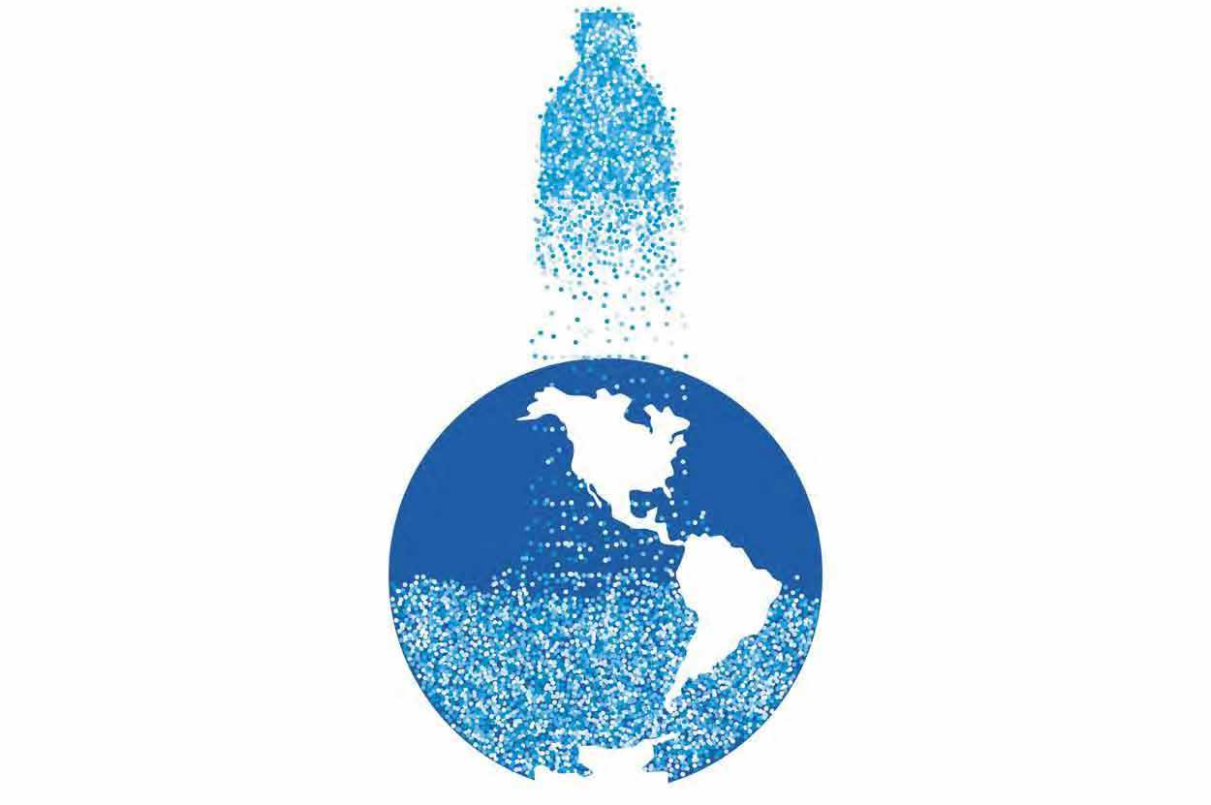
VKM
Vitenskapskomiteen for mat og miljø
Norwegian Scientific Committee for Food and Environment



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Microplastics; occurrence, levels and implications for environment and human health related to food

Opinion of the Steering Committee of the Norwegian Scientific Committee for Food and Environment

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Preparation of the opinion

The Norwegian scientific Committee for Food and Environment (VKM) appointed a project group consisting of two VKM members, eight external experts, and a project leader from the VKM secretariat to answer the Terms of Reference of this VKM self-initiated project.

Authors of the opinion

The members of the project group contributed to the drafting of the opinion (in alphabetical order after chair of the project group):

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VKM would like to thank the referees Senior lecturer Bethanie Carney Almroth (Department of Biological and Environmental Sciences, University of Gothenburg) and Researcher Iurgi Imanol Salaverria-Zabalegui (Department of Biology, Norwegian University of Science and Technology) for reviewing and commenting on the manuscript. VKM emphasises that the two referees are not responsible for the content of the final opinion. In accordance with VKM's routines for approval of a risk assessment, VKM received their comments before evaluation and approval by the Steering Committee, and before the opinion was finalised for publication.

Competence of VKM experts

Persons working for VKM, either as appointed members of the Committee or as external experts, do this by virtue of their scientific expertise, not as representatives for their employers or third-party interests. The Civil Services Act instructions on legal competence apply for all work prepared by VKM.

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Summary

The steering committee of VKM has self-initiated a mandate for an opinion on microplastics based on recently published international and/or national reports complemented with literature from December 2016 to February 2019. The mandate requested a summary of the state of knowledge on the presence of microplastics in the environment and the implications for the ecosystem, terrestrial and aquatic organisms, food production and human health. An overview of main national and international ongoing initiatives was also requested, and highlighting of data gaps where specific Norwegian data was needed.

VKM appointed a working group consisting of two VKM members and eight external experts (two are former VKM-members), in addition to a project leader from the VKM secretariat to write the assessment.

Introduction

Microplastics are global contaminants and have been ubiquitously detected in water, atmosphere, sediments, soils, sewage sludge, biota, and foodstuff, primarily as a result of degradation and fragmentation of larger plastic debris (secondary microplastics). Fragmentation occurs as plastic debris turns brittle due to weathering, especially as a result of solar photodegradation. Due to a large variation in material composition and environmental conditions, the fragmentation kinetics and processes are poorly understood, so there are no reliable estimates of the time to embrittlement of different types of plastics. Nano- and microplastics originally manufactured to be that size (primary microplastics) contribute to a lesser extent. Plastics contain a mixture of chemicals added during manufacture and may also ab/adsorb and act as vectors for persistent, bioaccumulative and toxic contaminants (PBTs) and microorganisms from the environment.

Microplastics have been subject to several recent reviews and risk assessments from international authorities which address both potential environmental and human health effects. (EFSA Panel on Contaminants in the Food Chain (CONTAM) on the presence of nano- and microplastics in food, with particular focus on seafood in 2016 (Alexander et al., 2016), a technical paper on the status of knowledge on microplastics in fisheries and aquaculture from Food and Agriculture Organization of the United Nations (FAO) (Lusher et al., 2017b), and a scientific perspective on microplastics in nature and society (SAPEA, 2019)).

Interpretation of mandate and methods

The summary of knowledge is based on the assessments done by EFSA (2016), FAO (2017) and SAPEA (2019), together with the outcome of a systemic literature search done in May 2018, back to back with the EFSA report. In February 2019, an updated literature search was performed, searching for scientific literature published between May 2018 and February 2019. The literature searches were performed in four different databases (Medline, Scopus, Web of Science and Embase) and resulted in 1330 and 786 unique hits, respectively. Some papers were judged not relevant to the mandate and excluded, and many of the papers were judged to have rather poor quality. Thus a set of quality scores was defined and used, and this resulted in a final acceptance of 270 papers from the search (see chapter 2 and appendix for details on publication selection, quality scoring, and data extraction and

treatment). With the accepted papers from the search as a base for quality secured knowledge and data, more or less conceptual human and environmental risk assessments were attempted. In the human risk assessment, VKM addressed only oral exposure and uptake via the gastrointestinal tract. Uncertainties in each step of the risk assessments were addressed, and knowledge gaps identified. The same quality assured data and knowledge were also used to summarize briefly sources, uses and transport of microplastics with particular focus on Norwegian conditions when excisting knowledge and available data made it possible.

An overview of the main national and international ongoing initiatives is based on information provided by the Norwegian Research Council and corresponding European sources.

To answer to the mandate's request for a summary of knowledge from the recently published reports, and scientific literature, on contamination by microplastics, VKM has started each chapter throughout the report with a short summary of the reports from EFSA (2016), FAO (2017) and SAPEA (2019), followed by any updated knowledge that was found in the literature, on the topic in question. Any conclusions that could be drawn from the literature on the topics addressed in the chapters, and in the terms of reference (TOR), are given in the end of the summaries. These are referred in chapter 12.

Summary of results and discussion

In general:

- VKM acknowledges that there is no international agreed upon definition of nano- and microplastics. Actions should be taken to facilitate a common terminology, taking into account the need for flexibility and adaptiveness as the science evolves. VKM support the proposal of Hartmann *et al.* (Hartmann et al., 2019) which defines nano- and microplastics as
    - consisting of synthetic or heavily modified natural polymers,
    - being solid and insoluble in water at 20°C,
    - being between 1 and 999 µm in size in their largest dimension (for microplastics)
    - nanoplastics is defined as being less than 999 nm (0.999 µm) in size in their largest dimension
- VKM acknowledges that many different approaches are used to study microplastics depending on the matrix of interest. While this is inherent to an evolving field of research, this also poses a challenge to risk assessment as data comparability is limited.

Analytical methods used to characterise occurrence and levels

EFSA (2016) and FAO (2017), and even SAPEA (2019) concluded that there was an urgent need for development and refinement of analytical methods for identification and characterisation of nano- and microplastics in different matrices.

In the present assessment, almost 60% of the scientific papers identified in the literature search (see chapter 2 for details) were not included in the data analysis as they were not of an acceptable quality. This highlights the requirement for researchers to carry out appropriate quality assessment/quality control (QA/QC). Analytical methods should include: sufficient replicates, analytical confirmation of microplastics, determination of recovery rates, blank controls, calculation and consideration of uncertainties/confidence levels.

VKM found:

- Different methodologies are used for sampling across matrices (water, sediment, biota), and a combination of methods is used to separate microplastics from the matrix and remove other matrix components.
- Current methods have certain limitations, in particular with regard to QA/QC.

- VKM acknowledges that techniques are under development to detect and identify increasingly smaller microplastics through automated methods. However, as methods become more complex and sensitive, they have higher chances of procedural contamination, and studies must be quality assured throughout. Further, these methods (e.i. uFTIR, FPA-uFTIR, uRAMAN/RAMAN) are very costly and, thus, unavailable for the larger scientific community.
- VKM acknowledges that quality assurance, method validation, and reporting across all methods are of variable quality. Improving those should become a focus.
- VKM concludes that transparent and good quality reporting is important to generate datasets relevant and usable for risk assessment. For example, if researchers would report the details and uncertainties of their method more transparently, this will allow for harmonisation and better comparability across methods and studies.
- VKM concludes that matrix analyzed as well as reporting metrics are often not suitable for risk assessment. From a food safety perspective, qualitative and quantitative data on the levels of microplastics in the edible tissues of seafood are requested.
- VKM recommends an international harmonisation of microplastics sampling, sample processing, analytical methods and reporting to be initiated for improvement of the quality and comparability between studies. Such harmonisation must not necessarily result in international standards because it will take time to develop and agree on those. A more pragmatic and short-term goal will be the development of quality criteria that the international scientific community agrees upon.

Methods used in experimental studies

EFSA (2016) did not address experimental methods and designs. FAO (2017) and SAPEA (2019) reported a lack of ecological relevance in current experimental designs both with regard to size and shape of nano- and microplastics used as well as exposure concentrations. Acute test scenarios with low ecological relevance was identified as a major uncertainty.

VKM found:

- The experimental designs commonly employed in nano- and microplastic effect studies are currently not well adapted to test the specific toxicity of different plastic materials.
- Most of the laboratory studies are performed using much higher concentrations than are found in the environment, very small spherical microplastics, which are not representative of environmental nano- and microplastics and relatively short exposure times. Thus, it is uncertain to what extent the experimentally derived toxicity data apply to the natural environment. This limits the reliability in a risk assessment.
- Laboratory studies will also need to be adapted to better reflect the natural environment by acknowledging the presence of, and interaction with, naturally occurring nano- and microparticles. Thus, conclusions about effects on the natural environment, that are based on current laboratory experiments are uncertain, and should be confirmed.

➢ VKM acknowledges that although there has been a recent movement towards longer exposure durations, more environmentally relevant test conditions, and the use of particle shapes and particle condition (weathered particles) more representative of those currently identified in the environment, there is still much to be asked for regarding ecological relevance of current tests.

Levels of microplastics

EFSA (2016), FAO (2017) and SAPEA (2019) concluded that available data on nano- and microplastics in the environment and food were mainly qualitative and that quantitative data are very limited. They also stated that there were serious difficulties in data comparisons due to methodological limitations.

VKM found:

- The present literature search revealed some data inconsistencies across the Nordic environment. Most data available are related to surface and subsurface water, and marine biota. There is limited data from freshwater and terrestrial compartments as compared to the marine compartment.
- The use of methods which have not been adequately validated, further complicates the data comparability. There is still no consensus on how data is reported across studies, both in terms of particle sizes and concentrations (i.e., metrics), furthering the difficulties in comparisons. Consequently, this lack of robust estimates on microplastic quantities as well as regional differences in abundance is a source of uncertainty. With many of the investigations implementing visual identification as the only step for microplastic identification/confirmation, this may lead to misestimations of microplastics levels, especially when researchers using confirmatory steps report errors in identification rates reaching 70%.

➢ VKM concludes that data available on levels of microplastics in the Norwegian environment are mostly from the marine compartment (surface and subsurface waters and biota). Limited data only are available from freshwater and terrestrial compartments.

- VKM concludes that very limited data of acceptable quality are available on levels of microplastics in foods. Importantly, many relevant food categories (meat, vegetables, dairy products) have not been investigated at all.

Sources, transport, distribution and fate of microplastics in the environment

EFSA (2016), FAO (2017) and SAPEA (2019) did not go into any details with regard to sources, release and fate of nano- and microplastics. SAPEA (2019) stated that the fate of microplastics in soils and atmosphere was unknown. For information on these issues it is referred to GESAMP (Koehler et al., 2015).

VKM found:

- More information has emerged on freshwater systems than in previous reports. Researchers are still far from understanding the sources, transport processes and sinks of nano- and microplastics on land. This is also true from the transfer of plastics from terrestrial to aquatic systems.
- Marine systems still appear to be the ultimate sink for microplastics in the environment. However, as this will happen on geological time scales freshwater and terrestrial systems are also important recipients and reservoirs of microplastics pollution.
- There is not enough information on sources to infer the quantities/relative contributions of microplastics released by and in Norway.
- From the overview of sources which could contribute to the input of microplastics to the Norwegian and Nordic environment we are able to infer potential sources but currently there is not enough empirical data available for interpretation. MEPEX (2014) provides estimations and assumptions, but further data on sources is required (Sundt et al., 2014).

- VKM concludes that further information is required to understand sources and transport of microplasics in the Nordic/Norwegian environment, and efforts should focus on terrestrial and freshwater systems to increase the knowledge similar to that of the marine systems.

Biofilms and rafting

EFSA (2016) and FAO (2017) both recognized that plastic debris can act as a substrate for diverse microbial communities, including pathogens, but concluded that the relevance to human health still remains unknown. Microbial contamination of microplastic was basically not covered by SAPEA (2019).

VKM found:

- Microplastics biofilms have unique microbial community structures compared to the surrounding environments.
- Microplastics can serve as vectors for microorganisms that are potentially pathogenic to humans, animals or plants.

- Opportunistic human pathogens have been found to be enriched in microplastic biofilm.
- Microplastics biofilms are considered possible hotspots for horizontal gene transfer.
- Several studies have suggested that the plastisphere may contribute to the spread of antibiotic resistance.

➢ VKM concludes that the available information on microplastic biofilms does not provide sufficient basis to characterize potential effects on human health.

Human hazard assessment

EFSA (2016) highlighted that a general lack of information on toxicokinetics and toxicity of nano- and microplastics in human exists. FAO (2017) does not specifically address toxicokinetics in humans, nor does it refer toxicity studies of relevance for human risk assessment. SAPEA (2019) acknowledges that the human microplastics toxicity is uncertain.

VKM found:

- The few studies relevant for human hazard assessment that have become available since EFSA's assessment in 2016 used pristine nano- and microparticles. However, micro- and nano-sized particles present in food are generally not pristine, and the relevance of studies on pristine particles for toxicity of weathered particles present under natural exposure conditions is unknown. The same unceratainty applies for ecotoxicological studies.

➢ VKM concludes that the available information does not provide sufficient basis to characterize potential toxicity in humans.

Environmental hazard assessment

EFSA (2016), FAO (2017) and SAPEA (2019) did not specifically address the toxicokinetics of nano- and microplastics in an environmental context. FAO (2017) stated that little information was available on the internal distribution.

EFSA (2016) did not assess the environmental impacts of nano- and microparticles, while FAO (2017) briefly summarised available knowledge on species relevant to fisheries and aquaculture, especially mollusks, crustaceans and fish. SAPEA (2019) took a qualitative look on the hazards based on published reviews and stated that microplastics can induce physical and chemical toxicity and induce adverse effects on the food consumption, growth, reproduction and survival in a range of species.

VKM found:

- A wide range of species are capable of ingesting nano- and microplastics.
- Translocation from the gastrointestinal tract to organs has been claimed but the extent to which this occurs is unclear due to potential experimental artefacts. Thus, the toxicokinetics of nano- and microplastics remain largely unknown.

- The present systematic literature search extracted toxicity data from 122 peer-reviewed publications.
- Histological evidence of physical injuries caused by nano- and microplastic ingestion are reported by several authors but have been criticised for poor quality. VKM supports this criticism.
- The effects of nano- and microplastics may be the result of a caloric restriction caused by the presence of non-digestible particles. Very few studies actually account for this by analysing the effects caused by non-plastic particles. This, however, would be needed to differentiate between general particle and specific plastic effects.
- The present assessment did not investigate the capacity of nano- and microplastics to act as vectors for hydrophobic contaminants (HOCs) quantitatively, but recognises that contaminant transfer is bi-directional and can either increase or decrease contaminant body burden depending on polymer type, environmental conditions and chemical fugacity gradients. The relative importance of nano- and microplastics as carrier of HOCs is currently estimated to be low compared to other media.
- Species sensitivity distributions (SSDs) using numerical as well as mass-based lowest observed effect concentrations (LOECs) have been constructed from 63 studies covering 39-40 species.
- The predicted no effect concentrations (PNEC) for nano- and microplastics based on the SSDs are 0.14 µg/L (95% confidence interval: 0.04-0.64 µg/L) for mass-based concentrations and 71.6 particles/L (95% confidence interval: 3.45-1991 particles/L) for numerical concentrations.
- These estimates compare reasonably well with previous risk assessments. The somewhat lower HC_5 (PNEC) may be a result of the more extensive and recent dataset used by VKM. HC_5 = hazard concentration 5% level.
- From the SSDs, there is no clear pattern regarding particularly sensitive taxa or levels of biological organisation affected.
- The toxicity data for nanoplastics mainly determine the HC_5 when using mass-based concentrations probably because of their mass-to-particle-number ratio. Accordingly, the HC_5 derived from numerical concentrations is dominated by data from larger microplastics. This highlights that the choice of dose metric affects the hazard assessment.

➢ VKM concludes that the environmental hazard assessment has two major limitations: First, it is pragmatic in a sense that all available toxicity data were included. Second, it treats all nano- and microplastics as one entity, which is clearly ignoring their physico-chemical heterogeneity. The reason not to perform a more differentiated hazard assessment was that this would have resulted in very small datasets. Instead, VKM aimed at gathering as much information as possible.

Summary human exposure

EFSA (2016) and FAO (2017) confirms that microplastics have been found in many seafood species intended for human consumption. However, quanitative data are missing. SAPEA (2019) states that there is sufficient published evidence to say that microplastics occur in bottled water and foodstuff. However, the actual levels are uncertain due to methodological limitations.

- VKM affirms that still very limited data of acceptable quality are available on levels of nano- and microplastics in foods. Thus, VKM concludes that an exposure assessment for human exposure to nano- and microplastics can not be done.

Summary environmental concentrations

EFSA (2016) and FAO (2017) did not define MECs or PECs and did not perform any environmental exposure assessement. SAPEA (2019) refers MEC or PECs from three peer-reviewed articles, but does not define any own MEC or PEC, and did not perform an own exposure assessment.

VKM found:

- Exposure data are still limited and only aggregated levels of large microplastics are reported. Accordingly, the levels of smaller microplastics being underestimated.
- MECs of microplastics were derived from cumulative distributions for the measured environmental concentrations in aquatic ecosystems on a global scale, and a regional scale directly relevant to Norway (Atlantic, Arctic, North Atlantic, North Sea).

- VKM affirms that there is still limited data of acceptable quality on levels of nano- and microplastics in the environment. Most data are available from aquatic ecosystems. MECs were derived from cumulative distributions of the measured concentrations in surface and water columns globally or from locations relevant to Norway.

Summary of conclusions

Human risk characterisation

EFSA (2016), FAO (2017) and SAPEO (2019) conclude that since there is a general lack of exposure and hazard data, the risk of nano- and microplastics to human health cannot be evaluated.

- VKM concludes that the available information does not provide sufficient basis to characterise potential toxicity in humans, based on oral expsosure solely, and that the occurrence data in food is not sufficient to estimate the exposure, and, thus, the risk from micro- and nanoplastics exposure could not be characterised.

Environmental risk characterisation

EFSA (2016) and FAO (2017) do not perform environmental risk characterisation.

SAPEA (2019) concludes that high quality risk assessment is not yet feasible and that there is a need for adequate risk assessment methods that take into consideration the different nature of nano- and microplastics compared to chemical contaminants, as well as their role in a multiple stressor environment. They concluded that an environmental risk of nano- and microplastics were low on a global scale, but that a few very polluted locations existed where a risk may exist.

VKM found:

- The risk characterisation attempted in this report must be considered provisional due to large data gaps. It was only performed for aquatic ecosystems taking into account nano- and microplastics in the surface water and the water column.
- Comparing the PNEC with PECs in different scenarios resulted in risk characterisation ratios (RCRs) of 5.41×10^{-6}, 2.80×10^{-3} and 1.455 for 95, 50 and 5% of locations on a global scale.
- Thus, the environmental risks on nano- and microplastics are low for most locations as the RCRs are well below 1 in most scenarios.
- For the 6% most heavily polluted locations, the RCR is estimated to exceed 1, implying a risk from nano- and microplastics exists at those places.
- When considering only marine ecosystems relevant to Norway, the overall risk is low.
- However, for the highest microplastic levels reported from the Nordic countries (North Sea, Sweden), the RCR is close to 1. This implies that there is a very small margin of safety at Nordic locations that are heavily polluted with microplastics.
- This assessment has a number of limitations that need to be taken into account when interpreting its results.

➢ VKM concludes that available information does not provide sufficient basis to perform a high quality characterisation of risk to the environment by nano- and microplastics. Thus, the attempted present risk characterisation must be considered provisional due to large data gaps. Moreover, it was only performed for aquatic ecosystems (surface water and the water column). On a global scale, the environmental risks are low and only for the 6 % most heavily polluted locations a risk is implied. For marine ecosystems relevant to Norway, the overall risk is also low. For the most heavily polluted locations in the North Sea and Sweden, a potensial risk exists.

Key words: VKM, Norwegian Scientific Committee for Food and Environment, microplastics, human risk assessment, environmental risk assessment, food/feed safety, environment, freshwater, terrestrial, marine water, biofilms, species sensitivity distributions

Sammendrag

I denne selvinitierte rapporten oppsummerer Vitenskapskomiteen for mat og miljø (VKM) kunnskap om forekomst av mikroplast i miljøet, og mulige effekter på økosystemer, terrestriske og akvatiske organismer, matproduksjon og menneskers helse. Rapporten gir også en oversikt over de viktigste pågående nasjonale og internasjonale forskningsprosjektene innen feltet, og synliggjør kunnskapshull spesielt der det er nødvendig med spesifikke norske data.

Hovedbudskapet er at det finnes mikroplast i alle deler av miljøet, og i mat, men at den vitenskapelige kvaliteten på data og kunnskap er for dårlig til at vi kan si noe sikkert om hvilke følger mikroplast har for human helse og miljø. Det mangler kvalitative og kvantitative data om nivåene i de fleste matvarer, og de få toksikologiske studiene som er gjort for å se på giftigheten til mikroplast for mennesker er lite relevante for vurdering av risiko. VKM har konkludert med at tilgjengelig kunnskap og data ikke gir tilstrekkelig grunnlag for å vurdere om inntak av mikroplast via mat påvirker human helse. Det er publisert flere økotoksikologiske studier, men også her er kvaliteten variabel og miljørelevansen er usikker. Selv om det er store kunnskapshull har VKM likevel forsøksvis gjort en pragmatisk risikovurdering for akvatisk miljø der alle tilgjengelige toksisitetsdata er benyttet, og alle nano- og mikroplastpartikler er behandlet som én enhet. Basert på denne sammenstillingen av data (artsfølsomhetsfordeling, SSD), konkluderer VKM med at det på globalt nivå er lav risiko, men at det for de 6 % mest forurensete stedene er indikert en risiko. For marine økosystemer som er relevante for Norge er den samlete risikoen lav. For de mest forurensete stedene i Nordsjøen og Sverige er det en potensiell miljørisiko fra nano- og mikroplast. VKM konkluderer også med at det er behov for mer kunnskap for å forstå kilder og spredning av mikroplast i norsk/nordisk miljø, spesielt terrestrisk- og ferskvannsmiljø.
Når det gjelder nanoplast er kunnskapen og datagrunnlaget enda dårligere enn for mikroplast. Dette skyldes hovedsakelig mangel på metoder til å bestemme forekomst og nivå.

Introduksjon

Mikroplast er en global forurensning, og er påvist både i vann, atmosfære, sedimenter, jordsmonn, kloakkslam, biota og matvarer. Mikroplast er først og fremst et resultat av at plast brytes ned og fragmenteres (sekundær mikroplast). Det skjer når plast blir porøst på grunn av miljøpåvirkning og UV-stråling (sollys). På grunn av stor variasjon i type plast og miljøforhold, er det vanskelig å få tilstrekkelig kunnskap om fragmenteringskinetikk og nedbrytningsprosesser. Det finnes ingen pålitelige estimater for hvor lang tid det tar før ulike typer plast blir sprø og porøse. Nano- og mikroplast som opprinnelig er produsert for å være i mikrostørrelse, (primær mikroplast) bidrar i mindre grad til forurensningen. Plast inneholder en blanding av kjemikalier som tilsettes under fremstillingen, og kan også absorbere og adsorbere, og fungere som vektorer for, persistente, bioakkumulerende og toksiske (PBT) forbindelser, samt mikroorganismer fra miljøet.

De siste årene har internasjonale myndigheter viet mikroplast mye oppmerksomhet, blant annet i rapporter og risikovurderinger som både ser på potensielle miljø- og helseeffekter (EFSA Panel on Contaminants in the Food Chain (CONTAM), om forekomst av nano- og mikroplast i mat, med særlig fokus på sjømat i 2016 (Alexander et al., 2016); en kunnskapsoppsummering om mikroplast i fiskerier og havbruk fra Food and Agriculture Organization of the United Nations (FAO) (Lusher et al., 2017b); og en rapport fra SAPEA som gir et vitenskapelig perspektiv på mikroplast i natur og samfunn (SAPEA, 2019).

Tolking av mandatet, og metoder

Den delen av vurderingen som er en kunnskapsoppsummering, er basert på vurderingene gjort av EFSA (2016), FAO (2017) og SAPEA (2019), sammen med resultatet av et systemisk litteratursøk som ble gjort i mai 2018, opp til, men ikke overlappende med, EFSA-rapporten. I februar 2019 ble det gjort et oppdatert litteratursøk der det ble søkt etter litteratur publisert mellom mai 2018 og februar 2019. Litteratursøkene ble utført i fire forskjellige databaser (Medline, Scopus, Web of Science og Embase) og resulterte i henholdsvis 1330 og 786 unike treff. Deler av litteraturen ble bedømt som ikke relevant for mandatet og derfor ekskludert. Mange av artiklene ble dessuten vurdert til å være av dårlig kvalitet. Det ble derfor definert et sett med kvalitetskrav. Dette resulterte i endelig inklusjon av 270 publikasjoner fra søket (se kapittel 2 og vedlegg for detaljer om utvelgelse av publikasjoner, kvalitetspoeng, dataekstraksjon og -behandling). Med de inkluderte artiklene som en base for kvalitetssikrede data og informasjon, forsøkte vi å gjøre risikovurderinger for mennesker og miljø. I vurderingen av risiko for human helse, så vi bare på oralt inntak, og opptak via mage- og tarmkanalen. I hvert trinn i risikovurderingen identifiserte vi usikkerhet og kunnskapsbehov. Vi brukte de samme kvalitetssikrede dataene og informasjonen til å oppsummere kilder, bruk og distribusjon av mikroplast. Vi hadde særlig oppmerksomhet på norske forhold, der eksisterende kunnskap og tilgjengelige data gjorde det mulig.

Oversikten over de viktigste pågående nasjonale og internasjonale prosjektene er basert på informasjon gitt av Norges forskningsråd og tilsvarende europeiske kilder.

For å svare på mandatets forespørsel om et sammendrag av kunnskap fra nylig publiserte rapporter og vitenskapelig litteratur om mikroplastforurensning, har VKM startet hvert kapittel i vurderingen med en kort oppsummering av rapportene fra EFSA (2016), FAO (2017) og SAPEA (2019), etterfulgt av den oppdaterte informasjonen som ble funnet i litteraturen, om det aktuelle temaet. Eventuelle konklusjoner som kan trekkes fra litteraturen om emnene som er behandlet i kapitlene, og i mandatet, blir gitt i slutten av sammendragene. Disse er referert i kapittel 12.

Sammendrag av resultater og diskusjon

Generelt

- VKM påpeker at det ikke finnes noen internasjonal omforent definisjon av nano- og mikroplast. Det bør iverksettes tiltak for å enes om en felles terminologi, der det tas hensyn til behovet for fleksibilitet og tilpasning etter hvert som vitenskapen utvikler seg. VKM støtter forslaget fra Hartmann *et al.* (2019) som definerer nano- og mikroplast som partikler
    - bestående av syntetiske eller betydelig modifiserte naturlige polymerer
    - som er faste og ikke-løselige i vann ved 20°C
    - som er mellom 1 og 999 µm i størrelse i sin største dimensjon
    - Nanoplast defineres som partikler mindre enn 999 nm (0,999 µm) i størrelse i sin største dimensjon
- VKM påpeker at det brukes mange ulike tilnærminger for å studere mikroplast, avhengig av hva slags omgivelser den finnes i. Selv om dette må forventes i et forskningsfelt som er under utvikling, utgjør dette en utfordring for risikovurdering av mikroplast ettersom det begrenser mulighetene for å sammenligne data.

Analytiske metoder som brukes til å karakterisere forekomst og nivåer

EFSA (2016) og FAO (2017), og også SAPEA (2019), konkluderte med at det var et presserende behov for utvikling og forbedring av analysemetoder for identifisering og karakterisering av nano- og mikroplast i forskjellige typer prøver.

Nesten 60 prosent av de vitenskapelige artiklene som ble identifisert i litteratursøket for VKMs vurdering, ble ikke inkludert på grunn av for dårlig kvalitet. Det synliggjør kravet til forskere om å utføre relevante kvalitetsvurderinger/kvalitetskontroller (QA/QC). Analytiske metoder bør omfatte: tilstrekkelig med replikater, analytisk bekreftelse av mikroplast, bestemmelse av utvinningsgrader, relevante kontroller («blanks»), beregning og vurdering av usikkerhet/konfidensnivå.

VKM fant:

- Det brukes ulike metoder for prøvetaking på tvers av matriser (vann, sediment, biota), og en kombinasjon av metoder for å skille mikroplast fra matrisen og fjerne andre matrisekomponenter.
- Metodene som brukes har visse begrensninger, spesielt med hensyn til vurdering og kontroll av kvalitet.

- VKM påpeker at teknikker for å kartlegge og identifisere stadig mindre mikroplast gjennom automatiserte metoder er under utvikling. Når metodene blir mer komplekse og følsomme, har de imidlertid større sjanser for prosedyreforurensning, og studier må kvalitetssikres gjennomgående. Metodene er dessuten svært kostbare og dermed utilgjengelige for en stor del av det vitenskapelige miljøet.
- VKM påpeker at kvalitetssikring, metodevalidering og rapportering for alle metodene er av variabel kvalitet. Det bør være fokus på forbedring.

- VKM konkluderer med at det er viktig med transparent rapportering av høy kvalitet for å generere datasett som er relevante og anvendelige for risikovurdering. Det vil gi rom for harmonisering og bedre sammenlignbarhet på tvers av metoder og studier.
- VKM konkluderer med at matrisene som er analysert og mengdeangivelser/måleenheter som er benyttet, ofte ikke er egnet for risikovurdering. Fra et mattrygghetsperspektiv etterspørres kvalitative og kvantitative data om nivåene av mikroplast i sjømat.
- VKM foreslår at det settes i gang en internasjonal harmonisering av prøvetakingsmetoder for mikroplast, prøveprosessering, analysemetoder og rapportering for å forbedre kvaliteten og sammenlignbarheten mellom studier. Slik harmonisering må ikke nødvendigvis resultere i internasjonale standarder, fordi det vil ta tid å utvikle og bli enige om disse. Et mer pragmatisk og kortsiktig mål vil være utvikling av kvalitetskriterier som det internasjonale vitenskapelige samfunnet er enige om.

Metoder brukt i eksperimentelle studier

EFSA (2016) tok ikke opp eksperimentelle metoder og studiedesign. FAO (2017) og SAPEA (2019) rapporterte om manglende økologisk relevans i nåværende studiedesign, både med hensyn til størrelse og form på nano- og mikroplast som ble brukt, og på eksponeringskonsentrasjoner. Bruk av akutte testscenarier med lav økologisk relevans ble identifisert som en stor usikkerhetsfaktor.

VKM fant:

- Utformingen av eksperimentelle studier som ofte brukes i nano- og mikroplasteffektstudier er foreløpig ikke godt tilpasset for å teste den spesifikke toksisiteten til forskjellige plastmaterialer.
- De fleste laboratorieundersøkelser er utført ved å bruke mye høyere konsentrasjoner enn det som finnes i miljøet, veldig små, sfæriske mikroplastpartikler som ikke er representative for nano- og mikroplast i miljøet, og relativt korte eksponeringstider. Dermed er det usikkert i hvilken grad de eksperimentelt avledede toksisitetsdataene gjelder for det naturlige miljøet. Dette begrenser påliteligheten i en risikovurdering.
- Laboratorieundersøkelser må også tilpasses for bedre å reflektere det naturlige miljøet ved å ta hensyn til tilstedeværelsen av, og samhandling med, naturlig forekommende nano- og mikropartikler. Konklusjoner om effekter på det naturlige miljøet som er basert på dagens laboratorieeksperimenter, er derfor usikre og bør bekreftes.

- VKM påpeker at selv om utviklingen går i retning av lengre eksponeringsvarighet, mer miljørelevante testforhold, og en utforming av partikler og partikkeltilstand («nedbrytingspartikler») som er mer representative for de som per i dag er identifisert i miljøet, er det fortsatt et stort forbedringspotensial for at aktuelle tester er relevante for human- og miljørisikovurderinger.

Nivåer av mikroplast

EFSA (2016), FAO (2017) og SAPEA (2019) konkluderte med at tilgjengelige data om nano- og mikroplast i miljø og mat hovedsakelig var kvalitative, og at kvantitative data er svært begrensede. De uttalte også at det var store problemer med datasammenligning på grunn av metodologiske begrensninger.

VKM fant:

- Litteratursøket avslørte en del inkonsistens i dataene fra det nordiske miljøet. De fleste tilgjengelige data er relatert til overflatevann og vannsøyle, og marin biota. Det er begrenset med data fra ferskvann og jord sammenlignet med marine omgivelser.
- Bruken av metoder som ikke har blitt tilstrekkelig validert, kompliserer datasammenligningen ytterligere. Det er fremdeles ingen enighet om hvordan data rapporteres på tvers av studier, hverken når det gjelder partikkelstørrelser eller konsentrasjoner (dvs. måleenheter), noe som forsterker vanskelighetene med å sammenligne. Denne mangelen på robuste estimater for mengder av mikroplast, så vel som regionale nivåforskjeller, er kilder til usikkerhet. Mange av undersøkelsene benytter visuell identifikasjon som det eneste trinnet for mikroplastidentifikasjon/-bekreftelse. Dette kan føre til feilvurderinger av mikroplastnivåer. Dette understrekes av at forskere som bruker bekreftende trinn rapporterer feil i identifikasjonsgraden på opptil 70 %.

- VKM konkluderer med at tilgjengelige data på nivåer av mikroplast i norsk miljø stort sett er hentet fra marine omgivelser (overflaten, overflatevann og biota). Det er begrenset med tilgengelige data for ferskvann og jord.
- VKM konkluderer med at det er svært begrenset med tilgjengelige data av akseptabel kvalitet for nivåer av mikroplast i matvarer. Mange relevante matvaregrupper (kjøtt, grønnsaker, meieriprodukter) er ikke undersøkt i det hele tatt.

Kilder, spredning, distribusjon og skjebne for mikroplast i miljøet

EFSA (2016), FAO (2017) og SAPEA (2019) gikk ikke inn på detaljer med hensyn til kilder, frigjøring og skjebne for nano- og mikroplast. SAPEA (2019) uttalte at skjebnen til mikroplast i jord og atmosfære var ukjent. For informasjon om disse problemstillingene henviser VKM til GESAMP (Koehler et al., 2015).

VKM fant:

- Det har nå kommet mer informasjon om nano- og mikroplast i ferskvannssystemer enn det som har vært kjent i de tidligere rapportene. Forskere er fremdeles langt fra å forstå hva som er kildene til nano- og mikroplast, hvordan den distribueres og hvor nano- og mikroplast samles på land. Det gjelder også for overføring av plast fra terrestriske til akvatiske systemer.
- Marine systemer ser fortsatt ut til å være den ultimate «endestasjonen» for mikroplast i miljøet. Siden dette gjelder for en geologisk tidsskala, er ferskvann og landbaserte systemer likevel viktige mottakere og reservoarer for mikroplastforurensning.
- Det finnes for lite informasjon om kilder til å utlede hvilke mengder/relative bidrag til mikroplastforurensning som frigis av og i Norge.
- Fra oversikten over mulige kilder som kan bidra til tilførsel av mikroplast til det norske og nordiske miljøet, kan vi peke ut potensielle kilder, men foreløpig er det ikke nok empiriske data tilgjengelig for tolkning. MEPEX (2014) gir noen estimater og antagelser, men det er nødvendig med ytterligere data om kilder (Sundt et al., 2014).

- VKM konkluderer med at det er nødvendig med mer kunnskap for å forstå kilder og spredning av mikroplast i det nordiske/norske miljøet. Innsatsen bør rettes mot land- og ferskvannssystemer for å heve kunnskapsnivået om disse til samme nivå som for de marine systemene.

Biofilm og rafting

EFSA (2016) og FAO (2017) anerkjente begge at plastrester kan fungere som et substrat for ulike mikrobielle samfunn, inkludert patogener, men konkluderte med at det fremdeles er ukjent hvilken betydning det har for menneskers helse. Mikrobiell forurensning av mikroplast ble i praksis ikke dekket av SAPEA (2019).

VKM fant:

- Biofilmer som vokser på mikroplast har unike mikrobielle samfunnsstrukturer sammenlignet med miljøet rundt.
- Mikroplast kan tjene som vektorer for mikroorganismer som potensielt er sykdomsfremkallende for mennesker, dyr eller planter.
- Opportunistiske humane patogener er funnet å være beriket i biofilm som vokser på mikroplast.
- Biofilmer som vokser på mikroplast anses som mulige «hotspots» for horisontal genoverføring.
- Flere studier har antydet at «plastisfæren» kan bidra til spredning av antibiotikaresistens.

- VKM konkluderer med at tilgjengelig informasjon om biofilmer på mikroplast ikke gir grunnlag for å beskrive potensielle effekter for human helse.

Vurdering av fare for mennesker

EFSA (2016) fremhevet at det generelt er mangel på informasjon om toksikokinetikk og toksisitet av nano- og mikroplast hos mennesker. FAO (2017) så ikke spesielt på toksikokinetikk hos mennesker, og viser heller ikke til toksisitetsstudier av relevans for risikovurdering for mennesker. SAPEA (2019) erkjenner at toksisiteten av mikroplast for mennesker er usikker.

VKM fant:

• De få studiene som er relevante for vurdering av fare for mennesker, og som har blitt tilgjengelige siden EFSAs vurdering i 2016, brukte syntetiserte, ikke-forvitrete nano- og mikropartikler. Imidlertid er plastpartikler av nano- og mikrostørrelse som finnes i mat, vanligvis forvitret, og relevansen av studier som benytter ikke-forvitrete partikler for å se på toksisiteten av de partiklene som er til stede under naturlige eksponeringsforhold, er ukjent. Den samme usikkerheten gjelder for økotoksikologiske studier.

- VKM konkluderer med at tilgjengelig informasjon ikke gir tilstrekkelig grunnlag til å karakterisere potensiell toksisitet hos mennesker.

Vurdering av fare for miljø

EFSA (2016), FAO (2017) og SAPEA (2019) tok ikke spesielt opp toksikokinetikken til nano- og mikroplast i miljøsammenheng. FAO (2017) uttalte at det fantes lite tilgjengelig informasjon om toksikokinetikk.

EFSA (2016) vurderte ikke miljøkonsekvensene av nano- og mikropartikler, mens FAO (2017) kort oppsummerte tilgjengelig kunnskap om arter som er relevante for fiskerier og havbruk, spesielt bløtdyr, krepsdyr og fisk. SAPEA (2019) tok et kvalitativt blikk på farene basert på publiserte oppsummeringsartikler, og uttalte at mikroplast kan indusere fysisk og kjemisk toksisitet og indusere skadelige effekter på matinntak, vekst, reproduksjon og overlevelse hos en rekke arter.

VKM fant:

- Et bredt spekter av arter er i stand til å få i seg nano- og mikroplast.
- Det har blitt hevdet at nano- og mikroplast overføres fra mage-tarmkanalen til organer, men i hvilken grad det skjer er uklart på grunn av potensielle eksperimentelle usikkerheter. Dermed er toksikokinetikken til nano- og mikroplast stort sett ukjent.
- I denne vurderingen er det hentet ut toksisitetsdata fra 122 fagfellevurderte publikasjoner, basert på det systematiske litteratursøket (beskrevet i kapittel 2).
- Flere forfattere rapporterer histologiske bevis på fysiske skader forårsaket av inntak av nano- og mikroplast, men artiklene har blitt kritisert for dårlig kvalitet. VKM støtter denne kritikken.
- Effekter av inntak av nano- og mikroplast kan være et resultat av en kaloribegrensning forårsaket av tilstedeværelse av partikler som ikke er fordøyelige. Svært få studier tar hensyn til dette, men det er nødvendig for å skille mellom generelle partikkeleffekter og spesifikke plasteffekter.
- Denne vurderingen undersøkte ikke muligheten til nano- og mikroplast til å fungere som vektorer for hydrofobe forurensninger (HOC) kvantitativt, men erkjenner at overføring av forurensning er to-veis og kan enten øke eller redusere kroppsbelastning av forurensningen avhengig av polymertype, miljøforhold og kjemiske fugasitetsgradienter. Den relative viktigheten av nano- og mikroplast som bærer av HOC er foreløpig estimert til å være lav sammenlignet med andre medier.
- Artsfølsomhetsfordelinger (SSD-er) basert på både numeriske og massebaserte «lavest observerte effekt konsentrasjoner» (LOEC), er konstruert fra 63 studier som dekker 39-40 arter.
- De «predikerte ingen-effektkonsentrasjonene» (PNEC) for nano- og mikroplast, basert på SSD-ene, er 0,14 ug/L (95% konfidensintervall: 0,04-0,64 ug/L) for massebaserte konsentrasjoner og 71,6 partikler/L (95% konfidens intervall: 3,45-1991 partikler/L) for numeriske konsentrasjoner.
- Disse estimatene stemmer rimelig godt overens med tidligere risikovurderinger. Den noe lavere HC_5 (PNEC) kan være et resultat av det mer omfattende og oppdaterte datasettet som brukes av VKM. HC_5 = farekonsentrasjon 5% nivå.
- Fra SSD-ene er det ikke noe klart mønster angående spesielt sensitive taxa eller nivåer av biologisk organisering som er berørt.

