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## Presence of microplastics and nanoplastics in food, with particular focus on seafood

### EFSA Panel on Contaminants in the Food Chain (CONTAM)

#### Abstract

Following a request from the German Federal Institute for Risk Assessment (BfR), the EFSA Panel for Contaminants in the Food Chain was asked to deliver a statement on the presence of microplastics and nanoplastics in food, with particular focus on seafood. Primary microplastics are plastics originally manufactured to be that size, while secondary microplastics originate from fragmentation. Nanoplastics can originate from engineered material or can be produced during fragmentation of microplastic debris. Microplastics range from 0.1 to 5,000  $\mu\text{m}$  and nanoplastics from approximately 1 to 100 nm (0.001–0.1  $\mu\text{m}$ ). There is no legislation for microplastics and nanoplastics as contaminants in food. Methods are available for identification and quantification of microplastics in food, including seafood. Occurrence data are limited. In contrast to microplastics no methods or occurrence data in food are available for nanoplastics. Microplastics can contain on average 4% of additives and the plastics can adsorb contaminants. Both additives and contaminants can be of organic as well of inorganic nature. Based on a conservative estimate the presence of microplastics in seafood would have a small effect on the overall exposure to additives or contaminants. Toxicity and toxicokinetic data are lacking for both microplastics and nanoplastics for a human risk assessment. It is recommended that analytical methods should be further developed for microplastics and developed for nanoplastics and standardised, in order to assess their presence, identity and to quantify their amount in food. Furthermore, quality assurance should be in place and demonstrated. For microplastics and nanoplastics, occurrence data in food, including effects of food processing, in particular, for the smaller sized particles ( $< 150 \mu\text{m}$ ) should be generated. Research on the toxicokinetics and toxicity, including studies on local effects in the gastrointestinal (GI) tract, are needed as is research on the degradation of microplastics and potential formation of nanoplastics in the human GI tract.

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**Keywords:** microplastic, nanoplastic, food, seafood, occurrence

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## Summary

Following a request from the German Federal Institute for Risk Assessment (BfR), the EFSA Panel for Contaminants in the Food Chain (CONTAM Panel) was asked to deliver a statement on the presence of microplastics and nanoplastics in food, with particular focus on seafood.

With regard to additives and chemical contaminants, this statement includes information up to the possible transfer of these substances into edible tissues and an estimation of the human exposure.

Although there is no legislation for microplastics and nanoplastics as contaminants in food, there are a broad range of European Union (EU) policies and legislation with regard to marine litter, covering sources and impacts and a number of EU initiatives, relevant to marine litter, including microplastics.

### *Microplastics*

There is no internationally recognised definition of microplastics. For this statement, they are defined as a heterogeneous mixture of differently shaped materials referred to as fragments, fibres, spheroids, granules, pellets, flakes or beads, in the range of 0.1–5,000  $\mu\text{m}$ . A distinction can be made between primary and secondary microplastics. Primary microplastics are plastics that were originally manufactured to be that size while secondary microplastics originate from fragmentation of larger items, e.g. plastic debris.

Methods for identification and quantification of microplastics in food, including seafood, have been reported in literature. However, in some of the studies, quality assurance to avoid contamination from the air and equipment is not described, and it is not always clear how a particle is identified as being a 'plastic'. The methods described for microplastics include one or more of the following steps: (i) extraction and degradation of biogenic matter; (ii) detection and quantification (enumeration); and (iii) characterisation of the plastic. Some of the described methods for degradation of the biogenic matter have the drawback that some plastics are degraded to a certain degree. Enumeration is performed by examining the samples with the naked eye or with the aid of a microscope. In the literature, microplastics have been classified or named in several ways, including microfibrils, film spherule, and fragment bead, film. Advanced techniques for the characterisation and identification of the type of plastic are by Fourier transform infrared spectrometry (FT-IR) and Raman spectrometry. Another technique to obtain structural information of the plastic is pyrolysis-gas chromatography/mass spectrometry (GC/MS). Identification is performed by comparison with standard spectra or pyrograms of plastic.

There is no available literature on the fate of microplastics during the processing of seafood. Humans will most often eat cleaned seafood, e.g. fish, where the gastrointestinal tract (GI) is not included. As most of the microplastics will be found in the GI tract, gutting will decrease the exposure compared to eating whole fish. This does not apply to shellfish and certain species of small fish.

Microplastics are likely to originate from other sources than the food itself, e.g. processing aids, water, air or being release from machinery, equipment and textiles, although there is no available literature on this issue. It is therefore possible that the amount of microplastics increases during processing. The effect of other processes, e.g. cooking and baking, on the content of plastics is not known.

Experimental evidence in marine organisms indicates that microplastics have the potential to be transferred between trophic levels. Fish meal has some use in poultry production and pig rearing, hence, microplastics may end up in non-marine foods. Limited data are available on the occurrence of microplastics in foods. Available data are from seafood species, such as fish, shrimp, and bivalves, and also in other foods such as honey, beer and table salt. In studies where the content of microplastics in seafood species has been determined, the microplastic content is given in different units, e.g. number of particles/marine organism or number of particles/g wet weight so it is not always possible to compare results. The concentration of microplastics in marine species is determined in the stomach, GI or the whole digestive tract. In fish, the average number of particles found per fish is between 1 and 7. In shrimp, an average of 0.75 particles/g is found. In bivalves, the average number of particles is 0.2–4 (median value)/g. Average content of microplastics reported for honey are 0.166 fibres/g and 0.009 fragments/g. In beer, fibres, fragments and granules have been found at the following amounts 0.025, 0.033 and 0.017 per mL, respectively. For table salts, microplastic content of between 0.007 and 0.68 particles/g have been found.

Microplastics can contain on average 4% of additives and the plastics can adsorb contaminants. Both additives and contaminants can be of organic as well of inorganic nature and they can be determined using universally accepted analytical methods. Trophic transfer of contaminants, e.g. persistent organic pollutants (POPs), has been reported and biomagnification has been shown. The

main plastic additives and adsorbed contaminants for which some information is available comprise phthalates, bisphenol A, polybrominated diphenyl ethers, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). Concentrations of up to 2,750 ng/g of PCB and 24,000 ng/g of PAHs have been found in microplastic deposited at beaches. Information on metals is scarce and data on other chemical contaminants are lacking.

Bivalves, such as mussels, are eaten without removal of the digestive tract, and thus represent a conservative scenario of microplastic exposure for all fish and other seafood. As an example, the exposure to microplastics was calculated after consumption of a 225 g portion of mussels. Using the highest amount of microplastics found in mussels, this would give an exposure of 900 pieces of microplastic. Assuming spherical microplastics with a diameter of 25  $\mu\text{m}$  and density of 0.92  $\text{g}/\text{cm}^3$ , the exposure would be 7  $\mu\text{g}$  of plastics. Based on the above estimate and considering the highest concentrations of additives or contaminants in the plastics reported and complete release from the microplastics, the portion of mussels would have a small effect on the exposure to PCBs (increase < 0.006%), PAHs (increase < 0.004%) and bisphenol A (increase < 2%).

There is a lack of information on the fate of microplastics in the GI tract. The available data on toxicokinetics only include absorption and distribution, whereas no information is available on metabolism and excretion. Only microplastics smaller than 150  $\mu\text{m}$  may translocate across the gut epithelium causing systemic exposure. The absorption of these microplastics is expected to be limited ( $\leq 0.3\%$ ). Only the smallest fraction (size < 1.5  $\mu\text{m}$ ) may penetrate deeply into organs. There is a lack of knowledge about the local effects of microplastics in the GI tract, including microbiota. Toxicological data on the effects of microplastics as such are essentially lacking for human risk assessment.

For microplastics, it is recommended that analytical methods should be further developed and standardised, in order to assess their presence, identity and to quantify their amount in food. Quality assurance should be in place and demonstrated. Occurrence data in food, including effects of food processing, in particular, for the smaller sized particles (< 150  $\mu\text{m}$ ) should be generated in order to assess dietary exposure. Research on the toxicokinetics and toxicity, including studies on local effects in the GI tract, are needed, in particular, for the smaller sized particles. Research on the degradation of microplastics and potential formation of nanoplastics in the human GI tract are needed.

### *Nanoplastics*

Based on the internationally recognised definition of nanomaterials, nanoplastics can be defined as a material with any external dimension in the nanoscale or having internal structure or surface structure in the nanoscale (0.001–0.1  $\mu\text{m}$ ).

In general, there is very little or no information with regard to nanoplastics for all the areas covered in this Statement.

Nanoplastics can be produced during fragmentation of microplastic debris and can originate from engineered material used, for example in industrial processes.

No analytical methods exist for identification and quantification of nanoplastics in food, thus data on the occurrence in foods are completely lacking. It is expected that the analytical strategy that applies to nanomaterials in general will be applicable.

There is no available literature on the fate of nanoplastics during the processing of seafood. Nanoplastics are likely to originate from other sources than the food itself, e.g. processing aids, water, air or being release from machinery, equipment and textiles, although there is no available literature on this issue. It is therefore possible that the amount of nanoplastics increases during processing. The effect of other processes, e.g. cooking and baking, on the content of plastics is not known.

There is a lack of information on the fate nanoplastics in the GI tract. The available data on toxicokinetics only include absorption and distribution, whereas no information is available on metabolism and excretion. It is not known whether ingested microplastics can be degraded to nanoplastics in the GI tract. Some engineered nanomaterials have shown toxic effects, however, toxicity data for nanoplastics are essentially lacking for human risk assessment and it is not yet possible to extrapolate data from one nanomaterial to the other. Nanoplastics can enter cells; the consequences for human health are unknown.

For nanoplastics, it is recommended that analytical methods should be developed and standardised, in order to assess their presence, identity (including shape) and to quantify their amount in food. Quality assurance should be in place and demonstrated. Occurrence data in food should be generated in order to assess dietary exposure. Research on the toxicokinetics and toxicity are needed.

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## 1. Introduction

### 1.1. Terms of Reference as provided by the requestor

In accordance with Art (29) of Regulation (EC) No 178/2002, the Federal Institute for Risk Assessment (BfR) asks the European Food Safety Authority (EFSA) to provide a scientific opinion on the presence of plastic microparticles and nanoparticles in food, with particular focus on seafood.

In particular, the opinion should:

- 1) deliver an extensive review of the available information on the presence of plastic microparticles and nanoparticles in food, including their potential hazards to human health;
- 2) identify the main data gaps to be filled for the performance of a comprehensive assessment on the risks to human health related to the presence of plastic microparticles and nanoparticles in food, in particular seafood;
- 3) propose research recommendations to fill the data gaps identified under (2).

### 1.2. Interpretation of the Terms of Reference

For plastic microparticles, the term microplastics will be used throughout the statement. There is no universal harmonised term defining the dimensions of microplastics. In the literature, they are generally considered to comprise of a heterogeneous mixture of differently shaped materials referred to as fragments, fibres, spheroids, granules, pellets, flakes or beads, in the range of 0.1–5,000 µm.

The European Union (EU) adopted a definition of a nanomaterial in 2011 to provide a common basis for regulatory purposes across all areas of EU policy (Recommendation on the definition of a nanomaterial (2011/696/EU<sup>1</sup>)). Its provisions include a requirement for review in the light of experience and of scientific and technological developments and the European Commission (EC) is expected to conclude the review in 2016. According to the Recommendation a

“nanomaterial” means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1–100 nm. In specific cases and where warranted by concerns for the environment, health, safety or competitiveness the number size distribution threshold of 50% may be replaced by a threshold between 1 and 50%.

