A critical review of microbiological colonisation of nano- and microplastics (NMP) and their significance to the food chain

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1. Summary

The Food Standards Agency commissioned a critical review of the scientific evidence concerning the diversity of microorganisms that colonise nano- and microplastics (NMP), key pathways by which microbiologically contaminated NMP could enter the food chain from environmental sources and the risk these pose to the consumer. The review was also to consider interesting traits such as anti-microbial resistance (AMR), virulence genes, the formation of biofilms and changes to microbiomes.

A literature search using pre-agreed search terms was carried out in 2020 using peer-reviewed research literature and accessible grey literature.

The review provides evidence that plastics are found throughout every aspect of our lives, and this results in vast amounts of NMP making their way into the environment. Millions of tonnes have entered the environment since the 1950s, most of it is still there and it is steadily breaking down into smaller parts. These NMP interact with the environment and provide a new substrate for biofilms to form. These biofilms can contain human pathogens and support horizontal gene transfer (HGT) including AMR genes.

The NMP themselves can enter the human food chain via various routes and it is possible that they could transfer pathogens into the gut, but there is a scarcity of data that would allow comprehensive assessment of these outcomes. There are many areas of NMP research with few or no data, and where studies exist, there is a range of approaches to quality assurance, sample collection, size range, analysis and quantification which make detailed comparisons impossible.

Data gaps noted in this review include an absence of data about viruses on NMP, no epidemiological data, many food types have not been assessed for the presence of NMP at all, and there is a lack of studies that include similar sized non-NMP particles in the same environment. The data become even more sparse at the nano end of the scale due to difficulties of working with, and analysing for, nano plastic particles.

2. Executive summary

Plastics have become an important component of everyday life since the start of their mass production in the 1950s. Over the last 70 years, production has increased exponentially for a multitude of polymer types to a global virgin plastics (i.e. plastics newly manufactured from raw materials) production of more than 400 million tonnes annually. Concurrently, plastic has become an important part of global waste flows. As of 2015, 6300 million tonnes of plastic waste had been generated, 79% of which has accumulated in landfills, or the natural environment. As these plastics are exposed to environmental factors such as UV radiation, and physical or biotic abrasion, items fragment into smaller particles, however, the durable nature of many polymers prevents them from being broken down to biologically accessible compounds. Plastic particles smaller than 5 mm are known as microplastics while those smaller than 100 nm tend to be classed as nanoplastics. Both types are combined in this review under the term nano- and microplastics (NMP).

The aim of this review was to see whether there was evidence that NMP act as vectors of pathogens into humans, consider the scientific evidence around the diversity and amount of microorganisms that colonise NMP, the key pathways by which these microbiologically-contaminated NMP enter the food chain from the environment, and the risk that these pose to the consumer. Literature searches for this review were carried out using a combination of database searches, the application of expert knowledge of existing literature, and the use of team-member networks.

A list of search terms was agreed by consensus between the review delivery team and the Food Standards Agency (FSA). The search terms were developed to be inclusive so that as many relevant articles were found as possible and results were limited to articles published since 1980. The Web of Science was selected for the peer-reviewed literature search by consensus with the project team and FSA due to its broad coverage of scientific literature. Searches were carried out on 3rd February 2020. We also searched databases that contain records for different types of grey literature; GreenFile , OpenGrey, BASE, British Library Theses, Library Hub Discover and OATD. In total, over 10,000 documents were considered, and then

refined to 305 specifically included in the report. The review considered scientific articles published between 1980 and 3rd February 2020. However, there has been an increased focus on plastic-related research in recent years and it is acknowledged that there have been relevant papers published after 3rd February 2020. Whilst the authors have included a few recent key publications, it was not possible to include all of them within this review due to timelines.

Mismanaged plastic waste is expected to continue growing even if a range of feasible prevention and intervention techniques are employed. The most widely used synthetic plastics are low- and high-density polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polystyrene (PS) and polyethylene terephthalate (PET). Altogether, these plastics represent ~90% of total world production.

Much of the work published so far on NMP has focussed on limited parts of the marine environment and then a few additional areas with some research, including drinking water, soils and air. Coastal marine areas and areas near to urbanised rivers tend to have higher levels of NMP than more open sea areas. Where converging currents result in large rotating bodies of water, called gyres, this also acts to concentrate floating plastics particles, including NMP. There is a vast amount of plastic litter in the world's oceans, but the amounts reported vary quite dramatically, depending on techniques used and assumptions that drive the generation of global estimates. Varied approaches to sampling, analytical and enumeration methodology and the consequent issues this raised in terms of quality assurance and comparing study data are a feature throughout the report.

Some research on NMP focussed on its ability to provide a substrate for biofilm formation. Surface roughness and hydrophobicity are key characteristics dictating how NMP will interact with the microbial community, and these features can change depending on the amount of weathering that the plastic has undergone. It is possible that polymer type is another determining factor, but the body of evidence was too small to be certain. Many studies fail to consider non-plastic substrates in the same environment, and so the reported NMP effects may in some cases just be small particle effects. It would appear that biofilm communities on NMP have increased rates of horizontal gene transfer (HGT) compared with free-floating communities, and this may include anti-microbial resistance (AMR) genes. However, the data are

limited and suffer from a lack of non-plastic comparators. Another critical impact of biofilms formed on NMP may be the production of olfactory signatures that include algal-derived compounds such as dimethyl sulphide (DMS). Marine grazers and some of their seabird predators are reported to use complex chemosensory cues involving DMS to mediate foraging behaviour and consumption of marine plastic debris.

Biofilms may play an important part in how NMP move in the environment. Some particles sink permanently once biofilms form, whilst others might sink, lose the biofilm and refloat several times. This has implications for NMP fate and transport in the environment.

The review confirmed that there was a general lack of scientific papers on the potential pathways by which NMP can enter into the human food chain. Both airborne and terrestrial movement of NMP were considered as ways of NMP reaching food. There have also been some studies on inhalation of airborne NMP. While the inhalation pathway is not part of the food chain (and so potentially out of scope for this review), this is a potentially important pathway for airborne NMP and so was included in this review as a comparison to foodborne NMP. There was a limited number of papers investigating NMP in food. Drinking water, fish and shellfish, and salt were the main food commodities addressed in the published research, with a few supplementary studies on beer, honey, milk etc. The shortage of data regarding potential NMP contamination of many types of food might mean that ingestion of NMP is currently underestimated. Currently, exposure to airborne NMP via inhalation appears to be a more critical route of human intake, but more data about a wider range of food and drink might change this balance.

Looking across the various food chain pathways the data have many gaps. For terrestrial systems (soil, plants etc.), the small amount of published research appears to show NMP contamination in most areas. Tested drinking water and water sources all appear to contain NMP. Fish and shellfish have the most data, but tests on fish often only consider the digestive tract which is not normally eaten and is also where microplastics would be expected to be concentrated. Shellfish studies frequently use shellfish prior to cleaning or depuration. Studies of fish and shellfish at point of sale or point of consumption are rare. Information is even more scarce for less well

studied parts of the food chain and investigations looking specifically at processed foods are even rarer.

There are differences between the sampling methodology, sampling size ranges, information measured and reported, instrumentation, analytical approach and reported quality assurance information which make comparisons between studies complicated. Smaller particles are more likely to be capable of transmigrating into edible tissues, but plastics at the nano end of the size spectrum are also progressively harder to detect as their size reduces. It is not clear whether these small particles are capable of carrying active pathogens with them.

Finally, we have attempted to pull together the information on global presence of NMP in the environment, how they interact with the microbial community and how they reach the human food chain, to develop an understanding of possible transfer of pathogenic organisms into the food chain. Research has shown that human pathogens have definitely been found on NMP; there are relatively few studies in this area, but those published have demonstrated enrichment of AMR genes, increased HGT processes (such as mobile genetic elements and plasmid transfer) and dysbiosis (gut disfunction) as a possible outcome of NMP in the gut. Data on the effect of viruses on NMP are very limited, and no studies data linking pathogens, NMP and disease were found.

Overall, the scale of NMP in the environment has been shown to be a large and growing problem. Association between NMP and pathogenic organisms such as *Vibrio* spp*, Pseudoalteromonas* spp and *Aeromonas salmonicida* has been demonstrated in research, but the amount of data is low and there is a lack of data comparing association with similarly sized, non-plastic items. Research that quantifies dietary NMP intake is not available for most food and drink categories. From the identified studies involving foods, most have been carried out on fish and shellfish, with a very small fraction considering foods at point-of-sale or point-of-consumption. From the data available so far, it can be inferred that pathogens might reach the human food chain in association with NMP but the consequences of this, and comparison to non-plastic vectors, have been insufficiently studied to create a clear picture of risk. Data gaps and data paucity exist in all aspects of NMP research, but key areas requiring further data include toxicological impacts of NMP, impacts on

gut microflora, presence in a wide range of food and drinks, the importance of NMP size and type, and whether NMP can act as vectors for all pathogens, or just specific ones. A list of identified data gaps is presented at the end of the review.

3. List of abbreviations

Acronym	Full Definition
AA-OXO	Artificially Aged OXO
ABS	Acrylonitrile butadiene styrene
AMR	AntiMicrobial Resistance
ARB	Antibiotic Resistant Bacteria
ARG	Antibiotic Resistance Genes
CL	Cellulose
CP	Cellophane
DEHP	Diethylhexyl phthalate
DLVO	Derjaguin-Landau-Verwey-Overbeek theory of colloid stability
DMS	Dimethyl sulphide
EFSA	European Food Safety Authority
EPS	Extracellular Polymeric Substances
FSA	Food Standards Agency
FTIR	Fourier-transform infrared spectroscopy
GI	Gastrointestinal
H_2Pc	Phthalocyanine
HAB	Harmful Algal Bloom
HDPE	High-density PE
HGT	Horizontal Gene Transfer
LDPE	Low-density PE
LOD	Limit of detection
MGE	Mobile Genetic Elements
MRG	Metal Resistance Genes
MT	Metric tonnes
NMP	Nano- and microplastics
NY	Nylon
OTU	Operational Taxonomic Units
OXO	PE with pro-oxidant
PA	Polyamide

Acronym	Full Definition
PAA	Polaryl amide
PAM	Polyacrylamide
PAN	Polyacrylonitrile
PBO	Polybenzoxazoles
PBT	Polybutylene terephthalate
PC	Polycarbonate
PCL	Polycaprolactone
PDAP	Polydiallyl phthalate
PDMS	Polydimethylsiloxane
PE	Polyethylene
PES	Polyester
PET	Polyethylene terephthalate
PEVA	Poly (ethylene-vinyl acetate)
PHBV	Poly(3-hydroxybutyrate-co-3-hydroxyvalerate
PI	Polyimide
PMPS	Polymethyl pentene
POM	Polyoxymethylene
PP	Polypropylene
PPD	p-Phenylenediamine,
PPTA	Poly-p-phenylene terephthalamide
PS	Polystyrene
PTFE	Polytetrafluoroethylene
PU	Polyurethane
PUR	Polyurethane
PVA	Poly (vinyl alcohol)
PVC	Polyvinyl chloride
PVS	Polyvinyl siloxane
RA	Rayon
SBR	Styrene butadiene rubber
UV	Ultraviolet
WWTP	Waste-water treatment plant

4. Introduction

The main objective from the Food Standards Agency's (FSA) Strategic Plan for 2015-2020 was to protect the public from unacceptable risks which could arise from the consumption of food (including risks caused by the way in which it is produced or supplied) and otherwise to protect the interest of consumers in relation to food. This includes reducing foodborne disease to ensure 'food is safe'. As shown in this review, nano- and microplastics (NMP) are a global concern as environmental contaminants and have been found in aquatic and terrestrial environments and are ingested by both food and non-food producing animals and can potentially contaminate crops. However, a current evidence gap exists in the actual risk to consumers. This may involve potentially adverse effects on the human gut microbiome posed by exposure to microplastic particles colonised with microorganisms, including those carrying antimicrobial resistance (AMR) genes which enter the food supply chain.

Attempting to address this key evidence gap, in 2019, the FSA commissioned Cefas to produce a critical literature review of the scientific evidence by considering both peer-reviewed and grey literature in this area. Of main concern was the diversity of microorganisms that colonise NMP (including agglomerates of NMP), the key environmental pathways by which these microbiologically contaminated NMP could enter the food chain via (such as water, soil and air) and the risk these pose to the consumer. The review was also to consider interesting traits such as AMR and virulence genes and the formation of biofilms and dysbiosis in environmental matrices (for example, soil or sediment) and in organisms.

The outcome is relevant to the FSA's Science, Evidence and Information (SEI) Strategy for 2015-2020 as it will improve understanding of the potential microbiological hazards posed by NMP in the food chain and indicate whether further research is needed to address these.

Plastics are valuable resources with numerous societal benefits. Worldwide plastics production reached about 367 million tonnes in 2020, a slight decrease of 0.3% compared to 2019. Despite the Covid-19 pandemic, global levels of production and

demand for plastics remain stable, while Europe shows a decline in demand due to the direct impact of the pandemic. The decrease in demand was strongly driven by a decline in production in two main application sectors: packaging and automotive [1]. It has been estimated that between 4.8 and 12.7 million tonnes of plastics enter the marine environment annually from land with rivers as main pathways [2], [3], causing plastics to form a large proportion of marine litter.

According to their size, plastic fragments can be classified in macro- and mesoplastics (> 5 mm), microplastics (MP, <5 mm) and nanoplastics (NP, with a range size from 1 nm to 1 µm) [4], [5]. Distinction can also be made between **primary** nano- and microplastics directly entering the environment within their size classes and **secondary** nano- and microplastics resulting from the degradation process of larger debris by isolated or combined effects of environmental factors such as UV radiation, physical or biotic abrasion [6] . The two groups will be combined in this review under the term nano-and microplastics (NMP).

NMP have become an important research topic in the study of plastic pollution (Figure 4.1) and have been reported in atmospheric, sediment, water and biota samples globally [7], [8].

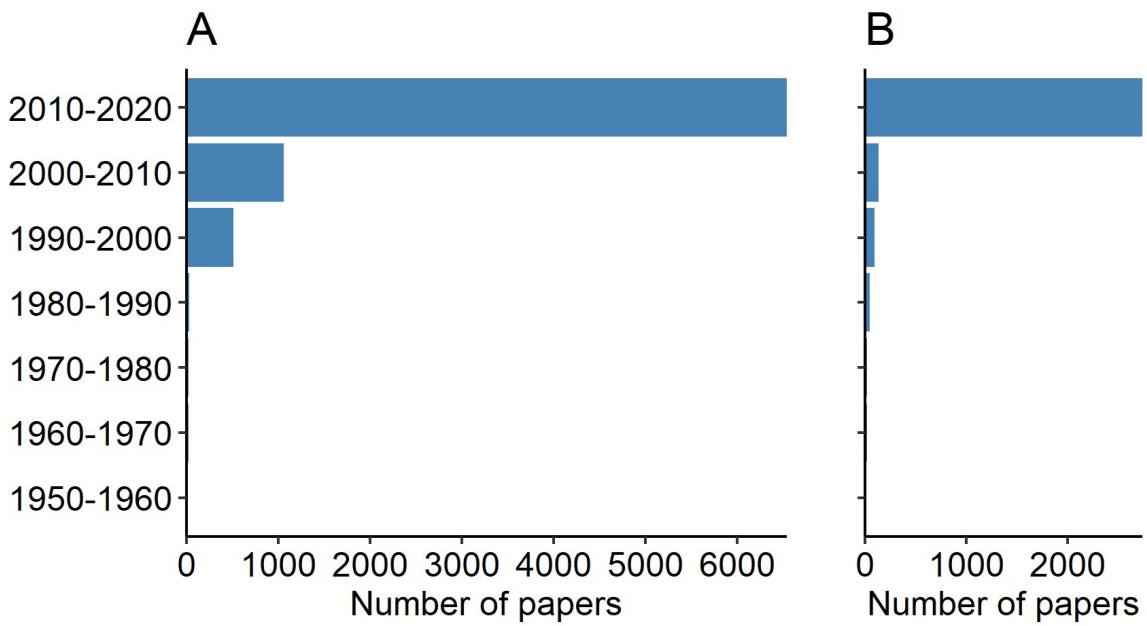

Figure 4.1: The number of scientific publications published within 10-year periods from 1950 to 2020 on (A) plastics and (B) microplastics. Adapted from Rochman (2020) [9].

The ubiquity of NMP in the environment has led to increased concerns about their potential entry into food chains. NMP have previously been shown to be ingested by marine organisms with some evidence of bioaccumulation in tissues [10]. As a result, potential related risks to human food safety have also been the focus of several studies, especially on the consumption of marine food sources [11]. It has also been reported that microplastics have been found in human faeces [12]. This demonstrates that not only are NMP found in the environment, but they can contaminate the food supply chain and be ingested by consumers.

Additional to the physical impacts of NMP in the environment, NMP are known to be able to sorb and concentrate harmful contaminants such as hydrophobic organic compounds (HOCs), plastic additives and heavy metals, with potential for their transfer to marine and terrestrial organisms following ingestion [13], [14]. However, it has been suggested by the Food and Agriculture Organization (FAO) that transfer of sorbed co-contaminants and additives from ingestion of plastic particles would be negligible due to the low dietary exposure to such contaminants [11]. This suggestion was also supported by several studies based on sorption/desorption kinetics studies and bioaccumulation models which concluded that the transfer of sorbed contaminants by marine life would be negligible compared to other routes of exposure (i.e. via water and contaminated prey) [15]–[17] . However it is unclear whether the transfer of plastic additives from ingested NMP is of concern due to their high concentration added during manufacture in some cases [18], [19].

NMP can also act as vectors for human pathogens [20], [21]. There is also some evidence that environmental NMP can build up biofilms over time which can result in complex communities of microorganisms [22]. The durability of NMP, which allows them to persist for long periods of time in the environment, makes them a potentially stronger vector than naturally occurring particles. Additionally, many NMP are neutrally to positively buoyant which keeps them in the water column, or at the surface, allowing for rapid transport by currents and winds with potential for the colonisation of even pristine areas [23].

This is potentially a concern for human health because it is known that various human pathogens have been found associated with NMP and biofilms (for example, *Pseudomonas monteilii and Pseudomonas mendocina*) [24]. Furthermore, as

microorganisms form a biofilm on plastic fragments, they may also interact with other microorganisms through HGT [24]. This process could lead to an increased occurrence of AMR in the organisms colonising microplastics destined to reach our food sources [25]. While there is a growing body of evidence that NMP can provide new microbial niches in aquatic environments [24], large knowledge gaps remain regarding their significance to the food chain with unknown associated risks to the consumer, this is the focus of this study.

The main aim of this report was to produce a critical review of microbiological colonisation of NMP and its significance to the food chain. This was achieved by: summarising the available scientific evidence (including peer-reviewed and grey literature sources) related to the diversity of microorganisms that colonise microplastics; outlining the key pathways by which these microbiologically contaminated microplastics can enter the food chain from environmental sources (for example, from water, soil and air) and identifying the associated risks these may pose to human health for the consumer. This critical review also identifies key evidence gaps that urgently need to be addressed as well as giving some recommendations for future work.

The main objectives of this report were to evaluate the existing literature regarding: i) the environmental burden of NMP, ii) how microorganisms interact with NMP, iii) the pathways that microbiologically colonised NMP enter the human food chain, and iv) the microbial risk to consumers following ingestion of colonised NMP. These objectives were achieved by splitting the review into four discrete work packages for each area of investigation and a final work package to bring all the review sections together in a single document (as presented here). Each work package was assigned a team as follows:

Work package 1: An Overview of NMP in the Environment

- Lead: Craig Baker-Austin
- Team: Adil Bakir, Tamara Galloway, Ceri Lewis

Work package 2: Interactions between NMP and Microorganisms

- Lead: Karen Thorpe

- Team: Will Gaze, David Walker

Work package 3: Pathways of colonised NMP into the human food chain

- Lead: Andy Smith
- Team: Adil Bakir, Emiline Quill, Josie Russel, Nanne van Hoytema

Work package 4: NMP specific microbial risks to consumers

- Lead: Craig Baker-Austin
- Team: Will Gaze, David Walker, Ceri Lewis

Work package 5: Synthesis report

- Lead: David Walker
- Team: Craig Baker-Austin, Andy Smith, Adil Bakir, Sharron Ganther, Nanne van Hoytema

5. Review methodology

The literature searches for this review were carried out using a combination of database searches, the application of expert knowledge of existing literature and the use of team-member networks.

All search results were saved in a bibliographic database created with Microsoft Excel. This database was used during the sifting process to record those references that were kept or rejected. Following sifting, the references kept were stored in a Mendeley Group to allow for easy sharing between the project team and for easy citations in the report.

5.1 Database searching

5.1.1 Search terms

Before literature searches were carried out, a list of search terms was agreed by consensus between the review delivery team and the Food Standards Agency (FSA). To achieve this, search terms were initially developed separately for each of the four review sections by the teams working on those sections. The search terms were then reviewed by the rest of the project team and FSA before being finalised. Each section of the review had its own set of search terms to allow the search results to be categorised between the review sections. This allowed the results to be easily separated later on in the sifting process and to be reviewed by topic experts.

The search terms are listed in Appendix 1 in the syntax required for the Web of Science advanced search function. The same search terms were used in all databases (including those for grey literature searches) but using the appropriate syntax for each database. The search terms were developed to be inclusive to ensure that as many relevant articles were found as possible. However, due to the broad scope of this review, it was also necessary to include exclusion terms within the searches to limit the results that were not relevant. These exclusion terms are listed with the search terms in Appendix 1 as those preceded with the “NOT” operator. When searching the databases, this review considered scientific articles published between 1980 and 3rd February 2020. However, there has been an

increased focus on plastic-related research in recent years and that the authors of this review acknowledge that there have been papers published after 3rd February 2020. Whilst we have tried to include a few recent key publications, it was not possible to include all of them within this review due to timelines. The search terms are listed in Appendix 1.

5.1.2 Peer reviewed literature

To search for the peer-reviewed literature used in this review, the Web of Science database was used. Web of Science was selected for this purpose by consensus with the project team and FSA due to its broad coverage of scientific literature and the universal access available to members of the project team. Web of Science searches were carried out on 3rd February 2020.

All of the databases within Web of Science were searched for this review. Using the search terms developed previously, searches were carried out individually for each review section using the “advanced search” feature of Web of Science using the appropriate syntax.

For each search that was conducted, the results were exported to Excel for inclusion in the bibliographic database. After all Web of Science searches were completed, duplicate entries in the bibliographic database were removed.

The total number of unique search results from the Web of Science searches was 8,706.

5.1.3 Grey literature

Grey literature, which can be defined as literature which is not controlled or published by publishing organisations [26], can be a useful source of information that may not be available in the peer-reviewed literature. Due to its nature, there is no single database that provides a comprehensive catalogue of topic specific grey literature resources. It was therefore necessary to search several databases that contain records for different types of grey literature. These included GreenFile, OpenGrey, Bielefeld Academic Search Engine (BASE), British Library Theses (BLT), Library Hub Discover (LHD) and Open Access Theses and Dissertations (OATD).

Search results were exported to the Excel based bibliography and duplicate entries were deleted.

The total number of new search results (i.e. those that were not duplicates of the Web of Science results) from the grey literature database searches was 1,293. This brought the total number of unique articles found in databases to 9,999.

Using the review section specific search terms, the number of results for each section are outlined in Table 5.1.

Table 5.1: Number of search results from Web of Science and other literature databases by review section.

Section title	Web of Science	Green File	OpenGrey	BASE	BLT	LHD	OATD	Number of database results
An Overview of NMP in the Environment	547	785	12	75	10	123	24	1,384
Interactions between NMP and microorganisms	6,057	292	12	232	1	71	7	6,368
Pathways of colonised NMP into food chains	3,574	487	8	176	1	19	15	3,750
NMP specific microbial risks to consumers	598	93	0	76	0	1	5	654
Articles overlapping sections	2,070	330	4	121	2	28	12	2,157

5.1.4 Other literature

In addition to formal searches for literature on databases, other literature considered suitable was included in the review. These literature sources included articles that were discovered during the review process or papers known to the review team that were not found in the database searches.

The total number of additional articles considered for review that were not in the database searches was 107. This brought the total number of articles considered for review to 10,106.

5.2 Literature sifting

Once the searches were completed and the results added to the bibliographic database, the results were manually sifted to remove any results that were not relevant to the review.

The sifting process followed a series of stages. At each stage, more detail was reviewed for each article before being either rejected or taken through to further sifting or review.

The initial sift was based solely on the title of each article. At this stage a single member of the project team read the titles of all the articles and removed only those articles that were obviously not relevant to this review, without any need for expert knowledge on the subject.

As a result of the first sift, a total of 1,634 articles were taken forward for further review.

At the second stage of sifting, the remaining literature was split into those articles relevant to each of the review sections. A further sift, based on both the article titles and the abstracts, was then carried out within the teams working on the relevant review section. This allowed a more expert level of sifting to be carried out on the remaining literature. A total of 886 articles were taken forward after this stage of sifting.

During the title and abstract sifting stages, articles were included based on the following criteria:

- Literature dealt with micro and nano plastics
- Evidence and data presented in the literature were generated or assessed using robust application of scientific principles.
- The literature contained original data or evidence and was not simply a repetition or review of evidence from another source.
    - An exception to this rule was required for Section 6, which was largely a review of existing reviews, and only included recent original research articles.
- The literature discussed micro and nano plastics in the context of pollutants and not as products in themselves.
    - For example, papers that dealt with nano fibres in the context of their use as a filter substrate would not be relevant.

During the title and abstract sifting stages, articles were excluded based on the following criteria:

- Literature not published in English
- Literature was published prior to 1st January 1980.
- Literature dealt exclusively with macro plastics.
    - With the exception of discussing concepts for biofilm formation on plastic substrates.
- Literature was a repetition of literature already included.

Following these sifts, articles were read in detail before being accepted or rejected for review based on the discretion of the review section teams according to their relevance to the review section.

A total of 334 unique articles were reviewed, some of which were included in multiple sections. These included a combination of those articles found in the literature databases that remained after sifting and those that were discovered independently of the databases and kept in the review at the discretion of the review team. The number of articles reviewed for each review section are outlined in Table 5.2.

Table 5.2: Number of articles reviewed in each review section.

Section title	Number of articles reviewed
An overview of NMP in the environment	66
Interactions between NMP and microorganisms	77
Pathways of colonised NMP into food chains	145
NMP specific microbial risks to consumers	36

6. An Overview of NMP in the Environment

6.1 Introduction

In this overview chapter we undertook a review of the knowledge on the abundance, distribution, composition, and movement of NMP in the environment.

