Emerging environmental contaminants and human health: Risk assessment of dietary exposure to microplastics

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Abstract

Microplastics (MPs) are an emerging contaminant ubiquitous in the environment. There is growing concern regarding potential human health effects. A major human exposure route is hypothesised to be the dietary pathway via ingestion of contaminated food. A risk assessment perspective was employed, which is the standard approach for human health protection regarding food safety. It is comprised of the four interconnected evidence-based steps of hazard identification, hazard characterization, exposure assessment and risk characterization. Existing scientific data were collected via the execution of scoping, systematic and rapid reviews, using state of the art, robust methodology. Quantitative meta-analysis and meta-regression analyses were also employed. Two bespoke novel risk-of-bias tools were developed and implemented in the execution of the reviews for the standardized quality appraisal of the studies.

Seventy-two studies were included in the systematic reviews on food contamination from three categories. The majority of the samples were contaminated in varying levels: 0-4889 MPs/L in drinking water, 0–10.5 MPs/g in seafood and 0–1674 MPs/kg in salt, thus establishing the dietary ingestion route for MP human exposures. According to the exposure assessment modelling, the estimated levels for MP dietary aggregate exposures could be as high as 3.6 million MPs per year.

Seventeen studies were included in a rapid review focusing on human cell *in vitro* MP toxicological effects. Four biological endpoints displayed MP-associated effects: cytotoxicity, immune response, oxidative stress and barrier attributes. Irregular shape was found to be the only MP characteristic predicting cell death, along with the duration of exposure and MP concentration (μg/mL). Minimum concentrations of 10 μg/mL (5–200 μm), had an adverse effect on cell viability, and 20 μg/mL (0.4 μm) on cytokine release, effectively constituting thresholds of adverse effects. The preliminary comparison of the levels of the thresholds and the exposures reveals that human health could be at risk due to MP dietary exposures.

Further high-quality research using standardized methods is needed to cement the scientific evidence on MP contamination and human exposures. On the other hand, serious data gaps exist regarding toxicodynamics and toxicokinetics which are necessary for a complete toxicological profile.

Οὐ δεῖ δὲ πᾶν πρόβλημα οὐδὲ πᾶσαν **θέσιν** ἐπισκοπεῖν, ἀλλ᾽ ἣν ἀπορήσειεν ἄν τις τῶν λόγου δεομένων καὶ μὴ κολάσεως ἢ αἰσθήσεως.

Ἀριστοτέλης, Τοπικῶν Α΄, Κεφάλαιον ΙΑ΄, 350 π.Χ.

Not every problem, nor every **thesis**, should be examined, but only one which might puzzle one of those who need argument, not punishment or perception.

Aristotle, Topics, Book 1, part 11. 350 B.C.E.

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Author's declaration

I confirm that this work is original and that if any passage(s) or diagram(s) have been copied from academic papers, books, the internet or any other sources these are clearly identified by the use of quotation marks and the reference(s) is fully cited. I certify that, other than where indicated, this is my own work and does not breach the regulations of HYMS, the University of Hull or the University of York regarding plagiarism or academic conduct in examinations. I have read the HYMS Code of Practice on Academic Misconduct, and state that this piece of work is my own and does not contain any unacknowledged work from any other sources

Publications arising from this thesis

DANOPOULOS, E., JENNER, L., TWIDDY, M. & ROTCHELL, J. M. 2020a. Microplastic contamination of salt intended for human consumption: A systematic review and meta-analysis. SN Applied Sciences, 2, 1950.
(presented in sections 3.1, 3.2 and Chapter 4)

DANOPOULOS, E., JENNER, L. C., TWIDDY, M. & ROTCHELL, J. M. 2020b. Microplastic contamination of seafood intended for human consumption: A systematic review and meta-analysis. Environmental Health Perspectives, 128, 126002.
(presented in sections 3.1, 3.2 and Chapter 5)

DANOPOULOS, E., TWIDDY, M. & ROTCHELL, J. M. 2020c. Microplastic contamination of drinking water: A systematic review. PLOS ONE, 15, e0236838.
(presented in sections 3.1, 3.2 and Chapter 6)

DANOPOULOS, E., TWIDDY, M., WEST, R. & ROTCHELL, J. M. 2021. A rapid review and meta-regression analyses of the toxicological impacts of microplastic exposure in human cells. Journal of Hazardous Materials, 127861
(presented in sections 3.3, 3.4 and Chapter 7)

In the above four publications I was responsible for: conceptualization, methodology, software, formal analysis, investigation, data curation, writing the original draft and edits, visualization and project administration. In Danopoulos et al., 2020a, 2020b and 2020c Lauren Jenner was the second reviewer for the search strategy screening process and Jeanette Rotchell acted as the third-party arbitration and as an expert adviser on microplastics. Maureen Twiddy was the second reviewer for the rerun of the searches for Danopoulos et al., 2020b. Maureen Twiddy and Jeanette Rotchell also acted as supervisors and provided input into the conceptualisation of the studies, and methodological input into the design of the systematic reviews, and feedback and editing for all four papers. Robert West provided feedback for Danopoulos et al., 2021 especially for software and statistical analysis. Jeanette Rotchell also did the funding acquisition.

AKOUESON, F., SHELDON, L. M., **DANOPOULOS, E.**, MORRIS, S., HOTTEN, J., CHAPMAN, E., LI, J. N. & ROTCHELL, J. M. 2020. A preliminary analysis of microplastics in edible versus non-edible tissues from seafood samples. Environmental Pollution, 263, 114452.
(presented in section 3.6)

In the paper by Akoueson et al. (2020) I was responsible for conceptualization, formal analysis, writing - original draft; especially for the risk assessment part of the paper.

Chapter 1. Introduction

A contaminant is a substance that is either found in an environment where we wouldn't expect to find it or at greater concentration than usual. A pollutant is a contaminant that is proven to have adverse effects to organisms. Therefore by definition "all pollutants are contaminants, but not all contaminants are pollutants" (Chapman, 2007: 492). Emerging environmental contaminants (EECs) or contaminants of emerging concern, as they are sometimes referred to, are a collection of heterogeneous substances that share a similar level of uncertainty (Halden, 2015, Richardson and Kimura, 2017, Sauvé and Desrosiers, 2014) about their origin, distribution, accumulation and most importantly health effects (Lei et al., 2015).

In most cases, EECs are expected to have some kind of adverse effect but the evidence to upgrade them to pollutants or the severity of these effects is not enough to do so. The term 'emerging' is either time-dependent or importance-dependent, or both, and is used interchangeably in the literature. The list of EECs is inherently ever-changing following research and scientific/technological advances (Browne et al., 2007). The list of currently prominent EECs includes the three substances that were the starting point for this thesis: microplastics (MPs), estrogens/xeno-estrogens and three-dimensional (3D) printer dust. The three substances have substantial overlapping attributes and effects that are illustrated in the course of this chapter.

MPs, estrogens as well as health inequalities were discussed in the Annual Report of the Chief Medical Officer 2017, "Health Impacts of All Pollution - what do we know?". The report highlights the lack of evidence around human exposure, hazards and clear causal relationships between MPs and estrogenic substances and anticipated health effects. The report proposes the exposome concept as an appropriate environmental health paradigm for linking environmental pollution and human health effects (Figure 1). It brings together data on exposures coming from measuring, modelling and biological doses. Furthermore, the report illustrates and stresses the importance on gaining knowledge around health inequalities in terms of health outcomes (Department of Health and Social Care, 2018).

1.1. Emerging Environmental Contaminants

The following section includes a brief introduction to the three EECs that were the initial exploratory focus of this thesis, namely MPs, estrogens and 3-dimensional (3D) printer dust. These three families of components are presented in the context of being an EEC.

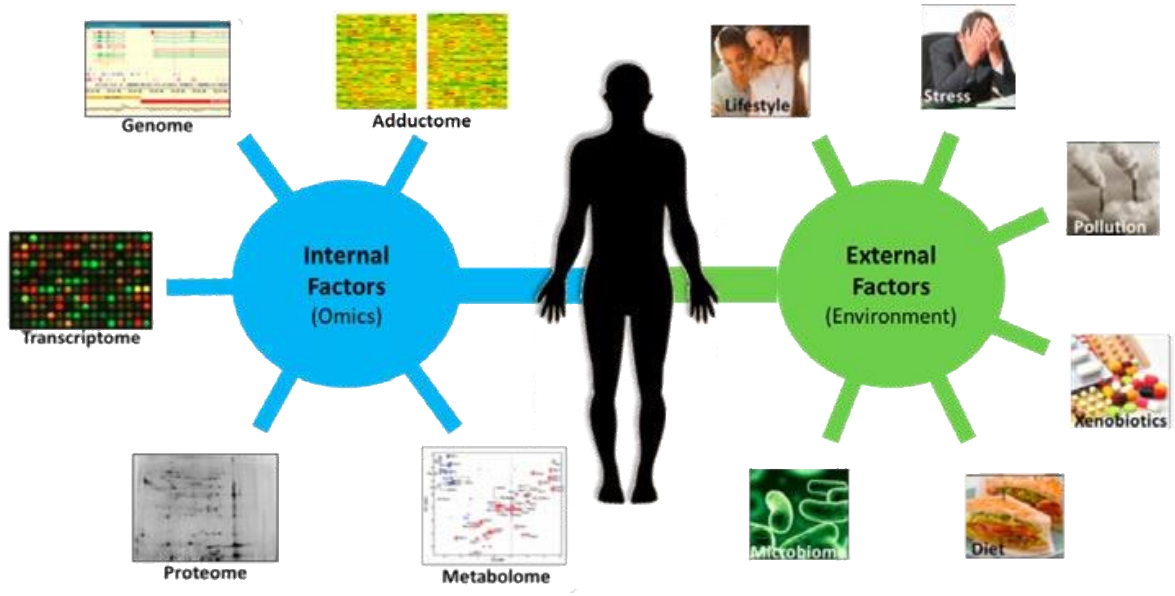


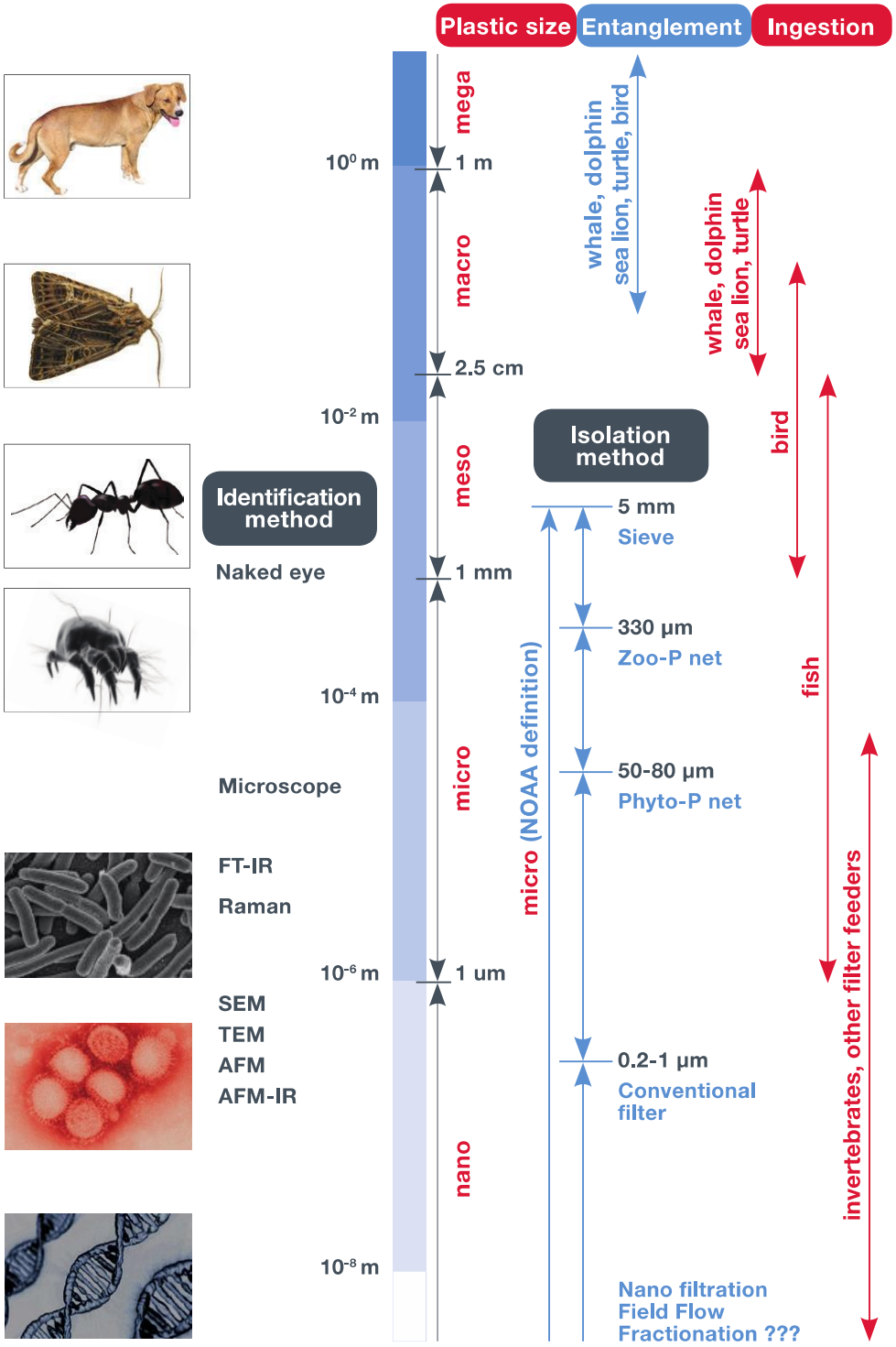
Figure 1. The exposome concept (Department of Health and Social Care, 2018: chapter 8, page 7)

1.1.1. Microplastics (MPs)

The term MPs was coined in 2004 by Thompson et al. (2004). MPs are broadly defined as synthetic polymeric particles < 5 mm in diameter (Frias and Nash, 2019, GESAMP, 2015b, 2016), often also including nanoplastics (NPs) which are < 100 nm (Amy Lusher et al., 2017). Although this definition has been used for a lot of studies, another more rigorous size description has been proposed by the Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP, 2015b, GESAMP, 2016, Arthur et al., 2009). This description catalogues plastics into 5 categories: mega (> 1 m), macro (1 m to 2.5 cm), meso (2.5 cm to 1 mm), micro (1 mm to 1 µm) and nano (< 1 µm). (see Figure 2). Other studies have proposed multiple step characterization criteria for the identification of MPs including chemical composition, solid state, solubility, size, shape, structure, colour and origin (Hartmann et al., 2019). For the purpose of this thesis the size definition of < 5 mm (NPs < 100 nm) was applied to assure the inclusion of all relevant scientific literature and evidence.

MPs are diverse, originating from the wide variety of plastics produced for household products, construction material and industrial applications. They can be classified into two categories according to their origin: primary (intermediate feedstock, pellets/ resin, by-products) and secondary (fragmentation and degradation); some studies propose a third distinct category, the tertiary products which would only include the preproduction pellets (Carbery et al., 2018, Karlsson et al., 2018). This classification can be very helpful since it could indicate the potential source of dispersion for MPs into the environment and therefore identify possible mitigation actions (GESAMP, 2016). The types of plastic that are most

commonly produced and used around the world are polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polystyrene (PS and EPS), polyurethane (PUR), polyethylene terephthalate (PET), acrylonitrile butadiene styrene (ABS) and styrene acrylonitrile (SAN) (Figure 3). (Engler, 2012, Bouwmeester et al., 2015, GESAMP, 2015b, Plastics Europe, 2017, 2020, 2019).



FT-IR Fourier-transform infra-red spectroscopy, Ramon Spectroscopy,
SEM scanning electron microscopy, TEM transmission electron microscopy,
AFM atomic force microscopy, AFM with IR

Figure 2. Size categorization of plastics (GESAMP, 2015a: 5)

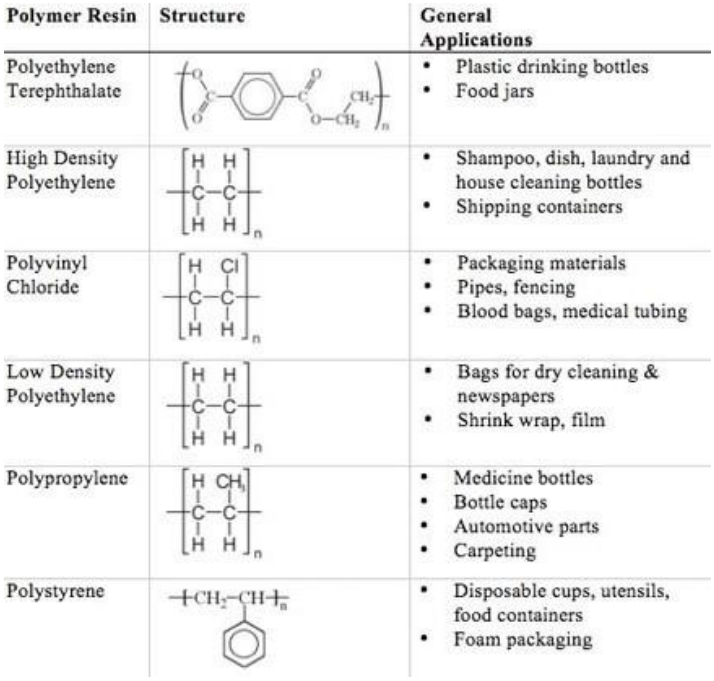


Figure 3. Chemical structure of plastics (Quora, n.d.)

MPs are extremely persistent particles; over time they have contaminated all compartments of the environment and have become ubiquitous. Marine environments are especially affected due to the amount of plastic waste they receive (Burns and Boxall, 2018, Gourmelon, 2015, Li et al., 2016). The degradation of plastic waste in the sea is the major source of MP contamination (Eriksen et al., 2014). The generation of plastic waste and mismanagement of its disposal is expected to triple by 2060, reaching 155–265 million metric tonnes per year (Lebreton and Andrady, 2019). The distribution of MPs in the environment has been researched and documented in numerous studies around the world. They have been identified in varying concentrations and compositions in sea water (Avio et al., 2017, Barrows et al., 2018), fresh water (Lin et al., 2018, Xiong et al., 2018, Wang et al., 2018), sediments (Zhang et al., 2016, Bergmann et al., 2017, Chunfang Zhang et al., 2019), seagrass (Jones et al., 2020), the atmosphere (Cai et al., 2017, Jenner et al., 2021), food (Akoueson et al., 2020, Seth and Shriwastav, 2018, F. Z. Wu et al., 2020), drinking water (Mintenig et al., 2019, Shruti et al., 2020) and biota across different trophic levels: including bivalves (S. Y. Zhao et al., 2018), crustaceans (F. Zhang et al., 2019), cnidarians (Devereux et al., 2021), fish and mammals (Lusher et al., 2015, Nelms et al., 2018). MPs have been found in various parts of organisms such as the gastrointestinal (GI) tract (Sun et al., 2019), liver (Collard et al., 2017a), gills (Feng et al., 2019), and flesh (Akoueson et al., 2020, Karami et al., 2017c). Therefore, MPs appear to be abundantly present in the environment and humans are constantly exposed to them.

MPs could affect organisms via direct and indirect pathways. Three potential mechanisms of exposure, uptake and effect have been identified: ingestion, inhalation and dermal absorption (Lijun Wang et al., 2017, Li J. et al., 2018). The major proposed exposure route is via the food web as dietary exposure (Bouwmeester et al., 2015, Gallo et al., 2018, Karbalaei et al., 2018, Smith et al., 2018, Waring et al., 2018). The first step towards understanding their significance for humans is establishing the exposure routes and quantifying exposures. The potential health effects could come from the MPs causing physical or chemical damage. The effects that are currently being investigated can come from the plastics primary components (polymers) or the additives that are added to enhance their attributes (plasticisers), such as bisphenol A (BPA) which has already been proven to be toxic to humans (Gore et al., 2015). MPs can also act as transporting vectors. Plastic has shown the ability to sorb persistent, bio-accumulative and toxic substances which can later be leeched from it (Engler, 2012, Hartmann et al., 2017, Koelmans et al., 2016, Seltenrich, 2015). Finally, MPs have proven to be a good substrate to be colonized by microorganisms; effectively transporting them and dispersing them into novel environments (Arias-Andres et al., 2018, Keswani et al., 2016). NPs present a somewhat different behaviour resulting from their ability to cross membranes possibly delivering substances to different locations than the aforementioned uptakes. They might become cellular vectors due to their nano size, thus transporting substances into cells (GESAMP, 2015b). MPs can thus be considered either the primary hazard or a pathway for a hazard, both linked to human health.

There are several ongoing research projects on the effects of MPs on humans. The logical and obvious assumption being that since they are abundantly present in the environment they will make their way into the human body too; via dietary or non-dietary ingestion, inhalation etc. (Carbery et al., 2018, Thompson et al., 2009, Halden, 2010, Wright and Kelly, 2017, Smith et al., 2018, Keswani et al., 2016, Prata, 2018). The presence of MPs has been confirmed in human stools (Schwabl et al., 2019), while more recent studies have found MPs in human colectomy samples (Ibrahim et al., 2021), human placenta (Ragusa et al., 2021) and human lung tissue (Amato-Lourenço et al., 2021). Human health effects related to MP exposures, and indeed the levels of MPs in human subjects, are only recently being investigated but there is a growing body of literature to support evidence of uptake (Abbasi et al., 2018, Gallagher et al., 2015, Schwabl et al., 2019) and detrimental impacts (Dong et al., 2020, Gallo et al., 2018, Stock et al., 2019). Recently reported potential human effects include gastrointestinal and liver toxicity (Chang et al., 2020, Wenfeng Wang et al., 2019) as well as neurotoxicity (Prüst et al., 2020). The key identified exposure route is ingestion (along with inhalation) (Chang et al., 2020, Hale et al., 2020), with seafood being a major medium of exposure (van Raamsdonk et al., 2020, Yung-Li Wang et al., 2020). Key toxic

mechanisms include cytotoxicity via oxidative stress (Chang et al., 2020), gene expression alteration and genotoxicity (Yung-Li Wang et al., 2020), changes to the gut microbiota (van Raamsdonk et al., 2020), metabolism disorders and inflammatory reactions (Chang et al., 2020). Evidence comes from animal studies and human cell lines. Although the findings are in some cases contradictory (van Raamsdonk et al., 2020) and further research is undoubtedly needed, there is also no evidence that MPs human exposure is safe (Leslie and Depledge, 2020).

The contamination of food intended for human consumption, with this emerging risk and the possible effects on health, has raised concern in the scientific community (Barboza et al., 2018, Diepens and Koelmans, 2018, Santillo et al., 2017, Waring et al., 2018) as well as among stakeholders (GESAMP, 2015b, 2016) and policy makers globally (EFSA, 2016). There is a growing body of evidence regarding effects in aquatic animals, but health effects on humans are still unclear (Karbalaei et al., 2018, Sharma and Chatterjee, 2017, Smith et al., 2018). There is a clear need to address this emerging risk and promptly implement mitigation strategies for the protection of the environment and human health. In addition to food ingestion, atmospheric MP contamination presents an additional pathway for MP human exposures (Chen et al., 2020), related to direct exposures via inhalation (Wright et al., 2020) and indirect exposures via non-dietary ingestion routes of hand-to-mouth behaviour (Gasperi et al., 2018), inadvertent ingestion (Abbasi et al., 2018) and occupational exposures (Gallagher et al., 2015). The focus of this thesis is the human dietary exposures via the ingestion pathway.

A growing body of evidence has been established regarding the presence of MPs in food and drinking water and a number of reviews have been published (Cox et al., 2019, Hantoro et al., 2019, Toussaint et al., 2019, Welle and Franz, 2018). However, to date, all the reviews that have been published so far are neither systematic nor follow a meta-analysis approach. Another significant issue in the field of MPs is that consensus has not been achieved yet on the methods used to sample, test, analyse, measure, classify and report MPs both in environmental science and in toxicology. A number of reviews are available regarding the sampling methods that are primarily used (Mai et al., 2018, Shim et al., 2017, Hanvey et al., 2017, Zobkov and Esiukova, 2018). This lack of consensus brings about difficulties in validating and aggregating results from different studies and reviews.

The diverse MP inherent characteristics create a lot of difficulties in research. **Diverse composition**: there is a wide variety of polymers, in many cases the productions details of polymers (e.g. which plasticizers are used) are not known since they are classified as

sensitive and not in the public domain. Different polymers could have different behaviour when they enter the environment e.g. different degradation/ fragmentation durations. **Diverse physical characteristics**: e.g. size, colour, shape, buoyancy, density, solubility. The characteristics of MPs vary between test MPs used in labs and MPs extracted from environmental samples creating relevance issues (Connors et al., 2017). In addition, reporting is not consistent, so a lot of data gaps have been created (Ogonowski et al., 2018). Another issue is the absence of **reference material**. Currently there is an absence of reliable and generally accepted reference MPs. Different labs used different test MPs creating problems with results comparability and reliability.

1.1.2. Estrogens

Estrogens are a group of chemically similar sex hormones synthesized in all vertebrates (Barrington, 2017). Estrogens in humans govern the development of the female reproductive system and their secondary sexual characteristics as well as regulating the menstrual cycle (Johnson, 2013). The three major estrogen derivatives that are naturally produced in the human body are Estrone (E1), Estradiol (E2) and Estriol (E3) (see Figure 4) (The Hormone Health Network, 2018). Phytoestrogens and mycoestrogens are natural occurring estrogen like substances. They are plant and fungi derivatives respectively (Xueyan Chen et al., 2016). Beside these natural compounds there are also synthetic compounds (Adeel et al., 2017) which are produced in large scale to substitute estrogens and are often used as medication for hormone therapy (contraception, hormone replacement, infertility etc.).

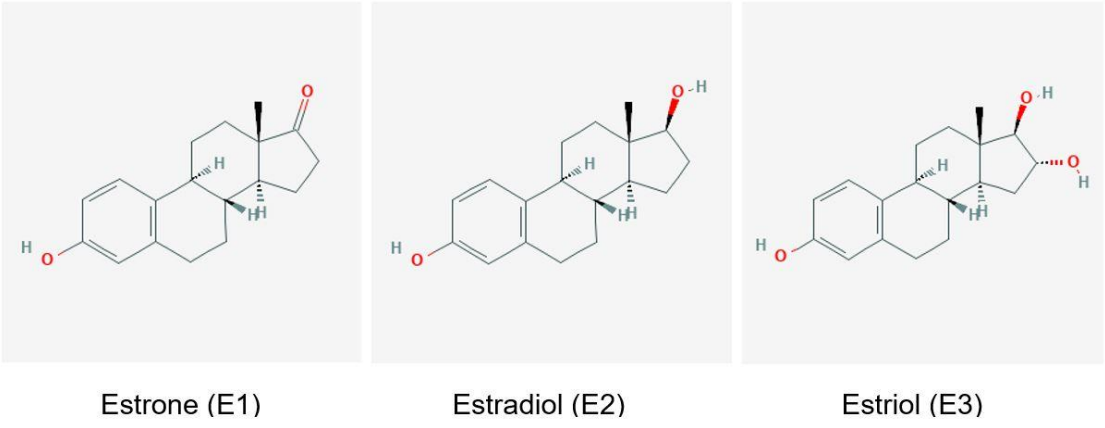


Figure 4. Estrogen chemical structure (PubChem, n.d.-c)

Xenoestrogens is a group of substances which although are not estrogens themselves, when found in the human body have estrogen-like behaviour or estrogen related effects (Oyelowo, 2007). Examples of xenoestrogens are brominated flame retardants, phthalates and Bisphenol A; a substance also mentioned in the MPs section as a plasticiser (Figure 5).

Xenoestrogens incidentally possess estrogen-related attributes and enter the human body through the route of environmental contamination including the food web. They are part of the endocrine disruptor chemicals that are defined by their ability to alter mechanisms of the endocrine system (Caserta et al., 2008). In many cases whether the mechanism of action that ultimately causes the health affect is estrogenic or not is ambiguous (Gore et al., 2015, Maqbool et al., 2016).

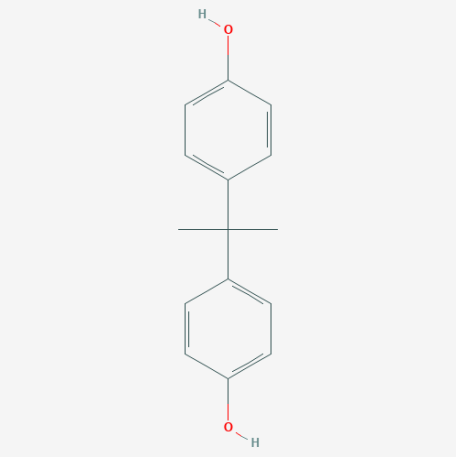


Figure 5. Bisphenol A chemical structure (PubChem, n.d.-b)

In recent years it has been widely recognized that the estrogens and xenoestrogens found in the human body are linked to a wide variety of adverse health effects including breast cancer, ovarian cancer, uterine cancer, fallopian tube cancer, infertility or reduced fertility, reduced fecundability, anovulation, premature ovarian failure, primary ovarian insufficiency, PCOS (Polycystic Ovary Syndrome), obesity, heart disease, osteoporosis, dementia (vascular), birth defects and sex disorders (Bidgoli et al., 2011, Newbold et al., 2009, Gore et al., 2015, Cruz et al., 2014, Z. Wang et al., 2017: etc.). Exposure to these contaminants has been documented as early as during foetal life (Gaspari et al., 2011). Some researchers propose to list estrogen as a "toxic organic pollutant" (Adeel et al., 2017). These contaminants can have synergistic effects with other chemicals and compounds producing estrogenic mediated effects to the human body (Andersson et al., 2011). Their emerging significance is also illustrated by the fact that estrone (E1) and the synthetic estrogens 17-alpha-ethinylestradiol (EE2) and 17-beta-estradiol (E2) are included in European Union's first and the second "Watch list of substances for Union-wide monitoring in the field of water policy" (Decision (EU) 840, 2018).

1.1.3. 3D printer dust

The advent of 3D printing technology has been revolutionary in engineering and other disciplines. 3D printers are an additive manufacturing technology which can be defined as a "process of joining materials to make objects from three-dimensional (3D) model data,

usually layer upon layer, as opposed to subtractive manufacturing methodologies" (ASTM, 2012: 12). They are being used in large scale in industry and as desktop printers for domestic use. Most 3D printers use the same technology which is called fused filament fabrication (FFF) or fused deposition modelling (FDM) (trademark term) (Brenken et al., 2018, Guo and Leu, 2013). There is a wide variety of filaments, the most common are ABS (acrylonitrile butadiene styrene) (Figure 6) and PLA (poly-lactic acid) (Figure 7) (Azimi et al., 2016).

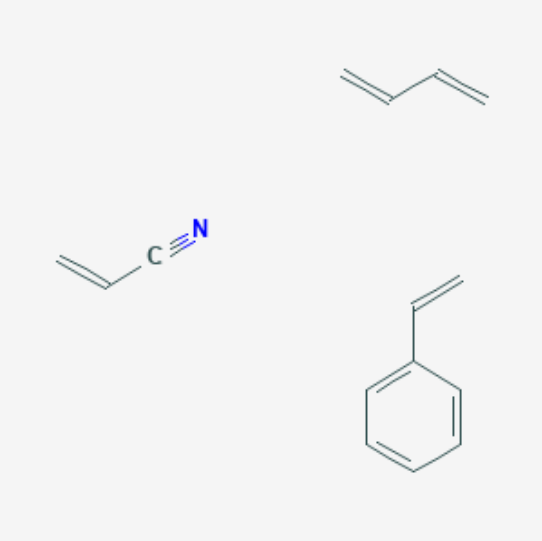


Figure 6. Acrylonitrile butadiene styrene (ABS) chemical structure (PubChem, n.d.-a)

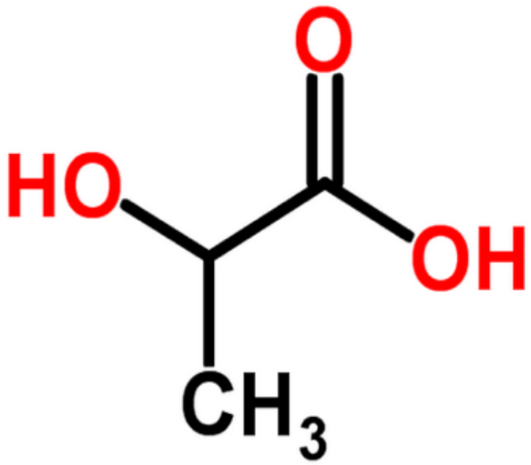


Figure 7. Poly-lactic acid (PLA) chemical structure (Polymer Properties Database, 2015)

Research has shown that during the printing process particles are produced as a by-product (Vance et al., 2017, Mendes et al., 2017). The majority of them are ultra-fine particles (Byrley et al., 2018). Ultrafine particles are less than 100 nm in diameter (or < 0.1 μm) which makes them of nanoscale size. The difference between nanoparticles and ultra-fine particles is that nanoparticles are produced intentionally thus being engineered nanomaterials

(ECETOC, 2013). If the ultrafine particles are of polymeric composition these are NPs, but in a different setting. Figure 8 illustrates the overlap in term sizes. There is increasing research interest around the possible effects of nanoparticles and ultra-fine particles (Rui Chen et al., 2016). Adverse side effects have started to be documented in relation to 3D printer dust, mainly regarding the respiratory system (Randolph, 2018, Chan et al., 2018).

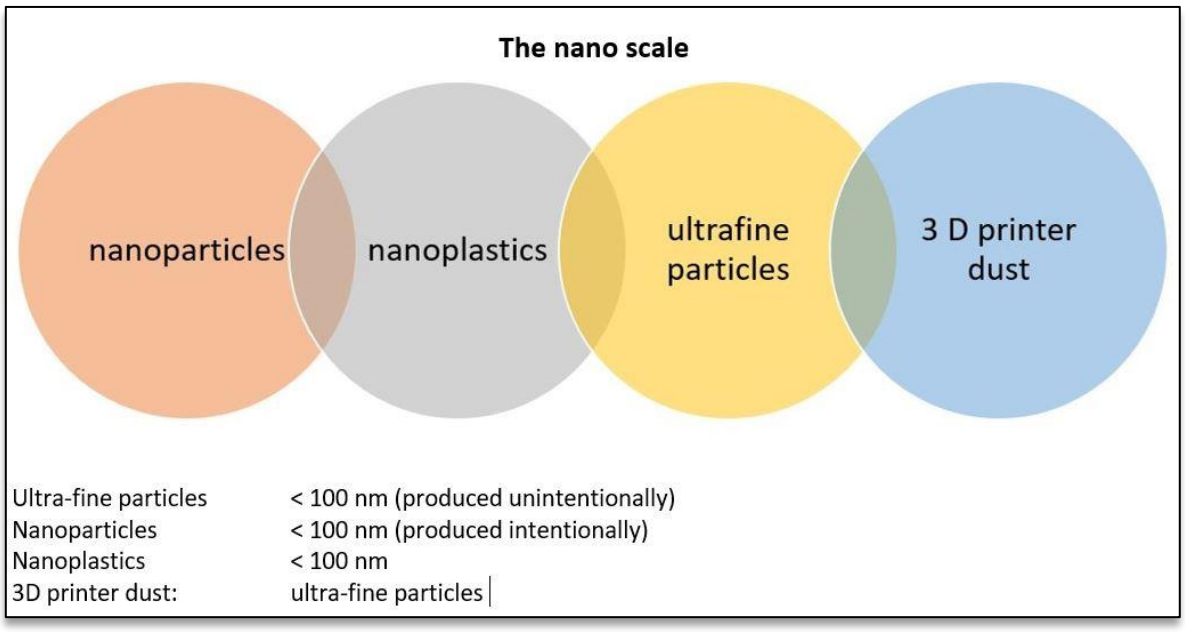


Figure 8. Particle size overlap in the nano scale

1.2. Risk Analysis

Risk analysis consists of three pillars: risk assessment, risk management and risk communication (WHO & IPCS, 2010). Risk assessment is the first and central part of the analysis and its outcomes are a qualitative or quantitative expression of the likelihood of the hazard to cause harm (FAO and WHO, 2009). The aim of risk assessment is to evaluate the hazards, exposures and potential harms posed by an agent. It consists of identifying, collecting and integrating information on the human health hazards of an agent, the human exposures to the agent, and the relationships between exposures, doses and related adverse health effects (WHO & IPCS, 2010). This information can come from multiple sources, including published scientific evidence as well as primary research. Although human health is the focus, epidemiological data are not the sole input. Animal *in vivo* and *in vitro* studies, human *in vitro* studies and *in silico* studies may be used.

Risk assessment can be executed even when complete information is lacking, and can be performed in a way to be protective and not underestimate the actual risk to public health. When certain inputs are missing, estimations and expert judgement can be used to substitute for them, while the introduced uncertainty is assessed and characterised formally (WHO &

IPCS, 2010). MPs are an EEC for which there is much concern around their potential risk to the wider environment, in many different ecosystems, and to humans. The execution of a risk assessment is imperative to better understand and estimate this risk. A conceptual framework incorporating the environmental health paradigm and MP human risk assessment is illustrated in Figure 9.

Hazard classification differs depending on the intended purpose and the field it is used for. In order to classify hazardous chemicals under the Globally Harmonized System of Classification and Labelling of Chemicals (GHS), three classes are used: physical hazards, health hazards and environmental hazards, each comprised of several categories of hazardous properties (UN, 2019). The United States Environmental Protection Agency (EPA) proposes six categories of environmental hazards: chemical, radiation, physical, microbiological/biological, nutritional and socio-economic (EPA, 2017a). On the other hand, in food safety risk analysis hazards are classified in three categories: chemical, physical and microbiological (ISO, 2018, Wallace, 2015, Council Regulation (EC) No 178/, 2002). Categorization is shifted depending on the focus. In the first case the focus is on the effect the hazard can have whereas in the second and third the focus is on what the hazard is. According to the principals proposed by the World Health Organization (WHO) and the International Programme on Chemical Safety (IPCS), which have also been adopted by the European Food Safety Authority (EFSA) and the U.S. Food and Drug Administration, there are four main steps in undertaking a risk assessment: hazard identification, hazard characterization (or dose response), exposure assessment and risk characterization. (EFSA, n.d., FDA, 2002, WHO, 1999, FAO and WHO, 2009). The risk assessment processes are discussed in detail in the methodology and methods chapter (sections 3.5 and 3.7).

1.3. Food safety and risk assessment

In the modern world, food safety is managed in terms of hazards and risk analysis. The main legislative framework in the UK is comprised of Regulation 178/2002 of the European Union (Council Regulation (EC) No 178/, 2002) and the national legislation that incorporates its provisions in English law (The Food Safety and Hygiene (England) Regulations, 2013, The General Food Regulations, 2004). According to this framework, food hazards are the agents that have the potential to cause a health effect and can be classified in three categories: biological, chemical and physical. Consequently, risk is defined as the function of the likelihood of the hazard to have a health effect and the severity of the effect. Risk analysis comprises three processes that aim to protect human life and health: risk assessment, risk

management and risk communication. It is the responsibility of each country to enforce this legislation and implement these processes.

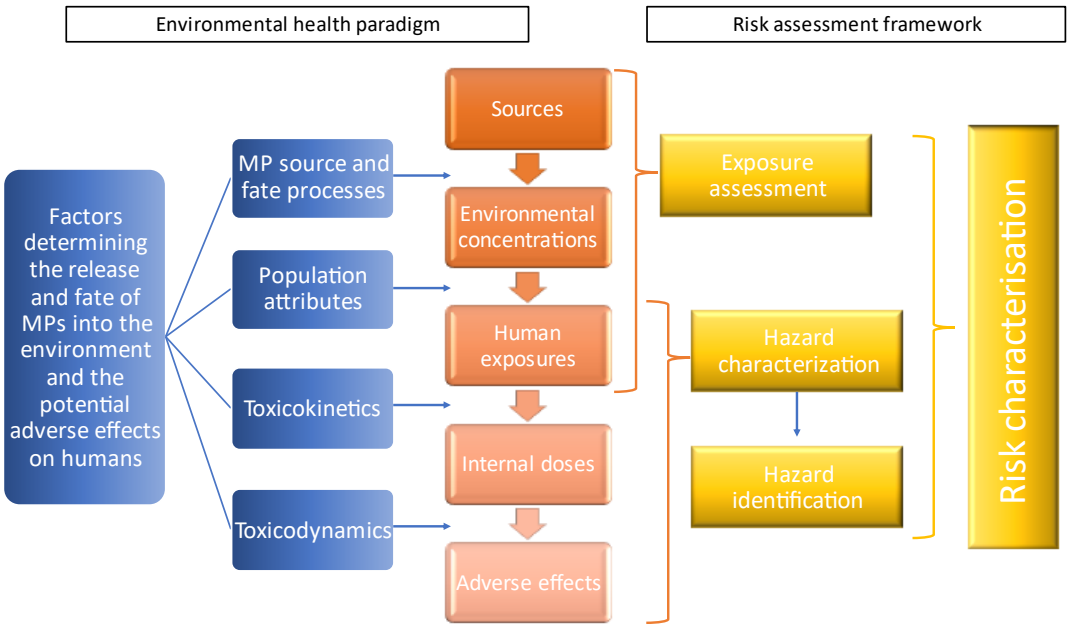


Figure 9. Environmental heath paradigm and human risk assessment framework. Adapted from WHO & IPCS (2010)

In this view, risk management is not only scientifically important but it is also a matter of law. The implementation of risk analysis occurs at different levels and the responsibility varies. On a European Union level, the responsible authority focusing on scientific guidance and consultation is the EFSA and in the UK, the Food Standards Agency (FSA). It should also be noted that risk assessment must be based on current scientific, evidence-based knowledge (Council Regulation (EC) No 178/, 2002). Other pieces of regulation that complete the EU Framework on Food Contaminants are Regulation 315/93/EEC on procedures for contaminants in food as well as Regulation 1881/2006/EC on maximum levels for contaminants in foodstuffs. Although MPs and NPs are not mentioned directly in them they are covered in the general provisions of the law (European Council, 1993, European Commission, 2006). In addition, the recent guidance around nanoscience and nanotechnologies in the food chain provides a comprehensive framework for these emerging contaminants (EFSA (SC), 2018).

1.4. Aims and objectives

Following the completion of a series of scoping reviews (ScRs) focusing on the health effects of the three EECs (see sections 2.2.2 and 2.2.3) a decision was made to focus only on MPs and execute a MP human health risk assessment (see section 2.5). The aims of a human

health risk assessment would be to estimate the risk to a specific population of humans (general or sub-population) that has been exposed to an agent, taking into consideration the characteristics of both the agent and the population at hand (IPCS, 2004). The assessment can be used retrospectively or prospectively for past, present or even future exposures and effects (WHO & IPCS, 2010, Solomon et al., 2008). Since the aims and objectives of this thesis follow the main structure of the risk assessment process they are presented within this context:

Hazard identification is the process by which the specific hazards of MPs are identified. For MPs, one can argue that it can fall in two categories. When referring to MPs as inert agents, they would be classified as physical hazards. On the other hand, when referring to their chemical properties (inherent or additives) or vectors of contaminants, they would be classified as chemical hazards (EFSA, 2016). This thesis will focus on both physical and chemical hazards. The substances that might have contaminated the MPs after they are released in the environment are beyond the scope of the thesis as this is a separate area of MP research in its own right. **Hazard characterization or dose response** is the step in which cause and effect are examined. Scientific evidence must be acquired that demonstrates the causal link between the hazard and the adverse human health effect. If a causal link can be established the next task is to try and set safe exposure levels. **Exposure assessment**: There are two main tasks in this step; first to assess the presence of the hazard in food in terms of concentrations and physicochemical characteristics; second, to assess the groups of people that are likely to consume the specific food and consequently, be exposed to the hazard. It may just be the general public or a specific group e.g. children. **Risk characterization**: The final step is to assess the likelihood of the hazard to have an adverse effect on our health. In doing so, the exposure levels are looked at against the safe exposure levels (EFSA, n.d.). There are different approaches that can be used; two of the most prominent for food-borne hazards are the no observed adverse effect level (NOAEL) and the benchmark dose (BMD) approach (EFSA, 2017a) (see section 3.7.3).

Chapter 2. Literature reviews

2.1. Scoping reviews

The initial overarching aim of the thesis was to examine whether there was enough evidence to build a causal relationship between one of the EECs in question and specific adverse human health effects. This was attempted, in the first instance, by the means of a set of ScRs on the EECs' health effects and the EECs' distribution in the environment. The ScRs were used to map the existing data and appraise their quality. The ScRs' findings were actively used to determine the route that the thesis took while they also informed key parts of the subsequent systematic reviews as detailed in Chapter 3. In the interest of brevity, only the ScR for the MPs' health effects and environmental distribution is reported herein in full. For the rest of the ScRs, a only a summary of the findings is reported.

2.1.1. Methods/ Design

As these were planned as scoping studies, limitations were adopted for the searches. All searches were executed on two databases: MEDLINE and the Web of Science Core Collection. The decision was made on the basis that the first one is medicine-orientated and the second one has a broader scientific discipline range. It is also in line with the time limitations set for the undertaking and concept of a ScR (Arksey and O'Malley, 2005). The timespan for the studies was set from 2008 until the time of the searches, which was October 2018 to January 2019. The timespan decision coincides with the "emerging" characteristic of the contaminants. The studies' publication was in the English language. The studies were evaluated against specific eligibility criteria (see below). The criteria for the ScRs were broad since scoping aims to map the existing literature and is therefore intentionally not focused in the way a systematic review would be (Armstrong et al., 2011). All available types of studies were included (primary and secondary), peer reviewed and not. There was no geographic, population nor outcome limitation. Finally, all possible routes of exposure were included for the health effects' ScRs and all sample type/ sampling methods for the distribution' ScRs.

The results of the searches were screened in two levels. In the first level, only titles and abstracts were taken into consideration. In the second level, the full test of the studies was assessed against the eligibility criteria of the ScRs. For each of the ScRs a spreadsheet was created to chart the extracted data from each of the included studies. The data extracted and tabulated differed depending on whether it was for health effects or distribution of MPs. Charting of the studies was used to enable easy comparison and reporting the results (Higgins and Green, 2011). The synthesis of the data was executed for each of the ScRs

separately. Synthesis focused on quality assessment and summarizing the available literature.

2.2. Emerging Environmental Contaminants (EECs) human health effects scoping reviews

2.2.1. Microplastics' human health effects scoping review

Out of the 3,206 studies returned by the searches, 20 were found to be relevant to be included in the review. The process is illustrated in a flow diagram (Figure 10) based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) methodology (Moher et al., 2009). Eight were primary studies and the rest (12) secondary. Out of the secondary studies one was not peer-reviewed (opinion article) and the remaining 11 were reviews but none of them fulfilled the criteria to be a systematic review. The full search strategy can be found in Appendix 1. a.

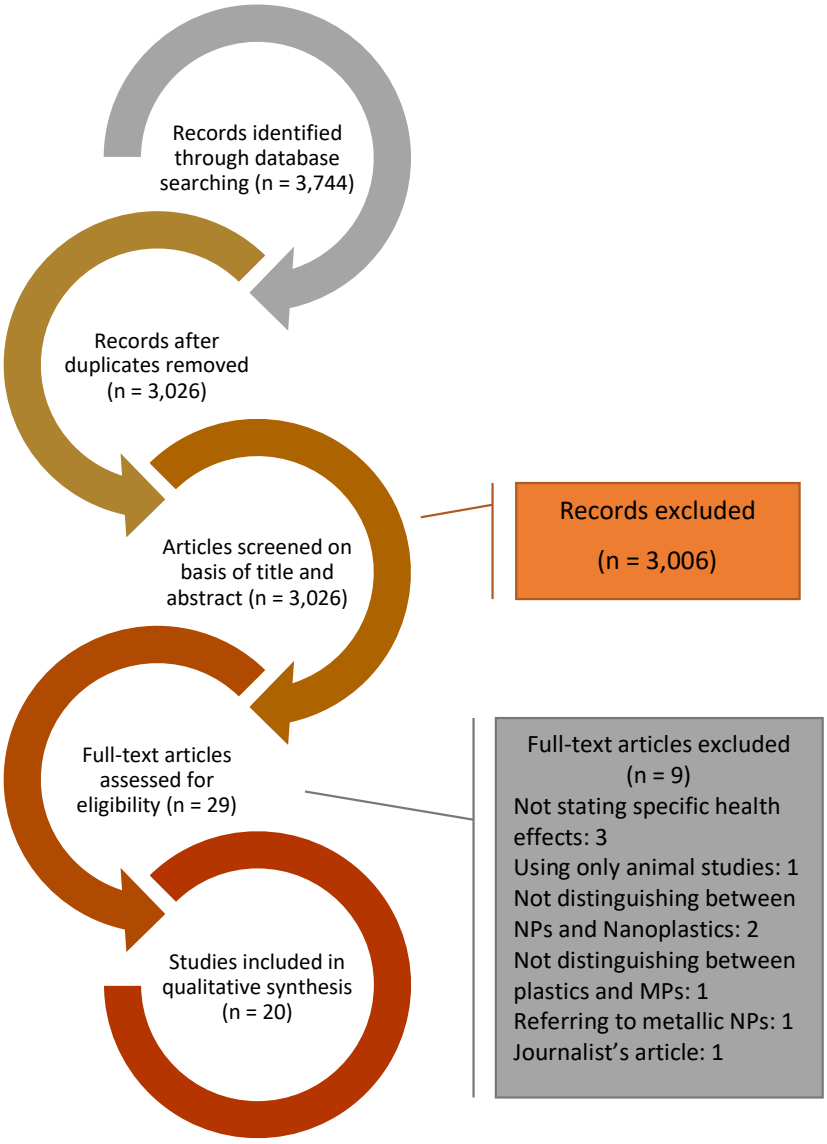


Figure 10. Flow diagram for the MP human health effects scoping review

2.2.1.1. Primary studies included in the scoping review

Human Cell studies: Three studies used human cell as the model. Their results were contradictory regarding the exerted toxicity and the underlying toxicity mechanism. A study by Schirinzi et al. (2017) on polyethylene (PE) and polystyrene (PS) MPs effects on cerebral and epithelial human cells concluded that oxidative stress was the probable mechanism for their toxicity but did not find a significant effect on cell viability. In contrast, the Mishra et al. (2018) study on polystyrene nano-spheres reported hazardous effects on human red blood cells (RBCs) and lymphocytes, mentioning specific genotoxic and cytotoxic effects. Cytotoxic effects included haemolytic effect via mechanical damage of RBCs at concentrations of 75 and 100 μg/mL. The genotoxic effects were mononucleation (48–62%), binucleation (14–20%), trinucleation (16–26%), and multinucleation (6–18%) of the lymphocytes after being treated with polystyrene nano-spheres' for 24 hours. A third study by Magrì et al. (2018) involved PET NPs that were manufactured in a lab using a laser ablation technique and were used to measure NP effects on human Caco-2 intestinal epithelial cells. They did not detect toxic effects in the short term, but they found that nano-PET could cross the gut barrier with a high propensity, thus raising concerns about the possible long-term effects and their potential to act as mediators for other pollutants. Unfortunately, they did not define what they meant by short term and long term. They measured the kinetic uptake profile for the plastic nanoparticles for up to 24 hours and their bio-persistence for up to two months. Caco-2 cytotoxicity was measured after 24, 72 and 96 hours of exposure while gut barrier crossing was measured at 1, 5 and 9 days of exposure. The studies used slightly different concentrations for their experiments ranging from 0.05 - 10 μg/mL (Schirinzi et al., 2017), 1 - 30 μg/mL (Magrì et al., 2018) and 50 - 100 μg/mL, (Mishra et al., 2018).

Mishra et al. (2018) did not justify why they chose the specific concentrations. Schirinzi et al. (2017) stated that they considered concentrations lower than the ones that are usually tested in research, "such as those that can be considered by incidental exposure" (2017: 580) without providing further explanations. Finally, Magri et al. (2018) stated that they used the concentrations that are typically used in nanotoxicological *in vitro* studies but did not reference them.

Appraisal of the studies indicated that their reporting was lacking in many aspects. Although the studies might have been of high quality, reporting failed to provide vital information on how key decisions were made on their protocol. Advanced synthesis of their results was not possible due to the heterogeneity of the data across studies. Research in this area was in the

very early stages, but consensus on key aspects must be achieved if the value and longevity of "vanguard" studies is to be protected.

Dust studies: Three studies focused on dust. Abbasi et al. (2018) conducted their study in Iran and reported that MPs contributed notably to the effects that urban and industrial dust had on health (morbidity and toxicity) by presenting oxidative potential. The samples came from two sites: one urban and one industrial, situated next to a major gas field. The urban site was in the city of Asaluyeh in southern Iran and the industrial site was in the nearby Pars Special Energy/Economic Zone (plants and refineries). The population of the city and the industrial site was about 75,000. A total of 31 samples were used, taken over 8-day periods. 15 samples were street dust and the rest (16) were suspended dust. The study identified both inhalation and ingestion as an uptake route but make no claims of specific health effects. Their results for the ingestion route were calculated using average and acute (short-term) exposure scenarios from relevant literature (Harris and Harper, 2004; Dehghani et al., 2017; US EPA, 2002 in Abbasi et al., 2018). The estimation of the inhalation route effects was attempted by evaluating the oxidative potential. The rationale around the choice of specific locations, the number of the samples or the periodicity of sampling is not provided. The lack of this information does not allow for evaluation of the relevance of their results in other settings and if their results could be used as a guide.

Wang et al (2017) focused their research on phthalic acid esters (PAEs) found in street dust in an urban environment (n=58 samples). The PAEs origin are plasticizers used for plastic enhancement (≈80%) and non-plasticizers for a wide range of consumer products. Ingestion and dermal absorption were found to be the main uptake routes; inhalation was also considered. The models to calculate daily intakes were based on the United States Environmental Protection Agency's (EPA) assessment guidance (EPA 1989, 1996, 2001 in Lijun Wang et al., 2017). The study concluded that the majority of the PAEs in street dust came from plasticizers. Neither non-cancer nor cancer risk was found to be increased by PAEs. The researchers acknowledged that both risks were underestimated due to lack of reference doses and cancer slope factors, respectively.

The third study used samples (n=410) from children's bedrooms in urban homes (n=332) and rural homes (n=78) from the areas of Tianjin and Cangzhou in China (Sun et al., 2017). The aim was to measure phthalates and their health effects. Di-(2-ethylhexyl) phthalate (DEHP) which was used in PVC products was one of the three major phthalates to be identified in the settled dust. The study found that the DEHP concentrations were almost 10 times higher in urban areas. An association was found between exposure to higher

concentrations of phthalates and health outcomes, namely asthma and allergy. The health status of the children in the study was assessed via questionnaires. There was no information on the type or the content of the questionnaire or how they were processed. Furthermore, the statistical analysis used for the results was not reported, so the nature of the association cannot be determined. With regard to the health effects the authors only provided two tables with ratios for 10 different respiratory and dermal symptoms and diagnoses for six substances. Unfortunately, the palpable lack of important methodological information of the study diminished its value.

Occupational study: The only cohort study included in the ScR was the update of an occupational exposure study by Gallagher et al. (2015) on female workers in the synthetic textile industry undertaken in Shanghai, China. The methods included questionnaires to attain their occupational history, followed by exposure assessments conducted by specialized personnel. The cohort ran from 1989 to 2006 following a sample of n=267,000 for cancer incidence. The first follow up of the cohort examined exposures for more than 10 years and did not find any associations (Wernli et al., 2006). This study was not included in the results because it did not fulfil the time span criterion. The aim of the second update was to examine the possible associations for extended duration of more than 20 years. The prevalence of cancer in the sample revealed that exposure to synthetic fibre dust for long durations (> 20 years) could increase stomach cancer risk. Specifically, hazard ratio (HR) for < 10 years exposure was 0.9, for 10-20 years 1.1 and for > 20 years 1.2 (1.1 – 1.4 for CI 95%). The study did not provide concentrations for the exposures to synthetic fibre, as these were not available from the start of the cohort in 1989. This is recognized by the authors as a limitation of the study.

Food study: The last of the primary studies included in the ScR looked at the presence of MPs in commercially available salts intended for human consumption in 8 different countries (Karami et al., 2017a). The study claimed that the concentrations of the detected MPs taken together with the global daily sodium consumption illustrate that the health impacts would be negligible. The possible health effects that were taken into consideration were “micro injuries” physically caused by MPs and toxicity by absorbed persistent organic pollutants. Assumptions and extrapolation were made from relevant literature. The estimates took into account only MPs with a size > 149 μm due to technical limitations. The authors recognize that this is a limitation of the study.

2.2.1.2. Secondary studies included in the scoping review

Eleven reviews were included in the ScR. The quality of the reporting was identified as poor. None of the reviews provided the methodology that was used, the number of studies nor the number of the samples included. Only one review provided a search strategy and described the resources that were used (Smith et al., 2018). The studies in this part of the ScR are reviewed according to their focus on an uptake or exposure route.

All uptake/exposure routes: Wright and Kelly (2017) categorised the health effects into physical and chemical. Chemical effects are subsequently divided into the ones caused by endogenous chemicals of the MPs and exogenous chemicals. The physical effects included "inflammation, genotoxicity, oxidative stress, apoptosis, and necrosis" (2017: 6640), which in turn could lead to "tissue damage, fibrosis and carcinogenesis" (2017: 6640). The exogenous chemicals often involve priority pollutants. Particle toxicity was proposed to be explained through the oxidative stress paradigm on the assumption that all plastics enclose reactive oxygen species. The example of wear particles coming from prosthetic implants was used to illustrate non-immunological effects of MPs (Willert et al., 1996, Urban et al., 2000 in Wright and Kelly, 2017, Doorn et al., 1996). The corona that can be formed on MPs was also mentioned in relation to its ability to influence particle uptake and toxicity (Evans et al., 2002, Lundqvist et al., 2008 in Wright and Kelly, 2017).

Regarding the GI tract and the air tracts, Wright and Kelly (2017) stated that the desorption rates of exogenous chemicals from MPs to the human body was expected to be enhanced compared to sea water desorption. Desorption, as the opposite of absorption, refers to the rate that the chemicals would leach out of the MPs. This means that transfer of the exogenous chemicals from MPs to the human body could be more readily available and more potent than to sea water. They went on to raise the question on the potential overall contribution to the bioaccumulation of priority pollutants in the human body. Concerning the Microbiome, Wright and Kelly (2017) noted that biofilms created on the surface of MPs could include harmful human pathogens. They argued that the presence of MPs in the GI tract and in human air tracts would alter the local conditions thus affecting the immune responses with unknown results. The review provided no evidence on concentrations, exposure limits or time limits with regard to the health effects. The authors made a number of human health claims based on research done on fish, animals and the environment in general. In many cases they did not make it clear when they were citing animal and environmental studies and made extrapolations to humans without providing a justification of rationale. The review was very ambitious in attempting to combine research from different disciplines.

Nevertheless, the heterogeneity of the subjects taken together with the absence of reporting on the methodology used for the review diminish its quality and usefulness.

The non-peer reviewed opinion paper by Vethaak and Leslie (2016) drew from the GESAMP (2015b) report and the work by Galloway (2015) to state that lung and gut injury, cell damage, chemical bioavailability enhancement and infection by pathogens were possible health effects. They stated that their claims are based on human cell studies and animal models but specific evidence was not provided. Galloway (2015), although relevant, was not included in the results of the present ScR as it is a chapter of a book.

The review by Karbalaei et al (2018) argued that the potential health effects came from the endogenous and the exogenous chemicals sorbed by the MPs. They stated that health effects such as potential carcinogenicity and reproductive abnormalities have been linked to specific plastic polymers (PET, PS, and PVC) and made a "logical" leap from plastics and MPs, but this was not backed up by evidence. The authors also reported on the MPs' attributes as pathogen and parasite vectors citing the aforementioned review by Vethaak and Leslie (2016). They also referred to three occupational studies; two on nylon flock workers (Boag et al., 1999, Eschenbacher et al., 1999) and a questionnaire-based study on 3-D printer users (Chan et al., 2017), reporting respiratory symptoms and interstitial lung disease. Finally, they made a case for the anticipated health effects coming from BPA, which is a plasticizer. These reported alterations in liver, reproductive and brain function effects as well as obesity and cardiovascular disease were based on a review paper on the use of plastic products (Srivastava and Godara, 2013) which could not be accessed nor read as it is not written in English. Karbalaei et al (2018) failed to connect BPA health effects to evidence related to MPs. The paper extrapolated or rather, interpolated from plastics and did not take into consideration the difference in scales.

A review of the impact of NPs by da Costa et al (2016) argued that the potential effects could be attributed to the ability of NPs to cross biological barriers, such as cell membranes, but also to their morphology which may induce the accumulation and the amplification of other pollutants. The specific effects mentioned in this review were limited to the possible effects coming from the absorbed chemicals such as Polychlorinated biphenyls (PCBs) and Polybrominated diphenyl ethers (PBDEs) which are linked to reproductive disorders. The paper also looked at three studies that focus on cell and cell membrane interaction with NPs reporting adverse effects to cell function and viability.

A short review paper by Sharma and Chatterjee (2017) stated that the prolonged use of personal care products that include MPs will ultimately cause skin damage. Furthermore, taking a rather free interpretation of the first GESAMP report (2015b) they argue that the ingestion of MPs "can cause alteration in chromosomes which lead to infertility, obesity and cancer. In case of women, estrogenic mimicking chemicals can cause breast cancer." (2017: 21542). The GESAMP report makes no such clear causal associations.

A discussion piece by Gallo et al (2018) focused on marine MPs. They reported a variety of health effects: "DNA damage, changes in gene and protein expression, cell clotting, necrosis, apoptosis, proliferation and loss of cell viability, oxidative stress, increased Ca ions, inflammation and bone osteolysis, to lesions in organs" (Gallo et al., 2018: 7). All of the effects are based on the report by Lusher et al. (2017) for the Food and Agriculture Organization of the United Nations on "Microplastics in fisheries and aquaculture". They are all drawn from a single table in the report (Table 1) and refer to medical literature regarding MPs and NPs from inhalation or surgical plastic material. The literature used to create this table spans from 1994 to 2011 and is comprised of 17 papers (Table 2). The table in the report categorises the effects according to the size and the type of the plastic which is not mentioned in the Gallo et al (2018) paper. Nevertheless Lusher et al. (2017) concluded that the risk of toxicity via the oral uptake route could not be evaluated due to lack of experimental data.

Table 1. Details of MPs and test models used in the Lusher et al. (2017: Table 6.2) report.

Medical literature on impact of microplastics and nanoplastics originating from inhalation and surgical materials at various levels of biological organization

Level of biological organization	Particle type and size	Effect	Reference
Macromolecules	PE 100 nm–30 µm PS 50 nm–4.7 µm PMMA 1 µm–2 µm PC 1 µm–55 µm	DNA damage, changes in gene and protein expression	Gelb *et al.*, 1994; Brown *et al.*, 2001; DeHeer *et al.*, 2001; Gretzer *et al.*, 2002; Petit *et al.*, 2002; Ingram *et al.*, 2004; Clohisy *et al.*, 2006; Kaufman *et al.*, 2008; Markel *et al.*, 2009; Huang *et al.*, 2010; Hallab *et al.*, 2012; McGuinness *et al.*, 2011; Samuelsen *et al.*, 2009; Smith and Hallab 2010; Pearl *et al.*, 2011
Organelles*	PMMA 10 µm	more micronuclei	Zhang *et al.*, 2008
Cells	PS 20 nm–4.7 µm PE 300 nm–10 µm PMMA 2 µm–35 µm PS 20 nm–200 nm PS 60 nm–200 nm	cell clotting, necrosis, apoptosis, proliferation and loss of cell viability Oxidative stress Increased Ca ions	Gelb *et al.*, 1994; Brown *et al.*, 2001; Gretzer *et al.*, 2002; Bernard *et al.*, 2007; Fröhlich *et al.*, 2009; Samuelsen *et al.*, 2009; Hallab *et al.*, 2012; McGuinness *et al.*, 2011
Tissues	PE 600 nm–21 µ, PMMA 1 µm–35 µm	inflammation and bone osteolysis	Gelb *et al.*, 1994; Clohisy *et al.*, 2006; Markel *et al.*, 2009; Pearl *et al.*, 2011
Organs	PMMA 1 µm–10 µm	lesions	Zhang *et al.*, 2008; Pearl *et al.*, 2011

*An organelle is a specialized subunit within a cell (e.g. mitochondria) with a specific function.
PE (Polyethylene), PS (Polystyrene), PMMA (Poly(methyl methacrylate)), PC (Polycarbonate).

Finally the review by Kole et al. (2017) reported on health effects related to the wear and tear of tyres. Health effects were categorised according to the uptake route of inhalation and ingestion. The inhalation effects were the human respiratory symptoms mentioned by Wright and Kelly (2017), namely: cardiac oxidative stress, cell toxicity, acute respiratory responses. The stated effects from ingestion were potential toxic effects and local inflammatory effects. The study concluded that the wear and tear of tyres may contribute to the global burden on health caused by particulate matter as described by WHO. In order to reach this conclusion, they used national estimates for the volume of wear and tear of tyres from eight countries. Kole et al. (2017) recognized that there were no relevant studies on wear and tear of tyres. They used an analogy to micro- and nano- particles to justify the inhalation effects and animal studies for the ingestion effects.

Table 2. Further characteristics of the studies included in Table 1.

Study	**MPs**	**Model**
Gelb et al. (1994)	polymethylmethacrylate (PMMA) particulate debris	subcutaneous rat air-pouch model
Brown et al. (2001)	ultrafine polystyrene particles	female Sprague–Dawley rats
Gretzer et al. (2002)	polystyrene particles (PS; 105/mL)	human monocytes
Petit et al. (2002)	polyethylene particles	J774 macrophages
Ingram et al. (2004)	polyethylene wear particles	murine macrophages from C3H/hej mice
Clohisy et al. (2006)	spherical PMMA particles	mice
Kaufman et al. (2008)	ultra-high molecular weight polyethylene	primary human macrophages
Markel et al. (2009)	ultra-high molecular weight polyethylene	gene production and inflammatory osteolysis in a mouse model.
Z. Huang et al. (2010)	ultra-high molecular weight polyethylene	human macrophages (THP-1) and human mesenchymal stem cells (MSCs)
Hallab et al. (2012)	ultra-high molecular weight polyethylene, polyetherether-ketone	differentiated human macrophages (THP-1; ATCC, Rockville, MD) and primary human monocytes
McGuinnes et al. (2011)	polystyrene latex nanoparticles	human blood and platelets
Samuelsen et al. (2009)	polystyrene particles	female Balb/*c*A mice
Smith and Hallab (2010)	polycarbonate-urethane (PCU), ultra-high molecular weight polyethylene	human monocyte cell line (THP-1)

Pearl et al. (2011)	polymethylmethacrylate (PMMA) particles	macrophage cell line RAW 264.7 from BALB/c mice
Zhang et al. (2008)	polymethylmethacrylate (PMMA) particles	osteoclastogenic bone marrow cultures from C57BL/6J mice
Bernard et al. (2007)	ultrahigh-molecular-weight polyethylene (UHMWPE) wear particles	human neutrophils
Fröhlich et al. (2009)	carboxyl polystyrene nanoparticles	human endothelial cell line EAhy926

Dietary uptake: Four reviews focus on the uptake of MPs through the food chain. Bouwmeester et al (2015) drew from nanotechnology to reported that although effects on the immune system and the barrier capacity of the GI tract are expected the overall impact could not, at that time, be evaluated. Waring et al (2018) cited the effects on human cells observed in the Schirinzi et al (2017) study while also citing some of the work included in the aforementioned review by Wright and Kelly (2017). The review by Smith et al. (2018) categorised the effects as physical and chemical. They also cited Wright and Kelly's (2017) stated effects. Drawing from mammalian modelling research, they reported effects related to cell viability and the immune system (Hussain et al., 2001). They also used, as evidence to support their conclusions, the review by Lusher et al. (2017) and the second GESAMP (2016) report on cell toxicity. Regarding oral exposure to NPs, the review reports that their effects include "cardiopulmonary responses, alterations of endogenous metabolites, genotoxicity, inflammatory responses, oxidative stress, effects on nutrient absorption, gut microflora, and reproduction" (2018: 381).

Inhalation uptake: Two studies focused on the respiratory uptake route. The review by Prata (2018) focused on airborne MPs and their effects. The conclusions were drawn from occupational studies in the synthetic textile, flock, Vinyl chloride (VC) and PVC industries. The reported effects were: respiratory symptoms, increased cancer risk in relation to synthetic fibre dust, PVC and VC exposure, interstitial lung disease, flock's disease, restrictive lung disease and undifferentiated airway and interstitial lung disease. The review used studies that were published over a very wide time period going as far back as 1975 using almost "historic" studies. The review by Sauler and Gulati (2012) also drew from occupational studies in the nylon flock industries reporting chronic respiratory symptoms, pulmonary disease and interstitial lung disease. Unfortunately, both reviews did not provide a lot of detail around the occupational exposures; specific substances, concentrations, time of exposure, work specifications, etc. Therefore, the reviews cannot be used to draw safe

conclusions around occupational effects. The overall quality of the primary studies and the reviews included in this ScR was poor; regarding the primary studies, recurring issues include poor reporting on methodology and lab protocols and justification for the concentrations used.

2.2.2. 3-D printer dust health effects scoping review

Out of the 994 studies 21 were potentially eligible based on title and abstract screening. The full search strategy can be found in Appendix 1. b. The selected studies were downloaded for further review. Six of them were finally included (Figure 11). The inclusion criterion for this ScR was that the study made claims for specific health effects deriving from exposure to 3D printer emitted particles or nanoparticles. Five were primary studies and one secondary. None of the studies refer to "3D printer dust", but to particles, nanoparticles and ultra-fine particles emitted from 3D printers. Furthermore, there is no categorization between physical and chemical effects. All the 3D printers reported in the studies are using Fused Deposition Modelling (FDM) and Fused Filament Fabrication (FFF) technology.

Summary: Six studies were included in this ScR: five primary and one secondary. The study by Guemperlein et al (2018) was the only one that used laboratory tests as well as self-reported symptoms to support the claimed health effects. They found no significant acute health effects for a short time exposure of one hour. Chan et al (2018) looked at occupational exposure and reported a high percentage of the participants (59%) experiencing respiratory symptoms at least once a week in a duration of a year. An association was also discovered between working more than 40 hours per week in a 3D printer environment and developing asthma or allergic rhinitis. A case report by House et al (2017) also claimed an asthma effect. Two studies used their primary findings on emission rates to estimate exposures and predict health outcomes. Kim et al (2015) stated that 3D printing emissions can be harmful. They reported the toxicity of the nanoparticles but did not mention specific health effects caused by them. Azimi et al. (2016) estimated that the ultrafine particles (UFP) and volatile organic compounds (VOCs) measured emissions did present implications for human health. Azimi et al. (2017) also undertook a secondary data analysis of their previous data to model emission scenarios and time-varying concentrations by the use of modelling software and argued that the expected health effects would be similar to the ones attributed to outdoor UFPs.

Research in this area was found to be in the very early stages. A major issue was that in some of the studies a clear distinction between the different emissions and subsequent evoked

effects coming from the operation of 3D printers was not highlighted. Another issue was that the studies did not provide information on the chemical composition of the filaments they used or the size range of the resin.

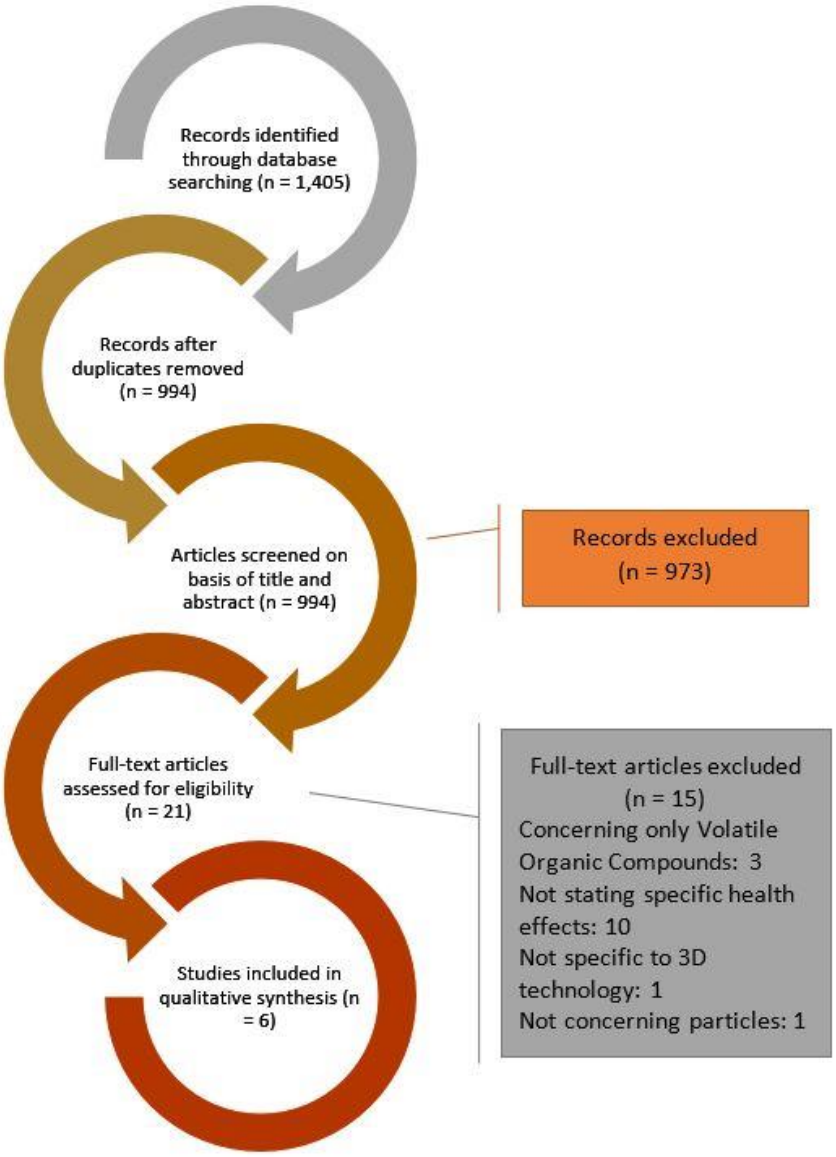


Figure 11. Flow diagram 3D printer dust's health effects scoping review

2.2.3. Estrogens' human health effects scoping review

After title screening of 4,149 studies 56 went on to the next phase of the ScR. The full search strategy can be found in Appendix 1 c. The articles were downloaded, and all the content was screened. After abstract and full-text review, 33 studies were included. There were no duplicates because Web of Science interface offers the option to search multiple databases at the same time, in this case the Web of Science Core Collection and MEDLINE, and the interface automatically omits duplicates.

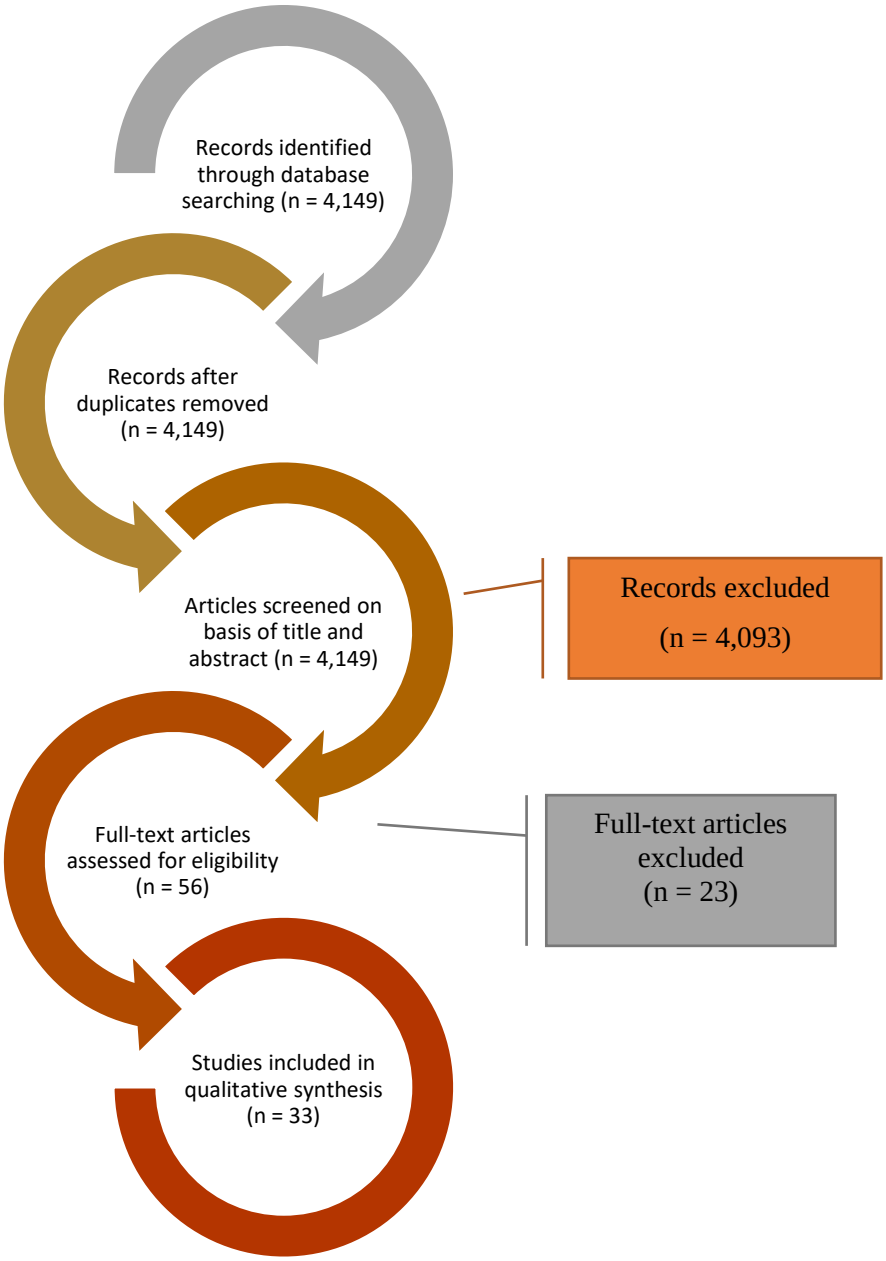


Figure 12. Flow diagram for the estrogens' health effects scoping review

There were 12 primary studies, 19 reviews, of which one was a systematic review, and two editorial articles. Two of the primary studies come from Canada, one from China, one from USA, one from Japan, one from Iran and the remaining six from Europe. The reviews included studies from all over the world. The process is illustrated in a PRISMA flow chart (see Figure 12). The papers were firstly categorized and reviewed according to the study type (primary, systematic review, and reviews) and consequently according to the compound/s they focused on. Due to the large number of included compounds, it was decided that this was the best way of illustrating and assessing the literature.

Summary: Starting with the four primary studies on BPA, Dominguez et al. (2008) reported on ovarian function effects but only in very high doses. Bouskine et al. (2009) found that

BPA at low doses could "interfere with the developmental programming of fetal germ cell proliferation and/or differentiation when they cross the placenta" (2009: 1053). Zhang et al. (2017) reported that BPA in low doses promoted growth thus stimulating proliferation of cancer cells. The concentration they used was much higher than Bouskine et al. (2009) and they might not be environmentally relevant. Finally, Andra et al. (2015) found a preliminary association between human exposure to monochlorinated BPA and the development of type II diabetes mellitus.

There are four primary studies focusing on PCBs. Brucker-Davies et al. (2010) reported mild effects on the health of infants, connected to delivery and neo-natal growth. Gallo et al. (2016) found that lighter and lower chlorinated PCBs, are associated with "increasing the probability of not ovulating in women with known exposure" (2016: 416) thus impairing reproductive function. Felty et al. (2010) stated that exposure to environmentally relevant levels of PCBs could induce the mechanisms of inflammation and adhesion thus affecting endothelial cell dysfunction leading to pulmonary vascular lesions. Andersson et al. (2011) found that PCB126 could affect hypertension as it could cause dysfunction to the human endothelial cells that are related to hypertension. The PCBs' studies focused on different compounds thus making comparison between them not appropriate. Felty et al. (2010) and Andersson et al. (2011) studies both looked at PCB126 but in relation to different effects.

The remaining four primary studies focused on endocrine disrupting chemicals (EDCs) and xenoestrogens. Gaspari et al. (2011) looked at EDCs; they did not specify which compound/s they focused on. They stated that partial androgen insensitivity syndrome (PAIS) might be related to prenatal xenoestrogens exposure. Bidgoli et al. (2011) reported that exposure to xenoestrogens could affect the expression of the aryl hydrocarbon receptor (AhR) which in turn leads to the development of "premenopausal breast cancer in Iranian women" (2011: 2429). Teixeira et al. (2015) found significant associations between xenoestrogen levels and metabolic abnormalities concluding that the presence of xenoestrogens in the plasma of premenopausal women could be "a predictor of 10-year cardiovascular disease risk" (2015: 1792). Suzuki et al. (2012) found a significant negative correlation between the levels of MEHP in maternal urine and the anogenital index AGI 1, meaning that prenatal environmental exposure to DEHP could negatively affect the development of the human reproductive system of males. The reporting in all the primary studies is detailed at large.

The systematic review by Kay et al. (2013) focused on phthalate esters, female development and the reproductive system. The review looked at both human and animal studies. They concluded that evidence did not exist on associations between phthalates and endometriosis,

breast cancer and effects on puberty, however "the epidemiological literature supports a weak potential relationship between phthalate exposure and subfertility, pregnancy loss, pre-term birth and decreased birth weight that merits further investigation" (Kay et al., 2013: 215).

There were five reviews on BPA included in the ScR. Ben-Jonathan et al. (2009) stated that BPA might be the most important endocrine disruptor contaminant that affects metabolism worldwide. Similarly, Vom Saal et al. (2012) concluded that developmental exposure to BPA can contribute to becoming obese later in life. Bloom et al. (2016) looked at BPA's relation to ovarian steroidogenesis but concluded that the available literature did not provide enough evidence for a conclusive risk assessment. Leonardi et al. (2017) stated that BPA could affect female patients with precocious puberty as well as premature thelarche. Wang et al. (2017) looked at the carcinogenic effect of BPA and argued that BPA should be characterised as a carcinogen due to its estrogenic and non-estrogenic activities that both accelerate the development of breast cancer.

There were seven reviews that looked at EDCs. Sikka and Wang (2008) argued that the real extent of the EDCs' effects on human were questionable. Similarly, Caserta et al. (2008) stated that the available data were not sufficient to support a causal relationship between exposure to EDCs and effects to the female reproductive system but enough to warrant further studies. On the other hand Hauser et al. (2015) found low epidemiological but high toxicological evidence linking exposure to phthalate and male infertility as well as prenatal exposure to PBDEs and cryptorchidism. They also reported a modest association between exposure to PBDEs and testicular cancer as well as an association between exposure to phthalates and lower T concentrations, concluding that EDCs had a substantial contribution to the overall male disorders and diseases. The review/executive summary for "the Endocrine Society's Second Scientific Statement on Endocrine-Disrupting Chemicals" (EDC-2) by Gore et al. (2015: 593) reported on a wide range of human health effects. EDCs were reported to be obesogens and diabetogens and possibly cardiovascular disruptors; both male and female reproductive systems were found to be vulnerable to EDCs; several types of cancers were hypothesised to be linked to EDC environmental exposures; EDCs had effects on prostate cancers; EDCs could disrupt thyroid function; exposure to EDCs was linked to neurodevelopmental and neurobehavioral issues for long term exposures. The Dogan and Simsek (2016) review stated that the mechanisms for the EDCs' effects were more complicated than what has so far been recorded and as such the association between them and cancers could not easily be established. Gibson and Saunders (2014) stated that a causal link between exposure to EDCs and cancer had not been established yet, but EDC

exposure in utero could have an effect on the birth weight and metabolism which in turn gave rise to risk of endometrial cancer. Hu et al. (2012) stated that exposure to estrogens and EDCs during early life gave rise to prostate cancer vulnerability in later life. Maqbool et al. (2016) stated that EDCs had reproductive and developmental effects, carcinogenicity, thyroid system effects, obesity/ diabetes, cardiovascular system effects and nervous system effects. They noted that the most prominent effects are observed in the nervous system while they also had effects on all the above areas.