The International Organization for Standardization (ISO) (ISO, 2015) has defined the term nanomaterial as a material with any external dimension in the nanoscale or having internal structure or surface structure in the nanoscale. Nanoscale is defined as ranging from approximately 1 to 100 nm (0.001–0.1 µm). Nanoparticles are defined as nanoobjects with all three external dimensions in the nanoscale where the lengths of the longest and the shortest axes of the nanoobject do not differ significantly. If the dimensions differ significantly (typically by more than three times), terms, such as nanofibre, may be preferred to the term nanoparticle.

In this assessment, the term nanoplastics will be used throughout the statement to indicate any plastic material in the size range complying with the above ISO definition.

With regard to additives and chemical contaminants, the statement includes information up to the possible transfer of these substances into edible tissues and an estimation of the human exposure.

## 2. Methodologies

### 2.1. Collection and appraisal of literature

#### 2.1.1. Strategy for literature search

For the present evaluation, the EFSA Panel for Contaminants in the Food Chain (CONTAM Panel) considered literature made publicly available up to and including 01 February 2016. A comprehensive search for literature was conducted for peer-reviewed original research pertaining to the presence of microplastics and nanoplastics in food, with particular focus on seafood. The search strategy was designed to identify scientific literature on microplastics and nanoplastics in food, covering the

<sup>1</sup> Commission Recommendation 2001/696/EU of 18 October 2011 on the definition of nanomaterial. OJ L 275, 20.10.2011, p. 38–40.

following areas: methods of analysis, chemistry, processing, occurrence, exposure, toxicity, mode of action, toxicokinetics and human observations (see Appendix A for more details).

The literature search was not restricted to publications in English language, however, literature in other languages was only considered if an English abstract was available. The first literature search was performed in July 2014 and has since been updated in March 2015, October 2015, December 2015 and 1 February 2016. Web of Science<sup>2</sup> and Pubmed<sup>3</sup> were identified as databases appropriate for retrieving literature for the present evaluation. The references resulting from the literature search were imported and saved using a software package (EndNote<sup>4</sup>), which allows effective management of references and citations. Additionally, reviews and relevant scientific evaluations by national or international bodies were also considered.

### 2.1.2. Appraisal of studies

Information retrieved has been reviewed by the CONTAM Working Group on the presence of microplastics and nanoplastics in food, with particular focus on seafood, using expert judgement. Any limitations of the information used are clearly documented in this opinion.

## 3. Assessment

### 3.1. Background information

#### 3.1.1. Microplastics

Microplastics have been subject to several recent reviews (Barnes et al., 2009; Andrady, 2011; Browne et al., 2011; EC, 2011; Law and Thompson, 2014; Wang et al., 2016), with the most comprehensive recent being by Bouwmeester et al. (2015) and GESAMP (2015) which also addressed potential human health effects.

A distinction can be made between primary and secondary microplastics (see Figure 1). Primary microplastics are originally manufactured to be that size and include industrial 'scrubbers' used to blast clean surfaces, plastic powders used in moulding, microbeads in cosmetic formulation as well as spherical or cylindrical virgin resin used during production of plastic products (GESAMP, 2015).

Secondary microplastics are the predominant form and originate from fragmentation of plastic debris floating in the oceans through prolonged exposure to ultraviolet (UV) light and physical abrasion. Secondary microplastics can originate from land-based or sea-based sources. Sea-based sources include fishing equipment and sewage from ships. Land-based sources could be plastic bags, packaging materials or waste from plastic industry. Biofouling of these small-sized fragments causes them to sink to the sea floor at all depth from intertidal to abyssal environments. Microplastics have been detected in a large variety of zooplanktonic organisms and also in higher trophic levels, both invertebrates and vertebrates which are exposed either directly or via lower trophic levels. It has been estimated that the total amount of secondary microplastics emission to the marine environment is 68,500–275,000 tonnes per year (EU, 2016). This can be divided into coastal emission of 54,300–145,000 tonnes per year; inland emission of 500–20,000 tonnes per year and marine emission of 13,700–1,110,000 tonnes per year.

Release of microplastics into the terrestrial environment occurs from personal care products like toothpaste and cleaning agents and textile fibres (e.g. clothes through washing). They are transported to sewer systems, which are not able to remove these particles and thus may enter the marine environment. Other sources are paints and tyres (GESAMP, 2015; EU, 2016).

In addition, atmospheric transport has to be considered as a route of microplastic contamination (Bouwmeester et al., 2015). The top three of polymer types reported in microplastics are polyethylene (PE), polypropylene (PP) and polystyrene. On average, 4% of the weight of plastics is additives (Bouwmeester et al., 2015) and can be both organic and inorganic substances. About half of these additives are plasticisers, such as phthalates, but alkylphenols and bisphenol A also occur. Titanium dioxide nanoparticles as well as barium, sulfur and zinc have been examples of inorganic additives found in microplastics (Fries et al., 2013). Polymers usually also contain remnants of the monomers.

<sup>2</sup> Web of Science (WoS), formally ISI Web of Knowledge, Thomson Reuters. <http://thomsonreuters.com/thomson-reuters-web-of-science/>

<sup>3</sup> PubMed, Entrez Global Query Cross-Database Search System, National Center for Biotechnology Information (NCBI), National Library of Medicine (NLM), Department of the National Institutes of Health (NIH), United States Department of Health and Human Services. <http://www.ncbi.nlm.nih.gov/pubmed/>

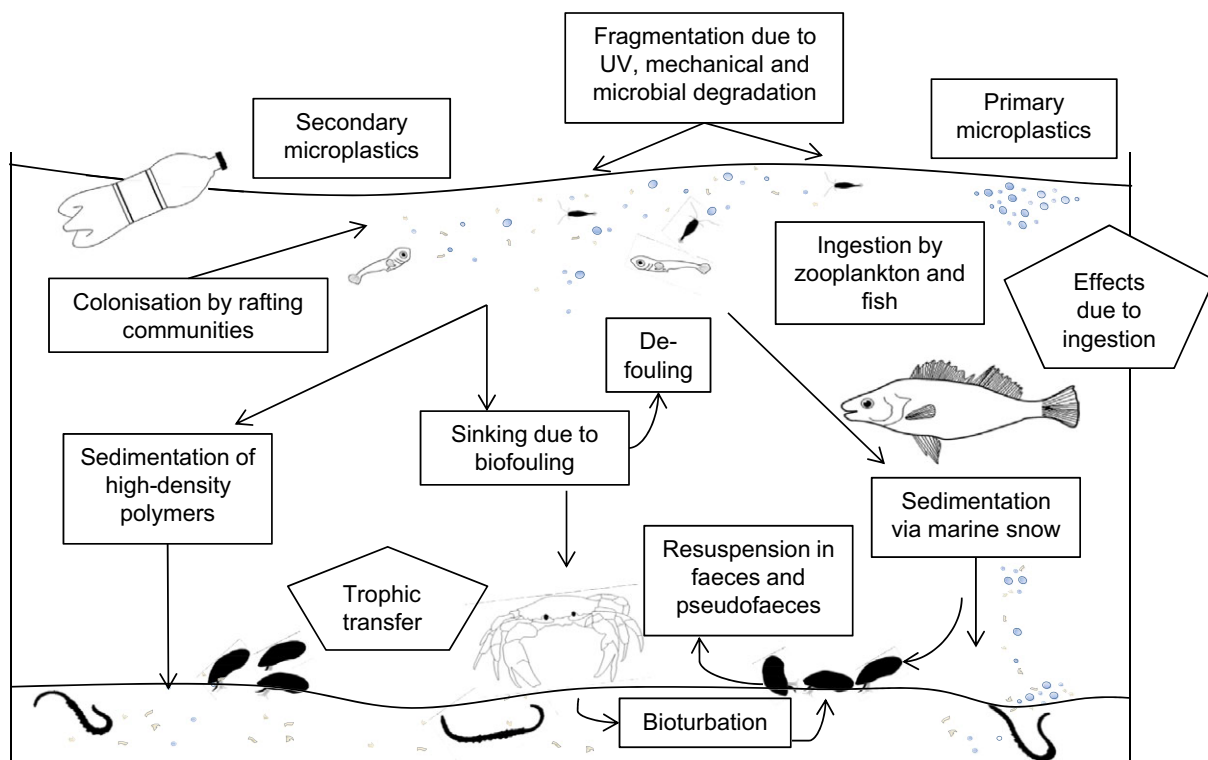
<sup>4</sup> EndNote X5, Thomson Reuters. <http://endnote.com/>

Persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and organochlorine pesticides, which are generally hydrophobic, preferentially adsorb to the surface of the particles, and because of the particle's high surface to volume ratio, the amount adsorbed per gram of plastic may be high (see Sections 3.5 and 3.6 for further details).

In addition, inorganic substances, e.g. metals in water adsorb to microplastics where they may concentrate (see Section 3.5 for further details).

### 3.1.2. Nanoplastics

There is little doubt that nanoplastics will be produced during fragmentation or weathering of microplastic debris (Andrady, 2011; Koelmans et al., 2015). Laboratory experiments showed degradation of polystyrene disposable coffee cup lids with formation of nanoplastics over time (Lambert and Wagner, 2016). Possibly, microbial degradation could also play a role, because several hydrocarbon-degrading microorganisms have been identified to thrive on plastic debris in the oceans (Zettler et al., 2013). The size distribution of floating plastic material in the oceans also suggests that continued fragmentation of microplastics into nanoplastics may occur (Cozar et al., 2014). Finally, engineered nanoplastics are used in a variety of industrial processes and will therefore turn up in the environment (GESAMP, 2015). However, to date, analytical methods for nanoplastics have not been sufficiently developed to confirm their presence in the environment or food chain (Koelmans et al., 2015).



**Figure 1:** Potential pathways for the transport of microplastics and their biological interactions (Wright et al., 2013. © Elsevier)

## 3.2. Legislation, initiatives and assessments

### 3.2.1. Legislation

Although there is no legislation for microplastics and nanoplastics as contaminants in food, there are a broad range of EU policies and legislation with regard to marine litter, covering sources and impacts.

The marine strategy framework directive (MSFD), Directive 2008/56/EC<sup>5</sup> aims to achieve Good Environmental Status (GES) of marine waters in the EU, by 2020. Member States (MS) are required to

<sup>5</sup> Directive 2008/56/EC of the European Parliament and of the Council of 17 June 2008 establishing a framework for community action in the field of marine environmental policy (Marine Strategy Framework Directive). OJ L 164, 25.6.2008, p. 19–40.



develop marine strategies that should lead to achieve GES. Article 3(5) of the directive, defines GES as 'the environmental status of marine waters where these provide ecologically diverse and dynamic oceans and seas which are clean, healthy and productive'. Annex I of the Directive lists 11 qualitative descriptors for determining GES. Descriptor 10 focuses on marine litter and considers that GES will be achieved when 'properties and quantities of marine litter do not cause harm to the coastal and marine environment. To support implementation of the Directive, detailed reports for each descriptor were prepared by task groups; (JRC, 2010). On 1 September 2010, Commission Decision 2010/477/EU<sup>6</sup> was adopted, detailing criteria and indicators to be used by MS for each descriptor; for which, two criteria (see 10.1 and 10.2, below) and four indicators (see 10.1.1–10.1.3 and 10.2.1, below) are provided for descriptor 10:

The distribution of litter is highly variable, which needs to be taken into consideration for monitoring programmes. It is necessary to identify the activity to which it is linked including, where possible, its origin. There is still a need for further development of several indicators, notably those relating to biological impacts and to micro-particles, as well as for the enhanced assessment of their potential toxicity.