Research into microplastics has expanded rapidly in recent years and they have been found globally in the atmosphere of major cities and remote mountain ranges [27], in beaches, agricultural lands, and remote conservation areas [28], [29], in freshwater in the world's major rivers and lakes [30] and in the ocean from the tropics to the poles and down into the deepest trenches [31]–[33]. As microplastics entered all habitats on earth, organisms began to interact with them. Microplastics can be present in high concentrations in plants , including edible fruits, leaves and roots [34]. A study by Li *et al.* [35] proposed a mechanism for the uptake of NMP in crop plants by the "crack-entry" pathway, via the apoplastic transport system. Animals can ingest microplastics directly because they mistake them for normal food particles and inadvertently trap them as they filter particles or actively select them besides their normal food sources, or indirectly through consumption of microplastic contaminated foods. Microplastics have been found in the gastro-intestinal tract and tissues of many species, in particular fish and molluscs [36]. Effects of microplastics ingestion on organism health and fitness are hard to determine at concentrations which they encounter naturally in the environment (environmentally-relevant concentrations and exposures) and evidence remains inconclusive [37]. There is growing evidence suggesting that microplastics can sorb and transport chemicals into animal tissues [38].

Various estimates of both the abundance and geographical extent of plastic contamination have been produced in the last two decades. These studies demonstrate that contamination is geographically widespread, longstanding and likely to increase in the future [39], [40]. Much of the research has focussed on the aquatic environment so far, with more attention on marine than on freshwater, resulting in most of the data relating to the marine environment. NMP are released at

differing rates into the environment both as virgin plastics and as plastics undergoing a spectrum of weathering and degradation processes, which break down by various physical and chemical processes and vary in both time and space. Numerous marine “hotspots” of contamination, including enclosed marine systems, coastal zones and gyres, have been widely identified and studied. Plastic debris is an extremely diverse material, composed of many different polymers at different weathering states and of different shapes and sizes [7]. Little is known about the transformations of plastics in seawater (for example, how these are broken down), including the time scales of degradation and its ultimate sinks [41].

Overall, although marine NMP have been the focus of substantial scientific research, the extent of microplastic pollution in other areas of the natural environment, such as rivers, lakes, soil and air, and their environmental interactions, remains poorly understood. An increasing body of evidence suggests that microplastic contamination in the environment is ubiquitous with various studies identifying plastic particles in pristine environments such as National Parks, Arctic snow and Mount Everest [42]–[45]. This suggests that non-marine food chains will be affected, but such research has either not been carried out or is not available yet. Increasingly, the complexity of microplastic pollution is being recognized, resulting in a paradigm shift reflecting the importance of this group of contaminants. Microplastic contamination and the cycling of these contaminants between different environmental compartments is being progressively viewed within a wider framework analogous to more complex cycles present in nature, such as the nitrogen or carbon cycle, and encompassing factors at all levels of biological organization, from molecular to landscape scales.

6.2 Background and methodology

Since the mass production of plastics began in the 1940s, microplastic contamination of the marine environment has been a growing problem [39] and is cause for particular concern due to overall abundance combined with durability and persistence, particularly in marine settings [46]. Although this research area has received a significant amount of recent interest, this is not a new topic with early reports on the occurrence of plastics in the marine environment being traced back to

the early 1970s [47]. NMP are a major source of anthropogenic (human-derived) contamination in the natural environment, and globally. As such, NMP are now considered an urgent ecological, animal health and human health concern. As plastic pollution in the environment continues to increase, there are growing concerns regarding the potential role of these pollutants as a potent and persistent human health risk, particularly from food sources.

This section provides a concise overview of the latest understanding of these areas and how they might influence biological uptake of NMP into the food chain. We provide an evaluation of where NMP are found; the sources of NMP; the type of plastics from which NMP are made; the residence time of NMP in the environment and NMP agglomeration. Relevant information on the quantity, number of particles and total mass of NMP were also gathered and synthesized for this aspect of the report. This short synthesis review utilised the most relevant review articles and primary literature and was updated using the most contemporary studies, including those published in 2019 and early 2020 and which have subsequently not been reviewed elsewhere.

Primary studies and metareviews/literature papers focussing on key areas set out in this chapter (sources/quantity, abundance of NMP, types of plastics, how NMP influence uptake, where NMP are found etc.) were given a priority during evaluation of the literature identified during the early review stages. Grey literature in this area was also considered. Approximately 200 of these papers were read, and to make the synthesis review as succinct as possible, around 50 of the most relevant papers were selected for inclusion in this chapter. A synthesis report on this analysis was then compiled and sent for review to Dr Ceri Lewis (University of Exeter) in December 2020. Feedback from this review was then amalgamated in January & February 2021.

The study by Cozar *et al.* [48] presented one of the first global mapping efforts to identify hotspots of NMP pollution in the global oceanic environment. This study, among others [49]–[52], has demonstrated that globally there are wide variations in the amount of microplastics observed across different marine settings. For example, the surface waters of the North-western Pacific Ocean are extensively polluted by microplastics, with concentrations ranging from 640 to 42,000 items/km^2 depending

on the action of currents [49], [50]. Semi-enclosed ocean systems, such as the Mediterranean (which have large sources of NMP and limited mixing), represent one of the most contaminated marine systems studied on Earth [51]–[53]. Generally, microplastic levels are significantly higher in coastal waters adjacent to highly urbanized areas compared with rural areas [50]. In a study by Song *et al.* sampling the seawater off the coast of South Korea, the mean microplastic abundance in urban coastal areas was as high as 1051 particles/m^3, compared to 560 particles/m^3 in rural coastal areas [54]. NMP have also been identified in both the Arctic [42], [43] and Antarctic [44], [45], highlighting the ubiquity of these contaminants globally.

Converging surface currents in oceanic gyres are responsible for the global distribution of plastics on the ocean surface [41], [48], [55]. These gyres effectively concentrate positively buoyant plastics into “garbage patches” and are considered hotspots for the presence of plastic pollution in the open ocean system [41], [56]. Deep pelagic waters within marine ecosystems dwarf all other available living space on Earth and growing evidence demonstrates that plastic is accumulating within the animals, bottom sediments, and trenches of the deep-sea system [57]. Increasingly there is interest beyond the surface contamination of microplastics in the marine environment. A recent study by Pabortsava and Lampitt [32] sampled more widely throughout the top 200 m of the ocean’s water column, using techniques that capture smaller particles, and estimated that the combined mass of just the three most-littered plastics (PE, PP and PS) of 32–651 μm in size in the Atlantic Ocean alone is between 11.6 and 21.1 million tonnes. This is ten times higher than previous estimates made using surface trawl data alone. This number still does not account for the plastic debris that has been transported downwards to the seabed, having either fouled and sunk or have become incorporated into aggregates (naturally occurring material in the ocean) such as marine snow.

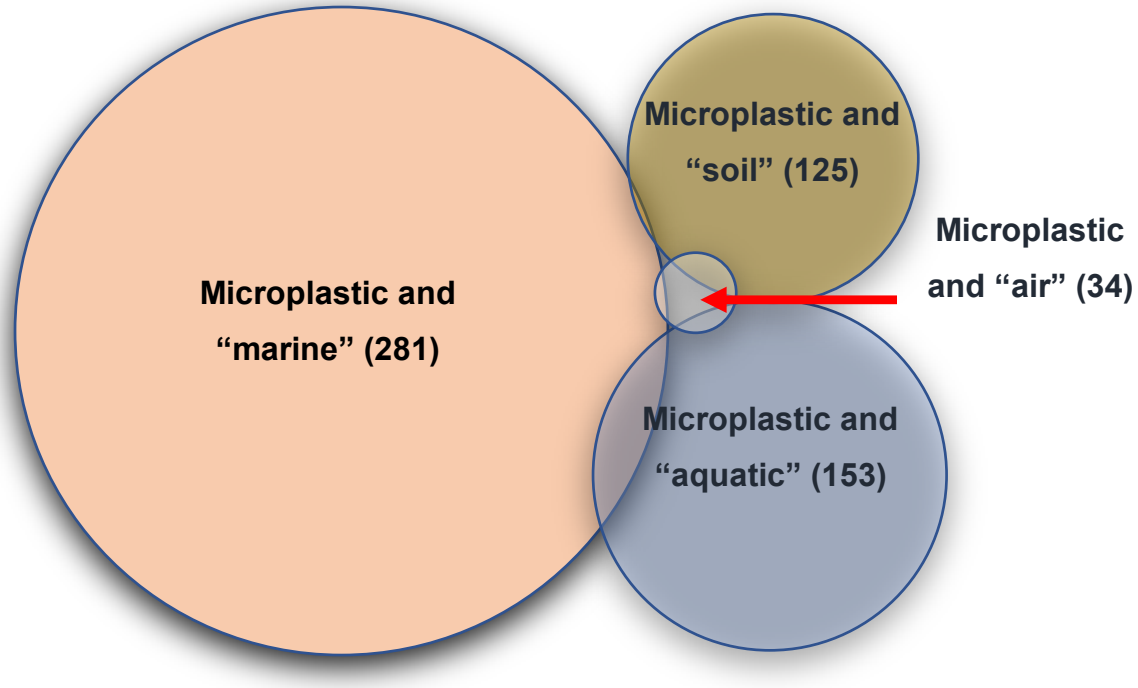

Figure 6.2 Published scientific studies with the term "microplastic" in the title alongside other environmental search terms (until Feb 2021) available on PubMed. Figure generated 26th February 2021.

6.3 Quantity, number of particles and total mass of NMP

One of the most important aims of marine plastic pollution research is to accurately estimate current microplastic abundances and to predict the future abundance in the ocean environment [40], [58]. There are a variety of different estimations with regards to the quantity of NMP present in the natural environment, with a majority of published studies focusing on the marine and aquatic environments [50].

To be able to quantify NMP, a suite of sampling techniques has been developed that allow the presence of small (<~300 µm) plastic debris to be determined. These include: beach combing; sediment sampling; marine trawls; marine observational surveys and biological sampling [39]. Plastic pollution is now considered a ubiquitous problem globally in the marine environment [40] with an estimated 15 – 51 trillion microplastic particles floating on the surface of the world's oceans [41]. However, this likely only represents ~1% of the 4.8-12.7 MT yr^{-1} (metric tonne) of plastics

thought to enter global oceans annually [59], with the majority of microplastics eventually sinking via fouling, flocculation and egestion amongst a variety of processes [60].

Several studies have attempted to provide the global mass of plastics in the ocean system, using data generated via surface-trawling plankton net-based approaches. Cózar *et al.* [48] estimated that there were 7–35 thousand tonnes of plastic waste in the ocean system. Eriksen *et al.* [56] estimated the level of contamination to be slightly higher at around 66 thousand metric tonnes. A substantially higher global burden of plastic contamination was estimated by van Sebille *et al.* [41] which indicated that there were 93 to 236 thousand metric tonnes of waste globally. Indeed, the variety of figures quoted by these different authors outline some of the difficulties in providing a cohesive and universally accepted guide as to the levels of plastic waste currently circulating in the global oceanic system. It has been noted that although a ubiquitous environmental contaminant, accurate quantitative estimates on the global abundance and weight of floating plastics are still limited [61].

Accurately mapping the microplastic abundance in the actual ocean has proven difficult, largely because insufficient measurements are available for all the world's oceans [40]. The lack of standardised sampling protocols, methodologies for appropriate analysis and standardised units to ascribe MPs abundance are yet to be internationally standardised [62]. Agamuthu *et al.* [63] noted that plastics represent the major constituent of marine debris, accounting for between 50% and 90% of the total marine debris found globally. Consumer plastics frequently end up in the world oceans, resulting in the presence of more than 100 million particles of macroplastics in only 12 regional seas worldwide, and with 51 trillion particles of microplastic floating on the ocean surface globally [63].

Other differing estimates on NMP have been presented by several authors. These have noted that there are approximately 5 trillion plastic particles present in our oceans, from macro to micro size [56], [64]. Several studies have shown how the abundance of NMP has increased significantly over time. Law *et al.* [65] showed a 10-fold increase of 18,160 to 189,800 pieces of plastic km^2 in the eastern Pacific Ocean[65], [66]. It was estimated that plastic fragments accounted for 60–80% of

total marine debris and >90% of floating particles in 2008 [67], making them the predominant components of marine debris. Barrows *et al.* [68] showed, using a citizen scientist driven study of 1-litre grab samples, that the global microparticle average was 11.8 ± 24.0 particles L^{-1} (mean ± SD). This is approximately three orders of magnitude higher than global model predictions. It should also be noted that recent research is reinforcing the data showing that the number of microplastics reported is irrevocably linked to the mesh size used to retain them. Current figures are likely to be substantially under reporting the actual situation [69]. There is also some current debate on what contaminants could and should be considered "microplastics" [70]. If plastic production and waste generation continue to grow at current rates, the annual mass of mismanaged waste has been projected to more than double by 2050 [71].

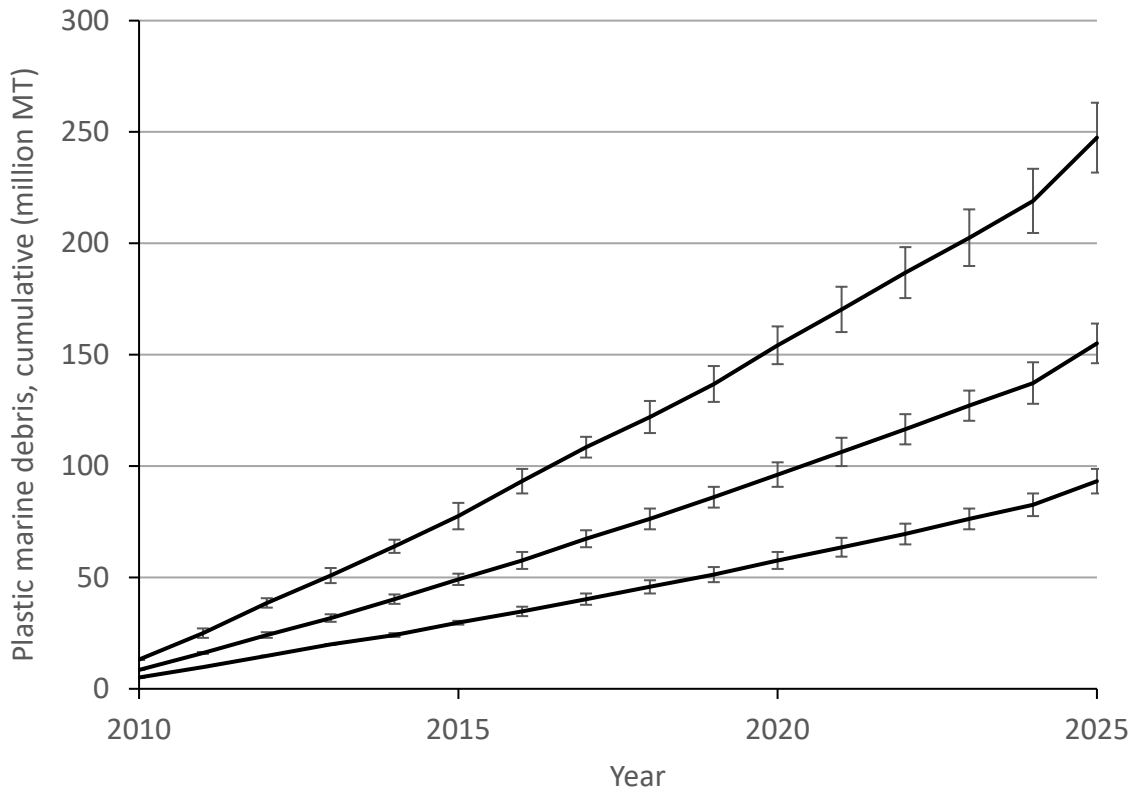

Figure 6.3 Estimated mass of mismanaged plastic waste (millions of metric tonnes) input to the ocean by populations living within 50 km of a coast in 192 countries, plotted as a cumulative sum from 2010 to 2025. Figure adapted from Jambeck *et al.* [2]. Estimates reflect assumed conversion rates of mismanaged plastic waste to marine debris (high, 40%; mid, 25%; low, 15%). Error bars

were generated using mean and standard error from the predictive models for mis-managed waste fraction and percent plastic in the waste stream.

Several studies have attempted to predict how plastic pollution of the marine environment may change in the future. Jambeck *et al.* [2] (Figure 6.2) noted that without waste management infrastructure improvements, the cumulative quantity of plastic waste available to enter the ocean from land is predicted to increase by an order of magnitude by 2025 [2]. More recently, Lau *et al.* [58] attempted to estimate the effectiveness of interventions to reduce plastic pollution and used modelled stocks and flows of municipal solid waste and four sources of microplastics through the global plastic system for five scenarios between 2016 and 2040. They found that implementing all feasible interventions reduced plastic pollution by 40% from 2016 rates and 78% relative to “business as usual” (i.e. no additional interventions to reduce plastic pollution) in 2040. However, even with immediate and concerted action, 710 million metric tonnes of plastic waste cumulatively entered aquatic and terrestrial ecosystems.

6.4 Types of plastics from which NMP are made and residence times

Currently, the most widely used synthetic plastics are low- and high-density PE, PP, PVC, PS and PET. Altogether, these plastics represent ~90% of the total world production [72], [73]. Likewise, the most commonly detected polymer types found in marine systems include PP, PE, PVC, PS, as well as polytetrafluoroethylene (PTFE) [50]. Most synthetic polymers are buoyant in water (for example, PE and PP) [73] and the surface properties of plastic are thought to play an important part in determining its ecological impacts [74].

Plastic debris is an extremely diverse material, composed of many different polymers at different weathering states and of different shapes and sizes [7]. Generally, NMP are classified into two groups, primary NMP and secondary NMP [7]. Primary NMP are microscopic pieces of plastic that are purposefully manufactured for specific applications, such as pellets for industrial production and microbeads. Secondary NMP are produced indirectly from the breakdown of larger plastic waste or debris, both at sea and on land. These fragments are broken down by a variety of processes

including degradation by sunlight, oxidation or physical friction, and by the action of microorganisms further degrading the plastic. The diversity and complexity of sources is reflected in the diversity of NMP characteristics (shape, size, density, polymer type).

Transport is likely to be affected by particle size, density and shape as well as processes such as fouling and aggregation-sedimentation. Using high-resolution spatial and temporal data in the US, Brahney *et al.* [29] provided evidence of both global and regional microplastic transport. The authors note that this “microplastic cycle” is akin to global biogeochemical cycles (nitrogen, carbon, and water). While this analogy may be questionable, this study provided compelling evidence that the transport of NMP between different environmental compartments is a very complex process and we are only now beginning to understand the level of this complexity. In marine waters, horizontal and vertical transport of particles occurs, influenced by wind as well as water movement. Turbulent mixing can transport positively buoyant plastic down for tens of metres and microplastics of buoyant polymers such as PP and PE, which should float as virgin (unfouled) particles, have now been reported at depths down to 5000 m in ocean sediment and in the guts of deep-sea organisms. A recent estimation that 99% of plastic entering the oceans will eventually reach the ocean floor included buoyant polymers. In the marine environment plastics degrade through physical, chemical and biological processes [72], [75]. However, plastic fragments are generally considered persistent environmental contaminants, potentially lasting hundreds to thousands of years in the aquatic environment [47], [76].

6.5 Sources of NMP in the environment

Between 1960 and 2000, the world production of plastic resins increased 25-fold, while recovery of the material remained below 5% [67]. From 2010 to 2016, global plastic production increased 26% from 334 to 422 Mt [71], [77]. In total, the proportion of plastics in solid waste grew from 10 to 12% globally, reaching 242 Mt in 2016 alone [78], [79]. It is projected that plastic wastes accounted for 33 billion tonnes globally by 2015 [80]. Approximately 80% of plastic in the oceans is estimated to have derived from land-based sources or entry points [81], [82]. These

sources of litter and NMP are varied, and for the more highly researched marine environment, include beaches, rivers, storm water runoff, aquaculture and fisheries, shipping transport, and atmospheric outfall [7]. In 2016, the U.S. population produced the largest mass of plastic waste of any country in the world and had the largest annual per capita plastic waste generation of the top plastic waste–generating countries (>100 kg). The countries with the next highest plastic waste generation were also those with the highest populations such as India and China. The EU-28 countries collectively generated more plastic waste than either India or China, despite having only ~40% of the population. Even in the EU-28, the per capita plastic waste generation rate was approximately half that of the United States [77].

Table 6.3 Countries with the highest waste generation in 2016. Table from Law *et al.* [65]. Calculations using data reported in [62], with a *refined estimate for the United States. EU-28 countries are reported collectively.

Country	Plastic waste generation (metric tonnes)	Total waste generation (metric tonnes)	Plastic in solid waste (%)	2016 Population (millions)	Per capita plastic waste generation (tonne)
*United States	42,027,215	320,818,436	13.1	323.1	130.09
United States	34,020,748	263,726,732	12.9	323.1	105.3
EU-28	29,890,143	243,737,466	11.7	511.2	54.56
India	26,327,933	277,136,133	9.5	1,324.50	19.88
China	21,599,465	220,402,706	9.8	1,378.70	15.67
Brazil	10,675,989	79,081,401	13.5	206.2	51.78
Indonesia	9,128,000	65,200,000	14	261.6	34.9
Russian Federation	8,467,156	59,585,899	14.2	144.3	58.66
Germany	6,683,412	51,410,863	13	82.3	81.16
United Kingdom	6,471,650	32,037,871	20.2	65.6	98.66
Mexico	5,902,490	54,151,287	10.9	123.3	47.86
Japan	4,881,161	44,374,189	11	127	38.44
Thailand	4,796,494	27,268,302	17.6	69	69.54
Republic of Korea	4,514,186	18,576,898	24.3	51.2	88.09
Italy	3,365,130	29,009,742	11.6	60.6	55.51
Egypt	3,037,675	23,366,729	13	94.4	32.16
France	2,929,042	32,544,914	9	66.9	43.81
Pakistan	2,731,768	30,352,981	9	203.6	13.42
Argentina	2,656,771	18,184,606	14.6	43.6	60.95
Algeria	2,092,007	12,378,740	16.9	40.6	51.59
Malaysia	2,058,501	13,723,342	15	30.7	67.09
Spain	1,832,533	20,361,483	9	46.5	39.42

6.6 How NMP might influence biological uptake and agglomeration

NMP are similar in size to plankton that form the base of the food chain [83]. The smooth, hydrophobic surfaces of virgin (unfouled) plastics have no net charge, but this rapidly changes once in seawater as organic matter, biomolecules, nutrients, and bacteria, as well as hazardous hydrophobic contaminants, quickly sorb (gather/aggregate on a surface) to the polymer surface [38]. This sorption of biological materials produces a unique 'ecocorona' [74]. which, as demonstrated by ecotoxicology studies, can influence both biological uptake of nanoparticles and their fate within tissues and cells [38]. The ecocorona has a distinct chemical profile compared to virgin plastics, with a differing surface charge, aggregation pattern and reactivity of the material.

Ramsperger *et al.* [84] showed that environmentally exposed microplastic particles are internalized significantly more often than pristine microplastic particles into macrophages. They subsequently identified biomolecules forming an ecocorona on the surface of microplastic particles, suggesting that environmental exposure promotes the cellular internalization of microplastics into human cells. There is however, a lack of data on the cellular mechanisms of microplastic internalization from fresh water and salt water and even terrestrial environments [84]. Microplastic is a complex, dynamic mixture of polymers and additives, to which organic material and contaminants can successively bind, increasing the density and surface charge of particles and changing their bioavailability and toxicity [74].

Numerous studies have been performed to investigate the impacts of microplastics on marine biota. The ingestion of microplastics has been documented for vertebrate and invertebrate marine species, see review by Ivar do Sul and Costa [73] and the potential routes of entry into the human food chain are covered later in this report. There is a growing body of evidence of microplastic uptake by the commercial seafood and aquaculture shellfish species *Mytilus edulis* and *Crassostrea gigas*. Microplastics were recovered from the soft tissues of both species. At time of human consumption, *M. edulis* contains on average 0.36 ± 0.07 particles g^{-1} (wet weight), while a plastic load of 0.47 ± 0.16 particles g^{-1} wet weight was detected in *C. gigas*.

As a result, the annual dietary exposure for European shellfish consumers can amount to 11,000 microplastic particles per year [85].

A recent metareview of microplastic contamination of seafood indicated that molluscs collected off the coasts of Asia were the most heavily contaminated, coinciding with reported trends of microplastic contamination in the sea [86]. Limited field evidence from higher trophic level organisms in a variety of habitats suggests that trophic transfer of microplastics may be a common phenomenon and occurs concurrently with direct ingestion [87]. For microorganisms, trillions of these floating microplastics particles represent a huge surface area for colonization [88]. When multiple particles, such as NMP, cells, detritus and mineral particles collide, they may attach to one another, forming an agglomerate [75], [89]. Marine aggregates often readily incorporate NMP and information of how and why these mixed matrices are transported through marine food webs are now being more fully understood. For instance, laboratory generated marine snows have been shown to transport microplastics of different shapes, sizes and polymers away from the water surface and enhance their bioavailability to benthic organisms. The study demonstrated that the incorporation of NMP into marine snows increased microplastic bioavailability for mussels, where uptake increased from zero to 340 microplastic particles individual^{-1} for free microplastics to up to 1.6×10^5 microplastic particles individual^{-1} when incorporated into snows [60].

6.7 Summary

There has been an enormous increase in scientific and societal interest in the microplastics issue recently. It is clear that plastic contamination in the environment is widespread, with the majority of published scientific studies focussing on marine and aquatic systems (which form the basis of many of the studies presented here). Various estimates of both the abundance and geographical extent of plastic contamination have been produced recently, particularly in the last two decades. Many of these studies demonstrate that plastic contamination is geographically widespread, longstanding and likely to increase in the near future. Numerous “hotspots” of contamination have been identified, however areas presumed as pristine have shown the presence of plastic contamination, highlighting the

environmental ubiquity of these pollutants. Many studies are now finding clear evidence of microplastic uptake and subsequent contamination of key species present in the human food chain.

6.8 Data gaps

Throughout the review process, a variety of important data gaps and data limitations were identified in various published studies. Recurring data gaps identified by the different studies are detailed below:

- There is currently a lack of standardised and universally applied methods (including analytical methods) to collect data such as NMP concentrations in surface waters.
- The need for an internationally accepted definition regarding NMP. Some studies for instance include cellulose-based polymers, whilst many do not. There is the need to provide clear, concise, and universally accepted definitions.
- A huge number of studies have looked at the fate of NMP in the marine environment. However, there has been less emphasis on other environments (for example, aquatic, soil/terrestrial etc, see Figure 6.1). This situation appears to be changing rapidly, with a large increase in published studies in the last year alone. Similarly, there are few data on NMP in “pristine” environments. Again, this is changing with various studies in the last year alone on NMP in the Arctic, Antarctic, National Parks etc.
- Little is known about the transformations of plastics in seawater, including the time scales of degradation and its ultimate sinks (end point) [41], as well as potential to present further ecotoxicological hazards [90]. In this regard there is a lack of standardised, laboratory-based data to study the long-term fate of NMP. This should include both laboratory and environmental studies that can provide insights into the specific processes of degradation coupled to the health risks associated with these pollutants.
- Although the presence of NMP have been widely reported in certain food commodities (see Chapter 5), there are very few detailed studies on these at ecosystem-levels. For instance, there are many published studies that have

identified the presence of microplastics in seafood matrices, and also in the wider marine environment, but the presence of microplastics across the entire marine ecosystem is poorly understood.