Two reviews focused on environmental estrogens. Newbold et al. (2009) found that exposure to estrogenic chemicals during early development had effects on weight gain and obesity. Cruz et al. (2014) stated that exposure to environmental estrogens early in development could alter the epigenetic programming thus affecting the development of ovaries and their function. A review by Adeel et al. (2017) examined the fate and effects of mammalian estrogens (estrone, E2 and estriol). The review stated that estrogens had been proven to promote cardiovascular diseases and raised the risk for cancer and prostate cancer. It was concluded that human health was indeed disrupted by estrogens, that there was a link between estrogens and breast cancer for specific populations and that they should be classified as toxic organic pollutants. There are two reviews that focused on both natural and synthetic compounds. Bonds and Midoro-Horiuti (2013) stated that estrogens influence the immune cells and played a role in asthma by affecting airway mechanics and inflammation in the lungs. Liu and Sun (2018) stated that regarding DHEA, there might be a link to insulin sensitivity in females whereas, regarding E2 the evidence from epidemiological studies were contradictory.

The reviews were poorly reported. Only one of the reviews provided information on how many studies are included and how they were identified and selected (search strategy and inclusion/exclusion criteria), and none if their quality was somehow assessed. In addition, a lot of the reviews did not mention which specific compound they are referring to but rather use more generic terms such as EDCs. The papers that did, unfortunately did not provide specific exposures and in some cases not even uptake routes. In addition, a lot of the papers made claims or disputed claims on human health effects by using both epidemiological and animal studies, and in some cases only animal studies without acknowledging the obvious limitations or even the fact that they are basing their conclusions on animal studies. There are two editorial articles included in the ScR. One focused on male reproductive health (Handelsman and Cooper, 2013) and one on breast cancer (Darbre and Fernandez, 2013). Handelsman and Cooper (2013) did not provide a clear conclusion but urged readers to be highly critical of the research mentioning a wide range of issues: small sample sizes,

introduction of bias, unavailability of semen samples from the general population, lack of evidence to connect estrogenic pollution to falling sperm counts etc. Darbre and Fernandez (2013) argued that although various environmental estrogens had been examined in relation to breast cancer they are often seen in isolation while the reality is that humans are exposed to a variety of these compounds throughout their lives.

2.3. Microplastics environmental scoping reviews

The ScR on the environmental distribution of MPs began as one review but eventually it became clear that it was imperative to divide it into two separate but interconnected ones. The overall full search strategy can be found in Appendix 1. d. The aim of the ScRs was to identify primary studies which provided data on the existence of MPs in the environment in a setting that humans may be exposed to them. The first ScR focuses on MPs in food for human consumption; the second focuses of MPs in the environment.

Although the first three ScRs, on the health effects of the three EECs, included both primary and secondary (reviews) studies, in these ScRs only primary studies are included. This decision was made based on the aforementioned aim. Additional inclusion/exclusion criteria for these reviews were that specific MPs' identification methods have to be employed. Recent consensus around the study of MPs supports that mere observation of particles with the naked eye or through the use of a conventional microscope is not accurate and can lead to under or over-reporting (Rocha-Santos and Duarte, 2015, Strungaru et al., 2019, Shaoliang Zhang et al., 2019). For example, in a MPs case study by Bergmann (2015), only 1.4% of the particles that had been evaluated as possible MPs by visual observation were later verified as MPs by Fourier-transform infrared spectroscopy (FT-IR). The use of an additional technique is therefore imperative to verify observation and to identify particles that are too small to be classified by observation characteristics alone (Mai et al., 2018, Elert et al., 2017). The analytical techniques that are accepted for these ScRs are: Fourier-transform infrared spectroscopy (FT-IR), Raman spectroscopy (RM), pyrolysis gas chromatography/ mass spectrometry (Pyr-GC-MS) and scanning electron microscopy plus energy-dispersive X-ray spectroscopy (SEM/EDS). All of them are able to identify the polymer type thus identifying a particle as of plastic origin or not. A short description of the techniques permissible within the eligibility criteria is provided in section 2.4, to elaborate on why they are widely accepted and to highlight their main differences.

2.3.1. MPs in food for human consumption scoping review

For the MPs in food ScR, additional eligibility criteria were adopted. These exclusion/inclusion criteria contour the purpose of this ScR which was to map studies that provide evidence for human exposure to MPs via the ingestion route. There are a number of studies that look at the existence of MPs in sea life for various species and also in various parts of their body. For example, there is extensive research of the presence of MPs in fish's GI tract; most often in the stomach (e.g. Baalkhuyur et al., 2018, Brate et al., 2016, Alomar et al., 2017 etc.). A series of decisions were made to address the heterogeneity of the studies. First, only commercially relevant species of sea life were to be included (seafood); second if a study focused on the GI tract of a type of seafood, it would only be included if the species of the seafood is small and usually eaten whole with the GI tract intact e.g. anchovies (*Engraulidae*). It should be highlighted that a lot of the included studies did not aim to research human exposures, nevertheless they provide important relevant data.

The development of the eligibility criteria for the ScRs guided the criteria for the subsequent systematic reviews in the same topic. The search strategy that was used was common for both the environmental and food ScRs and produced 3,280 results. After the title and abstract screening, 72 studies were downloaded for full text assessment resulting to 25 of them being included in the ScR (see Figure 13). The results in the studies are presented in different metrics according to the sample in question. Most studies referred to number of MPs per individual or weight, usually g, while some just provided the percentage of the samples positive for MPs presence. The key data that were extracted from the included studies can be seen in Table 3.

All studies have common, shared steps in their methods which are: acquiring the samples; using a technique to treat the samples in order to extract the particles (e.g. digestion); visually examine the particles (with or without a microscope); examine the particles (all or part of them) using one accepted MPs identification method. Each of the steps must be considered in order to appraise and compare the studies. It should also be noted that all studies included additional procedures according to their aims and objectives. These were size measurements of the particles, categorization according to shape, colour of form, comparison to other environmental samples (e.g. sediments) etc.

2.3.1.1. Sampling

The focus was both on the sampling technique and the sampling location. Sampling differed depending on the sample (live samples, ready to eat products etc.). The choice of the

sampling location depended on what the research's aims were. For example, one of Li J. et al. (2016) aims' was to establish a difference in mussel (*Mytilus edulis*) contamination according to the general contamination of the areas they come from, accordingly, they chose areas known to be slightly or highly contaminated. For the purposes of this review, sampling locations and the rationale for their selection, mattered in the sense of them being random, targeted or even representative of their respective environments.

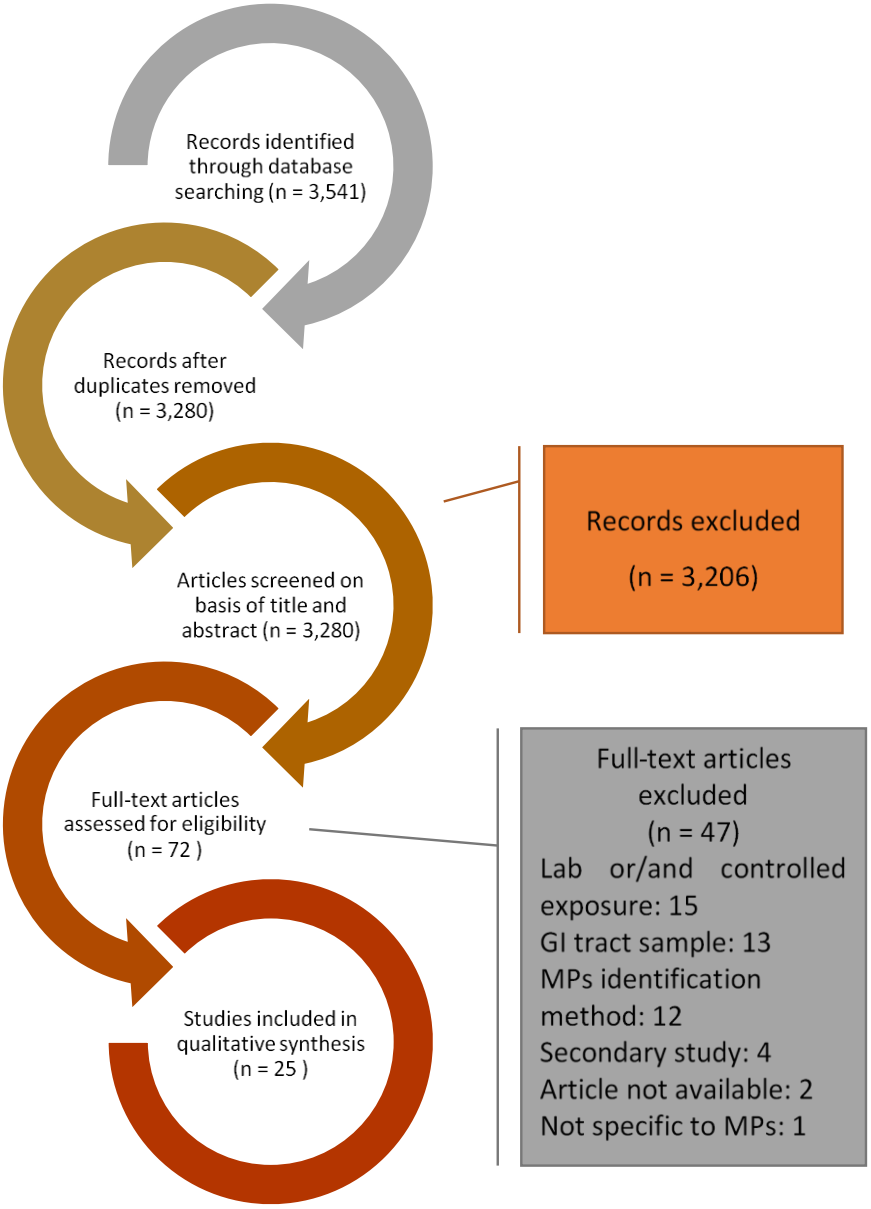


Figure 13. Flow diagram for MP in food for human consumption scoping review.

In the case of seafood, for many studies the exact location of where the samples were initially caught or collected is not known. This can be attributed to the type of the sample, as in the case of canned fish (Karami et al., 2018) or to the design of the study, as in the case of the study by Li J. et al. (2015) which was looking at the contamination of commercial bivalves,

not connected to their origin but the end consumers. It can also be due to poor reporting as in the case of the honey study by Muhlschlegel et al. (2017) which did not provide information of how they acquired their samples. Review of the data led to the conclusion that, at this point, they cannot safely be used to produce indexes on MPs content by location.

2.3.1.2. Microplastics extraction procedure

The importance of the extraction procedure lies in the effectiveness of the technique as well as its potential to have destructive effects on the MPs thus affecting the results (Munno et al., 2018). There are several details around these procedures that should be pointed out. First, the heterogeneity between the samples needs to be acknowledged. It is not reasonable that the same technique can be used for different samples. In addition, since this was a rather new area there was no consensus on the preferred technique for each of the samples. In the case of shrimps, Bour et al. (2018) used the technique developed by Avio et al. (2015b) for fish tissue with some modifications, Carreras-Colom et al. (2018) preferred the technique by Castejón et al. (2015) for crabs (*Brachyura*), while Bordbar et al. (2018) didn't report on their extraction procedure.

In the case of mussels and bivalves in general every study apart from one exception used a different extraction technique for their samples. Jinfeng Ding et al. (2019) and Li J. et al. (2018) developed their own techniques which they used in their studies with small modifications. The rest of the studies used a heterogeneous mix of techniques. Brate et al. (2018) used a variation of the technique by Dehaut et al. (2016) developed for seafood, Digka et al. (2018) employed the technique by Mathalon and Hill (2014) for *M. edulis* (mussels), Khoironi et al. (2018) used a procedure based on based on Li J. et al. (2016) also for mussels; Li H. X. et al. (2018) (Li Heng-Xiang) used the technique by Li J. et al. (2015) (Li Jiana) for bivalves; Van Cauwenberghe and Janssen (2014) used a variation of the Claessens et al. (2013) method for *M. edulis* while Teng et al. (2019) used the Munno et al. (2018) for organic matrices.

Regarding the salt samples Yang et al. (2015) was the first to develop a method. Their technique was later used by Iniguez et al. (2017) and Seth and Shriwastav (2018). Kim et al. (2018) also used their method but combined it with the slight variation developed by Iniguez et al. (2017), as did Gundogdu (2018) who combined it with the method developed by Karami et al. (2017a). Finally, the Karami et al. (2017a) method was based on their previous method developed for fish (Karami et al., 2017b).

Table 3. MPs in food studies scoping review data and characteristics

Study (location)	**Sample**	**Sample**	**MPs extraction procedure**	**identification method** (% of the specimen)	**MPs positively identified**	**Procedural blank samples**	**Findings**
Gundogdu (2018), Turkey	Salt	Table salt (sea salt n=5, lake salt n=6, rock salt n=5)	Yang et al. (2015) and Karami et al. (2017a)	Observation and RM (% not specified)	Not Specified	Yes	MPs content is sea salt was 46 ± 12.6 item/kg, in lake salt 37.5 ± 14.1 item/kg and in rock salt 11.8 ± 1.2 item/kg.
Iniguez et al. (2017), Product of Spain		Table salt (sea salt n=16, well salt n =5)	Their own procedure based on Yang et al. (2015)	Observation and FT-IR spectroscopy (% not specified)	7% not identified	Yes	MPs content was 50–280 MPs/kg salt. Well salt samples ranging from 115 to 185 particles/ kg and sea salt samples from 50 to 280 particles/ kg.
Karami et al. (2017a), Product of Australia, France, Iran, Japan, Malaysia, New Zealand, Portugal, and South Africa		Table salt (sea salt n=14, lake salt n=2, unidentified n=1)	Their own procedure based on Karami et al. (2017b)	Observation and RM (100%, 72 particles)	41.6% plastic polymers	Yes	MPs content per salt sample ranged from 0 to 10 MPs/ kg.

Kim et al. (2018), Product of China, Korea, Thailand, Philippines, India, Vietnam, Indonesia, France, Italy, UK, Australia, Germany, Bulgaria, Belarus, Romania, Croatia, USA, Brazil, Pakistan, Senegal		Table salt (sea salt n=28, rock salt n=9 and lake salt n=2)	Variation of Yang et al. (2015) and Iniguez et al. (2017).	Observation and FT-IR spectroscopy (100%, 10,723 particles)	91% synthetic MPs	Yes	MPs content in sea salts was 0−13 629 MPs/kg (average = 675 ± 2560), in rock salt 0−148 MPs/kg (average = 38 ± 55); and in lake salt 28−462 MPs/kg (average = 245 ± 307).
Seth and Shriwastav (2018), India		Table sea salt N=8	Variation of Yang et al. (2015)	Observation, FT-IR spectroscopy (% not specified)	Not specified	Yes	MPs content ranged from 103 ± 39 to 56 ± 49 MPs/kg of salt. Their total mass concentration was also estimated as 63.76 µg/kg of salt.
Yang et al. (2015), China		Table salts N=15 (brands) (sea, lake and rock/well)	Their own procedure	Observation, FT-IR spectroscopy (% not specified)	84.9% MPs	Yes	MPs content was 550−681 particles/kg in sea salts, 43−364 particles/kg in lake salts, and 7−204 particles/kg in rock/well salts.

Brate et al. (2018), Norway	Bivalve molluscs	Mussels *Mytilus spp (M. edulis, M. trossulus* and *M. galloprovincia -lis*) N=332	Variation of Dehaut et al. (2016)	Observation and FT-IR spectroscopy (25%, 224 of 894)	94%	Yes	Mussels contain MPs, with an overall average of 1.5 (±2.3) particles/ individual and 0.97 (±2.61) particles/g (wet weight).
Digka et al. (2018), Greece		Mussels *Mytilus galloprovincial is* N=80	Variation of Mathalon and Hill (2014)	Observation and FT-IR spectroscopy (20%)	Not specified	Yes	Frequency of occurrence of ingested MPs was 46.3%. Average content of MPs was 1.9 ± 0.2 MPs/individual.
Jinfeng Ding et al. (2019), China		Mussels and clams, *M. galloprovincia -lis* n=20, *Ruditapes philippinarum* n=10, *Mactra veneriformis* n=10	Ding et al. (2018)	Observation, FT-IR spectroscopy (100%) and SEM (% not specified)	Not specified	Yes	MPs content measured for *M. galloprovincialis* in two sampling sites: Qingdao 0.16 ± 0.13 MPs/g, Dongying 0.42 ± 0.26 MPs/g, for *Ruditapes philippinarum* 0.74 ± 0.54 MPs/g, and for *Mactra veneriformis* 0.31 ± 0.27MPs/g (wet weight).
Khoironi et al. (2018), Java Sea, Indonesia		Mussels *Perna viridis* N=30	Procedure based on (Li J. et al., 2016)	Observation, SEM and Electron Dispersive X-Ray (EDX) (% not specified)	Not specified	No	For mussels that breed in high saline waters (36 ppb) the content of MPs was 5 MPs/0.25 g. In lower salinity (33 ppb) water the content was 2 MPs/0.25 g. At brackish environment (31 ppb) it was 1 MP/0.25 g.

Li H. X. et al. (2018), China		Oysters *Saccostrea cucullata* N= 330	Li J. et al. (2015)	Observation and FT-IR spectroscopy (% not specified)	89.2% plastic polymers (of 139 analysed)	Yes	The content of MPs ranged from 1.4 to 7.0 items per individual or from 1.5 to 7.2 items per g tissue wet weight.
Li J. et al. (2018), U.K.		Mussels *M. edulis* N=246	Li J. et al. (2016)	Observation and FT-IR spectroscopy (13%, 138 of 1048)	50% MPs	Yes	In mussels sampled from the coastal locations (n=162) the content of MPs ranged from 0.7 to 2.9 items/g tissue (wet weight). In the supermarket bought mussels (n=72) live mussels contained 0.9 MPs/g, and cooked mussels contained 1.4 items/g (n=12).
Li J. et al. (2016), China		Mussels *M. edulis* (wild) N=390	Li J. et al. (2015)	Observation and FT-IR spectroscopy (8.5%, 129 of 1519)	84.5% plastic particles	Yes	The average content of MPs was 2.2 MPs/g. The average content of MPs was 2.7 MPs/g in wild mussels (n=222) and 1.6 MPs/g in farmed mussels (n=168).
Li J. et al. (2015), Shanghai, China		Nine species of marine bivalves: *Scapharca* n=6, *Tegillarca* n=18, *Mytilus* n=18, *Patinopecten* n=6, *Alectryonella* n=18, *Sinonovacula*	Their own procedure	Observation and FT-IR spectroscopy (% not specified)	Not specified	Yes	The content of MPs varied from 2.1 to 10.5 items/g (wet weight) and from 4.3 to 57.2 items/individual. The highest content by weight was found in *Sc. subcrenata* at 10.5 items/g. The highest by individual was found in *Patinopecten yessoensis* at 57.2 items/individual.

		n=6, *Ruditapes* n=24, *Meretrix* n=18, *Cyclina* n=30.					
Teng et al. (2019), China		Oysters *Crassostrea gigas, Crassostrea angulate, Crassostrea hongkongensis* and *Crassostrea sikamea* N=510	Variation of Munno et al. (2018)	Observation, FT-IR spectroscopy (25%, 301 of 1218)	94% MPs	Yes	The average content of MPs was 0.62 items/g (wet weight) or 2.93 items/individual.
Van Cauwenberghe and Janssen (2014), Germany, France		Mussels *M. edulis* n=36 and *Oysters Crassostrea gigas* n=10	Variation of Claessens et al. (2013)	Observation and RM (% not specified)	Not specified	Yes	In *M. edulis* the average MPs content was 0.36 ± 0.07 particles/g of soft tissue (wet weight). In *Crassostrea gigas* it was 0.47 ± 0.16 particles g-1 w.w.
Bordbar et al. (2018), Greece	Crusta-cean	Shrimp *Plesionika narval* (stomachs) N=2411	Not specified	Observation and FT-IR spectroscopy (at least a 10%)	Not specified	No	Ingested plastics were found in 146 shrimp stomachs, corresponding to 5.93% of all examined stomachs. (No data on concentrations).
Bour et al. (2018), Norway		Shrimp *Crangon allmanni* N= 20	Variation of Avio et al. (2015b).	Observation and FT-IR spectroscopy (% not specified)	Not specified	Yes	The frequency of MPs occurrence was 65%. The average content was 2 MPs/individual.

Carreras-Colom et al. (2018), Balearic Basin		*Shrimp* Aristeus *antennatus* N=148	Castejón et al. (2015)	Observation and FT-IR spectroscopy (100%)	54%	No	A total of 58 out of 148 (39.2%) individuals contained MPs inside their stomachs. (No data on concentrations)
Collard et al. (2017a), Gulf of Lions (Mediterranean Sea)	Fish	Anchovies *Engraulis encrasicolus* n=10 Sardines *Sardina pilchardus* n=2, (livers)	Collard et al. (2015)	RM (100%)	100%	Yes	In *E. encrasicolus* nine MPs were found in eight of the ten analysed livers. In *S. pilchardus* and in *Clupea harengus* MPs were found in three out of the four analysed livers.
Digka et al. (2018), Greece		Sardines *Sardina* pilchardus n=36, *Pagellus erythrinus* n 19, *Mullus barbatus* n=25.	Variation of Mathalon and Hill (2014)	Observation and FT-IR spectroscopy (20%)	Not specified	Yes	Frequency of occurrence of ingested MPs was 47.2% in sardines 42.1% in common pandoras and 32.0% in red mullets. Average content of MPs 1.8 ± 0.2 items/individual in sardines, 1.9 ± 0.2 items/individual in common pandoras, and 1.5 ± 0.3 items/individual in red mullets.
Karami et al. (2018), Product of Canada, Germany, Iran, Japan,		Canned sardines and sprats, N=20 (brands)	Karami et al. (2017b)	Observation and RM (100%)	28.6% plastic polymers	Yes	No MPs were found in the filling liquids. MPs were found in only two brands: Iran sample #5=1 MPs/sample (sprat) and Russia sample #19=1 MP/sample (sprat).

Latvia, Malaysia, Morocco, Poland, Portugal, Russia, Scotland, Thailand, and Vietnam							
Karami et al. (2017c), Malaysia		Packed dried mullet, croaker, mackerel and anchovy *C. subviridis*, *J. belangerii*, *R. kanagurta*, and *S. waitei*, n=30 per species.	Karami et al. (2017b)	Observation and RM (100%)	59.0% plastic polymers	Yes	Seven MPs were found in the excised organs and 29 MPs in the eviscerated flesh: *C. subviridis* 16 MPs/sample, *J. belangerii* 16 MPs/sample, *R. kanagurta* 3 MPs/sample, *S. waitei* 1 MPs/sample.
Monia Renzi et al. (2019), central Adriatic Sea, Italy		*Sardina pilchardus* and *Engraulis encrasicolus* (Stomach samples) N=80	Avio et al. (2015b) and Nuelle et al. (2014)	Observation, FT-IR spectroscopy (% not specified)	Not specified	Yes	In *S. pilchardus* the MPs content was found at 4.63 MPs/individual and in *E. encrasicolus* at 1.25 MPs/individual.
Muhlschlegel et al. (2017), Switzerland	Honey	Honey N=5	Variation of Liebezeit and	Observation, FT-IR spectroscopy and	Not specified	Yes	Black particles count (between 1760/kg and 8680/kg), white transparent fibres (between 132/kg and 728/kg), white

			Liebezeit (2013)	RM (% not specified, 15 particles for FT-IR and 28 for R)	transparent particles (between 60/kg and 172/ kg), coloured fibres (between 32/kg and 108/kg), and coloured particles (between 8/kg and 64/kg). (Not characterised as MPs).

Note: FT-IR, Fourier-transform infrared spectroscopy; N, total sample size; n, sub-sample size when provided; RM, Raman spectroscopy; SEM, scanning electron microscopy; w.w. wet weight.

This interconnection between the methods can be seen in Figure 14. Regarding the fish samples Collard et al. (2017a) used a method they previously developed (Collard et al., 2015) while Monia Renzi et al. (2019) combined the methods by Avio et al. (2015b) and Nuelle et al. (2014). Finally, Mohsen et al. (2019) used the popular method developed by Karami et al. (2017a) and Muhlschlegel et al. (2017) used a variation of Liebezeit and Liebezeit (2013) which was developed for honey.

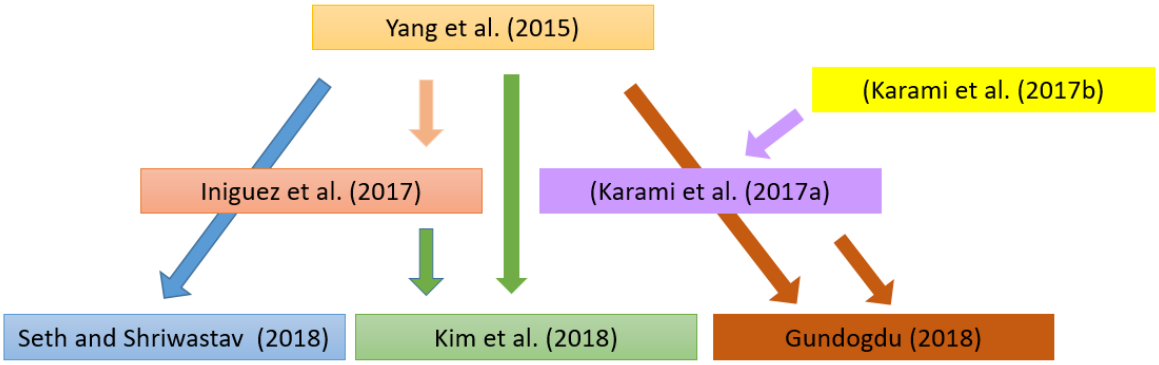


Figure 14. MPs extraction methods for salt samples.

2.3.1.3. Visual examination of the samples

All the studies incorporated visual examination in order to primarily characterize the particles/MPs before subjecting them to the identification procedure. This step becomes more important in the studies that didn't examine 100% of the recovered particles in the following step, but only a part of them. This visual examination, in the majority of the studies, involved the use of a microscope doubled with a camera to photograph the particles. The particles were then described in terms of their physical characteristics including: morphology (fibre, line, flake, sphere, fragment, bead, etc.), colour or even the absence of biological structure. In addition, some studies utilized more techniques such as checking if the particles can be cracked or not (Mohsen et al., 2019). Visual identification of the particles is quite open to interpretation. This step is highly likely to introduce bias since the decision of whether a particle is of plastic origin or not, is based on the operator's judgement, experience and ability. In this sense, the introduction of human error is more than possible.

2.3.1.4. Chemical identification of the MPs

Although all the studies used one of the identification techniques mentioned in the eligibility criteria, there are still differences between them. Regarding the use of the FT-IR, there are several issues. There are different modes built into the instrument that the researchers could chose to use e.g. attenuated total reflection (ATR) (see section 2.4). In addition, they can combine the instrument with a microscope (m or μ FT-IR) to allow them to identify smaller

particles. Once the instrument has produced the output, the spectrum needs to be compared with spectra from existing libraries. There are two issues here: first, the choice of the libraries, and second, the percentage above which the researchers consider as a valid match to a polymer. For example Kim et al. (2018) accepted matches better than 70% and stated that their decision was based on the previews studies by Lusher et al. (2013) on fish and Woodall et al. (2014) on marine plastic debris. Digka et al. (2018) accepted an 80% match but did not provide a rationale. Jinfeng Ding et al. (2019) reported matching above 89% but didn't mention having a pre-decided level of similarity. Iniguez et al. (2017) and Muhlschlegel et al. (2017) did not report on their accepted degree at all.

In the case of RM, some researchers chose to use the default settings of their instrument (confocal hole size, slit size, integration times per second, etc.) while others customized them. Most studies used commercially available spectra libraries to compare the results using software (Collard et al., 2017a, Karami et al., 2018, Muhlschlegel et al., 2017, Karami et al., 2017a, Karami et al., 2017c). Collard et al. (2017a) did not report on which libraries they used, while Van Cauwenberghe and Janssen (2014) did not report what they compared their spectra to. Finally, Gundogdu (2018) chose to produce their own reference spectra for comparison to their samples.

The Marine Strategy Framework Directive, MSFD Technical Subgroup on Marine Litter (2013), of the European Commission, proposes all samples with matches < 60% be dismissed, matches between 60 and 70% be individually examined for similarities with known polymers and matches > 70% be accepted without further examination. This proposal is for FT-IR and RM and could be used to standardize the method. The SEM technique provides more uniformity since the results refer to elemental composition which is more definite than a spectrum. However, it must be stressed that the SEM technique is more time consuming and expensive than FT-IR and RM. It should be acknowledged that all identification methods are extremely time-consuming (some more than others) and it is not always possible to examine 100% of the particles.

2.3.1.5. Quality control/ quality assurance

The ubiquitous existence of MPs in every environment has been well documented. Even in the relatively controlled environment of the lab the danger of sample contamination is still high. The use of contamination prevention measures coupled with the use of procedural blank samples and/or controls is therefore imperative, effectively constituting quality control (QC) and quality assurance (QA) measures. Contamination prevention measures target the

protection of samples from the time of sampling until the end of their processing in the laboratory. Procedural blanks are used to validate that the contamination prevention measures in the laboratory have been effective, meaning that no extra MPs were added during the experiments, or alternatively allow any identified to be taken into account when interpreting the data obtained. Three of the included studies did not report on using procedural blank samples thus there is no guarantee that their results have not been affected by contaminated samples, namely Bordbar et al. (2018), Carreras-Colom et al. (2018), and Khoironi et al. (2018).

2.3.1.6. Conclusions

Looking at the different steps it becomes obvious that differences in the methods can affect the final results. Starting from sampling and across all the aforementioned steps, the use of standard techniques based on protocols specific to sample types would help protect against the introduction of bias and would make comparisons across studies feasible. Furthermore, the studies did not use the same units to report their results. As shown in Table 3, some studies only reported the percentage of the MPs presence and not the estimated concentrations, other reported the concentration per individual animal while others per weight. The studies in Table 3 were presented according to the sample type for easier comparison.

After the completion of the qualitative analysis of the papers a preliminary attempt was made to collate the quantitative data in order to calculate human exposures via dietary ingestion using descriptive statistics and a statistical summary approach. Calculating the consumption was not possible for all studies; only for the studies that provided enough primary data for the necessary calculations. This exercise in not reported herein for brevity. Nevertheless, it helped to understand how MP food contamination can be modelled and presented in the context of human exposures.

2.3.2. Microplastics in environmental compartments scoping reviews

For the purposes of this ScR, environmental compartments are defined as any setting in the environment where humans may be directly exposed to MPs. As aforementioned, the terms used in the search strategy for this ScR was shared with the previous ScR (section 2.3.1). Following the title and abstract screening, 261 studies were downloaded for full text assessment. The initial strategy for the ScR was to include all studies that provide evidence on MPs distribution in the environment. After reviewing the downloaded studies, 134 met the general eligibility criteria. Unfortunately, the vast majority of the studies provided

information on sea water, lakes, rivers and sediments in a context that direct human exposure could not be established. Therefore, a decision was made to include only the studies that did provide such evidence, thus reducing the number of the included studies to five, as illustrated in the flow diagram (Figure 15).

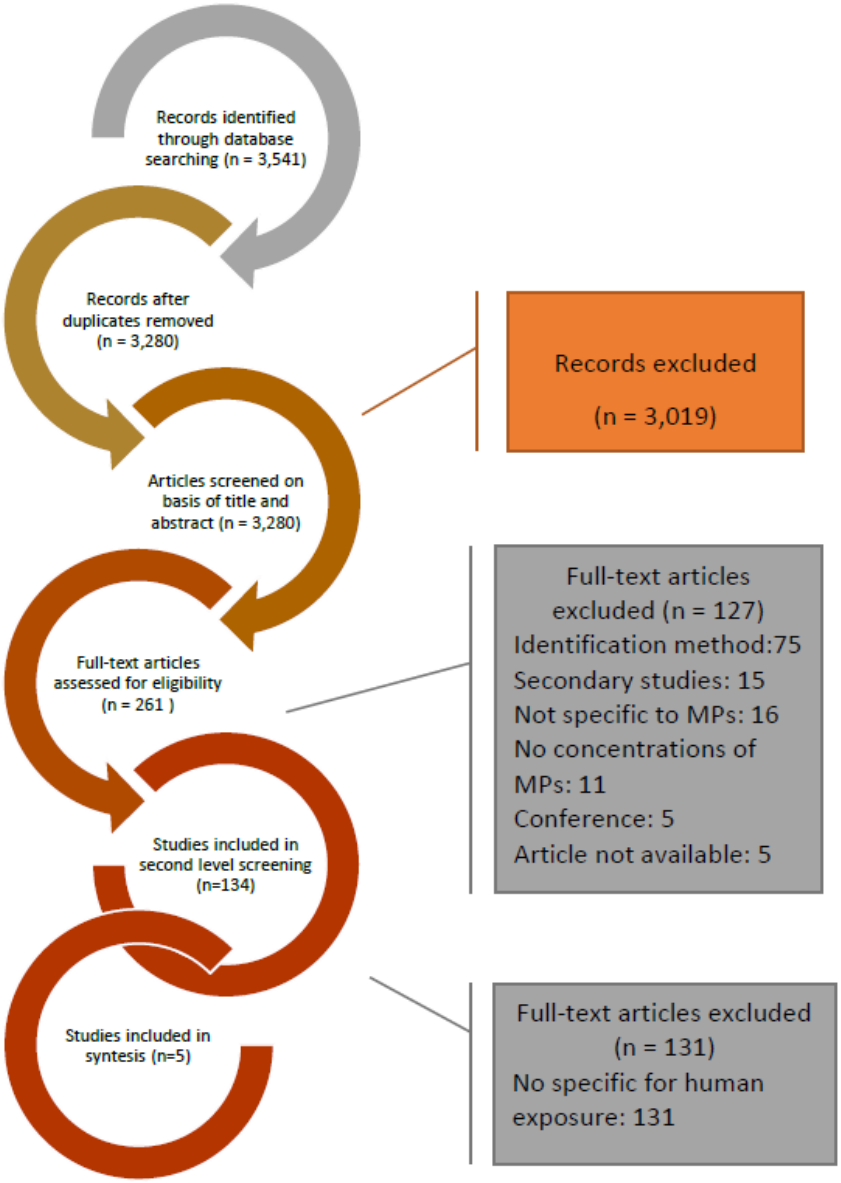


Figure 15. MPs in the environment flow diagram

The procedure that the studies use to measure the MPs follows the same logic and steps as the food studies. The main difference was that there is less of a need to digest the samples in order to obtain the MPs, thus these studies used filtering as the medium of extracting the MPs. All studies used procedural blank samples, evidencing QC/QA measures. The studies can be divided into two categories: atmospheric and water, as shown in Table 4.

2.3.2.1. Atmospheric studies

Regarding the atmosphere, the aim of the two studies was different. Cai et al. (2017) focused on the inhalation uptake route and report their results in particles per m^2 per day while Dehghani et al. (2017) focused on ingestion of MPs and report in particles per g of dry dust. Due to these fundamental differences, comparison between them was not appropriate. Dehghani et al. (2017) used their findings to calculate MPs ingestion using their data in conjunction with what they cited as the recommended mean value of soil ingestion (200 mg day^{-1}) by the U.S. Environmental Protection Agency (EPA), citing a review draft from 2000; aimed at child-specific exposures. The authors state that EPA recommends mean values for adults too (100 mg day^{-1}), but that value is not mentioned in the review. This external review draft has a *do not cite or quote* label on it, and it has been superseded by the final report published in 2002 and by various other publications (EPA, 2002a). The current recommendations by EPA are quite different; the central tendency for soil and dust ingestion for the general population is 40 – 50 mg/day for ages 6 months – 12 years, and 20 mg/day for ages 12 years through adult; upper percentile 100 mg/day and 60 mg/day, respectively. These recommendations can be found in the latest Exposure Factors Handbook of 2011, which has been updated for soil and dust ingestion in 2017, and refers to a 45% and 55% mix of soil and dust (EPA, 2017b). In view of the above, the findings of the Dehghani et al. (2017) study cannot be taken into consideration. A discovery in the Cai et al. (2017) study was that during the identification process, using μ-FT-IR, they were able to identify the spectrum for 91.5% of the fibres they processed and from them only 28% were positively identified as of polymeric origin. These findings validate the standpoint that without the use of an instrument-based identification technique, over- or under-reporting is very possible.

2.3.2.1. Drinking water studies

Three studies focused on drinking water. Mintenig et al. (2019) reported concentrations in the magnitude of 0.7 MPs m^{-3} (1000 L); their sampling sites were water treatment plants (WTPs) and they tested treated and untreated water. The untreated water came from wells (at least 30 m deep). No major fluctuation across their samples was found, reporting a range of 0 to 7 MPs m^{-3}. It could be assumed that this groundwater would be protected from atmospheric MPs contamination. Pivokonsky et al. (2018) also focused on WTPs and sampled untreated and treated water reporting an extremely higher range between 338 and 3605 MPs L^{-1}. However, these plants did not use groundwater sources.

Table 4. Microplastics in the environment scoping review studies

Study (location)	**Sample**	**MPs identification method (% of the sample)**	**MPs positively identified**	**Procedural blank samples**	**Findings**
Cai et al. (2017) Dongguan, China	Atmospheric fallout	Observation and μ-FT-IR spectroscopy (20% of fibres and 100% of the rest shapes)	Fibres: 91.5% identified and 23% were MPs, rest shapes: 84.6% were MPs	yes	The average concentration of MPs in the three sites was 36 ± 7 particles/m2/day.
Dehghani et al. (2017) Tehran metropolis, Iran	Urban dust	Observation and SEM-EDX (20 out of 2649 MP particles)	Not specified	Yes	Minimum and maximum MPs concentration ranged from 83 ± 10 particles/30 g dry dust to 605 ± 10 particles/ 30 g dry dust.
Mintenig et al. (2019) Germany	Groundwater from wells (at least 30m deep)	FT-IR imaging (100%)	Not specified	Yes	MPs concentrations ranged from 0 to 7 MPs/m^{-3}, overall mean 0.7 MPs/m^{-3}.
Panno et al. (2019) USA	Groundwater from springs and wells (< 65m deep)	Observation and pyr-GCMS 20 out of 274 (7%)	Four out of 20 (20%)	Yes	Median concentration 6.4 particles/L and a maximum of 15.2 particles/L.
Pivokonsky et al. (2018) Czech Republic	Water from two water reservoirs and one river.	Observation and FT-IR (25%, for > 10 μm) and micro RM (25% for 1-10 μm), SEM-EDX (30-50 particles from each filter)	Not specified	Yes	MPs average abundance ranged from 1473±34 to 3605±497 MPs per L^{-1} in raw water and from 338±76 to 628± 28 MPs per L^{-1} in treated water.

Note: FT-IR, Fourier-transform infrared spectroscopy; pyr-GCMS, pyrolysis gas chromatography mass spectrometry; SEM-EDX, Scanning Electron Microscopy - Energy Dispersive X-Ray Spectroscopy.

The three WTPs included in the study processed water coming from two open water reservoirs and one river. The significance of this difference is that the water is therefore exposed to MPs contamination. On the other hand, Panno et al. (2019) sampled only untreated water from springs and shallow wells. Their findings were in the magnitude of 6.4MPs/L. There is a striking difference in the results of the three studies. There were also major differences in the MPs extraction protocol the studies used (see Appendix 2). Mintenig et al. (2019) used a multistep procedure before observing the particles, including chemical digestion and multiple filtration, using filters of 3 μm twice and 0.2 μm once. Pivokonsky et al. (2018) also used chemical digestion to remove organic material but only used a two-level filtering of 5 μm and 0.2 μm. Panno et al. (2019) only used a 0.45 μm filter and no digestion. Taking into consideration that all three studies used a validated procedure to identify their MPs and the fact that they used procedural blank samples, it appears that the extensive treatment of the samples in the Mintenig et al. (2019) study coupled with the fact that their samples are groundwater from deep wells could explain the difference in the magnitude of their results. It should also be noted that Mintenig et al. (2019) concluded that due to the amount of the contamination of their procedural samples, the MP content of their water samples should be attributed to sample handling.

2.3.2.2. Conclusions

As in the case of the food studies, consensus on the sampling and processing protocol would be extremely beneficial, not only to the quality of future studies but also as a tool to help us compare and appraise existing studies. Across the studies a rather important piece of information was missing. Although the studies that have been included in these two ScRs used one of the validated MPs identification procedures, the vast majority of them did not report if, and how, the results of that process informed their final findings. One would assume that they used those results to circumscribe their findings to only the particles of polymeric origin. Nevertheless, it is not clearly stated in the papers that they did so.

2.4. MPs identification techniques

2.4.1. Fourier-transform infrared spectroscopy (FT-IR)

The technique is based on identifying the polymer based on the infrared (IR) absorption spectra which is characteristic for each chemical component (Bergmann, 2015). It can be executed in three different modes “attenuated total reflection (ATR), reflectance and transmission” (Strungaru et al., 2019: 122). Additional techniques include reflectance micro-FT-IR (or μ-FT-IR) and focal plane array detector-based micro FT-IR (Löder et al., 2015, Harrison et al., 2012). The different techniques offer different levels of certainty under

different circumstances. For example, ATR FT-IR is mostly used for larger samples (> 500 μm) while micro-FT-IR, which utilizes a microscope, is used for smaller particles that cannot be seen with a naked eye. FT-IR analysis can detect particles down to a size of approximately 20 μm and it has been used widely in MPs related research with very good results (Löder et al., 2015). The spectrum produced during the measurement is compared to already known spectra from polymers that are available in commercial libraries. The comparison is done automatically by the use of software. The results are reported with a level of % match to the known spectra libraries.

2.4.2. Raman micro-spectroscopy (RM)

RM also utilizes a spectroscopic technique to identify the polymer. The main difference with FT-IR is that it can detect the composition of even smaller samples. It has been reported to detect polyamide at a size of 1μm (Oßmann et al., 2018). The technique produces a spectrum, and the final results are decided by comparing the spectrum against a known one. Another difference with FT-IR is that in some studies the researchers chose to custom-make their own spectra for evaluation, while others use commercially available libraries (Araujo et al., 2018). The comparison is done manually or via software. RM can also provide additional information for the samples such as absorbed organic of inorganic components by the polymers (Strungaru et al., 2019).

2.4.3. Pyrolysis-GC/MS

Pyrolysis-gas chromatography (GC) in combination with mass spectrometry (MS) identifies the chemical composition of the particles by "analysing their thermal degradation products" (Fries et al., 2013: 1951). It a destructive technique, but because the pyrolysis procedure is done sequentially, using different temperatures, components such as plastic organic/inorganic additives, can be detected in one sample (Fischer and Scholz-Böttcher, 2017). The reports of this thermo-analytical technique are pyrograms created by the mass spectrometer for all the pyrolysis products (polymers and additives). Pyrograms are then compared to pyrograms of known polymers that can be custom-made by the researchers or obtained from literature and it is done manually. This technique can run samples down to 100 μm (Strungaru et al., 2019, Fischer and Scholz-Böttcher, 2017). An advantage of this technique is that it also provides the mass of the particles.

2.4.4. Scanning electron microscopy (SEM)

This technique is being used in different configurations such as coupled with energy dispersive X-ray spectroscopy (EDS) and environmental scanning and microscopy-energy

dispersive X-ray spectroscopy (ESEM-EDS). They all use the surface morphological characteristics of the sample in order to identify the chemical composition (Rocha-Santos and Duarte, 2015). The SEM-EDS set up produces images that can be evaluated according to the known morphology of components as well as an elemental analysis which can link the sample to a polymer (Eriksen et al., 2013). The ESEM-EDS can be used to identify the atomic composition of the sample by producing images as well as obtaining the atomic number of the component under examination (Vianello et al., 2013). The analysis can go as far as nano-size and is the only one of the four that can do so.

2.5. Scoping reviews conclusions and project trajectory

The ScRs findings highlight a range of possible health effects from these emerging contaminants. The abundance, and the robustness of the evidence regarding estrogens and xenoestrogens, arguably, classifies them as contaminants that have already emerged. On the other hand, the evidence on 3D printer dust was found to be sparse and not appropriate for further analysis at this point. Regarding MPs, the evidence on health effects was controversial, due to the limitations of the existing studies. Nevertheless, the data did point at adverse health effects. As a result, MPs were taken forward as the EEC that most warranted further research. The findings of the two environmental ScRs were extremely helpful and laid the basis of the protocol of the systematic reviews on MP food contamination as well as the development of a systematic tool to appraise the quality of MP environmental research (see section 3.2.7). This enabled pinpointing which were the important details and differences in every aspect of a study starting from inception and finishing on the publication of the scientific paper.

The initial objective of the project was to develop an epidemiological model and indices for the EEC's health effects in relation to distribution and health inequalities. An alternative objective was also proposed, in the case that the data were sparse or inappropriate, which was to generate policy recommendations and guidelines for the EECs in question. In light of the findings of the ScRs the original objective of the project was modified, and the thesis focused on a risk assessment perspective for MPs and human health.

Chapter 3. Methodology

3.1. Systematic review methodology

Systematic reviews and meta-analyses bring together evidence from multiple studies in a standardized, transparent and reproducible manner. They seek to identify, evaluate and summarise evidence of all relevant individual studies in order to answer a specific research question (Higgins et al., 2019). Systematic reviews' methods and methodology were key in validating the underlying hypothesis of this thesis; allowing the variables of the conceptual model to be replaced with known data (see section 3.7.1.1). Although the use of systematic reviews and meta-analysis is common in health and medical sciences and their merits are widely recognized, they have only relatively recently started to be used in other disciplines such as environmental science. The aim of this contribution was to use well-established methodology and further develop it to accommodate the needs of MPs research.

The systematic reviews presented in Chapters 4, 5 and 6 are based on the methodology proposed by the Cochrane Collaboration (Higgins et al., 2019) and the Centre for Reviews and Dissemination (CRD) of the University of York (CRD, 2009). The methods adopted for reporting the systematic reviews and developing the protocol used to guide them was governed by the PRISMA statement (Moher et al., 2009) and the PRISMA-P (protocol) statement (Moher et al., 2015) as well as their accompanying guidelines (Liberati et al., 2009, Shamseer et al., 2015). PRISMA is a widely accepted method in the health disciplines primarily developed and used to systematically review health care topics. It provides a framework for planning and executing a systematic review starting from the construction of a solid protocol (Moher et al., 2015). The Systematic Reviews involved the following main stages: research evidence identification, selection of studies, extraction of data, quality assessment of the data and data synthesis (CRD, 2009). All the sections were documented with the help of the PRISMA tools (flow diagram and checklist) (Moher et al., 2009).

A search strategy was constructed using the experience gained by the ScRs (see Chapter 2) to refine and expand the search terms. After reviewing the results of the searches executed for the ScR on MP environmental distribution more terms were added in the search strategy to ensure the inclusion of all available scientific data (see Appendix 1. a,d). Papers were examined against the inclusion and exclusion criteria. Eligibility criteria were informed by the results of the ScRs, which can be seen as a way of piloting the selection process (Khan et al., 2003). Piloting ensured that the criteria were clear and unambiguous, thus promoting consistency between individual reviewers. Data extraction charts constructed in Microsoft Excel for the ScRs were used as a template for the data extraction, with both numerical and

text data extracted. The quality assessment of the papers included in the systematic reviews was executed on an individual and on an overall level. An integral part of any systematic review is the assessment of the studies' validity (reporting, internal and external), comprising a detailed assessment of their quality from inception to publication in order to evaluate the introduction of systematic error. This process is called a Risk of Bias (RoB) assessment and uses a checklist approach to promote an objective assessment. The RoB assessment is made on the published or readily available material, so is reliant on how well the studies are reported. RoB was assessed in a consistent and reproducible manner (see section 3.3.7.2). Due to the focus and the nature of these systematic reviews, the available tools in the literature were not appropriate. Because systematic reviews are common in medical, health and social sciences, most available RoB tools are focused on these areas and use metrics to appraise studies in these fields. For example, the ROBINS-I tool (Sterne et al., 2016) is widely used RoB tool for non-randomised studies but is specifically tailored to health intervention studies. To achieve a standardized way of critically appraising the studies, a bespoke tool for assessing RoB (checklist) was developed to meet the needs of the specific systematic reviews (see Section 3.2.7). The synthesis of the data was executed using a narrative synthesis (qualitative), i.e. textual analysis of the relationships between scientific evidence and an appraisal of its robustness, accompanied by meta-analysis (quantitative) when it was deemed to be meaningful. (CRD, 2009). The choice in using qualitative and/or quantitative synthesis did not depend only on the form of the data but also on their quality (see Chapters 4, 5 and 6).

3.2. Systematic review methods

Preliminary searches identified literature relating to only three different food groups: salt, seafood and drinking water. In order to consider the evidence in a robust manner, one overarching search strategy was developed, and searches undertaken, but the resulting literature was synthesized in three linked but separate systematic reviews, one for each food group. In this way, sample heterogeneity was addressed in a comprehensive way, at the onset of the reviewing process. The methods presented below were followed for all three food themes and further details for each of them are provided when needed.

3.2.1. Protocol and registration

The protocol (Danopoulos et al., 2019) was developed according to the PRISMA-P guidelines (Moher et al., 2015, Shamseer et al., 2015). It was designed to include available research on all food categories (salt, drinking water, seafood) which were determined by the

preceding ScR. It is registered on the international prospective register of systematic reviews (PROSPERO), registration number: CRD42019145290, and is available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42019145290).

3.2.2. Eligibility Criteria

The primary division of environmental studies according to their design is into observational and experimental studies. Both of these categories can be further divided. For the purposes of this review, only descriptive and analytic observational study designs (not experimental) are included since the focus is on uncontrolled environmental exposures (Centre for Evidence-Based Medicine, 2019). Uncontrolled refers to not conducting an experiment under controlled conditions, not to the use of control samples (Eberhardt and Thomas, 1991).

3.2.2.1. Inclusion Criteria

- Only primary studies were included. The reviews and reports that were discovered during the search were used to check and validate the searches by comparing the results to their reference lists.
- There was no time limit on publication date. All databases were searched from launch date. Databases are reported in section 3.3.3 (information sources). Although the term MPs was coined in 2004 by Thompson et al. (2004) studies that use more descriptive terms could also be included (see section 3.3.4, search strategy).
- The definition for MPs that was accepted is: particles of plastic material of a size up to 5 mm; the definition does not include NPs which are in the nano scale (< 100 nm) (GESAMP, 2015b, GESAMP, 2016). Although more robust definitions for MPs have been proposed more recently (Hartmann et al., 2019, Frias and Nash, 2019) this broader definition was used to assure the inclusion of all relevant scientific literature.
- Only studies were included that reported on food samples as defined by Regulation (EC) No 178, 2002 "any substance or product, whether processed, partially processed or unprocessed, intended to be, or reasonably expected to be ingested by humans. 'Food' includes drink, chewing gum and any substance, including water, intentionally incorporated into the food during its manufacture, preparation or treatment" (Council Regulation (EC) No 178/, 2002: 2).
- Studies reporting on samples that have not been collected as food but are regularly consumed as such; since the aim was to collect data on the presence of MPs in media in the environment as well as in products that are already 'on the shelf'.

- Studies that looked at the presence of MPs in aquatic life regardless of the species of the organism (e.g. fish, mollusc, crustacean etc.) or the part of the body that MPs are reported to be found in e.g. gills, GI tract, liver, flesh etc.
    - Only commercially relevant species of aquatic life were to be included (seafood).
    - If a study focused on the GI tract of a type of seafood, it was only included if the species of the seafood was small and it is reasonable to assume that it is usually eaten whole with the GI tract intact (e.g. anchovies, shrimps).
- Studies reporting on samples that were not collected as food, but are regularly consumed as such (e.g. mussels), were included.
- Studies must have used one of the following four validated processes for the identification of MPs: Fourier-transform infrared spectroscopy (FT-IR), Raman spectroscopy (RM), pyrolysis gas chromatography/ mass spectrometry (Pyr-GC-MS) and scanning electron microscopy plus energy-dispersive X-ray spectroscopy (SEM/EDS).
- Studies must have used procedural blank samples to validate that the samples have not been contaminated after/during their collection.
- All sampling locations around the world and all sampling procedures were included.
- For the meta-analysis part of the systematic review only studies that reported specific abundance/ concentrations of MPs were included. All the measuring units were included.

3.2.2.2. Exclusion criteria

- Commentaries, opinion pieces, proceedings of conferences, editorials, non-peer reviewed reports.
- Studies that reported on food samples that do not conform to the definition above (Council Regulation (EC) No 178/, 2002: 2).
- Studies that did not report the process for the identification of MPs.
- Studies that reported on the process of the identification of MPs using a process other than the four listed above.
- Studies that did not explicitly report the use of procedural blanks to validate post-collection processes.
- Only articles published in the English language were included.

3.2.3. Information sources

The following online databases/sources were searched from launch date: MEDLINE (OVID interface, 1946 onwards), EMBASE (OVID interface, 1974 onwards) and Web of Science core collection (Web of Science, 1900 onwards) using free text. The thesaurus medical subject heading (MeSH) was also used in MEDLINE. MEDLINE and EMBASE were used as specialized data bases for medical and biomedical sciences, and the Web of Science core collection as an interdisciplinary tool. In addition, the reference lists of the reviews that were discovered were searched, as well as the reference lists of relevant published reports. In some instances, authors of papers were contacted in order to obtain missing information and data from published studies. A detailed list of the authors that were contacted can be found in Appendix 3.

3.2.4. Search

For all three systematic reviews, the searches were executed twice. The first one was run at the start of the review process and the second one before the end of each systematic review so that the most recently published papers would be included. The initial search was executed on the 10th of July 2019. The second search was executed for the drinking water theme on the 3rd of June 2020, for salt on 10th of September 2020, and for seafood on 5th of October 2020. The search strategy was informed by the ScR that was previously undertaken (section 2.3.1). The number of possible hits was high as the search strategy maximised sensitivity rather than specificity. The strategy was first developed for MEDLINE and EMBASE (OVID interface) using free text and MeSH and then the syntax was adapted for the Web of Science interface. The search strategy is presented in Appendix 4, a and b.

3.2.5. Study selection

The selection assessment process of the studies was conducted in a standardized manner. EndNote (X 9.2) software was used to extract, de-duplicate, and manage the citations of the articles that were identified through the search strategy. The screening questions were developed according to the eligibility criteria. The eligibility criteria and the screening process were previously used and validated in the ScR. The studies were screened in two levels. An initial screening of titles and abstracts was conducted independently by two reviewers according to the inclusion/ exclusion criteria. Discrepancies between the reviewers were resolved by third-party arbitration, using an expert in the field.

For the studies that met the inclusion criteria, full papers were downloaded for the second level (full text) screening. The reasons for excluding the studies were recorded and are

reported in the results chapters (see Chapters 4-6). The second reviewer screened 20% of the full text studies in order to validate the process. The second level screening process identified the studies to be included for the meta-analysis and the ones for the narrative analysis. Additional inclusion/ exclusion criteria were applied for the inclusion of a study in the meta-analysis as described in the Eligibility Criteria section. At this stage, the studies were divided into the three food and drinking water themes in order to examine the data in a cohesive and comparable manner. In this way, sample heterogeneity could be addressed in a comprehensive way and findings could be synthesised in a meaningfully. The following processes of the systematic review were executed in the same way for all the themes.

3.2.6. Data Extraction

The data extraction process was based on a form developed, used and validated by the ScR. Since the extraction procedure had been validated during the ScR, it was carried out by one reviewer for the systematic reviews. Excel was used to record and structure the data extraction. For each of the included studies, the following information was extracted: sampling (geographic location of the sampling site/s, geographic coordinates of sampling site/s, date of sampling, sampling method), sample characteristics (sample kind and type, number of samples), sample analysis (sample replicates, MPs extraction procedure, visual identification method, composition identification method, percentage of sample which underwent composition identification method, library used for spectral analysis, percentage match index for polymer identification), results of procedural blank samples and the results of the analysis (identified type of polymer, MPs' content). In the case that the specific content of the MPs was not reported but only their presence or absence, this information was also extracted to be used in the narrative systematic review. If more than one type of sample is included in one paper, all the relevant information was extracted separately for each sample type.

A number of studies in which the required information was not clearly reported were identified. In some studies, the data were not presented at all, in others, data were presented in a statistical form that could not be used for the review. Finally, in some studies the data were only available in figures and graphs. To obtain usable data, the corresponding author of each paper was contacted in order to obtain the primary data. A maximum of three emails were sent to the corresponding author of the paper. Six out of the 18 authors that were contacted, provided the data for the salt food theme and six out of the 11 for the seafood theme (Appendix 3). Where additional information was not provided by the authors, papers were only included in the narrative systematic review and were excluded from the meta-

analysis. During the data collection process, specific attributes of the data (e.g. sample n, sample type) were examined in order to avoid duplicate publication of the same data. One duplicate publication was identified in the studies by Phuong et al. (2018a) and Phuong et al. (2018b) where only the first publication of the data was included in the systematic review.

3.2.7. RoB in individual studies

RoB was assessed in a consistent and reproducible manner. Due to the focus and the nature of the systematic reviews, the available tools in the literature were not appropriate. To achieve a standardized way of critically appraising the studies, a bespoke tool for assessing RoB (checklist) was developed using the experience gained by the ScRs (see Chapter 2). The construction of the RoB tool was based on up-to-date scientifically robust methodology by the Cochrane Collaboration, which is the leading scientific body in the field of systematic reviews of interventions (Higgins et al., 2011, 2019, 2021). The guidelines set by the CRD (2009), were also used. More specifically, the advice on systematic reviews of adverse effects was particularly helpful, since most systematic reviews focus on positive effects which is not the context of this systematic review. The quality of reporting section was developed according to the STROBE Statement—checklist regarding items that should be included in reports of observational studies (von Elm et al., 2007) and the recommendations of the Agency for Healthcare Research and Quality U.S. Department of Health and Human Services (West et al., 2002). In addition, the principles laid down by the Environmental-RoB Tool (E-RoB), which was developed by Bilotta et al. (2014) to be used for evidence in environmental science, were also taken into consideration. E-RoB was adapted from the Cochrane Collaboration's tool for assessing RoB in randomised trials (Higgins et al., 2011). The bespoke RoB assessment tool rates the studies across four domains: study design, sampling, analysis and reporting with a final overall assessment (Table 5). The tool comprises a checklist with questions covering all aspects of experimental protocol development, execution and reporting.

In accordance to relevant guidance (Higgins et al., 2019) the use of scales and scores (numerical) for the assessment was avoided. Instead, for each of the entries a question was formulated in order to prompt a response that was used as the support for the judgement. For each item in the tool there are two entries: the answer, with additional notes when needed, and the rating. In the 'answer' entry a copy of the text from the study on which the decision is made on is provided, allowing transparency on how the decision was made.

Table 5. Environmental Risk of Bias (RoB) tool template

Domain	**no**	**Question**	**Answer**	**Notes**	**Rating** (high, low, unclear)
Internal validity					
Appropriateness of study design to the research objective	1	Is the design appropriate for the questions of the study?			
Sampling					
Sample method	2	Has the method been used in other studies?			
	3	Is the method validated?			
	4	Are there precautions in place to protect further contamination of the sample?			
Sample location	5	Is there a rationale available?			
	6	Is the location appropriate?			
Sample randomization	7	Is the sampling method guarantying randomization of the sample?			
Use of procedural blank samples	8	Are the results of the procedural blank samples reported?			
Use of replicate samples	9	Is the study using replicate samples?			
	10	How many?			
Analysis					
Particles extraction method	11	Is the method used by other studies?			
	12	Is the method validated?			
Particles identification method	13	Is the method one of the four validated methods?			
Amount of sample analyzed for composition.	14	How much of the sample has been analyzed?			
Particle composition match to the library of choice	15	Is the match > or < 60% match?			
Library of choice (type, kind)	16	Is the library made by the lab or is it a commercial library?			
	17	Is one library or more being used?			

Statistical analysis	18	Is the statistical analysis appropriate for the sample?		
Interpretation	19	Has the interpretation of the results been based on the outcomes of the analysis?		
Quality of reporting				
Methodology	20	Have the methods used in the study been reported in detail?		
Limitations	21	Have the study recognized limitations?		
				overall rating

The rating of the studies for each entry, domain and overall study is: high risk, low risk or unclear RoB. RoB assessment was done both on the study and on specific outcome level. This allowed for the direct comparison of RoB rating of a specific domain of the study against a specific outcome. For example, when reviewing the sampling methodology, the sampling domain RoB rating is more relevant that the overall RoB rating. For the majority of the items in the tool the rating 'high' and 'low' is based on a yes/no answer or a numerical value. The rating 'unclear' is assigned when the study does not report sufficient information to make a judgment or when the associated risk is unknown. In order to achieve maximum transparency all items are discussed in detail in the RoB tool explanation/elaboration section in Appendix 5.

3.2.7.1. Weighting of domains and questions

A rating is given to each of the 21 items of the RoB tool, subsequently, a rating is given to each of the four domains based on the rating of the individual items in it and finally the overall rating is given according to the domains rating. In order to decide the weighting of the individual entries in the checklist, three experts in the field were contacted and asked to provide their top three entries/questions of the table as the most important factors to judge the studies' RoB. All three experts concentrated on four questions: 4, 8, 13 and 15. The questions focus on two topics. First, the prevention of sample contamination and its validation by the use of procedural blank samples. Second, the use of a validated method for identifying the composition of the particles and how a spectra library will be employed to do so. This expert opinion on the importance of individual entries of the RoB tool informed the rating of the domains as well as the overall rating of the studies. Both reviewers used the RoB tool, accompanied by the explanation/elaboration guidance to perform the appraisal of the studies. The appraisal was done independently in order to evaluate the standardization

and accuracy of the tool across reviewers. The rating of the studies was a complete match between the reviewers, thus verifying the effectiveness of the RoB tool.

3.2.8. Summary measures

The primary outcome was the presence of MPs in the sample and a quantitative measure of it (if available) for the quantitative analysis. For the meta-analysis, the focus is the MP content of the sample. Additionally, the size of the sample (n), the mean value, the standard deviation (SD) and/or the range of MP content in each type of sample in each study was also extracted. Additional information of interest was the method that was used for the extraction of the particles from the sample; the percentage of the sample (n) that was analysed for polymeric composition; the results of polymeric composition, expressed in percentage of identified MPs per n; and further details surrounding the MPs polymeric composition identification procedure. These were extracted and are discussed in the synthesis of the findings when appropriate.

3.2.9. Synthesis of results (planned methods of analysis)

Different units of measurement for MPs content were used across the studies and within the food themes. The selection of the unit of measurement varied but a rationale was not necessarily provided. Therefore, this might have been due to the type of the sample, the chosen methodology, instrumental limitations, the authors' choice of preference etc. Examples of different units include MPs/g or Kg, MPs/mL or l (litre), MPs/individual organism using the wet weight (w.w.) or the dry weight. All different units were extracted, and effort was made to standardise the units, when it was appropriate, and the necessary data to do so were available. In the studies where the mean value was not provided but the individual data for the samples were available, the values were calculated using the standard formulae for mean and SD. For pooling the results of different samples in the same studies the formulae for combining groups proposed by the Cochrane handbook (Equation 1) (Higgins and Green, 2011) was used. The formulae can be used to combine two groups into a single sample size (N_1+N_2), mean (M_1+M_2) and SD (SD_1+SD_2). If more than two groups were to be combined the formulae was applied sequentially combining the data of one group at a time.

When needed, the conversion of the five-number summary (sample minimum and maximum, median, lower and upper quartile) to the quantities needed for this review, was made using the methods and calculator developed by Shi et al. (2020). The calculator draws

on the methods developed by Luo et al. (2018) for the estimation of the mean of the sample and the methods by Wan et al. (2014) for the estimation of the SD.

Equation 1. Formulae for combining groups (Higgins and Green, 2011: Table 7.7.a).

	Group 1 (e.g. males)	Group 2 (e.g. females)	Combined groups
Sample size	N_1	N_2	$N_1 + N_2$
Mean	M_1	M_2	$\frac{N_1M_1 + N_2M_2}{N_1 + N_2}$
SD	SD_1	SD_2	$\sqrt{\frac{(N_1 - 1)SD_1^2 + (N_2 - 1)SD_2^2 + \frac{N_1N_2}{N_1 + N_2}(M_1^2 + M_2^2 - 2M_1M_2)}{N_1 + N_2 - 1}}$

3.2.9.1. Meta-analysis

For the quantitative synthesis of the results from different studies a meta-analysis model was used (Higgins et al., 2021). Models in meta-analysis are used either to quantify the effect of an intervention or the presence of a risk factor. In the case of measuring an effect, the quantification relies on comparison of the intervention effect to the effect of a control condition or the absence of one. In the case of quantifying the presence of a risk factor, which is the focus of this review, the quantification relies on comparison to its absence (Veroniki et al., 2016). Although the minimum number of studies required for meta-analysis is two (Borenstein, 2009), a small number of included studies can limit the strength of the results (Konstantopoulos and Hedges, 2019). This limitation was explored throughout the meta-analysis.

The effect estimate for each study was calculated by weighing their results using the inverse of the variance method, which is the standard weighing scheme (Chen and Peace, 2013). In order to collate and quantify the data, random-effects meta-analysis models were used (Higgins et al., 2019). Random-effects models were preferred over fixed-effects models as it was assumed the samples did not share one common true effect size that was influenced equally by the same factors, but a distribution of true effect sizes (Chen and Peace, 2013, Harrer et al., 2019b, Veroniki et al., 2016). The DerSimonian-Laird t^2 estimator was used for all the random effects models (DerSimonian and Laird, 1986, 2015), as this accounts for variations both within and between studies. The Higgins I^2 test and the Chi^2 Cochran's Q Statistic were used to assess statistical heterogeneity (Higgins and Thompson, 2002, 2003). The I^2 test is the percentage of variability in the effect size that is not produced by sampling

error. The Cochran's Q Statistic refers to the null hypothesis of homogeneity and is expressed in Chi^2 and p value. (Higgins et al., 2003).

The source of between-study statistical heterogeneity was investigated by examining statistical outliers and an influence analysis of studies. Statistical outliers were defined as studies where the 95% confidence interval (CI) of their effect size estimate, as calculated by the random-effects model, did not overlap with the 95% CI of the pooled effect size estimate (Harrer et al., 2019b). Statistical outliers of extremely large effects were specifically targeted to account for and avoid overestimations (where the lower bound of 95% CI of the study was higher that the upper bound of 95% CI of the pooled effect). To test the influence of individual studies the models were re-run without these outliers, and the two pooled effect size estimates compared. To further test the influence of every study, the models were re-run excluding one study each time to assess each study's influence on the pooled effect size (Harrer et al., 2019b). Influence diagnostics included the Higgins I^2 test and Q values (Baujat et al., 2002, Higgins and Thompson, 2002, Higgins et al., 2003) and the contribution to the pooled effect size (Viechtbauer and Cheung, 2010). The results of the influence analysis were examined numerically and graphically.

Methodological and sample heterogeneity were explored using sub-group analysis employing a fixed-effects (plural) model (mixed-effects model) (Harrer et al., 2019b). R (version 3.6.0) (R Core Team, 2019), was used for all calculations and models executing all analysis via RStudio, (version 1.2.1335) (RStudio Team, 2018), using the additional packages meta (version 4.9-7) (Schwarzer, 2019), metaphor (version 2.1-0) (Viechtbauer, 2010), dmetar (Harrer et al., 2019a), robvis (McGuinness and Kothe, 2019) and ggplot2 (Hadley, 2016). Each dataset was assessed separately in order to determine whether they were suitable for meta-analysis in terms of heterogeneity. The maps that were used to synthesize and illustrate the results geographically were created in ArcGIS Desktop (version 10.8).

The results of the meta-analysis are presented as a summary of the mean effect (MP content) with a 95% confidence interval (CI) and p value. The random-effects estimate and its CI provides the best estimate of the average effect while the corresponding p value refers to the probability of the observed effect being attributable only to chance (Higgins et al., 2021). Meta-analysis was found to be meaningful and appropriate only for the two food themes of salt and seafood. The rationale is reported in the results sections (see Chapters 4-6).

3.2.9.2. Narrative analysis/ statistical summary

All the studies were also reported in a statistical summary of effect combined with a systematic narrative analysis. The analysis was undertaken according to the guidelines set down by the CRD (2009). Regarding the statistical summary of effect, when the range was not stated explicitly, the minimum and the maximum reported MP content was used. When the results were expressed on a different mass scale, they were homogenised into the same scale for ease of comparison. Methodological heterogeneity was assessed in terms of the overall design of the study, focusing on sample type, and the method used for the particle extraction from the samples. Overall assessment of the certainty of the evidence was based on the GRADE framework (Higgins et al., 2019) and the Environmental-GRADE (Bilotta et al., 2014) across five domains, categorized into four certainty ratings: high, moderate, low and very low. The assessments are presented in a summary of results table for each food theme.

3.2.10. RoB across studies

Publication bias was assessed using funnel plots, in which symmetry implies that publication bias was not present, based on the justification that a representative range of possible effect sizes have been published. In order to investigate the possibility of missing information, the precision of the effect estimate was investigated (Liberati et al., 2009). To do so, funnel plots combined with the Egger's test were used, recognizing that this test only picks up bias in small studies (Egger et al., 1997).

3.3. Rapid systematic review methodology

The methodology used for the rapid review (Hamel et al., 2021, Garritty et al., 2020) was based on a simplified, accelerated version of the systematic review guidelines (as discussed in section 3.1) and used a protocol based on the PRISMA-P guidelines (Moher et al., 2015, Shamseer et al., 2015). Acceleration was accomplished by omitting specific methods as detailed in the following section (3.4).

3.4. Rapid systematic review methods

Only experimental study designs were eligible for inclusion. No publication date limits were set. Only studies that used human-cell models to test any toxicity effects from MPs were included. When a study also used animal cells, these outcomes were not included in the review. Studies that focused only on NPs (< 100nm) were not included. MPs were defined to have a size range from 100 nm to 5 mm (Amy Lusher et al., 2017). When a study tested both MPs and NPs, only the results for the former were included.

Web of Science core collection (1900 onwards) and MEDLINE (1950 onwards) were searched. The reference lists of any relevant reviews discovered, were searched. The last search was executed on the 19th of March 2021. Search terms included: microplastic, human cell (Appendix 6). Study screening was executed at two levels and the screening questions were developed according to the eligibility criteria. In the first level, only titles and abstracts were reviewed. For studies that met the inclusion criteria, full papers were downloaded for the second-level screening. The reasons for excluding any studies at the second level of screening were recorded and reported in the results. Data extracted were: test MP characteristics (size, origin, shape, polymer, density), test cell model characteristics (origin, cell density), MP concentration of applied dose (in any quantified unit), duration of exposure, biological endpoint, test, biological marker and outcomes.

3.4.1. Synthesis of the results

The primary outcomes of interest were toxicity descriptors concerning all possible biological endpoints, expressed either quantitatively or qualitatively. Each study included multiple outcomes testing a range of experimental conditions. Different methodologies and methods were used across studies. Similar biological endpoints, tests and biological markers were grouped to achieve the best possible relevance and comparability. All outcomes were synthesized and explored in a narrative analysis (CRD, 2009, Higgins et al., 2019, Liberati et al., 2009, Moher et al., 2009).

Quantitative results were explored via meta-regression, modifying the approach of Borenstein (2009) and dose–response thresholds were reported in a statistical summary. The initial protocol for the rapid review included a traditional meta-analysis design using mixed-effects models (random and fixed-effects) to collate scientific data. Unfortunately, a meta-analysis was not possible as effect sizes were not reported, buth only the statistical significance of the effect at certain probability thresholds (for further information see Chapter 7).

A novel meta-regression analysis was used instead to explore and assess the relationship between certain predictors, namely, the experimental characteristics (from now on termed covariates) and the dependent variable (effect size) which in this case was the binary outcome of whether a statistically significant difference from the results of the negative control samples (using probabilistic analysis) was detected or not, from now on denoted as SIG. (statistically significant) and N. SIG. (not statistically significant). One limitation of

the analysis was that unit weights were assigned to the studies as the precision of their respective effect estimate was not known.

Meta-regression can be seen as an extension of sub-group analysis in meta-analysis and as such it allows for the simultaneous investigation of multiple covariates (numerical and categorical) and the effect size (Higgins et al., 2019); it is similar to logistic regression with the main difference being that outcomes come from different studies. The relationship between covariates and outcome is measured by estimating the probability of class, where class is the binary outcome, 0 or 1 (Osborne, 2015). In order to achieve meaningful analysis grouping and comparison, results were collated, in the first instance, by biological endpoints and then by the reported outcome, where it was possible and appropriate. A series of simplifications were applied on the covariates for coherence (see Appendix 7).

The main outcomes of the logit model were the intercept and the regression coefficient estimates (β) which accompanied by a p value informed us as to the effect of the covariate on the outcome. All analysis was performed in R (version 4.1.1) (R Core Team, 2019) using RStudio (version 1.2.1335). A series of diagnostic tests were used to evaluate the logit models. Multi-collinearity was assessed by calculating the Variance Inflation Factor (VIF) value (Craney and Surles, 2002, Thompson et al., 2017). The overall performance of the models was judged by the prediction error of the coefficients in the model, which was calculated using the MASS package in R (Venables and Ripley, 2002). Predictions of both outcomes were also reported in a contingency table. Linearity between the covariates and the logit of the outcome were explored graphically. Extreme values and influential values were detected by visualizing the Cook's distance values (Osborne, 2015) and examining the standard residual errors (Menard, 2002).

All-subset logistic regression was also used to detect the best possible combination of covariates to predict the outcome. The criterion to determine the best-subset model was the Akaike Information Criterion (AIC), which is the Residual Deviance adjusted for the number of parameters in the model. Sensitivity analysis was also carried out by reducing or expanding the data frame to target specific covariates. Furthermore, multilevel logistic modelling was used to account for the heterogeneity caused by the data clustered within different studies. The multilevel models used a random intercept representing the nesting of the data in the studies. The level-1 covariates were the experimental characteristics and have intrinsic meaning. Generalized linear mixed models were fitted: fixed and random effects for level-1 variables and random effects for the intercept (Sommet and Morselli, 2017). The maximum likelihood method (with a Laplace Approximation to calculate the likelihood) was

used. There are two major implications of the multilevel model: first, that the log of the odds of the outcome can vary between clusters (level-2) and, second, that the effect of the level-1 covariates is allowed to vary from cluster to cluster.

Four steps are usually included in a multilevel analysis (Aguinis et al., 2013). In this analysis, three steps were used: first, a null (empty) model was created which did not include any of the level-1 predictors but allowed intercepts to vary across clusters and calculated the intraclass correlation coefficient (ICC) which quantifies the proportion of the variation between the clusters in the total variation. ICC can take values from 0 to 1. A value close to 0 indicates that a multilevel approach is not needed since the observed outcomes do not depend on cluster, whereas a value > 0 indicates that the level-2 variable can explain the heterogeneity of the outcome across clusters. Second, a model that included a random intercept and a fixed slope, which examines the variation of the level-1 effects between clusters, was fitted. Slopes were not allowed to change between clusters and the model assessed the direct cross-level effects. Third, random intercept and random slope/s models were fitted to understand the variance of slopes across clusters. This variance is connected to the variation of the effect of the level-1 covariates on the outcome from one cluster (study) to the other. Analysis was performed in R (R Core Team, 2019) using the additional package of lme4 (Bates et al., 2015). The overall assessment of the certainty of the evidence for each study was guided by the five domains of the GRADE framework (Higgins et al., 2021) and classified into four certainty ratings: high, moderate, low and very low.

3.4.2. Risk of Bias (RoB) assessment

Although a number of RoB tools exist (e.g. Higgins et al., 2019, Hooijmans et al., 2014, Schaefer and Myers, 2017, Whaley et al., 2020, Woodruff and Sutton, 2014), a novel tool was needed for application in the field of MP toxicological studies to address the specific issues arising in this particular field. The development of the MP toxicological RoB tool (MP-tox-RoB) has been informed by the US National Toxicology Program's Office of Health Assessment and Translation (OHAT) (OHAT, 2019) RoB tool, guidelines by US EPA (2018) under the Toxic Substances Control Act (TSCA) risk evaluations and the previously developed RoB tool for MP environmental research (section 3.2.7). The principles underpinning its development are those that govern the Cochrane systematic reviews of interventions (Higgins et al., 2019, Sterne et al., 2016). The MP-tox-RoB tool is intended for the appraisal of studies employing experimental study designs. It is structured by domains covering study design, execution and reporting, and includes signalling questions identifying possible routes for the introduction of bias. There are eight domains

tailored to MPs research with 31 signalling questions: test MP and model information, test design, MP exposure characteristics, quality assurance/control and confounding, outcome assessment, analysis, result reporting and other sources of bias followed by an overall rating (Table 7). Some questions use numeric answers, others use a more nuanced, response format (yes, probably yes, no, probably no and no information). These responses were then translated into a RoB rating of definitely low, probably low, probably high, definitely high (Table 6), with each domain rated individually to provide an overall rating of low, moderate, serious, or critical. The overall rating of each study was subsequently used to judge the inclusion of the study's evidence in the rapid review and the meta-regression.

Rating of each signalling question is connected to the response given. The domain level rating however is assigned by judging the rating of all questions within the domain and comparing them to the state-of-the-science across all the included studies in the review. The domain-level rating is connected to the confidence of the studies' results. When one domain is rated as critical, the overall rating cannot be other than critical. When one part of the study is compromised to that degree, the whole study is considered to be compromised. When the majority of domains is rated as serious RoB, then the overall rating must be critical. MP-tox-RoB is not based on static scales but scientific judgement and the currently available body of evidence. In this sense, the tool will be continuously evolving since the standard of each study is measured against other similar studies and not a 'gold standard'. As new studies become available the standard will inevitably shift, aiming to become increasingly higher as studies' quality enhance. It is essentially a state-of-the-science approach not a gold-standard approach. According to FAO and WHO (2009) all toxicological studies that are used in the process of a risk assessment concerning a food hazard should be assessed in terms of design and conduct which is in line with the RoB tool developed and used for this RR.

Table 6. Responses to signalling questions and rating in the MP-tox-RoB tool

Response to signalling question	**Rating of each signalling question**	**Domain and overall rating**
Yes	Definitely low RoB	Low RoB
Probably yes	Probably low RoB	Moderate RoB
Probably no/ No information	Probably high RoB	Serious RoB
No	Definitely high RoB	Critical RoB
Not applicable	Not applicable/rated	-

Table 7. MP-tox-RoB, toxicological Risk of Bias tool Checklist

Domain		Question	Response	Justification	Response rating
Test MP information	1.1	Were the MPs' source and identity provided?			
	1.2	Were the MPs characteristics verified by analytical methods?			
Test model	1.3	Was the test model's identity and origin reported?			
	1.4	Is the model appropriate for the possible routes of MP exposure?			
	1.5	Has the model been used by other MP studies?			
	1.6	Were replicates used?			
Test design	2.1	Were negative controls used?			
	2.2	Were positive controls used?			
	2.3	Did the study use validated tests/assays?			
	2.4	Were the methods of the tests/assays reported?			
MP exposure characteristics	3.1	Was the preparation, storage and administration of the test MPs reported?			
	3.2	Were the concentrations or doses of MPs reported?			
	3.3	Were the concentrations or doses of MPs appropriate?			
	3.4	Was the number and spacing of concentrations appropriate?			
	3.5	Were the durations of exposures reported?			
	3.6	Were the durations of exposure appropriate?			

Quality assurance/ quality control	4.1	Were there precautions for the protection of the test substance/s and the test model/s in place?			
	4.2	Were the negative control samples tested for MPs cross-contamination at the end of the experiment?			
Confounding	4.3	Did the study report potential confounding or modifying variables?			
	4.4	If yes, did the study use appropriate experimental or analytical methods to control them?			
Outcome assessment	5.1	Was the assessment methodology reported?			
	5.2	Was the assessment methodology appropriate?			
	5.3	Was the assessment methodology consistent across study groups?			
	5.4	Was analysis of the samples blinded?			
	5.5	Were analytical or measurement limitations reported?			
Analysis	6.1	Was statistical methodology reported?			
	6.2	Was statistical analysis appropriate?			
	6.3	Were the biological endpoints described and justified?			
	6.4	Were the criteria for the tests/essays reported?			
Results reporting	7.1	Was there a comprehensive reporting of the results?			
Other sources of bias	8				

3.5. Risk assessment methodology

According to the principles proposed by the World Health Organization (WHO, 1999) and the International Programme on Chemical Safety WHO & IPCS (2010), the EFSA (n.d.) the U.S. Food and Drug Administration (FDA, 2002) and the EPA (2017a) there are four main steps in undertaking a risk assessment which follow the formulation of the problem. The methodology for these steps is provided in sections 3.5.1 to 3.5.4.

3.5.1. Hazard identification

Hazard identification is the process by which the agent's identity, and its hazardous properties are determined, drawing from all available evidence (Figure 16). In the context of chemicals in food, the main aims are to identify the nature of the possible health hazards for humans and the circumstances under which they may occur (FAO and WHO, 2009). One approach is that a weight of evidence is developed to characterize the level of evidence supporting the link between the agent and the effects (EPA, 2017a). Historically, the preferred and most reliable data came from human subject studies but in their absence data from other sources were used. Toxicology studies focus on how an organism absorbs and then reacts to an agent (toxicokinetics) as well as on the effects of the agent on the organism (toxicodynamics). Regarding the possible adverse effects, different paradigms of toxicity testing have been developed. Traditional paradigms are generally focused on apical endpoints, which look at effects on a 'larger scale' e.g. on organs, systems etc. while more recent paradigms focus on biological perturbations which are detected at the cellular, and sub-cellular level and can be the signal or the precursor of the eventual effect e.g. High-Throughput Screening (Krewski et al., 2010).

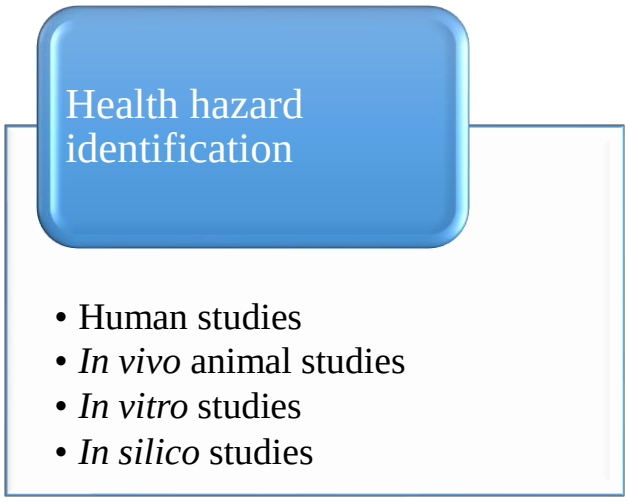


Figure 16. Hazard Identification sources of information

In the arena of risk analysis different practices are adopted by different scientists and organizations according to their aims and the hazard under examination. Hazard identification can focus on the agent's mode of action, which is the underlying mechanism that leads to an effect, it can focus on the effect itself, or it can focus on both (EPA, 2017a, FAO and WHO, 2009). For example, EPA current practice, when assessing a potential carcinogen, is to concentrate on the mode of action.

Hazards are predominantly classified according to their effects to human health. The hazardous properties of MPs are still under investigation. The Globally Harmonized System of Classification and Labelling of Chemicals (GHS) (UN, 2019) offers robust and internationally recognized guidelines on how to investigate, identify, categorize and communicate them. GHS was first published in 2003 and was developed to provide an internationally-harmonized approach of chemicals' labelling and classification regarding safety of their use, transport and disposal (UNECE, 2019). Its implementation was first adopted by World Summit on Sustainable Development (WSSD) in 2002, which was followed by numerous international organizations, governments, institutions and the industry. According to GHS (UN, 2019) health hazards of chemicals are categorized to: acute toxicity, skin corrosion/irritation, serious eye damage/eye irritation, respiratory of skin sensitization, germ cell mutagenicity, carcinogenicity, reproductive toxicity, specific target organ toxicity- single exposure, specific target organ toxicity- repeated exposure and aspiration hazard. The International Agency for Research of Cancer (IARC) proposes further classification of possible carcinogenic agents into four groups: carcinogenic to humans (Group 1), probably carcinogenic to humans (Group 2A), possibly carcinogenic to humans (Group 2B), not classifiable as to its carcinogenicity to humans (Group 3) (IARC, 2019). This further classification is required due the agents' special characteristics e.g. non-threshold effects and the implementation of the weight of the evidence approach (see section 3.7.5) (WHO & IPCS, 2010).

3.5.2. Hazard characterization/ dose response

This step involves examining the cause-and-effect relationship. Scientific evidence must be acquired that demonstrate a causal link between the hazard and the adverse human health effect. Response refers to the biological endpoint that is under examination, whether it is observed in a laboratory *in vitro* using human or animals cells/cell models, or *in vivo* (IPCS, 2009). The definition of dose in the setting of a risk assessment is discussed in the exposure assessment section (3.7.4). Dose-response assessment is the quantitative relationship between exposure to a hazard and the incidence of the response. This relationship between

the amount of a toxicant and the degree of response is observed consistently and as such it is a fundamental concept of toxicology (Klaassen et al., 2013).