- Ved bruk av massebaserte konsentrasjoner er det stort sett toksisitetsdataene for nanoplast som bestemmer HC_5, sannsynligvis på grunn av deres masse-til-partikkeltall-forhold. Følgelig er HC_5 avledet fra numeriske konsentrasjoner dominert av data fra større mikroplast. Dette fremhever at valget av måleenhet påvirker farevurderingen.

➢ VKM konkluderer med at miljøfarevurderingen har to hovedbegrensninger: For det første er den pragmatisk på den måten at alle tilgjengelige toksisitetsdata ble inkludert. For det andre behandler den all nano- og mikroplast som en enhet, og dermed ignorerer deres fysisk-kjemiske heterogenitet. Grunnen til at det ikke ble gjort en mer differensiert farevurdering, var at dette ville resultert i veldig små datasett. I stedet har VKM ønsket å samle så mye informasjon som mulig.

Human eksponering

EFSA (2016) og FAO (2017) slår fast at mikroplast er funnet i mange fiskearter som brukes som mat. De påpeker imidlertid at det mangler kvantitative data. SAPEA (2019) slår fast at mikroplast finnes flaskevann og i enkelte matvarer. Men at nivåene er usikre pga. metodologiske begrensninger.

➢ VKM påpeker at det fortsatt er begrenset med kvantitative data av tilstrekkelig kvalitet som viser nivåer av mikroplast i mat. Derfor konkluderer VKM med at en eksponeringsberegning ikke kan gjennomføres.

Miljøkonsentrasjoner

EFSA (2016) og FAO (2017) gjorde ikke eksponeringsberegninger for miljø. SAPEA (2019) refererte MEC eller PEC fra tre vitenskapelige artikler, men ikke definerte selv ingen, og gjorde heller ikke en egen eksponeringsberegning.

VKM fant:

- Eksponeringsdata er fortsatt begrenset. Kun aggregerte nivåer av større mikroplast er rapportert. Dette betyr at tilstedeværelse av mindre mikroplast er underestimert.
- Målte miljøkonsentrasjoner (MEC) av mikroplast ble utledet på en global skala fra kumulative fordelinger av MEC i akvatiske økosystemer, og tilsvarende på regionalt nivå direkte relevant for Norge (Atlanterhavet, Arktis, Nord Atlanteren og Nordsjøen)

➢ VKM konkluderer med at det fremdeles er begrenset med data av akseptabel kvalitet på nivåer av nano- og mikroplast i miljøet. Mest data er tilgjengelig for akvatiske økosystemer. MEC ble utledet fra kumulative fordelinger av målte konsentrasjoner i overflaten og vannsøylen globalt og fra lokaliteter relevante for Norge.

Sammendrag av konklusjonene

Risikokarakterisering – human

EFSA (2016), FAO (2017) og SAPEO (2019) konkluderte med at siden det er en generell mangel på eksponerings- og faredata, kan ikke risikoen for nano- og mikroplast for menneskers helse vurderes.

- VKM konkluderer med at tilgjengelig informasjon og data ikke er tilstrekkelig for å beskrive potensiell toksisitet av nano- og mikroplast for mennesker, som følge av oralt inntak. Videre er kunnskapen om forekomst og nivå av nano- og mikroplast i mat ikke tilstrekkelig til å estimere eksponeringen. En risikovurdering kunne derfor ikke gjennomføres.

Risikokarakterisering - miljø

EFSA (2016) og FAO (2017) utførte ikke miljørisikokarakterisering.

SAPEA (2019) konkluderer med at det ikke er mulig å gjennomføre risikovurdering av høy kvalitet, og at det er behov for risikovurderingsmetoder som tar hensyn til de spesielle egenskapene til nano- og mikroplast sammenlignet med kjemiske kontaminanter, samt hvordan de oppfører seg i et miljø med flere stressfaktorer. SAPEA konkluderte med at nano- og mikroplast utgjør lav risiko for miljøet på globalt nivå, men at det fantes noen få svært forurensede steder der det kan være en risiko.

VKM fant:

- Risikokarakteriseringen i denne rapporten må anses som midlertidig på grunn av store kunnskapshull. Den ble bare utført for akvatiske økosystemer der man har regnet med nano- og mikroplast i overflatevannet og vannsøylen.
- Sammenligning av PNEC med PEC i forskjellige scenarier resulterte i risikokarakteriseringsforhold (RCR) på 5,41x10-6, 2,80x10-3 og 1,455 for hhv. 95, 50 og 5 % av lokasjonene på global nivå.
- Miljørisikoen knyttet til nano- og mikroplast er således lav for de fleste områder, da RCR-ene er godt under 1 i de fleste scenarier.
- For de 6 % av lokalitetene som er mest forurenset, anslås RCR til å overstige 1, noe som innebærer en risiko fra nano- og mikroplast på disse stedene.
- Når man bare vurderer marine økosystemer som er relevante for Norge, er den samlede risikoen lav.
- For de høyeste mikroplastnivåene rapportert fra Norden (Nordsjøen, Sverige) er imidlertid RCR nær 1. Dette innebærer at det er en veldig liten sikkerhetsmargin i nordiske områder som er sterkt forurenset med mikroplast
- Denne vurderingen har en rekke begrensninger som må tas i betraktning når en tolker resultatene.

- VKM konkluderer med at tilgjengelig informasjon ikke gir tilstrekkelig grunnlag for å utføre en høykvalitets risikokarakterisering av nano- og mikroplast for miljøet. Dermed må denne forsøksvise risikokarakteriseringen anses som midlertidig på grunn av store kunnskapshull. Dessuten ble den bare utført for akvatiske økosystemer (overflatevann og

vannsøyle). På globalt nivå er miljørisikoen lav, og bare for de 6 % mest forurensede stedene indikeres det en risiko. For marine økosystemer som er relevante for Norge er den samlede risikoen også lav. For de mest forurensede stedene i Nordsjøen og Sverige, er det en potensiell risiko.

Abbreviations and acronyms

ADME	absorption, distribution, metabolism, elimination
ALDFG	abandoned, lost and discarded fishing gear
ARG	antibiotic resistance genes
EFSA	European Food Safety Authority
FAO	Food and Agriculture Organization of the United Nations
FPA-FTIR	focal-plane-array Fourier-transform infrared spectroscopy
FTIR	Fourier-transform infrared spectroscopy
GESAMP	Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection
HC_5	hazardous concentration (for 5% of species)
HOC	hydrophobic contaminants
IR	infrared
LOD	level of detection
LOEC	lowest observed effect concentration
LOQ	level of quantification
MEC	measured environmental concentration
PBT	persistent bioaccumulative and toxic contaminant/pollutant
PEC	predicted environmental concentration
PET	polyethylene terephthalate
PNEC	predicted no-effect concentration
PP	popypropylene
PS	polystyrene
PVC	polyvinyl chloride
QA/QC	quality assurance/quality control
RCR	Risk characterisation ratio
ROS	reactive oxygen species
SEM	scanning electron microscopy
SPM	suspended particulate matter

SSD	species sensitivity distribution
TOR	terms of reference
VKM	Norwegian Scientific Committee for Food and Environment (Vitenksapskomiteen for Mat og Miljø)
WWTP	wastewater treatment plant

Background to the opinion

There is global interest in the impact of plastic debris on land, in waterways and in the ocean. Small plastic particles designated as "microplastics" are widespread in the environment, they are also found in organisms and may have harmful effects at an individual level and on ecosystems. Global consumption of plastics is increasing, and global emissions are likewise expected to increase. Plastics that are released into the environment may slowly degrade into smaller pieces, from macroscale to microscale plastics, which will further fragment into nanoscale. Notwithstanding their fragmentation, some polymers can remain in the environment for many years and can be transported over long distances. Plastics contain a mixture of chemicals added during manufacture and may also ab/adsorb and act as vectors for persistent, bioaccumulative and toxic contaminants (PBTs) and microorganisms from the environment.

International and national media are drawing attention to the microplastics issue, and there are growing concerns on microplastics in the human food chain.

Microplastics have been subject to several recent reviews and risk assessments from international authorities, which address both potential environmental and human health effects. Prior to the development of this document a statement from the EFSA Panel on Contaminants in the Food Chain (CONTAM) on the presence of nano- and microplastics in food, with particular focus on seafood (Alexander et al., 2016), and a technical paper on the status of knowledge on microplatics in fisheries and aquaculture from Food and Agriculture Organization of the United Nations (FAO) (Lusher et al., 2017b) were available. After the VKM review process had started, the Science Advice for Policy by European Academies consortium (SAPEA) published a scientific perspective on microplastics in nature and society, which was the basis for recommendations by Europe's Chief Scientific Advisors (SAPEA, 2019).

VKM has noted a growing public and scientific concern and a corresponding demand for information and interpretation. Furthermore, possible national implications for food resources, environment and human health effects have not been assessed. Thus, VKM consider it necessary to provide a summary of the state of the science on microplastics and their potential implications for the environment and food safety in Norway.

Terms of reference

The steering committee of VKM has self-initiated a mandate for an opinion on microplastics based on recently published international and/or national reports complemented with literature from December 2016 to February 2019. The opinion will summarise the state of knowledge on the presence of microplastics in the environment and the implications for the ecosystem, terrestrial and aquatic organisms, food production and human health. It will also elucidate any specific Norwegian conditions additional to the information available in the recently published reports.

The opinion shall

- Present a summary of knowledge from the recently published reports and scientific literature on contamination by microplastics with a focus on the use and release of microplastics in Norway.

- Contribute to improved understanding about sources and effects of microplastic pollution.

- Provide an overview of main national and international ongoing initiatives, and highlight data gaps where specific Norwegian data is needed.

1 Introduction

The annual global plastic production has increased steadily and has reached 348 million tons in 2017 (PlasticsEurope, 2018). Recent estimates indicate that the mass of plastic released to the environment will reach 250 million tons by 2025. The majority of plastics are used as packaging and in construction, with smaller proportions used in a range of other applications, including the automotive industry, agriculture, and for electrical and electronic components (Koehler et al., 2015).

Microplastics are global contaminants and have been ubiquitously detected in the water, atmosphere, sediments, soils, sewage sludge, biota, and foodstuff, primarily as a result of degradation and fragmentation of larger plastic debris (secondary microplastics). Fragmentation occurs as plastic debris turns brittle due to weathering, especially as a result of solar photodegradation. Due to a large variation in material composition and environmental conditions, the fragmentation kinetics and processes are poorly understood, so there are no reliable estimates of the time to embrittlement of different types of plastics. Nano- and microplastics originally manufactured to be of small sizes (primary microplastics) contribute to a lesser extent to the total amounts of microplastics.

Following the input from point and diffuse sources, transport of plastic debris and microplastics results in a highly heterogenous distribution in the environment. Key compartments include ocean and freshwater surface waters, and ocean and freshwater sediments, and soils. Hot spots for microplastics include the oceanic gyres as well as some highly populated areas.

Microplastics are particles that in many ways behave like natural micro-sized particles, with the latter being more abundant. Microplastics can be ingested by biota, given that many microplastics have the same size as plankton and other food items. While a potential for trophic transfer is speculated, environmental studies are yet to confirm this. The presence of microplastics in foodstuff, including seafood, bottled water, honey and sea salt, has been reported in a number of studies.

As outlined in the terms of reference, this report tries to draw conclusions upon available knowledge and information on microplastic levels and effects that are relevant to Norwegian conditions.

Below, VKM summarises the findings and knowledge gaps identified by three recently published international assessments on microplastics. Added to this, the assessment was based on recently published peer reviewed papers from the comprehensive literature search described in Chapter 2, as well as several relevant recent reports.

1.1 Previous published assessments

1.1.1 EFSA (2016)

In 2016, the European Food Safety Agency (EFSA) delivered a statement on the presence of nano- and microplastics in food with particular focus on seafood (Alexander et al., 2016). In their conclusions, EFSA highlighted that there was a lack of a recognized definition of microplastics and that the majority of data was available for the marine environment. The

majority of microplastics were found in the digestive system of fish, which is mostly discarded before consumption, at least in Norway. There was insufficient information to assess the human exposure to microplastics from consumption of seafood other than fish, and other food. EFSA called for further studies, as there was too little data to infer possible consequences of seafood consumption related to microplastics. EFSA also noted that there was currently no legislation for the presence of microplastics in food. The report indicated that microplastics, in addition to the polymer, may contain both organic and inorganic additives, and that microplastic polymer can absorb contaminants and also serve as a vehicle for growth of microorganisms, including pathogens. Using a conservative estimation, EFSA suggested that microplastics in seafood would contribute little to the overall exposure to additives or contaminants in humans. EFSA highlighted that toxicokinetic data were lacking for a human risk assessment related to nano- and microplastics. There was lack of information on the fate of ingested microplastics in the gastrointestinal tract and possible systemic uptake and distribution to other organs. It appeared that particles < 150 µm to a limited extent may pass the intestinal wall and only the smallest fraction (< 1.5 µm) penetrates into other organs. The report recommended that analytical methods should be further developed, standardised and quality assured to assess the actual presence, identity and quantity of microplastics in food.

1.1.2 FAO (2017)

In 2017, the Food and Agricultural Organisation (FAO) published their in-depth review of scientific knowledge on microplastics in fisheries and aquaculture (Lusher et al., 2017b). The microplastic contamination has been on-going since the 1950s when high volume industrial polymer production started. They reviewed the occurrence of microplastics in the aquatic environment and pointed out that it is widespread and variable in both sea and freshwater environments. The sediments appear to be more contaminated than the water and many aquatic organisms contain microplastics. FAO highlighted that microplastics have been found in many species intended for human consumption, including wild and farmed mollusks, crustaceans and fish. Whereas trophic transfer of microplastics takes place under laboratory conditions, this has not been observed in the natural environment. Because most microplastics reside in the lumen of the gastro-intestinal tract and not in the tissues, accumulation through the food web is not considered likely. Further, scientific evidence has outlined numerous pathways of microplastics exposure via food, including evidence of microplastics in species contributing to the global marine fisheries. However, there is still insufficient knowledge on the distribution, content and nature (chemical composition and size) of microplastics in aquatic organisms consumed as food. It was noted that only microplastics below 150 µm may pass the gastro-intestinal barrier and only those below 20 µm may penetrate to a significant extent into tissues of mammals. The report explained that the risk of microplastics ingestion by consumption of seafood is reduced when gastro-intestinal tracts are removed from organisms prior to consumption. Furthermore, a worst-case estimate of microplastics exposure following a portion of mussels would have a negligible effect (<0.1% of total dietary intake) on chemical exposure to certain polybutylenterephthalates and plastic additives.

1.1.3 SAPEA (2019)

In 2018, the European Commission's Scientific Advice Mechanism tasked the Science Advice for Policy by European Academies (SAPEA) consortium to prepare an Evidence Review Report on microplastics. The resulting SAPEA report was published in April 2019 (SAPEA, 2019).

The report is based on a systematic literature search. However, because of time constrains and the large number of publications, the experts decided to perform a review of reviews. It provides a comprehensive overview of the state of the science and debate on nano- and microplastics and clearly differentiates between what is known and what is uncertain or unknown.

The main conclusions from the realm of natural sciences are:

- Microplastics are ubiquitous in all environmental compartments. Here, knowledge exists on microplastics concentration on the ocean surface and to a lesser degree into freshwater systems. Quantitative data on microplastics in air and soil are lacking.
- There is limited but sufficient evidence demonstrating the presence of microplastics in drinking water and foodstuff. The actual levels are uncertain due to methodological limitations.
- Nano- and microplastics can induce physical and chemical toxicity in marine and freshwater biota based on laboratory studies. A major shortcoming is that most studies are either performed with very high concentrations (currently not found in the environment) or very small nano- and microplastics for which no or limited exposure data exists.
- Beyond classical toxicity, nano- and microplastics can have ecological impacts by altering the environmental matrix (e.g., shading), geochemical processes (e.g., nutrient cycling) or transporting pathogens and invasive species. These ecological impacts are widely postulated but largely unexplored.
- The impacts on nano- and microplastics on human health are uncertain although limited knowledge from occupational exposures points towards potential respiratory effects. The sources and levels of human exposure to nano- and microplastics remain poorly understood.
- Microplastics contain and sorb chemicals that can become bioavailable for biota. Compared to chemical exposures from other sources (e.g., food), the contribution of microplastics are considered minor.
- At present, an environmental risk from nano- and microplastics are low on a global scale but there are a few heavily-polluted locations at which a risk may already exist.
- In the future, if emission continues to increase, an environmental risk of nano- and microplastics are probable and potentially wide-spread.
- Based on this, "The evidence [...] supports the position that, even though 'high quality' risk assessment is not yet feasible, action to reduce, prevent and mitigate pollution with nano- and microplastics is suggested to be needed."

The SAPEA report also highlights a number of challenges and research gaps:

- No information is available on the abundance, fate and risks of nanoplastics, mainly because methodologies for their detection in the environment are missing.

- To enable data comparability, there is a need to improve, validate and harmonise detection methods for nano- and microplastics. The same is true for their toxicity testing.
- There is a need for adequate risk assessment methods that take into consideration the different nature of nano- and microplastics compared to dissolved chemicals as well as their role in a multiple stressor environment.

The SAPEA report also covered social and behavioral sciences, which is out of scope for VKM. The Chief Scientific Advisors to the European Commission used this Evidence Review Report to make recommendations as to political measures (SAPEA, 2019).

1.2 Interpretation of the terms of reference

The summary of knowledge from the recently published reports and scientific literature on contamination by microplastics and the possible implications for human and environmental health is based on the assessments done by EFSA (2016), FAO (2017) and SAPEA (2019) together with the outcome of a systemic literature search done in May 2018, and an update in February 2019. The search resulted in numerous papers, however, the quality of many of them was judged to be poor based on a set of predefined quality criteria. A set of quality scores were therefore defined and used for final inclusion of papers (see Chapter 2 for details). With the accepted papers as a base for quality assured information and data, more or less conceptual human and environmental risk assessments were attempted. In the human risk assessment, VKM addressed only oral exposure and uptake via the gastrointestinal tract. In doing so uncertainties in each step of the risk assessments were addressed and knowledge gaps identified. The same quality assured data and information were also used to summarize briefly sources, uses and transport of microplastics with particular focus on Norwegian or Nordic conditions when available knowledge made it possible.

An overview of the main national and international ongoing initiatives is based on information provided by the Norwegian Research Council and corresponding European sources.

1.3 Physical and chemical characterisation of microplastic pollution

1.3.1 Definition and description of microplastics

EFSA (2016), FAO (2017) and SAPEA (2019) all pinpointed that there were no international agreed upon definition of microplastics.

Microplastic research is rapidly evolving, and currently lacks a common terminology (Hartmann et al., 2019). Many studies describe microplastic pollution without clearly defining it. It is generally agreed that guiding principles for a definition of microplastics should be based on consensus as well as pragmatism, and in line with Hartmann *et al.*, the following defining criteria seem adequate, and VKM supports the following definition of microplastics:

Plastic debris can be defined as objects consisting of synthetic or heavily modified natural polymers as an ingredient that, when present in natural environment without fulfilling an intended function, are solid and insoluble in water at 20°C.

While the chemical composition and presence of additives (see Chapter 16, Appendix II) in plastic debris may significantly influence their toxicity, the key characteristics that are considered relevant to this opinion to further describe microplastics include size, shape, colour and origin.

1.3.2 Particle size, shape, colour and origin

The term "microplastics" is frequently used to indicate tiny plastic fragments with an upper size limit of 5 mm. The upper limit 5 mm was proposed by a group of marine environmentalists at the international workshop in 2008 to focus the microplastics discussion on "possible ecological effects other than physical blockage of gastrointestinal tracts". According to GESAMP (Working group 40; plastics and microplastics in the ocean) "A more scientifically rigorous definition of plastic pieces might refer to nano-, micro-, meso-, macro and mega-size ranges". In 2017, a microplastic-related standardisation was discussed in an *ad hoc* working group "Microplastic" of the ISO Technical Committee ISO/TC 61/SC 5/AHG 1. The group produced a Working draft ISO/TR 21960 that defines microplastics as particles with a size between 1 and 1000 µm. Closely related to microplastics size definition is the concept of dimension (e.g., diameter, width, length) and particle shape, which still is not agreed upon. The frequently used upper limit of <5 mm in all dimensions is based on the generally accepted cut-off and US controls on microbeads, while the European Chemicals Agency (ECHA) have widened the scope to include <5 mm in any dimension. In January 2019, a group of environmental scientists (Hartmann et al., 2019) proposed a size definition in order to unify the terminology as follows, where the largest dimension of the object determines the category, seeTable 1.3.2-1.

In the scientific literature, some authors precisely describe the largest dimension of the debris, while others do not specify which dimension is attributed. For spherical particles such as pellets it does not matter, while for example in case of fibres where cross sections are in the range of some micrometers, while their length is in the millimeter scale, it would be crucial because it leads to different estimates of microplastics abundance. Another source of uncertainty is lack of robust estimates regarding absolute quantities and regional differences in microplastics abundance, because larger debris accounts for high mass (weight), while smaller debris accounts for a high numerical value. Thus, comparisons of quantities between larger and smaller debris must consider both units.

Table 1.3.2-1 Suggested size definitions (Hartmann et al., 2019).

Term definition	Largest dimension
Nanoplastics	1 to <1000 nm
Microplastics	1 to <1000 µm
Mesoplastics	1 to <10 mm
Macroplastics	1 cm and larger

Microplastics can vary considerably in shape from spherical to long and thin fibres, and from plain to irregular (Table 1.3.2-2). Colour may be a useful characteristic to identify potential sources, although the origin might not easily be deduced. In a biological context, some

coloured plastic objects may be more or less likely to be mistaken as food. With regards to particle origin, microplastics are typically categorised as either primary or secondary particles, reflecting whether the particle was originally produced in that size range or formed through fragmentation. Particle origin is particularly important from a regulatory perspective in order to target mitigation measures.

Table 1.3.2-2 Shapes and structures (Hartmann et al., 2019).

Shape	Description
Spheres	Spherical
Cylindrical	Rod-shaped
Fragment	Irregularly shaped
Film	Planar shape
Fibre	Significantly longer in one dimension

1.4 Methods for sampling and analysis of microplastics

Researchers approach the study of microplastics in environmental samples in several ways, and methods applied vary depending on the matrix of interest. An overview of methods for sampling, processing and analysis is given in Table 1.4-1.

Table 1.4-1 Overview of sampling, processing and analysis methods for plastics in the environment. Adapted from (Koehler et al., 2015); (Crippa et al., 2019) and (Ryan et al., 2019). MP = microplarticle, QA/QC = quality assurance/quality control, SEM = Scanning electron microscopy.

	Sampling	Processing	Analysis	Reporting
Terrestrial	Few investigations, no advanced methods published	Visual separation used most commonly for larger particles	Visual (with or without microscope) most common to describe particle morphology (shape, size, colour)	Number of MPs per g/kg
Freshwater	Surface sampling methods and pumps introduced			Number of MPs per l/m3 of sample
Marine	Surface sampling nets most common for water samples, cores and grabs most common for sediments	Density separation for sediment	Dyes	Number of MPs per g/kg, $l/m^3/m^2$
Biota	Hand collection, trawling, market sampling, mainly as monitoring campaigns	Organic matter removal when high organic content Biota are dissected for target tissues or processed whole, sometimes depuration before processing	Spectroscopic approaches for polymer identity Spectroscopy with a microscope can work with smaller particles, including SEM for details of surface area and porosity	Number of MPs per g tissue/per individual
Challenges and limitations	Often not validated using spiked samples and recovery rates, net samples pre-define lower size limits of samples	Loss of particles due to the processing methods used. Risk of contamination from lab equipment and air	Each technique has own advantages and limitations, visual only really suitable for particles >500 µm, risk of misidentification	Different reporting units make comparisons difficult
QA/QC	Hard to control for contamination in the field Lack of QA/QC	Limitations of methods chosen are not discussed and therefore cannot be compared across studies	Visual analysis is user subjective and could vary between researchers	Data extrapolations are common from sample sizes of g to kg

1.5 Summary

- VKM acknowledges that there is no international agreed upon definition of nano- and microplastics. Actions should be taken to facilitate a common terminology, taking into account the need for flexibility and adaptiveness as the science evolves. VKM support

the proposal of Hartmann *et al.* (Hartmann et al., 2019), which defines nano- and microplastics as

- consisting of synthetic or heavily modified natural polymers,
- being solid and insoluble in water at 20°C,
- being between 1 and 999 µm in size in their largest dimension (for microplastics)
- nanoplastics is defined as being less than 999 nm in size in their largest dimension

- VKM acknowledges that many different approaches are used to study microplastics depending on the matrix of interest. While this is inherent to an evolving field of research, this also poses a challenge to risk assessment as data comparability is limited.

2 Methodology

This report used the knowledge obtained by EFSA (2016), FAO (2017) and SAPEA (2019) as a starting point. In May , VKM did a systematic literature search for newer literature, that is, literature that had been published after EFSA did their literature search in 2016. The last update was until end of February 2019. These searches are described in more detail in Chapters 2.1 and 2.4.

2.1 Literature search

A systematic literature search was performed in May 2018 in collaboration with a research librarian from the National Institute of Public Health, Oslo, Norway. The search terms were based on the search made by EFSA in 2016, but search terms irrelevant to the mandate for this opinion were removed and special search terms related to Norway was added, as were relevant environmental terms to meet the ToRs. The search dated back-to-back with the EFSA search. The search was performed in four different databases; Medline, Scopus, Web of Science and Embase, and resulted in 1330 unique hits. The full search strategy is included in Chapter 15, Appendix I.

After an initial round of screening to exclude publications obviously irrelevant to the mandate, all the titles and abstracts were screened in an independent, blinded manner by two members of the project group, using Rayyan, a web application for systematic reviews (Ouzzani et al., 2016). A flowchart for the literature search is given in Figure 2.1-1.

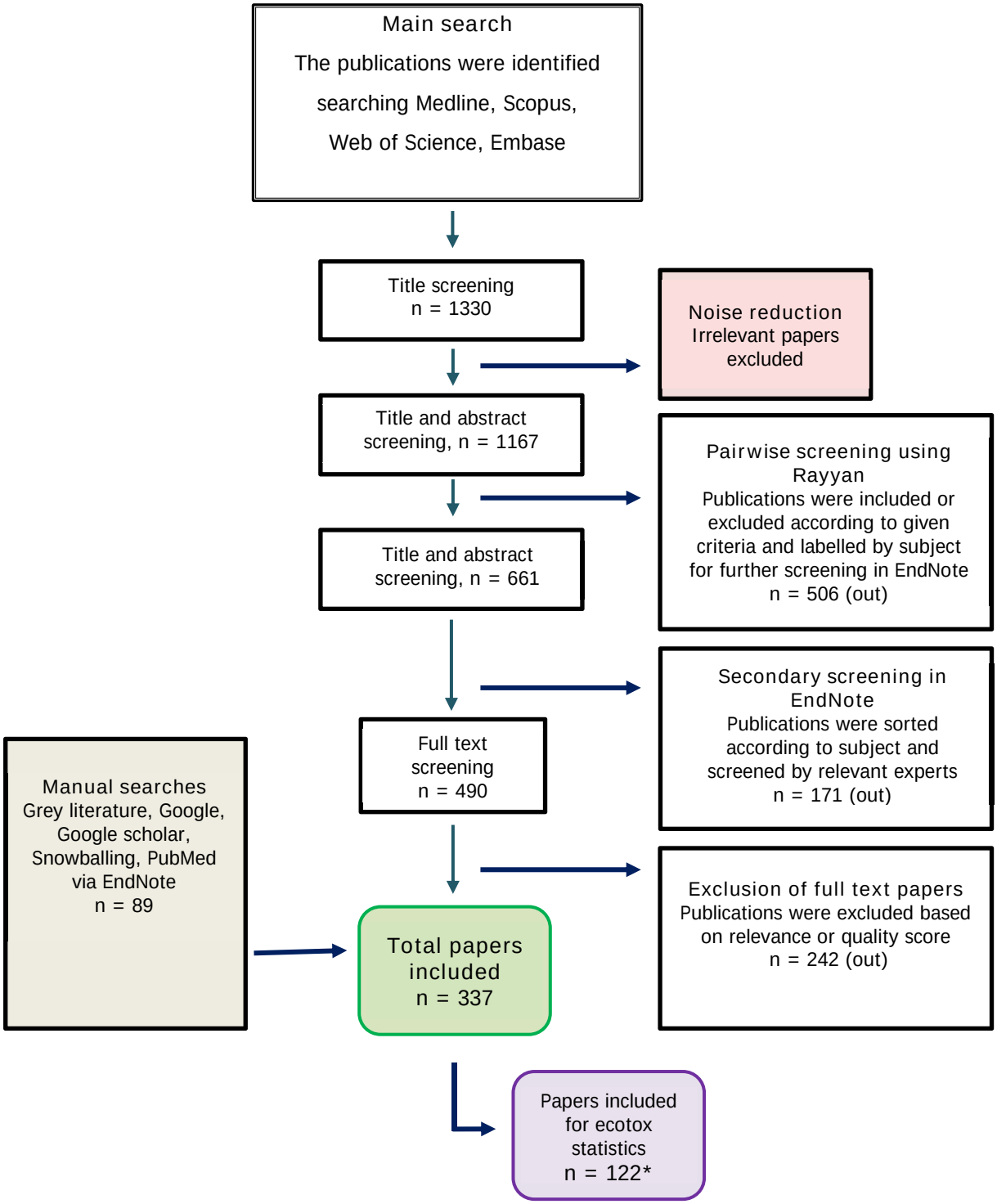


Figure 2.1-1 Flowchart for the systematic literature search on microplastics performed in May 2018. *) The 122 papers included for ecotox statistics were exclusively from the library-search (≠ manual searches), 15 were from the updated search performed in February 2019, see Chapter 2.5.

2.2 Publications selection

The selection of publications in the original systematic literature search was based on the following inclusion/exclusion criteria:

2.2.1 In general

Papers related to nanoplastic and/or microplastic (based on the terminology used in the papers): Include

Papers related to mesoplastic and/or macroplastics (size > 5mm): Exclude

All papers regarding material science and modifications of polymers: Exclude

All papers regarding microbial degradation, waste management and clean-ups on an industrial scale: Exclude

2.2.2 For occurrence and levels

Papers from Europe (including the Baltic Sea, the North Sea, the Northern Atlantic, the Arctic and the Mediterranean Sea): Include

Papers from all other geographical areas: Exclude

Due to lack of data for freshwater areas, papers from all geographical areas were included for papers on freshwater.

Included papers were categorised as either ecotoxicology, human toxicology, freshwater, levels, methods, microbiology, degradation and/or fate, review or 'other'. All the titles were then reviewed one more time by experts in each particular field. Papers that were irrelevant to the mandate were then excluded based on expert judgement.

2.3 Quality scoring

2.3.1 Occurrence and levels

For the papers included in the occurrence and levels chapters, full text papers were screened and given a quality score. The quality score was based on the following seven criteria:

1. Chemical validation of visual results
2. Appropriate sample size and/or number of replicates
3. Procedural controls were used, such as blanks or airbourne particle moniotring
4. Extraction efficiencies of methods, where methods were tested for processing ability (i.e. spiking a sample with a known microplastic and reporting the recovery rates), or where methods were tested for their effects on known plastics (i.e. degradation)
5. Results corrected based on procedural blanks
6. Calculations of uncertainties/confidence levels, such as limit of detection/limit of quantifications (LOD/LOQs)
7. Consideration of uncertainties by authors, including but not limited to:
    - Understanding of sampling bias
    - Sample location

- Sample size
- Depuration
- Extrapolation of results

For each criterium that was met, 1 point was awarded and papers were catagorised based on a quality score out of 7. Poor =1-2; Acceptable = 3-4; Good = 5-6; Excellent = 7. Papers that were catagorised as Poor were then excluded.

2.3.2 Ecotoxicology

For the papers included in the ecotoxicology category, full text papers were screened and given a quality score. The quality score was based on the following six criteria (based on Connors *et al.*, (Connors et al., 2017)):

1. Use of appropriate control (reference particle other than plastic)
2. Are the particles well-characterised (size distribution, surface charge, confirmation of polymer type by e.g., FT-IR, if commercial plastic was used)?
3. Was the particle preparation technique and stability of suspensions reported?
4. Has sedimentation of dense particles been considered when filter-feeding organisms have been used? For example, by use of e.g., a rotating plankton wheel?
5. Analytical verification of test concentrations?
6. Have findings been interpreted accurately, without conjecture beyond experimental limits ("overselling")?

For each criterium that was met, 1 point was awarded. Papers that scored less than 3 in total were then excluded. Papers that scored 3 or more in total could be excluded based on expert judgement. Such decision were justified in each case in Table 18-1 in appendix IV.

2.4 Data extraction

Each study of acceptable quality, containing quantitative toxicity data, was carefully examined. Parameters like experimental concentrations, lowest observed effect concentration (LOEC), polymer type, shape, size and endpoint were collected in a spreadsheet (see Table 2.4-1 for a full list of collected parameters). Generally, the data extraction for a particular study was performed factorially, meaning that LOEC data for a particular endpoint was retrieved for each combination of particle shape, particle size, polymer type and exposure duration. However, there were a few cases when this was not possible due to the vast amount of data generated by gene expression or enzyme activity profiling. In these cases, we chose a pragmatic approach and did not extract LOEC values for each individual gene or enzyme. Instead, the lowest LOEC for any one of these endpoints was extracted. Hence, in these cases, the toxicity, operationally defined as the LOEC, was defined by the most sensitive specific endpoint in enzyme activity and gene expression category. Importantly, we did not evaluate whether an effect was adverse or not because this would require a subjective judgment that biases the extraction. For example, reproduction in the water flea *Daphnia magna* may increase at low doses of a toxicant which might be interpreted as a positive response (Ogonowski et al., 2016; Stanley et al., 2013). However, this effect may be transient. Moreover, increased reproduction can also be a

temporary stress response to low food availability whose net negative effects only become visible over extended periods of time (Jager et al., 2013).

Due to the diversity of biological endpoints reported, we classified the endpoints hierarchically into two categories: (1) the level of biological organization (Galloway et al., 2017) and (2) subcategories corresponding to the biomarker type. Taxonomical information on the model species, as well as the typical habitat these species occupy in the natural environment (marine, freshwater, brackish or terrestrial), was retrieved from the World Register of Marine Species (WORMS) (http://www.marinespecies.org/).

Table 2.4-1 List of parameters extracted from reviewed ecotoxicological studies. MP = microparticle. PE = polyethylene, PS = polystyrene, PP = popypropylene.

Parameter category	Parameter	Explanation	Note
Particle characteristic	Size range (min-max)	Reported size range of MP (particle diameter) used in the experiment	Studies that have used ground plastic sometimes lack a well-defined size range. Size ranges of fibres were not extracted due to ambiguous size definitions.
Particle characteristic	Polymer type	Type of polymer (e.g. PS, PE or PP)	
Particle characteristic	Particle shape	Shape of the MP used in the experiment (sphere, fibre or fragment)	
Test characteristic	Unit	Dose metric (e.g. mg/L, number of MP/L, g MP/g sediment)	
Test characteristic	Concentration range	Range of test concentrations	
Test characteristic	Exposure method	Exposure pathway, e.g. suspension in water, mixed in soil or oral administration (MP mixed in food)	
Test characteristic	Endpoint	The reported endpoint	
Test characteristic	Endpoint class	Endpoint type	
Test characteristic	Endpoint category	Level of biological organisation	
Test characteristic	LOEC	LOEC: the lowest statistically significant concentration different from the control treatment	
Test characteristic	Duration (days)	Experimental duration	Exposure could be intermittent, but the most common exposure method was by the administration of a single dose lasting the entire duration of the experiment.

Parameter category	Parameter	Explanation	Note
Test characteristic	Food present (y/n)	Indicates whether food was provided during the exposure	Food is normally not provided in acute tests, but this is common in longer chronic exposures.
Test characteristic	Reference particle	Indicates whether a non-plastic particle was used in the control treatment	
Test characteristic	n concentrations	Number of concentrations tested	
Test species characteristic	Species	Test species	
Test species characteristic	Organism, organ, tissue, cell	Target of investigation. Indicates the biological target of a particular endpoint	
Test species characteristic	Feeding strategy	Factor indicating typical feeding mode of the test species	Some species are able to feed using several feeding modes. The typical mode was reported.
Test species characteristic	Life stage	Life stage of test species	In some cases, the experimental duration extended over several life stages.

2.4.1 Data treatment

To make LOEC values comparable across studies that had used different dose metrics, we converted mass-based concentrations to numerical concentrations and vice versa, unless both measures were reported originally. To convert the metrics, we assumed a spherical microplastic shape and calculated the individual particle weight using either the reported polymer density or a nominal density-value retrieved from Scientific Polymer Products Inc. (https://scientificpolymer.com/density-of-polymers-by-density/) along with the mean particle diameter. Unless the mean diameter was reported by the original authors, we calculated the mean particle size based on the reported size range. However, we only accepted size ranges that were within one order of magnitude between the smallest and the largest reported particle diameter to restrict conversion bias. Because we used a spherical approximation of the particle volume, we had to exclude studies where fibres or particles with undefined or too wide size ranges were used, from the unit conversions. However, if mass-based or numerical concentrations were provided originally, the raw data was still extracted and used for downstream analysis. Studies that consisted of mixed polymer exposures were excluded from conversion because of difficulties in determining the density of the mixture.

Ultimately, this resulted in a set of studies in which mainly filter-feeding organisms had been exposed to particle suspensions directly via the water phase. Omnivores and visual predators were also represented to a lesser extent.

2.5 Updated literature search

In February 2019 an updated literature search was performed, using the same search strategy as before, searching for literature published between May 2018 and February 2019. The search resulted in 786 unique hits. These publications were screened based on titles and/or abstracts by two members of the project group. Only titles that fulfilled the original inclusion criteria, and covered topics where more data were needed, were included. 101 papers were read in full-text by an appropriate expert. The total number of papers added after this search was 22.

Due to the great production of papers in this field, VKM decided to include peer-reviewed references published later than February 2019 where relevant in Chapter 3, 4, 5, 7 and 8. However, a prerequisite for including such papers was that they passed the quality assessment described in section 2.3. All studies included in the ecotox statistics that are used for the environmental risk characterization were exclusively from the systematic literature search.

3 Analytical, experimental and sampling methods

In the following Chapter, the different methods applied in the papers that were acquired, selected and quality checked as described in Chapter 2, are discussed in detail. This is done in an attempt to demonstrate the large variation in many of the parameters used.

3.1 Methods used to characterise occurrence and levels

EFSA (2016) states that methods for the identification and quantification of microplastics in food including seafood are available but recommend that analytical methods should be refined for microplatics, developed for nanoplastics, and standardised.

FAO (2017) do not discuss the methodology used in the report, but include them in the annex. The annex summarises the methods used, but does not make recommendations.

SAPEA (2019) in their report does not focus on summarizing analytical methods, but highlight the lack of detection methods for nanoplastics and the need to improve the quality and comparability of methods by means of an international harmonisation.

In the following section the different methods applied in the above described papers are broken into methods for sampling, processing and analysis and then matrix type: water, sediment, biota, wastewater and food products.

3.1.1 Methods for sampling

Methods for sampling in different matrices

Water

Water samples are collected in many ways, including pumping and filtering, bulk samples, using nets (including manta, neuston, bongo) to sample a given area or volume of the surface water or water from the water column (Figure 3.1.1-1). Of the methods of sampling applied for water in all aquatic matrices, sampling using net is the most common approach. The advantage of using nets is fast filtering of large water volumes to obtain a concentrated sample. The mesh size and opening of the net will determine the composition of the obtained samples. Unfortunately, in many studies the sampling parameters are not always reported in full, with often only mesh size being reported. Three hundred µm mesh sizes are generally used, but studies have begun introducing smaller mesh sizes to target smaller particles (Dris et al., 2018). However, this introduces a further risk of clogging and procedural error from contamination of smaller airborne particles, especially when nets are rinsed and prepared on the deck of a vessel and reduces the speed of the tow. Many of these studies therefore exclude fibres from the analysis. When sampling is conducted using nets the obtained data often have a much higher size cut off (limit of detection) than pumps

as this is a produce of the sample mesh used. Furthermore, towing duration, water currents and the presence of biomass in the sampled area can influence the effectiveness of nets.

Alternative methods have been sought including dip sampling using buckets/glass jars, pumps or passive samplers. Pump or bulk water samples allow researchers to collect samples and determine microplastics with a lower size limit, this method has been applied for offshore research and instruments are being designed to work on research infrastructures (such as offshore research vessels, monitoring platforms and continuous plankton recorders) for continuously monitoring microplastics in this way (Conchubhair et al., 2019). Passive samplers and glass jars have only been applied to marine investigations. There has been some discussion on the appropriate sample volume. For bottled water, it has been highlighted that single bottles are not appropriate to understand microplastics contamination due to the low frequency of occurrence (Koelmans et al., 2019). Ten thousand litres has been recommended as a minimum. However, sampling small volumes of wastewater is appropriate as these samples can contain excessive amounts of particles and it is not feasible to sample larger volumes. Size limits of microplastics can be imposed by the sampling apparatus and must be included when reporting.

Sediment

Methods for sampling sediments and soil are reliant on their content of organic material and fine particulate matter, e.g., whether sediment is terrestrial, shoreline or benthic (Figure 3.1.1-1). Sediment samples collected on shorelines and on land tend to be collected by hand, using a quadrat with a scoop or shovel. In most cases samples are collected from the top surface sediments down to 5 cm. Samples from benthic sediments are normally collected with grabs or corers. The advantages of using core samplers allow researchers to look at depth stratification within a sample. Currently, there is no recommended method for sampling benthic sediments to obtain a representative sample. A similar method has been applied to Arctic ice using ice corers (Peeken et al., 2018). Sediment samples tend not to have a lower size limit imposed as samples are collected in bulk and often subsampled when processed in the laboratory but further discussion around this is required.

Biota

Biota (mainly fish and invertebrates) from both marine and freshwater systems are sampled most commonly using trawls and nets. Hand fishing or hand collection of shoreline individuals is common in marine systems. Electrofishing was used in one study in the marine environment. For large marine mammals, sometimes scat is sampled.

Food products

These are generally sampled from points of sale or from places of production. The sample sizes tend to vary between investigations and the number of replicate samples varies and is often low due to limited throughput, which limits comparability (no figure included since there are few data points).

