Microplastics in the aquatic food chain

Sources, measurement, occurrence and potential health risks

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Summary

Pollution of the environment with plastics is a growing problem, and is expected to persist for hundreds to thousands of years. As a result microplastics, plastic particles with particle sizes smaller than 5 mm, are ubiquitously present in the aquatic food chain. Taking into account that trophic transfer of microplastics has been demonstrated, consequences for food safety need to be considered. The present literature review shows that the information needed for such an evaluation is not available yet.

Analytical methods for microplastics in foods are lacking: methods have been specifically developed for sediments. They consist of subsequent filtering steps and characterisation of the composition of the microplastics by Infrared spectroscopy (FTIR). These methods will need to be adapted for measuring foods. Microplastic contents of relevant foods, fish and shellfish, have not been systematically quantified; only one brief report showed their presence in mussels collected along the Belgian coast.

Toxicity data of microplastic particles are not available. Toxicity data of nanoplastics, particles < 0.1 µm, which also are present in the aquatic environment, are largely incomplete. In contrast to microplastic particles, there is concern that nanoplastic particles may reach all organs and translocate through cellular membranes. Leachable additives from microplastics, able to disrupt endocrine function, and adhering contaminants such as PCBs are of concern. The adhering contaminants have been shown to accumulate up to $10^6$ fold in the particles.

For an evaluation of the health risks of microplastics in foods, dietary exposure has to be known. However, microplastic contents of fish and shellfish, foods expected to contain microplastics, are not available and will have to be determined, after appropriate analytical methods for foods have been developed. These are the first steps needed for a future evaluation of the potential health risks of food microplastics. In addition, basic research is needed to explore the toxicity of the plastic particles and the potential transfer of additives and contaminants from the particles to body tissues.
1 Introduction

In 1997 it was discovered that in the middle of the Pacific Ocean, thousands of miles away from civilization, a high concentration of plastic bits and pieces was present in an area as large as the surface of Portugal, Spain and France together. This ‘plastic soup’ is collected by ocean currents, and five of such large patches of plastic garbage have been identified in our oceans including the Arctic and Antartic (1-3). The early concern was that these plastics could not only choke and starve (through accumulation in stomachs) wildlife, but also transport a wide variety of non-indigenous and potentially harmful organisms around the planet. More recent studies have examined the degradation of these plastics, and showed the build-up of billions of microplastic fragments in sediments worldwide (4), and their uptake in the food chain (5-8).

This plastic pollution is persistent, will very likely increase in future years, and might potentially have an impact on food safety. Hitherto, a comprehensive evaluation of microplastics in relation to food safety has not been performed. This review presents a literature study on the measurement and occurrence and of microplastics in foods, their toxicity, and the potential health risks of microplastics in foods. Major aim of the report is to describe our current knowledge, to identify knowledge gaps and as a consequence, to formulate priorities for food safety related research.
2 Sources of plastic litter

2.1 Production, use and fate of plastics

The production of plastics is a proxy for the amount of plastic litter that eventually may appear in the environment. Plastic production has strongly increased during the last 50 years, and after a dip around 2008-2009, world production reached a new record of 265 million tonnes (265 x 10^9 kg) in 2010 (Figure 1), consuming about 8% of the world oil production. Rapidly developing Asian countries constitute the world’s largest potential growth area. Use of plastic materials in North America and Western Europe reached about 100 kg kg per capita per year in 2005 and is expected to increase to around 140 kg per capita per year by 2015, whereas in Asian countries a growth from 20 to 36 kg is predicted (1).

A major part of the yearly plastic production comprises disposable packaging materials and other short-lived products that are discarded within a year of manufacture (9).

![Figure 1](source_image)

Therefore, waste management but also improper human behaviour are important determinants of the amount of plastic litter in the environment. Plastic recycling and re-use vary greatly, even in developed countries. As an example, in 2009 a number of European countries recycled more than 84% of used plastics, whereas other European countries only recycled 25% or less. In many developing countries only a small percentage of the plastic produced is recycled (1). Unfortunately, reliable and comparable data on the generation, collection and management of waste are very scarce (10).