- There is a lack of data on microplastic contamination and the cycling of these contaminants between different environmental compartments. The complexity of NMP transitions in the environment is now only starting to be appreciated, encompassing factors at all levels of biological organization (for example, studies addressing aspects of microplastics from transcriptional, genomic and cellular, organismal, community and ecosystem-level scales). However, large data gaps exist regarding key parts of this cycle, for example what proportions move from different environmental compartments and over what timescales.

7. Interactions between NMP and Microorganisms

7.1 Introduction

Once in the environment, plastics (and NMP) immediately start to interact with the microorganisms they encounter. The degree to which microorganisms interact with any surface can vary widely, from transient contact to the development of complex structures, known as biofilms, consisting of microorganisms and associated extracellular matrices [91]. Where microorganisms are not physically attached to surfaces, they may be relatively easily removed by water or air currents. However, the formation of complex biofilms increases the ability of microorganisms to remain attached to surfaces [92].

Microbial associations with plastic marine debris were noted in the first published report on NMP in the ocean [93]. Since then, the presence of biofilms on the surfaces of plastics recovered from a range of environments has been reported and these plastic-associated communities are suggested by a number of studies to be ‘distinct; from the surrounding environment [94]–[98] and, in some cases, to include a high number of pathogenic species [99], [100]. Further, there are reports that plastics may be selective for enrichment of species that are resistant to antimicrobials [101]. This has raised concerns about the potential risks associated with exposure to the plastic-associated microbial communities if NMP enter the food chain. The influence of biofilm formation on the fate and behaviour of plastic debris has also been reported and there are suggestions that the presence of a biofilm can influence the fragmentation of plastics into NMP [102] and alter the buoyancy and transport of particles through aquatic ecosystems [103]–[105]. Furthermore, because complex biofilms can play a protective role, reducing the susceptibility of the attached microorganisms to mortality caused by changes in the physical environment such as pH fluctuation, ultraviolet radiation and temperature [106] or by exposure to antimicrobials [107] this could have important implications for increasing the risk of pathogens associated with the NMP being transported across greater distances. There is also evidence that biofilms can increase the potential uptake of plastics into food chains by releasing chemicals that stimulate feeding activity by

planktivorous species and, in turn, increasing the frequency of plastic ingestion by zooplankton and the organisms (for example, seabirds) that predate on them [108]–[110]. If organisms that are consumed by humans also predate on zooplankton that have ingested plastics this could provide a potential pathway for plastic particles and their associated microbial communities entering the human food chain. Understanding interactions between microorganisms and NMP is therefore important to understanding the potential health risks associated with exposure to NMP.

Following a title and abstract sift, 77 original peer-reviewed research papers were reviewed to address the following objectives:

- Identify the key factors governing colonisation of plastics (and NMP);
- Determine whether there is evidence for selection of pathogens on plastics;
- Assess evidence of anti-microbial resistant bacterial colonisation of microplastics and potential horizontal gene transfer
- To assess the influence of biofilm formation on the fate and behaviour of NMP.

7.2 Key Factors Governing Colonisation of Plastics (and NMP)

Biofilm formation is a highly regulated biological process which follows a series of stages including 1) the rapid acquisition of a thin film of organic molecules, known as the conditioning film, on the surface, 2) physical attachment of microorganisms to the surface, 3) the formation of microcolonies, 4) development into mature and complex biofilms and 5) dispersal from the surface [111, p.]. Development of a biofilm can be influenced by the physicochemical properties of both the substrate surface and the microorganism (such as hydrophobicity); the physical characteristics of a surface, such as roughness, hardness and surface charge [112] and the chemical and electrical signalling within and between cells [113], [114]; as well as by environmental factors such as pH, salinity, temperature and hydrodynamics [92], [113].

Investigations into the composition of bacterial communities on plastics collected from a range of environments are in general agreement that the bacterial phyla

Proteobacteria and Bacteroidetes form the main groups within plastic debris-colonising communities [96], [98], [115], [116] and several studies report enrichment of bacterial species involved in degradation of hydrocarbons [94], [115], [117]–[122]. However, there is less agreement regarding other bacterial phyla. For example, members of the Erythrobacteracea and Rhodobacteraceae families were reported to be enriched on PP and PVC samples in the Yellow Sea and South China Sea [116], whilst members of the bacterial phyla Planctomycetes, Chloroflexi and Cyanobacteria were more abundant on plastic fragments collected in the North Adriatic Sea [115] and on mesoplastics (typically between 5 and 10 mm in at least one dimension) collected in the North Atlantic Gyre [120]. As plastics found in the environment are reported to be diverse in terms of their chemical composition, surface properties and particle size. The influence of these factors on microbial colonisation are considered.

7.2.1 Evidence for an influence of surface properties

Surface hydrophobicity, the degree to which a surface repels water, has been highlighted as a key property governing bacterial colonisation, particularly during the first stages of colonisation, with studies using bacterial isolates showing greater bacterial adhesion occurring on hydrophobic surfaces than hydrophilic ones [123]–[126]. In studies where substrates have been exposed to a mixture of species with varying substrate affinities and competitive abilities, surface hydrophobicity has also been observed to influence bacterial community structure [127]. In their study, Ogonowski *et al.* [127] did not observe any correlation between substrate hydrophobicity and area-specific bacterial abundance, when they compared bacterial community assemblages on five substrates (PE, PP, PS, as well as cellulose particles and inert glass beads) exposed to ambient Baltic bacterioplankton. However, they did observe a correlation between substrate hydrophobicity and bacterial composition suggesting that the differences in surface hydrophobicity resulted in species sorting due to differences in the adhesive capacity of bacteria [127]. Similarly, Xu *et al.* [116] observed that the bacterial community structures of biofilms diversified with an increase in exposure time due to changes in surface hydrophobicity over time [116]. These latter studies highlight the importance of using complex communities containing mixture of species, rather than bacterial isolates

with uniform cell size and adherence patterns, to understand the factors that may influence biofilm formation in environmental matrices.

Surface roughness has also been shown to influence bacterial adhesion to surfaces through influencing surface hydrophobicity. Gong *et al.* [128] demonstrated that low-density PE (LDPE) NMP pre-treated with UV-irradiation (to increase surface roughness) were less hydrophobic than untreated LDPE NMP and more susceptible to microbial adhesion and biofilm formation. These findings were consistent with an earlier study in which Hossain *et al.* [129] compared colonisation dynamics on weathered LDPE and PP and non-weathered high-density PE (HDPE), LDPE, PP and PS incubated in lake water microcosms inoculated with *Acinetobacter calcoaceticus*, *Burkholderia cepacia*, and *Escherichia coli.* Following an 8-week incubation, bacterial abundance was observed to be higher on the pre-weathered plastics. This was attributed to the increased surface roughness (and less hydrophobic surfaces) of the weathered plastics at the start of the exposure, compared to the non-weathered plastics. The increased tendency for biofilms to form on rough surfaces, even where the process of increasing the roughness of the surface has reduced the material's surface hydrophobicity, indicates that surface roughness may have a more important role in enhancing biofilm formation than hydrophobicity.

In contrast to the above studies, Cai *et al.* [112] found that surface hardness was a key factor influencing biofilm formation. They compared short-term (10-minute incubation) and long-term (4 and 16-hour incubation) adhesion of bacteria onto PE, PP, PET and PVC and found that more bacteria adhered to the softer PE and PVC surfaces than attached to the harder PP and PET. Surface charge (zeta potentials), hydrophobicity/hydrophilicity and roughness were determined to be comparable between the four different types of plastic and so were not considered to be dominant factors affecting bacterial adhesion of plastic surfaces. The surface hardness of PE and PVC, however, was far lower than that of either PP or PET. As PP and PET showed lower cell adhesion than PE and PVC, this led the authors to conclude that surface hardness was the key factor dominating the adhesion of bacteria onto the plastic surfaces with plastics with a low surface hardness being more favourable for bacterial adhesion than plastics with a high surface hardness.

Surface charge is also reported to be important in influencing biofilm formation. The combination of repulsive electrostatic forces and attractive van der Waals forces between cells and the settlement surface (as described by the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory of colloid stability [130], [131]) can limit attachment by microorganisms. Cells that can overcome this repulsive layer may come into direct contact with the surface and become attached. However, the ability of cells to do this depends on their shape as well as their ability to produce extracellular substances to disrupt the repulsive layer with cells with a smaller radius being better able to penetrate the repulsive layer while expending less energy. Roy *et al.* [132] used colloidal filtration and DLVO theory to compare bacterial attachment to PE and nylon fibres. They found that attachment of bacterial cells was higher for nylon than PE. The DLVO profile of the bacterial attachment on both fibres revealed that PE has a higher energy barrier, indicating that high energy barriers resist the deposition of bacteria. The free energy in a system also affects the attachment of microorganisms to surfaces [133], [134]. In general, surfaces with low surface energy are hydrophobic, whereas those with high surface energy are hydrophilic. If the overall free energy in a system is reduced when a cell contacts a surface it will usually remain attached. This means that surfaces that have low surface energy (hydrophobic surfaces such as plastics) may have a greater potential to adsorb microorganisms than those with high surface energy [133] where other, more dominant physicochemical factors (such as roughness) are not confounding factors.

Surface polarity, the degree to which a material's surface is made up of polar molecules, can also influence biofilm formation on plastics, with increased polarity resulting in increased primary colonisation of plastics by microorganisms. Dussud *et al.* [97] compared colonization of polyolefin-based plastics, including PE, PE with pro-oxidant (OXO), artificially aged OXO (AA-OXO), and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) incubated in aquariums with natural circulating seawater for up to 6 weeks. They observed that colonisation increased with increasing polarity (AA-OXO had the highest polarity and biofilm formation, followed by OXO and then PE with the lowest polarity and biofilm formation) for plastics with a similar roughness; colonisation was also higher for PHBV than for PE (PHBV is more polar and has a higher surface roughness than PE). As the biofilm matured, PE and OXO

eventually hosted a homogenous cluster, but the community structures on the two biodegradable polymers (AA-OXO and PHBV) continued to change over time.

7.2.2 Evidence for polymer type as an influence

Different polymer types possess unique surface properties which can influence biofilm formation. For example, PVC has been shown to be more susceptible to bacterial adhesion than PP [112] because the specific surface area of uneven PVC is larger than that of PP, which has a smooth surface and PVC has a lower bond energy (due to the chlorine atom which is a strong electron-withdrawing group) making it vulnerable to attack and breakage. Li *et al.* [135] compared bacterial community composition on five types of plastic debris deployed in situ, in the Haihe Estuary, for up to 6 weeks. They found that bacteria showed selectivity toward different polymer types with *Bacillus* & *Shewanella* being identified as marker genera for PVC, *Bdellovibrio* and *Lewinella* for PP, *Faecalibacterium* and *Veillonella* for PE, *Pseudoalteromonas* and *Alteromonas* for PS, and *Alcanivorax* for polyurethane (PU). Rosato *et al.* [136] also compared colonisation dynamics on pristine NMP (PE, PET, PS, PP and PVC) incubated in anaerobic microcosms containing marine sediment. They found that all NMP were rapidly colonised (within 2 weeks). The cell densities decreased depending on the plastic substrate with the highest cell density found on PVC followed by PE then PET then PP and PS with the lowest cell density. After 28 weeks, biomass concentration was an order of magnitude higher on PVC than other NMP. Differences in community richness and organisation were also observed between the different types of NMP; whilst biofilm communities on PET, PS and PP did not change over time, a reduction in community richness and an increase in community organisation was observed on PE indicating that a fraction of the biofilm community was able to become dominant over time, causing a loss of biodiversity. Conversely, an increase in community richness, with limited fluctuations in community organisation were observed on PVC indicating that an increase of biodiversity took place without affecting the functional organization of the community.

In contrast to the above studies, investigations comparing the bacterial composition of biofilms found on different types of plastic debris recovered from aquatic environments, do not report a significant influence of polymer type on bacterial community structure [95], [137]. In field studies, an influence of substrate specificity

on biofilm composition may be masked because the history and age of the collected material is unknown. However, Xu *et al.* [116] also found no evidence for an influence of polymer type on the bacterial diversity of biofilms formed on PP and PVC deployed for up to 12 months in China's offshore waters suggesting that species sorting may only be detectable during the early stages of colonization [138].

7.2.3 Evidence for an influence of particle size

Zhang *et al.* [139] found that the bacterial communities colonising NMP recovered from a cotton field were significantly different in structure from those in the surrounding soil, plant litter and macroplastics. The NMP enriched the bacterial groups involved in their own biodegradation and keystone species found on the NMP included *Acidobacteria*, *Chloroflexi*, *Gemmatimonadetes* and *Bacteroidetes*. As the particle size of the NMP was much smaller than the plant litter or larger plastic residues the increased specific surface area of the NMP in contact with the surrounding soil may have allowed the NMP to support more complex interactions between bacterial groups than would occur on macroplastics or plant litter. The larger specific area of NMP may also release larger amounts of additive small molecular compounds that may contribute to niche sharing between active taxa in bacterial communities [140]. However, as noted by the authors, a second explanation may be that because NMP remain in the soil longer than macroplastics or plant litter there is more time for mature, complex biofilms to form relative to other substrates. Differences in microbial community structure (including eukaryotes, bacteria and archaea), have also been reported for micro and macroplastics collected from marine environments [94], [120]. However, because the age and history of the micro and macroplastics are unknown it is possible that this was a consequence of duration of exposure, rather than size.

For smaller plastic particles, within the size range of 0.3-5 mm, no effect of size on the diversity of the colonising bacterial communities was observed for NMP collected from the Bay of Brest [141]. Similarly, in a controlled exposure, Gong *et al.* [128] did not observe any difference in community richness and diversity between 1000 and 5000 nm LDPE particles exposed to lake water for up to 8-weeks. Studies comparing colonisation of smaller particles (within the NMP range) however, appear to be lacking.

7.2.4 Environmental Influences

The composition of plastic-associated communities is reported to vary between geographical locations and between seasons due to variations in ambient water conditions across space and time[98], [105], [116], [118], [119], [122], [142]–[145]. Exposure of plastic debris in the Haihe Estuary revealed that nutrients (total nitrogen and total phosphorus) and salinity were the main factors influencing biofilm growth, while salinity was the primary factor affecting bacterial diversity [135]. In a freshwater lake, differences in the biofilm growth rate and presence of distinct algae compositions within the biofilm between different seasons, were attributed to differences in temperature, nutrient levels and suspended solids in the lake water [105]. In the lake study, biofilm formed more rapidly on the surface of PP plastics during spring and summer (3 days) compared to autumn (6 days) and winter (9 days). These effects were attributed to higher temperatures, elevated nutrient levels, and long illumination durations in the summer months.

Oberbeckmann *et al.* [122] demonstrated that nutrient levels and salinity are also important for determining substrate specific influences on the structure of bacterial communities. They compared bacterial communities on HDPE, PS and wooden pellets exposed for 2 weeks at 7 different stations in the coastal Baltic Sea, in the estuary of a river and in the effluent basins of a waste-water treatment plant (WWTP). Corresponding water communities (free-living and attached to naturally occurring particulate material) were also sampled. NMP-specific assemblages were identified but the degree of specificity depended on the ambient environmental conditions. In areas with lower nutrient concentrations (and high salinity) there was a clear differentiation between plastic, wood and particle-attached water communities and significant differences between the HDPE and PS-assemblages. At stations with higher nutrient concentrations no major differences were detected between the communities associated with different substrates. These effects were attributed to the influence of nutrient concentrations on the acquisition of a conditioning film; the more nutrients that are available, the quicker a conditioning film and primary biofilm can develop and the faster a less substrate specific biofilm can establish. Consideration of ambient water quality is therefore essential to understanding dynamics of colonisation and composition of the biofilm.

7.3 Evidence for selection of pathogens on plastics

In 2013, Zettler *et al.* [94] reported that *Vibrio* spp. dominated the plastisphere community on a PP sample collected from the North Atlantic, constituting nearly 24% of bacterial operational taxonomic units (OTU). Since then, members of the genus *Vibrio* have also been found on plastic debris at a Scottish beach [146], on marine plastics from the Belgian North Sea [96], on microbeads from the China coastline [116] and Baltic Sea [147] and on 13% of NMP particles found close to the coast, and occasionally offshore, in the North Sea [148]. Members of the genus *Vibrio* and *Pseudoalteromonas* were found on plastic fragments recovered from The Bay of Brest in France [141], Haihe Estuary [135] and Sungo Bay in China [149]. Both *Vibrio* spp. and *Escherichia coli* were found on nurdles (plastic pellets roughly 5 mm in diameter used in the production of plastic products) in the Forth Estuary, Scotland [100] and on plastic fragments recovered from Guanabara Bay, Brazil [150]. The bacterial fish pathogen *Aeromonas salmonicida* was detected on the surface of NMP fragments collected from the North Adriatic Sea [115]. *Arcobacter* spp were found on plastic fragments in sediments from the Humber Estuary, UK [151]. Pathogenic taxa (including *Comamonas, Agrobacterium, Brevundimonas, Acinetobacter, Sphingobacterium, Wautersiella, Chryseobacterium, Flavobacterium, Bacillus*, and *Clostridium*) have also been found on the surfaces of NMP recovered from lake water [128]. These reports have raised concerns regarding the potential for plastic particles to act as a vector for transporting harmful pathogens within and between environmental compartments.

In other studies, whilst potentially pathogenic bacteria have been found on NMP, the prevalence has been considerably lower. For example, in [98] Jiang *et al.* identified bacterial taxa associated with human and animal pathogens on NMP recovered from intertidal locations around the Yangtze Estuary in China. They found abundances were low with *Pseudomonas* spp comprising <0.01%. *Vibrio* spp. comprised <0.4% and were only found on plastics collected from one location (Xiangshan Bay). Similarly, Debroas *et al.* [120] only detected 1 OTU, representing 0.14% of the total reads, belonging to the genus Vibrio on plastics collected from the North Atlantic

Gyre, supporting earlier investigations in which Harrison *et al.* [151] and Bryant *et al.* [117] did not detect *Vibrio* spp. on the surface of plastics in coastal sediments.

Most studies reporting the presence of pathogenic species on plastic debris, however, do not include non-plastic comparators which makes it difficult to determine whether the surfaces of plastics are more likely to harbour pathogenic species than other surfaces. For example, enrichment of *Vibrio* spp. in aquatic environments has been reported to be common on wooden debris, plants and other natural surfaces [152]. In the few studies where non-plastic comparators have been included, there is evidence that plastics are more likely to enrich potential pathogens than other surfaces in some studies, but not others. For example, Oberbeckmann *et al.* [122] investigated the abundance of potential pathogens on PE and PS NMP incubated for 2 weeks in a range of environmental conditions, from freshwater (waste-water treatment plant) through to marine (coastal Baltic Sea). The genus *Vibrio* (*Vibrionaceae*) was found in the plastic-attached communities, but higher abundances were found in both the wood and water associated communities. Similarly, members of the family *Enterobacteriaceae* were detected on PE and PS at two locations with the WWTP basin but relative abundances (<0.5%) were low in relation to levels in the associated water communities. In contrast, McCormick *et al.* [118] found that sequences representing the genus *Arcobacter* were significantly more abundant on NMP than organic material. Similarly, Wu *et al.* [137] found a higher abundance of pathogenic bacterial families, such as *Pseudomonadaceae* and *Moraxellaceae*, on NMP collected from the Haihe Estuary compared to the surface water and sediments. Analysis of metabolic pathways, including pathogenic potential and stress tolerance indicated that the pathogenicity from NMP (0.43) was higher than that from surface water (0.21) and sediment (0.37) further suggesting an accumulation of pathogenic bacteria on NMP.

7.4 Antimicrobial resistant bacterial colonisation of microplastics and potential horizontal gene transfer

Twelve papers were identified during the title and abstract sift that specifically dealt with AMR colonisation, selection for AMR or changes in gene transfer frequency. Of

these studies, most simply reported colonisation of NMP by AMR bacteria often with no non-NMP comparator (for example, naturally occurring particles within the same size range as NMP), making conclusions regarding the significance of the studies difficult to ascertain given that AMR bacteria are ubiquitous in all environments including ancient permafrost, isolated cave systems and polar regions. This section of the review does not go into the detail of individual AMR phenotypes or genotypes reported as they are diverse and numerous, rather the focus is on evidence for colonisation of plastics versus other substrates by AMR bacteria and or selection or HGT of resistance genes.

7.4.1 Studies focusing on colonisation of NMP by AMR bacteria

Zhang *et al.* [101] investigated the enrichment of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARG) on the surfaces of NMP in a mariculture system in China. Most NMP were PET (75% of collected NMP) which carried 6.40 x 10^6 - 2.48 x 10^8 cultivable ARB, which was between 100 and 5000 times the abundance found in water samples. 16S rRNA amplicon sequencing demonstrated these bacteria belonged to *Vibrio, Muricauda* and *Ruegeria* genera illustrating resistance to several antibiotic classes. The study concluded that NMP are hazardous pollutants for the enrichment of ARB, however as no comparator substrates were analysed it is difficult to determine whether NMP are any more hazardous than any other particle in water. It is well known that, for example, sediments are associated with a bacterial load several orders of magnitude greater and any particle, natural or manmade, is likely to be colonised by bacteria including ARBs.

A study of marine plastics in the North Pacific Gyre found that bacterial diversity and diversity of ARGs and metal resistance genes (MRG) were greater on plastics than in water samples [153]. There were no significant differences in the abundance or diversity of ARGs and MRGs between macroplastics and NMP. Statistical analyses suggested bacterial community composition was the determining factor of the ARG but not MRG profiles. This study did not have a non-plastic substrate comparator and did not compare plastic associated microbiomes with water column communities

collected at the same location. A separate study of NMP in Antarctic land litter [154] found AMR bacteria colonising plastics, but no comparator substrates were analysed. A further study in Chinese aquaculture systems [155] found that NMP had higher bacterial densities than associated water samples and that NMP had distinct bacterial and ARG signatures compared to water.

Whilst most studies do not compare AMR on NMP to AMR on non-plastic surfaces, one study by Wu *et al.* [156] compared colonisation of PVC micro pellets, quartz particles and leaves. All substrates were washed and air dried, prior to incubation in river water collected from Northern China. Results showed bacterial biomass decreased to the greatest extent on leaves, followed by NMP and the least bacterial biomass was found on quartz. Substrates were colonised by distinct bacterial communities, with greater similarity observed between NMP and quartz compared to leaf biofilm. Of the 515 OTUs (roughly equivalent to species) found within biofilm communities, 14 were specific to NMP and the authors suggest this is evidence of a "plastisphere" supporting several other studies comparing colonisation of different substrates. This is an interesting finding although to date the numbers of studies characterising biofilms on multiple substrates is relatively small, so it may be too early to conclude that there is a NMP specific microbiome. ARG diversity mirrored bacterial diversity with greatest diversity on leaves, followed by NMP and the lowest diversity on quartz. ARG abundance in biofilms was 3 times greater than in river water bacteria suggesting that ARGs were enriched in biofilms. The NMP biofilm possessed a unique ARG profile with some genes only found on NMP associated bacteria. Opportunistic pathogenic pseudomonads were found on NMP and it is well known that these organisms carry resistance determinants and are able to withstand xenobiotic stress.

A final study of NMP in farmland soils in China found that larger more highly weathered NMP adsorbed more antibiotics and heavy metals and had higher prevalence of mobile genetic elements (MGE) associated with AMR [157]. Increased duration of vegetable production was also associated with increased concentration of antibiotics and MRGs on the surface of NMP. No comparisons with bulk soil or other substrates were done so it is not possible to conclude anything regarding the significance of NMP other than some variation in diversity and AMR was observed

with size and weathering which may be a function of bacterial density. There was no causal link between antibiotic concentrations sorbed to NMP and ARGs determined.

7.4.2 Selection for AMR on NMP

A small number of papers investigated the role of NMP in selection for AMR. The hypothesis being that NMP can adsorb micro-pollutants, including antimicrobials, therefore providing a substrate that may increase selection pressure for AMR. Ma *et al.* [158] investigated the effect of tetracycline and NMP on selection in the annelid worm *Enchytraeus crypticus* microbiome. An experiment was performed using tetracycline at 10mg L^{-1} to which sterilised oats were added with or without nanoscale PS at 1000mg kg^{-1}. The paper concluded that exposure to PS and tetracycline increased ARG diversity and abundance in the *E. crypticus* microbiome. However, the data appear to suggest that 14 days after the NMP-tetracycline exposure was removed the differences between tetracycline and tetracycline-NMP treatments was minimal. NMP themselves appeared to have some selective effect at seven days, but this was less marked at later time points. In addition, the concentrations of tetracycline used are relatively high, towards the maximum found in animal faeces and amended soil, so the applicability to lower residue concentrations found in most environments is unclear. Perhaps the most interesting finding was the fact that NMP themselves appeared to have some selective effect for AMR although this may be species sorting rather than *de novo* selection for resistance (i.e. AMR may be associated with bacteria more able to colonise NMP rather than selecting for AMR itself).

A further recent study published in 2020 by Wang *et al.* [159] investigated adsorption of antibiotics on NMP (polyethylene) in river, estuarine and marine water. Results indicated that NMP can enrich antibiotics, ARGs and microbes from the surrounding water. There was less adsorption of antibiotics with increasing salinity. Microbial diversity and AMR varied with salinity and antibiotic treatment. Concentration of antibiotics used was 20 µg L^{-1} which is greater than generally found in aquatic environmental compartments but is in the same order of magnitude as concentrations present in heavily contaminated wastewater effluents [160] and significantly below concentrations present in faecal waste [161]. Significant adsorption of sulfamerazine was observed from 12.13 µg g^{-1} in marine water to 37.55

μg g^{-1} in freshwater. Adsorption of tetracycline, chloramphenicol and tylosin was much lower with only chloramphenicol showing any differences with salinity. Antibiotics were added to the microcosms weekly throughout the experiment so any attached bacteria would have been subject to selection from dissolved as well as adsorbed antibiotic. Diversity and salinity appeared to be linked, with greater similarity between treatments at each salinity than by treatment across salinities. Selection for ARGs was greatest in river water, particularly for sulphonamide and tetracycline with little or no effect on specific gene enrichment by tylosin. When water and NMP ARG prevalence was compared, prevalence was lower on NMP than in the water column in all cases. In summary this paper appears to suggest a strong salinity effect on diversity and on sorption of some antibiotics and that community structure varies between attached and pelagic communities. There was no non-NMP control so whether these trends are specific for NMP relative to other substrates is not known. However, the overall findings did not demonstrate any greater selection in NMP associated AMR bacteria than in water column bacteria.