If a causal link can be established the next task is to try and set acceptable exposure levels expressed as guidance and/or guideline values (Figure 17). Guidance values come from toxicological and epidemiological evidence and refer to the levels of a compound below which the health risk posed to a person is not appreciable e.g. acceptable daily intake (ADI) and tolerable daily intake (TDI). TDIs are used for contaminants while ADIs are used when the agent can be controlled e.g. residues of pesticides in food (FAO and WHO, 2009). Guideline values are media-specific and implement the guidance values to specific media of exposure e.g. food, air (WHO & IPCS, 2010). Values are dependent both on the route of exposure and the duration of exposure. The dose response data that are used in the dose-response analysis come from *in vivo* and *in vitro* studies on animals and/or human subjects.

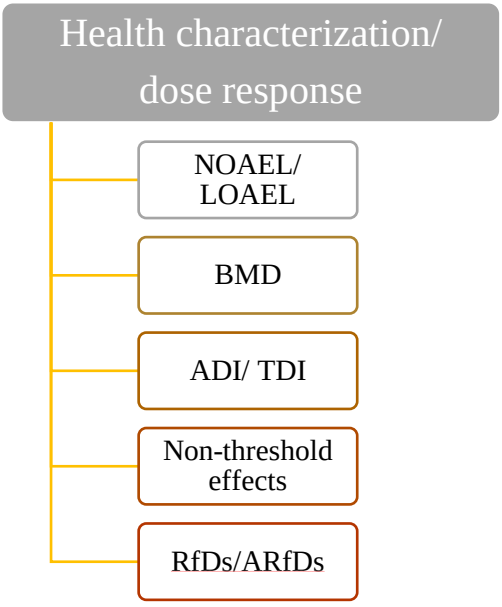


Figure 17. Dose response metrics. Note: ADI, acceptable daily intake; ARfDs, Acute Reference Doses; BMD, Benchmark dose; LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect-level; TDI, tolerable daily intake; RfDs, Reference Doses

3.5.3. Exposure assessment

In this step, the levels of the hazard in different media and in different exposure pathways is assessed relating to specific populations that are likely to be exposed (Table 8). In addition, the duration of the exposures as well as the intensity is taken into consideration. It is evident that these two steps of hazard characterisation and exposure assessment are complementary to each other.

Table 8. Exposure assessment process

Exposure assessment	Evaluate	Source
		Intensity
		Frequency
		Duration
		Route of exposure
		Uptake rate
		Dose or internal dose
	Quantify	Measurement at point of contact
		Scenario evaluation
		Biomarkers of exposure
	Exposure setting	General environment
		Occupational
		Consumer products

3.5.4. Risk characterization

The final step is to assess the likelihood of the hazard to have an adverse effect on health, expressed in a statement of risk that can be either qualitative or quantitative. In doing so, the exposure levels are looked at against the guidance/guideline values (EFSA, n.d.). There are different approaches that are used depending on the medium of exposure as well as the available data and evidence (Figure 18). For example in food borne hazards two of the most frequently used values are the NOAEL and the BMD approach (EFSA, 2017a). In broad terms, the risk of the hazard is a function of the availability of the agent in the environment the level that a population is exposed to it and its toxicity (WHO & IPCS, 2010).

3.5.1. Tiered approach

Risk assessment in all four steps can be applied in different tiers according to the specific aims and objectives and the available evidence and resources. Environmental risk assessments (ERAs) are, by design, tier processes (SETAC, 2018, Solomon et al., 2008, 2016). Tiers are used to manage the complexity of the risk assessment and to manage

resources relevant to realistic expectations for the problem at hand. For example, in a tier one risk assessment, the measured concentration levels of an agent would be compared to screening levels, which are the concentration levels of the agent that are not associated to a harmful effect (SETAC, 2018). If the measured concentration levels are below the screening levels, then adverse effects are not to be expected and the risk assessment can stop there. If adverse effects are possible then further analysis is needed in a next tier. Moving upwards from tier to tier the complexity of the data as well as accuracy and precision increases while predictions become more realistic (Figure 19) (Solomon et al., 2008).

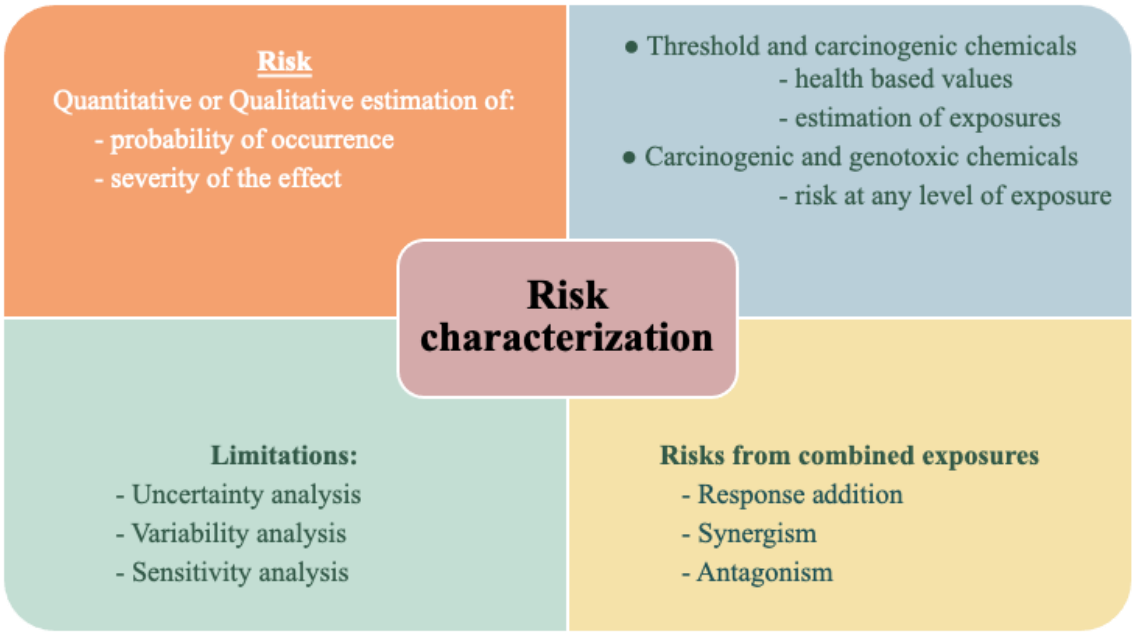


Figure 18. Risk characterization process

Different tiers are proposed by organizations for different purposes. The Organisation for Economic Co-operation and Development (OECD, 2012) uses a three-tier classification for the assessment of chemicals within the Cooperative Chemicals Assessment Programme: initial, refined and comprehensive. Each of the tiers represent biological hazard endpoints. The initial assessment would include one or two species using short-term toxicity tests, the refined would employ long-term toxicity tests (chronic and/or sub-chronic) including more species, and the comprehensive would use data from field studies for the assessment of effects (OECD, 2012).

WHO & IPCS (2010) propose four tiers for human health risk assessment: screening, adaptive, modelling/ field-based and de novo. The tiers represent the source and the level of detail of the evidence (qualitative and/or quantitative) as well as the extend of new data produced in the course of undertaking the analysis. In the screening, adaptive and modelling/field-based tiers only existing data are used. The difference in the adaptive tier is

that in the hazard identification and the exposure assessment steps, data are adjusted to local conditions of the area/population of interest. Modelling/field-based tier utilizes modelling or original measurements for the exposure assessment. The de novo tier uses originally produced data in all the steps of the risk assessment. As evident, analysis becomes more rigorous moving from tier to tier.

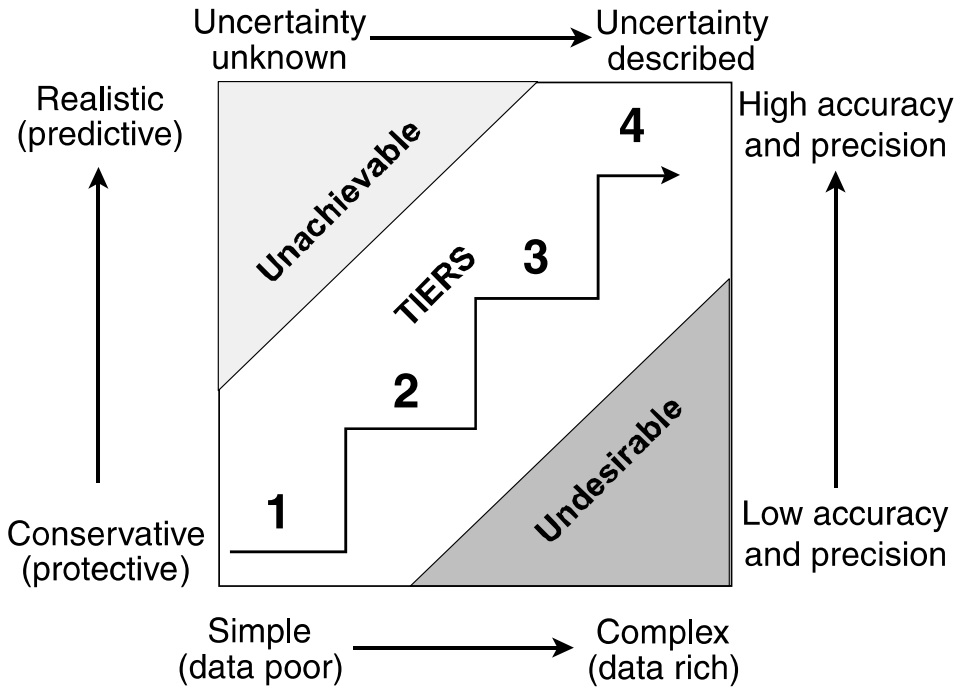


Figure 19. Tiers in environmental risk assessment process (Solomon et al., 2008: 4).

Human health MPs risk assessment presents a number of challenges. MPs are a collection of particles which often have more differences that similarities (Hartmann et al., 2019). To begin with, consensus on their definition is still lacking (Frias and Nash, 2019, Hartmann et al., 2019). Quantitative risk characterization is largely based on defining the dose-response relationship between exposure to the compound and the effect on the organism. When examining MPs, the first challenge is defining the exposure substance. MPs include particles of variant polymeric composition in the micro scale (< 5mm). In addition to polymers they can also be made up by a large amount of plasticizers (polymeric additives), while they have also shown the ability to sorb chemicals they come in contact with when they remain in the environment as well as be a good substrate for microbial colonization (GESAMP, 2015b, 2016, Hartmann et al., 2017, Koelmans et al., 2016). At the onset of the risk assessment, it is imperative to determine which substance is being assessed in other words ‘framing the question’. Other factors causing further complications is that there are different methods used to identify and characterize MPs currently used by the scientific community which

introduces methodological heterogeneity in the available evidence thus diminishing their strength and generalizability (ECETOC, 2019, SAPEA, 2019, Connors et al., 2017).

It has been argued that the quality of the available data to evaluate and communicate the impact of MPs is insufficient (Gouin et al., 2019). In an attempt to use only the best available data, the results of the systematic reviews and the rapid review, will be used to inform the risk assessment process. EPA has recently endorsed the use of systematic reviews as an effective tool for identifying, assessing and integrating evidence for risk assessments (EPA, 2019b). Throughout the risk assessment process, the weight-of-evidence approach is used as well as the precautionary principle (Kriebel et al., 2001, EFSA (SC), 2017, WHO, 2009)

The weight-of-evidence approach includes three major steps: "1) Assembling the evidence, 2) Weighing the evidence, and 3) Integrating the evidence" (EFSA (SC), 2017: 10). In many ways, this approach is similar to the undertaking of a systematic review, with or without a meta-analysis (Higgins and Green, 2011). Both are frameworks that try to achieve transparency and reproducibility while avoiding bias. The collected data are judged according to their reliability, relevance and consistency, which is how data are also assessed in the phase of quality assessment in the systematic review. The outcome of the weight-of-evidence approach will ultimately affect the uncertainty of the results (SCENIHR, 2012). The initial definition of the precautionary principle states that precautionary measures should be taken even when the cause and effect relationships are not fully established (Jackson and Steingraber, 1999). In the context of the protection of the environment, the precautionary principle is the cornerstone of environmental policy and law (Kriebel et al., 2001). The underlying drivers are that in many cases the response to modern environmental and public health issues cannot be as fast as the issues arise. Furthermore, hazards cannot always be identified and controlled. Therefore, the precautionary principle proposes that risk should be avoided even when its likelihood seems small (O'Riordan and Cameron, 2013).

The process of the risk assessment is an iterative process which allows for multiple feedback loops (FDA, 2002). The aim of the risk assessment is not only to produce robust well-informed outcomes but also to communicate the results keeping in mind the welfare of the public. A major component of the risk assessment procedure is establishing an exposure/ uptake route. Looking at the experimental data and the data from the literature review (Chapter 2) regarding the presence of MPs in food and water it becomes clear that there is enough evidence to support human exposure to MPs through the ingestion uptake route and to establish food and drinking water as a vector of MPs into the human body.

3.6. Emerging risk identification

In the face of continuous introduction of novel hazards in food and feed chain, EFSA has adopted the terms of emerging issue and emerging risk. "An emerging issue can be defined as one that has very recently been identified and merits further investigation (EFSA, 2012a: 8), while emerging risk is defined as a "risk resulting from a newly identified hazard to which significant exposure may occur or from an unexpected new or increased significant exposure and/or susceptibility to a known hazard" (EFSA, 2007a: 1). The promotion of an emerging issue to an emerging risk goes through a process that addresses their special characteristics called the Emerging Risk Identification (ERI) procedure. ERI is a Strengths, Weaknesses, Opportunities and Threats (SWOT)-based analysis which is widely used in strategic management (Bull et al., 2016). This approach also incorporates the European regulation for the Registration, Evaluation, Authorization and restriction of Chemicals (REACH) (EFSA, 2014). The process is comprised of three steps:

1. Identification of the emerging issue
2. Identification of data sources and data collection
3. Evaluation of emerging risk (EFSA, 2018)

The evidence for the ERs should be related to a specific indicator and longitudinal trends (EFSA, 2007a). The indicator could be a measurement and/or an observation coming from new research data. The criteria for the evaluation include "novelty, soundness, imminence, scale and severity" (Table 9) (EFSA, 2018: 6).

Table 9. Evaluation criteria for the identification of emerging risks (EFSA, 2018: 16)

1. Novelty	Has a new hazard been identified? If so, which one and how? Hazard known, but re-emerging, either in the same or in another matrix
2. New or increased exposure	Has a possible exposure through the food/feed chain to the new hazard been identified? If so, who could be exposed to the hazard?
3. New susceptibility	Could the possible exposure to the new hazard lead to adverse health effects in (vulnerable) subgroups of the population?
4. Soundness	What is the reliability of the source of information? What is the amount of existing knowledge underpinning the proposed issue?
5. Imminence	How soon it is estimated that the potential emerging hazard will manifest in the food and feed, environment? How soon is it estimated that this emerging health risk will manifest in the population? What is the expected time scale for development of the risk?
6. Severity	What could be the severity of effects on human, plant and animal health in terms of, e.g. magnitude of symptoms, morbidity, mortality, number of individuals affected and potential economic impact.
7. Scale	What is the rate at which it is spreading (i.e. temporal and spatial dimensions)? What is the number of people (animals, plants) and Member States (maximum geographical area) potentially exposed to this hazard (i.e. spatial scale)? What is the maximum duration and or frequency of the potential effects (i.e. temporal scale)?
8. Risk management issue	Is it already subject to risk management measures and or controls?

In order to address the weaknesses of the existing evidence frameworks such as the DPSRI model could be employed in order to provide better results (Kristensen, 2004). The approach that was used to determine whether MPs and NPs should be deemed a food-borne emerging risk is illustrated in Figure 20. The outcome of the ERI procedure was that MPs and NPs are indeed an emerging risk and thus a full risk assessment must be undertaken. This evidence-based decision was informed by the findings the MP food ScR (section 2.3) which illustrated that MPs have entered the human food web. A number of food items were found to be contaminated with MPs in varying levels.

3.6.1. Conceptual model for MPs and NPs in seafood

It has already been established that seafood is an emerging risk in terms of introducing MPs into the human body (section 2.3.1, 3.6). Taking this finding further, a conceptual model was developed that took into consideration the unique characteristics of MPs and NPs found in seafood intended for human consumption (Figure 21). The aim was to illustrate the "decision tree" approach implemented for MPs in this food theme. The model was informed by relevant guidelines and models that are already used for other contaminants and for assessing environmental risks and drives (FDA, 2002, EFSA (SC), 2018, EFSA (SC), 2017, SCENIHR, 2012). As shown in Figure 21, all the steps feed into the final step which is the risk characterization. Within this step, all the evidence collected and described in the previous steps are collated, leading to a quantitative assessment of the risk by the use of a statistical model (in the absence of substantial data, a qualitative assessment and a gap analysis is selected). Part of this process was also the exercise of validating the statistical model and conducting an uncertainty/ influence analysis (FDA, 2002). The available data can roughly be divided into three categories: exposure information, characteristics of the contaminants and adverse health effects.

A major component of the risk assessment procedure is establishing an exposure/ uptake route. Looking at the experimental data and the data from the literature review regarding the presence of MPs in seafood and in other types of food it becomes clear that there is enough evidence to support human exposure to MPs through the ingestion uptake route and to establish food as a vector of MPs into the human body. The findings of a risk assessment could also provide proof that the MPs do not pose a risk to human health. The emerging risk identification process as well as the conceptual framework have been published as part of a primary research, review and risk assessment scientific paper (Akoueson et al., 2020).

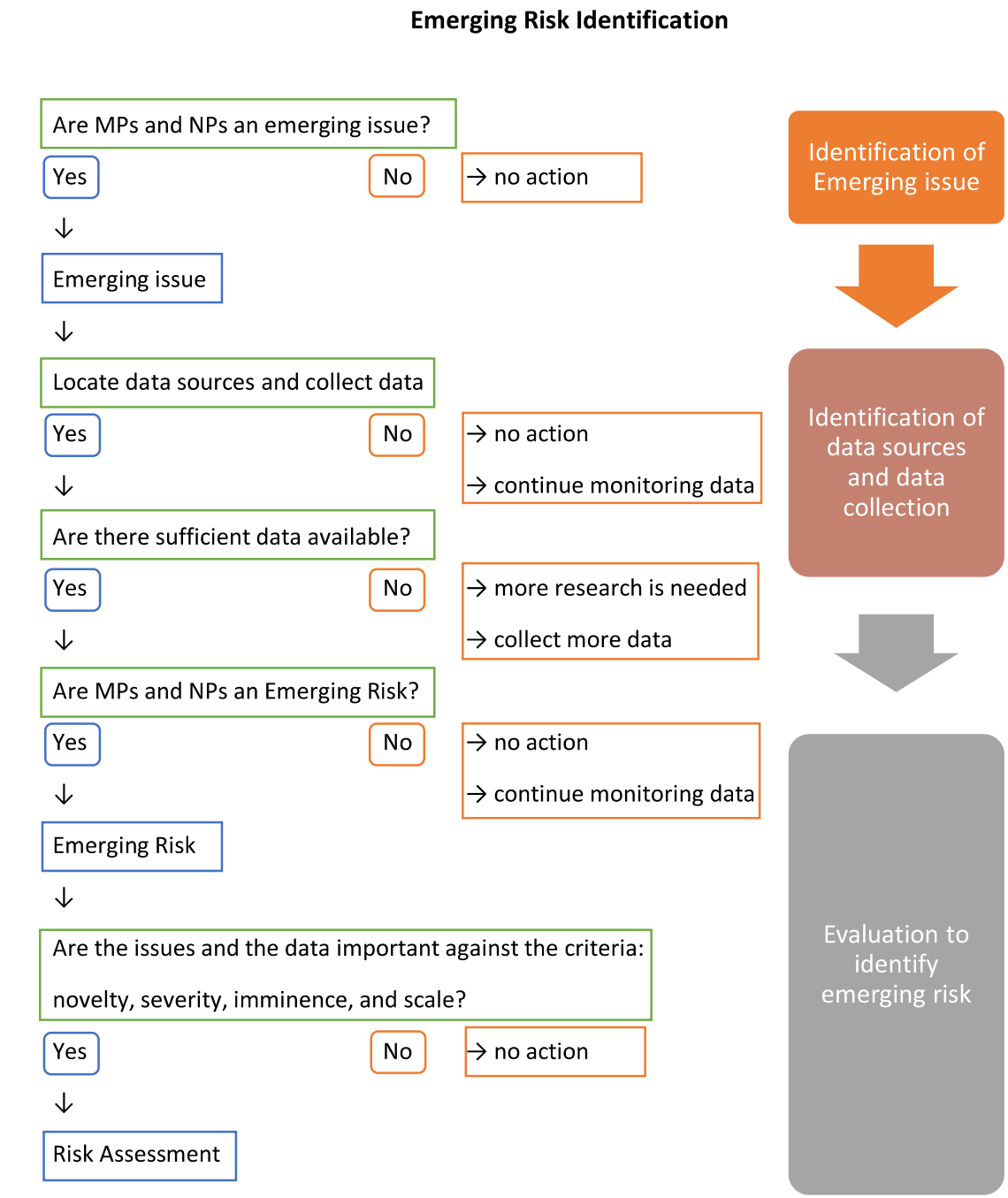


Figure 20. Emerging Risk Identification (ERI) procedure diagram for MPs (and NPs)

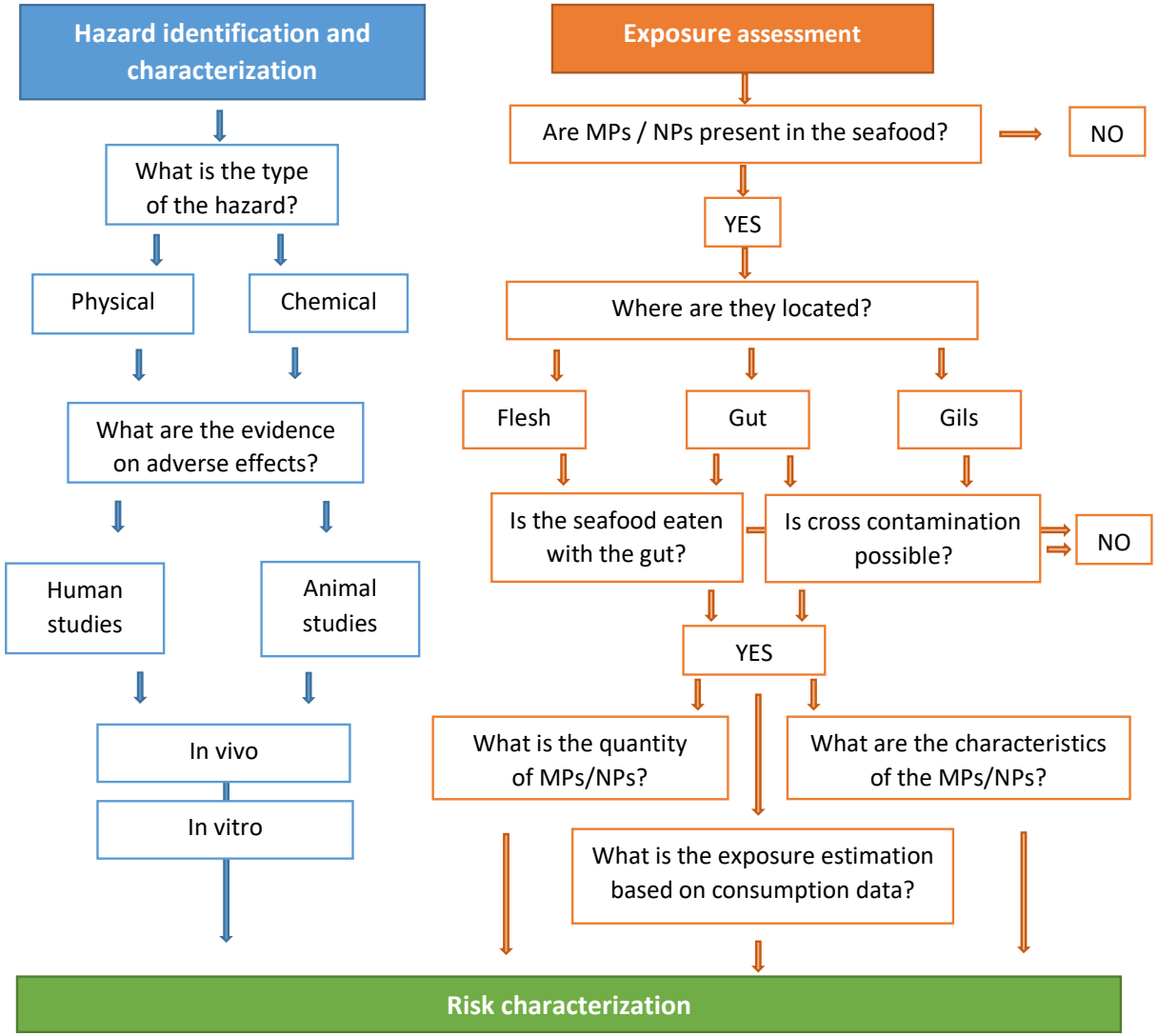


Figure 21. Risk assessment diagram for MPs (and NPs) in seafood

3.7. Risk assessment methods

3.7.1. Question formulation

The aim of this risk assessment is to evaluate the risk posed to the general population from exposure to MPs via dietary ingestion. If data for more specific populations are available, they will be used for the adaptive tier (see section 3.5.1). The risk assessment will examine MPs as both a chemical hazard and a physical hazard. Regarding the chemical hazard the risk assessment will only focus on the MPs' inherent chemical components not the chemicals that they might absorb when found in the environment. The desired outcomes are the qualitative and quantitative risk characterization of MP human environmental exposures.

The definition that is used for the MPs is the one proposed by the European Chemicals Agency: **"microplastic** means a material consisting of solid polymer-containing particles, to which additives or other substances may have been added, and where ≥ 1% w/w of particles have (i) all dimensions 1nm ≤ *x* ≤ 5mm, or (ii), for fibres, a length of 3nm ≤ *x* ≤ 15mm and length to diameter ratio of > 3. Polymers that occur in nature that have not been chemically modified (other than by hydrolysis) are excluded, as are polymers that are (bio)degradable." (ECHA, 2019: 29). The only difference from this definition is that the lowest end of particle size will be 100 nm and every particle below this threshold will be termed NPs and will not be part of the risk assessment.

There is an abundance of definitions around MPs since the term was coined in 2004 (Thompson et al., 2004). This definition was preferred as it is a regulatory definition developed taking into consideration recent scientific advice (Hartmann et al., 2019), definitions found in legislation (Microbead-Free Waters Act of 2015) as well as internationally recognized standards (ISO, 472:2013) with a view to address a regulatory body (EU). One of the aims of any risk analysis is to be used by policy makers and other stakeholders, in these terms this definition is more fit for purpose than others.

The sources of MPs can be either primary (intermediate feedstock, pellets/ resin, by-products) or secondary (fragmentation and degradation) (Carbery et al., 2018, Cole et al., 2011, Frias and Nash, 2019, Hartmann et al., 2019, Karlsson et al., 2018). The origin of MPs is important for the risk management in the regulatory perspective since responsibility for pollution and remediation actions can be assigned (Brennholt et al., 2018, ECHA, 2019).

Two environmental routes of exposure have currently been proposed:

- Ingestion:
    - dietary via food for human consumption, which includes drinking water and
    - non-dietary via dust, soil (e.g. hand-to-mouth behaviour)
- Inhalation.

These routes of exposure have been established by numerous studies, reviews and reports (Bouwmeester et al., 2015, EFSA, 2016, Gallo et al., 2018, GESAMP, 2016, Karbalaei et al., 2018, Amy Lusher et al., 2017, Prata, 2018, Sauler and Gulati, 2012, Schirinzi et al., 2017, Smith et al., 2018, Waring et al., 2018, Wright and Kelly, 2017, etc.). The presence of MPs has been verified in the human digestive tract (Ibrahim et al., 2021), in human stool (Schwabl et al., 2019), in human lung tissue (Amato-Lourenço et al., 2021) and in human blood (Leslie et al., 2022). A third environmental exposure route has also been proposed via dermal absorption but currently there is no evidence to support it (BfR, 2014). Another recognized exposure route (not environmental) to MPs is via the degradation of medical prosthetics that are entirely made of or contain plastic (Doorn et al., 1996, Minoda et al., 2003, Urban et al., 2000, Willert et al., 1996). This exposure constitutes a distinct paradigm for human MPs exposures that warrants a separate risk assessment. The focus of this risk assessment is only the dietary ingestion route. The choice to focus only on the dietary ingestion route was made due to its importance and the limited timeframe of the project.

Humans are presumed to be exposed to MPs via multiple media. Nevertheless, at this point in time, scientific evidence on specific MPs concentrations in media of exposure are limited. Likewise, the exposure scenarios used in exposure modelling are limited to the existing scientific evidence and include only a part of the hypothesised media. It must be noted that these are not the only possible routes, pathways and medium of exposure to MPs but the ones that are currently supported by robust scientific evidence as evaluated by the systematic reviews (see sections 3.1 and 3.2).

The focus of the risk assessment is the general population thus addressing the omnipresent MPs contamination of the environment and therefore the ubiquitous human exposures. Specific subgroups might be more exposed based on their geographical habitat, occupation, cultural background etc. while some could be in more risk due to age, gender, pre-existing conditions etc. Effort was made to highlight these subgroups throughout the risk assessment, according to the available data that are specified in the exposure assessment chapter (section 8.3).

Exposures of the general population can be acute, sub-chronic, chronic/life-long and intermittent according to the setting (IPCS, 2009). For example when examining exposures via inhalation of general populations living in a specific geographical location, as in the studies by Abbasi et al. (2018) and Lijun Wang et al. (2017), chronic exposures were considered. On the other hand, when examining occupational exposures, as in the study by Gallagher et al. (2015), sub-chronic exposures were considered. The life stage during which exposures occur could also be significant as highlighted in the study by Sun et al. (2017) which examined dust found in children's bedrooms. The inhalation route is used as an example. Both these time parameters are taken into consideration in the risk assessment process.

A tiered approach to risk assessment is adopted (WHO & IPCS, 2010). Tier 1 and 2 (screening and adaptive) is to be implemented for all media of exposure. Tier 3 (modelling) is implemented for the dietary ingestion uptake route using the results of the systematic reviews on MPs contamination of food intended for human consumption (Chapters 4-6).

3.7.1.1. Conceptual model

Conceptual models are used regularly in the risk assessment process as a planning tool. They can be used to illustrate the possible sources of the contaminant/s, the routes and the pathways and the media involved in the exposure. A framework is created demonstrating the links between the source of the contaminant and the possible exposure points. In developing the conceptual model seven dimensions are taken into consideration (EPA, 2014):

- Characteristics of the population at risk
- Sources of the contaminant
- Characteristics of the contaminant
- Characteristics of exposure (pathways, fate, transport, exposure routes)
- Characteristics of the adverse health effect endpoints
- Temporal characteristics of exposure
- Toxicokinetic characteristics

The conceptual model for MPs in seafood can be found in section 3.6.1 (Figure 21). This framework can also be implemented for other media of dietary exposures. Figure 22 illustrates the framework for MP contamination of drinking water and the potential routes for human exposures that were considered for the systematic review (Chapter 5).

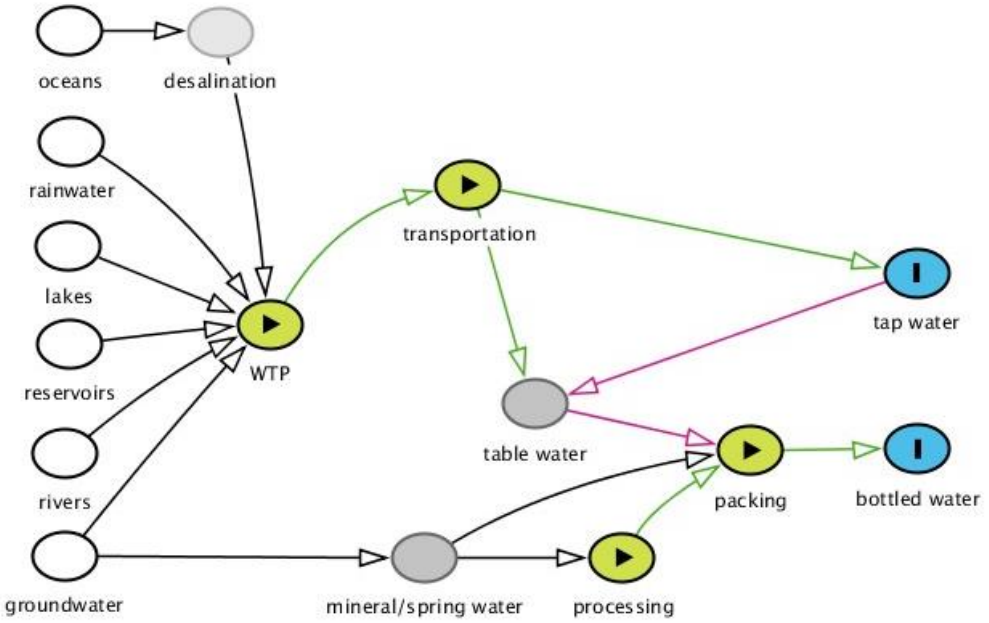


Figure 22. Conceptual framework for MP in drinking water human exposures. WTP, water treatment plant

An ERA framework that takes into consideration the issues stemming from the nature of MP was proposed by (Gouin et al., 2019). The framework highlights how analytical processes for the identification of MP and the consideration of the fate of MP in nature are linked to the risk assessment process.

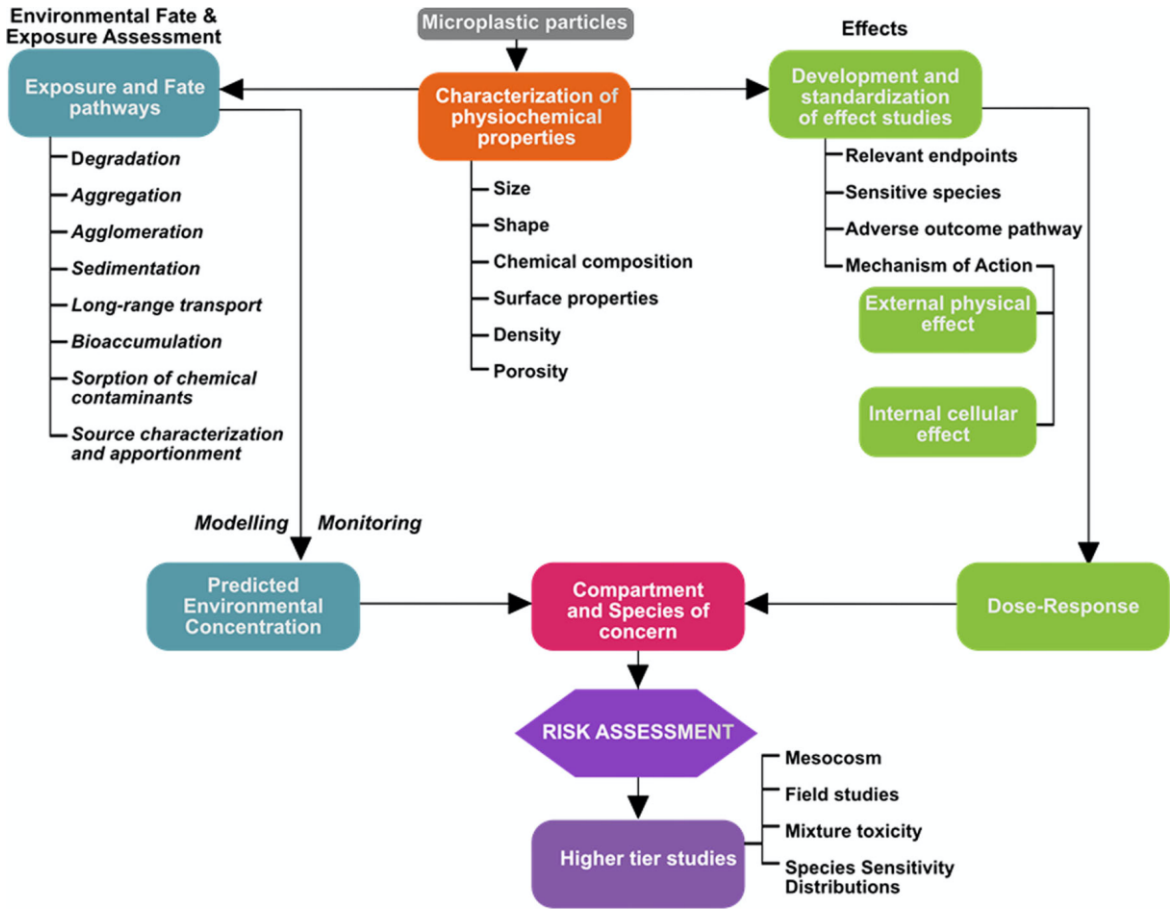


Figure 23. Environmental risk assessment framework for MPs (Gouin et al., 2019: 2091)

3.7.2. Hazard identification

In the case of MPs, one can argue that they can fall in two categories. If MPs are examined as inert agents and the focus is on their physical characteristics (shape, size etc.) they would be classified as physical hazards. On the other hand, if the focus is on their chemical properties (inherent or additives) or vectors of contaminants, they would be classified as chemical hazards (EFSA, 2016). In any case, chemical and physical characteristics of MPs must be examined in conjunction because they are interlinked. For example, the size of MPs is directly related to their uptake by the human body (EFSA, 2016). MPs are a collection of particles varying in size, shape and chemical composition. The accepted definition (see section 3.7.1) is not enough for the purposes of the risk assessment. Their characteristics must be identified and described in detail. The results of the systematic reviews on MPs contamination of food and drinking water include a comprehensive description of the MPs chemical and physical characteristics (Chapters 4-6).

A key part of hazard identification is the exploration of the relationship between external doses and biological effects (EPA, 2014). This relationship depends on the disposition of the contaminant in terms of toxicokinetics and toxicodynamics, which is, in turn, defined by the behaviour and fate of the contaminant upon its contact with the human body. Toxicokinetics can be described in the four stages of absorption, distribution, metabolism and excretion (ADME), also discussed in the exposure assessment section (3.7.4). Toxicodynamics describes the interaction of the substance within the body at the molecular, cellular, tissue and organ level (Klaassen et al., 2013). ADME is affected by the substance's physicochemical characteristics and/or structural properties such as size, molecular weight and water or lipid-solubility (Duffus et al., 2009). Absorption is the process of transfer from the site of administration into the human body. For the ingestion route that would be the crossing of the gut barrier. Some substances may only reach as far as the epithelium, for others the uptake may continue to the lumen or the gut wall, while some cross the barrier altogether. The distinction between them is key as, for certain substances, this will dictate the bioavailability of the substance (Duffus and Worth, 2006). Distribution refers to the transfer of the substance or its metabolites to the rest of the body. It is measured as the rate of distribution and the extent of distribution. Metabolism is the structural change of the substance in order to be eliminated from the body, often termed biotransformation or detoxification. In some cases, the metabolism process of the metabolites affects and even enhances the toxic effects. Finally, excretion includes all the processes that participate in the elimination of the substance from the body, usually involving its transformation into biological waste products (urinary of faecal excretion) (Klaassen et al., 2013). *In vitro*

experiments on human cells have the limitation that the ADME processes are bypassed. On the other hand, they can provide vital information on mechanistic characteristics, response mechanisms and metabolic pathways as long as toxicokinetics are taken into consideration for the dose determination (also see exposure assessment section 3.7.4) (FAO and WHO, 2009). ADME processes and the related MPs characteristics also relate with the other risk assessment stages of dose-response and exposure assessment as discussed in sections 3.7.3 and 3.7.4.

The hazardous properties of a substance are examined in two levels: apical endpoint and toxicity mechanisms (Jeong and Choi, 2019). Apical endpoints focus on the caused effect while toxicity mechanisms focus on the MPs mode of action leading to the caused effect. The design and the execution of the ScR on MPs health effects (section 2.2.1) and the rapid review and meta-regression analyses of the toxicological impacts of MP exposure in human cells (Chapter 7), was tailored for the needs of the hazard identification process. The strengths and limitations of the data is reported, as well as data gaps.

3.7.3. Hazard characterization/dose-response relationship

The output of traditional hazard characterization is a qualitative or quantitative description of the agent's hazardous properties expressed in an estimation of risk or a development of guidance and guideline values, set in the specific situation of interest according to the aims of the risk assessment (WHO & IPCS, 2010, FAO and WHO, 2009). Guidance and guideline values are developed by international organizations as well as national authorities. In the case of EECs, such values may not yet be available or may be under examination. Hazard characterization of MPs therefore, would include the development of health-based guidance values (HBGVs) based on available toxicological and/or epidemiological evidence which would provide the estimate of the safe levels of human exposure considering intakes from the dietary ingestion route of exposure. In turn, the guidance values would be used to determine the guideline values specific to the media of interest. For food contaminants the term "tolerable" instead of "acceptable" is generally used to describe the HBGVs and they are expressed in terms of tolerable daily intakes (TDI). When there is still uncertainty around the levels of exposure and effects, a provisional intake is proposed. According to FAO and WHO (2009), if the food contaminant has been shown to accumulate in the body the provisional maximum tolerable daily intake (PMTDI) should be used, but when accumulation is expected, the provisional tolerable weekly (PTWI) and monthly (PTMI) intake should be used.

Currently, there is no epidemiological evidence concerning MPs exposures. The only available scientific evidence comes from animal *in vivo* and *in vitro* studies, as well as human cell *in vitro* studies. For the purposes of this risk assessment, hazard characterization is informed by the results of the rapid review and meta-regression analyses of the toxicological impacts of MP exposure in human cells, which was one of the two available alternatives to epidemiological data (see Chapter 7).

Dose-response analysis will employ modelling of the available data to examine whether they can be used to estimate human relevant doses (FAO and WHO, 2009). Dose response modelling can be divided into two major activities : dose-response information analysis and use of the results to reach a conclusion (IPCS, 2009). Modelling follows a six-step process which consists of selecting the data, selecting the appropriate model, choosing the statistical linkage between the model and the data, estimating the parameters of the model (dose-response analysis) and finally, implementing the model and evaluating the results of the analysis (FAO and WHO, 2009).

The approach of determining dose-response values is mainly divided into two categories for threshold effects and non-threshold effects or non-linear and linear dose-responses, respectively (EPA, 2017a, WHO & IPCS, 2010). The information on which category they fall into comes from the hazard identification process outputs in terms of the end points under evaluation (section 7.2). Carcinogenic and genotoxic agents have a linear relationship with risk of cancer. Cancer can be considered both a threshold and non-threshold effect according to the underlying mode of action (IARC, 2020). Threshold approaches are only used to evaluate nongenotoxic mechanisms of carcinogenicity (Klaassen et al., 2013). Non-threshold means that they can occur at any level of exposure. On the other hand, for threshold effects it is possible, in theory, to determine a specific value below which adverse effects would not be expected (WHO & IPCS, 2010). Typically, as doses (or concentrations) increase the measured effects also increase.

Dose-response relationships can be different for the same agent for different effects and different populations. They can describe relationships on an individual organism level (individual or graded dose-response relationship) or on a population of organisms level (quantal dose-response relationship). Since these relationships are virtually infinite, toxicological studies focus on specific effects and subjects in order to be more effective. Following the identification of all available studies on MPs adverse effects (relatable to human health) the effect or the precursor of an effect that occurs at the lowest dose/concentration will be identified as the critical effect (CrEf) (EPA, 2017a). The CrEf

will effectively drive the risk assessment, under the assumption that if it is averted, human health will not be at risk. The most robust scientific data and the most relevant route of exposure should be used to inform the CrEf (Klaassen et al., 2013). These assessments are carried out within the rapid review process (see section 3.4).

Different approaches may be used to characterize the threshold responses. The most often used are the NOAEL, the lowest-observed-adverse-effect level (LOAEL) or the BMD. For regulatory purposes, when there are several NOAELs available, the focus is on the highest one, thus determining the highest dose at which no statically or biologically significant adverse effect was detected. When a NOAEL has not been determined experimentally, the LOAEL is used instead as the highest dose that was tested (EPA, 2017a). These thresholds inform the point of departure for calculating lower exposure doses. NOAELs are usually used to calculate further risk assessment calculations such as the reference doses (RfDs) and the ADI or the TDI (Klaassen et al., 2013). These are the levels of exposure that are considered to be acceptable or tolerable. The BMD is a dose that causes the benchmark response (BMR), which is a predetermined change in the rate of the effect. The BMR is usually set at a 10 or 5% change in the response rate compared to the response of the control group and is connected to the characteristics of the data.

In order to determine the NOAEL, the dose-response relationship (model) is expressed as:

$$R(D) = \begin{cases} 0 \; if \; response \; (R) \; at \; the \; dose \; (D) \; is \; not \; significantly \; different \\ from \; the \; response \; of \; the \; control \\ 1 \; if \; response \; (R) \; at \; the \; dose \; (D) \; is \; significantly \; different \\ from \; the \; response \; of \; the \; control \end{cases}$$

Equation 2

The statistical linkage between them is the pairwise statistical test that was used by each study to execute their analysis and compare dose groups and control groups (IPCS, 2009). The next step of parameter estimation is done to assess the point of departure. The selection is based on the dose below which all R(D) was 0 and above which all R(D) was 1, therefore this procedure is based on the assumption that all doses below the NOAEL will not be significant and all doses above the NOAEL will be significant. This can be expressed as:

$$NOAEL = D_{NOAEL},$$

where:

$$R\ (D) = 0\ for\ all\ D \leq D_{NOAEL}$$

and

$$R\ (D) = 1\ for\ all\ D > D_{NOAEL}.$$

In the absence of dose-response data coming strictly from human subject studies, animal and *in vitro* studies can be used. Nevertheless, when extrapolating from animal, human or *in vitro* trial results to the general human population a series of uncertainty factors (UFs) need to be taken into consideration in order to avoid underestimation of risks. In a non-linear quantitative expressed dose-response (e.g. RfD) or reference concentration (RfC)) the numerical expression of UFs that has historically been used to account for uncertainty due to variability between animals and humans in an order-of-magnitude expression of 10-fold as is the UF to account for variability between humans (Klaassen et al., 2013). (EPA, 2017a, FAO and WHO, 2009). An additional UF could also be used to address possible experimental limitations/ shortcomings. In the absence of evidence to calculate a NOAEL the 10-fold factor can be used to extrapolate from the LOAEL (EPA, 2017a). There is also a recommendation to use an additional 10-fold factor to create infant and children thresholds for pesticides (EPA, 1996). On the other hand, modifying factors (MFs) can be used to reduce the magnitude of the UFs when there are available data which evidence that there is a stronger relevance between animal and human subjects. For example, if there are evidence that the toxicokinetics of a substance is very similar between the experimental animal and humans, the UF can be reduced from 10 to 3 (Klaassen et al., 2013). The equation that can be used to express UFs and MFs is:

$$RfD\ (or\ ADI) = \frac{NOAEL(or\ LOAEL\ or\ BMDL)}{UF\ \times\ MF}$$

Equation 3

Effort is put into using more detailed UFs instead of the 10-fold correction, based on toxicokinetic and toxicodynamic studies. These studies provide more detailed and relevant information for the toxicological characteristics of the substances under examination. The use of more specific UFs could drastically reduce the uncertainty in the calculation of thresholds. WHO and IPCS (2005a) propose the use of the following UFs introducing the concept of chemical specific adjustment factors:

- 2.5 for toxicodynamic differences between animals and humans
- 4 for toxicokinetic differences between animals and humans

- 3.16 for toxicodynamic variability in humans
- 3.16 for toxicokinetic variability in humans.

The BMD is an alternative to the NOAEL approach and its implementation follows a similar path, but it is far more statistically intensive. After the data selection is completed, a dose-response (regression) model must be chosen such as the log-logistic, log-normal and Weibull models, which must be appropriate for the data and the characteristics of the response e.g. continuous, binary, discrete (Ritz et al., 2016). The statistical linkage in this case is usually defined by the statistical distribution of the response data (e.g. binomial distribution for quantal data), but simpler linkages can also be used (IPCS, 2009). Parameter estimation is based on the nature of the data, the model that is used and the aims of the modelling. Several different parameters can be fitted in statistical software (e.g. R) and comparisons can be run to choose the ones that produce the best fit (Ritz et al., 2016). According to the EPA (2012) the BMR for both continuous and quantal data is 10%, while (EFSA, 2017b) proposes a 5% BMR for continuous data. The results of the estimated BMD model is a confidence interval, the lower confidence limit of the BMD (BMDL) is taken to calculate the HBGVs. UFs and MFs are applied as for the NOAEL method.

There are arguments on the use of both approaches, NOAEL has been criticized for not taking into consideration the dose-response curve, being dependent on sample size and dose selection and using only a tested experimental dose (EFSA, 2017b). On the other hand, BMD has been criticized for producing shallow dose-responses, being affected by the spacing of the applied doses and its use might be limited by the format of the available data (Klaassen et al., 2013, EFSA, 2017b). The BMD approach has advantages over NOAEL in that it can extrapolate beyond the experimental doses by using modelling and that it can better incorporate uncertainty and sample sizes (IPCS, 2009). Nevertheless, the choice between them is, in many cases, determined by the available data, since each approach requires specific data format. It should be noted that another key difference between them, as well as an source of criticism, is that NOAEL can sometimes not be a solely statistical procedure, and the risk assessor is, on occasion, allowed to make expert decisions (EFSA, 2017b).

A method to overcome the limitations of the non-threshold approaches in defining an actual dose is the use of the margin of exposure (MOE) approach for substances that are both carcinogenic and genotoxic (EFSA, 2012b). The method was developed by the EFSA for contaminants in the food chain. The MOE approach uses a reference point from the dose-response relationship observed in an animal study, which is a dose causing a low cancer incidence. This reference point is then compared to the human dietary exposure estimates

and is expressed as a ratio of these two factors. No correction factors are usually applied. The approach is not confined for the non-threshold substances but can also be used when the available scientific evidence is not sufficient. Low MOE values show that the NOAEL values in animal subject are close to human exposure levels. For example, values below 100 have prompted further investigations by regulatory stakeholders (Klaassen et al., 2013).

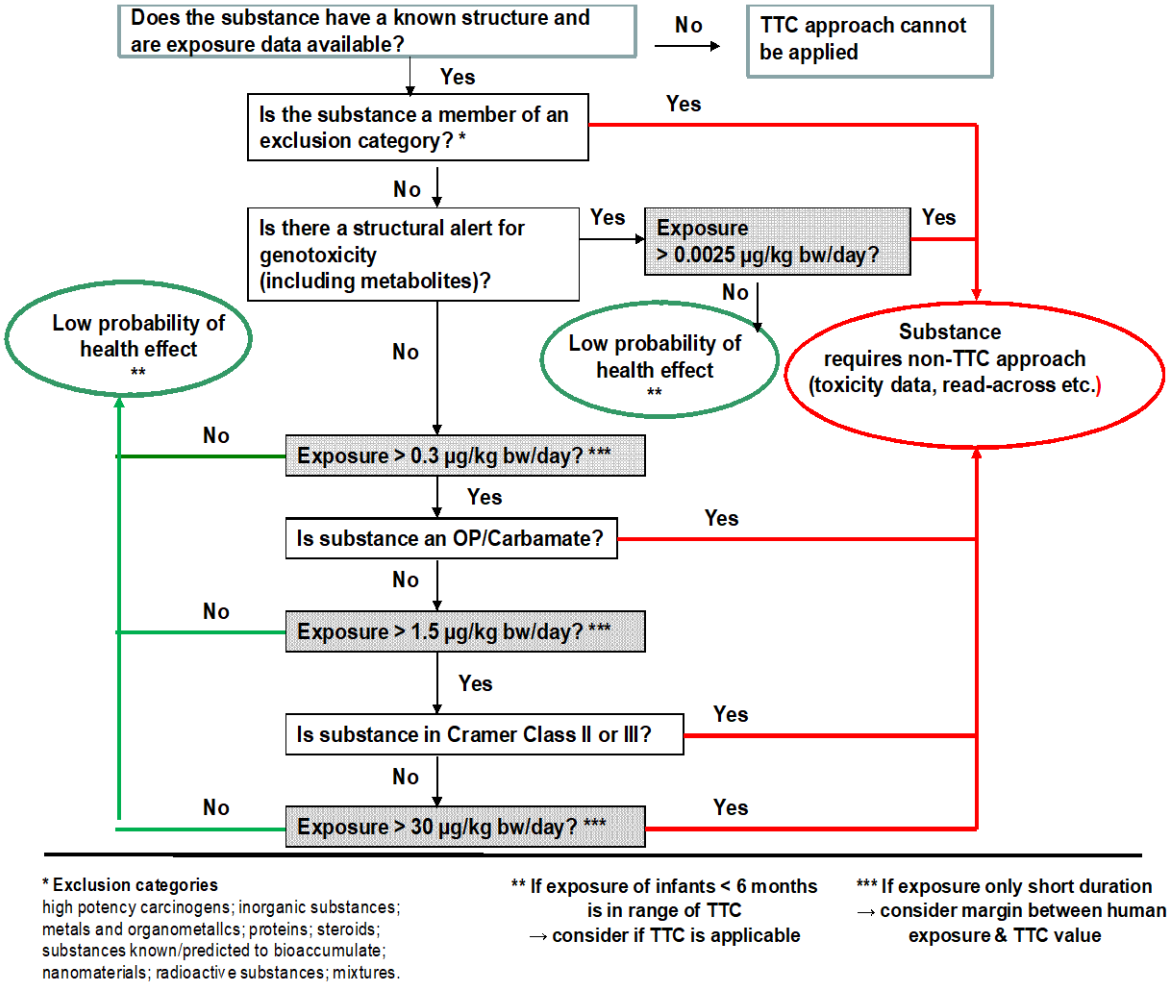


Figure 24. Generic scheme for the application of the threshold of toxicological concern (TTC) approach (EFSA (SC), 2012: 51)

A further method that can be used in the absence of sufficient data to formulate a HBGV, is the use of the screening tool of termed threshold of toxicological concern (TTC) (EFSA, 2012b, FAO and WHO, 2009). The TTC approach is based on the chemical structure of the substance and the human dietary exposure estimates. TTC can be used for both threshold and non-threshold assessments and is of use when the toxicity data for a chemical are limited (EFSA (SC), 2019). Generic human exposure threshold values (TTC values) are established depending on the structure and then these values are compared to the exposures. If they are not exceeded, it is assumed that there is very low probability of adverse effects (EFSA (SC), 2012). TTC can be used as priority setting screening tool or to establish if further

investigations are warranted. The TTC approach is based on classifying chemicals into categories, first developed by Cramer et al. (1978). Chemicals are classified into three categories (Class I, II and III) according to their chemical structure and whether they occur naturally in food and in the human body (EFSA (SC), 2019). EFSA (SC) (2012) has summarized the TTC process in a generic scheme illustrated in Figure 24.

3.7.4. Exposure assessment

The outputs of an exposure assessment are whether people come in contact with a hazardous agent, the quantified level of exposure (magnitude), the media of exposure, the route of exposure, the duration and the frequency of exposure (EPA, 2017a, 2019b, WHO & IPCS, 2010). Besides determining the characteristics of exposure, two other elements should be considered in the process of exposure assessment:

- Information on how the exposures can be reduced.
- Information on changes of the exposures over time.

3.7.4.1. Exposure characteristics

The exposure characteristics that must be determined within this process are:

- Pathways of exposure
- Routes of exposure
- Media of exposure
- Magnitude of exposure
- Duration/frequency of exposure

They can either be estimated directly by measurements on biological media or indirectly by considering the measured concentrations of the hazard in the environment and modelling regarding human intake. Biological media that are used to measure the internal dose of an agent include blood, urine, faeces, saliva etc. Currently there is only one study providing information of this kind which detected MP in human stools (Schwabl et al., 2019).

In this risk assessment, the parameters are informed indirectly by the results of the systematic reviews (see Chapters 4-6) using MPs concentration evidence in the exposure media from observational studies and creating relevant environmental exposure scenarios and modelling. The ScRs (see section 2.3.1 and 2.3.2.1) identified evidence coming from primary studies and reviews on MP contamination of food and drinking water, in the first instance. Aggregate (combined) exposures scenarios from multiple routes (ingestion and inhalation),

pathways and media can be estimated as shown in Figure 25 (EPA, 2019b, FAO and WHO, 2009), to better describe real-world situations according to the available data. According to the focus and the scientific evidence collected for this risk assessment only the aggregate dietary pathway via ingestion will be explored.

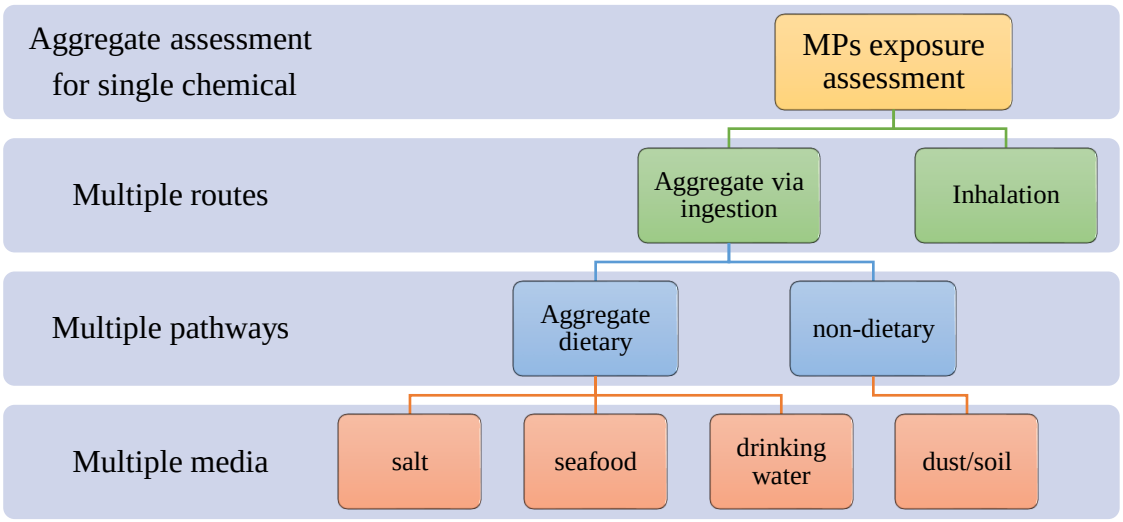


Figure 25. Aggregate exposure assessment framework for MPs.

3.7.4.2. Uncertainty/variability of the data

The data coming from observational environmental studies are expected to introduce a level of uncertainty and variability. Uncertainty refers to the internal and external validity of the studies. Both aspects are assessed by the methodology and methods implemented in the systematic reviewing process, in the RoB assessment of individual studies and the overall assessment of the certainty of the evidence across all studies per medium (see Chapters 4-6) (Higgins et al., 2019). The quantification of uncertainty of the data is expressed in the meta-analysis section of the systematic reviews as confidence intervals (95% CI). On the other hand, variability of the data can be attributed to natural differences that are to be expected in any environmental sampling and analysis of a population or medium and are expressed in individual studies in SD or standard error (SE). Variability has also been taken into consideration in the meta-analysis and the narrative analysis sections of the systematic reviews. In this risk assessment, the level of exposure, derived from environmental studies, is expressed as the concentration of MPs per mass or volume, depending of the medium (food, drinking water).

3.7.4.3. Exposure modelling

In order to estimate exposures, the data identified are used in modelling. Models that are used in the context of environmental exposure assessments can be defined as "a simplification of reality that is constructed to gain insights into select attributes of a particular physical, biological, economic, or social system" (NRC, 2007: 31). Computational models are comprised by two interconnected processes: the construction of a conceptual model that describes all the aspects/factors of exposure (see section 3.6.1) and the derivation of a mathematical model to express that conceptualization (EPA, 2019b). Models in exposure assessments can help:

- Analyse complex real-world processes for which empirical data cannot be collected or do not exist at this moment.
- Extrapolate to populations for which data do not exist to:
    - estimate environmental concentrations,
    - estimate exposure factors.
- Attempt retrospective of future extrapolations based on informed scenarios.
- Integrate available data of exposures to develop estimates that are consistent with current scientific knowledge.
- Evaluate potential reduction of exposures coming from the implementation of specific policies. (EPA, 2009, 2019b, Lobdell et al., 2011).

The computational models can range from more simple deterministic models to more complex probabilistic models. The choice of model reflects the aims of the risk assessment and the available data. The main difference is that deterministic models estimate single outcomes based on single value model parameters, whereas probabilistic models predict a range of probable exposures (probability distribution). Deterministic models are often used for a screening-level risk assessment which are used to examine whether an agent can pose a risk to human health (EPA, 2019b). These models can use average values across populations to estimate the average exposure of the individual. When the input values are realistic, the exposure estimates computed by the deterministic models will fall in the high end of the anticipated exposure distribution. Both approaches use the same parameters: concentration of the agent, intake rate, duration of exposure. According to the aims of this risk assessment, which fall in the screening-level, deterministic models are used in the first instance.

Modelling includes all the different media for the dietary pathway of the ingestion route of exposure in conjunction with the expected duration of exposure, expressed on a basis of

daily and yearly exposures. Two types of models are used: human exposure models and dose estimation models. The human exposure models will be used to calculate the prediction of the exposure to MPs i.e. the external dose (intake), while the dose estimation model will be used to predict the internal doses resulting from the exposure to MPs (uptake).

Environmental exposures and the consequent health effects are not uniformly distributed across different populations. Vulnerability and susceptibility characteristics might affect the resulting health risks. Possible vulnerability factors are related to culture, lifestyle, diet, geography and socioeconomic statues, while susceptibility factors can be related to gender, age or life-stage, genetics and health status (EPA, 2019b). Effort is made to differentiate exposures in different sub-populations especially by age group (e.g. children and adults) following a measurement-based approach, when the necessary data to do so were available.

To model ingestion exposures, published data from international organizations will be used such as: the Food and Agriculture Organization of the United Nations (FAO, 2020) for seafood consumption, the European Food Safety Authority (EFSA NDA, 2019), the U.S. Department of Health and Human Services and U.S. Department of Agriculture (2015) for salt consumption and (WHO, 2017, 2020b) for salt and drinking water consumption. Due to the lack of scenario- and location-specific data for exposure factors across all media under consideration, the default values provided by the aforementioned organizations and agencies will be used.

Computation of the human exposure models and assessment of variability, uncertainty (not to be confused with the same terms used in the uncertainty/variability of the data section) and data quality follows the guidelines by WHO (IPCS, 2005, IPCS, 2008) and EPA (2019b). Variability refers to the inherent differences between the input of any model such as dietary patterns and population characteristics. Uncertainty is caused by the lack of evidence for the necessary inputs of the model as well as the limitation of models to describe an open system which is susceptible to effects by unknown factors, and their limitation in simulating the extremely complex processes relating to human environmental exposures (EPA, 2019b). The uncertainty of the models used are described in terms of: scenario, parameter and model uncertainty (EPA, 2003).The choice of the appropriate model for this exposure assessment was based on guidance by EPA (2009), (2019b), the IPCS (2005) and the EFSA (2007b).

3.7.4.4. Human exposure models

The exposure rate can be in practice combined under one mathematical expression:

$$\text{Exposure rate} = \frac{\text{concentration x contact rate x duration}}{\text{body weight x averaging time}}$$

Equation 4

where:

- concentration is the amount of the agent per mass or volume,
- contact rate is the mass or volume of the medium coming to contact with the human body,
- exposure duration is the time period of exposure to the agent,
- body weight is the average body weight of the population under examination, and
- averaging time is the time of exposure that would be relevant to the specific health risk characterization (WHO & IPCS, 2010)

Similarly for the dietary exposure assessment, the equation can be expressed as:

$$Dietary\ exposure = \frac{\sum(Concentration\ of\ chemical\ in\ food) \times\ food\ consumption}{\text{Body weight (kg)}}$$

Equation 5

(FAO and WHO, 2009).

In this risk assessment the variation of the equations proposed by EPA (2019b) will be used:

$$E_{ing} = (C_{ing})(IR)$$

Equation 6

where:

- E_{ing} is the ingestion exposure (MPs or mass per time),
- C_{ing} is the concentration of the chemical in food or other exposure media (mass of MPs or MPs per mass of medium or mass of chemical per volume of medium), and
- IR is the ingestion rate (mass of medium ingested during the exposure per time).

The equation can be used for both acute (up to one day) and chronic exposures by adding the temporal variation. Exposure rates must be expressed in the same form (unit of measurement) as the output of the hazard characterization process. In the case of MPs a particular challenge is presented since MPs concentrations in the media are in many cases expressed in particles per mass of volume of the medium while doses in toxicological studies are expressed in mass. Effort is made to transform all measurements to the same unit where possible.

3.7.4.5. Dose estimation model

The profile of hazard exposure can be described as a journey in the human body dependent on the four processes of ADME (EPA, 2019b) (see section 3.7.2). Therefore, the exposure becomes dose once it has entered the human body. Regarding MP exposure, an important distinction must be noted. The final MPs uptake by the human body might be less than the MP intake through ingestion and inhalation. A large amount of MPs is expected to 'pass through' the gastrointestinal system and be expelled thus reducing the final intake dose. Similarly, MPs could be expelled from the respiratory system by one of the available defence mechanisms (structural, secretory, cellular etc.) (Canto et al., 1994).

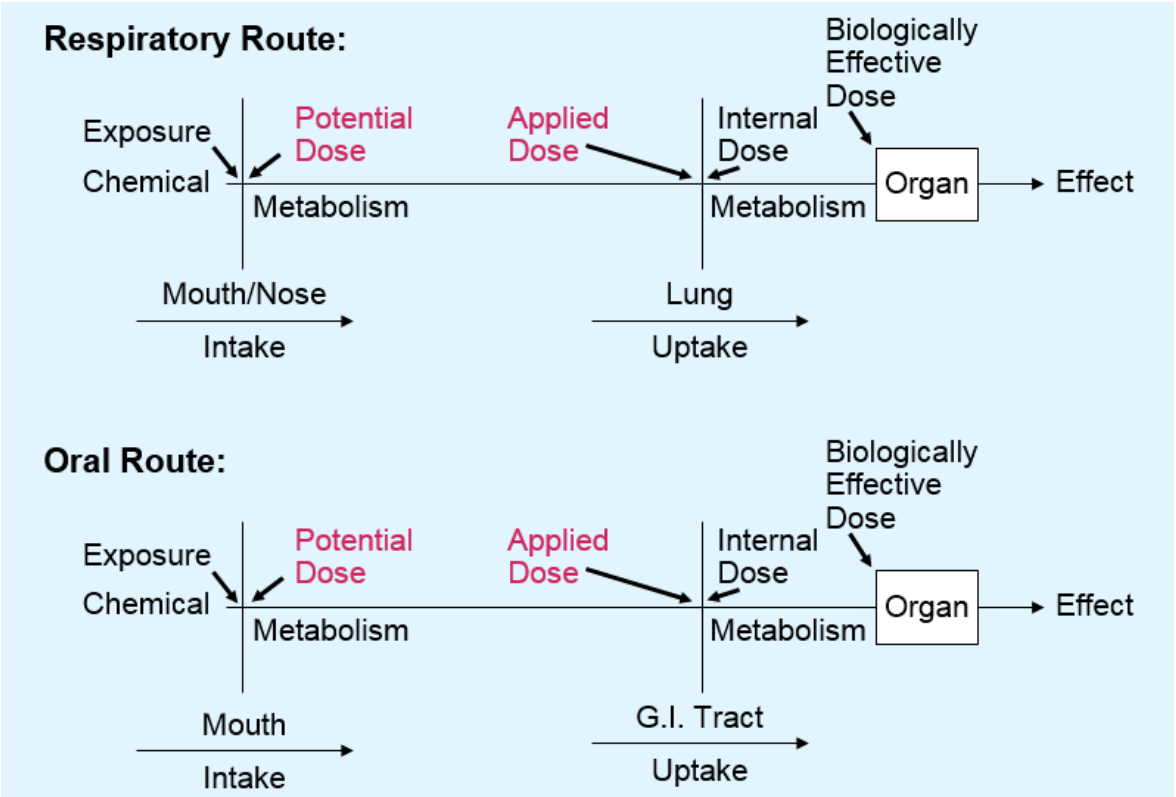


Figure 26. Schematic of Exposure/Dose Terms for the oral route (EPA, 2019b: 11)

Two parameters must be examined here: the amount of MPs that could remain in the human body; whether the duration of time that the MPs remain in the body is enough for them to cause an effect. Exposure doses can be demarcated to applied, potential, internal (or absorbed)/delivered. Potential is the dose that is taken into the body via ingestion and inhalation, applied is the dose that is available for absorption and internal/ delivered are the doses that finally remain in the body (see Figure 26) (EPA, 2019b).

The endpoint of exposure science is the dose that is delivered at the location where the toxicity pathway is initiated thus triggering the health effect. WHO proposes a narrower separation to external (or administered) and internal doses (FAO and WHO, 2009). Regarding dietary exposures, the intake refers to the external dose, the amount that is systemically available would be the internal dose and the target or tissue dose is the amount that is present in the tissue of interest (IPCS, 2009).Ingestion is possibly the most important route of absorption for foreign compounds (Timbrell, 2009). Only particles of sizes < 150 μm are expected to be able to pass the gut barrier and cause systemic exposure with limited absorption (≤ 0.3%) and even smaller to have the ability to translocate to other organs (< 1.5 μm) (EFSA, 2016). MPs that exceed these size cut-offs are not automatically eliminated from the exposure assessment but will be associated with different location endpoints in the body. In order integrate an absorption factor for MPs via the ingestion route according to their size these two equations are proposed:

$$E_{ing} = \left(C_{ing} \times AF_{ing.a}\right)(IR)$$

Equation 7

where: $AF_{ing.a}$ is the absorption factor calculated as the proportion of particles that are < 150 μm, and

$$E_{ing} = \left(C_{ing} \times AF_{ing.b}\right)(IR)$$

Equation 8

where: $AF_{ing.b}$ is the absorption factor calculated as the proportion of particles that are < 1.5 μm. These equations estimate possible internal doses. The conceptual model for dose modelling regarding aggregate dietary exposures is illustrated in Figure 27.

3.7.4.6. Uncertainty/variability of the exposure models

Uncertainty can described in the three categories of scenario, parameter and model (EPA, 2019b). Variability is due to the inherent characteristics of the system. A sensitivity analysis will be implemented in order to understand the influence of factors for both data and decision

uncertainty. Regarding data uncertainty and variability, this process will help determine which parameters drive the results of the risk assessment. Regarding decision uncertainty, the analysis will assess both the choice of data and choice of model.

3.7.5. Risk characterization

Risk characterization is the last of the four steps of risk assessment. Within this step the outputs of the exposure assessment and the hazard characterization are brought together. Health-based values or MOE results are used as the output of the hazard characterization step. Different approaches can be used when examining substances that present linear and non-linear dose-responses, while for genotoxic and carcinogenic substances, threshold values are not appropriate (FAO and WHO, 2009). Risk characterization can be qualitative or qualitative according to the available data.

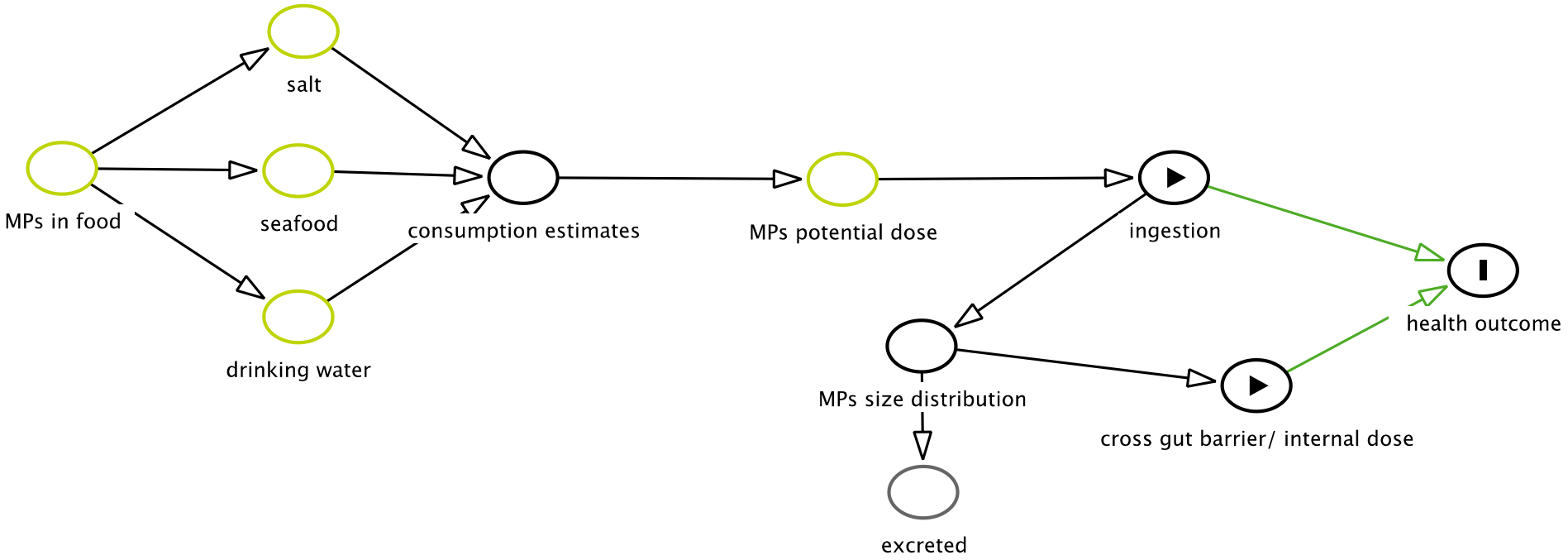


Figure 27. Conceptual model for MP dietary aggregate exposure and dose modelling.

Chapter 4. Microplastic contamination of salt intended for human consumption; Systematic review and meta-analysis results.

This chapter is based on a manuscript that was submitted for publication to the journal SN Applied Sciences (Danopoulos et al., 2020a).

4.1. Study selection

The search strategy produced 2467 citations after duplicates were removed (see section 3.2.5). The details of the study selection procedure are illustrated in the PRISMA flow chart in Figure 28. During the first-level screening, 2307 citations were removed based on their title and abstract as not meeting the criteria for this review.

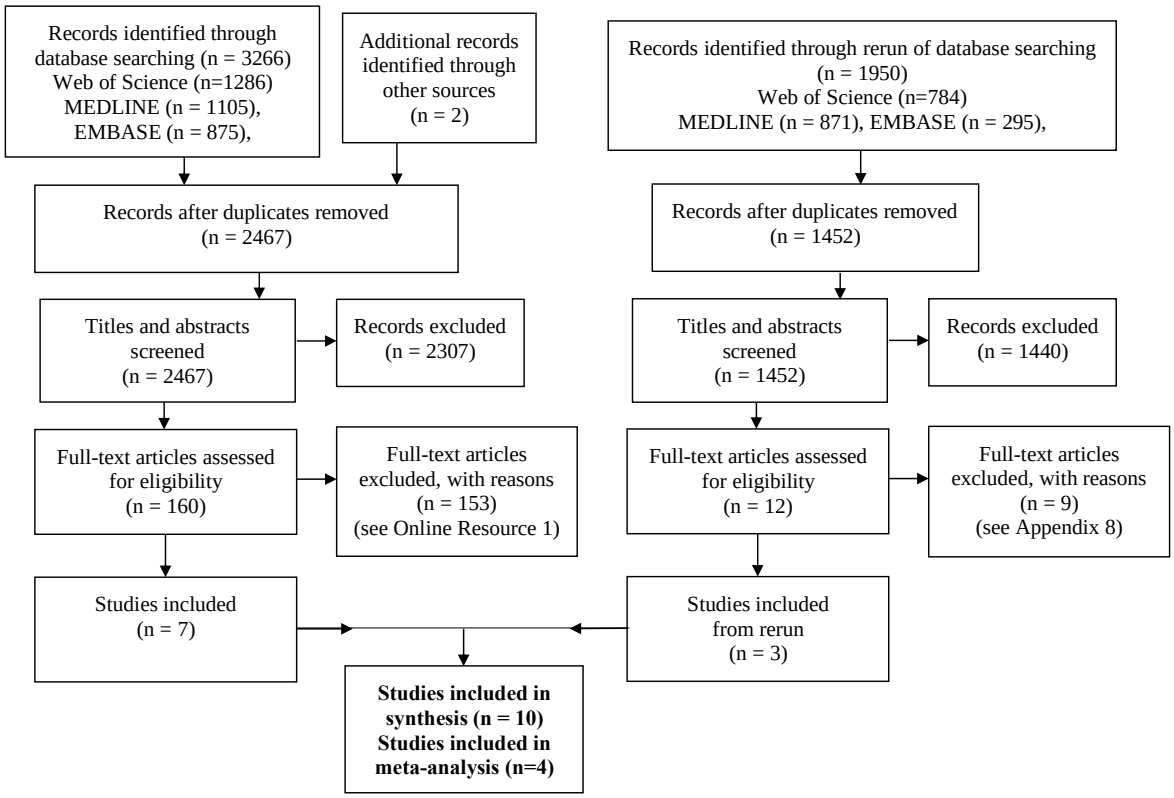


Figure 28. PRISMA flow diagram of screening process for salt studies.

In the second-level screening, the whole text of the paper was evaluated against the eligibility criteria and 112 studies were discarded; the reasons for exclusion can be found in Appendix 8. Studies on three different food themes was identified: salt, seafood, and drinking water. A total of seven studies were included in this salt review (of 48 studies identified across all three food themes). When the searches were re-run, (see section 3.3.4) three more studies were included after the first and second level screening (Figure 28), resulting in ten studies (Gundogdu, 2018, Iniguez et al., 2017, Karami et al., 2017a, Kim et al., 2018, Lee et al., 2019, Renzi and Blaskovic, 2018, M. Renzi et al., 2019, Sathish et al., 2020, Seth and

Shriwastav, 2018, Yang et al., 2015) finally included in this systematic review. All ten studies were included in the systematic review and four in the meta-analysis.

4.2. Study characteristics

Study characteristics for the salt studies are presented in Table 10. The design of all the studies was observational (non-analytic) (Centre for Evidence-Based Medicine, 2019). Their aim was to examine the prevalence of MPs in commercial salt in specific countries or globally. The outcomes are presented as average content of MPs per mass (g or kg of sample) (n=6) and/or range of MPs per mass (n=10). In terms of the salt origin, four different sources/procedures were considered. In total, n=164 different salt brands were analysed across the seven studies: n=110 sea salt, n=15 rock salt, n=10 lake salt, n=12 well salt, and n=17 table salts of unidentified source (Table 10). The importance of the origin lies predominantly in the nature of the raw material itself, as well as the different procedure used to acquire it, namely evaporation or mining (rock or solution (well)) (EUsalt, 2019). Two authors were contacted and asked for additional unpublished information but did not respond.

4.3. Risk of bias (RoB) within studies

The studies were individually appraised using the RoB assessment tool across four domains and assigned an overall rating (Appendix 9). The judgement for each of the studies is recorded in the tool, accompanied by relevant text from the studies, where appropriate (see section 3.2.7). Study design was found to be of low risk across all studies. The domain with the most "High RoB" was "reporting" while the domain with the most "Unclear RoB" was "analysis" (Figure 29). The studies with overall high RoB were those of Renzi and Blaskovic (2018), Karami et al. (2017a) and Sathish et al. (2020). The results of the assessment are discussed and addressed in the synthesis part of the review.

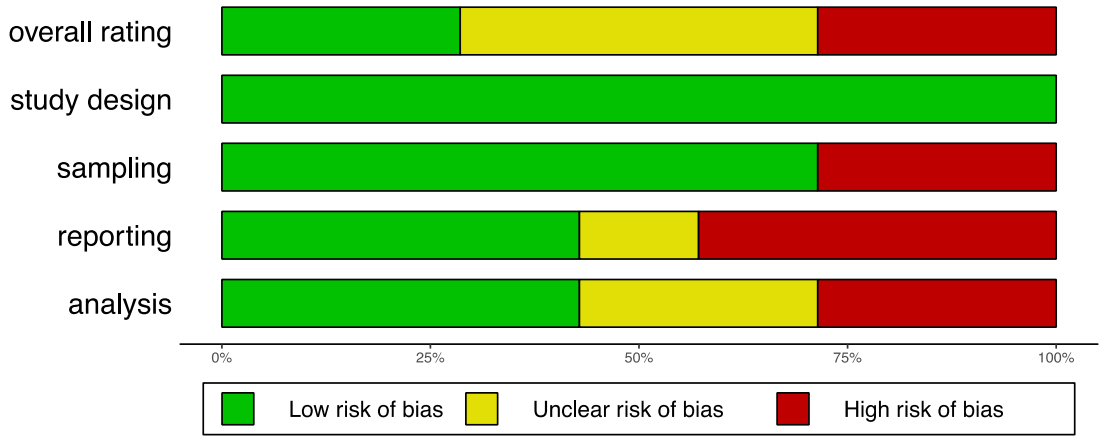


Figure 29. Risk of Bias assessment for salt studies. Rating scale is: Low, Unclear and High. It is expressed in% of the studies that are included in the review.

Table 10. Salt studies characteristics

Author, year	Geographic location	Sample	Number	MPs extraction procedure	Filter pore size (µm)	MPs identification method	Reported outcome
Gundogdu (2018)	Turkey	Salt: sea lake rock	N=16 n=5 sea n=6 lake n=5 rock (packs of 500–750 g)	Yang et al. (2015) and Karami et al. (2017a)	0.2	m-RM	Mean MPs content per mass with SD
Iniguez et al. (2017)	Spain	Salt: sea well	N=21 brands n=16 sea n=5 well (~1 kg per pack)	Yang et al. (2015)	5	FT-IR	Mean MPs content per mass with SD
Karami et al. (2017a)	Product of Australia, France, Iran, Japan, Malaysia, New Zealand, Portugal, and South Africa,	Salt: lake sea unidentified	N=17 brands n=14 sea n=2 lake n=2 unidentified (200-400g per pack)	Karami et al. (2017b)	8	RM	MPs content range per mass
Kim et al. (2018)	Product of China, Korea, Thailand, Philippines, India,	Salt: sea rock lake	N= 39 brands n=28 sea n=9 rock n=2 lake	Yang et al. (2015) and Iniguez et al. (2017)	2.7	FT-IR	Mean MPs content per mass with SD

	Vietnam, Indonesia, France, Italy, UK, Australia, Germany, Bulgaria, Belarus, Romania, Croatia, USA, Brazil, Pakistan, Senegal						
Lee et al. (2019)	Taiwan	Salt: sea rock	N=11 products n=10 n=1 (4.4 kg)	Karami et al. (2017a), Yang et al. (2015)	5	m-FT-IR	Mean MPs content per mass with SD
Renzi and Blaskovic (2018)	Products of Italy and Croatia	Salt: sea	N=11 brands n=6 Italian n=5 Croatian (mass not specified)	their own method	0.45	m-FT-IR	Mean MPs content per mass with SD
M. Renzi et al. (2019)	Products of Italy and Croatia	Salt: sea	N=11 brands n=6 Italian n=5 Croatian (mass not specified)	Renzi and Blaskovic (2018)	0.2	m-FT-IR	MPs content range per mass
Sathish et al. (2020)	India	Salt: sea well	N=14 brands n=7 n=7 (250 g of each type)	Yang et al. (2015)	0.8	FT-IR	Mean MPs content per mass with SD

Seth and Shriwastav (2018)	India	Salt: sea	N=8 brands (~ 1 kg)	Yang et al. (2015)	0.45	m-FT-IR	MPs content range per mass with SD
Yang et al. (2015)	China	Salt: sea lake rock	N=15 brands Sea, lake and rock (not specified n for each) (240 - 500 g per pack)	Their own procedure	5	m-FT-IR	MPs content range per mass

Note: FT-IR, Fourier-transform infrared spectroscopy; N, total sample size; n, sub-sample size (when available or appropriate); RM, Raman spectroscopy

Table 11. Salt studies MP content and polymeric composition.