Plastic fragments of cleaning agents (scrubbers) (4) personal care products (11), and textile fibres shed from clothes during washing (12) are litter sources that are hard to control by waste management. These fragments, called microplastics, are transported to sewer systems, but waste treatment plants are currently not able to remove these products, because they escape the filtering process (12, 13). In addition, not all sewage water will pass through a sewer treatment plant on its way to rivers and oceans. An estimation of the per capita use of microplastics in personal care...
products in the US population is about 1 g per year (14). However, this is very likely an under-
estimation because these microplastics probably are mainly derived from washing-clothes, rather than
from cleaning agents or personal care products. The proportion of polyester and acrylic fibers in
microplastics in sewage effluents suggests a predominant textile origin (13).

2.2 Diversity of plastics composition

The major components of plastics are synthetic polymers, and the composition of the plastics highly
depends on their intended use. Plastic films for packaging materials (plastic bags, plastic sheets)
mainly consist of low-density polyethylene. These products are easiest to escape into the environment
as wind-blown debris, and are likely the major component of terrestrial plastic litter (2). PET
(polyethylene terephtalate) is the major component of plastic bottles. Textile fibers have a high
content of polyester, and will additionally contain acrylic polymers (13). Polyethylene is by far the
largest produced synthetic polymer, and comprises more than 40% of the plastics produced (Table 1).

Polymers are not the only constituents of plastics. Many other chemicals, named additives, are
combined with the polymers in order to shape the desired physical properties of the plastics. These
include plasticizers, inert fillers, flame retardants, surfactants, additives to prevent oxidation and to
enhance resistance to UV radiation and high temperatures, pigments, dispergents, lubricants,
antistatics, nanoparticles or nanofibers, biocides, and fragrances (15). These additives may have a
high impact on the environment because of their large production volumes (Table 2) and the known or
suspected toxicity of many of these compounds. Approximately 4% of the weight of plastics are
additives, and about half of it are plasticizers (16). The additives in plastics have been shown to leach
out during the life cycle of the product (17), and toxicity of leaching to aquatic life has been
demonstrated (18).

<table>
<thead>
<tr>
<th></th>
<th>% total production</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Density Polyethylene (LDPE)</td>
<td>22.3</td>
</tr>
<tr>
<td>High Density Polyethylene (HDPE)</td>
<td>20.4</td>
</tr>
<tr>
<td>Polypropylene (PP)</td>
<td>13.8</td>
</tr>
<tr>
<td>Polyethylene Terephtalate (PET)</td>
<td>9.9</td>
</tr>
<tr>
<td>Polystyrene (PS)</td>
<td>9.0</td>
</tr>
<tr>
<td>Polyvinyl Chloride (PVC)</td>
<td>5.7</td>
</tr>
<tr>
<td>Other</td>
<td>19.0</td>
</tr>
<tr>
<td>Polystyrene (PS)</td>
<td></td>
</tr>
<tr>
<td>Polycarbonate (PC)</td>
<td></td>
</tr>
<tr>
<td>Polyester (PES)</td>
<td></td>
</tr>
<tr>
<td>Polyamides (PA)</td>
<td></td>
</tr>
</tbody>
</table>
3 Plastic litter in the environment

3.1 Routes of distribution

Land-based sources of plastic litter in the oceans are numerous: poorly managed burials in landfills, riverine transport, untreated sewage, inadequate industrial control, storm discharges, wind-blown debris, recreational use of coastal areas, and tourist activities (2). Probably these are the most important sources of marine plastic litter, but important regional variations exist. More litter is found closer to population centres, and the proportion of consumer plastic items such as bottles, shopping bags and personal hygiene products is higher here (1). Ocean-based sources such as shipping and fisheries are important sources in the East Asian Seas region and the southern North Sea. In addition, recreational vessels, cruise liners, merchant shipping, oil and gas platforms and aquaculture contribute to the litter in oceans (1).

The buoyancy and persistence of the plastic material together with ocean circulation greatly affect the distribution of plastic litter over the oceans. Plastic debris is transported by ocean currents and will tend to accumulate in a limited number of sub-tropical convergence zones or gyres. Therefore, it may turn up thousands of miles away from civilization. Computer model simulations, based on satellite-tracked floats since the early 1990s, suggest that the plastic litter may remain in the gyres for many years (1).

Although most plastics that enter the marine environment are buoyant and float on the sea surface, there are numerous reports of sunken plastic debris of all kinds that have settled to the sea floor at all depths from inter-tidal to abyssal environments. The mechanisms by which these materials may reach the deep sea floor are poorly understood, but increased density due to fouling by bacteria, algae, shellfish and other organisms will play a role. Once they have reached the sea floor, particularly in deeper and still waters, they will stay there for ages. The sea floor can thus be considered as the ultimate sink for plastic debris (19).