Other sample matrices

Wastewater is sampled, in most instances, using a pump to collect a composite sample over a defined period. Atmospheric microplastics are sampled using a passive sampler to monitor

fallout. Although few studies exist, soil is sampled using similar approaches as used for sediments.

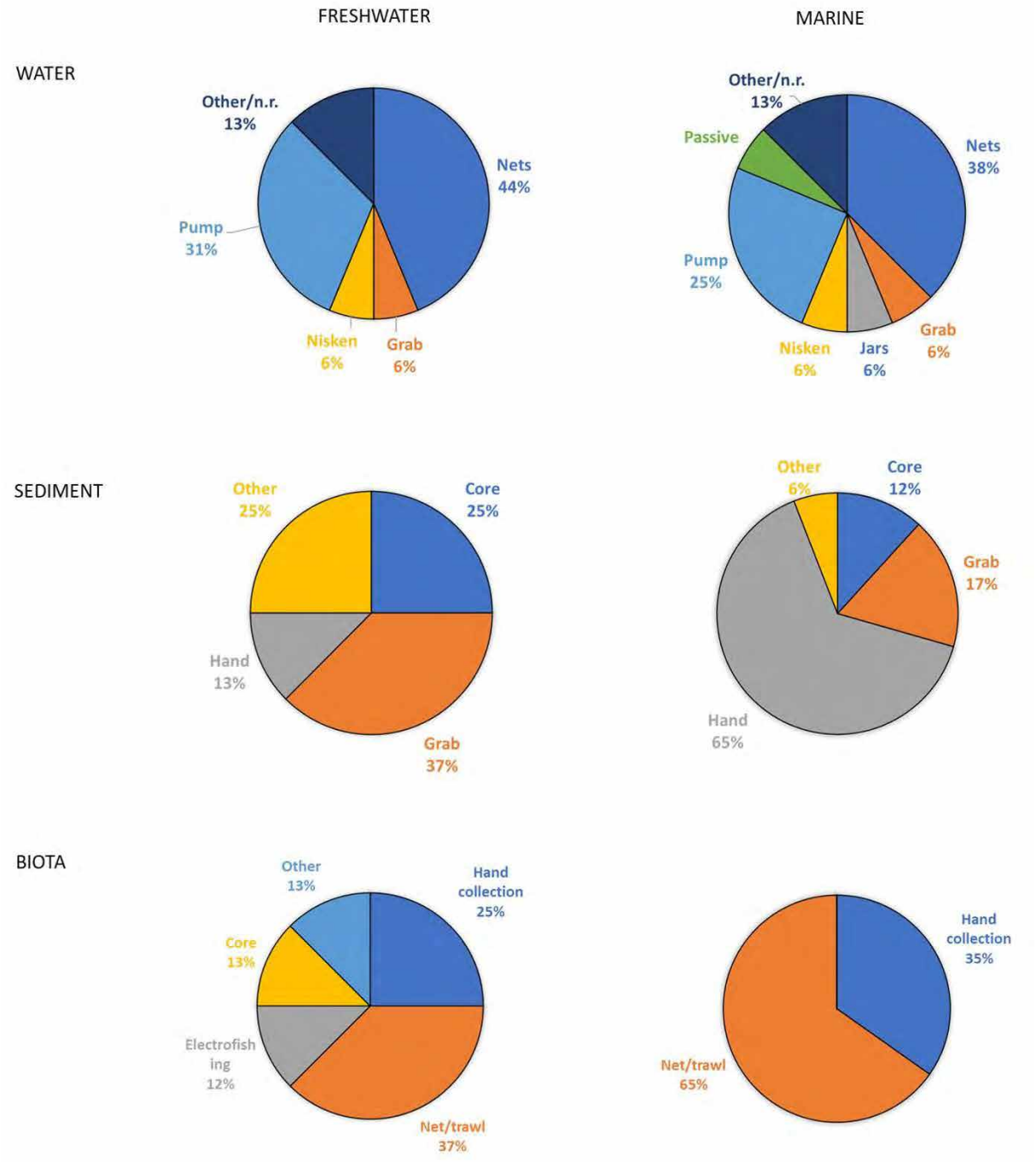


Figure 3.1.1-1 Sampling methods used to collect samples for microplastic analysis. Note that there are a limited number of terrestrial studies and these are therefore not included in this figure.

3.1.2 Methods for processing

There are four main categories for processing collected samples: filtering, sieving, density separation (using a salt solution to separate buoyancy material from non-buoyant material),

and digestion of organic matter (using acids/alkaline/enzymes to remove organic material). Modifications are required for different matrices. The four categories are described below (after the different matrices).

Methods for processing in different matrices

Water

Water can be processed in different ways depending on the level of organic matter present in the sample (Figure 3.1.2-1). For example, samples of drinking water can be filtered directly for visual and/or chemical analysis. This is the same for samples with low plankton content. Large material can be sieved out, isolated in a stepwise process using either density separation or digestion, or a combination of methods. Samples with high amounts of organic material, e.g., spring bloom marine and freshwater samples, tend to require more processing. The same is true for water samples containing a high content of (in)organic suspended particulate matter.

Sediment

Sediment samples can be processed in different ways depending on grain size and organic matter content (Figure 3.1.2-1). For example, samples can be sieved and sorted visually if there is little organic material or the sediment grain size is smaller than the lower limit of detection, although visual sorting methods are time consuming and introduce a visual bias (Löder and Gerdts, 2015). Density separation is more common than digestion methods when working with sediments, while combinations of density separation and chemical or enzymatic digestion methods are often applied to extract and purify microplastic from samples. As with water samples, sediment may require complex methods as high levels of (in)organic suspended particulate matter can be present. For example, freshwater and urban marine samples can have high organic matter contents (i.e., sewage outlets and deposition sites with low currents) and can be very difficult to prepare (Haave et al., 2019; Lusher et al., 2018a).

Biota

Biota samples are generally analyzed in two approaches. Either the digestive tract (mostly fish) is dissected and its content is analyzed directly or further processed (Figure 3.1.2-1). Alternatively, soft tissues of biota, mostly mussels, are digested as a whole. Digestion methods similar to those employed for water and sediment and can be carried out in combination with each other (e.g., (Avio et al., 2017)). Limitations of only using visual observation include human error and any particle less than 500 µm requires confirmation with analytical chemistry.

Other sample matrices

Wastewater samples, including influent, effluent and sludge, tend to be processed in the same manner as water and sediment samples, respectively. Low volumes of influent and effluent can be filtered, although this raises the question of representativeness. Furthermore, samples require sterilization, which can further introduce sample contamination or affect sample composition. Most samples are treated in a stepwise manner, using density separation or digestion. Density separation can be applied, and has been applied using NaCl,

$ZnCl_2$, or NaI. Digestion with hydrogen peroxide also sterilises the sample. Atmospheric microplastics are commonly not further processed but directly analyzed. Food and drink products tend to be processed in the same way as water samples by filtering.

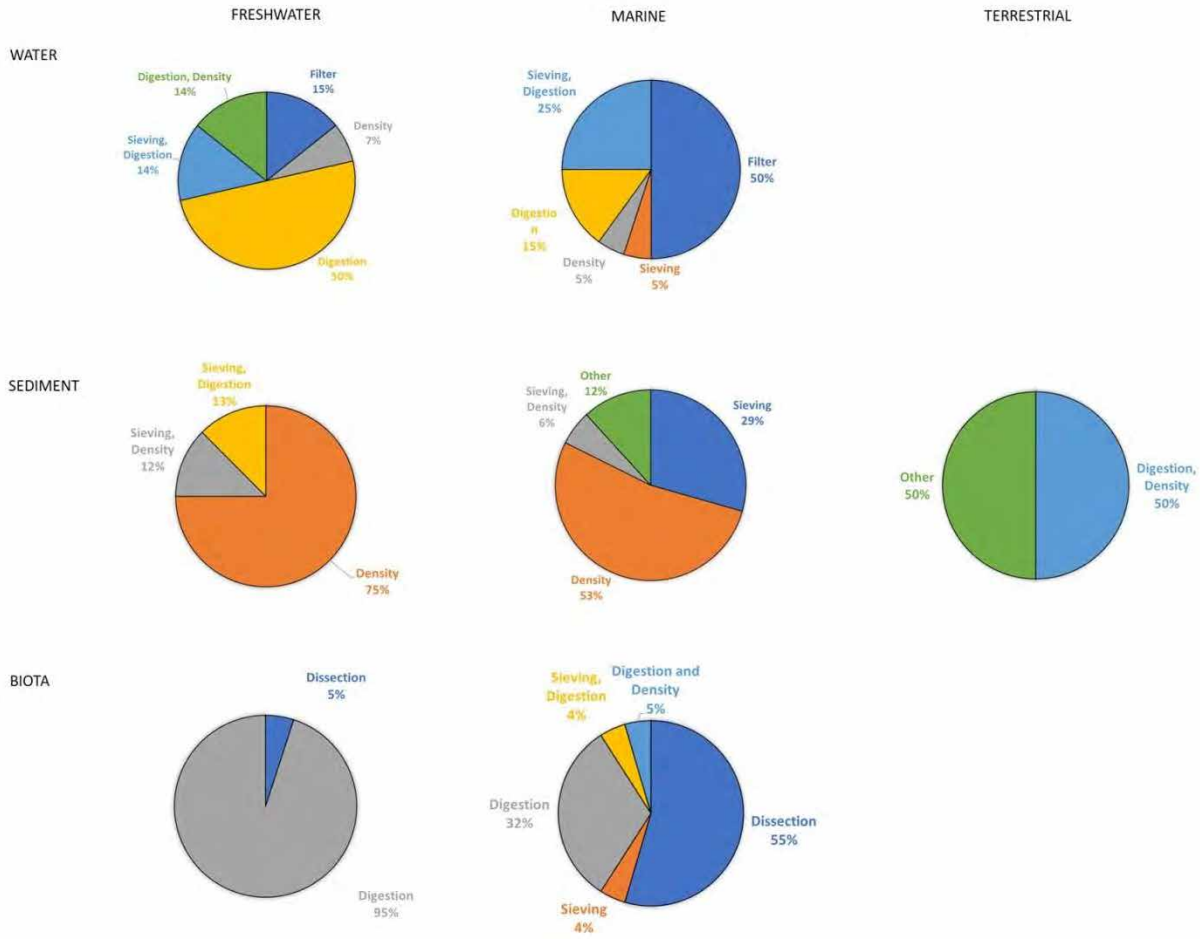


Figure 3.1.2-1 Processing methods used for microplastic analysis.

Categories for processing

Sieving

Metal sieves can be used to sieve sediment (wet or dry) and water samples. Sieving introduces a lower size limit, as the mesh becomes a cut off for capturing smaller samples. Sieving can be used to introduce size categories for sample analysis and to reduce bulk samples in readiness for processing with density separation or digestion agents.

Density separation

Many density solutions have been used globally for the separation of plastics from samples (see review in Lusher *et al.,*(Lusher et al., 2017a)). The specific density of most plastics ranges from 0.8 to 1.7 g/cm^3 with the exception of expanded polystyrene (0.05 g/cm^3) and Teflon/PTFE (2.1-2.3 g/cm^3). As the density of sand is generally around 2.6 g/cm^3, liquids with a density somewhat higher than plastics can be used to separate microplastics from

sediment matrices: plastics with a density lower than the solution will float, whilst denser material (mostly nontarget material) will sink. NaCl is commonly used as it is cheap, accessible and has a low hazard. The density of NaCl is 1.2 g/cm^3 which means that particles with a lower density will float. Common plastics, which fall into this category, include polypropylene (0.85-0.92 g/cm^3), polyethylene (0-89-0.98 g/cm^3) and ethylene vinyl acetate (0.94-0.95 g/cm^3). This solution is by far the most commonly used method for density separation (Figure 3.1.2-2). Heavier solutions of NaI or $ZnCl_2$ (1.6 – 1.8 g/cm^3) have also been applied to sediments, and can increase the extraction of dense particles such as polyamides, acrylics, PET, PS (not expanded) and polyurethanes. Heavier salt solutions are now commonly used, along with other denser salts to isolate particles which sink in water, but require careful handling, as they are expensive and toxic to aquatic organisms. Some methods using density separation can be carried out using specifically designed apparatus, such as elutriation devices and sediment separators, which have been shown to have high extraction efficiencies.

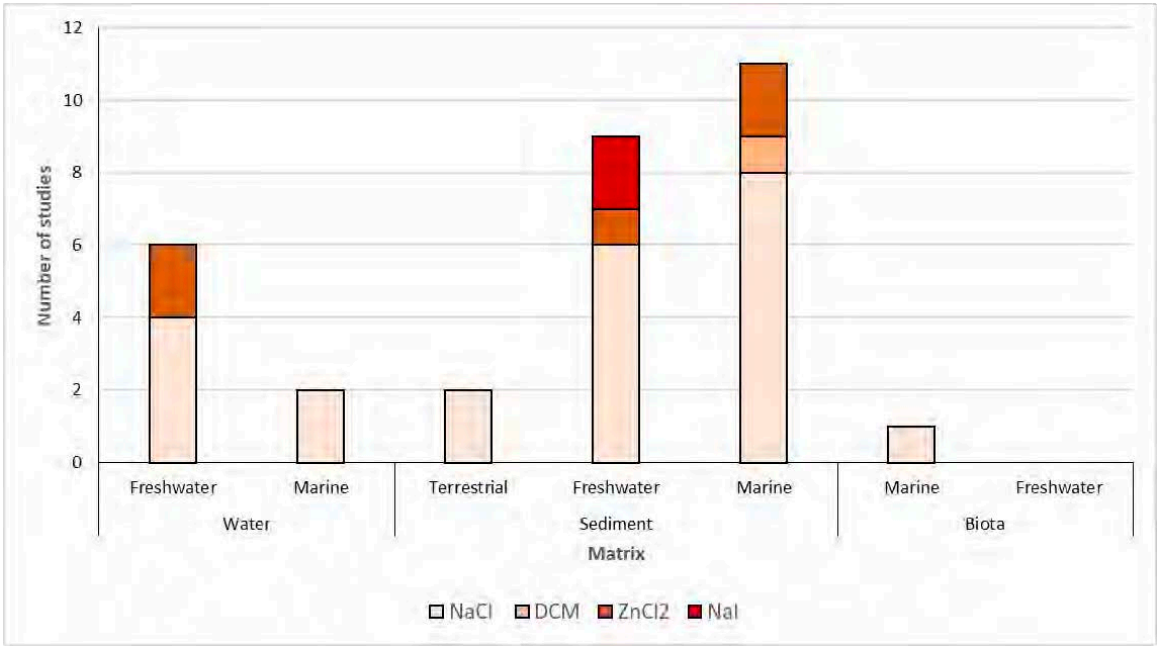


Figure 3.1.2-2 The use of different density solutions for processing environmental matrices. NaI = sodium iodide, DCM = dichloromethane, $ZnCl_2$ = zinc chloride, NaCl = sodium chloride

Digestion

Digestive procedures used also vary between studies (Figure 3.1.2-3). They can be divided between acid, alkaline and enzymatic digestion. Acid digestion has been discouraged in many recent reviews because it can damage plastics at high concentrations and temperatures. Wet oxidation using hydrogen peroxide with or without iron catalysts (Fenton's reagent) has emerged as an effective procedure for working with complex matrices (e.g., (Hurley et al., 2018b)). Enzymatic digestion procedures tend to be more costly and require several sampling steps which may not be suitable for long-term monitoring schemes. Some of the enzymes used include cellulose, protease K, corolase, protease, amylase, and lipase.

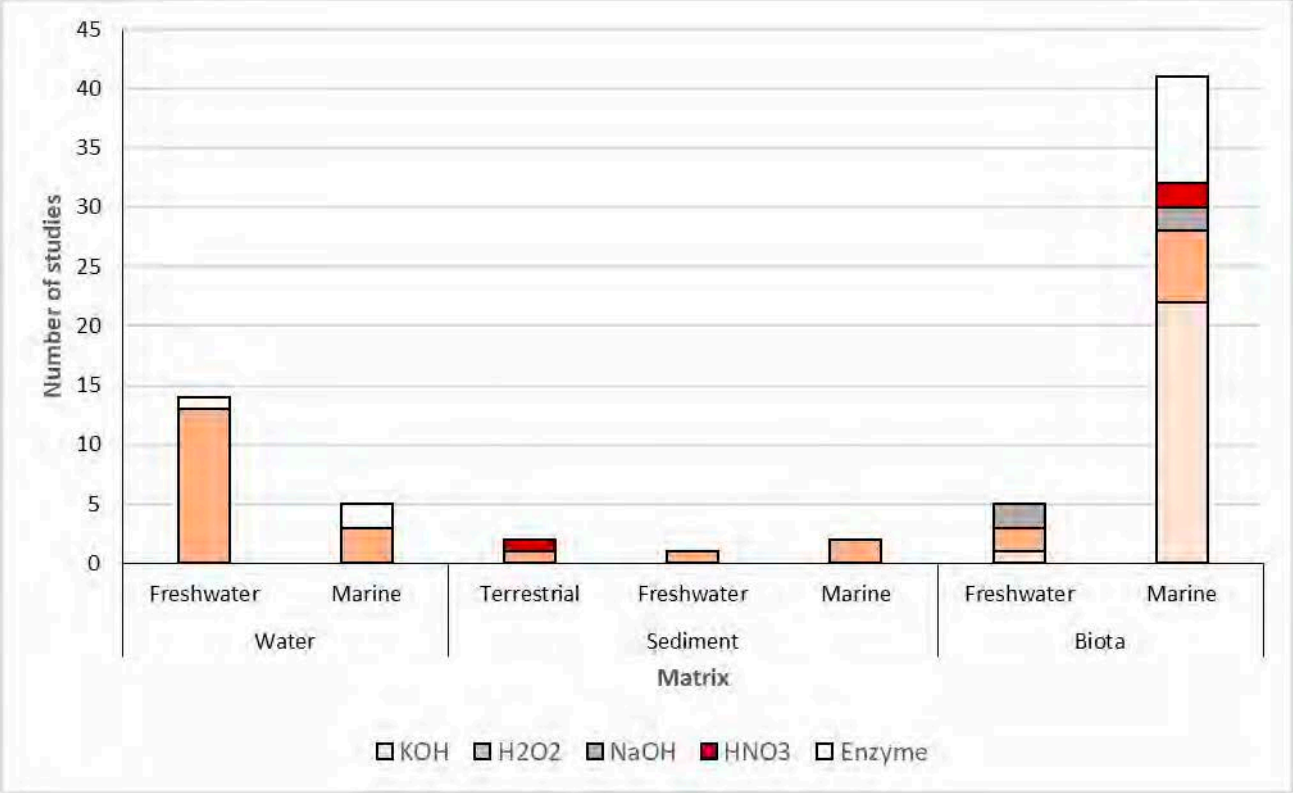


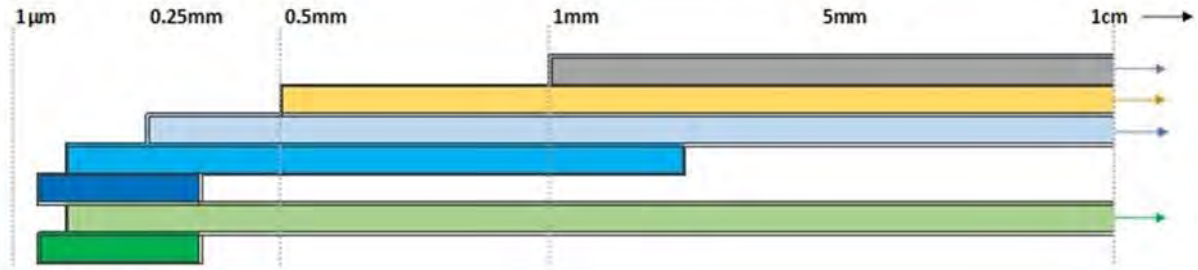
Figure 3.1.2-3 Purification steps used to remove organic matter for microplastics analyses, in selected articles for this study (2016-2018). KOH = potassium hydroxide, H_2O_2= hydrogen peroxide, HNO_3 = nitric acid.

Filtration

Once samples are processed, they are generally transferred onto filters and dried for further analysis. The type and pore size of filters vary between studies. Filtered samples allow researchers to move onto visual or spectroscopic identification of particles (see below).

3.1.3 Methods for analysis

Once samples have been transferred to filters, the number of microplastics can be assessed. Researchers can use characteristics, including morphology, polymer composition and density, to identify the microplastics. Figure 3.1.3-1 below shows an overview of methods used for the identification and subsequent verification of plastic particles from different size ranges.



	Advantages	Limitations
Visual	Fast Inexpensive	Does not provide material composition Subjective, relative to experience Requires verification
Hot needle	Fast Inexpensive	Possible sample loss Subjective - based on visual ID Not all polymers react to heat Does not identify specific polymers
FTIR	Quite fast Comparable as widely used method Library search functions available A less expensive alternative to µFTIR	Possible sample loss due to hand picking Subjective - based on visual ID Time consuming IR detection is limited by the size of the particle
µFTIR	Comparable as many researchers use it Library search functions available Lower detection limit than standard FTIR	Some subjectivity when used for single particles Time consuming when working with simple particles Expensive instrumentation
FPA-FTIR	Less subjective Highly automated More quantitative than other methods Lower detection limits Can scan for multiple polymers at once	Very expensive instrumentation Not fully automated or optimised Time consuming with extended periods of sample preparation and data handling Upper limit for particle size.
µRAMAN/RAMAN	Particle isolation not required Low detection limit Comparable as many researchers using it	Can be subjective, based on visual ID Time consuming for each sample Not easy to work with fibres Limited spectra libraries when compared to FTIR Expensive instrumentation

Figure 3.1.3-1 Methods used for the identification and subsequent verification of plastic particles from different size ranges.

Visual identification

Visual identification is based on the morphological and physical characteristics of particles whereas chemical characteristics are determined by more advanced analytical techniques. Visual identification is most widely used, but without analytical confirmation it is impossible for researcher to confirm the identity of a particle as plastic with certainty. Difficulties in visual determination of polymers increase with decreasing particle size. Visual identification is rapid and does not require many resources besides training and experience. Recent studies on the quality of microplastic analysis have highlighted the lack of further analytical confirmation as a limitation of some of the early microplastic studies, see (Hermsen et al., 2018) because it may result in false-positive as well as false-negative detects. Plastics can be classified visually by their morphological characteristics: size, shape, and colour (Hartmann

et al., 2019), although this is known to produce bias (Bergmann et al., 2017). Size is typically based on the longest dimension of a particle and size categories can be used where appropriate. When reporting microplastics shape, researchers tend to use five main categories, although the nomenclature used varies between research groups. Finally, colours are often reported across a wide spectrum; colour differentiation is subjective, and visual identification of microplastics cannot be based on colour alone. Characteristics such as flexibility or shine may also be elusive, since natural polymers can also be both shiny, transparent and flexible. Visual methods should be combined with chemical or spectroscopic methods to confirm the polymers, especially when focusing on smaller sized particles (<500 µm). Some studies use a hot needle, three which are included in this report, but this is not a sophisticated method and is widely discouraged.

Chemical analyses

Chemical analyses are used to confirm the identity of the polymers (Figure 3.1.3-2). FTIR, Raman and other IR spectroscopy are the most commonly used method to identify microplastics. IR methods compare the IR absorbance or transmission of a sample to known spectra. Databases and libraries are used to assign and identify to unknown particles. There are many various instruments for IR including near IR, µFTIR, FPA-µFTIR but by far the single point measurements using ATR-FTIR are the most common.

Raman spectroscopy can be carried out on single particles or through micro spectroscopy (µRaman) and is a popular technique that allows the identification of different polymers in a sample.

Pyrolysis GC-MS is used to identify the chemical composition of microplastics through thermal decomposition and the analysis of gaseous products. This method is emerging and can provide researchers with a mass-based data output but does not provide information on number of particles.

Scanning electron microscopy (SEM) can be used to visualise the surface morphology of microplastics but it is not a commonly used method.

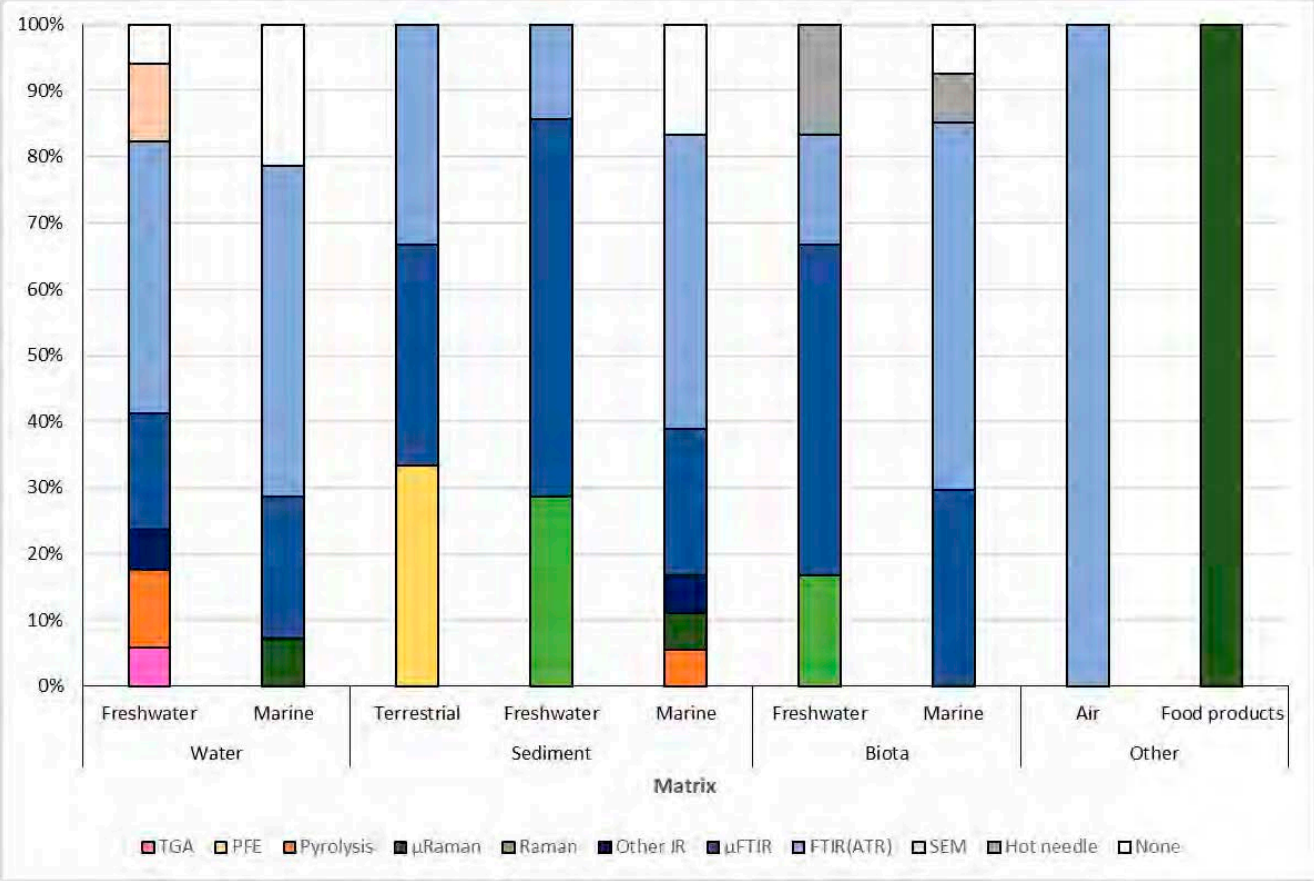


Figure 3.1.3-2 Analytical techniques applied within studies to identify suspected microplastics. TGA – Thermogravimetry; PFE- pressurised fluid extraction; SEM- Scanning electron microscopy.

Size limitations of applied approaches

Each method introduces their own size limits, which are imposed in the laboratory due to sample processing, such as sieving or filtering. In addition, size limits to visual identification exist. Here, the researcher should not report particles less than 500 µm unless using further analytical procedures to confirm that the particles are polymeric. Interestingly, the studies included in our data did not analytically confirm potential microplastics which were <500 µm (Figure 3.1.3-3). Most worryingly is that researchers use no validation of particles <100 µm in some studies. Without confirmation of these particles as anthropogenic, or plastic, researchers may be inflating results based on misidentification. For all studies with a lower size limit than 500 µm and above the size of the particle was confirmed.

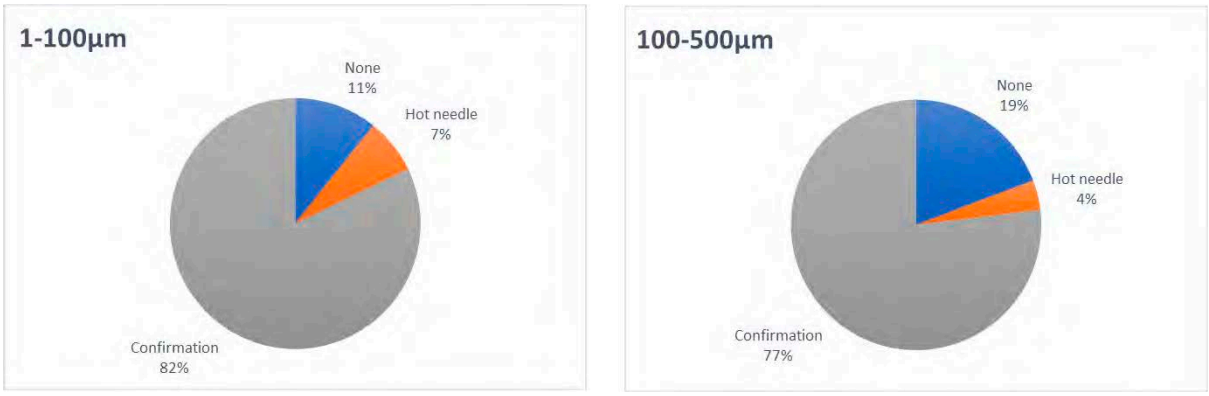


Figure 3.1.3-3 Use of confirmatory techniques when working with microplastics. The citatory confirmation includes both Raman and FTIR analysis on all or a subsample of microplasics.

Metrics used to report microplastic concentrations

Different metrics for reporting microplastic levels are used for different sample matrices and studies. Often either numerical or mass-based concentrations are reported per volume or area of sample. This complicates the comparability of the available data. Figure 3.1.3-4 shows the different metrics in the studies included in this report. Some standardisation can be made within matrices, for example the studies reporting levels per m^2 and km^2 can be standardised to the lowest denominator, m^2. Similarly, levels per litre and m^3 can be standardised to m^3. Interestingly, all of the studies reporting levels per m^2 or km^2 are those which were conducted using surface sampling nets. This is because the study design looks at the surface area of the net covered. However, there is an ongoing discussion on how realistic this is, as the net does sample a volume. The same applies to sediment samples, and levels per km^2 can be standardised to m^2, g could be standardised to kg, and there is discussion regarding how researchers extrapolate their results when working with small samples.

More importantly, the conversion between numerical and mass-based concentrations is hardly possible and there is ongoing debate on which is more relevant. From a toxicological perspective, numerical concentrations are preferred because these represent the number of microplastics biota are exposed to. However, mass-based concentrations may be more suitable for modelling flows etc. For concentrations reported per area or volume the question is easier to resolve: Microplastics are always sampled from a volume of a sample and, thus, levels per volume should not be reported. Importantly, the reporting metrics of course depend on the specific research question and should be adapted as such. To enable comparability, nonetheless, reporting levels in multiple metrics or at least providing the information needed for conversion (e.g., volume of a manta net, depth of sediment sample) is recommended.

The most common metric for reporting microplastics in biota is number of microplastics per individual, although researchers have now started reporting per g of tissue alongside per individuals. Much of the data is reported in terms of monitoring, thus the number of microplastics per g/l/individual is appropriate, but it is not useful for risk assessments.

In terms of a risk assessment, the reporting metrics are often not suitable from a food safety perspective because data needs to be reported for a particular matrix. For example, the number of microplastics per individual fish does not provide information on the number of particles in the edible tissues. The most common data available are regarding digestive tracts, which in many instances are removed before consumption by humans. However, data presented per gram of mussels is appropriate. Mussels are normally processed whole and thus values per individuals or

per gram are reported, which can be directly used for toxicity data as the whole individuals are consumed.

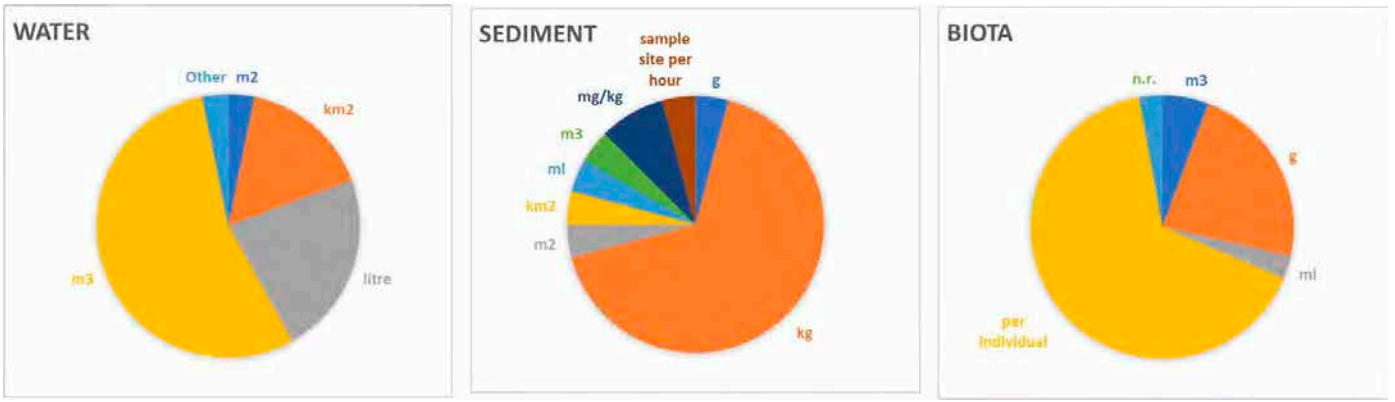


Figure 3.1.3-4 Different reporting units used between investigations.

3.2 Methods used in experimental studies

EFSA (2016) does not address experimental methods and designs.

FAO (2017) identifies a mismatch between experimental exposures and *in situ* conditions. Experimental studies tend to employ test concentrations orders of magnitude higher than those found in the environment. The size and shape of nano and microplastic also differs in experiments compared to those found in the environment. A predominance of poorly replicated and acute test scenarios with low ecological relevance is identified as a major uncertainty.

SAPEA (2019) also reports a lack of ecological relevance in current experimental designs. The working group reports a strong bias towards the use of high concentrations of small spherical polystyrene beads in experiments, which are not representative of current environmental conditions, where polymeric fibres dominate at considerably lower concentrations.

In the following section, the experimental designs of 122 reviewed ecotoxicological studies involving nano- and microplastic are described and discussed. Only the studies that passed a set of predetermined quality critera have been included in the analysis (Chapter 2.3.2).

3.2.1 Experimental designs used in the nano- and microplastics literature

Exposure conditions and species

Due to the lack of standardised test methods for nano- and microplastics most ecotoxicological studies have adopted standardised test protocols established for soluble chemicals (OECD, n.d.). Based on our analysis, we identified exposure via the water phase as the most common exposure method (86 of 122 studies) and filter feeders as the most common type of test organism (49 of 122 studies) (Fig. 3.2.1-1). In these laboratory experiments, nano- and microplastics are introduced to the test system once, at the beginning of the exposure (static exposure). Exposures where the test medium is replaced over the course of the test duration are also common (semi-static). Although these procedures are acceptable for soluble chemcials they introduce several problems when particles are added to the test system. Firstly, nano- and microplastics either aggregate at the water surface or sink out of suspension depending on the density of the polymer, and secondly, the physical interaction between nano- and microplastics and other particles such as algae leads to aggregation phenomena, which affect the size of particles in suspension. This, in turn affects the bioavailable fraction which ultimately can lead to non-monotonic dose-response relationships. When planktonic organisms are used, the problems of uneven exposure can be overcome by the use of a plankton wheel (Gerdes et al., 2019; Ogonowski et al., 2018). However, we did not find any ecotoxicological studies in our literature search that applied this approach.

Another way to control and deliver a precise dose is to supply a known amount of nano- and microplastics mixed with the food (Paul-Pont et al., 2018). However, this type of dosing is realtively uncommon (16 of 122 studies) and mostly applies to studies involving fish that are fed fixed and controlled food rations (Asmonaite et al., 2018; Rummel et al., 2016) or prey

containing nano- and microplastics (Mattsson et al., 2017). Studies on sediment and soil dwelling organisms are even rarer (8 of 122 studies) despite the fact that these habitats are important sinks of nano- and microplastics (Chapters 5.3.2 and 5.3.3). The effects on terrestrial biota have been largely neglected so far but interest is now increasing, particulary for soil dwelling organisms (Huerta Lwanga et al., 2016; Jemec Kokalj et al., 2018; Lei et al., 2018; Rodriguez-Seijo et al., 2017).

Figure 3.2.1-1 Number of studies showing the use of different exposure methods to various organism groups classified by their feeding modes. The category "mix" represents organisms which are not restricted to one particular feeding mode or organisms that have switched feeding strategies throughout the course of the experimental duration.

Experimental controls

The complex nature of plastic materials (different sizes, shapes, densities, and physicochemical characteristics) makes it difficult to isolate specific properties and test them robustly. Failing to understand which aspects of the exposure links to a specific observed adverse effect hinders the identification of particularly toxic nano- and microplastics and the greater understanding of nano- and microplastic effects on wildlife. Despite this complexity, at least two key issues should be considered in ecotoxicological test designs: (1) the fact that the presence of non-nutritional particles in the environment is a natural state and the addition of any non-food particle will dilute the quality of the food, which, can affect most of the commonly measured ecotoxicological endpoints, and (2) the leaching of material specific chemicals can have toxic effects of their own with different modes of toxic action. These,

aspects are, however, rarely considered in experiments. We found only six studies that used particles other than nano- and microplastics in control treatments to control for food dilution effects (Ogonowski et al., 2016; Peda et al., 2016; Redondo-Hasselerharm et al., 2018; Rist et al., 2016; Straub et al., 2017; Tosetto et al., 2016) and five studies that tested the effects of leachates separately (Huerta Lwanga et al., 2016; Martinez-Gomez et al., 2017; Paul-Pont et al., 2016; Redondo-Hasselerharm et al., 2018; Zhao et al., 2017). VKM acknowledges that more studies aiming at describing effect mechanisms are warranted.

Nano- and microplastics characteristics

The most common size range of nano- and microplastics used in ecotoxicological studies is between 10 and 100 µm (interquartile range, IQR = 15-59 µm). However, it is evident that there is a preponderant use of pristine, spherical and small (<10 µm) nano- and microplastics with polystyrene or surface-functionalised polystyrene being the most common polymers used (Fig. 3.2.1-2). The average size of polymer spheres used in experiments ranged 0.02-1755 µm in diameter (IQR = 0.1-10 µm), with 75% of studies using nano- and microplastics < 10 µm. This pattern is consistent with earlier studies (Lenz et al., 2016).

A greater diversity of polymer types and shapes can be found with studies that use larger microplastics where 95% of the studies using fibres or fragments > 16.5 µm (IQR = 48-238 µm). This is probably related to the difficulty in generating fibres and fragments in smaller sizes for experiments (Cole, 2016; Ogonowski et al., 2016).

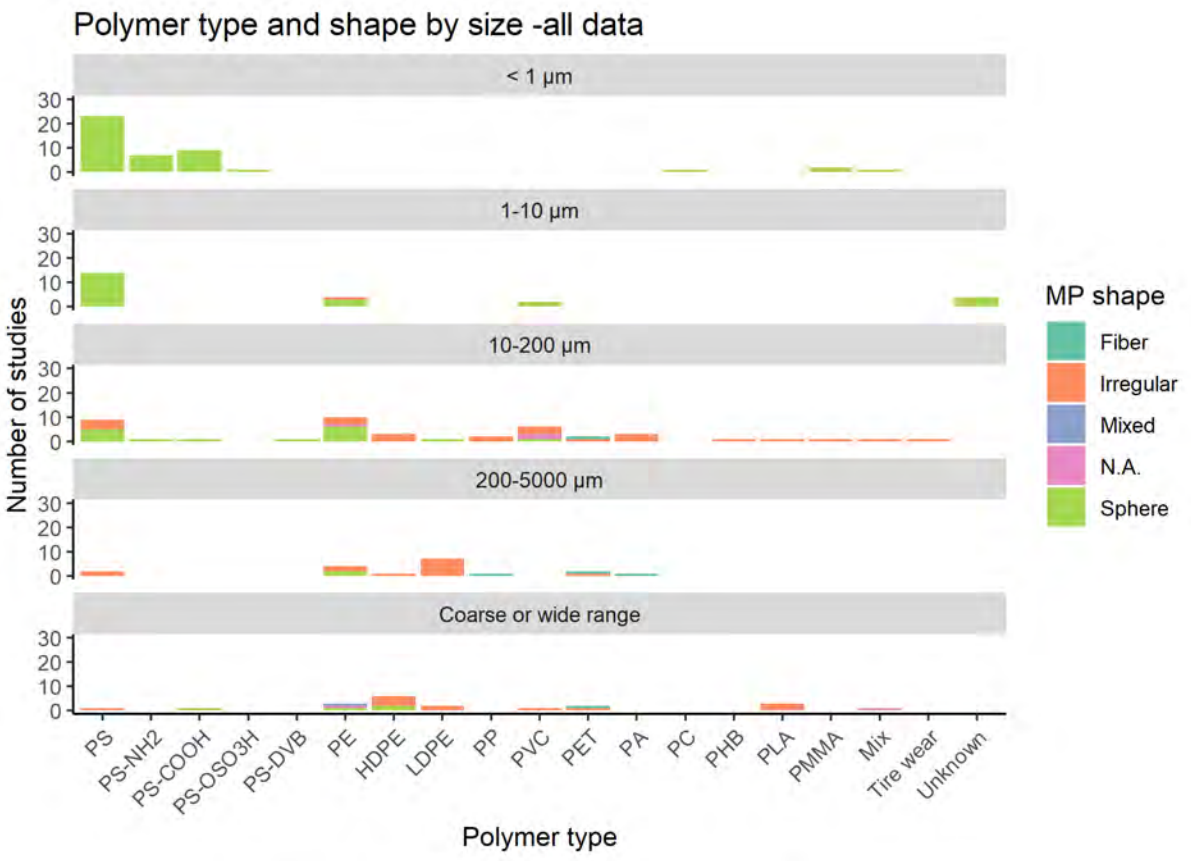


Figure 3.2.1-2 Size class, polymer type and shape of microplastics used in the full set of reviewed ecotoxicological studies (2016-2019). Studies reporting undefined size ranges or spanning across more than one size class have been excluded. Studies on fibres often fall into these categories and are therefore underrepresented. The size classes are arbitrary.

Ecotoxicological endpoints

Regarding the levels of biological organisation (Figure 3.2.1-3), the majority of nano- and microplastic studies target standard life-history parameters at the individual and/or population level such as mortality, growth, reproduction and body condition. Various oxidative stress biomarkers at the molecular and cellular levels as well as histopathological alterations at the organ level are also quite common (Figure 3.2.1-4 and 5). Studies targeting higher order levels of greater ecological relevance are very rare, which is likely a result of the inherently higher experimental complexity, need for more resources and a longer experimental duration.