Author (year)	Salt sample type	N[a]	Mean MPs/kg	SD	Range MPs/kg	MPs size range	Composition per salt origin	Composition all samples	Shape
Gundogdu (2018)	sea	5	46	12.6	16-84	20 µm-5 mm	PU (25%)	PE (22.9%)	fragment > film
	lake	6	37.5	14.1	8-102		PE (35.3%)		
	rock	5	11.8	1.2	9-16		PP (100%)		
Iniguez et al. (2017)	sea	16	124.06	56.43	50-280	30 µm-3.5 mm	n/r	PET (83.3%), PP (6.7%), PE (3.3%)	fibres
	well	5	139	26.24	115-185				
Karami et al. (2017a)	sea	14			0-10	160 µm-980 µm	n/r	PP (40.0%), PE (33.3%), PET (6.66%), polyisoprene/PS (6.66%), PAN (10.0%), NY6 (3.33%)	fragment > filament > film
	lake	2							
	unidentified	2							
Kim et al. (2018)	sea	28	675	2560	0-13629	100 µm-5 mm	PE (35%), PP (30%), PET (30%)	Not specified	fragment > fibre > film
	rock	9	38	55	0-148		PET (41%), PE (26%), PP (23%)		

[a] n refers to number of brands

Note: CP, cellophane; n/r, not reported; NY6, nylon 6; PA, Polyamide; PAN, Polyacrylonitrile; PB, Polybutylene; PE, Polyethylene; PEI, Polyetherimide; PET, Polyethylene terephthalate; PP, Polypropylene; PS, Polystyrene; PU, Polyurethane; PVC, Polyvinyl chloride

4.4. Results of individual studies

The results of the individual studies are presented in tabular form in Table 11 grouped by sample origin where possible. The results of the Iniguez et al. (2017) study were pooled for the overall sea and well salts using the Higgins and Green (2011) formulae for combining groups. The results of the Lee et al. (2019) study were pooled for the sea salt samples using the standard mean and SD formulas. The results of the Renzi and Blaskovic (2018) study were expressed in MPs/g and they were converted to MPs/kg to facilitate comparison between studies. All studies provide ranges of MP content. Grouping the samples according to country of origin was not possible due to the lack of necessary data from some of the papers as discussed earlier.

4.5. Synthesis of results: Meta-analysis

Only the four studies that provide sample size, mean MP content and the corresponding SD are included in the meta-analysis models (Table 12). The results of the study by Sathish et al. (2020) were excluded from the meta-analysis as the study was rated of high RoB (Appendix 9), as discussed in the narrative analysis.

Table 12. Salt studies included in the meta-analysis

Author (year)	Sample type	n	Mean MPs/kg	SD	RoB[a]
Gundogdu (2018)	sea	5	46	12.6	Low
	lake	6	37.5	14.1	
	rock	5	11.8	1.2	
Iniguez et al. (2017)	sea	16	124.06	56.43	Unclear
	well	5	139	26.24	
Kim et al. (2018)	sea	28	675	2560	Low
	rock	9	38	55	
	lake	2	245	307	
Lee et al. (2019)	sea	10	9.5	6.1	Low

[a] Rob: Risk of Bias assessment

All three studies present different results depending on the origin of the salt (sea, lake, rock, well). For the purposes of the meta-analysis, it is not reasonable to assume that samples of different origin should be considered the same. To test this assumption statistically, a subgroup analysis using a fixed-effects (plural) model (or mixed-effects model) was conducted (Harrer et al., 2019b). The results of the model are illustrated in a forest plot (

Figure 30) (Fagerland, 2015). The forest plot shows the standardized mean difference (SMD) results for each salt type by origin (calculated weighted effect estimate) and the corresponding confidence interval (CI 95%) which is the range of values that is expected that the true effect to lie in.

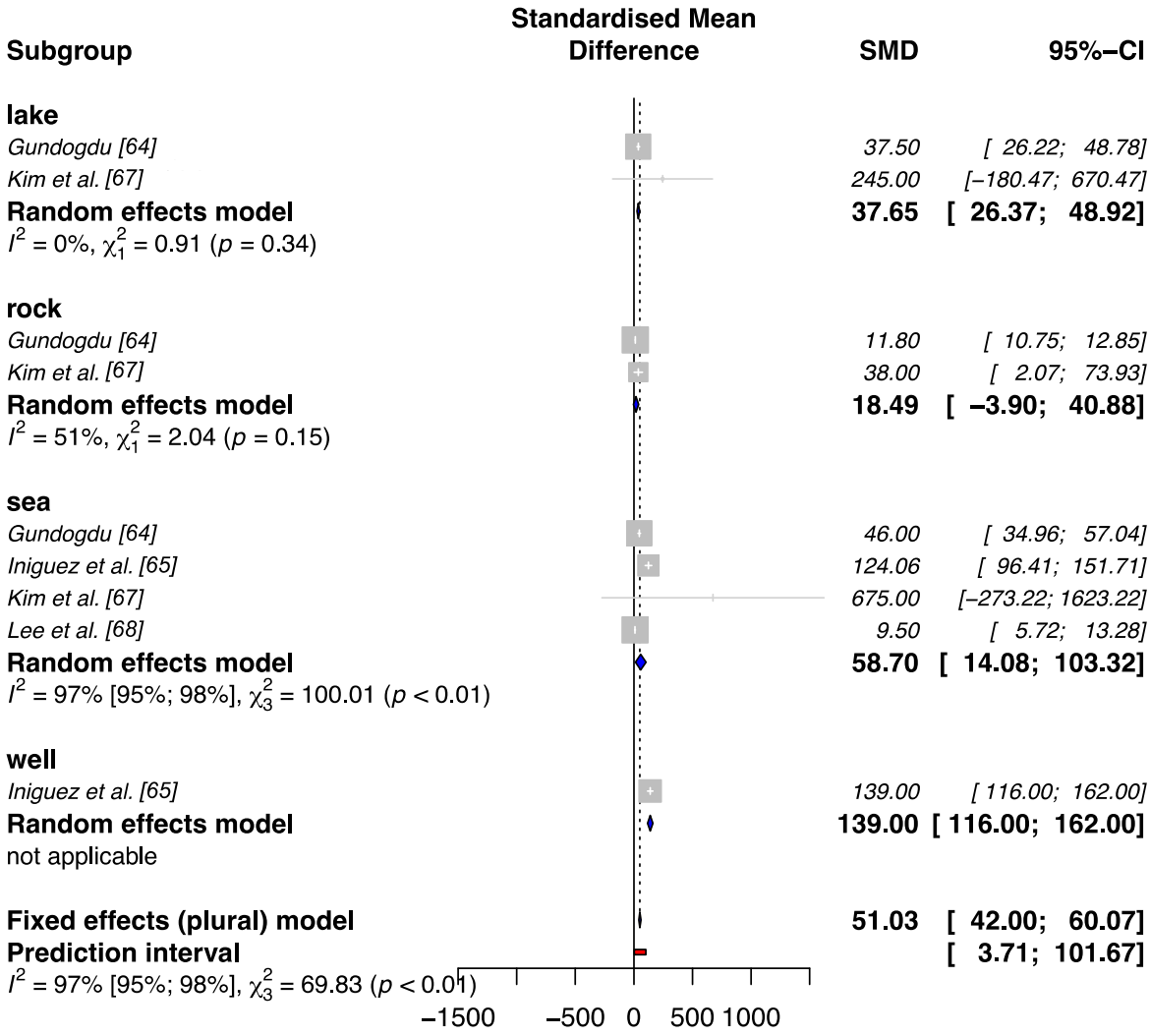


Figure 30. Subgroup analysis for all four origins of salt. The x axis represents the standardized mean difference (SMD) expressed in MPs/kg. The vertical line is the line of null effect where MP content is 0. The grey boxes represent the pooled effect estimate and the lines the confidence interval (CI) 95%. The size of the boxes is proportional to the study weight. The diamonds are the combined point estimates and CI for each of the subgroups. The dotted line is the overall pooled effect for all subgroups with a corresponding diamond. The red box is the prediction interval PI 95%.

The results of the random effects for salts of the same origin and the result of the fixed effect model for the four pooled different origins are also illustrated. The pooled effect for each subgroup ranges from 18.49 MPs/kg to 139 MPs/kg for rock salts and well salts,

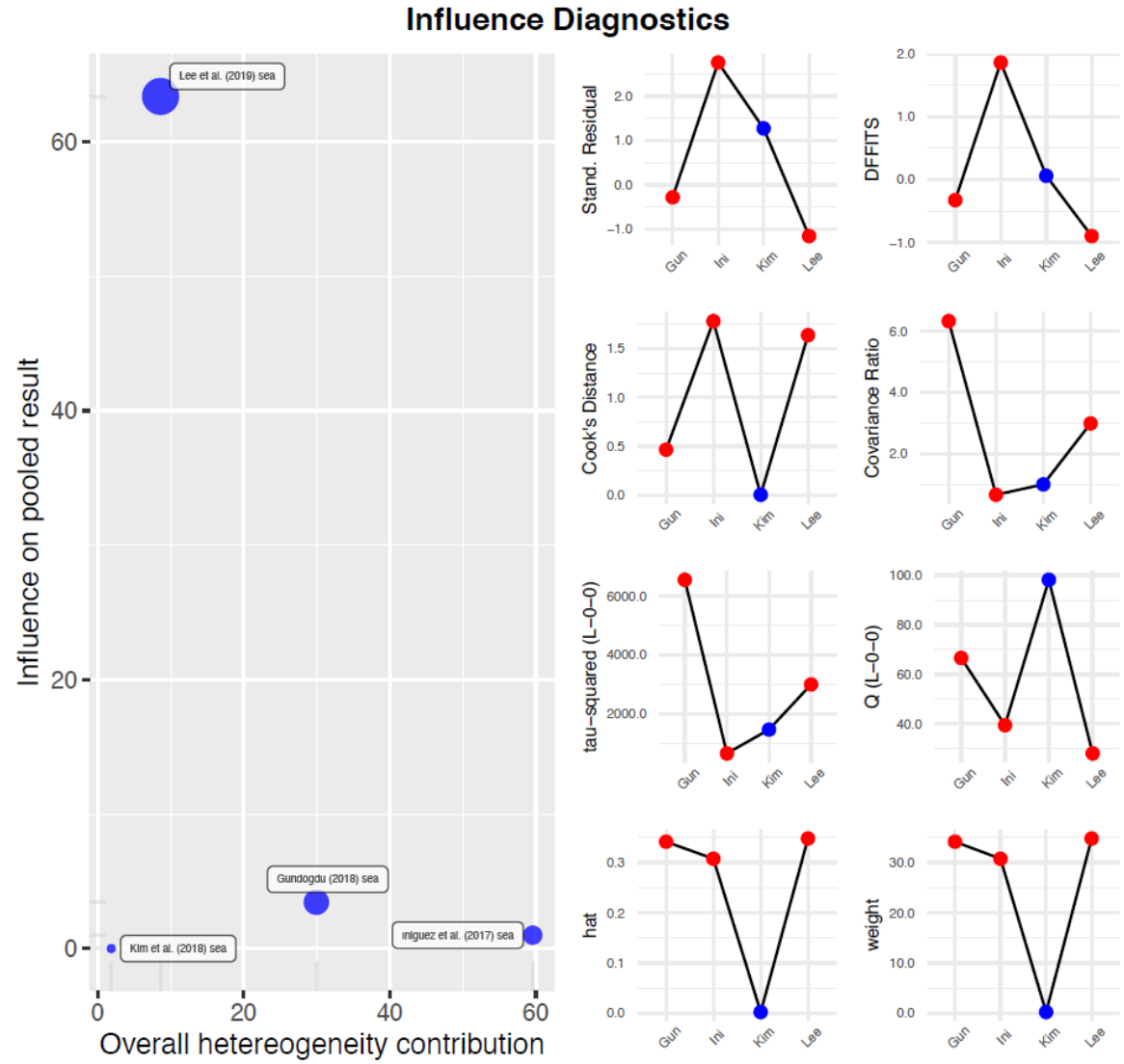
respectively. The results of the subgroup analysis regarding heterogeneity were Chi^2=69.83, p<0.01 showing a statistical significance between the samples of different origin and I^2=97% (high heterogeneity). Both findings support the samples being analysed separately according to origin. The results of the subgroup analysis are interpreted taking into consideration the small number of studies.

Consequently, separate random-effects models were fitted for the sea salt samples (n=59, four studies); the lake salt samples (n=8, two studies); and the rock salt samples (n=14, two studies). Regarding the sea salt samples, the summary mean content was 58.7 MPs/kg (95% CI 14.08 to 103.32, p=0.0099). There was a high statistical heterogeneity of the pooled effect, I^2 =97%, and Chi^2=100.01, p< 0.0001, as evidenced by the wide (95%) CI.

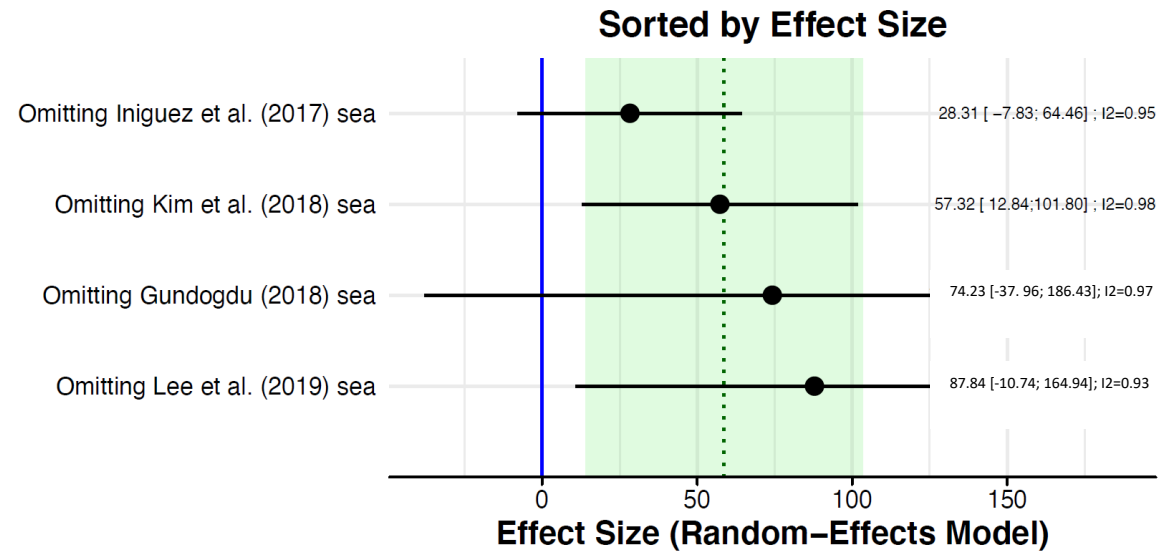
A major difference between the studies, which can be seen in Table 12, is that Kim et al. (2018) report a very large SD while the other three studies (Gundogdu, 2018, Iniguez et al., 2017, Lee et al., 2019) report much smaller ones. This could be attributed to the fact that Kim et al. (2018) had more samples and these came from multiple countries while the other three studies use samples from one country. It should be noted that the Kim et al. (2018) results are heavily influenced by an outlier. This means that results herein are also being influenced by this outlier. To statistically detect the origin of the heterogeneity, a sensitivity analysis was run but no outliers were detected. Consequently, an influence analysis was fitted the results of which can be found in Figure 31 A-C. The results of both analyses were inconclusive which led us to include all four studies. RoB was also examined in a sensitivity analysis but was similarly inconclusive.

Regarding the two lake salt studies (n= 8 samples), the overall content was computed at 37.65 MPs/kg (95% CI 26.37 to 48.92, p< 0.0001). The heterogeneity is extremely low I^2=0% and Chi^2=0.91, p=0.3393. In this case, there is no need to explore heterogeneity further. The rock studies meta-analysis provided an overall estimate of MP content of 18.49 MPs/kg (95% CI -3.9 to 40.88, p=0.1056). The heterogeneity is moderate I^2=51% and Chi^2=2.04, p=0.1532. Regarding RoB, both studies in the lake and rock group were rated as “low”.

A.


Influence Diagnostics
Influence on pooled result
Overall hetereogeneity contribution
Lee et al. (2019) sea
Gundogdu (2018) sea
Iniguez et al. (2017) sea
Kim et al. (2018) sea
Stand. Residual
DFFITS
Cook's Distance
Covariance Ratio
tau-squared (L-0-0)
Q (L-0-0)
hat
weight
Gun
Ini
Kim
Lee

B.


Sorted by Effect Size
Omitting Iniguez et al. (2017) sea
Omitting Kim et al. (2018) sea
Omitting Gundogdu (2018) sea
Omitting Lee et al. (2019) sea
28.31 [−7.83; 64.46] ; I2=0.95
57.32 [12.84;101.80] ; I2=0.98
74.23 [-37. 96; 186.43]; I2=0.97
87.84 [-10.74; 164.94]; I2=0.93
Effect Size (Random−Effects Model)

C.

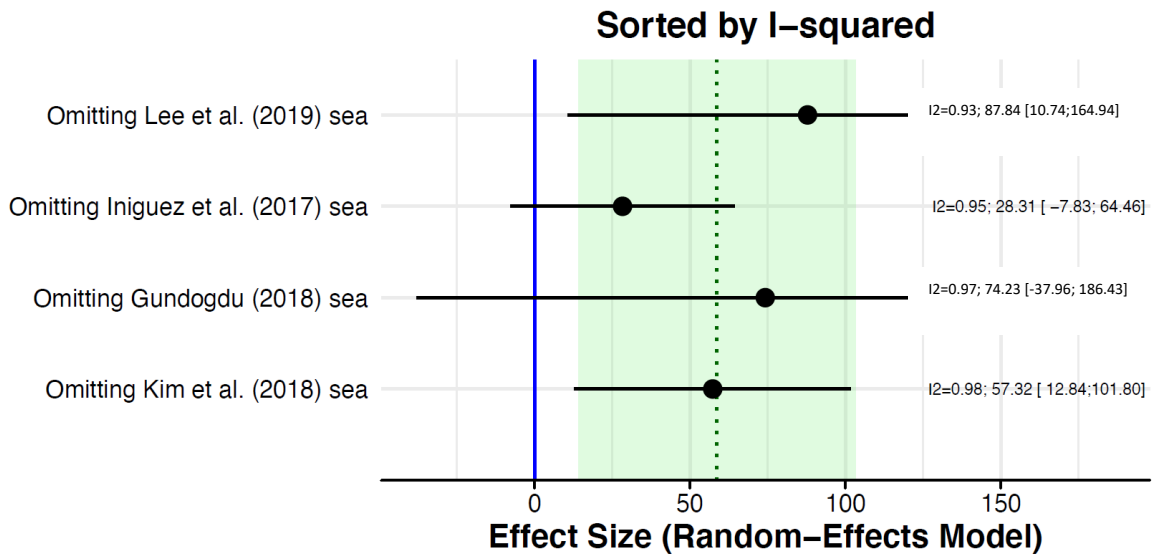


Figure 31. Influence analysis for meta-analysis of sea-salt data. (A) Influence analysis Baujat Plot of random-effects model. The horizontal axis illustrates statistical heterogeneity as measured by Cochran's Q statistic. The vertical axis illustrates the influence on the pooled result. Influence analysis forest plots of random-effects model using the leave-one-out method, sorted by (B) effect size estimate, expressed as microplastics per kg (MPs/kg) and 95% confidence interval (CI) and (C) heterogeneity expressed in I^2. The pooled effect is recalculated each time leaving out one study. In both figures results are ordered from low to high.

4.6. Risk of bias (RoB) across studies

In order to explore RoB across studies (publication bias), a series of funnel plots (Borenstein, 2009) were explored (Appendix 11, A-C). As can be seen in Figure 32, the asymmetry of the distribution for all the salt studies is caused by the two results on the left hand side of the plot; these are the Kim et al. (2018) study results for lake and sea salt samples. This study has already been observed to disproportionally affect the meta-analysis due to extreme size effects. The studies in the white background do not have statistically significant effect sizes. The results of the Egger's test were intercept = 4.441 (1.501-7.381 CI, p = 0.02264). The p value for the Egger's test is significant which means that there is notable asymmetry in the funnel plot. The results of the Egger's test should be interpreted with caution since the number of the studies is too small (< 10) to draw safe conclusions.

This systematic review has set stringent methodological eligibility criteria that have led to a large number of studies being excluded. Studies with lower methodological rigor tend to report higher results due to overestimation of MP content. Therefore, it can be assumed that the symmetry at the bottom of the funnel plot would have been better had these studies been

included, but they would also be statistically non-significant for the effect size. Hence, it is reasonable to assume that the asymmetry is not due to non-reporting bias.

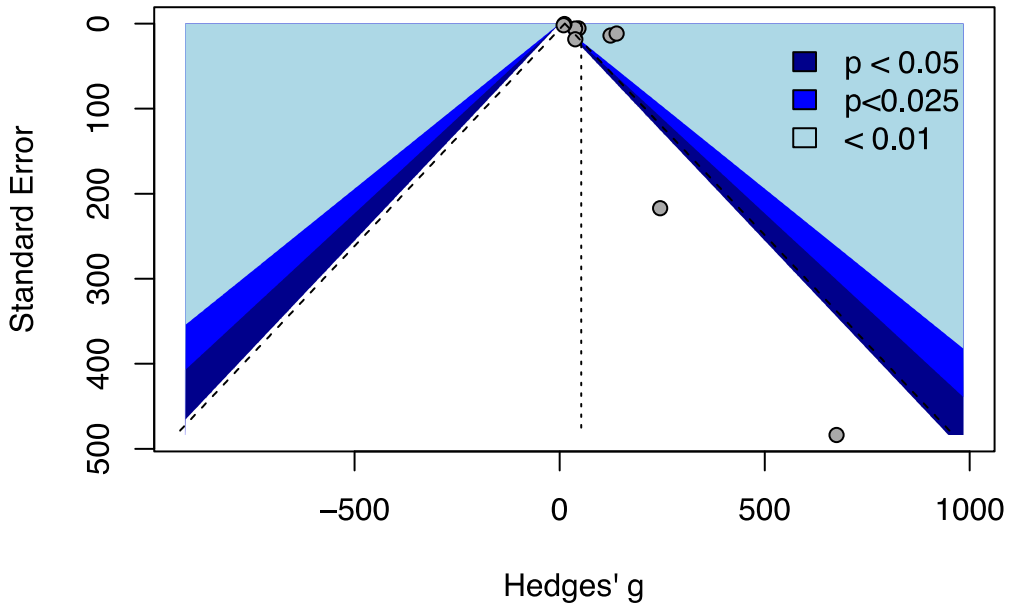


Figure 32. Publication risk of bias funnel plot for all salt origins. Content expressed in MPs/kg salt. Dots represent individual studies. The vertical dotted line represents the pooled effect size. Diagonal lines represent pseudo 95% confidence limits

4.7. Statistical summary of effects/ narrative analysis

The effect size for the summary is the range of MP content (MPs/kg), which has been reported by all the included studies (Table 11). Sample heterogeneity, in terms of origin, is primarily addressed again by grouping the samples according to their origin (sea, lake, rock and well). Taking into consideration the ranges of MP content reported by the studies (without any weighting), the MP content is 0 to 31,680 MPs/kg for sea salt, 0 to 462 MPs/kg for lake salt, 0 to 204 MPs/kg for rock and for well salt (Figure 33).

Regarding the results of MP content in sea salt, the study by Renzi and Blaskovic (2018) stands out. They report mean contents of 5,400 and 28,900 MPs/kg and ranges of 1,570 - 8,230 and 27,130 – 31,680 MPs/kg for Italian and Croatian marine salts, respectively. The range reported by Kim et al. (2018) is similar (0−13,629 MPs/kg) but the mean is much lower at 675 MPs/kg salt. Kim et al. (2018) also highlight that they identified one outlier sea salt sample in their analysis and reported a reduced range of 0-1,674 MPs/kg when excluding it. Renzi and Blaskovic (2018) state that extremely high values might be due to human error during visual particle identification and the increased level of pollution in the areas where the salt's raw material is collected. However, the lower mean content for the Italian salts is eight times higher than the closest reported mean content. This study was one of five (Iniguez

et al., 2017, Karami et al., 2017a, Lee et al., 2019, Renzi and Blaskovic, 2018, M. Renzi et al., 2019) that did not use digestion in the particle-extraction procedure and the one of the three (Lee et al., 2019, Renzi and Blaskovic, 2018, M. Renzi et al., 2019) that did not use a density-separation technique to separate MPs from non-polymeric particles. Their analysis protocol fails to report important information: the number of replicates they used, the results of their procedural blank samples to account for after-sampling contamination and whether the results of the procedural blanks were subtracted from the final results. In addition, they do not report the specifics around the polymer composition identification: how many particles they identified with the help of m-FT-IR as a fraction of their sample, the spectral library they used, the acceptance rate for a particle to be considered of polymer origin (usually set above 60%) and it was the only study that did not report results on the polymer composition of the MPs. In the light of these reporting omissions, the results and conclusions should be interpreted with caution. In their later work (M. Renzi et al., 2019) they identified extremely lower content of 70 – 320 MPs/kg of salt (in the size fraction of 10 – 150 μm) and recognize that systematic composition analysis is necessary to avoid overestimations. Kim et al. (2018) also report high ranges compared to the other studies. They state that differences in the analytical processes and samples might be the cause of these variations. However, the highest difference is caused by a reported outlier sample.

At the other end of the extreme ranges, Karami et al. (2017a) reports a range of 0-10 MPs/kg. They stated that they had a high proportion of non-identified particles (29.1%), while a quarter of the sample was identified as pigments. This study employed a two-step filter extraction procedure, without digestion, which resulted in only a fraction of the extracted particles (size particles > 149 μm), being considered in the results. This could have led to a significant underestimation of the MP content, a limitation the authors acknowledge. The size of particles included is important because the number of MPs increases as their size decreases (Cozar et al., 2014, 2015, Ter Halle et al., 2016). Therefore, including only smaller size MPs could affect the estimate of overall MP content disproportionally. This study did not analyse the procedural blank samples. Instead, the filters were weighed and change in their weight was used to account for post-sampling contamination. This procedure is not common practice and cannot be seen as adequate in verifying the protection of their samples. Thus, it is not known if and how contamination of the samples has affected the results of this study. Similarly, the study by Sathish et al. (2020) did not report any details surrounding the procedural blank samples including their results.

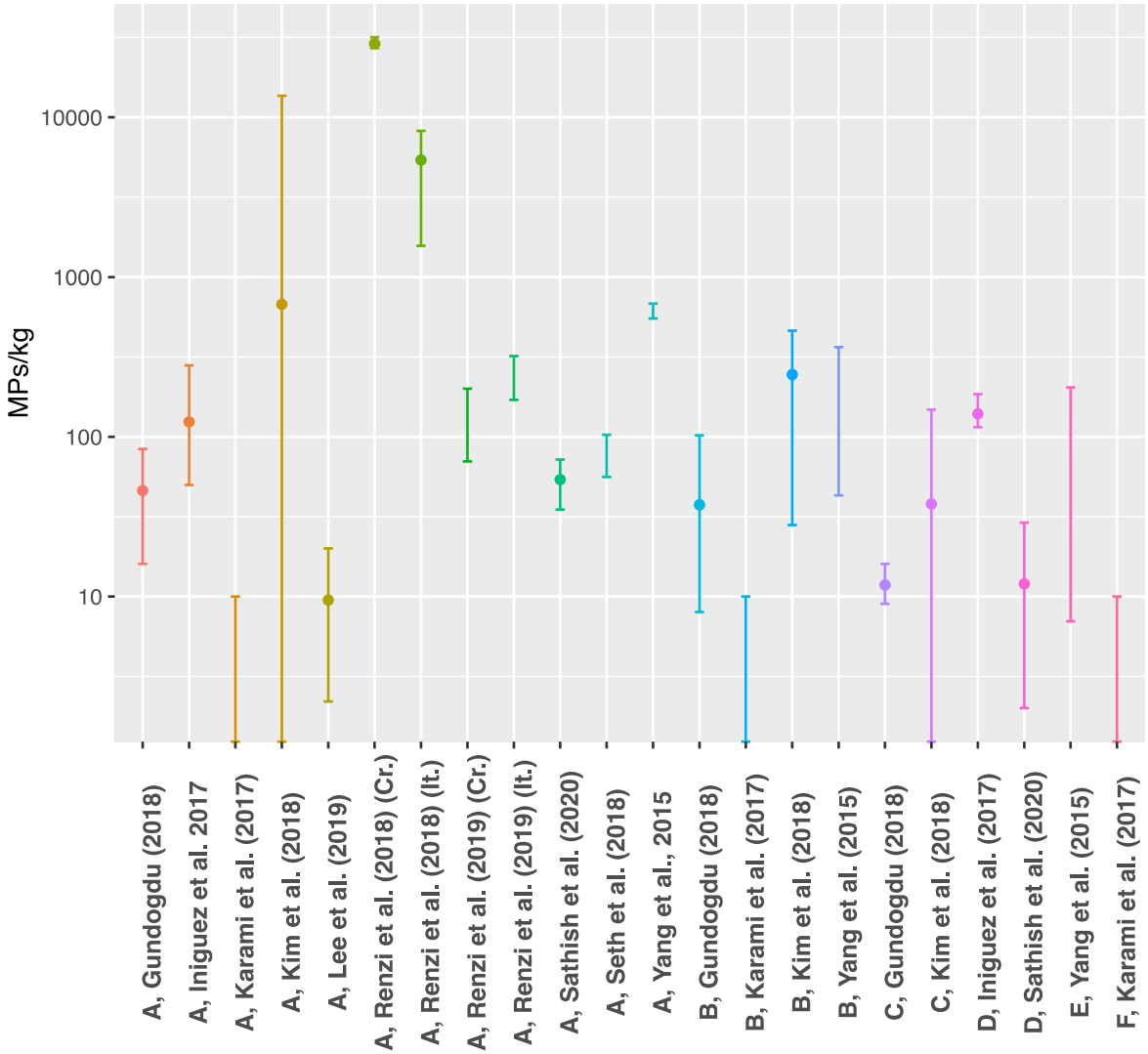


Figure 33. MP content in salt from all origins expressed in $\log_{10}$ for ease of comparison. The points in the graph represent the mean values of MP content for the studies that report it, whiskers represent the reported ranges of MPs/kg. A: sea salt, B: lake salt, C: rock salt, D: well salt, E: rock/well salt, F: unidentified origin

The studies by Renzi and Blaskovic (2018), Karami et al. (2017a) and Sathish et al. (2020) were rated as of high RoB (Appendix 9). It should also be noted that the results of the study by M. Renzi et al. (2019) cannot be directly compared nor collated with the rest of the studies because the design of the study targeted the specific size fraction of 10 – 150 μm. Removing the three studies that were rated of high RoB studies from the results as well as the outlier of the Kim et al. (2018) study (as suggested by the authors (Kim et al., 2018)) the distribution of the ranges decreases (Figure 34), and the MP content range narrows to 0 – 1,674 MPs/kg of sea salt.

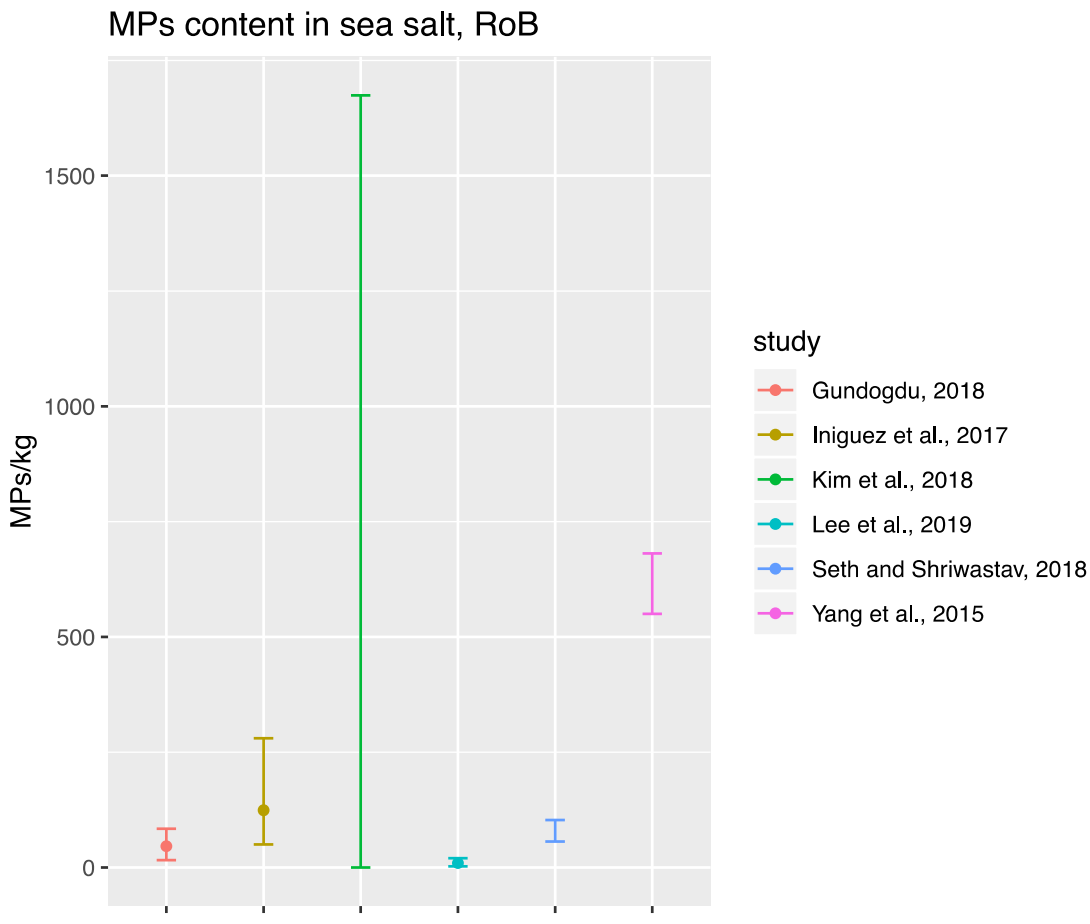


Figure 34. Sea salt MPs content range incorporating the Risk of Bias (RoB) rating. Only studies by Gundogdu (2018), Iniguez et al. (2017) and Lee et al. (2019) report the mean, illustrated by points on the graph.

Lake salt MP content ranges exhibit the same pattern. Karami et al. (2017a) and Kim et al. (2018) report the lowest and highest ranges, respectively. Besides the narrowed size fraction, there is another factor that might play a part in the underrepresentation of MPs in the Karami et al. (2017a) study, which is the absence of a digestion step in the particle-extraction process which the other three studies use. Omitting this study from the results only narrows the range to 8-462 MPs/kg of lake salt. Regarding the rock and well salt studies, Yang et al. (2015) present one combined result for both origins that cannot be directly compared to the result for well salt from the Iniguez et al. (2017) study, while the results of the Sathish et al. (2020) study are omitted due to high RoB rating (Appendix 9). The rest of the studies report fairly similar results.

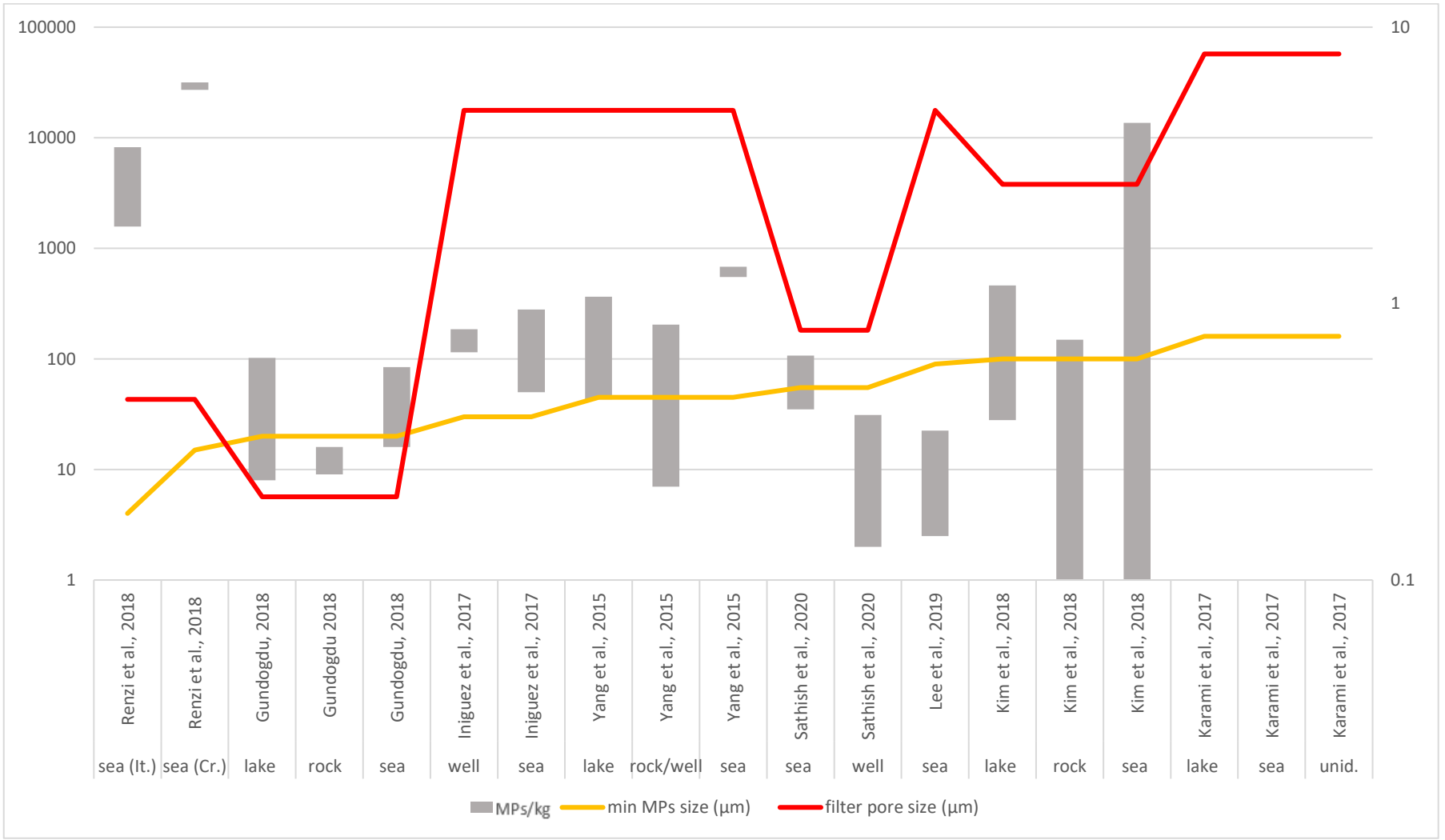
Across the studies, the minimum size of identified MP particle by the studies ranged from 4 μm to 160 μm (Table 11) and could have been directly affected by two experimental parameters: the pore size of the filters used for the extraction of the particles and the technical abilities/limitations of the technology used for the composition identification. Filters of different pore sizes were used ranging from 0.2 μm to 8 μm, representing the minimum cut

off size, while two of the studies also used a maximum cut off size of > 149 μm (Karami et al., 2017a) and < 150 μm (M. Renzi et al., 2019), as previously noted (Table 10). FT-IR and RM can analyse particles in the range of 40 μm and 10 μm, respectively, but when they are coupled with microscopes their technical specifications are enhanced to analysing particles in the size of 10 (m-FT-IR) μm and 1 μm (m-RM) (Araujo et al., 2018, Bergmann, 2015, Harrison et al., 2012, Löder et al., 2015, Oßmann et al., 2018, Strungaru et al., 2019). The relationship between these parameters and the MP content are illustrated in Figure 35 a and b where it is illustrated that there is only a weak negative trend between the size of the identified MP and the content in the samples which could be attributed to the small number of studies and other confounding parameters previously discussed.

The association between the size of the measured MPs particles and their content is also highlighted in the systematic review of drinking water MP contamination in Chapter 5 herein. Fragment was the most commonly discovered shape of MPs across all studies followed by fibre (Table 11). Across all the studies that analyses salt from different origins, the pattern of MP content found is that sea salts exhibit the highest content, followed by lake and then rock. This pattern can be attributed to the corresponding environmental contamination of the raw material i.e. natural brine from a sea or lake or man-made brine from wells which is an open (and exposed) system, compared to a closed and largely protected system of underground rock salt.

There are only two studies (Iniguez et al., 2017, Sathish et al., 2020) that sampled well salt (n=12) and one study (Yang et al., 2015) that sampled rock/well salt (n=unknown). Although these studies use samples that come from underground there is a key difference between them. Well salt is derived from brine that has been artificially produced by pumping water in underground salt sources. The brine is then evaporated in open lakes or in closed circuits. The samples used in the studies by Iniguez et al. (2017) and Sathish et al. (2020) come from artificial brine that has been evaporated in open lakes and is therefore exposed to further environmental pollution. On the other hand, the Yang et al. (2015) study does not differentiate between the two types (rock and well) and it is not possible to determine whether the salt has been exposed to environmental conditions or not. The higher MP content in the Iniguez et al. (2017) study could be attributed to these processes.

A.


100000
10000
1000
100
10
1
10
1
0.1
Renzi et al., 2018
Renzi et al., 2018
Gundogdu, 2018
Gundogdu 2018
Gundogdu, 2018
Iniguez et al., 2017
Iniguez et al., 2017
Yang et al., 2015
Yang et al., 2015
Yang et al., 2015
Sathish et al., 2020
Sathish et al., 2020
Lee et al., 2019
Kim et al., 2018
Kim et al., 2018
Kim et al., 2018
Karami et al., 2017
Karami et al., 2017
Karami et al., 2017
sea (It.)
sea (Cr.)
lake
rock
sea
well
sea
lake
rock/well
sea
sea
well
sea
lake
rock
sea
lake
sea
unid.
MPs/kg
min MPs size (µm)
filter pore size (µm)

B.

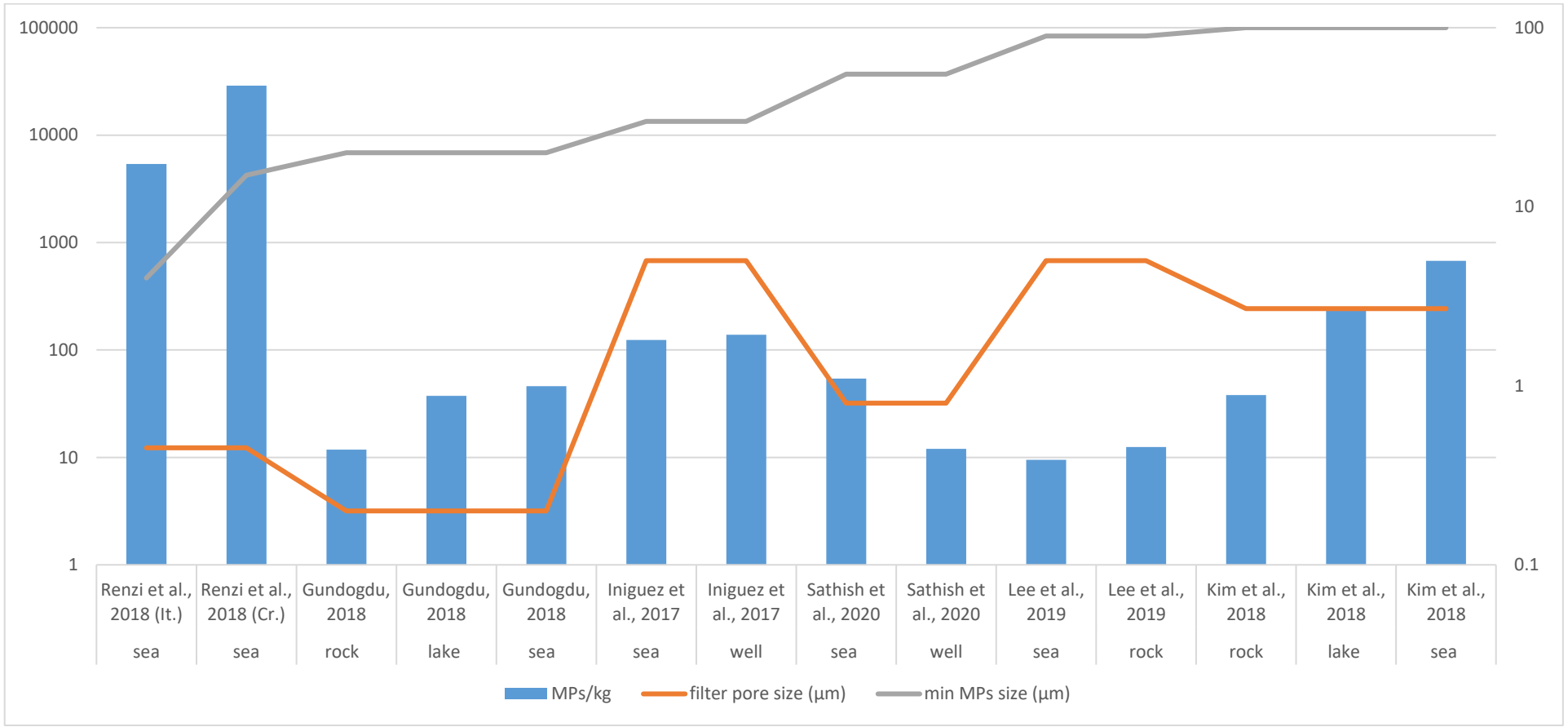


Figure 35. A. Relationship between the ranges of MP content (MPs/kg of salt), pore filter size and minimum reported MP particle size. Both axes are illustrated in $\log_{10}$ scale. B. Relationship between the mean MP content (MPs/kg of salt), pore filter size and minimum reported MP particle size. Both axes are illustrated in $\log_{10}$ scale.

In terms of polymeric composition, four studies (Gundogdu, 2018, Kim et al., 2018, M. Renzi et al., 2019, Yang et al., 2015) differentiate between salts of different origin, five studies (Iniguez et al., 2017, Karami et al., 2017a, Lee et al., 2019, Sathish et al., 2020, Seth and Shriwastav, 2018) do not and one study (Renzi and Blaskovic, 2018) does not report any results (Renzi and Blaskovic, 2018). The most prevalent polymers across all studies and origins were polypropylene (PP) and polyethylene (PE) followed by polyethylene terephthalate (PET) (Table 11). The most commonly found polymer in sea salt was PE and PET, in lake salt PE and in rock/well salt PET. In addition, it is important to highlight that Yang et al. (2015) included the compounds cellophane (CP) and cellulose (CL) in their MPs results while the rest of the studies did not. Kim et al. (2018) did consider CP but reported that this was not detected in their samples.

Five studies collected samples from one country (Gundogdu, 2018, Iniguez et al., 2017, Sathish et al., 2020, Seth and Shriwastav, 2018, Yang et al., 2015) (four in the continent of Asia (Gundogdu, 2018, Sathish et al., 2020, Seth and Shriwastav, 2018, Yang et al., 2015)) and two studies (Renzi and Blaskovic, 2018, M. Renzi et al., 2019) described samples for two countries (both in Europe). Three studies (Karami et al., 2017a, Kim et al., 2018, Lee et al., 2019) examined MPs salt contamination in multiple countries. Karami et al. (2017a) examined samples of salt produced in different eight countries but available through the Malaysian market (n=17 brands). They did not attempt a comparison between countries and did not report their results in a usable form by country. Likewise, Lee et al. (2019) sampled brands that were available in the Taiwanese market and possibly also coming from third counties but did not report further details. In contrast, Kim et al. (2018) who also analysed salts from different countries (n = 21), purchased most of the samples in the countries that they were produced in (n = 17), thus allowing them to extrapolate to a global pattern. The study reports a comparison between Asia (1028 ± 3169 MPs/kg) and all other continents (39 ± 9 MPs/kg). A pattern did not emerge for the MP content in salt between different countries or continents.

4.8. Summary of evidence

The results of the systematic review are presented in the summary of evidence table (Table 13) which integrates the meta-analysis, the statistical summary and the narrative analysis as well as the overall rating of the evidence according to the GRADE methodology and the E-GRADE tool (Bilotta et al., 2014, Higgins et al., 2019). The GRADE certainty framework assessment for the salt studies evidence is presented in detail in Appendix 10. In brief, RoB rating did not downgrade the certainty of the evidence as the high RoB studies were

ultimately excluded from synthesis in both meta-analysis and statistical summary results. Heterogeneity in meta-analysis was only found to be high in the salt outcomes and as such only these were downgraded. Data were not downgraded in the domains of indirectness, imprecision and publication bias. Regarding the three upgrading domains, large effects and dose response did not apply in these studies, while the lack of confounders resulted in upgrading all studies by one grade.

Table 13. Summary of evidence of salt studies.

MP content in salt intended for human consumption

Samples: commercially available salt of all origins **Setting: global** **Measure: MP content**				
Origin	Number of studies	Outcomes [a]		Certainty of the evidence [b]
		Average MPs/kg content [c]	95% CI	
Sea salt	4	58.70	14.08 to 103.32	⊕⊕⊖⊖ Low [d]
Lake salt	2	37.65	26.37 to 48.92	⊕⊕⊕⊖ Moderate
Rock salt	2	18.49	-3.9 to 40.88	⊕⊕⊕⊖ Moderate
Well salt	1	139.00	26.24 (SD)	
		Range of MPs/kg content [e]		
Sea salt	5	0 – 1674		⊕⊕⊕⊖ Moderate
Lake salt	4	8 - 462		⊕⊕⊕⊖ Moderate
Rock/well salt	4	0 - 204		⊕⊕⊕⊖ Moderate

[a] The three studies that were rated as of high RoB: Renzi and Blaskovic (2018), Karami et al. (2017a) and Sathish et al. (2020) are not included in the summary of evidence. The outlier sample of the Kim et al. (2018) study is not included.

[b] All studies are upgraded due to the absence of confounders.

[c] Meta-analysis.

[d] Due to high heterogeneity.

[e] Statistical summary.

Certainty rating symbols are according to Higgins et al. (2019):
High ⊕⊕⊕⊕, Moderate ⊕⊕⊕⊖, Low ⊕⊕⊖⊖, Very low ⊕⊖⊖⊖.

4.9. Discussion

At the time this study was published (Danopoulos et al., 2020a), it was the first systematic review addressing MP contamination of salt intended for human consumption. Ten studies

were reviewed, which, in total, analysed 164 different salt samples/brands coming from 28 different countries. Four studies were included in the meta-analysis, and all ten studies are included in the statistical summary of effects and narrative analysis. MPs were present in the majority of the examined samples from all four origins (sea, lake, rock and well), with levels varying significantly across studies from 0 to 1674 MPs/kg of salt. The studies are of moderate to low quality (Table 13). The review provides robust evidence of ubiquitous salt MPs contamination.

Narrative analysis detected a number of issues in the methodology of the studies in all stages. The quality of the existing evidence was explicitly appraised in order to move forward discussions around MPs in food intended for human consumption. Major issues concern the use of different processes for the extraction of particles from the samples and the following identification of their composition as well as poor reporting. Well-reported studies would allow for more effective comparison across the studies and increase confidence in the conclusions. In this fast developing field, consensus is needed in order to achieve consistency in how MPs are extracted (Silva et al., 2018, Cannon et al., 2016, Filella, 2015, Hermsen et al., 2018, Hidalgo-Ruz et al., 2012, Hong et al., 2017, Kedzierski et al., 2019, Li et al., 2018, Mai et al., 2018, Miller et al., 2017, Shim et al., 2017) what is measured (GESAMP, 2015b, Frias and Nash, 2019, Hartmann et al., 2019) and how it is reported (von Elm et al., 2007, West et al., 2002, Kase et al., 2016, Kentin, 2018, Klimisch et al., 1997). Improving the quality of reporting is key to creating a more robust methodology.

A recent review by Peixoto et al. (2019) on MPs pollution in commercial salts has reviewed the same studies plus one study that did not meet the eligibility criteria for this systematic review. However, the Peixoto et al. (2019) review is not systematic, nor claims to be, and does not attempt to collate the results of the studies. They report a maximum potential yearly ingestion of 36135 particles coming from salt, which is driven by the results of the study by Renzi and Blaskovic (2018) that have not been included in this analysis. Similarly, the review by Qun Zhang et al. (2020) estimates MPs human exposures reporting a maximum of 7.3×10^4 MPs per year which is also derived by the results of the Renzi and Blaskovic (2018) study. The review by Cox et al. (2019) included salt in their analysis and reported 0.11 MPs/g content, using data from four studies. Cox et al. (2019) used one of the studies (Karami et al., 2017a) which was omitted from the results of this review due to methodological issues. The Cox et al. (2019) review does not differentiate between salt origin in their results and do not report projected MPs consumption coming only from salt but from a range of foodstuffs. Nevertheless, the magnitude of MP content they report is similar to this review. The MP exposure assessment results based on the findings of this

systematic review are presented in section 8.3.1. Lee et al. (2019) also included a review in their study reporting an annual intake of 537.4 MPs (10.5 g salt per daily consumption) which is in the same range of the statistical summary results but more than two times higher from the meta-analysis results (see section 8.3.1). This could be attributed to the use of descriptive statistics as opposed to the use of statistical modelling that weighs results according to the sample size and SD. The quality of the results of this systematic review has been improved by excluding evidence coming from studies that did not meet the a priori eligibility criteria set in the protocol, as well as, studies that were rated as of high RoB according to the RoB assessment tool.

Polymeric composition of MPs varied across the studies including: PET, PP, PE, polyamide (PA), polyurethane (PU) etc., among others, (Table 11). The most prevalent polymers were PP and PE which were also the most produced and used in the past decades (Plastics Europe, 2008, 2017, 2018, 2019) further supporting the connection between the mismanagement of plastic waste and environmental pollution.

Regarding sea salt, it would be expected that the MP content would follow the level of MPs contamination in the sea or ocean of origin. However, this was not found. The limited number of studies included and methodological heterogeneity between studies may have distorted this association. Additionally, this review did not focus on detecting how the salt was contaminated but instead on the level of contamination in salt "on the shelf" ready to be consumed. In terms of country of origin, a pattern of MP content did not emerge.

4.9.1. Strengths and Limitations

This is the first systematic review and meta-analysis that collates evidence from multiple studies to estimate the MP content in salt for human consumption to extrapolate to human MPs uptake from this specific foodstuff. It provides a robust and realistic assessment of MPs in salt by bringing together evidence from multiple studies that have been thoroughly assessed in a systematic and standardized manner. Quantification of human exposure to MPs is the first step of an informed, evidence-based risk assessment of the risk posed by this emerging risk factor. The bespoke quality assessment tool constructed for this review can be used in future reviews to assess robustness of research. It can also be used as a guide to inform future researchers on common issues identified in this field. A limitation of this review is that the conclusions that can be drawn are limited by the small number of studies as well as heterogeneity in the samples and the methods used by different studies. Three studies were considered as of high RoB and they were excluded from the summary of

evidence. The incomplete reporting of the results by a number of studies invalidated their use in the meta-analysis and in a validation process.

4.10. Chapter conclusions

The presence of MPs in food intended for human consumption and in human stool has been documented (Schwabl et al., 2019). Although the possible effects to humans are still to be explored (Bucci et al., 2020, Mishra et al., 2018, Schirinzi et al., 2017, Wright and Kelly, 2017), given the international concerns about the potential effects of MPs on human health, more research is urgently needed on the impact of MPs in salt and other foodstuffs. From a food safety perspective, when and if MPs are proven to be agents that have the potential to cause adverse human health effects they will be classified as food hazards. Therefore they will be included in any food safety risk assessment and management system, such as the Hazard Analysis and Critical Control Point (HACCP) management system (Wallace, 2015), as a possible chemical or physical risk factor, conforming to current food safety legislation (Council Regulation (EC) No 178/, 2002). Salt is included in a vast array of foodstuffs, raising the issue of MPs being transferred to different foods and acting as vehicles for the distribution of MPs thus possibly making it a major food safety issue. Given the global nature of food consumption and the export of salt around the world, this needs investigation.

It is essential to quantify and assess the exposures from all available routes (ingestion, inhalation) and sources and then use it as a risk-assessment framework to bring together current scientific knowledge from animal studies (Avio et al., 2015a, Nelms et al., 2018, Ribeiro et al., 2017, Setala et al., 2016, Sussarellu et al., 2016) and human studies (Mishra et al., 2018, Schirinzi et al., 2017, Magrì et al., 2018) to investigate the potential causal link. This hazard characterization can then be used in conjunction with the exposure assessment to produce the risk characterization of MPs which will ultimately inform us of the likelihood that this hazard will adversely affect human health (EFSA, 2018, 2019, FAO and WHO, 2009, FDA, 2002). Further research is needed in order to establish exposure routes as well as exposure doses for a complete risk assessment of MPs (FAO and WHO, 2009, WHO and IPCS, 2005b). The outcomes of this study can be used by policy makers to address exposures to this emerging contaminant.

Chapter 5. Microplastic contamination of drinking water; Systematic review and meta-analysis results.

This chapter is based on a manuscript that was submitted for publication to the journal PLOS ONE (Danopoulos et al., 2020c).

5.1. Study selection

2467 citations were identified by the search strategy, after duplicates were removed, and 2307 citations were dismissed in the first-level screening based on their title and abstract as illustrated in Figure 36 (see section 3.2.5). During the second-level screening, the full papers were scrutinized, and 112 studies were removed with reasons (Appendix 8) and seven were included. When the searches were re-run (see section 3.3.4), five more studies were included after the first and second level screening (Figure 36), resulting in 12 studies (Kankanige and Babel, 2020, Mason et al., 2018, Mintenig et al., 2019, Oßmann et al., 2018, Pivokonsky et al., 2018, Schymanski et al., 2018, Shruti et al., 2020, Strand et al., 2018, Tong et al., 2020, Wiesheu et al., 2016, M. Zhang et al., 2020, Zuccarello et al., 2019a) finally included in this systematic review.

5.2. Study characteristics

All the studies included analysed water readily available for human consumption. The study characteristics are presented in Table 14. Six studies used samples of bottled water (BW) (table and mineral) and six studies used tap water (TW). The overall sample size for BW was n=91 brands (n=435 bottles) and for the TW, n=155 samples. All studies used different techniques to extract particles from their samples. One study used FT-IR (Mason et al., 2018), three studies used m-FT-IR (Mintenig et al., 2019, Strand et al., 2018, M. Zhang et al., 2020), one study used RM (Tong et al., 2020), four used m-RM (Oßmann et al., 2018, Schymanski et al., 2018, Wiesheu et al., 2016, Shruti et al., 2020), one both FT-IR and RM (Kankanige and Babel, 2020), one used both m-FT-IR and m-RM (Pivokonsky et al., 2018) and one SEM-EDX (Zuccarello et al., 2019a) to identify the composition of the extracted particles. Ten of the studies reported the results by MP particles per volume, one provides only the range of MP content and one the frequency of occurrence.

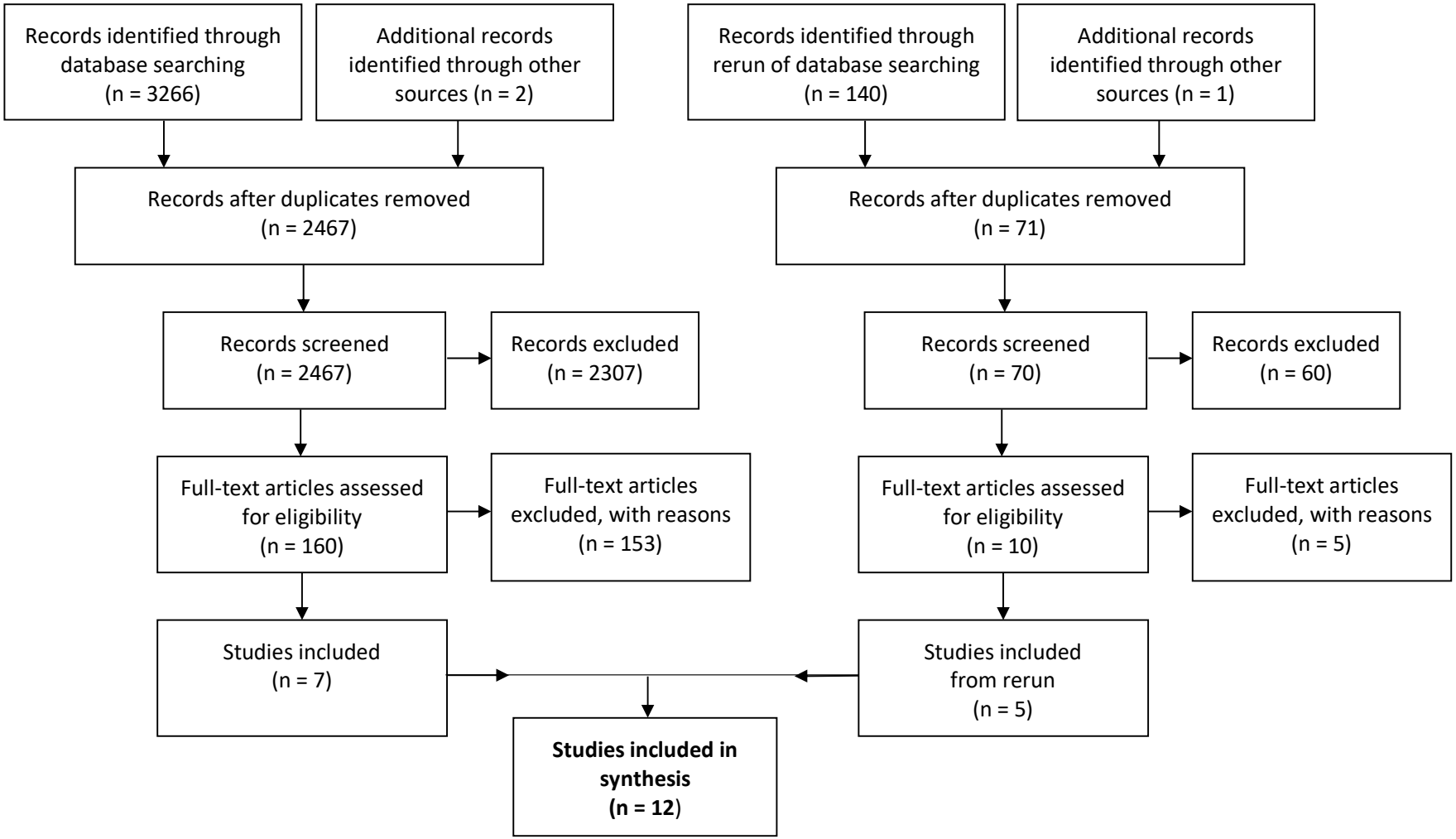


Figure 36**.** PRISMA flow diagram of screening process for drinking water studies.

Table 14. Drinking water study characteristics.

Study	Year	Geographic location	Sample	N	MPs extraction procedure	MPs identification method	Reported outcome
Mason et al. (2018)	2018	Brazil, China France, Germany, India, Indonesia, Italy, Lebanon, Mexico, UK, USA.	BW: table and natural mineral	N=259 bottles 11 brands: 9 brands 500–600 mL per bottle, 2 brands 0.75–2 L per bottle n=253 plastic bottles n=6 glass bottles	Their own procedure	FT-IR (particles > 100 μm)	Mean MPs content per volume
Mintenig et al. (2019)	2019	Germany	TW: Groundwater from wells	N=24 samples n=9 raw (8 m^3) n=15 drinking (32 m^3)	Mintenig et al. (2017)	m-FT-IR	Mean MPs content per volume and frequency of occurrence
Kankanige and Babel (2020)	2020	Thailand	BW: Spring and tap	N=95 n=65 PET single use (still) n=30 glass (carbonated) (10 brands, total 43.23 L)	Maes et al. (2017)	FT-IR and RM	Mean MPs content per volume with SD
Oßmann et al. (2018)	2018	Germany	BW: mineral	N=32 n=12 PET reusable n=10 PET single use n=9 glass reusable n=1 glass single use (21 brands, 0.5 – 1 L per bottle)	Oßmann et al. (2017)	m-RM	Mean MPs content per volume with SD

Pivokonsky et al. (2018)	2018	Czech Republic	TW: WTPs[a] from open reservoirs	N=36 (1 L per sample)	Anderson et al. (2017), Leslie et al. (2017) and Mintenig et al. (2017)	m-FT-IR for particles > 10 µm m-RM for particles 1–10 µm	Mean MPs content per volume with SD
Schymanski et al. (2018)	2018	Germany	BW: mineral	N=38 n=15 returnable plastic bottles n=11 single-use plastic bottles n=3 beverage cartons n=9 glass bottles (volume range 700-1500 mL)	their own method	m-RM	Mean MPs content per volume with SD
Shruti et al. (2020)	2020	Mexico	TW:	N=42 (3 L x 3 *per site*)	Liebezeit and Liebezeit (2014), Kosuth et al. (2018), Schymanski et al. (2018)	m-RM	Mean MPs content per volume with SD
Strand et al. (2018)	2018	Denmark	TW	N=17 n=9 private households n=3 private workplace n=5 private or public institutions	Strand (2018)	m-FT-IR	Frequency of occurrence
Tong et al. (2020)	2020	China	TW	N=38 (2 L *per site*)	their own procedure	RM	Mean MPs content per volume with SD

Wiesheu et al. (2016)	2016	Germany	BW: mineral	n=1 water (3 L)	their own procedure	m-RM	MPs content range per volume
M. Zhang et al. (2020)	2020	China	TW	N=7 (4.5 L x 3 per site)	their own procedure	m-FT-IR	Mean MPs content per volume with SD
Zuccarello et al. (2019a)	2019	Italy	BW: Mineral still and sparkling	N=10 (10 brands, 500 mL per bottle)	their own procedure	SEM-EDX	Mean MPs content per volume with SD

[a] Water treatment plants

Note: BW, bottled water; FT-IR, Fourier-transform infrared spectroscopy; MPs, microplastics; n/s: not specified; RM, Raman spectroscopy; SEM-EDX, Scanning Electron Microscopy - Energy Dispersive X-Ray Spectroscopy; TW, tap water

5.3. Risk of bias (RoB) within studies.

RoB was assessed in a systematic way using the RoB tool (see section 3.2.7). The results of the assessment are illustrated in Appendix 12 and Figure 37. Two studies were assessed as of high RoB (Zuccarello et al., 2019a, Wiesheu et al., 2016) and three of unclear RoB (Strand et al., 2018, Shruti et al., 2020, Tong et al., 2020). The RoB assessment is used in the analysis part of the review.

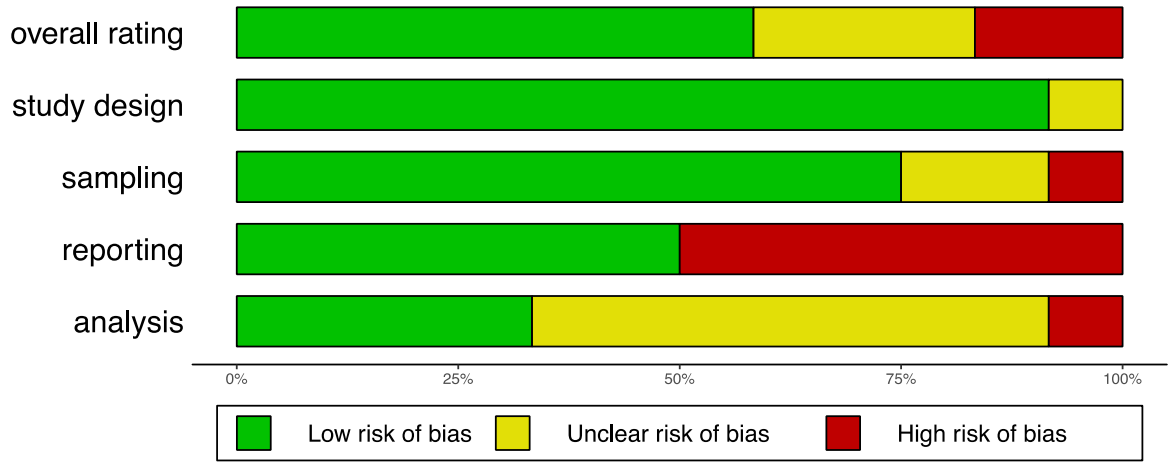


Figure 37. Risk of Bias (RoB) assessment across all water studies.

5.4. Results of drinking water MP contamination

The results are presented in Table 15 as two categories of TW and BW. The results from Mintenig et al. (2019) were converted from MPs/m^3 to MPs/L content for ease of comparison to the remaining studies. Mason et al. (2018) divided the results in two sections: one including particles ≥ 100 μm that were verified as MPs through FT-IR spectral analysis and particles < 100 μm that were only tagged using Nile Red solution to dye them. In line with the eligibility criteria, only the results of the FT-IR verified particles will be included in this review. Visual observation for the identification of MP particles can lead to under- or overestimations (Strungaru et al., 2019). The use of instruments which identify the chemical composition in a standardized way based on a physical or electronic output (spectra, pyrograms etc.) exclude the introduction of human error and enable reproducibility and transparency of the results. Regarding studies other than BW, when results were presented for both untreated and treated water, only the latter are presented, since the focus of the review is the expected human exposures, which relates to the water that is readily available for human consumption.

Table 15. Drinking water studies results

Study, Year	Sample type	n		Sample volume	MPs/L	±SD	Range MPs/L	Samples containing MPs (%)	Polymers	Shape
Mintenig et al. (2019)	TW: Ground-water from wells	N=24	n=9 raw n=15 drinking	8,000 32,000	0.0007		0 – 0.0007	42	Polyester 62%, PVC 14%, PA and epoxy resin 9%, PE 6%	fragments[a]
Pivokonsky et al. (2018)	TW: from WTPs[b]	N=36	WTP1 n=12	1 L per sample	443	10		100	PET 41%, PP	fragments > fibres > spherical
			WTP2 n=12		338	76			PET 62%, PP	
			WTP3 n=12		628	28			PET 26%, PP, PE 24%	
Shruti et al. (2020)	TW	N=42	metro stations water fountains	3 L x 3 per site	18	7	5 ± 2 to 91 ± 14	100	PTT, epoxy resin	fibres > fragments
Strand et al. (2018)	TW	N=17	n=9 private households	50 L for each sample	< 0.58			24	PP 50%, PS 25%, PET 25%	fragments
			n=3 private workplaces							
			n=5 private or public institutions							
Tong et al. (2020)	TW	N=38	private households	2 L per site	440	275	0 to 1247	95	PE 26.8%, PP 24.4%, co PE-PP 22.0%, PPS 7.3%, PS 6.5%, PET 3.3%	fragments > fibres > spheres

M. Zhang et al. (2020)	TW	N=7	private households	4.5 L x 3 per site	0.7	0.6	0.3 to 1.6	100	Rayon, PET, PE, PS, Polyester, PAA, PMPS, PIS	fibres > fragments
Kankanige and Babel (2020)	BW: Spring and tap	10 brands, 95 bottles	n=65 PET single use bottles	10 brands: total 43.23 L	140	19		100	PET 28.4%, PE 24.2%, PP 18.1%, PA 7.2%, PVC 4.4%	fibres > fragments
			n=30 glass bottles		52	4				
Mason et al. (2018)	BW: table and mineral	11 brands, 259 bottles	n=253 plastic bottles	9 brands: 500–600 mL per bottle 2 brands: 0.75–2 L per bottle	10.4[c] (≥100 µm) 315 (6.5-100 µm)		0-14	93	PP 54%, Nylon 16%	fragments > fibres > films
			n=6 glass bottles							
Oßmann et al. (2018)	BW: mineral	21 brands, 32 bottles	n=12 PET reusable bottles	0.5 – 1 L per bottle	4889	5432		Not specified	PET, PP, PET and olefin, PE	Not specified
			n=10 PET single use bottles		2649	2857			PET, PET and olefin, PP, PE	
			n=9 glass reusable bottles n=1 glass single use bottle		6292 3074[d]	10521 2531[d]			PE, PP, Styrene-Butadiene, PET	
Schymanski et al. (2018)	BW: mineral	38 brands, 38 bottles	n=15 returnable plastic bottles	700-1500 mL	118	88	28-241	100	PET 84%, PP 7%, PE 5%, PA 2%	fragments

			n=11 single-use plastic bottles		14	14	2-44			
			n=3 beverage cartons		11	8	5-20			
			n=9 glass bottles		50	52	4-156			
Wiesheu et al. (2016)	BW: mineral	1 brand	n=1	3 L	1 in the sample[e]			Cannot confirm contamination	PET	fibres
Zuccarello et al. (2019a)	BW: Mineral still and sparkling	10 brands, 10 bottles	n=10 plastic bottles	500 mL per bottle	5.42 X 10^7	1.95 X 10^7	3.16 X 10^7 to 1.1 X 10^8	100	Not specified	Not specified

[a] fibres were not taken into consideration, [b] Water Treatment Plant, [c] only particles ≥ 100 µm were verified with FT-IR, [d] without outlier, [e] only fibres counted

Note: PP, polypropylene; PVC, polyvinyl chloride; PA, polyamide (nylon); PE, polyethylene; PET, polyethylene terephthalate; PS, polystyrene; PTT, poly trimethylene terephthalate; PPS, polyphenylene sulphite; PAA, polyacrylic acid; PMPS, poly (methyl phenyl siloxane); PIS, poly (isoprene)

5.5. Tap water

Six studies (Mintenig et al., 2019, Pivokonsky et al., 2018, Strand et al., 2018, Shruti et al., 2020, Tong et al., 2020, M. Zhang et al., 2020) sampled and analysed TW that was readily available to consumers via a public service. The percentage of samples containing MPs across the studies ranged from 24% to 100% and the MPs content from 0-1247 MPs/L. The most common shapes identified were fragments and second most common was fibres. A key difference between the samples is that Pivokonsky et al. (2018) used water coming from surface waters (reservoirs), which are open aquatic systems exposed to contamination, while Mintenig et al. (2019) used water from underground and therefore protected sources. Shruti et al. (2020) used water from a variety of sources but the majority came from local aquifers. Strand et al. (2018), Tong et al. (2020) and M. Zhang et al. (2020) did not provide information on the origin of the water. It is reasonable to assume that water quality before it entered the WTP would vary and directly affect the quality of the water after processing (Di and Wang, 2018).

Four of the studies (Shruti et al., 2020, Tong et al., 2020, M. Zhang et al., 2020, Pivokonsky et al., 2018) provided the necessary data to attempt a meta-analysis. In order to test whether the results were appropriate for meta-analysis, the statistical heterogeneity was measured using a Higgins I^2 test (Higgins and Thompson, 2002), calculated using R (version 3.6.0) (R Core Team, 2019), executing all analysis via RStudio, (version 1.2.1335) (RStudio Team, 2018), and using the additional packages meta (version 4.9-7) (Schwarzer, 2019), metaphor (version 2.1-0) (Viechtbauer, 2010), dmetar (Harrer et al., 2019a), robvis (McGuinness and Kothe, 2019) and ggplot2 (Hadley, 2016). A random-effects model was fitted (Chen and Peace, 2013, Harrer et al., 2019b) and heterogeneity was found to be high, I^2 = 99.8% (chi^2=1301.43, $p < 0.0001$). The results of the model were SMD=96.5751 (CI 95% 76.3584 to 116.7919), $p < 0.0001$, as shown in Figure 38.

In order to detect the origin of heterogeneity, a series of random-effects models were fitted excluding two studies (Tong et al., 2020, Pivokonsky et al., 2018) that were identified as statistical outliers. The exclusion of the studies did not improve heterogeneity which remained high (I^2 = 99.9%, chi^2= 737.11, $p< 0.0001$). Although the effect size was significantly reduced to 9.3393, the confidence interval extended below 0 (95% CI -7.6144 to 26.2929), p=0.2803. Excluding the high RoB study (Tong et al., 2020) and refitting the model still detected high heterogeneity I^2 = 99.8%, chi^2 1204.79, $p< 0.0001$. Therefore, the data were found to be inappropriate for meta-analysis. Heterogeneity was either caused by

clinical (sample) or methodological variability (Higgins et al., 2019) and is further discussed in the narrative analysis section.

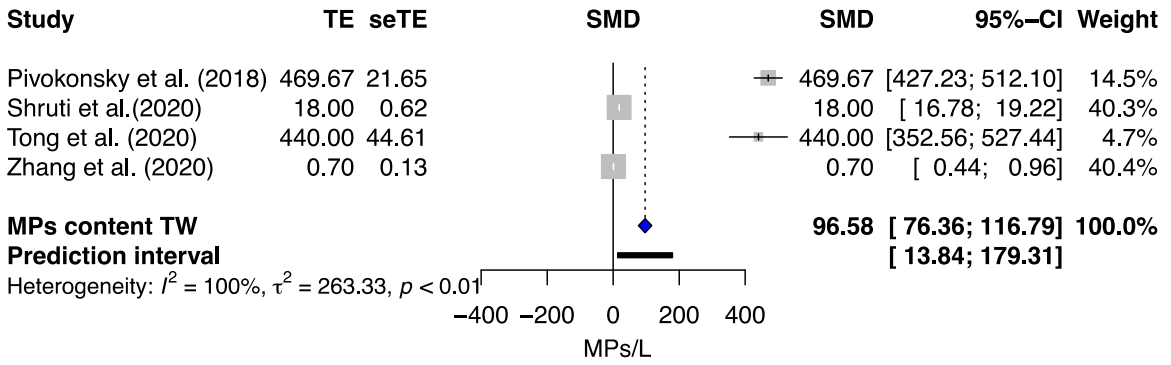


Figure 38. Forest plot for random-effect model, MP content in TW studies. The x axis represents the standardized mean difference (SMD) expressed in microplastics per litre (MPs/L). TE is the MP content reported by each study and seTE is the calculated standard error (SE). The vertical line is the line of null effect where MP content is 0. The grey boxes represent the pooled effect estimate and the whiskers the CI 95%. The size of the boxes is proportional to the study weight. The diamond is the combined point estimate and CI 95%, and the dotted line is the overall pooled effect. The black box represents the 95% prediction interval.

5.5.1. Sample treatment/ particle extraction

The experimental protocol for the extraction of particles differed between the six studies in terms of sample collection, treatment and filtering. Mintenig et al. (2019) filtered the water directly at the sampling sites using stainless steel filter cartridges (3 μm) and then further treated the residue on the filters at the lab. A solution of hydrochloric acid was used to dissolve inorganic material, such as calcium carbonate and iron precipitates, followed by a second filtering through another 3 μm stainless steel filter. The residue was treated again using hydrogen peroxide before the third and final filtration on 0.2 μm aluminium oxide filters. An additional density separation step was used for the raw water samples, employing a zinc chloride solution to remove further iron oxide particles. Strand et al. (2018) also filtered the samples at the sampling sites but using a stainless-steel filter with absolute filtering ability of 11-12 μm. The sample was then treated using a solution of acetic acid. For the collection of the particles used for the spectral analysis, a backwashing procedure with detergent solution was used, this was pre-filtered water and then ethanol under vacuum suction on an Anodisc filter (0.2 μm). Four studies (Pivokonsky et al., 2018, Shruti et al., 2020, Tong et al., 2020, M. Zhang et al., 2020) collected the samples in bottles and then transported them to the lab for processing. Pivokonsky et al. (2018) used wet peroxide oxidation and heat treatment at 75 °C for digestion, followed by a double filtration through 5 μm and then 0.2 μm membrane filters (PTFE). Tong et al. (2020) used hydrochloric acid

for digestion followed by filtering through 0.2 μm aluminium oxide filters. In contrast, Shruti et al. (2020) and M. Zhang et al. (2020) did not treat the samples prior to filtering, using 0.22 μm and 0.45 μm pore size filters respectively. The difference in the pore size of the filters used in the different stages reflects the sizes of the particles extracted which were subsequently further analysed for composition identification, and has thus directly affected the measured MP content. On the other hand, the use of a digestion step to dissolve particulate matter is employed only by some of the studies to extract water impurities and optimize the filtration process.

5.5.2. Spectral analysis

Differences in the methodology of the studies were identified while important information such as the number of extracted particles and the number of particles that were analysed for composition were not reported (Appendix 13). Three studies used FT-IR for spectral analysis, while Pivokonsky et al. (2018) also used RM for the smaller size range of 1-10 μm. One study used m-FT-IR, one RM and one m-RM. A key difference between them is the technical limitation of the instrument regarding the minimum particle size detected. FT-IR and RM technical specifications are in the range of 40 μm and 10 μm, respectively. When these methods are used in conjunction with microscopes, it becomes possible to analyse particles down to the size of 10 μm (m-FT-IR) and 1 μm (m-RM) (Araujo et al., 2018, Bergmann, 2015, Harrison et al., 2012, Löder et al., 2015, Strungaru et al., 2019). Mintenig et al. (2019) and M. Zhang et al. (2020) analysed 100% of the filters' surface, Pivokonsky et al. (2018) about 25% of the sample and Strand et al. (2018) 10% of the filter but coming from only three out of the 17 sampling sites/samples. Shruti et al. (2020) and Tong et al. (2020) did not report the amount of the sample analysed.

None of the studies reported the final number of particles analysed and only Strand et al. (2018) reported the success rate of conclusive identification (44%) and the proportion that was identified as MPs (3%). Only the two studies by Pivokonsky et al. (2018) and M. Zhang et al. (2020) reported the similarity index for the spectral analysis, 80% and 70%, respectively. Although scientific guidance on the particles that need to be analysed does not exist, it is reasonable to assume that larger proportions would lead to more robust results. Mintenig et al. (2019) did not analyse the fibres at all. Although a larger number of fibres were discovered compared with 'particles' in the samples, spectral analysis was not utilised because the fibre presence was attributed to their presence as post-sampling contamination. Fibres are a high proportion of MPs and their complete exclusion from the results might have resulted in an underestimation of MP content.

5.5.3. Particle size

The key difference in the studies' protocol is the size of the particles identified and verified via spectral analysis and is directly connected to the extraction process and the composition identification process used. Shruti et al. (2020) only analysed particles > 500 μm, Mintenig et al. (2019) ≥ 20 μm, Strand et al. (2018) and M. Zhang et al. (2020) ≥ 10 μm, Pivokonsky et al. (2018) ≥ 1 μm, while Tong et al. (2020) did not report the minimum size. The study by Pivokonsky et al. (2018) reported the highest MP content ranging from 338 ±76 to 628 ±28 MPs/L and stated that 25-60% of the MPs were in the range of 1-5 μm and 30-50% in the range of 5-10 μm. Tong et al. (2020) reported content in the same magnitude of 440 ±275 MPs/L, and state that MPs < 50 μm were significantly dominant. It must be noted that Tong et al. (2020) used only Nile Red dying and visual identification for the determination of particle size in a reported range of 3-4453 μm. The results from these two studies present a noteworthy difference. When the MPs' size range is taken into consideration it becomes clear that this variance could be attributed to the fact that the other four studies were not able to detect that same range of sizes (Figure 39). In addition, it should be noted that although Strand et al. (2018) state that particles were measured down to 10 μm, the majority of the results were based on particles ≥ 100 μm. The inverse relationship between the size of MPs and their abundance is further supported by the findings of Shruti et al. (2020) who reported that 75% of the particles were in the range of 100 μm - 1 mm, M. Zhang et al. (2020) who reported that 46% were in the range of 500 μm - 1 mm and Mintenig et al. (2019) who found that all particles were in the range of 50 - 150 μm.

5.6. Bottled water

Six studies samples BW (Table 3). Kankanige and Babel (2020) sampled spring and TW, Mason et al. (2018) sampled table and mineral water and the rest of the studies sampled only mineral water. Three different container materials were selected: plastic (single-use and reusable), glass and carton. MPs content ranged from 0 to 1.1 X 10^8 MPs/L across all containers. The percentage of samples containing MPs ranged from 92% to 100%. Fragments and films were the most commonly identified shapes. Meta-analysis was attempted using the results from four of the studies (Schymanski et al., 2018, Kankanige and Babel, 2020, Zuccarello et al., 2019a, Oßmann et al., 2018) which provided the necessary data. Statistical heterogeneity as measured by Higgins I^2 test in a random-effects model was found to be high, I^2=99.4%, chi^2=1593.72, p=0. The results of a random-effects model were SMD=67.9031 (95% CI 22.3353 to 113.4708), p=0.0035 as shown in Figure 40. Heterogeneity remained high I^2 = 99.5%, chi^2= 1516.46, p < 0.0001, even when the high RoB study by Zuccarello et al. (2019a) was excluded.

