

The State of the Art on the Potential Human Health Impacts of Microplastics and Nanoplastics

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ENVIRONMENTAL PROTECTION AGENCY

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- Office of Radiation Protection and Environmental Monitoring
- Office of Communications and Corporate Services

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EPA RESEARCH PROGRAMME 2014–2020

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EPA Research Report

Prepared for the Environmental Protection Agency

by

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The EPA Research Programme addresses the need for research in Ireland to inform policymakers and other stakeholders on a range of questions in relation to environmental protection. These reports are intended as contributions to the necessary debate on the protection of the environment.

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Executive Summary

Global plastic production has increased considerably since the mid-20th century, with 400 million tonnes of plastic produced annually. Plastic waste in Ireland predominantly ends up in landfill, although some waste is incinerated, recycled or lost to the environment. Plastic is accumulating in water bodies, where it can persist for several hundred years, degrading into smaller particles known as microplastics (MPs) and nanoplastics (NPs). Humans are ubiquitously exposed to MPs and NPs from everyday sources such as drinking water, food, synthetic fibres and the atmosphere. This report is formed from an extensive literature review that addresses three fundamental questions: (1) What are the routes of human exposure to MPs and NPs? (2) What is the range and mid-level of human exposure (and how do we measure it)? and (3) What are the potential human health impacts of MPs and NPs? A brief review of current policies and legislation regarding plastic and plastic waste was also completed.

The PECO (Population, Exposure, Comparison and Outcome) framework was used to develop an effective search protocol for the review. To be eligible for inclusion in this review, we required research articles to be original articles written in English, French, Arabic or German and published from 1999 to 2019 in peer-reviewed journals or government or non-governmental reports. All study designs were considered, including studies conducted on humans, aquatic animals, experimental models and mammalian tissues. Publications were included if the outcomes measured linked to routes of exposure and human health impacts. The search strategy employed a combination of advanced search techniques, while a standard data collection form was designed to systematically extract data from each study.

The report finds that the major routes of human exposure to MPs and NPs examined in the literature are inhalation, ingestion and dermal contact, with most studies focusing on the ingestion of MPs and NPs. In the case of oral exposure, the majority of published studies examined the presence of MPs and NPs in food sources, primarily in shellfish and fish, and there is strong evidence to show that humans are

consuming MPs and NPs as part of a seafood diet. Fewer studies have examined the presence of MPs and NPs in other foodstuffs such as honey, chicken, sugar, salt, teabags, milk, seaweed, and some fruit and vegetables. Further research is needed to evaluate the presence of MPs and NPs in major food groups, which should also include beef, poultry, dairy, fruit, vegetables and grains. Similarly, there is strong evidence that humans are ingesting MPs and NPs in drinking water. More recently, atmospheric fallout of MPs was reported as a possible inhalation exposure pathway, and further studies are required.

The key characteristics for MP/NP analysis fall into two groups: physical (size, shape and colour) and chemical (plastic type). MP/NP analysis is complex, consisting of extraction, isolation, identification and quantification techniques. Any method that reliably measures both physical and chemical characteristics is preferable, and often a combination of multiple methods is required – usually microscopy followed by spectroscopic or thermal analysis. Many different analytical methods are used throughout the literature, and their performance for MP/NP characterisation needs to be optimised as each method has specific limitations. Therefore, standard protocols must be developed and validated in order to detect, identify and quantify MPs and NPs, particularly when they are present in complex environmental, biological or food matrices.

Owing to a lack of studies, there is insufficient information assessing the level of risk in humans following exposure to MPs and NPs, and reliable estimation of the total mean daily levels of exposure is as yet lacking. In humans, occupational exposure among textile workers is associated with respiratory disease, lung cancer and large bowel cancer, while MPs have also been found in lung tumours. MPs may cause harm to humans via physical and chemical pathways; however, there is a lack of direct evidence linking MPs to adverse effects on human health. No studies have directly measured the levels of MPs and NPs retained in human tissues following exposure to or the translocation of MPs and NPs from the site of entry to distal tissues such as liver, kidney and muscle.

Most research on the toxic effects of MPs has focused on aquatic organisms, with several studies evaluating the adverse effects of MPs in experimental models and *in vitro* mammalian systems. Adverse effects in these

model systems include immune modulatory, cytotoxic, genotoxic and neurotoxic effects. Given these findings, further studies in human populations and model systems are a priority.

1 Introduction

1.1 Background

Plastics are typically synthetic organic polymers created by the process of polymerisation of monomers extracted from hydrocarbons. Plastic is cheap to manufacture and is a highly versatile, lightweight material that has countless applications in all aspects of everyday life, including food packaging, consumer products, medical devices and construction (Andrady and Neal, 2009). Large-scale plastic production began in the 1950s and has increased enormously since then, from 1.9 tonnes in 1950 to an estimated 360 million tonnes globally in 2018, of which 62 million tonnes was produced in Europe (Plastics Europe, 2019). Around 40% of the plastic produced is for packaging, with these items generally designed for a single use before disposal (Geyer *et al.*, 2017). Plastic pollution is of critical concern not only because of increased production and disposal, but also because the biodegradability of plastic is low, and plastic materials can persist for hundreds of years in marine and terrestrial environments (Auta *et al.*, 2017). There are an estimated 4.85 trillion microplastic (MP) (<5 mm) particles in the global ocean (Ockelford *et al.*, 2020), while it is estimated that between 75,000 and 300,000 tonnes of MP particles is released into the environment annually in the European Union (EU) (EC, 2018). The first priority, and most fundamental problem to be tackled, is undoubtedly the reduction in the production and use of plastics, along with increased recycling and environmentally safe disposal of plastics (Rist *et al.*, 2018). This could have a significant impact on the levels of secondary MPs and nanoplastics (NPs) from the breakdown of larger plastic items. We must also develop technologies that remove MPs from our environment and find innovative ways of recycling. Given the persistent widespread use of plastics, it is important that we also understand the potential risks of MPs and NPs to human health (Wright and Kelly, 2017).

MPs are generally defined as plastic pieces smaller than 5 mm in diameter (Andrady, 2017), while NPs are defined as measuring between 1 and 100 nm (EFSA, 2016). Primary MPs are small plastic particles specifically manufactured for a particular purpose

(Cole *et al.*, 2011) and are found in products such as pre-production resin pellets, microbeads in cosmetics, toothpaste, powders for textile coatings and drug delivery systems (Koelmans, 2015; Shim *et al.*, 2018). NPs are increasingly manufactured for use in products such as paints, adhesives, drug delivery vehicles and electronics (Koelmans, 2015). Secondary MPs and NPs result from the fragmentation of larger plastics, constituting the major source of MPs and NPs in the terrestrial and aquatic environments (Jiang, 2018; Murphy *et al.*, 2016).

The ubiquitous nature of MPs and NPs in the environment and in consumer products inevitably leads to human exposure to these particles via ingestion, inhalation or absorption through the skin. MPs have been detected in shellfish (Cho *et al.*, 2019), fish (Karami *et al.*, 2018), drinking water (Koelmans *et al.*, 2019; Schymanski *et al.*, 2018), table salt (Renzi and Blašković, 2018), honey and sugar (Liebezeit and Liebezeit, 2013), beer (Liebezeit and Liebezeit, 2014), sushi nori (Q. Li *et al.*, 2020), fruit and vegetables (Oliveri Conti *et al.*, 2020) and chicken (Huerta Lwanga *et al.*, 2017). It follows that human exposure can result from ingestion of these products (Gallo *et al.*, 2018). Sludge from wastewater treatment is also a potential source of human exposure (Corradini *et al.*, 2019; Edo *et al.*, 2020). When sludge is spread onto agricultural land as fertiliser, MPs and NPs retained in the sludge can pass from the soil through to freshwater, and to groundwater sources. In addition, dried sludge debris can be carried by the wind. Similarly, MPs and NPs can be carried in onshore winds in sea salt aerosols (Vignati *et al.*, 2010), potentially exposing marine workers or individuals residing in coastal regions (Wright and Kelly, 2017); however, the major sources of airborne microscopic particulates, whether at sea or on land, are generally considered to be terrestrial (K. Liu *et al.*, 2019). Airborne MP/NP particulates have been detected widely, with associated risk of inhalation exposure; sources include indoor and outdoor dust, tyres and synthetic fibres from textiles (Dris *et al.*, 2017; C. Liu *et al.*, 2019; Prata, 2018). Human exposure by the dermal route is mainly attributed to the application of personal care products such as cosmetics and creams; however, evidence of

transdermal transmission is lacking (Guerranti *et al.*, 2019; Praveena *et al.*, 2018).

The implications of exposure to MPs and NPs for human health are not well understood because very little is known about how humans absorb MPs and NPs when exposed via food or air or when applied to the skin. In addition, to date only a small number of studies have evaluated NPs in sources of exposure because validated and standardised sampling and analytical methodologies are lacking. Some studies have reported the capacity of NPs to be adsorbed onto algae (Bhattacharya *et al.*, 2010) and to penetrate marine species such as mussels (Wegner *et al.*, 2012), oysters (Ward *et al.*, 2019) and fish (Chae and An, 2017). The trophic transfer of NPs from algae to fish via zooplankton has also been described (Cedervall *et al.*, 2012); however, no peer-reviewed study has yet unambiguously demonstrated the presence of NPs in other food products, and studies have yet to report NPs in air or sources of inhalation or dermal exposure (Alexy *et al.*, 2019).

There are four main potential mechanisms by which plastic particles could affect human health: leaching of additives from the plastic; release of pollutants adsorbed onto the plastic during its lifetime; impacts from biofilms on plastic surfaces; and, finally, through effects directly attributable to the plastic particles themselves (Bouwmeester *et al.*, 2015). The last is the main focus of this report. Evidence of an interaction of plastic particles with human tissues and cells is limited. Given their diverse physicochemical characteristics (size, shape, solubility, surface charge, surface reactivity and energy band structure), MPs and NPs could potentially cause a variety of types of tissue damage (L. Wang *et al.*, 2017). The large surface area of MPs and NPs can lead to release of toxic compounds, adding to the oxidative stress and cytotoxicity to individual cells, and may facilitate translocation of MPs and NPs to human and animal tissues (Galloway, 2015). Plastics can contain toxic chemicals (hazardous monomers, additives such as plasticisers and benzene) and are a potential source of release of endocrine-disrupting compounds, which have known adverse effects on human reproductive function as well as potential effects on metabolic, immune and nervous functions (Hahladakis *et al.*, 2018; Hirai *et al.*, 2011). Phthalate-type plasticisers have been linked to a myriad of human health impacts, including epigenetic modulation, reproductive toxicity

in both women and men, insulin resistance and type II diabetes, overweight and obesity, skeletal anomalies, allergies, asthma and cancer (Benjamin *et al.*, 2017). Polyethylene terephthalate (PET), polystyrene (PS) and polyvinyl chloride (PVC) have been linked with the release of potentially carcinogenic compounds and related adverse effects on cell viability and inflammatory gene expression *in vitro* (Rodrigues *et al.*, 2019). However, the focus of this report is on the impact of the particles themselves, rather than any leachable or extractable plastics.

There is evidence that synthetic fibres have an adverse impact on respiratory function in textile workers (Ghio *et al.*, 2006), and MPs have been identified in lung tumour samples from humans (Prata, 2018), indicating the potential for plastics to cause pulmonary cytotoxicity (Dong *et al.*, 2020). There have been several investigations of the adverse effects of MPs on aquatic organisms. Studies in animal models have given some insight into the possible risks of MP/NP exposure in humans: like endocrine-disrupting compounds, MPs and NPs can elicit a range of biological responses that affect reproductive, metabolic, immune and nervous functions (Patisaul *et al.*, 2018). However, the physical effects, cellular changes and alterations in biochemical pathways induced by MPs and NPs remain poorly understood (Desforges *et al.*, 2014). Plastic particles could cause lung and gut tissue damage, and very fine particles in particular could cross cell membranes, the blood–brain barrier and the human placenta (Hesler *et al.*, 2019). The consequences of this exposure on human health are not yet well understood and, given the potential impact on multi-organ systems, it is critical for future research to more fully understand the impacts of human exposure.

1.2 Purpose of the Report

Scientists, policymakers and the public are becoming increasingly concerned about the ubiquity of MPs and NPs and the uncertainties surrounding their impacts, hazards and the risks they pose to human health (Völker *et al.*, 2019). The purpose of this Environmental Protection Agency (EPA)-funded desk study was to perform a critical evaluation of the literature on the potential human health risks associated with exposure to MPs and NPs in the environment and to summarise how current

knowledge might influence policy and research needs by identifying gaps and prioritising areas for future research. This report was created to communicate the important research findings to key stakeholders.

1.3 Scope of the Report

This report critically reviews the current evidence of the effects of environmental exposure to MPs and NPs on human health, presenting evidence on potential routes of exposure such as inhalation, ingestion and dermal contact (Chapter 3). The current state-of-the-art methods available for the detection and characterisation of MPs and NPs were reviewed and recommendations are made regarding optimal detection methods and study protocols that could be utilised in future studies (Chapter 4). Information on the health impacts of MPs and NPs in humans is largely limited to exposure levels measured in air, food, water and personal care products, with some studies linking synthetic fibres to health outcomes in humans, mainly in the context of occupational exposure by textile workers (Chapter 5). However, studies have examined the impact of MPs and NPs on aquatic

organisms, animal models and mammalian *in vitro* systems as models to predict potential health risks to humans. Although NPs are considered in this report, at present there is insufficient information available for an in-depth evaluation. Despite the fact that the number of publications is growing exponentially in this field, there is a wide knowledge gap that needs to be filled, and knowledge is not growing at the rate needed to understand the potential health effects. The health impact of additives, leachables, extractables or toxic compounds associated with plastic particles is not within the scope of this report, as there are already a number of comprehensive review articles on this subject.

The project team addressed three main research questions, reflected in the structure of the report: (1) What are the routes of human exposure to MPs and NPs (Chapter 3)? (2) What is the range and mid-level of human exposure and how do we measure it (Chapter 4)? and (3) What are the potential human health impacts of MPs and NPs (Chapter 5)? A brief review of current policies and legislation is provided in Chapter 6, followed by a final summary with a list of recommendations.

2 Methodology

2.1 Review Approach

The PECO (Population, Exposure, Comparison and Outcome) framework was used for each of the three central research questions as a tool to develop an effective search protocol (Akram *et al.*, 2019; Yates *et al.*, 2019). This framework was used to define the pillars of each question and to determine key search terms in order to identify the relevant published literature. The study population comprised humans without geographical, gender, ethnicity or age restrictions. However, for question 3, given the dearth of studies in human populations, the review was expanded to include studies in aquatic animals, animal models and *in vitro* studies involving mammalian tissues. The review considered inhalation, ingestion and dermal contact as the major routes of human exposure. To investigate the level of human exposure, the review examined the range of and overall exposure levels to MPs and NPs from inhalation, dermal or dietary sources (question 1). Methods of detection were examined, and particle characteristics such as size, chemical characterisation and hydrophobicity were recorded (question 2). When examining the possible health effects of human exposure to MPs and NPs, the literature was searched for evidence of translocation of MPs and NPs into the lung, gastrointestinal tract and skin (question 3). All data were tabulated to facilitate comparison, for example of the possible health effects associated with high versus low exposure or different particle sizes. The outcomes of the study included data showing the major routes of human exposure, current techniques that can identify the presence of MPs and NPs in different environmental sources and the evidence linking MPs and NPs to potential health impacts.

2.2 Inclusion/Exclusion Criteria

Eligibility criteria were based on study design, participant, outcome, timeframe and language. Studies that met the following criteria were included: (1) original articles published in peer-reviewed journals, (2) government and non-government reports, (3) observational studies (i.e. cohort, case-control and cross-sectional) and experimental, meta-analysis,

systematic review and ecological studies, (4) studies conducted on human subjects, aquatic animals, animal models and mammalian tissues, (5) studies in which outcomes measured were related to routes of exposure to MPs and NPs and impacts on human health and (6) studies published in the last 20 years (with some minor exceptions in the medical field that were dated prior to the year 2000). Studies were screened if written in English, French, Arabic or German. We excluded hypothesis papers, commentaries and letters to the editor or other non-primary research that did not present unique or new data. Studies dating from 2000 up to May 2020 were included in the review, with some exceptions at final review stage (July 2020) to reflect the most up-to-date research/policy.

2.3 Literature Search and Data Management

The use of multiple databases [PubMed, Elsevier, Science Direct, Springer, ACS (American Chemical Society) Publications, ISI Web of Science, Scopus, Sage, Google Scholar, Research Gate and Mendeley] and resources (journals and grey literature) were employed in order to increase the sensitivity of the search and identify all relevant articles. The Dublin City University subject matter expert librarian was consulted in the preparation of the search protocol. Titles, abstracts and full-text articles of potentially relevant studies were screened. The search string was appropriately adapted for each of the selected databases, and the search strategy employed a combination of the following advanced techniques: concepts, keywords, Boolean operators and thesaurus. The following list is a sample of the keywords utilised (where possible, a thesaurus was used to include a wider array of keywords): MP(s), NP(s), routes of human exposure, inhalation, dermal, ingestion, pathways, occupational exposure, level of exposure, concentration, range, particle size, chemical characterisation, hydrophobicity, techniques for quantifying MPs and NPs in human tissues, lung, gastrointestinal tract, absorption, uptake, translocation, bioaccumulation, human health effects, food, fish,

mussels, tea, seaweed, honey, water, beer, salt, fruit, vegetables, air, atmosphere and tyres/tires. The Boolean operators used included AND (link different concepts), OR (expand a term), NOT (exclude a term from the search) and parentheses.

A standard data collection form was designed to systematically extract data from each study. Any duplicate studies were removed. The full text of selected papers was explored in depth to ensure that only relevant papers were included in the review. A summary table was generated in Microsoft Excel and included the following: details of publication (i.e. first author's name, year of publication, study

location, study design, type of study and study period), characteristics of the study population (i.e. age and sex of studied participants, the sample size), exposure (types of exposure, level of exposure, measurements of exposure and outcome) and tables reviewed by the project team. Data were analysed for associations (systematic review and meta-analyses) and context (e.g. via descriptive statistics, narrative and descriptive spatial analyses) (Diepens and Koelmans, 2018). Study outcomes were summarised using narrative and quantitative methods. The extracted data were tabulated under the appropriate sections and included in the report.

3 Major Routes of Human Exposure to Micro- and Nanoplastics

3.1 Introduction

Humans are ubiquitously exposed in their daily environment to MPs and NPs, and analysis of the literature indicates that human exposure to MPs and NPs can arise via three main routes: ingestion, inhalation and dermal contact (Lehner *et al.*, 2019). There may be stratification in exposure based on geographical, occupational or lifestyle/dietary factors. Some occupations are particularly susceptible to exposure, for example textile workers may be exposed to synthetic fibres (Mastrangelo *et al.*, 2002), while differences in levels of exposure can be attributed to a reliance on food sources with higher contamination levels, such as shellfish (Santillo *et al.*, 2017), or to living in an urban versus rural environment (Zhang *et al.*, 2020). Exposure to MPs and NPs via inhalation, ingestion or dermal contact could potentially affect human health (Karbalaie *et al.*, 2018); however, it is not clear which route presents the greatest risk. No studies have directly measured human exposure; all studies to date have focused on the sources of MPs and NPs as a measure of exposure (Revel *et al.*, 2018). This section provides a comprehensive review of the literature, outlining studies that have measured different sources of exposure, and provides a list of research gaps that should be addressed.

3.2 Oral Exposure

Ingestion is likely to contribute significantly to human exposure to MPs and NPs, as their presence has been quantified in a variety of common foods and beverages consumed daily (De-la-Torre, 2020; Galloway *et al.*, 2017).

3.2.1 Drinking water

MPs and NPs in tap water and bottled water may represent a major route of human exposure (Lehner, 2019). MPs have been detected in virtually all water sources, and many traditional drinking water treatment plants are not designed to remove them. In this section, the occurrence of MPs and NPs in drinking water sources and removal of MPs and NPs from

drinking water treatment plants are summarised. A comprehensive, World Health Organization (WHO)-commissioned systematic review of the literature up to August 2018 on MPs in drinking water was published in mid-2019 (Koelmans *et al.*, 2019), which was further consolidated into a larger WHO report on MPs in drinking water in late 2019 (Marsden *et al.*, 2019); some of the major points are summarised here.

Approximately 83% of the population of Ireland is linked to a municipal drinking water supply, with the remaining 17% relying on private drinking water supplies such as household wells or private group water schemes. The majority (more than 75%) of private drinking water supplies rely on wells or springs (EPA, 2020). In public drinking water supplies, surface water accounts for 80% of drinking water sources, with 13% attributed to groundwater and 7% to springs (EPA, 2019). Drinking water treatment plant conditions, which differ geographically and between public and private supplies, could potentially have an impact on the levels of MPs and NPs in drinking water supplied to households and businesses, while drinking water source (groundwater, surface water or spring) may also have an impact. Average annual bottled water consumption in Ireland is estimated at 58.5 litres per capita (Statista, 2020), with approximately 63% of the population buying bottled water frequently (Kantar Media, 2017). Bottled water source and processing method may have an impact on MP/NP levels in the water, while re-use of plastic capped water bottles could potentially lead to increased MP/NP ingestion (Winkler *et al.*, 2019).

Drinking water sources

MPs and NPs have been widely detected in drinking water sources, including groundwater and surface waters. Sea or brackish water, which is treated by desalination, is not included as a drinking water source in this section, as thermal or membrane-based desalination processes would be expected to remove all source-related MPs and NPs. However, it should be noted that aspects of the desalination process (e.g. shearing of membranes, plastic piping, pump

elements) could contribute to the presence of MPs and NPs in desalinated drinking water; this is an area in which there have been no studies to date. However, MPs have been detected post reverse osmosis treatment in wastewater treatment plants (Ziajahromi *et al.*, 2017), indicating the potential for presence of MPs and NPs in drinking water in countries where desalinated water is distributed.

Fifty studies on the presence of MPs in drinking water and freshwater were assessed for scientific quality, with only four of those studies regarded as meeting all of the quality parameters applied (Koelmans *et al.*, 2019). The highest-ranked studies in terms of quality were included in the WHO report (Marsden *et al.*, 2019). A more recent review paper notes the difficulty in comparing studies owing to lack of standardisation in reporting of methods and quantification, but does not apply the same quality control parameters to reported results (C. Li *et al.*, 2020). An EPA-funded study makes reference to an unpublished study on MPs in Irish drinking water sources, with up to 6.5 MP particles per litre in untreated private well water samples and 1.6 particles/L in public water supplies (Mahon *et al.*, 2017a); however, there is a lack of published studies on Irish drinking water sources.

Despite a variety of conflicting reports, the WHO report summarises that the concentration of MPs in drinking water sources is typically less than 5 particles/L (Marsden *et al.*, 2019), which is broadly in agreement in terms of order of magnitude with Mahon *et al.* (2017b).

