source of atrazine into the Red River in Manitoba. Neonicotinoid loadings also suggested inputs from both sides of the border. Like the Great Lakes, a large portion of the Red River watershed (nearly 70%) is located in the USA, but less work has been done to characterize pesticide inputs in this watershed (Challis et al. 2018).

Recommendations developed for regulatory risk assessment and monitoring of pesticides in Northern Europe by Stenrød et al. (2016) could perhaps apply to the diverse geographical, agricultural, and sociopolitical landscapes present across Canada, particularly with regard to interprovincial or international cooperation. Specifically, the authors call for establishing streamlined information sharing

platforms, evaluating current studies for their utility in risk assessment (and modifying accordingly in future studies), and characterizing the conditions present in each region to adapt local sampling programs within the umbrella of a larger coordinated program (Stenrød et al. 2016). Better cooperation and coordination in monitoring efforts across government, academic, and grass-roots organizations will improve the quality and availability of pesticide data, helping to optimize use of limited resources across the vast expanse of Canadian waters.

4.6 Habitat and Food Web-Mediated Effects on Fish

For active ingredients that are not acutely toxic to fish but instead exert greater toxicity towards plants and insects, there is generally a need to better understand whether impacts to primary producers and invertebrates will translate to indirect effects on fish populations. These indirect effects are more difficult to attribute to pesticide exposure. Given that trends in pesticides are moving towards more targeted chemistries, often with plant or invertebrate targets, greater toxicity towards habitat structural species (aquatic plants), and prey species including phytoplankton, insects, crustaceans, and/or molluscs compared with fish may be expected for many active ingredients. This was observed often in the review, but potential impacts to fish populations as a result of impacts to lower trophic levels are unclear.

The pulsed nature of pesticide use and thus input into local receiving waters necessitates consideration of chronic or sub-chronic endpoints in these sensitive organisms, as well as integrating an evaluation of recovery (Alvarez et al. 2019; Kattwinkel et al. 2015; Raby et al. 2018c). Repeated applications, mixtures, and timing (e.g., are sensitive life stages present?) should also be considered in study design to replicate conditions in the field, and models represent potential tools to help elucidate indirect effects to fish.

4.7 Current-Use and Legacy Pesticides in the Arctic

Recent studies suggest that current-use pesticides can be detected in Arctic media, but concentrations are typically relatively low compared to legacy compounds (e.g., DDT, chlordane, PCBs; Balmer et al. 2019, Brown et al. 2018, Cabrerizo et al. 2018, 2019). Generally, the physical-chemical properties common among the current-use pesticides measured in the Arctic are: high octanol-air partitioning, intermediate lipid solubility, low air-water partitioning (allowing long-range transport, perhaps some movement via ocean currents), and moderate to low water solubility (Balmer et al. 2019; summarized for currently discussed pesticides in Table 4). Modelling exercises reported by Balmer et al. (2019) suggest that nitrapyrin, picloram, nitrofen, 2,4,6-trichlorophenol, and dinoseb have the potential to reach the Arctic via long-range transport, but these have not yet been investigated in Arctic media. Balmer

et al. (2019) also reported that seven new current-use pesticides have been measured in Arctic media since 2010 – MCPA (2-methyl-4-chloro-phenoxyacetic acid), metribuzin, pendimethalin, phosalone, quizalofop-ethyl, tefluthrin, and trillate. Of these, MCPA, pendimethalin, and trillate are considered "high production volume chemicals" or those that are produced or imported at >1,000 t per year (Balmer et al. 2019). Sea-ice in the Arctic is a unique environmental compartment requiring additional consideration; it behaves as a lid over the ocean, potentially collecting pesticides which are released in a pulse when it melts (Bigot et al. 2017). In a study by Pućko et al. (2017), concentrations of dacthal, a pre-emergence herbicide used for control of grasses and some broad-leafed weeds, were deemed to pose a potential risk to Arctic marine organisms. Specifically, measured concentrations of dacthal in melt-pond water were much greater than those in seawater under ice, and the entry of the pulse into seawater was observed to coincide with spring blooms of under-ice phytoplankton, potentially posing a risk. To monitor which current-use pesticides may become contaminants in the Arctic, strategic environmental monitoring should be ongoing and increased effort in modeling which pesticides may reach the Arctic would be beneficial.

5 Conclusions

The specific active ingredients reviewed represent some of the most widely applied and detected pesticides in Canadian waters based upon the available sales and monitoring information. As would be expected, pesticide classes generally exhibited aquatic toxicity consistent with their uses and targets: herbicides were typically most toxic to algae or macrophyte species, insecticides were highly toxic to invertebrate species, and fungicides were toxic across taxa. As such, monitoring for effects in the aquatic environment should also be strategic to determine baseline conditions and changes in those organisms or classes most likely to be affected by the active ingredient(s) of interest. Generally, we found that toxicity data were available to support regulatory review, but gaps exist in our understanding of fate, species sensitivity distribution, and/or surface water concentrations for many of the active ingredients identified as among the top-priority active ingredients. It should be noted that the top-priority pesticides highlighted in this review represent a snapshot in time, and this exercise can and should be revisited to reflect changing use patterns, toxicity and monitoring data availability, and regulation. In addition, our selection of the top 7 national priority pesticides does not preclude consideration of the balance of the 55 active ingredients screened into the review, especially those scoring in the top half or third of the list. Some of these may be particularly relevant under mixture scenarios or where their local or regional use may be high. Through this review, it became clear that there are many opportunities for collaborations across Canadian provincial and federal agencies, as well as with academic and industry partners, to fill important data gaps that have been identified here and elsewhere. Doing so will support informed pesticide use in Canada and ongoing efforts to avoid unintended impacts to the Canadian aquatic environment.

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