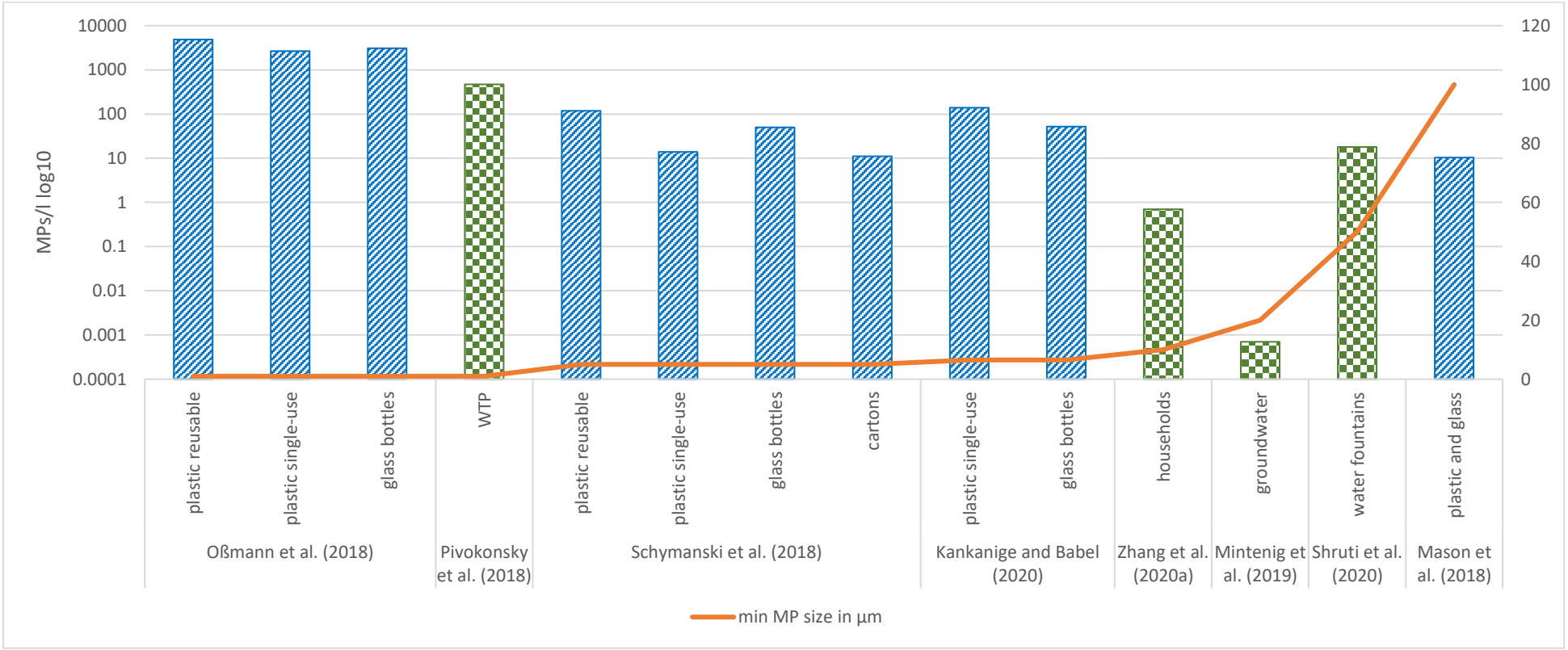


Figure 39. MP content in tap water (TW) and bottled water (BW). MP content (MPs/L) is illustrated in the left-hand side y axis in $\log_{10}$ scale. BW: diagonal stripes, TW: chequerboard, Minimum particle size included in each study is illustrated in the right-hand side y axis. Studies by Tong et al. (2020), Wiesheu et al. (2016) and Zuccarello et al. (2019a) were not included because they were rated as of high riks of bias (RoB). Note: WTP, water treatment plant

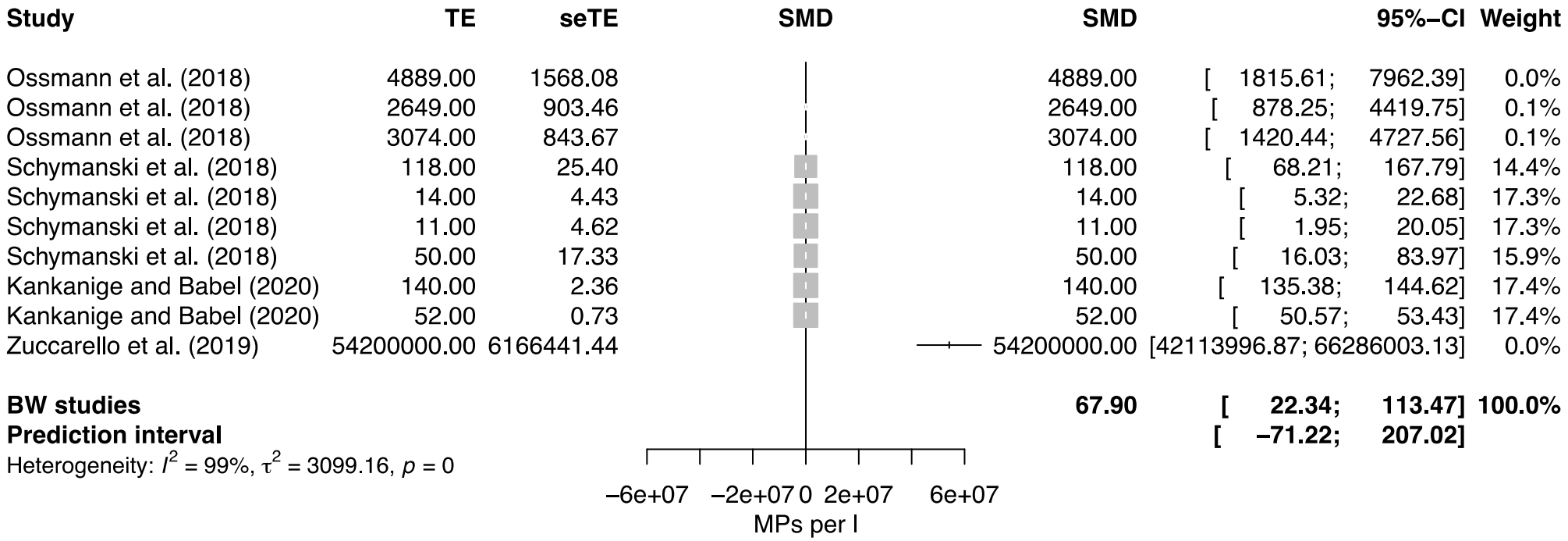


Figure 40. Forest plot bottled water random-effects model. The x axis represents the standardized mean difference (SMD) expressed in microplastics per litre (MPs/L). TE is the MP content reported by each study and seTE is the calculated standard error (SE). The vertical line is the line of null effect where MP content is 0. The grey boxes represent the pooled effect estimate and the whiskers the CI 95%. The size of the boxes is proportional to the study weight.

Examining the four different types of containers separately in a mixed-effects subgroup analysis (Chen and Peace, 2013, Harrer et al., 2019b), statistical heterogeneity within the groups still remained high I^2 from 84% (glass bottles) to 100% (plastic reusable) (Figure 41). The pooled effect estimate was accompanied by a 95% confidence interval which included negative values for all categories, further showing that meta-analysis was not appropriate. The results of the analysis showed that pooling of the data was not appropriate. The origin of heterogeneity is addressed in the narrative analysis.

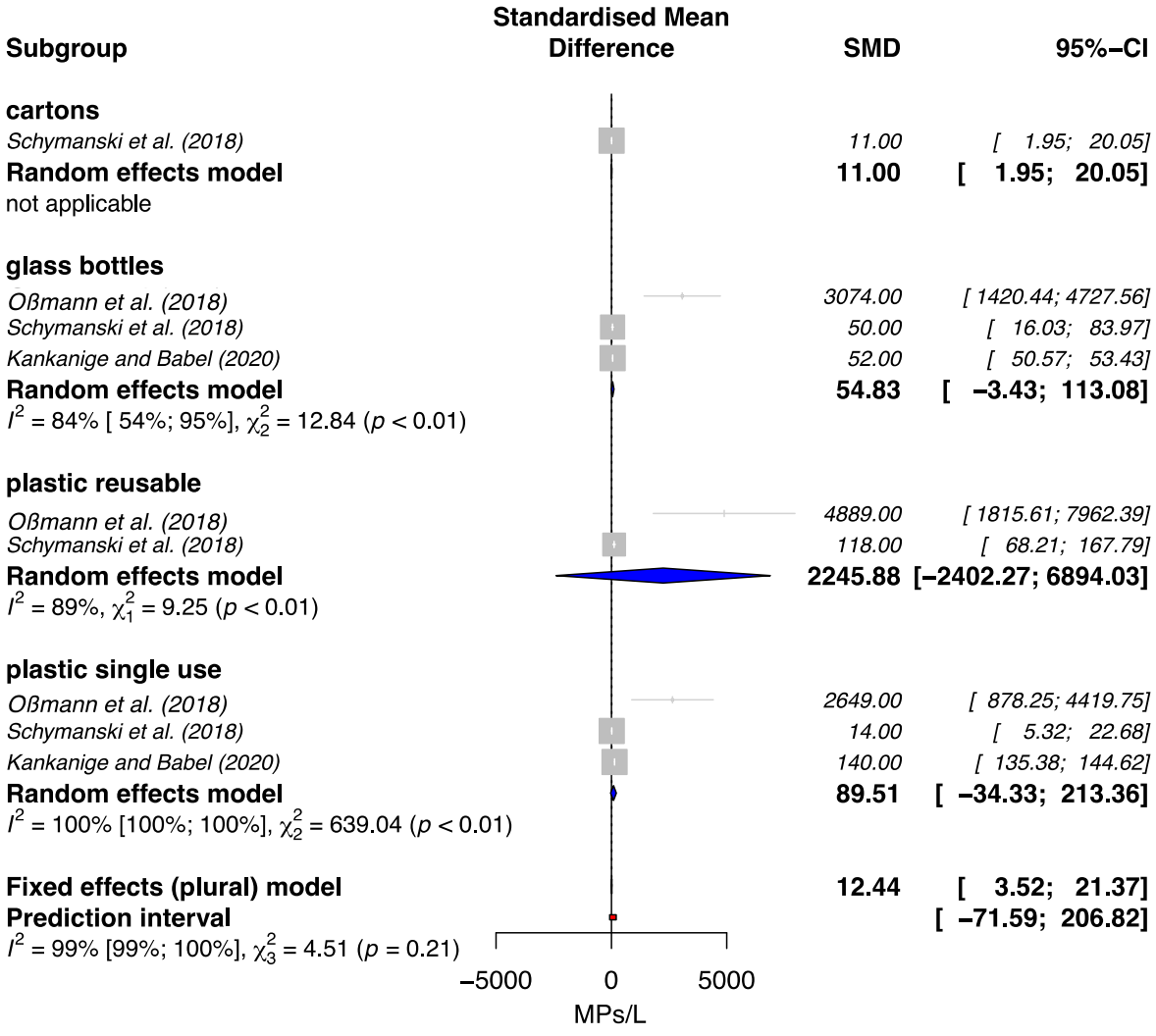


Figure 41. Subgroup analysis. Bottled water samples. The x axis represents the standardized mean difference (SMD) expressed in MPs/kg. The vertical line is the line of null effect where MP content is 0. The grey boxes represent the pooled effect estimate and the lines the confidence interval (CI) 95%. The size of the boxes is proportional to the study weight. The diamonds are the combined point estimates and CI for each of the subgroups. The dotted line is the overall pooled effect for all subgroups with a corresponding diamond. The red box is the prediction interval PI 95%.

5.6.1. Sample treatment/ particle extraction

Four studies (Mason et al., 2018, Schymanski et al., 2018, Wiesheu et al., 2016, Kankanige and Babel, 2020) did not use a digestion process. Mason et al. (2018) used glass-fibre filters (1.5 μm pore size), Schymanski et al. (2018) used gold-coated poly-carbonate filters (3.0 μm pore size) while both studies by Kankanige and Babel (2020) and Wiesheu et al. (2016) used cellulose nitrate filters (0.45 μm pore size). Oßmann et al. (2018) implemented a digestion process using an ethylene diamine tetra-acetic acid tetrasodium salt (EDTA) solution then followed by a density separation (flotation) step via a detergent solution of sodium dodecyl sulphate (SDS) and filtration through aluminium-coated polycarbonate membrane filters (0.4 μm pore size). Zuccarello et al. (2019a) did not employ a digestion nor a filtration process, opting for a newly developed method to target MPs < 10 μm, which differs significantly from previous studies and cannot thus be directly compared to the rest of the studies. The alternative approach used nitric acid and a high temperature incubation (60° C for 24 hours) for mineralization of the samples to remove carbon-based particles. This was followed by vortexing, centrifuging, addition of dichloromethane, resuspension using acetonitrile and drying. The sample was then deposited on an aluminium and copper alloy stub to be coated with gold before SEM-EDX analysis (Zuccarello et al., 2019b). The methods used by this study have already been highlighted (Oßmann et al., 2019) under the reporting and verification sections of the analytical methods which was partially addressed by a corrigendum of the authors (Zuccarello et al., 2019b). The scientific base of the process employed is a publication that is not available in English (Sosna et al., 2003) and therefore cannot be assessed, as well as a second publication (Roch and Brinker, 2017) concerning MPs extraction method from the GI tract of fish. The latter describes a different method (two-step digestion process using sodium hydroxide and nitric acid, followed by filtration, density separation and verification by visual identification alone, that subsequently targets MPs of a completely different size of >100 μm).

5.6.2. Spectral analysis

Schymanski et al. (2018) examined the largest number of particles in RM spectral analysis, analysing 100% of the particles or a maximum of 1000 (in the 5-10 μm size fraction) on each of the filters, corresponding to each of the 38 samples (Appendix 14). The verified MP particles ranged from 0.03 to 10.7% of the analysed particles, using a ≥ 70% spectral similarity index. Kankanige and Babel (2020) analysed 100% of the extracted particles (> 50 μm), using FT-IR and a 60% spectral similarity index, verifying 45.8% of them as MPs. RM analysis was used for particles of the lower range of 1-10 μm but these findings are not

reported in the details of the analysis. Mason et al. (2018) also used FT-IR but only for particles ≥ 100 μm and examined around 1000 particles which was almost 50% of the particles extracted, using a ≥ 70% similarity index verifying 40% of the particles as MPs. Oßmann et al. (2018) on the other hand, did not provide information on the number of extracted particles, reporting the analysis of 4.4% of the surface of each filter using RM, but not reporting how many were finally verified as MPs. Oßmann et al. (2018) did not use an automated software option in which spectral similarity is calculated automatically but a mix of semi-automated methods. In this sense, a standardized spectral similarity index was not utilised, which might have introduced experimental error into this protocol. Wiesheu et al. (2016) only analysed the one fibre extracted from the samples isolated, not providing further details on the methods employed.

Zuccarello et al. (2019a), (Zuccarello et al., 2019b) used SEM-EDX for the identification of MPs. No digestion or filtration process for the extraction of the mineral water impurities was employed. The authors suggest that the mineralization process extracts all carbon-containing particles that are not plastic. This removal needs to be done with near unit efficiency due to the fact that typical concentrations of carbonates in mineral water exceed, by many orders of magnitude, the reported MP concentrations in BW samples in other studies. The specificity of this method has not been proven as mentioned in the previous section. The aim of the method was to quantify the number of MPs per volume in the size range of 0.5 – 10 μm and a further objective was to calculate the mass of MPs per volume, using the density of the plastic bottles containing the water. The reported validation of the process used is weak in that the mass of MPs per volume was measured in three samples spiked with MPs (whose size was not reported), and then a calculation of MPs per volume was conducted, which is the opposite way round to the calculation made with the unknown samples and may introduce systematic error.

5.6.3. Particle size

Mason et al. (2018) used FT-IR only for particles ≥ 100 μm but reported that 95% of particles were between 6.5 and 100 μm. The MP content for all sizes was 325 MPs/L, whereas for particles ≥ 100 μm it was only 10.4 MPs/L. In addition, it was not clear what maximum size cut-off was employed. Kankanige and Babel (2020) used FT-IR for particles ≥ 50 μm but extrapolated the findings to the smaller size range 6.5 – 50 μm, reporting MPs contents of 140 ±19 MPs/L for plastic bottles and 52 ±4 MPs/L for glass bottles. The size range of 6.5 – 20 μm was identified as the most dominant. Schymanski et al. (2018) extracted and

analysed particles including even smaller sizes of ≥ 5 μm and reported that 80% of the verified MPs were in the range of 5 and 20 μm, with MP contents of 14 ±14 MPs/L for single use plastic bottles, 118 ± 88 MPs/L for reusable plastic bottles, 11 ± 8 MPs/L for carton and 50 ± 52 MPs/L for glass bottles. Oßmann et al. (2018) decreased the size of the included particles to ≥ 1 μm reporting much higher MP contents of 2649 ± 2857 MPs/L for single use PET bottles, 4889 ± 5432 MPs/L for reusable PET bottles and 6292 ± 10521 MPs/L for glass bottles. The same authors also highlight that more than 95% of MPs were smaller than 5 μm and 50% smaller than 1.5 μm. Zuccarello et al. (2019a) focused on the 0.5-10 μm size range, reporting high concentrations of 5.42 ± 1.95 X 10^7 MPs/L. Although the size range of the identified MPs (1.28 – 4.2 μm) is similar to the Oßmann et al. (2018) study (> 1 μm), the results differ by a factor of 11000, further highlighting the possible quality issues of the study. The results of the Wiesheu et al. (2016) study on MPs content were inconclusive. As can be seen in Figure 39, as the size of the identified particles decreases, the MP content increases significantly.

5.7. Discussion

Twelve studies were systematically reviewed, which collectively analysed more than 40000 L of TW and 435 bottles of BW (table and mineral water). It would not be appropriate to collate the evidence from the twelve studies included in this systematic review due to key differences that were identified in the experimental protocols and high sample heterogeneity. In addition, the lack of key information (e.g. SE, SD) and high statistical heterogeneity hinder the execution of meta-analysis in an attempt to quantify MP content. RoB was found to be low in the majority the studies. Two studies were rated as of high RoB and therefore the results of these are excluded. The study by Zuccarello et al. (2019a) was rated high RoB in the two domains of sampling and reporting, while the study by Wiesheu et al. (2016) was rated high RoB in the domains of analysis and reporting.

All studies reported some level of MP contamination. Samples positive for contamination ranged from 24-100% in TW and 92-100% for BW. Comparing the results between the different water origins, specifically between the two studies (Oßmann et al., 2018, Pivokonsky et al., 2018) that targeted similar MP sizes of minimum 1 μm, MP content was higher in BW (plastic and glass bottles) than TW (Figure 39). Therefore, current evidence suggests that there are higher rates of MP contamination in BW compared with TW, both in terms of frequency and quantity. This variation will inevitably drive the level of potential MP human exposures according to the product that is consumed. The consumption of TW

or BW can depend on geographical, cultural, economic, or even lifestyle parameters. In many cases it is not a choice altogether since there are several places around the world where TW is not available, suitable or preferable for consumption thus forcing people to use alternatives.

According to its primary origin BW is divided into table, spring and natural mineral water. Specific regulations govern their categorization according to their origin and the processes that they are allowed to undergo before being bottled (e.g. Council directive 98/83/EC, 1998, Directive 2009/54/EC, 2009, The Natural Mineral Water Spring Water and Bottled Drinking Water (England) (Amendment) Regulations, 2018). Water from different categories will vary in quality depending on the initial water quality, and the processes they are subjected to ensure food safety, transportation and packaging. Regarding the primary origin of BW, Mason et al. (2018) analysed table and mineral BW, and Kankanige and Babel (2020) tap and spring BW, but did not report a comparison between the different water origins which could shed some light on the possible differences. Both natural mineral and spring water come from underground water sources, in principle, protected from pollution and are bottled *in situ*. In contrast, bottled table water can come from any source, including municipal mains (TW), as long as it conforms to water safety specifications (Council directive 98/83/EC, 1998). Comparison between the different origins could provide evidence and insights regarding the source of MP contamination; whether it is found in the raw material or is introduced during processing or even after packing.

The methodology used in the studies varied in both sampling and analysis. Standardization of the experimental protocols is key in order to increase confidence in the quality of the studies and certainty of the evidence. The first step in obtaining comparable and trustworthy results is the use of a verified composition identification process, which was employed by all of the studies included in this review. Not using such a process has been proven to lead to gross under- or over-estimations (Mai et al., 2018, Strungaru et al., 2019, Shaoliang Zhang et al., 2019). Even with all the studies using either FT-IR, RM or SEM-EDX, there were still differences in the spectral similarity index, the number and proportion of the particles analysed, and the spectral library used. Furthermore, poor reporting hindered the assessment of the experimental protocols' effectiveness; only one study (Kankanige and Babel, 2020) reported how many particles were retrieved from the extraction process and only four (Kankanige and Babel, 2020, Mason et al., 2018, Schymanski et al., 2018, Wiesheu et al., 2016) reported how many particles were analysed for composition identification.

The most significant difference in the methods is the size of the particles that were extracted from the samples and analysed for composition identification. Studies using FT-IR and RM were able to analyse particles down to 1 μm which significantly influenced the results. The degradation of MPs in the marine environment and the exponential increase of the number as the size decreases has been experimentally and mathematically explored (Cozar et al., 2014, 2015, Ter Halle et al., 2016). This would suggest that the same fragmentation pattern may also apply to other aquatic environments as well.

On the other hand, only seven (Mintenig et al., 2019, Oßmann et al., 2018, Pivokonsky et al., 2018, Shruti et al., 2020, Tong et al., 2020, Wiesheu et al., 2016, M. Zhang et al., 2020, Zuccarello et al., 2019a) of the twelve studies reported the upper limit of the range in MP size. The importance of defining and reporting the size range of the identified MPs has a double significance as follows. As a methodology parameter it is connected to the quantified MP content results. As a food contamination parameter, it is indicative of the potential health effects. MPs < 1.5 μm are characterized as more dangerous since they are, in theory, capable of crossing the gut epithelium, further progressing into the human body and thus possibly causing an adverse health effect (EFSA, 2016).

Differences in sample size were striking, ranging from 36 to 32000 L (per study) for TW and 3 to (>)130 L for BW. At the moment, methodological consensus concerning sample size does not exist. Koelmans et al. (2019), in a recent review, proposed a minimum of 1000 L for TW and 500 L for BW. In the first instance, sample size is dictated by the objectives and design of the study which in many cases are a function of the available resources (EPA, 2000, 2002b). Sample size should be directly connected to the contaminant under examination. The volume of the samples as well as the sampling frequency can only be set when there is enough evidence to support what a meaningful MP content is. Meaningful being expressed in terms of food safety linked to human health and what is considered to be ‘wholesome and clean’ water intended for human consumption, which is the requirement of relevant European regulations and universal standards (Council directive 98/83/EC, 1998, Council Regulation (EC) No 178/, 2002, WHO, 2017). At the moment, there is not enough evidence to formulate an informed guideline for sampling sizes, nevertheless scientific experience points to larger sample sizes being more robust and reliable (Zhang, 2007).

Another area of importance is quality assurance of sampling and sample handling to avoid cross contamination via airborne MPs. This issue was addressed by the RoB assessment tool in the sampling domain. In addition, only studies that employed blank procedural samples

to account for this type of experimental error were included (A. L. Lusher et al., 2017, Silva et al., 2018). The lack of detailed information on the results and the significance of procedural blank samples downgraded the quality of the study as assessed by the RoB assessment tool. The bespoke RoB tool used did not employ scales to rank the studies as done by other reviews in the field (Koelmans et al., 2019) but is a domain-based evaluation according to the guidance of leading methodology regarding systematic reviews (Higgins et al., 2019). The use of scales in RoB assessment is explicitly discouraged as research experience has shown that they can be unreliable (Higgins and Green, 2011).

Seven studies used samples from Europe (3 TW, 4 BW), three from Asia (2 TW, 1 BW), one from North America (TW), and one from multiple continents (BW) (Table 14). The highest MPs content are reported in Europe for both TW and BW. Regarding TW, the highest reported MPs content for Europe and Asia were in the same magnitude but almost 25 times higher than those reported for the samples from North America. In BW, the maximum reported MPs content in Europe was 35 times higher than that reported in Asia. However, it is not clear if this is due to the number of existing studies and the varying methodology employed or to various factors external to this review such as the geographical origin of the water, the possible differences in the treatment of TW and BW around the world, the differences in legislation governing drinking water standards, equipment and materials used during transportation, processing and packaging etc. Recent research has shown that MP contamination of the environment is directly linked to waste management, which is compromised in developing countries (Burns and Boxall, 2018, Jambeck et al., 2015). In this sense, it would be reasonable to expect higher MPs contamination of potable water in these countries, where further research is needed. In terms of polymeric composition, PET and PP were the most prevalent polymers identified in BW. The differences between the polymeric composition in the various BW studies can be attributed to the different origin of the water, processing, the material used for packaging but also to the different particle sizes the studies extracted and analysed since degradation rates between polymers vary (Hartmann et al., 2017, 2019). In TW, polymeric composition varied with PET and PP present along with polyester, PTT and rayon. This may possibly be due to the wide geographical and environmental origin of the water samples. Rayon is a man-made but not synthetic fibre and is not included in most MP research. It should be noted that the most produced and used polymers for the last 15 years have been PE and PP, whose prevalence would be anticipated to be the highest in terms of environmental contamination although geographical variation is expected (Plastics Europe, 2008, 2017, 2018, 2019).

Fragments and fibres were the prevalent MP shape in both categories, highlighting an agreement in the findings across all studies. Polymeric composition and shape characteristics can be used as guides to the origin of MPs as well as to focus future toxicological research.

A recent review by Koelmans et al. (2019) has recently addressed the issue of MPs contamination of drinking water. Koelmans et al. (2019) focused not only on drinking water but also on freshwater MP contamination and experimental methodology and did not attempt quantitative collation of the evidence. The study assessed the quality of the studies using a bespoke rating system, focusing on different aspects of experimental design and execution using a scoring system. The use of scoring scales in quality assessment is explicitly discouraged by the Cochrane Collaboration, which is the leading body of systematic reviews, as research experience has shown that they can be unreliable due to the lack of justification for the ratings (Higgins and Green, 2011, 2019, Page et al., 2018, Whiting et al., 2016). The World Health Organization (WHO) delivered a report (2019) based on a commissioned systematic review by Koelmans et al. (2019), yet the authors make no claim that it is systematic, nor is there a description of the relevant review methods utilised, such as the existence of a published protocol. A MP exposure assessment via the consumption of drinking water, based on the findings of this systematic review, is presented in section 8.3.2.

5.8. Strengths and limitations

At the time this review was published (Danopoulos et al., 2020c), it was the first systematic review focusing on MP contamination of water intended for human consumption. The review was based on a protocol which was created beforehand, outlining the methodology used throughout. The protocol ensures that bias is not introduced. In addition, the quality of studies was assessed using a systematic RoB tool tailored to the needs of the review, addressing every stage of design, execution and reporting of research. The review was limited to a narrative analysis and did not include a meta-analysis due to high sample, experimental and statistical heterogeneity as well as poor reporting in a fraction of the studies. The majority of the studies were assessed to be low RoB.

5.9. Chapter conclusions

Research methodology in the field of MPs environmental contamination has advanced in recent years, especially with the use of FT-IR and RM validation of particle characteristics, but is still lacking in quality and robustness.

The systematic review identified specific areas where further development and standardization is needed:

- Sampling methodology: sampling size, location, frequency, instruments, quality assurance, procedural blanks, replicate samples.
- Registry of all relevant sample characteristics when available: brand, geographical and environmental origin, volumes, production dates, information on water treatment and additives.
- Particle extraction process specifications: sample volumes, chemicals used for digestion and density separation, type and pore size of filters.
- Spectral analysis:
    - Use of one of the currently validated methods: FT-IR, RM, SEM, Pyr-GC-MS and SEM-EDS.
    - Proportion of extracted particles for analysis.
    - Spectral similarity index and which spectral libraries are used (bespoke or commercially available).
- Post-sampling handling: measures to protect cross-contamination and use of procedural blank samples in all experimental aspects to ensure effectiveness and account for experimental errors.
- Detailed reporting of all aspects of research including design, execution and statistical analysis.

In terms of future research there is a clear need for research on MP contamination of drinking water in countries beyond Europe where there is less data. Comparison between table water, natural mineral and spring waters to detect differences is another area that has not been explored. The additional exposure pathway via the use of MP contaminated water for incorporation into food also merits further research.

As this review shows, there are still relatively few studies examining MP contamination in drinking water, and levels vary significantly. The presence of MP in human stool samples has recently been verified (Schwabl et al., 2019), although the effects on human health are still under examination (Abbasi et al., 2018, Gallagher et al., 2015, Gallo et al., 2018, Schwabl et al., 2019, Wright and Kelly, 2017). Given the amount of water humans drink and its use for incorporation into food, a clearer understanding of the levels of MP present in drinking water is needed, in order to better assess the risks that MPs in water present. Quantification of MPs human exposures is an integral part of the exposure assessment in the

wider frame of a risk assessment to determine the likelihood of MPs having adverse human health effects (SAM, 2019, SAPEA, 2019).

The findings support the omnipresent MPs contamination of drinking water. Current food and drinking water safety regulation and standards around the world (ISO, 2018, Wallace, 2015, Council Regulation (EC) No 178/, 2002) adopt the precautionary principle (Kriebel et al., 2001, Jackson and Steingraber, 1999) on food safety risk management. The principle dictates that in the face of scientific uncertainty concerning possible harmful effects, after an initial assessment of available evidence has been completed and a comprehensive risk assessment is anticipated, risk management measures must be adopted in order to ensure the protection of health. The weight of the current evidence suggests that the time may have come to implement protective measures against the ingestion of MPs.

Chapter 6. Microplastic contamination of seafood intended for human consumption; Systematic review and meta-analysis results

This chapter is based on a manuscript that was submitted for publication to the journal Environmental Health Perspectives (Danopoulos et al., 2020b).

6.1. Study selection

The initial searches led to 2467 publications following the removal of duplicates (see section 3.2.5). On the first level screening, 2307 citations were excluded on the basis of their title and abstract. For the second level screening, the full text of the remaining 160 studies were evaluated and a total of 34 studies that analysed seafood samples met the eligibility criteria set for this review (see PRISMA flow diagram, Figure 42). The update of the searches (see section 3.3.4) identified 16 more studies eligible for the review, bringing the total number of included studies to 50.

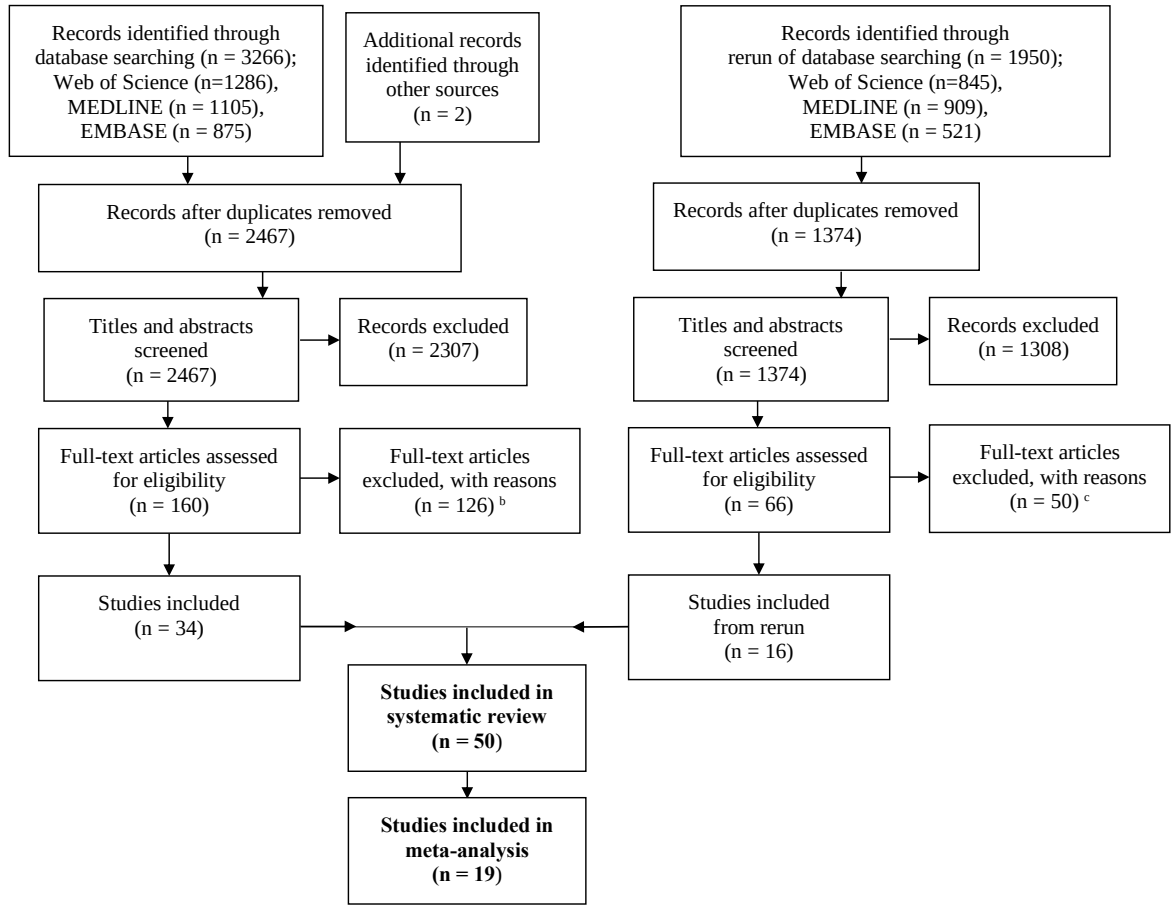


Figure 42. PRISMA flow diagram of screening process for seafood studies.

6.2. Study characteristics

All the studies included are environmental field studies employing descriptive and analytic observational study designs, sampling and analysing four phyla: molluscs, crustaceans, fish and echinoderms (Table 16). Eight studies analysed organisms coming from more than one phylum. Twenty-three studies sampled only molluscs, 15 only fish, three only crustaceans and one only echinoderm. Five studies sampled both molluscs and crustaceans, two molluscs and fish, and one comprised molluscs, crustaceans and fish. The study characteristics are presented in Table 16. Twenty-eight studies used samples from Asia, 13 from Europe, four from the Americas, two from Africa, one from Australia/Oceania and two from more than one continent (and their coasts). The overall sample size for fresh fish was n=1,269 (n=665 anchovies, n=274 sardines, n= 240 painted comber, n=20 sand lance, n=19 bogue, n=19 seabass, n=12 haddock, n=10 plaice, n=10 mackerel), dried fish n=120 (n=30 mackerel, n=30 croaker, n=30 mullet, n=30 anchovies) and canned fish n=842 (n=608 sprat, n=184 sardines, n=45 tuna, n=5 mackerel). For the rest of the seafood, the overall sample size was n=4,543; molluscs n=3,882 (n=1,728 mussels, n=1,015 oysters, n=702 clams, n=171 sea snails, n=166 scallops, n=100 cockles), crustaceans n=451 (n=262 shrimps, n=139 crabs and n=50 barnacles) and echinoderms n=210. Two studies did not provide the exact sample size; Qu et al. (2018) reported n~760 mussels and F. Z. Wu et al. (2020) reported 10-20 samples for each species, while Teng et al. (2020) did not report sample sizes at all. Species for all samples are presented in Table 16. An additional phylogenetic tree is provided for the molluscan species in Appendix 15 to facilitate reference to nomenclature. Sample size fluctuated between the studies. Although a 'gold standard' does not exist, as yet, for the number of samples for such environmental studies, many used $n \geq 5$ per species, while others raised that to $n \geq 30$. Only three studies in the review used less than five organisms per species (Abidli et al., 2019, Collard et al., 2017a, F. Zhang et al., 2019).

FT-IR was used by 72% (n=36) of the studies as the preferred method for identifying the chemical composition of the particles, followed by RM used by 20% (n=10) (Table 16). One study used both methods, while the other three combined the use of FT-IR and SEM. Twenty-three different particle extraction processes were used (Table 16 and Appendix 16). The most common method was that developed by Li J. et al. (2015) used by 11 studies. The method uses a hydrogen peroxide (30% H_2O_2) treatment for the digestion of the samples, which is followed by a density separation step using a saline (NaCl) solution and filtration.

Table 16. Study characteristics for seafood studies.

Authors	Geographic location	Sample Phylum/ Class	Sample Species (common name)	Sampling location	Habitat	N	n	MPs extraction procedure	MPs identification method	Outcome
Abidli et al. (2019)	Tunisia	Bivalve molluscs	*Mytilus galloprovincialis* (mussel)	Environment	Wild	42	15	Li H. X. et al. (2018)	FT-IR	Mean MPs content per mass with SD
			Ruditapes decussatus (clam)				24			
			Crassostrea gigas (oyster)				3			
		Gastropod molluscs	*Hexaplex trunculus* (sea snails)			18	9			
			Bolinus brandaris (sea snails)				*9*			
Akhbarizadeh et al. (2020)	Iran	Fish	*Thunnus tonggol* (longtail tuna)	Market (canned)	N/A	50	25	Karami et al. (2017c)	RM	Mean MPs content per mass with SD
			Thunnus albacares (yellowfin tuna)				20			
			Scombermorus commerson (mackerel)				5			
Akoueson et al. (2020)	Scotland	Fish	*Melanogrammus aeglefinus* (haddock)	Market	N/A	42	12	Li J. et al. (2018)	FT-IR	Mean MPs content per mass

	Greece		*Dicentrarchus labrax* (seabass)				10			and individual with SD
	Iceland		*Pleuronectes platessa* (plaice)				10			
	Scotland		*Scromber scombrus* (mackerel)				10			
	Chile	Bivalve molluscs	*Zygochlamys patagonica* (scallops)			20	10			
	Scotland		*Pecten maximus* (scallops)				10			
Baechler et al. (2020)	USA	Bivalve molluscs	*C. gigas* (oysters)	Environment	Farmed	283	141	developed their own	FT-IR	Mean MPs content per mass and individual with SD
			Siliqua patula (razor clams)		Wild		142			
Birnstiel et al. (2019)	Brazil	Bivalve molluscs	*Perna perna* (mussels)	Environment	Farmed	20	10	Van Cauwenberghe et al. (2015)	FT-IR	MPs content range per mass with SD
					Wild		10			
Bour et al. (2018)	Norway	Crustace-an	*Crangon allmanni* (shrimp)	Environment	Wild		20	Avio et al. (2015b) and Dehaut et al. (2016)	FT-IR	Frequency of MPs occurrence
		Bivalve molluscs	*Ennucula tenuis* (mussel)				12			

Brate et al. (2018)	Norway	Bivalve molluscs	*M. edulis* (mussels)	Environment	Wild	332	N/A	Dehaut et al. (2016)	FT-IR	Mean MPs content per mass and individual with SD
			M. trossulus (mussels)				N/A			
			M. galloprovincialis (mussels)				N/A			
Cho et al. (2019)	South Korea	Bivalve molluscs	*C. gigas* (oyster)	Market	Farmed	240	60	Karami et al. (2017b)	FT-IR	Mean MPs content per mass and individual with SD
			M. edulis (mussel)				60			
			Tapes philippinarum (clam)				60			
			Patinopecten yessoensis (scallop)				60			
Collard et al. (2017a)	Mediterranean Sea, English Channel	Fish	*Engraulis encrasicolus* (anchovies)	Environment	Wild	15	13	Collard et al. (2015)	RM	Frequency of MPs occurrence
			Sardina pilchardus (sardines)				2			
Collard et al. (2017b)	English Channel, Mediterranean Sea and North-	Fish	*E. encrasicolus* (anchovies)	Environment	Wild	40	20	Collard et al. (2015)	RM	Mean MPs content per individual
			S. pilchardus (sardines)				20			

	eastern Atlantic									
Digka et al. (2018)	Northern Ionian Sea.	Bivalve molluscs	*M. galloprovincialis* (mussels)	Environment	Wild/ Farmed		80	Mathalon and Hill (2014)	FT-IR	Mean MPs content per individual with SD
		Fish	*S. pilchardus* (sardines)		Wild		36			
Ding et al. (2018)	China	Bivalve molluscs	*Chlamys farreri* (scallop)	Market	Farmed	115	50	developed their own	FT-IR	Mean MPs content per mass and individual
			M. galloprovincialis (mussel)	Market	Farmed		50			
				Environment	Wild		15			
Jinfeng Ding et al. (2019)	China	Bivalve molluscs	*M. galloprovincialis* (mussel)	Market	N/A	40	20	developed their own	FT-IR and SEM[e]	Mean MPs content per mass and individual with SD
			Ruditapes philippinarum (clams)				10			
			Mactra veneriformis (clams)				10			
Ding et al. (2020)	China	Bivalve molluscs	*M. galloprovincialis* (mussel)	Market	N/A	120	10	Ding et al. (2018) and Jinfeng Ding et al. (2019)	FT-IR	Mean MPs content per mass and individual with SD
			Perna viridis (mussel)				10			
			R. philippinarum (clam)				20			
			C. gigas				20			

			(oyster)							
			Sinonovacula constricta (clam)				20			
			Scapharca subcrenata (clam)				20			
			Meretrix Lusoria (clam)				20			
		Gastropod molluscs	*Busycon canaliculatu* (sea snail)			20	20			
Fang et al. (2018)	Bering Sea and Chukchi Sea	Bivalve molluscs	*Astarte crenata* (clams)	Environment	Wild	57	28	Digestion: Dehaut et al. (2016) and Phuong et al. (2018a) Floatation/filtration: Li J. et al. (2015)	FT-IR	Mean MPs content per mass with SD
			Macoma tokyoensis (clams)				29			
		Gastropod molluscs	*Retifusus daphnelloides* (sea snails)			43	24			
			Latisipho hypolispus (sea snails)				19			
		Crustace-an	*Pandalus borealis* (Arctic shrimp)			80	21			
			Chionoecetes opilio (snow crab)				59			

Feng et al. (2019)	China	Fish	*Thryssa kammalensis* (rednose anchovy)	Environment	Wild	19		Dehaut et al. (2019), Foekema et al. (2013), Hermsen et al. (2018) and Karami et al. (2017b)	FT-IR	Mean MPs content per mass and individual with SD
Feng et al. (2020)	China	Echinoderm ata	*Strongylocentro-tus intermedius* (sea urchin)	Environment	Wild	210	N/A	Foekema et al. (2013) and Karami et al. (2017b)	FT-IR	Mean MPs content per mass and individual
			Temnopleurus hardwickii (sea urchin)				N/A			
			Temnopleurus reevesii (sea urchin)				N/A			
			Hemicentrotus pulcherrimus (sea urchin)				N/A			
Hermabessiere et al. (2019)	France	Bivalve molluscs	*M. edulis* (mussels)	Environment	Wild	200	100	Dehaut et al. (2016)	RM (no fibres)	Mean MPs content per mass with SD
			C. edule (cockles)				100			
Hossain et al. (2020)	Bangladesh	Crustace-an	*Metapenaeus monocerous* (brown shrimp)	Environment	Wild	30	20	Li J. et al. (2015) and Su et al. (2016)	FT-IR	Mean MPs content per mass with SD
			Penaeus monodon (tiger shrimp)				10			

Karami et al. (2017c)	Malaysia	Fish	*Chelon subviridis* (greenback mullet)	Market (Packed dried)	N/A	120	30	Karami et al. (2017b)	RM	Frequency of MPs occurrence
			Johnius belangerii (belanger's croaker)				30			
			Rastrelliger kanagurta (*Indian mackerel)*				30			
			Stolephorus waitei (spotty-face anchovy)				30			
Karami et al. (2018)	Product of Canada, Germany, Iran, Japan, Latvia, Malaysia, Morocco, Poland, Portugal, Russia, Scotland, Thailand, and Vietnam	Fish	Canned sardines (species unknown)	Market (canned)	N/A	792[a]	184[a]	Karami et al. (2017b)	RM	Frequency of MPs occurrence
			Canned sprats (species unknown)				608[a]			
Leslie et al. (2017)	Netherlands	Bivalve molluscs	*M. edulis* (mussel)	Environment	Wild	26	20	Van der Horst (2011), (2013)	FT-IR	

			C. gigas (oyster)				6			Mean MPs content per mass
		Gastropod molluscs	*Littorina littorea* (sea snail)				10			
		Crustace-an	*Carcinus maenas* (crab)				10			
Li H. X. et al. (2018)	China	Bivalve molluscs	*Saccostrea cucullata* (oysters)	Environment	Wild	330		Li J. et al. (2015)	FT-IR	MPs content range per mass and individual
Li J. et al. (2018)	U.K.	Bivalve molluscs	*M. edulis* (mussels)	Environment	Wild	246	162	Li J. et al. (2016)	FT-IR	Mean MPs content per mass with SD
				Market	Farmed	84	54			
					Wild		30			
Li J. et al. (2016)	China	Bivalve molluscs	*M. edulis* (mussels)	Environment	Wild	390	222	Li J. et al. (2015)	FT-IR	Mean MPs content per mass and individual
					Farmed		168			
Li J. et al. (2015)	China	Bivalve molluscs	*Scapharca subcrenata (clams)*	Market	Wild/ Farmed	144	6	developed their own	FT-IR	Mean MPs content per mass with SD
			Tegillarca granosa (clams)				18			
			Alectryonella plicatula (clams)				18			

			R. philippinarum (clams)				24			
			Sinonovacula constricta (clams)				6			
			M. lusoria (clams)				18			
			Cyclina sinensis (clams)				30			
			M. galloprovincialis (mussel)				18			
			P. yessoensis (scallop)				6			
Lopes et al. (2020)	Portugal	Fish	*S. pilchardus* (sardine)	Environment	Wild	226	76	Dehaut et al. (2016)	FT-IR	Mean MPs content per individual with SD
			E. encrasicolus (anchovy)				131			
			Boops boops (bogue)				19			
McGoran et al. (2018)	Thames Estuary, U.K.	Crustace-an	*C. crangon* (brown shrimp)	Environment	Wild	116		Their own method (without digestion)	FT-IR	Mean MPs content per individual and frequency of occurrence

Naji et al. (2018)	Persian Gulf	Gastropod molluscs	*Amiantis umbonella* (sea snail)	Environment	Wild	30		Li J. et al. (2015)	FT-IR, SEM	Mean MPs content per mass
		Bivalve molluscs	*Amiantis purpuratus* (scallop)			63	30			
			Pinctada radiate (oyster)				33			
Nam et al. (2019)	Vietnam	Bivalve molluscs	*P. viridis* (mussel)	Environment	Wild	5		Phuong et al. (2018b)	FT-IR	Mean MPs content per mass and individual with SD
Phuong et al. (2018a)	French Atlantic coasts	Bivalve molluscs	*M. edulis* (mussel)	Environment	Wild/ Farmed	180	120	Phuong et al. (2018b)	FT-IR	Mean MPs content per mass and individual with SD
			C. gigas (oyster)				60			
Pozo et al. (2019)	Chile	Fish	*Strangomera bentincki* (sardine)	N/A	N/A	10		Lindeque and Smerdon (2003)	FT-IR	Frequency of MPs occurrence
Qu et al. (2018)	China	Bivalve molluscs	*M. edulis* (mussels)	Environment	Wild	~760	~430	Li J. et al. (2015)	FT-IR	MPs content range per mass and individual
			P. viridis (mussels)				~330			
Monia Renzi et al. (2019)	Adriatic Sea	Fish	*S. pilchardus* (sardines)	Environment	Wild	160	80	Nuelle et al. (2014) and	FT-IR	Mean MPs content

			E. encrasicolus (anchovies)				80	Avio et al. (2015b)		per individual
Su et al. (2018)	Middle-Lower Yangtze River Basin, China	Bivalve molluscs	*Corbicula fluminea* (Asian clams)	Environment	Wild	208		Li J. et al. (2015) and Su et al. (2016)	FT-IR	Mean MPs content per mass and individual with SD
Su et al. (2019)	China	Fish	*Lateolabrax maculatus* (seabass)	Environment	Wild	9		Jabeen et al. (2017)	FT-IR	Mean MPs content per mass and individual with SD
Sun et al. (2019)	Yellow Sea, China	Fish	*Setipinna taty* (anchovy)	Environment	Wild	380	20	Desforges et al. (2015)	FT-IR	Mean MPs content per individual
			Anchoviella commersonii (anchovy)				30			
			Engraulis japonicus (anchovy)				280			
			Ammodytes personatus (Sand lance)				50			
Tanaka and Takada (2016)	Tokyo Bay, Japan	Fish	*E. japonicus* (Japanese anchovy)	Environment	Wild	64		Foekema et al. (2013) and Rochman et al. (2015)	FT-IR	Mean MPs content per individual with SD

Teng et al. (2019)	China	Bivalve molluscs	*C. gigas* (oysters)	Environment	Farmed	306	N/A	Munno et al. (2018)	FT-IR	Mean MPs content per mass and individual
			C. angulate (oysters)				N/A			
			C. hongkongensis (oysters)				N/A			
			C. sikamea (oysters)				N/A			
Teng et al. (2020)	China	Fish	*Sardinella zunasi* (Japanese scaled sardine)	Environment	Wild	N/A		Munno et al. (2018)	FT-IR	Mean MPs content per mass and individual with SD
Thushari et al. (2017)	Gulf of Thailand	Bivalve molluscs	*Saccostrea forskalii* (oyster)	Environment	Wild		15	Claessens et al. (2013)	RM	Mean MPs content per mass with SD
		Gastropod molluscs	*Littoraria sp.* (periwinkle, sea snail)				50			
		Crustace-ans	*Balanus amphitrite* (barnacle)				50			
Van Cauwenberghe and Janssen (2014)	Germany	Bivalve molluscs	*M. edulis* (mussels)	Environment	Farmed	93	72	Claessens et al. (2013)	RM	Mean MPs content per mass with SD
			C. gigas (oysters)	Market			21			
Jun Wang et al. (2019)	South Yellow Sea,	Bivalve molluscs	*Acila mirabilis* (clams)	Environment	Wild		20	Claessens et al. (2013)	FT-IR, SEM	Mean MPs content

	Korea and China	Crustace-an	*C. affinis* (sand shrimps)				10			per mass with SD
Q. Wang et al. (2020)	China	Fish	*Konosirus punctatus* (spotted sardine)	Environment	Wild	58	44	Munno et al. (2018)	FT-IR	Mean MPs content per mass and individual with SD
			Thryssa mystax (Gangetic anchovy)				8			
			Sardinella zunasi (Japanese scaled sardine)				6			
Webb et al. (2019)	New Zealand	Bivalve molluscs	*Perna canaliculus* (mussel)	Environment	Wild	96		Claessens et al. (2013)	FT-IR	Mean MPs content per individual with SD and range of MPs per mass
F. Z. Wu et al. (2020)	China	Fish	*Larimichthys crocea* (large yellow croaker)	Environment	Farmed	N/A	10-20[b]	Li J. et al. (2015)	FT-IR	Mean MPs content per mass and individual with SD
			Konosirus punctatus (dotted gizzard shad)				10-20[b]			
		Bivalve molluscs	*Ostrea denselamellosa* (oyster)			N/A	10-20[b]			

			Sinonovacula constricta (razor clam)				10-20[b]			
		Crustacean	*Parapenaeopsis hardwickii* (shrimp)				10-20[b]			
F. Zhang et al. (2019)	China	Crustacean	*Oratosquilla oratoria* (shrimps)	Environment	Wild	136	64	Masura et al. (2015) for crustacean	FT-IR	Frequency of occurrence per individual
			O. kempi (shrimps)				1			
			Portunus trituberculatus (crabs)				30			
			Carcinoplax vestita (crabs)				18			
			Charybdis bimaculata (crabs)				15			
			Charybdis variegata (crabs)				4			
			Portunus gracilimanus (crabs)				3			
			Charybdis japonica (crabs)				1			

S. Y. Zhao et al. (2018)	Avery Point Dock, U.S.A.	Bivalve molluscs	*M. edulis* (mussels)	Environment	Wild	37		Zhao et al. (2017)	RM April samples FT-IR September samples	Mean MPs content per mass and individual with SD
Zhu et al. (2019)	China, Maowei Sea	Bivalve molluscs	*C. hongkongensis* (oysters)	Environment	Wild	20		Foekema et al. (2013) and Karami et al. (2017b)	FT-IR	Mean MPs content per mass and individual with SD
Zitouni et al. (2020)	Tunisia	Fish	*Serranus scriba* (Painted comber)	Environment	Wild	240		Dehaut et al. (2016) and Phuong et al. (2018b)	RM	MPs content per mass with SD

[a] 20 brands of canned fish were employed, 11 for sardines and 9 for sprats. Samples n was calculated based on the number of fish in one can per brand

[b] 10-20 per species (exact n was not reported)

Note: Outcome is the description of the microplastic (MP) content as reported by each study. FT-IR, Fourier-transform infrared spectroscopy; N, overall sample size expressed in number of organisms per phylum or class; n, sample size expressed in number of organisms per species, sampling location or habitat, accordingly; N/A, not available; SD, standard deviation; RM, Raman spectroscopy; SEM, scanning electron microscopy.

6.3. Risk of bias (RoB) within studies

The summary of the results of the RoB assessment are illustrated in Figure 43 (see section 3.2.7). The individual rating for each study across all domains is presented in Appendix 17; 13 studies (26%) were rated as having a high RoB, 11 (22%) an unclear RoB and the remaining 26 (52%) of having low RoB. The domain most often rated as of high RoB was 'reporting' (20 studies; 47%), and the domain that was most rated as unclear RoB was 'analysis' (20 studies; 47%). The most common issues were failure to report the results of the procedural blank samples (e.g. Hossain et al., 2020, Li H. X. et al., 2018, Thushari et al., 2017, Jun Wang et al., 2019, F. Z. Wu et al., 2020) and the specifications of the chemical composition analysis (e.g. Collard et al., 2017a, Monia Renzi et al., 2019). The domain of 'study design' was rated as of low RoB across all studies. Lack of space often precludes careful description of the sampling design development and this was not reported in any of the studies, but, the description of sampling activities was adequate to infer it. Further details of the RoB assessment are discussed in the narrative analysis and the results are used to inform both qualitative and quantitative analysis.

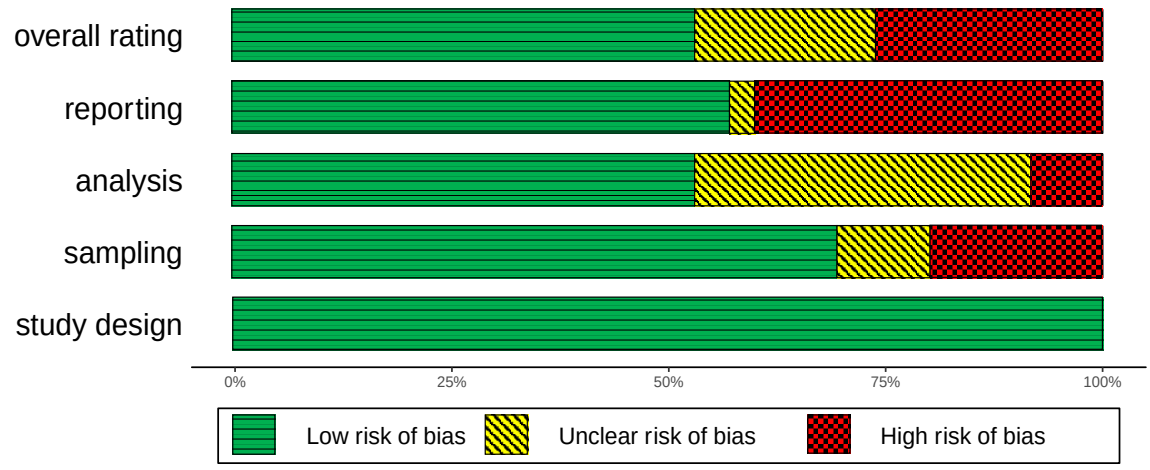


Figure 43. Risk of Bias (RoB) assessment for seafood studies. The three ratings are illustrated by percentage. Individual rating per study and per domain is provided in Appendix 17. Rating was executed according to the RoB tool (see section 3.2.7).

6.4. Contamination levels within seafood

The MP content results are presented in three tables, one for each phylum to facilitate comparison (Table 17, Table 18 and Table 19). The results for the echinoderms phylum (Feng et al., 2020) are presented in Table 17 along with the molluscan phylum. This is done for ease of tabular presentation since there is only one study which sampled echinoderms.

Studies appear in more than one table if their samples included more than one phylum of organisms. The MP content is expressed as the number of MP particles per gram of sample or per individual organism. Studies provided either the mean content (with or without the SD) or the range of content or both. Lopes et al. (2020) only reported the median and the interquartile range; the methods and calculator developed by Shi et al. (2020) were used to estimate the mean and SD. A minority of the studies only reported the frequency of samples being positive for MP contamination and were excluded from the statistical summary.

In terms of procedural blank samples results, 18% of the studies (n=9 out of 50) did not report their results, while a surprising 36% (n=18) reported that no MPs were found (Appendix 18). The 46% of the studies (n=23) that did report the discovery of specific MPs content in their blank samples used their results in different ways. Thirty-five percent of the studies (34.7%; n=8 out of 23) corrected their final findings against the results of the procedural blank samples, while an additional 8.6% (n=2) subtracted the absolute number of discovered MPs from their results. Twenty-six percent (n=6) considered the results to be negligible without offering justification to that effect, while 4.3% (n=1) did not make use of the results and did not provide an explanation. On the other hand, 13% of the studies (n=3) tested the significance of their results statistically and 8.6% (n=2) used the results to set detection limits. The remaining 4.3% (n=1) did not report if and how the results were used.

6.5. Molluscan studies

6.5.1. Statistical summary of effects and narrative analysis

Thirty-one studies analysed molluscs (Table 17), but only data from 27 studies (87%) were combined in a statistical summary. Four studies were excluded; Leslie et al. (2017) used a different approach for the analysis and reported their results as MP/g of dry weight, two studies reported results per individual organism (Birnstiel et al., 2019, Digka et al., 2018) and one reported frequency of MP occurrence (Bour et al., 2018).

The range of MP content for molluscs was 0-10.5 MPs/g of organism (wet weight). The means and ranges reported by the included studies were skewed towards the lower MP content. Sixteen studies reported values below 1 MPs/g and the remaining 11 reported values above 1 MP/g (Figure 44).

Table 17. Molluscan seafood MP content results.

Study	Geographic location	Sample species	Sample additional details	N	Mean MPs/g	±SD	Range MPs/g	Composition
Abidli et al. (2019)	Tunisia				1.03	0.36	0.70 ± 0.10 to 1.15 ± 0.02[a]	Fibres: PP 100%, Fragments: PP 60%, PE 40%, Films: PP 50%, PE 50%.
		Mytilus galloprovincialis		15	N/A			
		Ruditapes decussatus		24	N/A			
		Crassostrea gigas		3	1.48	0.02		
		Hexaplex trunculus		9	0.70	0.11		
		Bolinus brandaris		9	N/A	N/A		
Akoueson et al. (2020)	Scotland	*Zygochlamys patagonica*		10	0.29	0.10	0.16 - 0.47	PET, PE
	Chile	*Pecten maximus*		10	0.17[b]	0.9	0.06 - 0.35	
Baechler et al. (2020)	USA	*C. gigas*	Farmed	141	0.35	0.04	0.1 ± 0.02 to 0.85 ± 0.41	PET, acrylic, aramid
		Siliqua patula	Wild	142	0.16	0.02	0.09 ± 0.01 to 0.62 ± 0.33	
Birnstiel et al. (2019)	Brazil	*Perna perna*	Farmed	10			16.6 ± 6.6 to 31.2 ± 17.8[c]	Fibres: PA, Fragments: PMMA
		P. perna	Wild	10				
Bour et al. (2018)	Norway	*Ennucula tenuis*		12			41.1% [d]	PE 54%, PP 16.8%
Brate et al. (2018)	Norway	*Mytilus spp.*		332	0.97	2.61	0 - 7.9	CP 63.9%, “parking lot tar” and EVA foam 18.7%, PET 9.9%, acrylic 2.9%, PP 1.2%, PE 1%, PA< 1%

Cho et al. (2019)	South Korea	*C. gigas*		60	0.07	0.06	0 - 0.19	PE, PP, PS, and polyester accounting for > 80% of MPs
		M. edulis		60	0.12	0.11	0 - 0.35	
		Tapes philippinarum		60	0.34	0.31	0.03 - 1.08	
		Patinopecten yessoensis		60	0.08	0.08	0.01 - 0.17	
		all species		240	0.15	0.20		
Digka et al. (2018)	Northern Ionian Sea	*M. galloprovincialis*		80	1.9 [e]	0.2		75% PE, 12.5% PP, 12.5% PTFE
Ding et al. (2018)	China	*Chlamys farreri*	Farmed	50			3.2 - 7.1	CP, PP, PTFE.
		M. galloprovincialis	Farmed	50	3.17		2.0 - 12.8	
			Wild	15	2			
Ding et al. (2019)	China	*M. galloprovincialis*	Qingdao	10	0.16	0.13	0.16 - 0.74	RY 48.92%, PET 33.87%, CPE 9.68%, PTFE 4.84%, PS 2.15%, PE + PP 0.54%.
			Dongying	10	0.42	0.26		
		Ruditapes philippinarum	Qingdao	10	0.74	0.54		
		Mactra veneriformis	Dongying	10	0.31	0.27		
Ding et al. (2020)	China			140			0.8 to 4.4	Qingdao: RY 41.5%, PET, 16.4%, CPE, 11.8%, PVC, 10.3%. Xiamen: RY 44.4%, PVDF 24.2%, CPE 14.0%, PVC 6.8%, PET 5.1%
		M. galloprovincialis		10				
		Perna viridis		10				
		R. philippinarum		20				
		C. gigas		20				
		Sinonovacula constricta		20				
		Scapharca subcrenata		20				
		Meretrix Lusoria		20				

		Busycon canaliculatu		20				
Fang et al. (2018)	Bering Sea and Chukchi Sea	*Astarte crenata*		28	0.08	0.07	0 - 0.12	PA 46%, PE 23%, PET 18%, CP 13%.
		Macoma tokyoensis		29	0.03	0.05	0 - 0.08	
		Retifusus daphnelloides		24	0.12	0.07	0.05 - 0.13	
		Latisipho hypolispus		19	0.02	0.002	0.02 - 0.03	
Feng et al. (2020) [f]	China	*Strongylocentrotus intermedius* [f]	Wild	210	1			CP 36.65%, PET/Polyester 16.29% PE 14.03%, PP 13.12%, PP-PE 7.69%, PA 4.07%, RY 3.17%, PAN 2.71%, PU 1.36%, PVA-PE 0.90%
		Temnopleurus hardwickii [f]						
		Temnopleurus reevesii [f]						
		Hemicentrotus pulcherrimus [f]						
Hermabessiere et al. (2019) [g]	France	*M. edulis*	Le Portel	50	0.25	0.16	0.15 - 0.25	PE 36.8%, ABS 32.5% and SBR 26.3%, PP, PS, PET> 5%
			Baie des Veys	50	0.15	0.06		
		C. edule	Baie d'Authie	50	0.74	0.35	0.19 - 0.74	
			Baie des Veys	50	0.19	0.08		
Leslie et al. (2017) [i]	Netherlands	*C. gigas*	Eastern Scheldt	3	87			Not specified
			Rhine Estuary	3	30			
		M. edulis	Eastern Scheldt	10	105			
			Ter Heijde N. Sea	10	19			
		Littorina littorea	Eastern Scheldt	10	20			
Li H. X.et al. (2018)	China	*Saccostrea cucullata*		330			1.5 - 7.2	PET 34%, PP 19%, PE, 14%, PS, 8%, CP, 8%, PVC 6%, PA, 4%, EPS 3%

Li J.et al. (2018)	U.K.	*M. edulis*		162			0.72-2.89	Polyester 43%, RY 26%, CL 14%
			Edinburgh	12	1.23	0.25		
			Filey	18	2.55	0.44		
			Hastings-A	30	1.59	0.51		
			Hastings-B	18	2.37	0.90		
			Brighton	18	0.95	0.18		
			Plymouth	24	0.72	0.16		
			Cardiff	30	2.89	0.62		
			Wallasey	12	1.65	0.23		
		M. edulis	Supermarket live (farmed)	36	0.91	0.19		PP 17%, Polyester 17%, RY 17%, acrylic 13%, CL 9%, PE 4%, PGR 4%.
			Supermarket processed (farmed/wild)	48	1.37	0.24		
Li J. et al. (2016)	China	*M. edulis*	Wild	222	2.7		0.9 - 4.6	CP 41.1%, PET 16.3%, PTA 10.9%, POM 7%, PE 3.1%, PNMA 2.3%.
		M. edulis	Farmed	168	1.6			
Li J.et al. (2015)	China	*Scapharca subcrenata*		6	10.45	4.4	2.1 - 10.5	PE, PET, PA (no%)
		Tegillarca granosa		18	4.13	1.72		
		M. galloprovincialis		18	2.39	1.32		
		P. yessoensis		6	2.34	0.78		
		Alectryonella plicatula		18	5.77	1.28		
		Sinonovacula constricta		6	2.08	1.18		
		R. philippinarum		24	2.52	1.07		
		M. lusoria		18	4.19	1.19		

		Cyclina sinensis		30	3.98	1.38		
Naji et al. (2018)	Persian Gulf	*Amiantis umbonella*		30	~2			PE, PET, nylon (no%)
		A. purpuratus		30				
		Pinctada radiata		33				
Nam et al. (2019)	Vietnam	*P. viridis*	Wild	5	0.29	0.14		PP 31%, Polyester 23%, PE 15%, PVA 8%, PA 8%, Rubber 8%, PS 7%
Phuong et al. (2018a)	French Atlantic coasts	*M. edulis*		120	0.23	0.20		PP 47%, PE 38%
		C. gigas		60	0.18	0.16		PE ~50%, PP ~25%
Qu et al. (2018)	China	*M. edulis*		~430			1.52 - 5.36	PET 74%, RY, PE, PVC and PP
		P. viridis		~330				
Su et al. (2018)	China	*Corbicula fluminea*		208			0.3 - 4.9	Polyester 33%, PP 19%, PE 9%
			S1 Lake	N/A	0.72	0.19		
			S2 Lake	N/A	0.55	0.20		
			S3 River	N/A	4.88	2.31		
			S4 River	N/A	1.43	0.47		
			S5 River	N/A	2.21	0.77		
			S6 River	N/A	0.57	0.80		
			S7 River	N/A	0.86	0.48		
			S8 Lake	N/A	0.44	0.24		
			S9 Lake	N/A	0.29	0.26		
			S10 Lake	N/A	0.42	0.15		
			S11 Lake	N/A	0.42	0.07		
			S12 Estuary	N/A	1.11	1.10		
			S13 Estuary	N/A	2.71	0.20		
			S14 Estuary	N/A	0.99	0.57		

			S15 Lake	N/A	0.55	0.02		
			S16 Lake	N/A	0.78	0.13		
			S17 Lake	N/A	1.72	1.15		
			S18 River	N/A	1.22	0.53		
			S19 Lake	N/A	3.70	2.33		
			S20 Lake	N/A	2.19	1.32		
			S21 Lake	N/A	0.68	0.32		
Teng et al. (2019)	China	*C. spp.*		306	0.62	0.88	0.11 - 2.35	CP 41.34%, PE 22.97%
Thushari et al. (2017)	Gulf of Thailand	*Saccostrea forskalii*		15			0 - 0.57	PA, PET, PS (no%)
			Angsila	N/A	0.57	0.22		
			Bangsaen	N/A	0.37	0.03		
			Samaesarn	N/A	0.43	0.04		
		Littoraria sp.		50				
			Angsila	N/A	0.23	0.02		
			Bangsaen	N/A	0	-		
			Samaesarn	N/A	0.17	0.08		
Van Cauwenberghe and Janssen (2014)	Germany	*M. edulis*	no depuration	36	0.36	0.07		Not specified
			after depuration	36	0.24	0.07		
		C. gigas	no depuration	11	0.47	0.16		
			after depuration	10	0.35	0.05		
Jun Wang et al. (2019)	South Yellow Sea	*Acila mirabilis*		20	6.9	2.1		Not specified
Webb et al. (2019)	New Zealand	*Perna canaliculus*	Wild	96	0.03	0.04	0 to 0.48	PE, PA, acrylic, RY, nylon, PVA
F. Z. Wu et al. (2020)	China	*Ostrea denselamellosa* (oyster)	Farmed	10-20	0.31	0.10		CL, PET, PP, PE, PA, acrylonitrile

		Sinonovacula constricta (razor clam)		10-20	0.21	0.05		
S. Y. Zhao et al. (2018)	U.S.A.	*M. edulis*		37	0.6	1.2	0 - 5.1	PP 44.7%, polyester 21.2%, CL 11.8%, nylon 3.5%, PE 2.3%, PS 2.3%, etc.
Zhu et al. (2019)	China	*C. hongkongensis*		20	0.8	0.2	0.7 - 1.1	RY 50%, polyester 39%.

[a] calculated from MPs/kg, [b] not significantly different for the procedural blank results, [c] range MPs/individual organism, [d] frequency of MPs/ind. occurrence on the sample, [e] MPs/individual organism, [f] Echinodermata phylum, [g] expressed in mean ± 2 S.E (95% confidence interval), [i] total number of particles per gram of dry tissue. Note: The column studies additional details provides further sample characteristics appropriate for each study regarding sampling further geographic location, sampling origin (environment, market), habitat (wild, farmed) and sample further processing information (depuration). Studies reported either the mean MP content (with or without the SD) or the range of MP content or both. MP content is expressed as number of MP particles per gram of tissue (wet weight) unless otherwise stated. CL, cellulose; CP, cellophane; CPE, chlorinated polyethylene; EPS, expanded polystyrene; EVA, Ethylene-vinyl acetate; MPs, microplastics; N, sample size expressed in number of organisms; N/A, not available; PA, polyamide (nylon); PAN, polyacrylo-nitrile; PE, polyethylene; PET, polyethylene terephthalate; PGR, propylene glycol ricinoleate; PMMA, polymethyl methacrylate; PNMA, poly(N-methyl acrylamide); POM, polymerized oxidized material;PP, polypropylene; PS, polystyrene; PTA, Polyester terephthalic acid; PTFE, polytetrafluoroethylene; PU, polyurethane; PVA, polyvinyl alcohol; PVA-PE, poly-vinylacetate- ethylene; PVC, polyvinyl chloride; RY, rayon; SD, standard deviation.

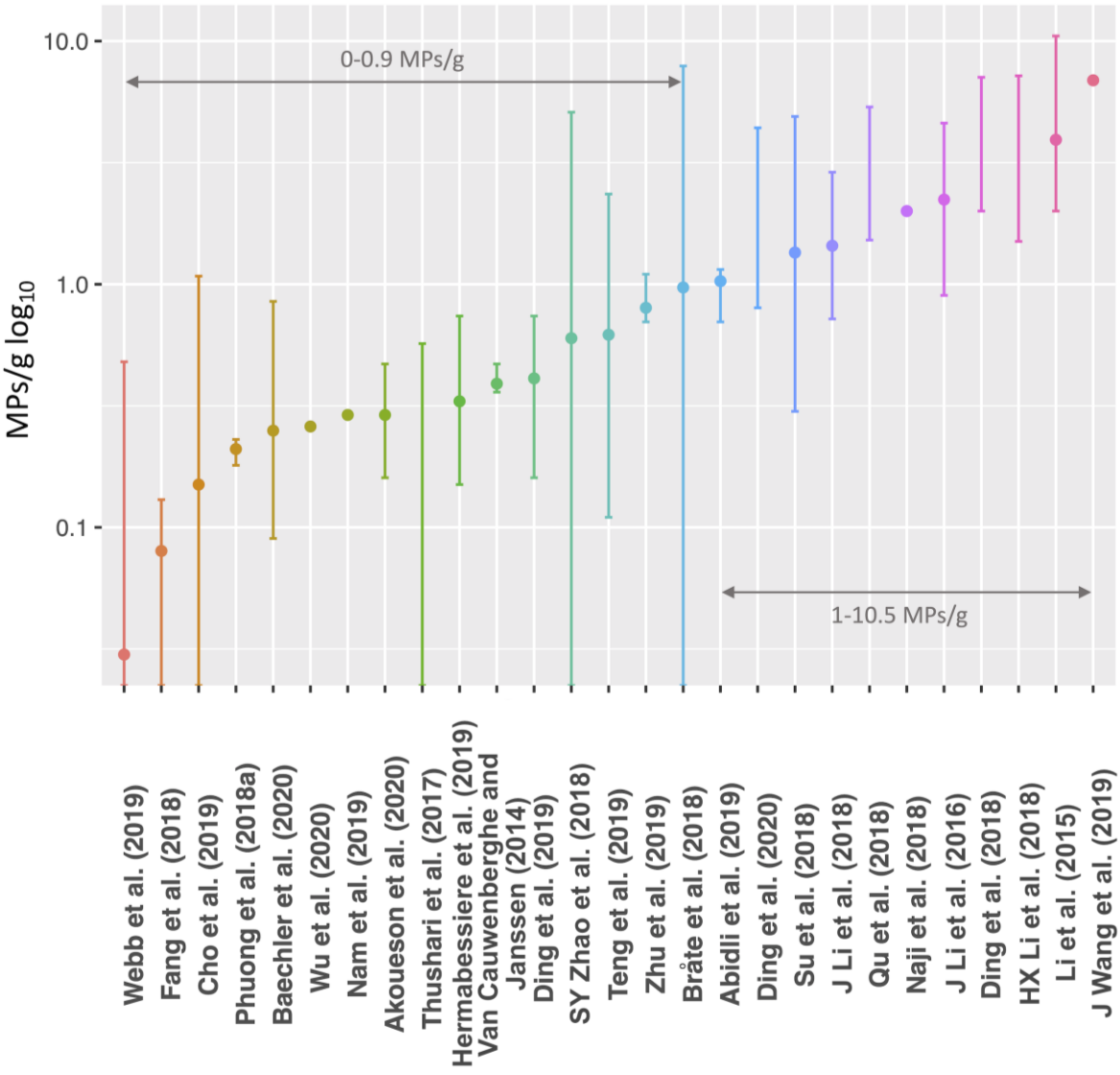


Figure 44. The overall microplastics per gram (MPs/g) content for molluscs illustrated in a $\log_{10}$ scale. Points represent mean MPs/g values for the studies, where reported. Whiskers represent the reported ranges of MPs/g.

Seven studies were rated as having a high RoB because they did not report the results for the analysis of their procedural blanks (Hermabessiere et al., 2019, Li H. X. et al., 2018, Thushari et al., 2017, Jun Wang et al., 2019, Webb et al., 2019, F. Z. Wu et al., 2020, S. Y. Zhao et al., 2018) (Appendix 17), an analysis step that was rated as one of the most important questions in the RoB assessment tool, since it can diminish the reliability of the reported results (see section 3.3.7). Five of these studies reported MP content above 1MP/g and the rest, below 1MP/g. The study by Baechler et al. (2020) was also found to have high RoB in the domains of analysis and reporting because the majority of the analysis details were not reported. The study reporting the highest MP mean content (6.9 MPs/g) (Jun Wang et al., 2019) and the study reporting the highest MP range of content (2-7.1 MPs/g) (Li H. X. et al., 2018) were both rated as having a high RoB in two domains: sampling and reporting. Jun Wang et al. (2019) was additionally rated as having an unclear RoB in the analysis domain.

Omitting these two studies from the statistical summary decreased the MP content to 0-7.2 MPs/g w.w.

In terms of geographical spread, 59.2% (n=16 out of 27) of the studies sampled organisms off the coasts of Asia, (52.6% of which were from China; n=10 out of 19), 18.5% (n=5) off the coasts of Europe, 11.1% (n=3) from the Americas, 3.7% (n=1) from Africa, 3.7% (n=1) from Australia/Oceania, and 3.7% (n=1) between the Americas and Asia (Table 17). Eighty-two percent of the studies (n=9 out of 11) that reported MP content above 1 MP/g came from the coasts of Asia. In contrast, only 20% of the studies (n=1 out of 5) from Europe reported MP content above 1 MP/g.

At least 15 different particle extraction procedures were reported. The procedures can be divided in three broad categories depending on the chemical compound used to digest the samples: H_2O_2, KOH (potassium hydroxide) and HNO_3 (nitric acid) (Appendix 16). There are further differences between these three categories such as time period and temperatures for digestion, the use of a density separation step and its specifications (physical/chemical), the use of further chemicals, and the pore size of the filters. Many studies poorly reported the procedure used, in some cases, missing crucial details of the analysis protocol. In terms of MP content, out of the 12 studies that used H_2O_2 for digestion (exclusively or not), 67% (n=8 out of 12) reported MP content above 1MP/g. In most cases the use of H_2O_2 was accompanied by a subsequent density-separation step (88% of the studies; n=7 out of 8), suggesting that they are more effective in extracting MPs from biota than the methods using KOH and HNO_3 for digestion.

Samples examined by the studies came either directly from the environment or from markets which opens up two associated issues: post collection MP contamination and the effects following any depuration period. It has been argued that depuration might be effective in extracting MPs from bivalves, with two studies testing this hypothesis (Birnstiel et al., 2019, Van Cauwenberghe and Janssen, 2014). Birnstiel et al. (2019) concluded that depuration (over a four-day period) significantly reduced MP content in their samples (*Perna perna*). Similarly, Van Cauwenberghe and Janssen (2014) found that a three-day depuration was effective in removing a large proportion of MP contamination (in *Mytilus edulis* and *Crassostrea gigas*).

Although the results of these two studies are promising in terms of the reduction of MP contamination, more research is needed to address a number of issues mainly around the methodology of the depuration procedure. For example, the time of depuration required may

vary between different species, and the use of seawater that has already been filtered specifically to target MPs, is also key. The effect of depuration cannot be assessed in this review since in most cases, when bivalves have been acquired from markets, it is not known whether they have undergone a depuration process or not. Therefore, it is not clear whether MP contamination after the collection of seafood has a significant effect, or if it is mitigated by depuration. Five studies collected samples only from markets (Akoueson et al., 2020, Cho et al., 2019, Jinfeng Ding et al., 2019, Ding et al., 2020, Li J. et al., 2015), three both from markets and the environment (Ding et al., 2018, Li J. et al., 2018, Van Cauwenberghe and Janssen, 2014) and the other 19 from the environment (Table 16). The samples collected directly from the environment had a broader range of MP content of 0.03 – 6.9 MPs/g than the samples collected from a market: 0.15 – 3.93 MPs/g (Table 17).

The importance of the source (farmed vs wild) has been highlighted in previous research (Mathalon and Hill, 2014). From the studies that collected molluscs only from market, only one reported sampling both farmed and wild organisms. Li J. et al. (2015) stated that MP content was significantly higher in farmed samples but did not report separate data for the MPs contents of the two groups. Ding et al. (2018) collected samples from markets and the environment but did not compare the two groups. Instead, they tested wild versus farmed organisms and reported farmed mussels contained more MPs (3.17 MPs/g) than wild (2 MPs/g). In contrast, Li J. et al. (2018) reported higher anthropogenic debris content in wild mussels per g (1.6 items/g) than farmed (1.1 items/g) (but more in farmed mussels per individual organism). The study by Van Cauwenberghe and Janssen (2014) only sampled farmed organisms. The results of the 19 studies that only collected environmental samples were contradictory. Four studies sampled both wild and farmed organisms of the same species. Li J. et al. (2016) found more MPs in wild mussels (2.7MP/g) than in farmed ones (1.6 MPs/g). Phuong et al. (2018a) reported higher detection rates for MPs in farmed samples (oysters 93%, mussels 90%) compared to the wild ones (oysters 80%, mussels 65%). Digka et al. (2018) did not detect a difference between the ingestion of MPs in wild (47.5%) and farmed (45%) mussels. Birnstiel et al. (2019) also found the wild mussels to be more contaminated than farmed, but this difference was not significant (ANOVA $F_{1,36}=0.006$, $p=0.94$). Out of the rest of the environmental studies, two analysed farmed organisms (Teng et al., 2019, F. Z. Wu et al., 2020) and the remaining 14 wild. No pattern between wild and farmed organisms emerged in a review of the data.

In terms of validating the chemical composition as actual MPs, ten studies (32%) did not report how many of the extracted particles were analysed for polymeric composition. The remaining 21 studies (68%) reported percentages ranging from 0.9 to 100%. Eight studies

(26%) analysed 100% of the particles (Cho et al., 2019, Ding et al., 2018, 2019, 2020, Nam et al., 2019, Phuong et al., 2018a, Webb et al., 2019, F. Z. Wu et al., 2020), one 80% (Hermabessiere et al., 2019) and the rest 0.9-36% (Appendix 19). Following on from this, it is noteworthy that 16 (52%) of the studies, once these particles have been isolated, did not state the percentage of similarity compared with the spectral library that was used as the level of acceptance.

In order to investigate the relationship between all these variables, a series of statistical tests were executed. Only seven studies reported all the variables needed for the analysis (Hermabessiere et al., 2019, Li J. et al., 2018, Li J. et al., 2016, Phuong et al., 2018a, Su et al., 2018, Teng et al., 2019, Zhu et al., 2019). Data was examined to detect if they were normally distributed by fitting a series of Shapiro-Wilk's tests (Ennos and Johnson, 2018). Pearson correlation analysis was used for the normally distributed data and Spearman correlation analysis for the data not normally distributed (Ennos and Johnson, 2018). There was a significant negative correlation between the MPs/g content, the percentage of the particles that were analysed, p=0.024, correlation coefficient R=-0.86 (Appendix 20. A) and the number of particles analysed, p=0.0004, R=-1 (Appendix 20. B). There was also a borderline significant positive correlation between the MP content and the similarity index of the spectral library, p=0.054, R=0.75 (Figure 45).

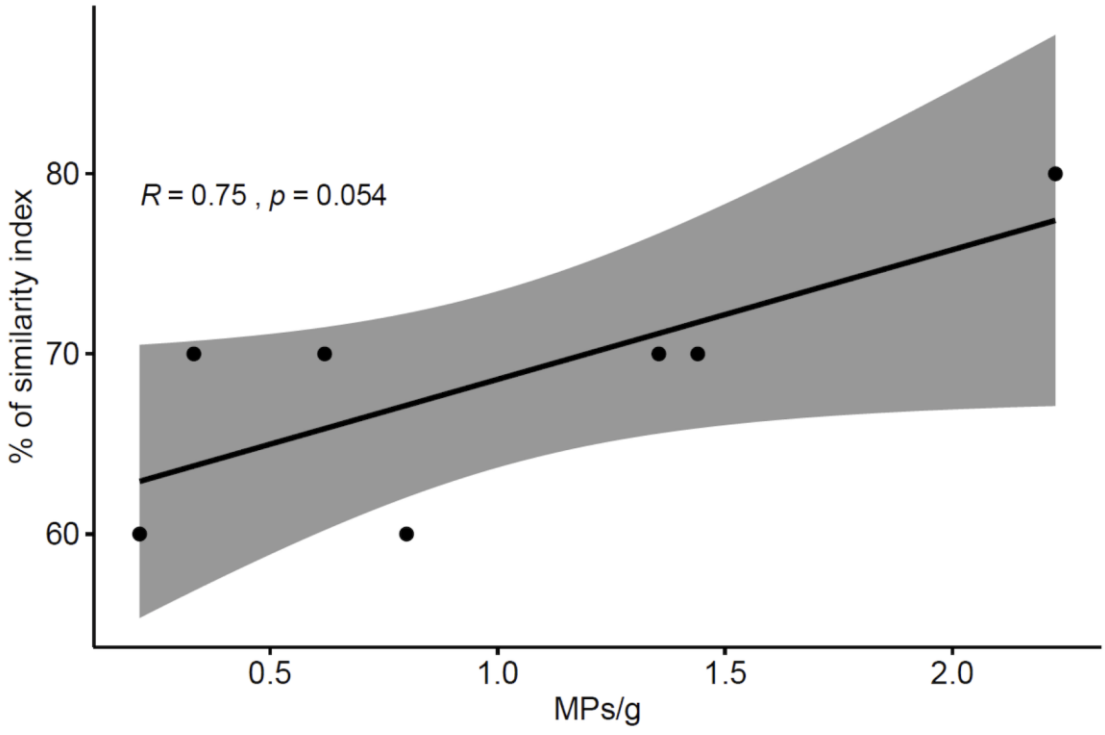


Figure 45. Pearson correlation analysis between the amount of microplastics per gram (MPs/g) in mussels and the percentage of similarity compared with the spectral library that has been used as the level of acceptance. R is the Pearson correlation coefficient with the corresponding p value.

No significant correlation (Spearman correlation analysis) was found between the percentage of the verified MPs and the percentage of the particles that were analysed (p= 0.1667); the number of particles analysed (p= 0.2357); nor the similarity index of the spectral library (p= 0.356). Ten percent of the studies (n=3 out of 31) did not report any results on polymeric composition of the particles (Leslie et al., 2017, Van Cauwenberghe and Janssen, 2014, Jun Wang et al., 2019) (see Table 17).

A key difference between the rest of the studies is that 53.6% (n=15 out of 28) reported to have found either cellulose, cellophane or rayon in their samples and reported them as part of the plastic material, while the other half did not. It is unclear whether this is because they were not considered plastic or because they were not found. Looking at the percentages of composition attributed to these materials, it became clear that their inclusion as MPs had a substantial effect to the MP content results. Across the studies that did not report cellulose related material, PE was the most abundantly discovered polymer, followed closely by PP. In the rest of the molluscan studies, CP was the most abundant material followed by PET, rayon and polyester. The type of library (commercial or made in-house) used for the spectral analysis is reported in Appendix 19. Sixteen studies used commercial libraries (also reporting specific which ones were used) two studies used libraries created in-house and 13 studies did not provide details of the library that was used.