3.2 Fragmentation: microplastics and nanoplastics

Prolonged exposure to UV light and physical abrasion cause the plastic items to fragment, despite the durability of the polymers (2, 4). Especially on shorelines, photo degradation will make plastic brittle and abrasion through wave action will enhance fragmentation. Fragmentation is of concern because these smaller fragments have the potential to be ingested by a much wider array of organisms, and additionally, they are difficult to remove from the environment. Plastic particles with a diameter smaller than 5 mm are generally designated as microplastics (1). Although it has not been quantified as yet, it is quite likely that also nanoscale particles are produced during weathering of plastic debris (20).

Microplastic particles were observed in water surface samples from the North Sea, Atlantic and Pacific Ocean and ranged from 80 µm to 2 mm (16, 21). Between 1998 and 2006 an increase in microplastics was measured on shorelines in the UK, and similar trends have been observed worldwide in shorelines and riverine environments (2, 16).

As has been mentioned before, microplastics from e.g. washing-clothes and personal care products will enter the environment directly. Via this route also engineered plastic nanoparticles will come into the ecosystem (20).
3.3 Non-polymeric chemicals (additives) and adhering contaminants of microplastics

An important concern relates to some of the non-polymeric compounds in microplastics. For an evaluation of their environmental impact, two types of processes have to be distinguished: 1) leaching of chemicals from the particles and 2) adsorption of persistent toxic contaminants that will accumulate in the particles over time (1).

Leaching to the ecosystem of additives but also of styrene monomers has been described (1, 17, 18, 22, 23). Both have been measured in landfill leachates, or in model experiments. Additives involved are plasticizers (phthalate esters), which form the largest group of polymer additives, organotin compounds, alkylphenols, and bisphenol A (Table 2).

**Table 2**

<table>
<thead>
<tr>
<th>Leachable chemicals from plastics (22, 23).</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Function</strong></td>
</tr>
<tr>
<td>Phthalates</td>
</tr>
<tr>
<td>monomethyl phthalate (MMP)</td>
</tr>
<tr>
<td>dimethyl phthalate (DMP)</td>
</tr>
<tr>
<td>diethylhexyl phthalate (DEHP)</td>
</tr>
<tr>
<td>butylbenzyl phthalate (BBzP)</td>
</tr>
<tr>
<td>monobutyl phthalate (MBP)</td>
</tr>
<tr>
<td>dibutyl phthalate (DBP)</td>
</tr>
<tr>
<td>Alkylphenols</td>
</tr>
<tr>
<td>trisnonylphenol phosphites (TNP)</td>
</tr>
<tr>
<td>nonylphenol (NP)</td>
</tr>
<tr>
<td>octylphenol (OP)</td>
</tr>
<tr>
<td>Bisphenol A (BPA)</td>
</tr>
<tr>
<td>Organotin compounds</td>
</tr>
<tr>
<td>mono- en dialkyltin carboxylates</td>
</tr>
<tr>
<td>tin mercaptans</td>
</tr>
<tr>
<td>tin sulphides</td>
</tr>
<tr>
<td>Polybrominated diphenyl ethers (PBDEs)</td>
</tr>
<tr>
<td>tetabromobisphenol A (TBBPA)</td>
</tr>
</tbody>
</table>

Microplastics can adsorb all kinds of toxic contaminants that are already present in seawater, river water and in sediments. Contaminants able to adsorb to these particles include polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), organochlorine pesticides (e.g. DDT, HCH), together with other persistent organic pollutants (1, 24). These contaminants generally are hydrophobic and therefore have a high affinity for microplastics that is orders of magnitude higher than that for water. In addition, the small particle size (high surface to volume ratio) of microplastics strongly increases the amount adsorbed per gram plastic. As a consequence, microplastics efficiently extract and concentrate contaminants, a phenomenon widely used in analytical chemistry, known as solid phase extraction (SPE). Virgin microplastics (ca. 500 µm) submersed in naturally contaminated seawater in Japan for about a week accumulated PCBs and dichlorodiphenyldichloroethylene (DDE), a metabolite of DDT, in concentrations up to 10⁵ - 10⁶ times that of the surrounding seawater (25). Concentrations found in these microparticles were comparable to those found in microplastics collected from the same area: PCBs, 4 – 117 ng/g; DDE, 0.2 – 3.1 ng/g. The mechanisms explained before, have caused a wide distribution of highly contaminated microplastics (Figure 2).