7.4.3 Increased horizontal gene transfer (HGT) on NMP

In addition to selection for AMR, it is well known that some stressors can increase the rate of HGT, which may increase transfer of ARGs. Arias-Andres *et al.* [140] quantified gene transfer rates of a green-fluorescent tagged broad-host range plasmid encoding trimethoprim resistance. They undertook a single species experiment *in vitro* in the presence and absence of PS 4mm^2 particles. They used communities from lake water and plastics colonised in lake water *in situ* before collection and plasmid transfer analysis *in vitro*. Communities from lake water and natural aggregates were also analysed for plasmid transfer frequency i.e. their ability to take up the resistance plasmid. In the single species experiment, transfer frequency was several orders of magnitude greater in cells on NMP compared to in the water column. This might be expected due to greater density and increased HGT that has been reported in biofilms and/or dense populations where cell-cell contact is more likely to occur. It was also observed that organic matter adsorption to NMP also increased plasmid transfer frequencies as has been observed for natural aggregates. In the second experiment with natural communities from NMP, water, and aggregates from a lake, the NMP associated communities consistently showed

greater transfer frequencies. These experiments used high bacterial donor frequencies but standardise recipient cell density and matrix controlling for these variables. Transconjugants (cells that acquired the plasmid) were diverse although dominated numerically by *Rheinheimera* (*Gammaproteobacteria*) and *Arthrobacter* (*Actinobacteria*) demonstrating transfer from *E. coli* to distantly related taxa.

7.5 Influence of biofilm formation on the fate and behaviour of NMP

Formation of biofilms on the surfaces of plastics may also be important for influencing their fate and behaviour in natural environments and so influence their persistence and potential to transport any harmful pathogens they may harbour. It is well documented that the formation of biofilms can influence the fate and degradation of plastics by accelerating the degradation process [94], [162]–[165]. However, biofilms can also increase the persistence of plastics through protecting them from ultraviolet radiation and photocatalysis either directly by 'shielding' the plastic from the light [166] or by decreasing their buoyancy resulting in sinking [142], [167]. Kooi *et al.* [104] modelled the influence of biofilm formation on the settling/rising of NMP particles in seawater. Five different polymer types were simulated; HDPE, LDPE, and PP which have a lower density than seawater and are initially buoyant plastics; and PVC and PS which are non-buoyant. The effects of size (0.1 µm to 10 mm) were also considered for PP, LDPE, and HDPE particles. Their model predicted that all particles could settle due to biofouling, irrespective of polymer type/initial buoyancy, but that particle size influenced the time the particles took to settle with larger particles starting to settle last. Because of this late settling onset, larger particles remain at the ocean surface longer than smaller particles. Also, larger particles, such as 10 mm and 1 mm particles, were predicted to oscillate at a fast rate and resurface with each oscillation. In contrast, smaller particles (≤ 10 µm) were not predicted to resurface. Over time, this would result in a size selective removal of smaller particles from the surface, but because they sink so slowly NMP could reside anywhere in the water column. These predictions supported an earlier report in which examination of the size distribution of plastic debris collected from ocean surfaces around the world showed a peak in abundance of fragments around 2 mm and a pronounced gap of fragments below 1 mm in size [48].

Kaiser *et al.* [103] compared the influence of biofouling on the sinking behaviour of PS (non-buoyant) and PE (buoyant) NMP incubated in estuarine and coastal waters. They observed that the sinking velocities of PS increased by 16% in estuarine water (salinity 9.8 ppt) and 81% in marine water (salinity 36 ppt) after 6 weeks of incubation. After this time, the sinking velocities decreased due to lower water temperatures and reduced light availability. However, biofouling did not cause PE to sink during the 14 weeks of incubation in estuarine water. In coastal water, PE started to sink after six weeks incubation due to colonisation by blue mussels *Mytilus edulis* which increased the weight of the particles and the sinking velocity continued to increase until the end of the incubation period. These observations led the authors to conclude that the development of a microscopic biofilm alone is not enough to cause sinking of buoyant NMP particles, and that attachment of fouling macro-organisms is also necessary to transport buoyant NMP through the water column.

The influence of biofilm formation on the behaviour of plastics in freshwater environments has received considerably less attention. However, in a recent study, Chen *et al.* [105] demonstrated an influence of biofilm formation on plastic buoyancy in freshwater lakes. Influence of the biofilm was observed to be seasonal and all buoyant plastics lost buoyancy during the summer but only a small portion lost buoyancy in other seasons. These effects of season were associated with differences in development and algal composition of the biofilm in difference seasons and results suggest that buoyant plastics will remain in the water column for longer periods during cold seasons.

Aggregation of NMP can also play an important role in the vertical transport of NMP from the water's surface to sediment. Lagarde *et al.* [168] demonstrated that interaction of PP and HDPE NMP with freshwater microalgae resulted in rapid colonisation of the NMP by the microalgae but differences were observed in the type of long-term colonization. For PP, some hetero-aggregates appeared in samples after 20 days of contact and their size increased throughout the experiment due to an increase of the adhesion of PP fragments between one another. The hetero-aggregates composition was estimated to comprise approximately 50% of PP fragments and 50% of microalgae, which led to a final density close to 1.2. For HDPE, the adhesion phenomenon appeared to be limited to the plastic surface and

did not lead to observable aggregation during the experiment. The authors attributed the different behaviours of the two polymers to differences in the types of extracellular polymeric substances (EPS) produced by the microalgae colonising the fragments, with the EPS on PP exhibiting more cohesive and sticking properties. Michels *et al.* [169] demonstrated that the aggregation of PS NMP with marine biogenic particles is also influenced by biofilm formation, with the presence of both a biofilm and biogenic particles causing pronounced aggregation. This suggests that NMP-associated biofilms could modify the vertical export of biogenic particles in the marine water column which could alter important biogeochemical processes and ecosystem services. Toxicity of NMP can also be influenced by agglomeration/aggregation behaviours of particles. Sendra *et al.* [170] observed that the toxicity of smaller (50 nm) PS particles to the marine diatom *Phaeodactylum tricornutum* was higher at 24 hours than the toxicity exerted by larger (100 nm) PS particles. However, after 72 hours, the 100 nm PS particles were more toxic than the 50 nm particles. This was attributed to the formation of a biofilm which caused agglomeration/aggregation of the smaller particles and so decreased their bioavailability.

Biofilm formation has also been suggested to alter the palatability/attractiveness of plastics to organisms. Biofilms emit infochemicals such as dimethyl sulphide (DMS) during phytoplankton grazing by zooplankton that can serve as a foraging cue for many pelagic marine organisms and could potentially increase likelihood of ingestion. Indeed, Procter *et al.* [110] recently demonstrated that infusion of NMP in a DMS solution acted as a stimulus for grazing by copepod *Calanus helgolandicus.* Grazing rates increased 0.7 to 3-fold compared to DMS-free controls. This supports an earlier investigation in which ingestion of pristine and aged 15 µm PS beads was compared in two species of copepods, *C. finmarchicus* and *A. longiremis*, [171]. The aged NMP were ingested by more individuals and at higher rates than pristine NMP for both species. Plastics have been shown to develop biofilms that emit a DMS signature [109]. Savoca *et al.* [109] analysed plastic ingestion data from 55 studies that sampled a total of 13,350 individuals among 25 procellariiform species of seabirds and found a positive relationship between DMS responsiveness and plastic ingestion frequency. DMS-responsive species were found to ingest plastic five times more frequently than non-DMS responsive species. As other marine organisms,

including those that are consumed by humans, may be lured to ingest plastic debris that have acquired a biofilm this suggests a need for future research that examines feeding cues associated with plastic ingestion.

Furthermore, the formation of the biofilm may attract organisms that play an important role in fragmentation of plastic debris to NMP. For example, detritivores, such as amphipods, ingest and shred natural organic matter and are fundamental to its breakdown in natural environments. Shredding of plastic carrier bags by the amphipod, *Orchestia gammarellus* has been shown in the laboratory and it is estimated that an entire bag could be shredded into approximately 1.75 million NMP pieces (average diameter 488.59 µm) by *O. gammarellus* [102]. Presence of a biofilm on the surfaces of plastic carrier bags increased the amount of shredding 4-fold, resulting in an average of 8.23 fragments per amphipod per day suggesting that *O. gammarellus* may be attracted by the presence of a biofilm which could be acting as a feeding cue.

7.6 Summary and further work

There is clear evidence that plastic debris are rapidly colonised by a diverse array of microorganisms, especially bacteria, once it enters the environment. Differences in the physico-chemical properties of different polymer types does appear to have an influence on early stages of colonisation. However, these appear to primarily result from differences in the surface properties of the plastics, especially hydrophobicity. As the biofilm matures, the influence of polymer type is less apparent. Evidence to date suggests that the surrounding environment exerts the greater influence in determining the structure of the biofilm community with nutrients and salinity being the main factors influencing biofilm growth and bacterial diversity.

There is some evidence that plastics-associated communities are distinct from those found on other substrates and that they may even harbour higher abundances of pathogenic organisms. However, because very few studies conducted to date have included non-plastic comparators caution should be taken in concluding that plastics pose a greater risk in this regard than other substrates. Future research is required employing micro- and nano-scale particles of multiple substrate types, including non-plastics, deployed in a broader range of environments and seasons than have

already been studied. These studies should also investigate whether any pathogens identified on the surfaces of the NMP are tightly attached to the surfaces [148], indicating a substrate specific preference for plastics or whether they are secondary colonisers that are simply hitching a ride on the surfaces of the plastics. Investigations into how the transport of NMP through different environmental compartments influences the structure and composition of microbial communities is also required to determine whether NMP could act as vectors to transport pathogens and whether the plastic-associated microbial communities protect pathogenic species.

The small amount of literature identified suggests NMP are colonised by antibiotic resistant bacteria (ARB) and that concentrations are higher than in the water column. However, biofilms commonly contain bacteria at higher densities than the surrounding water column regardless of whether they are ARB or not. Additionally, there is little evidence to suggest that NMP are any more likely to be colonised by ARB than other particle substrates. Several studies suggested that bacterial communities and AMR are different in NMP associated biofilms than on other substrates or in the water column although the available data for multiple substrates within single studies are limited. This is not unexpected as all substrate niches have distinct characteristics that will favour specific bacterial taxa. Evidence of preferential colonisation of NMP by opportunistic pathogens known to possess ARGs in their genomes was suggested by one study in the absence of antibiotics. The same study tested whether sorption of antibiotics to NMP would increase colonisation by, or selection for AMR. However, the evidence for the role of sorbed antibiotics on NMP was limited [159]. Where evidence for increased AMR associated with NMP does exist [158], the phenomenon appeared to be transient and may have been the result of differential colonisation rather than selection *per se*. One study identified increased plasmid transfer of trimethoprim resistance in bacterial communities present on NMP vs natural aggregates and also that HGT was greater in single species experiments in the presence of NMP. However, the latter may be attributed to increased density and opportunity for HGT, whereas the complex community study controlled for recipient cell density and substrate by conducting experiments on harvested biofilms under standardised filter mating conditions.

Future research should move on from simply characterising AMR associated with NMP. Analyses of AMR associated with NMP using well designed experimental approaches are required, testing the effects of different NMP and non-MP substrates on colonisation. Further experimental work is required to determine if NMP themselves, or sorbed antimicrobials, exert a selective effect for AMR through species sorting (differential colonisation) or *de novo* acquisition of AMR through mutation or HGT. Quantitative approaches are required to determine the contribution of NMP to total AMR loads in aquatic systems as well as their role in transmission of AMR to humans through direct ingestion or through the food chain. Significant volumes of water and associated particles are ingested during environmental exposures such as recreational use of bathing waters [172] and ingestion may also occur through internal or external contamination of food, including plants and animals as previously discussed. Filter feeding bivalves may pose a particular risk of food borne ingestion due to their capacity to concentrate particles, including plastics [173], from large volumes of water.

There is some, albeit limited, evidence that biofilms may also play an important role in attracting shredding organisms which could accelerate the fragmentation of larger plastic debris into NMP and so significantly increase the number of NMP in our waterways. Given that there is evidence that the biofilms may also release of volatile compounds such as DMS that may attract marine grazers that feed on the biofilm, and their predators, biofilms could play a significant role in influencing accidental ingestion of NMP. Future research should focus on furthering our understanding of the role biofilms play in influencing the attractiveness of plastics to a range of organisms to better inform on the risks of NMP entering human food chains via this route. Further, because biofilms can influence aggregation and sinking processes, they could also potentially increase the risk of organisms living in deeper ocean areas of becoming exposed to NMP and even potentially being infected by any harmful pathogens present on the NMP.

While some work has been conducted to investigate microbial eukaryotes on NMP [120], the majority of studies reviewed here solely investigated bacteria. Given that human pathogens are not limited to bacteria, future studies should also be conducted to investigate the role of NMP in the transport of eukaryotes and viruses.

8. Pathways of colonised NMP into the human food chain

8.1 Introduction

Plastic pollution has become one of the key global environmental issues. Global plastic production has reached more than 350 million tonnes annually [174] and over the last 60 years, more than 8300 million tonnes of plastic have been produced. Of that, 79% is estimated to have been landfilled, or lost to the environment [71]. The rapid expansion of global plastics use has happened due to a combination of low cost and weight, along with high durability and diversity of application due to the development of a myriad of polymers, all with differing properties. The low production cost and high durability of plastics have created a system where plastics are rapidly discarded but could potentially endure for centuries, especially in areas with low levels of light and oxygen like the ocean water column [76]. Actual lifespans of plastics are hard to determine as they have only been with us for 60 years.

While the chemical structures of plastics are highly durable, their larger structures can rapidly fragment due to ultraviolet (UV) radiation, physical stress and abrasion, or biological interactions, down to microscopic levels [175]. Plastics smaller than 5 mm are known as microplastics and those smaller than 100 nm are classed as nanoplastics [176], although exact size limits differ between publications. Such microscopic fragments can, for example, originate from degrading litter items, car tyres wearing down, synthetic clothing in the washing machine or flaking paints [177]. Besides plastics fragmenting down to microscopic levels, there are also plastic particles of that size that were specifically designed for use in for example personal hygiene and cleaning products or as pre-production pellets in manufacturing. These particles are known as primary microplastics, while those originating from larger items are secondary microplastics [177]. Irrespective of their origin, once microplastics have escaped into the environment, removing them becomes extremely difficult as they become integrated in soils, sediments, the particulate matter of water columns and are ingested by biota.

Research into microplastics has expanded rapidly in recent years and they have been found globally in the atmosphere of major cities and remote mountain ranges [27], in beaches, farmlands, remote conservation lands [28], [29], in freshwater in the world's major rivers and lakes [30] and in the ocean from the tropics to the poles and down into the deepest trenches [31]–[33]. As microplastics entered all habitats on earth, organisms started interacting with them. Microplastics in soils can be taken up by plants and end up in their tissues, including edible fruits, leaves and roots [34]. Animals can ingest microplastics because they mistake them for normal food particles, inadvertently trap them as they filter particles or actively select them besides their normal food sources. Microplastics have been found in the gastro-intestinal tract and tissues of many species [36]. Effects of microplastics ingestion on organism health and fitness are hard to determine at concentrations which they encounter naturally and evidence remains fragmented [37]. An additional effect which has received much attention is the ability of microplastics to transport sorbed toxic chemicals into animal tissues. However, estimations of natural microplastic abundances and their sorption capacity compared to naturally occurring particles, indicate that this forms a low health risk [15]. More information is required on the sorption capacities of NMPs especially in relation to concentrations that may be found in the environment as opposed to elevated concentrations used in some lab studies.

As microplastics are universally found, they are also part of our daily lives. Recent research has found that microplastics are present in food and water we ingest and the air we inhale [178]. These findings have led to abundant news items and general public concern, but reviews on the various pathways by which microplastics reach us are rare and research is developing rapidly. The size of particles is also important. Evidence suggests that ingested particles above 500 µm would not be capable of translocation through the gut wall. Transport into lymph and portal veins might be possible for particles of around 100 µm, whilst NMP would have to be 20 µm or smaller to move into organs [179].

It is, therefore, the aim of this review to describe the current published knowledge of all routes through which humans can take NMP into their bodies.

The articles from the reviewing process were assessed and allocated to categories according to the type of pathway that they represented. The categories developed purely from the papers that were returned, with no previous agenda. These are given the following nominal titles:

- Airborne
- Terrestrial
- Drinking water
- Shellfish
- Salt
- Fish
- Other Foods

Each category has been dealt with separately as they represent different pathways into the human food chain with different levels and types of research. Fish and Shellfish represent the largest categories in terms of number of published articles.

8.2 Pathways of airborne NMP into food chain

While human exposure to NMP from food and drink has been documented in several studies, very little is known about atmospheric pathways. Only twelve papers on airborne pathways were reviewed here. Studies can be classified between uptake from airborne vectors only [180] and the combined pathways through food, water and air [178]. Results indicated that the quantity of particles ingested via inhalation was as important as the quantity consumed via diet. Estimates of quantities of microplastics ingested from airborne sources varied greatly between studies highlighting the diversity of calculation approaches used. Table 8.1 presents a summary of the data on airborne NMP. Two main mechanisms have been identified for the human exposure of airborne NMP, namely direct ingestion and inhalation [181].

8.2.1 Deposition on food and ingestion

NMP deposited from the air have been found in the food chain, although only one study on the subject was found in this review. The study by Catarino *et al.* [182]

found low levels of microplastics in wild mussels for human consumption with an estimated consumption of 123 particles per year per capita in the UK representing a consumption up to 4620 particles per year per capita in countries with a higher shellfish consumption. However, this is relatively small compared with the estimated 13,731 to 68,415 particles per year per capita uptake of microplastics from food exposed to household dust fallout [182].

8.2.2 Inhalation of particles

Cox *et al.* [178] estimated the human consumption of microplastics focusing on the American diet involving the total consumption of microplastics for a diverse range of food sources (for example, seafood, sugar, honey, salt, alcohol, bottled water, tap water) and air. Intake from air, bottled water, and seafood accounted for main proportion of microplastic uptake by consumers. However, the high variation in reported atmospheric concentration of microplastics from the literature (Table 8.1) led to large variations in estimating uptake via inhalation. Daily inhalation of microplastics was generally comparable to the daily amount consumed indicating that inhalation is as an important vector of uptake as diet. Annual microplastics consumption was estimated to range from 39,000 to 52,000 particles, which is remarkably similar to estimates for inhalation of 35,000 to 62,000 particles annually [178].

Table 8.4 Reported atmospheric concentration of microplastics used to estimate uptake via inhalation.

Country	Environmental compartment	Rate of items	Particle concentration	Number of samples	Method	Particle type	Polymer type	Size (mm)	Ref.
Denmark	Apartment air	ns	1.7–16.2 m^{-3}	3	FPA-mFTIR-Imaging	Synthetic fragments and fibres	PES, PE, NY	<11	[180]
France	Indoor air	1586 – 11,130 items day m^{-2}	1.0–60 m^{-3}, 0.19–0.67 g	3 sites	ATR-FTIR and visual assessment	Synthetic (33%) and natural fibres (67%)	PP	50–3250	[183]
France	Outdoor air	ns	0.3–1.5 m^{-3} mean: 0.9 m^{-3}	1 site	ATR-FTIR and visual assessment	ns	ns	50-1650	[183]
France	Outdoor air (remote)	365 items day m^{-2}	ns	ns	Visual microscopy inspection and micro-Raman analysis	Fibre, film and fragment	PS, PE, PP, PVC, PET	<25-2600	[184]
Iran	Deposited urban dust	ns	88–605 per 30g dry dust	ns	Visual characterisation	Black and yellow granules	ns	250-500	[181]
Iran	Street and suspended dust samples	ns	1150 per 15 g	31	Visual characterisation	Micro rubbers and microplastics	ns	2–100	[185]
Turkey	Campus air	14.27 items g $L^{-1}m^{-3}$	ns	ns	ns	ns	ns	ns	[186]

Country	Environmental compartment	Rate of items	Particle concentration	Number of samples	Method	Particle type	Polymer type	Size (mm)	Ref.
Turkey	Bus terminal	23.95 items g $L^{-1}m^{-3}$	ns	ns	ns	ns	ns	ns	[186]

PES: polyester, PE: polyethylene, NY: nylon, PS; polystyrene, PET: polyethylene terephthalate, PVC: polyvinyl chloride, PP: polypropylene, ns: not specified

Gasperi *et al.* [187] investigated particle characteristics and their “breathability” with a differentiation between “inhalable” and “respirable”. Inhalable particles will enter the nose and mouth and deposit in the upper airway. “Inhaled” particles would reach and deposit in the deep lung. Other studies indicated that plastic fibres are durable and likely to persist in the lung [187], [188].

8.3 Pathways of terrestrial NMP into food chain

Similarly to airborne pathways, terrestrial pathways for NMP into the food chain are relatively understudied. A total of nine papers on terrestrial pathways were reviewed here. NMP are emitted to the terrestrial environment either by direct sources (for example, organic manure, sewage sludge, fertilizer) or from the degradation of bigger debris (for example, plastic mulching) where they can accumulate in high quantities. Zhang *et al.* [189] demonstrated that 72% of microplastics were associated with soil aggregates while 28% were being dispersed (i.e. not associated with soil aggregate fractions) suggesting high potential for particle mobilisation through irrigation and drainage [189]. It is therefore important to understand potential for migration and distribution of NMP from soils to food sources (for example, plant crops) with consequent impacts on human health.

Transfer of NMP from soils to crop plants have been demonstrated in a few studies [35], [190], [191]. Uptake, migration and distribution of NMP was demonstrated in wheat and an edible plant lettuce [190], [191]. Particle size was the main factor affecting uptake, with PS beads of 0.2 mm in size being more readily available than beads ranging from 1 to 7 mm in size. PS beads were transported from soils to plant with particles being present in roots, shoots and leaves of wheat [190]. PS beads (0.2 mm) were also transported from the roots to the stems and leaves via the apoplastic transport system, via the xylem where the presence of aggregates of PS beads was observed [191].

Lwanga *et al.* [192] also reported evidence for the transfer of plastic debris along a terrestrial food chain. They provided first-time evidence for microplastic transfer from soil to chickens. Microplastic concentrations increased from soil (0.87 ± 1.9 particles g^{-1}), to earthworm casts (14.8 ± 28.8 particles g^{-1}), to chicken faeces (129.8 ± 82.3 particles g^{-1}). Chicken gizzards contained 10.2 ± 13.8 microplastic particles, while no

microplastic was found in crops [193]. As no evidence of microplastic translocation is available, it is unclear whether NMP can be transferred to tissues. Micro and macroplastics were found in gizzards for human consumption that would represent a potential vector for the entry of microplastics in human diet. Gizzards are usually rinsed and cleaned before cooking removing the bigger proportions of solid particles and plastics. Published data about the type and abundance of NMP particles in soils and the country where the work was carried out are summarised in Table 8.2.

Table 8.5 Occurrence, abundance, and characteristics of microplastics in soils from the literature.

Continent	Country	Location	Abundance	Polymer type	Size (mm)	Ref.
Asia	Southern China	Dian Lake	7100 – 42,960 particles kg^{-1} (mean: 18,760)	Mostly fibres (92%), followed by fragments and films	95% between 1000 and 50	[189]
Central America	Campeche, SE Mexico	Home garden soil	870 ± 190 particles kg^{-1}	ns	~60% between 10 and 20 ~ 35% between 20-50 ~ 5% > 50	[193]
Oceania	Sydney, Australia	Industry areas	300 – 67,500 mg kg^{-1}	PE, PS, PVC	ns	[194]
Europe	Switzerland	Floodplain soils	5 mg kg^{-1}	ns	< 500 (diameter)	[195]
Europe	Southeast Germany	Agricultural farmland	Mean: 0.34 ± 0.36 particles kg^{-1} (0 – 1.25)	PE (62.5%), PP (25%), PS (12.5%)	> 2000	[196]

PE: polyethylene, PS; polystyrene, PP: polypropylene, ns: not specified

8.4 Pathways of NMP through drinking water

8.4.1 Sources of NMP in freshwater

In recent media coverage of microplastics in the human food chain, drinking water as a source of NMP has received major attention. Both bottled water and tap water have been researched, with microplastics found in both types of drinking water and their sources such as freshwater bodies and groundwater reservoirs. A first step in understanding the chain which brings these microplastics to human consumption is defining the sources of microplastics to freshwater.

A key source of microplastics to freshwater are WWTP. Although these facilities often remove more than 95% of microplastics from wastewater [197], a single WWTP can still be a source of tens of millions of particles per day [177]. Intense rainfall events may also overwhelm the handling capacity of facilities, causing direct discharge of wastewater to surface waters via overflows or misconnections, causing occasional spikes in microplastics' release to the freshwater environment. Residential wastewater is contaminated by microplastics from personal care products and estimates of personal use fall in the range of 7 grams per year per individual which adds up to 4130 tonnes per year for the European Union plus Switzerland and Norway [177]. Public awareness of this issue has driven campaigns in several countries for legislation on the use of microplastics in personal care products [198]. For example, the United Kingdom has banned the sale of products containing microbeads since 2018. The second major source of microplastics in residential wastewater are synthetic fibres released from laundry. A load of laundry can release millions of fibres dependent on the types of materials and machine, and age of clothing [199]. Mitigation methods such as filters, or mesh bags to trap fibres are available, but there is currently no organised approach to this source.

On roads, the wearing of car tyres and road paints can be substantial sources of microplastic release. If road run off is collected in sewers, it adds to the loads of wastewater treatment plants. Away from urban areas, road run-off can transport microplastics into soils or waterways directly [200]. There was also recent media attention regarding shredded tyres as part of artificial sport turf escaping into the

environment (for example, [201]), but there are estimates that the contribution of this source to surface waters is small compared to the wearing of car tyres on the road [202].

As part of plastics manufacturing, basic polymers are transported around the world as lentil-shaped pellets. These pellets can escape into the environment during transport, handling on site and storage and can comprise a significant fraction of the plastic contents of waterways [203].

In addition to these sources from which microplastics enter the environment, they can also originate from larger items of litter which enter the environment by individual littering, or due to poor waste management systems. Once in the environment, litter can fragment into microplastics due to physical stress and UV radiation, thereby adding to the microplastics load of freshwater systems [204].

See [177] for an in-depth review of sources of microplastics and estimates of their respective contributions.

8.4.2 Quantity of NMP in freshwater

Measurements of microplastics in freshwater have been carried out in many countries (Table 8.3) with concentrations found to range across 6 orders of magnitude. However, this is most likely due to use of different methodologies and specifically, size classes being investigated. Pivokonsky *et al.* [205] broke down their measurements of freshwater at inlets of drinking water plants into size classes and found that particles 1 - 10 µm comprised more than 90% of total particle number and particles > 100 µm comprised less than 2%. Koelmans *et al.* [206] reviewed the quality of published research into microplastic contamination of freshwater and drinking water and concluded that just 8% of studies scored positive on all quality criteria.

Table 8.6 NMP in freshwater (based in part on [206]).