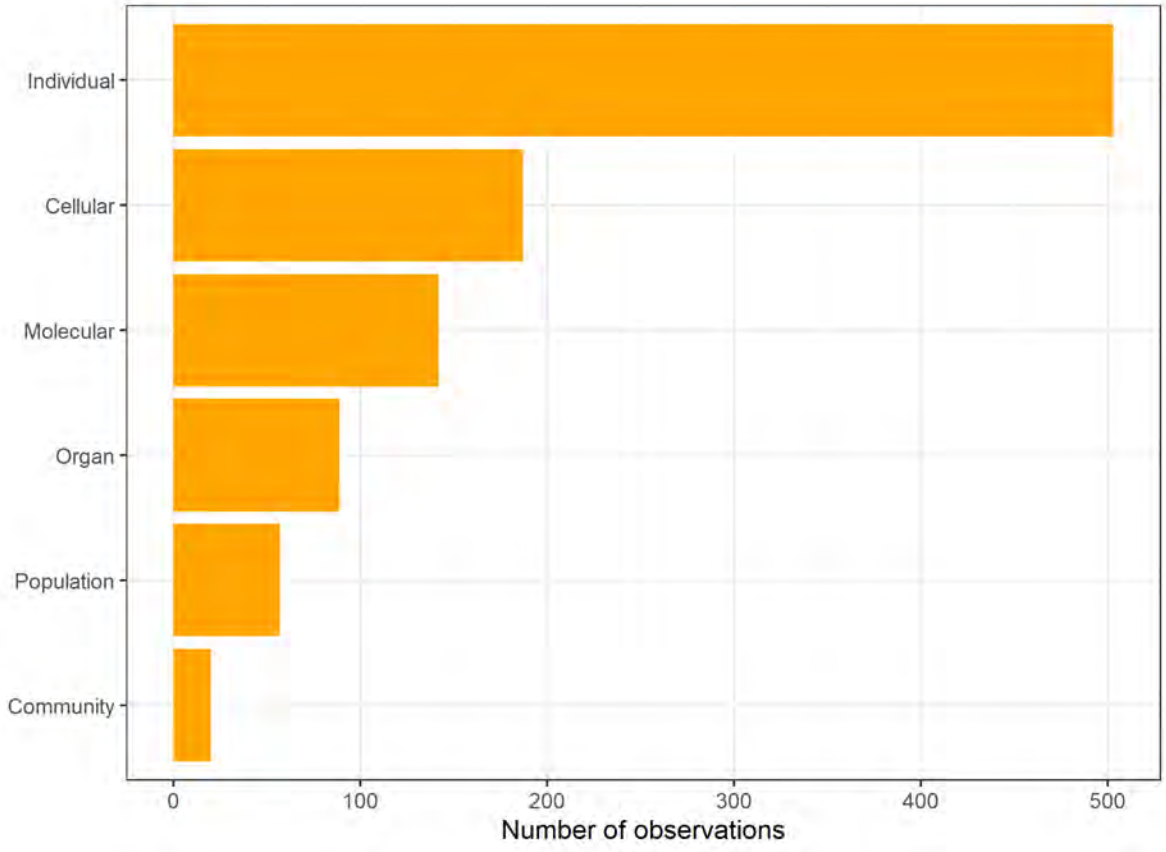


Figure 3.2.1-3 Total number of experimental observations per level of biological organisation. One observation is equal to a measured biological endpoint for a unique set of experimental conditions. There can be more than one endpoint and several experimental conditions within a particular study. For example, if mortality and growth had been studied for two different microplastics in two different organisms, then there would be 2 x 2 x 2 = 8 observations at the individual level in that study.

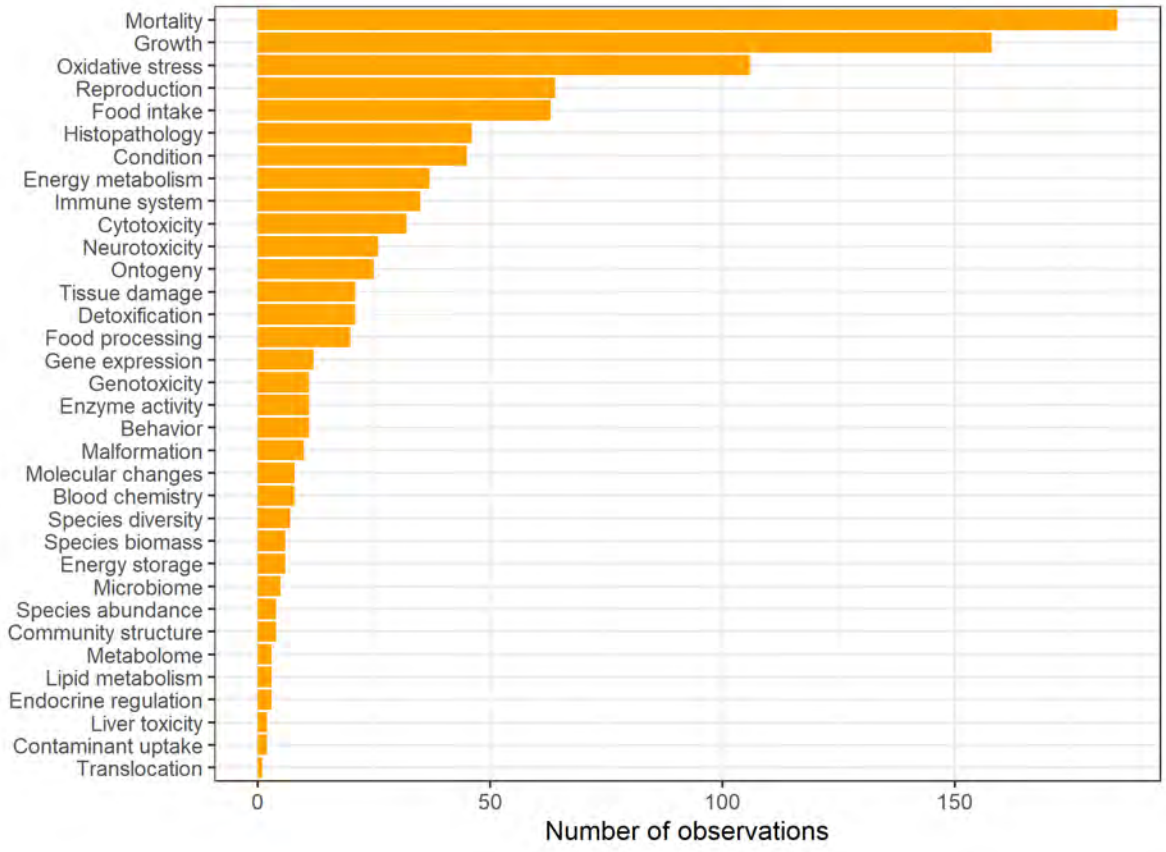


Figure 3.2.1-4 Total number of experimental observations per level of biological endpoint class. One observation is equal to a measured biological endpoint for a unique set of experimental conditions. There can be more than one endpoint and several experimental conditions within a particular study. For example, if mortality and growth had been studied for two different microplastics in two different organisms, then there would be 2 x 2 x 2 = 8 observations at the individual level in that study.

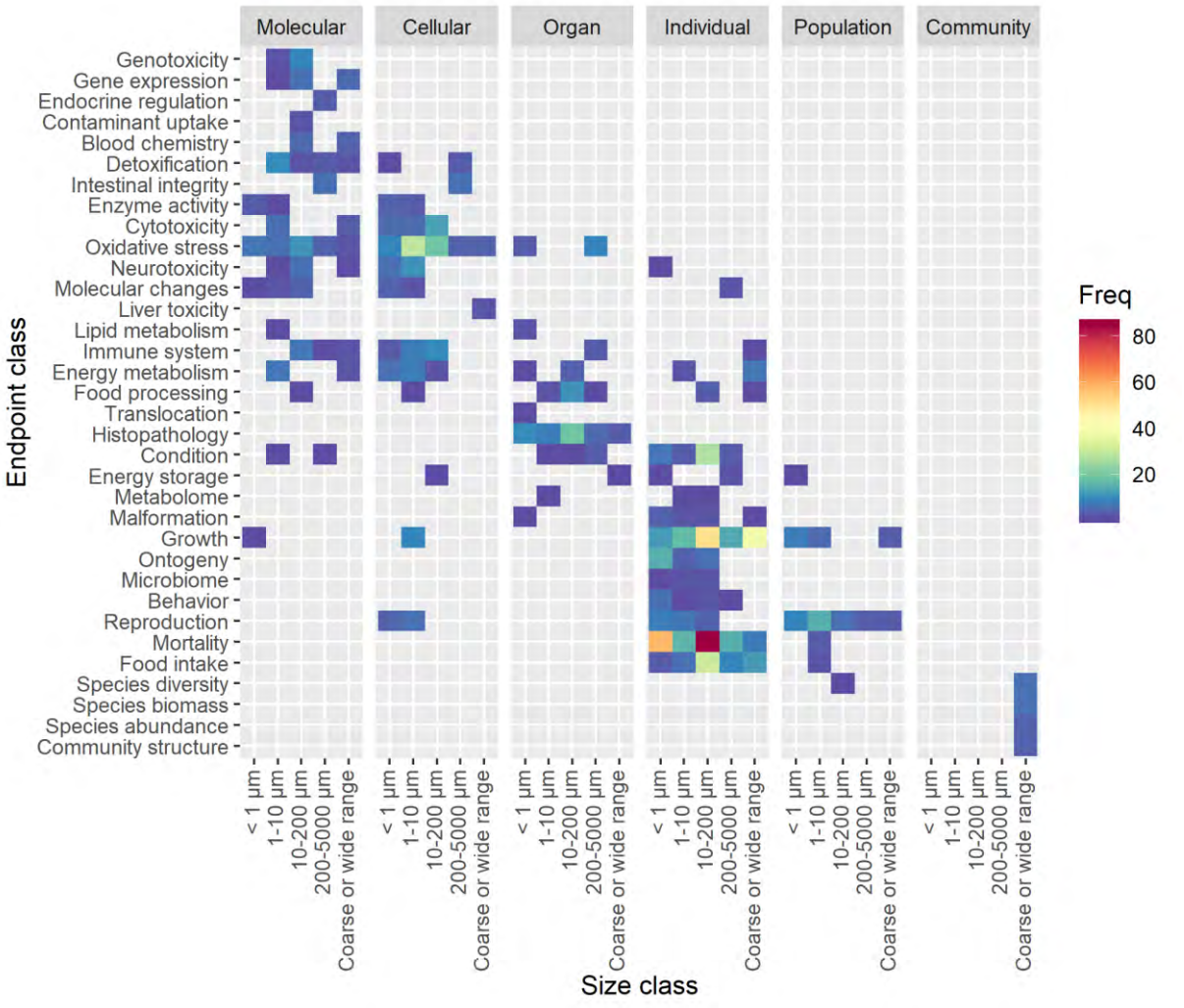


Figure 3.2.1-5 Heatmap showing the frequency of observations per endpoint class divided by particle size class and level of biological organisation. One observation is equal to a measured biological endpoint for a unique set of experimental conditions. There can be more than one endpoint and several experimental conditions within a particular study. For example, if mortality and growth had been studied for two different microplastics in two different organisms, then there would be 2 x 2 x 2 = 8 observations at the individual level in that study.

3.2.2 Methodological limitations in ecotoxicological studies

Although there has been a recent movement towards longer exposure durations, more environmentally relevant test conditions and the use of particle shapes and particle condition (weathered particles) better representative of those currently identified in the environment, there is still much to be asked for regarding ecological relevance of current test systems (Gouin et al., 2019; Lenz et al., 2016; Ogonowski et al., 2018). Several issues can be identified:

1. The presence of natural non-palatable microparticles is largely ignored despite the fact that these particles interact with nano- and microplastics, form aggregates and alter the bioavailability (Long et al. 2017, Michels et al. 2018).
2. The use of pristine plastic materials is not likely to be representative of environmental nano- and microplastics because chemicals will leach from the polymer (likely

decreasing the toxicity of plastics) and degradation processes will change the physicochemical properties (Gewert et al., 2015; Jahnke et al., 2017; Karlsson et al., 2018b).

3. Spherical nano- and microplastics are not suitable as model particles for nano- and microplastics because they are relatively rare in the natural environment.
4. Test concentrations in the larger size classes (>100 µm) are orders of magnitude higher than those reported from the environment (Chapter 10.2). At the lower end of the size spectrum, especially for particles <100 µm, little exposure data exists. Accordingly, realistic exposure levels cannot be established.
5. The lack of reference particles in control treatments precludes the identification of plastic-specific effect mechanisms.

3.3 Summaries

3.3.1 Analytical methods used to characterise occurrence and levels

EFSA (2016) and FAO (2017), and even SAPEA (2019) concluded that there was an urgent need for development and refinement of analytical methods for identification and characterisation of nano- and microplastics in different matrices.

In the present assessment, almost 60% of the scientific papers identified in the literature search (see chapter 2 for details) were not included in the data analysis as they were not of an acceptable quality. This highlights the requirement for researchers to carry out appropriate QA/QC. Analytical methods should include: sufficient replicates, analytical confirmation of microplastics, determination of recovery rates, blank controls, calculation and consideration of uncertainties/confidence levels. In summary:

- Different methodologies are used for sampling across matrices (water, sediment, biota), and a combination of methods is used to separate microplastics from the matrix and remove other matrix components.

➢ Current methods have certain limitations, in particular with regard to QA/QC.

- ➢ VKM acknowledges that techniques are under development to detect and identify increasingly smaller microplastics through automated methods. However, as methods become more complex and sensitive, they have higher chances of procedural contamination, and studies must be quality assured throughout. Further, these methods (e.i. uFTIR, FPA-uFTIR, uRAMAN/RAMAN) are very costly and, thus, unavailable for the larger scientific community.
- ➢ VKM acknowledges that quality assurance, method validation, and reporting across all methods are of variable quality. Improving those should become a focus.
- ➢ VKM concludes that transparent and good quality reporting is important to generate datasets relevant and usable for risk assessment. For example, if researchers would report the details and uncertainties of their method more transparently, this will allow for harmonisation and better comparability across methods and studies.
- ➢ VKM concludes that matrix analyzed as well as reporting metrics are often not suitable for risk assessment. From a food safety perspective, qualitative and

quantitative data on the levels of microplastics in the edible tissues (seafood) are requested.

- VKM recommends an international harmonisation of microplastics sampling, sample processing, analytical methods and reporting to be initiated for improvement of the quality and comparability between studies. Such harmonisation must not necessarily result in international standards because it will take time to develop and agree on those. A more pragmatic and short-term goal will be the development of quality criteria that the scientific community agrees upon.

3.3.2 Methods used in experimental studies

EFSA (2016) did not address experimental methods and designs. FAO (2017) and SAPEA (2019) reported a lack of ecological relevance in current experimental designs both with regard to size and shape of nano- and microplastics used as well as exposure concentrations. Acute test scenarios with low ecological relevance was identified as a major uncertainty.

VKM found:

- The experimental designs commonly employed in nano- and microplastic effect studies are currently not well adapted to test the specific toxicity of different plastic materials.
- Most of the laboratory studies are performed using much higher concentrations than are found in the environment, very small spherical microplastics, which are not representative of environmental nano- and microplastics and relatively short exposure times. Thus, it is uncertain to what extent the experimentally derived toxicity data apply apply to the natural environment. This limits the reliability in a risk assessment.
- Laboratory studies will also need to be adapted to better reflect the natural environment by acknowleddging the ubiquitous presence of and interaction with other, naturally occurring nano- and microparticles. Thus, conclusions about effects to the natural environment that are based on current laboratory experiments are uncertain and should be confirmed.

- VKM acknowledges that although there has been a recent movement towards longer exposure durations, more environmentally relevant test conditions and the use of particle shapes and particle condition (weathered particles) better representative of those currently identified in the environment, there is still much to be asked for regarding ecological relevance of current tests.

4 Levels of microplastics

EFSA (2016) identified that microplastics were found in wild-caught species including those consumed as seafood, focusing on fish and shellfish. It further stated that occurrence data was limited and there was no literature on the potential microplastic contamination of seafood during processing.

FAO (2017) said that at the time of writing very little information was available for freshwater and estuarine environments. Marine environments are globally contaminated with microplastics (detailed data in the FAO annex). Interactions of microplastics with biota were a focal point of the report. Species related to fisheries and aquaculture were highlighted but the report stated that there was no direct evidence of a trophic transfer of microplastics in wild species nor was there evidence from field studies that microplastics ingestion affected populations or communities.

SAPEA (2019) summarises what is known about the occurrence of microplastics in marine, coastal, freshwater and estuarine environments, wastewater, soils, air and biota as well as drinking water and food. The report mainly provides qualitative information that documents that microplastics are present with certainty in these compartments and matrices. SAPEA (2019) discusses several knowledge gaps (e.g., microplastic levels in the marine water column, the atmosphere and soil) and areas of uncertainty (e.g., the lack of information on microplastics <300 µm, limitations in data comparability).

The following chapter addresses studies published since 2016, with a focus on the Northern Europe and the Nordic environment. Several earlier reviews have shown global sources, global distribution and consequences for biota on a global scale. For data on a global scale we refer readers to the reviews on the environment published by GESAMP and the FAO (Koehler et al., 2015; Lusher et al., 2017b). These were heavily focused on the marine environment. For microplastics in terrestrial and freshwater systems, recent reviews are available (de Souza Machado et al., 2018a; Li et al., 2018; Mai et al., 2018).

4.1 Data analysis summary

Of the 247 papers initially selected to fall into the "Sources and Environmental Fate and/or Levels" category, 87 reported levels of microplastic, and were included in this Chapter, after being ranked related to their scientific quality (Figure 4.1-1). Of this, 66% investigated microplastics in the marine (n=57), 23% in the freshwater (n=20), and 8% in the terrestrial system (n=7). Only three studies covered multiple environments. 25 of these studies were relevant to the Nordic marine environment, with a breakdown per country corresponding to nine studies in the Baltic, six studies in the Arctic (two from Norway, two from Sweden), two in the North Atlantic, two from the North Sea, two from Scotland and single studies from Germany and Greenland.

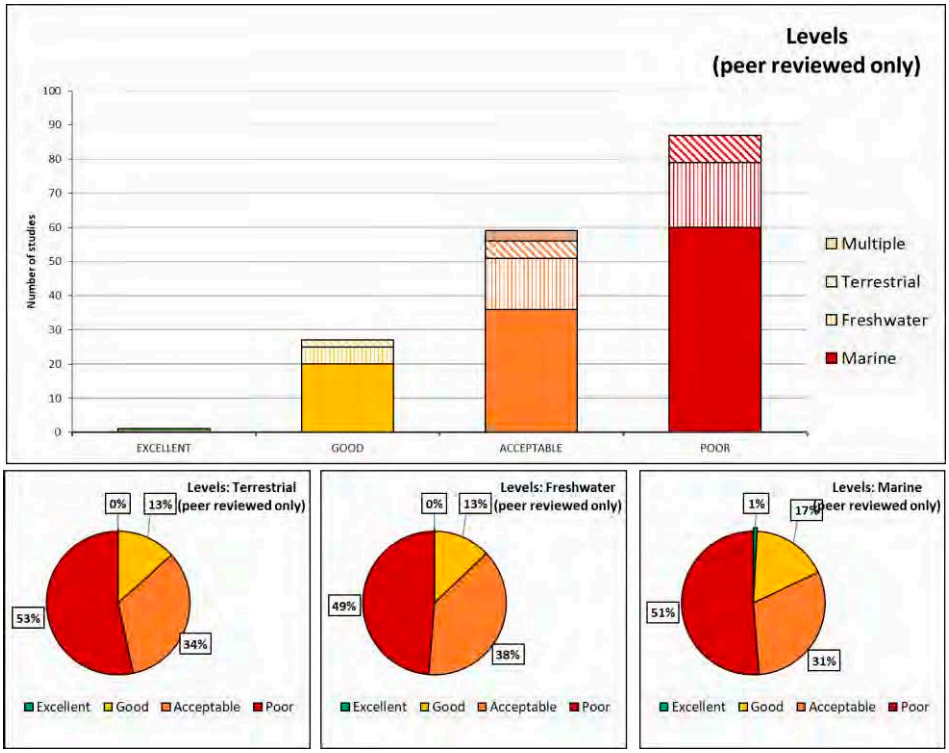


Figure 4.1-1 Quality assessment of peer-reviewed studies obtained from the original "Include" datasheet. Studies were rated with a score from 0 to 7 which were ranked into the following categories: Poor (0-2), Acceptable (3-4), Good (5-6), Excellent (7).

In addition to these peer-reviewed studies, there are several grey literature reports which have been published within the Nordic countries from 2016. This literature contains a significant proportion of data from the Nordic countries. In order to obtain a more detailed description of the Nordic environment, sources and sinks we refer to the recently published reports from the Nordic regions.

The body of evidence on microplastics in the Norwegian environment have been steadily growing, considering in 2014, MEPEX suggested we knew relatively little (Sundt et al., 2014). There have been two substantial reports detailing microplastics in the Nordic environment: one from the Nordic Council of Ministers (Bråte et al., 2017) and one from SINTEF (Booth et al., 2017).

4.2 Terrestrial environment

To date there are limited published reports on microplastics in the terrestrial environment. The following section shows the current data availability globally based on the quality assurance review.

4.2.1 Urban areas

There is no data available on microplastics in urban areas including road systems. microplastics originating in urban locations, including roadsides and storm drainage should be targeted to understand the sources and transport from terrestrial to aquatic environments. There is a single Norwegian Environment Agency Report available on

microplastics which can originate from road wear, but it contains no environmental data (Vogelsang et al., 2018b). Since our data analysis, a single publication has become available which discusses storm water runoff as a path for microplastics moving from roads to the environment (Liu et al., 2019). This study, conducted in Denmark, found that ponds serving highways and residential areas had lower microplastic concentrations than those close to industry and commercial areas (490-22894 items/m^3).

4.2.2 Landfill

Microplastics may end up in landfill following disposal of plastic material, the breakdown of plastics in landfill and the disposal of sludge containing microplastics (see below). At present, there is no data to confirm this hypothesis. Some recently published findings from China suggest that the generation, accumulation and release of microplastics in landfills is a long-term process (He et al., 2019). A single report produced on behalf of the Nordic Council of Ministers has investigated the occurrence of microplastics in landfill leachates in Norway, Finland and Iceland. The investigation looked at eleven landfills. They concluded that the lower microplastics count in samples from treated leachate suggests that local treatment has an impact on microplastic concentration (Praagh and Liebmann, 2019).

4.2.3 Agricultural soils

Initial investigations and reviews suggest that microplastics are present in agricultural soils (see (Hurley and Nizzetto, 2018; Rodríguez-Seijo et al., 2019). More data is required to validate this. The presence of microplastics in agricultural soils may pose implications for produce (Bosker et al., 2019; de Souza Machado et al., 2018b; van Weert et al., 2019).

To date, there are limited published reports on microplastics in the terrestrial environment. In 2017 Lusher *et al.,* published a report on behalf of the Norwegian Environment Agency investigating the potential release of microplastics into the terrestrial environment following the application of sewage sludge to land (Lusher et al., 2017d). The report investigated the presence of microplastics in sludge from a number of wastewater treatment plants (WWTPs) in Norway and calculated the annual release. The overall average microplastics concentration was 6077 items/kg dry weight (1701-19 837 items/kg) or 1 176 889 items/m^3 (470 270-3 394 274 items/m^3). They further estimated that, based on the average microplastic abundance and the present application of sewage sludge in Norway, over 500 billion microplastics are released into the environment via sewage sludge application each year, to agricultural soils, green areas and soil producers. It is therefore possible that sewage sludge contributes to the direct emission of microplastics to the environment.

4.2.4 Flood plains, marshes and wetlands

Terrestrial areas associated with freshwaters such as floodplains, marshes and wetlands have the potential to retain microplastics during flooding events and/or drainage. But they are also associated with catchment areas. Two relevant investigations were identified in this study: In the sediments of the Tejo estuary (wetland), microfibres were found in 100% of the sediments (Lourenco et al., 2017) and 90% of the Swiss floodplain soils contained microplastics (Scheurer and Bigalke, 2018). Recently published research shows that fish from urban wetlands are promising for biomonitoring in Australia (Su et al., 2019). There is currently no information available from the Nordic countries.

4.2.5 Air

Air has been discussed as one of the significant transport vectors of microplastics within and between terrestrial and aquatic environments. However, there is a lack of information on both the atmospheric transport and deposition of microplastics other than what has been reported for Paris, France, concerning atmospheric fall out in both indoor and outdoor settings (Dris et al., 2016a). The presence of plastics in air could lead to the contamination of pristine environments with plastics, but also the direct settling of particles into soil and water masses. Smaller microplastics (<500 µm) have been hypothesised to enter the soils through aeolian transport in a Swiss study (Scheurer and Bigalke, 2018). Outside of the geographical area included here, concentrations of microplastcs in atmospheric fall out in China were reported to range between 175 and 313 particles/m^2/day (Cai et al., 2017). There is currently no information available from the Nordic countries.

4.2.6 Biota

There is no information on the presence of microplastics in terrestrial biota to date. However, laboratory experiments have shown the ability for worms to take up particles following exposure (Huerta Lwanga et al., 2017) (see also Chapter 8). Earthworms can also transport microplastics within soil systems (Rillig et al., 2017).

4.2.7 Food and drink products

There is only one published investigation concerning the presence of microplastics in food and drink products which were accepted within this assessment. Schymanski *et al.* (Schymanski et al., 2018) compared microplastics in bottled water from different German producers (plastic bottles, glass bottles and beverage cartons). Microplastics of different size ranges were found in all types of products with an average microplastic content of 55 items/L in water from glass bottles, 118 items/L in returnable plastic bottles, 14 items/L in single-use plastic bottles, and 11 items/L in cartons. However, quality control found that the results were only significantly different from control samples in case of water bottled in returnable plastic bottles.

There has been one published report on microplastics in drinking water from Norway. This report for Norsk Vann was made available in late 2018 (Uhl et al., 2018). Over the course of the investigation, 72 triplicate samples from 24 water works were analyzed for the presence of potential microplastics. Anthropogenic microparticles were identified, although conclusive results were not drawn as the levels reported were below the detection limit and confirmation of plastic identity was not performed. Further research is required to focus on smaller levels of particles and the results require particle validation.

4.3 Freshwater

There is limited knowledge of microplastics from freshwater environments around the world. The following section shows the current data availability globally based on the quality assurance review. Freshwater systems are both transport vectors of microplastics between terrestrial and seawater systems, and they may also be sink areas for microplastics.

4.3.1 Streams, rivers and canals

Rivers and streams act as a transport route of microplastics between terrestrial environments (which includes aquatic ecosystems) to the oceans. Current data shows that microplastic contamination exists in rivers. There are three studies in Europe reporting values of microplatics in watercourses within urban areas. Levels in the Seine, Paris were 0.1 items/L (Dris et al., 2018); levels in Berlin, Germany ranged from 0.01 to 95.8 items/L (Schmidt et al., 2018); and levels in Amsterdam canals were higher, between 48 and 198 items/L (Leslie et al., 2017). However, results from other geographic regions are more variable. For example, the number of particles which has been seen in the Saigon River in Vietnam was higher than those reported for Europe (519 items/L; (Lahens et al., 2018)). However, in the Hudson River, USA, far fewer microfibres were reported (0.98 fibres/L), only 50% of which were synthetic (Miller et al., 2017). Streams in Chicago, Illinois had even lower concentrations (0.00048-0.001122 items/L; (McCormick and Hoellein, 2016)). It is evident that further research is required to quantify the distribution of microplastics in riverine systems as consequences may vary between local and more widespread implications.

River sediments have also been investigated for the presence of microplastics, for example in Shanghai, China ranged from 53 to 1600 items/kg (average 8.2 items/kg; (Peng et al., 2018)). Riverbed sediment in the UK was reported to contain a maximum concentration of 75 000 items/kg (Hurley et al., 2018a). Microplastics in lake sediments reached 2 783 items/kg in the tributary sediment of the Laurentian Great Lakes (Ballent et al., 2016b).

There is no information from the Nordic countries on the presence of microplastics in rivers, two Master's theses, which reported microplastics presence in Oslo rivers in 2016 (Bottolfsen, 2016; Buenaventura, 2017).

4.3.2 Lakes, dams and reservoirs

Lakes and dams around the world have been readily assessed for microplastics presence. However, many did not fulfil the quality requirements for this report. From this information, VKM assumes that these water bodies accumulate microplastics and are important sinks. For example, the lack of movement can lead to deposition of microplastics in the sediment. There have been two studies focused on Chinese lakes, including Lake Taihu with up to 25.8 items/L (Su et al., 2016) and Donghing and Hong Lakes with concentrations of 2.8 items/L (Wang et al., 2018). Despite being static, urban lakes may receive greater numbers of microplastics due to their vicinity to urban sources. However, there is limited information available in current literature. For example, almost 9 items/L have been reported (Wang et al., 2017). The Great Lakes in North America have also been investigated with tributaries feeding the lakes reported to have an average concentration of 1-9 items/m^3 (Ballent et al., 2016a). Two additional studies from the Lake Garda, Northern Italy (Imhof et al., 2018), Lake Winnipeg, Canada (Anderson et al., 2017) reported average concentration of 7.5 and 748 items/m^2, respectively. Two more studies from North America reported a maximum of 110 items/m^2 (Hendrickson et al., 2018) and a range between 127 and 1911 items/m^2 (Cable et al., 2017), although these data are presented relative to area whereas the other studies report concentrations per volume. Thus, a comparison is not possible.

In 2018, the first investigation of the presence of microplastics in two of Norway's largest freshwater ecosystems, Lake Mjøsa and Lake Femunden was published (Lusher et al., 2018a). Microplastics were identified in sediment from all sites in Lake Mjøsa and levels

varied depending on location to sources of anthropogenic influence including roads and boat harbors (range: 0-7.32 cumulative microplastics per gram).

4.3.3 Wastewater treatment plants (WWTPs)

Wastewater treatment plants can receive microplastic particles from domestic and industrial sewage systems. For example, these particles may be created during industrial processes, but can also come from the use of household cosmetics containing microplastic particles and the washing of textiles which produce fibres. Four studies on WWTPs were identified through the literature search (Table 4..2.3-1). All studies looked at effluent, two at sludge and two at influent. The methods of sample treatment were similar with density separation applied in two studies, digestion on its own and filtering on its own for the other two studies. During the water treatment process, microplastic concentration are higher in the influent (Leslie et al., 2017; Murphy et al., 2016), and the majority (<80%) is removed from the water phase during the multiple processing steps.

Leslie *et al.* (Leslie et al., 2017) showed a reduction in mean concentration of 73 items/L to 52 items/L in a Dutch WWTP from influent to effluent, and 94% reduction was shown across a Scottish WWTP (Murphy et al., 2016). Higher removal efficiencies were reported in a German WWTP which had a lower detection limit (9 000/m3; (Mintenig et al., 2017). Microplastics captured during wastewater treatment generally end up in the sludge. Sludge has been shown to contain varying levels of microplastic. As an example, Leslie *et al.* identified mean concentration of 650 items/L (range 370-930 items/L) in the Netherlands (Leslie et al., 2017). Sewage sludge can contribute to the direct application of microplastics to the terrestrial environment as it is applied directly to soils in agriculture.

Table 4.2.3-1 Summary of data collected from wastewater treatment plants with mean values reported.

Reference	Location	Method	Influent	Effluent	Sludge
Leslie et al., 2017	The Netherlands	Density, Visual, FTIR	73 per litre	52 per litre	650 per litre
Majewsky et al., 2016	Germany	Density, Digestion, TGA		240-1540 mg/m3	
Mintenig et al., 2017	Germany	Digestion/density, FTIR		0-9000 items/m3	100-24000 items/m3
(Murphy et al., 2016)	Scotland	Filtering	15.7 per litre	0.25 per litre	

4.3.4 Freshwater biota

Biota in all freshwater compartments (water, sediment) interact with microplastics, although the data available is scarce in comparison to the marine environment. One form of interaction, which is generally most studied across all compartments is ingestion. Fish are currently one of the most studied taxonomic group from the freshwater environment related to microplastic ingestion. For example, species studied were the Nile perch and Nile tilapia in Africa (Biginagwa et al., 2015), the common roach from the River Thames, UK (Horton et al., 2018) and goby and barbel fish from Switzerland (Roch and Brinker, 2017). Other taxonomic groups include oligochaetes (Hurley et al., 2017) and bivalves (Su et al., 2018). As an

example, Lourenzco *et al.* (Lourenco et al., 2017) found 97% of macroinvertebrates investigated at Tejo estuary contained at least 1 microfibre/individual. In addition, 74% of the shorebird feces analyzed in that area contained microfibres. This could indicate trophic transfer of microplastics, however, the authors reported that the difference in microfibre concentrations from shorebirds varied based on individuals rather than foraging strategies.

Table 4.3.4-1 Summary of data collected referring to microplastics identified in freshwater biota. Data displayed are mean values with range where possible. MP = Microplastic.

Species	Common names	Location	Mean MP/individual (range)	Reference
Lates niloticus	Nile perch	Africa	n.r.	Bignagwa et al., 2015
Oreochromis niloticus	Nile tilapia	Africa	n.r.	Bignagwa et al., 2015
Rutilus rutilus	Roach	UK	0.69 (0-6)	Horton et al., 2018
Neogobius melanostomus	Round goby	Switzerland	1	Roch et al., 2017
Barbus sp.	Barbel	Switzerland	1.25	Roch et al., 2017
Tubifex sp.	Tubifex worm	UK	125	Hurley et al., 2017
Corbicula fluminea	Asian clam	China	(0.2-12.5)	Su et al., 2018

4.4 Marine

There have been copious amounts of data collection regarding microplastics in the marine environment (e.g., reviews, and global investigations, (Barrows et al., 2018), for this reason the data presented in the following section focuses on Europe, with a main focus on the Nordic region. In addition, many models related to the oceanic distribution of microplastics were developed prior to this assessment (e.g., (Cozar et al., 2014; Eriksen et al., 2014; van Sebille et al., 2015)).

4.4.1 Coastal and brackish waters (includes estuarine and shorelines; pelagic and benthic)

In East Greenland, microplastics were identified in vertical tows (from 50 m to the surface) taken in from the foraging areas of little auks with average concentrations between 0.99 and 2.48 items/m^3 (Amelineau et al., 2016) which is similar to those reported by Morgana *et al.* (1-3 items/m3)(Morgana et al., 2018). There has been one baseline report from within the Oslofjord (Albretsen et al., 2018). Modeling suggests that Østfold (and the Bohuslän coast) are most prone to accumulation of waste coming from afar (through the North Sea or the Baltic Sea), while local discharges via the rivers are mainly a local problem for the coastal stretches (Albretsen et al., 2018).

Table 4.4.1-1 Summary of data collected referring to microplastics identified in coastal and brackish waters. Microplastic (MP) concentration is displayed as mean (range) unless otherwise stated.

Location	Compartment	Dominant plastics	MP concentration	Reference
Sweden	Surface water	Fibres	3.20 (0-14) items/m^3	Magnusson and Norén, 2011
Outer Oslofjord	Surface waters	Black fragments	9-217 items/m^3	Albretsen et al., 2018
Greenland	Surface/zooplankton samples	Fibres and fragments	0.99-2.48 items/m^3	Amelineau et al., 2018
Greenland	Surface water	Fragments	1-3 items/m^3	Morgana et al., 2018

4.4.2 Pelagic offshore

Some of the studies carried out in pelagic offshore waters indicate that these are dominated by microplastic fibres. For example, Barrows *et al.* (Barrows et al., 2018) found 91% of plastic particles identified in their global study to be fibres, with an average of 11.8 items/L. Currently, there is limited investigation on the presence of microplastics in the Nordic marine environment. Offshore waters have been studied in the polar region (Kanhai et al., 2018; Lusher et al., 2015) data from the Arctic suggests long range transport might be responsible for microplastics (Lusher et al., 2015).

Offshore waters in the Arctic have been studied for the presence of microplastics in three investigations (Cozar et al., 2017; Kanhai et al., 2018; Lusher et al., 2015). Concentrations in surface waters reported ranged from 0 to 320 items/m^2 (Cozar et al., 2017), whereas subsurface waters contained up to 7.5 items/m^3 (Kanhai et al., 2018). Areas such as the Barents Sea have been suggested as hotspots for microplastic occurrence, with some researchers referring to it as the 6th plastic gyre (Cozar et al., 2017).

Investigations in the Baltic Sea showed surface concentrations between 0.19 and 7.73 items/m^3 ($1.9x10^{-4}$ - $7.73x10^{-3}$ items/m^2; (Gewert et al., 2017)), whereas *Karlsson et al.* (Karlsson et al., 2017) found 48 items/L. Tamminga *et al.* (Tamminga et al., 2018) found lower levels of microplastics (fibres) in both pump (1.03 items/L) and surface (manta) net (0.07 items/m^3) samples.

The water masses in the North Atlantic and North Sea have been suggested as the potential main driver of plastics into the Nordic seas and the Arctic. Values from the offshore from Bay of Brest were reported as 2.4 x 10-4 items/m^3 (Frere et al., 2017) and 1.5 items/m3 in the North Sea (Maes et al., 2017). This is in accordance with the latitudinal gradient in the North Atlantic reported by Kanhai et al., (average 1.15/m^3; (Kanhai et al., 2017)). Microplastics have also been observed in pelagic deep-sea waters in the North Atlantic (Courtene-Jones et al., 2017).

Table 4.4.2-1 Summary of data collected referring to microplastics identified in pelagic offshore waters. Microplastic (MP) concentration is displayed as mean (range) unless otherwise stated.

Location	Compartment	Dominant plastics	MP concentration	Reference
Baltic Sea	Subsurface waters	Fibres and fragments	0.19 - 7.73 items m3	Gewart et al., 2017
Baltic Sea	Surface waters	Fibres and fragments	48 per litre	Karlsson et al., 2017
Baltic Sea	Surface waters	Fibres	0.04-0.09 (0.07) per m3	Tamminga et al., 2018
Arctic	Surface waters	Fragments and fibres	0-1.31 (0.34) items m3	Lusher et al, 2015
Arctic	Subsurface waters	Fibres	0-11.5 (2.0)	Lusher et al, 2015
Arctic	Subsurface waters	Fibres	0-7.5 (1.15) items m3	Kanhai et al., 2018
Arctic	Surface waters	Fragments	0- 320000 Per km2	Cozar et al., 2017
Arctic	Surface waters	Fibres	31.3 per litre	Barrows et al., 2018
North Atlantic	Subsurface waters	Fibres	0-8-5 (1.15)/m3	Kanhai et al., 2017
North Sea	Surface waters	Fragments	0-1,5 items/m3	Maes et al., 2017
Bay of Brest, North Atlantic	Surface waters	PE, PP, PS	2.4 x 10-4 items/m3	Frere et al., 2017
North Atlantic	Deep-sea	Fibres	70.8 items/m3	Courtene-Jones et al., 2017

4.4.3 Sea ice

Microplastics can also be captured and potentially accumulate in the sea ice, which means that sea ice can both be a collection site and a source of microplastics by ice melting (Obbard et al., 2014). In 2017, it was found that deep sea sediments from the Greenland Sea near the ice edge had high amounts of microplastics (Bergmann et al., 2017), which supports the theory of Obbard and colleagues. In addition, Peeken et al., (Peeken et al., 2018) reported a maximum of 12000000 microplastics per m^3 and suggested that sea ice acts as an important temporal sink and transport mechanism for microplastic.

4.4.4 Marine sediments (inshore/offshore)

Microplastics have been identified in sediments in the marine environment from beaches and coastal areas to the deep sea.

Coastal sediments (beach shorelines and intertidal zones) contain microplastics. One study investigating intertidal sediments from Orkney (Scotland, UK) reported mean concentrations of microplastics where 730 were particles and 2300 fibres per kg (Blumenroder et al., 2017). Similar concentrations were reported in the Tyrrhenian Sea which were lower than in the Adriatic Sea (1037/kg; (Fastelli et al., 2016)). Much lower concentrations were seen in Atlantic sediments (mean, 0.97/kg; Bay of Brest, France; (Frere et al., 2017).

There have been a few investigations looking at beach sediments in the Baltic (Graca et al., 2017; Hengstmann et al., 2018; Karlsson et al., 2017) which have shown varying levels of microplastics. Concentrations of microplastic from the west coast of Sweden averaged 27 particles/kg. Fibres (including polyester) were dominant on the shorelines of the Isle of Rugen, with a mean concentration of 88.1 particles per kg (Hengstmann et al., 2017). An investigation comparing different coastal structures (dunes vs. cliffs) found that there were similar concentrations. However, the number of microplastics decreased following storms in cliff areas (Graca et al., 2017). As with the previous study, fibres were the dominant particle type.

Offshore sediments are also contaminated with microplastics. They are considered one sink of microplastic contamination (Booth et al., 2017). Two examples include concentrations of microplastics in North Sea sediments ranging from 0-3146 particles/kg (Maes et al., 2017) and Hausgarten Observatory (2340-5570 m depth) where concentrations ranging from 42 to 6595 microplastic items/kg sediment d.w., with an overall mean concentration of 4356 (± 675 SE) items/kg (Bergmann et al., 2017). It should be noted that many of the studies use different approaches and the data cannot be directly compared.

The literature search conducted for this assessment did not pick up any Norway-specific investigations of microplastics in benthic sediments. Since the literature search was conducted, a single paper has been published. In Bergen, sediments from sites close to wastewater and depositions sites in the urban fjord of Bergen (Byfjorden) were investigated. Twenty different polymer types were identified, at concentrations from 12 000 to 200 000 particles/kg d.w. Most of these particles were <100 µm in size (Haave et al., 2019).

Additional information is available in reports for the Norwegian Environment Agency. Microplastics have been identified in coastal and offshore sediments in the Nordic marine environment. Using conservative estimates, relatively higher average concentrations of microplastics were identified in sediments from the central than the northern North Sea and the Barents sea (81 ± 93, 31 ± 40 and 21 ± 15 mg microplastics/kg dry sediment, respectively; (DNV-GL and NGI, 2018)). These values are similar to those reported for Arctic deep-sea sediments (Bergmann et al., 2017).

Table 4.4.4-1 Summary of data collected referring to microplastics identified in benthic sediment. Microplastic (MP) concentration is displayed as mean (range) unless otherwise stated.