### 10.1. Characteristics of litter in the marine and coastal environment

- Trends in the amount of litter washed ashore and/or deposited on coastlines, including analysis of its composition, spatial distribution and, where possible, source (10.1.1)
- Trends in the amount of litter in the water column (including floating at the surface) and deposited on the sea-floor, including analysis of its composition, spatial distribution and, where possible, source (10.1.2)
- Trends in the amount, distribution and, where possible, composition of micro-particles (in particular micro-plastics) (10.1.3)

### 10.2. Impacts of litter on marine life

- Trends in the amount and composition of litter ingested by marine animals (e.g. stomach analysis) (10.2.1).

This indicator needs to be developed further, based on the experience in some sub-regions (e.g. North Sea), to be adapted in other regions'.

With regard to cosmetic products, some of which may contain microplastics (or microbeads), the Regulation EC No 1223/2009<sup>7</sup> stipulates that 'a cosmetic product made available on the market shall be safe for human health'. The product should undergo a safety assessment, which takes into account the anticipated systemic exposure to individual ingredients in a final formulation.

On 28 December 2015, the United States passed the 'Microbead-Free Waters act of 2015'<sup>8</sup> to ban rinse-off cosmetics that contain intentionally-added plastic microbeads (from January 1, 2018), and to ban manufacturing of these cosmetics (from July 1, 2017). For cosmetics, that are over-the-counter drugs, the bans will be delayed by 1 year.

### 3.2.2. Initiatives and assessments

An ongoing, 7th Research Framework Programme (FP7) project, relevant to marine litter and food safety is ECsafeSEAFOOD,<sup>9</sup> (February 2013–January 2017) which aims to assess food safety issues in relation to priority contaminants present in seafood as a result of environmental contamination (including microplastics). The project will also contribute to descriptors 9 and 10 of the MSFD.

The International Council for the Exploration of the sea (ICES), on request from the Oslo and Paris Commission (OSPAR), have developed common monitoring protocols for plastic particles in fish stomachs and selected shellfish.<sup>10</sup>

In 2015, the Danish environmental protection agency published a report (Denmark EPA, 2015) on the occurrence, effect and sources of release to the environment of microplastics in Denmark.

<sup>6</sup> Commission Decision 2010/477/EU of 1 September 2010 on criteria and methodological standards on good environmental status of marine waters (notified under document C(2010) 5956). OJ L 232, 2.9.2010, p. 14–24.

<sup>7</sup> Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products. OJ L 342, 22.12.2009, p. 59–209.

<sup>8</sup> <https://www.congress.gov/bill/114th-congress/house-bill/1321/all-info>

<sup>9</sup> <http://www.ecsafeseafood.eu/>

<sup>10</sup> [http://www.ices.dk/sites/pub/Publication%20Reports/Advice/2015/Special\\_Requests/OSPAR\\_PLAST\\_advice.pdf](http://www.ices.dk/sites/pub/Publication%20Reports/Advice/2015/Special_Requests/OSPAR_PLAST_advice.pdf)

Initiatives and assessment by international organisations relevant to marine litter include the United Nations Environmental programme (UNEP) which in 2014 adopted a resolution on marine plastic debris,<sup>11,12</sup> noting the impact of marine litter (including plastics) on various areas, including potential risk to human health.

The Global partnership on Marine litter (GPML),<sup>13</sup> launched in June 2012 at Rio +20, seeks to protect the environment and human health by reducing and managing marine litter.

The Joint Group of Experts on the Scientific Aspects of Marine Environmental protection (GESAMP),<sup>14</sup> provides advice to UN organisations on pollution and other problems facing marine and environments. The GESAMP Working Group 40, on sources, fates and effects of microplastics in the marine environment recently published its global assessment report (GESAMP, 2015). With regard to risks to human health, it concluded:

'Although it is evident that humans are exposed to microplastics through their diet and the presence of microplastics in seafood could pose a threat to food safety (Van Cauwenberghe and Janssen, 2014), our understanding of the fate and toxicity of microplastics in humans constitutes a major knowledge gap that deserves special attention. Therefore, an analysis and assessment of the potential health risk of microplastics for humans should comprise dietary exposure from a range of foods across the total diet in order to assess the contributing risk of contaminated marine food items'.

### 3.3. Methods to identify and quantify

Methods for the determination of microplastics in foods, including seafood, are described hereafter. Specific methods for nanoplastics have not been described in the literature.

#### 3.3.1. Microplastics

Reference methods for sampling or analysis of microplastics in foods have not been described.

One of the crucial factors in the analytical determinations is to ensure that samples are not contaminated with microplastics from air, clothes, equipment or reagents used in the analysis. Precautions to avoid contamination comprise minimising contact with air as much as possible, e.g. covering of beakers, bottles, sampling equipment, etc., using filtered water and solutions, and careful cleaning of instruments (Liebezeit and Liebezeit, 2013, 2014; Lusher et al., 2013; De Witte et al., 2014; Sanchez et al., 2014; Van Cauwenberghe and Janssen, 2014). Air flow cabinets have also been used to prevent contamination (Foekema et al., 2013; Van Cauwenberghe and Janssen, 2014). Method blanks are essential to ensure analytical quality of the determinations. In some studies, method blanks without samples were subjected to the same treatment as samples. In Section 3.5 on occurrence, Table 1 indicates whether or not method blanks were used in the studies investigating the occurrence of microplastics in seafood and food.

The methods described for microplastics include one or more of the following steps:

- extraction and degradation of biogenic matter;
- detection and quantification (enumeration);
- characterisation of the plastic.

The determination of plastic additives, such as phthalates, bisphenol A, and polybrominated diphenyl ethers, and adsorbed contaminants, such as metals, PAHs and PCBs has been performed in microplastics by numerous authors (e.g. Frias et al., 2010; De Witte et al., 2014; Gauquie et al., 2015) with well-established techniques and which will not be further described here.

The sampling depends on the organism and the study. For instance, mussels were either collected at their natural growing sites, at farms or retail (Mathalon and Hill, 2014). Samples of beer, honey and sugar have been purchased at retail (Liebezeit and Liebezeit, 2013, 2014).

##### 3.3.1.1. Degradation of biogenic matter

Extraction of microplastics from foods can be achieved by the degradation or digestion of biogenic matter before detection and quantification. Several methods have been described. Degradation with

<sup>11</sup> <http://www.unep.org/chemicalsandwaste/Portals/9/Special%20Programme/UNEA%20Special%20Programme%20resolution%201-5%20and%20annex%20II.pdf>

<sup>12</sup> [http://www.unep.org/about/sgb/cpr\\_portal/Portals/50152/K1504068Doc6add4.pdf](http://www.unep.org/about/sgb/cpr_portal/Portals/50152/K1504068Doc6add4.pdf)

<sup>13</sup> <http://unep.org/gpa/gpml/gpml.asp>

<sup>14</sup> <http://www.gesamp.org/about>

30% H<sub>2</sub>O<sub>2</sub> has been used for mussels (Mathalon and Hill, 2014) and honey and sugar (Liebezeit and Liebezeit, 2013). 10% KOH has been used to completely dissolve the digestive tracts of different species of fish (Foekema et al., 2013). HNO<sub>3</sub> (22.5 M) has been used for the analysis of mussels (*Mytilus edulis*) and oysters (*Crassostrea gigas*) (Van Cauwenberghe and Janssen, 2014). A mixture of HNO<sub>3</sub> and HClO<sub>4</sub> was used for mussels (De Witte et al. (2014) and shrimp (Devriese et al., 2015).

Nuelle et al. (2014) compared H<sub>2</sub>O<sub>2</sub> (30% and 35%), NaOH (10, 20, 30, 40 and 50%) and 20% HCl for degradation of biogenic matter from animal or plant material that commonly can be found on a beach, e.g. feathers, bones, leafs. Also, the resistance of common polymers (polyvinyl chloride (PVC), polyethylene terephthalate (PET), nylon 6, acrylonitrile-butadiene-styrene, polycarbonate, polyurethane, PP, low-density polyethylene (LDPE), linear LDPE and high-density polyethylene (HDPE)) to degradation was tested. It was concluded that 30% H<sub>2</sub>O<sub>2</sub> achieved the best digestion (about 50%) of the biogenic matter. However, also changes in the size of some of the microplastics were observed and 35% H<sub>2</sub>O<sub>2</sub> destroyed some of the microplastics. HNO<sub>3</sub> (22.5 M), NaOH (52.5 M) and 30% H<sub>2</sub>O<sub>2</sub> were compared for the digestion of mussels (Claessens et al., 2013). HNO<sub>3</sub> performed better than the other two methods. Good recoveries of spikes of polystyrene spheres and fishing line fibres were obtained after HNO<sub>3</sub> digestion, whereas the nylon fibres were totally degraded upon acid extraction.

The HNO<sub>3</sub> method of Claessens et al. (2013) and the HNO<sub>3</sub>/HClO<sub>4</sub> method of De Witte et al. (2014) were used and compared to determine microplastics in mussels (*M. edulis*) from different waters in Europe (Vandermeersch et al. (2015). Although some differences existed, classification of particle types (fibre, particle, fragment and sphere) and total number of microplastics were not significantly different between the two methods.

The ICES/OSPAR protocol<sup>10</sup> suggestion was based on acid digestion and stated that it may underestimate some polymers.

Cole et al. (2014) compared treatment with HCl, NaOH and enzymes in zooplankton and showed that acid treatment was the least effective. Alkaline treatment caused physical damage and discoloration of microplastics (nylon, PE and unplasticised PVC) and several polyester fibres were lost. Instead, Cole et al. (2014) proposed the use of biogenic degradation with enzymatic treatment (protein kinase-K) that has no influence on the microplastics, e.g. size and at the same time degraded about 97% of the biogenic matter.

### 3.3.1.2. Detection and quantification (enumeration)

Visual examination of the isolated microplastics is mostly performed in every study to distinguish and separate microplastics from other materials, such as organic debris (shell fragments, animal parts, dried algae, etc.) and other items (metal, coatings, tar, glass, etc.). This is performed by the naked eye or with the aid of a microscope. 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. In many studies, dissection or stereomicroscopes were used (e.g. Claessens et al., 2013; Lusher et al., 2013; Devriese et al., 2015). If this visual-assisted microscopy is used, the lower size limit of detection is in the low micrometre range (Hidalgo-Ruz et al., 2012). Also scanning electron microscopic methods have been used (Murray and Cowie, 2011; Fries et al., 2013) and by this even smaller particles can be detected.

Degradation of biogenic matter prior to detection and quantification has not always been performed. Anastasopoulou et al. (2013) and Boerger et al. (2010), e.g. examined intestines and/or stomachs from different species of fish directly under a microscope. Boerger et al. (2010) sorted the contents into natural (plankton) and non-natural (plastic) items and the plastic items were described according to colour, length and shape (fragment, line, foam, pellet or film). Anastasopoulou et al. (2013) only stated that a quite high percentage of the litter consisted of plastic. Also, the digestive tract from gudgeon (*Gobio gobio*) has been analysed by visual inspection under a microscope (Sanchez et al., 2014) and it was stated that not only hard and coloured fibres but also other kinds of microplastic such as transparent fibres and pellets were recorded.