Country	Location	Sampling device	Particles per litre	Particle characterisation method	Particle type	Polymer type	Size class	Ref.
USA	Great Lake tributaries	Neuston net	0.05-32x10^{-3}	Visual inspection	Fibres, films, foams, fragments, pellets/beads	ns	>333 µm	[207]
USA	Lake Michigan	Neuston net	3.36-6.42x10^{-3}	Visual inspection and Pyrolysis-GCMS	Foam, film, fibre, fragment, pellet	PP, PS, PE	>333 µm	[208]
Italy	Lake Bolsena, Lake Chiusi	Manta trawl	0.82-4.41x10^{-3}	Nile red. UV-microscope. Subset of fibres verified with SEM	Fragments/spherules and fibres	ns	>300 µm	[209]
Vietnam	Saigon River fibres	Bulk sampling 300 mL	172-519	Microscopic inspection with image analysis software. 76 fibres (10%) were analysed by ATR FTIR.	Fibres	PET, PE, PP, PP, PS, PA, PVC, PE-PP copolymer, PP-vistalon, acrylic, polyepoxy, polyester, PE-ethyl acrylate	>2.7 µm	[210]
Vietnam	Saigon River fragments	Net	0.01-0.223	Microscopic inspection with image analysis software. 57 fragments (15%) were analysed by ATR FTIR.	Fragments	PET, PE, PP, PP, PS, PA, PVC, PE-PP copolymer, PP-vistalon, acrylic, polyepoxy, polyester, PE-ethyl acrylate	>300 µm	[210]

Country	Location	Sampling device	Particles per litre	Particle characterisation method	Particle type	Polymer type	Size class	Ref.
France	Seine river, Marne river	Plankton net	3-106x10^{-3}	Visual inspection	Fibres	ns	>80 µm	[211]
France	Seine river, Marne river	Plankton net	1-441x10^{-3}	Visual inspection, small subset of fibres checked with micro-FTIR spectroscopy	Fibres	PET, PP, PA, PET-PUR (and cellulosic fibres)	>80 µm	[212]
Portugal	Antuã river	Motor water pump	0.058-1.265	Visual inspection, Subsample of particles analysed with ATR-FTIR	Fragments, pellets, films, foam and fibres	PE, PP, PS, PET, PVA, EVA, PTFE, PMMA, PAE, SBR, cellulose acetate	>55 µm	[213]
China	Qingdao	Bulk sampling 50L x 3	0.2-0.7	Visual inspection and ATR-micro-FTIR	Fibres, fragments	PET, PE, PVC, PBT, PAA, PMPS, PVS, PAM, PDMS, PEVA, Nylon, PPD, POM, PBOPVA, PDAP, PS, PI, PCL, Rayon	>50 µm	[214]
China	Danjiangkou Reservoir	Teflon pump	0.5-15	Visual inspection, subset analysed by micro-Raman spectroscopy	Fibres, fragments, pellets, Styrofoam	PE, PP, PS	>48 µm	[215]

Country	Location	Sampling device	Particles per litre	Particle characterisation method	Particle type	Polymer type	Size class	Ref.
China	Taihu lake	Plankton net	3.4-25.8	Visual inspection, Subset (113/1805 particles) analysed with micro-FT-IR or SEM/EDS	Fibres, pellets, films and fragments	Cellophane, PET, PES, terephthalic acid, PP	>5 µm	[216]
Czech Republic	Unidentified reservoir 1	Bulk sampling 1L	1436-1504	SEM for number, size, morphology, FTIR and Raman spectroscopy for polymer ID	Fibres, spherical, fragments	PBA, PE, PMMA, PP, PS, PTT, PVC, PAM, PET, PPTA, Bakelite,	>1 µm	[205]
Czech Republic	Unidentified reservoir 2	Bulk sampling 1L	1772-1835	SEM for number, size, morphology, FTIR and Raman spectroscopy for polymer ID	Fibres, spherical, fragments	PBA, PE, PMMA, PP, PS, PTT, PVC, PAM, PET, PPTA, Bakelite, plasticizer DEHP	>1 µm	[205]
Czech Republic	Unidentified river	Bulk sampling 1L	3305-4179	SEM for number, size, morphology, FTIR and Raman spectroscopy for polymer ID	Fibres, spherical, fragments	PBA, PE, PMMA, PP, PS, PTT, PVC, PAM, PET, PPTA, Bakelite, plasticizer DEHP	>1 µm	[205]
Netherlands	Canal Amsterdam	Bulk sampling 2L	48-187	Visual inspection	Fibres, spheres and foils	ns	>0.7 µm	[217]

Country	Location	Sampling device	Particles per litre	Particle characterisation method	Particle type	Polymer type	Size class	Ref.
Germany	Groundwater	Sampling from tap through pre-rinsed cartridge filters	0-7x10^{-3}	Micro-FTIR	Fragments	PES, PVC, PA, epoxy resin, PE	>3 µm	[218]
USA	Groundwater, Illinois	Bulk sampling 2L	0-15.2	Visual inspection and Pyrolysis-GCMS	Fibre, fragment, foam, bead, or film	PE, 80% of samples lost during ID	>0.45 µm	[219]

DEHP: diethylhexyl phthalate, NY: nylon,PBO: Polybenzoxazoles, PBT: polybutylene terephthalate, PAA: polaryl amide, PAM: Polyacrylamide, PCL: Polycaprolactone, PDAP: Polydiallyl Phthalate, PDMS: Polydimethylsiloxane, PE: polyethylene, PES: polyester, PET: polyethylene terephthalate, PEVA: poly (ethylene-vinyl acetate) PI: Polyimide, , PMPS: polymethyl pentene, POM: Polyoxymethylene, PP: polypropylene, PPTA: poly-p-phenylene terephthalamide, PPD: p-Phenylenediamine, PS; polystyrene, PUR: polyurethane, PVA: Poly(vinyl alcohol), PVC: polyvinyl chloride, PVS: polyvinyl siloxane, ns: not specified

8.4.3 Quantity of NMP in drinking water

Freshwater sources of drinking water have frequently been shown to contain abundant levels of microplastics contamination as summarised in Table 8.4. Research in the Czech Republic found that drinking water treatment plants removed 70 – 83% of microplastics from water from reservoirs and a river, leaving a substantial amount of microplastics in drinking water to be distributed to the population [205]. It has also been found that the remaining microplastics fell into smaller size ranges which have been indicated as potentially more hazardous to human health. Analysis of microplastic removal by one of the largest drinking water treatment plants in China found that coagulants used in water treatment contained polyacrylamide, leading to concentrations of this polymer up to six times higher in treated water than raw water [220]. Research on microplastics presence in water along the drinking water purification and supply chain from groundwater to households in northern Germany found contamination in all steps along the chain, but results did not allow conclusions on where specific contamination might occur. Microplastic polymers found did match those used in components in the chain such as epoxy in tanks and PVC pipes [218]. Chlorine compounds are used in many countries to disinfect drinking water. These compounds can affect the mechanical properties of plastic materials used in many drinking water systems around the world. Such degraded pipes may well release microplastics into the drinking water supply, but direct observations of this are lacking [221].

Besides tap water, bottled water forms a vital drinking source in many places with plastic bottles forming a strong, but lightweight method of packaging. Microplastics have been found in many brands of bottled water [222]–[225] and glass bottles also contained microplastics. Cox *et al.* [178] combined available literature and concluded that bottled water contained on average 94.37 NMP/L and tapwater 4.23 NMP/L. Winkler *et al.* [226] investigated plastic water bottles and found a positive relation between repeated opening and closing of the lid and microplastics in the water, especially at higher frequencies of opening/closing which might be achieved by a person refilling a bottle designed for single use.

Table 8.7 NMP in drinking water, tap and bottle (based in part on [206]).

Country	Type and region	Sampling device	Particles per litre	Particle characterisation method	Particle type	Polymer type	Size class	Ref.
11 countries including the UK	Tapwater various countries	Bulk sampling 457-603 ml	0-61	Rose Bengal staining and visual identification	Fibres, fragments, film	ns	>2.5 µm	[227]
14 countries including England	Plastic-bottled water various countries	Per bottle	0-10390	Visual inspection, ATR FTIR on subsample.	Fragment, film, fibre, foam, pellet	PP, nylon, PS, PE, PES (polyester + polyethylene terephthalate), Azlon, polyacrylates, copolymers	>1.5 µm	[222]
Germany	Tapwater	Sampling from tap through pre-rinsed cartridge filters	$0\text{-}7x10^{-3}$	Micro-FTIR	Fragments	PES, PVC, PA, epoxy resin, PE	>3 µm	[218]

Country	Type and region	Sampling device	Particles per litre	Particle characterisation method	Particle type	Polymer type	Size class	Ref.
Germany	Plastic-bottled water Bavaria	Per bottle, 0.5 – 1.0 L	90-16634	ns	ns	PTFE, Poly(p-phenylenterephthalamid, PS, PP, PE, PET+Olefin, PS + Olefin, PET, PVC, PA, Poly(diallylisophthalat), polyester, styrene-butadiene-copolymer, tris(2,4-di-tert-butylphenyl)phosphite	>1 µm	[223]

Country	Type and region	Sampling device	Particles per litre	Particle characterisation method	Particle type	Polymer type	Size class	Ref.
Germany	Glass-bottled water Bavaria	Per bottle, 0.5 – 1.0 L	813-35436	Micro-Raman spectroscopy of 4.4% of filter	ns	PTFE, Poly(p-phenylenterephthalamid, PS, PP, PE, PET+Olefin, PS + Olefin, PET, PVC, PA, Poly(diallylisophthalat), polyester, styrene-butadiene-copolymer, tris(2,4-di-tert-butylphenyl)phosphite	>1 µm	[223]
Germany	Single-use plastic bottled water	Per bottle 700 - 1500 ml	2-44	Micro-Raman spectroscopy	ns	PES, PE, PP, PA	>1 µm	[224]
Germany	Re-usable plastic bottled water	Per bottle 700 - 1500 ml	28-241	Micro-Raman spectroscopy	ns	PES, PE, PP, PA	>1 µm	[224]
Germany	Glass-bottled water	Per bottle 700 - 1500 ml	4-156	Micro-Raman spectroscopy	ns	PES, PE, PP, PA	>1 µm	[224]

Country	Type and region	Sampling device	Particles per litre	Particle characterisation method	Particle type	Polymer type	Size class	Ref.
Germany	Cartons	Per carton 700 - 1500 ml	5-20	Micro-Raman spectroscopy	ns	PES, PE, PP, PA	>1 µm	[224]
Italy	Bottled water	Per bottle 500 mL	$3.16x10^6$-$1.1x10^8$	Scanning Electron Microscopy (SEM) coupled with an Energy Dispersive Detector (SEM-EDX)	ns	ns	0.5-10 µm	[225]
Czech Republic	Treated drinking water, 3 DWP	Bulk sampling 1L	266-659	SEM for number, size, morphology, FTIR and Raman spectroscopy for polymer ID	Fibres, spherical, fragments	95% of NMP: PAM, PE, PET, PP, PVC	>1 µm	[205]
China	Tapwater Qingdao	Bulk sampling 4.5L	0.3-1.6	Visual inspection and ATR-micro-FTIR	Fibres, fragments	Rayon, PET, PE, PS, PAA, PMPS, polyester, PI, PAM, PDMS, PCL-diol	>0.45 µm	[214]

NY: nylon, PAA: polaryl amide, PBO: Polybenzoxazoles, PAM: Polyacrylamide, PCL: Polycaprolactone, , PE: polyethylene, PES: polyester, PET: polyethylene terephthalate, PI: Polyimide, PP: polypropylene, PS; polystyrene, PTFE: Polytetrafluoroethylene, PUR: polyurethane, PVA: Poly(vinyl alcohol), PVC: polyvinyl chloride, ns: not specified

As with the data on microplastics in freshwater, the data available on microplastics in various types of drinking water show a wide range of values. The review by Koelmans *et al.* [206] found a range of particle numbers exceeding ten orders of magnitude, but also noted a lack of quality assurance across many of the studies. Some of the variation, as was found with studies into microplastics in freshwater, is most likely due to large differences in the study methods applied. For example, Kosuth *et al.* [227] used a basic dissection microscope and staining to visually determine microplastics counts, Ossman *et al.* [223] used membrane filtration and micro-Raman spectroscopy, whilst Zuccarello *et al.* [225] used a scanning electron microscope coupled with an energy dispersive detector. Different methods and different particle size ranges will produce different particle counts. To enable more useful comparisons between areas of contamination, the research field will require the application of standardised methodologies.

8.5 Pathways of NMP into food chain via shellfish

8.5.1 Nanoplastics

There is still no clear definition for nanoplastics according to their size. Nanoplastics have been defined as particles below <100 nm, however an upper size limit of 1000 nm has also been proposed [4]. In this review, a working definition of < 100 nm was selected. Nanoplastics have become subject to increased scrutiny in seafood in a food safety context [228]. To date, laboratory-controlled experiments have almost entirely been restricted to microplastics due to the technical difficulties for nanoplastic detection and laboratory contamination issues. Some studies have, however, investigated the ingestion and bioaccumulation of nanoplastics in brine shrimps and Mediterranean mussels [229].

Sendra *et al.* [230] reported the ingestion and bioaccumulation of PS nanoplastics (100 nm in size) to the brine shrimp *Artemia franciscana.* Presence of PS particles in the gut after a 24h depuration indicated that 24h was not long enough to eliminate the nanoplastics.

Al-Sid-Cheikh *et al.* [231] used radiolabelling techniques to demonstrate that nanoparticles at environmentally relevant concentrations were taken up by scallops,

and possibly translocated into the muscle as well as the expected hepatopancreas and gill.

Park *et al.* [232] investigated the accumulation of microplastics in the Mediterranean mussel *Mytilus galloprovincialis.* Experiments used PP particles of 53-63 µm diameter which decomposed into nano-sized particles during the experiment (diameter below 20 µm). NMP did accumulate in the gill, stomach, stylus sac, secondary duct and intestine of the mussels.

Translocation of NMP was suggested by Scanes *et al.* [233] with the detection of NMP below 2 mm in the haemolymph of the Sydney rock oyster *Saccostrea glomerata* following exposure under laboratory-controlled conditions.

8.5.2 Reported concentrations NMP in shellfish

NMP have been reported for shellfish globally (Table 8.5). Most studies have been primarily focusing on the detection and quantification of microplastics in mussels [85], [234]–[241] and oysters [85], [237], [240], [242]–[245].

Several studies compared the abundance of macroplastics in shellfish caught in the wild with shellfish sourced from aquaculture [239], [246]. Renzi *et al*. [239] did not report any significant differences in the abundance of NMP among wide mussels (*M. galloprovincialis*) and mussels issued from aquaculture. Li *et al*. [236] however, reported a significantly higher abundance of NMP in wild mussels (*M. edulis*) (1.6 items g-1 wet weight, 3.0 items individual^{-1}) from coastal sites, compared with (larger sized) farmed mussels from supermarkets (1.1 items g-1 wet weight, 4.7 items individual^{-1}). Depuration at the end of farming and the point of sale at a supermarket was suggested as an explanation for the reduction in NMP in mussels.

In contrast, Li *et al.* [234] detected higher levels of microplastic contamination in Chinese commercially bought bivalves (2.1 to 10.5 items g^{-1} wet weight). Similarly, higher microplastics levels were also reported for farmed clams (*Venerupis philippinarum*) relative to wild clams (ranging from 0.07 to 5.47 microplastics g^{-1} wet weight but with no significant difference in the mean values) in British Columbia, Canada [247].

8.5.3 Dietary uptake of NMP from shellfish

There a several methods available to measure seafood consumption in Great Britain. Seafish, a non-departmental public body in the UK [248], suggested that the Defra family food dataset represented an accurate and consistent benchmark for seafood consumption. Defra family food provides data on overall food consumption, with a breakdown by type of food. In 2015 (latest available data), this showed seafood consumption at just over 161g pp/pw. These data showed a 2% increase in seafood consumption from 157.83 grams per person per week in 2014 to 161.07 grams per person per week in 2015. This equates to around 8.4 kg/person/yr in 2015. The National Diet and Nutrition Survey by Public Health England shows an increase in fish consumption correlated to age, with actual consumption for adults in the same range as reported in the Defra family food data.

Seafood processing prior to commercialisation may have an impact on the abundance of NMP in seafood. Li *et al.* [236] reported a significantly higher abundance of NMP in processed mussels (*M. edulis*) (1.4 items g^{-1} wet weight) compared to live mussels (0.9 items g^{-1} wet weight) sourced from supermarkets in the UK. However, the live and processed mussels were harvested from different areas. This means that no direct comparison between live and processed mussels could be made. It is possible that the processing had no impact on the NMP content of the mussels and the differences were simply a result of differing levels of NMP in the waters of the harvesting areas. Renzi *et al.* [239] reported a reduction of 14% in the abundance of microplastics in mussels (*M. galloprovincialis*) after a cooking process as compared with raw ones.

Table 8.8 Occurrence and abundance of NMP in shellfish reported in the literature.

Organisms	Location	Species	Sample size (n)	Occurrence (%)	Number of items individual^{-1}	Number of items g^{-1} wet weight	Particle type	Polymer type	Size (mm)	Ref.
Anglerfish, snailfish, point-head flounder, acila, starfish, Cancer *gibbosulus,* ophiuroid, sand shrimp and decorator crab	South Yellow Sea, China	*Lophius litulon, Liparis tanakae, Cleisthenes herzensteini, Acila mirabilis, Luidia quinaria, Cancer gibbosulus, Ophiura sarsii, Crangon affinis, and Oregonia gracilis*	> 60	100	ns	1.7 – 47.0	Mostly fibres, fragments, and spherules	ns	< 500	[249]
Sea snail, rock seashells, clams, Gulf pearl-oyster	Persian Gulf	*Cerithidea Cingulate, Thais mutabilis, Amiantis umbonella, Amiantis Purpuratus, Pinctada radiata*	123	ns	3.5 – 17.7	0.2 - 20	Fibres (~58%), fragments, films and pellets	PE, PET, NY	ns	[250]

Organisms	Location	Species	Sample size (n)	Occurrence (%)	Number of items individual^{-1}	Number of items g^{-1} wet weight	Particle type	Polymer type	Size (mm)	Ref.
Commercial bivalves	Fishery market, China	*Sc. Subcrenata, T. granosa, My. galloprovincialis, P. yessoensis, A. plicatula, Si. constricta, R. philippinarum, Me. lusoria, C. sinensis*	144	ns	4.3 – 57.2	2.1 – 10.5	Mostly fibres, pellets	ns	< 250	[234]
Commercial molluscs	Lagoon of Bizerte, Northern Tunisia	*Mytilus galloprovincialis, Ruditapes decussatus, Crassostrea gigas, Hexaplex trunculus, Bolinus brandaris, Sepia officinalis.*	21	ns	ns	ns	Fibres, fragments and films	ns	ns	[240]
Crab	Indian River Lagoon system, Florida	*Panopeus herbstii*	90	ns	4.2	ns	Fibres (85%), beads, fragments	ns	ns	[242]

Organisms	Location	Species	Sample size (n)	Occurrence (%)	Number of items individual^{-1}	Number of items g^{-1} wet weight	Particle type	Polymer type	Size (mm)	Ref.
Crab	Corpus Christi Bay, TX.	*Callinectes sapidus*	39	36	0.87	ns	Fibres and fragments	Fibres; CL/RA blend, PES, acrylic, PS. Fragments : PET, PC	ns	[251]
Langoustine	North and West Scotland	*Nephrops norvegicus*	1450	67	ns	ns	Mainly fibres	NY, PP< PE, PVC	ns	[252]
Manila clam	Baynes Sound, British Columbia	*Venerupis philippinarum*	54 (27 farmed and 27 non-farmed)	ns	6.1 ± 2.5 – 15.4 ± 6.3	0.07 – 5.47	Fibres (90%)	ns	ns	[247]
Manila clam	Coastal British Columbia, Canada	*Venerupis philippinarum*	1330	ns	0.10 ± 0.10	0.16 ± 0.18 (dry weight tissues)	Mostly fibres	PES, NY, cellulosic fibres including cotton	ns	[243]
Mussel	Norwegian coastal waters	*Mytilus spp.*	332 (20 per site)	ns	1.5 ± 2.3	0.97 ± 2.61	Fibres (83%) and fragments (12%)	Cellulosic fibres, rubbery particles	< 1000	[238]

Organisms	Location	Species	Sample size (n)	Occurrence (%)	Number of items individual^{-1}	Number of items g^{-1} wet weight	Particle type	Polymer type	Size (mm)	Ref.
Mussel	Maricultured (central Adriatic Coast, Ligurian Sea Coast, and North East Sardinia) and natural mussels (Tyrrhenian Sea), Italy	*M. galloprovincialis*	10	ns	3.0 – 12.4	4.4 – 11.4	Fibres (90%), spherules (5.6%) and fragments (4.4%)	ns	750 - 6000	[239]
Mussel	Gulf of La Spezia (Ligurian Sea), Italy	*Mytilus galloprovincialis*	20	ns	ns	0.05 (dry weight)	ns	ns		[237]
Mussel	Coastal waters of China	*Mytilus edulis*	50 per site (22 sites)	ns	1.5 – 7.6	0.9 – 4.6	Fibres followed by fragments	ns	< 250	[235]
Mussel	Mussel farm in Germany	*Mytilus edulis*	72	ns	ns	0.36 ± 0.07	ns	ns	5 – 10	[85]
Mussel	French channel coast	*Mytilus edulis*	100	ns	0.76 ± 0.40	0.15 ± 0.06	Fibres and fragments	PE, PP, PS, ABS, PET, SBR	ns	[241]

Organisms	Location	Species	Sample size (n)	Occurrence (%)	Number of items individual^{-1}	Number of items g^{-1} wet weight	Particle type	Polymer type	Size (mm)	Ref.
Mussel	UK coastal waters	*Mytilus edulis*	162	100	1.1 – 6.4	0.7 – 2.9	Fibres, fragments, spheres, flakes	PES, PP, PE	73 - 4700	[236]
Oysters	Indian River Lagoon system, Florida	*Crassostrea virginica*	90	ns	16.5	ns	Fibres	ns	ns	[242]
Oyster	Gulf of La Spezia (Ligurian Sea), Italy	*Crassostrea gigas*	20	ns	ns	0.11 (dry weight)	ns	ns	ns	[237]
Oyster	Supermarket and originated from Brittany, France	*Crassostrea gigas*	22	ns	ns	0.47 ± 0.16	ns	ns	11 – 20	[85]
Oyster	Tuticorin coast in Gulf of Mannar in Southeast India	*Magallana bilineata*	ns	ns	6.9 ± 3.84	0.81 ± 0.45	Fibres (92%) and fragments	PE, PP	ns	[244]
Oyster	Lagoon of Bizerte, Northern Tunisia	*Crassostrea gigas*	ns	ns	ns	1.48 ± 0.02	Fibres, fragments and films	PP, PE	50 - 5000	[240]

Organisms	Location	Species	Sample size (n)	Occurrence (%)	Number of items individual^{-1}	Number of items g^{-1} wet weight	Particle type	Polymer type	Size (mm)	Ref.
Oyster	Coastline of China	*Crassostrea gigas, Crassostrea angulate, Crassostrea hongkongensis* and *Crassostrea sikamea*	At least 30 per site, 17 sites	84	2.93	0.62	Fibres, fragments, films and pellets	CP, PE, PET	20.34 – 4807.22	[253]
Oyster	Coastal British Columbia, Canada	*Crassostrea gigas*	1110	ns	0 – 3 Mean: 0.22 ± 0.28	0.04 ± 0.06 (dry tissue weight)	Mostly fibres	PES, NY, cellulosic fibres including cotton	ns	[243]
Oyster	Bahía Blanca Estuary (Southwestern Atlantic):	*Crassostrea gigas*	17	ns	Presence only	Presence only	Mostly fibres (91%), fragments, pellets and beads	ns	ns	[245]

ABS: Acrylonitrile-Butadiene-Styrene CL: cellulose, CP: cellophane, NY: nylon, PA: polyamide, PC: polycarbonate, PE: polyethylene, PES: polyester, PET: polyethylene terephthalate, PS: polystyrene, PVC: Polyvinyl chloride, RA: rayon, SBR: styrene butadiene rubber, ns: not specified

Table 8.9 Estimates of dietary NMP intake via consumption of shellfish in Tunisia. Data from [240].

Mollusc species	Dietary intake (item/person/year)
Mytilus galloprovincialis	24.50 – 2756.76
Ruditapes decussatus	43.73 – 4919.83
Crassostrea gigas	40.33 – 4537.44
Hexaplex trunculus	27.59 – 3104.45
Bolinus brandaris	22.73 – 2557.67

Table 8.6 shows how the number of items found per shellfish individual can be converted into a possible number of NMP items consumed through shellfish in the population. The data are from a study in Tunisia, with lower numbers based on the national average shellfish consumption, according to the FAO, whilst the higher numbers are based on annual consumption in fishing communities.

- Total number of papers in reference list: 19

8.6 Pathways of NMP into food chain via salt

From the overall review, 11 papers or reports dealt with microplastic content measured in salt, all published from 2015 onwards. There are several types of table salts which are derived from both marine and terrestrial origins and include sea salt, lake salt, rock salt, river and well salt. The salts come either from the evaporation process or from mining. This process is illustrated in Figure 8.1.

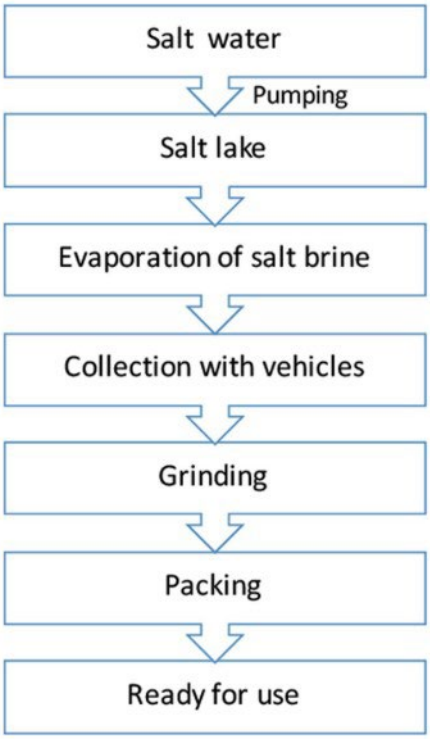

Figure 8.1 Scheme of salt manufacturing process [254]

Two of the papers were reviews [255], [256]. The other nine papers combined (n = 93) showed a wide range of microplastic particles (0-681) per Kg $^{-1}$ of salt. Lee *et al*. 2019 [255] produced a global review of microplastic contamination of table salts showing worldwide contamination issues. The results of a case study for salt produced in Taiwan showed that within 4.4kg of salt, 43 microplastic particles were detected (averages to 9.77 microplastic particles per kg). Global review found 94% of salt products contained microplastics with PET, PP and PE accounting for most of the particles. Looking across seven studies, the data show table salts to contain a mean of 140.2 microplastic particles/ kg. It was possible to estimate annual salt consumption of about 3.75 kg/ year, so salt could be causing ingestion of several hundred microplastic particles per human per year.

Peixoto *et al*. [256] reviewed data for salt and showed that microplastics have been found from 128 different commercial salt brands (sea and terrestrial origins), sourced from 38 countries that cover five continents.

Table 8.10 Occurrence, abundance, and characteristics of microplastics in salts from the literature.