It is impossible to estimate the environmental and health impact of these chemicals and contaminants, because knowledge on exposure levels is absent (26).
Figure 2  Concentrations of contaminants in microplastics in the North Pacific Central Gyre (solid diamonds) and the Japanese coast of the Pacific Ocean (open circles). PCBs: Polychlorinated biphenyls; DDE: p,p'-dichlorodiphenyl dichloroethene; PAHs: polyaromatic hydrocarbons; PBDEs: polybrominated diphenyl ethers; NP: nonylphenol; OP: octylphenol; BP: bisphenol. (22).
4 Measurement of microplastics

4.1 Introduction

Methods have been specifically developed for measuring microplastics in sediments and seawater. In general the following four main steps are distinguished: density separation, filtration or sieving, visual sorting and identification. Analytical protocols for quantification of microplastics in marine biota such as fish and mussels have not been published, but it may be expected that similar methods can be used once the microplastics have been extracted from these samples. All methodologies include a final step of visual separation of microplastics; in some cases the identity of the isolated microplastics was confirmed by an additional step such as Fourier Transform Infrared (FTIR) spectroscopy.

4.2 Separation and sorting of microplastics

The specific density of plastic particles can vary considerably depending on the type of polymer and the manufacturing process. Density values for plastics range from 0.8 to 1.4 g cm$^{-3}$ while typical densities for sand or other sediments that will be present in the gut of marine biota are about 2.65 g cm$^{-3}$. This difference is exploited to separate the lighter plastic particles from the heavier sediment grains by mixing samples with fresh water (27), seawater (28), a saturated NaCl or NaI solution (1.2 g cm$^{-3}$) (29), or a sodium polytungstate solution (1.4 g cm$^{-3}$) (30), and shaking it for a certain amount of time. Plastics that float in fresh water and seawater are polystyrene foam, high and low density polyethylene, and polypropylene. The plastics that float in the high-density sodium polytungstate solution include flexible and rigid polyvinyl chloride (PVC), polyethylene terephthalate (PET), and nylon (20, 27, 29).

Figure 3  Extracting microplastics from environmental media (31).

Figure 4  Extraction of microplastics from organisms (31).
Extraction of microplastics from organisms can be achieved using acid or enzymatic degradation techniques following isolation of the microplastics from the digests. Although not yet described in the literature, this method was recently used to show the presence of microplastics in blue mussels and lugworms (31).

Size separations of microplastics are achieved using filtration devises or sieves of variable mesh sizes. Filters mostly have pore sizes of 0.45, 1 and 2 µm, simply to collect all microplastics in a suspension. The sieves often have mesh sizes ranging from 0.038 to 4.75 mm and are used singly or in a cascade (28, 29, 32-34). For particles < 1 µm, chromatographic techniques may be used: Hydrodynamic Chromatography (HDC) (35) or Field Flow Fractionation (FFF) (36).

Finally, visual examination of the isolated microplastics remains an obligatory step. Careful visual sorting of residues is necessary to separate the plastics from other materials, such as organic debris (shell fragments, animals parts, dried algae, or sea grasses, etc.) and other items (metal, paint, coatings, tar, glass, etc.). This is done by direct examination of the sample by the naked eye or with the aid of a dissecting microscope (37-39). Due to the diversity of sources, there exists a wide variety of microplastics with multiple shapes, sizes, and origins. Categories used to describe microplastics are; source, shape, erosion and colour.

4.3 Identification of microplastics

Methods like characteristic smoke detection during combustion, solvent extraction, and density determination have been used to identify the polymers of microplastics (32). However, these techniques lack specificity and are prone to miss-identification, a problem that increases considerably with decreasing particle sizes. For that reason, the use of spectroscopic techniques is strongly recommended for small plastic fragments, because they are able to determine the chemical composition of unknown plastic fragments with high reliability.