In a study on Norway lobsters (*Nephrops novvegicus*), foregut (stomach) and midgut were removed from the animals and were examined by a microscope (Murray and Cowie, 2011). Identifiable hard foods as shells, fish bones, mud and algae were recorded. Any visible plastic present was also recorded and categorised in three groups: up to five strands; strands and ball; and ball. A ball was defined as when plastic strands had tangled into a ball with any algae present in the stomach, making individual plastic components difficult to quantify. Samples of plastic taken from the stomach contents were processed for viewing using microscopy.

Other studies first digested the samples before visual inspection by microscopy and sometimes enumeration of the number of microplastics (Foekema et al., 2013; Lusher et al., 2013; Mathalon and Hill, 2014; Van Cauwenberghe and Janssen, 2014; Devriese et al., 2015).

Microplastics have been classified or named in several ways. They were described as microplastic fibres (microfibrils) in the study by Mathalon and Hill (2014). In this study, microplastics that were smaller in diameter and brightly coloured were considered to originate from contamination. De Witte et al. (2014) and Devriese et al. (2015) classified the observed microplastic by colour and category (fibre, film spherule and fragment). In the study of Lusher et al. (2013), the items were described according to colour, length and shape (fragment, fibre, bead and film). In the study by Foekema et al. (2013), the number of plastics per fish was counted, and colour and shape were described. Particle sizes were measured at their largest cross-section. A selection of six items representing the major visually distinguishable classes were further analysed to obtain an impression of the polymer composition. Van Cauwenberghe and Janssen (2014), assigned the detected microplastics to one of five classes: 5–10 mm, 11–15 mm, 16–20 mm, 21–25 mm and > 25 mm.

### 3.3.1.3. Characterisation and identification of the plastic chemical composition

Simple methods for characterisation of the plastic material have been applied, such as the 'hot point test' in which a heated needle is used and if plastic, it will melt and the needle leaves a mark (De Witte et al., 2014; Devriese et al., 2015). No attempts were performed to identify the type of plastics.

Liebezeit and Liebezeit (2013, 2014) used a simple staining method to ascertain the nature of the coloured fibres and fragments in sugar, honey and beer. The particulates were stained with fuchsin and Rose Bengal. Synthetic fibres or fragments will not be stained as opposed to non-synthetic fibres/fragments. Non-stained material was referred to as microplastic. However, it was recognised that other methods, e.g. Fourier transform infrared spectrometry (FT-IR) or Raman spectroscopy should have been used to provide definite proof.

Advanced techniques for the characterisation and identification of the type of plastic are FT-IR (Foekema et al., 2013; Lusher et al., 2013), and Raman spectrometry (Murray and Cowie, 2011; Van Cauwenberghe and Janssen, 2014). Another technique to obtain structural information of the plastic is pyrolysis-gas chromatography/mass spectrometry (GC/MS) (Fries et al., 2013; Nuelle et al., 2014). Identification is performed by comparison with standard spectra or pyrograms of plastic.

### 3.3.2. Nanoplastics

Detection of nanoplastics in foods is challenging because the resolution or contrast between nanoplastics and the food matrix is very low, which severely hampers microscopic methods. As a result, imaging by electron microscopy, an obvious option for size-based detection of nanoplastics, would require in most cases prior isolation of the nanoplastics from the sample.

Methods for the determination of nanoplastics in foods have not been developed yet. It is expected that the analytical strategy that applies to nanomaterials in general will be applicable. This approach would require isolation of the nanoplastics from the food matrix, followed by size separation and detection, ideally including both identification and quantification (Rossi et al., 2014). Extraction of the nanoplastics from foods might be achieved by chemical digestion (using approaches similar to those described for microplastics) or enzymatic digestion. After isolation of the nanoplastics, size-based discrimination could be achieved by ultrafiltration or other separation methods such as flow field fractionation (FFF) and hydrodynamic chromatography (HDC). FFF might be used in combination with spectrometry for online detection; otherwise size fractions might be collected offline and studied further with mass spectrometry, to identify the chemical composition of the particles, or electron microscopy.

So far, characterisation of nanoplastics and polymer nanoparticles not embedded in complex matrices has been reported by transmission electron microscopy (TEM) (Velzeboer et al., 2014) and HDC combined with UV detection (Striegel and Brewer, 2012). Nanoparticle tracking analysis (NTA) has been used in laboratory studies on the degradation of polystyrene to nanosized particles (Lambert and Wagner, 2016) and, in principle, also dynamic light scattering (DLS) could be used in similar experiments. Scanning electron microscopy (SEM) with energy dispersive X-ray spectrometry (EDX) and FFF coupled to multiangle light scattering (MALS) with pyrolysis have also been reported (Bouwmeester et al., 2015). The combination of atomic force microscopy (AFM) and infrared (IR) spectroscopy can be used to characterise material in nanoscale including engineered polystyrene (Dazzi et al., 2012). However, the above-mentioned approaches and techniques will have to be developed for the detection of nanoplastics in foods.

### 3.4. Food processing

There are no studies about the fate of micro- or nanoplastics during the processing of seafood. Humans will most often eat cleaned seafood, e.g. fish, where the gastrointestinal (GI) tract is not included. As most of the microplastics will be found in the GI tract, gutting will decrease the exposure compared to eating whole fish. This does not apply to shellfish and certain species of small fish.

Micro- and nanoplastics are likely to originate from other sources than the food itself, e.g. processing aids, water, air or being released from machinery, equipment and textiles, although there is no available literature on this issue. It is therefore possible that the amount of micro- or nanoplastics increases during processing. The effect of other processes, e.g. cooking and baking, on the content of plastics is not known.

### 3.5. Occurrence

#### 3.5.1. Microplastics

##### 3.5.1.1. Trophic transfer in the marine food chain

Microplastics can be ingested by many marine invertebrates as the particles are similar in size to some species of plankton (Browne et al., 2008). Microplastics can also accumulate in sediment (Thompson et al., 2004), and may therefore be available to benthic species. On highly impacted beaches, microplastic concentrations (< 1 mm) can reach 3% by weight, and are a potential substrate for the adherence of organic contaminants (Wright et al., 2013), and colonisation by bacteria (Zettler et al., 2013).

In a laboratory study designed to mimic a coastal ecosystem, Setälä et al. (2016) compared the ingestion of microplastics in marine invertebrates with different feeding habits (bivalves, free-swimming crustaceans and benthic, deposit-feeding organisms). Microbeads (10 µm) were ingested by all organisms, with the highest quantities taken up in bivalves (*Mytilus tossulus* and *Macoma balthica*). The authors concluded that the ingestion of microplastics in marine invertebrates depended on the particle concentration and feeding mode.

Cole et al. (2013) showed that 13 zooplankton taxa had the capacity to ingest 1.7–30.6 µm polystyrene beads, with uptake varying by taxa, life-stage and bead-size. Similarly, Setälä et al. (2014) showed ingestion of 10 µm fluorescent polystyrene microspheres by mysid shrimp, copepods, cladocerans, rotifers, polychaete larvae and ciliates. Polystyrene microspheres (10 µm) were also ingested by polychaetes, bivalves, echinoderms and bryozoans (Ward and Shumway, 2004). Other invertebrates with a range of feeding strategies, including filter feeders (barnacles), deposit feeders (lugworms) and detritivores (amphipods, sea cucumbers), have been shown to ingest microplastics (Thompson et al., 2004; Browne et al., 2008; Graham and Thompson, 2009).

The common mussel (*M. edulis*) can ingest microplastic particles ranging in size from 2 to 10 µm (Ward and Targett, 1989; Ward et al., 2003; Browne et al., 2008).

Experimental evidence indicates that microplastics have the potential to be transferred between trophic levels. Farrell and Nelson (2013) demonstrated that trophic transfer occurs between mussels and crabs. Norway lobsters (*Nephrops norvegicus*) have shown to ingest microplastics via their food, although this did not reflect natural trophic level transfer as they were fed pieces of fish seeded with strands of polypropylene (Murray and Cowie, 2011). Plastic particles found in the scat of fur seals (*Arctocephalus* spp.) were speculated to have been ingested by lantern fish (*Electrona subaspera*), which is common prey for seals (Eriksson and Burton, 2003).

Fish meal has some use in poultry production and pig rearing, hence microplastics may end up in non-marine foods (Bouwmeester et al., 2015).

##### 3.5.1.2. Occurrence of microplastics in seafood and other foods

Microplastics have been reported in seafood (such as fish, shrimp, and bivalves) and also in honey, beer and table salt. Details of studies investigating the microplastic content in these foods are given below and in Table 1.

Boerger et al. (2010) reported that plastic fragments in the centimetre range were found in approximately one third of all fish (2.1 particles/fish) caught in the North Pacific Central Gyre.

In pelagic and demersal fish from the English Channel, the digestive tract of one third of the samples contained microplastics. On average 1.9 particles/fish were reported in the GI tract, ranging from 130 to > 5,000  $\mu\text{m}$  in size (Lusher et al., 2013).

Stomach contents of a variety of commercial fish species from Portugal contained on average  $1.40 \pm 0.66$  particles/fish ( $n = 52$ ), with particle sizes ranging 220–4,800  $\mu\text{m}$  (Neves et al., 2015).

Among the 535 fish collected in freshwater drainages and an estuary of the Gulf of Mexico, 8% of the freshwater fish and 10% of the marine fish had microplastics in their GI tract (Phillips and Bonner, 2015). Percentage occurrence of microplastics ingested by fish in non-urbanised streams (5%) was less than that of one of the urbanised streams (29%). Percent occurrence of microplastics by habitat (i.e. benthic, pelagic) and trophic guilds were similar.

The presence of plastic debris, indicated as anthropogenic debris, in the GI tract of fish on sale for human consumption, sampled from markets in Indonesia and California, USA, was assessed by Rochman et al. (2015). In Indonesian samples, microplastics were found in 28% of individual fish and in 55% of all the 11 species investigated (5.03 particles/fish). Similarly, in the USA, microplastics were found in 25% of individual fish (2.03 particles/fish) and in 67% of all the 12 species investigated. The microplastics recovered from fish in Indonesia were categorised as fragments, foam or film, whereas the microplastics recovered from fish in the USA were primarily fibres.

Rummel et al. (2016) investigated the occurrence of plastics, including microplastic, in pelagic (herring and mackerel) and demersal fish (cod, dab and flounder) from the North Sea and Baltic Sea. Plastic particles were detected in 5.5% of the fish examined, with 74% of all particles being in the microplastic (< 5 mm) size range (1–7 particles/fish) and almost 40% of the particles consisted of PE. Plastic ingestion was significantly higher in pelagic feeders compared to the demersal species (10.7% vs 3.4%). However, it is uncertain whether the fish examined in the various studies consumed the microplastics directly, or it was a result of trophic transfer.

Synthetic fibres with a size range of 200–1,000  $\mu\text{m}$  were detected in brown shrimp (*Crangon crangon*) from various locations at the English Channel. Fibres were found in 63% of the specimens and an average value of  $0.68 \pm 0.55$  microplastics/g ( $1.23 \pm 0.99$  microplastics/shrimp) was obtained (Devriese et al., 2015). Temporal differences were reported, with a higher microplastic uptake in October compared to March.