Continent	Country	Environmental compartment	Estimated human intake (NMP capita $^{-1}$ Yr^{-1})	Particles kg^{-1}	No. of samples	Particle characterisation protocol	Particle type	Polymer type	Size (mm)	Ref.
Europe	Italy	Marine (salt)	40.6-1,085.2	1.6- 8.2	6	Observations and µFT-IR	Fragments dominated	PP	4-2100	[239]
Europe	Croatia	Marine (salt)	50x that for Italy (above)	27.1- 31.6	5	Observations and µFT-IR	Fibres dominated	PP	15-4628	[239]
Europe	Spain	Marine (salt) and terrestrial	ns	50- 280	21	Stereo microscopy and FT-IR	Fibres	PET, PP, PE	3.5-30	[254]
Europe	France	Marine samples	37	0-1	6	Micro-Raman spectroscopy	Fragments dominated, filaments & films	PP, PET & PE	515- 171	[257]
Europe	Portugal	Marine samples	37	0-10	3	Micro-Raman spectroscopy	Fragments dominated, filaments & films	PET & PP	515-171	[257]
Europe	Turkey	Marine, lake and rock salt	64-302	Sea salt: 16-84 Lake salt: 8-102 Rock salt: 9-16	ns	Microscopy and Raman spectroscopy	ns	PE, PP	ns	[258]

Continent	Country	Environmental compartment	Estimated human intake (NMP capita $^{-1}$ Yr^{-1})	Particles kg^{-1}	No. of samples	Particle characterisation protocol	Particle type	Polymer type	Size (mm)	Ref.
Europe	Turkey	Marine, lake and rock salt	ns	Sea salt 56 Rock salt 28 Lake salt 63	ns	ns	ns	ns	ns	[259]
Asia	Iran	Lake samples	37	1	1	Micro-Raman spectroscopy	Fragments dominated, filaments & films	PP	515-171	[257]
Asia	Japan	Marine samples	37	0	1	Micro-Raman spectroscopy	Fragments dominated, filaments & films	PE & PET	515-171	[257]
Asia	China	Marine and terrestrial samples collected from supermarket table salt	1000	550-681 in sea salt 43- 364 in lake salt 7-204 in rock/ well salt	15	Stereo microscopy and μFT-IR	Fragments, fibres	PET & PE	majority of particles <200	[260]
Asia		Marine, lake and rock/ well salt	ns	Sea salt: 120-718 Rock salt: 0-14 Lake salt: 28	6	ATR FTIR	ns	PP, PE, PS, PET, PVC	100-5000	[261]

Continent	Country	Environmental compartment	Estimated human intake (NMP capita $^{-1}$ Yr^{-1})	Particles kg^{-1}	No. of samples	Particle characterisation protocol	Particle type	Polymer type	Size (mm)	Ref.
Asia	India	Coastal Salt pan stations	ns	ns	25	μFT-IR and AFM	Fragments, fibres and sheet	NY, CL, PE, PP	60% <100	[262]
Oceania	Australia	Marine samples	37	1-9	2	Micro-Raman spectroscopy	Fragments dominated, filaments & films	PE & PET	515-171	[257]
Oceania	New Zealand	ns	37	1	1	Micro-Raman spectroscopy	Fragments dominated, filaments & films	PE	515-171	[257]
Africa	South Africa	Marine samples	37	1	1	Micro-Raman spectroscopy	Fragments dominated, filaments & films	PET	515-171	[257]

ABS: Acrylonitrile-Butadiene-Styrene CL: cellulose, CP: cellophane, NY: nylon, PA: polyamide, PC: polycarbonate, PE: polyethylene, PES: polyester, PET: polyethylene terephthalate, PS: polystyrene, PVC: Polyvinyl chloride, RA: rayon, SBR: styrene butadiene rubber, ns: not specified

Kosuth *et al*. [227] compared 12 brands of sea salt in shops in Minneapolis, the origin was detailed on the product label and effort was made to purchase salt from different regions of the world.

Table 8.11 Summary of sea salts sampled by Kosuth *et al*. [227].

Salt ID	Minimum particles per 50 g	Maximum particles per 50 g	Mean particles per Kg	Standard deviation particles per Kg
North Sea Salt	0	7	66.6	3.61
Celtic Sea Salt 1	4	7	113	1.53
Celtic Sea Salt 2	4	20	187	8.19
Sicilian Sea Salt	9	13	220	2.31
Mediterranean Sea Salt 1	4	10	133	3.06
Mediterranean Sea Salt 2	3	11	144	4.16
Utah Sea Salt	4	8	113	2.08
Himalayan Rock Salt	13	37	367	12.7
Hawaiian Sea Salt	4	5	46.7	0.58
Baja Sea Salt	6	13	173	3.79
Atlantic Sea Salt	6	14	180	4.16
Pacific Sea Salt	22	51	806	15.3

Renzi *et al*. [263] looked at NMP in table salts from marine origin in Italy and Croatia. There was a significant correlation with number of particles found and total amount of general impurities recorded. PP fibres seem to dominate amongst the recorded shapes, but NMP in the form of granules and films were also found in Italy. In this study total averages ranged within 1.6-8.2 (Italian) and 13.5-19.8 (Croatian) items per gram of salt.

The paper of Iniguez *et al*. [254] looks at variable data from previous studies. Twenty-one samples of commercial table salt from Spain were analysed for microplastic (MP) content (sea salt and well salts) before and after packaging. NMP content was found to be 50-280 NMP/kg with no significant difference between samples. PET followed by PP and PE were the most frequently found polymers.

Karami *et al*. [257] analysed 17 salt brands from 8 countries. One was not found to contain microplastics while the others had between 1 to 10 NMP/ kg of salt. The most common polymer was PP (40%) and PE (33%). Fragments (63.8%) were the primary form followed by filaments (25.6%) and films (10.6%). The study did not look at particles less than 149 μm so this may be why numbers are so low compared to other studies.

Yang *et al*. [260] investigated 15 brands of sea salts, lake salts and rock/ well salts collected from supermarkets in China. Microplastic content was a lot higher in sea salts (550-681 particles/kg) compared to 43-364 particles/kg in lake salts and 7-204 particles/ kg in rock well salts. Fragments and fibres were the prevalent types of particles and microplastics less than 200um represented the majority of particles (55%) which were mostly PET, PE and cellophane. This was possibly the first documented study on sea salts. The sea salts collected were from very high densely populated areas.

Twenty-five types of sea salt samples were collected from salt pans along Tuticorin coast (South India) for the paper by Selvam *et al*. [262] . Microplastics <100um made up 60% of NMP. The most common polymers were found to be PP, followed by PE, nylon and cellulose. This study suggests that the accumulation rates of NMP is widely affected by urban activities, shore and coastal uses, wind and ocean currents.

Two of the papers focused on table salts from Turkey. Yurtsever [259] studied table salts procured in Turkey including rock salt, sea salt and lake salt and found particle counts to be 28,56 and 63 respectively. The paper lacks detailed methods and information about the context to ingestion. Gundogdu [258] looked at 16 different brands purchased in Turkey showing results of 16-84 item/ kg in sea salt, 8-102

item/ kg in lake salt and 9-16 item/ kg in rock salt. Using these values the estimated consumption rates were 249-302, 203-247 and 64-78 items per year retrospectively.

Kim *et al*. [261] looked at 39 salt brands from 16 countries on six continents. Microplastic particles per kg varied from 0-1674 in sea salt, 0-148 in rock salt and 28-462 in lake salt. Geospatially the study showed high microplastic content in Asian countries/regions and the study also showed significant linear correlation with plastic emissions from rivers (r^2 = 0.33; p=0.003) and with the microplastic levels in surrounding seawater (r^2 =0.45; p=0.021) both suggesting that sea salt can be a good indicator of microplastics in surrounding environments and help identify hotspots of environmental contamination.

8.7 Dietary uptake of NMP from fish

NMP in fish constitutes a large part of the published environmental data. However, for this food chain review we looked for data about the edible parts of fish rather than the gastro-intestinal (GI) tract which is not typically consumed. Initially this produced a list of 51 articles of interest, all published from 2016 onwards. During the more detailed review, 27 of the selected papers still turned out only to report on the GI tract or gills of fish. This is not a part of the fish usually considered as consumable, so these were put aside as not relevant directly to NMP entering the human food chain. Fish pathogens and human pathogens are adapted to very different host environments, and the epidemiological likelihood of a human pathogen surviving the transition through a fish GI tract, translocating through the stomach wall, and into the muscle tissue, still active and in numbers capable of causing infection is very small.

Four of the papers turned out not to have any data on specific fish-based MP content, in terms of human food chain risk. Nine papers were laboratory-based research papers looking at various aspects of microplastic interaction with fish, rather than measuring environmental concentrations. However, these still add information about possible pathways into the human food chain.

Twelve papers made an analysis of edible parts of fish in terms of microplastic or nanoplastic contamination. These were mainly investigating sections of fish muscle,

but there were also some studies of livers and one on fish skin, all of which potentially provide information on routes into the human food chain. Ten of these datasets were investigations concerning microplastic or nanoplastic content in muscle (or skin) with presence of microplastics varying from complete absence in 4 studies (3 from China), up to presence in 64.1% of fish in the study by Akhbarizadeh [264]. One study of 124 fish in Haizhou Bay, China, found 100% of samples had microplastics associated with the skin, with more particles in scale-less fish. Fish skin is often eaten as part of the normal consumption of fish in the diet.

Three studies looked at microplastic content in livers, showing presence in 0, 5 and 75-80% of samples.

Karami *et al*.'s paper [265] was one of few that investigated fish at point-of-sale, with clear human food chain implications [266]. They examined canned fish and their data showed that plastic polymers form a substantial proportion (28%) of the identified non-fish contents of the cans.

Table 8.12 Microplastics and nanoplastic research where NMP presence has been detected in edible parts of fish (muscle, liver or skin).

Location	Species	Sample size	Occurrence (%)	Number of items/ individual	Number of items/g	Particle type	Polymer type	Size (µm)	Ref.
Mediterranean Sea, Gulf of Lions	*Engraulis encrasicolus.L*	13	80 in livers	ns	ns	Particles, not fibres	Mainly PE	323±101	[267]
Mediterranean Sea, Gulf of Lions	*Sardina pilchardus*	2	75	ns	ns	Particles, not fibres	Mainly PE	124–438	[267]
Mediterranean Sea, Gulf of Lions	*Clupea Harengus*	2	75	ns	ns	Particles, not fibres	Mainly PE	124–438	[267]
Malaysia	*Rastrelliger kanagurta*	30/ species	10	0-3	Muscle (dried)	Fragments (85.7%), films (10.0%), filaments (4.08%)	PP (47.2%), PE (41.6%), PS (5.56%), PET (2.77%), NY6, (2.77%)	0.001->10000	[265]

Location	Species	Sample size	Occurrence (%)	Number of items/ individual	Number of items/g	Particle type	Polymer type	Size (μm)	Ref.
Malaysia	*Stolephorus waitei*	30/ species	5	0-3	Muscle (dried)	Fragments (85.7%), films (10.0%), filaments (4.08%)	PP (47.2%), PE (41.6%), PS (5.56%), PET (2.77%), NY6, (2.77%)	0.001->10000	[265]
Malaysia	*Chelon subviridis*	30/ species	30	0-3	Muscle (dried)	Fragments (85.7%), films (10.0%), filaments (4.08%)	PP (47.2%), PE (41.6%), PS (5.56%), PET (2.77%), NY6, (2.77%)	0.001->10000	[265]

Location	Species	Sample size	Occurrence (%)	Number of items/ individual	Number of items/g	Particle type	Polymer type	Size (µm)	Ref.
Malaysia	*Johnius belangerii*	30/ species	40	0-3	Muscle (dried)	Fragments (85.7%), films (10.0%), filaments (4.08%)	PP (47.2%), PE (41.6%), PS (5.56%), PET (2.77%), NY6, (2.77%)	0.001->10000	[265]
Musa Estuary, Persian Gulf	*Platycephalus indicus*	12	ns	21.8	0.59 muscle	Filamentous (71%)	ns	<100->1000	[268]
Musa Estuary, Persian Gulf	*Saurida tumbil*	4	ns	13.5	0.37 muscle	Filamentous (71%)	ns	<100->1000	[268]
Musa Estuary, Persian Gulf	*Sillago sihama*	17	ns	14.1	0.25 muscle	Filamentous (71%)	ns	<100->1000	[268]
Musa Estuary, Persian Gulf	*Cynoglossus abbreviatus*	11	ns	12	0.16 muscle	Filamentous (71%)	ns	<100->1000	[268]
France	*Squalius cephalus*	60 liver, 22 muscle	5 (liver), 0 (muscle)	1-2	Liver and muscle	Fibres	PE (3), PS (1)	147–567	[269]

Location	Species	Sample size	Occurrence (%)	Number of items/ individual	Number of items/g	Particle type	Polymer type	Size (µm)	Ref.
Global	*ns*	20	10	ns	Whole fish	Fragments (46.6%), films (26.6%), filaments (26.6%	PP (33.3%), PET(33.3 %), PE (16.6%), PVC (16.6%)	ns	[266]
Haizhou Bay, South Yellow Sea, China,	*Amblychaeturichthys hexanema*	23	100	9.3	0.08	ns	ns	973±803	[270]
Haizhou Bay, South Yellow Sea, China,	*Chaeturichthys stigmatias*	16	100	7.06	0.1	ns	ns	973±803	[270]
Haizhou Bay, South Yellow Sea, China,	*Odontamblyopus rubicundus*	23	100	8.3	0.12	ns	ns	973±803	[270]
Haizhou Bay, South Yellow Sea, China,	*Collichthys lucidu*	17	100	4.29	0.12	ns	ns	973±803	[270]
Haizhou Bay, South Yellow Sea, China,	*Cynoglossus semilaevis*	26	100	4.23	0.12	ns	ns	973±803	[270]
Haizhou Bay, South Yellow Sea, China,	*Thryssa kammalensis*	19	100	5.21	0.57	ns	ns	973±803	[270]

Location	Species	Sample size	Occurrence (%)	Number of items/ individual	Number of items/g	Particle type	Polymer type	Size (μm)	Ref.
NW Portugal, Atlantic Ocean	*Dicentrachus labrax*	50	32 (dorsal muscle)	0.54	0.4 ± 0.7	Fibres and fragments	ns	<100μm	[271]
NW Portugal, Atlantic Ocean	*Trachurus trachurus*	50	32 (dorsal muscle)	0.54	0.7 ± 1.3	Fibres and fragments	ns	<100μm	[271]
NW Portugal, Atlantic Ocean	*Scomber colias*	50	32 (dorsal muscle)	0.54	0.6 ± 0.8	Fibres and fragments	ns	<100μm	[271]
Persian Gulf, Iran	*Liza klunzingeri*	15	64.1% in muscle	ns	0.275	68% Fibres and 32% fragments	ns	Fibres 100->5000, fragments <50–100μm	[264]
Persian Gulf, Iran	*Platycephalus indicus*	20	64.1% in muscle	ns	0.178	68% Fibres and 32% fragments	ns	Fibres 100->5000, fragments <50–100μm	[264]
Persian Gulf, Iran	*Epinephelus coioides*	20	64.1% in muscle	ns	0.158	68% Fibres and 32% fragments	ns	Fibres 100->5000, fragments <50–100μm	[264]

Location	Species	Sample size	Occurrence (%)	Number of items/ individual	Number of items/g	Particle type	Polymer type	Size (μm)	Ref.
Hangzhou Bay and Yangtze Estuary, East China Sea	*Lateolabrax maculatus*	32	0 in muscle or liver	0	0	ns	PE, PP, PES	ns	[272]
Fujian Province, China	*Acanthopagrus latus*	20	0	0	0	Fibres fragments particles and films	PP, PA, PE, PS, PET, PVC, PAN, other	100-3000	[273]
Fujian Province, China	*Acanthopagrus latus*	20	0	0	0	Fibres fragments particles and films	PP, PA, PE, PS, PET, PVC, PAN, other	100-3000	[273]
Zhanjiang Mangrove Wetland, South China	*32 species*	120	0	0	Muscle and Liver	Pellets, films, fragments and particles	PE (35%), PET, (27.2%), PP, PS, PUR, PA and CP	20–5000	[274]
Han River, South Korea	*Cyrinus carpio*	2	0	0	0	Fibres and fragments	PTFE, PE and rayon (in GI tract)	>100	[275]

Location	Species	Sample size	Occurrence (%)	Number of items/ individual	Number of items/g	Particle type	Polymer type	Size (µm)	Ref.
Han River, South Korea	*Silurus asotus*	2	0	0	0	Fibres and fragments	PTFE, PE and rayon (in GI tract)	>100	[275]

CP: cellophane, NY6: nylon-6, PA: polyamide, PAN: Polyacrylonitrile, PC: polycarbonate, PE: polyethylene, PES: polyester, PET: polyethylene terephthalate, PP: polypropylene, PS: polystyrene, PTFE: Polytetrafluoroethylene, PUR: polyurethane, PVC: Polyvinyl chloride, ns: not specified.

There were nine papers covering laboratory-based studies of microplastics in fish investigating a broad range of aspects of microplastic interaction with the environment.

As early as 2016, Geppert *et al.* were looking at the question of what would happen if nanoplastics entered the human food chain [276]. They created a two-layer intestinal barrier model and demonstrated that it 'largely prevented' nano-polystyrene transport through the epithelium. Conversely, Parenti *et al*. [277, p.] found that 'nanoplastics', actually 500 nm beads, migrated through the gut epithelium. This caused behavioural changes (response to light and dark) and physiological changes which were similar to those found by Pitt *et al*. [278], who reported that nanoplastic PS in the diet of zebrafish caused physiological responses, but also transferred from mothers to their offspring.

The relatively small size of particle used by Parenti *et al.* is notable. Critchell and Hoogenboom [279] found that particle size was critical. The number of particles ingested and retained increased dramatically if the particles were reduced from 2mm to <300µm diameter. This was also found by Hoang and Felix-Kim [280] using smaller particles and a tighter size range of 63-75 µm and 125-150µm PE particles. Particles were also re-consumed where possible and had different properties after excretion.

Zhu *et al.* [281] carried out a chronic development study in Medaka which showed that 10 µm PS particles in the diet could lead to decreased fertility, along with physiological and pathological changes to the gut and kidneys. Other physiological changes were found by Zhang *et al.* [214]. Their study showed that microplastics in the diet enhanced the accumulation of the antibiotic roxithromycin, whilst reducing the neurotoxic effects. Oxidative damage was mitigated through assorted metabolic processes indicating that microplastics in the human food chain may also have unpredictable impacts on pharmaceutical metabolisation.

Nelms *et al.* [282] looked at the practicality of assessing microplastics in the diet by measuring microplastic abundance in the faeces of captive grey seals, top predators in some marine environments. They found NMP in over half of the samples and used metabarcoding to assess what species had been eaten. Hanachi *et al.* [283] investigated what was eaten and found that microplastics in fish meal were transferred proportionately to carp, indicating a clear possible step in transferring plastic along the

human food chain. This might not be true for all fish because Ryan *et al.* [284], used juvenile Blueback herring to show that microplastic particles in the water were avoided in preference for particles of food. Karbalaei *et al.* [285] also looked at microplastic content in fishmeal, but as a feedstock for other animals, with a clear pathway to the food chain for humans. Foods made from whole fish bodies had far fewer plastic particles than those composed of head, gills and viscera. The data suggest that the more commonly eaten sections of fish have very low numbers of plastic particles.

Apart from the studies relating to NMP found in edible sections of fish, and the laboratory studies, there were four other papers selected by the review process.

Rao [286], in the Indian Journal of Fisheries, produced a good broad summary of the situation with respect to micro and nanoplastic issues in fish as a foodstuff, but uses data from papers already reviewed here.

Hantoro *et al.* [287] reviewed coastal microplastics and included human health implications via food. The research estimates microplastics in the human diet via seafood but the numbers are skewed upwards by the inclusion of data for gastrointestinal (GI) tract plastics in fish. For Europe, the research suggested ingestion levels of 32 – 1060 NMP particles per person per year. The authors used the data to confirm that demersal fish tend to contain more microplastics than pelagic fish, raising risk levels if NMP can transfer pathogens from fish to humans.

Overall, the data in Table 8.9 for microplastics in the parts of fish generally considered edible are mixed. It is highly unlikely that human pathogens will transfer from fish GI tracts to edible parts of fish due to the widely different host types and the lack of evidence of this being part of the epidemiology for any human diseases. A wide range of percentage NMP occurrence from 0 to 100%, with an estimate of 0 to 0.7 plastic items per gramme or 0 to 21.8 plastic items per fish. The small number of studies with highly variable approaches, species, numbers of samples and results makes the generation of mean values a purely mathematical exercise with little real value in terms of assessing the actual hazard microplastics may present in seafood.

Data from Hantoro *et al.* and Karbalaei *et al.* [285] show that inclusion of fish GI tract data skews the perceived consumption hazards higher than they might be for the most commonly consumed muscle tissue. However, although the GI tract of fish is usually

discarded before consumption, the edible parts of fish could be contaminated by the NMP contained in the GI tract during food preparation or through translocation, raising potential health concerns (Collard *et al.*, 2017 [267]). This would potentially allow the transfer of pathogenic organisms to edible portions of the fish.

Using data from Barboza *et al.* [271] , and the recommendations of the European Food Safety Authority (EFSA) [288] regarding fish consumption, an estimate of microplastic exposure can be made, shown in Table 8.10

Table 8.13 Estimated human intake of microplastics from fish consumption based on the microplastics found in *Dicentrarchus labrax, Trachurus* and *Scomber colias* and on EFSA recommendations for fish consumption per week by children of different age groups, and adults or the general population.

Type of fish consumption	1 years	2-6 years	6-18 years	Greater than 18 years
Fish muscle/week	40g	50g	200g	300g
NMP items/week	2	3	11	16

8.8 NMP research into other food types and human food pathways

Published research into foods other than those already considered in this report is currently quite small in number, which makes assessment of dietary intake very difficult to accomplish with any degree of accuracy. Apart from number of particles, particle size is important too, both in terms of potential to transport microorganisms, but also in determining possible pathways once inside the gut. Where particles less than 150 µm are bioavailable to humans, particles less than 4 µm can be taken up by intestinal cells [289] and Ribeiro *et al.* [290] reported that particles more than 1µm have the ability to cross the lung epithelium whilst particles between 0.1 and 10 µm can be taken up into the gastrointestinal tract.

Bioreactivity is associated with decreasing particle size (increasing surface to volume ratio) and this was reported by Stock *et al.* [289] after an in-vitro digestion comparison of different materials. They, and others [291], [292] have identified the gap in

understanding and evidence associated with the mode of accumulation and translocation within humans.

The overall fate of microplastics within the human body following particle ingestion is still unknown and with sources coming from a variety of pathways simultaneously the accumulation rate is uncertain. Impacts on long term human health exposure to microplastics and any associated pathogen transfer needs further research [293].

Published data about microplastics in foods not considered in previous sections of this report are relatively low in number. Honey has been investigated and found to contain microplastics of various size ranges [294]. Honey and sugar were reported by EFSA [288] to contain approximately 32 fragments per kilogramme of product. However, the data reported for prevalence in honey may be overstated due to the lack of differentiation between natural and microplastic fragments in the method. Further work is required on honey to substantiate current evidence.

Beer has been reported to contain a range of concentrations of microplastics. Kosuth *et al.* [227] found that of 12 commercial brands, all tested positive, ranging from 1 to 14.3 particles per litre. Of these, 98.4% were fibres and the rest were fragments. In a similar study [295], artefacts and passive cross contamination were reported to contribute to a high level of false positives in beer, roughly equivalent to the nominally detected amounts in the beer. As with the data for honey, limitations of the methodology were a contributing factor. Santillo *et al.* [296] found that differentiating between plastic microfibres and natural cotton fibres proved challenging when interpreting microplastic samples.

Milk has also been studied on a small scale [297]. This study reported 100% presence of microplastics in 23 different retail milk samples, including 5 international and 3 national Mexican brands. They concluded that the source was likely to be the polymers used for ultrafiltration and microfiltration process during production.

Seaweed is a key component for some foods, and species that have been investigated contain microplastics on their surfaces [298], [299]. Biofilms and pathogenic microorganisms do not appear to have been investigated.

A 2016 UN report [300] documented over 800 animal species contaminated with plastics via ingestion or entanglement, although not all of these would be consumed by humans.

8.9 Summary of Pathways of colonised NMP into the human food chain

The most obvious finding from this review is that the amount of data that directly relates to human food chain items specifically is very small. Research into point of sale and point of consumption food is either not carried out or not yet published for most food types. The few exceptions are covered in this report and include fish, shellfish, water and salt, with some limited data on honey, milk and beer. Apart from the general lack of data on presence and amount of NMP contamination in the diet, there is also a lack of information about what this might mean in terms of the transfer of pathogens to human hosts.

Where there is data, the variation in technology, from Fourier-transform infrared spectroscopy (FTIR) and x-ray photoelectron spectroscopy [301] to traditional staining and filtration [295] provides inconsistent and sometimes incomparable data for levels of microplastic contamination.

Standardised extraction procedures and analytical methods are required if we are to understand the level of microplastic contamination in the human diet [296], [302]. Harmonised protocols would help an understanding of microplastic abundance both in the environment and foodstuffs.

Contradictory or missing information on the effect of various types of processing on the burden of microplastics in food also needs further investigation. Patchy and inconsistent data lead to a situation where farmed fish or seafood have been described as both having higher and lower microplastic levels than those of equivalent wild-caught species [247], [303]. Additional evidence would help to establish why this apparent anomaly exists, but fish and shellfish already seem to be attracting more attention from researchers whilst many potential sources of microplastics entering the food pathway have no published data at all. The burden on produce in retail outlets, for example, is far less well described than seafood, salt or even beer. Retail sampling

or supply chain sampling would provide evidence to bridge this gap at the point of sale to human consumers. Some research into a representative wide variety of food products would advance our understanding of human food chain risk, beyond those few items for which there is current data, including alternative protein sources such as plant-based products, or more novel insect-protein- based foods.

There is a definite need for more data about the potential transfer of pathogens into the human food chain with NMP as the vector. This is obviously linked to the general scarcity of data about NMP in foods and drinks at all, particularly at the point of consumption. These data are essential to start to assess the overall long-term impacts on public health and needs to be part of the big picture which includes the potential transfer of pathogens, but also the physical interactions of small nanoplastics with tissues and any toxicological mechanisms related to the polymers and associated chemicals.

9. NMP specific microbial risks to consumers

9.1 Introduction

There is now a gamut of published peer-review studies that indicate the potential for NMP to provide novel environments on which microorganisms can settle and enter the food chain. As such there are concerns about what effect microorganisms associated with these contaminants could potentially have on human health. Recent studies of marine NMP have shown that established fish and shellfish pathogens including *Vibrio* spp, are found in NMP biofilm communities [115], [141]. In addition, NMP may act as a long-distance transport vector for these pathogens and therefore aid the spread of diseases in the marine environment. If human pathogens also settle on NMP, then the probability of them entering the human food chain may be increased. We have reviewed the existing literature associated with NMP and microbial pathogens to determine whether there is any evidence that links the consumption of foods contaminated with NMP to human disease risk.