6.5.2. Meta-analysis of MP content results

Two molluscan classes were included: bivalves and gastropods, constituting six molluscan families: clams, cockles, mussels, oysters, scallops and sea snails (Table 16). The data for all the species of the same family per study were combined resulting in 32 different sample datasets from 19 studies (Appendix 21). Sample heterogeneity between the classes and families was assessed in sub-group analyses using mixed-effects models which showed no significant difference between the overall effect between the two classes (Q=0.82, p=0.37) but a significant difference between the six families (Q=33.73, p< 0.01) (Figure 46). Sub-group analysis was also used to identify whether further sample characteristics and methods variability might have affected heterogeneity.

A significant difference was also identified between samples that were collected directly from the environment (n=23) and those collected indirectly from a market (n=9) (Q=29.33, p< 0.01) (Appendix 22), coinciding with the findings of the narrative analysis concerning this sample characteristic. Significant differences were identified between the 16 different geographical origins of the samples, Q=698.52, p< 0.01 and the three different RoB ratings

Q=15.42, p< 0.01 (Appendix 22). In light of these results, analyses using random-effects models were fitted separately for each of the six families of molluscs. In doing so, the heterogeneity between the different families of molluscs could be addressed. Further characteristics were explored within each family analysis separately.

The effects that the habitat and feeding parameters in terms of farmed versus wild organisms could not be modelled due to the lack of information since one study (Jinfeng Ding et al., 2019) did not report this characteristic and two studies (Phuong et al., 2018a, Li J. et al., 2015) collected both farmed and wild organisms and did not provide differentiated results (see Appendix 21).

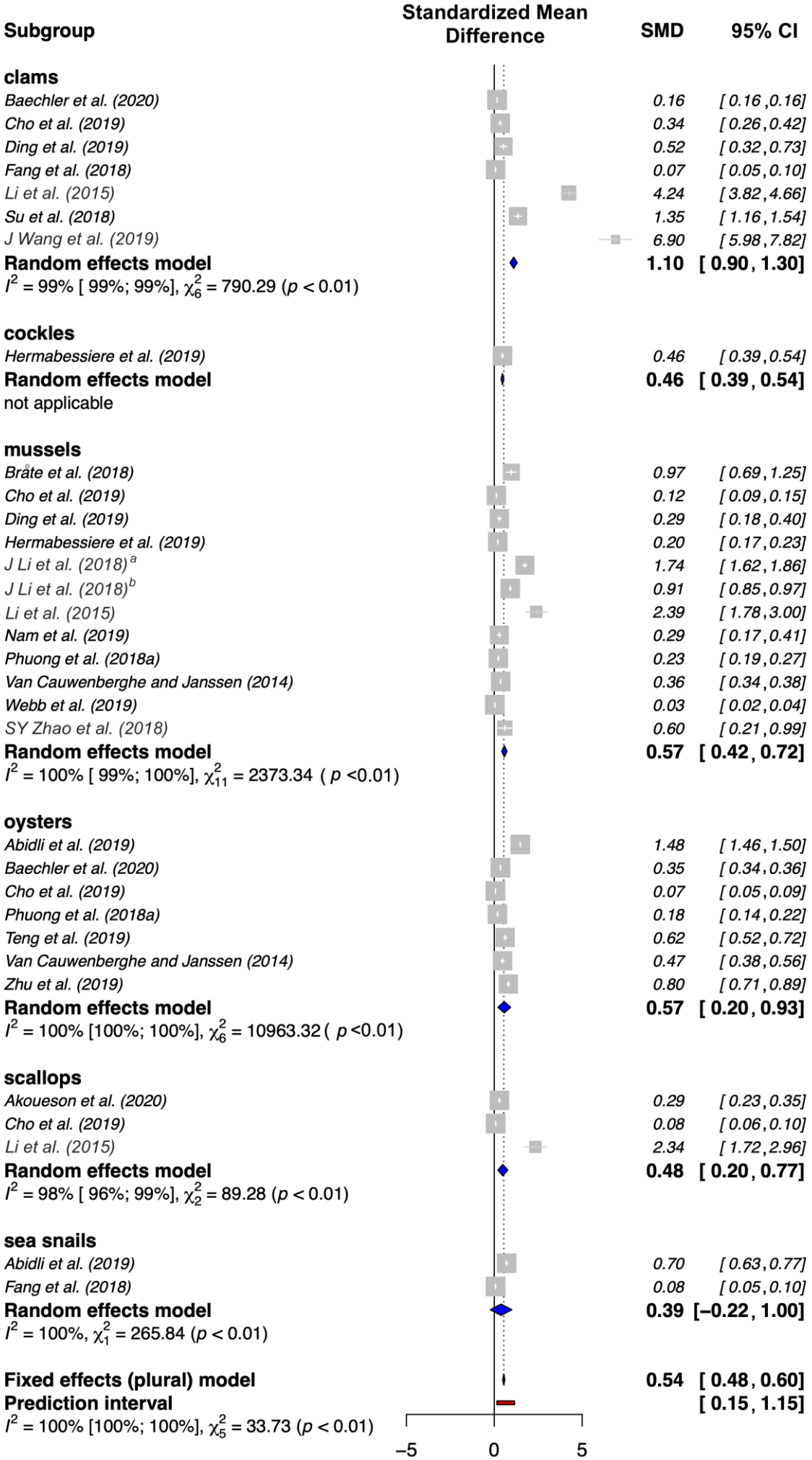

Subgroup
Standardized Mean Difference
SMD
95% CI
clams
Baechler et al. (2020) 0.16 [0.16 , 0.16]
Cho et al. (2019) 0.34 [0.26 , 0.42]
Ding et al. (2019) 0.52 [0.32 , 0.73]
Fang et al. (2018) 0.07 [0.05 , 0.10]
Li et al. (2015) 4.24 [3.82 , 4.66]
Su et al. (2018) 1.35 [1.16 , 1.54]
J Wang et al. (2019) 6.90 [5.98 , 7.82]
Random effects model 1.10 [0.90 , 1.30]
I^2 = 99% [99%; 99%], χ^2_6 = 790.29 (p < 0.01)
cockles
Hermabessiere et al. (2019) 0.46 [0.39 , 0.54]
Random effects model 0.46 [0.39 , 0.54]
not applicable
mussels
Bråte et al. (2018) 0.97 [0.69 , 1.25]
Cho et al. (2019) 0.12 [0.09 , 0.15]
Ding et al. (2019) 0.29 [0.18 , 0.40]
Hermabessiere et al. (2019) 0.20 [0.17 , 0.23]
J Li et al. (2018)[a] 1.74 [1.62 , 1.86]
J Li et al. (2018)[b] 0.91 [0.85 , 0.97]
Li et al. (2015) 2.39 [1.78 , 3.00]
Nam et al. (2019) 0.29 [0.17 , 0.41]
Phuong et al. (2018a) 0.23 [0.19 , 0.27]
Van Cauwenberghe and Janssen (2014) 0.36 [0.34 , 0.38]
Webb et al. (2019) 0.03 [0.02 , 0.04]
SY Zhao et al. (2018) 0.60 [0.21 , 0.99]
Random effects model 0.57 [0.42 , 0.72]
I^2 = 100% [99%; 100%], χ^2_{11} = 2373.34 (p <0.01)
oysters
Abidli et al. (2019) 1.48 [1.46 , 1.50]
Baechler et al. (2020) 0.35 [0.34 , 0.36]
Cho et al. (2019) 0.07 [0.05 , 0.09]
Phuong et al. (2018a) 0.18 [0.14 , 0.22]
Teng et al. (2019) 0.62 [0.52 , 0.72]
Van Cauwenberghe and Janssen (2014) 0.47 [0.38 , 0.56]
Zhu et al. (2019) 0.80 [0.71 , 0.89]
Random effects model 0.57 [0.20 , 0.93]
I^2 = 100% [100%; 100%], χ^2_6 = 10963.32 (p <0.01)
scallops
Akoueson et al. (2020) 0.29 [0.23 , 0.35]
Cho et al. (2019) 0.08 [0.06 , 0.10]
Li et al. (2015) 2.34 [1.72 , 2.96]
Random effects model 0.48 [0.20 , 0.77]
I^2 = 98% [96%; 99%], χ^2_2 = 89.28 (p < 0.01)
sea snails
Abidli et al. (2019) 0.70 [0.63 , 0.77]
Fang et al. (2018) 0.08 [0.05 , 0.10]
Random effects model 0.39 [–0.22 , 1.00]
I^2 = 100%, χ^2_1 = 265.84 (p < 0.01)
Fixed effects (plural) model 0.54 [0.48 , 0.60]
Prediction interval [0.15 , 1.15]
I^2 = 100% [100%; 100%], χ^2_5 = 33.73 (p < 0.01)
–5
0
5

Figure 46. Forest plot for sub-group analysis between six molluscan families using a mixed-effects model (random-effects model for studies within each category and fixed-effect model between family categories). Studies were weighted using the inverse of the variance method (Chen and Peace, 2013) The x axis represents the standardized mean difference (SMD) expressed in microplastics per gram (MPs/g). The vertical line is the line of null effect where MP content is 0. The grey boxes represent the pooled effect estimate and the lines the confidence interval (CI) 95%. The size of the boxes is proportional to the study weight. The diamonds are the combined point estimates and CI for each of the subgroups. The dotted line is the overall pooled effect for all subgroups with a corresponding diamond. The red box is the prediction interval PI 95%. Li J. et al (2018) a, samples collected from environment; Li J. et al (2018) b, samples collected from market.

6.5.2.1. Clams

Seven studies that analysed clams were included in the meta-analysis (Figure 46). The model revealed high statistical heterogeneity of the pooled effect: I^2 =99.2% and Chi^2= 790.29, p< 0.01. Two statistical outliers of extremely large effects were detected: Li J. et al. (2015) and Jun Wang et al. (2019); the overlap between the 95% CIs between the individual studies and the pooled results of the model are presented in the forest plot in Figure 46. An influence analysis revealed that they were also the most influential studies in terms of heterogeneity (I^2) and overall effect (Appendix 23; A, B) (Viechtbauer and Cheung, 2010). Two studies were rated as of high RoB (Baechler et al., 2020, Jun Wang et al., 2019). Fitting the model without these studies increased the MP content from 1.1 MPs/g to 1.25 MPs/g (95% CI 0.70 to 1.79, p< 0.01) but did not affect heterogeneity (Appendix 24). Therefore, the results of the statistical outlier test, the influence analysis and the RoB rating justified the exclusion of the Baechler et al. (2020) and the Jun Wang et al. (2019) data from the meta-analysis. A sub-group analysis using a random effects model also revealed that there was a significant difference between the five countries/regions included in the meta-analysis (Q= 274.41, p< 0.01), the use of FT-IR (n=6) and RM (n=1) (Q= 58.16, p< 0.01) and the source of the samples (environment, n=5; market, n=2) (Q=44.96, p< 0.01) (Appendix 22).

6.5.2.2. Mussels

Eleven studies reporting mussel MP content were included in the meta-analysis. The analysis did not include the results of the processed mussel samples coming from supermarkets in the Li J. et al. (2018) study nor the samples “after depuration” in the Van Cauwenberghe and Janssen (2014) study in order to improve the homogeneity of the data. The mean content was 0.57 MPs/g (95% CI 0.42 to 0.72. p< 0.01) with a high heterogeneity: I^2=99.5%, Chi^2=2373.34, p< 0.01 (Figure 46). The two studies by Li J. et al. (2015) and Li J. et al. (2018), were determined to be statistical outliers of extremely large effects (Figure 46). An influence analysis also identified the same studies as the most influential studies in terms of contribution to the effect size (Appendix 25; A) while the study by Webb et al. (2019) was

found to be the major contributor to the heterogeneity I^2 (Appendix 25; B) and a major influence to the pooled result (Appendix 26). The geographical origin of the samples was also found to be associated with significant differences in the MP content (Q=949.96, p< 0.01), but no significant differences of the source of the samples was found (environment, n=9; market, n=3) (Q=0.38, p=0.54) (Appendix 22). The influence in choice of FT-IR (n=8), RM (n=3) or both (n=1) also revealed a significant difference, Q=12.21, p< 0.01 (Appendix 22), where the use of FT-IR was associated with higher MP content. The RoB rating analysis showed that there was a significant difference between the three ratings (Q=13.11, p< 0.01). Consideritng these results, in order to improve the quality of the data, a model was fitted omitting the results of the three studies rated as of 'high RoB' (Hermabessiere et al., 2019, Webb et al., 2019, S. Y. Zhao et al., 2018).

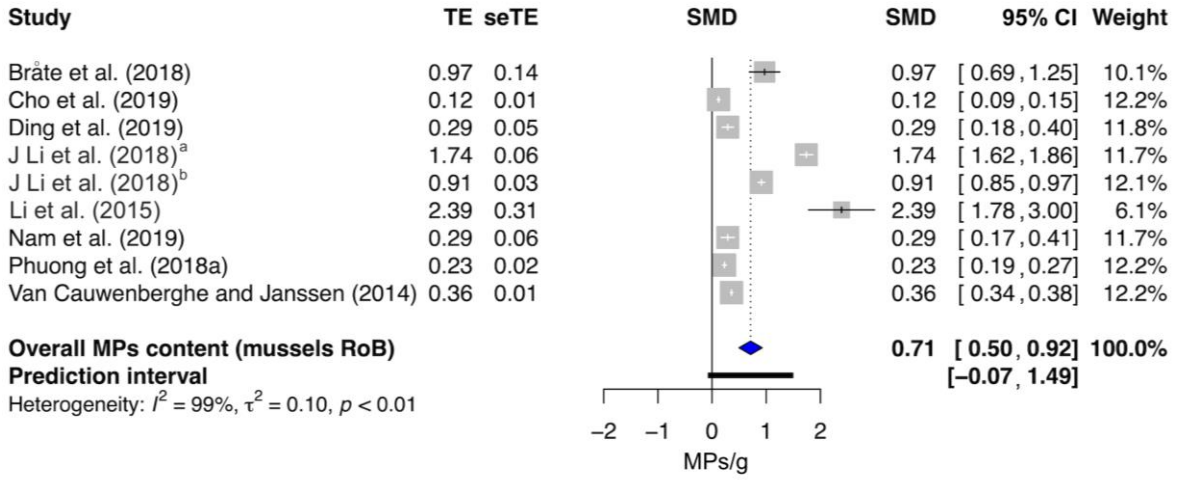


Figure 47. Forest plot for random-effects model results for mussels without the two high risk of bias (RoB) studies (Hermabessiere et al., 2019, S. Y. Zhao et al., 2018). The x axis represents the standardized mean difference (SMD) expressed in microplastics per gram (MPs/g). TE is the MP content reported by each study and seTE is the calculated standard error (SE). The vertical line is the line of null effect where MP content is 0. The grey boxes represent the pooled effect estimate and the whiskers the confidence interval (CI) 95%. The size of the boxes is proportional to the study weight. The diamond is the combined point estimate and CI 95%, and the dotted line is the overall pooled effect. The black box represents the prediction interval PI 95%. Li J. et al (2018) a, samples collected from environment, Li J. et al (2018) b, samples collected from market.

The results of the model are shown in Figure 47, where MP content was 0.71 MPs/g (95% CI 0.50 to 0.92, p< 0.01), and heterogeneity was high (I^2=99.3%, Chi^2=1170.31, p< 0.01). Although Li J. et al. (2015) and Li J. et al. (2018) were identified as a statistical outliers and the major influencers of the effect size, they were not omitted from the analysis because they were rated as having low RoB. Therefore, it was assumed that the difference in their results

was due to variability in the measurements rather than methodological or experimental factors.

6.5.2.3. Oysters

Seven studies were included in the oysters' meta-analysis (Figure 46). The mean content was 0.57 MPs/g (95% CI 0.20 to 0.93, p< 0.01). Heterogeneity was high (I^2=99.9%, Chi^2=10963.32, p< 0.01). One study (Abidli et al., 2019) was detected as a statistical outlier of extremely large effects (Figure 46), which was also rated as having unclear RoB. An influence analysis identified the same study to be the primary influencer in terms of I^2 heterogeneity and effect size results (Appendix 27; A, B). Excluding this study from the model resulted in reduced mean content of 0.41 MPs/g (95% CI 0.25 to 0.57) with high heterogeneity (I^2=99.6%, Chi^2= 1308.55, p< 0.01).

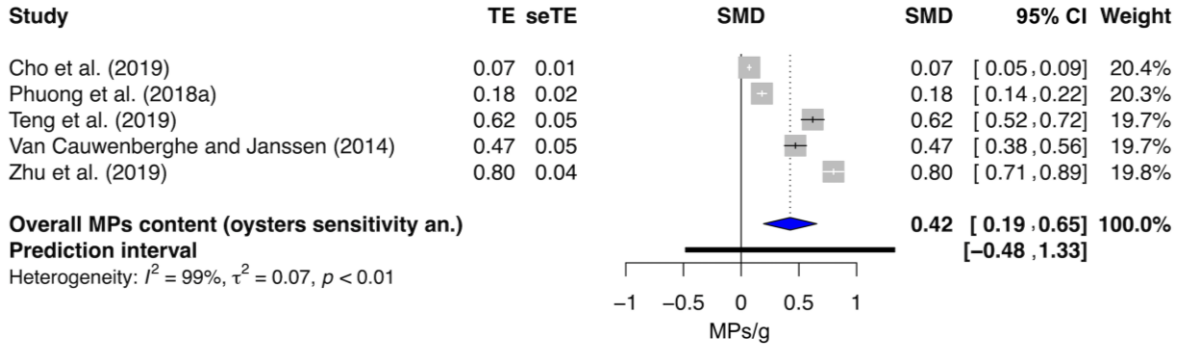


Figure 48. Forest plot for random-effects model for oysters, sensitivity analysis results without the high RoB study (Baechler et al., 2020) and the statistical outlier of extremely large effects (Abidli et al., 2019). The x axis represents the standardized mean difference (SMD) expressed in microplastics per gram (MPs/g). TE is the MP content reported by each study and seTE is the calculated standard error (SE). The vertical line is the line of null effect where MP content is 0. The grey boxes represent the pooled effect estimate and the whiskers the confidence interval (CI) 95%. The size of the boxes is proportional to the study weight. The diamond is the combined point estimate and CI 95%, and the dotted line is the overall pooled effect. The black box represents the prediction interval PI 95%.

One study was rated as having high RoB (Baechler et al., 2020). Excluding this study from the model resulted in a higher content of 0.60 MPs/g with a broader 95% CI (-0.06 to 1.26), p=0.07 and high heterogeneity (I^2=99.9%, Chi^2=10570, p< 0.01). Excluding both studies from the model in a further sensitivity analysis, justified by the previous findings, resulted in a mean content of 0.42 MPs (95% 0.19 to 0.65, p< 0.01) and high heterogeneity (I^2=99.1%, Chi^2=432.73, p< 0.01) (Figure 48). Sub-group analysis showed that there was a significant difference between the six different countries/regions of the samples

(Q=10866.76, p< 0.01). No significant difference was found between the use of FT-IR (n=5) and RM (n=2) (Q=1.33 *p*=0.25) nor between the origin of the sample (environment, n=5; market, n=2) (Q=1.78, *p*=0.18) (Appendix 22). The results of the sub-group analysis were interpreted with caution due to the low number of the studies in a similar manner to the clams' family analysis.

6.5.2.4. Scallops/ Sea snails

Three studies were included in the scallops' meta-analysis and the mean content was 0.48 MPs/g (95% CI 0.20 to 0.77, p< 0.01) with high heterogeneity (I^2=97.8%, Chi^2=89.28, p< 0.01) (Figure 46). All studies were rated as of low RoB. The study by Li J. et al. (2015) was identified as a statistical outlier of extremely large effects (Figure 46). Further influence and sub-group analysis were not appropriate due to the limited number of studies.

The results of the two studies on sea snails were not found to be appropriate for meta-analysis (Figure 46). The confidence intervals for this family included negative values (95% CI -0.22 to 0.99) and the statistical heterogeneity was extremely high (I^2=99.6%). Therefore, the studies were only included in the statistical summary and the narrative analysis.

After the completion of the separate analysis for each family of molluscs, a random effects model was fitted again including studies for all families but excluding the five high RoB studies (Baechler et al., 2020, Hermabessiere et al., 2019, Jun Wang et al., 2019, Webb et al., 2019, S. Y. Zhao et al., 2018) (Appendix 28). The mean content was 0.78 MPs/g (95% CI 0.58 to 0.97, p< 0.01) and heterogeneity was still high (I^2=99.8%, Chi^2=14491.45, p< 0.01). The results of this model represent the best estimation for MP content of all molluscan families.

6.5.3. Publication bias

The RoB across studies was examined using funnel plots (Borenstein, 2009), plotted separately for the different families of molluscs (Appendix 29; A-D). The results of the Egger's test of the intercept show that the asymmetry was not substantial for clams: p=0.07, oysters: p=0.58 and scallops: p= 0.09, but was substantial for mussels: p< 0.01 (Egger et al., 1997). The power of the Egger's test was lower for the clams, oysters and scallops as the number of the included studies was less than 10. The robustness of the eligibility criteria of the review might have excluded studies that would possibly improve the symmetry of the funnel plots. Regarding the crustacean and the fish studies their results were not expressed

in a way that they could be statistically appraised. Publication bias is addressed in the statistical summary/narrative analysis.

6.6. Crustacean studies

Nine studies sampled crustaceans (Table 18) where three reported the frequency of MP detection. McGoran et al. (2018) reported that only 6% of their samples tested positive for MP contamination, F. Zhang et al. (2019) reported the level to be 25%, while the study by Bour et al. (2018) elevated the level to 65%. All three studies were rated as having an unclear RoB in the domains of sampling, analysis and reporting (Appendix 17). Regarding the remaining six studies, the study by Leslie et al. (2017) could not be used for comparison due to the methodological issues in the particle extraction protocol (as mentioned above). Therefore, the statistical summary included five studies (Fang et al., 2018, Hossain et al., 2020, Thushari et al., 2017, Jun Wang et al., 2019, F. Z. Wu et al., 2020). The range of MP content was from 0.14 ±0.08 to 8.6 ±2.6 MPs/g (Figure 49). Four of these studies were rated as having a high RoB (Appendix 17) and could account for the major difference in these results.

Three of these studies have already been appraised in the molluscan analysis previously (Thushari et al., 2017, Jun Wang et al., 2019, F. Z. Wu et al., 2020). The study by Hossain et al. (2020) was found to have high RoB in the domain of sampling and unclear RoB in the domains of analysis and reporting (Appendix 17) as they did not report vital information of their analysis such as the results of the procedural blank samples. Regarding the particle extraction process, McGoran et al. (2018) did not use any type of digestion but dissected samples in 1-cm sections and examined them under a dissection microscope. This approach may have significantly affected the findings in that visual inspection in 1-cm dissections may not be adequate to discover and identify particles that can be less than 1-cm long. Three chemicals were used for digestion of the samples H_2O_2 (37.5% of the studies; n=3 out of 8), HNO_3 (25%; n=2), KOH (25%; n=2), combination of KOH and H_2O_2 (12.5%; n=1) and 50% of the studies (n= 4) followed the digestion with a density separation process (Appendix 16).

Five studies (56%) sampled from the broader area off the coasts of Asia (Hossain et al., 2020, Thushari et al., 2017, Jun Wang et al., 2019, F. Z. Wu et al., 2020, F. Zhang et al., 2019), one between Asia and America (Fang et al., 2018) and the rest from Europe (33%) (Bour et al., 2018, Leslie et al., 2017, McGoran et al., 2018) (Table 18). All studies included in the statistical summary came from Asia and the Americas. All studies used samples

collected directly from their habitat and all samples were wild apart from one (F. Z. Wu et al., 2020), while 89% (n=8 out of 9) used FT-IR for spectral analysis.

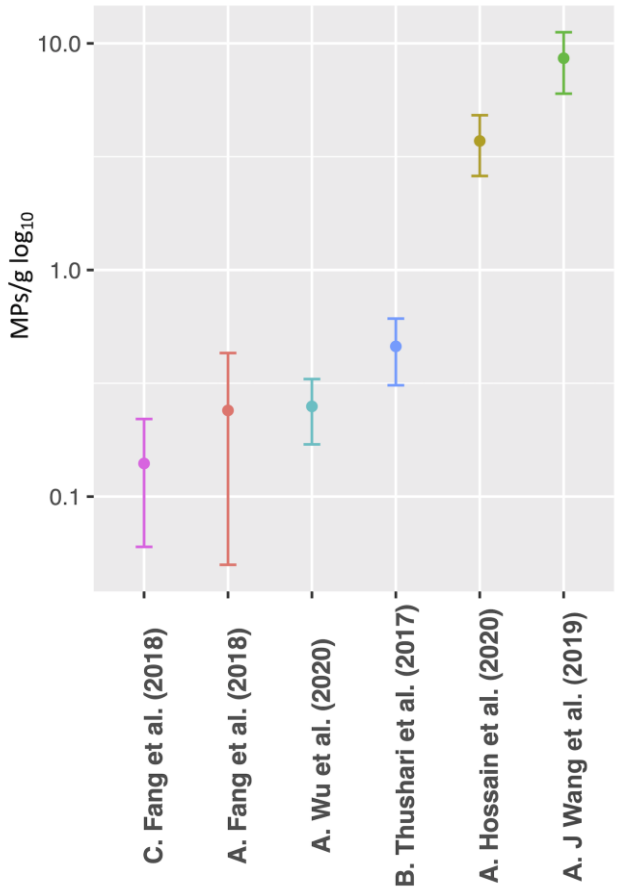


Figure 49. The overall microplastics per gram (MPs/g) content for crustacean families of (A) shrimps, (B) barnacles and (C) crabs; illustrated in a $\log_{10}$ scale. Points represent mean MPs/g values and whiskers represent the corresponding standard deviations (SD). The results of Hossain et al. (2020) and Thushari et al. (2017) have been pooled per family and species, respectively.

In terms of polymeric composition, the most abundant were PE and PA followed by PP and PET (Table 16). Fifty-six percent of the studies (n=5) did not report the similarity index of the spectral library (Fang et al., 2018, Leslie et al., 2017, McGoran et al., 2018, Thushari et al., 2017, Jun Wang et al., 2019), and only 44% (n=4) reported the proportion of extracted particles analysed for composition (Fang et al., 2018, Leslie et al., 2017, McGoran et al., 2018, F. Z. Wu et al., 2020). Therefore, executing correlation analysis was not possible due to the lack of data.

Table 18. Crustacean seafood MP content results.

Study	Geographic location	Sample	N	Mean MPs/g	±SD	Freq.	Composition
Bour et al. (2018)	Norway	*Crangon allmanni*	20			65%	PE 54%, PP 16.8%
Fang et al. (2018)	Bering Sea and Chukchi Sea	*Chionoecetes opilio*	59	0.14	0.08		PA 46%, PE 23%, PET 18%, CP 13%
		Pandalus borealis	21	0.24	0.19		
Hossain et al. (2020)	Bangladesh	*Metapenaeus monocerous*	20	3.87	1.05		PA, RY
		Penaeus monodon	10	3.40	1.23		
Leslie et al. (2017)	Netherlands	*Carcinus maenas*	9	0			Not specified
McGoran et al. (2018)	U.K.	*C. crangon*	116	1[a]	0	6%	polyester 33%, nylon 20% and PP 15%
Thushari et al. (2017)	Gulf of Thailand		50				PA, PET, PS (no%)
		Balanus amphitrite[b]	N/A	0.57	0.22		
		B. amphitrite[c]	N/A	0.37	0.03		
		B. amphitrite[d]	N/A	0.43	0.04		
Jun Wang et al. (2019)	South Yellow Sea, Korea and China	*Crangon affinis*	10	8.6	2.6		Not specified
F. Z. Wu et al. (2020)	China	*Parapenaeopsis hardwickii*	10-20	0.25	0.08		CE, PE
F. Zhang et al. (2019)	China	*Oratosquilla oratoria*	64			25%	PET 65%, PP 10%
		O. kempi	1				

		Portunus trituberculatus	30				
		Carcinoplax vestita	18				
		Charybdis bimaculata	15				
		C. variegate	4				
		P. gracilimanus	3				
		Charybdis japonica	1				

[a] MPs/individual organism, [b] sampling site: Angsila, [c] sampling site: Bangsaen, [d] sampling site: Samaesarn

Note: Studies reported MP content results either as the mean MP content (with or without the SD) or the frequency of samples positive for MP presence. MP content is expressed as number of MP particles per gram of tissue (wet weight) unless otherwise stated. Freq., frequency of samples positive for MP presence; CP, cellophane; MPs, microplastics; N, sample size expressed in number of organisms; PA, polyamide (nylon); PE, polyethylene; PET, polyethylene terephthalate; PP, polypropylene; RY, rayon; SD, standard deviation.

The statistical summary was based on five studies, four of which were rated as having a high RoB and therefore the confidence in those results was deemed to be low. Sample heterogeneity could not be assessed in depth due to the small number of studies. However, variability was identified throughout the research protocols as in the molluscan studies.

The available data on crustaceans were not found to be appropriate for meta-analysis. There were only three studies (Fang et al., 2018, Hossain et al., 2020, Jun Wang et al., 2019) that provided the necessary data (Table 18). These analysed two different families (shrimps and crabs) comprising three different species; Shrimps: *Crangon affinis, Metapenaeus monocerous, Pandalus borealis, Penaeus monodon*; crabs: *Chionoecetes opilio*, making it unreasonable to collate data with such sample heterogeneity.

6.7. Fish studies

Eighteen studies analysed fish, with four reporting the discovery of MPs in the samples, or the rate of discovery (Collard et al., 2017a, Karami et al., 2017c, 2018, Pozo et al., 2019) (Table 19). Two studies (Akhbarizadeh et al., 2020, Karami et al., 2018) used canned samples (whole fish) and one (Karami et al., 2017c) used dried fish (flesh and organs) (Table 16). Akhbarizadeh et al. (2020) reported 1.28 ±0.04 MPs/g in canned tuna. Karami et al. (2017c), (2018) did not report MP content (Table 19). These samples had undergone substantial processing and therefore it would not be reasonable to pool data including them as the fish might have been exposed to airborne MPs contamination in some part of processing. From the remaining 13 studies, seven reported MP content per mass, with a range of 0-11.9 MPs/g (Appendix 30), six reported MP content only per individual organism and three reported MP content expressed both per mass and per individual organism, with a range of 0.23-22.21 MPs/ind. (Appendix 31). Only three of the studies reported the weight of the samples used (Digka et al., 2018, Monia Renzi et al., 2019, Sun et al., 2019) allowing a conversion from MP content per individual to MP content per mass (Appendix 32).

All the studies apart from one (Akoueson et al., 2020) collected organisms directly from the environment, while one study did not report the origin of their samples (Pozo et al., 2019). Sixty-one percent of the samples (n=11 out of 18 studies) were wild organisms (Table 16). Regarding the particle extraction process, 39% used KOH (n=7 out of 18), 22% used H_2O_2 (n=4), 17% (n=3) a combination of KOH and H_2O_2, 11% (n=2) used a combination of NaClO (sodium hypochlorite) and CH_3OH (methanol), 5% (n=1) used HNO_3 and 5% (n=1) the enzyme Proteinase-K (Appendix 16). Forty-four percent (n=8 out of 18) combined the digestion with a density separation process. Sixty-seven percent (n=12) used FT-IR and 33%

(n=6) RM. Fifty percent of the samples came from Asia (n=9), 33% from Europe (n=6), 5.6% (n=1) from Africa, 5.6% (n=1) from South America and 5.6% (n=1) from multiple continents (Table 19).

There were seven studies that sampled anchovies (six species, see Table 16) reporting a range of 0.35 to 22.21 MPs/individual. The highest MP content (22.21 ±1.7 MPs/ind.) was reported by Feng et al. (2019). It was the only study that used the gut, gills and skin of the samples for analysis, reporting a significant difference of MPs in the different tissues of gut and gill ($F = 39.911$, $df = 2$, $p=0.001$). They did not report the MP content per tissue, per species and therefore the direct comparison with the rest of the studies would be inappropriate. Feng et al. (2019) attributed the higher MP content to the highly polluted sampling area of Haizhou Bay, the habitat and the feeding habits of the species (*Thryssa kammalensis*). Excluding this study brings the range to 0.35 to 2.3 MPs/ind. The study reporting the second higher MP content was Tanaka and Takada (2016). This study was rated as having an 'unclear' RoB due to missing information regarding sampling and analysis (Appendix 17). The higher amount of MP content could also be attributed to the fact the samples came from Tokyo Bay, which is situated off the highly urbanized and industrialized Tokyo metropolitan area.

Six studies sampled sardines (three species, see Table 16) reporting a range of 0.23 to 4.63 MPs/individual. The relatively high value of 4.63 MPs/individual was reported by the Monia Renzi et al. (2019) study, which was rated as having a high RoB. Information was not reported regarding sampling and analysis, the most important being the use of replicate samples, and any details around the composition identification process. Excluding this high RoB study brings the range to 0.23 to 3.71 MPs/ind. Out of the four studies that only reported MPs/ind., only two reported on the size of them (weight). The Monia Renzi et al. (2019) study used considerably larger samples (20.22 g ± 4.2) than Digka et al. (2018) (9.63 g ± 1.46), which would account for the higher MP content per individual. All the studies that sampled anchovies and sardines used the stomach or whole GI tract of the organism for the analysis.

Four studies sampled flesh of larger fish. Two studies reported the absence of MP contamination in seabass (*Lateolabrax maculatus*) (Su et al., 2019), in yellow croaker (*Larimichthys crocea*) and dotted gizzard sand (*Konosirus punctatus*) (F. Z. Wu et al., 2020), while Akoueson et al. (2020) did not discover MP content significantly different from the procedural blank samples results. Only the study by Zitouni et al. (2020) reported a content of 2.9 ±1.54 MPs/g in painted comber (*Serranus scriba*).

Table 19. Fish MP content results.

Study	Geographic location	Sample	N	MPs/g	±SD	MPs/ ind.	±SD	Frequency	Composition
Akhbarizadeh et al. (2020)	Iran			1.28	0.04				PET 36.6%, PS 17.6%, PP 13.5%, PS-PP 10.2%, PS-PET 7.9%, nylon 7.1%, PVC 3.9%, LDPE 3.2%
		Thunnus tonggol	25[a]	0.15	0.05				
		T. albacares	20[a]	0.10	0.04				
		Scombermorus commerson	5[a]	0.15	0.03				
Akoueson et al. (2020)	Scotland	*Melanogrammus aeglefinus*	12	1.07[b]	0.12				CL 62%, PET 19%, CP 15%, polyolefin 4%
	Greece	*Dicentrarchus labrax*	10	1.04[b]	0.07				CL 43%, CP 14%, PET 11%,
	Iceland	*Pleuronectes platessa*	10	1.31[b]	0.11				PE 41%, PET 14%, CL 14%, CP 9%
	Scotland	*Scromber scombrus*	10	0.58[b]	0.10				PET 25%, CL 25%, CP 25%, PP 9%, PA 8%, PAN 8%
Collard et al. (2017a)	Mediterranean Sea	*Engraulis encrasicolus*	13					9 MPs found in 8 of the 10 livers	PE
	English Channel	*Sardina pilchardus*	2						
Collard et al. (2017b)	Mediterranean Sea, Bay of Biscay	*E. encrasicolus*	20			0.85			PE 37%, PP 26%, PET 16%, PAN 7%, PS 5%, PA 5%, PEG 2%, PBMA 2%.
	English Channel, Bay of Biscay	*S. pilchardus*	20			0.53			
Digka et al. (2018)	Northern Ionian Sea	*S. pilchardus*	36			1.8	0.2		PE 55.5%, PP 27.7%, PET 5.5%, PS 5.5%, PTFE 5.5%.
Feng et al. (2019)	China	*Thryssa kammalensis*	19	11.19	1.28	22.21	1.70		CP 33.5%, PP 15%, PE 13%, nylon 8.0%, PET 4.5%

Karami et al. (2017b)	Malaysia	*Chelon subviridis* (Packed dried)	30					29 MPs in flesh 7 MPs in organs	PP, 47.2%, PE 41.6%, PS 5.56%, PET 2.77%, NY6 2.77%
		Johnius belangerii	30						
		Rastrelliger kanagurta	30						
		Stolephorus waitei	30						
Karami et al. (2018)	Product of Canada, Germany, Iran, Japan, Latvia, Malaysia, Morocco, Poland, Portugal, Russia, Scotland, Thailand, and Vietnam	sardines and sprats (canned, unknown species)	20[c]					MPs found in 35% of sample	PP 33.3%, PET 33.3%, PE 16.6%, PVC 16.6%
Lopes et al. (2020)	Portugal	*S. pilchardus*	76			0.23	0.04		PP 21%, PE 16%, CL 16%, RY 13%, styrene/acrylic copolymer 11%, polyacrylate 8%, nylon-6 4%, PET 4%, polymeric epoxy plasticizer 4%
		E. encrasicolus	131			0.5	0.6		
		Boops boops	19			0.34	0.6		
Pozo et al. (2019)	Chile	*Strangomera bentincki*	10					MPs found in 30% of sample	PET 75%, PE 25%
Renzi et al. (2019)	Adriatic Sea	*S. pilchardus*	80			4.63			PP 50%, PVC 30%, PTFE 10%, PA 10%
		E. encrasicolus	80			1.25			PVC 93%, PET 7%
Su et al. (2019)	China	*Lateolabrax maculatus*	9	0	0				
	China	*Setipinna taty*	20			0.35			

Sun et al. (2019)		*Ammodytes personatus*	50			0.54			Organic oxidation polymers 40%, PE 22%, PA 11%
		Anchoviella commersonii	30			0.40			
		Engraulis japonicus	280			0.39			
Tanaka and Takada (2016)	Tokyo Bay, Japan	*E. japonicus*	64			2.3	2.5		PE 52%, PP 43.3%, PS 2%, E/P 2%, E/P/D 0.7%
Teng et al. (2020)	China	*Sardinella zunasi*	N/A	0.77	1.42	2.84	1.93	MPs found in 78.8% of sample	CP 61.0%, PET 29%, PP 6%, PA 2.4%, PAN 1.6%
Q. Wang et al. (2020)	China	*Konosirus punctatus*	44	0.12	0.14	3.71	3.39		CP 77.5%, PET 16.9%, PP 2.5%, PAN 0.9%, PE 0.5%, PVAc 0.5%, PA 0.4%, PS 0.4%, PB 0.2%, PC 0.2%
		Thryssa mystax	8	0.09	0.05	1.65	1.39		
		Sardinella zunasi	6	0.02	0.02	0.74	0.76		
F. Z. Wu et al. (2020)	China	*Larimichthys crocea*	10-20	0	0				RY, PP, PA, AN, PET
		Konosirus punctatus	10-20	0	0				
Zitouni et al. (2020)	Tunisia	*Serranus scriba* (Painted comber)	240	2.90	1.54				PEVA, HD-PE, LD-PE), PA or nylons, PEMA

[a] cans of fish, [b] not significantly different for the procedural blank results, [c] brands (4 cans per brand, 2 - 30 fish per can

Note: Studies reported MP content results either as the mean MP content (with or without the SD) or the frequency of samples positive for MP presence. MP content is expressed as number of MP particles per individual organism. CL, cellulose; CP, cellophane; E/P, ethylene/propylene copolymer; E/P/D, ethylene/propylene/diene terpolymer; HD, high-density; LD, low-density LDPE, low density polyethylene; MPs, microplastics; N, sample size expressed in number of organisms, NY6 nylon-6; PA, polyamide (nylon); PAN, polyacrylonitrile; PB, polybutene; PBMA, poly (butyl methacrylate); PC, polycarbonate; PE, polyethylene; PEMA, polyethylene-co-methyl acrylate PEG, polyethylene glycol; PET, polyethylene terephthalate; PEVA, Polyethylene-vinyl- acetate; PP, polypropylene; PS, polystyrene; PTFE, polytetrafluoroethylene; PVAc, polyvinyl acetate; PVC, polyvinyl chloride; SD, standard deviation.

This study was rated as having unclear RoB in two domains of sampling and analysis and high RoB in the domain of reporting (Appendix 17) resulting in an overall high RoB. The main factor was the unclear reporting of the procedural samples results. Therefore, the results of the study were excluded from the statistical summary. F. Z. Wu et al. (2020) was also rated as of high RoB due to the lack of reporting of the procedural blank samples results.

Regarding the MPs polymer composition, the most prevalent polymers for fish were PE and PP followed by PET and CP (Table 19). Forty-four percent of the studies (n=8 out of 18) did not report on the accepted similarity index to the spectra library, while 39% (n=7) did not report how many suspected MP particles they analysed (Appendix 33).

Comparison between the species and/or the different body parts used for analysis and the geographical origin was hindered because not all studies reported the MP content per mass but only MPs per individual organism. MP content was associated with the part of the organism used for analysis and the RoB rating. Methodological heterogeneity identified in sampling and analysis was similar to the molluscan and crustacean studies.

Five studies (Akoueson et al., 2020, Feng et al., 2020, Su et al., 2019, Q. Wang et al., 2020, Zitouni et al., 2020) provided the necessary data for meta-analysis of MP content per mass and five (Digka et al., 2018, Feng et al., 2020, Lopes et al., 2020, Tanaka and Takada, 2016, Q. Wang et al., 2020) per individual organism but all of them sampled different families/species of fish (Table 16), which prevented comparison and therefore, meta-analysis was not attempted. One study (Feng et al., 2020) sampled the phylum echinodermata reporting a content of 0.82 MPs/ind. or 1 MP/g in the edible part (gonad) of sea urchins (4 species, see Table 16 and Table 17).

6.8. Summary of evidence

The summary of evidence table (Table 20) presents the results of the systematic review, integrating the meta-analysis results as well as the results of the statistical summary and the narrative analysis. The description of the certainty of the evidence as well as the justification for downgrading and upgrading evidence can be found in the certainty framework assessment in Appendix 34. In brief, RoB rating downgraded the certainty of the evidence only in the case of the crustacean studies since 80% of the studies included (n=4 out of 5) were rated as having a high RoB. Heterogeneity was high across all the families of organisms and downgraded all the evidence by one grade. Conversely, data were not downgraded regarding the three domains of indirectness, imprecision and publication bias, as the evidence was not found to be affected by these factors. As regards the three upgrading

domains, large effects and dose response did not apply in these studies, while all studies were upgraded by one grade due to the lack of confounders.

Table 20. Summary of effects from seafood studies

Seafood category	Number of studies	Outcomes	95% CI	Certainty of the evidence[a]
Average MPs/g content[b]				
Molluscs				Low[c]
Clams	5	1.25	±0.55	
Mussels	9	0.71	±0.21	
Oysters	5	0.42	±0.23	
Scallops	3	0.48	±0.29	
Overall	14	0.78	±0.2	
Range of MPs/g content[d]				
Molluscs	21	0-10.5		Moderate
Crustacean	2	0.1-8.6		Low
Range of MPs/ind. content[d]				
Fish				Moderate
Anchovies	6	0.35 - 2.3		
Sardines	6	0.23 - 4.63		
Lance	1	0.54		
Bogue	1	0.34 (±0.6 SD)		
Overall fish	9	0.23-4.63		
Echinodermata				Moderate
Sea urchins	1	0.82		
Range of MPs/g content				
Fish				Moderate
Anchovies	3	0.01-0.09		
Sardines	4	0.02-0.77		
Lance	1	0.08		
Comber	1	2.9 (±1.54 SD)		
Croaker	1	0		
Seabass	1	0		

Overall fish	10	0-2.9		
Echinodermata				Moderate
Sea urchins	1	1		

[a] all studies are upgraded due to the absence of confounders according to the results of the assessment of the certainty of evidence. Details for the assessment are provided in Appendix 34

[b] meta-analysis results

[c] due to high heterogeneity (see assessment of the certainty of evidence in Appendix 34)

[d] statistical summary results

Note: Microplastic (MP) content in global seafood samples (molluscs, crustacean, fish); Meta-analysis results and statistical analysis results; SD, Standard deviation; Certainty of the evidence rated according to Higgins et al. (2019).

6.9. Discussion

While this is not the first review on this topic, at the time it was published it was the first systematic review concerning MP contamination of seafood intended for human consumption (Danopoulos et al., 2020b). Two recent reviews (Hantoro et al., 2019, Toussaint et al., 2019) presented evidence of human exposure to MP through the consumption of seafood but did not critically collate evidence in order to quantify MP uptake. A recent review by Cox et al. (2019) reported MP content of 1.48 MPs/g for seafood, which is consistent with the higher end of the results reported here. The results of a MP exposure assessment via the consumption of seafood, based on the findings of this systematic review are reported in section 8.3.3. The review by Cox et al. (2019) included studies that were rejected by the screening process for this review. For instance, the studies by De Witte et al. (2014) and Davidson and Dudas (2016) were rejected because a particle composition identification process was not included. Using only visual observation for the identification of MP particles can lead to overestimations (Rocha-Santos and Duarte, 2015, Strungaru et al., 2019, Shaoliang Zhang et al., 2019). The inclusion of such studies in these reviews could explain this discrepancy.

Fifty studies were systematically reviewed, and the overall quality of the evidence was assessed as low to moderate (Table 19). RoB rating was correlated with fluctuations in the MP content results across all phyla. This suggests that the bespoke quality assessment tool was successful in detecting the most important parts of the studies' protocol and execution, from formulating the rationale to reporting of results. According to the meta-analysis, the MP content in molluscs was 0.78 MPs/g (95% CI 0.58 to 0.97) (Appendix 28). Meta-analysis was executed primarily separately for the different molluscan families to address sample and statistical heterogeneity. The range of MP content was found to be 0-2.9 MPs/g in fish, 0.1-8.6 MPs/g in crustaceans and 0-10.5 MPs/g in molluscs (Table 19), extrapolating to yearly

consumptions of 31-8323 MPs, 206-17716 MPs and 0-27825 MPs respectively (see Chapter 7, exposure assessment).

Seafood consumption between countries varies greatly. Countries that are the highest producers of seafood are not necessarily the ones that consume it. According to FAO (2020), Spain is the leading producer of mussels for human consumption, reaching 250000 tonnes per year, but it is not the highest consumer (42.38 kg/capita/year). China is also a leader in mussel production (600,000 tonnes), but a large proportion is used as fish food. Other major producers are Chile, Thailand and New Zealand (Guillen et al., 2019). Corrections for the calculation should include information on where the seafood is produced/caught and where it is consumed. Unfortunately, information at this level of granularity is not readily available. A recent study by Guillen et al. (2019) attempted to calculate the global seafood production and consumption footprint using FAO consumption data and modelling, reporting China to be the major global producer and consumer and also being self-sufficient for the most part (Guillen et al., 2019).

Other media have also been identified as vectors of MPs via the ingestion route with varying MP concentrations, such as sugar (0.44 MPs/g) (Cox et al., 2019), while as stated in Chapters 4 and 5, salt was found to have a MP content of 0-1674 MPs/Kg, TW 0-628 MPs/L and BW 0-4889 MPs/L. Further systematic reviews are needed to robustly assess MP contamination and human exposures from all food categories.

In terms of the most prevalent polymeric compositions in molluscs, discounting the studies that did not report cellulose-related material, PE was the most abundantly detected polymer, followed closely by PP. In the rest of the studies, CP was the most abundant material followed by PET, rayon and polyester; their reported MPs levels might have been inflated by the inclusion of these materials. In crustaceans the more prevalent polymers were PE and PA and in fish PE and PP. Consensus is needed in the definition of MPs since some studies included non-synthetic or semi-synthetic polymers in their results. Across the families of organisms, PE and PP were the most dominant, corresponding to the global plastic production trends (Plastics Europe, 2019). According to the European Plastics Industry Association, for the past 14 years, the plastics with the highest demand and distribution by resin have been PE (combined low and high density) followed by PP, PVC, PUR, PET and PS/EPS (Plastics Europe, 2008, 2017, 2018, 2019).

Narrative analysis showed that molluscan MP contamination skewed towards content of less than 1 MP/g and there seemed to be a correlation of higher MP values in samples from Asia.

A geographical variation in MP content was observed whereby a majority of studies (82%; n=9 out of 11) reporting an MP content above 1 MP/g were from the coasts of Asia, in contrast to only one study from Europe. It is important to note that this correlation might be artificial due to more research being conducted in Asia. However, a recent report by the Ocean Conservancy - McKinsey Center for Business and Environment (2015) argued that over 50% of plastic pollution of the oceans, originating from land, comes from five Asian countries (Jambeck et al., 2015). The pattern of MPs contamination of the oceans (surface/column water and sediments) has been the subject of intensive recent research but their results are contradictory (Li et al., 2019, Olivatto et al., 2019, Pan et al., 2019, Yu et al., 2018, etc., Chunfang Zhang et al., 2019, Jianmin Zhao et al., 2018). The systematic review on MP environmental occurrence by Burns and Boxall (2018) point to higher contamination close to urban and industrial coastal areas and rivers for surface waters. In contrast, other research and reviews report higher MP and plastic concentrations in the convergence zones of the subtropical gyres and higher concentrations in the open ocean than in coastal areas (Avio et al., 2017, Barrows et al., 2018, Cozar et al., 2014, Eriksen et al., 2014). Therefore, it is not yet possible to draw conclusions on geographical patterns of MP contamination and further research is needed.

The contamination of organisms is likely to be affected by the level of contamination of their environment, followed by their feeding habits and physiology. The differences in the amount of MPs between molluscs and the other two phyla can be attributed to the fact that they are filter or bottom feeders. Their physiology renders them a natural filtering system of the oceans, making them vulnerable to MP contamination. In fish, apparent organs for MPs aggregation include the GI tract and gills, which indeed were the focus of many of the studies (Digka et al., 2018, McGoran et al., 2018, Sun et al., 2019, Tanaka and Takada, 2016). On the other hand, MPs were not discovered by the studies that analysed the flesh of larger fish.

Sampling directly or indirectly from the environment and whether the organisms were wild or farmed, were recognized as important factors for their contamination. Regarding wild versus farmed organisms, analysis was inconclusive. A controlled environment might seem more protected against the contamination of farmed organisms, but if the farm is situated in a MP contaminated area, the water quality will have an impact. In addition, Karbalaei et al. (2020) identified MP contamination in three brands of commercial fishmeal; the use of such fishmeal could have cumulative effects in farmed seafood (Karbalaei et al., 2020). A significant difference was found between the molluscan families collected directly from the environment and those collected indirectly from markets, with the first found to be more

heavily contaminated with MPs. The depuration procedure that some molluscs are subjected to, before being commercially available, was proposed as one possible mitigating factor.

A wide range of methodological heterogeneity was detected across the studies regarding sampling and analysis. The size of the sampling regime has a direct effect to the power of the study in terms of both internal and external validity, that is, whether the results can be used to extrapolate to a general population (Higgins et al., 2019). Sampling size is inherently connected to the overall sampling design of the study and is a function of the project's objective, sampling approach, cost, environmental variability, and tolerable error (Environmental Protection Agency of the United States (EPA) 2000, 2002b, Zhang, 2007). The European Commission, through the Institute for Environment and Sustainability (IES, 2013), produced guidelines that raised the minimum amount of sampled specimens to 50 per species and age group, which was not reached by many of the studies in this review. It should be noted that this recommendation applies to monitoring the ingestion of litter by fish over time or between different locations. These guidelines speak to the need for more robust sampling. Furthermore, the majority of the studies did not use a robust sampling design, such as simple random, stratified or systematic but a judgemental sampling design. Judgemental sampling design should be avoided in environmental studies as it can affect the quality of the study and introduce bias (Zhang, 2007).

Results were associated with the different particle-extraction procedures and the specifications of the composition identification methods, highlighting the varying effectiveness of research protocols. It has been argued in recent reviews (Miller et al., 2017, A. L. Lusher et al., 2017, Silva et al., 2018) and method papers (Claessens et al., 2013, Collard et al., 2015, Dehaut et al., 2016), that the use of different chemical and physical treatments for the extraction of particles can influence the effectiveness of the procedure or even further degrade and damage the particles. Whilst the performance of these procedures is not the focus of this review, it highlights the methodological heterogeneity in the field and the need for consensus. These variations in methods are likely to affect results, under or over-representing MP content. Major differences were found in the processes that were implemented in order to extract possible MP particles from the tissue of the organisms, specifically in the use of different chemicals for the digestion of the samples and the use of a density-separation process.

Further important variations were identified in the composition identification process, in terms of the quantity of analysed particles and the specification of the analysis protocol. Following on from the extraction step, there was a lack of consensus on the percentage of

particles isolated that need to be analysed for composition in order to extrapolate safely to the whole sample. In most cases this would be a function of available time and resources, as composition analysis is time-consuming, labour intensive and expensive. Nevertheless, it can be assumed that the larger the proportion/number of the analysed particles, the higher the confidence in the results. The number/proportion of particles undergoing composition analysis should also be considered in relation to the percentage of particles confirmed as MPs, as well as the accepted percentage of similarity compared with the spectral library. Correlation analysis found that as the absolute number of particles and the proportion of particles analysed increased, the MPs/g content was reduced. This leads to the logical assumption that as the numbers of particles tested increase, the better the quality of the research protocol, and the less they are detected in samples. A further finding was that the use of higher spectral similarity indexes was found to be more robust. As the similarity index rose from 60% to 70% and 80%, the MPs/g content also rose. This suggests that as inclusion criteria become more stringent, higher MPs content is identified. One would expect that the lower the similarity index, the more particles would be confirmed as MPs, and thus the greater the MPs/g content would be observed. This is the opposite of what these results showed. In order to explore this further, correlation analysis was carried out between the percentage of the verified MPs and the rest of the variables (the percentage of particles that were analysed; the number of particles analysed; the similarity index of the spectral library) but no significant correlation was found. It should be noted that these results were only based on the results of seven studies, but this analysis can be repeated in the future when more data are available to produce more robust results.

RoB assessment revealed a few focal areas as the source of studies' weaknesses. The most frequently recognized issue was the use of procedural blank samples and the reporting, or not, of their results. In some cases (8.6%; n=2 out of 23, Appendix 18), studies that did report the results, did not further clarify how the results were used, while in many studies (26%, n=6 out of 23), the authors reported that the amount of MPs discovered in procedural samples was inconsequential without offering any more evidence to their conclusion (e.g. statistical tests). The specifics around their use also varied greatly in terms of the number of samples used, whether they tested the reagents used in the experiments etc.

Recent reviews by Hermsen et al. (2018) and Koelmans et al. (2019) proposed quality assessment systems for MPs research regarding biota samples and water samples, respectively, similar to the RoB tool used herein. Both reviews identified a lot of variability in methods and recognize the need for harmonization and transparency in methodology and reporting. There is an evident need for harmonization and/or standardization in all aspects

of the research protocols to increase confidence in the results (Hartmann et al., 2019). There is a subtle but significant difference between the two terms. Although they both refer to reducing the variations in the methodology, harmonization is less stringent and allows some variation while standardisation implies complete absence of variations. Standardisation cannot be achieved throughout all aspects of scientific experimental protocols, but best practises for analytical procedures and quality assurance and control tools can be set as the minimum standard for designing, executing and reporting experiments (Johnson et al., 2020). The lack of such harmonized methods hinders the acquisition of reliable and reproducible data. This need is also highlighted by current interlaboratory efforts to achieve these goals by the Joint Research Centre (JRC, 2019) of the European Commission, the German Federal Institute for Materials Research and Testing (BAM) and the Vrije Universiteit (2019). The findings of this review coincide with recent reviews by Hermsen et al. (2018) and Koelmans et al. (2019) who in proposing quality assessment systems for MPs research also identified a lot of variability in methods and the need for harmonization and transparency in reporting.

Statistical heterogeneity, which is the quantified variability of data, is the product of clinical and/or methodological variability among the studies of the meta-analysis (Higgins et al., 2019, Rücker et al., 2008). Clinical heterogeneity refers to the variability of the sample characteristics, and methodological heterogeneity refers to the variability of methods. Measuring the statistical heterogeneity in meta-analysis can be used to evaluate if all the studies are measuring the same thing. Meta-analyses, as part of systematic reviews, were initially used in the health sciences, and are still often used to measure and compare effect sizes of interventions. In the health sciences, and elsewhere, the effect sizes might refer to an effect that can be measured objectively e.g. a biological marker in a blood sample, or to an effect that is measured differently in individual studies e.g. a likert scale. In this review, the effect measure of interest (MP content) was a tangible physical measure, and it is possible to be confident that the studies are indeed measuring the same thing. Although the methods for analysis of MPs varied across the studies, the eligibility criteria set in the protocol offer a minimum but very important harmonization of the methods used to identify MPs. Specifically, in order to strengthen the confidence that all the studies measure the same thing (i.e. MPs), the use of a chemical composition identification method using on state-of-the-art technology was set as an inclusion criterion. Furthermore, heterogeneity can inform whether it is appropriate to combine data from different studies (Borenstein, 2009). The wide scope of this review predetermined that the diversity of the included studies would be high. Diversity existed regarding both sample characteristics (e.g. more than 40 species of molluscs, see Appendix 15) and the studies' methods (e.g. 23 different particle extraction

processes, see Appendix 16). Nevertheless, the studies were judged to be homogeneous enough to produce a meaningful summary. This decision was based on the similarity of the physiological characteristics of the sample population as well as the intended use of the organisms as seafood. Heterogeneity was recognized before the execution of the meta-analysis and was partially addressed by using random-effects models instead of fixed-effect models. Throughout the meta-analysis applied to the molluscan families, statistical heterogeneity as measured by the I^2 value was found to be high. The confidence in the I^2 values was limited due to the small number of studies. All attempts to decrease heterogeneity by excluding highly influential studies and statistical outliers were not successful. Sub-group analysis showed that significant differences existed between the geographical origins of the samples across all the different molluscan families. Therefore, there is a high probability that the residual heterogeneity was caused by diversity in the geographical origin of the samples. Meaning that the variations in the level of MP content in seafood, across the studies that were included in the meta-analysis, might be attributed to the geographical location of the sampling i.e. the habitat of the samples, as discussed above. The inclusion of further studies in an updated meta-analysis in the future, could inform us to that effect.

Human health effects related to MP exposures, and indeed the levels of MPs in human subjects, are only recently being investigated but there is a growing body of literature to support evidence of uptake (Abbasi et al., 2018, Gallagher et al., 2015, Schwabl et al., 2019) and detrimental impacts (Dong et al., 2020, Gallo et al., 2018, Stock et al., 2019). Recently reported potential human effects include gastrointestinal and liver toxicity (Chang et al., 2020, Wenfeng Wang et al., 2019) as well as neurotoxicity (Prüst et al., 2020). The key identified exposure route is ingestion (along with inhalation) (Chang et al., 2020, Hale et al., 2020), with seafood being a major medium of exposure (van Raamsdonk et al., 2020, Yung-Li Wang et al., 2020). Key toxic mechanisms include cytotoxicity via oxidative stress (Chang et al., 2020), gene expression alteration and genotoxicity (Yung-Li Wang et al., 2020) changes to the gut microbiota (van Raamsdonk et al., 2020), metabolism disorders and inflammatory reactions (Chang et al., 2020). Evidence comes from animal studies and human cell lines. Although the findings are in some cases contradicting (van Raamsdonk et al., 2020) and further research is undoubtedly needed, there is also no evidence that MPs human exposure is safe (Leslie and Depledge, 2020). Seafood is an important source of protein for populations around the world, and it may be time to implement the precautionary principle (Kriebel et al., 2001), based on the existing scientific evidence and take steps in policy, industry and society to minimize human exposures to foodborne MPs where possible.

6.9.1. Strengths and limitations

This systematic review collates the evidence from multiple studies and estimates human MP exposures via seafood consumption. The review used robust methodology and a bespoke RoB assessment tool to appraise the quality of the studies. Although heterogeneity was acknowledged throughout the review, the strategies used to remediate it had limited success. Extrapolating to human MP uptake through seafood was based only on the species for which evidence was available thus affecting the external validity of the results. More evidence coming from a wider range of commercial species around the world is needed to formulate a more comprehensive understanding of MP contamination levels.

6.10. Chapter conclusions

Fundamentally, the majority of studies included in this review found MPs in the seafood samples. The data support the hypothesis that seafood is a major verified vector for human exposure to MPs. The levels of MP contamination varied in different phyla of organisms from fish (0 to 2.9 MPs/g) to echinodermata (1 MPs/g), crustaceans (0.1 to 8.6 MPs/g) and molluscs (0-10.5 MPs/g). A key finding of this work is the need for harmonization and standardization of methods and procedures throughout the research process, starting from sampling design through to reporting. The bespoke RoB assessment tool used in this review and the narrative analysis along with the GRADE certainty framework identified the following areas that would benefit from improvement, clarification and further research:

- In order to reduce RoB, there is a need for overall methodological improvement in study design (sampling and analysis) and execution.
- Sampling design must be linked to the aim of the study and a rationale should be provided, particularly for sample size and location.
- High standards of laboratory practices should be followed to avoid post-sampling contamination.
- Use and detailed reporting of procedural blank samples to account for post-sampling MP contamination.
- There is a need for harmonization of the procedure that is used to extract particles from tissues of the organisms as varying effectiveness can significantly affect results and hampers comparisons across studies.
- The use of a verified technique for the identification of the composition of the particles is imperative to avoid over or under-representation. In particular, a consensus is needed in the definition of MPs since some studies include non-synthetic and/or semi-synthetic polymers in their results.

- Consensus is needed for the protocol of the composition identification process; proportion of particles analysed, which spectra library is used, and what minimum accepted similarity index to the spectra library is allowed.
- Consensus on the definition of MPs in terms of size, perhaps also related to body compartment exposure/uptake characteristics.
- Reporting should include details of organisms' characteristics, such as weight, to make conversion to other units and comparison between studies possible.

Further research is needed on the effectiveness of depuration on the mitigation of MP contamination of seafood.

Chapter 7. Toxicological impacts of microplastic exposure in human cells; Rapid review and meta-regression analyses results

This chapter is based on a manuscript that was submitted for publication to the Journal of Hazardous Material (Danopoulos et al., 2021). The methodology and methods are provided in sections 3.3 and 3.4.

7.1. Study selection

Database searches identified 166 publications, and a further two were identified from searching the reference lists of relevant reviews. During the first level screening 144 studies were excluded based on their title and abstract. The full text of 24 studies was then assessed and 17 met the eligibility criteria set for this rapid review. Eight of those studies were included in a quantitative meta-regression (Figure 50). The reasons for the exclusion of the studies in the second-level screening are provided in Appendix 36.

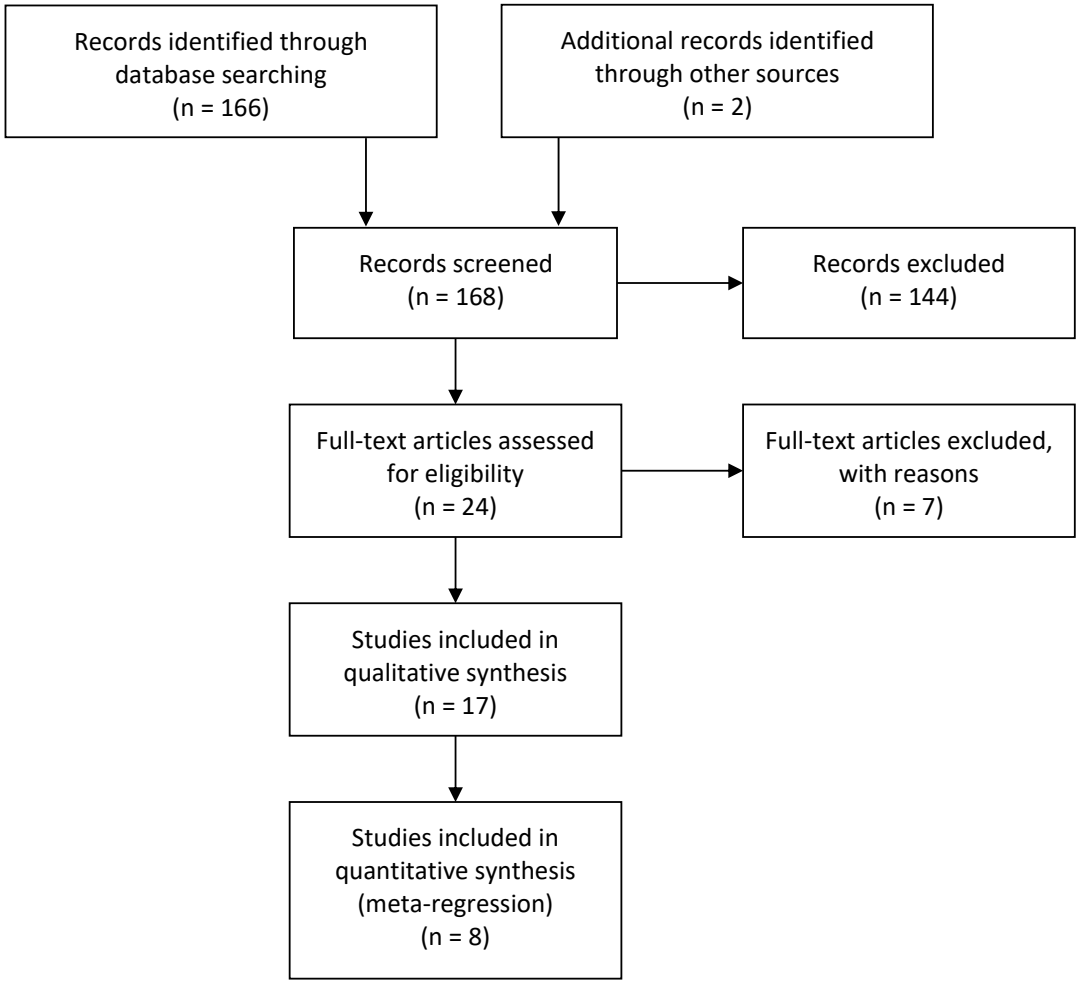


Figure 50. PRISMA flow diagram of the screening process for MPs toxicological human cell studies

7.2. Study characteristics

The characteristics of the studies are presented in Table 21. In order to facilitate the presentation of this versatile data frame, the biological endpoints have been grouped in five categories: cytotoxicity, immune response, oxidative stress, barrier attributes and genotoxicity, as illustrated in Figure 51. For the majority of the outcomes, grouping was straightforward as the studies reported similar endpoints using widely used, commercially available testing kits. When this was not the case, the endpoints were grouped within the most relevant group according to the extracted body of evidence. In order to ensure grouping of the biological endpoints was meaningful, expert advice was sought from two toxicologists. The studies used 15 different human cell models and co-cultures, testing 10 different polymers, using more that 30 different tests/biological markers. Full test conditions and results are presented in a spreadsheet, the file can be found in the supplementary material (SM 2) of the paper Danopoulos et al. (2021).

The studies used 28 test MPs: 16 primary and 11 secondary, while the origin of one test MPs was not defined (Shijin Wu et al., 2020). The primary test MPs were spherical (13 out of 16) and powders (three out of 16); the secondary MPs (11) were all consisting of irregular shapes. Seven out of the 17 studies did not use spherical MPs. Choi et al. (2020), Han et al. (2020), Hwang et al. (2019) and Lehner et al. (2020) used secondary, randomly-shaped, in-house produced MPs. Choi et al. (2021) used both spherical, primary MPs (HDPE) and randomly-shaped, secondary MPs (LDPE). Stock et al. (2021) also used a combination of primary, commercially sourced microspheres (PE) and powders (PE, PT, PVC) as well as secondary, grounded powders (PP). Liu et al. (2020b) used both primary, spherical PS MPs and secondary, irregularly shaped MPs.

All the studies, apart from Lehner et al. (2020) and Liu et al. (2020a) used a variation of a ball-mill method to create their secondary MPs. Lehner et al. (2020) used a combination of methods applying cryogenic temperatures followed by milling, while Liu et al. (2020a) used a digestion process to mimic the digestive tract. Shijin Wu et al. (2020) did not report the origin nor the shape of the MPs they used.

Four studies (Choi et al., 2020, Choi et al., 2021, Han et al., 2020, Hwang et al., 2019) reported only the size ranges used in the experiments, while 10 studies provided the exact sizes (Brown et al., 2001, Dong et al., 2020, Goodman et al., 2021, Hesler et al., 2019, Hwang et al., 2020b, Liu et al., 2020b, Stock et al., 2019, Qiangqiang Wang et al., 2020, Wu et al., 2019, Shijin Wu et al., 2020), one study (Lehner et al., 2020) provided the MP size

distributions (D10, D50 and D90). One study (Schirinzi et al., 2017) provided a range value for one of the test MPs (PE) and a specific size for the other (PS). One study (Stock et al., 2021) provided ranges for two test MPs (PE 1-4, 10-20 µm) accompanied by the mean diameter, as measured in the laboratory via SEM, for those and the remaining test MPs (PP, PET, PVC and PE 90 µm). The overall size range was 0.1 to 282 µm.

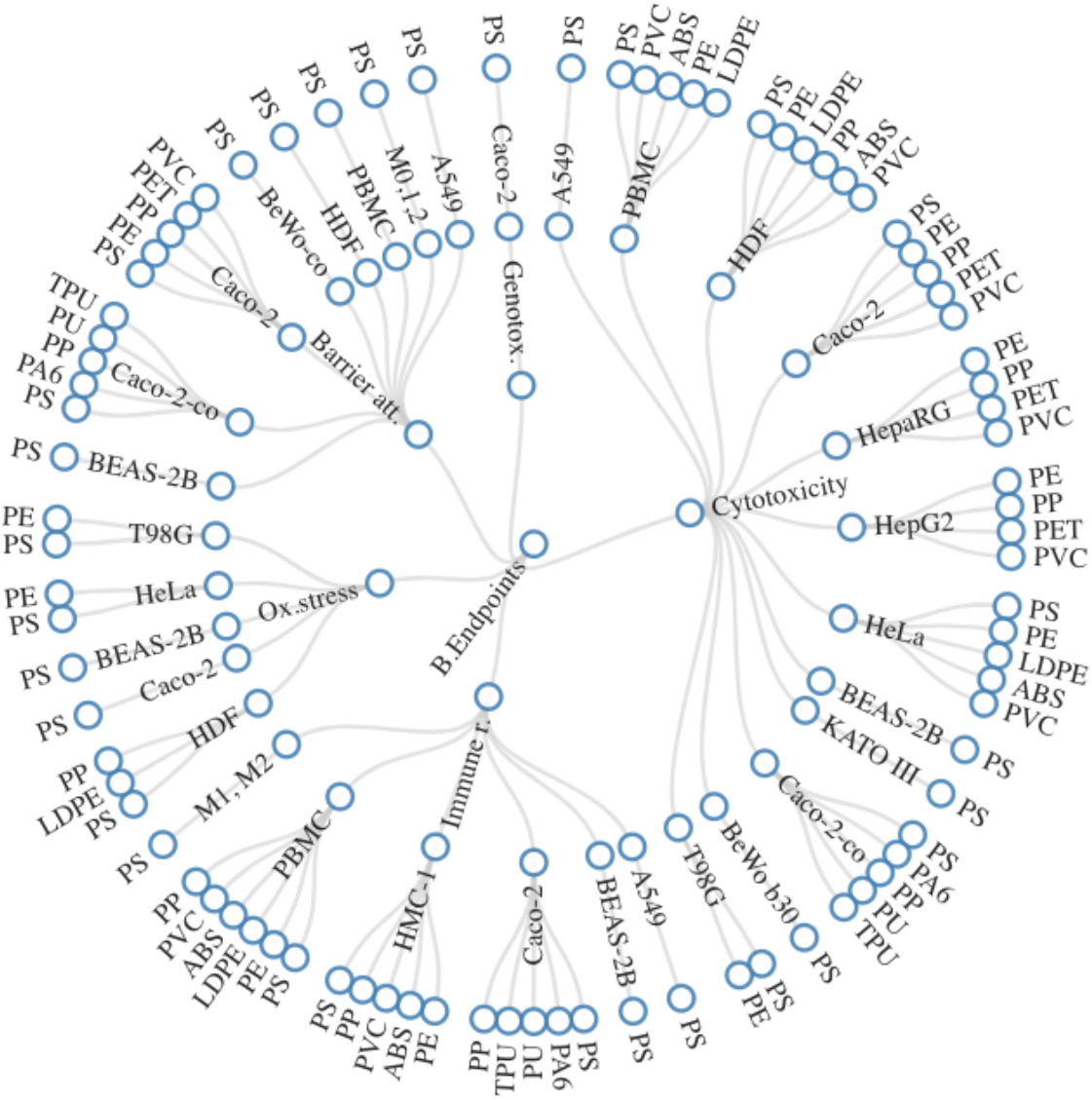


Figure 51. Biological endpoints, cell models and test MPs polymers used in the cumulative experiments reported by all studies. Note: ABS, acrylonitrile butadiene styrene; A549, adenocarcinomic human alveolar basal epithelial cells; Barrier att., Barrier attributes; BEAS-2B, human lung epithelial cells; BeWo b30, human placental choriocarcinoma cell line; Caco-2, human adenocarcinoma cell line; co, coculture; Genotox., Genotoxicity; HDFs, human dermal fibroblasts; HeLa, cervical cancer cells; HepaRG, human hepatic cells; HepG2, Human Caucasian hepatocyte carcinoma cells; HMC-1, the human mast cell line-1; Immune r., Immune response; KATO III, gastric cancer stem cells; LDPE, low-density polyethylene; M0,1,2, macrophages; Ox. Stress, Oxidative stress; PBMCs, peripheral blood mononuclear cells; PA6, polyamide; PE, polyethylene; PP, polypropylene; PS, polystyrene; PU, polyurethane; T98G, human glioblastoma multiforme cells; TPU, polyurethane

Table 21. Study characteristics for microplastic (MP) toxicological human cell studies.

Study	Polymer	Origin	Particle size (µm)	Shape	Cell model	Biological endpoint
Brown et al. (2001)	PS	primary	0.202 and 0.535	Spherical	A549	Immune response
Choi et al. (2020)	PS	secondary	5−25, 25−75 and 75−200	Randomly shaped	PBMCs	Cytotoxicity [a]
					RBC-removed PBMCs	Immune response
					KATO III cells	Cytotoxicity
					HeLa cells	Cytotoxicity
					HDFs	Cytotoxicity, Oxidative stress
Choi et al. (2021)	HDPE	primary	1–10, 50 (45-53), and 100 (90-106)	Spherical	PBMCs	Cytotoxicity, Immune response
					HMC-1 cell line	Immune response
	LDPE	secondary	25-75 and 75-200	Randomly shaped	HeLa	Cytotoxicity
					HDFs	Cytotoxicity, Oxidative stress

(Dong et al., 2020)	PS	primary	1.72 ± 0.26	Spherical	BEAS-2B cells	Cytotoxicity, Oxidative stress, Immune response, Barrier integrity, Predictive biomarker for COPD
Goodman et al. (2021)	PS	primary	1 and 10	Spherical	A549	Cytotoxicity, Cell proliferation, Internalization
Han et al. (2020)	PVC	secondary	25-75 and 75-200	Irregular	PBMCs	Cytotoxicity, Immune response
					HMC-1 cell line	Immune response
	ABS				HDFs	Cytotoxicity
					HeLa cells	Cytotoxicity

Hesler et al. (2019)	COOH - PS	primary	0.5, (0.4658 ± 0.0102)	Spherical	Co-culture: Caco-2 and HT29-MTX-E12	Cytotoxicity, Barrier integrity, Translocation, Uptake
					BeWo b30 cell line	Cytotoxicity
					Co-culture: BeWo and HPEC- A2 cells	Barrier integrity, Translocation, Uptake
					p53-sensitive reporter cell line	Genotoxicity
Hwang et al. (2019)	PP	secondary	~20 and ~200 (25–200)	Various shapes	PBMCs	Immune response
					HDFs	Cytotoxicity, Oxidative stress
					HMC-1 cell line	Immune response
Hwang et al. (2020b)	PS	primary	0.460, 1, 3, 10, 40 and 100	Spherical	HDFs	Cytotoxicity, Uptake
					PBMCs	Cytotoxicity, Immune response, Uptake
					HMC-1 cell line	Immune response

Lehner et al. (2020)	PA6	secondary	72 [b]	Fragments	Co-culture: Caco-2/HT29-MTX/ MDM/MDDC	Cytotoxicity, Immune response, Barrier integrity
	PU (hardened)		253 [b]			
	TPU (ester)		264 [b]			
	PP (Sun)		282 [b]			
Liu et al. (2020b)	PS	primary	0.1 and 5	Spherical	Caco-2 monolayer model	Barrier integrity, Permeability, Oxidative stress, Paracellular and trans-membrane transport, Immune response
	t-PS [c]	secondary	0.4402 [d]			
(Schirinzi et al., 2017)	PE	primary	3–16 (with NPs 0.1 – 0.6)	Spherical	T98G cells	Cytotoxicity, Oxidative stress
	PS	primary	10 (with NP 0.04 – 0.25)	Spherical	HeLa cells	Cytotoxicity, Oxidative stress
Stock et al. (2019)	PS	primary	1, 4, 10	Spherical	Caco-2 cell line	Cytotoxicity, Uptake
					Co-culture (mucus) model: Caco-2 cells and HT29-MTX-E12 cells	Uptake
					Co-culture: (M-cell) model: Caco-2 cells and Raji B	Uptake

					M0 macrophages (from THP-1 cell line), M1 and M2 M1, M2 [e]	Uptake Macrophage polarization
Stock et al. (2021)	PE	primary	2.2 (1-4), 16.5 (10-20)	Spherical	Caco-2 cells	Cytotoxicity
	PE	primary	90.1 [f]	Powder	HepaRG	Cytotoxicity
	PP	secondary	67.1 [f]	Powder		
	PET	primary	60 [f]	Powder	HepG2	Cytotoxicity
	PVC	primary	136.5 [f]	Powder		
					Caco-2 model	Uptake
Qiangqiang Wang et al. (2020)	PS	primary	0.3, 0.5, 1, 3, 6	Spherical	Caco-2	Cytotoxicity, Oxidative stress, Uptake
Wu et al. (2019)	PS	primary	0.1 and 5	Spherical	Caco-2 cells	Uptake, Cytotoxicity, Oxidative stress, Barrier integrity
Shijin Wu et al. (2020)	PS	n/r	5	n/r	Caco-2 cells	Cytotoxicity, Oxidative stress, Gene expression alteration

[a] cytotoxicity was accessed via cell viability unless stated otherwise, [b] median size, [c] original and transformed via a digestive process to mimic human digestive processes, [d] 100 nm transformed size: 440.2 nm, 5μm transformed size: not reported (n/r), [e] M0 macrophages differentiated from THP-1 cell line, exposed to MPs, and then polarized to M1 and M2, [f] polydisperse, mean diameter provided in the source, [g] spherical according to the manufacturer Microparticles GmbH.

Note: ABS, acrylonitrile butadiene styrene; A549 adenocarcinomic human alveolar basal epithelial cells, BEAS-2B, human lung epithelial cells; BeWo b30, human placental choriocarcinoma cell line; Caco-2, human adenocarcinoma cell line; COOH, carboxy-modified surface; COPD, chronic obstructive pulmonary disease; CPS, Carboxylated polystyrene; HDFs, human dermal fibroblasts; HeLa, cervical cancer cells; n/r, not reported; HepaRG, human hepatic cells; HepG2, Human Caucasian hepatocyte carcinoma cells; HMC-1, the human mast cell line-1; HPEC-A2 cells, SV40-transformed microvascular human placental venous endothelial cells; HT29-MTX-E12, a mucus-secreting subclone from colon adenocarcinoma HT29 cells differentiated into mature goblet cells; KATO III, gastric cancer stem cells; MDM, human blood monocyte-derived macrophages; MDDC, dendritic cells; M-cell, Microfold cells; M0,1,2, macrophages; NIH/ 3 T3, murine fibroblast cell line; NP, nanoplastics; n/r, not reported; PBMCs, peripheral blood mononuclear cells; PA6, polyamide; PE, polyethylene; PP, polypropylene; PS, polystyrene; PU, polyurethane; p53, sensitive reporter cell line based on the human liver carcinoma cell line; Raji B, human lymphocytes cells; RBC, red blood cells; T98G, human glioblastoma multiforme cells; THP-diff., THP-1 cells differentiated into macrophages; THP-1, human monocytic cell line; t-PS, digestive tract transformed PS-MPs; TPU, polyurethane ; U937, human histocytic lymphoma cells

7.2.1. Conversion of MPs mass to particle number

All the studies apart from one (Stock et al., 2019) used the mass of the particles to denote the MP concentrations of the dose used in the experiments. Of the 17 studies included in the analysis, eight attempted to convert the concentrations to another metric. Brown et al. (2001) and Goodman et al. (2021) reported concentrations in both mg/mL and MPs/mL, while Stock et al. (2019) expressed the concentrations in MPs/mL, pg/mL, μm^2/mL and μm^3/mL. None of the three studies reported their method for the conversions. Choi et al. (2020) and Choi et al. (2021) used the basic volume to mass conversion assuming that the particles were cubes, although they used spherical and randomly shaped MPs. Dong et al. (2020) is one of the two studies that reported the concentration by surface area (cm^2) and stated that the mass concentration can be converted to particle concentration by multiplying by 5.12×10^3, but did not provide any rationale for this conversion. Han et al. (2020) proposed the averaging of volumes and densities across MPs to calculate exposures in MPs/mL. Hwang et al. (2020b) used the more specialized equations proposed by Connors et al. (2017).

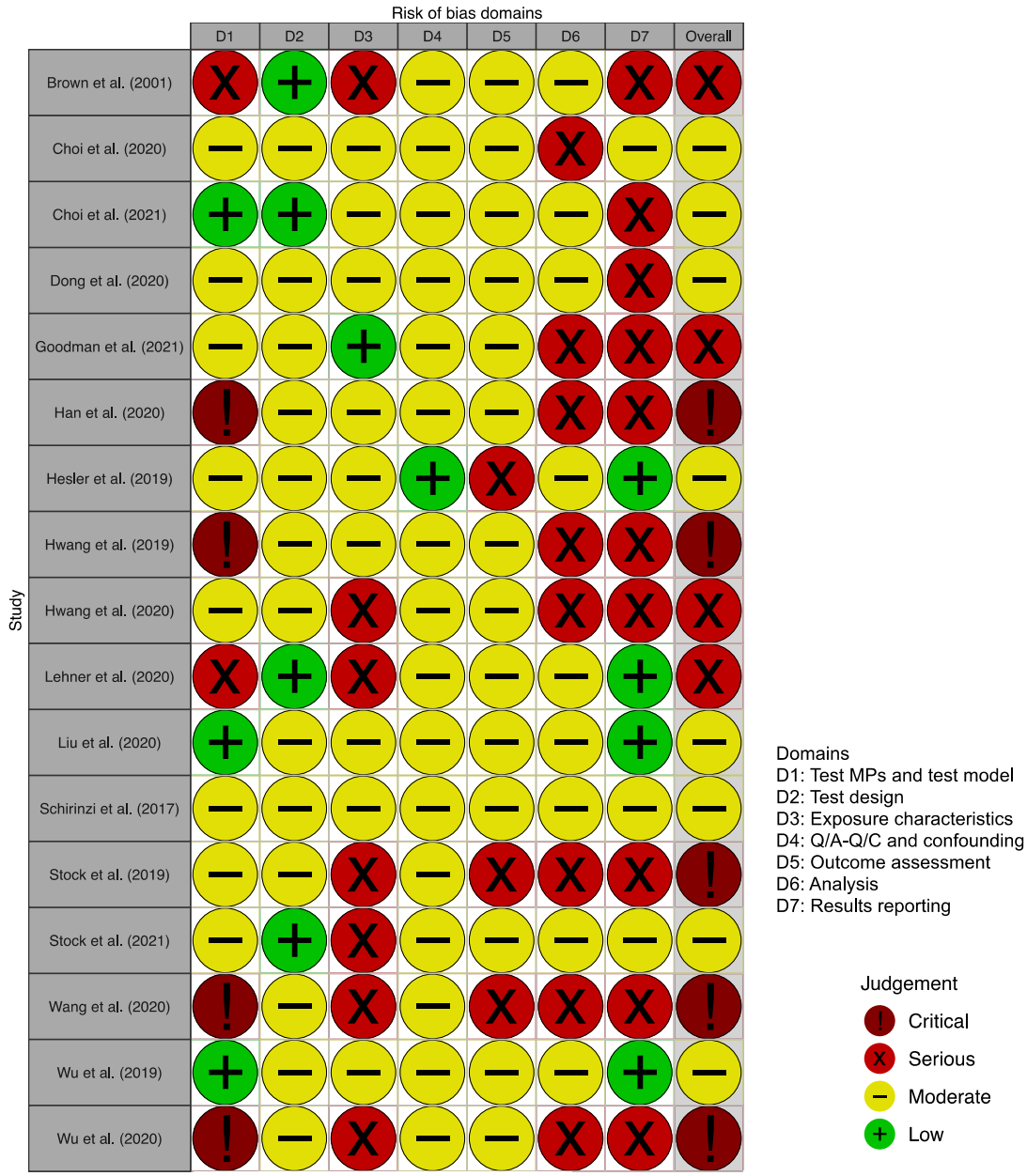
For the purposes of this review, a conversion was used for any concentrations reported in the toxicity studies (μg/mL) where studies did not supply both metrics (of either the amount or the mass), to the metrics commonly used within the environmental studies (MPs/mL). The rationale for this approach was that more details were available for the substances, as they have been handled in a controlled environment. This conversion is therefore an estimation of what is used, primarily, to detect whether the order of magnitude used in toxicity studies is relevant to the results reported by environmental studies. It must also be noted that the concentrations expressed by surface area (cm^2) could not be converted nor directly compared to the rest of the units. No method exists for the conversation of the concentration of irregularly shaped MP from μg/mL to MPs/mL or vice versa. Therefore, the equation by Connors et al. (2017) for converting MP mass concentration to abundance concentration was used for both spherical and irregularly shaped MPs. The equation is an extension to the basic relationship between size, weight and density. When the conversions were reported by the studies, those concentrations were used. When the studies did not report the density of the polymer, the standard density reported in literature was used: PE ≈ 0.940 g/cm^3, PP ≈ 0.905 g/cm^3 (Plastics Europe, 2021), and PS ≈ 1.053 g/cm^3 (Mark, 1999).