Microscopic synthetic fibres ranging from 200  $\mu\text{m}$  up to 1,500  $\mu\text{m}$  size were detected in the soft tissues of samples of wild and commercial mussels (*M. edulis*, *Mytilus galloprovincialis*, *M. edulis/galloprovincialis hybrid form*) collected from Belgian coasts (three groynes and three quayside locations) and three Belgian supermarkets (De Witte et al., 2014). Black, red, blue, purple, translucent, transparent, orange, green and yellow fibres, with the most common size class being 1,000–1,500  $\mu\text{m}$ , were detected. The number of total microplastics varied from 0.26 to 0.51 fibres/g of mussel. A higher prevalence of orange fibres at quaysides was put in relation to fisheries activities.

In samples of mussels (*M. edulis*), reared in the North Sea, and Pacific oysters (*C. gigas*), reared in the Atlantic Ocean, an average content of 0.36 and 0.47 particles/g, respectively, was detected (Van Cauwenberghe and Janssen, 2014). After a 3-day depuration period, the microplastic content decreased to 0.24 and 0.35 particles/g in mussels and oysters, respectively. Depuration resulted in the removal of all (mussels) or the majority (oysters) of the largest microplastics (i.e. > 25 mm in length); in mussels, the most abundant microplastics present after gut depuration were the particles ranging from 5 to 10  $\mu\text{m}$  (50%), while in oysters, the most abundant particles were those in the size ranges 11–15  $\mu\text{m}$  (30%) and 16–20  $\mu\text{m}$  (33%).

Higher amounts of microplastics were found in nine species of Chinese commercial bivalves, ranging 2.1–10.5 particles/g (Li et al., 2015). The particle sizes ranged 5–5,000  $\mu\text{m}$ , with 60% of the microplastics in the range of 5–250  $\mu\text{m}$ . Multiple types of microplastics, including fibres, fragments and pellets, occurred in the tissue of all bivalves. Fibres were the most common microplastics and consisted of more than half of the total microplastics in most cases.

In Pacific oysters (*C. gigas*) on sale for human consumption and sampled from markets in California (USA), microplastics were found in 33% of individual shellfish sampled (Rochman et al., 2015). The average length of all fibres recovered from oysters was 5,500  $\mu\text{m}$  and the width ranged 20–50  $\mu\text{m}$ .

Mussels (*M. edulis*) collected at six locations along the French–Belgian–Dutch coastline, after being submitted to a 24 h-clearance in order to allow complete gut emptying, were found to contain on average  $0.2 \pm 0.3$  particles/g (size range 20–90  $\mu\text{m}$ ), with a maximum value of 1.1 particles/g (Van Cauwenberghe et al., 2015). To account for potential artefacts due to airborne contamination, microplastic fibres were excluded from counting and thus the microplastic concentrations reported could be underestimated.

Honey samples of different origin, mostly from Germany, were found to contain coloured fibres and fragments (Liebezeit and Liebezeit, 2013). In honey, fibre counts ranged from 0.04/g to 0.66/g, (mean value of  $0.17 \pm 0.15/g$ ), and fragments counts were less abundant (0.009 fragments/g). An environmental origin, that is particles having been transported by the bees into the hive, or having been introduced during honey processing or both, was suggested. Fibres and fragments were also identified by the same authors in commercial sugar samples. In addition, granular, non-pollen material was observed in both honey and sugar samples.

Fibres, fragments and granular material assumed to be microplastics, were determined in 24 German beer brands (Liebezeit and Liebezeit, 2014). In all cases, contamination was found, with counts ranging from 0.002 to 0.079 fibres/mL, from 0.012 to 0.109 fragments/mL and from 0.002 to 0.066 granules/mL, with a high variability between individual samples and samples from different production dates. The possible origins of these foreign materials were speculated to be airborne atmospheric particles, materials used in the beer production process, unwanted impurities on bottle surfaces and particle contamination of raw materials used for beer production.

In 15 brands of table salt from China, the microplastics content was 0.55–0.68 particles/g in sea salts, 0.043–0.36 particles/g in lake salts, and 0.007–0.20 particles/g in rock/well salts (Yang et al., 2015). In sea salts, fragments and fibres were the prevalent types of particles compared with pellets and sheets. Particle sizes ranged 45–4,300  $\mu\text{m}$ , and microplastics < 200  $\mu\text{m}$  accounted for 55% of the total. The most common types of plastics were PET, followed by PE and cellophane in sea salts. The abundance of microplastics in sea salts was significantly higher than that in lake salts and rock/well salts possibly indicating that marine products, such as salt, are particularly subject to contamination from microplastics.

**Table 1:** Occurrence of microplastics in seafood and food

Food type	Microplastic average content (SD)	Method of analysis	Reference
<i>Fish</i>			
Mesopelagic (five species) and epipelagic (one species) fish, North Pacific Central Gyre	2.1 (5.8) particles/fish (n = 235) Size: > 10,000 $\mu\text{m}$ (cm range, 1–10 cm)	Stomach contents, detection microscope Method blanks not indicated	Boerger et al. (2010)
Pelagic and demersal fish, English Channel	1.90 (0.10) particles/fish (n = 184), of 504 fish, 184 had microplastics Size: 130 to > 5,000 $\mu\text{m}$	Digestive tract contents, detection naked eye, microplastics removed with tweezers confirmation with FT-IR Method blanks not indicated	Lusher et al. (2013)
Commercial fish, 26 species, Portuguese coast, seven locations	$1.40 \pm 0.66$ particle/fish (n = 52; 17 out of 26 species sampled) Size: 220–4,800 $\mu\text{m}$	Stomach contents, detection microscope, microplastics removed with tweezers, confirmation (subset) with FT-IR Method blanks not indicated	Neves et al. (2015)
Commercial fish from fish markets in California (USA) (12 species) and Sulawesi (Indonesia) (11 species)	California: 2.03 (2.71) <sup>(a)</sup> particles/fish, mainly fibres Sulawesi: 5.03 (6.43) <sup>(a)</sup> particles/fish, mainly fragments, film, foam Size: average 6,300 (SD 6,700) $\mu\text{m}$	Digestive tract contents, extraction/digestion with KOH, microscope (detection limit: > 500 $\mu\text{m}$ ) Method blanks used	Rochman et al. (2015)
Pelagic (two species) and demersal (three species) fish, North Sea, Baltic Sea	1–7 particles/fish (n = 16) Size: < 5,000 $\mu\text{m}$	Gastrointestinal tract contents, filter through sieve (500 $\mu\text{m}$ ), microscope, confirmation with FT-IR Method blanks not indicated	Rummel et al. (2016)

Food type	Microplastic average content (SD)	Method of analysis	Reference
<i>Shrimp</i>			
Brown shrimp ( <i>Crangon crangon</i> ), Southern North Sea, English Channel, 16 locations	0.75 (0.53) <sup>(a)</sup> particles/g wet weight (n = 165) Size: 200–1,000 µm	Extraction/digestion with HNO <sub>3</sub> /HClO <sub>4</sub> , detection/counting microscope, confirmation with hot point test Method blanks used	Devriese et al. (2015)
<i>Bivalves</i>			
<i>Mytilus edulis</i> , commercial mussels, from three Belgian supermarkets. Wild mussels, from Belgian groynes (three locations) and quaysides (three locations)	0.37 (0.22) <sup>(a)</sup> particles/g wet weight (n = 9) Size: 200–1,500 µm	Extraction/digestion with HNO <sub>3</sub> /HClO <sub>4</sub> , detection/counting microscope, confirmation with hot point test Method blanks used	De Witte et al. (2014)
Commercial bivalves: <i>Mytilus edulis</i> , from one location (mussel farm), <i>Crassostrea gigas</i> , from one location (supermarket)	<i>M. edulis</i> : 0.36 (0.07) particles/g wet weight (n = 72) <i>C. gigas</i> : 0.47 (0.16) particles/g wet weight (n = 21) Size: 5–25 µm (55–100%), > 25 µm (0–45%)	Extraction/digestion with HNO <sub>3</sub> , detection/counting microscope, confirmation (subset) with Raman Method blanks used	Van Cauwenberghe and Janssen (2014)
Commercial bivalves (9 species), from a fish market in China	Median 4.0, range 2.1–10.5 particles/g (n = 9) Size: 5–250 µm (60%), 5–5,000 µm (40%)	Extraction/digestion with H <sub>2</sub> O <sub>2</sub> , floatation with NaCl, filtered over 5 µm, detection/counting microscope, confirmation (subset) with µ-FT-IR Method blanks used	Li et al. (2015)
Oysters ( <i>Crassostrea gigas</i> ) commercial, from fish markets in California (USA)	1.8 (1.72) <sup>(a)</sup> particles/oyster (n = 4) Size (mainly fibres): average 5,500 (SD 5,800) µm	Extraction/digestion with KOH, microscope (detection limit: > 500 µm) Method blanks used	Rochman et al. (2015)
<i>Mytilus edulis</i> , French-Belgian-Dutch coastline, six locations	0.2 ± 0.3 particles/g (size range 20–90 µm) Size: 20–90 µm	Extraction/digestion with HNO <sub>3</sub> , detection/counting microscope, confirmation (subset) with Raman Method blanks not indicated	Van Cauwenberghe et al. (2015)
<i>Honey</i>			
19 samples, mostly from Germany, from local supermarkets (eight) or producers (11)	0.166 (0.147) fibres/g (n = 19) Size: 40–9,000 µm 0.009 (0.009) fragments/g (n = 19) Size: 10–20 µm	Filter through sieve (40 µm), digestion with 30% H <sub>2</sub> O <sub>2</sub> , detection counting microscope, confirmation by staining with fuchsin Method blanks not indicated	Liebezeit and Liebezeit (2013)
<i>Beer</i>			
24 German beer brands	0.025 (0.021) fibres/mL (n = 24) 0.033 (0.018) fragments/mL (n = 24) 0.017 (0.016) granules/mL (n = 24) Size: not given	Filtered through sieve (0.8 µm), detection/counting microscope, confirmation with Rose Bengal (organic matter) Method blanks used	Liebezeit and Liebezeit (2014)



Food type	Microplastic average content (SD)	Method of analysis	Reference
<i>Table salt</i>			
15 Chinese brands, from local supermarkets	Sea salts: 0.550–0.681 particles/g (n = 5) Lake salts: 0.043–0.364 particles/g n = 5) Rock/well salts: 0.007–0.204 particles/g (n = 5) Size, (all salts): 45–4,300 $\mu\text{m}$	Dissolved in water, digestion with 30% $\text{H}_2\text{O}_2$ , filtered (5 $\mu\text{m}$ ), detection counting microscope, confirmation with $\mu\text{-FT-IR}$ Method blanks not indicated	Yang et al. (2015)

n: number of samples containing microplastics.; FT-IR: Fourier Transform Infrared spectrometry.  
(a): value calculated from paper.

### 3.5.1.3. Chemical and microbial contamination

#### *Chemical contamination*

Organic contaminants in microplastics may either be introduced during manufacture or adsorbed from the seawater (Teuten et al., 2009). Plastic can concentrate contaminants up to the order of  $10^6$  (Mato et al., 2001), thereby acting as a potential source and vector for these chemicals. In oceans and near coastal areas, concentrations of PCBs,<sup>15</sup> PAHs and organochlorine pesticides (1,1-dichloro-2,2-bis(chlorophenyl)ethylene (DDE)), ranging from 1 to 200 ng/g, 4 to 10,000 ng/g and 0.1 to 250 ng/g, respectively, have been found (Bouwmeester et al., 2015). Globally, in microplastics deposited at beaches, even much higher concentrations have been detected: PCBs 0.01–2,750 ng/g; PAHs 90–24,000 ng/g; 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT) and analogues (1,1-dichloro-2,2-bis(p-chlorophenyl)ethane (DDD), 1,1-dichloro-2,2-bis(chlorophenyl)ethylene (DDE)) 2–1,061 ng/g.<sup>16</sup> Organic contaminants, such as PCBs, have been shown to transfer from plastic to sediment-dwelling organisms (Teuten et al., 2007) and streaked shearwater chicks (Teuten et al., 2009).