Location	Compartment	Dominant plastics	MP concentration	Reference
Hausgaten, Arctic	Offshore	Fragments	42-6595 per kg (4365)	Bergmann et al., 2017
Longyearbyen, Svalbard	Shoreline	Fibres and particles	6.3 kg-1	Sundet et al., 2015
Scarpa flow, Scotland	Offshore	Fibres	2300 items per kg	Blumenroder et al., 2017
Plymouth, UK	Beach sediment	Pellets	29.3-144.1 items per kg	Coppock et al., 2017
Brest, France	Inshore	Fibres	0.97 items per kg	Frere et al., 2017
Baltic Sea	Inshore	Fibres	0-27 items per kg	Graca et al., 2017
Baltic Sea	Beach sediment	Fibres	88.1 items per kg	Hengstmann, et al., 2018
North Sea	Inshore	Fibres	100-3600 items per kg	Leslie et al., 2017
North Sea	Offshore	Fibres, spheres	0-3146 items per kg	Maes et al., 2017
Baltic Sea	inshore	Fibres	443.3 items per m3	Railo et al., 2018
Norwegian Sea	Inshore	Fragments	1-50 kg'1	DNV-GL and NGI, 2018
Bergen Fjord	Inshore	Fragments	12,000 to 200,000 particles kg−	Haave et al., 2019
Greenland Sea, Adventfjord, Svalbard	Coastal sediment	Fibres	9.3 kg-1	Sundet et al., 2015

4.4.5 Marine biota

Microplastic ingestion by marine biota has been documented in a few species from the Norwegian marine environment, although knowledge on occurrence of microplastics in biota is still limited. Based on the detection of plastic and microplastics in cod and mussels from the Norwegian coast (Brate et al., 2018; Brate et al., 2016), it is clear that Norwegian biota, similarly to biota around the world, interact with a variety of different types of plastics.

Fish

In a study of plastic in the stomach of cod from six stations along the Norwegian coast (particles >0.15 mm), it was found that 3% of the cod had plastic in the stomach. Cod from the Finnmark coast did not have microplastics above this size in the stomach, while it was found in 27% of the cod in the Bergensfjord area (Brate et al., 2016). Furthermore, there was great variation in the shape, size and material composition of the plastic that was found. Low levels of ingestion have been observed in polar cod and big eye sculpin from the Arctic

near Svalbard and the East coast of Greenland (Table 4.4.5-1). Long term data series using fish as an indicator of microplastics found there to be no increase in the number of particles identified over three decades (Beer et al., 2018).

Bivalves

Recently, microplastics (particles over 0.07 mm) were found in mussels (Mytilus sp.) from 14 of 15 stations along the Norwegian coast, with an average of 1.5 microplastic particles per individual (Brate et al., 2018).

Benthic invertebrates

Benthic invertebrates have only been investigated in the Baltic (Karlsson et al., 2017), the North sea coast (Karlsson et al., 2017) and the Rockall Trough in the Atlantic (Courtene-Jones et al., 2017). There are also reports from the Pacific Arctic (Fang et al., 2018).

Table 4.4.5-1 Studies of microplastics in marine fish with relevance to Nordic environments. MP = microplastics. MP/individual refers to the average number of microplastics identified in digestive tracts (stomach and/or intestines) of individuals.

Species	Common name	Location	Mean MP/individual (range)	Reference
Boreogadus saida	Polar cod	Arctic	(0-1)	Kühn et al., 2018
Gadus morhua	Cod	Norway	n.r.	Bråte et al., 2016
Gadus morhua	Cod	Baltic	(0-1)	Budimir et al., 2018
Clupea harengus ***Sprattus sprattus***	Herring and Sprat	Baltic	1.15	Beer et al., 2018
Clupea harengus	Herring	Baltic	(0-1)	Budimir et al., 2018
Gasterosteus aculeatus	Stickleback	Baltic	(0-1)	Budimir et al., 2018
Mulitple	Mesopelagic fish	North Sea	0.13 (0-3)	Lusher et al., 2016
Gadus morhua Limanda limanda ***Platichthys flesus*** ***Clupea harengus*** ***Scomber scombrus***	Cod, dab, flounder, herring, mackerel	North Sea	(0-3)	Rummel et al., 2016
Triglops nybelini	Sculpin	Greenland	(0-1)	Morgana et al., 2018
Boreogadus saida	Polar cod	Greenland	1.1 (0-2)	Morgana et al., 2018
ammodytes tobianus	Sand eel	Celtic Sea	1.75	Welden et al., 2018
Not reported	Fish larvea	English channel	0-2	Steer et al., 2017
Clupea harengus ***Sprattus sprattus*** ***Limanda limanda*** ***Merlangius merlangus***	Atlantic Herring, Sprat, Common Dab, and Whiting	North Sea	0-1	Hermsen et al., 2017

Table 4.4.5-2 Studies of microplastics in bivalves with relevance to Nordic environments. MP = microplastics.

Species	Location	Mean MP/individual (range)	Reference
Mytilus sp.	Norway	1.5 (0-6.9)	Bråte et al., 2018
Mytilus sp.	Scotland	Per gram: 0.086	Catarino et al., 2018
Mytilus sp.	North Sea	5-37 per 5 individuals	Karlsson et al., 2017
Mytilus sp.	Baltic	0.4	Railo et al., 2018
Modiolus sp.	Scotland	Per gram: 3	Caterino et al., 2018

Table 4.4.5-3 Studies of microplastics in benthic invertebrates with relevance to Nordic environments. MP = microplastics.

Species	Location	Mean MP/individual (range)	Reference
Optiomusium lymani	Rockall Trough	1.153/g indi	Courtene-Jones et al., 2018
Hymenaster pelluciculus	Rockall Trough	1.582/g indi	Courtene-Jones et al., 2018
Colus jeffreysianus	Rockall Trough	0.678/g indi	Courtene-Jones et al., 2018
Asteria rubens	Pacific Arctic	0.17-9.73	Fang et al., 2018
Leptasterias polaris	Pacific Arctic	0.02-0.46	Fang et al., 2018
Ctenodiscus crispatus			
Latisipho hypolispus			
Retifusus daphnelloides			
Euspira nana			
Astarte crenata			
Macoma tokyoensis			
Ophiura sarsii			
Chionoecetes opilio			
Pandalus borealis			

Birds and mammals

Fulmars are probably the most studied seabird in terms of plastic ingestion worldwide (Van Franeker and Law, 2015), however, few recent publications emerged during our literature search. Fulmars have been identified as an indicator of plastics >1mm. Methods are being adapted for microplastics. Investigations of gular pouch contents of little auks (*Alle alle*) found concentrations of microplastic fibres and fragments to reach concentrations of 9.99 particles per individual in Greenland (Amelineau et al., 2016). Marine mammals have also been investigated for microplastic pollution, and mammals from the North Atlantic, stranded or bycaught in Irish waters have shown that particles are found throughout digestive tracts an can be compared between species (Lusher et al., 2018b). This form of opportunistic sampling is yet to be put in place for Nordic countries but looks promising, especially when deep diving species, such as beaked whales appear to be more sucseptable for plastic ingestion. These species are reported to strand most often in Nordic waters.

4.5 Summary

EFSA (2016), FAO (2017) and SAPEA (2019) concluded that available data on nano- and microplastics in the environment and food were mainly qualitative and that quantitative data are very limited. They also stated that there were serious difficulties in data comparisons due to methodological limitations.

VKM found:

- The present literature search revealed some data inconsistencies across the Nordic environment. Most data available are related to surface and subsurface water, and marine biota. There is limited data from freshwater and terrestrial compartments as compared to the marine compartment.
- The use of methods which have not been adequately validated, further complicates the data comparability.
- There is still no consensus on how data is reported across studies, both in terms of particle sizes and concentrations (i.e., metrics), furthering the difficulties in comparisons. Consequently, this lack of robust estimates on microplastic quantities as well as regional differences in abundance is a source of uncertainty.
  With many of the investigations implementing visual identification as the only step for microplastic identification/confirmation, this may lead to misestimations of microplastics levels, especially when researchers using confirmatory steps report errors in identification rates reaching 70%.

- VKM concludes that data available on levels of microplastics in the Norwegian environment are mostly from the marine compartment (surface and subsurface waters and biota). Limited data only are available from freshwater and terrestrial compartments.
- VKM concludes that very limited data of acceptable quality are available on levels of microplastics in foods. Importantly, many relevant food categories (meat, vegetables, dairy products) have not been investigated at all.

5 Sources, transport, distribution and fate of microplastics in the environment

EFSA (2016) mentioned the release of microplastics to the terrestrial and aquatic environment through the use of personal care products, cleaning agents and textile fibres, but does not go into detail on the fate or transports of microplastics into the environment and refers instead to reviews such as GESAMP (Koehler et al., 2015). Atmospheric transport is mentioned as a route of microplastics dispersal.

FAO (2017) does not go into detail on the sources of microplastics in the environment either but focuses on aquaculture and fisheries. Fisheries and aquaculture are reliant on plastics, including ropes and netting, packaging, buoys and also boat paints and antifouling coats. These items may act as a source of microplastics if they fragment during use, or are lost to the environment and broken down (such as abandoned, lost and discarded fishing gear, ALDFG). There is some discussion regarding the distribution of microplastics in aquatic environments related to fisheries and aquaculture.

SAPEA (2019) only touches briefly on the fate of microplastics, in line with the limited state of knowledge. While microplastic emissions occur from multiple primary sources and secondary processes (degradation of larger items) to all environmental compartments, the actual fate in the environment (i.e., degradation and transport) is highly context-dependent and often poorly understood. For microplastic transport, numerical modelling has been significantly advanced in recent years, but lack appropriate validation with empirical data. The fate of microplastics in soils and the atmosphere remains unknown, according to SAPEA (2019).

5.1 Sources

The identification of sources of microplastics is crucial to reduce their impact on the environment. Microplastic sources can loosely be aligned to the categories of primary and secondary microplastics. 'Primary' referring to those that are deliberately produced to be used in the micro-scale, whereas 'secondary' are those which breakdown during use or in the environment once discarded (see Chapter 3.2).

5.1.1 Land

Land is the main source of microplastics. After all, all plastics ever produced were first created in factories situated on land. A common source of microplastics is pre-production of plastic pellets used for making plastic products leaking directly into the environment from production site or are spilled during transportation. A study by Karlsson *et al.* (Karlsson et al., 2018a) found that the number of plastic pellets is correlated to vicinity to production sites. Microplastics can also be released from households and industry when they are used in cosmetics and personal care products (Guerranti et al., 2019), industrial abrasive scrubbers,

and paints. Microplastics generated by washing synthetic materials will release fibres during the washing cycle. Most of these materials will make their way in the sewage system, and end up at wastewater treatment plants; either being removed, or passing through to be released into aquatic systems (De Falco et al., 2019). Sources of microplastics can also be devided into land-based and marine-based. An overview of the different sources and how they interact is shown in Figure 5.1-1, and described in the following sections.

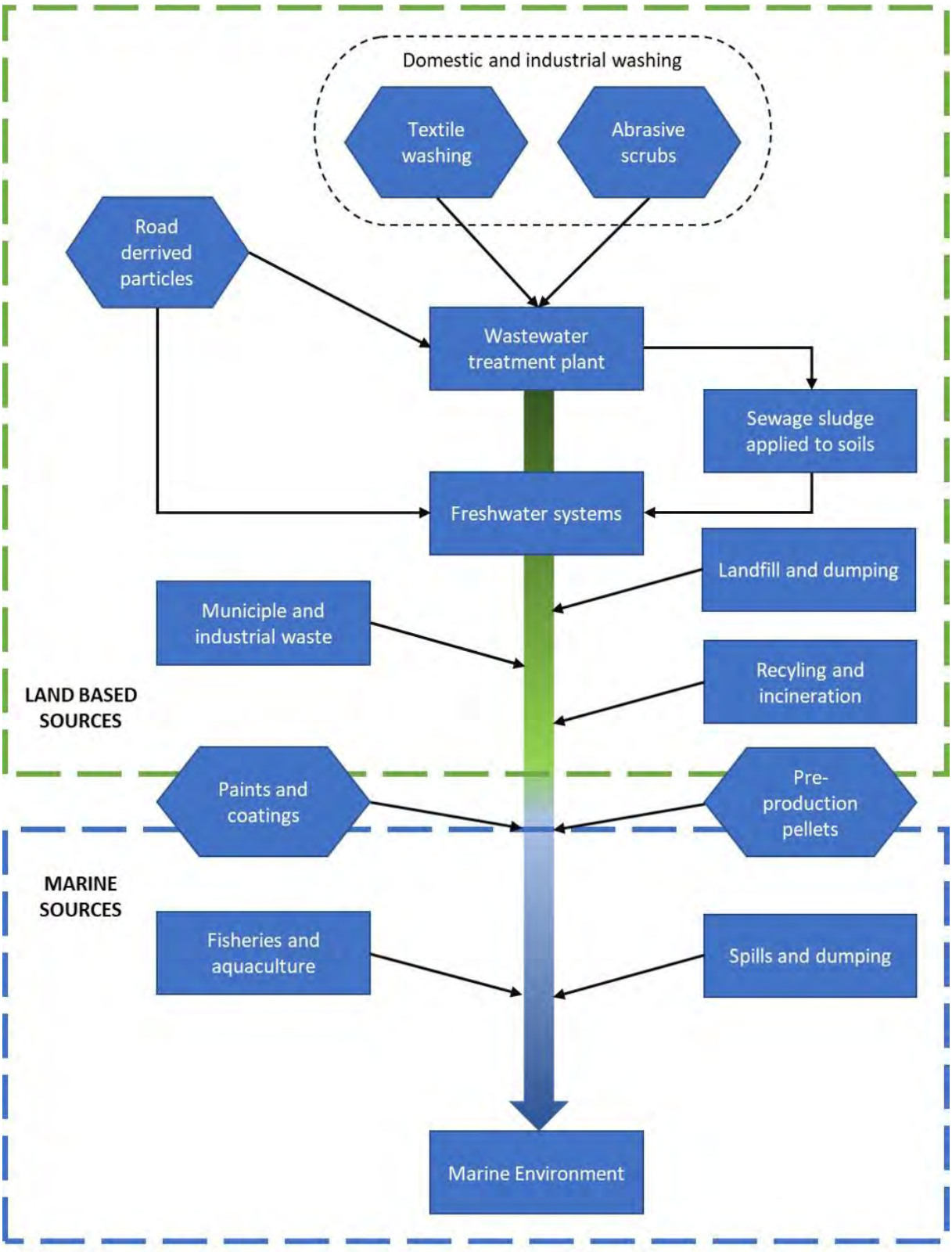


Figure 5.1-1 An overview of land-based and marine-based sources of microplastics.

Materials that reach sewage systems, including the primary microplastics, but also large items which have also been flushed, such as Q-tips, will end up in wastewater treatment plants, where they either enter the treatment process or bypass directly into aquatic

environments via stormwater overflows, thus making wastewater treatment facilities a reservoir for microplastics. Importantly, most state-of-the-art wastewater treatment plants equipped with tertiary treatment effectively remove >90% of microplastics via sedimentation. However, the application of sewage sludge as fertilizer in agriculture will result in an emission of microplastics. As was seen in an investigation of Norwegian WWTPs, an estimated 500 billion microplastics from sewage sludge may be applied to the terrestrial environment per year (Lusher et al., 2017c).

In addition, farming and agriculture have been discussed as a potential source of microplastics due to the use of plastic mulching films (Hurley and Nizzetto, 2018) and the microplastics contamination of compost and fertilizer (Weithmann et al., 2018). Microplastic derived from agriculture may accumulate in soils or can be transported to other compartments via run-off or atmospheric dispersal.

Roads, vehicles and buildings act as sources of microplastics during use but also through maintenance. Tires and road paint can be broken down and release microplastics directly to the vicinity of roads, furthermore, salt which is applied to roads in winter can contain large volumes of microplastics which is a direct source to terrestrial ecosystems (Vogelsang et al., 2018a). Tires not only act as a source during use: During the recycling processes they are shredded to granules and utilised as infill for artificial turf, or as paints for playgrounds and polymer modified asphalt (Lassen, 2015). The wear and tear of these materials will cause such particles to move into the surrounding environment and may be moved further by wind and rain.

Plastics in municipal waste generally have three routes for processing: recycling, incineration, or landfill. Sometimes, they can escape these systems and enter freshwater and terrestrial environments (He et al., 2019). Accidental littering as well as deliberate dumping of macroplastics is another source of microplastics if these materials degrade and fragment.

Ship breaking yards and other industrial facilities situated along rivers or on shorelines can leak microplastics. Their activities can lead to the input of microplastics, such as paints and plastics used in shipping, fishing and aquaculture (Barua et al., 2018).

5.1.2 Sea

Sources of microplastics at sea include all infrastructure operating in the oceans, from small fishing vessels to semi-permanent structures including oil and gas platforms. There are many sources of plastics from within fisheries and aquaculture (Lusher et al., 2017b). The most widely discussed of these sources is ALDFG which can fragment into smaller and smaller plastics once no longer in operation as fishing materials, but also by fishing gear, as it erodes. Loss of microplastics during shipping, including the transport and spillage of plastic production pellets, could be attributed to a source at sea, although it is hard to identify if they are released at sea or have been transported to the ocean by river and loss on land.

5.2 Transport and distribution

5.2.1 Land

Run-off and atmospheric dispersal can transport plastics from land into freshwater or marine ecosystems. There are very few publications available on the topic, although there has been

some effort to create theoretical assessments (Hurley and Nizzetto, 2018; Nizzetto et al., 2016; Siegfried et al., 2017)). Waterways and lakes may be sinks of microplastics, especially their sediments. Streams and rivers are also transporting microplastics to the ocean see (Lebreton et al., 2017; Schmidt et al., 2017). Along the way they may be transported and released through wastewater systems.

5.2.2 Air

Light and buoyant microplastics can be picked up by atmospheric currents and transport them from sources of input. Further research into this mode of transport is required. There are very few publications available on the topic and effort to create theoretical assessments is required (Allen et al., 2019; Dris et al., 2016b; Klein and Fischer, 2019).

5.2.3 Freshwater systems

Wastewater treatment plants receive inputs from a wide variety of uses including domestic and industrial. They also collect rain and storm water, as well as road runoff. Therefore, there are many different types of microplastics which they can distribute. Of the studies included in this review, we can see that there is a distinct reduction in the numbers of microplastics in the effluent compared to the influent, showing the WWTPs can remove particles rather than releasing them directly, e.g., Leslie *et al.* (Leslie et al., 2017). However, what is removed is often incorporated into the sludge, which is then directly released into the environment again when it is applied as a fertilizer to soil (Corradini et al., 2019).

Material which is released in the effluent tends to be heavily weighted with microplastics derived from cosmetics and other personal care products (Carr et al., 2016). This is probably related to the size of these particles being smaller than the filtering mechanisms within the treatment plant. Removal efficiently can depend on the design of the plant (Mahon et al., 2017). In addition, rainfall events can cause the influent to a WWTP exceed the handling capacity of the plant, or the sewer system, resulting in the discharge of untreated wastewater into rivers, lakes and coastal areas. It has been highlighted that these events may have a significant input of microplastics to the environment (Kataoka et al., 2019).

Streams and rivers are efficient modes of transport for plastics into the environment. Plastics of lighter densities can float and be transported with the current whereas more dense particles, or those which have become bio-fouled, are likely to sink and become incorporated into the sediment. Riverine systems will ultimately reach the ocean and release particles to ocean currents to continue the oceanographic distribution. The role of freshwaters as a major source of microplastics to the marine environment should not be neglected (Sighicelli et al., 2018). More information of river catchment areas and plastic contamination is needed.

5.2.4 Marine systems

Coastal systems

The transport of microplastics into coastal systems can be influenced by tides and inshore currents. Tidal cycles can move plastics onto beaches, but also move them offshore, turning beaches far away into reservoirs for microplastic pollution. For example, beaches in the Canary Islands have high levels of plastic debris, mainly composed of pellets. These pellets are far from sources of input, some of the nearest production sites are across on mainland

Europe and USA. Therefore, these pellets have been transported long distances in surface currents (Herrera et al., 2018).

Offshore systems

The oceans have many oceanographic features which can facilitate the transport of microplastics, these include the large ocean gyres, mesoscale eddies and upwelling locations. Mass movement of water masses with varied salinities and density can facilitate plastic movement. Little information on the dispersal of microplastics facilitated by these systems are available at present, but there is much which can be interpreted from studies on larger plastics items, e.g. (Brach et al., 2018).

Deep sea systems

Masses of water flows through troughs in the ocean, and the deep sea can cause the movement of microplatics from near shore environments to the deep sea.

5.2.5 Biota (flora and fauna)

Aside from ingesting microplastics (see Chapter 4, Levels of microplastics), biota in all ecosystems can facilitate the transport of microplastics. Many studies have shown the ability for animals to ingest plastics and rapidly egest them (Ory et al., 2018; Ward et al., 2019) (see also Chapter 8.1, Toxicokinetics in wildlife). Similar to the dispersal by sea birds and terrestrial animals, feeding in areas with plastic pollution, and egesting in other areas, can transport microplastic pollution. In a marine context, mesopelagic fish which feed on microplastics in surface waters return to the mesopelagic feed during the day. If they egest particles at deeper depths, they can encourage movement between water bodies (Lusher et al., 2016), furthermore microplastics incorporated into fecal pellets are very dense and therefore sink rapidly to benthic environments (Porter et al., 2018). Similar can be said for deep diving marine mammals (Lusher et al., 2018b) and epibenthic organisms (Choy et al., 2019). Individuals which have also eaten plastics, which themselves are eaten can act as vectors for the movement of plastics through the food web (Lusher et al., 2016).

5.3 Fate

The fate of microplastics in the environment depends on factors including chemical structure, additives, polymeric nature, ecological impacts, fragment and biofouling.

5.3.1 Land

The fate of microplastics in terrestrial environments is probably the least studied. Microplastics which are applied to land as a fertilizer are likely to remain to a large part in the soil, provided they are not transported by wind and rain. Currently the presence of plastics in soils are little studied and ongoing research through the EU funded Waters JPI: IMPASSE aims to illustrate the consequences of plastics in soil (see Chapter 12.3).

5.3.2 Freshwater

Fate of microplastics in the freshwater systems are less understood than marine systems. Sinks of microplastics in the freshwater environments include the sedimentation of particles

to sediments. Here, they can become further incorporated within the sediment either through physical processes or bioturbation. Sediment cores from rivers and lakes can provide interesting data on the potential for plastics to accumulate over time (Lusher et al., 2018a). Furthermore, lake sediments tend to have more stable transportation rate which is slower than in the water column (Nel et al., 2018) allowing a direct correlation between the distance from source to pollution levels in sediments (Su et al., 2016).

5.3.3 Marine

The fate of microplastics in the marine environment are still mostly unclear as many routes of transport are still to be justified and most of the proposed end points of plastics are complicated to sample. Benthic sediment has been identified as the major sink for microplastics in the marine environment (Booth et al., 2017). Shorelines and beaches may see an accumulation and burial of microplastics in sand, however this transitory zone will constantly be receiving new water transported material or looser surface particles within the surf zone. Thus, they act as a reservoir for plastic debris (Fok et al., 2017). Benthic sediments can receive particles that become biofouled and denser when they end up on the sediment, further sedimentation can lead to an accumulation, and bioturbation will encourage mixing within sediment layers. The deep sea has been seen to contain high numbers of microplastics. Microplastics have been found in all locations of the marine environment where investigations have been undertaken.

5.4 Summary

EFSA (2016), FAO (2017) and SAPEA (2019) did not go into any details with regard to sources, release and fate of nano- and microplastics. SAPEA (2019) stated that the fate of microplastics in soils and atmosphere was unknown. For information on these issues it is referred to GESAMP (Koehler et al., 2015).

VKM found:

- More information has emerged on freshwater systems than in previous reports. Researchers are still far from understanding the sources, transport processes and sinks of nano- and microplastics on land. This is also true from the transfer of plastics from terrestrial to aquatic systems. There is not enough information on sources to infer the quantities/relative contributions of microplastics released by and in Norway. Marine systems still appear to be the ultimate sink for microplastics in the environment. However, as this will happen on geological time scales freshwater and terrestrial systems are also important recipients and reservoirs of microplastics pollution.
- From the overview of sources which could contribute to the input of microplastics to the Norwegian and Nordic environment we are able to infer potential sources but currently there is not enough empirical data available for interpretation. MEPEX provides estimations and assumptions but further data on sources is required (Sundt et al., 2014).

- VKM concludes that further information is required to understand sources and transport of microplasics in the Nordic/Norwegian environment, and effort should focus on terrestrial and freshwater systems to increase the knowledge similar to that of the marine systems.

6 Biofilms and rafting

EFSA (2016) and FAO (2017) both recognize that plastic debris can act as a substrate for diverse microbial communities, including pathogens, but conclude that the relevance to human health still remains unknown.

SAPEA (2019) basically does not cover microbial contamination of microplastic.

The main concern about biofilms on plastic debris is that pathogenic microorganisms and antibiotic resistance genes (ARG) can be spread long distances and to new ecological niches with potencial high impact for both the environment and human health. Another interesting health related effect of microplastics on microbial diversity is reported by Lu *et al.* (Lu et al., 2018a). They found significant changes in the richness and diversity of the gut microbiota in the cecums of polystyrene microplastic-treated mice, resulting in dysbiosis (Lu et al., 2018a). These results may indicate that polystyrene microplastics could modify the gut microbiota composition in mice. The relevance of this finding may be low in the context of the levels and types of microplastics present in the environment, and overall the possible health risks of microplastics to mammalian gut microbiota is not known.

6.1 The plastisphere

Microplastics can be colonized by different types of microbial communities (Kettner et al., 2017; Oberbeckmann et al., 2018) and can thus be considered as specific niches for microbial life, commonly termed plastisphere (Keswani et al., 2016). A high diversity of microorganisms have been detected on microplastics, raising questions about the role of microplastics as a novel ecological niche for potentially pathogenic (Arias-Andres et al., 2018b; Kirstein et al., 2016) or invasive (Maso et al., 2016) microorganisms. As microplastics can be transported horizontally and vertically over long distances in aquatic systems, they might be vectors for spreading of attached pathogenic bacteria and fungi, harmful algae and invasive species (Arias-Andres et al., 2018b; Keswani et al., 2016; Kirstein et al., 2016; Maso et al., 2016).

However, the potential role of microplastics as a vector for distinct microbial assemblages or even pathogenic bacteria is hardly understood (Oberbeckmann et al., 2018). The main question is if microbial biofilms remains stable on microplastics over a prolonged period of time and various environmental conditions and whether microplastics thus could serve as a vector for potential pathogenic microorganisms. Microorganisms attached to the microplastics can potentially also play a significant role in their degradation.

6.2 Microbial diversity

Several studies on microbial attachment to various types of microplastics have been performed. Microplastic bacterial assemblages are reported to have lower taxon richness, diversity, and evenness than those on other substrates (McCormick et al., 2016). Wu *et al.*

(Wu et al., 2019) revealed through high-througput sequencing of 16sRNA that biofilm on microplastic had an unique community structure, and suggested that microplastic is a novel microbial niche. Functional potential and taxonomic composition of plastic-associated microbes versus planktonic microbes found in the open ocean are reported (Bryant et al., 2016), and the bacteria inhabiting plastics harboured distinct metabolisms from those present in the surrounding water (Debroas et al., 2017). Furthermore, there are indications that microplastic selects for taxa that may degrade plastic polymers (e.g., *Pseudomonas*) and common human intestinal pathogens (e.g., *Arcobacter*) (McCormick et al., 2016).

Oberbeckman *et al*. investigated how different *in situ* conditions contribute to the composition and specificity of bacterial communities on microplastics (PS and PE vs. wooden pellets) (Oberbeckmann et al., 2018). They concluded that the surrounding environment prevailingly shapes the biofilm communities, but that some microplastic-specific assemblage factors exist.

Kesy et al. compared the taxonomic composition of the biofilms on PA and chitin and found that they did not differ (Kesy et al., 2017). No potential pathogens was detected exclusively on polyamide. However, after 7 days of incubation of the biofilms in seawater, the species richness of the PA assemblage was lower than that of the chitin assemblage (Kesy et al., 2017).

Potentially pathogenic microorganisms can actually be considered hitchhikers in plastic-associated microbial communities (Kirstein et al., 2016; Shen et al., 2019). Several studies confirms the indicated occurrence of potentially pathogenic bacteria on marine microplastics (Foulon et al., 2016; Kirstein et al., 2016), among them *Vibrio* spp. and *Aeromonas salmonicida* (Imran et al., 2019). Wu *et al.* (Wu et al., 2019) detected two opportunisitic human pathogens (*Pseudomonas monteilii* and *Ps. mendocina*) and one plant pathogen (*Ps. syringae*) only in the microplastic biofilm, but not in biofilms formed on natural substrates. Furthermore, the potential human pathogens *Vibrio parahaemolyticus*, *V. vulnificus* and *V. cholerae* associated with floating microplastics (polyethylene, polypropylene and polystyrene) was reported from North and Baltic sea (Kirstein et al., 2016). Metabolic pathway analysis suggested adaptations of such bacterial assemblages to the plastic surface colonization lifestyle (Jiang et al., 2018).

There are only very few studies on how microplastics affect fungal communities. However, Kettner *et al.* explored the diversity of fungi attached to PE and polystyrene (PS) particles in different aquatic systems and a wastewater treatment plant (Kettner et al., 2017). They found that the fungal communities on microplastics differ from the mycobiota in the surrounding water and on wood as natural substrate. Members of Chytridiomycota, Cryptomycota and Ascomycota dominated the fungal assemblages, suggesting that both parasitic and saprophytic fungi thrive in microplastic biofilms. These fungal taxa might benefit from microplastic pollution in the aquatic environment with yet unknown impacts on their worldwide distribution, as well as biodiversity and food web dynamics at large (Kettner et al., 2017).

6.3 Formation and stability of biofilms

The development and stability of microbial biofilms in natural environments need to be explored further. Most of the research on these topics have so far only been performed on bacterial biofilm formation after short-term exposure or on floating plastic. However, in a study of bacterial and fungal communities on polyethylene plastic sheets and dolly ropes during long-term exposure on the seafloor, none of the typical features of a late stage biofilm were displayed (De Tender et al., 2017). Foulon *et al.* (Foulon et al., 2016) observed a longer bacterial attachment (6 d) on irregular microparticles compared to smooth particles (<10 h), but complete decolonization of all particles eventually occurred. These results indicate that biofilm formation is severely hampered in the natural environment where most plastic debris accumulates.

6.4 Antibiotic resistance

Antibiotic resistance of bacteria can be acquired from other bacteria through horizontal gene transfer or through mutations of antibiotic targets. Biofilms create an environment that protect bacteria from effects of antibiotics. Therefore, biofilm formation is considered an antibiotic resistance mechanism in bacteria (Imran et al., 2019). Multiple resistance against antibiotics belonging to cephalosporins, quinolones and beta-lactams were demonstrated in bacteria from a macro-plastic piece stranded on the shores in King George Island (South Shetlands, Antarctica) (Lagana et al., 2019). Furthermore, metagenomic analyses have revealed microplastic with broad -spectrum and distinctive resistome (Wu et al., 2019). Plastic can be transported long distances, and several studies have thus suggested plastics as possible vectors for the spread of antibiotic resistance (Arias-Andres et al., 2018b).

Heavy colonization of microplastics by bacteria commonly associated with antibiotic resistance made Oberbeckman et al. suggest microplastics as a possible hotspot for horizontal gene transfer (Oberbeckmann et al., 2018). This theory is supported by Arias-Andres *et al.* (Arias-Andres et al., 2018b), who demonstrated increased frequency of plasmid transfer in bacteria associated with microplastics compared to free-living bacteria or bacteria in natural aggregates. Furthermore, it has been demonstrated that microplastics in an aquatic environment can adsorb antibiotics (sulfadiazine, ciprofloxacin, amoxicillin, trimethoprim and tetracycline) on their surfaces (Imran et al., 2019).

The plastisphere may thus contribute to the spread of antibiotic resistance, which consequently could affect the diversity and ecology of aquatic microbial communities on a global scale and consequently also long-range dispersion and entry into food chain (Arias-Andres et al., 2018b) and (Imran et al., 2019).

6.5 Wastewater and sewage sludge

The composition of microplastic-attached bacterial assemblages in domestic wastewater have been shown to differ from that of assemblages in water and sediment and supports domestic wastewater as a point source of microplastic (e.g., gastrointestinal taxa) (Hoellein

et al., 2017). As microplastic particles promote persistence of typical indicators of microbial anthropogenic pollution in natural waters, their removal from treated wastewater should consequently be prioritised (Eckert et al., 2018).

6.6 Biodegradation

The microbial biofilms can also have a significant role in biodegradation of microplastics. Both bacteria and fungi have been found to form efficient consortiums for degrading weathered plastics in seawater (Morohoshi et al., 2018; Paco et al., 2017; Syranidou et al., 2017) and soil (Huerta Lwanga et al., 2018).

6.7 Carbon dynamics

Functional differences between microplastic-associated and pelagic microorganisms in different freshwater lake types have been demonstrated (Arias-Andres et al., 2018a)b). Consequently, increasing microplastic pollution has the potential to globally impact carbon dynamics of pelagic environments by altering heterotrophic activities (Arias-Andres et al., 2018a)b).

6.8 Summary

EFSA (2016) and FAO (2017) both recognized that plastic debris can act as a substrate for diverse microbial communities, including pathogens, but concluded that the relevance to human health still remains unknown. Microbial contamination of microplastic was basically not covered by SAPEA (2019).

VKM found:

- Microplastics biofilms have unique microbial community structures compared to the surrounding environments.
- Microplastics can serve as vectors for microorganisms that are potentially pathogenic to humans, animals or plants.
- Opportunistic human pathogens have been found to be enriched in microplastic biofilm.
- Microplastics biofilms are considered possible hotspots for horizontal gene transfer.
- Several studies have suggested that the plastisphere may contribute to the spread of antibiotic resistance.

➢ VKM concludes that the available information on microplastic biofilms does not provide sufficient basis to characterize potential effects on human health.

7 Human hazard assessment

7.1 Toxicokinetics

EFSA (2016) highlights that there is a general lack of information on the toxicokinetics of nano- and microplastics. An important question is whether they translocate across the intestinal wall and become available for internal, systemic exposure. The epithelium of the intestinal wall is a barrier to microplastics excluding transcellular transport. The size of the channels in the paracellular pathway is less than 1.5 nm, hence uptake of microplastics via this pathway is not expected. Uptake by lymphatic tissue of the Peyer's patches appears to occur in a size range of 0.1-150 µm as observed in various species, including humans. The absorption appears to be small, only in the order of 0.04-0.3%. Studies using *in vitro* intestinal cell models found 0.45% of microplastics to translocate. Very little is known about the distribution of microplastics after translocation. Particles >1.5 µm are not expected to enter blood capillaries of internal organs. The largest particles absorbed are likely to stay in the local lymphatic tissue.

EFSA also summarises the uptake kinetics of nanoplarticles in the gut epithelium. Most information is obtained from studies on polystyrene nanoparticles. Reported intestinal translocation studies show that many types of nanoparticles cross the epithelium, but that there is no simple relation between uptake and size, shape and composition. Various *in vitro* intestinal models show highly variable uptake of polystyrene nanoparticles NPs of different sizes. It was noted that complicating factors were interaction and coating with intestinal material such as lipids, carbohydrates and proteins. In general, injected nanoparticles of various compositions are widely distributed to different organs and are capable of crossing biological barriers. In an *ex vivo* human placenta model, polystyrene nanoparticles in the size range 50-240 nm were taken up by the placenta. Transfer across the placenta was more effective for small particles. EFSA did not identify studies on nanoplatics other than polystyrene particles.

FAO (2017) does not specifically address toxicokinetics in humans, but state that only microplastics below 20 µm may pass the gastro-intestinal barrier and penetrate to a significant extent into tissues of mammals.

SAPEA (2019) states that ingestion of contaminated food and drinking water and inhalation appear to be relevant routes of exposure. Much can be learnt about the latter from studies on occupational hazards and inhalation toxicology.

In relation to food- and feed safety, oral exposure is the relevant exposure route. For cosmetics, also the dermal route may be of relevance, in particular for nanoplastics. In this assessment, VKM will address only oral exposure and uptake via the gastrointestinal tract.

Reinholz *et al.* (Reinholz et al., 2018) published the only study relevant for nano- and microplastic toxicokinetics according to our literature search (Chapter 2). They investigated the process by which polystyrene nanoparticles (100 nm) are transported through layers of Caco-2 cells as a model for gut-blood transition and used mass spectrometry to characterise the metabolome adhering to transversed nanoparticles. The results indicated that a large

portion of the particles are directed to the lysosomes, whereas a smaller fraction undergoes transcytosis. The rate of transcytosis was determined to be around 0.2%, regardless of the concentration of nanoparticles (75–600 µg/mL). The authors stated that nanoparticles trapped inside intestinal cells will be excreted by normal cell shedding.

7.2 Toxicity studies

EFSA (2016) summarises the toxicity for nano- and microplatics, and indicates that toxicity of chemicals released from the particles, for instance additives used in the plastic production and persistent chemicals from the environment, is already well-documented elsewhere. Regarding the toxicity of the nano- and microplastics as such, EFSA does not identify any peer-reviewed rodent or in vitro studies of relevance for human risk assessment of oral exposure. EFSA also summarises the toxicity in wild marine animals, where, for instance, inflammatory responses after nano- and microplastics exposures are observed. Since there is a general lack of experimental data, the risk of toxicity of nano- and microplastics after oral uptake in humans could not be evaluated by EFSA.

FAO (2017) does not refer toxicity studies of relevance for human risk assessment.

SAPEA (2019) acknowledges that the human microplastics toxicity is uncertain. Theoretical mechanisms have been proposed, but not investigated.

The following studies relevant for toxicity of micro- and nanoplastics published after 2016 were identified by VKM in the literature search described in Chapter 2.

7.2.1 *In vivo* studies

Lu *et al.* (Lu et al., 2018b) studied whether polystyrene particles in nano-size range could affect gut microbiota and hepatic lipid metabolism in mice. Groups of five-weeks old ICR mice (8 mice per. group) were exposed to pristine polystyrene particles of size 0.5 or 50 µm at concentrations of 100 µg/L or 1000 µg/L in drinking water for five weeks. A concentration of 1000 µg/L corresponds to 1456 x 10^{10} particles/L with size 0.5 µm and 1456 x 10^{4} particles/L with size 50 µm. The control group received water. VKM calculated that the concentrations of 100 and 1000 µg/L correspond to 15 and 150 µg/kg body weight (bw), respectively, based on a conversion factor from drinking water of 0.15 (EFSA 2012). In the 1000 µg/L group, there was a slower bw increase from week 3 (0.5 µm) and 4 (50 µm). Relative liver weight and relative epididymal fat weight was also decreased in the high dose groups. This was accompanied by decreased level of hepatic and serum triglycerides and total cholesterol and increased hepatic pyruvate. Mucus secretion decreased in all the treated groups. Microbiota composition in faeces and cecum also changed at phylum and genus levels. No substantial difference regarding particle size was reported.

Mattsson *et al.* (Mattsson et al., 2017) mixed positively charged polystyrene-NH_2 nanoparticles of 53 nm and 180 nm and algae (*Scenesdesmus sp, diameter approximately 25 µm*) in water for 24 h, after which *Daphnia magna* were added to the media. After 2 h, the daphnids were collected, washed, and then 60 individuals were fed to three fish (Crucian carp, *Carassius carassius*) in each aquarium every third day for 67 days. The positively charged polystyrene-NH_2 nanoparticles were selected because they were toxic to *D. magna*.

On the 62th day, only unexposed daphnids were fed to the fish, and feeding time was recorded. The authors reported presence of the nanoparticles in the fish brains measured by hyperspectral microscopy after freeze-drying and homogenisation in PBS buffer, and behavioral changes in the fish regarding their feeding activity that was dependent on particle size (slower feeding in the 53 nm group and faster feeding in the 180 nm group). On a broad scale, the findings indicate that nanoparticles can be transported through the food chain and may affect the ecosystem. It also indicates that such nanoparticles may have the potential to reach the human brain and other tissues. However, potential artefacts due to a leaching of the fluorescent dye need to be taken into account as recently reported for daphnids (Schur et al., 2019) and zebrafish (Catarino et al., 2019).

Rafiee *et al*. (Rafiee et al., 2018) analyzed potential neurobehavioral effects of pristine polystyrene nanoparticles (0, 1, 3, 6 and 10 ng/kg bw, gavage) in adult rats treated for 35 days. The particle size was 38.92 nm (average d_h). No effects were observed in the five different behavior tests performed. There were no treatment-related effects on body weight.

7.2.2 *In vitro* studies

Schirinzi *et al.* (Schirinzi et al., 2017) investigated the oxidative stress of nano- and microparticles of polyethylene and polystyrene (50 ng/mL to 10 µg/mL) among several other nanomaterials (metal, metal oxide, carbon) in the human epithelial cell line HeLa and the human cerebral cell line T98G. The polyethylene microsphere beads (3-16 µm) used were in mixture with 100-600 nm nanoparticles. Polystyrene microparticles (10 µm) were accompanied by 40-250 nm nanoparticles. Cell viability was not affected. ROS generation was significantly increased with polyethylene microparticle exposure in T98G cells, but the effects was not dose-dependent. With polystyrene microparticles ROS was induced by the highest dose in both cell lines.

7.3 Summary

EFSA (2016) highlighted that a general lack of information on toxicokinetics and toxicity of nano- and microplastics in human exists. FAO (2017) does not specifically address toxicokinetics in humans, nor does it refer toxicity studies of relevance for human risk assessment. SAPEA (2019) acknowledges that the human microplastics toxicity is uncertain.

VKM found:

- The few studies relevant for human hazard assessment that have become available since EFSA's assessment in 2016 used pristine nano- and microparticles. However, micro- and nano-sized polystyrene particles present in food are generally not pristine, and the relevance of studies on pristine particles for toxicity of weathered particles present under natural exposure conditions is unknown. Similar applies for ecotoxicological studies.

➢ VKM concludes that the available information does not provide sufficient basis to characterize potential toxicity in humans.

8 Environmental hazard assessment

8.1 Toxicokinetics in wildlife

EFSA (2016) does not specifically address the toxicokinetics of nano- and microplastics in an environmental context.

FAO (2017) discusses the interactions of nano- and microplastics with biota and conclude that the available laboratory studies confirm the ingestion of nano- and microplastics by a diverse array of marine organisms, including protists, copepods, annelids, echinoderms, cnidaria, amphipods, decapods, isopods, mollusks, fish and birds. While ingestion is the most studied exposure route, less information is available about the internal distribution of microplastic. In bivalves, few studies report the transfer to the haemolymph and lysosomal system. In crustaceans, microplasticss have been detected in the haemolymph of the green crab after oral exposure. However, only a very small fraction translocated. Less data is available for fish, for which a transfer of nano- and microplastic from the gastrointestinal tract to the liver has been reported by two studies.

SAPEA (2019) does not specifically address the toxicokinetics of nano- and microplastics in an environmental context.

In an absorption, distribution, metabolism, elimination (ADME) framework, only the absorption of nano- and microplastics, typically called tissue translocation, has been studied. Very little knowledge is available on the distribution of nano- and microplastics in the body; the metabolism and elimination has not been addressed. This is probably because nano- and microplastics are unlikely to be metabolised and eliminated in a classical, chemical sense.

8.1.1 Ingestion by aquatic and terrestrial organisms

Most experimental studies focus on the uptake/ingestion of nano- and microplastics. Typically, these studies focus on ingestion as a route of exposure and investigate the presence of nano- and microplastics in the digestive tract of an organism. This is mostly done qualitatively using fluorescence microscopy, but quantitative approaches also exist. The latter quantify fluorescence as a marker of ingested nano- and microplastic (Rist et al., 2017) or count the ingested particles after digestion of the organisms (Scherer et al., 2017). In general, a wide range of taxa across many trophic levels in both terrestrial and aquatic systems ingest nano- and microplastics in experimental set-ups. Although exposure may also occur via ventilation and dermal uptake, ingestion is considered the main route of exposure. Ingestion takes place either directly or indirectly where direct ingestion refers to the consumption of microplastics as a result of active feeding while indirect consumption, or so-called trophic transfer, is a result of secondary ingestion of nano- and microplastic-contaminated prey.