7.3. Risk of bias

The results of the RoB assessment are presented in, Table 22 and in Figure 52. Five of the studies were found to be of critical RoB and their results were omitted from the narrative

and the meta-regression analysis. All the studies were assessed to have a RoB above the rating of low, implying that they all suffered from deficiencies in some aspect. The only domain where critical RoB rating was assigned was the test MPs and test model. Four studies (Han et al., 2020, Hwang et al., 2019, Qiangqiang Wang et al., 2020, Shijin Wu et al., 2020) did not provide information on the origin or identification of the basic test material, whether MPs or cells.

Table 22. Risk of bias (RoB) assessment results for MP toxicological studies



The domain with the highest serious RoB rating was results reporting, where a series of issues were noted. For example, Choi et al. (2020) stated that cell death was not affected following a 1-day exposure to PS particles, but in a results figure, a significant difference

($p < 0.01$) is reported for the dose with MP concentration of 1000 µg/mL for the 5-25 µm size. Hwang et al. (2020b) reported, in the methods section, the use of four sizes of PS particles (460 nm, 1 µm, 3 µm, 10 µm) and six concentrations of PS MPs (1, 10, 100, 500, and 1,000 µg/mL) for the cytotoxicity tests. However, in the results section for the PBMCs, only three sizes (460 nm, 3 µm, 10 µm) were reported and an additional concentration of 0.5 µg/mL is reported. Stock et al. (2019) did not report all the doses used for the cytotoxicity assays. In the supporting information of the paper (Figure S4), four doses for each of the three particle sizes are reported but not all of them. From the figures included in the results (Fig. 3, S1, S2, and S3, of the paper), it appears that for the sizes of 1 and 4 µm, more than four doses were used but not all reported. In addition, the conclusion states that the sizes of 4 and 10 µm particles were non-toxic, but the corresponding figures suggest that only the 10 µm size appears to have no significant impact.

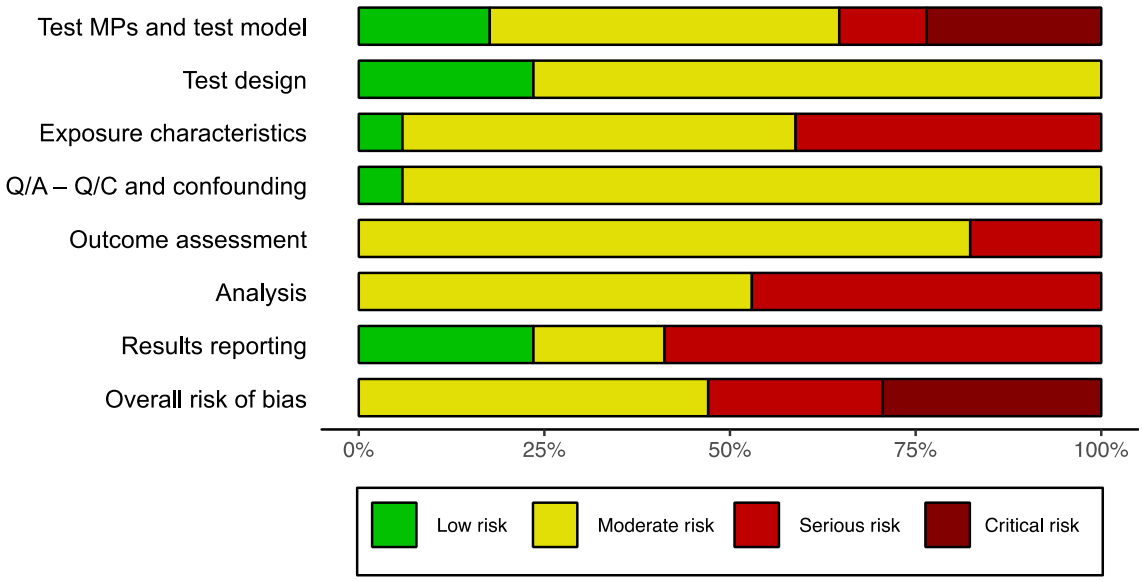


Figure 52. Risk of Bias (RoB) assessment rating results for MP toxicology studies. The four ratings are illustrated by percentage. Individual rating per study and per domain is provided in Table 22. Rating was executed according to the RoB tool. Note: MPs, microplastics; Q/A, quality assurance; Q/C, quality control.

7.4. Synthesis

In accordance with the aims and objectives of this rapid review, the results of the studies are presented by the biological endpoint that was under examination (Figure 51). When studies examined more than one biological endpoint, the outcomes are discussed separately. The majority of studies reported their results only graphically. Therefore, the only "quantitative" results that could be extracted for all the experimental conditions was the binary outcome

SIG. and N. SIG. It should be noted that some of the studies also reported in the figures the level of the detected significance ($p < 0.05$, 0.01 or 0.001); these results are reported in SM2 in Danopoulos et al. (2021). Certain outcomes, especially those related to cell barrier behaviour (e.g. MP uptake), were only discussed qualitatively and are explored in a narrative analysis. None of the studies provided the raw results, hindering traditional meta-analysis approaches. In addition, the majority of studies did not report the exact number of repeated tests and replicates for each experimental condition, while there was also ambiguity as to the density of the cells. All these pieces of information are vital for the execution of more in-depth analysis. It should also be noted that seven out of the 17 studies did not report the use of positive control samples (Goodman et al., 2021, Hesler et al., 2019, Liu et al., 2020a, Schirinzi et al., 2017, Stock et al., 2019, Qiangqiang Wang et al., 2020, Shijin Wu et al., 2020). Positive control samples are commonly used as an additional step to test the efficiency of the experimental process. There was a complete absence of quality assessment and quality control (QA/QC) reporting for cross contamination of test material and test models by airborne MPs. Only one study (Prietl et al., 2014) reported that they examined the test material for contamination with substances that could interfere with the experiments such as endotoxins. Stock et al. (2021) was the only study to include a limit of detection (LOD) method for each particle type, thus incorporating a quality assurance step into the experiments.

Only about a quarter of the studies (Choi et al., 2020, Choi et al., 2021, Han et al., 2020, Hwang et al., 2020a) used data from environmental studies to provide a rationale for the concentrations of MPs used in their experiments. The exposure to MPs on a weekly basis was largely the starting point for calculating exposures for longer period of times. Choi et al. (2020) applied estimated exposures for life-long exposures and used data from drinking water MPs contamination (Mason et al., 2018), while Choi et al. (2021) and Han et al. (2020) used data for various food categories (Cox et al., 2019). Apart from using data on food and water contamination, Hwang et al. (2020a) also included data for personal care products and assumed that using a facial scrub product which contains MPs can lead to MPs intake, which has no scientific basis. They state that intake of PS MPs from personal care or biomedical products is 4,594 – 94,500 per 5 mL of product per day. The study by Napper et al. (2015) is cited, which provides these data but refers to the quantities of MPs released by a product to the environment and not the intake of MPs by humans. Dermal absorption of MPs has been proposed as a possible route for MPs exposure, but it has yet to be proven. According to the current practice in toxicology studies in the field of MPs, 1 mg/mL was used as the

maximum acceptable MP concentration of the applied dose referring to life-long dietary exposures.

In terms of mode of exposure, the majority of the studies considered the ingestion route. Three studies focused on the inhalation route. Dong et al. (2020) used two doses with MP concentrations of 10 and 100 μg/cm^2: one for general public and one for occupational exposures but did not offer a rationale. The lower dose (10 μg/cm^2), however, is in line with data from environmental studies (Wright et al., 2020). Goodman et al. (2021) also stated that the MP concentrations considered for the doses (0.05 - 100 μg/mL) represented urban and industrial exposures but did not offer a justification. Brown et al. (2001), on the other hand, argued that although the MP concentration of the doses (1000 μg/mL) were larger than those found in ambient air, they were used to account for the susceptibility of the population that is ordinarily affected by ultra-fine particle inhalation. Four rather obvious but important parameters of the test MP and the test exposure must be noted. When the size, and, therefore, the mass per particle of the test MPs remains the same, increasing the concentration of the exposure (μg/mL) also increases the number of particles in the concentration (MPs/mL). If the size of the test MPs is increased, and the concentration of the exposure (mg/mL) is kept the same (as with the previous size of the test MPs) the number of particles in the concentration (MPs/mL) will inevitably decrease. Furthermore, when comparing different polymers with varying densities, the same concentration (μg/mL) contains more MPs/mL as the density of the polymer decreases. The relationship between these three variables must be taken into consideration in any attempt to analyse the data from the toxicology studies. The key distinction is whether to hypothesise that the MP effect is related to the mass of the dose, and therefore inextricably linked to the delivered volume of the substance, or to the number of particles which might also be linked to other parameters of the substance such as the surface charge. The shape of the test MP both affects the volume - mass relationship and the number of particles, and is, moreover, connected to surface characteristics of the test substance and possible physical MP effects. Untangling the mechanistic origin of possible MP effects is necessary in order to understand the overall toxicological behaviour of MPs.

7.5. Cytotoxicity

7.5.1. Narrative analysis

Sixteen studies examined cytotoxicity effects on human cells after exposure to MPs (Table 1). Five of the studies (Han et al., 2020, Hwang et al., 2019, Stock et al., 2019, Qiangqiang Wang et al., 2020, Shijin Wu et al., 2020) were rated as of critical RoB and were excluded

from further analysis (Table 2). Cytotoxicity was measured in terms of cell viability, cell proliferation, metabolic activity or cell barrier damage, with several studies looking at more than one of these expressions (Table 1). The studies used 11 different cell models, tested nine polymers of two shapes and origins, ranging from 0.1 to 282 μm. Applied doses ranged from MP concentrations of 0.01 to 100000 μg/mL while 14 tests/ biological markers were used. Two studies (Dong et al., 2020, Lehner et al., 2020) expressed the MP concentrations of applied doses as μg/cm^2, ranging from 1 to 1305.5 and the results could not be directly compared with the rest of the studies. All the details can be found in SM2 in Danopoulos et al. (2021). The results can be broadly grouped by the reported outcome of the applied tests. Six different tests reporting cell viability rates compared with negative control samples (CCK-8, HCA assay, LIVE/DEAD kit, MTS assay, MTT assay, WST-1 assay), were used by seven studies. Significant results were reported for exposure to MPs of five different polymers (LDPE, PE, PP, PS and PVC), of spherical and irregular shape, of primary and secondary origin, with a size range of 0.5 to 137.5 μm and applied doses of MP concentrations between 0.01 and 100000 μg/mL, exposed for 24 and 96-hour durations. Goodman et al. (2021) also used an MTT assay but reported the absorbance of MTT, instead of cell viability, as a measure of cellular metabolic activity (cell proliferation). Significant results were reported for every condition tested (PS MPs, sizes 1 and 10 μm, concentrations 0.05 to 100 μg/mL). Goodman et al. (2021) argued that the sole use of MTT assays for measuring cell proliferation and cell viability can introduce error, since, when used for prolonged exposure duration, metabolic activity and cell numbers cannot be disentangled and, accordingly, used further tests to verify results. Cell proliferation was examined by measuring the expression of the Ki67 marker reporting reduced ability. Goodman et al. (2021) also used Trypan Blue exclusion and Calcein-AM/FACS assays, and reported little cytotoxicity of the exposed cells, but did not report significance levels. Dong et al. (2020) used the Trypan Blue exclusion assay reporting significant results only for PS MPs (1.72 μm) at concentrations of 10, 100 and 1000 μg/cm^2. Enzymatic activity of caspase-3, 8 and 9 (reported as fold change) was measured by one study (Stock et al., 2021) as a secondary measure of cytotoxicity (for their contribution to the cell apoptosis pathway) and reported significant results only on caspase-8 activity at concentrations of 50000 μg/mL for PE MPs (2.2 μm) and PP MPs (67.1 μm) confirming the results obtained from corresponding MTT assays. Two studies (Lehner et al., 2020, Wu et al., 2019) measured the release of LDH as a measure of integrity of the cell membrane and one (Liu et al., 2020b) of the monolayer as related to cytotoxicity and all reported not significant results.

7.5.2. Meta-regression: Cell viability

Logistic regression modelling and multilevel modelling was used to examine the relationship between the variables of the experimental characteristics and the outcome of the cytotoxicity tests. Seven studies (Choi et al., 2020, Choi et al., 2021, Hesler et al., 2019, Hwang et al., 2020b, Schirinzi et al., 2017, Stock et al., 2021, Wu et al., 2019) expressed results in terms of cell rate viability (using six different tests: CCK-8, HCA, Live/Dead kit, MTS, MTT, WST-1) and were found to be similar enough to be grouped for a meaningful meta-regression analysis. It should also be noted that Choi et al. (2021) did not report the results of eight samples regarding the exposure of HeLa cells to LDPE and therefore, the data were not included in the synthesis. The characteristics of covariates that were explored, coming from the seven studies that reported the rate of cell viability (310 data points), are presented in Table 23.

Table 23. Covariates explored in meta-regression models for the cell viability (cytotoxicity) biological outcome.

Covariate	**Type**	**Values**
Cell model	factor	10 levels
Cytotoxicity test	factor	6 levels
Polymer	factor	6 levels
Shape	factor	3 levels
Size (μm)	numerical	0.1 – 137.5
Dose (μg/mL)	numerical	0.01 – 100,000
Dose (MPs/mL)	numerical	1 – 362,746,309,041
Duration of exposure (h)	integer	12, 24, 96
Outcome	factor	2 levels

The first step in this analysis, which used such a diverse data frame with many covariates, was to present the data visually to examine distributions and detect possible relationships (Ennos and Johnson, 2018). A series of observations were made by examining Figure 53 A-D, where three of the categorical covariates (cell model, cytotoxicity test, test polymer) and one integer covariate (duration) are presented. The most-used cell model was HDFs followed by PBMCs (Figure 53.A), the most-used test was CCK-8 followed by the MTT assay (Figure 53.B), the most-used test polymer was PS followed by PE (Figure 53.C) and the most-used exposure time was 24 hours (Figure 53.D). The exposure of 12 hours had no significant results (Figure 53.D). The relationship of the covariates of origin and shape are illustrated in Figure 54 and Figure 55. Out of the test MPs of primary origin (207), 69.5% (144) were

spherical and the remaining 30.5% (63) were of irregular shape. Unsurprisingly, 100% of the secondary test MPs were of irregular shape. All spherical MPs were of primary origin, and all irregularly shaped MPs were of secondary origin. This overlap was taken into consideration in the analysis.

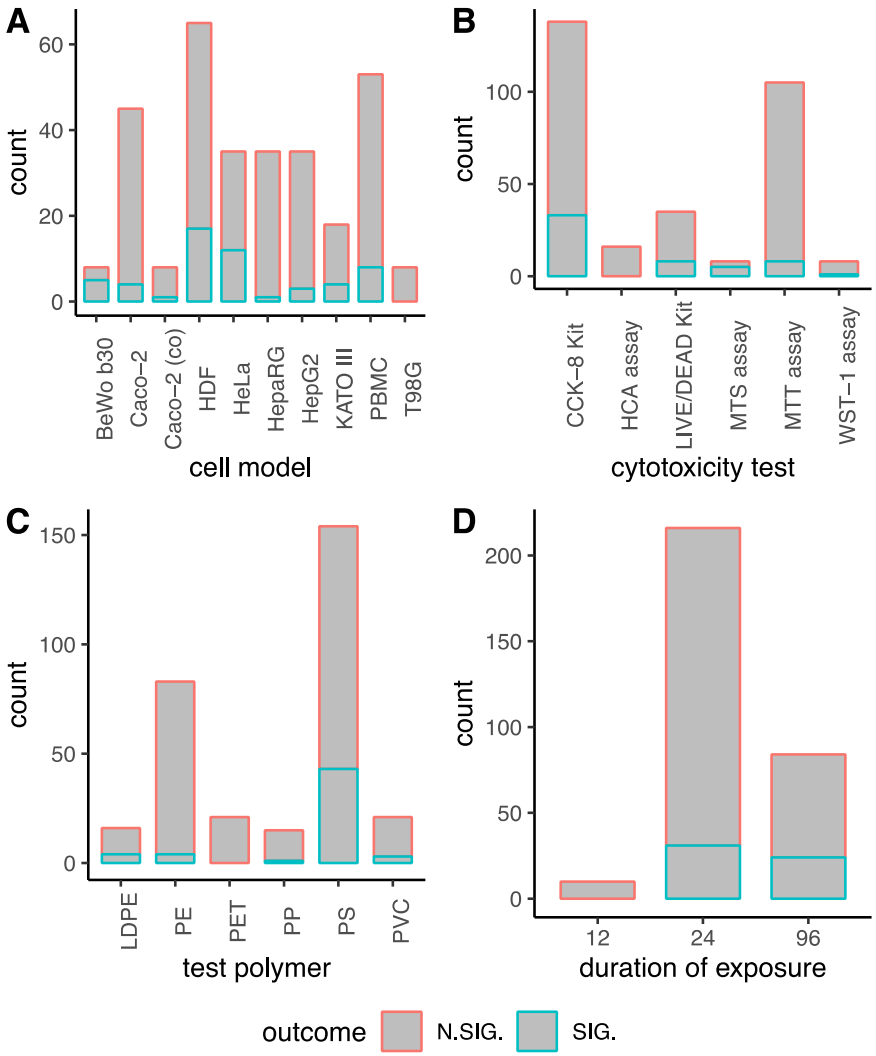


Figure 53. Distribution of the categorical covariates for the cell viability biological endpoint in the studies included in the meta-regression analysis; (A) cell model, (B) cytotoxicity test, (C) test polymer, and (D) integer covariate of duration of exposure. The outcome of significance results for the cell viability (cytotoxicity) biological outcome are highlighted in red/blue outlines. Note: BeWo b30, human placental choriocarcinoma cell line; Caco-2, human adenocarcinoma cell line; CCK-8, cell counting kit 8; co, coculture; HCA, high content analysis; HDFs, human dermal fibroblasts; HeLa, cervical cancer cells; HepaRG, human hepatic cells; HepG2, Human Caucasian hepatocyte carcinoma cells; KATO III, gastric cancer stem cells; LDPE, low-density polyethylene; LIVE/DEAD kit, viability/cytotoxicity test; MTS assay, colorimetric cell proliferation assay kit; MTT assay, cellular metabolic activity colorimetric assay; N.SIG., not significantly different outcome as compared to the control; PBMCs, peripheral blood mononuclear cells; PE, polyethylene; PET, Polyethylene terephthalate; PP, polypropylene; PS, polystyrene; PVC, polyvinyl chloride; T98G, human glioblastoma multiforme cells; SIG,. significantly different result as compared to the control; WST-1 assay, cell proliferation assay

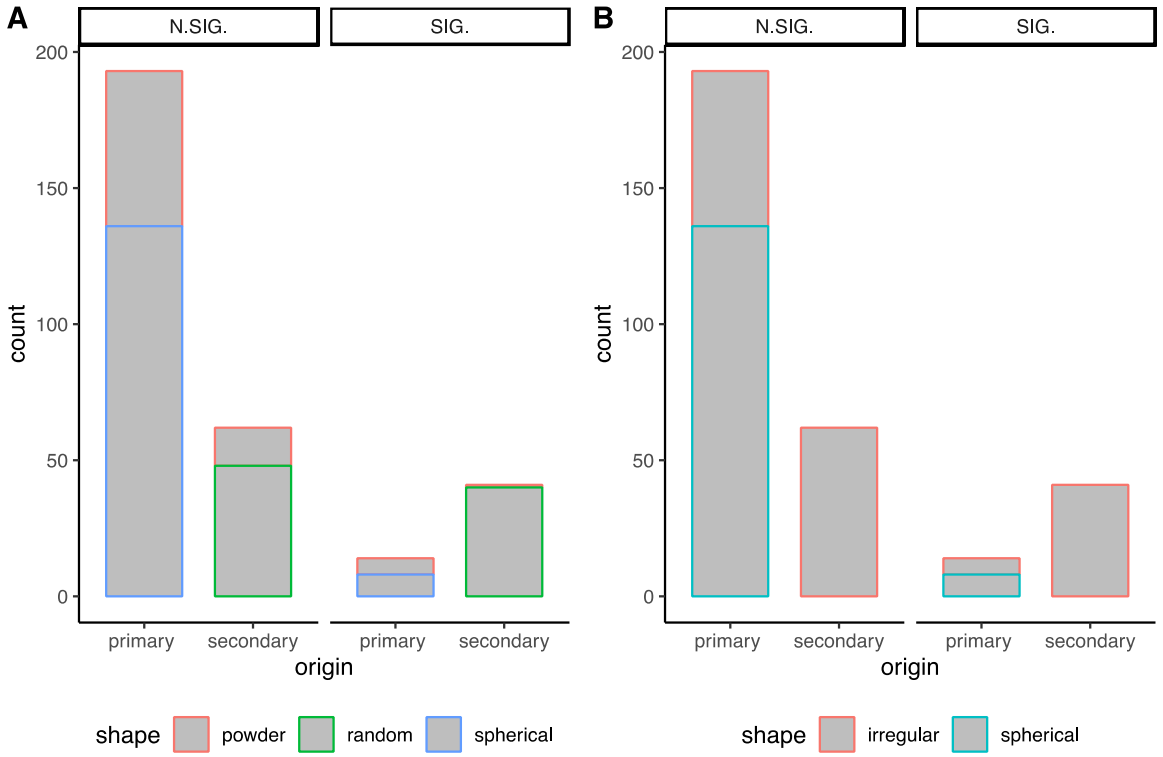


Figure 54. Distribution of the categorical covariates origin and shape and the outcome of significant and non-significant results for the cell viability (cytotoxicity) biological outcome. (A) three shape categories; spherical, random and powder, (B) two shape categories; irregular and spherical (irregular includes both random and powder shapes). N.SIG., not significantly different outcome as compared to the control; SIG,. significantly different outcome as compared to the control.

Regarding the significant reported outcomes for the primary MPs (14), these were spherical (57%, 8 out of 14) and irregular (43%, 6 out of 14) shaped MPs. A relationship between secondary MPs of irregular shape and toxicity was observed. The distribution of the numerical covariates was examined statistically using the Shapiro test followed by a skewness test (Table 24). All the data were found to be not normally distributed and present moderate to high skewness, so the Spearman correlation test was used to detect correlations. Normality of the independent variables is not an assumption for logistic regression (Osborne, 2015).

The numerical covariates correlation tests are presented in Figure 56. A significant positive correlation (ρ=0.386, $p< 0.05$) was detected between the size of the MPs and the applied concentrations expressed in mass/mL, while a significant negative correlation (ρ=-0.687, $p< 0.05$) was found between the size and the concentrations expressed in MPs/mL. Finally, a significant positive correlation (ρ=0.316, $p< 0.05$) was also found between the doses of test MPs expressed in concentrations of mass and particle number. This trend was also identified

when the binary outcome (SIG., N.SIG.) was tested separately as shown in Figure 56. These correlations were also taken into consideration in the next parts of the analysis. A basic assumption in logistic regression is that all variables must be independent and should not be highly correlated with each other. Multicollinearity could reduce the effectiveness of the model (Stoltzfus, 2011). The existing conceptual and statistical correlations between the three numerical covariates dictate that not all three can be included in the same model.

Table 24. Shapiro–Wilk test and skewness test results for the three numerical covariates for the cell viability (cytotoxicity) biological outcome.

	Size (µm)	Dose (µm/mL)	Dose (MPs/mL)
Shapiro test W	0.84643	0.50091	0.077311
p value	< 0.001	< 0.001	< 0.001
Skewness test	0.6181766 (data moderately symmetrical)	2.680669 (data high skewed)	14.00301 (data high skewed)

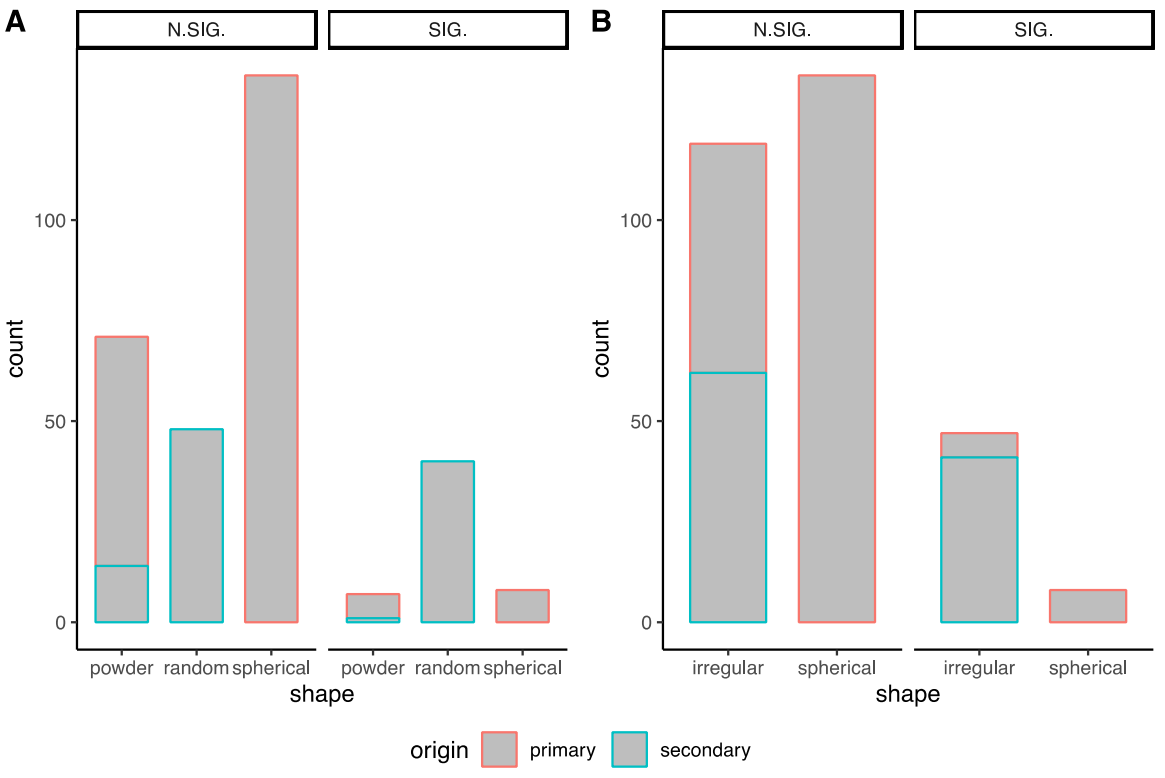


Figure 55. Distribution of the categorical covariates shape and origin and the outcome of significant and non-significant results for the cell viability (cytotoxicity) biological outcome. (A) three shape categories; spherical, random and powder, (B) two shape categories; irregular and spherical (irregular includes both random and powder shapes). N.SIG., not significantly different outcome as compared to the control; SIG,. significantly different outcome as compared to the control.

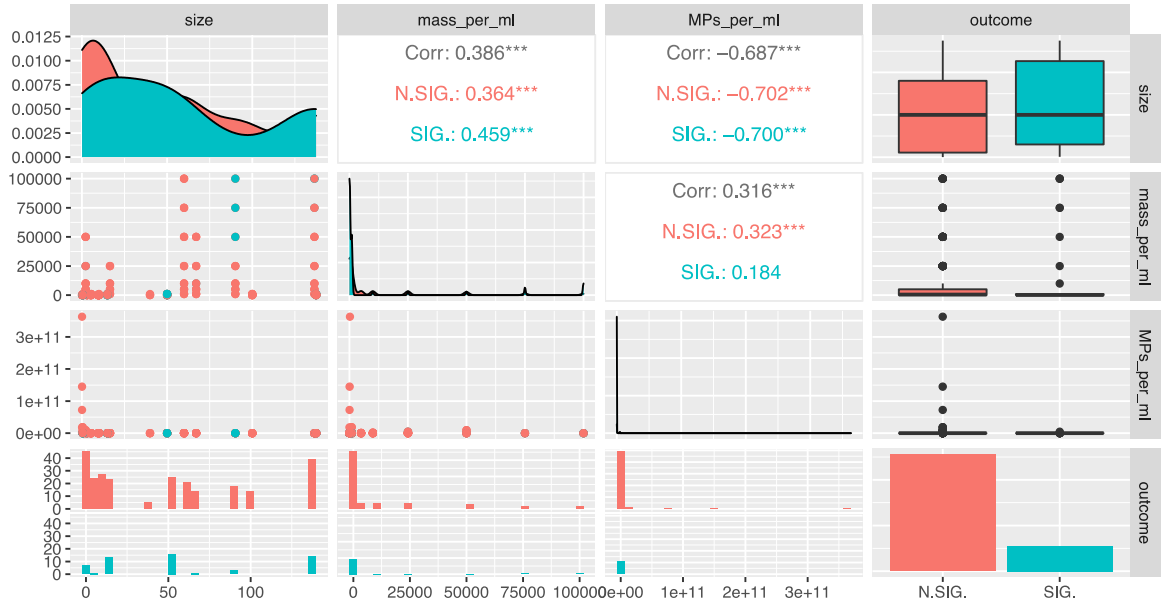


Figure 56. Correlogram between the numerical covariates and the outcome for the cell viability (cytotoxicity) biological outcome. The scatterplots for each pair of numerical covariates are displayed on the left part, Spearman correlation test results are displayed on the right, the diagonal shows the covariates' distribution, *, **, and *** indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. Note: N. SIG.: not significant difference as compared to the control, SIG.: significant difference as compared to the control, Corr.: Spearman rank corelation ρ. Blue: SIG, Red: N. SIG.. MP size in µm. MP concentration expressed in both µg/mL and MP/mL.

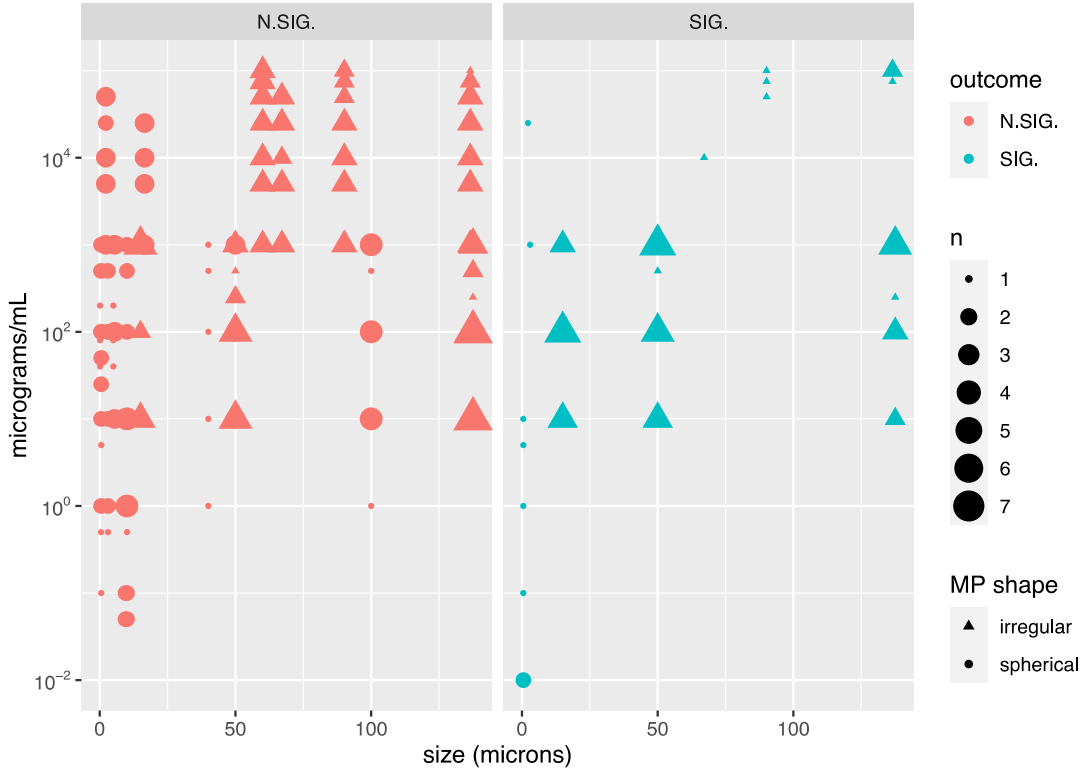


Figure 57. Distribution of test MPs characteristics of concentration (µg/mL) and size (µm) for the cell viability (cytotoxicity) biological outcome. N denotes how many times the same experimental conditions were tested by studies. SIG. statistically significant outcome as compared to the control, N.SIG. not statistically significant outcome as compared to the control.

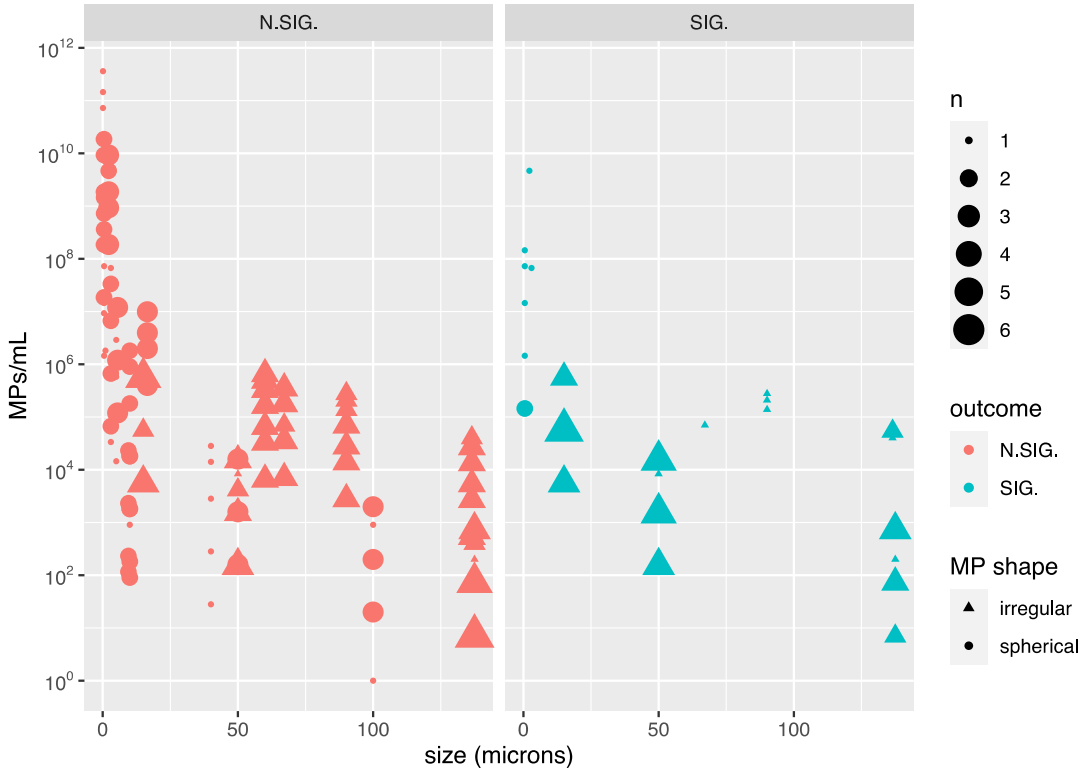


Figure 58. Distribution of test MPs characteristics of dose (MPs/mL) and size (µm) for the cell viability (cytotoxicity) biological outcome. MPs/mL are expressed in $\log_{10}$ scale. n denotes how many times the same experimental conditions were tested by studies. SIG. statistically significant outcome, N.SIG. not statistically significant outcome.

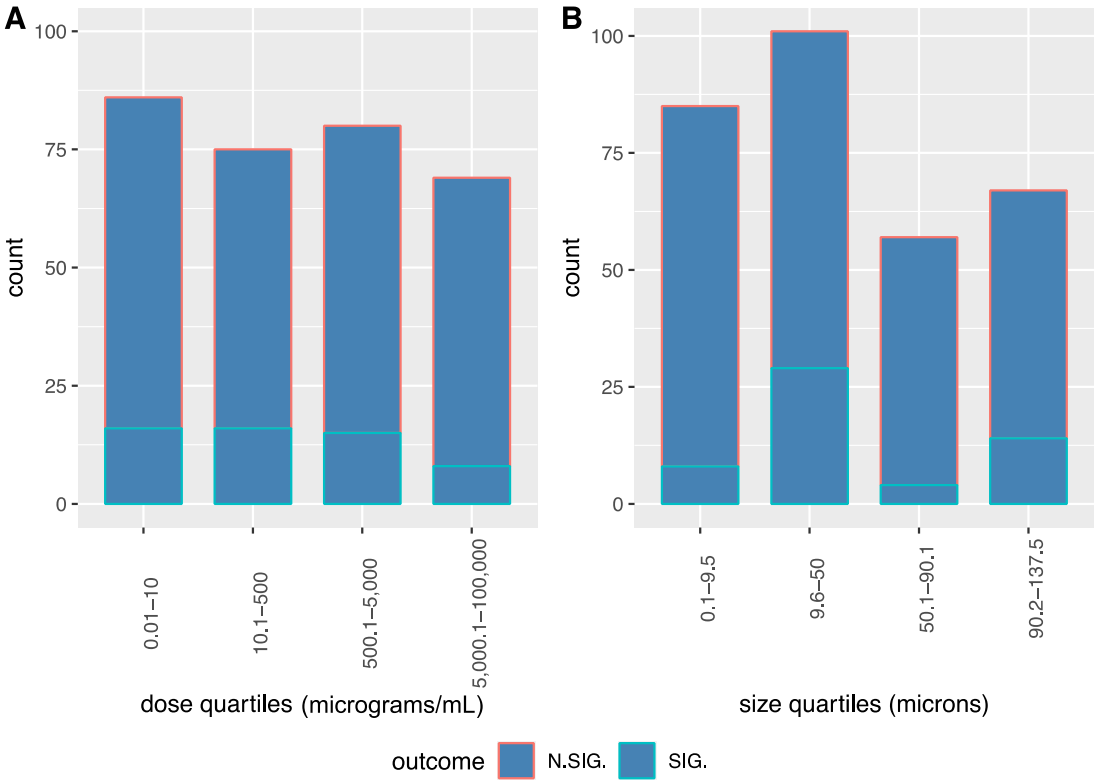


Figure 59. (A) MP dose range (µg/mL) and (B) MP size range binned into quartiles for the cell viability (cytotoxicity) biological outcome. N.SIG., not significantly different outcome as compared to the control; SIG,. significantly different outcome as compared to the control

Another important parameter was the range of sizes and concentrations that have been tested. As shown in Figure 57 and Figure 58, the majority of testing was focused on the smaller size range of MPs where many different concentrations were tested. On the other hand, when looking at the doses tested, their distribution, expressed in MPs/mg (Figure 58), was more skewed than when expressed in μg/mL (Figure 57). This under-representation in doses (sizes and concentrations) can also be detected by observing the quartiles illustrated in Figure 59, where the number of tests has been allocated in quartiles.

7.5.2.1. Regression models

The relationship between experimental conditions and the outcomes was explored through regression models. Two models were fitted in the first instance: one including the MP concentration expressed in μg/mL and one in MPs/mL. The first model showed a better fit as both the residual deviance (RD) and the AIC values were lower: RD 156.7 as against 168.04 (null 289.82), AIC 202.7 as against 214.04. Therefore, all consecutive models only included the covariate of MP concentration expressed in μg/mL, also recognizing that the MPs/mL metric is an estimation of the concentrations. The first configuration of the model included all covariates. Three estimate coefficients (secondary origin, MTS assay and WST-1 assay) were not defined because of singularities. Using the alias(x) function (in R) revealed that all three are highly correlated and linearly dependent with a number of other covariates. Removing these covariates from the model did not affect the fit as the RD rose from 156.7 to 157.57 while AIC was reduced from 202.2 to 197.57 indicating a better fit. The difference between the two models was not significant when compared using a likelihood ratio test (ANOVA, $p > 0.05$). It should also be noted that, as previously explored, there was an overlap between the covariates shape and origin, so both could be explored, to an extent, by keeping one in the model. VIF was found to be < 3 for all the six remaining covariates so the conclusion was that there was not strong multi-collinearity between the covariates (Craney and Surles, 2002, Thompson et al., 2017). Ten regression coefficient estimates were found to be statistically significant, seven coming from the cell model covariate, one from MPs characteristics and two from experimental characteristics. One coefficient was categorical (irregular shape, $\beta=5.913$, $p < 0.001$), one numerical (MP concentration in μg/mL, $\beta=0.00005$, $p < 0.01$) and one integer (duration, $\beta=0.02$, $p < 0.01$). The powder shape exhibited a much lower effect size ($\beta=0.669$) and it was not found to be statistically significant ($p > 0.05$). In order to examine the covariate of origin, a further model was fitted excluding the shape covariate which caused the multicollinearity. All the same regression coefficient estimates were found to be statistically significant (seven cell models,

concentration and duration) with marginally different effect sizes, plus the secondary origin (β=5.894, p< 0.001). The AIC was found to be reduced slightly from 197.5 to 195.75 and the fit of the model did not significantly improve (ANOVA, p> 0.05). All the irregularly shaped MPs in the dataset were secondary and all the spherical were primary, only the powders came from both sources. In order to explore this relationship, a model was fitted where the characteristics of shape and origin were merged into four categories: primary-spherical, primary-powder, secondary-powder, secondary-irregular and only the estimation coefficient for secondary-irregular MPs was found to be statistically significant (β=5.537, p< 0.01). In this model the polymer covariate could not be included due to multicollinearity. Following these results, the choice was made to go forward with the model that included only shape and not origin.

Regarding the cell model covariate, seven out of the 10 cell models had statistically significant regression coefficient estimates. Ranked by effect size, Caco-2 cells exhibited the highest prediction of cell death (β=-4.6, p< 0.05), followed by HepG2 cells (β=-4.9, p< 0.05), HDFs (β=-5.53, p< 0.001), HeLa cells (β=-5.88, p< 0.001), HepaRG cells (β=-6.47, p< 0.05), PBMCs (β=-7.2, p< 0.001) and KATO III cells (β=-8.12, p< 0.001), as compared to the reference class of BeWo cells (β=-0.63, p=0.55). To summarise, the cell model used, the MP characteristic of irregular shape (secondary origin) and the experimental characteristics of MP concentration and duration of exposure predicted the toxic outcome.

The classification prediction accuracy of the model was 89.4%, indicating the overall performance of the model. In order to examine the usefulness of the model, it is important to determine how accurately it can predict the outcomes (SIG./N. SIG.) (Ennos and Johnson, 2018). A data frame was created to show whether the model correctly assessed the outcome for each data point, these predictions are shown in a classification table (Table 25). These show the model correctly predicted the “N. SIG.” outcome at a rate of 93.3% and the “SIG.” outcome at a rate of 63.6%.

Table 25. Validity of predicted probabilities of the full model for the cell viability (cytotoxicity) biological outcome.

Outcome	Predicted		
Observed	N. SIG.	SIG.	Correct
N. SIG.	242	13	94.9%
SIG.	20	35	63.6%
Overall correct			89.4%

The linearity assumption was tested by creating a series of scatterplots to determine if there was a linear relationship between the numerical covariates and the logit of the outcome. As illustrated in Figure 60, the linearity assumption was not met, which might have caused the covariates to affect the model results disproportionally. The all-subset logistic regression method was subsequently used in an attempt to identify the subset of covariates that produced the best performing logit model. The best-subset model excluded the covariates of polymer type and size from the model, indicating that they hindered the model's performance. The residual deviance of the model was 168.02 (d.f. 296) and the AIC 196.2, showing a slight improvement in only the AIC value. VIF was found to be < 3 for all of the remaining covariates.

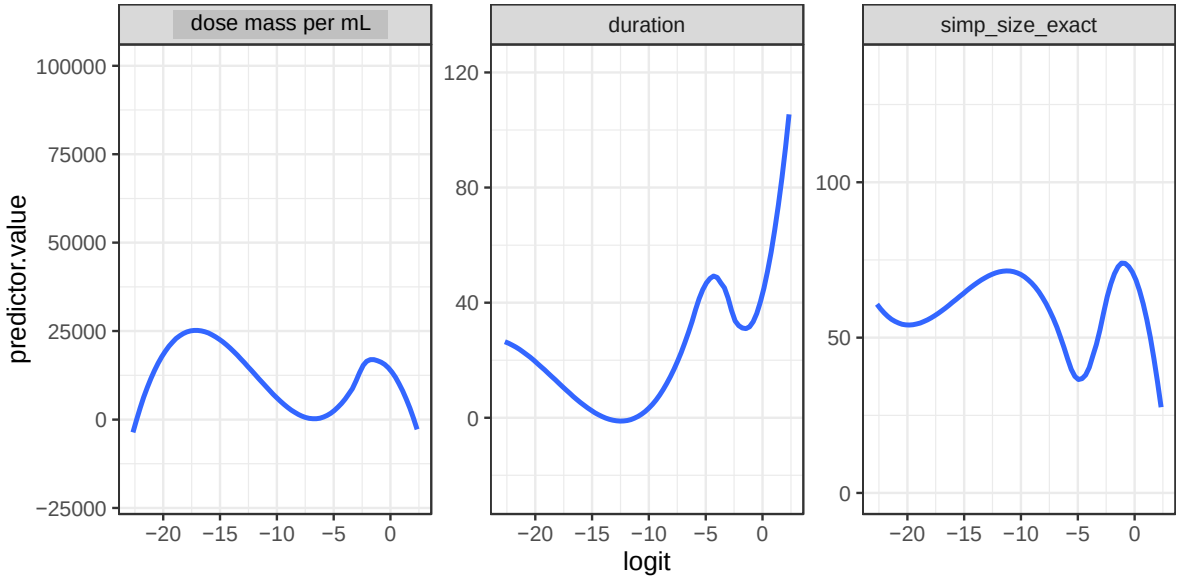


Figure 60. Linearity test between the numerical covariates and the logit of the outcome in the full model for the cell viability (cytotoxicity) biological outcome.

The classification prediction accuracy was calculated at 88.1% indicating that the performance of the best-subset model was not compromised, while the model was simplified by reducing the number of the covariates. The aim of the all-subset process was to find a less complex model without compromising accuracy. The predictions of the outcomes are shown in a classification table (Table 26).

In the best-subset model (as in the previous model), the regression coefficient estimate was found to be statistically significant for a number of covariates. Seven of the types of cell models had statistically significant large effect sizes, indicating that specific cells were more vulnerable to reduced viability due to MP exposure than others. The second covariate that stood out was shape. According to the model, irregular- (randomly) shaped MPs of

secondary origin displayed a larger effect size (β=5.334, p< 0.001) than spherical MPs of primary origin, while powder MPs had a smaller effect size (β=-0.05578), but the regression coefficient estimate was not statistically significant (p> 0.05). Two further coefficients: duration and MP concentration (μg/mL) had statistically significant results but small effect sizes β=0.0233 (p< 0.01) and β=0.0000379 (p< 0.01), respectively.

Table 26. Validity of predicted probabilities of the best-subset model for the cell viability (cytotoxicity) biological outcome.

Outcome	Predicted		
Observed	N. SIG.	SIG.	Correct
N. SIG.	238	17	93.3%
SIG.	20	35	63.6%
Overall correct			88.1%

The best-subset model also improved the linearity between the numerical covariates and the logit of the outcome, as shown in Figure 61, but did not change it substantially. In order to compare the full and the best-subset model, a likelihood-ratio test was performed (ANOVA) which found that the fitness of the best-subset model did not significantly improve (χ^2=-10.5, Df=-6, p> 0.05) compared to the full model, while it did improve compared to the null model (χ^2=121.8, Df=13, p< 0.001). The Cook's distance values were used to visualise the most extreme values (Figure 62) (Osborne, 2015). Although extreme values were depicted in Figure 62, in order to examine whether the values were also influential covariates, the standard residual error was examined and was found to be at acceptable levels (< 3) (Figure 63) (Menard, 2002). Following this examination, the conclusion was that no influential outliers were found in the data set.

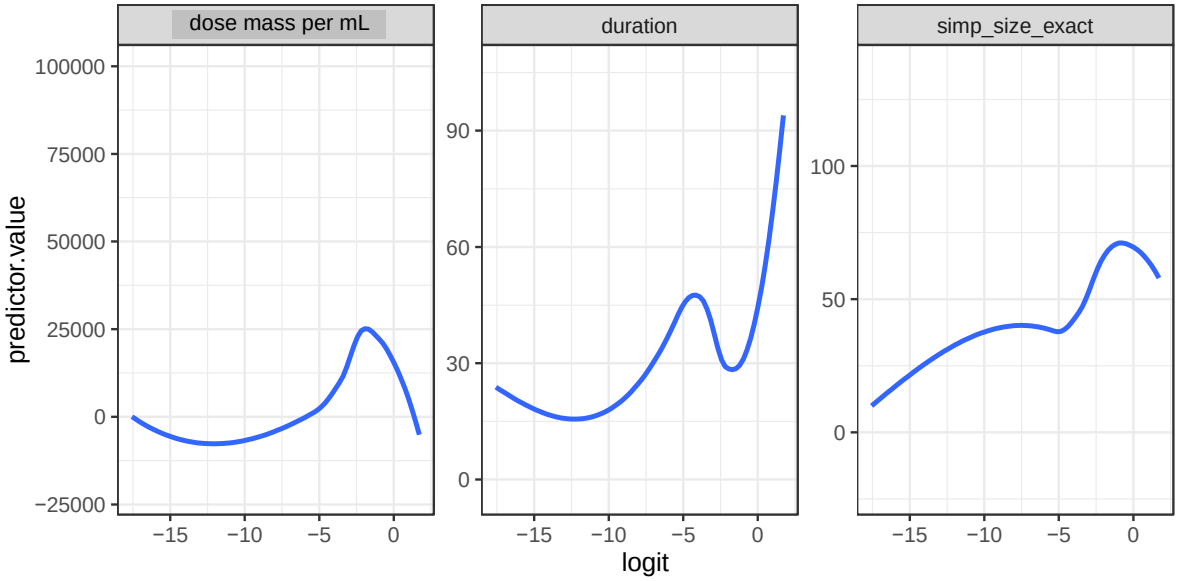


Figure 61. Linearity test between the numerical covariates and the logit of the outcome in the best-subset model for the cell viability (cytotoxicity) biological outcome.

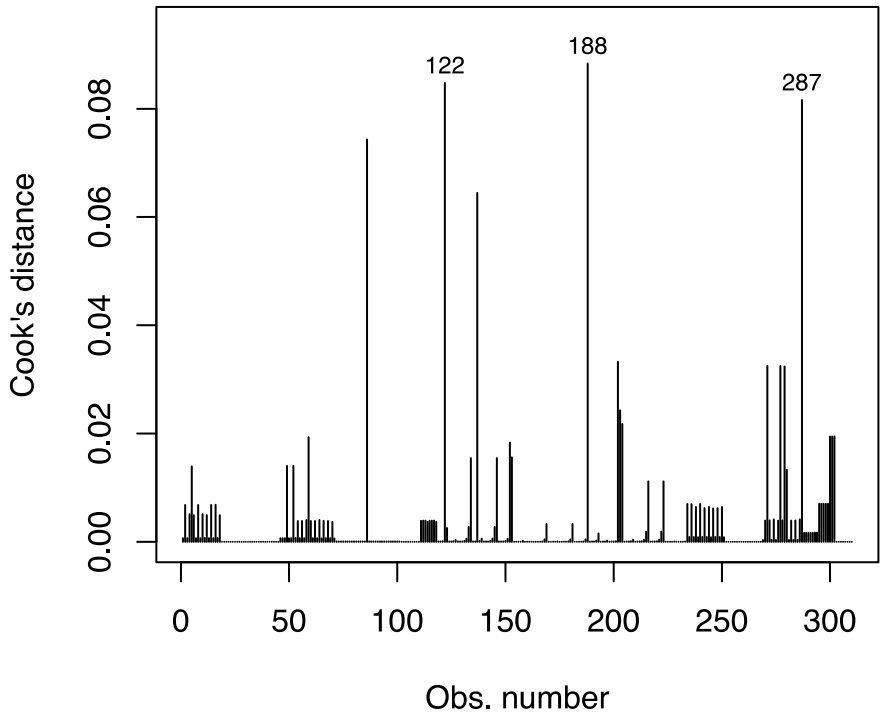


Figure 62. Cook's distance values, best-subset model for the cell viability (cytotoxicity) biological outcome.

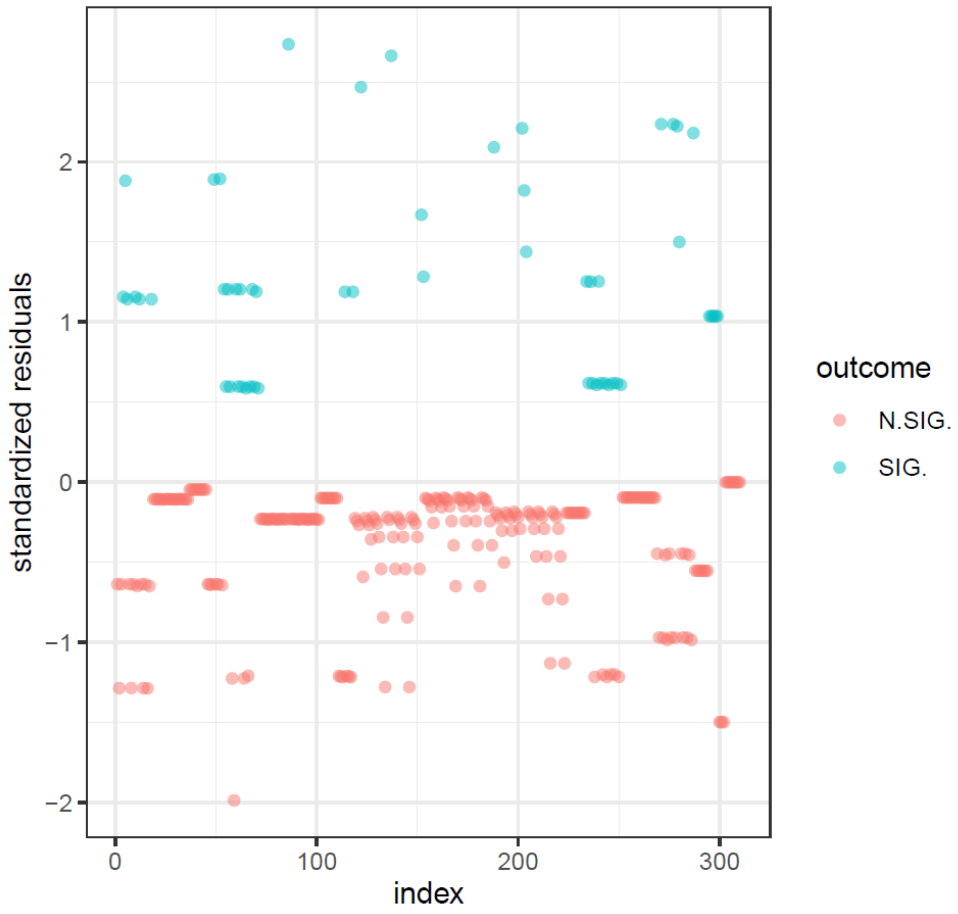


Figure 63. Standardised residuals of the best-subset model for the cell viability (cytotoxicity) biological outcome. N.SIG., not significantly different outcome as compared to the control; SIG,. significantly different outcome as compared to the control

7.5.2.2. Sensitivity analysis

In order to examine if the relationship between the covariates and the outcomes still held when the cell model characteristic was removed, the logit model was fitted again only for the HDF cell model data, which was the largest cell model subgroup in the data frame (65 data points). Only the covariates indicated by the all-subset process (shape, duration, MP concentration) were used in this model in order to achieve as direct a comparison as possible. In this data frame, only two of the three shape categories are included (spherical and random). Once again, the relationship between shape and outcome is statistically significant, as the spherical test MPs of primary origin were found to be less likely (β=-5.514, $p< 0.001$) than irregular MPs of secondary origin to have a SIG. outcome. The duration covariate was also found to be marginally statistically significant (β=0.03, p=0.05). A further model was fitted for the next largest data frame grouped by the cell model, which was PBMC cells (53 data points). A weak relationship between the concentration of MPs (μg/mL) and the outcome was found to be significant (β=0.003, $p< 0.05$), while the trends of duration and shape (and origin) were detected but were not found to be significant: β=0.03, p=0.06 and β=-0.21, p=0.99, respectively. The third largest data frame grouped by the cell model was Caco-2 cells (45 data points). Unfortunately, no study tested irregularly-shaped test MPs so the relationship could not be examined. Five studies were rated as of critical RoB (Table 22). The effectiveness of the RoB rating could not be assessed due to missing data. The covariate of test MP shape was not reported or reported ambiguously by two studies (Hwang et al., 2019, Shijin Wu et al., 2020), test MP origin was not reported by one study (Shijin Wu et al., 2020) and the duration of exposure was not reported for a fraction of their experiments by one study (Hwang et al., 2019).

7.5.2.3. Multilevel models

The failure of the linearity assumption could be attributed to the heterogeneity of the data frame being extracted by seven different studies, the heterogeneity of the experimental conditions across the studies and the inability to weight the studies. To account for the heterogeneity caused by the clustering of the data in studies, multilevel logistic regression models were fitted. First a null model was fitted. The ICC of the null model was 0.41, meaning that 41% of the variations in the outcome could be attributed to the clustering of the data in the seven studies. Next a random intercept and fixed slope model was fitted. The model included all the covariates that were used in the full logistic regression model: cell model, polymer, shape, duration, size (μm) and MP concentration (μg/mL), plus a random intercept to account for the clustering of the data by study. The multilevel model had the

same results in terms of prediction of coefficient estimates and accompanying p values. The same results were also generated when the multilevel model used only the three covariates included in the best-subset model: cell model, shape, duration and MP concentration (μg/mL), plus a random intercept for the studies. The fact that the results remained the same in the multilevel modelling can be attributed to the results of the random-effects variance for the studies' 1-level grouping. The variance was 0, which means that the variation between the clusters could be explained by the residual variance. In addition, it could also be related to the small number of clusters.

Random-intercept and random-slope multilevel models were also fitted. The random-slope variance was tested for all the covariates, one at a time. A likelihood ratio test was executed to compare each model with the fixed-slope model, where the deviance of the models was compared as a measure of fitness. None of the random-slope models were found to improve in a statistically significant manner from the fixed-slope model. It should also be mentioned that it was not conceptually hypothesised that there would be a difference of the covariates' effects between studies.

7.6. Immune responses

7.6.1. Narrative analysis

Ten studies considered immune responses to MP exposure (Table 1), examining different outcomes broadly divided into release of histamine, release of (pro-) inflammatory cytokines and myokines (IL-1β, 2, 6, 8,10, MCP-1, TNF-α), gene expression of cytokines (*IL-8* and *MCP-1*) and differentiation of THP-1 cells into macrophages and polarization. Three studies (Han et al., 2020, Hwang et al., 2019, Stock et al., 2019) were rated of critical RoB and were excluded from analysis, two further studies expressed MP concentrations as μg/cm^2 (Dong et al., 2020, Lehner et al., 2020) and as such could not be directly compared with the rest of the studies. The release of cytokines/myokines was measured using ELISA and gene expression via RT-PCR and results were reported using quantitative measures by comparison to negative control samples. A wide range of experimental designs was used: five cell models, seven polymers, three shapes, two origins, two tests, nine biological markers, MP sizes ranging from 0.202 to 283 μm, durations from 2 to 96 hours and MP concentrations from 1 to 1000 μg/mL and from 10 to 1305.5 μg/cm^2. The full experimental details and the results can be found in SM2 in Danopoulos et al. (2021). Five studies reported results of significant immune response effects as follows. Although nine biological markers were tested, only four were found to be significantly affected by MPs exposure. Choi et al.

(2020) found that exposure to irregularly shaped PS MPs significantly affected the release of IL-6 and TNF-α at MP concentrations as low as 100 μg/mL, while all experiments had a 24-hour duration. Choi et al. (2021) reported that the same biological markers were significantly affected by spherical PE and irregular LDPE MPs at MP concentrations of 500 – 1000 μg/mL, for 96-hour experiments. Hwang et al. (2020b) reported the same markers being affected by spherical PS MPs ranging from 0.46 to 10 μm at a MP concentration of 500 μg/mL, for 4-hour and 96-hour exposures. Finally, Liu et al. (2020b) reported that IL-8 and MCP-1 release were affected by irregular PS MPs (0.404 μm) at a very low MP concentration of 20 μg/mL, for 96-hour durations. It should be noted that Liu et al. (2020b) was the only study examining MCP-1 but other studies measured IL-8. Dong et al. (2020) reported that both IL-6 and IL-8 were affected by spherical PS MPs (1.72 μm) at MP concentrations of 10 and 1000 μg/cm^2, after 24-hour exposures.

7.6.2. Meta-regression: Cytokine release

Four studies (Choi et al., 2020, Choi et al., 2021, Hwang et al., 2020b, Liu et al., 2020b) that examined the release of cytokines using ELISA techniques were included in the analysis, comprising 136 data points. The studies expressed the results in terms of release amount (pg/mL) compared to the control samples and measured six different cytokines. The characteristics of covariates that were explored are presented in Table 27.

Table 27. Covariates to be explored in the meta-regression models of cytokines release.

Covariate	**Type**	**Values**
Cell model	factor	2 levels
Polymer	factor	3 levels
Biological marker	factor	6 levels
Shape	factor	2 levels
Origin	factor	2 levels
Size (μm)	numerical	0.44 – 137.5
Dose (μg/mL)	numerical	1 – 1000
Dose (MPs/mL)	numerical	1 - 9299196554
Duration of exposure (h)	integer	4, 24, 96
Outcome	factor	2 levels

The categorical covariates are illustrated in Figure 64 A-D. A few preliminary observations can be made from inspection of the figures. The most used cell model was PBMCs followed by Caco-2 (124 and 12 out of 136, respectively) (Figure 64.A). PS was the most used test polymer, followed by PE and LDPE (102, 18 and 16 out of 136, respectively) (Figure 64.B). The duration of exposure most frequently adopted was 96 hours (Figure 64.C), and two of the immune responses under examination have no SIG. outcomes (Figure 64.C).

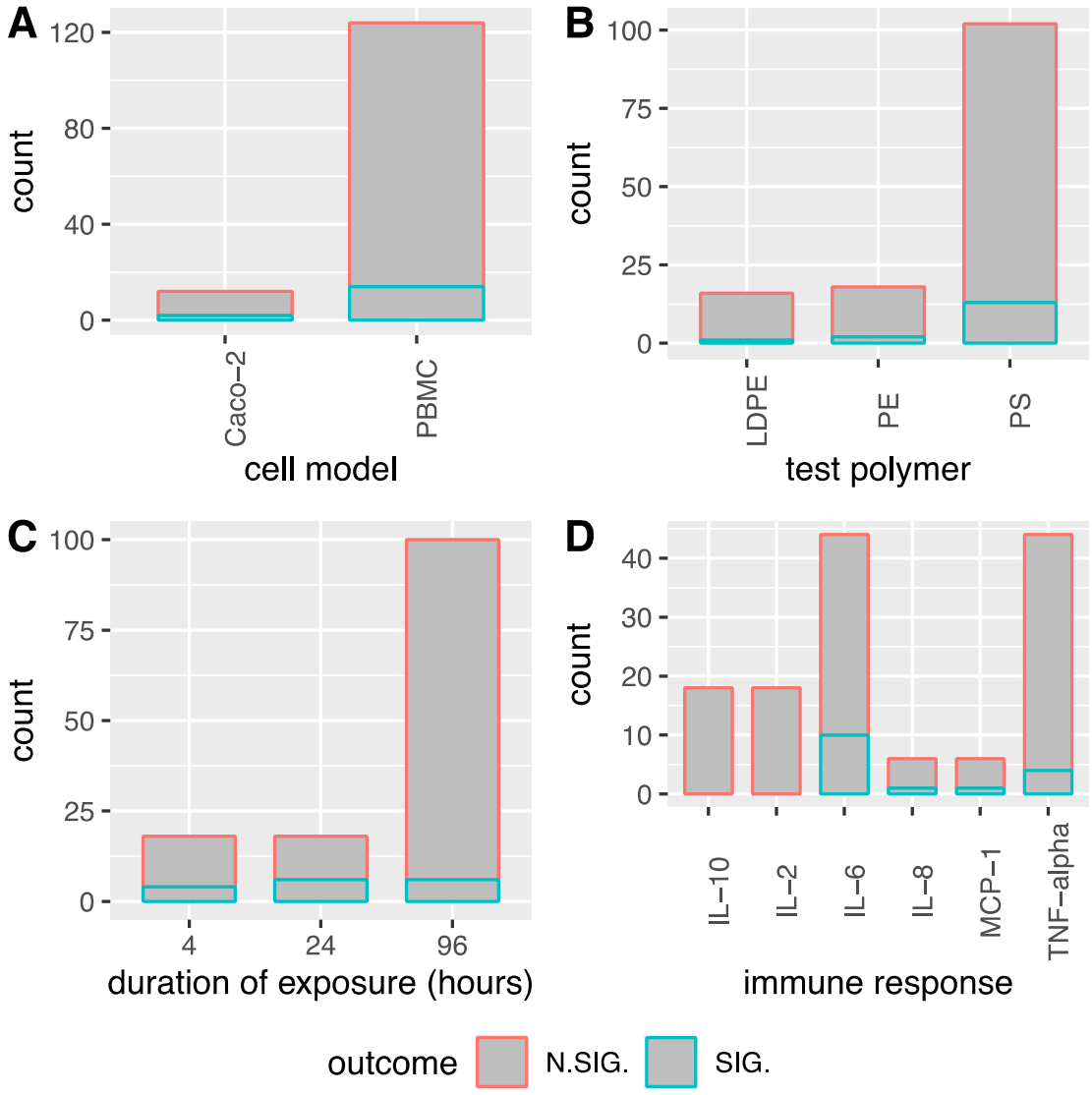


Figure 64. Distribution of the categorical covariates for the cytokine release biological endpoint between cell model (A), test polymer (B), duration of exposure (C), the immune response (D), and the outcome of significant and non-significant results. Note: Caco-2, human adenocarcinoma cell line; CCK-8, cell counting kit 8; co, coculture; IL-, interleukin; LDPE, low-density polyethylene; MCP-1, Monocyte chemoattractant protein-1; N.SIG., not significantly different outcome as compared to the control; PBMCs, peripheral blood mononuclear cells; PE, polyethylene; PS, polystyrene; SIG,. significantly different result as compared to the control; TNF-α, Tumour Necrosis Factor alpha

Figure 65 shows the relationship between the origin and shape covariates, where it is evident that all the primary MPs that were tested were spherical, and all of the secondary MPs were of irregular shape. Thus, only one of the covariates could be included in the analysis but describe both MP characteristics. The distribution of the numerical covariates was examined statistically using the Shapiro test followed by a skewness test (Table 28).

Table 28. Shapiro test, and skewness test results for the three numerical covariates for the cytokines release biological outcome.

	Size (µm)	Dose (µm/mL)	Dose (MPs/mL)
Shapiro test W	0.79909	0.71625	0.23948
p value	< 0.001	< 0.001	< 0.001
Skewness test	0.9460778 (data moderately symmetrical)	1.414324 (data high skewed)	5.140994 (data high skewed)

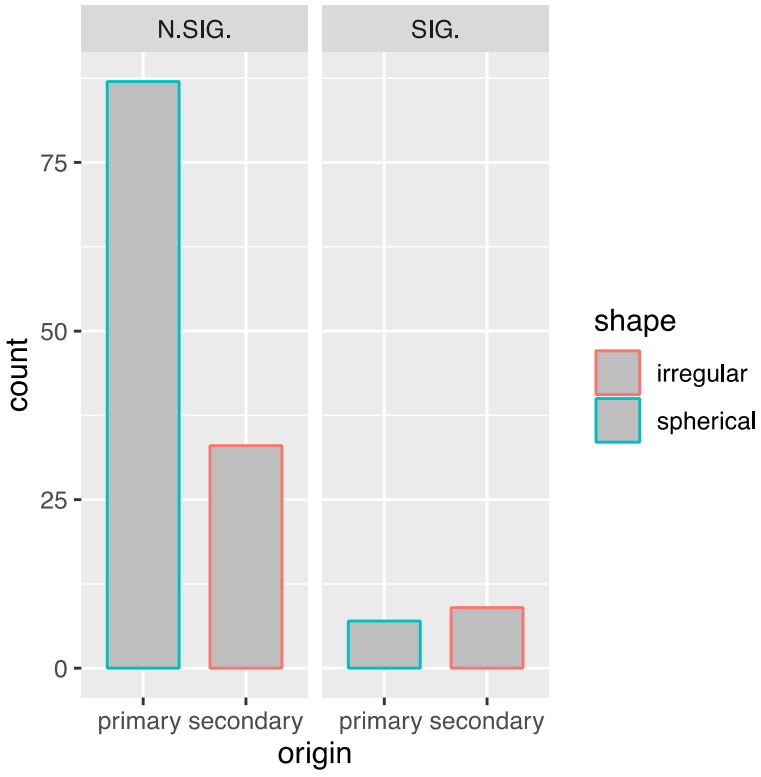


Figure 65. Distribution of the categorical covariates origin and shape and the outcome of significant and non-significant results for the cytokines release biological endpoint. N.SIG., not significantly different outcome as compared to the control; SIG,. significantly different outcome as compared to the control

All data were found to be not normally distributed and present moderate to high skewness. The Spearman correlation test was used to detect correlations. A not significant positive correlation (ρ=0.12, p=0.15) was detected between the size of the MPs and the applied dose expressed in MP concentration of μg/mL, while a significant negative correlation (ρ=-0.872, p< 0.05) was found between the size and the concentrations in MPs/mL. Finally, a significant positive correlation (ρ=0.265, p< 0.05) was also found between the doses of test MPs expressed in concentrations of mass and particle number.

The same trend was also identified when the binary outcome was tested separately as shown in Figure 66. As noted in the cytotoxicity analysis, the conceptual and statistical correlations between the three numerical covariates dictate that not all three can be included in the same model. The ranges of the sizes and MP concentrations that have been tested in this data frame are illustrated in Figure 67 and Figure 68. Similar to the cytotoxicity data frame (see previous section), testing focused on the smaller MP size, while the range and distribution of MP concentrations was better covered in doses expressed in μg/mL than MPs/mL.

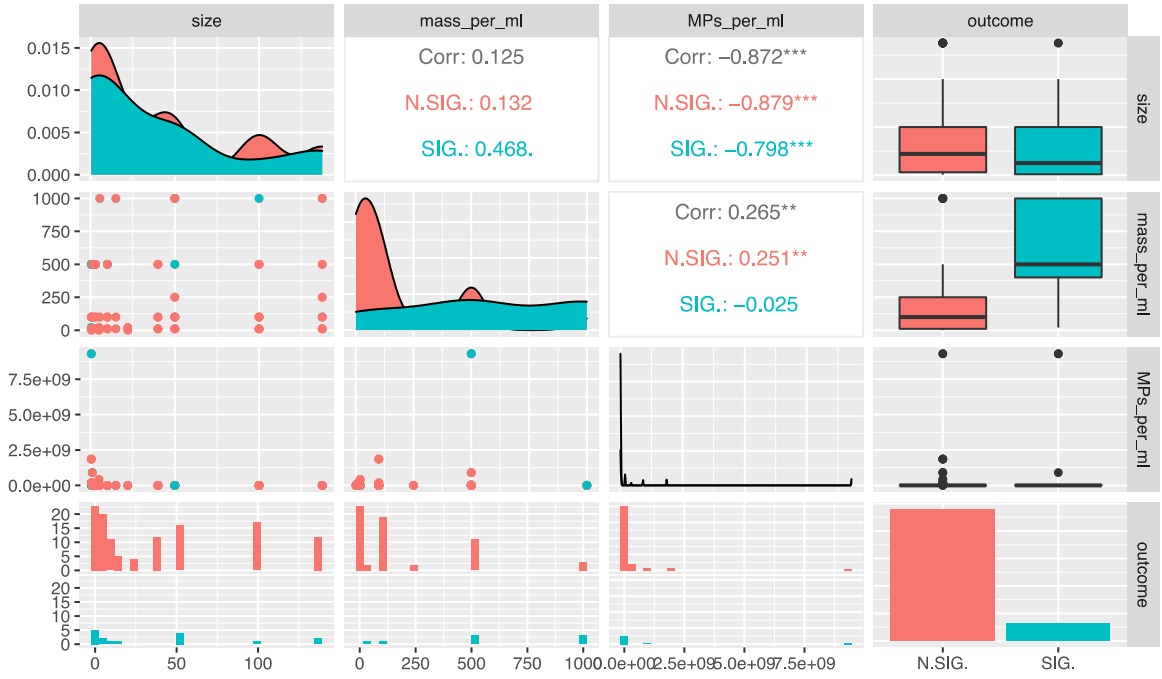


Figure 66. Correlogram between the numerical covariates and the outcome for the cytokine release biological endpoint. The scatterplots for each pair of numerical covariates are displayed on the left part, Spearman correlation test results are displayed on the right, the diagonal shows the covariates' distribution, *, **, and *** indicate p < 0.05, p < 0.01, and p < 0.001, respectively. N.SIG.: not significant, SIG.: significant, Corr.: Spearman rank correlation ρ. Blue: SIG, Red: N. SIG.

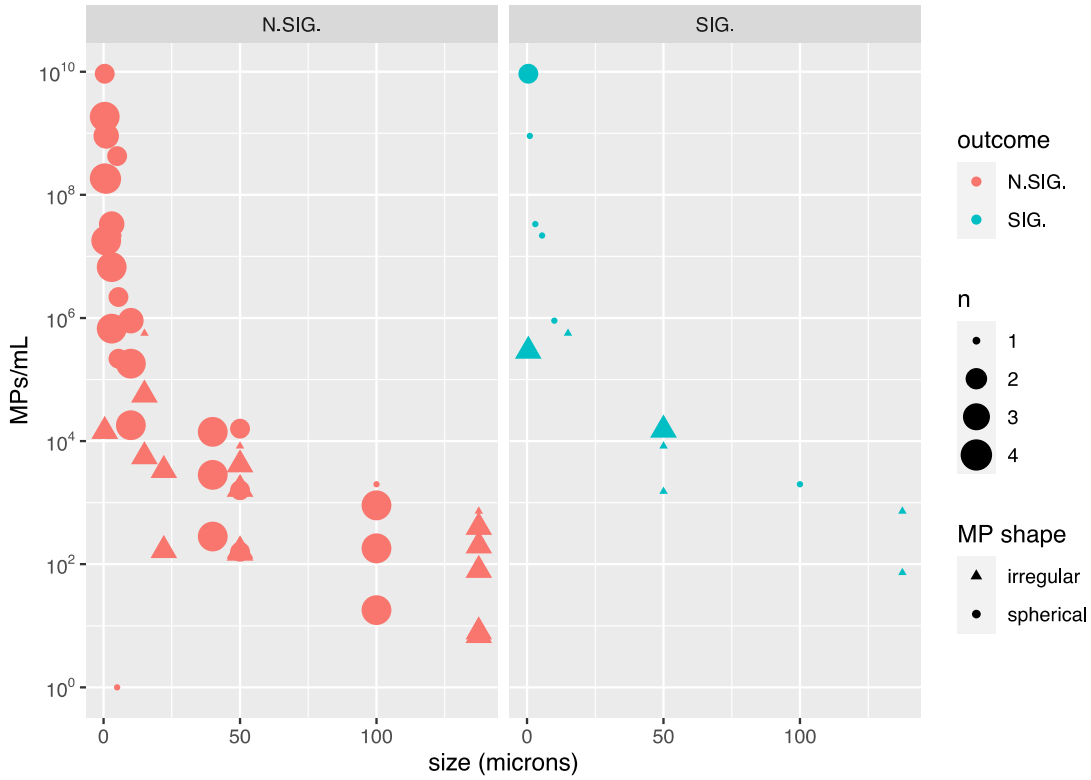


Figure 67. Distribution of test microplastic (MP) characteristics of dose (MPs/mL) and size (µm) for the cytokines release biological endpoint. MPs/mL expressed in log10 scale. n denotes how many times the same experimental conditions were tested by studies. SIG. statistically significant outcome, N.SIG. not statistically significant outcome.

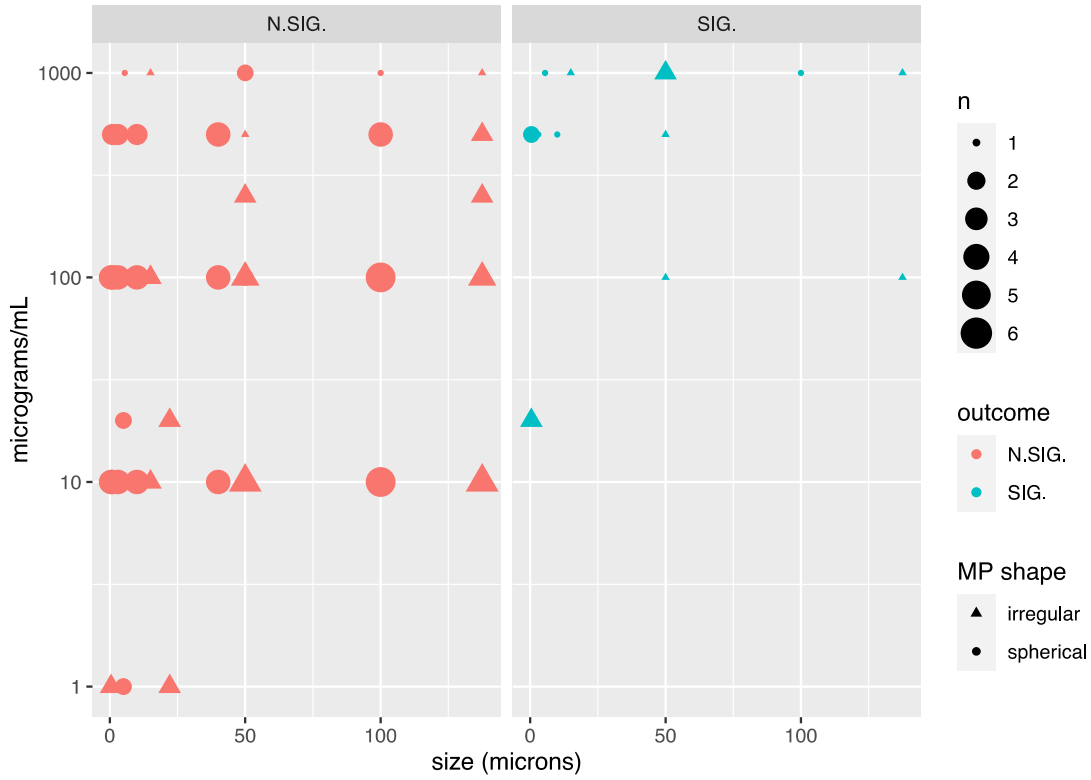


Figure 68. Distribution of test microplastic (MP) characteristics of dose (µg/mL) and size (µm) for the cytokines release biological endpoint. n denotes how many times the same experimental conditions were tested by studies. SIG. statistically significant outcome, N.SIG. not statistically significant outcome.

7.6.2.1. Regression models

The model was first fitted with all the covariates on Table 27, but two coefficients (secondary origin, MCP-1 test outcome) were not defined because of singularities, as they were highly correlated and linearly dependent on shape, cell model and test outcomes. Excluding the two covariates and refitting the model affected the residual deviance only marginally (55 from 49.1, null dev.= 98.5) nor did it notably change the AIC (73 from 75). It must be noted again that all primary MPs were spherical and all secondary were irregularly shaped. Only one regression-coefficient estimate was found to be statistically significant: MP concentrations expressed in μg/mL (β= 0.004, $p< 0.05$), but when testing for multicollinearity by calculating the VIF value, three covariates were found to exceed 5 (cell model, duration and dose in MPs/mL) and one almost 10 (duration) indicating a problematic amount of collinearity present. As the correlation between the MP concentrations expressed in μg/mL and in MPs/mL was already conceptually (and statistically) known, two models were fitted one excluding μg/mL and one excluding MPs/mL. The outcomes of the model revealed that by excluding MPs/mL, all the covariates had VIF values below 2, while, when excluding μg/mL, VIF values continued to be above 5 for three covariates (cell model, duration and MP concentration) which indicates high multi-collinearity. Therefore, the decision was made to proceed without the covariate of dose expressed in concentrations of MPs/mL, also recognizing that this metric is an estimation of the concentrations. The model results showed two regression coefficient estimates as statistically significant, concentration (μg/mL) (β=0.005, $p< 0.05$) and duration (β= -0.03, $p< 0.05$). The shape and origin covariates were not found to be statistically significant but spherical primary MPs (as opposed to irregular shape secondary MPs) did have a negative association with the outcome displaying a larger effect size of β=-1.15. The all-subset regression method was consequently applied, which indicated that the best-subset model excluded the polymer, shape and size covariates. The best-subset model found the three remaining covariates to be statistically significant estimates: duration (β=-0.03, $p<0.05$), PBMC cell model (β=-3.2, $p< 0.05$) and concentration (μg/mL) (β=0.004, $p<0.05$). VIF value was <2. Comparing the two models, the residual deviance marginally increased from 61.072 to 64.578, but the AIC decreased from 77.072 to 72.578 in the best-subset model. The overall prediction accuracy was higher for the full model at 91.2% than the best-subset model 89.7%, so the exclusion of the covariates somewhat affected the performance of the model. The predictions for each outcome for the full and the best-subset model are shown in classification tables (Table 29 and Table 30). Both models were better in predicting the N.SIG. outcome (98.3%) than the SIG. outcome (37.5% and 25%) but the overall prediction accuracy was very high (91.2% and 89.7%).

Table 29. Validity of predicted probabilities for the full model on cytokine release biological outcome.

Outcome	Predicted		
Observed	N. SIG.	SIG.	Correct
N. SIG.	118	2	98.3%
SIG.	10	6	37.5%
Overall correct			91.2%

Table 30. Validity of predicted probabilities for the best-subset model on cytokine release biological outcome.

Outcome	Predicted		
Observed	N. SIG.	SIG.	Correct
N. SIG.	118	2	98.3%
SIG.	12	4	25%
Overall correct			89.7%

Apart from the multicollinearity, which was tested for each model individually, further diagnostics were executed to test the basic assumptions of logistic regression. The linearity assumption was examined through a series of scatterplots to detect if there was a linear relationship between the numerical covariates and the logit of the outcome. As shown in Figure 69 and Figure 70, the linearity is improved in the best-subset model but is still not fully linear. The most extreme values were visualized using the Cook's distance values (Figure 71) (Osborne, 2015). The standard residual error for all the covariates were at acceptable levels (< 3) as illustrated in Figure 72 (Menard, 2002).