Tap water

Pivokonsky *et al.* (2018) measured the efficiency of removal of MPs of conventional coagulation, clarification and filtration processes in three drinking water treatment plants and found values of between 70% and 82%, findings that were confirmed by other studies (Mintenig *et al.*, 2019). However, other drinking water treatment processes based on coagulation–ultrafiltration were found to have removal efficiencies below 40% (Ma *et al.*, 2019). Lack of consistency and efficacy across drinking water treatment technologies could potentially lead to the presence of MPs and NPs in public and private drinking water supplies (Novotna *et al.*, 2019). The presence of MPs and NPs in drinking water is reported to be slightly higher in developed regions such as the USA and the EU than in less developed regions (Table 3.1). The most

Table 3.1. MPs/NPs in tap water

Number of samples	Location	Detection technique	Range/mean (particles/L)	Particle diameter (µm)	Reference
159	14 countries	Microscopy	0–60.9	100–500	Kosuth <i>et al.</i> , 2018
1	Cuba	Microscopy	7.17 (mean)	100–500	Kosuth <i>et al.</i> , 2018
24	Ecuador	Microscopy	0–9.04	100–500	Kosuth <i>et al.</i> , 2018
3	England	Microscopy	3.66–13	100–500	Kosuth <i>et al.</i> , 2018
1	France	Microscopy	1.82 (mean)	100–500	Kosuth <i>et al.</i> , 2018
2	Germany	Microscopy	0–1.82	100–500	Kosuth <i>et al.</i> , 2018
17	India	Microscopy	0–20	100–500	Kosuth <i>et al.</i> , 2018
21	Indonesia	Microscopy	0–10.8	100–500	Kosuth <i>et al.</i> , 2018
1	Ireland	Microscopy	1.83 (mean)	100–500	Kosuth <i>et al.</i> , 2018
1	Italy	Microscopy	ND	100–500	Kosuth <i>et al.</i> , 2018
16	Lebanon	Microscopy	0–23.3	100–500	Kosuth <i>et al.</i> , 2018
8	Slovakia	Microscopy	0–10.9	100–500	Kosuth <i>et al.</i> , 2018
2	Switzerland	Microscopy	0–5.47	100–500	Kosuth <i>et al.</i> , 2018
26	Uganda	Microscopy	0–12.7	100–500	Kosuth <i>et al.</i> , 2018
33	USA	Microscopy	0–60.9	100–500	Kosuth <i>et al.</i> , 2018
16	Denmark	µ-FTIR	2–45	0.45	Strand <i>et al.</i> , 2018
17	Denmark	µ-FTIR	4–30	<100	Strand <i>et al.</i> , 2018
1	Germany	Spectroscopy	0–11	<100	Kniggendorf <i>et al.</i> , 2019

FTIR, Fourier-transform infrared spectroscopy; ND, not detected.

comprehensive study was performed by Kosuth *et al.* (2018), who reported the presence of MPs in 81% of 159 samples tested; the majority were fibres (98.3%) that were smaller than 5 mm in diameter, and the number of particles ranged from 0 to 61 per litre (mean 5.45 particles/L). The presence of MPs in tap water has been attributed to the abrasion of PVC and high- or medium-density polyethylene (PE) pipes and fittings used in the drinking water system (Mintenig *et al.*, 2019). Further study within and between regions is warranted.

Similar studies have found that MPs are widely present in tap water in Germany (Kniggendorf *et al.*, 2019) and Denmark, where 100% of drinking water comes from groundwater sources (Strand *et al.*, 2018). Interpretation of the data in terms of the reliability of the analyses can be affected by the sample collection method; in some studies, the tap was allowed to run prior to taking the sample, in contravention of standard compliance methods for drinking water sampling in the EU and Ireland.

Bottled water

Relatively few studies that measure the presence of MPs in bottled water have been published. One such study found that 93% of bottled water contained MPs, with fragments and fibres being the most common forms (Mason *et al.*, 2018; Oßmann *et al.*, 2018, 2019; Zuccarello *et al.*, 2019). The mean number of MP particles per litre ranged from 0 to > 10,000, while particle size ranged from 0.45 to 100 µm (Table 3.2).

These results show variability not only in the individual bottles from different locations but also in individual bottles among the same lot and brand. This could be due to different water sources, different processes at bottling facilities or the conditions and/or length of time involved in shipping from bottling facilities to purchase locations prior to analysis within the laboratory (Mason *et al.*, 2018). However, it should be noted that quality control and reproducibility across studies are not guaranteed. For example, the study by Zuccarello *et al.* (2019) was heavily criticised for its method and data analysis (Oßmann *et al.*, 2019). In one study, water bottled in glass from a single source was found

Table 3.2. Summary of available data on MPs detected in bottled water

Bottle type (number of samples)	Location	Detection technique	Mean ± SD concentration (particles/L)	Particle diameter (µm)	Particle shape	Reference
Plastic (1)	Germany	µ-Raman spectroscopy	NA	0.45	Fibres	Wiesheu <i>et al.</i> , 2016
Reusable PET (12)	Germany	µ-Raman spectroscopy	2649±2857	1 to > 10	Spheres, fibres	Oßmann <i>et al.</i> , 2018
Single-use PET (10)	Germany	µ-Raman spectroscopy	4889±5432	1 to > 10	Spheres, fibres	Oßmann <i>et al.</i> , 2018
Glass (9)	Germany	µ-Raman spectroscopy	3074±2531	1 to > 10	Spheres, fibres	Oßmann <i>et al.</i> , 2018
Single-use glass (1)	Germany	µ-Raman spectroscopy	6292 ± 10,521	1 to > 10	Spheres, fibres	Oßmann <i>et al.</i> , 2018
Reusable plastic (15)	Germany	µ-Raman spectroscopy	14 ± 14	5 to > 100	Fibres	Schymanski <i>et al.</i> , 2018
Single-use plastic (11)	Germany	µ-Raman spectroscopy	118 ± 88	5 to > 100	Fibres	Schymanski <i>et al.</i> , 2018
Cartons (3)	Germany	µ-Raman spectroscopy	50 ± 52	5 to > 100	Fibres	Schymanski <i>et al.</i> , 2018
Glass (9)			11 ± 8			
Single-use plastic (259)	19 locations	FTIR and Nile Red staining	4.15 (range 0–14)	6.5–100	Fragments, fibres, pellets, films, foam	Mason <i>et al.</i> , 2018
Single-use glass (6)	USA	FTIR and Nile Red staining	204 (range 9–516)	6.5–100	Fragments, fibres, pellets, films, foam	Mason <i>et al.</i> , 2018
Plastic (10)	Italy	SEM-EDS	656.8 ± 632.9	2.44	Fragments	Zuccarello <i>et al.</i> , 2019

FTIR, Fourier-transform infrared spectroscopy; PET, polyethylene terephthalate; SEM-EDS, scanning electron microscopy and energy-dispersive X-ray spectroscopy.

to have lower numbers of MP particles (204/L) than the same brand of water in single-use plastic bottles (1410/L) (Mason *et al.*, 2018). Similar findings have been reported in other studies (Oßmann *et al.*, 2018; Schymanski *et al.*, 2018), suggesting that the type of packaging can influence the number of MP particles present in the water and that may be subsequently ingested directly by the consumer. However, other studies have reported significant amounts of MPs in water from glass bottles, which may be the result of abrasion between the soft plastic bottle cap (and sealing) and the neck of the bottle (Oßmann *et al.*, 2018), an effect that is particularly apparent after multiple uses (Winkler *et al.*, 2019). Exposure to MPs may also depend on the level of bottle shaking prior to drinking (Zuccarello *et al.*, 2019); however, in a study by Winkler *et al.* (2019), PET bottles themselves were not found to release MPs under the influence of mechanical stress. The level of MPs in bottled water is also influenced by abrasion or brittleness of bottles over time, and repeated use of plastic bottles results in a significant increase in the amount of MPs present (from 14 ± 14 to 118 ± 88 particles/L) (Schymanski *et al.*, 2018). In repeatedly used bottles, MPs detected consisted of 84% PET and 7% polypropylene (PP), attributable to the bottle and the cap material, respectively.

WHO reports that plastic particles in drinking water do not currently appear to pose a risk to human health, but qualified this by acknowledging that its conclusion was based on limited information, calling for greater research on the issue (Marsden *et al.*, 2019). A closer examination of bottled water production, cleaning and filling processes and re-use is therefore required. In addition, in order to compare studies, a comparative analysis of the experimental conditions associated with water sampling is required to avoid contamination of MPs and NPs during the sampling process.

3.2.2 Food chain

MPs can have chemical, physical and biological impacts on organisms that ingest them directly through food and water, while also potentially affecting organisms that ingest them indirectly through the consumption of contaminated prey (Farrell and Nelson, 2013; Wright and Kelly, 2017). Ingestion may serve as a potential source through which human consumers become exposed to these particles (Revel *et al.*, 2018). The majority of studies examining the presence

of MPs and NPs in food have investigated their presence in shellfish (Table 3.3) and fish (Table 3.4). A small number of studies have investigated their presence in other foodstuffs including salt (Table 3.5), beer, honey, sugar, teabags, chicken, seaweed, fruit and vegetables (Table 3.6).

While not within the scope of this report, it is worth noting that wastewater treatment removes 90% of MPs; however, given the large volumes treated, wastewater is thought to be a significant contributor to MPs and NPs in the aquatic and terrestrial environments. This could potentially contribute to human exposure to MPs and NPs in the food chain. MPs and NPs are known to become entrained in sewage sludge as part of the settlement process during wastewater treatment, and in many countries, including Ireland, up to 80% of wastewater biosolids produced are used as agricultural fertiliser (Mahon *et al.*, 2017a) rather than being diverted to landfill (Wagner *et al.*, 2014). Large volumes of industrial biosolids are also land spread in many countries (Rigby and Smith, 2014), which could also lead to the transmission of MPs and NPs. Surface runoff and percolation to groundwater sources can lead to MPs and NPs' entry to the aquatic environment, with an impact on drinking water sources and freshwater or marine habitats (Ghirardini and Verlicchi, 2019; Ziajahromi *et al.*, 2017).

Shellfish

Bivalves such as mussels, oysters, clams and shrimp are filter feeders and thus process relatively large amounts of seawater containing MPs and NPs during feeding. Larger crustaceans such as lobster and crab feed on these bivalves and therefore can indirectly ingest MPs and NPs through their diet. Consumption of seafood can be an indirect route of human exposure to MPs and NPs, in particular bivalves, which are consumed whole, without intestine removal. For these reasons, it is worthwhile assessing the level of contamination and the characteristics of the MPs and NPs in bivalves to understand the potential risks to human health (J. Li *et al.*, 2019).

The presence of MPs in shellfish has been reported in studies from numerous countries in all continents except Australia, where no studies are available. These studies examined shellfish sampled from their natural habitat or from supermarkets and

aquaculture farms, as well as samples of shellfish that were exposed to MPs and NPs under experimental conditions (Table 3.3). While most studies focused on blue mussel (*Mytilus edulis*) and oyster (*Crassostrea gigas*), other studies investigated MPs in clam (*Venerupis philippinarum*), rock oyster (*Saccostrea forskalii*), sea snail (*Littorina littorea*), brown shrimp (*Crangon crangon*), Norwegian lobster (*Nephrops norvegicus*) and crabs (*Carcinus maenas* and *Eriocheir sinensis*). All samples tested positive for the presence of MPs, with significant variation between studies in particle size (5–5000 µm) and abundance [0.014–20 particles per gram wet weight (g w/w)]; however, most studies reported levels <20 particles/g w/w (Table 3.3). The highest level of MPs (20 particles/g w/w) were found in bivalve samples from coastal and aquaculture farms in China (Khoironi *et al.*, 2018), followed by samples from the Netherlands, Italy, Greece, the UK, and other Chinese samples (Catarino *et al.*, 2018; Courtene-Jones *et al.*, 2017; Digka *et al.*, 2018; Karlsson *et al.*, 2017; J. Li *et al.*, 2016; Renzi *et al.*, 2018).

Widespread presence of MPs has been reported in bivalves sold for consumption (Cho *et al.*, 2019; Davidson and Dudas, 2016; De Witte *et al.*, 2014; J. Li *et al.*, 2016; L. Li *et al.*, 2019; Rochman *et al.*, 2015; Van Cauwenberghe and Janssen, 2014; Vandermeersch *et al.*, 2015a) as well as in those sampled under field conditions (De Witte *et al.*, 2014; Van Cauwenberghe *et al.*, 2015). Ingestion of MP particles of different sizes and shapes by filter feeders has also been examined in laboratory settings (Browne *et al.*, 2011; Cole *et al.*, 2011; Thompson *et al.*, 2009; Ward *et al.*, 2019). MP uptake in mussels from their natural habitat was compared with uptake by farmed mussels, with some studies reporting higher levels in farmed shellfish (Mathalon and Hill, 2014; Vandermeersch *et al.*, 2015b) and others reporting higher levels in shellfish from natural habitats (Davidson and Dudas, 2016; J. Li *et al.*, 2015; Van Cauwenberghe and Janssen, 2014). Contamination of bivalves by MPs may depend on the place of culture: most farmed seafood is cultured in seawater and, as a result, these filter feeders are exposed to any pollutant present, including MPs and other particles, in the same way as their wild counterparts.

Analysis of MPs detected in five wild shellfish species in Iran confirmed the presence of PE, PET and nylon (Naji *et al.*, 2018), while PE, PP, PS and polyester were the dominant MPs in samples from a South

Korean market (Cho *et al.*, 2019). A more recent study on oysters employing micro-Raman spectroscopy identified PS, PE, PP, poly(bisphenol A carbonate), rayon and polyacrylate (Martinelli *et al.*, 2020). Higher concentrations of MPs were detected in a predatory species of molluscan shellfish (sea snails), suggesting trophic transfer of MPs in the food chain (Naji *et al.*, 2018), while position in the water column was shown to affect the type of polymer found in the shellfish, with PS found in oysters and mussels cultured in the upper layer, and polyester increasing in clams and scallops cultured in the middle and bottom layers of the water column (Cho *et al.*, 2019). These studies point to the need to establish spatial and long-term trends in MP contamination to identify high-risk areas. However, it is important to note that comparability between studies is difficult owing to the lack of a standardised approach to MP detection and identification; even with advanced analytical techniques, only 2% of microparticles found in oysters were found to actually be MPs, with the rest found to consist of cellulose derivatives, shell fragments, biological or proteinaceous material, salts, minerals and gypsum (Martinelli *et al.*, 2020). Analytical methods are further discussed in Chapter 4.

In addition to MPs, humans can be exposed to NPs through the food chain by consuming contaminated shellfish. Intestinal uptake of 30- and 100-nm-diameter polystyrene NPs in mussels (*Mytilus edulis*) has been demonstrated (Wegner *et al.*, 2012). Polystyrene NPs (24 nm) can be transported through the aquatic food chain from algae, through zooplankton, to fish (Cedervall *et al.*, 2012). Carboxylated polystyrene particles (40 nm) were found to accumulate in the digestive tract of sea urchin embryos (*Paracentrotus lividus*), although whether this was also true for laminated polystyrene particles was less clear (Della Torre *et al.*, 2014). The scarcity of detection methods for NPs in tissues makes them difficult to study, which is a concern given that smaller particles are more likely to be taken up by human cells. Depuration (the process of transferring the shellfish to clean water to allow purification of physical impurities such as sand and grit) has been shown to reduce MP content in wild and farmed mussels by 29–47% after 3 to 4 days' (Birnstiel *et al.*, 2019; Van Cauwenberghe and Janssen, 2014), although no reduction in MP concentration was found after 2 hours' depuration (Rist *et al.*, 2019). Eighty-five per cent of MP particles > 100 µm were removed from Pacific oysters after 72 hours' depuration (Graham *et al.*, 2019).

Table 3.3. Summary of available data on MPs detected in shellfish species

Species	Sampling location	Detection technique	Concentration (particles/g w/w), range or mean \pm SD	Particle diameter (μm)	Reference
Mussels					
<i>Mytilus edulis</i>	Germany (market)	μ -Raman spectroscopy	0.36 \pm 0.07	5–10	Van Cauwenberghe and Janssen, 2014
	Canada (market)	Microscopy	2.79–3	NA	Mathalon and Hill, 2014
	Canada (coast, wild)		7.42		
	Belgium (coast, wild)	Microscopy	0.26–0.51 0.35	1000–1500	De Witte <i>et al.</i> , 2014
	France (market)	Microscopy	0.06 \pm 0.13	NA	Vandermeersch <i>et al.</i> , 2015a
	Netherlands (aquafarm)		0.32 \pm 0.22		
	France, Belgium and Netherlands (coast, wild)	μ -Raman spectroscopy	0.2 \pm 0.3	20–90	Van Cauwenberghe <i>et al.</i> , 2015
	China (coast, wild)	FTIR	0.9–4.6	<250	J. Li <i>et al.</i> , 2016
	China (coast, wild)	μ -FTIR	1.52–5.36	250–1000	Qu <i>et al.</i> , 2018
	Netherlands (coast, wild)	μ -FTIR	13.2	10–300	Leslie <i>et al.</i> , 2017
	UK (aquafarm)	Microscopy	12.6	NA	Catarino <i>et al.</i> , 2018
	UK (coast, wild)	μ -FTIR	3 \pm 0.9		
	UK (coast, wild)	Microscopy	1.05–4.44	1220	Courtene-Jones <i>et al.</i> , 2017
	Netherlands (coast, wild)	Microscopy	37	30–2000	Karlsson <i>et al.</i> , 2017
	France (coast, wild)	μ -FTIR	0.18 \pm 0.16 0.23 \pm 0.2	50–100	Phuong <i>et al.</i> , 2018
	UK (coast, wild)	μ -FTIR	0.7–2.9	5–250	J. Li <i>et al.</i> , 2018
	UK (market)		0.9		
	USA	FlowCAM	39 \pm 15	<5000	Woods <i>et al.</i> , 2018
	South Korea (market)	μ -FTIR	0–0.35	<300	Cho <i>et al.</i> , 2019
	France	μ -Raman spectroscopy	0.59–0.86	<5000	Hermabessiere <i>et al.</i> , 2019
<i>Mytilus galloprovincialis</i>	Spain (market)	Microscopy	0.04 \pm 0.09	NA	Vandermeersch <i>et al.</i> , 2015b
	Spain (coast, wild)		0.15 \pm 0.33		
	Italy (aquafarm)		0.25 \pm 0.26		
	Italy (coast, wild)		0.05 \pm 0.11		
	Portugal (coast, wild)		0.34 \pm 0.33		
	Italy (market)	Microscopy	4.4–11.4	1700–1900	Renzi <i>et al.</i> , 2018
	Italy (coast, wild)		7.2		
	Greece (aquafarm)	FTIR	2.5 \pm 0.3	100–500	Digka <i>et al.</i> , 2018
	Greece (coast, wild)		5.3 \pm 0.5		
	Italy	μ -FTIR	0.62–0.63	20–40	Gomiero <i>et al.</i> , 2019
	Tunisia	FTIR-ATR	0.703 \pm 0.011	100–1000	Abidli <i>et al.</i> , 2019
<i>Perna viridis</i>	Brazil (coast, wild)	Microscopy	1	20–5000	Santana <i>et al.</i> , 2018
	China (coast, wild)	SEM-EDS	4–20	51–232	Khoironi <i>et al.</i> , 2018
	India	DXR Raman spectroscopy	1.8–3.2	5–30	Naidu, 2019
<i>Mytilus modiolus</i>	UK (Coast, wild)	μ -FTIR	0.09 \pm 0.03	NA	Catarino <i>et al.</i> , 2018
<i>Ruditapes philippinarum</i>	China (market)	μ -ATR-FTIR and SEM-EDS	0.74 \pm 0.54	57–8639	Ding <i>et al.</i> , 2019

Table 3.3. Continued

Species	Sampling location	Detection technique	Concentration (particles/g w/w), range or mean \pm SD	Particle diameter (μ m)	Reference
Oysters					
<i>Crassostrea gigas</i>	France (market)	μ -Raman spectroscopy	0.47 \pm 0.16	16–20	Van Cauwenberghe and Janssen, 2014
	USA (market)	Microscopy	2.1 \pm 1.71	2270–15,840	Rochman <i>et al.</i> , 2015
	France (coast, wild)	μ -FTIR	0.18 \pm 0.1	50–100	Phuong <i>et al.</i> , 2018
	Netherlands (coast, wild)	FTIR	2.4–10.9	10–5000	Leslie <i>et al.</i> , 2017
	Tunisia	FTIR-ATR	0.7–1.4	100–1000	Abidli <i>et al.</i> , 2019
	South Korea (market)	μ -FTIR	0–0.19 0.07 \pm 0.06	100–200	Cho <i>et al.</i> , 2019
	China	μ -FTIR	2.41–9.08 2.93	< 1500	Teng <i>et al.</i> , 2019
	USA	μ -Raman spectroscopy and μ -FTIR	0.02–0.3	20–13,000 (majority 20–150)	Martinelli <i>et al.</i> , 2020
<i>Saccostrea forskalii</i>	Thailand (wild)	Raman spectroscopy	0.2–0.6 0.57 \pm 0.22	NA	Thushari <i>et al.</i> , 2017
<i>Saccostrea cucullata</i>	China	FTIR	1.5–7.2	20	Li <i>et al.</i> , 2015
<i>Crassostrea hongkongensis</i>	China	μ -FTIR	3.2–8.6	NA	Zhu <i>et al.</i> , 2019
<i>Pinctada radiata</i>	Iran (wild)	FTIR	0.2 (estimated mean)	10–5000	Naji <i>et al.</i> , 2018
Clams/oysters					
<i>Venerupis philippinarum</i>	China (wild)	FTIR	0.62	NA	Teng <i>et al.</i> , 2019
	Canada (farm)	Microscopy	0.07–5.47 1.7 \pm 1.2	NA	Davidson and Dudas, 2016
	Canada (wild)		0.9 \pm 0.		
<i>Amiantis umbonella</i>	Iran (wild)	FTIR	2 (estimated mean)	10–5000	Naji <i>et al.</i> , 2018
<i>Amiantis purpuratus</i>	Iran (wild)	FTIR	2.5 (estimated mean)	10–5000	Naji <i>et al.</i> , 2018
<i>Ruditapes philippinarum</i>	China (market)	μ -ATR-FTIR and SEM-EDS	0.16–0.42	57–8639	Ding <i>et al.</i> , 2019
<i>Tapes philippinarum</i>	South Korea (market)	μ -FTIR	0.03–1.8 0.34 \pm 0.31	100–200	Cho <i>et al.</i> , 2019
Scallop					
<i>Patinopecten yessoensis</i>	China (market)	μ -FTIR	10.5	5–250	Li <i>et al.</i> , 2015
<i>Patinopecten yessoensis</i>	South Korea (market)	μ -FTIR	ND	5–250	Cho <i>et al.</i> , 2019
Periwinkle					
<i>Littorina littorea</i>	Netherlands	Microscopy	20	300–5000	Leslie <i>et al.</i> , 2017
<i>Littorina littorea</i>	Thailand	Raman spectroscopy	0.17–0.23	ND	Thushari <i>et al.</i> , 2017
Brown shrimp					
<i>Crangon</i>	Channel area and North Sea	Microscopy	0.68 \pm 0.5	200–1000	Devriese <i>et al.</i> , 2015
Crab					
<i>Carcinus maenas</i>	UK	Microscopy	0.24	NA	Farrell and Nelson, 2013
<i>Eriocheir sinensis</i>	Poland	Microscopy	NA	0.5–5mm	Wójcik-Fudalewska <i>et al.</i> , 2016

ATR, attenuated total reflectance; DXR, dual X-ray; FlowCAM, flow cytometry and microscopy; FTIR, Fourier-transform infrared spectroscopy; ND, not determined; SEM-EDS, scanning electron microscopy and energy-dispersive X-ray spectroscopy.

Fish

MPs have been detected in a wide range of commercially relevant fish species, including canned sardines, dried fish, anchovies, herring, grey gurnard, whiting, horse mackerel, haddock, Atlantic mackerel, cod and sea bass (Table 3.4), with global spread. Particles were of a similar size (5–5000 µm) to those found in shellfish; however, there was significantly less variation in particle concentration between individual fish (0 to 50 µm per fish). Several studies have found that demersal fish species (which live close to the sea floor, where plastic debris accumulates) contain more MPs than pelagic species, which inhabit sunlit water (Jabeen *et al.*, 2017; Vendel *et al.*, 2017). When fish feed on benthic prey that are found on the seabed, they may swallow some sediment together with the prey, and thus also ingest plastics (Bellas *et al.*, 2016). Only one study found NPs in the liver, blood, gall bladder and kidney of *Oryzias latipes* 7 days after experimental exposure (Kashiwada, 2006).