Trophic transfer has been demonstrated both experimentally and *in situ* which also has led to concerns and speculation regarding biomagnification and consequences for food safety and human health. However, for this to occur there needs to be a net transfer of nano- and microplastics from the external environment to the systemic circulation of the animal

(bioaccumulation). The evidence for bioaccumulation in its strict sense is however poor. In fact, what is frequently referred to as bioaccumulation is the ingested dose, that is, the amount of nano- and microplastic found in the gastrointestinal tract and not inside the body. This is a common misconception also recognised within the field of nanotoxicology (Bour et al., 2015). Accordingly, the term bioaccumulation should not be used to describe the presence of nano- and microplastics in the gastrointestinal tract and ingestion can be used instead.

In aquatic invertebrates, the ingestion kinetics are rapid, and equilibrium is reached within few hours, especially in filter feeders (Dawson et al., 2018; Rist et al., 2017). The same is true for the excretion/egestion, although fewer studies are available. Importantly, the uptake depends on the autecology of the species, the presence of food as well as on the microplastic properties (Scherer et al., 2017). The retention times vary depending on the length and complexity of the gastrointestinal tract as well as the presence of food. However, egestion also depends on the size and shape, with larger, spherical microparticles generally being egested faster and at the same rate as natural food particles compared to nanoparticles or irregular and fibrous particles (Au et al., 2015; Ogonowski et al., 2016).

8.1.2 Tissue translocation

It is generally believed that particles larger than a few micrometers cannot pass biological barriers (Gustafson et al., 2015; He and Park, 2016; Zhu et al., 2013) while smaller particles can be actively transported across the epithelial cell layer in the gut lumen via specialised cells, by either endocytosis, phagocytosis or transcytosis (McClements, 2014). Indeed, several studies have indicated that nanoparticles translocate to tissues. For example, translocation to the liver and the circulatory system has been reported to be rather common in both fishes and bivalves (Asmonaite et al., 2018; Jovanovic, 2017; Karami et al., 2017; Paul-Pont et al., 2016; Peda et al., 2016) and one study even reported transport across the blood-brain barrier in fish fed amidine functionalised polystyrene nanoparticles (Mattsson et al., 2017).

The state of the science on tissue translocation of nano- and microplastics has recently been reviewed by Triebskorn *et al.* (Triebskorn et al., 2019). In total, 31 studies investigated the phenomenon covering 18 species, mainly fish, crustaceans and mollusks. Similar to other aspects, most studies are performed using spherical polystyrene nano- and microplastics. Twentyone out of 31 studies report a tissue translocation of nano- and microplastics. In fish, nano- and microplastics are mostly detected in fatty tissues, such as brain, liver and embryo yolk. The same is true for crustaceans, in particular daphnids, in which nano- and microplastics appear to accumulate in lipid droplets or embryos. In contrast, nano- and microplastics translocating in mollusks have been mostly investigated and detected in the haemolymph.

Importantly, Triebskorn *et al.* (Triebskorn et al., 2019) highlight methodological challenges when studying nano- and microplastics translocation: The histological evidence for translocation has in several cases been poor and criticised by other researchers (Baumann et al., 2016; Paul-Pont et al., 2018; Tang, 2017). Most studies use fluorescently labelled nano- and microplastics that can be localised using advanced microscopy. This may result in false-positive results as recent studies with daphnids (Schur et al., 2019) and zebrafish (Catarino et al., 2019) demonstrate. As the fluorescent dye is not covalently bound to the nano- and

microplastics, it can leach, and will, because of its lipophilic properties, partition to lipid rich tissues. In the light of these findings, previous reports on tissue translocation have to be carefully revisited and alternative detection methods are needed. One approach will be the use of nano- and microplastics with a metal core (Mitrano et al., 2019) or radiolabeling (Lanctot et al., 2018).

8.2 Toxicity in wildlife

EFSA (2016) does not assess the environmental impacts of nano- and microparticles.

FAO (2017) briefly summarises the available knowledge on species relevant to fisheries and aquaculture, especially mollusks, crustaceans and fish.

SAPEA (2019) takes a qualitative look on the hazard of microplastics based on published reviews. The report states that microplastics can induce physical and chemical toxicity and induce adverse effects on the food consumption, growth, reproduction and survival in a range of species. SAPEA (2019) also highlights the discordance between exposure and hazard data: Toxicity studies are often performed using high concentrations of very small microplarticles for which the environmental levels remain largely unknown. Knowledge is generally lacking regarding the toxicity on terrestrial biota and plants, population-level effects and their long-term ecological impacts. In terms of chemical toxicity, SAPEA (2019) concludes based on the available evidence that an additional exposure of biota to hydrophobic, persistent organic pollutants sorbed to microplastics is probably low. Thus, their contribution to toxicity is low because other sources of exposure to these compounds (especially from the diet) are dominating.

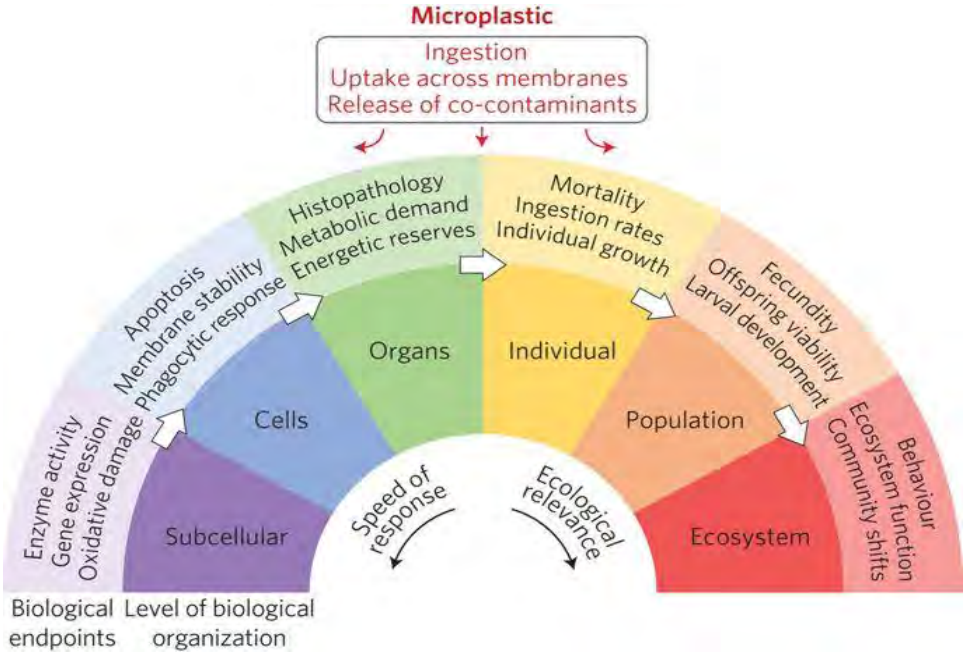


Figure 8.2-1 Organisation of biological endpoints according to level of biological organization (taken from Galloway *et al.* (Galloway et al., 2017)).

As more data on the toxicity of nano- and microplastics are generated, one approach is to organise that information according to levels of biological organisation, as done for other pollutants (Fig. 8.2-1) (Galloway et al., 2017). Another, very similar approach is to apply the framework of Adverse Outcome Pathways (Jeong and Choi, 2019).

VKM followed that logic in such that the hazard information is organised according to levels of biological organisation (see Fig. 8.2.1-2). On the basis of its systematic literature search, VKM selected 122 studies to be included in the assessment of the environmental toxicity of nano- and microplastics. From these, information on the biological effects were extracted and evaluated.

8.2.1 Biological effects

The effects of nano- and microplastic exposure have been observed at all levels of biological organisation (fig. 8.2.1-1 to 2) ranging from alterations in gene expression profiles, changes in enzymatic activity, lipid metabolism, induction of inflammatory responses to higher level effects such as behavioral changes, growth and reproduction. VKM acknowledges the presence of lower level effects and their potential importance in delineating molecular and cellular effect mechanisms. However, due to high test concentrations, many biomarker responses likely indicate exposure more than adverse effects at the individual or higher levels of organisation (Forbes et al., 2006). Therfore, we have chosen to only focus on the higher levels of effects and histological evidence which has direct bearing on individual performance and fitness.

8.2.2 Effects on food acquisition and life history traits

The most commonly detected and prominent effects caused by nano- and microplastics are changes in life history traits like mortality, growth and fecundity (Fig. 8.2.1-1, 8.2.1-2). These endpoints are intimately linked to energy intake and hence the nutritional status of the exposed organism. When nano- and microplastics are administered together with food in laboratory experiments, they effectively reduce the nutritional quality of the food by dilution with particles of low or no nutritional value. In non-selective filter or deposit feeders, this can induce compensatory behaviors like increased ingestion rate in earth worms (Huerta Lwanga et al., 2016), zooplankton (Ogonowski et al., 2016) and fish (Rochman et al., 2017) or decreased ingestion rate as a protective measure often seen in certain species of bivalves (Green et al., 2017; Xu et al., 2017). In either case, these alterations negatively affect the energy budget of the organism, which leads do changes in condition and fitness (Gardon et al., 2018; Sussarellu et al., 2016). It is however not well established whether these effects are specific to nano- and microplastics since similar effects can be induced by any particle of low nutritional value, like sand, silt, clay, or cellulose (Appelby and Scarratt, 1989; Chapman et al., 2017; Newcombe and Macdonald, 1991; Yang et al., 2017). Comparing the effects of natural particles and nano- and microplastics, Ogonowski *et al.* (Ogonowski et al., 2018) concluded that, when the differences in experimental designs, test concentrations and particle sizes between nano- and microplastics and suspended sediment studies were considered, the effects were of similar character and magnitude.

Figure 8.2.1-1 Number of observed effects/no effects at the species level per feeding guild and exposure matrix. The category "mix" represents organisms which are not restricted to one particular feeding mode or organisms that have switched feeding strategies throughout the course of the experimental duration.

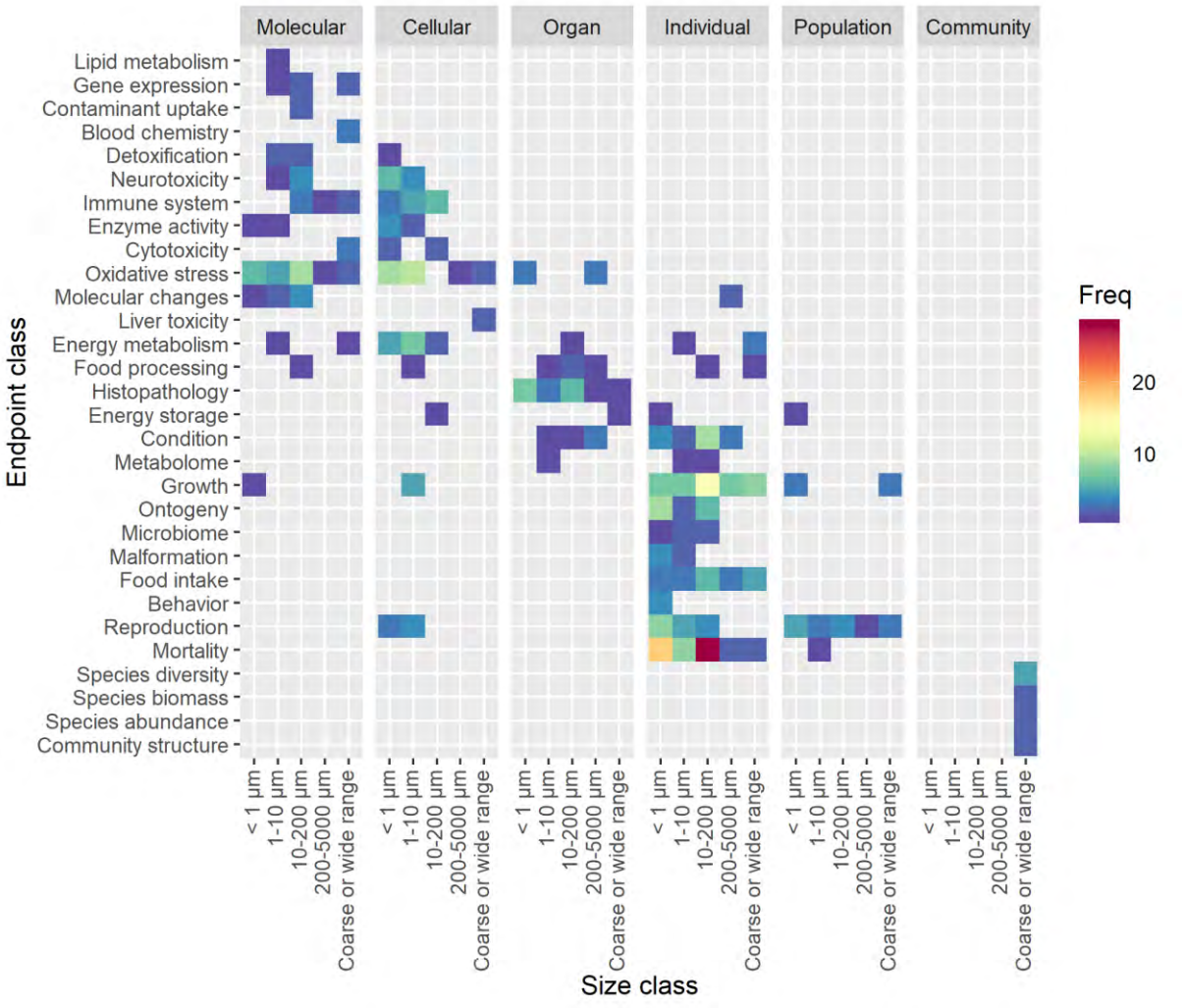


Figure 8.2.1-2 Heatmap showing the frequency of observed effects per endpoint class divided by particle size class and level of biological organisation. One observation is equal to a measured biological endpoint for a unique set of experimental conditions. There can be more than one endpoint and several experimental conditions within a particular study. For example, if mortality and growth effects have been observed for two different microplastics in two different organisms, then there would be 2 x 2 x 2 = 8 observations at the individual level in that study.

Histopathology

Histological investigations nano- and microplasticshave mainly targeted fish exposed to nano- and microplastics via food or different types of bivalves exposed to nano- and microplastics suspensions. However, the results of these studies are ambiguous. The most prominent case of histological damage was reported by Pedà *et al.* (Peda et al., 2016) where severe inflammation, tissue damage and necrosis of the gastrointestinal tissue was observed in juvenile sea bass (*Dicentrarchus labrax*) exposed to > 300 µm PVC fragments. Qiao *et al.* (Qiao et al., 2019) observed moderate effects on the gut epithelial layer and villi of adult zebra fish exposed to 5 µm polystyrene spheres (*Danio rerio*) while Romano *et al.* (Romano et al., 2018) only found slight thickening of the mucosal epithelial layer in juvenile silver barb (*Barbodes gonionotus*) exposed to a polydisperse suspension of PVC fragments (D90 = 310 µm). In contrast, Ašmonaitė *et al.* (Asmonaite et al., 2018) did not observe any histological changes in the gastrointestinal tract of rainbow trout (*Oncorhynchus mykiss*) exposed to

100-400 µm pristine PS fragments. Due to the diversity of polymers, sizes and shapes and exposure concentrations used in the experiments it is difficult to single out the factors factor that could explain these differences. However, in the studies by Pedà et al. and Romano et al. where the same polymer was used (PVC), it is likely that non-characterised plastic additives contributed to the adverse effects since PVC is known to contain high levels of unbound plasticizers (Navarro et al., 2010). Conversely, the lack of histological effects in the study by Romano et al. could be explained by the fact that additives actively were removed by leaching prior to the exposure, although a significantly shorter exposure time also could have contributed to this difference.

In bivalves, the histological effects are less pronounced. Rochman *et al*. (Rochman et al., 2017) observed slightly increased tubular dilation in the digestive gland of the Asian clam *Corbicula fluminea* when exposed to either polyethylene (PE), polyethylene terephthalate (PET), polystyrene (PS) or polyvinyl chlorid (PVC) fragments for 28 days compared to a particle free control. Others have reported epithelial detachment in the gametes of the pearl oyster *Pinctada margaritifera* at relatively low exposure concentrations (0.25 µg/L) to 6 and 10 µm virgin PS spheres and more general physical lesions throughout the body of *Mytilus galloprovincialis* (not statistically significant compared to a particle free control) exposed to 32 µg/L virgin PS spheres for 14 days. Many of these histological alterations were linked to elevated immunological responses at the molecular and cellular levels, indicating a reaction to the sudden nano- and microplastics exposure. However, Sussarellu *et al.* (Sussarellu et al., 2016) did not observe any histological changes in the Pacific oyster (*Crassostrea gigas*) after a two month exposure to 23 µg/L of 2 and 6 virgin PS spheres.

Importantly, the quality of histological work in toxicity studies in general (Wolf and Maack, 2017) and in nano- and microplastics in particular, is often poor (Baumann et al., 2016). One reason for that is the lack of histopathological training of researchers. Accordingly, histopathological data should be interpreted by trained experts.

8.2.3 Microplastics as vectors for contaminants

It is widely recognised that many different types of chemicals, both those internally embedded in the polymer during production as well as those sorbed from external sources in the environment can transfer from nano- and microplastic to biota via ingestion (Batel et al., 2016; Ma et al., 2016; Pittura et al., 2018). The transfer is however bi-directional (Gerdes et al., 2018; Koelmans et al., 2013) and depends on the chemical fugacity which is context dependent (Mohamed Nor and Koelmans, 2019). Although the importance of nano- and microplastics as vectors for chemical pollutants to biota has been much debated, it is now generally believed that even though nano- and microplastics could act as vectors of pollutants, they will only have a negligible contribution to chemical exposures compared to other sources, especially the diet (Besseling et al., 2018; Koelmans et al., 2016). VKM supports this line of reasoning.

8.3 Hazard assessment based on the compiled data

Species sensitivity distributions (SSDs) are commonly used in risk assessments to describe the sensitivity of different species to a specific chemical compound, to identify the most sensitive species and to derive environmental quality criteria. This approach has also recently

been used to assess the risk of nano- and microplastics (Adam et al., 2019; Besseling et al., 2019; Burns and Boxall, 2018; Everaert et al., 2018). In the following section, we have analyzed a large compilationon of nano- and microplastics toxicity data on the basis of our systematic literature search (see Chapter 2.).

On the basis of data published between 2016 and 2018, we estimated the HC_5, that is, the hazard concentration at which nano- and microplastics adversely affects 5% of species (see chapters 2.4.1 and 2.4.2 for details on the data collection and selection). The HC_5 was 0.14 µg/L (95% confidence interval: 0.04-0.64 µg/L) for mass-based concentrations and 71.6 particles/L (95% confidence interval: 3.45-1991 particles/L) for numerical concentrations. Because of the abundance of data from 39-40 species, spanning multiple taxa, VKM did not apply an additional assessment factor to derive predicted no effect concentrations (PNECs). The PNECs, thus, are equivalent to the HC_5s reported above and can be considered conservative estimates.

Given the uncertainties in these estimates, HC_5s (PNECs) are in accordance with previously published reports (Table 8.3-1). Compared to the HC_5s derived by Besseling et al. (2019) and Burns & Boxall (2018), the values derived by VKM are one to four orders of magniture lower, especially regarding the numerical HC_5. The main reason for this is that these previous assessments used a limited set of toxicity data that was not collected in a systematic way. In contrast, the current estimate includes by far the largest dataset and is based on a systematic literature search. It, thus, aligns well with the results of the probabilistic risk assessment by Adam et al. (2019) which is the most comprehensive study published to date. Again, the HC_5 (PNEC) derived for numerical concentrations in this report is somewhat lower, although the confidence bands overlap. The reason for this can be the inclusion of marine species in this assessment (Adam et al. used only freshwater species).

Table 8.3-1 Comparison of the HC_5 from this report and previous reports.

Study	HC_5 [µg/L]	PNEC [µg/L]	HC_5 [item/L]	PNEC [item/L]
VKM (this assessment)	0.14 (0.04-0.64)	0.14 (0.04-0.64)	71.6 (3.45-1991)	71.6 (3.45-1991)
Adam et al. (2019)	0.08 (0.04-0.11)*	0.08 (0.04-0.11)*	740 (610-1300)*	740 (610-1300)*
Besseling et al. (2019), correction for microplastics	1.67 (0.09–32.6)	-	1015 (191-10223)	-
Besseling et al. (2019), correction for nanoplastics	5.4 (0.93-31)	-	-	-
Burns & Boxall (2018)	-	-	64000	-
Everaert et al. (2018)	-	-	33.3 (0.36-13943)	6.65 (0.07-28000)
Zhang et al., (2019)	-	-	24.6	4.92

*25-75 percentile was used instead of the confidence interval.

8.3.1 Relevant studies

For the PNEC derived from numerical concentrations, the SSD, Figure 8.3.1-1 A, is driven by the following studies reporting the highest toxicity (see Table 8.3.1-1).

Table 8.3.1-1 Overview of the studies reporting the highest toxicity of nano- and microplastics. AF=assessment factor.

Study	Species	Endpoint	LOEC	AF	Est. NOEC
Romano et al. (2018)	*Barbodes gonionotus*	Enzyme activity, histopathology	0.5 mg/L → 127 items/L	20	0.025 mg/L → *6.36 items/L*
Xu et al. (2017)	*Atactodea striata*	Feeding activity	1000 items/L	20	*50 items/L*
Gardon et al. (2018)	*Pinctada margaritifera*	Scope for growth	0.00025 mg/L → 888 items/L	2	0.000125 mg/L → *444 items/L*
Reichert et al. (2018)	Stony corals	Necrosis, bleaching	4000 items/L	2	2000 items/L
Gray & Weinstein (2017)	*Palaemonetes pugio*	Mortality	50000 items/L	20	2500 items/L
Ding et al. (2018)	*Oreochromis niloticus*	Enzyme acitvity	0.001 mg/L	20	0.00005 mg/L
Gambardella et al. (2017)	*Amphibalanus amphitrite*	Enzyme acitvity	0.001 mg/L	20	0.00005 mg/L
Gambardella et al. (2017)	*Artemia franciscana*	Enzyme acitvity	0.001 mg/L	20	0.00005 mg/L
Gardon et al. (2018)	*Pinctada margaritifera*	Scope for growth	0.00025 mg/L	2	0.000125 mg/L
Zhao et al. (2017)	*Caenorhabditis elegans*	Behaviour, reporduction	0.01 mg/L	20	0.0005 mg/L
Gambardella et al. (2018)	*Dunaliella tertiolecta*	Growth	0.001 mg/L	2	0.0005 mg/L
Gambardella et al. (2018)	*Brachionus plicatilis*	Swimming behaviour	0.001 mg/L	20	0.00005 mg/L
Gambardella et al. (2018)	*Paracentrotus lividus*	Swimming behaviour	0.001 mg/L	20	0.00005 mg/L

In a study with silver barb *Barbodes gonionotus*, Romano *et al.* (Romano et al., 2018) exposed fry over 96 h to 0.2, 0.5 and 1 mg/L PVC microplastics. They used irregular, polydisperse particles in the size range of 0.1-1000 µm with 90% of the particles being <310 µm. 20 one-month old fish per 10 L aquarium (dechlorinated tap water) were exposed to PVC microplastics suspended in 10 mL ethanol. A negative and solvent control were included. Each treatment had three replicates (aquaria). The water was exchanged completely every day and fish were fed *ad libidum* during the experiment. The endpoints include whole body histology (n = 6 per treatment) and trypsin/chymotrypsin activity (n = 3 per treatment). The authors report no overt histological changes but a significant thickening of the mucosal epithelial layer in the intestine in fish exposed to 0.5 and 1 mg microplastics/L. This is probably a response to physical irritation. In addition, they report a significantly increased trypsin/chymotrypsin activity in those treatment groups which might be related to an increased digestive response. Accordingly, the LOEC in this study is 0.5

mg/L (or 127.3 microplastics/L after VKM conversion) for histological changes and enzyme activity. Noteably, both endpoints represent mild effects which might be due to the very short exposure duration. Nonetheless, a chronic irritation of the intestine as well as a constant secretion of proteolytic enzymes might induce more severe effects over a longer period.

Xu *et al.* (Xu et al., 2017) investigated the effect of PS microplastics on the marine clam *Atactodea striata*. The clams were exposed to polydisperse, irregular particles in the size range of 63-250 µm over 14 day at concentrations of 10 and 1000 particles/L. The water was renewed and the mussels were fed with green algae daily on weekdays. Ten individuals were used per replicate but it remains unclear, how many replicates per treatment were used. Clearance rate (feeding activity), absorption efficiency (organic content of the faeces) and respiration rate were investigated after 1, 5 and 10 days of exposure. The authors report a significantly lower feeding activity in clams exposed to 1000 microplastics/L (or 2.11 mg/L after VKM conversion) compared to the control (LOEC). They argue that the two-fold decrease is not due to a dilution effect because algea were much more abundant than microplastics in the experiment. They also speculate that a reduction of filtering might be an adaptive reponse to decrease the uptake of microplastics. As with the previous study, this effect may be considered mild and transient (if the exposure would be removed). However, long term reduction of feeding may result in more severe downstream effects.

Gardon *et al.* (Gardon et al., 2018) exposed the Pearl Oyster *Pinctada margaritifera* to 0.25, 2.5 and 25 µg/L spherical PS microplastics (mixture of 6 and 10 µm beads) over two months. They used six oysters per 20-L tank (replicate) and four tanks per treatment in a flow-through system constantly supplying a mixture of microplastics and algae diet. Clearance rates and oxygen consumption were determined after one month (n = 4) to calculate ingestion rates, assimilation efficiencies and energy budgets. Shell growth, gonad size and gametogenesis was also analysed. The assimilation efficiency was significantly reduced in oysters exposed to 25 µg microplastics/L (88800 microplastics/L after VKM conversion) while the scope for growth based on the energy budget was significantly reduced in the 0.25 µg/L treatment (888 microplastics/L according to VKM conversion). Accordingly, the latter represents the lowest LOEC observed in this study that ranks third in the SSDs for numerical as well as mass-based concentrations.

Reichert *et al.* (Reichert et al., 2018) investigated the effect of PE microplastics (37-163 µm, irregular shape) on small-polyp stony coral species of the genera Acropora, Pocillopora, and Porites. They used a concentration of 0.1 mg/L corresponding to circa 4000 particles/L in the water column (bioavailable) and exposed the corals (one 45-L tank per treatment) over four weeks in a semi-static system (20% water renewal every other day). The study looked at different parameters for coral health and reported microplastics effects on tissue necrosis (*A. humilis*, *A. millepora*, *P. cylindrica*) and bleaching (*P. damicornis*, *P. verrucosa*). Here, the affected coral surfaces were associated with higher levels of microplastics attachment. As only one concentration was used, the concentration in the water phase (4000 particles/L) represents the LOEC.

In a study with the daggerblade grass shrimp (*Palaemonetes pugio*), Gray and Weinstein (Gray and Weinstein, 2017) investigated the impacts of eleven different sizes (30-165 µm) and three shapes (spheres, fragments, fibres) of PE, PP and PS microplastics. They exposed grass shrimps (n = 40) to 50000 particles/L over three hours in 600-mL glass beakers and

monitored microplastics uptake and residence times (not relevant for hazard assessment) as well as mortality over 96 hours. An increased mortality of ≥ 20% was reported for spherical PE and PS microplastics (75, 82, 116, 165 µm), PP fibres (34, 93 µm) and PP fragments (93 µm). Accordingly, the LOEC in this short-term toxicity study was 50000 particles/L.

For the SSD derived from mass-based LOECs, Figure 8.3.1-1 B, the following studies reported the highest toxicity and, thus, determined the HC_5:

In their study, Ding *et al.* (Ding et al., 2018) exposed red tilapia (*Oreochromis niloticus*) to 0.1 µm, PS nanoplastics over 14 days. They used spherical nanoplastics at concentrations of 1, 10 and 100 µg/L corresponding to $1.8x10^9$, $1.8x10^{10}$ and $1.8x10^{11}$ particles/L and 12-15 fish per replicate (30-L tanks, three per treatment). After 0, 1, 3, 6, 10 and 14 days of exposure, two to three fish were sampled for biochemical analysis, including enzyme activities and EROD/BFCOD. Over the time course, all biomarkers changed significantly. The acetylcholine esterase (AChE), BFCOD and EROD activities were decreased, mainly after three and six days. This effect did not persist until the end of experiment, at which only the lowest concentration (1 µg/L) had an significant effect. In contrast, the SOD activities remained elevanted in all treatments from day 6 onward. MDA content as a marker of oxidative stress was significantly affected at day 1 but this effect disappeared with the latter time points. Accordingly, the most consistent effect was observed for SOD activities and the resulting LOEC is 1 µg/L. Importantly, it needs to be taken into account that changes in enzyme activity may not necessarily translate into more severe effects.

The study by Gambardella *et al.* (Gambardella et al., 2017) ranks second in the SSD for mass-based LOECs. They studied the impact of 0.1 µm, spherical PS nanoplastics on larval stages of *Amphibalanus amphitrite* barnacles and the brine shrimp *Artemia franciscana*. After an acute exposure (24 and 48 h) to 0.001, 0.01, 0.1, 1 and 10 mg/L, the authors investigated the impacts on mortality, swimming speed and enzyme activity (cholinesterases, catalase). While significant effects on swimming were reported for higher concentrations, exposure to the lowest concentration (0.001 mg/L) significantly affected the AChE and catalase activities in both species. Although not consistently concentration-dependent, the effect on enzyme activities thus determines the LOEC. As stated above, it is important to keep in mind that these represent rather mild effects and it remains uncertain whether changes in enzyme activites will translate to more substantial effects.

As with the numerical LOECs, the study by Gardon *et al.* (Gardon et al., 2018) with pearl oysters ranks third in the SSD with mass-based concentrations (see above for details). The LOEC for the microplastics impacts on scope for growth is 0.00025 mg/L. In contrast to the two studies above, Gardon *et al.* performed a chronic, long-term study with population-relevant endpoints. It should thus be considered more relevant for a risk assessment.

Zhao *et al.* (Zhao et al., 2017) studied the transgenerational toxicity of PS nanoplastics (0.1 µm) in the nematode *Caenorhabditis elegans*. L1-larvae were exposed to 1, 10, 100, 1000, and 10000 µg/L until adulthood (circa 4.5 d) and intestinal ROS production, locomotion behavior and brood size were investigated. In addition, the unexposed offspring was investigated. Significant microplastics effects were observed for all enpoints. However, ROS production, behavioural change and reproduction were affected in a concentration-dependent manner with an exposure concentration of 10 µg/L representing the LOEC.

Although the authors reported increased intestinal permeability already at 1 µg/L, this endpoint was excluded due to inconsistent reporting and uncertainties associated with the method of measurement (whole body fluorescence from nile red stained PS nanoplastics). Importantly, similar but less pronounced effects were observed in the unexposed offspring of exposed *C. elegans*.

Gambardella *et al.* (Gambardella et al., 2018) looked at the impact of spherical PS nanoplastics (0.1 µm) on a range of marine species, including the bacterium *Vibrio anguillarum*, the green microalga *Dunaliella tertiolecta*, the rotifer *Brachionus plicatilis* and the sea urchin *Paracentrotus lividus*. All species were exposed to 0.001, 0.01, 0.1, 1 and 10 mg nanoplastics/L under conditions specific to the species. The highest toxicity was reported for algae (growth inhibition), rotifers and sea urchins (both increased swimming behaviour) exposed to the lowest concentration (0.001 mg/L). Since the LOEC for *D. tertiolecta* was unique for this study it was chosen to represent the study in the SSD. Again, the exposures were acute and short-term and the results should be interpreted in that context

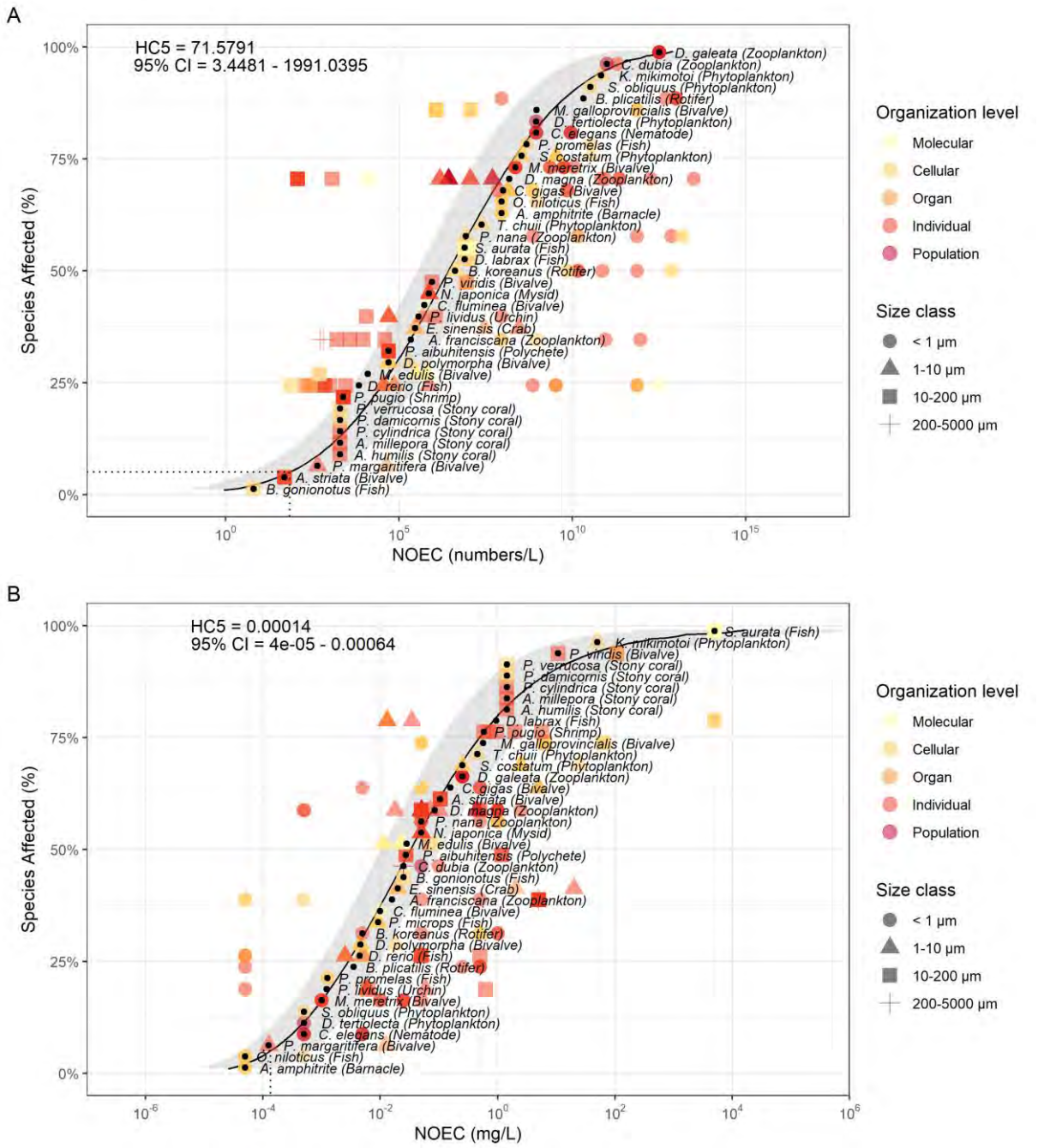


Figure 8.3.1-1 Species sensitivity distribution for species exposed via the water phase. Data is presented as either numerical (A, items/L) or mass-based (B, mg/L) concentrations. Black points represent the geometric mean of the NOECs reported for a particular species if this species occurred in more than one study. If a species was unique to one study but several NOECs were reported, the black dot represents the minimum NOEC. The symbols show all NOECsNOECs recorded for a particular species. The colour code indicatesindicates the level of biological organisation at which the NOECNOEC was observed. The shapes indicate the size class of nano- and microplastics used in a particular study. The black line represents the weighted fitted regression curve (weighted average of several different distributions) and the grey band represents the bootstrapped.

8.3.2 General observations

There is no clear pattern in the species distribution that suggests any type of sensitivity grouping by taxonomy using both dose metric approaches. For example, fish, bivalve and zooplankton species seem to span a wide range of sensitivities. However, one species seems to be particularly sensitive using both dose metrics; the pearl oyster *Pinctada margaritifera* displayed decreased scope for growth when exposed to rather low concentrations of 6 and 10 µm polystyrene spheres (Gardon et al., 2018). This can probably be explained by its general adaptation to oligotrophic and low turbidity tropical waters (Yukihira et al., 1999).

When LOEC is expressed in mass-based concentration the SSD is driven by toxicity data for nanoplastics. This pattern is, however, reversed if LOEC is expressed as numerical concentrations. This makes sense in such that high numerical concentrations of nanoplastics will translate to low mass-based concentrations compared to larger particles. Accordingly, the lower end of the numerical SSD is dominated by larger microplastics. As it remains so far unknown which dose metric is toxicologically more relevant, we prefer to report both, mass-based and numerical HC_5s. However, most exposure data (see Chapter 4) is reported as numerical concentrations.

Regarding the level of biological organisation that nano- and microplastics exposures affect, there is not a clear trend either. Contrasting the common assumption that molecular and cellular endpoints are more sensitive, the lower end of the SSDs is determined by LOECs derived from different levels, including individual and population level effects.

8.3.3 Physicochemical properties affecting toxicity

The mechanisms of nano- and microplastic toxicity are not well understood but it is likely that the toxicity is both intrinsic (effects that occur inside the cell due to chemical interactions at the molecular level, i.e. a plastic-specific effect) or extrinsic (system dependent effects caused by the mere presence of a non-nutritious particle) (Gouin et al., 2019). Although mechanistic studies are relatively rare in the nano- and microplastic field and most ecotoxicological studies are maladapted to distinguish intrinsic from extrinsic toxicity, there is some evidence to suggest that some physicochemical properties of nano- and microplastics are indeed important drivers of toxicity.

Size is one of the obvious factors determining toxicity: Smaller sizes with greater total surface area are generally believed to induce higher toxicity (Jeong and Choi, 2019) although others have reported the opposite effect (Gray and Weinstein, 2017). However, to examine the size effect without a proper understanding of the modes of toxic action it is important to test several different dose metrics in parallel. This is because we do not know whether the particle number, mass, surface area or volume is relevant. We found only one study that addressed this issue explicitly testing different exposure metrics (Mattsson et al., 2017), see Chapter 7.2.1.

Particle shape is another important factor of toxicity (Scherer et al., 2017). Spherical beads that are common in ecotoxicological experiments have been used as tracer particles to study the feeding ecology of bivalves and zooplankton for decades because they are processed at similar rates as natural food particles (DeMott, 1988; Ward et al., 2019). In contrast, irregular fragments and fibres, which are more common in the environment are retained

longer in the gastrointestinal tract which is suggested to cause elevated toxicity (Au et al., 2015; Ogonowski et al., 2016).

Another important factor in nano- and microplastic-biota interactions is surface charge. At the cellular level, Canesi et al. (Canesi et al., 2016) observed effects on lysosomal stability, cytochrome C reduction, and phagocytotic activity in bivalve hemocytic cells following 1 mg/L of 50 nm, positively charged polystyrene-NH_2 spheres. The effects were further amplified by the protein corona formed on the nanoplastic surface in the presence of haemolymph serum, suggesting increased toxicity due to promoted cell recognition by the NP-protein complex. At the individual level, similar effects were reported for *D. magna* exposed to daphnia-exudate conditioned PS-NH_2 and PS-COOH nanoplastic where the protein-corona increased the retention time of both PS-types but where PS-NH_2 induced a higher mortality compared to PS-COOH nanoplastic (Nasser and Lynch, 2016). Manfra *et al.* (Manfra et al., 2017) reported a dose-dependent mortality caused by 50 nm PS-NH_2 in the rotifer *Brachionus plicatilis* but no adverse effects when exposed to negatively charged PS-COOH nanoplastic of similar size (40 nm). The differences in toxicity were linked to surface-charge dependent aggregation where negatively charged nanoplastic tended to form large homo-aggregates, likely lowering the net bioavailability. Positively charged NP formed a much more stable suspension with 10 times smaller aggregates, indicating that particle interactions and suspension stability are important drivers of bioavailability and associated adverse effects.

8.3.4 Limitations in the hazard assessment

VKM acknowledges that the research on the toxicity of nano- and microplastics is still in a relatively early stage. Accordingly, scientific standards (in terms of quality and best practice) are currently forming. Thus, the results of many studies needs to be interpreted in this context and with caution (see Chapter 3.3.2). One such example is the overabundance of acute toxicity data derived from short-term experiments (mortality typically after few hours to days). This may serve as a point of departure for further research but is of very limited value for knowledge generation and risk assessment.

While the science evolves rapidly, there is a need to summarise and evaluate the available knowledge (European Commission, 2019). Because of the limited number of studies, VKM decided to make use of most available data and construct the SSDs for the hazard assessment pooling all species and endpoints. Accordingly, the hazard assessment contains data of different type and quality that might affect the SSDs. However, when looking at the studies reporting the lowest toxicity and, thus, driving the PNEC estimates, acute and chronic data are represented as are studies that can be considered of a high quality. Accordingly, the HC_5 (PNEC) estimates should be relatively robust.

However, although the derived HC_5 (PNEC)-values may be robust as a global average, the appropriateness of pooling of species with different ecological adaptations into a single SSD can be questioned. Since it yet has not been established to which extent the observed effects of nano- and microplastic exposure are specific to plastic materials, the default assumption (null hypothesis) should be that the effects are driven by the presence of non-nutritious particles, which also are naturally ubiquitous in the environment. It is likely that communities will respond to such stressors differently in different habitats due to local adaptations and species sensitivities can be driven by other factors, such as feeding strategies and hormone systems. Hence, the derived PNECs derived by VKM and others

following similar procedures (Adam et al., 2019; Besseling et al., 2019; Burns and Boxall, 2018) may be appropriate in some environments but not in others. A more detailed analysis based on specific communities and species constellations which also takes into account background levels of suspended solids would therefore provide more realistic PNECs (Struijs et al., 1997).

One reason for pooling all available data was that VKM was interested in observing patterns regarding the specific impacts of particle size on different taxa and levels of biological organisation. While this was not observed, a more in-depth analysis of material- and taxon-specific hazards would be interesting (e.g., by constructing SSDs for specific taxonomic groups or classes of microplastics), but is beyond the scope of this assessment. However, it is important to highlight that assessing "microplastics" as one entity is clearly ignoring their heterogeneity (Lambert et al., 2017; Rochman, 2019). Accordingly, future hazard assessments need to identify physico-chemical, as well as biological, properties that can be used to group microplastics in meaningful way.

8.4 Summary

EFSA (2016), FAO (2017) and SAPEA (2019) did not specifically address the toxicokinetics of nano- and microplastics in an environmental context. FAO (2017) stated that little information was available on the internal distribution.