Microplastics are composed of a variety of polymers (Table 1). Several methods have been employed to characterise the microplastic polymers. Infrared (IR) spectroscopy is most commonly used to compare the IR spectrum of an unknown plastic sample with reference spectra of known polymers (20). Other types of spectroscopy applied are FTIR (27, 30, 33, 40, 41) and near-infrared spectroscopy (42). Over the last few years, a number of applications in life and material sciences has shown that single spot characterization using FTIR micro-spectroscopy and imaging are powerful techniques for the chemical characterization of particles with sizes down to ca. 10 µm (43, 44). With focal plane array detectors, even smaller particles (1-5 µm) may potentially be characterized. As an example, up to 30 single-spot spectra were taken of a black particle; spectra (Figure 5b) of three of these spots (Figure 5a) indicated that the sample was quite homogeneous. For unequivocal chemical particle characterisation and identification, an appropriate infrared spectral database needs to be set up. A range of common polymers like PP, PE, and polyester can be identified by these techniques (13). Another technique, Raman spectroscopy (7) also gives information about the crystalline structure of the polymer (45).

4.4 Quantification of microplastics

Quantitative data of microplastics are expressed in different units, mainly mass and abundance. For environmental samples like sand, sediment and water surface, the most commonly used units for mass are ‘grams of microplastics per m2’ and for abundance ‘microplastic items per m2’ (or items cm−2). For water column samples, mass values are quantified in ‘milligrams per m3’, while abundance is reported as ‘items per m3’. For marine biota as fish and mussels the results are often expressed as ‘pieces of microplastic per animal’. Because of these different units comparisons of data between papers is often difficult.
Figure 5  (a) Microscopic image of a particle. (b) Corresponding mid-infrared spectra of three selected spots (1,2,3) of the sample. Scale bar 100 µm (46).
5 Microplastics in the food chain

5.1 Microplastics in organisms

Microplastics have been detected at the base of the food web in a large variety of planktonic organisms such as zooplankton, chaetognatha, larval fish, copepods, and salps. Also higher trophic levels both invertebrates such as polychaetes, crustaceans, echinoderms, bryozoans, and bivalves and vertebrates such as fish, seabirds and marine mammals are known to ingest microplastics, either directly or via lower trophic levels (47). Altogether, hundreds of species of animals have been documented to contain or ingest microplastics (4, 16, 48, 49).

Mussels have the ability to select particles for uptake. It is not completely known whether selection is based on physical or chemical properties of the particles, nor whether selection is passive or active (50). Exposure of blue mussel to a size range of model HDPE particles (0 - 80 µm, Gaussian distribution) showed that particles were taken up by epithelial cells of the digestive gland, using a semiquantitative method based on polarized light microscopy (47). Studying particles (algae, bacteria) in the range of 2 – 10 µm in various mussel species, it was found that particles > 4 µm were completely retained, whereas the retention efficiency for smaller particles varied between 35 and 70% (51). Model experiments in the blue mussel (Mytilus edulis) with polystyrene microspheres (3 and 10 µm) showed that these accumulated in the gut and were translocated to the circulatory system (52). The smaller particles were more abundant in the circulation, and elimination of a short-term pulse exposure was > 48 days. Simultaneous exposure of blue mussel to 10, 30 and 90 µm microplastic particles (identity of the particles not given) showed that only the 10 µm particles were able to translocate to the circulatory system (53). Thus, these experiments confirm the findings of Brown et al. (52) that translocation of microplastics is inversely related to particle size: 10 µm may be the upper limit for translocation. It was found that blue mussels (Mytilus edulis) and lugworms (Arenicola marina) in a natural environment contained microplastics. Tissue concentrations in mussels were around 2 particles/gram of tissue. Lugworms contained 3 particles/gram of tissue (54). Scallops were able to distinguish between particles of different sizes (range 5 – 20 µm) and densities (range 1.05 – 2.5 g/ml), retaining larger particles longer than smaller ones, and lighter particles longer than denser ones (55). This ability to reject small, dense particles may be of benefit to reject non-feed particles such as silt, but unfortunately will not favour excretion of microplastics.

Apart from microplastics, also nanoplastics may enter the food chain. Uptake of 100 nm polystyrene nanoparticles in mussels has been demonstrated (56, 57). In model experiments it was shown that polystyrene nanoparticles (20 nm) were adsorbed to single-celled green algea (2 – 10 µm) (58). Such a mechanism would enhance the absorption of these bound nanoparticles in scallops, as protein-coated polystyrene particles (6 µm) were retained longer than uncoated particles (59). It was shown that polystyrene nanoparticles (24 nm) were transported through an aquatic food chain from algea, through zooplankton to fish (60).