When humans consume fish, such as anchovies and sardines, that are typically consumed whole, they take in the fish gastrointestinal tract (GIT), where MPs accumulate, although accumulation in the gut has been shown to be transient (Grigorakis *et al.*, 2017). Pellini *et al.* (2018) reported that 80% of *Solea solea* specimens from the northern and central Adriatic Sea contained MP particles in the GIT, with similar mean numbers detected in in 2014 (1.73 ± 0.05 MP particles per fish) and 2015 (1.64 ± 0.1 MP particles per fish). Plastics are more abundant in the intestine of fish than in the stomach (Jabeen *et al.*, 2017), and therefore studies should include analysis of the entire digestive tract in order to obtain a realistic indication of the total amount of plastics to which humans are actually exposed. It should also be noted that fish waste (such as the stomach and intestines) is often diverted to animal feed, potentially leading indirectly to human ingestion (Bouwmeester *et al.*, 2015).

Although MPs detected in fish are mostly located in the GIT, there is evidence of direct human exposure through the consumption of fish muscle (Wright and Kelly, 2017). Studies measuring MP content in fish muscle are much rarer than studies of fish liver, gut and gill. It is crucial to investigate MPs in fish muscle, despite the fact that the potential for MP accumulation potential is lower in muscle than in liver or gill (Collard *et al.*, 2018; Monikh *et al.*, 2019; Neves *et al.*, 2015).

Accumulation of MPs in muscles has been found to be higher in benthic fish (demersal fish that reside on the sea floor) than in pelagic species (Akhbarizadeh *et al.*, 2018; Ding *et al.*, 2019). Important factors affecting the level of MP accumulation in fish muscle, apart from fish ecology such as feeding mode, habitat and fish size, include the level of MP contamination in seawater, human activity near the water source, and location (Akhbarizadeh *et al.*, 2018). Karami *et al.* (2018) suggested that MPs can reach fish muscles directly from skin, gills and the GIT. MPs have also been detected in the guts, skin, muscle, gills and liver of demersal and pelagic fish (Abbasi *et al.*, 2019; Ding *et al.*, 2019), and it has been demonstrated that MPs can translocate from the GIT to the gills and liver of young and adult zebra fish (*Danio rerio*), a common prey fish (L. Lu *et al.*, 2019). MP translocation is also documented in European sea bass and in the common goby (*Pomatoschistus microps*) (de Sá *et al.*, 2018). Parameters that might influence the translocation of MPs and NPs across the gut epithelium include particle size, dye and the composition, charge and molecular weight of the component plastics; however, the processes that control MP translocation from the gut to other organs including fish muscles are not yet fully clear, and further studies are required (Collard *et al.*, 2018; Rainieri and Barranco, 2019).

Salt

MP debris in salt has been reported in a variety of studies since 2015 (Table 3.5). One study found MPs in 12 brands of commercial sea salt tested from six grocery stores in the USA. The average number of particles detected was 212 per kilogram, with a range of 46.7–806 particles/kg (Kosuth *et al.*, 2018). Similarly, a Spanish study examining 21 brands of sea salt reported values ranging from 50 to 280 MP particles/kg (Iñiguez *et al.*, 2017). The highest levels of MPs detected in salt (13,500–19,800 particles/kg) were reported in a study examining Croatian salts, with slightly lower levels detected in Italian salts (Renzi and Blašković, 2018). These studies indicate that contamination of commercial salts for human consumption is very common. It is reasonable to deduce that the MPs in sea salts primarily arise from seawater; MP concentrations in sea salts were significantly higher than in lake and rock/well salts, indicating that marine products such as salt are

Table 3.4. Summary of available data on MPs detected in fish species

Species	Location	Tissue	Detection technique	Concentration (particles/g w/w), range or mean \pm SD	Particle diameter (μ m)	Reference
Pelagic fish						
<i>Symbolophorus californiensis</i> , <i>Cololabis saira</i> , <i>Myctophum auro lanternatum</i> , <i>Loweina interrupta</i> , <i>Hygophumre inhardtii</i> , <i>Astronesthes indopacifica</i>	North Pacific Ocean	Stomach	Microscopy	1–83 2.1 \pm 5.78	1–2700	Boerger <i>et al.</i> , 2010
<i>Merlangius merlangus</i>	English Channel	GIT	FTIR	1.9 \pm 0.10	1000–2000	Lusher <i>et al.</i> , 2013
<i>Mullus barbatus</i> , <i>Merluccius</i> sp.	Adriatic Sea	GIT	FTIR	1–1.78	10–5000	Avio <i>et al.</i> , 2015
<i>Argyrosomus regius</i> , <i>Brauma brama</i> , <i>Dentex macrophthalmus</i> , <i>Merluccius merluccius</i>	Portugal	GIT	FTIR, microscopy	0.09–1	217–4810	Neves <i>et al.</i> , 2015
<i>Xiphias gladius</i> , <i>Thunnus thynnus</i> , <i>Thunnus alalunga</i>	Mediterranean Sea	Stomach	Microscopy	4–16	<5000	Romeo <i>et al.</i> , 2015
<i>Engraulis japonicas</i>	Japan	Digestive tract	FTIR	2.3 \pm 2.5	10–500	Tanaka and Takada, 2016
<i>Argyrosomus regius</i> , <i>Dentex dentex</i> , <i>Dentex gibbosus</i> , <i>Diplodus annularis</i> , <i>Liza aurata</i> , <i>Pagellus acarne</i> , <i>Pagellus erythrinus</i> , <i>Pagrus pagrus</i> , <i>Scomber japonicus</i> , <i>Trachurus mediterraneus</i>	Turkey	GIT	FTIR	1–10.25 2.36	<656	Güven <i>et al.</i> , 2017
<i>Rastrelliger kanagurta</i> , <i>Spratelloides gracilis</i> , <i>Siganus canaliculatus</i>	Indonesia	GIT	Microscopy	0.3–1.1	100–4500	Rochman <i>et al.</i> , 2015
<i>Rastrelliger kanagurta</i> , <i>Stolephorus waitei</i>	Malaysia	Viscera, gills	Raman spectroscopy	1–2	0.001 to > 10	Karbalaei <i>et al.</i> , 2019
<i>Hyporhamphus intremedius</i> , <i>Coilia ectenes</i> , <i>Lateolabrax japonicas</i> , <i>Psenopsis anomala</i> , <i>Sillago sihama</i> , <i>Psenopsis anomala</i> , <i>Liza haemotocheila</i> , <i>Larimichthys crocea</i>	China	GIT	Microscopy, FTIR	1.1–4.6	4–5000	Jabeen <i>et al.</i> , 2017
<i>Alepes djedaba</i> , <i>Sphyræna jello</i>	Persian Gulf	Muscle	Microscopy SEM	0.57–0.8	< 100–5000	Akhbarizadeh <i>et al.</i> , 2018
<i>Sphyræna viridensis</i>	Greece	Stomach	Microscopy	42 \pm 20.5	<5000	Miliou <i>et al.</i> , 2016
<i>Trachurus mediterraneus</i>				28 \pm 19.5		
<i>Sardina pilchardus</i>	Spain	GIT	Microscopy	1.43 \pm 0.79	<5000	Ferrer <i>et al.</i> , 2016
<i>Engraulis encrasicolus</i>				1.18 \pm 0.4		
<i>Sardina pilchardus</i> , <i>Engraulis encrasicolus</i> , <i>Clupea harengus</i>	France	Liver	Raman spectroscopy	1–9	124–438	Collard <i>et al.</i> , 2018
<i>Gardus morhua</i> , <i>Scomber scrombus</i>	Germany	GIT	Microscopy, ATR-FTIR, FTIR	0.19 \pm 0.61	<5000	Rummel <i>et al.</i> , 2016
<i>Sprattus sprattus</i>	Netherlands	GIT	ATR-FTIR	2	>20	Hermesen <i>et al.</i> , 2017
<i>Osmerus eperlanus</i>	UK	GIT	Microscopy ATR-FTIR	0.2 \pm 0.42	<5000	McGoran <i>et al.</i> , 2017
<i>Atherinopsis californiensis</i> , <i>Engraulis mordax</i> , striped bass, <i>Morone saxatilis</i> , <i>Ophiodon elongates</i> , <i>Oncorhynchus tshawytscha</i>	USA	GIT	Microscopy	0.1–1.6	NR	Rochman <i>et al.</i> , 2015

Table 3.4. Continued

Species	Location	Tissue	Detection technique	Concentration (particles/g w/w), range or mean \pm SD	Particle diameter (μ m)	Reference
<i>Gadus morhua</i>	Canada	GIT	Microscopy	1–2	> 1000	Liboiron <i>et al.</i> , 2016
<i>Opisthonema oglinum</i> , <i>Rhinocardinia bahiensis</i> , <i>lycengraulis grossidens</i> , <i>Atherinella brasiliensis</i> , <i>Poecilia vivipara</i>	Brazil	GIT	Microscopy	0.03–0.35	NR	Vendel <i>et al.</i> , 2017
<i>Decapterus muroadsi</i>	Chile	GIT	Microscopy ATR-FTIR	2.5 \pm 0.4	< 1100	Ory <i>et al.</i> , 2018
<i>Benthoosema pterotum</i> , <i>Mauroliticus mucronatus</i> , <i>Vinciguerria mabahiss</i>	Saudi Arabia	GIT	FTIR	0–1	2000	Baalkhuyur <i>et al.</i> , 2018
<i>Engraulis japonicus</i> , <i>Decapterus maruadsi</i>	Yellow Sea	Digestive tract	μ -FTIR	1.2	< 5000	Sun <i>et al.</i> , 2019
<i>Acanthopagrus latus</i> , <i>Chelon affinis</i> , <i>Perca fluviatile</i> , <i>Konosirus punctatus</i> , <i>Sillago sihama</i>	China	GIT, gill	μ -FTIR	GIT: 2–5.3 Gill: 0–3	NR	Zhu <i>et al.</i> , 2019
<i>Gadus morhua</i> , <i>Merlangius merlangus</i> , <i>Melanogrammus aeglefinus</i> , <i>Clupea harengus</i> , <i>Trachurus trachurus</i> , <i>Eutrigla gurnardus</i> , <i>Scomber scombrus</i>	North Sea	GIT	Microscopy	1–4	< 5000	Foekema <i>et al.</i> , 2013
<i>Boreogadus saida</i>	Arctic Ocean	Stomach	μ -FTIR	0–22	< 5000	Kühn <i>et al.</i> , 2018
<i>Scomber scombrus</i>	UK	Digestive tract	FTIR	0.58 \pm 1.05	1500–2000	Nelms <i>et al.</i> , 2018
<i>Spratus spratus</i>	Baltic Sea	GIT	Microscopy	1–3	100 to > 500	Beer <i>et al.</i> , 2018
<i>Sardina pilchardus</i> , <i>Engraulis encrasicolus</i>	Italy	Stomach	Microscopy	1.25–4.63	40–2220	Renzi <i>et al.</i> , 2019
<i>Pseudosciaena polyactis</i>	China (market)	Muscle, stomach	μ -ATR-FTIR and SEM-EDS	0.58 \pm 0.83	57–8639	Ding <i>et al.</i> , 2019
<i>Scomberomorus cavalla</i> , <i>Rhizoprionodon lalandii</i>	Brazil	Stomach	Microscopy	2–6	1000–5000	Miranda and de Carvalho-Souza, 2016
Demersal fish						
<i>Scyliorhinus canicula</i> , <i>Merluccius merlucciu</i> , <i>Mullus barbatus</i>	Spain	Stomach	Microscopy	1.56 \pm 0.5	380–3100	Bellas <i>et al.</i> , 2016
<i>Limanda limanda</i> , <i>Platichthys flesus</i>	Germany	GIT	ATR-FTIR, FTIR	0.03 \pm 0.18	< 5000	Rummel <i>et al.</i> , 2016
<i>Chelon subviridis</i>	Malaysia	Viscera, gills	Raman spectroscopy	2	< 5000	Karami <i>et al.</i> , 2018
<i>Mugil cephalus</i> , <i>Terapon jarbua</i> , <i>Sebasticus marmoratus</i> , <i>Photopectoralis bindus</i> , <i>Thamnaconus septentrionalis</i> , <i>Oxyeleotrix marmorata</i>	China	GIT	Microscopy, FTIR	3–7.2	4–5000	Jabeen <i>et al.</i> , 2017
<i>Pleuronichthys cornutus</i> , <i>Liza haematocheila</i>	China (market)	Muscle, stomach	μ -ATR-FTIR and SEM-EDS	0.35 \pm 0.27	57–8639	Ding <i>et al.</i> , 2019
<i>Boops boops</i>	Greece	Stomach	Microscopy	15.4 \pm 3.2	< 5000	Miliou <i>et al.</i> , 2016
<i>Lithognathus mormyrus</i> , <i>Mullus barbatus</i> , <i>Mullus surmuletus</i> , <i>Nemipeterus randali</i> , <i>Pomadasy incises</i> , <i>Sciaena umbra</i> , <i>Sparus aurata</i>	Turkey	GIT	FTIR	1.44–3	< 656	Güven <i>et al.</i> , 2017
<i>Boops boops</i>	Spain	GIT	Microscopy	2.47–4.89	< 5000	Nadal <i>et al.</i> , 2016

Table 3.4. Continued

Species	Location	Tissue	Detection technique	Concentration (particles/g w/w), range or mean \pm SD	Particle diameter (μ m)	Reference
Demersal fish						
<i>Mullus surmuletus</i> L.	Spain	GIT	FTIR-microscopy	0.34 \pm 0.07	<5000	Alomar and Deudero, 2017
<i>Platycephalus indicus</i> , <i>Saurida tumbil</i> , <i>Sillago sihama</i> , <i>Cynoglossus abbreviatus</i>	Persian Gulf	GIT	Microscopy SEM-EDS	0.16–15	100	Abbasi <i>et al.</i> , 2018
<i>Stellifer brasiliensis</i> , <i>Stellifer stellifer</i>	Brazil	GIT	Microscopy	0.33–0.52	NR	Dantas <i>et al.</i> , 2012
<i>Boops boops</i>	Spain	GIT	Microscopy	1.46 \pm 0.66	NR	Ferrer <i>et al.</i> , 2016
<i>Mullus surmuletus</i>	Portugal	GIT	FTIR	1.66 \pm 0.57	<5000	Neves <i>et al.</i> , 2015
<i>Mugil cephalus</i>	South Africa	GIT	Microscopy	3.8 \pm 4.7	0.2–15	Naidoo <i>et al.</i> , 2016
<i>Luciopimelodus pati</i>	Argentina	GIT	Microscopy	18.5 \pm 18.9	<5000	Pazos <i>et al.</i> , 2017
<i>Diapterus auratus</i> , <i>Diapterus rhombeus</i> , <i>Eugerres beazilanus</i> , <i>Symphurus tessellatus</i>	Brazil	GIT	Microscopy	0.02–0.97	NR	Vendel <i>et al.</i> , 2017
Riverine and lake fish						
<i>Neogobius melanostomus</i>	USA	Digestive tract	FTIR	10–13 (\pm 1.6)	1500–3300	McNeish <i>et al.</i> , 2018
<i>Neogobius melanostomus</i> , <i>Barbus barbatus</i>	Germany	GIT	FTIR	1.25 \pm 0.5	264–2907	Roch and Brinker, 2017

ATR, attenuated total reflectance; FTIR, Fourier-transform infrared spectroscopy; SEM-EDS, scanning electron microscopy and energy-dispersive X-ray spectroscopy.

Table 3.5. Summary of available data on MPs detected in salts

Location (number of samples)	Detection technique	Concentration (particles/kg), range or mean	Particle diameter (μ m)	Reference
China (15)	μ -FTIR	7–718	< 100 to > 1000	Yang <i>et al.</i> , 2015
Australia (2), France (6), Japan (1), Malaysia (1), New Zealand (1), Portugal (3), South Africa (1)	μ -Raman spectroscopy	0–10	160–980	Karami <i>et al.</i> , 2017a
Spain (21)	FTIR	50–280	30–3500	Iñiguez <i>et al.</i> , 2017
Australia (1), Brazil (1), Bulgaria(1), Belarus (1), China (4), Germany (2), Hungary (1), Pakistan (1), Philippines (1), Senegal (1), Sicilian Sea (2), USA (1), UK (1), Vietnam (2)	Microscopy Raman spectroscopy	0–130	100–5000	Kim <i>et al.</i> , 2018
Atlantic Ocean (1), Celtic Sea (2), Himalaya (1), Mediterranean Sea (2), Mexico (1), North Sea (1), Pacific Ocean (1), Sicilian Sea (1), USA (1)	Microscopy	47–806	40–5000	Kosuth <i>et al.</i> , 2018
Turkey (5)	Raman spectroscopy	8–102	< 100 to > 1000	Gündoğdu, 2018
India (8)	μ -FTIR	56–103	500–2000	Seth and Shrivastav, 2018
Croatia (5)	μ -FTIR	13,500–19,800	15–4628	Renzi and Blašković, 2018
Italy (6)		22–594	4–2100	
Taiwan (667)	FTIR	342	89.7–1474.9	H. Lee <i>et al.</i> , 2019

FTIR, Fourier-transform infrared spectroscopy.

particularly susceptible to contamination with MPs (Gündoğdu, 2018; Yang *et al.*, 2015). In the case of rock salt, the source is less obvious and contamination is most likely the result of processing (Gündoğdu, 2018). MP content has been found to be reasonably independent of the salt brand and packaging process (Seth and Shrivastav, 2018). Commercial salts are used daily on a global scale to prepare food, and humans ingest relatively small amounts of salt in several food items. Consumption of MPs in sea salt may therefore constitute a long-term, but low-level, source of exposure in humans.

Other food and beverage items

Humans may also be exposed to MPs and NPs via the consumption of other food and beverage products such as beer, honey, sugar, chicken and plastic teabags (Table 3.6). While several studies have reported MPs in beer (Liebezeit and Liebezeit, 2014) and honey (Liebezeit and Liebezeit, 2013, 2015; Mühlischlegel *et al.*, 2017), only one study has reported MPs in chicken (Huerta Lwanga *et al.*, 2017), sugar (Liebezeit and Liebezeit, 2013), teabags (Hernandez *et al.*, 2019) and, most recently, milk (Kutralam-Muniasamy *et al.*, 2020). The presence of

Table 3.6. Summary of available data on MPs detected in foodstuffs

Matrices	Location (number of samples)	Detection techniques	Concentration, range or mean \pm SD	Particle diameter (μ m)	Reference
Beer	Germany (24)	Microscopy	2–109 particles/L	0.8	Liebezeit and Liebezeit, 2014
	USA (12)	Microscopy	0–14.3 particles/L 4.05 particles/L	100–500	Kosuth <i>et al.</i> , 2018
Milk	Mexico	μ -Raman spectroscopy	3–11 particles/L 6.5 particles/L	100–1000	Kutralam-Muniasamy <i>et al.</i> , 2020
Chicken	Mexico (5)	Microscopy	0.0102 \pm 0.0138 particles/kg	100–1000	Huerta Lwanga <i>et al.</i> , 2017
Honey	France, Italy, Spain, Mexico (19)	Microscopy	Fibres: 40–660 particles/kg; 166 \pm 147 particles/kg Fragments: 0–38 particles/kg; 9 \pm 9 particles/kg	10–40	Liebezeit and Liebezeit, 2013
	Germany (47)	Microscopy	Fibres: 10–336 particles/kg Fragments: 2–82 particles/kg	40	Liebezeit and Liebezeit, 2015
	Switzerland (5)	μ -Raman spectroscopy	32–728 particles/kg	10–20	Mühlischlegel <i>et al.</i> , 2017
Sugar	Germany (5)	Microscopy	Fibres: 217 \pm 123 particles/kg Fragments: 32 \pm 7 particles/kg	10–40	Liebezeit and Liebezeit, 2013
Teabags	Canada	FTIR XPS	11.6 \times 10 ⁹ MPs/teabag 3.1 \times 10 ⁹ NPs/teabag	1–1000	Hernandez <i>et al.</i> , 2019
Nori seaweed	China	Microscopy and μ -FTIR	900–3000 particles/kg dw 1800 \pm 700 particles/kg dw	5–500	Q. Li <i>et al.</i> , 2020
Apple	Italy (6)	SEM-EDS	1.955 \times 10 ⁸ \pm 1.28687 \times 10 ⁸ particles/kg	2.17 (median) (1.56–3.19)	Oliveri Conti <i>et al.</i> , 2020
Pear	Italy (6)	SEM-EDS	1.895 \times 10 ⁸ \pm 1.05 \times 10 ⁸ particles/kg	1.99 (median) (1.87–2.59)	Oliveri Conti <i>et al.</i> , 2020
Broccoli	Italy (6)	SEM-EDS	1.26 \times 10 ⁸ \pm 0.8 \times 10 ⁸ particles/kg	2.10 (median) (1.86–2.95)	Oliveri Conti <i>et al.</i> , 2020
Lettuce	Italy (6)	SEM-EDS	0.51 \times 10 ⁸ \pm 0.25 \times 10 ⁸ particles/kg	2.52 (median) (2.18–2.78)	Oliveri Conti <i>et al.</i> , 2020
Carrot	Italy (6)	SEM-EDS	1.019 \times 10 ⁸ \pm 0.44 \times 10 ⁸ particles/kg	1.51 (median) (1.36–2.00)	Oliveri Conti <i>et al.</i> , 2020

dw, dry weight; FTIR, Fourier-transform infrared spectroscopy; SEM-EDS, scanning electron microscopy and energy-dispersive X-ray spectroscopy; XPS, X-ray photoelectron spectroscopy.

MPs in nori seaweed, which is often consumed as a foodstuff, has also been reported (Q. Li *et al.*, 2020). Although it is unclear to what extent MPs and NPs can be attributed to the factory processing, laboratory studies show a significant likelihood of adsorption of MPs to seaweed in the marine environment (Gutow *et al.*, 2016; Sundbæk *et al.*, 2018). Both fibres and fragments were found in all food tested, ranging from 0.8 to 1000 µm, and a wide range of abundances were detected. Although the concentration of MPs varied among foodstuffs, the most alarming amount was reported in plastic teabags (Table 3.6), far in excess of the amounts detected in any other matrix. The finding of MPs in common foodstuffs indicates that micro-sized synthetic polymers are contaminating the human environment to a large extent (Kosuth *et al.*, 2018).

MPs were reported to be present in higher levels in 24 German beers than in nine US beer brands. The significant difference between the studies may be attributable to different brewing/processing customs and regulations in Germany and the USA. MPs were speculated to originate from airborne atmospheric particles, materials used in the production process, impurities on bottle surfaces or particle contamination of raw materials used in beer production (Kosuth *et al.*, 2018; Liebezeit and Liebezeit, 2014). Applying good principles of hygienic industrial design may reduce the levels of MPs in beer and other processed beverages.

Honey samples from different countries (France, Italy, Spain, Mexico, Switzerland and Germany) were found to contain coloured MP fibres and fragments in concentrations ranging from 10 to 728 particles/kg (Liebezeit and Liebezeit, 2015) (Table 3.6). Sources of MPs were tentatively identified as environmental, such as transport of atmospheric MPs by the bees into the hive or introduction during the honey processing itself. The ubiquitous presence of airborne particles may also contribute to the overall particle burden of honey (Mühlschlegel *et al.*, 2017). Studies also reported black carbon particles originating from the smoking of the hives, a common practice in bee-keeping to calm bees before harvesting (Liebezeit and Liebezeit, 2013; Mühlschlegel *et al.*, 2017).