EFSA (2016) did not assess the environmental impacts of nano- and microparticles while FAO (2017) briefly summarised available knowledge on species relevant to fisheries and aquaculture, especially mollusks, crustaceans and fish. SAPEA (2019) took a qualitative look on the hazards based on published reviews and stated that microplastics can induce physical and chemical toxicity and induce adverse effects on the food consumption, growth, reproduction and survival in a range of species.

VKM found:

- A wide range of species are capable of ingesting nano- and microplastics.
- Translocation from the gastrointestinal tract to organs has been claimed, but the extent to which this occurs is unclear due to potential experimental artefacts. Thus, the toxicokinetics of nano- and microplastics remain largely unknown.
- The present systematic literature search extracted toxicity data from 122 peer-reviewed publications (2016-2019).
- Histological evidence of physical injuries caused by nano- and microplastic ingestion are reported by several authors but have been criticised for poor quality. VKM supports this criticism.
- The effects of nano- and microplastics may be the result of a caloric restriction caused by the presence of non-digestible particles. Very few studies actually account for this by analysing the effects caused by non-plastic particles. This, however, would be needed to differentiate between general particle and specific plastic effects.
- The present assessment did not investigate the capacity of nano- and microplastics to act as vectors for hydrophobic contaminants (HOCs) quantitatively, but recognises that

contaminant transfer is bi-directional and can either increase or decrease contaminant body burden depending on polymer type, environmental conditions and chemical fugacity gradients. The relative importance of nano- and microplastics as carrier of HOCs is currently estimated to be low compared to other media.

- Species sensitivity distributions (SSDs) using numerical as well as mass-based LOECs have been constructed from 63 studies covering 39-40 species.
- The predicted no effect concentrations (PNEC) for nano- and microplastics based on the SSDs are 0.14 µg/L (95% confidence interval: 0.04-0.64 µg/L) for mass-based concentrations and 71.6 particles/L (95% confidence interval: 3.45-1991 particles/L) for numerical concentrations.
- These estimates compare reasonably well with previous risk assessments. The somewhat lower HC_5 (PNEC) may be a result of the more extensive and recent dataset used by VKM.
- From the SSDs, there is no clear pattern regarding particularly sensitive taxa and levels of biological organisation affected.
- The toxicity data for nanoplastics mainly determine the HC_5 when using mass-based concentrations probably because of their high mass-to-particle-number ratio. Accordingly, the HC_5 derived from numerical concentrations is dominated by data from larger microplastics. This highlights that the choice of dose metric affects the hazard assessment.

➢ VKM concludes that the environmental hazard assessment has two major limitations: First, it is pragmatic in a sense that all available toxicity data were included. Second, it treats all nano- and microplastics as one entity which is clearly ignoring their physico-chemical heterogeneity. The reason not to perform a more differentiated hazard assessment was that this would have resulted in very small datasets. Instead, VKM aimed at gathering as much information as possible.

9 Exposure assessment

9.1 Human exposure

EFSA (2016) focuses on the presence of nano- and microplastics in food, with particular focus on seafood, and states that microplastics are found in wild-caught species, including those consumed. However, they confirm that quantitative data are lacking.

FAO (2017) highlights that microplastics have been found in many species intended for human consumption, including wild and farmed mollusks, crustaceans and fish. However, there is still insufficient knowledge on the distribution, content and nature (chemical composition and size) of microplastics in aquatic organisms consumed as food.

SAPEA (2019) states that there is sufficient published evidence to say that microplastics occur in bottled water and foodstuff. However, the actual levels are uncertain due to methodological limitations, and hence, the human exposure to microplastics cannot be assessed due to the lack of data (especially on foodstuff other than sea food).

With reference to Chapter 4 "Levels of microplastics", VKM affirms that still very limited data of acceptable quality are available on levels of nano- and microplastics in foods. Thus, VKM concludes that an exposure assessment for human exposure to nano- and microplastics can not be done.

9.2 Measured and predicted environmental concentrations

EFSA (2016) does not define any measured or predicted environmental concentrations (MEC or PEC), and did not perform any environmental exposure assessement.

FAO (2017) does not define any MEC or PEC, and did not perform any environmental exposure assessement.

SAPEA (2019) refers MEC or PECs from three peer-reviewed articles, but does not define any own MEC or PEC, and did not perform an own exposure assessment.

The exposure data is limited in such that most studies report aggregated levels for large microplastics consisting of a mixture of multiple polymers. Accordingly, the levels of smaller microplastics are probably underestimated. This is supported by the fact that studies investigating smaller microplastics report the highest levels (Figure 9.2-1). One can safely assume that smaller microplastics are much more abundant in aquatic ecosystems than larger particles. Thus, the MECs used in this assessment would be higher when considering small microplastics.

Exposure data for other compartments and systems are scarce. However, recent evidence suggests that the microplastics levels are high in those, too (e.g., marine and freshwater sediments, atmosphere, sea ice).

In the present assessment VKM used the cumulative distributions for the measured environmental concentrations, MECs, (Figure 9.2-1) to estimate the environmental levels of microplastics in aquatic ecosystems on a global scale (Table 9.2-1). Here, most locations (95 %) would have levels of >0.0004 particles/L, half (50 %) of >0.2 particles/L and the most polluted locations (5 %) would have >104 particles/L (Table 10.2-1). Considering only the regional scale directly relevant to Norway (Atlantic, Arctic, North Atlantic, North Sea), the MECs range from 0 to 48 particles/L.

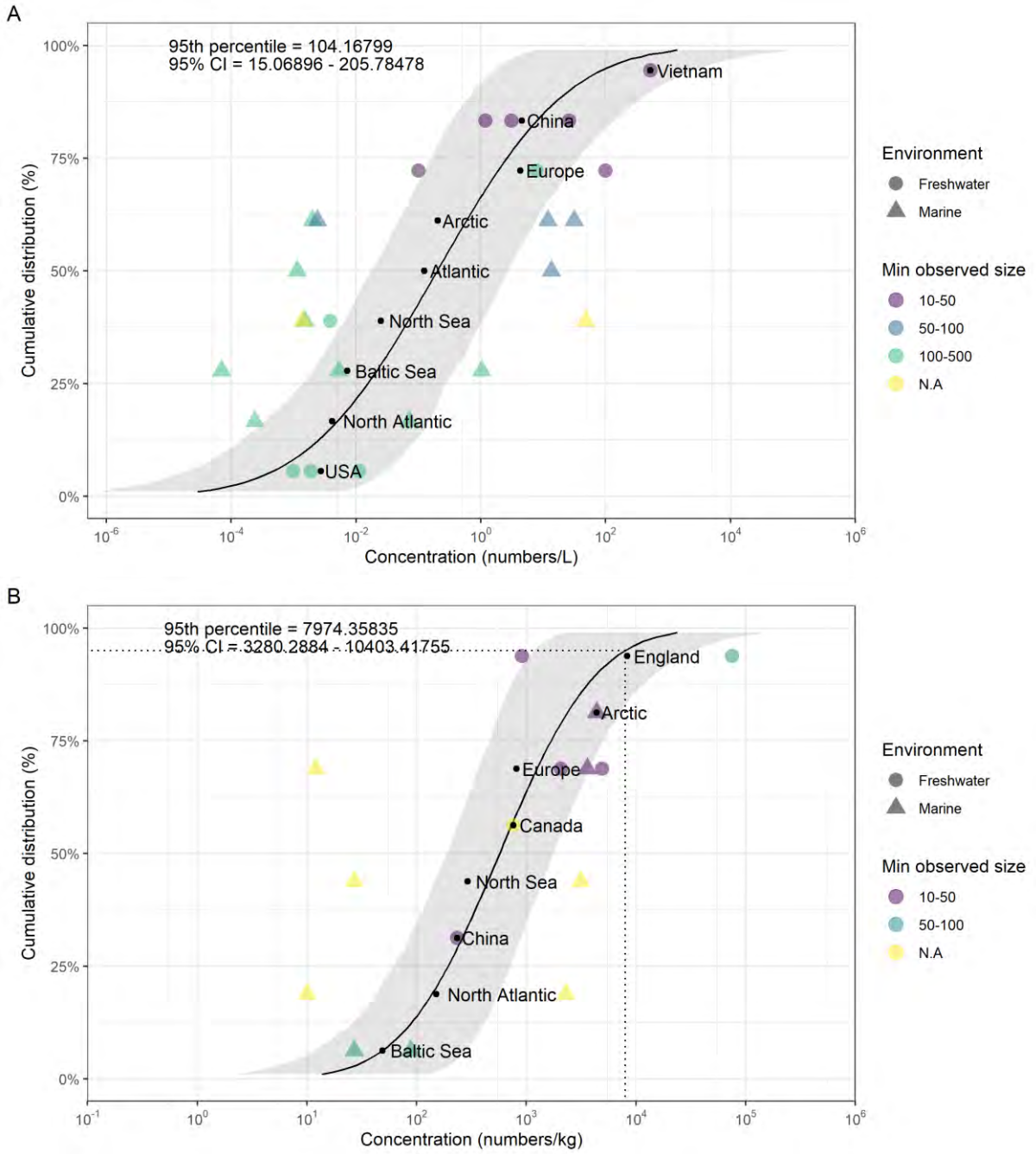


Figure 9.2-1 Cumulative distribution of numerical microplastic concentrations reported from freshwater and marine environments in water [surface or watercolumn] (A) and sediment (B). Black points are the geometric mean of values reported within a geographical area. Coloured points show the raw data from one or more studies presented by the minimum observed size (the lowest detection limit). The black line represents the fitted lognormal regression curve. The 95th percentile shows the concentration below which 95% of the data resides.

Table 9.2-1 Environmental levels of microplastics in the water column and sediments estimated based on the fraction of affected habitats.

Fraction of locations (percentiles)	**MECs (particles/L)** **(95 % confidence interval)**	**MECs (particles/kg)** **(95 % confidence interval)**
95 % (5 % percentile)	0.0004 (0.0002-0.0025)	40.91 (30.57-94.86)
75 % (25 % percentile)	0.0155 (0.0075-0.0538)	193.8 (133.8-319.2)
50 % (50 % percentile)	0.2008 (0.0866-0.4848)	571.2 (397.2-816.9)
25 % (75 % percentile)	2.6067 (0.8412-5.8627)	1684 (950.2-2311)
5 % (95 % percentile)	104.17 (15.069-205.79)	7974 (3409-11770)

9.3 Summary

9.3.1 Summary human exposure

EFSA (2016) states that microplastics are found in wild-caught species, including those consumed. However, they confirm that quantitative data are lacking. FAO (2017) highlights that microplastics have been found in many species intended for human consumption,. SAPEA (2019) states that there is sufficient published evidence to say that microplastics occur in bottled water and foodstuff. However, the actual levels are uncertain due to methodological limitations

- VKM affirms that still very limited data of acceptable quality are available on levels of nano- and microplastics in foods. Thus, VKM concludes that an exposure assessment for human exposure to nano- and microplastics can not be done.

9.3.2 Summary environmental concentrations

EFSA (2016) and FAO (2017) did not define MECs or PECs and did not perform any environmental exposure assessement. SAPEA (2019) refers MEC or PECs from three peer-reviewed articles, but does not define any own MEC or PEC, and did not perform an own exposure assessment.

VKM found:

- Exposure data are still limited and only aggregated levels of large microplastics are reported. Accordingly, the levels of smaller microplastics being underestimated.
- MECs of microplastics were derived from cumulative distributions for the measured environmental concentrations in aquatic ecosystems on a global scale, and a regional scale directly relevant to Norway (Atlantic, Arctic, North Atlantic, North Sea).

- VKM affirms that there is still limited data of acceptable quality on levels of nano- and microplastics in the environment. Most data are available from aquatic ecosystems. MECs were derived from cumulative distributions of the measured concentrations in surface and water columns globally or from locations relevant to Norway.

10 Risk characterisation

10.1 Human risk characterisation

Neither EFSA (2016), FAO (2017) nor SAPEA (2019) attempt to perform quantitative human risk characterisations. Moreover, they conclude that since there is a general lack of exposure and hazard data, the risk of nano- and microplastics to human health cannot be evaluated.

The available information from experimental animals does not provide sufficient basis to characterise potential toxicity in humans. The occurrence data in food is not sufficient to estimate the exposure, and thus any risk from micro- and nanoplastics exposure could not be characterised.

10.2 Environmental risk characterisation

EFSA (2016) does not assess the environmental impacts of nano- and microparticles, and hence does not perform any environmental risk characterisation.

FAO (2017) focuses on knowledge on microplastics in fisheries and aquaculture, and hence does not perform any environmental risk characterisation.

SAPEA (2019) concludes that high quality risk assessment is not yet feasible and that there is a need for adequate risk assessment methods that take into consideration the different nature of nano- and microplastics compared to chemicals contaminants as well as their role in a multiple stressor environment. They concluded that an environmental risk of nano- and microplastics were low on a global scale, but that a few very polluted locations existed where a risk may exist.

Bearing in mind the limitations of the hazard and exposure assessment (see 8.3.3 and 9.2), both can be compared to evaluate the risks nano- and microplastics pose to natural environments. As mentioned previously, such risk assessment must be considered provisional given the data gaps regarding the hazards of larger microplastics and environmental levels of smaller microplastics and nanoplastics. In addition, the pragmatic pooling of all available data is neglecting the heterogeneity of nano- and microplastics in terms of their physico-chemical properties.

Using cumulative distributions for the MECs (9.2), VKM estimated the environmental levels of microplastics in aquatic ecosystems on a global scale. Here, most locations (95 %) would have levels of >0.0004 particles/L, half (50 %) of >0.2 particles/L and the most polluted locations (5 %) would have >104 particles/L (Table 10.2-1). Considering only the regional scale directly relevant to Norway (Atlantic, Arctic, North Atlantic, North Sea), the MECs range from 0 to 48 particles/L.

This data can be used to derive risk characterisation ratios (RCRs) by dividing MECs by the PNEC. In scenarios covering 95, 50 and 5 % of the most polluted locations, the RCRs are

$5.41x10^{-6}$, $2.80x10^{-3}$ and 1.455, respectively (Table 10.2-1). The MEC distribution can also be used to estimate which fraction of locations is at risk (RCR ≥1). This is the case for 6.1 % of locations which are most polluted. In the region relevant to Norway, the RCRs range from $2.79x10^{-5}$ to 0.67 depending on whether the median MEC or a worst-case scenario (highest MEC) is used.

The mean RCRs from the global analysis imply that the environmental risks of nano- and microplastics is low for the majority of locations (RCRs <1). However, the RCR estimate exceeds 1 for about 6 % of the most heavily polluted locations. Thus, this assessment implies that in those areas nano- and microplastics pose an environmental risk. This is in line with the assessment of Adam *et al.* (Adam et al., 2019) who found RCRs >1 for Asian locations with a 0.4 % probability. The same is true for marine ecosystems on a regional scale that is relevant for Norway: The RCR derived from the median MEC (0.002 particles/L) is well below 1. However, the maximum concentration reported from the North Sea in Sweden (Karlsson et al., 2017) approaches the PNEC resulting in a RCR close to 1. Thus, the margin of safety for heavily polluted locations in the vincinity to Norway is very low.

Table 10.2-1 Risk characterisation ratio (RCR) estimates for different scenarios.

Scenario	PNEC	PEC*	RCR
Covering 95 % of locations	71.6	0.0004 (0.0002-0.0025)	$5.41x10^{-6}$ ($2.51x10^{-6}$-$3.48x10^{-5}$)
Covering 75 % of locations	71.6	0.0155 (0.0075-0.0538)	$2.16x10^{-4}$ ($1.04x10^{-4}$-$7.52x10^{-4}$)
Covering 50 % of locations	71.6	0.2008 (0.0866-0.4848)	$2.80x10^{-3}$ ($1.21x10^{-3}$-$6.77x10^{-3}$)
Covering 25 % of locations	71.6	2.6067 (0.8412-5.8627)	0.036 (0.012-0.082)
Covering 5 % of locations	71.6	104.17 (15.069-205.79)	1.455 (0.210-2.874)
Norwegian region (median MEC)	71.6	0.002	$2.79x10^{-5}$
Norwegian region (maximum MEC)	71.6	48	0.670

*PEC = MEC (as we did not apply an extra assessment factor

This provisional risk characterisation has a number of limitations. First and foremost, by lumping together all hazard and exposure data, we are obviously comparing data that should not be compared were more specific data available. As an example, we are comparing hazard data from marine species exposed to PS nanoplastics to exposure data for large microplastics from freshwater systems consisting of a mixture of multiple polymers. However, until reasonable criteria for grouping nano- and microplastics in more specific classes become available, this represent a pragmatic approach.

Second, the exposure data is limited in such that most studies report aggregated levels for large microplastics, only. Accordingly, the levels of smaller microplastics are probably underestimated. This is supported by the fact that studies investigating smaller microplastics report the highest levels (Figure 9.2-1). One can safely assume that smaller microplastics are much more abundant in aquatic ecosystems than larger particles. Thus, the MECs used in this assessment would be higher when considering small microplastics and the risk would be underestimated. To give an example, conservatively assuming the levels of small microplastics are one order of magnitude higher than based on what we currently know would result in ten-fold higher RCRs.

Third, there is a disconnect between the metrics used to generate hazard data (mostly mass-based concentrations) and the ones used to report environmental levels (usually aggregated numerical concentrations). The conversion of one to the other is possible for hazard data but involves a range of assumptions that introduces additional uncertainty. A conversion is impossible for most MECs because the size and the polymer of the individual microplastics are hardly reported. If mass-based MECs could have been used for risk characterisation, this might have resulted in very different RCRs given that hazard data from different species determine the PNEC.

Fourth, this evaluation only considered toxicity data. However, nano- and microplastics may have other than toxicological impacts. The transport of pathogens is one example (see Chapter 6.2), the change of habitat structures or geological processes is another. However, knowledge about these ecological impacts are limited and could not be assessed by VKM.

Fifth, this evaluation does consider aquatic ecsystems, specifically the water column, only. This is because hazard and exposure data for other compartments and systems are scarce. However, recent evidence suggests that the microplastics levels are high in those, too (e.g., marine and freshwater sediments, atmosphere, sea ice, terrestrial ecosystems, agricultural regions, etc.).

Sixth and most importantly, nano- and microplastics pollution is evaluated in isolation in this and previous assessments. Plastic particles in aquatic ecosystems (as in all other compartments) are always part of a larger fraction of particulate matter in the same size range that consists of a multitude of natural and synthetic materials (Scherer et al., 2017). Accordingly, the important yet unanswered question is whether nano- and microplastics just add to the environmental impacts of suspended particulate matter (SPM) in general. This would be the case if their hazard would be similar to other SPM. In this case, it would be important to determine whether plastic particles represent a significant part of SPM. Alternatively, the hazards of nano- and microplastics may be greater than that of other SPM. While this remains to be demonstrated (Backhaus and Wagner), in that case, a separate risk assessment is warranted. In any case, the environmental (and health) risks of nano- and microplastics need to be assessed in the larger context of SPM.

10.3 Limitations to the risk characterisation

The assessment has a number of limitations that need to be taken into account when interpreting its results:

- Pooling all available data is too simplistic yet pragmatic,
- most environmental levels refer to surface water concentrations which are not utilised by most species in ecotoxocological studies, likely resulting in overestimated risks,
- exposure data for small microplastics are scarce probably resulting in an underestimation of risks,
- The use of different metrics in the literature made it necessary to convert data which results in additional uncertainty,
- mass-based environmental concentrations are scarce, and cannot be converted to numerical concentrations, making those unavailable to risk assessment,
- Ecological impacts besides toxicity (pathogens, habitat change) are potentially relevant but were not considered in the assessment due to lacking data
- The risk on nano- and microplastics is assessed in isolation from the larger fraction of (suspended) particulate matter they are part of. It remains to be demonstrated whether plastic particles are more toxic than other particles occurring in abundance in the environment.

10.4 Summaries

10.4.1 Risks to human health

EFSA (2016), FAO (2017) and SAPEO (2019) conclude that since there is a general lack of exposure and hazard data, the risk of nano- and microplastics to human health cannot be evaluated.

- VKM concludes that the available information does not provide sufficient basis to characterise potential toxicity in humans, and that the occurrence data in food is not sufficient to estimate the exposure, and thus the risk from micro- and nanoplastics exposure could not be characterised.

10.4.2 Risk to the environment

EFSA (2016) and FAO (2017) do not perform environmental risk characterisation.

SAPEA (2019) concludes that high quality risk assessment is not yet feasible and that there is a need for adequate risk assessment methods that take into consideration the different nature of nano- and microplastics compared to dissolved chemicals as well as their role in a multiple stressor environment. They concluded that an environmental risk of nano- and microplastics were low on a global scale, but that a few very polluted locations existed where a risk may exist.

VKM found:

- The risk characterisation attempted in this report must be considered provisional due to large data gaps. It was only performed for aquatic ecosystems taking into account nano-

and microplastics in the surface water and the water column. Thus, this assessment has a number of limitations that need to be taken into account when interpreting its results

- Comparing the PNEC with PECs in different scenario resulted in risk characterisation ratios (RCRs) of 5.41×10^{-6}, 2.80×10^{-3} and 1.455 for 95, 50 and 5 % of locations on a global scale.
- Thus, the environmental risks on nano- and microplastics are low for most locations as the RCRs are well below 1 in most scenarios.
- For the 6 % most heavily polluted locations, the RCR is estimated to exceed 1, implying a risk on nano- and microplastics exists at those places.
- When considering only marine ecosystems relevant to Norway, the overall risk is low.
- However, for the highest microplastic levels reported from the Nordic countries (North Sea, Sweden), the RCR is close to 1. This implies that there is a very small margin of safety at Nordic locations that are heavily polluted with microplastics.
- This assessment has a number of limitations that need to be taken into account when interpreting its results.

➢ VKM concludes that available information does not provide sufficient basis to perform a high quality characterisation of risk to the environment by nano- and microplastics. Thus, the attempted present risk characterisation must be considered provisional due to large data gaps. Moreover, it was only performed for aquatic ecosystems (surface water and the water column). On a global scale, the environmental risks are low and for the 6 % most heavily polluted locations a risk is implied. For marine ecosystems relevant to Norway, the overall risk is also low. For the most heavily polluted locations in the North Sea and Sweden, a potensial risk exists.

11 Uncertainties

- The quality of microplastics detection depends largely on the quality of the method used for the identification (efficiency, sensitivity, accuracy, reproducibility) and the quality of the selection of representative test samples. Uncertainty when using analytical methods is also due to difficulties to handle particle brittleness, in avoiding biofouling to interfere with the signal, or due to the particle size being too small to be analyzed

- Methods used for sampling, extraction, purification, characterisation and identification of microplastics are not standardised. This makes it difficult or impossible to compare different studies and difficult to assess human and ecological exposure risks

- There is no consensus in how to report size and number of particles. This causes lack of robust estimates regarding absolute quantities and regional differences in microplastics abundance

- In this assessment, the use of different metrics in the literature made it necessary to convert data which results in additional uncertainty

- Most of the laboratory studies are performed using much higher concentrations than are found in the environment, or using very small or spherical microplastics, which are not representative of the types of particles found in the environment. Thus, it is uncertain to what extent these conditions apply to the natural environment. This limits the reliability of the risk assessment

- The environmental relevance of laboratory studies is not clear. Occurrence does not always equate to impact, and just because an effect is seen in the laboratory does not mean that the effect will occur in the real environment. Conclusions about effects for the natural environment that are based on laboratory experiments are uncertain

- Interactions between chemical pollutants and microplastics are reasonably understood, but their interaction remains difficult to predict in nature

- Because of the limited number of studies, VKM decided to make use of most available data without in-depth quality evaluation and construct the SSDs for the hazard assessment pooling all species and endpoints. Accordingly, the hazard assessment contains data of different type and quality that might affect the SSDs

- VKM assessed microplastics as one entity, hereby clearly ignoring their heterogeneity, including physico-chemical as well as biological properties that may be of importance for toxicity

- Most environmental levels refer to surface water concentrations which are not utilised by most species in ecotoxocological studies, likely resulting in overestimated risks

12 Conclusions and answers to the terms of reference

12.1 Answers to bulletpoint 1 and 2 in the terms of reference

In general,

- VKM acknowledges that there is no international agreed upon definition of nano- and microplastics. Actions should be taken to facilitate a common terminology, taking into account the need for flexibility and adaptiveness as the science evolves. VKM support the proposal of Hartmann *et al.* (Hartmann et al., 2019) which defines nano- and microplastics as
    - consisting of synthetic or heavily modified natural polymers,
    - being solid and insoluble in water at 20°C,
    - being between 1 and 999 µm in size in their largest dimension (for microplastics)
    - nanoplastics is defined as being less than 999 nm in their highest dimension

Further, to answer to the mandate's request for a summary of knowledge from the recently published reports, and scientific literature, on contamination by microplastics, VKM starts each chapter throughout the report with a short summary of the reports from EFSA (2016), FAO (2017) and SAPEA (2019), followed by any updated information that was found in the literature, on the topic in question. Any conclusions that could be drawn from the literature on the topics addressed in the chapters, and in the terms of reference (TOR), are given in the end of the summaries. These are referred below.

- VKM acknowledges that many different approaches are used to study microplastics depending on the matrix of interest. While this is inherent to an evolving field of research, this also poses a challenge to risk assessment as data comparability is limited.
- VKM acknowledges that techniques are under development to detect and identify increasingly smaller microplastics through automated methods. However, as methods become more complex and sensitive, they have higher chances of procedural contamination, and studies must be quality assured throughout. Further, these methods (e.i. uFTIR, FPA-uFTIR, uRAMAN/RAMAN) are very costly and, thus, unavailable for the larger scientific community.
- VKM acknowledges that quality assurance, method validation, and reporting across all methods are of variable quality. Improving those should become a focus.
- VKM concludes that transparent and good quality reporting is important to generate datasets relevant and usable for risk assessment. For example, if researchers would report the details and uncertainties of their method more transparently, this will allow for harmonisation and better comparability across methods and studies.
- VKM concludes that matrix analyzed as well as reporting metrics are often not suitable for risk assessment. From a food safety perspective, qualitative and quantitative data on the levels of microplastics in the edible tissues (seafood) are requested.
- VKM acknowledges that although there has been a recent movement towards longer exposure durations, more environmentally relevant test conditions and the use of particle shapes and particle condition (weathered particles) better representative of those

currently identified in the environment, there is still much to be asked for regarding ecological relevance of current test.

- VKM concludes that data available on levels of microplastics in the Norwegian environment are mostly from the marine compartment (surface and subsurface waters and biota). Limited data only are available from freshwater and terrestrial compartments.
- VKM concludes that very limited data of acceptable quality are available on levels of microplastics in foods. Importantly, many relevant food categories (meat, vegetables, dairy products) have not been investigated at all.
- VKM acknowledges the need for an international harmonisation of microplastics sampling, sample processing, analytical methods and reporting to be initiated for improvement of the quality and comparability between studies. Such harmonisation must not necessarily result in international standards because it will take time to develop and agree on those. A more pragmatic and short-term goal will be the development of quality criteria that the scientific community agrees upon.
- VKM concludes that further information is required to understand sources and transport of microplastics in the Nordic/Norwegian environment, and effort should focus on terrestrial and freshwater systems to increase the knowledge similar to that of the marine systems.

12.2 Answers to bulletpoint 2 in the terms of reference

- VKM concludes that the available information on microplastic biofilms does not provide sufficient basis to characterize potential effects on human health.
- VKM concludes that the available information does not provide sufficient basis to characterise potential toxicity in humans.
- VKM concludes that the environmental hazard assessment has two major limitations: First, it is pragmatic in a sense that all available toxicity data were included. Second, it treats all nano- and microplastics as one entity which is clearly ignoring their physico-chemical heterogeneity. The reason not to perform a more differentiated hazard assessment was that this would have resulted in very small datasets. Instead, VKM aimed at gathering as much information as possible.
- VKM affirms that there is still limited data of acceptable quality on levels of nano- and microplastics in the environment. Most data are available from aquatic ecosystems. MECs were derived from cumulative distributions of the measured concentrations in surface and water columns globally or from locations relevant to Norway.
- VKM concludes that the available information does not provide sufficient basis to characterise potential toxicity in humans, and that the occurrence data in food is not sufficient to estimate the exposure, and thus the risk from micro- and nanoplastics exposure could not be characterised.
- VKM concludes that available information does not provide sufficient basis to perform a high quality characterisation of risk to the environment by nano- and microplastics. Thus, the attempted present risk characterisation must be considered provisional due to large data gaps. Moreover, it was only performed for aquatic ecosystems (surface water and the water column). On a global scale, the environmental risks are low, and for the 6 % most heavily polluted locations a risk is implied. For marine ecosystems relevant to

Norway, the overall risk is also low. For the most heavily polluted locations in North Sea and Sweden, a potensial risk exists. Ongoing initiatives

12.3 Answers to bulletpoint 3 in the terms of reference - Norway

12.3.1 The Research Council of Norway (RCN)

RCN are funding multiple projects related to microplastics. Summaries of the major research projects are given below, listed by research programme. In addition, the RCN are funding, or co-funding, a large number of "Innovation Projects for the Industrial Sector", many of which aim at reducing plastic waste, with more or less focus on microplastics, and/or biodegradable plastic. These are not included here. Nor are projects funded under "PES2020 Support for the Establishment of Project Proposals Directed towards Horizon 2020", as these are not considered "ongoing". The project period and amount of funding is given in Table 14.1.1-1.

Programme «Marine ressurser og miljø» (MARINFORSK)

JPI Oceans[1] - Direct and indirect ecotoxicological impacts of microplastics on marine organisms (PLASTOX) (257479). SINTEF, NTNU and NILU. The PLASTOX project will investigate the ingestion and ecotoxicological impact of microplastics (MPs), together with the persistent organic pollutants (POPs), metals and plastic additive chemicals associated with them, on key European marine species and ecosystems. The influence of MP physicochemical properties (e.g. size, shape, surface area and composition) on these processes will be evaluated. PLASTOX aims to bridge the current gap between laboratory assessment using commercially available feedstock MPs and the additive-loaded and degrading MPs which dominate the marine environment. Macro-sized plastic debris collected from the marine environment will be used to generate fully characterized MPs derived from real marine litter. PLASTOX seeks to generate a clearer understanding of the adsorption and desorption of organic and inorganic pollutants to MPs using a range of common POP and metal contaminants, as well as common plastic additives. PLASTOX will investigate MP uptake through ingestion and other routes following controlled exposures. The potential for MP accumulation in tissues of marine organisms through transport across the gut and cell boundaries will be studied and attempts made to quantify MP accumulation using state of the art analytical approaches. MP accumulation will be linked to the physicochemical properties of MPs and comparisons drawn between different species. The acute and sublethal ecotoxicological effects of MPs will be assessed on marine phyto- and zooplankton. Using data and competence generated in these studies, a more detailed understanding of the potential for MP transfer between trophic levels, and the subsequent impacts this may have, will be obtained. The knowledge generated about MPs in the marine environment will be summarized in a guidance document and serve as a strong evidence base for development of future legislation and remedial efforts.

JPI Oceans - Ephemare (EU-project) Ecotoxocological effects of microplastics in the marine ecosystems (257902). UiO. EPHEMARE targets (1) the uptake, tissue distribution, final fate and effects of MPs in organisms representative of pelagic and benthic ecosystems, and (2) the potential role of MPs as vectors of model Persisten pollutants (PPs)

[1] JPI Oceans: Common European Programme; Joint Programming Initiative for Productive Seas and Oceans

that readily adsorb to their surfaces. The consortium, of true trans-European composition (16 partners from 10 countries), includes experts in biological effects of marine pollutants at molecular, cellular, physiological and organismic levels, up to-date singular facilities for aquatic toxicity testing under strict QA/QC conditions, and some of the world leading teams in MPs research. The EPHEMARE multidisciplinary consortium will allow identification of operational biomarkers with potential for MP detection in the environment. The composition and capacities of the partnership allow in-depth studies on fundamental mechanisms underlying these effects. The main focus of the current sub-project is sediment-dwelling organisms, including laboratory and field studies. In addition, a field survey will be performed to compare the situation in the Skagerrak with other coastal areas in Europe (including the Mediterranean).

Microplastics: Long-term Effects of plastics and Additive Chemicals on marine organisms (MicroLEACH) (295174): NIVA (in collaboration with partners from institutions in Norway, the Netherlands, England and Australia). This project will investigate the long-term effects of MPs to a range of environmentally and commercially relevant marine species from the Norwegian environment and identify the importance of plastic chemical additives to the observable effects. To improve environmental relevance, studies will be conducted with irregular shaped MP particles and fragments generated by cryo-milling post-production and post-consumer MP using realistic and future predicted concentrations. A detailed characterisation of test materials will be conducted using state-of the-art methods in addition to leaching studies of plastic additive chemicals under environmentally relevant conditions (e.g. seawater, synthetic gut fluid). Through innovative approaches, the ecotoxicity studies will attempt to distinguish between long-term effects derived directly from MPs and those resulting from the associated additive chemicals to identify potential risks at the individual and ecosystem level. MicroLEACH will also study MPs uptake, accumulation and elimination routes in test species and determine the associated toxicokinetics to facilitate an improved interpretation of effect data. The project will culminate in a series of trophic transfer-level studies to investigate the potential for bioaccumulation/biomagnification of MPs and associated additives in a Norwegian marine food-web. In a final step, the experimental data will be used to identify test systems for future development of regulatory guidelines for effect assessment of MPs and additive chemicals. MicroLEACH will establish a communication platform towards multiple stakeholders to ensure dissemination of the knowledge generated, with a focus on public engagement, to increase awareness regarding marine litter and ensure a better protection and mitigation of the marine ecosystem.

Evaluating the fate, effects and mitigation measures for microplastic fibre pollution in aquatic environments (MICROFIBRE) (268404): SINTEF. The MICROFIBRE project will investigate the uptake and impact of microplastic fibres (MPFs), develop and apply laboratory methods for simulating environmental MPF degradation, and establish a decision support framework for polymer material selection with low environmental impacts. To reflect the ubiquitous nature of MPF pollution, the project will focus on freshwater, temperate marine and polar marine ecosystems. The fate and effects of MPFs will be studied using environmental conditions and species representing these ecosystems, including species from different trophic levels and a broad range of acute and sublethal endpoints. MICROFIBRE will bridge the current gap between laboratory assessment using commercially available feedstock plastic materials and the degraded plastic materials

that dominate aquatic environments. Degraded MPFs will be generated using simulated UV and physical weathering of pristine reference materials in the laboratory. The role of degradation on the adsorption of persistent organic pollutants to MPFs and the implications for subsequent toxicity will also be investigated. To help identify potential mitigation measures, factors influencing the release of MPFs from synthetic clothing during washing will be studied. The project design aims to provide a basis for conducting comparisons between species, effects, ecosystems and MP degradation, with the goal of establishing a framework for enabling stakeholders (e.g. industry, consumers and regulators) to make informed material selections with low environmental impacts. The project brings together three of the key actors and stakeholders (research, industry and NGOs) in addressing and understanding the implications of MPF emissions to inland and marine waters.

Digestion and maternal/paternal transfer of microplastic contaminants in Atlantic cod (*Gadus morhua*) food web (PlasticCod) (255267): NOFIMA AS. The PlasticCod project will review potential sources of microplastic pollutants and contaminants at 10 selected locations along the coast of Norway, and further screen the abundances of chemical contaminants that are accumulated into microplastic particles in a desorption study at these locations. Ingestion of plastic particles have shown to effect the gastrointestinal system, while plastic additives and organic toxins accumulated in microplastics could possibly be absorbed, give physiological harm and/or be transferred through the food web or to the next generation. Microplastic pollution could therefore be a threat to the future production and sustainability of the marine ecosystem. The present project will study the effects of microplastic particles and selected organic toxins on the ecosystem of Atlantic cod (*Gadus morhua*) through studies of food web transfer of microplastics contaminants from zooplankton to cod larvae, and maternal transfer from cod broodstocks to larvae (long term effects), in addition to study of physiological effects on individual level (short term effects). The project results will be utilized in development of prospective biodynamic modelling tools that can simulate the impacts of contamination with microplastics as well as plastic-associated chemicals on individuals, populations and the food web of marine ecosystem. Further, results from this project will also be useful for the policymakers in formulating better policies.

Development of bidegradable materials to reduce the effect of ghost fishing in the Norwegian deep-sea gillnet fisheries (255568): SINTEF. Lost, abandoned, and/or discarded fishing gear (LADFG) is an internationally recognized problem that causes unwanted ghost fishing; pollution of the marine foodweb with plastics; alterations to the benthic environment; and a variety of costs related to clean-up operations and impacts on business activities. In response to this problem, a large number of international organizations and agreements now focus on LADFG, and numerous national and local-level initiatives have been implemented around the world. To date, Norway is the only country in the world that has a program for the systematic annual retrieving of LADFG from the most intensively fished areas. Since this program started the total number of retrieved gillnets has reached 18,300 nets (approx. 494 km). The retrieving operations are however highly demanding because of operation depth (500-1000m), strong currents in the areas, and the uncertainties associated with the accuracy of the lost gear's position. In the last decade, a large number of R&D projects with biodegradable EnPol gillnets to reduce the impact of ghost fishing have been carried out by Samsung Fine Chemicals and research institutions in Korea. These gears have been tested in 13 different fisheries, and include gillnetting and potting for round,

flatfish, shrimps, octopus and crabs, eels. The results of these experiments have shown that the fishing efficiency of these gears is similar to those made of synthetic fibres (nylon, polyethylene and polypropylene). Currently 21 kind of fishing gears have been developed and a total of 370 Korean coastal vessels are using biodegradable fishing gears on a regular basis. This project brings togheter Korean and Norwegian institutions to develop biodegradable gillnets for the most important deep-water gillnet fisheries in Norway. The main objective is to develop bio-degradable gillnets as a responsible fisheries management measure for reducing ghost fishing and pollution of plastics in the environment.

JPI Oceans – Defining the baselines and standards for microplastic analyses in European waters (BASEMAN) (257432): HI. A fundamental issue precluding assessment of the environmental risks arising from MP is the lack of standard operation protocols (SOP) for MP sampling and detection. Consequently there is a lack of reliable data on concentrations of MP and the composition of polymers within the marine environment. Comparability of data on MP concentrations is currently hampered by a huge variety of different methods, each generating data of extremely different quality and resolution. Although MPs are recognized as an emerging contaminant in the environment, currently neither sampling, extraction, purification nor identification approaches are standardised, making the increasing numbers of MP studies hardly -if at all- comparable. BASEMAN is an interdisciplinary and international collaborative research project that aims to overcome this problem, and address the two major themes the JPI-Oceans pilot call "Ecological aspects of MP in the marine environment": 1) "The validation and harmonisation of analytical methods" which is indispensable for 2) "Identification and quantification of MP". BASEMAN's project outcomes will equip EU and national authorities with the tools and operational measures required to describe the abundance and distribution of MP in the environment. Such tools will permit evaluation of member state compliance with existing and future monitoring requirements.

JPI Oceans - How microplastic weathering changes its transport, fate and toxicity in the marine environment (WEATHER-MIC) (257433): NGI. Understanding the hazards posed by microplastics in the sea requires understanding the changes they undergo as a result of various environmental weathering processes, like UV exposure, biofilm growth and physical stress. These processes will influence parameters such as their brittleness, density, size and surface charge, which can in turn affect their environmental fate as the microplastics undergo fragmentation, aggregation and ultimately sedimentation or mineralization. Changes that lead to fragmentation or mineralization into benign fragments or molecules will reduce potential hazards; though changes that lead to the production of problematic size fractions (e.g. that can accumulate in gills) and release toxic chemicals will increase potential hazards. Similarly, the influence on mobility of plastics and the contaminants they contain are wide-ranging. The WEATHER-MIC project assembles a multidisciplinary consortium of European experts from five institutes and four countries (UFZ Germany, ACES Sweden, NGI Norway, Fraunhofer IKTS Germany and KUL Belgium) that together will develop novel tools to tackle the complex implications of weathering of microplastics in a holistic manner. The toolbox of analytical and (eco)toxicological methods, models,and new knowledge that WEATHER-MIC seeks to establish and validate in case studies for the Baltic Sea and Oslo Harbor will consist of: fingerprinting methods to track microplastic weathering (ACES), mechanisms of chemical release from microplastics (ACES, UFZ, NGI), advanced particle imaging methods to investigate size distribution and

morphological changes with weathering (IKTS), improved understanding of ecological information on the biofilm that accumulates on microplastics and its trophic transfer (ACES), hydrodynamic models to account for changes in sedimentation and transport with microplastic fragmentation-aggregation (KUL, NGI), and toxicity profiles for weathered microplastics (UFZ).

JPI Oceans – Defining the baselines and standards for microplastic analyses in European waters (BASEMAN) (257434): NIVA. A fundamental issue precluding assessment of the environmental risks arising from MP is the lack of standard operation protocols (SOP) for MP sampling and detection. Consequently there is a lack of reliable data on concentrations of MP and the composition of polymers within the marine environment. Comparability of data on MP concentrations is currently hampered by a huge variety of different methods, each generating data of extremely different quality and resolution. Although MPs are recognized as an emerging contaminant in the environment, currently neither sampling, extraction, purification nor identification approaches are standardised, making the increasing numbers of MP studies hardly -if at all- comparable. BASEMAN is an interdisciplinary and international collaborative research project that aims to overcome this problem, and address the JPI-Oceans pilot call "Ecological aspects of MP in the marine environment". BASEMAN teams experienced MP scientists (from different disciplines and countries) to undertake a profound and detailed comparison and evaluation of all approaches from sampling to identification of MP.

Programmes «Marine ressurser og miljø» (MARINFORSK)/«Havet og kysten» (HAVKYST)

JPI Oceans - Defining the baselines and standards for microplastics analyses in European waters (BASEMAN) (257435): NILU. A fundamental issue precluding assessment of the environmental risks arising from MP is the lack of standard operation protocols (SOP) for MP sampling and detection. Consequently there is a lack of reliable data on concentrations of MP and the composition of polymers within the marine environment. Comparability of data on MP concentrations is currently hampered by a huge variety of different methods, each generating data of extremely different quality and resolution. Although MPs are recognized as an emerging contaminant in the environment, currently neither sampling, extraction, purification nor identification approaches are standardised. BASEMAN is an interdisciplinary and international collaborative research project that aims to overcome this problem, and address the JPI-Oceans pilot call "Ecological aspects of MP in the marine environment". BASEMAN's project outcomes will equip EU authorities with the tools and operational measures required to describe the abundance and distribution of MP in the environment.

Micro- and nanoplastic impacts on the marine environment (MIME) (225203): NIVA. This project will seek to establish whether the presence of micro- and nano-sized plastic particles in the marine environment impact on marine organisms. This will be evaluated for both the particles themselves and the additives and adhered chemicals that may be associated with them. There will be special focus placed on establishing where microplastics enter the food-chain and whether they will be translocated. Experiments will be performed that are pertinent to both Arctic and bordeal species. Additives can account for up 20% of a plastic and their presence and the environmental factors that control their release and persistence will be evaluated. An important factor regarding the presence of

microplastics in the environment is that they will interact with other contaminants and a key part of this project will be to evaluate how the environment affects the interactions of microplastics with known hazardous substances and whether microplastic vectors may pose an as yet unrecognised risk to seafood safety by affecting hazardous substance uptake.