Trophic transfer of POPs, for example dioxins, PCBs and polybrominated diphenyl ethers, within the marine food webs, is well-documented and has been reported to be associated with oceanic plastics in some cases (Ogata et al., 2009) and biomagnification of POPs has been shown (Hu et al., 2005). The extent of trophic transfer is dependent on characteristics including the octanol–water partition coefficient ( $K_{ow}$ ) and metabolic transformation rate of the compound (Wan et al., 2005). Other factors to consider for the transfer of microplastic-associated POPs are organism-dependent gut retention times, and the fraction of consumed microplastics that are capable of moving across the gut epithelium and into other tissues or organs.

Fossi et al. (2012) found that 56% of surface neustonic/planktonic samples from the Mediterranean Sea contained microplastic particles. Concentrations of the phthalate di-(2-ethylhexyl) phthalate (DEHP) in planktonic samples from the Ligurian Sea and Sardinian Sea (both in the Mediterranean) were  $18 \pm 44$  ng/g and  $23 \pm 33$  ng/g, respectively. Levels of the metabolite mono-(2-ethylhexyl) phthalate (MEHP) in plankton from the Ligurian Sea and Sardinian Sea were  $12 \pm 124$  ng/g and  $40 \pm 42$  ng/g, respectively. The mean concentration of MEHP in the blubber of stranded fin whales (*Balaenoptera physalus*) in the Mediterranean was 58 ng/g (Fossi et al., 2012).

Inorganic contaminants, such as metals, can also be adsorbed to microplastics in the aquatic environment. Beached pellets collected along the southwestern shores of Britain contained metal concentrations similar to (and in some cases exceeding) those in local estuarine sediments (Holmes et al., 2012). The profile and characteristics of metals adsorbing to PE beads differed somewhat between freshwater and seawater (Holmes et al., 2014). Beached pellets had higher equilibrium partition constants relative to water for several metals than virgin plastic beads from the same polymer (Holmes et al., 2012, 2014). Similarly, adsorption of copper and zinc from seawater was higher for aged (sun-exposed) PVC and polystyrene fragments than for their virgin counterparts (Brennecke et al., 2016). The partitioning coefficients for copper and zinc binding to aged PVC beads in seawater were 850 and 200, respectively, compared with 33 and 32 for virgin PVC pellets (Brennecke et al., 2016). A comparison of metal adsorption of five different materials of virgin microplastic polymers

<sup>15</sup> International Union of Pure and Applied Chemists (IUPAC) numbers 66, 101, 110, 149, 118, 105, 153, 138, 128, 187, 180, 170, 206.

<sup>16</sup> <http://www.pelletwatch.org/maps/map-1.html>

(PET, HDPE, PVC, LDPE, PP) deployed for 12 months in the San Diego Bay area resulted in similar concentrations of most adsorbed metals (aluminium, chromium, manganese, iron, cobalt, nickel, zinc, cadmium and lead) that were measured regardless of the plastic type (Rochman et al., 2014a). The only exception was cadmium, which adsorbed remarkably less to HDPE than to the other four polymers. Also, the deployed microplastics beads continued to adsorb metals throughout the 12-month deployment period. The uniformity of metal adsorption to plastic beads of quite different chemical composition might suggest that the metals bind to ligands on the biofilm and this would also explain the continuous long-term adsorption of metals as the biofilm grew (Rochman et al., 2014a). The uniformly observed greater metal adsorption to beached pellets compared to virgin microplastics could similarly be, at least in part, due to deposition of organic material from biofilm on the former (Brennecke et al., 2016; Rochman et al., 2014a). Weathering may also contribute by potentially increasing the surface area of the plastic particles (Holmes et al., 2012, 2014; Rochman et al., 2014a; Brennecke et al., 2016).

Microplastics could be a vehicle for metal transport in marine and freshwater environments because they have the potential to adsorb considerable concentrations of metals and may remain suspended for long periods of time, allowing distribution with water movements. No studies were identified that have assessed the contribution of metals adsorbed to microplastics in food.

#### *Microbial contamination*

It has been documented that plastic debris can act as a substrate for diverse microbial communities (Harrison et al., 2011, 2014; Zettler et al., 2013; McCormick et al., 2014). Microorganisms, including plastic decomposing organisms and pathogens have been shown to colonise microplastics. Furthermore, in the ocean such communities have been shown to be distinct from microbial communities in the surrounding surface water (Zettler et al., 2013). However, the relevance to food and the consequences to human health are unknown.

### **3.5.2. Nanoplastics**

There is no available information on nanoplastics.

## **3.6. Exposure**

### **3.6.1. Microplastics**

It is evident from Section 3.5, that data on the content of microplastics in food are scarce. For fish, only data on microplastics in the digestive tract are available, and the digestive tract normally is discarded and not consumed. The quantity of microplastics in the edible portion is likely to be negligible for consumer exposure. Bivalves that are filter feeders, such as mussels, accumulate microplastics. In addition, as opposed to fish, their digestive tract is eaten. Therefore, their consumption represents a conservative scenario of dietary exposure to microplastics from seafood in general. Lucas et al. (1995) determined portions sizes of mussels eaten by 21–25 French female volunteers when visiting the cafeteria of a large hospital/research centre, and found that a portion of mussels was on average 200 g (without shells). Assuming that generally men eat 25% more than women, an average adult is estimated to consume 225 g of mussels. According to Table 1, Chinese mussels contained the highest number of microplastics: median value 4 particles/g (Li et al., 2015). Thus, consumption of such a portion of Chinese mussels (225 g) would lead to ingestion of about 900 plastic particles. Assuming spherical particles with an average particle size diameter of 25  $\mu\text{m}$  (Van Cauwenberghe and Janssen, 2014) and a density of 0.92  $\text{g}/\text{cm}^3$  (density of LDPE, the most common polymer type of microplastics (Bouwmeester et al., 2015)), these 900 plastic particles would represent 7  $\mu\text{g}$  of plastics. According to the study on several beer brands by Liebezeit and Liebezeit (2014) (see Section 3.5, Table 1) most of the samples contained low quantities of microplastics.

#### **3.6.1.1. Persistent organic pollutants adhered to microplastics**

An estimate of the exposure to POPs via microplastics ingestion can be obtained from the microplastics occurrence data in bivalves of Li et al. (2015), the amount of microplastics from the consumption of 225 g of Chinese mussels and concentration data on POPs in microplastics. In a conservative scenario, the highest concentrations of organic pollutants measured in microplastics on a global scale would give a prediction of the highest exposure expected. In microplastics deposited at beaches, the highest concentrations have been detected: PCBs up to 2,750  $\text{ng}/\text{g}$  and PAHs up to

24,000 ng/g.<sup>16</sup> In this conservative scenario, the microplastics would lead to ingestion of about 19 pg of the measured PCBs<sup>15</sup> and 170 pg PAHs. In the latest EFSA evaluation on the monitoring of dioxins and PCBs in food and feed available on the European market (EFSA, 2012), EFSA estimated an average exposure to non-dioxin-like PCBs of 0.3–1.8 µg PCBs per day (for a person of 70 kg). Concerning PAHs, EFSA estimated for average EU consumers a median exposure of 3.8 µg per day (EFSA, 2008). Thus, even if it is assumed that the PCBs and PAHs will be completely released from the microplastics, consumption of these mussels would have a small effect on the exposure to PCBs (increase < 0.006%) and PAHs (increase < 0.004%).

### 3.6.1.2. Additives in microplastics

On average, 4% of the weight of the plastics predominantly found in microplastics are additives (see Section 3.1). Therefore, using the same mussel example as above, this portion with 7 µg of microplastics would contain about 0.28 µg of additives (4% of 7 µg of plastic). If bisphenol A is used as an example of an additive, this mussel portion would contain 0.28 µg of bisphenol A. In a conservative scenario we could assume that bisphenol A would be completely released from the microplastic. EFSA estimated an average bisphenol A exposure of adults from dietary and non-dietary sources of 0.19–0.20 µg/kg bw per day (EFSA CEF Panel, 2015). So a 70-kg adult would ingest on average about 14 µg bisphenol A per day. Consequently, the bisphenol A originating from the microplastics of the mussels would only contribute to about 2%, and would be small. The exposure to other additives from microplastics is not expected to be substantially different.

### 3.6.2. Nanoplastics

Because data on nanoplastics in foods are not currently available, exposure cannot be estimated.

## 3.7. Toxicokinetics

Humans may be exposed to micro- and nanoplastics via inhalation and ingestion or topically. There is a lack of information on fate of micro- and nanoplastics in the GI tract. In the case of dietary intake, important questions are whether after ingestion micro- and nanoplastics are confined to the gut lumen or whether translocation across the gut epithelium takes place. Translocation would imply that internal organs and tissues are exposed to these particles. Whether nanoplastics can be formed from degradation of microplastics under the conditions of the human GI tract is not known. The available data on toxicokinetics only include absorption and distribution, whereas no information is available on metabolism and excretion.

### 3.7.1. Uptake kinetics of microplastics

The epithelium of the gut wall represents an important barrier to microplastics, excluding direct transcellular transport. The paracellular route of uptake is also not possible, given that the maximal functional pore size of the connecting tight junction channels is only about 1.5 nm (Alberts et al., 2002). However, uptake via lymphatic tissue, specifically via the microfold (M) cells in the Peyer's patches (Galloway, 2015) after which phagocytosis may occur, or via endocytosis, might be possible. Specific data for microplastics are limited. Particle size is one of the most important factors in determining the extent and pathway of uptake. The upper particle size limit for endocytosis is about 0.5 µm (Yoo et al., 2011). Phagocytosis by macrophages is believed to occur with particles > 0.5 µm (Yoo et al., 2011). The upper size limit for phagocytosis obviously is dictated by the volume of the macrophage. Phagocytosis of 1, 5 and 12 µm polymethacrylate and polystyrene particles was demonstrated in peritoneal macrophages after intraperitoneal injection in mice (Tomazic-Jezic et al., 2001). Probably the Peyer's patches rich in M-cells are the predominant sites of absorption of microplastics (Galloway, 2015).

Translocation across the mammalian gut into the lymphatic system of various types and sizes of microparticles of various composition, ranging from 0.1–150 µm, has been demonstrated in studies involving different species, including humans (size of particles: 0.2–150 µm), dogs (3–100 µm), rabbits (0.1–10 µm) and rodents (30–40 µm) (reviewed by Hussain et al., 2001). PVC particles (5–110 µm) have been detected in the portal vein of dogs (Volkheimer, 1975).

The intestinal absorption of microplastics appears to be small. In various rodents, only 0.04–0.3% of the latex particles (2 µm) used were absorbed (Carr et al., 2012). Similar limited absorption (about 0.2%) of polylactide-co-glycolide microparticles (3 µm) was measured *in vitro* using human mucosal colon tissue mounted in an Ussing chamber. The mucosal colon tissue of patients with inflammatory

bowel disease, showed increased transport (0.45% as compared to 0.2% in healthy controls) due to increased permeability of the gut (Schmidt et al., 2013).