One study reported the contamination of sugar by MPs (Liebezeit and Liebezeit, 2013), with evidence that unrefined cane sugar had the highest amount of synthetic fragments and fibres present, while

a Mexican study (Huerta Lwanga *et al.*, 2017) of chicken gizzards for human consumption found 10.2 ± 13.8 MPs per gizzard. Although this study, carried out in a Mexican village, showed that MPs are capable of entering the terrestrial food chain on chickens living in gardens extensively polluted with plastic waste, there is no evidence that these particles translocated to the chicken muscle, which is the part most commonly consumed by humans (Huerta Lwanga *et al.*, 2017).

One study examining four brands of teabags demonstrated that a single plastic teabag releases 11.6 billion MP particles and 3.1 billion NP particles into hot water (Hernandez *et al.*, 2019). The level of particles released related only to the teabag packaging, as tea was removed from the teabags prior to the study. As part of this same study, *Daphnia magna* was exposed to various doses of the teabag-derived MPs and NPs, and dose-dependent behavioural and developmental effects were observed; however, in all of these cases more research is needed, as conclusions cannot be drawn from only one study.

The first study of the presence of MPs in fruit and vegetables found that the concentration of MP particles < 10 µm ranged from 52,050 to 233,000 particles/g, with apples having the highest levels and lettuces the lowest. Overall, fruits were found to be more highly contaminated than vegetables (Oliveri Conti *et al.*, 2020). The levels of MPs found in these foods are extremely high and definitely cause for concern. However, as this is the first study of its kind (and using a patented methodology for quantification, based on just 0.1 g of homogenised samples), it will be important that replication and comparison with other methodologies are also undertaken by more research groups.

3.2.3 Soil and crops

Plastic contamination of soil has been widely studied, as the use of plastics in agriculture, for example in silage films, greenhouses and polytunnels, and plastic-containing fertilisers such as mulch or biosolids, has been encouraged for many years (Qi *et al.*, 2020; Weithmann *et al.*, 2018). Fibres have been detected in soils up to 15 years after the application of biosolids (Zubris and Richards, 2005), and even soils without

agricultural plastic applications have been found to contain macro- and microplastics (Piehl *et al.*, 2018). MPs and NPs can enter the soil environment either directly (through fertiliser or mulch application, in irrigation water or through atmospheric deposition) or indirectly (through the degradation of macroplastics or plastic films). MPs can be transported through the soil by earthworms and along preferential flow patterns (Rillig *et al.*, 2017), and are typically concentrated in earthworm castings (Huerta Lwanga *et al.*, 2017). As a result, a downward transport of MPs and NPs to groundwater is possible, as is runoff to surface waters. Polystyrene NPs (PS-NPs) have been shown to impact on soil microbiota and enzyme activity, which can potentially impact crop health (Awet *et al.*, 2018). There are concerns about the potential for toxicity to crops, potentially causing damage, and about PS-NPs being taken up into the plant roots in particular, which is the portion intended for human consumption. There have been very few studies on the presence of MPs and NPs in crops themselves (Rillig *et al.*, 2019). A very recent study on uptake of PS-NPs by wheat showed root to shoot movement of NPs, with presence in the crop itself confirmed by 3D laser confocal scanning microscopy (LCSM) and scanning electron microscopy (SEM) (Lian *et al.*, 2020).

3.2.4 Pharmaceutical packaging

Plastic materials are widely employed to make medical items such as solution containers, transfusion sets, transfer tubing, drug packaging and devices (Jenke, 2007). Plastic containers and pharmaceutical packaging are primarily made from PE, PP, PVC, PS, nylon (polyamide), nitrile and PET. Polyester fibres and plastic particles have been found in pharmaceutical products (Shearer, 2003). Leaching of plastics from the pharmaceutical packaging is well documented, and the industry is well regulated for leachates and extractables (Jenke, 2015), but there are no studies available that investigate the presence of MPs and NPs in either pharmaceuticals for oral ingestion, such as tablets or liquids, or in therapies intended for parenteral use (injection). It is worth noting that there is significant consumption of food supplements or nutraceuticals; it would be expected that similar issues could be faced for this sector. There are no studies on these type of products in the literature, and this significant gap should be addressed.

3.3 Inhalation Exposure

Human exposure to MPs and NPs through inhalation can result from exposure to indoor and outdoor dust, airborne particulate matter (PM), sea salt aerosols and dried sludge debris carried by the winds. It is possible that exposure risk via inhalation could be stratified by setting, for example coastal versus agricultural, or urban versus suburban; however, more evidence is required to support this hypothesis (Dris *et al.*, 2015; Prata, 2018; Wright and Kelly, 2017).

Airborne MPs and NPs come from various sources, including synthetic plastic fibres from textiles released during washing and during tumble or air drying (Carney Almroth *et al.*, 2018). It has been estimated that fleece microfibre clothing contributes 0.0012 wt% per wash when air dried, and up to 3.5 times this value when tumble dried (Pirc *et al.*, 2016). In addition, MPs and NPs from dried sludge or sea spray may become airborne (Koelmans *et al.*, 2019). Although cigarette butts are a well-known source of MPs (particularly cellulose acetate) in the environment (Wright *et al.*, 2015), there are no studies or evidence to date to suggest that either cigarette smoking or vaping contributes to inhalation of MPs and NPs.

The atmospheric fallout of MPs has been investigated in urban and suburban sites and, following long-term monitoring, large numbers of fibres were detected (Cai *et al.*, 2017; Dris *et al.*, 2015). The suburban site systematically showed fewer fibres than the urban site, which is not surprising given that the urban site is more densely populated (Cai *et al.*, 2017; Dris *et al.*, 2017). Of the fibres analysed, 67% were made from natural fibres such as cotton and wool, whereas the remaining fibres contained plastic polymers (Dris *et al.*, 2017). A similar study performed in China examining atmospheric fallout of MPs showed a dominance of synthetic fibre-like particles (Cai *et al.*, 2017).

Tyre and road wear particles (TRWPs) could also contribute to the difference in the level of airborne MP particles observed in urban and rural areas. Although mass loss from tyres is typically estimated at between 10% and 20%, only a small fraction of the tyre wear and tear particles generated become airborne (between 0.1% and 10%), with the larger particles (> 10 µm) being deposited on or near the road. Three to seven per cent of PM < 2.5 µm in size (PM_{2.5}) in air is estimated to consist of micro-sized particles

resulting from tyre wear and tear (Kole *et al.*, 2017), while TRWP concentrations in PM₁₀ (< 10 µm in size) are estimated to be similarly low. Abbasi *et al.* (2019) analysed MPs and microrubbers (MRs) in air and street dust and found that MR concentrations were significantly higher in deposited dust than in airborne dust. The street dust samples were obtained from 15 different sites where MPs accounted for 78% of the total particles measured while MRs accounted for 22%. Other studies have demonstrated that average airborne TRWP concentration typically ranged from 0.05 to 0.70 µg/m³ (Panko *et al.*, 2013; Unice *et al.*, 2019). TRWP concentration in air has been associated with traffic load and population density; more research is needed to distinguish between TRWP and other sources of MPs in air in high-traffic areas.

Human exposure to airborne MPs in indoor versus outdoor air has been compared in several studies (Table 3.7). One such study (Dris *et al.*, 2017)

measured fibres in air in three indoor sites and one outdoor site and detected significantly fewer fibres in indoor air (range 0.3–1.5 fibres/m³) than in outdoor air (1.0–60.0 fibres/m³). In another study in Iran (Abbasi *et al.*, 2019), fibres in street dust collected from 15 sites in urban and industrial areas were examined; they were attributed to textiles such as clothing and soft furnishings, were mainly spherules (74%) and films (14%), and were predominantly white-transparent in colour (>50%). A recent experimental study using a breathing thermal manikin showed the presence of fibres at concentrations of between 1.7 and 16.2/m³ in the lungs of the manikin, which supports the possibility that MPs can enter the lungs through inhalation (Vianello *et al.*, 2019). Outdoor MPs may be readily transported into a house on shoes or enter ventilated buildings as airborne particulates (Catarino *et al.*, 2018). The health impacts arising from inhalation of MPs in the urban versus rural setting are unclear, and warrant further investigation.

Table 3.7. Summary of available data on MPs detected in matrices where inhalation could be a source of exposure

Matrix	Location (number of samples)	Detection technique	Concentration, range or mean	Particle diameter (µm)	Reference
Atmospheric fallout	France (2)	FTIR	2–355 particles/m ² /day 110±96 particles/m ² /day	50–600	Dris <i>et al.</i> , 2016
	China	FTIR	0–602 particles/m ² /day	50–1000	Zhou <i>et al.</i> , 2017
	China (3)	SEM, µ-FTIR	175–313 particles/m ² /day	NR	Cai <i>et al.</i> , 2017
Indoor and outdoor air	Iran (10)	Microscopy	Outdoor: 88–605 particles/m ³	250–500	Dehghani <i>et al.</i> , 2017
	France (2)	FTIR	Indoor: 1–60 particles/m ³ Outdoor: 0.3–1.5 particles/m ³	50–600	Dris <i>et al.</i> , 2017
	Denmark (3)	FPA-µFTIR	Indoor: 1.7–16.2 particles/m ³	4–398	Vianello <i>et al.</i> , 2019
	Iran (15)	SEM	Outdoor: 0.3–1.1 particles/m ³	2–100	Abbasi <i>et al.</i> , 2019
	China (39)	µFTIR	Indoor: 1550–120,000 mg/kg Outdoor: 212–9020 mg/kg	200	C. Liu <i>et al.</i> , 2019
	UK (3)	Nile Red staining, FTIR	Indoor: 5–10 particles/m ³	<500	Catarino <i>et al.</i> , 2018
	Europe (outdoor)	Microscopy	Outdoor: 365±69 particles/m ² /day	<25–2600	Allen <i>et al.</i> , 2019
PM	Japan	Py-GC-MS	0.16 µg/m ³	NR	Unice <i>et al.</i> , 2019
	Europe, USA, Japan (81)	Py-GC-MS	0.05–0.70 µg/m ³	NR	Panko <i>et al.</i> , 2013
	Dried sludge	USA	Microscopy	NR	NR
USA		Microscopy	1.5–4	NR	Zubris and Richards, 2005
USA		Microscopy	5.6–62.7/km ²	NR	Zylstra, 2013

FPA, focal plane array; FTIR, Fourier-transform infrared spectroscopy; NR, not reported; Py-GC-MS, pyrolysis–gas chromatography–mass spectrometry; SEM, scanning electron microscopy.

3.4 Dermal Exposure: Personal Care Products and Cosmetics

Microbeads have been used to replace natural exfoliating materials in personal care and cosmetic products (PCCPs) such as hand cleanser, soap, toothpaste, shaving foam, bubble bath, sunscreen, shampoo and facial scrub (Browne *et al.*, 2011; Dubaish and Liebezeit, 2013; Fendall and Sewell, 2009; Gregory, 1996; Zitko and Hanlon, 1991), and studies have measured MPs in several supermarket products from China, Malaysia and the USA (K. Lei *et al.*, 2017; Praveena *et al.*, 2018) (Table 3.8).

In the USA, per capita consumption of MPs in PCCPs has been estimated to be 2.4 mg of microbeads per day, which suggests that the US population may be sending about 263 tonnes per year of PE MPs to wastewater each year (Gouin *et al.*, 2011). Despite significant public and media attention, an EU-funded study estimated the contribution of microbeads in PCCPs to marine debris to be as low as 4.1% (Sherrington *et al.*, 2016). Dermal contact with MPs is not considered to be a significant route of human exposure, as particles larger than 1 µm are not expected to penetrate the skin (Lademann *et al.*, 2004). However, dermal contact with products containing NPs below 100 nm could potentially represent a human health risk, and this requires further study (Hernandez *et al.*, 2017; Revel *et al.*, 2018). The EU has asked the European Chemicals Agency (ECHA) to prepare a dossier with the view to restricting the intentional use of MPs in products of any kind under REACH (Registration,

Evaluation, Authorisation and Restriction of Chemicals) regulations (EC, 2018). ECHA published a draft dossier on restricting the intentional addition of microplastics to products in January 2019. ECHA's Committee for Risk Assessment and Committee for Socio-Economic Analysis is currently formulating its opinions on the dossier, which, after public consultation, will be sent to the European Commission. With the agreement of the European Council and Parliament, EU-wide restrictions on certain products containing intentionally added microplastics could be in place by 2022. In the case of some products, longer lead-in times before implementation of restrictions will be permitted to allow time for reformulation. It is estimated that the proposed restrictions will reduce emissions by at least 90% and prevent the release of 500,000 tonnes of microplastics over the 20-year period following their introduction (ECHA, 2020). Timelines for consultation may change in the light of delays due to COVID-19.

3.5 Conclusion and Gaps

The presence of MPs in food for human consumption is a global problem, and we are vulnerable to MP exposure through the consumption of seafood and other food items, as well as through other routes such as inhalation and dermal exposure (Avio *et al.*, 2015; Dris *et al.*, 2017; Kosuth *et al.*, 2018; Liebezeit and Liebezeit, 2013; Praveena *et al.*, 2018; Van Cauwenberghe and Janssen, 2014). The few studies to have measured NPs in food have reported particle diameters of <0.1 µm (Nguyen *et*

Table 3.8. Summary of available data on MPs detected in personal care products and cosmetics

Product (number of samples)	Microbeads concentration (beads/g product), range or mean	Diameter (µm)	Reference
Facial cleanser (6)	919–18,906	8–2000	Napper <i>et al.</i> , 2015
Facial scrub (1)	9906	363–945	Cheung and Fok, 2017
Facial scrubs (9)	5219–50,391	24–800	
Body scrubs (12)	482–15,058	500–2000	Guerranti <i>et al.</i> , 2019
Toothpaste (1)	2500	90–600	Carr <i>et al.</i> , 2012
Shower gel (10)	1.08–5.34	110–970	K. Lei <i>et al.</i> , 2017
Facial scrub (3)	300 billion per gram	24–52	Hernandez <i>et al.</i> , 2017
Facial cleanser (1)	124	183±93	Jemec Kokalj <i>et al.</i> , 2018
Personal care products (10)	NR	3–145	Praveena <i>et al.</i> , 2018
Facial cleanser (9)	NR	60–800	Chang, 2015
Personal care products	50,391	0.3–1000	Guerranti <i>et al.</i> , 2019

al., 2019). Only a few studies have analysed the plastic composition of the MPs and NPs contained in food, and have identified the following plastic types: PE, PP, PET, PS, PVC, polyester, polyacrylates and nylon. MP particles are mainly reported to be spheres, fragments, fibres or granules (Ding *et al.*, 2019). The shapes of NP particles occurring in the environment are relatively unknown, as methods to detect these nano-sized polymers are not as yet widely available (Stapleton, 2019); however, shape-dependent uptake into human cells is likely (Poma *et al.*, 2019). The primary route of human exposure to MPs and NPs in the environment may be oral, potentially from the consumption of plastic in teabags, food or drinking water. MP/NP ingestion has been observed in a range of animals of commercial interest that are consumed by humans, including fish, bivalves and crustaceans; it is recommended that shellfish undergo extended depuration prior to human consumption. These studies raise concerns regarding MP/NP-contaminated food and the potential effects on human health (Rochman *et al.*, 2015; Vandermeersch *et al.*, 2015a). Additionally, MPs and NPs have been detected in atmospheric fallout and dust, suggesting a possible source of inhalation exposure (Dris *et al.*, 2015). Finally, cosmetics that are applied dermally are also a source of human exposure to MPs and NPs (Guerranti *et al.*, 2019). While there is good evidence for the presence of MPs and NPs in shellfish and fish, there is a need for further studies measuring exposure to all other routes, given the lack of published reports. In particular, studies are required to examine the presence of MPs and NPs in human tissues following direct exposure, investigating if they are retained in organs (lung, skin and gut), are taken up by cells and/or can alter cell function (Lusher *et al.*, 2013).

The following list of gaps has been identified:

- There is a major gap in our knowledge of MP and NP levels in foods, particularly major food groups such as beef, poultry, dairy, fruit, vegetables and grains.
- It is possible that the amount of MPs and NPs in food/beverages increases during industrial processing, arising from processing aids or the release of water or air from machinery, equipment or packaging. However, there are no available studies on this issue. The effect of other processes such as cooking and baking on the content or characteristics of MPs and NPs is unknown.
- An average estimated daily intake of MPs and NPs should be calculated.
- There is a lack of information on the type of plastic, plasticisers and contaminants found in MP and NP particles – so chemical risk cannot currently be determined.
- Studies to date have measured exposure indirectly by analysing the presence of MPs in food, air or cosmetics; no studies have measured direct uptake. There is a critical need to develop methods or study design to evaluate actual human intake. There is also a need to determine whether MPs and NPs can translocate from the gut, skin or lungs to other organ tissues in humans (as is now evident in fish).
- Limited data are available on levels of MPs and NPs in air. Thus, the sources, transport mechanisms and human health impacts of MPs and NPs in air remain poorly understood.
- The lack of studies on MPs and NPs in pharmaceutical products for ingestion or injection needs to be addressed.
- There is a need for validated and standardised sampling and analytical methodologies.

4 What Is the Range and Mid-level of Human Exposure (and How Do We Measure It)?

4.1 The Range and Mid-level of Human Exposure

Humans can be exposed to MPs and NPs through the consumption of food products or drinking water, inhalation of air or application of personal care products (Vethaak and Leslie, 2016). Furthermore, human health effects are likely to depend on exposure concentrations. The transfer of MPs and NPs to humans may be influenced by the chemical characteristics of the MPs and NPs. Given our current knowledge, it is impossible to estimate the levels of exposure accurately. However, in this report we have estimated the average MP and NP consumption per person per year, for a limited number of exposure sources (Table 4.1). After reviewing the scientific literature, it appears that teabags, followed by bottled water, seafood, salt, honey and sugar, have relatively high levels of MPs compared with other sources. Owing to data gaps in MP and NP research, particularly for dermal and inhalation routes of exposure, there is insufficient information at present to comprehensively assess exposure concentrations. An understanding of levels of human exposure to MPs and NPs is critical in order to begin to predict the potential toxicological health effects.

4.1.1 Estimated daily intake of MPs by ingestion

The estimated daily intake of MPs ($\mu\text{g/L}$ or $\mu\text{g/kg bw/day}$) from drinking water and bottled water can be calculated using an equation described by Arena *et al.* (2015). It is estimated that the average person ingests more than 5800 particles per year from tap water, salt and beer sources, with the largest contribution coming from tap water (88%) (Kosuth *et al.*, 2018), and ingestion of 37–1000 plastic particles per year attributable to the consumption of sea salt (Karami *et al.*, 2017a; Yang *et al.*, 2015).

Based on an assumption that humans ingest MPs via the consumption of seafood and fish, we can also predict the number of particles to which they are

exposed. The annual dietary exposure of European shellfish consumers is estimated to reach 11,000 MPs per year (Van Cauwenberghe and Janssen, 2014); however, this will vary depending on location and dietary habits. The dietary exposure of Chinese shellfish consumers will be much higher than that of European consumers because the former consume more seafood (Van Cauwenberghe *et al.*, 2015). The annual MP dietary intake per capita among the Tunisian population was estimated to vary between 22.73 and 43.73 items per person per year and was largely attributed to the consumption of *Bolinus brandaris* and *Ruditapes decussatus* (Abidli *et al.*, 2019). Among fishermen and shellfish harvesters from the town of Bizerte, this figure was even higher, between 2557.67 and 4919.83 items per person per year, and was attributed to consumption of the same mollusc species (Abidli *et al.*, 2019). In the UK, MP ingestion by humans via consumption of mussels has been estimated at 123 MP particles person per year (Catarino *et al.*, 2018), while Spain, France or Belgium have a higher consumption rate of mussels (3.08 kg per person per year) (Lusher *et al.*, 2017a). A study conducted by Naji *et al.* (2018) in the Persian Gulf showed that the dietary exposure of regional consumers to MPs can amount to approximately 4800 particles per person per year.

Canned sardines and sprats present a low exposure, with a maximum of only three plastic particles per can (Karami *et al.*, 2018). Furthermore, MPs detected in fish are mostly located in the gut (Foekema *et al.*, 2013; Rochman *et al.*, 2015; Romeo *et al.*, 2015), which is usually removed before the fish is consumed. It is important that the amount of MPs and NPs in fish muscle tissue consumed by humans is analysed to more accurately determine the MP exposure.

4.1.2 Estimated intake of MPs by inhalation

The risk of plastic ingestion via mussel consumption was found to be minimal compared with fibre exposure from household dust fallout during a meal (13,731–68,415 particles per person per year) (Catarino *et al.*,

Table 4.1. Estimated daily intake of MPs by ingestion

Source	Estimated MP/NP concentration		Estimated exposure (particles per person per year)		Predicted exposure (Ireland), particles per person per year	References
Source	Range	Mean	Range of exposure	Mean		
Drinking water	0–10.97 particles/L	5.3 particles/L	1984–5800 particles	4400	2321	Kosuth <i>et al.</i> , 2018; Oßmann <i>et al.</i> , 2018; Schymanski <i>et al.</i> , 2018; Marsden <i>et al.</i> , 2019
Beer	0.79 particles/L	13.32 particles/L	0–1800 particles	520	1545	Kosuth <i>et al.</i> , 2018
Teabags	11.6 billion MP particles per teabag; 3.1 billion NP particles per teabag			21,170 billion MP particles; 5657 billion NP particles (based on consumption of five cups/day)	21,170 billion MPs 5657 billion NPs/y	Hernandez <i>et al.</i> , 2019
Milk	3–11 particles/L	6.5 particles/L	219–803 (based on consumption of 200 mL milk/day)		475	Diaz-Basantes <i>et al.</i> , 2020; Kuttralam-Muniasamy <i>et al.</i> , 2020
Shellfish	0.2–10.5 particles/g	0.36 ± 0.07 particles/g	1800–11,970	110,000	1806	FAOSTAT, 2013; De Witte <i>et al.</i> , 2014; Mathalon and Hill, 2014; Van Cauwenberghe and Janssen, 2014; Vandermeersch <i>et al.</i> , 2015b; J. Li <i>et al.</i> , 2016; J. Li <i>et al.</i> , 2018; Renzi <i>et al.</i> , 2018; Abidli <i>et al.</i> , 2019; Cho <i>et al.</i> , 2019
Fish	0.117–311 particles (based on weight of average fish portion of 170 g)	NR	0.819–2177 (based on average US consumption of 5.3 kg of fish per person per year)	NR	0.59–1648 (based on 5.3 kg of fish per person per year)	EFSA <i>et al.</i> , 2016; FAO, 2017
Chicken	NR	0.0102 particles/kg	NR	0.2124	NR	Huerta Lwanga <i>et al.</i> , 2017
Sugar	32–217 particles/kg	NR	832–5642 (based on average US consumption of 71 g/day)	NR	Range 1174–7963 (based on average Irish consumption of 100 g/day)	Liebezeit and Liebezeit, 2013
Salt	7–806 particles/kg	212 particles/kg	40–680	180	386.9	Mathalon and Hill., 2014; Yang <i>et al.</i> , 2015; Karami <i>et al.</i> , 2017a; Kosuth <i>et al.</i> , 2018
Air	2–355 particles/m ²	66.33 particles/m ²	353–2429	1063	NR	Dris <i>et al.</i> , 2016, 2017

NR, not recorded.

2018), making inhalation another important pathway of human exposure to MPs and NPs. Vianello *et al.* (2019) evaluated the exposure of humans to indoor airborne MPs using a breathing thermal manikin and

found that all samples assessed were contaminated with MPs, at concentrations between 1.7 and 16.2 particles/m³, which is the only evidence that MPs could potentially be retained in the lungs.

4.1.3 *Estimated daily intake of MPs by dermal exposure*

The per capita exposure to MPs found in personal care products for the US population has been estimated at approximately 2.4 mg per day (based on the usage of PE MP beads used in personal care products), suggesting that the US population may account for an estimated 263 tonnes per year of PE MP (Gouin *et al.*, 2015). Given that the use of microbeads in these products is being banned, this source of exposure may not be as important as digestion and inhalation.