Several research areas were considered as part of this chapter:

- Dysbiosis (for example, a deleterious change in the microbiome of an individual caused by a specific stress)
- The presence of pathogenic microorganisms on NMP and evidence of novel and non-monitored pathogens on NMP
- Evidence of NMP as hotspots for gene exchange and AMR
- NMP and human disease risk/evidence of disease

9.2 Dysbiosis

Previous sections have established that microplastics have been identified in food consumed by humans and exposure to NMP via ingestion could lead to adverse human health effects [304]. As well as the direct impact from increased pathogen exposure, NMP have been shown to affect the natural gut microbiome in mammals. This phenomenon is known as dysbiosis and has been reviewed recently [305] in the

context of effects to wildlife. The gut microbiome has been shown to have a significant impact on overall health of individuals [306], [307] and so chronic disruption of this community can have significant impacts on the long-term health of individuals. Currently there are few human and epidemiological data on this phenomenon. Some studies have utilised zebrafish models to determine role on NMP on altering gut microflora [308], [309]. Kurchaba *et al.* [310] studied NMP impacts on zebrafish larvae. They found gut microflora disrupted by NMP exposure, with evidence of an increased abundance of *Bacteroidetes* in NMP fish, a combination frequently found in intestinal pathologies. Thus, it appears that acute NMP exposure can increase oxidative stress and dysbiosis, which may render the animal more susceptible to certain diseases. Qiao *et al.* [311] studied the influence of different NMP size and shape on gut microflora in zebrafish. They found induced gut microbiota dysbiosis and specific bacteria alterations, which provided novel insights into the potential mechanism of microplastics causing intestinal toxicities in fish. In addition, Jin *et al.* [312] found that in zebrafish exposed to NMP, the abundance of *Bacteroidetes* and Proteobacteria decreased significantly and the abundance of *Firmicutes* increased significantly in the gut after 14-day exposure to 1000 µg/L of both sizes of PS NMP. Jin *et al.* [308] found that high doses of PS NMP induced gut microbiota dysbiosis, intestinal barrier dysfunction and metabolic disorders in mice. Similarly, Lu *et al.* [313] found that polystyrene MP could modify the gut microbiota composition and induce hepatic lipid disorder in mice models. Luo *et al.* [314] investigated maternal polystyrene microplastic exposure also using a murine model. They noted hepatic lipid accumulation was observed in adult F1 mice, especially in the female mice used in the study group. These results suggest that maternal microplastic exposure during gestation and lactation increases the risk of metabolic disorder suggest the potential long-term hazards microplastic contamination and exposure. More recently, Li *et al.* [315] assessed the effect of exposure to different amounts of polyethylene microplastics (6, 60, and 600 µg/day for 5 consecutive weeks) in a murine model. Treatment with a high concentration of microplastics increased the numbers of gut microbial species, bacterial abundance, and diversity. However, the authors noted that the intestine (colon and duodenum) of mice fed high-concentration microplastics showed obvious inflammation and higher expression of genes associated with

inflammation. They suggest that polyethylene microplastics can induce intestinal dysbacteriosis and inflammation.

9.3 Presence of pathogenic microorganisms

A variety of human pathogens have been found on microplastics across a range of environmental compartments (marine water, aquatic systems, soil etc.). Many of these studies have been published in the last decade and the majority have focussed on the study of the presence of these pathogens in marine systems. For microorganisms, trillions of floating microplastics particles represent a huge surface area for colonization [316]. The attachment of harmful microalgae to macroplastic debris was observed by Masó *et al.* [317]. A more recent study by Zettler *et al.* [94], first described the 'plastisphere', that highlighted the potential for marine microplastics to house distinct communities of bacteria on their surfaces. There have been many reports of the presence of numerous pathogenic eukaryotes and bacteria on both macro- and microplastic surfaces from across oceanic regions [38]. *Vibrio* spp., in particular, have been found in high abundances within plastisphere communities, particularly in the summer months [94], [101], [141], [318], [319] (summarised in Table 1). Numerous studies have suggested that *Vibrio* spp., not all of which are pathogenic, are generally sparse in the open ocean, preferring more estuarine salinities. Yet strikingly high numbers of *Vibrio* spp. have been reported on microplastic from the mid-North Atlantic Ocean [94], [319]. Given the long-distance dispersal potential of floating microplastics, it raises the important question as to whether the increasing amount of plastic waste in global oceans provides greater opportunities for *Vibrio* spp, and other pathogens to be transported and transmitted to potential hosts, leading to increased outbreaks of disease, compared to the opportunities provided by other natural particles [38]. There are sparce epidemiological data in this context. However the spread of pathogenic *Vibrio* spp. and their associated outbreaks have increased recently [320]. Earlier in this report we noted that few studies have adequately addressed the comparative abundance of potentially pathogenic bacteria on NMP compared to the surrounding environment. As such, the total abundance of pathogenic microorganisms on particles of microplastic, compared to other, natural particles, may actually be similar [38]. This hypothesis is supported by one recent meta-analysis which concluded that the median relative abundances of a variety of potentially pathogenic species found on

microplastic across the North Sea, the Baltic Sea and the Yangtze Estuary were comparable with those present on natural particles sampled within the same regions and at the same time [321].

Bowley *et al.* [38] addressed whether microplastics can act to increase the risk of pathogen transfer and disease occurrence, other than by simply providing increased availability of floating particles, and provided a framework (summarised in Figure 9.2) of additional considerations that should be taken into account in this context in marine settings: the attachment processes and microbial interactions (for example,, rates of HGT) on the particle surface; the rate and distance of transport of pathogen colonised particles across oceans, and whether the plastisphere changes as plastics transit through different oceanographic regions; vertical transport processes to the benthos, where ingestion and trophic transfer occurs; the uptake and retention of particles into mariculture organisms and the likelihood of disease transfer occurring as a result and which of these are more critical risks influencing the overall risk to human consumers.

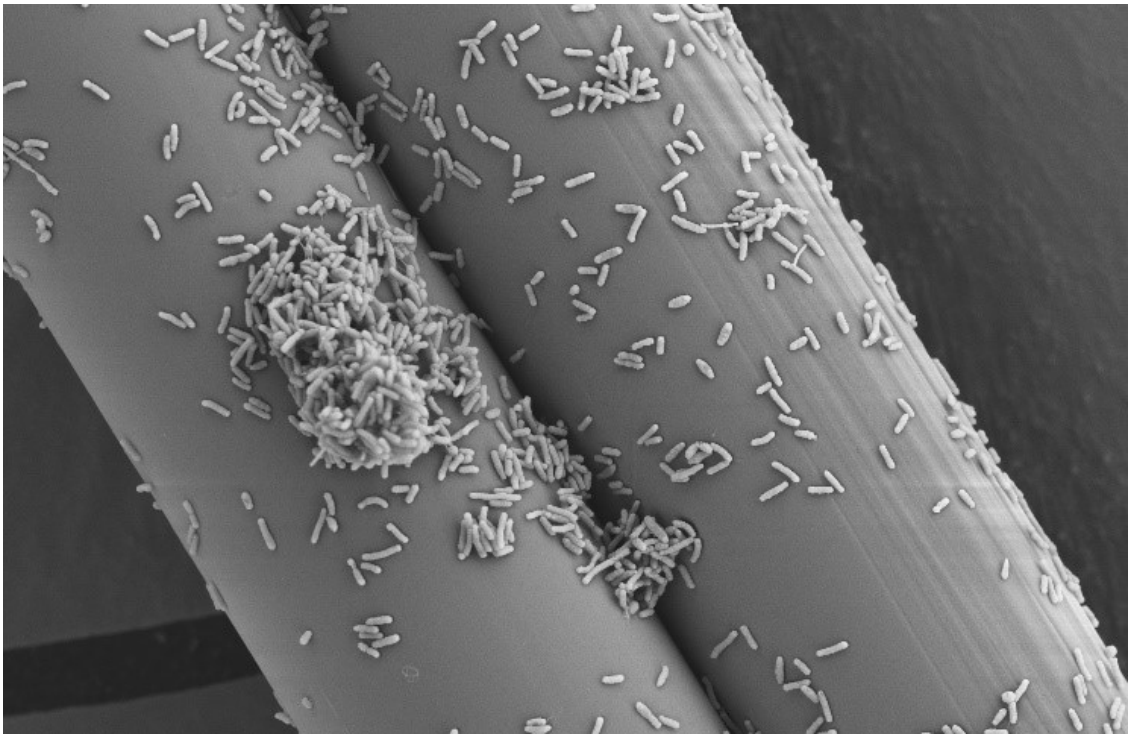

Figure 9.4 Scanning electron micrograph image of the attachment of the foodborne pathogen *Vibrio parahaemolyticus* to microplastic fragments. Picture courtesy Jake Bowley (University of Exeter).

Table 9.14 Bacterial pathogens on different marine plastics. Summary of the current published studies reporting the presence of potential pathogens on both environmental and in situ macro- and microplastic. Courtesy Bowley *et al.* [38].

Potential pathogen	Plastic type	Plastic morphology	Location
Vibrio parahaemolyticus	PE, PP	PE fibres, PE fragments, PE films, PP fragments	North/Baltic Sea
Aeromonas salmonicida	Undetermined	Fragments	Northern Adriatic Sea
Vibrio spp. (*V. splendidus*), *Pseudoalteromonas* spp.	PE, PP, PS	Fragments	The Bay of Brest (France)
Vibrio spp. and *Escherichia coli*	Undetermined	Nurdles	Forth Estuary (Scotland)
Vibrio spp.	Undetermined	Fragments (75%)	Haihe Estuary (China)
Vibrio spp.	PP, PVC	Microbeads	China coastline
Vibrio spp.	PE, PS	Microbeads	Baltic Sea
Vibrio spp., *Pseudoalteromonas, Shewanella* spp.	Undetermined	Film	Haihe Estuary (China)
Pseudomonas alcaligenes	Unknown	Unknown	Singapore coastline
Arcobacter spp.	LDPE+	Fragment	Humber Estuary (UK)
Escherichia coli and *Vibrio cholerae, Vibrio vulnificus, Vibrio mimicus*	PE, PP, PET	Fragments	Guanabara Bay (Brazil)
Vibrio spp. *Pseudoalteromonas* spp. and *Alteromonas* spp.	Undetermined	Fragments	Sungo Bay (China)
Tenacibaculum spp., *Phormidium* spp. and *Leptolyngbya* spp.	Undetermined	Undetermined	Western Mediterranean Sea
Vibrio spp.	PET	Plastic bottle	North Sea

9.3.1 Non-monitored pathogens

Naik *et al.* [322] studied the source and vector for metals, antibiotics, toxic chemicals, pathogenic bacteria (*Vibrio cholerae*), and Harmful Algal Bloom (HAB)-forming dinoflagellates through analysis of ballast water (seawater taken into the ballast tanks

of a ship to maintain its stability). They suggest that microplastics in ballast waters serve as 'hotspots' for the development and spread of multiple drug-resistant human pathogens through co-selection mechanisms. Likewise, Kirstein *et al.* [99] analysed surface water and microplastic fragments (northwest Europe) and discovered potentially pathogenic *Vibrio parahaemolyticus* on a number of microplastic particles, for example, PE, PP, and PS from the North/Baltic Sea. This study confirms the indicated occurrence of potentially pathogenic bacteria on marine microplastics and highlights the need for detailed biogeographical analyses of marine microplastics. Silva *et al.* [150] studied the presence of pathogen and indicator species on NMP in Brazilian coastal waters. Plastic debris with high coliform contents were found, while their respective water samples had only low titres. No correlations were observed, however, between the amounts of bacteria and the chemical compositions of the plastic debris. The authors noted that this study suggests that NMP could act as effective dispersal mechanism for pathogen and indicator species. Conversely, Kesy *et al.* [323] studied the bacterial composition of NMP and chitin after feeding to blue mussels (*Mytilus edulis*). The experiments revealed that egested particles were rapidly colonised by bacteria from the environment, but the taxonomic composition of the biofilms on polyamide (PA) and chitin did not differ. No potential pathogens could be detected exclusively on PA in this particular study. There is, to date, almost no published research work assessing the presence of viral pathogens on NMP.

9.4 Evidence of NMP as hotspots for gene exchange and AMR

AMR is recognised as a critical global issue and there is increasing acceptance that the natural environment plays a role in the persistence and evolution of clinically relevant resistances. Given that biofilms are known to be ideal environments for the horizontal transfer of genes [324], it is possible that consumption of NMP and their associated biofilms could increase the prevalence of harmful genetic traits such as antibiotic resistance in human gut microflora. This may, therefore, synergistically increase risk to consumers from not only consuming higher numbers of increasingly virulent pathogens, but also by not being able to treat infections using antibiotics (to which they may become resistant).

A review of studies in this area revealed very few published reports that clearly demonstrate the role of NMP with a particular emphasis on food, foodborne pathogens, and human health disease outcomes. In the absence of published studies in this area, we have highlighted below some of the most relevant studies that provide examples in food production systems, or mechanistic evidence of potential risks, with a subsequent discussion on the methodological limitations of these particular studies.

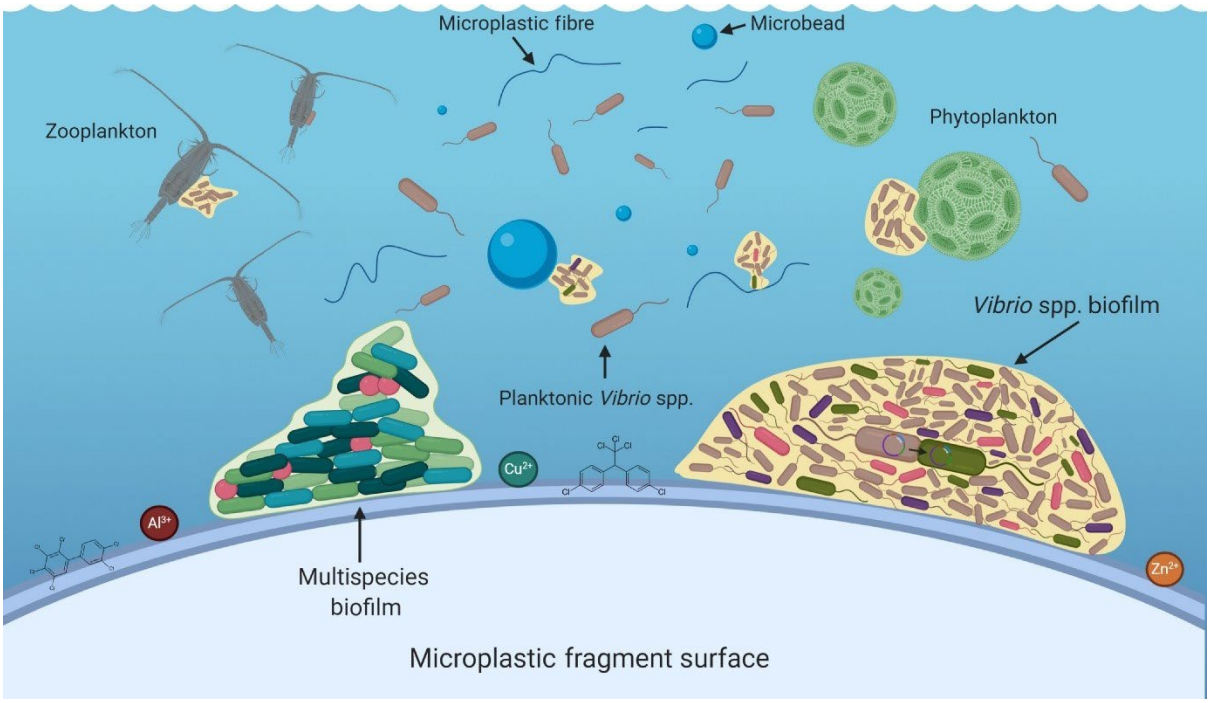

Figure 9.5 Potential interactions of microorganisms on plastic fragments. Courtesy Bowley *et al.* [38].

Oberbeckmann *et al.* [122] investigated how different *in situ* conditions contribute to the composition and specificity of NMP-associated bacterial communities in relation to communities on natural particles, ranging from marine (coastal Baltic Sea) to freshwater (WWTP) conditions. They discovered no enrichment of potential pathogens on NMP. However, the abundant colonization of NMP in a wastewater treatment works by certain bacteria commonly associated with antibiotic resistance suggests NMP as a possible hotspot for HGT.

In one of the few studies that looked at the presence of AMR in a primary food production setting, Zhang *et al.* [101] investigated the enrichment of ARB and ARGs on the surfaces of microplastics in a mariculture system in China. Molecular analysis

of these samples, utilising 16S rRNA amplicon sequencing, demonstrated these bacteria belonged to a taxonomically diverse group of bacteria, including *Vibrio* spp., *Muricauda* and *Ruegeria* genera and illustrated resistance to several antibiotic classes. In particular, a higher percentage of antibiotic resistance was observed to penicillin, sulfafurazole, erythromycin and tetracycline on NMP-associated samples. Unfortunately, this study is largely anecdotal, as only non-comparator substrates were analysed. An additional study in a Chinese aquaculture setting by Lu *et al.* (2019) [155] found that NMP had higher bacterial densities than associated water samples and distinct bacterial and ARG signatures compared to water samples, and that microplastics might be an important reservoir of ARGs in aquaculture settings. In particular, the absolute abundances of mobile genetic elements that can drive AMR exchange (in this instance, the class-1 integron *intI1*) on microplastics were higher than those in water by 2-3 orders of magnitude.

Arias *et al.* [325] studied the frequency of plasmid transfer in bacteria associated with NMP. They found that this plasmid transfer was higher when compared to bacteria that are free-living or in natural aggregates. Moreover, increased gene exchange occurred in a broad range of phylogenetically-diverse bacteria. The results indicate a different activity of HGT in NMP biofilms, which could affect the ecology of aquatic microbial communities as well as the spread of antibiotic resistance. The same authors also studied aquatic bacteria using a model antibiotic resistance plasmid, comparing communities that form biofilms on microplastics vs. those that are free-living. Utilising gene-transfer experiments, the authors demonstrated an increased frequency of plasmid transfer in bacteria associated with microplastics compared to bacteria that are free-living or in natural aggregates. A study in China assessed the fate and persistence of NMP and antibiotic resistance in farmland soils [157]. The authors found that NMP from soil after long-term vegetable cultivation, with larger size, or with stronger weathering adsorb more antibiotics and heavy metals and cause more mobile genetic elements, which can contribute to antibiotic resistance on the surface of the NMP. An analysis of the literature suggests that laboratory-based studies in this area are rare and are generally limited in scope. Wu *et al.* [156] incubated biofilm on microplastics and two natural substrates (rock and leaf) under a controlled environment to investigate the differences of bacterial community structure, ARG profiles and ARG bacterial hosts between biofilms on three types of substrates.

Metagenomic analyses further revealed microplastic biofilm with broad-spectrum and a distinctive resistome. Specifically, according to taxonomic annotation of ARG bacterial hosts, two opportunistic human pathogens (*Pseudomonas monteilii, Pseudomonas mendocina)* and one plant pathogen (*Pseudomonas syringae*) were detected only in the microplastic biofilm, but not in biofilms formed on natural substrates. Likewise, Li *et al.* [325] demonstrated that PA particles can serve as a carrier of antibiotics in the aquatic environment, but this carriage is likely restricted in marine (saline) settings.

9.5 NMP and human disease risk/evidence of disease

Various studies and meta-reviews have shown potential disease risks associated with NMP in humans. These published studies show entry points and in some studies specific toxicological impacts of NMP on human health, including respiratory systems, digestive and excretory system, and the central nervous system among others (see review by Campanale *et al.* [326]). Conversely, there are currently few data or published studies on NMP and microorganisms associated with specific human disease risks or disease outcomes. This is a clear data gap and requires further research. For example, whilst attachment of certain pathogens (for example, *Vibrio* spp.) and other potential foodborne pathogens to microplastic is well evidenced, the overarching effects that this may cause for any potential transfer to human, for example, via foodborne exposure such as bivalve aquaculture, are yet to be described [38]. However, many of the factors likely to drive foodborne disease risk have been identified and outlined, including the following:

- The presence of key foodborne pathogens on and in association with NMP [38];
- The determination of potential virulence of attached strains (in a few studies [99]);
- The presence of ARG and the potential for AMR gene spread on NMP [101], [155]; and
- Studies that assess and quantify NMP contamination of key food commodities (for example, seafood [287], [294], [326]) coupled with data on human uptake from consumption.

9.6 Summary

In summary, pathogens of human health relevance have been found on NMP and NMP evidenced from a variety of published scientific studies. Most of the published research in this area assessed through WP4 were focussed on marine settings. However, there are also studies providing insights into this phenomenon in aquatic and soil-focussed studies. Importantly, a variety of human pathogens have been found on microplastics in environmental settings, but we do not know their pathogenicity and virulence potential or what, if any, human pathogen transmission occurs via this potential route of exposure [38].

With regards to microbial resistances, many of these studies suggest that NMP can be colonised by antibiotic resistant bacteria and that densities are frequently higher than in the surrounding natural environment (again, frequently marine and aquatic settings). There is, however, a lack of robust experimental data to demonstrate this phenomenon in laboratory settings. There is also little evidence and few published studies to suggest that NMP are any more likely to be colonised by antibiotic resistant bacteria than other particle substrates. Numerous studies have demonstrated enriched AMR gene diversity on microplastics in the environment. However, many of these studies are anecdotal in nature, without appropriate comparators or laboratory-based work to underline and support the mechanistic nature of these findings. Similarly, albeit through a smaller number of published studies, enhanced gene exchange (HGT) on NMP has also been demonstrated. Of the studies assessed here, few dealt with direct food production systems, which represents a key data gap.

In conclusion, there is emerging evidence, albeit limited, that human pathogens can effectively colonise NMP and also that NMP can be effectively colonised by AMR bacteria. There are limited data (backed by robust experimental approaches and published studies) to suggest that HGT may occur more frequently on NMP than in surrounding natural substrates. There are currently few published quantitative approaches that determine the contribution of NMP to total AMR loads in aquatic systems as well as their role in transmission of AMR to humans through direct ingestion or through the food chain. There is a clear lack of published evidence regarding dysbiosis in humans, although there are a variety of studies addressing this in other model organisms (for example, zebrafish, mice). To date, there is almost no

published research assessing the presence of viral pathogens on NMP, which is surprising given the importance of viral pathogens in foodborne disease. There are also few current data and published studies regarding impacts of NMP on pathogens with human health outcomes. With a clear lack of epidemiological data in this context, this is an important data gap.

Future work could include a risk-based analysis of certain foodborne commodities coupled to microbiological and epidemiological investigations. This broader and interdisciplinary analysis is required to more clearly disentangle the potential for certain high-risk foods (for example, bivalve shellfish) to cause disease, coupled to laboratory-based studies to outline the role as well as mechanistic process of microbes in this process.

10. Summary and Discussion

In this report we have outlined the specific methodology that was used to sift available documents into a bibliography of manageable size that suited the purpose of the review. This had to be time bounded with such a dynamic field producing new research and reviews on a daily basis. The boundaries that delimited the documents we considered have produced a snapshot of the situation at a given time, covering research from 1980 to 2020 searchable via Web of Science and specified grey literature databases.

Chapter 3 put the current available knowledge about NMP in general into context before looking into the potential pathways of pathogens into the human food chain. Much of the work so far has focussed on some parts of the marine environment, with very patchy datasets other areas, including drinking water, soils and air. Within marine zones, coastal areas and areas near to urbanised rivers tend to have higher levels of NMP than more open sea areas, although where converging currents result in gyres, this also acts to concentrate floating plastics particles, including NMP. There is a vast amount of plastic litter in the world's oceans, but the amounts reported vary quite dramatically, depending on techniques used and assumptions that drive the generation of global estimates. This issue in determining the effect of methodology on reported data was a feature throughout the report.

Mismanaged plastic waste is expected to continue growing even if a range of feasible prevention and intervention techniques are employed. the most widely used synthetic plastics are low- and high-density PE, PP, PVC, PS and PET. Altogether, these plastics represent ~90% of the total world production.

Chapter 4 of the report looked at the number of papers that deal with interaction between NMP and microorganisms, showing the capability for biofilms to form on NMP. Surface roughness and hydrophobicity are key aspects dictating how NMP will interact with the microbial community, and these features can change depending on the amount of weathering that the plastic has undergone. It is possible that polymer type is another determining factor, but the body of evidence is too small to be certain. Many of the studies fail to consider non-plastic substrates in the same environment, so the reported NMP effects may in some cases just be small particle effects. It would

appear that biofilm communities on NMP engage in HGT, and this may include AMR genes, but the amount of data is very small currently, and again suffers from a lack on non-plastic comparators.

Biofouling (the accumulation of bacteria, algae, plants etc on submerged material) may play an important part in how NMP moves in the biogeochemical cycle. Smaller particles might sink once they are fouled, whereas larger ones might sink, lose the biofilm and refloat several times. This has implications on NMP fate. Another critical impact of biofilms forming on NMP may be the production of DMS by the grazing microbial community. It is thought that this may increase NMP intake by seabirds, amphipods, etc that are stimulated to feed by the biofilm.

Chapter 5 dealt with pathways of NMP into the human food chain and showed the general paucity of research data in this field. The review considered airborne and terrestrial movement of NMP as ways of NMP reaching food, or the human body via inhalation, but the actual range of food with reasonable amounts of data was very small. Drinking water, fish and shellfish, and salt made up the main body of data, with a few supplementary studies on beer, honey, milk etc. This shortage of data for a wider body of food groups might mean that the importance of airborne NMP is overemphasised. Currently airborne NMP appear to be in similar numbers to the entire amount consumed via food and drink.

Terrestrial systems (soil, plants, etc) seem to be contaminated generally, although data are too scarce for more than a generalisation. Tested drinking water and drinking water sources all appear to contain microplastics. Tests on fish and shellfish often include, or only consider parts of the animal not normally eaten and where microplastics would be expected to be concentrated. Studies looking at edible material specifically are rare, and even rarer are studies of fish and shellfish at point of sale or point of consumption. As these are the most widely studied foodstuffs for NMP content this illustrates the large data gaps that appear as soon as the food chain is considered. There is also the largely unexplored potential for NMP to be deposited in the home, which has not been systemically studied as a key source of these contaminants [182].

Issues with sampling methodology, sampling targets, what is measured and reported, instrumentation, etc make comparisons between studies complicated. Smaller particles are more likely to be capable of transmigrating into edible tissues, but plastics at the nano end of the size spectrum are also progressively harder to find in analysis as the size reduces. If particles did move into edible tissues, and were detectable, we then an understanding of what the toxicological or physiological consequences might be is required. This subject was out of scope for this review, but could be the subject of a further review.

Finally, we attempted to pull together the information of global presence of NMP in the environment, how they interact with the microbial community and how they reach the human food chain, to consider the situation of possible transfer of pathogenic organisms into the food chain. Human pathogens have definitely been found on NMP; AMR genes and HGT processes have been found and dysbiosis (gut microbiome disfunction) has been demonstrated as a possible outcome of NMP in the gut. Data on viruses on NMP are very limited, and data linking pathogens, NMP and human disease are also minimal or missing completely.