It is increasingly clear that microplastics are ubiquitously present in the aquatic food chain. Because of the persistent nature of microplastics, it can be predicted that trophic transfer will lead to biological accumulation: the next trophic level will have higher concentrations (16, 61). This could also include transfer and accumulation of contaminants (20). However up until now, accumulation of microplastics or their adhering contaminants in animals has not been quantified.

5.2 Microplastics in foods

To date, quantitative data on the occurrence of microplastics in foods have not been systematically reported in the published literature. However, there is no doubt that bivalves such as mussels and a variety of fishes, sea fish as well as riverine fish, will contain microplastics. The vast amounts of fish
meal produced, are for a large part used in aquaculture, with some use in poultry production and pig rearing. So, food sources of microplastics will be restricted to fish and shellfish.

Recently, for the first time, an attempt was made to quantify microplastics in blue mussels (Mytilus edulis) sampled in a natural environment at six locations along the French-Belgian-Dutch coast. All samples turned out to contain microplastics. Tissue concentrations in mussels were around 2 particles/gram of tissue. These tissue concentrations were low compared to the concentrations present in their environment: the seawater contained about 80 particles/liter and the sediment 40 particles/kg (54). The experimental method used is depicted in Figures 3 and 4, and was based on acid digestion, followed by density separation, filtration, visual microscopic detection, and identification by Raman spectroscopy. Browne et al. exposed blue mussels to polystyrene microparticles of 3 and 10 µm for 3h (65 000 particles/liter seawater), and measured 0.2 particles per µl of haemolymph, using fluorescence microscopy (52). Assuming that an average mussel weighs 5 g and contains 70% water (equated with haemolymph), its content of microplastics can roughly be estimated at 100 particles/gram. These very limited data of microplastics in mussels suggest that contents may vary between 1 and 100 microparticles/gram. It is clear that microplastic contents may vary depending on their concentration and distribution of particles sizes in the seawater.
6 Human impact of microplastics

6.1 Toxicity of microplastics

The literature on microplastics and microparticles is not always clear about the particle sizes involved. In the following discussion, it is useful to make a distinction between microplastics (particle sizes of 0.1 – 5000 µm) and nanoplastics (particles < 0.1 µm). A further distinction will be made in separating the effects of the particles as such, and those of the leachable additives and adhering persistent contaminants.

6.1.1 Microplastic particles

Kinetics
Translocation of various types and sizes of microparticles across the mammalian gut into the lymphatic system has been demonstrated in dozens of studies involving humans (size of particles: 0.2 – 150 µm), dogs (3 – 100 µm) rabbits (0.1 – 10 µm) and rodents (30 nm – 40 µm) (62). These data show that uptake is not very discriminative regarding size and composition of the particles. However, vastly contrasting reports exist on the upper size limits of particles capable of being absorbed and the magnitude of such translocation (62). Major sites of entry are probably the M-cell rich Peyer’s patches in the intestine. In dogs it was shown that PVC particles (5 – 110 µm) also appeared in the portal vein, and thus will reach the liver (63). Because of the size of the particles, Volker (63) excluded translocation through the epithelial layer, and suggested paracellular transport. However, the paracellular pathway is controversial given that junctions between cells are between 7 and 20 nm in diameter (62). Using 2 µm latex particles in various rodents, it was shown that intestinal transport appeared to be small (0.04 – 0.3%)(64). Human mucosal colon tissue mounted in an Ussing chamber also showed very limited transport (< 0.1%) of microparticles (3 µm), however, in patients with inflammatory bowel disease, transport tended to be higher (65).

Via lymph, microparticles may enter the circulation. Entrance into organs is governed by particle size: particles > 1.5 µm will clog the smallest capillaries which are only a few µm in diameter (66). Phagocytosis by macrophages may occur in the circulation (as well in the intestinal lumen), which is believed to occur with particles > 0.5 µm. Endocytosis would be a way of particle transport through cell membranes into cells. Again particle size governs this process: the upper particle size limit for endocytosis generally is 0.5 µm (66). However, it was found that endothelial cells may also internalise large particles of 5 µm by endocytosis (67, 68). Particles > 0.2 µm will be eliminated via the splenic filtration system (66).