4.2 **Measuring Human Exposure to MPs and NPs**

4.2.1 *Introduction*

MPs and NPs are heterogeneous particles that can differ in their physical and chemical properties, shape, size, plastic types, surface chemistry and hydrophobicity (Smith *et al.*, 2018). In order to determine the levels of exposure it is important to identify optimal analytical tools. Analytical procedures for determining MPs and NPs in samples consist of extraction, isolation (or separation), identification and quantification (or classification). Conventionally, visual sorting and extraction from tissue with the naked eye has been used to identify large MPs (Heo *et al.*, 2013; Jensen *et al.*, 2019; Rummel *et al.*, 2016) based on physical characteristics such as size (range 1–5 mm), shape and colour (Devriese *et al.*, 2015; Nadal *et al.*, 2016; Romeo *et al.*, 2015; Taylor *et al.*, 2016; Vendel *et al.*, 2017). However, this method is highly susceptible to human error. MPs trapped within tissues or contaminated with organic matter cannot be visualised and, as a result, acid, oxidative, alkaline or enzyme-based digestion must be performed. There is no uniform protocol for quantification of MPs and NPs in solid matrices (tissues, soils, etc.), which makes it difficult to compare studies employing different methods (Z. Wang *et al.*, 2018).

Because the size of MP/NP particles varies widely, determining accurate levels of exposure demands the quantification of particles according to size (J. Lee *et al.*, 2013). Methods of doing so vary according to the matrix of interest, such as water, sediment or biota, and the techniques used in the extraction, enumeration and identification. Techniques vary greatly, even within

the same matrix, making comparison between studies difficult (Hermsen *et al.*, 2018; Hidalgo-Ruz *et al.*, 2012; Lusher *et al.*, 2017a). The standardisation of protocols to extract and characterise these particles is an urgent scientific priority. There is also a need to develop methods to detect NPs, particularly in human tissues, as, given their size, they are more likely to be taken up by human cells and interact with them at the cellular level. In this section, the current techniques used for sample processing and detection and quantification of MPs and NPs are summarised. Recommendations are made on the optimal methods available and future developmental needs for new analytical tools are identified.

4.2.2 *Sieving and filtration*

In order to be quantified and characterised, MPs from liquid samples are often separated by density flotation through salt addition [usually sodium chloride (NaCl) or sodium iodide (NaI)] and flotation, filtration through size fractionation or sieving through size exclusion. Filtering or sieving is the most commonly used method for separation of MPs from water samples and from plastic containing supernatants resulting from density separation of sediment samples. Stainless-steel sieves or glass fibre filters are used instead of plastic tools to minimise procedural contamination. Rinsing is always required after each sieving or filtration (Mason *et al.*, 2016). The pore size of filter or of sieve meshes can vary greatly. Pore or mesh size determines the lower size of MPs detected. However, small pore or mesh sizes may also result in quick obstruction by organic and mineral matter. Disadvantages of this sieving method include, but are not limited to, easy blocking of sieve holes, difficulty in obtaining wide-range size fractions, and lengthy sample processing time (Vianello *et al.*, 2019). Thus, a standardised pore or mesh size should be defined. To save filtration time, stacks of sieves or filters with different aperture sizes may be used to fractionate MPs. A standardised series of reasonable size fractions is urgently needed to better compare the occurrence of MPs from the same size fraction.

4.2.3 *Density separation: flotation and elutriation*

Density separation is widely used to isolate low-density particles from higher-density sand, mud,

sediment and other sample matrices (Mai *et al.*, 2018). Many MPs, such as PP and PE, have lower densities than seawater ($\approx 1.10 \text{ g/cm}^3$). High-density plastics, e.g. PVC, have densities up to 1.40 g/cm^3 or greater, depending on additives and attached biofilms. Various high-density solutions have been employed to isolate MPs from environmental matrices. The density separation with a hypersaline solution that separates plastic by difference in their density is a method that has been employed to extract MPs from sediments (Thompson *et al.*, 2009) such as marine sediment (Morét-Ferguson *et al.*, 2010) with varying degrees of success, with recoveries as low as 30% for smaller polystyrene particles $< 100 \mu\text{m}$ in biosolids and sludge with zinc chloride (ZnCl) flotation (Wang *et al.*, 2018).

A wide range of brine solutions are used for density separation, including NaCl, ZnCl, NaI and zinc bromide (ZnBr_2), as well as canola (rapeseed) oil, owing to the hydrophobic properties of plastics. Density separation methods using these solutions have a number of advantages, such as being inexpensive (except for NaI) and easy to implement; in addition, the materials required are stocked in most laboratories. Following separation, sample analysis is possible using Fourier-transform infrared spectroscopy (FTIR) and FTIR and Raman spectroscopy (FTIR/Raman). Density separation can be time-consuming; for example, a 5 M NaCl solution needs to be left overnight, although canola oil requires only 2 hours (Crichton *et al.*, 2017). The recovery rate varies depending on the solution used, with ZnBr_2 presenting the highest recovery rate, at 99%, followed by canola oil (96%), NaCl (85–95%) and NaI (83%) (Nuelle *et al.*, 2014). The most favoured solution is NaCl, which is inexpensive and non-hazardous, and has a density of approximately 1.20 g/cm^3 (Fries *et al.*, 2013).

Density separation using NaCl is recommended by the National Oceanic and Atmospheric Administration (Mausra and Foster, 2015). It is appropriate to use the NaCl solution to extract low-density MPs such as PE ($0.917\text{--}0.965 \text{ g/cm}^3$), PP ($0.85\text{--}0.94 \text{ g/cm}^3$) and PS ($1.04\text{--}1.1 \text{ g/cm}^3$). However, for separation of denser MPs such as PVC ($1.3\text{--}1.7 \text{ g/cm}^3$) and PET ($1.4\text{--}1.6 \text{ g/cm}^3$), saturated NaCl solutions are less efficient, which can lead to underestimation in the quantification of MPs (Zobkov and Esiukova, 2018).

More efficient instruments or solutions such as surfactants could be developed to achieve better recovery at lower cost and with less environmental

impact. As most MPs are hydrophobic, surfactants can be used to separate MPs from water (Shen *et al.*, 2019). The extraction efficiency relies on the shape, size and origin of MPs and the extraction method. Future research should focus on achieving higher extraction efficiency and identifying universal standard protocols.

4.2.4 Digestion

Digestion is a process required to remove organic material that would interfere with the analysis of the MPs and NPs in the environmental samples. Samples collected from the environment inevitably contain dense amounts of naturally occurring organic materials such as zooplankton, phytoplankton, remnants of aquatic organisms or biofilms (e.g. brown algae or bacterial film) attached to the surface of plastic particles. This can lead to overestimation of environmental concentrations of particles and increase the number of particles subjected to further analysis. Thus, there is a need to create a simple method of digestion capable of reducing organic matter without affecting the structural or chemical integrity of plastics (Felsing *et al.*, 2018; Miller *et al.*, 2017). Multi-step pretreatments are used to ensure complete removal of biofilms and organic materials attached to the surface of MPs in environmental samples, which subsequently aids visual observation and spectral identification. To date, four major classes of digesting agents have been employed to eliminate organic materials, namely acids (De Witte *et al.*, 2014; Van Cauwenberghe and Janssen, 2014; Van Cauwenberghe *et al.*, 2015), bases (Foekema *et al.*, 2013; Rochman *et al.*, 2015), oxidative agents (Collard *et al.*, 2018; Nuelle *et al.*, 2014) and enzymes (Cole *et al.*, 2011) (Table 4.2).

Acid digestion

Chemical digestion with simple and/or mixtures of strong acids such as nitric acid (HNO_3), hydrochloric acid (HCl) and perchloric acid (HClO_4) is a useful treatment to remove biological material from organic samples, thus facilitating isolation of plastic particles (Brandon *et al.*, 2016; Catarino *et al.*, 2018; Karami *et al.*, 2017b; Naidoo and Glassom, 2019; Vandermeersch *et al.*, 2015b). However, some plastics (e.g. nylon, PET) have low resistance to acids and may be degraded; HNO_3 is particularly destructive (Lusher *et al.*, 2017b). Higher temperatures can

Table 4.2. Summary of extraction methods of MPs/NPs

Method	Plastic degradation (verified for)	Advantages	Disadvantages	References
Density separation: flotation and elutriation				
NaCl	PS, PA, PP, PVA and PE	Inexpensive Easy methodology Low chemical hazards Ability to use FTIR/Raman following separation Easily accessible – common in laboratories	Time intensive – multiple density separations must occur Lower recovery rates (85–95%) Applicability to all sample types unknown	Quinn <i>et al.</i> , 2017
NaI	PS, PA and PVC	As above except relatively expensive	Lower recovery rates (83%) Applicability to all sample types unknown Time-intensive – multiple density flotations required	Crichton <i>et al.</i> , 2017
ZnBr ₂	PP, LDPE, PE, HDPE, PS, PA, PVC and PET	As above Recovery rates of 99%	Not confirmed for application to non-sediment samples Time-intensive – multiple density flotations required	Quinn <i>et al.</i> , 2017
Canola oil	PS, PVC, ABS, PES and PA	As above Recovery rates high (96.1%), especially for PVC (high-density)	Not confirmed for application to non-sediment samples Additional cleaning step must be applied to allow for FTIR/Raman spectroscopy	Crichton <i>et al.</i> , 2017
Acid digestion				
HClO ₄	Unknown	Easy methodology Overnight digestion Easily accessible – common in laboratories	Recovery rates showed a loss of product after treatment Expensive Ability to use FTIR/Raman spectroscopy following separation unknown Applicability to all sample types unknown High chemical hazards – corrosive acid Often needs to be heated/boiled to digest biologically rich samples, which could result in loss of plastics	Vandermeersch <i>et al.</i> , 2015b
HCl	Alteration to PET and PVC following treatment	Easy methodology 12-hour digestion Easily accessible – common in laboratories	Recovery rates showed a weight change after treatment Expensive Ability to use FTIR/Raman spectroscopy following separation unknown Applicability to all sample types unknown	Brandon <i>et al.</i> , 2016; Karami <i>et al.</i> , 2017b
HNO ₃	Alteration to PS and PA following treatment	Easy methodology Overnight digestion Easily accessible – common in laboratories Relatively inexpensive Ability to use Raman spectroscopy following treatment	High chemical hazards – corrosive acid Often needs to be heated/boiled to digest biologically rich samples, which could result in loss of plastics Ability to use FTIR/Raman spectroscopy following separation unknown Applicability to all sample types unknown	Naidoo <i>et al.</i> , 2016; Catarino <i>et al.</i> , 2018

Table 4.2. Continued

Method	Plastic degradation (verified for)	Advantages	Disadvantages	References
Alkaline digestion				
NaOH	Unknown	Easy methodology Low chemical hazards Relatively inexpensive Ability to use FTIR/Raman spectroscopy following separation Easily accessible – common in laboratories	Lengthy digestion time of 3 weeks May be necessary to heat sample, which may cause loss of plastics Applicability to all sample types unknown	Dehaut <i>et al.</i> , 2016; Catarino <i>et al.</i> , 2018
KOH	Loss of PET; yellowing of PA Degradation of LDPE, CA and PA	As above Short digestion time of only 24 hours Recovery rates show no change in weight	Recovery rates reported only by weight, not abundance Applicability to all sample types unknown Known to leave behind reaction residue on plastics; may hinder FTIR if not cleaned Some chemical hazards	Karami <i>et al.</i> , 2017b
Oxidative digestion				
FeSO ₄ (catalyst)	Unknown	Easy methodology Relatively inexpensive Short digestion times (< 1 hour) Recovery rates > 85% Low chemical hazards Easily accessible – common in laboratories	Ability to use FTIR/Raman spectroscopy following separation unknown Applicability to all sample types unknown Often needs to be heated/boiled to digest biologically rich samples, which could result in loss of plastics	Karami <i>et al.</i> , 2017b
H ₂ O ₂	Degradation of PA; colour change of PET	Easy methodology Relatively inexpensive Short digestion times (< 1 hour) Recovery rates > 85% Ability to use FTIR/Raman spectroscopy following separation Can be applied to all sample types	J. Li <i>et al.</i> , 2015	
Enzymatic digestion				
Proteinase K	Unknown	Short digestion time of ≈ 3 hours Low chemical hazards Ability to use FTIR/Raman following separation	Recovery rates unknown Applicability to all sample types unknown Relatively very expensive (US\$448/100g) Methodology more complex than simple acid digestion Not common in laboratories	Karlsson <i>et al.</i> , 2017
Corolase 7089	PET, HDPE and PA	Fast methodology of ≈ 1 hour Recovery rates of 93% Ability to apply FTIR/Raman following separation	Need to heat sample to 60°C – may result in loss of plastic Applicability to all sample types unknown Not common in laboratories	Ziajahromi <i>et al.</i> , 2017
Trypsin	PET, HDPE, PVC, PP, PS and PA	Short digestion time of 30 minutes Low chemical hazards	Recovery rates unknown Ability to use FTIR/Raman spectroscopy following separation unknown Applicability to all sample types unknown Effect on plastic types unknown Very expensive (US\$4210/100g) Methodology more complex than simple acid Not common in laboratories	Courtene-Jones <i>et al.</i> , 2017

Table 4.2. Continued

Method	Plastic degradation (verified for)	Advantages	Disadvantages	References
Other				
Pulsed ultrasonic extraction	PVC, PE, PP, PS, PET and fibres	Easy methodology Relatively inexpensive No chemical hazards Easily accessible – common in laboratories Fast methodology (≈6 minutes)	Applicability to all sample types unknown Recovery rates unknown	Wagner <i>et al.</i> , 2014, 2017

PES, Polyethersulfone.

accelerate the digestion process. For example, at 80°C HNO₃ (55%), digestion of fish tissue is up to 26 times faster; however, temperatures above 60°C can destroy some MPs (Naidoo *et al.*, 2016). Desforges *et al.* (2014) suggested that a range of plastics should be subjected to HNO₃ isolation techniques to determine whether they survive the digestion protocol. Methods using acid digestion are relatively easy, and the materials required for the process are generally common in most laboratories. The process is lengthy, taking 12–14 hours, and MP recovery rates showed a decrease in product weight after treatment. HNO₃ is highly corrosive and therefore may be unsuitable if the sample requires analysis by FTIR/Raman spectroscopy following separation (Brandon *et al.*, 2016; Catarino *et al.*, 2018; Karami *et al.*, 2017b; Naidoo and Glassom, 2019; Vandermeersch *et al.*, 2015b). HCl seems to be the least effective in treating large quantities of biological material (Maes *et al.*, 2017). Nonetheless, Karami *et al.* (2017b) found that HCl (37%) at 25°C had a digestion efficiency >95%, although it caused PET to melt. This difference in the findings of different studies may reflect different protocols, with variations in concentration and temperature affecting digestion efficiency. Thus, acid digestion should be used with caution, as it may lead to underestimation of MPs in environmental samples.

Alkaline digestion

Alkaline digestion with sodium hydroxide (NaOH; 2–10 M) or potassium hydroxide (KOH; 10%) (Dehaut *et al.*, 2016; Zhao *et al.*, 2017) is the most commonly reported alternative to acid digestion. However, alkaline digestion may also damage or discolour plastics (Qiu *et al.*, 2016), leave oily residues and

bone fragments (Dehaut *et al.*, 2016) or result in tissue residues being deposited on plastic surfaces. These negative effects complicate characterisation by spectroscopy (Wagner *et al.*, 2014). Like acid digestion, the methodology is relatively easy, with low chemical hazards, and uses materials that are common in most laboratories. The digestion time required is shorter for KOH than for NaOH, 12 hours compared with 3 weeks. Both chemicals degrade MPs but NaOH has been reported to lead to the degradation of polycarbonate (PC) and PET (Dehaut *et al.*, 2016), while KOH may cause discoloration of nylon, PE and uPVC (unplasticised PVC), and degradation of nylon, polyester, PE, PC, PET, PVC, low-density polyethylene (LDPE) and cellulose acetate (CA) (Karami *et al.*, 2018; Kühn *et al.*, 2017; Maes *et al.*, 2017). Both NaOH and KOH have been used to remove MPs from organic matter (Minténig *et al.*, 2018). KOH solution has been shown to be more damaging at higher temperatures (Karami *et al.*, 2017b), and the optimum conditions for the extraction of MPs from biota have been reported to be 10% KOH at 60°C (Avio *et al.*, 2015; Foekema *et al.*, 2013; Karami *et al.*, 2017b). However, the efficacy of KOH in extracting MPs from sludge or soil has not yet been examined in detail. Hard parts and fats are not fully digested by alkali solutions, so alkali-insoluble compounds in soils would not be removed (Watteau *et al.*, 2018).

Oxidative digestion

Hydrogen peroxide (H₂O₂; 30–35%) is an oxidising agent able to digest organic matter. Like other types of digestion, the methodology is both easy and relatively inexpensive and can be applied to all

sample types; in addition the digestion time required is short (< 1 hour) and recovery rates are relatively high (> 85%). However, H₂O₂ oxidation has been shown to negatively impact extraction efficiencies of PS MPs < 100 µm in size from soil and biosolids (Wang *et al.*, 2018). MP samples separated from organic matter using H₂O₂ can be characterised by FTIR/Raman spectroscopy. H₂O₂ is more efficient than NaOH and HCl in dissolving organic matter, and causes little to no degradation of plastics, although it can cause colour fading (Nuelle *et al.*, 2014; Zhao *et al.*, 2017). Nuelle *et al.* (2014) reported resistance of PVC, PET, nylon, acrylonitrile butadiene styrene (ABS), PC, polyurethane (PUR), PP, LDPE, LLDPE (linear LDPE) and high-density polyethylene (HDPE) to H₂O₂, with some discoloration, while other studies have reported reduced recovery of nylon-6 (NY6) and nylon-66 (NY66). Colour alteration of PET fragments has been reported with H₂O₂ treatments at high temperatures (50°C) for prolonged periods (96 hours) (Brandon *et al.*, 2016; Karami *et al.*, 2017b). It must be mentioned that H₂O₂ could also complicate optical analysis rather than facilitate it, as natural particulates can then no longer be distinguished from plastics that are whitish or transparent.

FeSO₄ (Fenton's reagent) can also be used to remove organic matter such as MPs from organic-rich wastewater samples, although it has been shown to work best with H₂O₂ (Dyachenko *et al.*, 2017; Tagg *et al.*, 2016). FeSO₄ is effective in destroying organic components, such as highly chlorinated aromatic compounds or inorganic compounds, that are typically resistant to H₂O₂, and therefore may prove more effective in removing all organic components from complex environmental substrates (Pignatello *et al.*, 2006).

Enzymatic digestion

Enzymatic digestion is a biologically specific means of hydrolysing proteins and breaking down tissues (Cole *et al.*, 2014) and is preferred over other digestion methods because it has a negligible effect on plastics (Table 4.3) (Catarino *et al.*, 2018; Cole *et al.*, 2014). However, enzyme efficiency will vary with the type of organic material present in the sample (Courtene-Jones *et al.*, 2017). Trypsin can be used to extract MPs from biological samples, providing a rapid, cost-efficient and effective method of separation

(Courtene-Jones *et al.*, 2017), while the use of Corolase 7089 (AB Enzymes GmbH, Darmstadt, Germany) is associated with high recovery rates (93% ± 10%) when separating MPs from soft tissue such as muscle tissue (Catarino *et al.*, 2018). The best performance is achieved with proteinase K, with the highest recovery rate (97%) and no observed degradation effects on the plastics in subsequent Raman analysis (Karlsson *et al.*, 2017). However, it should be noted that some enzyme digestion techniques rely on the presence of oxidising reagents, such as sodium perchlorate or H₂O₂ that can degrade plastics (Cole *et al.*, 2014; Karlsson *et al.*, 2017).

Conclusion and other digestion methods used

Other, less common, methods of extracting MPs from organic matter have been reported in the literature. These include the use of microwaves, which seem to damage MPs (Karlsson *et al.*, 2017), and ultrasonication, which is useful in combination with other methods such as improved digestion of sludge using NaOH (Jin *et al.*, 2018). The use of sodium hypochlorite (NaClO; 24.8 g/L) was reported as an efficient method in the digestion of fish stomach contents without affecting plastics, but potentially causing discoloration (Collard *et al.*, 2018). Other studies have also suggested that a combination of methods, for example acid and alkali digestion used sequentially (e.g. NaOH and HNO₃), can provide good digestion of biological material and high recovery rates (95%), with few changes to MP characteristics (Roch and Brinker, 2017). NaCl flotation followed by H₂O₂ digestion resulted in successful extraction of MPs from fish guts, with good recovery rates (80–90% dependent on particle size and class; Avio *et al.*, 2015). While digestion is not necessary for drinking water, a digestion step is required for surface water and wastewater samples, in which high organic matter concentrations hamper the selection and (visual) identification of particles.

4.2.5 Physical characterisation of MPs

It is difficult to identify MPs of various sizes, shapes, and plastic types fully and reliably from complex environmental matrices using a single analytical method. This identification is also complicated by the requirement to first separate the MPs from organic matter or from sediment, which can alter

their characteristics. In general, MP analysis consists of two steps: physical characterisation of potential plastics (e.g. microscopy) followed by chemical characterisation (e.g. spectroscopy) for confirmation

of plastics (Table 4.3). The first examination of the sample is frequently performed by visual observation, which can be achieved through simple naked-eye observation or assisted by optical microscopy (Song

Table 4.3. Comparison of different MP detection techniques

Method	Procedure	Application/advantages	Limitations/disadvantages	References
Visual identification	Use of microscope to observe the composition	MPs' source, degradation stage, type, colour and shape of particles can be detected easily Simple, fast and easy	Size limitation of greater than 1 mm Time-consuming with high rate of error (>20%) No chemical contribution High false-positive rate High possibility of missing small and transparent plastic particles No plastic composition data	Hidalgo-Ruz <i>et al.</i> , 2012
Nile Red staining	Use of fluorescence or UV radiation for visualisation and image processing	Easy methodology Relatively inexpensive Low chemical hazards Easily accessible – common in laboratories Fast (\approx 1 hour) Ability to use FTIR/Raman spectroscopy following separation	Not actually a separation method; still a need to implement additional techniques While quick, could add on time to methodology depending on actual separation technique chosen Recovery rates unknown Applicability to all sample types unknown	Maes <i>et al.</i> , 2017; Hengstmann and Fischer, 2019
Thermal analysis	Comparison of plastic origin with their characteristic combustion products	Allows simultaneous analysis of plastic type and additive chemicals (Py-GC-MS)	Only small particles can be manipulated Destructive analysis Limited plastic identification (DSC) Complex data (Py-GC-MS)	Fries <i>et al.</i> , 2013
Raman spectroscopy	Sample is irradiated with laser wavelengths in the range 500–800 nm	Facilitates the detection of even the smallest MPs Accurate method of identifying the abundance and types of MPs No possibility of false-positive data as all plastic-like particles can be chemically identified Detection of plastics down to 1 μ m in size Non-destructive analysis Non-contact analysis	Fluorescent samples excited by the laser cannot be measured Expensive instrument Laborious work and whole-particle identification is time-consuming Interference by pigments	Song <i>et al.</i> , 2015
FTIR spectroscopy	Infrared radiation causes molecular vibrations	An ideal technique for the identification of MPs with highly specific distinct band patterns No possibility of false-positive data as all plastic-like particles can be chemically identified Low false-negative rate Non-destructive analysis Detection of plastics down to 10 μ m in size Automatic mapping	Due to high absorption rate, black particles are not detectable Expensive instrument Laborious work and whole-particle identification is time-consuming Contact analysis (ATR)	Harrison <i>et al.</i> , 2012; Talvitie <i>et al.</i> , 2017
SEM-EDS	Microscopy coupled with elemental composition analysis	High-resolution imaging enables identification of chemical elements Ability to differentiate from non-plastic particles due to signal brightness from inorganic elements	Cannot always distinguish between plastic and non-plastic particles Expensive instrument; requires skilled operator	Wagner <i>et al.</i> , 2017; Ding <i>et al.</i> , 2019;

et al., 2015). The second step reveals surface texture and structural information that allows for the identification of ambiguous particles (Shim *et al.*, 2017). More characteristics that distinguish MPs from other particles include colour, shape and surface texture.