Not much is known on the distribution of microplastics after absorption, but it is known that microparticles  $> 0.2 \mu\text{m}$  that appear in lymph will be eliminated through the splenic filtration system into the gut (Yoo et al., 2011), whereas microparticles in the blood will be removed in the liver by bile, and finally excreted via faeces. Particles  $> 1.5 \mu\text{m}$  are not expected to enter the capillaries of organs, so they will not penetrate into organs (Yoo et al., 2011).

Considering the many factors affecting absorption, such as size, composition, surface charge and hydrophilicity, it is difficult to predict the uptake of the particles.

Summarising, *in vivo* human data on the absorption of microplastics are not available. Mammalian studies have detected microparticles with sizes up to  $150 \mu\text{m}$  in lymph, whereas one study detected PVC particles ( $110 \mu\text{m}$ ) in the portal vein. Thus very likely, microplastics  $> 150 \mu\text{m}$  are not absorbed, and only local effects on the immune system and inflammation of the gut are to be expected. The smaller ones ( $< 150 \mu\text{m}$ ) may lead to systemic exposure, but available data show that absorption was limited ( $\leq 0.3\%$ ). Only the smallest fraction (size  $< 1.5 \mu\text{m}$ ) may penetrate deeply into organs.

### 3.7.2. Uptake kinetics of nanoplastics

A special concern of nanoparticles is their ability to translocate across the lung and gut epithelium, resulting in systemic exposure. Most of the uptake data are obtained with a large variety of nanoparticles, and not specifically with nanoplastics. Polystyrene nanoparticles have been used as model particles for some decades in mammalian *in vivo* and *in vitro* studies. The estimated oral bioavailability of 50 nm polystyrene nanoparticles varied between studies from 0.2% to 2% (Walczak et al., 2015) to 7% (Jani et al., 1990). As with microplastics, there does not seem to be a simple relation between uptake, size and composition of the nanoparticles (Jani et al., 1990, 1992; Hillery et al., 1994; Hillery and Florence, 1996; Hussain et al., 1997, 2001; Walczak et al., 2015). Highly variable uptakes of polystyrene nanoparticles (50–500 nm) have been reported in various *in vitro* intestinal models ranging from 1.5 to 10%, depending on nanoparticle size, surface chemistry and type of *in vitro* model (des Rieux et al., 2007; Kulkarni and Feng, 2013; Walczak et al., 2015). In a direct comparison of the movement of engineered carboxylated polystyrene nanoplastics of 50 and 200 nm across a coculture of Caco-2 (enterocyte-like), HT29-MTX (goblet cell-like) and Raji B (M cell-like) cells, it was found that the transport of 50 nm particles was about two orders of magnitude faster than that of 200 nm particles (Mahler et al., 2012). Furthermore, transport of 200 nm polystyrene particles was temperature dependent and greatly dependent on the presence of M cells while movement of 50 nm particles was independent of these variables (Mahler et al., 2012). Collectively these results indicate that the 200 nm particles were transported by M cells through an energy-dependent process, such as endocytosis, and that the 50 nm particles may have crossed the *in vitro* epithelium via a paracellular route.

In studying the uptake of nanoplastics, the lumen of the GI tract is a complicating factor. Ingested nanoparticles will not remain in a free form in the lumen, and hence absorption may be affected. Nanoparticles can interact with a wide range of molecules, such as proteins, lipids, carbohydrates, nucleic acids, ions, and water present in the GI tract (EFSA Scientific Committee, 2011). Interactions with proteins surround the particles with a so-called 'corona' of proteins (Lundqvist et al., 2008). Polystyrene nanoparticles may form complex coronas that change over time depending on the local environment (Tenzer et al., 2013). The protein corona has been shown to be affected in an *in vitro* model mimicking human digestion causing significantly increased translocation (Walczak et al., 2015). In addition, the dissolved organic matter present in natural waters will adsorb onto the surface of the nanoplastics. The interactions of dissolved organic matter with metal (oxide) nanoparticles has recently been reviewed (Philippe and Schaumann, 2014) showing that it greatly affects agglomeration and deposition.

Once nanoparticles have been absorbed, whole body distribution has been shown. For example, after intravenous injection of various sized gold nanoparticles (10–250 nm) in rats, the smallest particles appeared to be widespread and were found in the liver, spleen, heart, lungs, thymus, reproductive organs, kidney, and even in the brain (i.e. crossed the blood–brain barrier). The largest particles were mainly found in the liver and spleen (De Jong et al., 2008). Some nanoparticles are capable to cross biological barriers and potentially access, e.g., the brain, the testes, the fetus. Using an *ex vivo* human placental perfusion model, fluorescent polystyrene particles with diameters from 50 to 240 nm were found to be taken up by the placenta in a size-dependent manner (i.e. the

transplacental transfer was greater for smaller particles) (Wick et al., 2010). Particles sized 500 nm were mainly retained in the maternal circulation or placental tissue, with a low concentration detected in the fetal circulation.

Summarising, translocation across the epithelium has been demonstrated for many types of nanoparticles which may result in access to many organs, including the brain. In addition to the blood–brain barrier, the placental barrier may also be crossed. Nanoplastics, other than polystyrene particles, have not been studied yet, and it should be realised that uptake and toxicity very much depend on the chemical nature of the material along with size, shape and other physicochemical properties (EFSA Scientific Committee, 2009). Thus, extrapolations from studies on one kind of nanomaterial should be made with caution.

### 3.8. Toxicity of microplastics and nanoplastics

Apart from the already adequately documented toxicity of the chemical moieties that may be released from micro- and nanoplastics, the toxicity of the plastic particles themselves should be considered. In general, after oral ingestion the largest fraction (> 90%) of the ingested micro- and nanoplastics will be excreted via faeces. As described in Section 3.7, only plastic particles smaller than 150 µm (by definition the smallest microplastics and all nanoplastics) may translocate across the gut epithelium, causing systemic exposure.

No peer-reviewed papers on *in vivo* or *in vitro* toxicity studies of microplastics or nanoplastics in rodent species usually used for toxicity studies have been identified by the CONTAM Panel on which to base risk assessment for humans.

An *in vivo* chicken model was used to study effects of nanoplastic particles on uptake of iron (Mahler et al., 2012). A single dose of 2 mg/kg body weight (bw) of 50 nm carboxylated polystyrene particles resulted in a threefold suppression of iron absorption. Interestingly, in chickens orally dosed daily with the same polystyrene particles for 2 weeks prior to measurement of iron uptake, the iron absorption was significantly higher than in the unexposed control birds. The 2 weeks of exposure also caused an increase in the overall volume of villi in the duodenum and this was interpreted by the authors as a compensatory response to an impairment of nutrient absorption and attributed to the enhanced iron absorption (Mahler et al., 2012). In addition, exposure to 2 mg/kg bw per day resulted in periportal accumulation of heterophils and increased density of lymphoid follicles with active germinal centres in the spleen (Mahler et al., 2012). An *in vitro* study using human cell lines suggests that positively charged polystyrene nanoplastic particles can disrupt intestinal iron uptake. Exposure of a coculture of Caco-2 (enterocyte like), HT29-MTX (goblet cell like) and Raji B (M cell like) cells to 50 nm or 200 nm carboxylated polystyrene nanoplastic particles had large but complex effects on transport of iron into and across the epithelial cell layer (Mahler et al., 2012). A concentration of  $1.25 \times 10^{12}$  particles/mL of the 200 nm polystyrene particles stimulated iron transfer across the cell layer by 7.5-fold. Curiously, a lower exposure concentration ( $1.25 \times 10^{10}$  particles/mL) reduced transepithelial iron transport by about -1.7-fold. Exposure to 50 nm particles at  $2 \times 10^{13}$  particles/mL caused a twofold increase in uptake of iron into the cells and a fivefold increase in the transfer of iron across the epithelium. No effects were observed at lower concentrations of the 50 nm particles (Mahler et al., 2012).

Some further information on toxicity of microplastics and nanoplastics can be found in reports on studies of wild marine animals. Polystyrene microspheres ingested by mussels (*M. edulis*) were translocated from the gut into the circulatory system and persisted for over 48 days, however, no toxicological effects were observed despite the presence of microplastics in haemolymph and haemocytes (Browne et al., 2008). Conversely, granulocytoma formation (inflammation), increased number of haemocytes and decreased lysosomal stability were observed in mussels (*M. edulis*) 48 h, after uptake of plastic particles (1–80 µm) into vacuoles in the digestive gland (GESAMP, 2010). Rochman et al. (2014b) conducted a chronic 2-month dietary exposure study in Japanese medaka (*Oryzias latipes* (rice fish)), using plastic pellets. Female fish exposed to PE pellets expressed significantly less Chg H than the control. Della Torre et al. (2014) investigated disposition and toxicity of two different polystyrene nanoparticles in the early development of sea urchin embryos (*Paracentrotus lividus*). Embryos were exposed to either carboxylated polystyrene nanoparticles (PS-COOH) (40 nm) or amino-modified polystyrene nanoparticles (PS-NH<sub>2</sub>; 50 nm). Differences in disposition of the polystyrene nanoparticles were noted. PS-COOH accumulated inside the digestive tracts of the embryos, while PS-NH<sub>2</sub> was more dispersed. Exposure to PS-NH<sub>2</sub> was reported to be more embryotoxic compared to PS-COOH. Findings included thickening and abnormal proliferation of

the ectodermal membrane, incorrect location, incomplete or broken skeletal rods and fractured ectoderm. The authors suggest that the differences in the surface charge of the two polystyrene nanoparticles may cause the differences noted in toxicity. In a study by Cole and Galloway (2015), in Pacific oyster larvae, no effects were noted on larval growth or feeding capacity, following 8 days of exposure to PS-COOH or PS-NH<sub>2</sub> (1 and 10 µm).

In addition to these effects studied, it may be expected that micro- and nanoplastics will most likely interact with the immune system, not in the least because they can be taken up by phagocytic cells. In a study in mussels (*M. galloprovincialis*), decreased phagocytic activity caused by nanoplastics has been described (Canesi et al., 2015), but studies in other species are lacking. Although neither nanoplastics nor microplastics are categorised as chemicals, they may eventually have similar health outcomes involving the immune system, depending on the amount of the material that gains access to the immune system. As for chemicals, immunotoxicity of micro- and nanoplastics may potentially be associated with several adverse outcomes: 1) immunosuppression – decreased host resistance to infectious agents and tumours; 2) immune activation – increased risk of developing allergic and autoimmune diseases; and 3) abnormal inflammatory responses – unresolved inflammation – tissue or organ damage and dysfunction. However, such effects have so far not been reported. Furthermore, it may be expected that diseases related to GI tract could potentially be worsened, since most of the particles will be deposited in the GI tract and may interact with bioprocesses at that site (Powell et al., 2007; Handy et al., 2008), including those in microbiota.

Experiments in rodents on intraperitoneally injected or inhaled microplastics and nanoplastics beads collectively show that they activate T-cells and are phagocytosed by macrophages, which traffic the particles to the lymph nodes (e.g. Tomazic-Jezic et al., 2001; Blank et al., 2013). Some of these effects have been corroborated by *in vitro* studies (Seydoux et al., 2014) and were more pronounced with smaller plastic beads and differed between different polymers. Other potential effects based on *in vitro* studies have been reviewed by Galloway (Galloway, 2015).