Toxicity
In the current literature there are no reports on in vivo or in vitro toxicity studies of microparticles or microplastics. However, it can be predicted that microplastics present in the lumen will surely interact with its complex fluid through adsorptive reactions facilitated by their large-area surfaces and charge: larger proteins and glycoproteins will be adsorbed to the surface of the particles. This may affect the immune system and inflammation of the gut (69). However, it is not known yet whether this may lead to adverse effects.

6.1.2 Nanoplastic particles

Kinetics
Just like microparticles, nanoparticles are absorbed by M cells in Peyer’s patches, but in addition they may pass through intestinal cells by transcytosis or passive diffusion. As a result, absorption of nanoparticles (tested particle size 116 nm) may be 15 – 250 times greater than that of microparticles (70). Unlike microparticles, nanoparticles can enter the capillaries and thus can reach the organs of the body. After intravenous injection of various sized gold nanoparticles (10, 50, 100 and 250 nm) in
rats, the smallest particles appeared to be widespread and were found in the liver, spleen, heart, lungs, brain, thymus, reproductive organs, kidney, and even in the brain. The largest particles were mainly found in the liver and spleen (71). Their presence in the brain demonstrates that they were even able to pass through the very selective blood-brain barrier (72). There is only fragmentary information on excretion and elimination of the particles. As an example, in rats, 95% of the orally administered polymethyl metacrylate particles (120 nm) was eliminated after 2 days (70). The kinetic properties may differ substantially between various types of particles, and their physico-chemical properties (size, surface properties, chemical composition) will play a major role (72). Unfortunately, most studies have only been performed with metal and metal oxide particles, so there is a large gap of information (70).

**Toxicity**

Only limited information is available, mostly obtained with metal and metal oxide particles. In most studies, nanoparticles were administered in artificial dispersions and not via feed, which very likely greatly affects their absorption and behavior in the human body (72). Long-term studies are lacking. Again, physico-chemical properties are likely to be very important, but experimental data are very incomplete. Numerous in vitro studies have found that nanoparticles may induce oxidative stress at high concentrations. Some in vitro studies suggested genotoxic and inflammatory effects (70).

### 6.1.3 Leachable additives

Release of compounds from plastics depends on many factors, e.g. type of polymer (hydrophobic, hydrophilic), pore size, size of the chemical, pH and ionic strength of the leaching fluid (13, 22). It can also be expected that the particle size (surface to volume ratio) of microplastics plays an important role. However, leaching data from microplastics are not available.

Toxicity of major additives (Table 2) has been extensively studied, and there is some concern on disruption of endocrine function by phthalates, bisphenol A and polybrominated diphenyl ethers. These studies have been reviewed by Meeker et al. and Talsness et al. (23, 73). A recent UNEP/WHO report (74) on the state of the science of endocrine disrupting chemicals expressed concerns over endocrine disrupters because of the high incidence and increasing trends of many endocrine-related disorders in humans and of endocrine-related effects in wildlife populations. In addition, chemicals with endocrine disrupting properties linked to disease outcomes in laboratory studies, have been identified in the environment and in foods.

### 6.1.4 Adhering persistent contaminants

A major question is whether these contaminants can be released in the body. There is some evidence in support of uptake of adsorbed contaminants into tissues. Indirect evidence was found in seabirds, because the amount of plastic in the stomach was positively correlated with PCB concentrations in fat tissues (75). In an experimental setting in a species of seabird, ingestion of microplastics with adhering PCBs showed to increase PCB levels in the fat depots (22). These data support plastic-mediated transfer of contaminants to organisms.

Toxicity data of contaminants are well established and have been translated into food regulations of many authorities, e.g. European Union (76).

### 6.2 Potential risks of microplastics in foods

Fish and shellfish will very likely be the major sources of microplastic intake. Although it is well established that these foods will contain microplastics, there is only one very brief report on microplastic particle contents of muscles. So currently, dietary exposure to microplastics cannot be estimated. Because particle toxicity data of microplastics are not available, an estimation of the potential risks of microplastic particles in food is not yet possible.