Microscopy

Although some small plastics can be identified with the naked eye (Heo *et al.*, 2013), stereomicroscopy (or dissecting microscopy) is a widely used identification method for MPs whose size falls in the hundreds of microns range (Desforges *et al.*, 2014; Eriksen *et al.*, 2014). Sorting and identification allows classification of particles based on physical characteristics such as shape, colour and size. This method has many limitations in terms of accuracy, as non-plastic particles in a variety of colours can be mistaken for MP particles (Hanvey *et al.*, 2017). The error rate of visual sorting ranges from 20% to 70% (Eriksen *et al.*, 2013; Hidalgo-Ruz *et al.*, 2012), and increases with decreasing particle size. Eriksen *et al.* (2013) reported that approximately 20% of particles identified by visual observation as MPs were later revealed by scanning electron microscopy and energy-dispersive X-ray spectroscopy (SEM-EDS) to be aluminium silicate from coal ash, while Martinelli *et al.* (2020) reported that only 2% of particles extracted from oysters and originally described as MPs were subsequently found, upon further analysis, to be plastic.

Fluorescence staining methods provide a simple and sensitive approach to highlighting specific objects or structures in biological and medical studies, and the use of the lipophilic fluorescent dye Nile Red has gained acceptance for staining of MPs extracted from a variety of environmental matrices (Hengstmann and Fischer, 2019). This allows for a simple tool to visually identify plastics and improve the pre-selection method of particles to be submitted for chemical characterisation (Maes *et al.*, 2017; Shim *et al.*, 2016), and can be combined with ultraviolet (UV) photography or fluorescence microscopy and image analysis software techniques for fast throughput analysis. The advantages of staining with Nile Red are the short incubation time (10–30 minutes), the high recovery rate (>95%) and the possibility of subsequently using vibrational spectroscopy with or without a short cleaning step with bleach (Erni-Cassola *et al.*, 2017).

SEM can provide extremely clear and high-magnification images of MPs, facilitating their discrimination from, for example, glass fibres (Cooper and Corcoran, 2010). However, SEM does not enable identification of particles from their colour, and therefore this technique is recommended only for specific plastic particles (Shim *et al.*, 2017). Other advanced microscopy techniques have been used to identify plastic particles in specific cases. Polarised optical microscopy was successfully used to identify PE particles in laboratory accumulation and toxicity experiments (von Moos *et al.*, 2012); however, this method is not suitable for identification of opaque MPs.

4.2.6 Chemical characterisation of MPs

Although visual sorting is a time-saving method for the enumeration of MPs, technologies that are more reliable are used for evaluating their abundance and chemical composition. To date, potential MPs have mostly been identified using spectroscopic (Imhof *et al.*, 2012; K ppler *et al.*, 2016; L der and Gerdts, 2015) or thermal degradation analyses (Fries *et al.*, 2013). Particles sorted manually are mostly analysed using attenuated total reflectance (ATR), FTIR or μ -FTIR spectroscopy (Courtene-Jones *et al.*, 2017; Rummel *et al.*, 2016), and pyrolysis–gas chromatography–mass spectrometry (Py-GC-MS) (Fries *et al.*, 2013). Raman or μ -Raman spectroscopy is also applied.

Thermo-analytical techniques

Thermoanalytical methods such as differential scanning calorimetry (DSC), Py-GC-MS and thermogravimetric analysis (TGA) (often also coupled with mass spectrometry) are used for analysis of MPs by measuring changes in the physical and chemical properties of plastics depending on their thermal stability (D michen *et al.*, 2017). DSC is a useful method for studying the thermal properties of plastic materials. This method requires a different reference material to identify each plastic type (Casta eda *et al.*, 2014). Thermal analysis provides an alternative to spectroscopy for chemical identification of certain plastic types; however, it is a destructive method as samples are firstly thermally degraded, preventing subsequent additional analysis. DSC analysis is relatively simple and fast; however, the techniques are limited by the size of plastic particles. The method is

less applicable for mixtures with a high concentration of impurities (Ivleva *et al.*, 2017).

TGA-MS allows fast analysis and quantification of large quantities of the five most common plastics found in MPs (PE, PP, PS, polyamide and PET) in environmental samples, assuring the samples' composition representativeness, and without preselection of MPs in the samples (Dümichen *et al.*, 2017). Thermal analysis combined with GC-MS can simultaneously analyse additive chemicals in MPs. In addition to single particles, bulk samples can also be analysed with thermal analysis combined with GC-MS, which provides summed MP concentration data on a weight basis (w/w). However, information related to the number, size and shape of analysed MP particles is not provided by bulk analysis.

Py-GC-MS is a destructive technique that has also been described for the characterisation of MPs in terms of plastic type, by analysing their thermal degradation products (Fries *et al.*, 2013). This technique eliminates the need for pretreatment of samples, as the solid plastic sample is examined directly. In addition, Py-GC-MS is not sensitive to the shape, size and associated organic or inorganic contaminants of the analysed particles (Käppler *et al.*, 2016). Only a small amount of sample (100–500 µg) is needed for one measurement, which is an advantage of this method if that small amount is all that is available (Dümichen *et al.*, 2017); however, the technique is not recommended for the processing of large sample quantities (Nuelle *et al.*, 2014).

Fourier-transform infrared spectroscopy

FTIR spectroscopy can provide a unique infrared spectrum because different materials have different bond compositions, making it possible to identify an unknown substance by comparing its spectrum with that of a known substance. Because of its high reliability, FTIR has become one of the most commonly used techniques in the chemical characterisation of MPs recovered from environmental samples (Shim *et al.*, 2017; Silva *et al.*, 2018). FTIR spectroscopy is frequently used for the qualitative analysis of MPs (> 10 µm), as plastic particles are identified by their characteristic IR spectra (Frias *et al.*, 2010; Thompson *et al.*, 2009; Vianello *et al.*, 2019). Application of FTIR spectroscopy in analysing ultra-fine plastic particles (e.g. particle sizes < 1 µm) and classifying plastic type

from complex environmental samples is challenging and requires a skilled operator (Huppertsberg and Knepper, 2018).

Diverse FTIR techniques have been used in the characterisation of MPs; ATR-FTIR improves the information on irregular MPs, while transmission FTIR is applicable to thick or opaque samples (Courtene-Jones *et al.*, 2017). One advantage of FTIR reflectance spectroscopy is its non-invasive nature, as it allows samples to be analysed without destroying them (Harvey *et al.*, 2017). However, the disadvantage is the possibility of non-interpretable spectra due to refractive error (Harrison *et al.*, 2012). Small MPs require the use of micro-FTIR (µ-FTIR), which is FTIR spectroscopy combined with microscopy by using micro-FTIR and infrared bands (Song *et al.*, 2014). µ-FTIR instruments are expensive (Shim *et al.*, 2018). A further drawback is that preselection of particles is necessary. To avoid this requirement, the application of focal plane array (FPA) detectors with FTIR and comparison with a comprehensive spectral database, along with image analysis for physical characterisation, was developed by Primpke *et al.* (2017).

Raman spectroscopy

Raman, like FTIR, spectroscopy identifies plastics based on the energy absorption of characteristic functional groups. It is a suitable method for identification of the most common plastic types and identifies MP particles in different environmental samples with high reliability (Cole *et al.*, 2011; Imhof *et al.*, 2012; Murray and Cowie, 2011; Van Cauwenberghe *et al.*, 2015). Raman spectroscopy is a "surface technique"; thus, large, visually sorted MP particles can be analysed and the technique can be coupled with microscopy. Accordingly, micro-Raman spectroscopy allows for the identification of a broad range of size classes down to very small plastic particles of sizes below 1 µm, as the smaller diameter of the Raman laser beam facilitates the identification of MPs as small as a few micrometres in size (Cole *et al.*, 2014). One of the main limitations of Raman spectral analysis is the degradation of the sample following UV exposure (Lenz *et al.*, 2015). Spectroscopic methods are used to identify the specific chemical structure of plastics by comparing their absorption or emission spectra with reference spectra (Löder

and Gerdt, 2015); thus, the spectra of degraded plastics at different stages should be included in reference databases in order to enable more accurate identification of plastics in MPs (Araujo *et al.*, 2018). Raman spectroscopy is comparable to the FTIR method, including the requirement for expensive instrumentation.

Scanning electron microscopy and energy-dispersive X-ray spectroscopy

Analysis with energy-dispersive X-ray spectroscopy (EDS) is useful for identifying carbon-dominant plastics from inorganic particles (Z.-M. Wang *et al.*, 2017). The combined use of SEM and EDS is able to provide detailed information about the elemental composition of MPs and the inorganic additives they contain (Quinn *et al.*, 2017). Utilisation of SEM-EDS aids in further differentiating natural materials from MPs via imaging and elemental analysis, thereby reducing the number of particles needed for spectroscopic analysis (Fries *et al.*, 2013). However, SEM-EDS is expensive and sample preparation and examination require substantial time and effort, thus limiting the number of samples that can be processed simultaneously.

4.2.7 NP detection, characterisation and quantification methods

Several methods that have been used for the detection of nanomaterials, such as UV-Vis spectrometry, electron microscopy, field flow fractionation (FFF) and dynamic light scattering (DLS) techniques, are also useful in detecting NPs (von der Kammer *et al.* 2012). Electron microscopy is widely used in plastic science and has been used over the years to characterise several types of nano-sized plastic structures and particles (Gigault *et al.*, 2016). In addition, light scattering (LS)-based techniques such as DLS (Gigault *et al.*, 2016) and nanoparticle tracking analysis have been exploited to monitor the formation of NPs from fragmentation of plastics. In most cases, the procedure would require not only degradation of the matrix but also isolation/separation of the NPs from organic residues or other particulates for their proper identification. In this respect, hyphenated techniques such as FFF and hydrodynamic chromatography coupled to multiple detectors [e.g. multi-angle light scattering, (MALS) or UV-Vis absorbance] are quite

attractive as they allow separation of small molecules (e.g. matrix residues), particles and macromolecules in a considerable size range (around 1–1000 nm) ahead of detection by LS or absorbance (Shim *et al.*, 2018). Shim *et al.* (2017) used SEM-EDS to confirm the presence of NPs in abrasion experiments, while multiple-wavelength UV-Vis has been used to detect NPs in mussels (*Mytilus edulis*) (Wegner *et al.*, 2012). Velzeboer *et al.* (2014) used transmission SEM and conventional light microscopy to characterise pristine polystyrene particles and aggregates, respectively. Correia and Loeschner (2018) found that asymmetrical FFF coupled to MALS was suitable for the detection of NPs in fish, with a limit of detection of 52 µg/g fish for polystyrene NPs. Digestion of the organic matrix is necessary prior to analysis, and it was found that acid digestion caused aggregation of the PS NPs, whereas enzymatic degradation worked well. However, the method was found to be unsuitable for detection of PE NPs, i.e. a method that works for one set of NPs may not work for other polymer types. Methods for the detection of NPs are in the early stages of development as, to date, we are not aware of studies reporting established analytical methods to detect NPs in marine or freshwater systems. This is problematic for human and animal health as, given their size, these particles are more likely to translocate the cell membranes.

4.3 Conclusions and Gaps

The two key groups of characteristics for MP/NP analysis are physical (size, shape and colour) and chemical (plastic type) (Löder and Gerdt, 2015). Any method that reliably measures both, without affecting adversely any feature that is under scrutiny, is suitable for MP/NP analysis. Because it is difficult to determine both types of characteristics using only one analytical tool, a combination of methods is required (Ding *et al.*, 2019; Käßler *et al.*, 2016). The minimal cut-off size of the MPs and NPs is a critical factor to consider when selecting the identification method. Microscopy is an essential tool for measuring the physical characteristics of MPs particle by particle. When only large MPs are the target, microscopy can be used on its own to analyse the physical characteristics, together with an additional test to identify the plastics. When the size of the MPs is < 1 mm and the minimal cut-off size is tens of microns (Desforges *et al.*, 2014), microscopic analysis should be combined with

chemical analysis such as spectroscopic or thermal analysis. For reasons of ease of handling, analytical time and number of plastics that can be analysed, micro-ATR-FTIR spectroscopy is currently the method recommended for routine analyses of environmental samples. If the minimal cut-off size is a few microns (Shim *et al.*, 2017), Raman spectroscopy can be used to obtain better spectra from particles <20 µm in size (Cole *et al.*, 2014). Thermal analysis and automated mapping spectroscopy (e.g. FPA-FTIR) may be suitable for laboratory experimental samples of known plastic types. Although it has some advantages over µ-ATR-FTIR and Raman spectroscopy, small MP particles may still be missed or information lost in complex environmental samples with various unknown types of weathered plastics (Claessens *et al.*, 2011). These methods are not recommended for routine monitoring studies at present. Given the large variety of particles in terms of size, shape and composition, the adsorption of other pollutants and the dynamic change in their distribution in our environment depending on human activities, the development of fit-for-purpose standardised methods constitutes a challenge.

The following gaps in our knowledge have been identified:

- Methods of sampling and extracting MPs and NPs from environmental and biotic matrices need to be standardised and validated.
- There is a need to develop highly efficient analytical techniques to facilitate rapid and accurate identification and quantification of MPs and NPs that are validated for a range of different types of plastic.
- MPs and NPs vary in size, shape and composition, and a standard set of reporting criteria should be developed to facilitate inter-study comparisons.
- Standardised separation and detection methods are in an early stage of development and, to date, limited information is available on measurement methods with respect to NPs.
- Agreement on protocols for different plastics, which are standardised across Europe and potentially globally, is required.
- There is a need to develop standardised and validated methods to detect MPs and NPs in animal or human tissue.

5 Possible Health Effects of Exposure to MPs and NPs

5.1 Introduction

Studies examining the health impacts of plastics have predominantly focused upon individual chemical components such as phthalates and bisphenol A (BPA) (Choi *et al.*, 2017). There is strong evidence to suggest that these components affect a number of organ systems including the reproductive, endocrine, gastrointestinal and immune systems, leading to negative health impacts (Braun, 2017; Di Ciaula and Portincasa, 2019; Lee *et al.*, 2017; Robinson and Miller, 2015; Song *et al.*, 2016). However, human exposure is through contact with MPs and NPs that contain multiple and varied types of plastics in addition to other chemical components that could act in concert, leading to greater toxic effects. In the case of NPs, these fragments have a large specific surface area with stronger sorption affinities for toxic compounds, potentially leading to enhanced toxicity over time (Velzeboer *et al.*, 2014). No studies have examined the health impacts of MPs and NPs following human exposure, although there is some evidence linking larger synthetic fibres to occupational respiratory illness and cancer. While there is a dearth of studies on the health impacts of MPs in human populations, a number of preliminary studies in aquatic animals or experimental animal models and *in vitro* studies have provided some insight into the potential health impact following exposure to MPs and NPs. This chapter of the report will summarise these studies, providing evidence of the potential health impacts as well as identifying major gaps in the literature in order to provide recommendations for further studies.

5.2 Human Populations

In human populations there has been a rise in a number of diseases, such as reproductive, metabolic, neurodegenerative and immune disorders, that cannot be explained by genetic factors alone and to which changes in the environment must therefore be a contributory factor (Campbell, 2014; Stern *et al.*, 2020). Given the ubiquitous nature of MPs and NPs in our environment, exposure to MPs and NPs could be a possible explanation; however, their ubiquitous nature

also presents serious challenges when designing and carrying out human studies that examine the adverse health effects following exposure to MPs and NPs in human populations (Chen *et al.*, 2020; Prata, 2018). A number of studies have linked respiratory illness in textile workers to exposure to high levels of synthetic fibres, poly(vinyl chloride) dust and vinyl chloride (Barroso *et al.*, 2002; Daroowalla *et al.*, 2005; Kern *et al.*, 2000; Pimentel *et al.*, 1975). One study found that 4% of workers from nylon flock plants in the USA and Canada developed coughs, dyspnoea and reduced lung capacity (Eschenbacher *et al.*, 1999), while other studies have linked occupational exposure to synthetic fibres with pulmonary granulomatous lesions and respiratory functional abnormalities (Burkhart *et al.*, 1999; Turcotte *et al.*, 2013; Valic and Zuskin, 1977; Washko *et al.*, 2000). Pulmonary disease is most probably due to inhaled particles that act as haptens, causing an allergic reaction in individuals with a genetic predisposition to atopy. Indeed, allergic skin tests and nasal and inhalation provocation tests in nylon workers suggest that synthetic fibres are an important cause of occupational asthma among textile workers (Muittari and Veneskoski, 1978).

Flock and textile workers exposed to synthetic fibres have an increased risk of lung cancer when compared with non-exposed individuals (Kern *et al.*, 2000; Mastrangelo *et al.*, 2003, 2002; Pauly *et al.*, 1998; Vobecky *et al.*, 1978). The fibres, owing to their size, remain trapped in lung tissues, causing chronic inflammation that can lead to cancer development. Histopathological examination confirmed the presence of inhaled cellulosic and plastic fibres in 87% of tissue samples obtained from 114 individuals with different lung cancer types (Pauly *et al.*, 1998). Another study suggested a link between large bowel cancer and chronic exposure to persistent synthetic fibres in textile workers compared with other industrial workers within the plant that had low or no exposure (Mastrangelo *et al.*, 2002, 2003). Although in 2002 Mastrangelo *et al.* examined the incidence of tumours, histopathological types of tumour tissue and the distribution of cancer in the GIT in exposed and control groups, they did not look for the presence of synthetic fibres in the intestinal tumours. Occupation could be a particularly

useful indicator of exposure to MPs and NPs, and industrial workers, farmers or fishermen may be good cohorts in which to examine the health impacts of MPs and NPs in future studies (Kern *et al.*, 2000; Vobecky *et al.*, 1978).

Oral drug preparations can contain PS and PVC particles (< 150 µm), and studies have examined their translocation to gut tissue, providing us with some insight regarding the potential destination of MPs and NPs following oral ingestion. In humans, these drug particles traverse the GIT to reach the local lymph and circulatory system (Hussain *et al.*, 2001). Another study demonstrated the presence of PE particles up to 50 µm in abdominal lymph nodes, liver and spleen. Thompson *et al.* (2009) showed that particles translocated to the GIT are taken up by epithelial cells via endocytic processes. Factors that influenced uptake and translocation included particle size, surface charge, hydrophobicity and surface functionalisation. Blood cells can adhere to the plastic surface, forming a protein corona that also facilitates the uptake of particles (Thompson *et al.*, 2009). Although these studies focused on drug delivery systems, the evidence would suggest that MPs and NPs could potentially move from the primary exposure site to different organ tissues through similar processes.

5.3 Animal Models to Examine Potential Health Effects of MPs and NPs

5.3.1 Shellfish and fish

Given the difficulty in examining the impact of MPs in human populations, clues as to the possible harmful effects can be drawn from studies examining biological changes in fish and shellfish exposed to MPs and NPs under experimental conditions. We know that, in their natural habitat, shellfish and fish uptake and retain MPs and NPs in the gut (Collard *et al.*, 2018; Hantoro *et al.*, 2019; Smith *et al.*, 2018; W. Wang *et al.*, 2019). Studies in mussels have demonstrated that ingested PS microparticles can translocate from the gut to the haemolymph and haemocytes, causing increased haemocyte mortality (Browne *et al.*, 2008; von Moos *et al.*, 2012). PS microparticles also cause cellular oxidative imbalance, reduced feeding rates and neurotoxicity (Avio *et al.*, 2015; Paul-Pont *et al.*, 2016; Rist *et al.*, 2018; Wegner *et al.*, 2012). Other studies have shown that PS microparticles are

transferred into the lymphatic systems of mussels, inducing an immune response (Avio *et al.*, 2015; von Moos *et al.*, 2012), while one study reported cellular changes described as early granulocytoma formation, increased haemocyte numbers and decreased lysosomal membrane stability (Browne *et al.*, 2011). In contrast, a study showed that exposure of *M. edulis* to PS microparticles less than 10 µm did not have a significant impact on metabolism (Rist *et al.*, 2019).

Similar studies in grass shrimp have demonstrated that MP particles of various sizes and shapes can be ingested and ventilated (Gray and Weinstein, 2017), causing neurotoxic effects and oxidative stress (Gambardella *et al.*, 2017). Adult oysters experimentally exposed to PS microparticles for 2 months during a reproductive cycle presented feeding modifications and reproductive disruption, with significant impacts on offspring (Sussarellu *et al.*, 2016), while studies in clams exposed to PS microparticles for 7 days exhibited DNA damage, neurotoxicity and oxidative damage (Ribeiro *et al.*, 2019). A recent study examining salps exposed to high concentrations of MPs demonstrated potential impacts on the global biological pump. Salps release faecal matter, an essential carbon food source for organisms residing in or passing through greater sea depths where no photosynthesis takes place (Wieczorek *et al.*, 2019).

Fish, like shellfish, can retain MPs in their gut, and MPs have been shown to migrate to muscle and organ tissue (Abbasi *et al.*, 2018; Karami *et al.*, 2017c; Lusher *et al.*, 2017b; Vendel *et al.*, 2017), where they can have either neutral or harmful effects (Wang *et al.*, 2019; Wright *et al.*, 2013). A range of harmful effects observed in fish include neurotoxicity (Barboza *et al.*, 2018; Oliveira *et al.*, 2018), oxidative stress (Ding *et al.*, 2019; Oliveira *et al.*, 2018), reduced feeding (de Sá *et al.*, 2018; Welden and Cowie, 2016), intestinal damage (Pedà *et al.*, 2016), reduced allocation of energy for growth (Farrell and Nelson, 2013), reduced predatory performance (de Sá *et al.*, 2018), reduced swimming performance (Barboza *et al.*, 2018) and decreased fertilisation and larval abnormalities (Martínez-Gómez *et al.*, 2017). The intestinal responses of European sea bass, *Dicentrarchus labrax*, chronically exposed to MPs through ingestion, demonstrated histological changes in the intestine after 90 days (Pedà *et al.*, 2016). While the expression levels of cytochrome P450 1A1 gene,

which encodes a protein involved in the metabolism of endogenous substrates, was positively correlated with the number of microbeads in *D. labrax*, microbead ingestion had limited impact on sea bass larvae (Mazurais *et al.*, 2015). A recent systematic review examined 46 studies published from 2011 to 2019 examining the harmful effects of virgin MPs and NPs in fish. The study considered a number of factors including exposure to virgin MPs and NPs and the effects of associated contaminants and biofilms. In general, they concluded that virgin MPs and NPs less than 20 µm in size caused harmful effects in fish. However, the huge variation between studies makes direct comparisons difficult, highlighting the need for more systematic approaches with standardised protocols (Jacob *et al.*, 2020; Wieczorek *et al.*, 2019). Furthermore, many studies assessed the effects of concentrations that were much greater than would occur in the natural habitat. Such studies could identify a threshold that could predict levels of MP/NP pollution that would cause serious effects in the future in the marine environment. The levels of MP pollution in the marine environment are expected to increase as a result of projected increases in plastic production and the accumulation and slow degradation of MPs in the marine environments. Some studies have attempted to recreate natural conditions by exposing virgin MPs and NPs to degradative conditions that would naturally occur in the environment. These studies are important if we are to understand the effects of MPs and NPs in current environmental conditions (Wieczorek *et al.*, 2019).