Overall, the scale of NMP in the environment has been shown to be a large and growing problem. Association of NMP and pathogenic organisms has been demonstrated in research, but the amount of data is low, as is that linking dietary intake in most food and drink groups. Much of the data are associated with fish and shellfish but focus on inedible parts, or uncleaned stages of preparation. There is evidence that pathogenic organisms might reach the human food chain in association with NMP but the consequences of this, and comparison to non-plastic vectors, have not been researched in sufficient studies to create a clear picture of risk. Data gaps, and data paucity exist in all aspects of the study, but key areas include toxicological impacts of NMP, impacts on gut microflora, presence in a wide range of food and drinks, the importance of NMP size and whether specific pathogens are more likely to be vectored than others, and is this related to polymer type or size. A list summarising the data gaps is presented in the next chapter.

11. Evidence gaps and recommendations for further work

11.1 An overview of NMP in the environment

1. Sampling protocols, analytical methodologies, and the units of NMP abundance are yet to be standardized.
2. The need for internationally accepted definition regarding NMP. Some studies for instance include cellulose-based polymers, whilst many do not. There is therefore the need to provide clear, concise and universally accepted definitions.
3. A huge number of studies have looked at the fate of NMP in the marine environment, however more work is needed on other environments. This situation appears to be changing rapidly, with a large increase in these published studies in the last year alone. Data are also needed on NMP in “pristine” environments.
4. Little is known about the transformations of plastics in seawater, including the time scales of degradation and its ultimate sinks.
5. A lack of standardised, laboratory-based data on long-term fate of NMP.
6. Although the presence of NMP have been widely reported in certain food commodities (see Chapter 5), there are very few detailed studies on these at the ecosystem level.
7. There is a lack of data on the cycling of NMP contaminants between different environmental compartments. Analogous to the nitrogen or carbon cycle, the complexity of NMP transitions in the environment is now only starting to be appreciated, encompassing factors at all levels of biological organization and large data gaps exist regarding key parts of this cycle for example, what proportions move from different environmental compartments, and over what timescales.

11.2 Interactions between microplastics and microorganisms

1. There is a lack of standardised approaches to investigate the interactions between plastics and microorganisms. Lack of consistent characterisation of surface properties makes it difficult to identify key properties for colonisation.
2. There is a lack of inclusion of non-plastic substrates in controlled exposure experiments to better understand the factors influencing colonisation and to determine whether selection driven by plastic substrates is occurring.
3. Further work is needed to determine the role NMP-associated biofilms play in selecting for and/or transporting pathogenic bacteria.
4. Research needs to be expanded in the relatively new field of determining whether NMP themselves, or sorbed antimicrobials, exert a selective effect for AMR through species sorting or *de novo* acquisition of AMR through mutations or HGT.
5. Quantitative approaches are required to determine the contribution of NMP to total AMR loads in aquatic systems as well as their role in transmission of AMR to humans through direct ingestion or through the food chain.

11.3 Pathways of colonised NMP into the human food chain

1. There are few published studies to date, although this is a rapidly changing field of research, that cover the presence of NMP in foods and drinks that are ready for consumption. Much of the work is on an earlier stage, and some focus on foods at the point of consumption would help to establish what the risks to humans are.
2. There are very few papers that tackle NMP and pathogen transfer to humans. In many cases the preparation process of cooking, smoking, pickling, etc. would inactivate any pathogens that the NMP might have carried. A focus on foods and drinks that don’t use these processes prior to consumption would be the most likely to identify any areas of concern.
3. It would be difficult to robustly link pathogens on NMP to pathogens on human food and drink because the food will be coming from the same environment, and through the same processes as the NMP. Pathogens may be coming from another

source and attaching to the food and associated NMP at the same point. Research in this area needs to look at the food or drink as well as the NMP.

4. More research needs to be done on co-occurring NMP sized particles so that we can understand differences between things that NMP do, compared to things that small particles do. Most papers neglect this area of testing and reporting.
5. There is a lack of standardisation in analytical methodology and the reporting of sizes, quantities, limits of detection and other associated quality control information. This makes it difficult to compare studies and understand whether they are showing real differences or not. These discussions are taking place so it is hoped that in the near future some aspects of this data gap issue will be resolved.

11.4 NMP specific microbial risks to consumers

1. Data on the presence of viruses on NMP are currently lacking.
2. Research into human disease risks and NMP focuses on toxicological effects, but very little attention is focused on the potential role of associated microorganisms.
3. Few studies dealt with direct food production systems, which represents a key data gap.
4. Many studies regarding the presence of pathogens on NMP in environmental settings are purely anecdotal and lack robust controls such as comparisons to other substrates.
5. Similarly, studies on AMR tend to lack appropriate controls, making direct comparisons as well as ascertaining overall relevance to risk difficult to gauge.
6. There is a lack of published evidence regarding dysbiosis in humans, although there are studies in other model organisms (for example, zebrafish, mice). More studies also looking at specific groups that are “at risk” of dysbiosis (for example, sufferers of irritable bowel disease, Crohns disease etc) should also address the importance of microplastics in long-term health outcomes.
7. There are relatively few data regarding the impacts of NMP on pathogens and human health outcomes, including a clear lack of epidemiological data.

11.5 Recommendations for key evidence gaps to be considered

1. If we are to gain a comprehensive understanding of the scale of the issues presented by NMP, studies are required from a broader range of environments and matrices. This is true for all NMP related science investigated in this report.
2. Standardised methodologies and/or standardised reporting parameters would be a beneficial advance towards being able to compare studies, matrices and work at different institutions. The following points need global agreements or normalised approaches for best practice:
    a. Definitions of which polymers are defined as plastics;
    b. The size at which plastics are classified as nanoplastics;
    c. What are suitable mesh sizes for sampling.
3. Studies need to include suitable controls, such as similarly sized non-plastic particles to ensure that any impacts observed are due to NMP and not just due to small particles and fibres (regardless of material type), many of which occur naturally.
4. There are very few studies on the possible links between NMP and pathogens and their respective fates. Data on viruses, interrelationships between organisms and sorbed chemicals, AMR acquisition, and dysbiosis effects in compromised subjects are missing, or anecdotal.
5. Direct links to health outcomes are very sparse. A lot of information exists about presence of NMP, but there are few studies linking NMP with micro-organisations and their impact on human health.

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Appendix 1: Search terms

A sets of search terms were developed for searching databases for literature used in this review. Specific search terms were developed for each topic and were combined with a generic set of search terms that was used to identify literature on NMP generally.

To make the searches more manageable, multiple set of topic specific terms were developed for work packages 2 and 4 (Interactions between NMP and microorganisms; NMP specific microbial risks to consumers). The search results for these work packages were combined for each work package for sifting.

Below, the search terms are presented in the format used for searching the Web of Science database. The syntax of the search terms was altered appropriately for each database that was searched.

Work Package 1 (Chapter 6): An Overview of NMP in the Environment

TS=((sea OR ocean OR marine OR soil OR airborne OR atmosphere OR freshwater OR fresh-water OR aquatic OR river* OR estuar*) AND ((microplastic* OR micro-plastic* OR nanoplastic* OR nano-plastic* OR microfibre* OR microfiber* OR nanofibre* OR nanofiber* OR polymer fibre* OR polymer fiber* OR microbead* OR micro-bead* OR nano-bead* OR nanobead* OR glitter OR tyre crumb*) OR ((rubber OR polystyrene OR polyethylene OR polypropylene OR "polyethylene terephthalate" OR polyamide OR nylon OR polyurethane OR "polyvinyl chloride" OR PVC OR acrylic OR polymethylmethacrylate OR PMMA OR "poly(methyl methacrylate)") NEAR/1 (microparticle$ OR micro-particle$ OR particle$ OR nanoparticle$ OR nano-particle$))) NOT (fabricat* OR electrosp* OR biosynthetic OR wound dressing OR PEG OR polyethylene glycol OR mesh scaffold OR composite scaffold OR tissue engineer* OR polymerisation OR self-assembl* OR battery OR batteries OR magnet* OR alloy fibre* OR alloy fiber* OR wood OR encapsulat* OR spectroscop* OR nanorod OR synthesis OR microsphere* OR biomimetic OR fuel cell OR bioelectric* OR fibre$reinforc* OR fiber$reinforc* OR mo$lded OR mo$lding OR silver

nanoparticle$ OR polymeri?ation OR particle pattern* OR composite$ OR nanocomposite$ OR cement OR nanotube$))

Work Package 2 (Chapter 7): Interactions between NMP and microorganisms

Set A

TS=(((bacteria* OR fung* OR virus OR viral OR pathogen* OR microbe* OR microbial OR microbiome* OR microbiota OR micro-organism* OR microorganism* OR biofilm* OR protozoa* OR protist* OR dinoflagellate* OR diatom * OR yeast OR mo$ld) AND ((microplastic* OR micro-plastic* OR nanoplastic* OR nano-plastic* OR microfibre* OR microfiber* OR nanofibre* OR nanofiber* OR polymer fibre* OR polymer fiber* OR microbead* OR micro-bead* OR nano-bead* OR nanobead* OR glitter OR tyre crumb*) OR ((rubber OR polystyrene OR polyethylene OR polypropylene OR "polyethylene terephthalate" OR polyamide OR nylon OR polyurethane OR "polyvinyl chloride" OR PVC OR acrylic OR polymethylmethacrylate OR PMMA OR "poly(methyl methacrylate)") NEAR/1 (microparticle$ OR micro-particle$ OR particle$ OR nanoparticle$ OR nano-particle$))) **NOT** (fabricat* OR electrosp* OR biosynthetic OR wound dressing OR PEG OR polyethylene glycol OR mesh scaffold OR composite scaffold OR tissue engineer* OR polymerisation OR self-assembl* OR battery OR batteries OR magnet* OR alloy fibre* OR alloy fiber* OR wood OR encapsulat* OR spectroscop* OR nanorod OR synthesis OR microsphere* OR biomimetic OR fuel cell OR bioelectric* OR fibre$reinforc* OR fiber$reinforc* OR mo$lded OR mo$lding OR silver nanoparticle$ OR polymeri?ation OR particle pattern* OR composite$ OR nanocomposite$ OR cement OR nanotube$)) OR plastisphere)

Set B

TS=((AMR OR anti-biotic resistance OR anti-microbial resistance OR antibiotic resistance OR antimicrobial resistance OR drug resistance OR HMR OR metal resistance OR gene transfer OR trait transfer OR resistance transfer OR resistance

genes OR HGT OR ABR OR drug resistance OR integron OR plasmid) AND ((microplastic* OR micro-plastic* OR nanoplastic* OR nano-plastic* OR microfibre* OR microfiber* OR nanofibre* OR nanofiber* OR polymer fibre* OR polymer fiber* OR microbead* OR micro-bead* OR nano-bead* OR nanobead* OR glitter OR tyre crumb*) OR ((rubber OR polystyrene OR polyethylene OR polypropylene OR "polyethylene terephthalate" OR polyamide OR nylon OR polyurethane OR "polyvinyl chloride" OR PVC OR acrylic OR polymethylmethacrylate OR PMMA OR "poly(methyl methacrylate)") NEAR/1 (microparticle$ OR micro-particle$ OR particle$ OR nanoparticle$ OR nano-particle$))) **NOT** (fabricat* OR electrosp* OR biosynthetic OR wound dressing OR PEG OR polyethylene glycol OR mesh scaffold OR composite scaffold OR tissue engineer* OR polymerisation OR self-assembl* OR battery OR batteries OR magnet* OR alloy fibre* OR alloy fiber* OR wood OR encapsulat* OR spectroscop* OR nanorod OR synthesis OR microsphere* OR biomimetic OR fuel cell OR bioelectric* OR fibre$reinforc* OR fiber$reinforc* OR mo$lded OR mo$lding OR silver nanoparticle$ OR polymeri?ation OR particle pattern* OR composite$ OR nanocomposite$ OR cement OR nanotube$))

Set C

TS=(((plant* OR crop* OR agricultur* OR stomata OR root$) AND (bacteria* OR vector OR transport OR uptake)) AND ((microplastic* OR micro-plastic* OR nanoplastic* OR nano-plastic* OR microfibre* OR microfiber* OR nanofibre* OR nanofiber* OR polymer fibre* OR polymer fiber* OR microbead* OR micro-bead* OR nano-bead* OR nanobead* OR glitter OR tyre crumb*) OR ((rubber OR polystyrene OR polyethylene OR polypropylene OR "polyethylene terephthalate" OR polyamide OR nylon OR polyurethane OR "polyvinyl chloride" OR PVC OR acrylic OR polymethylmethacrylate OR PMMA OR "poly(methyl methacrylate)") NEAR/1 (microparticle$ OR micro-particle$ OR particle$ OR nanoparticle$ OR nano-particle$))) **NOT** (fabricat* OR electrosp* OR biosynthetic OR wound dressing OR PEG OR polyethylene glycol OR mesh scaffold OR composite scaffold OR tissue engineer* OR polymerisation OR self-assembl* OR battery OR batteries OR magnet* OR alloy fibre* OR alloy fiber* OR wood OR encapsulat* OR spectroscop* OR nanorod OR synthesis OR microsphere* OR biomimetic OR fuel cell OR bioelectric* OR fibre$reinforc* OR fiber$reinforc* OR mo$lded OR mo$lding OR silver

nanoparticle$ OR polymeri?ation OR particle pattern* OR composite$ OR nanocomposite$ OR cement OR nanotube$))

Work Package 3 (Chapter 8): Pathways of colonised NMP into food chains

TS=((food OR agriculture OR aquaculture OR farm OR fish OR shellfish OR drink OR meat OR poultry OR fresh produce OR salad OR vegetable* OR leafy greens OR seafood OR bivalve molluscs OR milk OR dairy OR bottled water OR tap water OR drinking water OR beverage OR graze OR forage OR eat OR ingest) AND ((microplastic* OR micro-plastic* OR nanoplastic* OR nano-plastic* OR microfibre* OR microfiber* OR nanofibre* OR nanofiber* OR polymer fibre* OR polymer fiber* OR microbead* OR micro-bead* OR nano-bead* OR nanobead* OR glitter OR tyre crumb*) OR ((rubber OR polystyrene OR polyethylene OR polypropylene OR "polyethylene terephthalate" OR polyamide OR nylon OR polyurethane OR "polyvinyl chloride" OR PVC OR acrylic OR polymethylmethacrylate OR PMMA OR "poly(methyl methacrylate)") NEAR/1 (microparticle$ OR micro-particle$ OR particle$ OR nanoparticle$ OR nano-particle$))) **NOT** (fabricat* OR electrosp* OR biosynthetic OR wound dressing OR PEG OR polyethylene glycol OR mesh scaffold OR composite scaffold OR tissue engineer* OR polymerisation OR self-assembl* OR battery OR batteries OR magnet* OR alloy fibre* OR alloy fiber* OR wood OR encapsulat* OR spectroscop* OR nanorod OR synthesis OR microsphere* OR biomimetic OR fuel cell OR bioelectric* OR fibre$reinforc* OR fiber$reinforc* OR mo$lded OR mo$lding OR silver nanoparticle$ OR polymeri?ation OR particle pattern* OR composite$ OR nanocomposite$ OR cement OR nanotube$))

Work Package 4 (Chapter 9): NMP specific microbial risks to consumers

Set A

TS=(((pathogen OR disease OR vector OR pathogens OR diseases OR vectors OR pathobiome) AND (food OR agriculture OR aquaculture OR farm OR fish OR shellfish OR drink OR meat OR poultry OR fresh produce OR salad OR vegetable* OR leafy greens OR seafood OR bivalve molluscs OR milk OR dairy OR bottled water OR tap water OR drinking water OR beverage OR graze OR forage OR eat OR ingest)) AND ((microplastic* OR micro-plastic* OR nanoplastic* OR nano-plastic* OR microfibre* OR microfiber* OR nanofibre* OR nanofiber* OR polymer fibre* OR polymer fiber* OR microbead* OR micro-bead* OR nano-bead* OR nanobead* OR glitter OR tyre crumb*) OR ((rubber OR polystyrene OR polyethylene OR polypropylene OR "polyethylene terephthalate" OR polyamide OR nylon OR polyurethane OR "polyvinyl chloride" OR PVC OR acrylic OR polymethylmethacrylate OR PMMA OR "poly(methyl methacrylate)") NEAR/1 (microparticle$ OR micro-particle$ OR particle$ OR nanoparticle$ OR nano-particle$))) **NOT** (fabricat* OR electrosp* OR biosynthetic OR wound dressing OR PEG OR polyethylene glycol OR mesh scaffold OR composite scaffold OR tissue engineer* OR polymerisation OR self-assembl* OR battery OR batteries OR magnet* OR alloy fibre* OR alloy fiber* OR wood OR encapsulat* OR spectroscop* OR nanorod OR synthesis OR microsphere* OR biomimetic OR fuel cell OR bioelectric* OR fibre$reinforc* OR fiber$reinforc* OR mo$lded OR mo$lding OR silver nanoparticle$ OR polymeri?ation OR particle pattern* OR composite$ OR nanocomposite$ OR cement OR nanotube$))

Set B

TS=((((gut OR intestin*) AND (flora OR microflora OR microbe OR microbes OR microbiome OR microbiota)) OR dysbiosis OR dysbacteriosis) AND ((microplastic* OR micro-plastic* OR nanoplastic* OR nano-plastic* OR microfibre* OR microfiber* OR nanofibre* OR nanofiber* OR polymer fibre* OR polymer fiber* OR microbead* OR

micro-bead* OR nano-bead* OR nanobead* OR glitter OR tyre crumb*) OR ((rubber OR polystyrene OR polyethylene OR polypropylene OR "polyethylene terephthalate" OR polyamide OR nylon OR polyurethane OR "polyvinyl chloride" OR PVC OR acrylic OR polymethylmethacrylate OR PMMA OR "poly(methyl methacrylate)") NEAR/1 (microparticle$ OR micro-particle$ OR particle$ OR nanoparticle$ OR nano-particle$))) **NOT** (fabricat* OR electrosp* OR biosynthetic OR wound dressing OR PEG OR polyethylene glycol OR mesh scaffold OR composite scaffold OR tissue engineer* OR polymerisation OR self-assembl* OR battery OR batteries OR magnet* OR alloy fibre* OR alloy fiber* OR wood OR encapsulat* OR spectroscop* OR nanorod OR synthesis OR microsphere* OR biomimetic OR fuel cell OR bioelectric* OR fibre$reinforc* OR fiber$reinforc* OR mo$lded OR mo$lding OR silver nanoparticle$ OR polymeri?ation OR particle pattern* OR composite$ OR nanocomposite$ OR cement OR nanotube$))

Appendix 2: Stakeholder workshop

As part of the review process, we held a workshop to present the findings to date and to discuss outcomes and data gaps with stakeholders. The workshop was originally intended to take place in London, but due to the COVID-19 pandemic, the presentations were pre-recorded and a live Q&A session was held with stakeholders. This was carried out online using a combination of YouTube and Microsoft Teams.

This proved to be a very successful strategy in a previous National Reference Laboratory (NRL) meeting, and it proved to be an excellent platform for this project as well.

Invitations were also sent out to attendees to record their own thoughts on the topic. However, we received no responses to this invitation.

On 9th March 2021, 5 videos were posted privately to YouTube. These were:

- Introduction (presented by Dr David Walker, Cefas)
- WP1 Overview of Microplastics in the Environment (presented by Dr Craig Baker-Austin, Cefas)
- WP2 Interaction of Microorganisms with microplastics (presented by Dr Karen Thorpe, Fera)
- WP3 Pathways of colonised NMP into food chains (presented by Dr Andy Smith, Cefas)
- WP4 NMP-specific microbial risks (presented by Dr Craig Baker-Austin, Cefas)

The links to these videos were sent out to all attendees so they could be watched between 9th and 11th March, prior to the Q&A session on 11th March.

On 11th March, we held a two-hour Q&A session on Microsoft Teams. This was attended by a total of 32 people. Of those who attended, 17 were linked to the project either directly (member of the project team), or indirectly (supporting the project, FSA or Cefas). Another 15 attendees were not linked to the project in any way. A breakdown of attendees by organisation is shown in Table A2.

Table A15: The number of attendees at the workshop by organisation

Organisation	Number of attendees
Centre for Environment, Fisheries & Aquaculture Science (Cefas)	11
Department of Environment, Food & Rural Affairs (Defra)	3
Food Standards Agency (FSA)	3
Environment Agency	2
University of Exeter	2
Food and Environment Research Agency (FERA)	2
Thames Water	2
Dwr Cymru Welsh Water	1
Grieve Strategic Ltd	1
Plymouth Marine Laboratory	1
Seafish	1
University of Southampton	1
University of Warwick	1
University of York	1

Knowledge gaps & discussion points

A list of knowledge gaps that have been identified so far in the individual work packages (WP) is shown in Table A3.

List of knowledge gaps identified in work packages 1 to 4.

Work package 1:

- Lack of standardised methods to collect data **for example,** on NMP in surface waters.
- Need for internationally accepted definition regarding NMP.
- Less emphasis on environments other than marine (but changing rapidly).
- Lack of data in pristine environments (but changing).

- Little known about transformation of plastics in seawater, including time scales of degradation and ultimate sinks
- Lack of standardised, laboratory-based data on long term date of NMP
- Many studies on NMP in food commodities, but few details studies at ecosystem level.
- Complex cycling of NMP in environment analogous to nitrogen and carbon cycle.

Work package 2:

- Standardised approaches for isolating DNA & for characterising surface properties of plastics.
- Inclusion of non-plastic 'comparator' substrates in controlled exposures for factors influencing colonisation substrate-driven selection.
- Experimental work on NMP associated biofilm’s role in selecting for and/or transporting pathogenic bacteria.
- Experimental work to determine if NMP or sorbed antimicrobials, exert a selective effect for AMR through species sorting or HGT.

Work package 3:

- Very few studies on non-seafood.
- Lack of data on point-of-sale and point-of-consumption.
- Lack of standardised sampling and analysis methods.
- Lack of consistency of size classes.
- Lack of information of human toxicity.

Work package 4:

- Studies on presence of pathogens on NMP tend to be anecdotal and lack relevant controls.
- AMR studies tend to lack appropriate controls, making risk analysis challenging.
- Very little evidence regarding dysbiosis. Only exists for model organisms.

- Few data on direct links or epidemiological data between NMP associated pathogens and human health outcomes.

During the Q&A session, attendees were given the opportunity to ask questions about the project and its findings. The key discussion points and additional knowledge gaps that were identified are outlined below.

- There are very few articles about biosolids and sludge and the impact on agriculture. These are a by-product from water treatment facilities and are often used as soil conditioners or fertilisers in agriculture. The presence of NMP in these products may be a route into the terrestrial food chain. It was suggested that BSI and CEN committees for standards for biosolids could be consulted to see if NMP were being considered in these standards.
- There is a gap in evidence about the impact that flooding has on the loading of NMP to agricultural soils. For example, where floodplains are used for agriculture, NMP deposited during a flood may introduce novel routes of NMP into the terrestrial food chain.
- More research is needed to study the effect that chemicals that leach from NMP surfaces have on the colonising microbial communities.
- There was some suggestion that NMP may have a many-fold increase in the level of sorption of contaminants relative to natural materials. However, there are very few studies comparing NMP with natural materials and so it is not known whether this is a significant observation. If it is significant, then what effect does this have on microbial communities and human health risks?
- Whether NMP act as transport vectors for pathogens and AMR microbes is still an area lacking strong evidence. Some have hypothesised that events such as the movement of one pathogenic strain from one continent to another could be aided by transport microbial colonised NMP in oceanic currents. However, not enough evidence currently exists to support or reject these hypothesises.
- Depending on the point in which NMP are introduced into the food chain, the methodology for detecting them may be different for the same food product. For example if steaks are contaminated by NMP during processing, then a food surface methodology would be most appropriate. If steaks are contaminated during the growth of cattle, then more destructive methods would be needed to

investigate the tissues of the steak. Therefore, when developing methods for NMP detection in food, it is important to consider the route of contamination.

- There is a potential conflict in methodologies between the collection of NMP from environmental matrices and the study of microbial communities that colonise them. Some methods for isolating NMP from environmental matrices rely on the degradation of biological material prior to characterisation. This may take the form of corrosion by a mixture of hypochlorite and strong bases. However, by its very nature, this same process will destroy and microbial communities and their genetic signatures. For further study of natural assemblages on NMP, isolation methods that are compatible with detection of microorganisms needs more research.
- One of the key issues that was brought up several times throughout the workshop was the small volume of data on many of the topics discussed. This means that when two studies are published that have conflicting conclusions, we do not know which is the outlier.
- Some studies suggest that one of the main routes of NMP into the food chain is from the air, for example, settling of dust particles on food preparation and serving surfaces. However, it is not clear what the relative risks are between consumption of NMP from the air is relative to the consumption of food otherwise contaminated with NMP. For example, if NMP originating from an agricultural setting contaminate foodstuffs, they may have more microorganisms associated with them than household airborne NMP. Therefore, while the overall dose of airborne NMP may be higher, the risk posed by a smaller doses of foodborne NMP may be greater. There are no empirical studies to support of reject these hypotheses.
- NMP accumulate in the environment over time. This is not only a direct effect of the continuous release of virgin NMP into the environment, but also as result of the degradation of macro-plastics into smaller particles. This means that a study in the 2020s concluding that there is little overall risk from environmental NMP may not account for an increasing load and therefore possible increasing risk in future decades.
- While not the direct topic of this project, it was noted that there is still very little evidence on the toxicity of NMP themselves and therefore what should be considered a high-risk dose.

- There is a lack of studies on bloodborne NMP, for example, serum or macrophage analysis. Such studies would help to answer questions regarding the movement of NMP across the gastrointestinal tract.
- Due to the relative novelty of NMP research, there are still some clear issues with the quality of evidence. This partially relates to the lack of standardised methods, as has already been noted in the review work packages. The development of guidance documents by ISO and in regional bodies should start to address this issue by providing a baseline for the quality of data that should be expected in publications on this topic.
- While development of standards for NMP data collection is underway, there are several organisations working towards this goal. Caution should therefore be used so that such standards do not end up conflicting with each other and causing further confusion.
- In addition to some studies being published with poor quality data, there is also the possibility that some studies with negative results are not being published. The difficulty of publishing negative results is not unique to the study of NMP and is a common problem across all of science.

Centre for Environment Fisheries & Aquaculture Science

Cefas

World Class Science for the Marine and Freshwater Environment

We are the government's marine and freshwater science experts. We help keep our seas, oceans and rivers healthy and productive and our seafood safe and sustainable by providing data and advice to the UK Government and our overseas partners. We are passionate about what we do because our work helps tackle the serious global problems of climate change, marine litter, over-fishing and pollution in support of the UK's commitments to a better future (for example the UN Sustainable Development Goals and Defra's 25-year Environment Plan).

We work in partnership with our colleagues in Defra and across UK government, and with international governments, business, maritime and fishing industry, non-governmental organisations, research institutes, universities, civil society and schools to collate and share knowledge. Together we can understand and value our seas to secure a sustainable blue future for us all and help create a greater place for living.

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