An estimation of the potential risks of the leachable additives and adhering persistent contaminants is also hampered by this absence of dietary exposure data. In addition, the bioavailability of
contaminants and additives in microplastics is poorly, if at all studied. However, toxicity data of most of these compounds are quite well known (see previous chapter 6.1). In the European Union, these toxicity data of contaminants have been translated into maximum allowed levels in foodstuffs (76). In the previous chapter 5.1 it was roughly estimated that mussels may contain between 1 and 100 microplastic particles/gram. Assuming a particle diameter of 10 µm, and particle density of 1 g/ml, the mass of this particle will be about 200 µg, and the mussels will contain between 0.2 and 20 mg plastic/gram. In Fig 2., PCB concentrations in microplastics were up to 300 ng/gram, and thus the mussel of our example would contain up to 6 ng PCBs/gram. Of course this calculation makes assumptions which should be founded by experimental results, the bioavailability is not accounted for, and the chosen concentration of PCBs is a worst case scenario. Nevertheless, a contribution of microplastic PCBs to dietary exposure does not seem unlikely, as maximum levels of non-dioxin-like PCBs mentioned in the EU regulation are up to 75 ng/gram (76).
Summary and key outcomes

Pollution of the environment with plastics is a growing concern, and because of the durability of plastics this problem is expected to persist for hundreds to thousands of years. Plastic production continues to grow at approximately 9% per year, which means that the quantity of plastics produced in the first 10 years of the current century will approach the total that was produced in the entire preceding century (77). As a result, microplastics, plastic particles with particle sizes < 5 mm, will be ubiquitously present in the aquatic food chain for many years. Because trophic transfer of microplastics undoubtedly takes place, food safety might be affected. An evaluation of the potential health effects of microplastics in foods has to distinguish between the effects of the particles and those of their additives and adhering contaminants. These are the key outcomes of this literature review:

Microplastics in foods
• Fish and shell fish will be the major food sources of microplastics
• Microplastic contents of these foods have not been systematically quantified; only one brief report showed their presence in mussels collected along the Belgian coast.

Toxicity of microplastic particles
• Because of their size, it is not very likely that transport through cellular membranes will occur.
• When entering the blood circulation via lymph, they cannot penetrate deeply into organs and will probably be eliminated via the spleen.
• They will behave quite differently from nanoplastic particles: about 1 µm seems to be a critical particle size for their fate in the body.
• Toxicity studies with microparticles or microplastics have not been reported
• Effects on the immune system and inflammation of the gut certainly need to be explored.

Toxicity of nanoplastic particles
• Absorption may be 15 - 250 times higher than that of microplastic particles.
• They have been shown to be able to translocate through cellular membranes
• They may even pass through the blood-brain barrier, and potentially also the placenta.
• They may reach and penetrate all organs.
• Data on toxicity are largely incomplete
• Toxicity will very likely be highly dependent on their physico-chemical properties, but how?
• Data on nanoparticles are almost exclusively limited to metal and metal oxide particles.

Toxicity of additives and adhering contaminants
• Toxicity of plastic additives as such has been extensively studied, and there is some concern on disruption of endocrine function
• Data on leaching of additives and desorption of contaminants from microplastics in the body are not available
• There is evidence for plastic-mediated transfer of PCBs to fat tissues
• Microplastics may concentrate contaminants such as PCBs up to 10^5 fold
• Toxicity data of contaminants are well established and have been translated into maximum allowed levels in foodstuffs

Health risks of microplastics in foods
Health risks cannot be evaluated yet because essential data are lacking:
• Dietary exposure to microplastics cannot be calculated, because contents of foods are lacking
• Particle toxicity is not known (microparticles) or largely incomplete (nanoparticles)
• Bioavailability of additives and contaminants in plastic particles is not known

A first estimation showed that a contribution of foodborne microplastic PCBs to dietary exposure does not seem unlikely
Measurement of microplastics in foods
- Methods have been designed for sediments. Methods for foods need to be developed

Research priorities for microplastics
Considering the many gaps in our knowledge, research on the potential health risks of food microplastics is needed. The following is proposed:
- Development of a quantitative method to measure microplastics in foods. The method should be able to distinguish between microparticles (0.1 – 5 µm) and nanoparticles (< 0.1 µm). The polymer composition of the particles should be determined. Determination of selected additives and adhering contaminants should be considered.
- Determination of the content of microplastics in fish and shellfish, including specification of their particle sizes
- Estimation of the dietary exposure with microplastics (including particle sizes)
- Determination of the extent of leaching of additives from microplastics in the digestive tract. For this, adequate approaches will have to be developed.
- Determination of the transfer of adhering contaminants to body tissues. For this, adequate approaches will have to be developed.
- Development of research on the toxicity of microplastic and nanoplastic particles
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Microplastics in the aquatic food chain

Sources, measurement, occurrence and potential health risks

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