In contrast to nanoplastics, the toxicity of engineered nanomaterials, such as metal and metal oxide particles, have been more widely studied and various toxic effects have been found, such as reactive oxygen species (ROS) production and associated inflammation, liver and kidney damage, secondary genotoxic effects and immune effects (reviewed by Bouwmeester et al., 2009). Again, extrapolations from such studies on engineered nanomaterials should be made cautiously, because it is known that toxicity depends on the chemical nature of the material along with size, shape, surface chemistry and charge, and other aspects (reviewed by Bouwmeester et al., 2009).

In conclusion, because of a general lack of experimental data, the risk of toxicity of micro- and nanoplastics after oral uptake in humans cannot be evaluated.

### 3.9. Observations in humans

No studies were identified that address the potential human health effects of microplastics ingested by humans through the food chain.

In an investigation of the effects of coarse bran on small bowel transit time in adults, microplastic beads (barium-labelled polyethylene (PE)) were used as a control. A single dose of 15 g PE in rice pudding hastened the arrival of the label at the colon to the same degree as coarse bran (McIntyre et al., 1997). Although this is a pronounced effect, the dose is highly unrealistic in terms of human exposure through the food chain.

### 3.10. Uncertainties

Uncertainties are indicated throughout the text and the recommendations highlight areas that will help to reduce these.

## 4. Conclusions

### 4.1. Microplastics

- There is no unambiguous and internationally recognised definition of microplastics and for the purpose of this statement they are defined as a heterogeneous mixture of differently shaped materials referred to as fragments, fibres, spheroids, granules, pellets, flakes or beads, in the range of 0.1–5,000 µm.

- Primary microplastics are plastics that were originally manufactured to be that size while secondary microplastics originate from fragmentation of larger items, e.g. plastic debris.
- Plastic additives and adsorbed contaminants can be determined using universally accepted analytical methods.
- The majority of information on microplastics concerns the marine environment.
- Experimental evidence indicates that microplastics have the potential to be transferred between trophic levels.
- The digestive tract of marine organisms contains the largest quantities of microplastics. However, this part is normally discarded before consumption. However, the digestive tract of bivalves, e.g. mussels is eaten. As an example, a conservative estimate of exposure to microplastics after consumption of a portion of mussels (225 g) would be 7 µg of plastics.
- Based on the above estimate and considering the highest concentrations of additives or contaminants in the plastics reported and complete release from the microplastics, the portion of mussels would have a small effect on the exposure to PCBs (increase < 0.006%), PAHs (increase < 0.004%) and bisphenol A (increase < 2%).
- Only microplastics smaller than 150 µm may translocate across the gut epithelium causing systemic exposure. The absorption of these microplastics is expected to be limited ( $\leq 0.3\%$ ). Only the smallest fraction (size < 1.5 µm) may penetrate deeply into organs.

#### 4.1.1. Datagaps

- Limited methods for identification and quantification are available. It should be noted that the described methods for degradation of biogenic matter in foods all have drawbacks as they also degrade the plastics to a certain degree.
- Only limited data are available on the occurrence of microplastics in foods. Available data are from fish, bivalves, crustaceans, honey, beer and salt.
- The main plastic additives and adsorbed contaminants for which some information is available comprise phthalates, bisphenol A, polybrominated diphenyl ethers, PAHs and PCBs. Information on metals is scarce. Data on other chemical contaminants are lacking.
- There is a lack of information on the fate of micro- and nanoplastics in the GI tract. The available data on toxicokinetics only include absorption and distribution, whereas no information is available on metabolism and excretion.
- There are no data on the effect of food processing on microplastics.
- There is a lack of knowledge about the local effects of microplastics in the GI tract, including microbiota.
- Toxicological data on the effects of microplastics as such, are essentially lacking for human risk assessment.

#### 4.2. Nanoplastics

- Based on the internationally recognised definition of nanomaterials, nanoplastics can be defined as a material with any external dimension in the nanoscale or having internal structure or surface structure in the nanoscale. Nanoscale is defined as ranging from approximately 1–100 nm (0.001–0.1 µm).
- Nanoplastics can be produced during fragmentation of microplastic debris and can originate from engineered material used for example in industrial processes.
- It is not yet possible to extrapolate data from one nanomaterial to the other.

#### 4.2.1. Datagaps

- No analytical methods exist for identification and quantification of nanoplastics in food, thus data on the occurrence in foods are completely lacking.
- It is not known whether ingested microplastics can be degraded to nanoplastics in the GI tract.
- Nanoplastics can enter cells; the consequences for human health are unknown.
- Some engineered nanomaterials have shown toxic effects, however, toxicity data for nanoplastics are essentially lacking for human risk assessment.
- For all other areas covered in this statement, there is also a lack of information with regards to nanoplastics.

## 5. Recommendations

### 5.1. Microplastics

- Analytical methods should be further developed and standardised, in order to assess their presence, identity and to quantify their amount in food. Quality assurance should be in place and demonstrated.
- Occurrence data in food, including effects of food processing, in particular for the smaller sized particles (< 150 µm) should be generated in order to assess dietary exposure.
- Research on the toxicokinetics and toxicity, including studies on local effects in the GI tract, are needed in particular for the smaller sized particles.
- Research on the degradation of microplastics and potential formation of nanoplastics in the human GI tract are needed.

### 5.2. Nanoplastics

- Analytical methods should be developed and standardised, in order to assess their presence, identity (including shape) and to quantify their amount in food. Quality assurance should be in place and demonstrated.
- Occurrence data in food should be generated in order to assess dietary exposure.
- Research on the toxicokinetics and toxicity are needed.

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## Abbreviations

AFM	atomic force microscopy
BfR	German Federal Institute for Risk Assessment
CONTAM	Contaminants in the Food Chain
DDE	1,1-dichloro-2,2-bis(chlorophenyl)ethylene
DDD	1,1-dichloro-2,2-bis(p-chlorophenyl)ethane
DDT	1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane
DEHP	di-(2-ethylhexyl) phthalate
DLS	dynamic light scattering

EDX	energy dispersive X-ray spectrometry
FFF	flow field fractionation
FP7	7th Research Framework Programme
FT-IR	Fourier transform infrared spectrometry
GC/MS	gas chromatography/mass spectrometry
GES	Good Environmental Status
GESAMP	The Joint Group of Experts on the Scientific Aspects of Marine Environmental protection
GI	gastrointestinal
GPML	Global partnership on Marine litter
HDC	hydrodynamic chromatography
HDPE	high-density polyethylene
ICES	International Council for the Exploration of the sea
IR	infrared
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemists
$K_{ow}$	octanol–water partition coefficient
LDPE	low-density polyethylene
MALS	multiangle light scattering
MEHP	mono-(2-ethylhexyl) phthalate
MSFD	Marine strategy framework directive
NCBI	National Center for Biotechnology Information
NIH	Department of the National Institutes of Health
NLM	National Library of Medicine
NTA	nanoparticle tracking analysis
OSPAR	Oslo and Paris Commission
PAH	polycyclic aromatic hydrocarbons
PCB	polychlorinated biphenyls
PE	polyethylene
PET	polyethylene terephthalate
POP	persistent organic pollutant
PP	polypropylene
PS-COOH	carboxylated polystyrene nanoparticles
PS-NH <sub>2</sub>	amino-modified polystyrene nanoparticles
PVC	polyvinyl chloride
ROS	reactive oxygen species
SEM	scanning electron microscopy
TEM	transmission electron microscopy
UNEP	United Nations Environmental programme
UV	ultraviolet
WoS	Web of Science

## Appendix A – Literature search

**Table A.1:** Literature search terms

<b>Chemistry and analysis</b>	
Search terms	TOPIC: "plastic particle*" OR microplastic* OR "micro plastic*" OR "micro sized plastic*" "micro-sized plastic* nanoplasic* OR "nano plastic*" OR "nano sized plastic*" "nano-sized plastic*" AND TOPIC: (chemistry OR analysis OR determination OR detection OR identification OR formation OR screening OR ELISA OR immune* OR GC OR GC-MS OR HPLC OR LC-MS OR ICP-MS)
<b>Occurrence, Exposure</b>	
Search terms	TOPIC: "plastic particle*" OR microplastic* OR "micro plastic*" OR "micro sized plastic*" "micro-sized plastic* nanoplasic* OR "nano plastic*" OR "nano sized plastic*" "nano-sized plastic*" AND TOPIC: (occurrence OR exposure OR assessment OR survey OR levels OR concentrate* OR marine OR aquatic OR sea OR seawater OR organisms OR plankton OR seafood OR fish OR sardines OR shrimp OR prawns OR crustacean OR bivalves OR mussels OR cephalopod OR squid OR octopus OR mammal OR bird OR drinking water OR water OR bottled water OR beverage OR food OR vegetable* OR fruit* OR grain OR cereal OR poultry OR chicken OR meat OR eggs OR milk OR pig OR feed OR livestock OR cattle OR cow)
<b>Contaminants/additives</b>	
Search terms	TOPIC: "plastic particle*" OR microplastic* OR "micro plastic*" OR "micro sized plastic*" "micro-sized plastic* nanoplasic* OR "nano plastic*" OR "nano sized plastic*" "nano-sized plastic*" AND TOPIC: (POPs OR dioxins OR transport)
<b>Processing</b>	
Search terms	TOPIC: "plastic particle*" OR microplastic* OR "micro plastic*" OR "micro sized plastic*" "micro-sized plastic* nanoplasic* OR "nano plastic*" OR "nano sized plastic*" "nano-sized plastic*" AND TOPIC: (transport OR processing OR reduction OR cooking OR roasting OR frying OR boiling OR baking OR storage OR storing OR brewing OR food OR beverage OR brewing)
<b>Toxicokinetics</b>	
Search terms	TOPIC: "plastic particle*" OR microplastic* OR "micro plastic*" OR "micro sized plastic*" "micro-sized plastic* nanoplasic* OR "nano plastic*" OR "nano sized plastic*" "nano-sized plastic*" AND TOPIC: (toxicokinetic* OR metabolism OR distribution OR excretion OR absorption OR distribution OR biomarker OR mode of action OR biotransformation OR elimination OR reduction OR detoxification OR transport)
<b>Toxicity</b>	
Search terms	TOPIC: "plastic particle*" OR microplastic* OR "micro plastic*" OR "micro sized plastic*" "micro-sized plastic* nanoplasic* OR "nano plastic*" OR "nano sized plastic*" "nano-sized plastic*" AND TOPIC: (toxicity OR toxi* OR acute OR subacute OR subchronic OR chronic OR mutagen* OR carcino* OR genotox* OR reprotox* OR nephrotox* OR neurotox* OR hepatotox* OR immunotox* OR immune* OR haemotox* OR hematotox* OR haematotox OR cytotox* OR develop* toxicity OR thyroid OR endocri* OR endocrine OR estrogen OR oestrogen OR poisoning OR incidental poisoning OR rat OR mouse OR lab animal OR animal* OR case studies)
<b>Human studies</b>	
Search terms	TOPIC: "plastic particle*" OR microplastic* OR "micro plastic*" OR "micro sized plastic*" "micro-sized plastic* nanoplasic* OR "nano plastic*" OR "nano sized plastic*" "nano-sized plastic*" AND TOPIC: (human adverse effects OR human biomarker OR biological marker OR case studies OR incidental poisoning OR poisoning OR human poisoning OR digestion)