5.3.2 Zebrafish

Approximately 80% of human disease-related genes can be linked to a zebrafish orthologue, making zebrafish an important model organism to understand human genetic studies (Phillips and Bonner, 2015). Zebrafish studies also have many advantages related to laboratory manipulation, and therefore zebrafish are considered a strong screening model system (Limonta *et al.*, 2019). Oxidative stress as measured by increased superoxide dismutase and catalase activity was induced in zebrafish after treatment with PS microparticles (Lu *et al.*, 2016; Veneman *et al.*, 2017; Wan *et al.*, 2019); however, PS microparticles also significantly decreased the activities of catalase and the content of glutathione, enzymes responsible for eliminating reactive oxygen species (Deng *et al.*, 2018). Furthermore, increased oxidative stress, which

is linked to reduced body length, has been found to be associated with reduced zebrafish larvae locomotion following MPs and NPs exposure (Q. Chen *et al.*, 2017). PS microparticles have also been linked to disruption of oogenesis, neurotoxicity, disrupted lipid metabolism (lipid metabolites of triglycerides and fatty acids) and energy metabolism (as measured by increased metabolites of adenosine triphosphate, adenosine diphosphate and adenosine monophosphate in response to larger particles only) in zebrafish (Lu *et al.*, 2016; Mak *et al.*, 2019).

In developing zebrafish, intestinal damage and toxicity of MPs is mostly down to their size rather than their composition, as polystyrene NPs (PS nanoparticles) accumulate in numerous organs, initially in the yolk sac, and then migrate to the GIT, gallbladder, liver, pancreas, heart and brain throughout development (Lei *et al.*, 2018). Significant physiological effects of PS nanoparticles include bradycardia and hypoactivity, suggesting that PS nanoparticles may induce organ toxicity specific to their developmental distribution pattern (Pitt *et al.*, 2018). Different sizes of PS microparticles could induce microbiome dysbiosis and changed metabolomics profiles in larval zebrafish (Wan *et al.*, 2019). Dysbiosis of gut microbiota was associated with physiological changes including the occurrence of inflammation (F. Zhang *et al.*, 2019), as PS microparticles increased the levels of immune inflammatory immune factors such as interleukin 1α (IL-1α), IL-1β and interferon as well as of their mRNA (Jin *et al.*, 2018). Other effects on the immune system included enhancement of the complement system, which is important in immunological recognition (Veneman *et al.*, 2017). The associations, along with interactions among the intestinal microbiota with mucous layer, bile acids and mucosal immune responses, reveal potential mechanisms by which the microbiota affects metabolism (Nieuwdorp *et al.*, 2014).

5.3.3 Mice and rats

A number of studies have examined the adverse effects of MPs in experimental mammalian models, such as mice and rats, that share genetic, biochemical and physiological similarities with humans (Perlman, 2016). Studies examining the bioaccumulation of MPs and NPs in rats and mice have highlighted the importance of surface charge, surface chemistry, size and type of coating material on the uptake and

accumulation of PS nanoparticles after oral exposure (Araujo *et al.*, 2018; Deng *et al.*, 2018; Hurley and Nizzetto, 2018). PS nanoparticles are reported to be translocated to the GIT and thence transported in the blood to a range of organs (Jani *et al.*, 1990), including the lung, testis, spleen, kidney, heart and liver (Cho *et al.*, 2019; Hussain *et al.*, 2001; Jani *et al.*, 1990; van der Zande *et al.*, 2012). Intravenously administered PS nanoparticles have been shown to accumulate and persist over a number of days in the placenta but not to translocate to the embryo (Mortensen *et al.*, 2019). However, the sites of greatest accumulation of PS nanoparticles are the walls of the stomach and intestine (Walczak *et al.*, 2015), which are the main sites of biodistribution after oral exposure (Hussain *et al.*, 2001; Jani *et al.*, 1990).

Exposure to PS nanoparticles induces changes in biomarkers and metabolomic profiles, reflecting disruption to energy and lipid metabolism as well as induction of oxidative stress and neurotoxicity, highlighting the potential health risk to mammals (Deng *et al.*, 2018, 2017). Studies have also revealed that co-exposure of mice to MPs and organophosphorous flame retardants induces greater oxidative stress and neurotoxicity, and enhanced disruption of amino acid metabolism and energy metabolism (Deng *et al.*, 2018). PS microparticles have been shown to modify the composition of the gut microbiota in mice by decreasing the secretion of mucin in the gut and inducing hepatic lipid disorder (L. Lu *et al.*, 2018). It is known that the gut microbiota has an essential role in modulating host metabolism and in the development of obesity in the host (Jin *et al.*, 2019). Other studies have reported gut microbiota dysbiosis, intestinal barrier dysfunction and metabolic disorders in mice exposed to 5- μm PS microparticles (L. Lu *et al.*, 2018). Exposure of pregnant mice to different sizes of polystyrene MPs increased metabolic disorder in their offspring, with 5- μm PS microparticles having a greater effect than 50- μm particles (Luo *et al.*, 2019). These results provided more information on the potential risks of MP/NP exposure as well as preliminary data on the possible adverse effects on human health.

5.4 *In Vitro* Models to Examine Potential Health Effects of MPs and NPs

In vitro model systems are inexpensive and provide a high-throughput means of quickly determining the

effects of MPs and NPs on human cells. Studies have demonstrated the impact of PS nanoparticles on hepatocytes (Johnston *et al.*, 2010), macrophages (Xia *et al.*, 2008), lung cells (Geys *et al.*, 2006) and gut cells (Thubagere and Reinhard, 2010), showing that nano-PS particle shape and size can influence cellular uptake and internalisation (Guarnieri *et al.*, 2011). Cellular uptake of MPs from 1 to 10 μm occurred in only a small fraction of particles, while cell differentiation was not altered (Stock *et al.*, 2019). Other studies have demonstrated rapid cellular uptake of PS nanoparticles less than 500 nm (Feng *et al.*, 2013; Fiorentino *et al.*, 2015). MPs are able to induce IL-6, tumour necrosis factor alpha and histamine, which can cause local immune responses in macrophage cell lines, and peripheral blood mononuclear cells are exposed to high concentrations of particles of 20 μm , but not 200 μm (Hwang *et al.*, 2019).

The presence of serum can also influence cellular uptake and internalisation, as coronated PS nanoparticles (particles covered with human plasma proteins that have affinity for NPs) are internalised more efficiently (Smith *et al.*, 2012). A study conducted on human blood cells and *Allium cepa* root tip cells exposed to virgin, coronated and isolated NPs from facial scrubs demonstrated that coronated NP particles were more genotoxic and cytotoxic to human blood cells than virgin or isolated particles, while virgin NPs and isolated NPs hindered root growth and caused chromosome aberration (Gopinath *et al.*, 2019). Therefore, adding serum to a culture medium may influence the experimental outcomes of *in vitro* studies, and serum-free medium should thus be used as part of the experimental design.

Human gastric cells are most likely the first cells that encounter NPs following ingestion of contaminated food. Studies of human gastric cells exposed to NPs found that exposure had severe consequences for cellular metabolism (Nowack *et al.*, 2012). PS nanoparticles had adverse effects on cell viability, inflammatory gene expression and the morphology of human gastric adenocarcinoma cells (Forte *et al.*, 2016). The effect of exposure to NPs on the absorption of iron was examined in an *ex vivo* chicken intestinal loop model, and it was found that 50-nm polystyrene carboxylated nanoparticles inhibited iron transport owing to disruption of the cell membrane, while chronic exposure caused remodelling of the intestinal villi,

which decreased the surface area available for iron absorption (Mahler *et al.*, 2012).

PS nanoparticles have cytotoxic and genotoxic effects on human cell lines *in vitro*, inducing autophagocytic and programmed cell death (Chiu *et al.*, 2014; Paget *et al.*, 2015). Exposure time, diameter and concentration of NPs are key factors that reduce cell viability, altering the cell cycle and inducing programmed cell death (Xu *et al.*, 2019). The activation of autophagy in cell lines was thought to be the result of PS nanoparticles interfering with energy metabolism (Lim *et al.*, 2019). Studies by Schirinzi *et al.* (2017) have demonstrated that oxidative stress is one of the mechanisms of cytotoxicity at the cellular level. In contrast, a study examining the effects of 50-nm and 0.5- μ m carboxylated-modified PS particles in a number of *in vitro* biological models found induction of acute toxicity or DNA damage (Hesler *et al.*, 2019),

5.5 Conclusion and Gaps

There is universal concern that MPs and NPs have the potential to cause serious health effects on human populations owing to their ubiquitous presence in our environment. Although there are studies linking synthetic fibres to lung disease and cancer in textile workers, there is a lack of direct evidence linking MPs and NPs to human health effects (Mastrangelo *et al.*, 2002, 2003; Turcotte *et al.*, 2013). Knowledge to date is limited to the results of experimental studies in aquatic models (Avio *et al.*, 2015; Hantoro *et al.*, 2019; Smith *et al.*, 2018), animal studies (Jin *et al.*, 2018; Lu *et al.*, 2018) and human cell lines (Forte *et al.*, 2016; Mahler *et al.*, 2012). These studies have shown that MPs and NPs have an impact on cell viability, inducing both autophagy and programmed cell death (Chiu *et al.*, 2014; Paget *et al.*, 2015). Other effects include the induction of oxidative stress (Schirinzi *et al.*, 2017), induction of inflammatory pathways (Forte *et al.*, 2016) and disruption of the gut microbiome (Jin *et al.*, 2018). MPs have also been found to be genotoxic (McCarthy *et al.*, 2011) and neurotoxic (Deng *et al.*, 2018), and to affect reproductive (Sussarellu *et al.*, 2016) and metabolic processes (Hesler *et al.*, 2019). The evidence concerning the effects of particle size and composition and transfer of adsorbed chemical pollutants on human tissues is incomplete, although a number of studies have found that MP and NP

particles have no adverse effects. The major issue is that there is huge variability in the experimental design of studies, with differences in the type, size and concentration of MPs and NPs used. Many studies have used virgin MPs that have not been exposed to the environmental stresses that occur in nature and have found that the smaller MPs undergo cellular uptake *in vitro* and have toxic effects. These discrepant results highlight the importance of developing tools to measure human exposure to MPs and NPs and internationally standardised and validated protocols.

The following gaps have been identified:

- There is an enormous knowledge gap regarding the uptake and fate of MPs and NPs in human tissues.
- There is an enormous knowledge gap regarding the impact of MPs and NPs on human health.
- Assessments of the toxicity of MPs and NPs on mammals remain limited, and more research is required to understand the impacts on human health.
- There is a need to understand the potential modes of toxicity of different sizes, shapes and types of MPs and NPs in different model systems.
- There is a need to standardise international protocols to examine the health-related effects of MPs and NPs.
- There is a need for studies to measure MPs and NPs present on human tissue. Tissue could be obtained from tissues banks or post mortem. The presence of MPs and NPs in blood and faecal samples could be measured once validated protocols are established.
- The potential human toxicity of NPs is poorly studied. Quantitative methods for the detection of NPs in biological samples will have to be developed. Future studies should further investigate the mechanism underlying acute and chronic NP toxicity, especially in animal models or human subjects.
- The kinetics and biodistribution of MPs post exposure is poorly studied. The *in vivo* persistence of MPs in different physiological environments is also unknown. Although there is evidence of inflammatory effects of plastic dust in animal models, it is not known if the findings of these studies also apply to humans.

6 Regulations and Legislative Aspects

The European plastic industry employs 1.5 million people and is worth €350 billion per year (Plastics Europe, 2019). It produces 25.8 million tonnes of plastic waste in Europe annually, approximately 8% of the 322 million tonnes produced globally. It is predicted that plastic waste will increase to 1800 million tonnes by 2050 (Gallo *et al.*, 2018; Wright and Kelly, 2017). Previously the EU exported 50% of its plastic waste to China for incineration; however, a change in policy means that in future the EU is required to develop a more robust circular economy without increasing the need for incineration plants (Malinauskaite *et al.*, 2017). Another challenge is the bioaccumulation of MPs and NPs in seafood, which has the potential to increase dramatically as the levels of MPs and NPs increase in our oceans. Given the potential food safety concerns, this could negatively impact the fishing industry (Gallo *et al.*, 2018). Approximately three billion people in the world rely on wild-caught or farmed seafood as their primary source of protein (Guillen *et al.*, 2019). The Irish fishing industry contributes €700 million per year to the national economy, providing 11,000 jobs for people living in coastal regions (EC, 2016). If MPs and NPs are linked to adverse human health effects, the safety of seafood as a food source will come into question, thereby potentially removing a valuable source of protein from the food chain and significantly affecting countries that rely on fish as a major source of animal protein.

MPs and NPs represent an important topic in European pollution policymaking, as it is estimated that it will cost €22 billion to correct environmental damage caused by plastic pollution by 2030 (Karapanagioti and Kalavrouziotis, 2019). It is essential that Member States take specific measures to address this and, in order to guide decision-makers to take corrective actions, it is important that governments identify the main contributing sources of MPs and NPs that are released into our environment and those that are the greatest threat to human health. Table 6.1 lists some of the directives related to water, food and air – the main sources of human exposure to MPs and NPs. It is clear that many of these directives

do not explicitly mention MPs and NPs, and it is therefore important that all relevant EU directives be revised to address this gap. Although there are no EU food directives, the European Food Safety Authority (EFSA, 2020, 2016) explicitly addresses NPs in its guidance on the risk assessment of the use of nanoscience and nanotechnology in the food chain with a particular focus on seafood. In addition, the European Commission's "Proposal for a Directive of the European Parliament and of the Council on the quality of water intended for human consumption" has recently (24 February 2020) been updated to include changes that make direct and specific reference to MPs (EC, 2020). This recast version requires Member States to adopt a methodology to monitor MPs and to put risk assessment in place.

A 2018 news article highlighted the problem of plastic content in animal foodstuffs that could also have the potential to propagate up the food chain; this should be addressed in current directives (Grant, 2018). Finally, although there are a number of EU directives on litter management, only the EU Marine Strategy Framework Directive (MSFD) (2008/56/EC) explicitly mentions MPs, setting out a requirement to measure MPs in the marine environment. The MSFD also requires Member States to determine whether their marine environments are in a state of good environmental status (GES) in respect of pollutants not causing significant harm to the marine environment (which includes plastics and microplastics) (EU, 2008). The Directive requires Member States to implement measures to maintain and/or improve the environmental status of their marine environments. Further research is needed to determine at what threshold MPs cause harm in the marine environment.

In 2018, the European Commission proposed new EU-wide rules in the form of Directive 2019/904 on the reduction of the impact of certain plastic products on the environment, targeting the 10 single-use plastic products most often found on Europe's beaches and in its seas and accounting for 43% of marine litter. The Fishing for Litter programme encourages fishing trawlers to voluntarily collect litter, and Irish trawlers

Table 6.1. Legislation and policies relating to Water, Food, Air and Litter (non-exhaustive)

Legislation/communication	General aims	Source	Explicit mention of MPs/NPs
Water/marine			
Proposal for a Directive of the European Parliament and of the Council on the quality of water intended for human consumption (recast). The most recent version is dated 24 February 2020	The revised proposal aims: to manage drinking water in a resource-efficient and sustainable manner to reduce energy use and unnecessary water loss to reduce the number of plastic bottles to improve people's confidence in tap water	Drinking water	Yes
European Drinking Water Directive (98/83/EC), revised February 2018 ^a	Protect human health from sources of contamination (applies to all treated and untreated water including tap water and all water used in any food production)	Drinking water	No
EU Water Framework Directive (2000/60/EC) ^b	Ongoing review of Directive (2019): maintain "good status" of aquatic ecosystems and wetlands identification and monitoring of anthropogenic pressures	Fresh water	No
EU Bathing Water Directive (2006/7/EC) ^c	Governs the quality of bathing water and safety of beaches To preserve, protect and improve water quality	Bathing water	No
Marine Strategy Framework Directive (2008/56/EC) ^d	Aims to achieve "good environmental status" by 2020 across Europe's marine environment Prevention of marine macro- and micro-sized litter Inputs and reduction of litter in the marine environment Requirement to measure macrolitter and MPs	Marine litter	Yes, MPs
Food			
European Food Safety Authority statement 2011 ^e	Guidance on the risk assessment of the application of nanoscience and nanotechnologies in the food chain with a particular focus on seafood	Food (seafood)	Yes, NPs
Air			
Ambient Air Quality Directive (2008/50/EC), amended 2015 (Directive 2015/1480/EC) ^f	Governs air quality Rules concerning reference methods, data validation and location of sampling points for the assessment of ambient air quality	Air	No
Litter			
Directive (EU) 2019/904 of the European Parliament and of the Council of 5 June 2019 on the reduction of the impact of certain plastic products on the environment ^g	Prevent and reduce the impact of certain plastic products on the environment Focus on aquatic environment, and on human health Promote the transition to a circular economy MPs do not fall directly within the scope of this Directive	Soil/water/air	Yes, MPs, but outside scope
EU Directive on packaging and packaging waste (2004/12/EC) ^h	To harmonise national measures concerning the management of packaging and packaging waste, enhancing environmental protection	Soil/water	No
Directive 2008/98/EC of the European Parliament and of the Council of 19 November 2008 on waste and repealing certain Directives (Revised 2018) ⁱ	Sets out measures to protect the environment and human health by: preventing or reducing the generation of waste preventing the adverse impacts of waste generation and management by reducing overall impacts of resource use and improving the efficiency of such use	Soil/water/air	No

Table 6.1. Continued

Legislation/communication	General aims	Source	Explicit mention of MPs/NPs
EU Directive on the landfill of waste (1999/31/EC) ^j	Revised version of directive to transpose Aims to prevent or minimise possible environmental negative effects from the landfilling of waste by introducing stringent technical requirements for waste and landfills	Soil/water/air	No
Directive (EU) 2019/883 of the European Parliament and of the Council of 17 April 2019 on port reception facilities for the delivery of waste from ships, amending Directive 2010/65/EU and repealing Directive 2000/59/EC ^k	Aims to reconcile the interests of smooth operation of maritime transport with the protection of the marine environment Plastic is mentioned with source attributed mostly to land-based activities	Water/marine	No
Commission Decision (EU) 2017/1219 of 23 June 2017 establishing the EU Ecolabel criteria for hand dishwashing detergents, hard surface cleaning products and industrial and institutional laundry detergents ^l	EU ecolabelled products cannot contain MPs	Water	Yes, MPs
Commission Regulation (EU) 2019/2023 of 1 October 2019 laying down ecodesign requirements for household washing machines and household washer-dryers pursuant to Directive 2009/125/EC of the European Parliament and of the Council, amending Commission Regulation (EC) No 1275/2008 and repealing Commission Regulation (EU) No 1015/2010 ^m	Not under consideration in this version; however, it is stated that revision by 2025 will examine the feasibility and appropriateness of new requirements for reducing MPs in the water outlet, such as filters	Water	Yes, MPs
Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) legislation, establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC	Asked by the Commission to prepare an Annex XV dossier regarding addition of MPs to consumer products	Soil/water	Not at present

^j<https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:01998L0083-20151027> (accessed 6 October 2020).

^khttps://eur-lex.europa.eu/resource.html?uri=cellar:5c835afb-2ec6-4577-bdf8-756d3d694eeb.0004.02/DOC_1&format=PDF (accessed 6 October 2020).

^l<https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32006L0007&from=EN> (accessed 6 October 2020).

^m<https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32008L0056> (accessed 6 October 2020).

ⁿhttps://europa.eu/european-union/topics/food-safety_en (accessed 6 October 2020).

^o<https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32015L1480> (accessed 6 October 2020).

^p<https://eur-lex.europa.eu/eli/dir/2019/904/oj> (accessed 6 October 2020).

^q<https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32004L0012> (accessed 6 October 2020).

^r<https://eur-lex.europa.eu/eli/dir/2008/98> (accessed 6 October 2020).

^s<https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A31999L0031> (accessed 6 October 2020).

^t<https://eur-lex.europa.eu/eli/dir/2019/883/oj> (accessed 6 October 2020).

^u<https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32017D1219> (accessed 6 October 2020).

^v<https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32019R2023> (accessed 6 October 2020).

Table 6.2. Measures to curb plastic waste and litter (extracted from the EU plastics strategy)^a

Measure	Milestones	Status
Actions to reduce single-use plastics	Public consultation to determine the scope of a legislative initiative on single-use plastics	Ongoing
Actions to tackle sea-based sources of marine litter	Development of measures to reduce loss or abandonment at sea of fishing gear	2018 onwards
	Development of measures to limit plastic loss from aquaculture	
Actions to monitor and curb marine litter	Monitor and map marine litter, including MPs, on the basis of EU-harmonised methods	2018 onwards
Actions on compostable and biodegradable plastics	Development of harmonised rules on labelling compostable and biodegradable plastics	2018 onwards
	Restrict the use of oxo-plastics via REACH	
Actions to curb MP pollution	Restrict the addition of MPs to products via REACH	Ongoing
	Reduce the unintentional release of MPs from tyres, textiles and paint	
	Develop measures to reduce plastic pellet spillage	
	Evaluate the Urban Waste Water Treatment Directive: MPs' detection and removal	

^a<https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:52018DC0028&from=NL> (accessed 6 October 2020).

have hauled 190 tonnes of marine litter from the sea over 3 years. The Plastics Strategy (adopted 16 January 2018) contains a wide range of legislative and non-legislative measures to reduce plastic (and MP) litter, and some of the milestones are outlined in Table 6.2. The strategy aims to ensure that all plastic packaging will be either reusable or recyclable in a cost-effective manner by 2030. The measures of this strategy are divided into four actions: (1) improving the economics and quality of plastics recycling; (2) curbing plastic waste and littering; (3) driving innovation and investment towards circular solutions; and (4) harnessing global action. Taking a holistic approach to tackling plastic waste will ensure that governments can employ many effective strategies to tackle this complex problem.

Governments all over the world have developed policies and legislation to reduce the use of lightweight plastic bags by banning their sale, by requiring shops to charge customers for them or by taxing the sale of bags (Clapp and Swanston, 2009). Germany and Denmark were early adopters of this policy, banning the provision of plastic bags in most retail stores in 1991 and 1994, respectively (Xanthos and Walker, 2017), and many countries in Africa, Asia and Europe have introduced bans since 2001 (Dikgang *et al.*, 2012). EU Member States are required to take measures to reduce annual average consumption of lightweight plastic bags to 90 bags per citizen by

December 2019 and to 40 per capita by December 2025 (Directive (EU) 2015/720). Ireland, in 2002, was the first EU Member State to introduce levies on plastic bag use, which resulted in a dramatic 90% drop in the use of plastic bags, reducing the number of bags in the environment by 90 billion annually. The levy generated significant funds (over €9 million), which were ring-fenced for environmental projects. Although the policy was successful in reducing plastic bag usage, funding for environmental projects from levies also fell. The Irish government must find alternative strategies to raise funds for these vital projects and, in 2021, is enforcing a “latte levy” with the aim of reducing the use of disposable cups, with plans to reduce the use of plastic plates, cutlery, straws, balloon sticks, cotton buds and food containers, offering an alternative revenue source.

Plastic bags aside, there have been limited interventions to reduce MPs, although since 2014 there has been a rapid proliferation in policies (Table 6.3) to reduce the use of microbeads in rinse-off cosmetic products (Xanthos and Walker, 2017). The EU is currently developing REACH regulations to restrict the intentional addition of MPs to products, with immediate bans proposed for rinse-off cosmetic and cleaning products containing plastic microbeads, and longer lead-in times for implementation of proposed bans on other products that contain MPs, to allow time for reformulation.