Programme JPI «Water challenges for a changing world» (JPIWATER)[2]

WaterWorks2015 – Impacts of MicroPlastics in AgroSystems and Stream Environments (IMPASSE) (271825): NIVA. While it is widely known that microplastics (MPs) in the ocean are a serious environmental problem, the threat posed by MPs in agricultural lands is almost entirely unknown. As much as 90% of MPs produced in industrialized countries may end up in sewage sludge. A sizeable fraction of this sewage sludge is spread on agricultural lands. We estimate the MP input to agricultural lands in Europe to be between 50000 and 175000 tonnes/year. This is especially alarming given the high concentrations of toxic compounds and endocrine disrupting substances with no safe level commonly found in MP. Effectively, sewage sludge application may be causing persistent, pernicious and almost totally ignored contamination of agricultural land. In IMPASSE, we propose to develop and communicate the new understanding of MP behavior in agrosystems which is urgently needed to avoid the potential of serious and long lasting environmental contamination. The highly interdisciplinary project includes risk communication, stakeholder engagement, ecotoxicology, catchment modeling, development of decision support tools, monitoring and experimental work needed to understand and then minimize threats associated with MPs in agrosystem

Programme «Bioteknologi for verdiskapning» (BIOTEK 2021)

Marine microorganisms for bioplastic production (MARPLAST) (270308): UiT – Norges Arktiske Universitet. The exposure to sunlight causes a disintegration of plastics into nanoparticles, which are ingested by marine organisms and has been shown to enter our food chain. The long-term effect of this is not fully understood, but the steady increase in microplastic concentration could result in dramatic effects on the vulnerable wildlife of the oceans and marine food supplies. It is therefore of immediate importance to develop novel types of polymeric materials that can be sustainably produced to address these environmental concerns. MARPLAST focuses on Polyhydroxyalkanoates (PHAs), a class of biodegradable bioplastics which are considered to be feasible replacements for current petroleum-based plastics. PHAs are polymers occurring in nature, produced among others by bacteria, and with properties similar to oil-derived polypropylene and polyesters, rendering them useful as an attractive biodegradable replacement. However, the naturally occurring PHA production pathways are not sufficiently understood, and currently known technologies for production are too costly to allow for a full-scale replacement. MARPLAST aims to develop and provide tools (bacteria, enzymes, and pathways) to enable efficient production of sustainable and biodegradable bioplastics from low-cost unexploited biomass. Focus will be on PHA-producing cold-adapted marine bacteria, with a range of properties that make them especially suitable for industrial applications. MARPLAST will utilize expertise from the University of Tromsø (Norway), University of Bucharest (Romania) and Umeå University (Sweden) to make important progress and contributions to the transition to a bio-based European economy.

[2] Common European Programme

Programme «South Africa - Norway co-operation on ocean research including **blue economy, climate change, the environment and sustainable enegergy"** (SANOCEAN)

Microplastics in wastewater as a carrier and dispersal route of antibiotic resistance in oceans (288073): GENØK. By seasonal sampling of influx and efflux water at waste water treatment plants, GENØK wants to investigate the types of microplastics released throughout the seasons, and to examine their affinity for biofilm formations and role in horisontal gene transfer. By comparing waste water treatment plants in South Africa and Norway, GENØK can inform policy makers in both continent as to the threat and effect of microplastic release into the environment when it comes to antibiotic resistance, and potential give science based advice on type of plastics used and on preferable waste water treatment strategies. In the first phase of the project, GENØK wants to sample seasonal (spring/autumn) influx and efflux water at 2 different treatment plants in each country - preferably with different waste water treatments and determine physical properties of the filaments and retention efficiencies of the treatments. Then GENØK seeks to describe the biofilms formed at the particles, by doing phylogenetic analyses based on 16s sequencing. Antibiotic resistance genes, plasmid specific genes and gene transfer elements determine the possibility for spread of ABres genes aided by biofilms and microplastics. Lab tests of the different types of microplastics found by sampling, may carry different potential for biofilm formation and HGT, and GENØK will test this by laboratory studies (transformation frequencies and biofilm affinity). Lastly, the outcome should be of great interest to policy makers in both countries and will provide science based advice on microplastic handling in the future.

Factors influencing the formation, fate and transport of microplastic in marine coastal ecosystems (FORTRAN) (287939): SINTEF. The FORTRAN project will investigate factors influencing the formation of nano- and microplastic particles from degradation of plastic marine litter, studying their subsequent fate and transport in marine coastal ecosystems. The project will use reference materials representing the most abundant types of plastic litter found in the marine environment. To investigate the influence of plastic additive chemicals on degradation processes, the reference materials will include plastic with differing additive contents and profiles. Methods will be developed to characterise and quantify the formation of nano- and microplastic particle degradation products and additive chemical leaching. FORTRAN will study the influence of plastic physicochemical properties, including additive chemical content, on microbial colonisation and biofilm formation. The role of biofilms on the vertical and lateral dispersal and transport of microplastic will also be investigated. Finally, FORTRAN will develop a series of models to predict plastic and microplastic degradation, biofilm formation and transport in the marine environment. The models will be developed and validated using data generated within the project. State of the art analytical and imaging instruments will characterise the test materials throughout the degradation and additive leaching studies. Conceptual modelling within FORTRAN will (i) act as a basis for designing laboratory experiments, (ii) utilise data generated within the project to develop a hydrodynamic model that will disperse simulated particles through the coastal zone. In situ validation of the model output will be conducted. FORTRAN will establish a communication platform towards multiple stakeholder groups to ensure dissemination of the knowledge generated in the project. Furthermore, public engagement and increasing public

awareness about the issue of marine litter and microplastic will be achieved through the activities of the Wildland Conservation Trust (in South Africa).

Emerging species for sea cucumber aquaculture (288536): MØREFORSKNING. Aquaculture is suggested as an alternative for producing sea cucumber to fulfil the market demand and reduce the fishing pressure on vulnerable wild local stocks. University of KwaZulu-Natal (UKZN) in South Africa and Møreforsking Ålesund (MFAA) in Norway will through the project "Emerging species for sea cucumber aquaculture" create new knowledge on biology and aquaculture of native species and provide valuable input to knowledge-based policies for management and conservation of this high-value marine resource. The two countries have a history of collaboration within marine sciences and this project will strengthen the exchange of expertise and joint research efforts. The research comprises studies of the species Parastichopus tremulus (Gunnerus, 1767), a potential candidate for sea ranching and integrated aquaculture in Norway, and three species from South Africa, Pentacta doliolum (Pallas), Thyone auriata (Q. & G.) and Holothuria cinerascens (Brandt), for which their potential in aquaculture is unknown. Existing knowledge on the biology and key life history parameters of the species is incomplete, and the project will obtain background information as to their occurrence, abundance and fishing methods, development of early life stages, growth in land-based cultivation systems, suitability as deposit feeders in integrated aquaculture systems (i.e. IMTA) and effects of marine pollution. Special attention will be given the effects of microplastics on sea cucumber behaviour and quality. The project is organised in six work packages covering the different scientific tasks as well as knowledge exchange, networking, dissemination and project management. The output is expected to benefit researchers and students at the partner institutions and their national and international network including industry stakeholders and management bodies.

Programme "Bionæringsprogram" (BIONÆR)

Grønt granulat for kunstgressbaner (Green granules for artificial turf) (281914): Norges Fotballforbund (Norwegian Football Association). The aim of the project is to develop a new «green» biodegradable granulate for artificial turf in Norway. The project will contribute to increased use of residual raw material (wood fibre). At the same time, a significant environmental problem, the spread of microplastics, could be reduced.

Programme «SSF – Svalbard Science Forum»

Uptake and effects of microplastics in Arctic bottom dwelling marine invertebrates (282535): Norsk polarinstititutt (Norwegian Polar Institute). This research aims to determine and quantify uptake and effects of different types of microplastics in key sediment dwelling invertebrates inhabiting the coastal seas of Svalbard. Investigations will involve longer term experimental exposures, which simulate different scenarios. The scenarios will represent background concentrations, polluted site concentrations and future "worst case scenario concentrations" relevant to the Arctic. Our knowledge on microplastic pollution in the Arctic is very limited. Recent investigations show that microplastic particles are found in surface waters around Svalbard with higher concentrations detected in sub surface layers, on the sea floor down to 2 500 m depth, frozen into the lower turbid layer of sea ice from the Arctic Ocean, and as larger plastic fragments in Arctic seabirds. Sewage and waste water are identified as important sources of microplastics to the marine environment in temperate areas. Sewage treatment is generally lacking in the Arctic, as well as in the larger settlements on Svalbard. Consequently

municipal, industrial and hospital wastewater is discharged directly into the sea. The relative importance of global, regional and local sources for microplastic pollution is currently unknown and the impact of microplastics on coastal marine organisms, ecosystems and resources in the Arctic remains yet to be determined.

Programme "Fri prosjektstøtte Matematikk, naturvitenskap og teknologi" (FRINATEK)

The Fate and Threat of Man-Made Polluted Particles (231736): NGI. Hydrophobic organic contaminants (HOCs) are often introduced into the environment alongside or within man-made particles. As examples, the Baltic Sea continues to be bombarded with dioxin loaded aerosols formed by industrial combustion, and the coast of Ålesund, Norway contains elevated levels of brominated flame retardants from microplastic particles. An increasing number of field observations are providing clues that these man-made particles regulate the exposure of HOCs to the local ecosystem, more so than natural sorbing phases do, contrary to the orthodox view of organic contaminant research. To address this, this project hypothesize that risks from man-made organic contaminants depend on the man-made particles that introduce them into the environment, particul arly for man-made combustion, petroleum and sewage residues, as well as nanoparticles and microplastics. Methods: The project seek to gain unprecedented, highly resolved profiles of dissolved HOCs and particulate bound HOCs along air-water-sediment transects in contaminated coastal areas. For this, the project have designed novel equipment that will simultaneously and passively sample dissolved and particulate-bound HOCs. This equipment takes full advantage of the most recent advances in passive sampling technology and marine technology design. Four, unique areas will be studied that vary in terms of how the HOCs are introduced: atmospheric deposition, microplastic pollution, river dredging and water treatment overflow. Impact: Combining HOC profiles, geochemical characterization and biogeochemical process modelling, unparalleled accounts of man-made particulate - pollutant dynamics will be obtained. This will shift the focus of HOC pollution research, improve risk and management guidelines, introduce novel technology and methods, inform on the remediation strategies for contaminated areas such as natural recovery, as well as be transferable to assessing emerging threats from nanoparticles and microplastics.

Table 12.3.1-1 Table shows type of funding, amount of money granted from the Research Council of Norway, and project period for each project.

Project number	Funding (in mill NOK)	Type of funding	Project period
295174	7	Research project	2018-2022
268404	10.1	Research project	2016-2020
225203	4.7	Research project	2012-2018
255267	9	Research project	2015-2019
255568	6.7	Research project	2015-2018
271825	4.5	Research project	2017-2020
288073	2	Research project	2018-2022
287939	1.5	Research project	2018-2022
288536	1.9	Research project	2018-2022
281914	0.2	Pre-project/feasibility study	2017-2018
257432	1.7	Other	2015-2019
257435	0.61	Other	2015-2018
257434	0.98	Other	2015-2019
257433	1.8	Other	2015-2019
231736	7.1	Other	2014-2018
257902	1.7	Other	2015-2018
257479	3.1	Other	2015-2019
270308	5.7	Other	2017-2020
282535	0.044	Other	2018

12.3.2 Norwegian Environmental Agency

The Norwegian Environmental Agency are currently updating an assessment of potential measures against microplastics, which will be completed in 2019. The aim of the project is to establish the status, and stake out the direction for the national work on microplastics. The Norwegian Environmental Agency will look at both sources (artificial turf, paint, etc.) and transport routes (e.g. drains, storm water, etc.). Part of the assignment is aimed specifically at sea-based sources (fisheries and aquaculture).

The Norwegain Environmental Agency is also involved in various kinds of international work. E.g. the HAV5 project, which is a collaboration with Russia, and AMAP (Artic monitoring assessment programme)under the Arctic Council. Norway is also responsible for following up action point 42 under Ospar's (Oslo-Paris Convention) action plan against marine litter; "Investigate and promote with appropriate industries the use of Best Available Techniques (BAT) and Best Environmental Practices (BEP) to develop sustainable and cost-effective solutions to reduce and prevent sewage and storm water related waste entering the marine environment, including micro particles".

The Norwegian Environmental Agency is also involved in the ECHA-processes described in Chapter 14.2.2.

12.4 Answers to bulletpoint 3 in the terms of reference - European Commission and EU member states

12.4.1 European Commision Research and Innovation

VKM have identified 10 projects funded by the European Union under the Horizon 2020 framework programme for research and innovation from 2014 to 2020, that focus on understanding and/or reducing microplastics. These are listed below, with acronyms and titles. Further information on the projects, including publications and deliverables can be found via the EU Open Data Portal (https://data.europa.eu/euodp/en/data/dataset/cordisH2020projects).

- GoJelly. GoJelly – A gelatinous solution to plastic pollution
- LimnoPlast. Microplastics in Europe's freshwater ecosystems: From sources to solutions
- EcoFLEXY. Innovative nanocellulose bioplastic film from fruit waste
- EnviroCaps. Enabling a future of safer laundry products and cleaner oceans
- MICROPATH. The fate and persistence of microplastics and associated pathogens in lowland rivers
- SULACHANGE. Microplastic-free Sulapac-material challenges plastic
- CLAIM. Cleaning litter by developing and Applying Innovative Methods in European seas
- CM. Prevention of Cosmetic-induced Non-Communicable Diseases and Micro Plastics entering Food Chains with the CosmEthics-Health App
- POSEIDOMM. Photchemistry at the Ocean's Surface: Effects and Interactions of Dissolved Organic Matter with Microplastics

- Freshwater MPs. The environmental fate and effects of microplastics in freshwater ecosystems

12.4.2 European Chemicals Agency (ECHA) – proposal for restriction of intentionally added microplastics

The European Commission requested ECHA in 2018 to prepare a proposal for restriction of intentionally added microplastics in the framework of the REACH regulation. This is done in the wider context of the EU plastics strategy. In March of 2019, ECHA published their proposal and opened a public consultation that will be open until 20 September 2019. The restrictions proposal addresses a wide range of uses of intentionally added microplastics. In the framework of the public consultation, further information has been requested on the use of granular infill material in synthetic turf in order to assess the implications and the possible need for a derogation. ECHA is also gathering information on the effectiveness of technical measures to prevent the loss of infill material from artificial turf pitches into the environment. ECHA's scientific committees for Risk Assessment (RAC) and Socio-economic Analysis (SEAC) will consider the information received as they consider their opinions on the restriction proposal, which will include their evaluation of the costs and benefits of the proposal and the need for transitional arrangements. The committees' opinions are planned to be finalised in early 2020, after which they will be sent to the Commission for decision-making. All factors, including the important role that sport fields play in promoting physical exercise, health and social inclusion, are taken into account in the decision-making process.

12.4.3 European Strategy for Plastics in a Circular Economy and The Single-Use Plastics Directive

A Europe-wide strategy on plastics was adopted by the European Commision in Januar 2018. The aim is to protect the environment from plastic pollution whilst fostering growth and innovation, turning a challenge into a positive agenda for the Future of Europe. Under the new plans, all plastic packaging on the EU market will be recyclable by 2030, the consumption of single-use plastics will be reduced and the intentional use of microplastics will be restricted.

The Single-Use Plastics Directive is an essential element of this strategy. After addressing plastic bags in 2015, the EU is now turning its attention to the 10 single-use plastic products and fishing gear that together account for 70% of the marine litter in Europe. The new rules will introduce:

- Plastic ban in certain products: Where alternatives are readily available and affordable, single-use plastic products will be banned from the market. The ban will apply to plastic cotton buds, cutlery, plates, straws, drink stirrers and sticks for balloons which will all have to be made exclusively from more sustainable materials instead. Single-use drinks containers made with plastic will only be allowed on the market if their caps and lids remain attached;
- Consumption reduction targets: Member States will have to reduce the use of plastic food containers and drinks cups. They can do so by setting national reduction targets, making alternative products available at the point of sale, or ensuring that single-use plastic products cannot be provided free of charge;
- Obligations for producers: Producers will help cover the costs of waste management and clean-up, as well as awareness raising measures for food containers, packets and wrappers

(such as for crisps and sweets), drinks containers and cups, tobacco products with filters (such as cigarette butts), wet wipes, balloons, and lightweight plastic bags. The industry will also be given incentives to develop less polluting alternatives for these products;

- Collection targets: Member States will be obliged to collect 90% of single-use plastic drinks bottles by 2025, for example through deposit refund schemes;
- Labelling Requirements: Certain products will require a clear and standardised labelling which indicates how waste should be disposed, the negative environmental impact of the product, and the presence of plastics in the products. This will apply to sanitary towels, wet wipes and balloons;
- Awareness-raising measures: Member States will be obliged to raise consumers' awareness about the negative impact of littering of single-use plastics and fishing gear as well as about the available re-use systems and waste management options for all these products.

For fishing gear, which accounts for 27% of all beach litter, the Commission aims to complete the existing policy framework with producer responsibility schemes for fishing gear containing plastic. Producers of plastic fishing gear will be required to cover the costs of waste collection from port reception facilities and its transport and treatment. They will also cover the costs of awareness-raising measures.

13 Data gaps

1. Human toxicity data

Data is lacking to provide a basis for characterisation potential toxicity of microplastics to humans. There is a general lack of information on the toxicokinetics of nano- and microplastics in humans. There is a lack of experimental data relevant to human toxicity.

2. Human exposure data

Data is lacking to perform human exposure assessment. There is a lack of data on the precence of microplastics in food and drinking water.

- Filling data gaps 1 and 2 will make a human risk characterization possible

3. Environemental toxicity data

Although a lot more data exist on environmental toxicity compared to human toxicity, data are still missing. Many experimental studies use relatively short exposure periods. Data on long-term effects are therefore lacking. The effect of microplastics in the environment is often investigated in relation to their size and concentration only. It is important to demonstrate if microplastics cause impacts that differ from those caused by natural particles. Definition of microplastics includes particles composed of different polymers with different chemical compositions and morphologies and containing a variety of chemical ingredients, but microplastics are often categorised into one contaminant group. Detailed reporting of chemical composition and categorisation of hazard data is needed. Most experimental studies use polystyrene particles. Polystyrene particles are not commonly found in environmental samples and therefore more studies investigating the effects of particles similar to those found in the environment are encouraged. There is limited knowledge about the effects of particle aging and fragmentation on the interaction with chemicals. Surface functional groups, size, shape, surface charge, buoyancy, and hydrophobicity influence microplastics uptake.

4. Data on levels of microplastics

Technical difficulties and the cost of sampling microplastics from benthic and pelagic habitats limit present knowledge of spatial and temporal distributions. Thus, data on the quantities of microplastis in these matrices is lacking. Data on levels in terrestrial environments are lacking. The presence of plastics in air could lead to the contamination of pristine environments with plastics, but also the direct settling of particles into soil and water masses. Data on microplastics in air is lacking. There is limited data on microplastics in freshwater.

5. Data on nanoplastics

Sampling and analysis methods of nanoplastics are not yet established and consequently, information on their sources, occurrence and fate is not available.

6. Environmental concentrations

The environmental concentations data is limited in such that most studies report aggregated levels for large microplastics consisting of a mixture of multiple polymers. Accordingly, data on the levels of smaller microplastics are lacking.

- ❖ Filling data gaps 3,4,5 and 6 will increase the quality of an environmental risk assessment

7. Information on sources and fate

There is lack of understanding of degradation and weathering processes for various plastics in various marine environments in terms of time scales for degradation, fragmentation, change of particle morphology and surface properties, biofouling as well as the formation of chemical mixture of degradation products. Estimation of continued degradation of plastics, vertical transport, toxicity, affinity to chemicals and uptake by marine organisms is inadequate. Degradation processes and rates of microplastics in deep water and sediment are unknown. Researchers are still far from understanding the sources, transport processes and sinks of nano- and microplastics on land. This is also true from the transfer of plastics from terrestrial to aquatic systems.

- ❖ The identification of sources and fate of microplastics is crucial to identify measures and actions to reduce their impact on the environment

8. Knowledge on microplastics as a vectors for organic pollutants and biofilms on plastic debris

Significant evidence for microplastics acting as a vector for organic pollutants into organisms has yet to be proven. Chemicals associated with microplastics include plastics additives, byproducts from manufacturing, degradation products and chemicals adsorbed from the environment. Thus, possible toxicological responses can be due to one specific substance or a combination of several. In-depth knowledge of these conditions is currently missing but is important to establish for designing of relevant hazard testing.

Microplastics can also serve as vectors for microorganisms that are potentially pathogenic to humans, animals or plants. The role of microplastics as vectors of animal pathogens should also be investigated as this potentially relates to food safety in food production systems eg aquaculture. Opportunistic human pathogens have been found to be enriched in microplastic biofilm. Microplastics biofilms are considered possible hotspots for horizontal gene transfer. Several studies have suggested that the plastisphere may contribute to the spread of antibiotic resistance.

- ❖ Knowledge of the role of microplastics as vectors for organic pollutants and biofilms on plastic debris is important to map possible devastating effects of microplastics other than direct toxicity of the microplastics itself

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15 Appendix I: Literature search

15.1 Literature search Microplastic in food and environment

Database: Web of Science
Dato: 15.05.2018
Antall treff: 962

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Kommentar: Søkestreng1 begrenset på Document types. (Fanger opp News item osv.)

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Database: Medline
Dato: 15.05.2018
Antall treff: 668

Ovid MEDLINE(R) Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) <1946 to Present>

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Database: Embase
Dato: 15.05.2018
Antall treff: 642

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	endocri* or estrogen or oestrogen or rat* or mouse or mice or "lab* animal*" or animal* or "case stud*" or ecotoxic* or inflammat* or ecosystem or reproduct* or algal or algae or behaviour* or behavior* or inhibition or survival or feeding* or filtrat* or "species composition" or diversity or biodiversity or disturbance or adaptation or germination or communit* or population or androgen or competition or "human adverse effect*OR human biomarker*" or "biological marker*" or "case stud*" or "incidental poison*" or poisoning or "human poison*" or "epidem* stud*" or degradat* or distribut* or gyre* or depositi* or fate or dispositi* or "sinking rates" or sphere* or fibre* or fiber* or bead or granul* or film or fragment* or filament* or foam or effluent or "waste water treatment plant" or WWTP or STP or sewage or stormwater or bioaccumulat* or biomagnificat* or bioconcentrat* or weathering or biodegradat* or "photo-oxidat*" or "photo oxidat*" or photodegradat* or "microbial population*" or biofilm* or rafting or "water treatment" or accumulate* or "filter feed*" or hotspot* or trophic* or "climate chang*" or fisheries or "farmed Fish*" or fjord or fiord or arctic or "drinking water treatment" or "artificial turf" or "car tyres" or "car tires" or "sludge-based fertiliz*" or "sludge based fertiliz*" or "sludgebased fertiliz*").tw.	
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	OR monitoring OR sediment* OR soil OR "food chain" OR concentrate* OR accumulate* OR arctic OR coral* OR salmon OR cod OR aquaculture OR agriculture OR plastisphere OR plastosphere OR dolphin* OR turtle* OR cetacean* OR "filter feeder*" OR pop* OR dioxin* OR transport* OR pollut* OR pbt OR pah* OR pcb OR metal* OR contaminant* OR additive* OR "brominate* compound*" OR "chlorinate* compound*" OR "fluorinate* compound*" OR pfos OR process* OR reducti* OR cooking OR roast* OR frying OR boiling OR bolding OR storage OR brewing OR fermentat* OR microwav* OR toxicokinetic* OR metabolism OR distribut* OR excret* OR absorption OR biomark* OR "mode of action*" OR biotransformat* OR digest* OR eliminate* OR reduct* OR detox* OR "membrane transport" OR egest* OR 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 cell* OR cytotox* OR develop* OR thyroid* OR hormone* OR ecd OR endocri* OR estrogen OR oestrogen OR rat* OR mouse OR mice OR "lab* animal*" OR animal* OR "case stud*" OR ecotoxic* OR inflammat* OR ecosystem OR reproduct* OR algal OR algae OR behaviour* OR behavior* OR inhibition OR survival OR feeding* OR filtrat* OR "species composition" OR diversity OR biodiversity OR disturbance OR adaptation OR germination OR communit* OR population OR androgen OR competition OR "human adverse effect*OR human biomarker*" OR "biological marker*" OR "case stud*" OR "incidental poison*" OR poisoning OR "human poison*" OR "epidem* stud*" OR degradat* OR distribut* OR gyre* OR depositi* OR fate OR dispositi* OR "sinking rates" OR sphere* OR fibre* OR fiber* OR bead OR granul* OR film OR fragment* OR filament* OR foam OR effluent OR "waste water treatment plant" OR wwtp OR stp OR sewage OR stormwater OR bioaccumulat* OR biomagnificat* OR bioconcentrat* OR weathering OR biodegradat* OR "photo-oxidat*" OR "photo oxidat*" OR photodegradat* OR "microbial population*" OR biofilm* OR rafting OR "water treatment" OR accumulate* OR "filter feed*" OR hotspot* OR trophic* OR "climate chang*" OR fisheries OR "farmed Fish*" OR fjord OR fiord OR arctic OR "drinking water treatment" OR "artificial turf" OR "car tyres" OR "car tires" OR "sludge-based fertiliz*" OR "sludge based fertiliz*" OR "sludgebased fertiliz*")) AND PUBYEAR > 2015) AND (EXCLUDE (DOCTYPE , "ed") OR EXCLUDE (DOCTYPE , "le"))	
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Kommentar: Søkestreng1 begrenset på Document types. (Fanger opp News item osv.)

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16 Appendix II: Polymer chemistry, additives, and physical-chemical properties

Natural and synthetic polymers play an essential and ubiquitous role in everyday life. A polymer is a large molecule made up of thousands of repeated linked units (monomers), each being a relatively simple low molecular weight molecule. The polymer-based industries started with naturally occurring materials such as cellulose and continued to develop by the chemical modification of natural polymers to achieve a better usability. A further development created entirely synthetic polymers because they opened possibilities for customizing properties for different end-use requirements. Polymer based materials can widely differ in structure, performance and durability depending on the type of polymer, type and quantity of additives and manufacturing method. Depending on end-use application, composition, properties, manufacturing process type of industry, etc. polymer based materials can be identified in the broad sense as shown in the figure below.

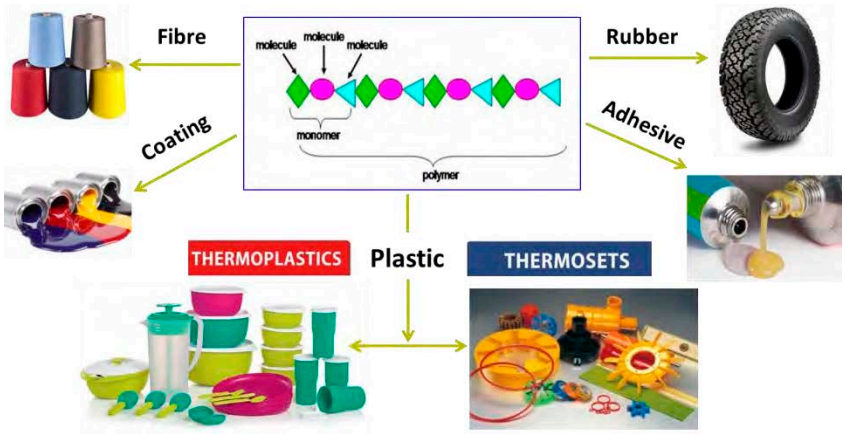

Figure 16-1. Polymer based materials

Rubbers or elastomers are soft and compliant long-chain polymers that are able to undergo large, reversible deformations. Elastomers are typically amorphous, network polymers with lower cross-link density than thermoset plastics. The term rubber is often used when referring to vulcanized material.

A fibre is defined as a structure whose length is much greater than its cross-sectional dimension. The common manufacturing process is spinning process in which the material is uniaxially drawn to obtain a very high level of crystallinity. The end-use performance of a fibre is to a large extent determined by the conditions employed in spinning, in addition to the particular chemistry of the polymer being spun. In addition, the extended list of chemicals is used during manufacture of textiles including pesticides, monomers, additives such as surfactants and detergents, solvents, dyestuffs, etc. Following a strict chemical

definition, not all fibres produced from polymers are plastics. However, among microplastic researchers, microfibres produced from polymers are generally considered microplastics.

A coating is a covering that is applied to the surface of an object. Paints and lacquers are polymer based coatings that mostly are used to protect the substrate and to be decorative. A single coating can be based on several polymers varying greatly in composition: polar and nonpolar vinyl polymers, urethanes, epoxies, polyesters, and alkyds. The goal is to better optimised, the rheology, wetting properties, reactivity, and chemical resistance of the coatings. As for the fibres, polymer based coatings in the micro size-reange are generally considered as microplastics, although they may not actually be "true" plastics.

An adhesive is a material that, by means of surface attachment, can hold together solid materials.

Chemical curing adhesives are based on a series of monomers which react chemically in order to produce polymer structures with thermoplastic, elastomers or thermosets properties.

A plastic is a "material which contains as an essential ingredient a high polymer and which, at some stage in its processing into finished products, can be shaped by flow". Plastics can be classified in many different ways e.g. by chemical structure, by polymerisation process, by their various physical properties, etc. One important classification is by the ability to be molded again and again. Thermoplastics can be molded several times without chemical changes while thermosets can only be melted and shaped once.

Polymer based materials have many different chemical and physical forms, such as cross-linked versus thermoplastic, crystalline versus amorphous, and rubbery versus glassy. Pure polymers are rarely used as commercial products but often contain different additives such as antioxidants, stabilisers, lubricants, processing aids, nucleating agents, colourants, and antistatic agents in small quantities or, in larger quantities, plasticizers and fillers. Quantity and type of additives in the formulation can significantly alter physical end chemical properties of the finished material and their ability to form micro-particles. In addition, non-intentionally added substances (NIAS), e.g. contaminants from raw materials (fossil fuels), metabolic products, etc. may be present. Normally the amounts of NIAS are insignificant, still their influence on toxicity should not be totally neglected.

The physical and chemical properties of polymers depend on multiple factors:

- Chemical nature of monomers (families of polymers). The back bones of polymers such as polythene, polystyrene and poly acrylates are made up of carbon-carbon bonds, whereas polymers such as polyamides, polyesters, polyurethanes, polysulfides and polycarbonates have also other elements (e.g. oxygen, sulfur, nitrogen) inserted along the backbone. Chemical nature of monomers affects many physical and chemical properties such as hydrophobicity, chemical resistance, water absorption, durability, etc.
- Number of monomeric units (molecular weight and its distribution). Molecular weight greatly affects the properties of the polymer. Longer polymer chains allow for stronger Van der Waal attractive forces between them and copious entanglement, resulting in better mechanical properties such as modulus, strength and fracture toughness.
- Monomeric functionality (branches and crosslinking). Functionality is the number of bonds that a monomer's repeating unit forms in a polymer with other monomers. In case of functionality of 2 a linear polymer is formed while functionality of 3 or more leads to a branching or cross-linking.

- Relative positions of the groups (tacticity and changes in shape). The arrangement of the pendant groups in a linear asymmetric polymer chain is called tacticity. Polymers with different arrangements of side groups can have significantly different properties e.g., the difference in glass transition temperature (T_g) of syndiotatic and isotatic polymethacrylates lies in the range of 112 K. Generally, tactic polymers are often to a significant part crystalline.
- Ordering the positions of the chain branches (crystallinity). Linear polymers have a greater amount of crystallinity compared to branched polymers. In amorphous polymers chains are completely random and irregularly interlaced in each other with no long-range order while crystalline arrangement has molecules arranged in distinct patterns. Crystallinity creates benefits in increased strength, stiffness, chemical resistance, and stability. Crystalline structures are generally opaque, while amorphous materials are transparent. Amorphous polymers have god flexibility and elasticity.

Processing of plastics is usually performed in several steps where the material is subjected to heat and mechanical shear. Finished products are exposed to oxygen, heat, sunlight, water and chemicals. Under these conditions, polymer chains can undergo degradation processes such as thermo- or photo-oxidation (e.g. polyethylene and polypropylene) or hydrolysis (polyesters, polyamides and polyurethanes). Thermo-oxidative degradation can be inhibited by adding suitable stabilisers and antioxidants. Saturated hydrocarbons do not absorb sunlight however, due to the presence of catalyst residues and carbonyl groups introduced during polymerisation and manufacturing processes polyolefins are sensitive to photo-oxidation if not protected by UV-stabilisers. Degradation leads to decrease in molecular weight and alteration of the chemical structure of the polymer as in turn leads to a change in the properties such as mechanical strength, flexibility, colour, water absorption etc.

One particular polymer can be an integral part of a wide range of materials. Polymer´s inherent properties can be further modified and enhanced by the variety of additives and manufacturing processes resulting in materials with various properties design for different applications. As evident from Figures 16-2 and 16-3, a variety of materials can be created based on the same polymer.

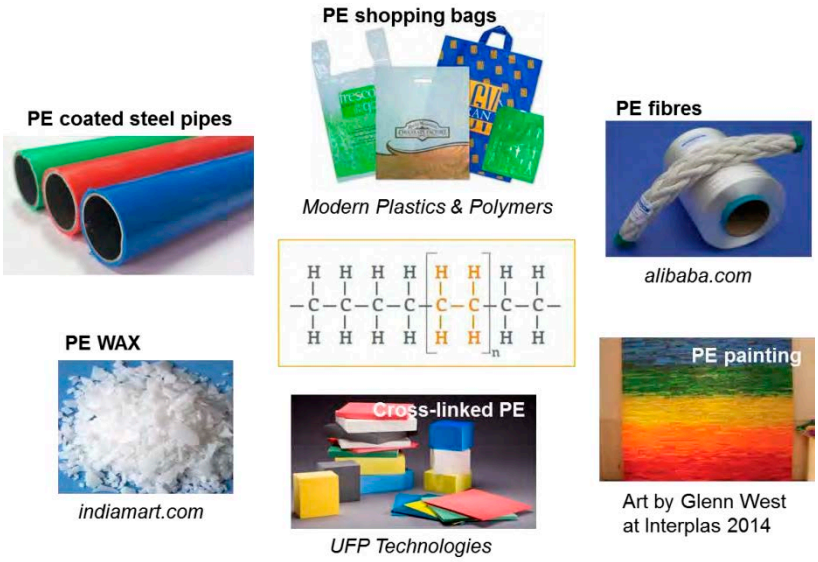

Figure 16-2. Various materials based on polyethylene (PE)

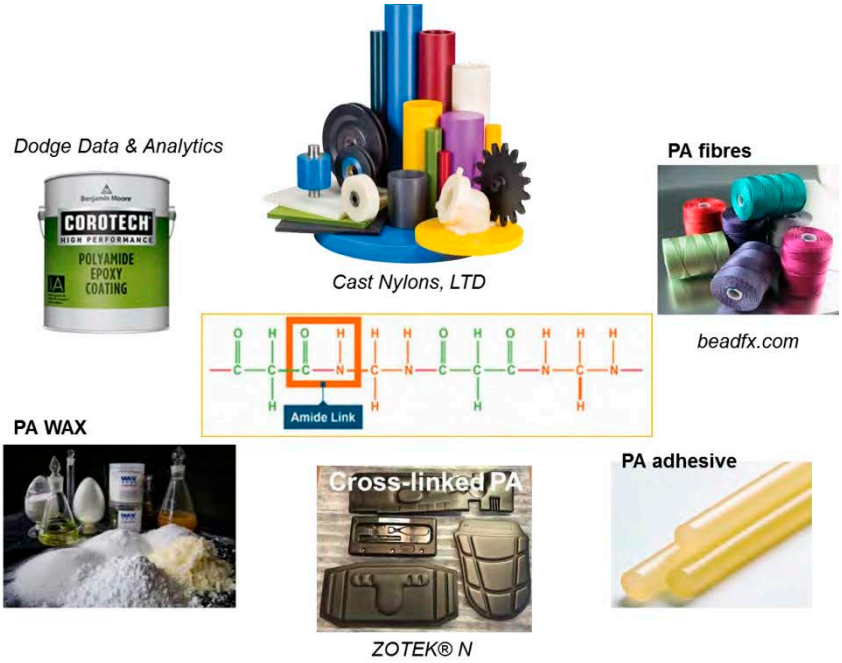

Figure 16-3. Various materials based on polyamide (PA)

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[1] ISO/TR 21960 - Working draft, 2017-08-28

17 Appendix III: A side note on large plastic items

Plastics have been identified in the Norwegian marine environment for a number of years, and although not specific to the mandate of this report, macroplastics are relevant as they can break down and form MPs. For a summary of large plastics on beaches we refer the readers to GESAMP, 2019: http://www.gesamp.org/publications/guidelines-for-the-monitoring-and-assessment-of-plastic-litter-in-the-ocean

Plastics can be input directly from the Norwegian coast or they may be transported long distances from the North Sea or the North Atlantic. numerical models have suggested that much of the plastic found along the Norwegian coast comes from far away. Furthermore, plastic litter found on seven Norwegian beaches using the OSPAR methodology corresponds to what is found on other beaches in Europe with this method (Pham et al 2014). In general, the amount of debris on beaches decreased to the north of Norway and is low in Svalbard. Large fishing nets are more common in Svalbard. Norway probably has the world's longest-continuous series of clean-up gear for lost fishing gear. Norwegian fishermen are obliged to report loss of fishing gear and annual approximately 200 reports are submitted.

As part of the MAREANO program's national mapping, marine debris including plastic is reported following video observation the continental shelf from Ålesund to Lofoten and from Lofoten and north (Buhl-Mortensen & Buhl Mortensen, 2017). One-third of the video recordings contained marine debris with an average value of 678 garbage units per km2, which corresponds to the estimated weight of 601 kg / km2. Fishery-related litter is 70-80%. Compared to the open sea (171 units / km2), higher density of trash is recorded near the shore shallower than 500 (2706 units / km2). For comparison, studies further south in Europe show levels of 200 units per km2, based on fewer observations (Pham et al. 2014). Additionally, IMR has monitored litter in the Barents Sea in cooperation with the Russian marine research institute PINRO, and reported figures are based on the period 2010-2016. Distribution and composition of garbage is reported from bycatch in pelagic trawl (collection in the upper 60 m), from bottom trawl, and visually observed in the sea surface in connection with whale counting. The study includes 2265 pelagic trawls, 1860 bottom trawls and surface traces between trawl stations. Garbage has during this period been recorded in 13% of the pelagic trawls (301 records) and in 33% of the bottom trawl collections (624 records) and 784 garbage units were visually observed. Plastic occurs at 72% in the surface, 94% in the body of water and 86% at the bottom (Grøsvik et al., 2018).

The presence of plastics in the environment raises concerns form organisms and plastics have specifically been identified in different species of biota including seabirds (e.g., Hammer et al., 2016; Trevail et al., 2015; others), fish (e.g., Bråte et al., 2016) and mammals *(Cuviers beaked whale, Bergen).*

18 Appendix IV: Excluded papers

Table 18-1 Of the papers labeled as "ecotox" 18 papers were excluded due to poor quality, and 41 were excluded because they were not relevant. 10 papers were judged to be of high enough quality, but were excluded for other reasons. These are described below. 122 papers were included for for the final ecotox statistics. Of these 122, 15 were from the updated search performed in February 2019. MP = microplastics.

Reference	Qualilty score	Reason for exclusion
L. Baumann, H. Schmidt-Posthaus, et al., Comment on "Uptake and Accumulation of Polystyrene Microplastics in zebrafish (Danio rerio) and Toxic Effects in Liver", Environmental Science & Technology, 50 22 (2016) 12521-12522.	Good	Comment. Not including results
Gandara e Silva, P. P., et al. (2016). "Leachate from microplastics impairs larval development in brown mussels." Water Research 106: 364-370.	Acceptable	Study of toxocity of virgin and beached pellets of mixed polymers on brown mussle embryo survival and development. Beached pellets more toxic than virgin pellets, meaning toxicity form adsorbed contaminants, not MP.
Pacheco, A., et al. (2018). "Toxicological interactions induced by chronic exposure to gold nanoparticles and microplastics mixtures in Daphnia magna." Science of the Total Environment 628: 474-483.	Good	No ecotox data. Authors investigare colour selectivity in fish. A preference for blue MP was found and was linked to the fishes' preference for blue copepods. I.e. the authors infer that blue MP are selectively eaten mistaking them for food items.
Peters, C. A., et al. (2017). "Foraging preferences influence microplastic ingestion by six marine fish species from the Texas Gulf Coast." Marine Pollution Bulletin 124(1): 82-88.	Good	Study looking at feeding ecology of several fish species and linking feeding mode to MP ingestion. Conclusion: Generalists tend to consume more MP compared to specialists. Ok study but does not provide effect data
Rummel, C. D., et al. (2016). "No measurable "cleaning" of polychlorinated biphenyls from Rainbow Trout in a 9 week depuration study with dietary exposure to 40% polyethylene microspheres." Environmental Science-Processes & Impacts 18(7): 788-795.	Acceptable	Depuration study in fish testing the capacity of of MP to clean an organism from PCBs. Inconclusive due to low statistical power.

Reference	Qualilty score	Reason for exclusion
Santana, M. F. M., et al. (2017). "Trophic transference of microplastics under a low exposure scenario: Insights on the likelihood of particle cascading along marine food-webs." Marine Pollution Bulletin 121(1): 154-159.	Acceptable	Study looking at trophic transfer from mussels to crab to puffer fish. Allowing for depuration time between feeding events precluded the accumulation up into the food web. No adverse effects were observed.
Welden, N. A. C. and P. R. Cowie (2016). "Long-term microplastic retention causes reduced body condition in the langoustine, Nephrops norvegicus." Environmental Pollution 218: 895-900.	Good	Ecdysis is the main route for MP removal in Nephrops and probably all crustaceans. Sheeding is a mechanism to remove MP. Implies that slow growth and low shedding rate promotes MP accumulation in the gut.
Wu, C. X., et al. (2016). "Sorption of pharmaceuticals and personal care products to polyethylene debris." Environmental Science and Pollution Research 23(9): 8819-8826.	Acceptable	Sorption kinetics of personal care chemicals and pharmaceuticals. PCPPs sorb to PE.
Al-Sid-Cheikh, M., et al. (2018). "Uptake, Whole-Body Distribution, and Depuration of Nanoplastics by the Scallop Pecten maximus at Environmentally Realistic Concentrations." Environmental Science & Technology 52(24): 14480-14486.	Good	Radiolabelled nano-PS. Distribution study in scallop. Good study but not relevant for table. 24 nm PS distributed in whole animal. Translocation confirmed and highest conc. in hepatopancreas. 250 nm PS limited mainly to GIT. Depuration quite fast in clean water. likely even higher if food had been provided
Caruso, G., et al. (2018). "Effects of microplastics on trophic parameters, abundance and metabolic activities of seawater and fish gut bacteria in mesocosm conditions." Environmental Science & Pollution Research 25(30): 30067-30083.	Acceptable	Tested the effects of PVC on gut microbiota in seabass. No community changes and changes in bacterial metabolic activity observed. Bentonite used as natural control in fish feed. Units not convertable for this table.