Table 6.3. Worldwide prohibitions on products containing microbeads (non-exhaustive)

Country/jurisdiction	Enforcement date for prohibition of manufacture	Enforcement date for prohibition of sale of certain products	Prohibited products
USA ^a	1 July 2017	1 January 2018	Rinse-off cosmetic products
Canada ^b	1 January 2018	1 July 2018	Rinse-off cosmetic products
Sweden ^c	No date published	1 July 2018 (products in stock before this date can continue to be sold in stores until 1 January 2019)	Rinse-off cosmetic
France ^d	No date published	1 January 2018 (no obligation to withdraw products placed on the market before 1 January 2018)	Rinse-off cosmetic
England ^e	9 January 2018	19 June 2018	Rinse-off cosmetic products
New Zealand ^f	7 June 2018	7 June 2018	Rinse-off cosmetic products/ abrasive cleaning products
Scotland ^g	19 June 2018	19 June 2018	Rinse-off cosmetic products
Wales ^h	30 June 2018	30 June 2018	Rinse-off cosmetic products
Northern Ireland ^{ij}	11 March 2019	11 March 2019	Rinse-off cosmetic
South Korea ^k	13 July 2017 (manufacture and import)	1 June 2018	Rinse-off cosmetic products
Italy ^l	Pending 2020	Pending 2020	Rinse-off cosmetic products
Ireland ^{m,n}	20 February 2020	20 February 2020	Rinse-off cleaning products, scouring agents, detergents, cosmetic products

^aMicrobead-Free Waters Act of 2015 (available online: <https://www.congress.gov/bill/114th-congress/house-bill/1321/text>) (accessed 6 October 2020).

^bMicrobeads in Toiletries Regulations (SOR/2017-111) (available online: <http://laws-lois.justice.gc.ca/PDF/SOR-2017-111.pdf>) (accessed 6 October 2020).

^chttps://docs.wto.org/dol2fe/Pages/FE_Search/FE_S_S009-DP.aspx?language=E&HasEnglishRecord=True&HasFrenchRecord=False&HasSpanishRecord=False&CatalogueIdList=231247,237432,88564,237417,237400,237343,237345,237344,98194,133373&CurrentCatalogueIdIndex=3&FullText (accessed 6 October 2020).

^d<https://chemicalwatch.com/50368/france-to-ban-MPs-in-some-cosmetics-products> (accessed 6 October 2020).

^eThe Environmental Protection (Microbeads) (England) Regulations 2017 (available online: http://www.legislation.gov.uk/uksi/2017/1312/pdfs/uksi_20171312_en.pdf) (accessed 6 October 2020).

^fWaste Minimisation (Microbeads) Regulations 2017 (available online: <http://www.legislation.govt.nz/regulation/public/2017/0291/latest/whole.html>) (accessed 6 October 2020).

^gEnvironmental Protection (Microbeads) (Scotland) Regulations 2018 (available online: <http://www.legislation.gov.uk/ssi/2018/162/contents/made>) (accessed 6 October 2020).

^hThe Environmental Protection (Microbeads) (Wales) Regulations 2018 (available online: <http://www.legislation.gov.uk/wsi/2018/760/contents/made>) (accessed 6 October 2020).

ⁱThe Environmental Protection (Microbeads) Regulations (Northern Ireland) 2018 (available online: <http://ec.europa.eu/growth/tools-databases/tris/en/search/?trisaction=search.detail&year=2018&num=205>) (accessed 6 October 2020).

^jhttps://www.daera-ni.gov.uk/sites/default/files/publications/daera/Microbeads%20Art%2032%283%29%20Legislation%20Consultation_0.pdf (accessed 6 October 2020).

^k<https://cosmetic.chemlinked.com/news/cosmetic-news/korea-banned-11-ingredients-relating-functional-cosmetics> (accessed 6 October 2020).

^l<http://ec.europa.eu/growth/tools-databases/tris/en/search/?trisaction=search.detail&year=2018&num=258> (accessed 6 October 2020).

^m<https://data.oireachtas.ie/ie/oireachtas/act/2019/52/eng/enacted/a5219.pdf> (accessed 6 October 2020).

ⁿ<http://www.irishstatutebook.ie/eli/2020/si/36/made/en/print> (accessed 6 October 2020).

7 Conclusion, Recommendations and Priorities

7.1 Conclusion

This EPA-funded desk study examined the impacts of MPs and NPs on human health by addressing three fundamental questions: (1) What are the routes of human exposure to MPs and NPs? (Chapter 3); (2) What is the range and mid-level of human exposure (and how do we measure it)? (Chapter 4); and (3) What are the potential human health impacts of MPs and NPs? (Chapter 5). This chapter will provide a brief summary of the key findings, with a list of the seven key recommendations that should take priority.

7.1.1 *What are the routes of human exposure to MPs and NPs?*

It is clear from the report that exposure to MPs and NPs through inhalation, ingestion and dermal exposure is ubiquitous. As countries ban microbeads in personal care products and detergents, dermal exposure will become less important. Although we have some knowledge of the levels of MPs and NPs in food, air and water (Avio *et al.*, 2015; Dris *et al.*, 2015, 2017; Kosuth *et al.*, 2018; Van Cauwenberghe and Janssen, 2014), diet is the most extensively studied source of exposure. Studies focused on dietary exposure are limited to seafood and water, with very few studies examining other food products (Cai *et al.*, 2017; Dris *et al.*, 2017; Praveena *et al.*, 2018). It is therefore difficult to determine the greatest sources of dietary exposure; current studies suggest plastic teabags, bottled water and seafood, although this may not be the case given the dearth of studies for other food products and the limited replication and validation of these studies. In addition, daily exposure levels for individuals cannot be calculated. Identifying populations exposed to high or low levels of MPs and NPs is critical to determine associations with health effects in human populations. Although some studies suggest that different locations and occupations can influence levels of exposure (Pimentel *et al.*, 1975), more studies are required to support this observation.

Knowledge of exposure levels and potential health effects is not the only requirement: MPs and NPs exhibit different characteristics, some of which

could potentially have health effects (Lusher *et al.*, 2013). Although we have a good understanding of the size and shape of MPs, the composition of plastic types is less clearly defined (Smith *et al.*, 2018). The composition of NPs in the environment is relatively unknown because of a lack of routine and standardised methods to detect them (Wagner *et al.*, 2014). Studies have confirmed the presence of MPs and NPs in marine species and water, giving rise to food safety concerns; however, the current lack of information and evidence around the link between the consumption of these products and human health means that we cannot fully assess this risk (Hantoro *et al.*, 2019). Finally, although studies have demonstrated that MPs and NPs are directly taken up by cells (and/or can alter cell function), this has not been demonstrated *in vivo* (Koelmans *et al.*, 2019; Lusher *et al.*, 2013; Smith *et al.*, 2018; Wright and Kelly, 2017). Studies showing the presence of MPs in humans are therefore inadequate owing to the lack of analytical tools that can demonstrate and measure the presence of MPs and NPs in human tissue or the migration of these particles to other organs from the point of entry.

7.1.2 *What is the range and mid-level of human exposure (and how do we measure it)?*

There are many state-of-the-art techniques to measure the presence of MPs and NPs in the environment through the analysis of physical (size, shape and colour) or chemical (plastic type) characteristics (Hidalgo-Ruz *et al.*, 2012). However, the measurement of particles presents many challenges, as MPs and NPs can be embedded in animal tissue, which needs to be digested to enable the particles to be isolated for analysis, or they are present in complex matrices such as soil, sludge, food products or wastewater that require different separation techniques. At present, there are no standardised and validated international protocols. Each digestion method has limitations, such as destroying or altering the characteristics of particles (Felsing *et al.*, 2018; Miller *et al.*, 2017). Some analysis requires a combination of multiple methods, which can be time-consuming and costly

(Hidalgo-Ruz *et al.*, 2012; Nuelle *et al.*, 2014). Some of the more sophisticated analytical tools are very expensive, incur high maintenance costs and require highly skilled personnel to operate (Nguyen *et al.*, 2019). Some methods are not recommended for routine monitoring owing to the number of steps in the process. Given the large variety of size, shape and composition of MPs and NPs, their capacity to adsorb other pollutants and dynamic changes in their distribution in our environment, the development of a fit-for-purpose, high-throughput standardised method constitutes a major challenge (Nguyen *et al.*, 2019). There are currently no methods that can measure MPs *in vivo*, as measurement in human tissues would require samples of tissue to be removed for analysis (Löder and Gerdts, 2015). In order to examine the health impacts in human populations, these challenges must be urgently addressed.

7.1.3 *What are the potential human health impacts of MPs and NPs?*

Studies documenting health effects of MPs and NPs are limited to a few that link exposure of textile workers to synthetic fibres with lung disease (Pimentel *et al.*, 1975), and some that have reported an association of synthetic fibres with lung (Burkhart *et al.*, 1999; Mastrangelo *et al.*, 2002; Washko *et al.*, 2000) and bowel cancer (Vobecky *et al.*, 1978). Only a few studies have measured MPs in lung tumours (Boag *et al.*, 1999). Studies in aquatic models, animal models and human cell lines all show that MPs and NPs have negative biological effects, including effects on cell viability (Chiu *et al.*, 2014; Paget *et al.*, 2015), the induction of oxidative stress (Schirinzi *et al.*, 2017) and inflammatory pathways (Forte *et al.*, 2016) and the disruption of the gut microbiome (Jin *et al.*, 2018). MPs have also been found to be genotoxic (McCarthy *et al.*, 2011) and neurotoxic (Deng *et al.*, 2018), and can affect the reproductive system (Sussarellu *et al.*, 2016) and metabolic processes (Hesler *et al.*, 2019). However, although there is compelling evidence that MPs and NPs have the potential to affect human health, there is a huge variation between these studies, with few linking defined plastic type (size, shape, colour, chemical composition) with these effects. Some preliminary studies suggest that the size of MPs and NPs may be an important factor in relation to harmful effects, as it is clear that the smaller MPs and NPs are better able to enter individual cells.

However, there is huge variation in the size and type of particles used in these studies, and some are of virgin and others of non-virgin MPs. In many studies the levels of MPs and NPs used are far greater than levels found in the environment, which would call into question the relevance of the findings of these particular studies. However, such studies do suggest that higher levels of MPs and NPs can have adverse effects, which makes monitoring environmental levels important. It is difficult to draw any firm conclusions, particularly around the types of plastics that have the greatest adverse effect on human health. More evidence is required on the effects of MP/NP size, composition and chemical transfer of adsorbed chemical pollutants on human cells and tissues.

7.2 Recommendations and Priorities

- There is a need for more research to address the gaps in knowledge, in particular an evaluation of the presence of MPs and NPs in major food groups such as beef, poultry, dairy, fruit, vegetables and grains, and in processed versus non-processed foods as well as other routes of oral exposure.
- There is a need to calculate average estimated daily intake of MPs and NPs in humans and to monitor this over time.
- A complete validation of all current methods to analyse different plastic types in sources of exposure is required so that the components of MPs and NPs that pose the greatest risk to human health can be determined.
- Studies in human populations are required to determine actual human intake of MPs and NPs and if these particles are translocated from gut, skin or lungs to other organ tissues. Complementary experimental studies to determine mechanisms of action using protocols that are standardised and reflect conditions in the field are also required.
- There is a need to standardise international protocols to extract MPs and NPs, analyse them physically/chemically and examine their health-related effect.
- Quantitative methods for the detection of NPs in biological samples will have to be developed. Agreement on protocols for different plastics, which are standardised across Europe and potentially globally, is required.

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Abbreviations

ABS	Acrylonitrile butadiene styrene
ACS	American Chemical Society
ATR	Attenuated total reflectance
BPA	Bisphenol A
bw	Body weight
DCU	Dublin City University
DLS	Dynamic light scattering
DSC	Differential scanning calorimetry
ECHA	European Chemicals Agency
EDS	Energy-dispersive X-ray spectroscopy
EPA	Environmental Protection Agency
EU	European Union
FFF	Field flow fractionation
FlowCAM	Flow cytometer and microscope
FPA	Focal plane array
FTIR	Fourier-transform infrared spectroscopy
FTIR/Raman	Fourier-transform infrared spectroscopy and Raman spectroscopy
GIT	Gastrointestinal tract
HDPE	High-density polyethylene
IL	Interleukin
LCSM	Laser confocal scanning microscopy
LDPE	Low-density polyethylene
LS	Light scattering
Micro-PS	Micro-polystyrene
MALS	Multi-angle light scattering
MP	Microplastic
Nano-PS	Nano-polystyrene
NP	Nanoplastic
PC	Polycarbonate
PCCP	Personal care and cosmetic product
PE	Polyethylene
PECO	Population, Exposure, Comparison and Outcome
PET	Polyethylene terephthalate
PM	Particulate matter
PP	Polypropylene
PS	Polystyrene
PS-NP	Polystyrene nanoplastic
PUR	Polyurethane
PVC	Polyvinyl chloride
Py-GC-MS	Pyrolysis–gas chromatography–mass spectrometry
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
SEM	Scanning electron microscopy
TGA	Thermogravimetric analysis
TRWP	Tyre and road wear particle
UV	Ultraviolet

AN GHNÍOMHAIREACTH UM CHAOMHNÚ COMHSHAOIL

Tá an Gníomhaireacht um Chaomhnú Comhshaoil (GCC) freagrach as an gcomhshaoil a chaomhnú agus a fheabhsú mar shócmhainn luachmhar do mhuintir na hÉireann. Táimid tiomanta do dhaoine agus don chomhshaoil a chosaint ó éifeachtaí díobhálacha na radaíochta agus an truaillithe.

Is féidir obair na Gníomhaireachta a roinnt ina trí phríomhréimse:

Rialú: Déanaimid córais éifeachtacha rialaithe agus comhlionta comhshaoil a chur i bhfeidhm chun torthaí maithe comhshaoil a sholáthar agus chun díriú orthu siúd nach gcloíonn leis na córais sin.

Eolas: Soláthraimid sonraí, faisnéis agus measúnú comhshaoil atá ar ardchaighdeán, spriocdhírthe agus tráthúil chun bonn eolais a chur faoin gcinnteoireacht ar gach leibhéal.

Tacaíocht: Bimid ag saothrú i gcomhar le grúpaí eile chun tacú le comhshaoil atá glan, táirgiúil agus cosanta go maith, agus le hiompar a chuirfidh le comhshaoil inbhuanaithe.

Ár bhFreagrachtaí

Ceadúnú

Déanaimid na gníomhaíochtaí seo a leanas a rialú ionas nach ndéanann siad dochar do shláinte an phobail ná don chomhshaoil:

- saoráidí dramhaíola (*m.sh. láithreáin líonta talún, loisceoirí, stáisiúin aistriúcháin dramhaíola*);
- gníomhaíochtaí tionsclaíocha ar scála mór (*m.sh. déantúsaíocht cógaisíochta, déantúsaíocht stroighne, stáisiúin chumhachta*);
- an diantalmhaíocht (*m.sh. muca, éanlaith*);
- úsáid shrianta agus scaoileadh rialaithe Orgánach Géinmhodhnaithe (*OGM*);
- foinsí radaíochta ianúcháin (*m.sh. trealamh x-gha agus radaiteiripe, foinsí tionsclaíocha*);
- áiseanna móra stórála peitрил;
- scardadh dramhuisece;
- gníomhaíochtaí dumpála ar farraige.

Forfheidhmiú Náisiúnta i leith Cúrsaí Comhshaoil

- Clár náisiúnta iniúchtaí agus cigireachtaí a dhéanamh gach bliain ar shaoráidí a bhfuil ceadúnas ón nGníomhaireacht acu.
- Maoirseacht a dhéanamh ar fhreagrachtaí cosanta comhshaoil na n-údarás áitiúil.
- Caighdeán an uisce óil, arna sholáthar ag soláthraithe uisce phoiblí, a mhaoirsiú.
- Obair le húdarás áitiúla agus le gníomhaireachtaí eile chun dul i ngleic le coireanna comhshaoil trí chomhordú a dhéanamh ar líonra forfheidhmiúcháin náisiúnta, trí dhírú ar chiontóirí, agus trí mhaoirsiú a dhéanamh ar leasúchán.
- Cur i bhfeidhm rialachán ar nós na Rialachán um Dhramhthrealamh Leictreach agus Leictreonach (DTLL), um Shrian ar Shubstaintí Guaiseacha agus na Rialachán um rialú ar shubstaintí a ídionn an ciseal ózóin.
- An dlí a chur orthu siúd a bhriseann dlí an chomhshaoil agus a dhéanann dochar don chomhshaoil.

Bainistíocht Uisce

- Monatóireacht agus tuairisciú a dhéanamh ar cháilíocht aibhneacha, lochanna, uisce idirchriosacha agus cósta na hÉireann, agus screamhuisec; leibhéil uisce agus sruthanna aibhneacha a thomhas.
- Comhordú náisiúnta agus maoirsiú a dhéanamh ar an gCreat-Treoir Uisce.
- Monatóireacht agus tuairisciú a dhéanamh ar Cháilíocht an Uisce Snámha.

Monatóireacht, Anailís agus Tuairisciú ar an gComhshaoil

- Monatóireacht a dhéanamh ar cháilíocht an aeir agus Treoir an AE maidir le hAer Glan don Eoraip (CAFÉ) a chur chun feidhme.
- Tuairisciú neamhspleách le cabhrú le cinnteoireacht an rialtais náisiúnta agus na n-údarás áitiúil (*m.sh. tuairisciú tréimhsiúil ar staid Chomhshaoil na hÉireann agus Tuarascálacha ar Tháscairí*).

Rialú Astaíochtaí na nGás Ceaptha Teasa in Éirinn

- Fardail agus réamh-mheastacháin na hÉireann maidir le gáis ceaptha teasa a ullmhú.
- An Treoir maidir le Trádáil Astaíochtaí a chur chun feidhme i gcomhar breis agus 100 de na táirgeoirí dé-ocsaíde carbóin is mó in Éirinn.

Taighde agus Forbairt Comhshaoil

- Taighde comhshaoil a chistiú chun brúnna a shainnaint, bonn eolais a chur faoi bheartais, agus réitigh a sholáthar i réimsí na haeráide, an uisce agus na hinbhuanaitheachta.

Measúnacht Straitéiseach Timpeallachta

- Measúnacht a dhéanamh ar thionchar pleananna agus clár beartaithe ar an gcomhshaoil in Éirinn (*m.sh. mórfheananna forbartha*).

Cosaint Raideolaíoch

- Monatóireacht a dhéanamh ar leibhéil radaíochta, measúnacht a dhéanamh ar nochtadh mhuintir na hÉireann don radaíocht ianúcháin.
- Cabhrú le pleananna náisiúnta a fhorbairt le haghaidh éigeandálaí ag eascairt as tairmí núicléacha.
- Monatóireacht a dhéanamh ar fhorbairtí thar lear a bhaineann le saoráidí núicléacha agus leis an tsábháilteacht raideolaíochta.
- Sainseirbhísí cosanta ar an radaíocht a sholáthar, nó maoirsiú a dhéanamh ar sholáthar na seirbhísí sin.

Treoir, Faisnéis Inrochtana agus Oideachas

- Comhairle agus treoir a chur ar fáil d'earnáil na tionsclaíochta agus don phobal maidir le hábhair a bhaineann le caomhnú an chomhshaoil agus leis an gcosaint raideolaíoch.
- Faisnéis thráthúil ar an gcomhshaoil ar a bhfuil fáil éasca a chur ar fáil chun rannpháirtíocht an phobail a spreagadh sa chinnteoireacht i ndáil leis an gcomhshaoil (*m.sh. Timpeall an Tí, léarscáileanna radóin*).
- Comhairle a chur ar fáil don Rialtas maidir le hábhair a bhaineann leis an tsábháilteacht raideolaíoch agus le cúrsaí práinnfhreagartha.
- Plean Náisiúnta Bainistíochta Dramhaíola Guaisí a fhorbairt chun dramhaíl ghuaiseach a chosaint agus a bhainistiú.

Múscaill Feasachta agus Athrú Iompraíochta

- Feasacht chomhshaoil níos fearr a ghiniúint agus dul i bhfeidhm ar athrú iompraíochta dearfach trí thacú le gnóthais, le pobail agus le teaghlaigh a bheith níos éifeachtúla ar acmhainní.
- Tástáil le haghaidh radóin a chur chun cinn i dtithe agus in ionaid oibre, agus gníomhartha leasúcháin a spreagadh nuair is gá.

Bainistíocht agus struchtúr na Gníomhaireachta um Chaomhnú Comhshaoil

Tá an gníomhaíocht á bainistiú ag Bord Iáinimseartha, ar a bhfuil Ard-Stiúrthóir agus cúigear Stiúrthóirí. Déantar an obair ar fud cúig cinn d'Oifigí:

- An Oifig um Inmharthanacht Comhshaoil
- An Oifig Forfheidhmithe i leith cúrsaí Comhshaoil
- An Oifig um Fianaise is Measúnú
- Oifig um Chosaint Radaíochta agus Monatóireachta Comhshaoil
- An Oifig Cumarsáide agus Seirbhísí Corparáideacha

Tá Coiste Comhairleach ag an nGníomhaireacht le cabhrú léi. Tá dáréag comhaltáí air agus tagann siad le chéile go rialta le plé a dhéanamh ar ábhair inní agus le comhairle a chur ar an mBord.

The State of the Art on the Potential Human Health Impacts of Microplastics and Nanoplastics



Authors: Imen Gdara, Jenny Lawler, Anthony Staines and Sandra O'Neill

Identifying Pressures

Plastic pollution is a global environmental problem. Human exposure to microplastics and nanoplastics (MPs/NPs) through inhalation, ingestion and dermal exposure is an emerging health concern. While it is possible to investigate the levels of exposure through food, air and water, it is not yet possible to estimate individuals' daily exposure levels, as exposure through all three routes has not been comprehensively investigated. Dietary exposure through seafood and water is the most extensively studied, and the data have given rise to food safety concerns. However, the lack of evidence around the effects of MPs/NPs in human populations means that we cannot fully assess the risk. Laboratory studies show that MPs/NPs affect cell function and could lead to a number of detrimental human health effects. There are many state-of-the-art techniques to measure the presence of MPs/NPs in the environment, but to date there are no standardised and validated international protocols to reliably measure MPs/NPs in different sources. Thus, studies investigating MPs/NPs in humans are lacking, simply owing to the lack of direct methods that demonstrate their retention in tissues in situ.

Informing Policy

It is critical that policies and recommendations are established to address the major challenges associated with MPs/NPs. The Irish Government effected a 90% reduction in plastic bag use by the introduction of plastic bag levies in 2002, and has since banned microbeads in cosmetic products and detergents. In 2021, Ireland plans to enforce a levy to reduce single-use cups, and in the future will ban plastic plates, cutlery, straws, balloon sticks, cotton buds and food containers. Given that water, food and air are the main sources of human exposure to MPs/NPs, it is vital that all relevant EU directives and national policies and guidelines are amended to explicitly include MPs/NPs. Ireland, as a Member State, must take specific measures to identify the main sources of MPs/NPs in the environment, specifically those that pose the greatest threat to human health. Once identified, the development of policies and practices to measure levels of exposure in our environment is critical to reduce the potential negative impact on human health.

Developing Solutions

The ubiquitous exposure of humans to MPs/NPs through inhalation, ingestion and dermal exposure makes designing studies to assess health effects challenging. While the routes of human exposure are clearly defined, there is a requirement for standardised fast-throughput methods and validated international protocols to enable measurement of exposure from different sources over time. There is a need to develop specific methods to measure MPs/NPs in human tissue and to identify populations with high-level exposure to facilitate investigations of the impact of MPs/NPs on human health. While laboratory studies link MPs/NPs to cancer and inflammatory, endocrine and reproductive disorders, there is a need for studies to link these effects to MP/NP size and composition and the chemical transfer of adsorbed chemical pollutants, so that any potential toxic substances can be banned for use in plastics. Until these studies are conducted, governments should revise directives and policies on food, water and air to include MPs/NPs, and every effort should be made to remove the potential for plastic to contaminate the food chain and our environment.