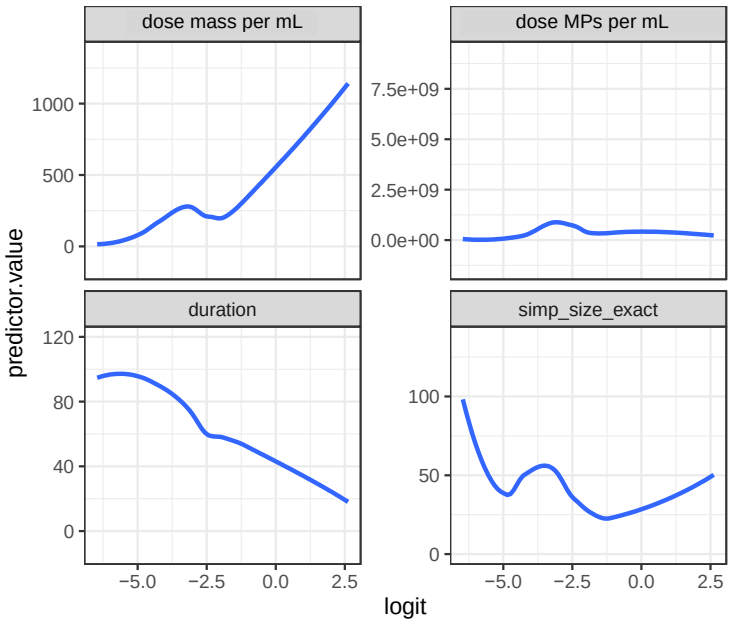


Figure 69. Linearity test between the numerical covariates and the logit of the outcome in the full model for the cytokines release biological endpoint.

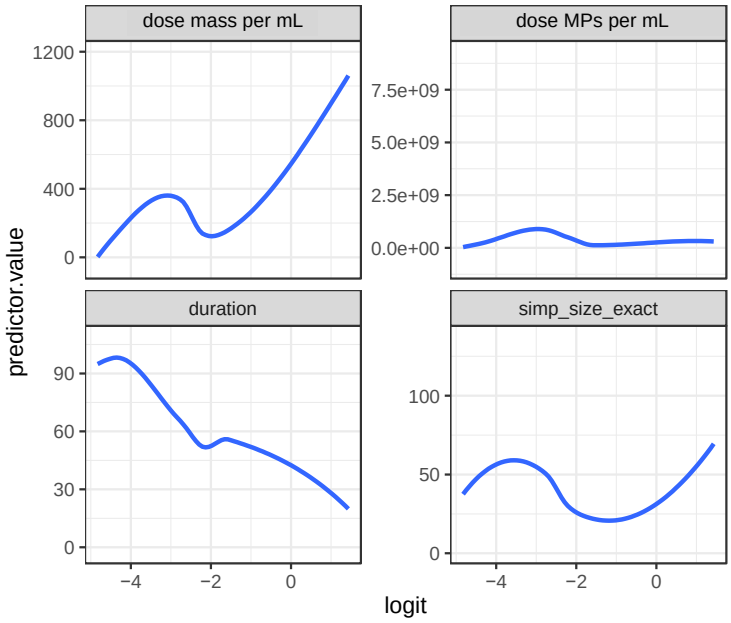


Figure 70. Linearity test between the numerical covariates and the logit of the outcome in the best-subset model for the cytokines release biological endpoint.

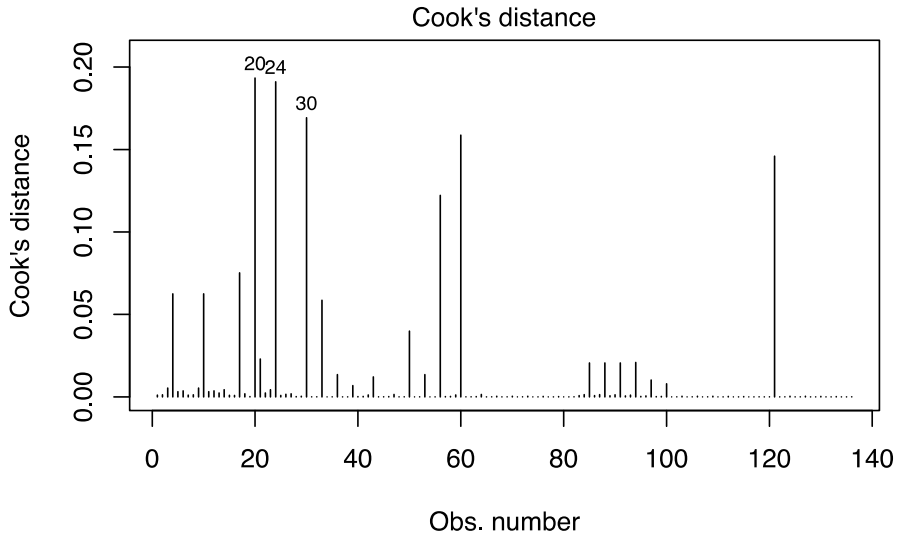


Figure 71. Cook's distance values, full model, for the cytokines release biological endpoint.

7.6.2.2. Sensitivity analysis

The biological-marker covariate was also fitted to detect if it was associated with the results. The cell-model covariate was excluded from this model as it presented singularities with the outcome. The regression-coefficient estimates were not statistically significant for any of the six biological markers. A further model was fitted for the largest subgroup of the data frame, categorized by biological marker. The IL-6 outcome was chosen with 44 data points and 12/32 distribution of outcomes (see SM2 in Danopoulos et al. (2021)). The model results showed that no coefficients were statistically significant, but VIF values were extremely high, pointing to strong multicollinearity. The last model to be explored was a subgroup of

the data frame that included only the PBMC cell models (124 data points) which was previously found to be a statistically significant predictor. The model could not express the covariate of origin due to singularities. The model excluding origin found MP concentration as the only statistically significant covariant (β=0.005, p< 0.05), while all VIF values were < 3.

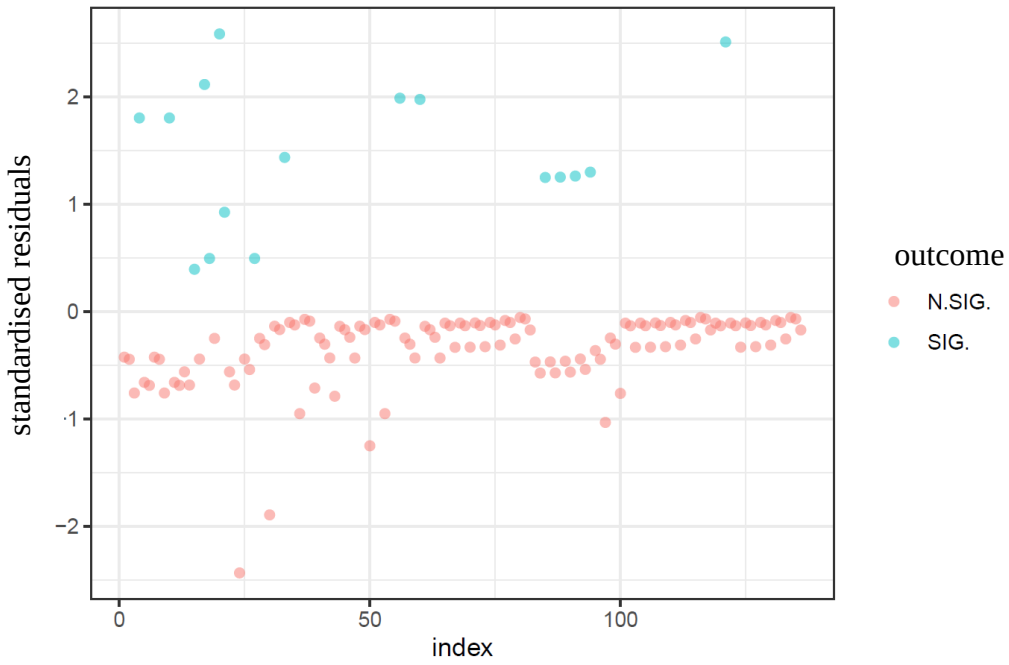


Figure 72. Standardised residuals of the best-subset model, for the cytokines release biological endpoint. N.SIG., not significantly different outcome as compared to the control; SIG,. significantly different outcome as compared to the control

The RoB influence could be tested in this data frame (184 data points). Three RoB categories were included in the RoB covariate: moderate, serious and critical. The two covariates of origin and test outcome could not be defined due to singularities and were not included in the model. When comparing the RoB model with the full model, four prediction coefficients were statistically significant, two similar to the RoB constrained model: duration (β= -0.029, p< 0.05) and MP concentration (β=0.002, p< 0.05) and a further two: spherical shape (β= -1.548, p< 0.05) and size (β= -0.015, p< 0.05), with VIF values < 2. The overall prediction accuracy was reduced to 88%, residual deviance 103.3 (null 138.65) and AIC 125.3. The all-subset regression method was used, which excluded the covariates of cell model and polymer, and retained the coefficients of duration (β= -0.018, p< 0.05), MP concentration (β=0.002, p< 0.05), spherical shape (β= -1.354, p< 0.05) and size (β= -0.014, p< 0.05), in the best-subset model, with marginally changed effect sizes and VIF < 2. Residual deviance

of the best-subset model was 110.43 and AIC 120.43. The overall prediction improved marginally at 88.5% but was still less than the restricted RoB model.

7.6.2.3. Multilevel models

Multilevel logistic regression models were subsequently fitted to account for the data clustering depended on the four studies included in the data frame. The ICC of the null model was 0.095, meaning that 9.5% of the variations in the outcome could be attributed to the clustering of the data in the four studies. The multilevel mixed model included fixed effects for the covariate and a random intercept for the four studies. The covariates used for the model were: cell model, polymer, shape, duration, size (μm) and MP concentration (μg/mL). The results were similar to the previous model. Consequently, a further model was fitted excluding the cell model covariate that was excluded by the all-subset regression process. This model also produced the same results. Random-slope, random-intercept models were also fitted testing one covariate at a time. Using the likelihood ratio test, none of the random-slope models were found to significantly improve from the fixed slope.

7.7. Histamine release, oxidative stress, genotoxicity

Histamine release was examined by four studies (Choi et al., 2021, Han et al., 2020, Hwang et al., 2019, Hwang et al., 2020b) (Table1). Each used one cell model (HMC-1), tested five different polymers and used two different tests (ELISA kit, histamine assay) (Figure 73). Only two studies (Han et al., 2020, Hwang et al., 2019) reported significant outcomes, and these were rated of critical RoB, therefore the data could not be explored in a meta-regression. The rest of the studies (Choi et al., 2021, Hwang et al., 2020b) tested two polymers PE and PS for sizes ranging from 5.5 to 100 μm and MP concentrations ranging from 10 to 1000 μg/mL for PE and 0.46 to 100 μm and MP concentrations of 500 μg/mL for PS, but all of the test MPs were of spherical shape.

Nine studies examined oxidative stress (Table 1). Excluding the three studies rated of critical RoB (Hwang et al., 2019, Qiangqiang Wang et al., 2020, Shijin Wu et al., 2020), two studies reported significant outcomes. Wu et al. (2019) reported a significant increase of intracellular reactive oxygen species (ROS) generation after exposure to spherical, 0.1 and 5 μm, PS MPs using Caco-2 cells at a MP concentration of 200 μg/mL and Dong et al. (2020) after exposure to 1.72 μm spherical PS MPs using BEAS-2B cells at a MP concentration of 1000 μg/cm^2. The results of the oxidative stress tests could not be analysed in meta-regression due to the small size of the data frame (44 data points), and the use of four

different measures of the outcome. Two studies examined genotoxicity (Table 1) and one was rated of critical RoB (Shijin Wu et al., 2020). The other study (Hesler et al., 2019) examined genotoxicity through testing a p53 reporter, exposing Caco-2 cells to spherical 0.5 µm PS MPs (up to 10 µg/mL), but all results were non-significant.

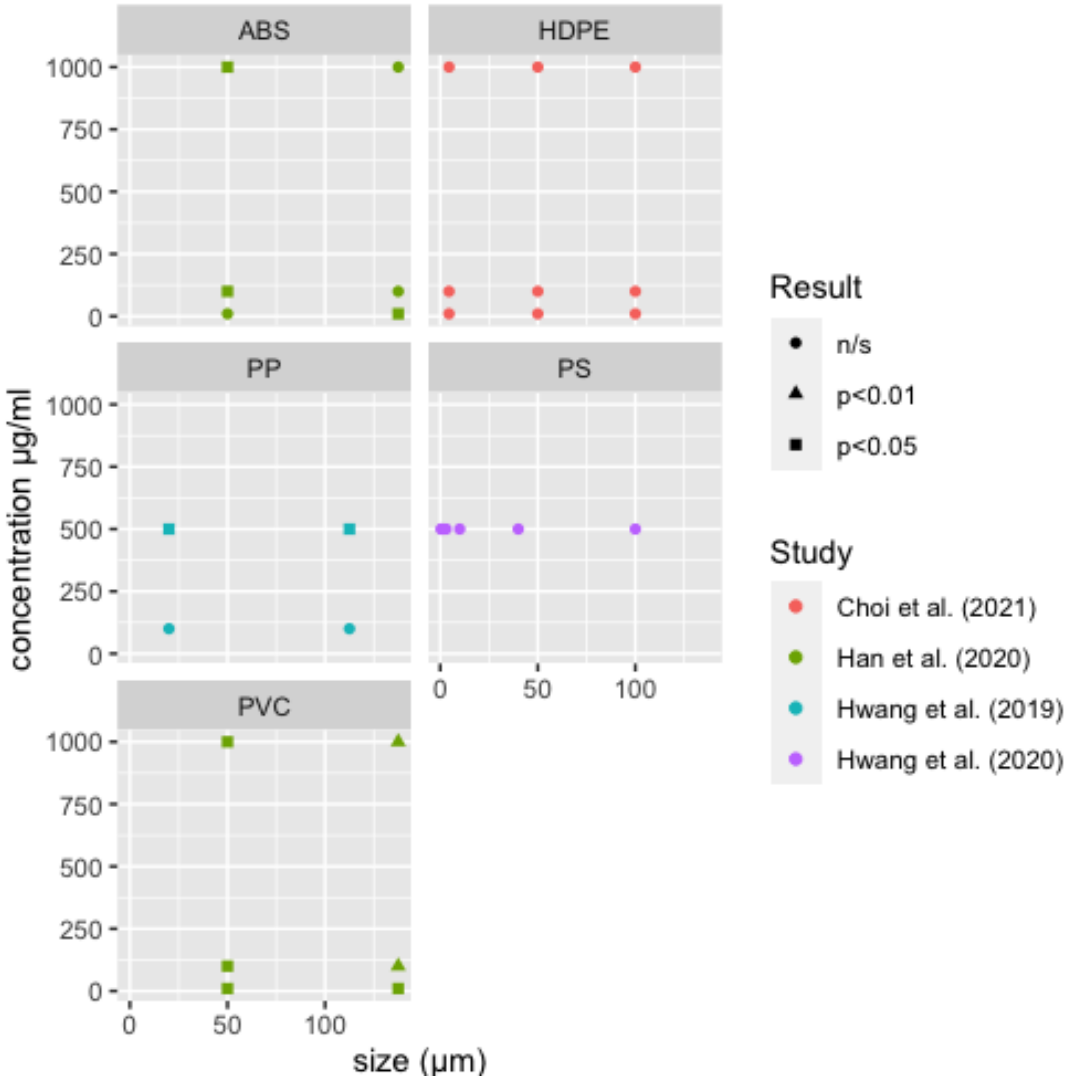


Figure 73. Histamine release tests grouped by test microplastics (MPs) polymer. All risk of bias rating was included. Note: ABS, acrylonitrile butadiene styrene; HDPE, high-density polyethylene; n/s, not significant; PP, polypropylene; PS, polystyrene; PVC, polyvinyl chloride

7.8. Cell barrier

Ten studies (Table 21) examined the cell-barrier behaviour, relating to either cell viability or a series of MP and cell-membrane or cell-model interactions: uptake (translocation, internalisation), barrier integrity, permeability and trans-membrane transport. Two studies (Liu et al., 2020b, Wu et al., 2019) focused on cell barrier attributes in terms of cytotoxicity and both used the relative release of LDH as the measure. No significant change to LDH

release after exposure to spherical and irregular PS MPs was reported. Barrier integrity was examined by three studies (Dong et al., 2020, Hesler et al., 2019, Lehner et al., 2020) by measuring the transepithelial electrical resistance (TEER) before and after exposure to MPs. Only Dong et al. (2020) reported a significant decrease in the barrier integrity after exposure to spherical PS MPs (1.72 μm) for 24 hours at two MP concentrations of 10 and 1000 μg/cm^2. The expression of the protein ZO-1, using an ELISA technique as a measure of disruption of the barrier, was also conducted, and a significant decrease of Z0-1 after the same exposures observed. Liu et al. (2020b) examined the permeability of the cell barrier and reported significant down-regulation of the expression of transmembrane transporters (*ABCC2, ABCG2*) after exposure to irregularly shaped MPs and spherical PS MPs (5 μm) at MP concentrations of 1 and 20 μg/mL for 96 hours. Liu et al. (2020b) was the only study that examined paracellular transport examining the expression of *ZO-1* and *Occludin* using qPCR, but only reported a significant down-regulation after exposure to NPs which is beyond the scope of this review. The quantitative barrier integrity / permeability results could not be analysed in meta-regression due to the small size of the data frame (34 data points) and the use of six different measures for the outcome.

MPs uptake/internalisation was examined by seven studies (Table 1) two of which were rated as of critical RoB (Stock et al., 2019, Qiangqiang Wang et al., 2020). The other five studies all used qualitative measures for examining MP cellular uptake. Hesler et al. (2019) stated that spherical PS MPs (0.5 μm) were internalised by both the co-cultures they used (Table 1) after a 24-hour exposure. Translocation of MPs was also detected in the apical but not in the basolateral compartment of the models. Stock et al. (2021) exposed MPs (PE, PP, PET, PVC) to a Caco-2 trans-well model in order to examine cell uptake via microscopic examination and fluorescence quantification of the cell membranes and reported that intracellular uptake was detected only for spherical, PE MPs (1-4 μm). Wu et al. (2019) reported that both sizes (0.1 and 5 μm) of spherical PS MPs entered the Caco-2 cells after a 12-hour exposure. Goodman et al. (2021) confirmed the internalisation of 1 μm spherical PS MPs for exposures from 24 to 96 hours via flow cytometry (Calcein AM and Ki67 assays) and phase-contrast microscopy, using A549 cells. Hwang et al. (2020b) did not report MP uptake results.

7.9. Characteristics of MP toxicological profile

The MP exposure characteristics that were examined in order to create a toxicological profile were size, surface area, shape, surface charge, chemical composition, MP concentration and

duration. Choi et al. (2020) concluded that both chemical and physical effects influenced the observed toxicity. Chemical effects were hypothesised to be related to the release of chemical reagents from the MPs, while the physical effects came from the direct damage of cellular membranes. Choi et al. (2020) stated that the effects were concentration-dependent, not MP size-dependent and noted that immune responses and ROS generation were observed after short-term (i.e. 24-hour) cultures and cell death after long-term cultures (i.e. after 96 hours). A subsequent study focused on the physical effects by using both spherical and irregularly shaped MPs (Choi et al., 2021), concluding that the observed toxicity was correlated with the ruggedness of the irregularly shaped MPs. In contrast, spherical MPs did not affect cell death but did induce immune responses in high MP concentrations.

Hesler et al. (2019) focused on acute toxicity and highlighted the range of toxicological effects on different cell models, noting that the sensitivity of cell models and co-cultures to MP exposure varies. Hesler et al. (2019) was one of the studies which examined whether MPs could cross biological barriers, reporting that the function of the intestinal and the placental barrier was not compromised. MPs did not cross the co-cultures, but internalization by cells was confirmed. The authors also did not exclude the possibility that long-term exposures (more than 24 hours) could have different results on uptake and detected different responses and behaviour between the two models when exposed to MPs. Furthermore, it was stated that responses were both size- and dose-dependent (MP concentration). Lehner et al. (2020) also used an intestinal model but found no cytotoxic or inflammatory responses. The size of the test MPs (50-500 μm) was proposed as a possible explanation for the absence of effects, which were much larger than the test MPs used by Hesler et al. (2019) (0.5 μm). It should also be noted that Lehner et al. (2020) was one out of two studies that did not use a dispersion of MPs but, rather, dry powder directly applied on the surface of the cells. Liu et al. (2020b) used a Caco-2 monolayer and examined the effects of two MPs: one primary and one secondary, processed to mimic the conditions of the digestive tract. Differences between the measured effects on toxicity and immune responses were detected and attributed to size and shape, especially on the corona that was created on the surface of the secondary test MPs. The shape change was hypothesised to have altered the Zeta potential value (surface charge) of the test MPs. It was not reported whether the MPs affected paracellular transport but an abnormality of transmembrane transport indices were reported. Stock et al. (2021) examined MP toxic effects as a result of intra-cellular interactions but concluded that cytotoxicity could not be associated to specific polymers or shapes but only to extremely high concentrations (> 10000 μg/mL) of large MPs exceeding the intracellular uptake limit

of < 10μm. Regarding particle uptake and transport, the only test MPs found to cross the model's barrier were in the size range between 1-4 μm which coincides with the pore size (3 μm) of the polycarbonate membrane which was integral to the model used.

Wu et al. (2019) tested two different sizes of MPs (0.1 and 5 μm) on Caco-2 cells and found differences in mitochondrial depolarization which was attributed to the accumulation of the smaller MPs in lysosomes. The larger MPs, on the other hand, could escape lysosomes, localize in other parts of the cells and cause more damage, further triggering depletion of ATP and inhibition of ABC plasma membrane transporter activity. A different mechanism was hypothesised for the smaller MPs, which might have acted as substrates of the transporters thus causing competitive inhibition resulting in the reduction of the ABC transporters' action.

Hwang et al. (2020b) stated that MPs (< 1 μm) at high concentrations (> 500 μg/mL) could be associated with innate rather than adaptive immune responses and suggested that cells might recognize them as pathogens. Other than that, no mechanism of toxicity has been proposed. Schirinzi et al. (2017) did not detect cytotoxic effects but did report significant effects on ROS generation which were proposed to be size-dependent, with no mechanism proposed.

Three studies focused on the inhalation route connected to the respiratory system (Brown et al., 2001, Dong et al., 2020, Goodman et al., 2021). Brown et al. (2001) initially hypothesised that inflammatory effects would be size-dependent but concluded that they were more likely connected to the MP surface area and their ability to generate oxidative activity. Dong et al. (2020) stated that the underlying mechanism for all the effects (cytotoxic and inflammatory) caused by MPs was the formation of ROS. Goodman et al. (2021) noted that there could be a difference between short-term and long-term exposures and highlighted that the effects of MPs in the lungs are likely to be cumulative for life-long exposures. These authors suggest that the observed effects (reduced proliferation, morphological/behavioural changes) are all likely initiated by a mechanical signal caused by the MP presence.

7.10. Statistical summary of evidence

In order to use the congregated data derived from all the studies in a way that is meaningful in the context of risk assessment, threshold values must be defined. Threshold values can be expressed as no observed adverse effect level (NOAEL) and/or lowest observed adverse

effect level (LOAEL), both relating to the level of exposure where no effect occurs (IPCS, 2009) (section 3.7.3). The choice of the appropriate data to be included in this part of the analysis were based on conceptual justification and the results of the meta-regression. In the paradigm of dietary and atmospheric exposures of humans to MPs there is a mix of polymers as illustrated by the systematic reviews on food and drinking water contamination (see sections 4.9, 5.7 and 6.9) and atmospheric studies (Jenner et al., 2021, Wright et al., 2020). In addition, according to the meta-regression (sections 7.5.2 and 7.6.2), polymer type was not found to be a significant predictor of the outcome. The structure of the analysis, following the overarching categorization by biological outcome, must be the cell model that was used in the experiments, which was found to be a significant predictor in the meta-regression of the cytotoxicity outcome (section 7.5.2), followed by the size of MPs, since different sizes can, in theory, reach different locations of the human body, and the applied dose (MP concentration). A secondary categorization of duration can also be applied. The structure of the data synthesis follows the categorization of cell model/ polymer/ size/ concentration/ duration. The results of food-related and atmospheric MP studies also indicate that only a small proportion of the MPs discovered were spherical (Danopoulos et al., 2020a, 2020b, 2020c, Jenner et al., 2021). Consequently, only the results of non-spherical test MPs will be included, in order to achieve the best possible analogue to the MPs currently found in the environment, readily available as contaminants for human exposures.

In the process of dose-response modelling, in order to ensure that the toxic responses are acknowledged across endpoints and subjects, the lowest observed levels can be used across cell models as a measure of the most sensitive cells (IPCS, 2009). Likewise, endpoints where clear dose-response is not present can be omitted. After examining the available data, lowest threshold values could only be defined for the endpoints of cytotoxicity, barrier integrity and immune responses. Regarding the oxidative stress biological endpoint, only non-significant values were reported for irregular MPs, (Table 31). Histamine responses and genotoxicity were only tested using spherical MPs.

Table 31. Highest applied MP doses resulting in non-significant oxidative stress effects after exposure to irregularly shaped MPs.

Cell model	**Biological marker**	**Polymer**	**Size**	**Dose (µg/mL)**	**Dose (MPs/mL)**	**Duration (hours)**
HDF	ROS[a]	LDPE				6
			50	1000	16643	

			137.5	1000	800	
Caco-2						
	GPx[b]	PS				96
			0.4402	20	290197	
			22.1	20	3360	
	OXSR1[b]	PS				96
			0.4402	20	290197	
			22.1	20	3360	

[a] reactive oxygen species (ROS) assay kit, [b] polymerase chain reaction (PCR) used. Note: Caco-2, human adenocarcinoma cell line; GPx, Glutathione Peroxidase; HDFs, human dermal fibroblasts; LDPE, Low-density polyethylene; OXSR1, oxidative stress responsive kinase 1; PS, polystyrene

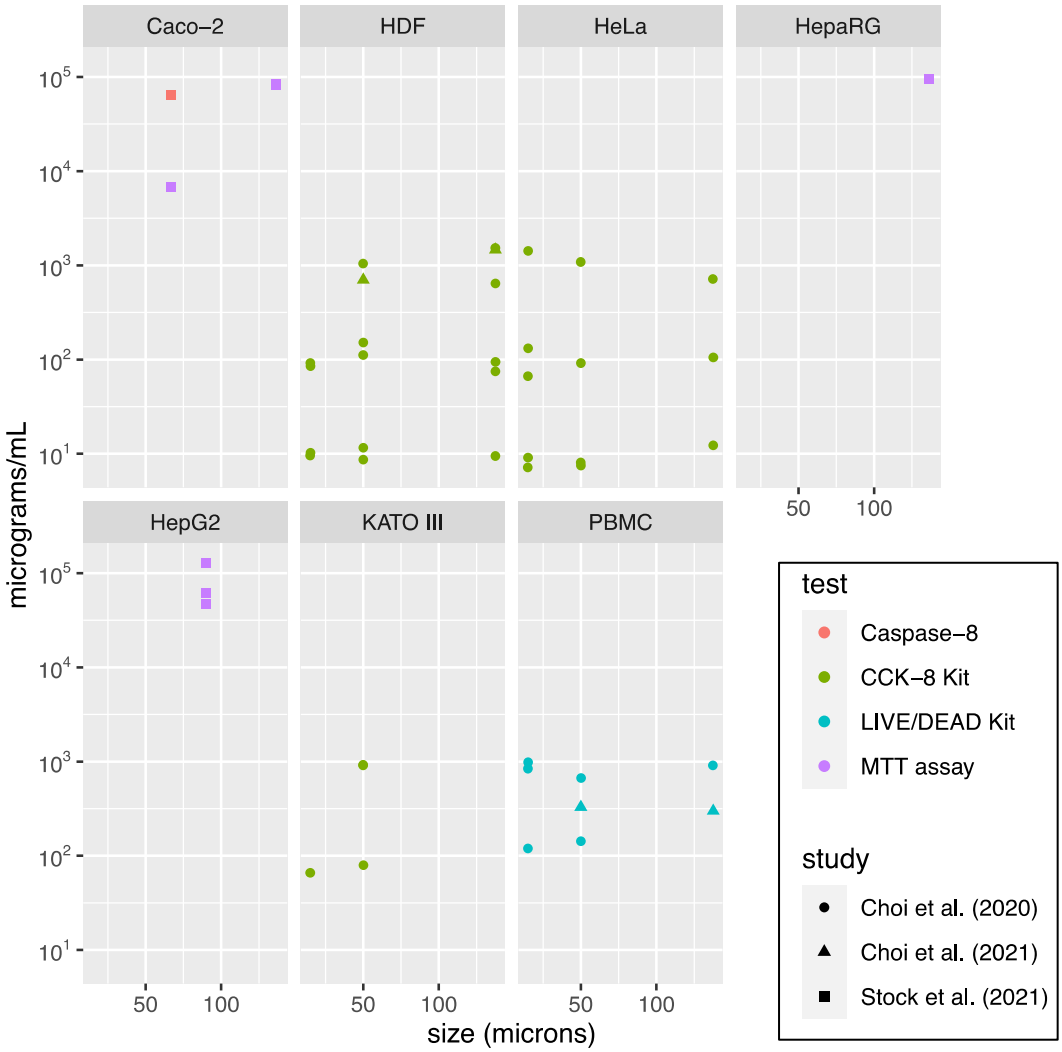


Figure 74. Applied MP doses that resulted in significant reduction of cell viability after exposure to non-spherical microplastics (MPs). Dose expressed in MP concentrations in µg/mL (log_{10} scale) and MP size in µm. Note: Caco-2, human adenocarcinoma cell line; CCK-8, cell counting kit 8; HDF, human dermal fibroblasts; HeLa, cervical cancer cells; HepaRG, human hepatic cells; HepG2, Human Caucasian hepatocyte carcinoma cells; KATO III, gastric cancer stem cells; LIVE/DEAD kit, viability/cytotoxicity test; MTT assay, cellular metabolic activity colorimetric assay; PBMCs, peripheral blood mononuclear cells

7.10.1. Cytotoxicity and barrier integrity

The results for all the non-spherical shaped MPs that significantly reduced cell viability are illustrated in Figure 74. The lowest doses that reduced cell viability significantly are presented in Table 32 categorized by cell model. The lowest MP concentration (of 10 μg/mL) was found to affect the HDF and HeLa cell models both in μg/mL and MPs/mL, while the smallest MPs (15 μm) affected HDF, HeLa, KATOIII and PBMC cells. One study (Liu et al., 2020b) measured the effects of MP exposure on the permeability of the cell barrier using a quantitative metric by evaluating transmembrane transporters (*ABCC2, ABCG2*) via qPCR assay (Table 32). A series of tests/biological markers investigations reported no significant results constituting a form of NOAEL, and these threshold values are presented in Table 33. Full results can be found in SM2 in Danopoulos et al. (2021).

Table 32. Lowest applied non-spherical microplastic (MP) doses resulting in significant reduction of cell viability after exposure to irregularly shaped MPs.

Cell model	**Test**	**Polymer**	**Size (μm)**	**MP concentration**		**Duration (hours)**
				μg/mL	MPs/mL	
Caco-2						
	MTT	PP	67.1	10000	70241	24
	Caspase-8	PP	67.1	50000	351205	24
	MTT	PVC	136.5	75000	40228	24
	qPCR	PS				96
			0.4402	20 [a]	290197	
			22.1	1 [b]	168	
HDF	CCK-8					
		PS				
			15	10	5630	24
			50	10	152	24
			137.5	10	7	96
		LDPE				
			50	1000	16643	24
			137.5	1000	800	24
HeLa	CCK-8	PS				
			15	10	5630	24
			50	10	152	24
			137.5	10	7	96
HepaRG	MTT	PVC	136.5	100000	53638	24
HepG2	MTT	PE	90.1	50000	138889	24
KATO III	CCK-8	PS				
			15	100	56306	24

			50	100	1520	24
PBMC	LIVE/DEAD kit					
		PS				
			15	100	56306	96
			50	100	1520	96
			137.5	1000	727	96
		LDPE				
			50	500	8321	24
			137.5	250	200	24

[a] qPCR of *ABCC2* gene expression was used to test cell membrane permeability, [b] qPCR of *ABCG2* gene expression was used to test cell membrane permeability. Note: Caco-2, human adenocarcinoma cell line; CCK-8, cell counting kit 8; HDFs, human dermal fibroblasts; HeLa, cervical cancer cells; HepaRG, human hepatic cells; HepG2, Human Caucasian hepatocyte carcinoma cells; KATO III, gastric cancer stem cells; LDPE, Low-density polyethylene; LIVE/DEAD kit, viability/cytotoxicity test; MTT assay, cellular metabolic activity colorimetric assay; PBMCs, peripheral blood mononuclear cells; PCR, polymerase chain reaction; PE, polyethylene; PP, polypropylene; PS, polystyrene; PVC, polyvinyl chloride

Table 33. Highest applied MP doses resulting in non-significant outcomes after exposure to irregularly shaped MPs for the cytotoxicity and barrier integrity biological endpoints. When more than one duration was used, the longest duration was included per experimental condition.

Cell model	**Test**	**Polymer**	**Size**	**Dose (µg/mL)**	**Dose (MPs/mL)**	**Duration (hours)**
Caco-2						
	MTT					
		PE	90.1	100000	277778	24
		PET	60	100000	654958	24
		PP	67.1	50000	351205	24
		PVC	136.5	50000	26819	24
	LY	PS	0.4402	20	290197	96
	ZO-1[a]	PS				
			0.4402	20	290197	96
			22.1	20	3360	96
	Occludin[a]	PS				
			0.4402	20	290197	96
			22.1	20	3360	96
	ABCC2[a]	PS				
			0.4402	1	14510	96
	ABCG2[a]					
			0.4402	20	290197	96
			22.1	1	168	96
	Caspase-3					24

		PE	90.1	100000	277778	
		PET	60	100000	654958	
		PP	67.1	50000	351205	
		PVC	136.5	100000	53638	
	Caspase-8					24
		PE	90.1	100000	277778	
		PET	60	100000	654958	
		PP	67.1	25000	175602	
		PVC	136.5	100000	53638	
	Caspase-9					24
		PE	90.1	100000	277778	
		PET	60	100000	654958	
		PP	67.1	50000	351205	
		PVC	136.5	100000	53638	
	LDH	PS				96
			0.4402	20	290197	
			22.1	20	3360	
Caco-2 (co)	LDH					48
		PA6	72	1028.58[b]	n/a	
		PP	282	1305.5[b]	n/a	
		PU	253	1263.25[b]	n/a	
		TPU	264	1098.02[b]	n/a	
	TEER					
		PA6	72	1028.58[b]	n/a	
		PP	282	1305.5[b]	n/a	
		PU	253	1263.25[b]	n/a	
		TPU	264	1098.02[b]	n/a	
HDF	CCK-8					
		PS				
			15	1000	563068	96
			50	1000	15202	24
			137.5	10	7	24
		LDPE				24
			50	500	8321	
			137.5	500	400	
HeLa	CCK-8	PS				
			15	1000	563068	24
			50	1000	15202	24
			137.5	1000	727	24
HepaRG						
	MTT					24
		PE	90.1	100000	277778	

		PET	60	100000	654958	
		PP	67.1	50000	351205	
		PVC	136.5	75000	40228	
	Caspase-3					24
		PE	90.1	100000	277778	
		PET	60	100000	654958	
		PP	67.1	50000	351205	
		PVC	136.5	100000	53638	
	Caspase-8					24
		PE	90.1	100000	277778	
		PET	60	100000	654958	
		PP	67.1	50000	351205	
		PVC	136.5	100000	53638	
	Caspase-9					24
		PE	90.1	100000	277778	
		PET	60	100000	654958	
		PP	67.1	50000	351205	
		PVC	136.5	100000	53638	
HepG2						
	MTT					24
		PE	90.1	25000	69444	
		PET	60	100000	654958	
		PP	67.1	50000	351205	
		PVC	136.5	100000	53638	
KATO III	CCK-8	PS				96
			15	1000	563068	
			50	100	1520	
			137.5	1000	727	
PBMC	LIVE/DEAD kit					
		PS				
			15	10	5630	96
			50	100	152	96
			137.5	100	73	96
		LDPE				
			50	250	8321	24
			137.5	500	400	24

[a] test via quick polymerase chain reaction (qPCR), [b] doses in μg/cm^2.

Note: Caco-2, human adenocarcinoma cell line; CCK-8, cell counting kit 8; HDFs, human dermal fibroblasts; HeLa, cervical cancer cells; HepaRG, human hepatic cells; HepG2, Human Caucasian hepatocyte carcinoma cells; KATO III, gastric cancer stem cells; LDPE, Low-density polyethylene; LIVE/DEAD kit,

viability/cytotoxicity test; LDH, lactate dehydrogenase; LY, lucifer yellow; MTT assay, cellular metabolic activity colorimetric assay; PBMCs, peripheral blood mononuclear cells; PCR, polymerase chain reaction; PE, polyethylene; PET, Polyethylene terephthalate; PP, polypropylene; PS, polystyrene; PU, polyurethane; PUR, polyurethanes; PVC, polyvinyl chloride;; TEER, transepithelial electrical resistance; TPU, polyurethane; ZO-1, Zonula occludens-1

A striking finding worth highlighting, is that in a small number of studies, the highest applied MP concentration per experimental condition was not the most effective, or not as effective in inducing a response within one of the biological endpoints. This phenomenon has been observed in three studies (Choi et al., 2020, Choi et al., 2021, Stock et al., 2021) within the results of two different cytotoxicity tests. When examining the MTT assay results for Caco-2 cells exposed to PP MPs of 67.1 μm, a significant result for the 10000 μg/mL dose, but not for the 25000 and the 50000 μg/mL doses, is reported for the same duration of exposure (Stock et al., 2021). The authors omit this from the discussion, stating that PP was non-toxic.

In another study, CCK-8 assay results for the HDF cells exposed to PS MPs of 15 μm, were significantly different for the 10 and 100 μg/mL doses but not the 1000 μg/mL dose, after a 24 hour duration (Choi et al. (2020). The same pattern was observed for the 50 μm sized MPs but not for the 137.5 μm sized MPs. Again, CCK-8 assay results for HeLa cells exposed to PS MPs (only for the two test MP sizes: 15 and 50 μm), and KATO III cells exposed to PS MPs (only for the 15 μm sized MPs) all using a 24 hour duration, show the same pattern (Choi et al. (2020). In contrast, in the same study, using the same cytotoxicity test, the same polymer but a different cell model, in this case PBMC, the highest MP concentrations were the most effective at inducing a biological response. Choi et al. (2020) attributed this non-linearity in the dose-response relationship to the physicochemical characteristics of MPs, proposing that MPs at high concentrations likely formed clusters, thus reducing their (physical) toxicity and leading to the linear toxicity pattern observed in the PBMC cells due to their greater sensitivity. This issue was also reported in a subsequent study using LIVE/DEAD assay results, when PBMC cells were exposed to 137.5 μm sized LDPE MPs for 24 hours, but no comment was made in the discussion (Choi et al., 2021).

Regarding spherical MPs, the same issue was highlighted following WST-1 and MTT assays, using Caco-2 and BeWo cells exposed to 0.5 μm PS MPs (Hesler et al., 2019) and Caco-2 cells exposed to 2.2 μm PE MPs (Stock et al., 2021). Stock et al. (2021), omit these results, concluding that PE MPs were non-toxic. Hesler et al. (2019), on the other hand, recognised that lower MP concentrations exhibited higher toxicity and referenced the work

by Vandenberg et al. (2012). The latter report that a non-linear dose-response relationship (non-monotonic) and low-dose effect of endocrine disrupting chemicals (EDC) is possible. It was not clear how EDC toxic mechanisms was related to MPs or if Hesler et al. (2019) attributed MPs toxic effects to chemical, instead of physical, interactions with the cells.

7.10.2. Immune response, cytokines

The release of four cytokines was found to be significantly affected after exposure to irregular MPs: IL-6, IL-8, MCP-1 and TNF-a (measured using an ELISA technique). In addition, gene expression of *IL-8* and *MCP-1* measured via qPCR, was found to be significantly altered (Figure 75). The lowest MP concentrations were found to affect the Caco-2 and PBMC cells (as shown in Table 34). The highest doses not to exhibit significant results are presented in Table 35.

Table 34. Lowest applied MP doses resulting in significantly altered cytokine responses after exposure to irregularly shaped MPs. ELISA technique used unless otherwise specified.

Cell model	**Cytokines**	**Polymer**	**Size (µm)**	**MP concentration**		**Duration (hours)**
				µg/mL	MPs/mL	
Caco-2	IL-8					
	MCP-1					
	IL-8 mRNA[a]					
	MCP-1 mRNA[a]					
		PS	0.4402	20	290197	96
PBMC		PS				24
	IL-6					
			15	1000	563068	
			50	100	1520	
			137.5	100	73	
	TNF-α					
		LDPE	50	500	8321	96
		PS	50	1000	15202	24

[a] polymerase chain reaction (PCR) analysis used.

Note: Caco-2, human adenocarcinoma cell line; IL-, interleukin; LDPE, Low-density polyethylene; MCP-1, Monocyte chemoattractant protein-1; PBMCs, peripheral blood mononuclear cells; PS, polystyrene; TNF-α, Tumour Necrosis Factor alpha

Table 35. Highest applied MP doses resulting in non-significant release of cytokines or alteration of their gene expression after exposure to irregularly shaped MPs. ELISA kit used unless otherwise specified. When more than one duration was used the longest duration of were included per experimental condition.

Cell model	**Cytokines**	**Polymer**	**Size**	**MP concentration**		**Duration (hours)**
				(μg/mL)	(MPs/mL)	
Caco-2						
	IL-1β					48
	TNF-α					
		PA6	72	1028.58[b]	n/a	
		PP	282	1305.5[b]	n/a	
		PU	253	1263.25[b]	n/a	
		PU	264	1098.02[b]	n/a	
	IL-8					
		PS				
			0.4402	1	14510	96
			22.1	20	3360	96
		PA6	72	1028.58[b]	n/a	48
		PP	282	1305.5[b]	n/a	48
		PU	253	1263.25[b]	n/a	48
		PU	264	1098.02[b]	n/a	48
	MCP-1					
	IL-8 mRNA[a]					
	MCP-1 mRNA[a]					
		PS				
			0.4402	1	14510	96
			22.1	20	3360	96
PBMC						24
	IL-6					
		LDPE				96
			50	500	8321	
			137.5	500	400	
		PS				
			15	100	56306	
			50	10	152	
			137.5	10	7	
	TNF-α					96
		LDPE				
			50	250	4160	
			137.5	500	400	
		PS				24
			15	1000	563068	

			50	100	1520	
			137.5	1000	727	

[a] polymerase chain reaction (PCR) analysis used, [b] dose in µg/cm^2. Note: Caco-2, human adenocarcinoma cell line; IL-, interleukin; LDPE, Low-density polyethylene; MCP-1, Monocyte chemoattractant protein-1; PA6, polyamide; PP, polypropylene; PS, polystyrene; PU, polyurethane; PBMCs, peripheral blood mononuclear cells; PS, polystyrene; TNF-α, Tumour Necrosis Factor alpha

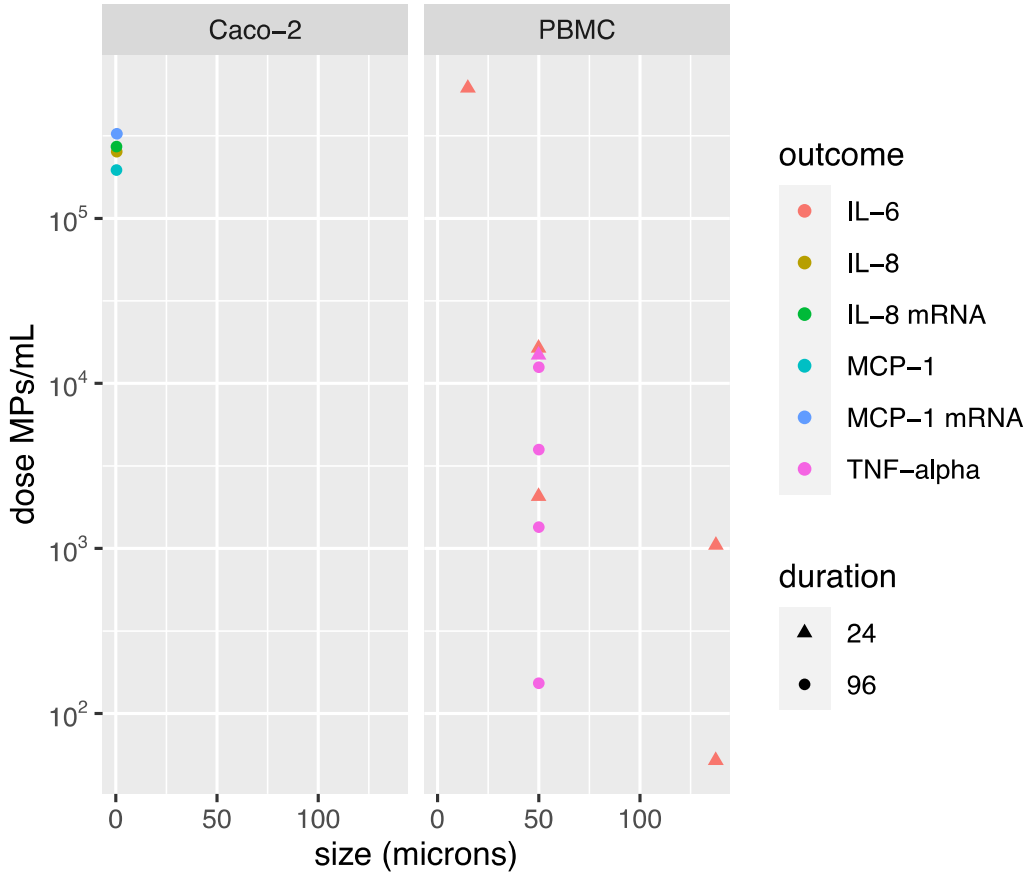


Figure 75. Applied MP doses that resulted in significant release (red colour) or alteration of gene expression (blue colour) of cytokines after exposure to irregularly shaped microplastics (MPs). Dose expressed in MPs/mL in $\log_{10}$ scale and MP size in µm. Note: IL-, interleukin; MCP-1, Monocyte chemoattractant protein-1; TNF-α, Tumour Necrosis Factor alpha.

7.11. Discussion

At the time this review was published (Danopoulos et al., 2021) it was the first rapid review focusing on MP toxicity on human cells and attempting a meta-regression approach to determine whether MPs are toxic to humans. A large number of recent reviews have examined the topic of MP toxicity with a broader scope, including animal *in vitro* and *in vivo* studies (Chang et al., 2020, Jacob et al., 2020, Kogel et al., 2020, Shi et al., 2021, Jeong and Choi, 2019, Rubio et al., 2020). Nevertheless, the scope of this review and meta-

regression is unique as the aim was to combine quantitative and qualitative data to inform the steps of hazard identification and dose-response within a risk assessment framework. Seventeen studies were included in the rapid review reporting on five biological endpoints: cytotoxicity, immune response, oxidative stress, barrier attributes and genotoxicity. Furthermore, seven studies were included in a meta-regression concerning cell viability (cytotoxicity) and four concerning cytokine release (immune response). The findings of this rapid review and meta-regression highlight that shape, origin, concentration and duration were the main drivers in cytotoxicity as measured by cell viability tests, while cells exhibited varying sensitivity to MP exposure. MP toxicity was linked to both physical and chemical effects across the different biological endpoints, but physical toxicity was prevalent.

7.11.1. Risk of Bias tool and overall quality of evidence

The bespoke MP-tox-RoB tool played a key function in the review process and meta-regression. Five out of the 17 studies were found to be of critical RoB and their findings have been excluded from the analysis, thus elevating the overall confidence in the findings. The tool can also be used in the wider setting of MP risk assessment in the stages of hazard identification and dose-response assessment. It is not a static but an intuitive grading tool that can adapt and follow the scientific evolution of MPs research. There was a great degree of heterogeneity observed in every aspect of the experimental design among the included studies. MP-tox-RoB can also be used by researchers as a guide for the design, execution and reporting of their project, thereby encouraging much-needed harmonization and standardization which is presently lacking and is greatly needed in all aspects of MPs research (Hartmann et al., 2019).

The overall certainty of the body of evidence was assessed guided by the GRADE framework (Higgins et al., 2021). The evidence was downgraded in the domain of RoB rating and was not downgraded regarding the four domains of heterogeneity/inconsistency of results, indirectness, imprecision and publication bias. In addition, the body of evidence was not found to meet the criteria for an upgrade according to the domains of large effects, dose-response or plausible confounding. Therefore, the overall certainty of the body of evidence was graded as low.

7.11.2. Polymer

PS was the most tested polymer, used by 12 studies, followed by PE and PP, each used in three studies. PVC was tested by two studies and all the remaining polymers (ABS, PA6,

PET, PU and TPU) were only tested by one study. Indeed, PS MPs have been found in abundance in the environment, especially in some atmospheric studies (Allen et al., 2019), but their popularity amongst toxicologists is not fully backed up by data. The polymers with the highest demand and distribution in the last decades (in Europe) have been PE, PP, PVC, PU, PET followed by PS (Plastics Europe, 2008, 2017, 2019, 2020). In the interest of examining more aspects of MPs contamination and targeting evidenced environmental exposures, more targeted polymer types must be examined. In the systematic reviews on MP contamination of food (Chapters 4 and 6) and drinking water (Chapter 5), the most abundant MP polymers as reported by 72 studies were PE, PP, PET and PA, the latter missing from the most popular list of the researchers. On the other hand, Lithner et al. (2011) attempted to rank the hazard of polymers based on the chemical composition of their monomers, ranking those exhibiting carcinogenic and mutagenic properties as the most hazardous. According to their findings the polymeric families of PUR, PAN, PVC, epoxy resins, and styrenic copolymers were the most hazardous. Since possible chemical effects from MPs are still under examination, testing of these specific polymers could inform us whether the effects of the monomers are still present in their descendent polymeric MPs.

It should also be noted that only five studies used a composition-identification method to either verify or identify the chemical composition of their test MPs. Two studies used Raman spectroscopy (Choi et al., 2020, 2021) and three used Fourier Transform Infrared spectroscopy (FT-IR) (Dong et al., 2020, Liu et al., 2020b, Wu et al., 2019). Along with pyrolysis, these are the three methods that are currently used by environmental MP studies as best practice to identify the chemical composition of particles that have been extracted from samples. There is currently an ongoing effort to create reference material for MP research in order to promote standardization between labs across the world. The use of these methods by toxicology studies (and report of the results) would assist in this process as well as promote transparency and reproducibility of their experiments.

The use of QA/QC measures are increasingly common practice in environmental MP studies but was completely absent in the toxicological studies. The combination of negative and positive control samples could be considered as a QA/QC measure to account for MP cross-contamination, regarding the outcome, but would not provide information on the possible distortion of the dose-response effect. The MP concentrations that have so far been used in the experiments are so large that additional cross contamination could be considered

negligible. In the future, as MP concentrations become lower, to better represent environmental exposures, the use of QA/QC will become increasingly important.

7.11.3. Morphological characteristics

The majority of MP found in nature are secondary MPs of irregular shapes, as evidenced by numerous studies in various environmental compartments (Burns and Boxall, 2018) as well as biota (Akoueson et al., 2020, Li J. et al., 2018). Spherical shapes are not absent, but they are the minority. In the interest of aligning actual environmental exposures and laboratory experiments, future MP toxicological research should be targeting secondary and irregularly shaped MPs rather that primary spheres. In addition, none of the studies tested MP fibres which is one of the most prevalent MP shapes found in the environment (Jenner et al., 2021, Huang et al., 2021). A further crucial aspect in using irregular MPs is that more and more studies hypothesise and have begun to verify, that the toxicological effects of MPs on cells are more physical than chemical. Shape is one the pivotal characteristics as highlighted by three studies in this review (Choi et al., 2020, Choi et al., 2021, Liu et al., 2020b). Liu et al. (2020b) further connected origin (secondary), shape and size with surface area and charge and the creation of a corona.

The only available characteristic connected to the origin of MPs was shape. Different weathering processes in nature and in the laboratory can affect MP characteristics such as porosity, shape, size, crystallinity, leaching and chemical properties (Sun et al., 2020), which may in turn affect their potential toxicity, unfortunately this level of detail was not available in the papers under review. All the secondary test MPs used by the studies were of irregular shape and produced in-house by either a variation of the ball milling method or digestion. Overall comparison between the methods was not possible in meta-regression, since the three included studies (Choi et al., 2020, Choi et al., 2021, Stock et al., 2021) that used secondary, non-spherical MPs, all produced them via ball milling. Furthermore, the level of detail that would be needed to review the methods' specification and to compare the physicochemical characteristics of the produced secondary MPs was not available by all studies. This is an important area that must be explored as more data become available.

The relationship between the origin and the shape of the test MPs was evident in every part of the synthesis and analysis. Including both covariates of origin and shape in the same regression model for cell viability was not possible due to multicollinearity. A series of models fitting the covariates consecutively revealed that shape was a better predictor that

origin. Out of the two shapes of secondary origin, only one produced significant results. The meta-regression findings on the cell viability results support the hypothesis that shape is one of the drivers of the exerted toxicity. The regression coefficient estimates of only one out of the three MP characteristics that were explored (polymer, size, shape) was found to be statistically significant. Irregular shape, as compared to spherical shape had the largest effect size (β=5.913) with the highest significance ($p< 0.001$), followed by two experimental conditions of duration (β=0.02, $p< 0.01$) and MP concentration expressed in μg/mL (β=0.00005, $p< 0.01$) and then the type of cell model (seven out of ten, see section 3.5.2.1). This trend was also discovered in all-subset and in multilevel modelling. The toxicity mechanism related to shape is discussed in section 4.5. On the other hand, cytokine release meta-regression modelling found that only MP concentration (μg/mL) and duration were the significant experimental characteristics as predictors of the outcome. The trend of the association between irregular shaped MPs of secondary origin and the outcome was still detected but it was not significant. In the cytokine release model experiments, the masking between origin and shape was complete and the disentanglement of the covariates was not possible.

The other striking finding of the meta-regression models was that the size of the test MPs was not a significant predictor of the outcome for both biological endpoints of cytotoxicity (cell viability) and immune response (cytokines release). Contrary to these results, four studies included in the review argued that the toxicological effects were somehow size-dependent (Hesler et al., 2019, Hwang et al., 2020b, Schirinzi et al., 2017), while one study further connected MPs size with surface area (Brown et al., 2001). Nevertheless, it should be noted that all the former studies tested only primary spherical MPs, further highlighting the need for testing secondary, irregularly shaped MPs to produce more representative, and environmentally relevant results.

Regarding MP size, there is scientific evidence, beyond human studies, that MPs < 20 μm could enter and translocate in the tissue of a wide range of biota (Hale et al., 2020), while others argue that particles of sizes < 150 μm are expected to be able to pass the human gut barrier and cause systemic exposure with limited absorption (≤ 0.3%) and only even smaller particles < 1.5 μm to have the ability to translocate to other organs (EFSA, 2016). Recent studies analysing human sample tissue reported the discovery of MPs in ranging sizes. In human colectomy samples, the size of identified MPs ranged from 800 to 1600 μm (Ibrahim et al., 2021), in human placenta from 5 to 10 μm (Ragusa et al., 2021) and in human lung

tissue from 1.6 to 5.58 μm (Amato-Lourenço et al., 2021). The differences in sizes could be attributed to the physiology of the tissues. This initial data on the size of MPs could guide the MP size ranges tested for toxicity.

7.11.4. Doses and relevance of environmental exposures

Only four out of the 17 studies referenced data produced by MP environmental studies to estimate the MP concentrations used in their experiments. There is currently an abundance of scientific data on the level of MP contamination on a wide range of environmental media, to which humans can be indirectly and directly exposed to, coming from primary studies, reviews, systematic reviews, meta-analyses and modelling. There is no reason for study designs not to use the data already available in the literature to inform their study designs.

Since all the experimental doses used in the studies included were administered directly on cells or cell models, the doses refer to internal or even target doses (see section 3.7.4.5). Six studies applied doses of MP concentrations in the range of 1000 and 100000 μg/mL which practically correspond to doses of several hundreds or even several millions of MPs particles, depending on the particle size. There is no scientific evidence to support such kinds of exposures, unless examining life-long exposures, which would then fundamentally alter the study designs in terms of durations. According to the MP exposure assessment, based on the results of the systematic reviews (see section 8.3.4), maximum annual MP exposures from consuming only two food categories (seafood and salt) and drinking water can reach up to 3.6 million particles, which are potential doses. Applying the average density of the test MPs (1.1 g/cm^3), used by studies herein, and assuming spherical shape, that level of annual exposures can be transformed to a dose of around 250 μg/mL of 5 μm sized MPs, or 250000 μg/mL of 50 μm MPs, which was the size of the test MPs averaged across all studies (48.5 μm). The level of these doses must be modified to represent not potential but internal doses. Scientific evidence is not available at this time on MP toxicokinetics in the human body but paradigms from other contaminants could potentially be applied (Dixit et al., 2003). Internal doses are unlikely to be greater than such potential doses, and the latter can be used, provided this caveat is made clear, as a starting point for determining the MP concentrations used in toxicological experiments.

The range of doses tested for the cell viability and cytokines release (Figure 57 - Figure 58 and Figure 67 -Figure 68, respectively) reveal further limitations of the currently available data. Disregarding polymer type, the cell viability doses (included in meta-regression modelling)

ranged in size from 0.1 μm to 137.5, but the majority of tests used the smaller sized MPs. One third of the tests (34%, 104 out of the 310 data points) involved test MPs in the range between 0.1 and 10 μm and although they used MP concentrations of 0.01 to 50000 μg/mL, 73% of the tests applied doses up to 100 μg/mL. Similarly, in the cytokine release tests although test MPs ranged from 0.4402 to 137.5 μm in size, almost half of them (46%, 62 out of 136 data points) used MPs up to 10 μm, and 71% of this fraction (44 of 62 data points) used doses up to 100 μg/mL. It is understandable that there a limit to the number of tests each study can execute and analyse connected to timeframes and available resources, nevertheless, in the future it would be useful that studies would target doses (MP sizes and concentrations) that have not been already tested by other studies in order to have a fuller picture of potential exposures. These data might also help us understand if indeed there is a break in the linear relationship between concentrations and outcomes that has been identified in a few studies regarding the cytotoxicity results, or if it is an artefact.

The conversion of the concentrations to MPs/volume or mass is necessary in order to establish two key parameters. Firstly, whether the concentrations used in the experiments were environmentally relevant in terms of the level of exposure (for a specific duration of exposure) and secondly whether these exposures are exceeded and under what circumstances. The reason that the conversion is necessary is that the majority of environmental studies that provide evidence of MP concentrations in various media use the MPs per volume or mass metric (Connors et al., 2017, Burns and Boxall, 2018). Attempting the conversion of the data coming from environmental studies is not feasible as the MPs extracted from the environment are a mixture of polymers with different chemical characteristics varying in size and shape. Details at that level are not available in environmental studies. This is a shortcoming that has been widely recognized and will be hopefully tackled in future research (Koelmans et al., 2019, Miller et al., 2021, Burns and Boxall, 2018).

7.11.5. MP mechanisms of toxicity and thresholds of adverse effects

Little information is available on the underlying toxicity mechanisms and the experimental conditions that drive MP toxic effects. Two recent reviews (Banerjee and Shelver, 2021, Yong et al., 2020) that focused on MPs (0.1 μm–5 mm diameter) and NPs (< 0.1 μm diameter) using human and animal *in vitro* and *in vivo* studies concluded that size, MP concentration, surface charge and duration were related to MP uptake and cell toxicity with varying effects amongst different mammalian cell models. Banerjee and Shelver (2021) also

reported that cell death mechanisms could be attributed to ROS generation, DNA damage and autophagy but pointed out that these mechanisms are interrelated and might trigger each other. Prüst et al. (2020), focusing on neurotoxicity, proposed that factors that could affect the potential toxicity (besides MP concentration and duration) was the temperature at which the exposure takes place, as well as the MP characteristics of size, hydrodynamic diameter and shape, affecting uptake, particle aggregation and surface area/internalization capacity, respectively. Different mechanisms have been proposed by the studies included in the current review. The heterogeneity of the test MPs, cell models and other experimental conditions do not allow a direct comparison. Nevertheless, MP shape is highlighted as an important MP characteristic in exerting toxicity (cell viability) by both narrative analysis and meta-regression. The shape of MPs has been hypothesised to affect cell behaviour and viability either directly or indirectly. There are different mechanistic level biochemical and physicochemical effects proposed. Rugged or even sharp shaped MPs can directly damage cell membranes upon contact, elucidating adverse effects (Choi et al., 2021). Shape, also related to surface area and surface charge, can affect MP movement, the relationship between MPs and between MPs and biological barriers, thus indirectly affecting cells. Surface charge can cause the MPs to aggregate resulting in particle agglomeration, effectively increasing their size and surface areas which in turn could affect cell uptake directly or indirectly by altering the electrostatic forces between MPs and cell membranes (Liu et al., 2020b). Agglomeration, which is more related to smaller sized MPs (< 0.5 μm), and movement are also affected by Brownian motion which is, in turn, depended on MP shape and size (Rist and Hartmann, 2018).

Wright et al. (2013) highlighted that the potential MP-induced adverse effects on the cellular and tissue level would vary according to MP shape; while also affecting MP uptake by marine organisms. Cellular shape-related effects were attributed to increased cellular uptake and the consequent apoptosis (Xinglu Huang et al., 2010). The contribution of MP shape to toxicity has also been explored in animal *in vivo* studies. Au et al. (2015) found that PE MPs (powder) were significantly less toxic to *Hyalella azteca* than PP fibres following acute exposures. Xia et al. (2021) reported that irregularly shaped secondary PVC MPs were more toxic to *Oryzias melastigma* embryos than primary PVC MPs in powder form. The importance of shape has also been highlighted by an ecological risk assessment study as follows. Jung et al. (2021), synthesised data from 32 *in vivo* animal studies, examining apical endpoints of toxicity on aquatic organisms, reporting that small (< 20 μm) non-spherical MPs may exert higher chronic ecotoxicity impacts than spherical MPs.

The paradigm of asbestos could offer some additional insight regarding the MP mechanisms of toxicity with respect to shape. Although the chemical composition of asbestos and MP particles is not similar, there is an overlap in the size ranges, they are both highly bio-persistent compounds, and a notable proportion of MPs are fibres. The size of the biologically critical asbestos fibres is considered as ≥ 5 μm, with a diameter ≤ 3 μm (WHO, 2000). A recent study by Amato-Lourenço et al. (2021) identified MPs in human lung tissue of 13 of the 20 cadavers that were autopsied. The mean particle size was 3.92 μm (±0.67) and the mean fibre length 11.23 (±1.96) μm. The majority of the MPs identified in the lung samples were fragments (87.5%) and the remainder, fibres (12.5%). While the underlying mechanisms of asbestos induced toxicity has been researched for decades, there are still significant knowledge gaps (Kuroda, 2021). Asbestos has been linked to various diseases of the lung, with cellular injury (and the consequent generation of oxidative stress) and inflammation response to exposure cited as the two initiating toxic mechanisms (Manning et al., 2002) (Brown et al., 2001, Dong et al., 2020, Goodman et al., 2021). On finding MPs in human lung tissues, Amato-Lourenço et al. (2021) proposed that MPs interaction with epithelial cell or macrophages could trigger pro-inflammatory effects. Relevantly for this review, the complex interaction between asbestos and cells/tissue is affected not only by dose and exposure duration, but also size, shape, chemical composition, the presence of metals, surface reactivity and crystallinity as well as bio persistence (Sanchez et al., 2009). The shape of fibres affect not only their potential to be inhaled, reach and remain in the lower parts of the lungs, but also their interaction and detrimental effects on macrophages, leading to long-term sustained inflammation (Manning et al., 2002). While MPs do not share the same toxicological profile as asbestos, lessons learned can be used to examine the findings herein that shape is an important component of MP toxicity.

In terms of LOAELs and NOAELs, different concentrations were effective for different biological endpoints and different cell models as summarised in the tables of section 7.10. Regarding quantitively assessed tests, doses using MP concentrations as low as 10 μg/mL had an adverse effect on cell viability and as low as 20 μg/mL on cytokine release, for irregularly shaped MPs. Oxidative stress effects were identified at doses of MP concentrations of 200 μg/mL and 1000 μg/cm^2 of spherical PS MPs. The highest MP concentration tested for histamine release with no observed effect was 1000 μg/mL of spherical PE MPs and the highest MP concentration for the genotoxicity biological endpoint with no observed effect was 10 μg/mL of spherical PS MPs. MPs uptake, examined qualitatively, was found to occur for only spherical MPs up to 5 μm in size. It should be

noted that only one study (Stock et al., 2021) also analysed cellular uptake using non-spherical MPs, but used a different size range (> 60 μm). Barrier integrity was reported to be affected after exposure to spherical PS MPs at MP concentrations as low as 10 μg/cm^2.

7.11.6. MP and human health effects; future risk assessment

The present and, arguably, the future of applied risk assessment and risk analysis is combining the best available scientific data coming from multiple studies, since commissioned, targeted studies are not always feasible or appropriate. Systematic reviews, rapid reviews and meta-analysis methodology is a very powerful and reliable tool which can be used to that end (NASEM, 2021). Nevertheless, the reliability and applicability of a systematic review is only as good as the studies it includes (Higgins et al., 2021). Unfortunately, in the present work, the overall certainty of the body of evidence was graded as low. In addition, none of the studies included in this review made their full data available. This omission has prohibited the execution of a meta-analysis and has limited the power of the meta-regression.

The outcome data that were used in the analysis were quantal (binary), therefore, information was only available on one degree of effect regarding the chance of incidence for each experimental exposure, thus limiting the understanding of the effects (IPCS, 2009). On the other hand, if raw data were made available, it could provide vital information on how the degree of effect changes when exposure characteristics change, providing a more comprehensive picture of the relationship. It is possible that the variability of the tests used for cell viability may have affected the summary of evidence, since there is no inter-comparability mechanism that can evaluate differences in the tests' sensitivity.

All the toxicological studies have been carried out under controlled conditions, in order to extrapolate from laboratory experiments to real-life environmental conditions, and from cell-based effects to system-based or whole organism effects. A series of adjustments must therefore be made within the risk assessment process. The intrinsic characteristics of MPs cause a further limitation of laboratory-based toxicological experiments as follows. MPs are detected in the environment/foodstuffs as a mix of polymers, so single-polymer exposures are not environmentally relevant. It also is known that MPs can absorb and later sorb various toxic substances (such as hydrophobic organic chemicals) (Hartmann et al., 2017) as well as additives (plasticisers) that have been added during production (e.g. bisphenol A) (Chang et

al., 2020) thus exerting synergistic toxicological effects, that are at this moment under examination (Hale et al., 2020).

7.12. Chapter conclusions

MP contamination is on the verge of being established as MP pollution. A risk analysis is essential in understanding the extend of the issue in terms of adverse effects posed to humans. In the absence of epidemiological data, *in vitro* toxicology studies can be used to delineate the molecular initiating event and the consecutive key events that lead to adverse effects in an adverse outcome pathways framework. This first rapid review has synthesised and appraised currently available data using a novel RoB tool. MP adverse effects in human cells have been confirmed by the majority of studies regarding four out of the five biological endpoints included in this review. Specifically, effects were reported concerning cytotoxicity, immune responses, barrier attributes and oxidative stress, although not always corresponding to environmentally relevant MPs regarding origin, shape and concentrations. Of the various MP characteristics explored, shape was found to be the single characteristic that significantly affects the cytotoxicity outcome. Out of the 10 different cell models used in the cell viability experiments, Caco-2 cells exhibited the highest association to MP effects. Furthermore, the experimental conditions that significantly affected both cytotoxicity and the induction of immune responses were MP concentration (μg/mL) and duration of exposure. Further physicochemical properties of the MPs under examination are needed to produce a fuller and more robust toxicological profile.

A series of recommendations on the design and conduct of future research will benefit upcoming risk assessments and the understanding of MP-related health effects in humans. Recommendations for future MP toxicological studies:

- Use of environmentally relevant doses based on data coming from MP environmental studies, e.g. below 250 μg/mL of 5 μm sized MPs, or 250000 μg/mL of 50 μm MPs corresponding to annual potential doses.
- Target doses (size and concentrations) that have not been the focus of testing to date (e.g. doses > 100 μg/mL for MPs < 10 μm and all environmentally relevant doses for MPs > 10 μm).
- Include secondary and irregularly shaped MP (not simply primary MP spheres for convenience of procurement)

- Test polymers that have been found to be prevalent in environmental samples/foodstuffs
- Use of FT-IR, Raman or other verified method to identify the chemical composition of the test MPs
- Use of QA/QC measures during and after experiments to verify results
- Use of the MP-tox-RoB as a set of guidelines for study design and reporting results
- Report the origin and characteristics of test MPs and cell models
- Report full data results (perhaps also lodged in a shared international repository) including
    - Number of repeated tests per experimental condition
    - Number of replicates
    - Cell density per experimental condition

More research is always needed to confirm existing results and complete the evidence gaps and the results of this rapid review and meta-regression can be used to guide future efforts. For instance, from the key findings herein, irregular shapes have biological impact, size is critical, and minimum doses of 10 μg/mL (5-200 μm) and 20 μg/mL (0.4 μm) resulted in cytotoxicity and caused immune responses, respectively, indicating that thresholds of effects are much lower than previously expected.

Chapter 8. Risk assessment

In this chapter the results of the scoping, systematic and rapid reviews, along with their meta-analysis and meta-regression components (as reported in Chapters 2 and 4-7) are synthesised to inform the four steps of the risk assessment.

8.1. Hazard identification

This is the initial step of the risk assessment where the nature of the possible health hazards is identified along with the circumstances under which they may occur (3.5.1). This step is informed by the results of the ScR on MP human health effects (section 2.2.1) and the results of the rapid review on *in vitro* MP toxicity on human cells (Chapter 7).

8.1.1. MP health effects scoping review- summary findings

In this section, a summary of the findings of the MP human health effects ScR is provided. The full analysis of the ScR and the appraisal of the included studies can be found in section 2.2.1. The ScR had a wider scope than the subsequent systematic and rapid reviews (Chapters 4-7), in that they included all possible routes of contamination, particles of a smaller size range than MPs, as well as evidence coming from animal studies (within reviews). The summary of the findings is presented here in order to provide an overview of the current understanding of MP hazard identification in direct relation to human health. The ScR included 20 studies, eight primary studies (three on human cells, three on dust, one occupational and one food study), 11 reviews and one opinion article.

With regards to the three human cell studies (see section 2.2.1.1), Schirinzi et al. (2017) reported that MPs did not have a significant effect on cell viability. Similarly, Magrì et al. (2018) did not find toxic effects in the short term (albeit a time span that was not defined), but argued for possible long-term effects and reported that NPs can cross the gut barrier. Mishra et al. (2018), on the other hand, reported specific genotoxic and cytotoxic effects associated to NPs. All three studies used different particle concentrations (that varied from 0.05 to 100 μg/mL) and sizes, thus making comparisons between the studies difficult. Only one of the cell studies (Schirinzi et al., 2017) was included in the subsequent rapid review (Chapter 7) based on the eligibility criteria (section 3.4).

Two of the airborne dust studies reported on a contribution of MPs to morbidity and toxicity via the inhalation and ingestion uptake routes (Abbasi et al., 2018), and an association between exposure to phthalates with asthma and allergies (Sun et al., 2017). The third dust

study reported that neither non-cancer nor cancer risk was found to be increased by PAEs (Lijun Wang et al., 2017). The occupational study focused on female workers in the synthetic textile industry considering non-dietary ingestion exposures (Gallagher et al., 2015). The prevalence of cancer in the sample population revealed that exposure to synthetic fibre dust for long durations (> 20 years) can increase stomach cancer risk. Finally, the food study (salt), reported that the number of identified MPs is too low to cause any significant impact to human health (Karami et al., 2017a). This food study was also included in the systematic review on MP contamination of salt (Chapter 4).

Eleven reviews were included in the ScR (see section 2.2.1.2). The review by Wright and Kelly (2017) reported on a wide range of health effects including "inflammation, genotoxicity, oxidative stress, apoptosis, and necrosis" (2017: 6640), which, in turn, can lead to "tissue damage, fibrosis and carcinogenesis" (2017: 6640). They also reported that MPs can serve as vectors for chemicals and pathogens to enter the human body. Vethaak and Leslie (2016) reported that lung and gut injury, cell damage, chemical bioavailability enhancement and infection by pathogens are possible health effects. Karbalaei et al. (2018) concluded that carcinogenicity and reproductive abnormalities as well as alterations in liver, reproductive and brain function, obesity and cardiovascular disease are possible health effects. da Costa et al. (2016) stated that reproductive disorders attributed to chemicals absorbed by MPs are possible effects. Sharma and Chatterjee (2017) stated that the prolonged use of personal care products that include MPs will ultimately cause skin damage and that the ingestion of MPs "can cause alteration in chromosomes which lead to infertility, obesity and cancer. In case of women, estrogenic mimicking chemicals can cause breast cancer." (2017: 21542). Gallo et al. (2018) reported a variety of health effects: "DNA damage, changes in gene and protein expression, cell clotting, necrosis, apoptosis, proliferation and loss of cell viability, oxidative stress, increased Ca ions, inflammation and bone osteolysis, to lesions in organs" (Gallo et al., 2018: 7). Kole et al. (2017) mentioned the health effects reported by Wright and Kelly (2017). Karbalaei et al. (2018) did not connect BPA health effects to evidence related to MPs.

Four reviews focused on the uptake of MPs specifically through the food chain. Bouwmeester et al (2015) stated that the potential impact could not be evaluated at that time. Waring et al. (2018) cited the effects reported by Schirinzi et al. (2017) and Wright and Kelly (2017). Smith et al. (2018) also cited Wright and Kelly (2017) as well as Lusher et al. (2017) and the second GESAMP (2016) report, stating that the possible health effects include

“cardiopulmonary responses, alterations of endogenous metabolites, genotoxicity, inflammatory responses, oxidative stress, effects on nutrient absorption, gut microflora, and reproduction” (2018: 381).

The two studies focusing on the inhalation uptake route reported respiratory symptoms, increased cancer risk in relation to synthetic fibre dust, PVC and VC exposure, interstitial lung disease, flock's disease, restrictive lung disease and undifferentiated airway and interstitial lung disease (Prata, 2018) and chronic respiratory symptoms, pulmonary disease and interstitial lung disease (Sauler and Gulati, 2012).

Looking at the reviews taken together, the almost complete lack of reporting on their methodology and methods makes room for unaccounted bias and impacts on their credibility as they cannot be replicated (see section 2.2.1.2). In many cases, extrapolation to human health effects was made from surrogate animal and environmental studies without acknowledging the lack of evidence to support them. There is a clear need for further, scientifically- and methodologically robust research in this area. Systematic review methodology can be used to synthesise existing scientific evidence in order to draw safer, more robust, conclusions on the possible human adverse effects from exposures to MPs. To this end, the rapid review on MP toxicity on human cells was executed; the full results are provided in Chapter 7.

8.1.2. Rapid review on MP human cell *in vitro* toxicity; MPs toxicodynamics

In the absence of epidemiological evidence, animal and *in vitro* studies can be used to draw inference for possible human health effects. In the first instance, human cell *in vitro* studies were the focus of this risk assessment. Animal *in vivo* and *in vitro* studies, although very important in a risk assessment process, were not explored due to resource limitations. The rapid review process identified 17 MP toxicological studies that used human cells. Five different biological endpoints were tested: cytotoxicity, immune response, oxidative stress, barrier attributes, and genotoxicity. All biological endpoints were found to be affected by MP exposure apart from genotoxicity. This does not necessarily mean that MPs cannot, or will not, exert genotoxic effects on human cells, but that the currently available scientific evidence (coming for the studies included in this review) do not support it. The details of the analysis and the results are found in Chapter 7.

Regarding toxicodynamics, evidence from the rapid review suggests that different mechanisms of toxicity might be involved. Both physical and chemical effects were related to the toxicity exerted (see section 7.9). According to the meta-regression analysis, adverse effects at the cellular level were found to be concentration- and duration-dependent for both cell viability and immune responses. Furthermore, cytotoxicity was found to be affected by the shape of the MPs, where irregularly shaped MPs were identified as significant predictors of cell death as compared to spheres and powders (see section 7.5.2.1). Another important discovery was that the 10 different cell models used for the cytotoxic experiments exhibited varying sensitivity to MP exposure. The same trend was not found in the immune responses analysis. Effects were exerted at different levels of exposure and for different sizes as discussed in section 8.2.

8.1.3. MPs toxicokinetics

Toxicokinetic evidence on how MPs pass through the human body is not available at this time. Paradigms from studies on plastic material that have been used for orthopaedic replacement prosthetics have demonstrated translocation of plastic particles to organs such as the liver, spleen and lymph nodes (Hicks et al., 1996, Urban et al., 2000, Minoda et al., 2003, GESAMP, 2015b). The effects of MPs are hypothesised to depend on their size, polymeric composition, additives (plasticisers), the chemicals that they might have absorbed from the environment, their chemical state and where they are located in the human body (Wright and Kelly, 2017, GESAMP, 2016).

The gastrointestinal (GI) system is composed of the organs that form the GI tract, as well as the pancreas, liver and the biliary system. The location of interest for the delivery of MPs is the GI system itself as well as further locations beyond the gut barrier; the principal site within the main digestive surface is the small intestine (Keshav et al., 2013). The absorption rate of MPs is likely to be affected by factors such as the size and shape of the particles, type of polymer, surface charge, hydrophilicity, the presence of food or other chemicals and the environment of the GI tract (e.g. gut bacteria) (EFSA, 2016, Galloway, 2015, Timbrell, 2009). As noted in the exposure assessment methods section (3.7.4.5), only MPs with a size < 150 μm are expected to be able to pass through the gut barrier with a limited expected absorption of $\leq 0.3\%$, while even smaller MPs (< 1.5 μm) are expected to be able to translocate to other organs (EFSA, 2016). Different mechanisms have been proposed to facilitate MPs crossing the gut barrier including phagocytosis and endocytosis, for particles with maximum sizes of 22 μm and 0.5 μm, respectively (EFSA, 2016, Timbrell, 2009). The

intestinal permeability can be further affected if intestinal barrier function has been compromised by gastrointestinal diseases and disorders such as inflammatory bowel disease (IBD), irritable bowel syndrome, non-alcoholic fatty liver disease (NAFLD) etc. (Vancamelbeke and Vermeire, 2017).

Special mention should be made regarding the potential for MPs to bioaccumulate. The term `bioaccumulation' refers to dissolved chemical contamination and is a term that has also recently been applied in MP research (Rochman et al., 2019). The term is used to describe the accumulation of a contaminant in an organism which occurs when the exposure and consequent uptake is larger than the amount that the organism is able to egest. Unfortunately, there is evidence that MPs can bioaccumulate in marine organisms and specifically within each trophic level (Miller et al., 2020). Indeed, this raises questions about the potential for bioaccumulation in the human body, which in turn shifts the focus of the risk assessment towards examining possible life-long exposures leading to cumulative internal doses and consequent effects.

8.2. Hazard characterization/ dose response

The first assumption that has to be made before using a dose-response relationship is establishing, within reasonable certainty, that there is a causal relationship between the agent and the effect (Klaassen et al., 2013); meaning that the response is the result of the exposure to the hazard. This can be difficult when the data come from epidemiological data where numerous factors, often confounding, must be considered and disentangled. In the case of *in vitro* experiments, establishing the causal relationship is more straightforward. The domain on QA/QC and confounding (section 3.4.2) that was used to appraise the toxicological studies addressed these issues (Chapter 7). The second assumption is that there is a relationship between dose administered and the magnitude of the response. The underlying assumptions are that there is an initiating molecular target site, that the dose at the target site is related to having a response and its degree, and that the dose at the target site is related to the administered dose (for *in vivo* assessments) (Klaassen et al., 2013). The traditional dose-response relationship can be violated by endocrine-disrupting chemicals which exhibit non-monotonic dose-response curves (Vandenberg et al., 2012). In other words, they might exert effects at low doses and not at high doses (Klaassen et al., 2013). The monomer, BPA, that was regularly used as a plasticizer has been proven to be such a substance (Vandenberg, 2014). Therefore, the possibility of non-monotonic MPs dose-response curves cannot be

excluded. MPs, as with other toxicants, have a family of dose-response relationships, one for each biological endpoint under examination.

In order to decide which were the appropriate methods to be used for the dose-response assessment and modelling, the six steps proposed by the IPCS (2009) for the risk assessment of chemicals (section 3.7.3) were employed. Initially, two approaches were examined: the BMD and the NOAEL approach. The available data for dose-response modelling were identified via a rapid review and meta-regression (Chapter 7) focusing on human cell MP *in vitro* toxicology experiments.

A series of issues was identified after the analysis of the data. Due to missing information, it was not possible to plot dose-response curves, as sample sizes and the results for each sample were not reported by the studies (section 7.2.1). The meta-regression analysis for the cytotoxicity (cell viability) endpoint findings were that test cell model, shape of the test MPs, dose and duration were the predictors of cell death. Conceptually, this means that, in using the data for further dose-response modelling, grouping must be done to address these different characteristics. Consequently, further datasets were created to test whether they would be sufficient for modelling. For example, a dataset was created with the cell viability tests on Caco-2 cells, using irregular test MPs, for 24-hour exposures and excluding the critical RoB rated studies. The dataset had further variability in other characteristics as it included four different types of polymers (PE, PET, PP and PVC) each having a unique particle size. Doses expressed in μg/mL ranged from 100 to 100000 but as different polymers and particles sizes were used within the same study, the doses were repeated for each polymer, resulting in a distortion of the results. In order to work around this problem, since polymer type was not found to be an important predictor of cell death, concentrations expressed in MPs/mL was used instead and polymer type was dismissed. This resulted in a data set with 26 data points, only three of which had statistically significant results. Furthermore, the relationship was non-monotonic, as described in section (7.10.1).

There was an attempt to fit dose-response modelling in R (version 4.1.1) (R Core Team, 2019) using RStudio (version 1.2.1335) and the additional package drc (Ritz and Streibig, 2005). A series of different models were fitted: two-parameters and three-parameters log-logistic and Weibull models, which are appropriate for the binomial responses (dependent variable) of the dataset (Ritz et al., 2016). Due to the very small size of the available data, missing information and the presence of non-monotonic relationships, it was decided that

dose-response modelling using the BMD approach was not appropriate for this dataset. This decision coincides with the EPA (2012) recommendations for the minimum dataset criteria necessary for calculating a BMD. It is anticipated that this analysis will be feasible in the future.

Consequently, the NOAEL/LOAEL approach was explored. This approach has lesser demands on data but also yields more constrained results (section 3.7.3). At least one dose with an effect and one without one is required. Examining the data again derived from the rapid review (Table 21), out of the five biological endpoint that were identified, effects were observed on four: cytotoxicity, immune response, oxidative stress and barrier attributes. The quantitative synthesis of the data was executed in a statistical summary for all endpoints (section 7.10), while meta-regression was executed on a subset of the data for cell viability (cytotoxicity) (section 7.5.2) and for immune responses (section 7.6.2). The quantitative synthesis was informed by the narrative analysis in both cases.

According to the rationale of the statistical synthesis (section 7.10), only the results of the non-spherical MPs were included, and LOAELs could be defined for only certain endpoints of cytotoxicity and barrier integrity (Table 32) and immune responses (Table 34). NOAELs could not be defined for the cytotoxicity endpoint due to the non-monotonic dose-response relationships that were identified (section 7.10.1).

On the other hand, linear relationships were found for the immune responses' endpoints. Regarding the experiments on Caco-2 cells, only two doses (1 and 20 μg/mL) were tested for two sizes (0.44 and 22.1 μm) of PS MPs for each of the four biological markers/tests used, so it would not be reasonable to base a NOAEL on them. Lehner et al. (2020) also used Caco-2 cells, but they expressed the MP concentrations in $\mu g/cm^2$ and all results were not significantly different from the control samples. In the case of the PBMCs, two sizes (50 and 137.5 μm) of LDPE MPS were tested, applying four doses for each of the two biological markers that were explored (IL-6 and TNF-α) and three sizes of PS MPs (15, 50 and 137.5 μm), testing three or four doses for the same markers (Figure 76). The results for the LDPE MPs included a significant result for one of the doses (500 μg/ML, 96h) and not significant results only for the remaining three (10, 100, 250 μg/mL, 96h), for only one of the two tested MP sizes (50 μm) and for one of the two tested markers (TNF-α). The results for the PS MPs included both results for all sizes for the IL-6 marker and for one size (50 μm) for the TNF-α marker, as shown in Figure 76.

The threshold values are used to determine the CrEf, which is the effect or the precursor of an effect that occurs at the lowest dose/concentration for each of the two biological endpoints. The threshold values are compared to the exposure levels to determine whether they can be exceeded and thus causing an adverse effect, posing risk to health.

For the derivation of heath-based values/reference doses (RfD), according to Equation 3, a correction UF of 10 needs to be applied to account for variability between humans (see section 3.7.3). The results of the application of the UF for cytotoxicity biological endpoint are presented in Table 36. For this endpoint, the calculation of a NOAEL is not appropriate as discussed above. Regarding the immune responses data, two UFs will be applied, one 10-fold for human variability and a further 10-fold to calculate the NOAELs as shown in Table 37.

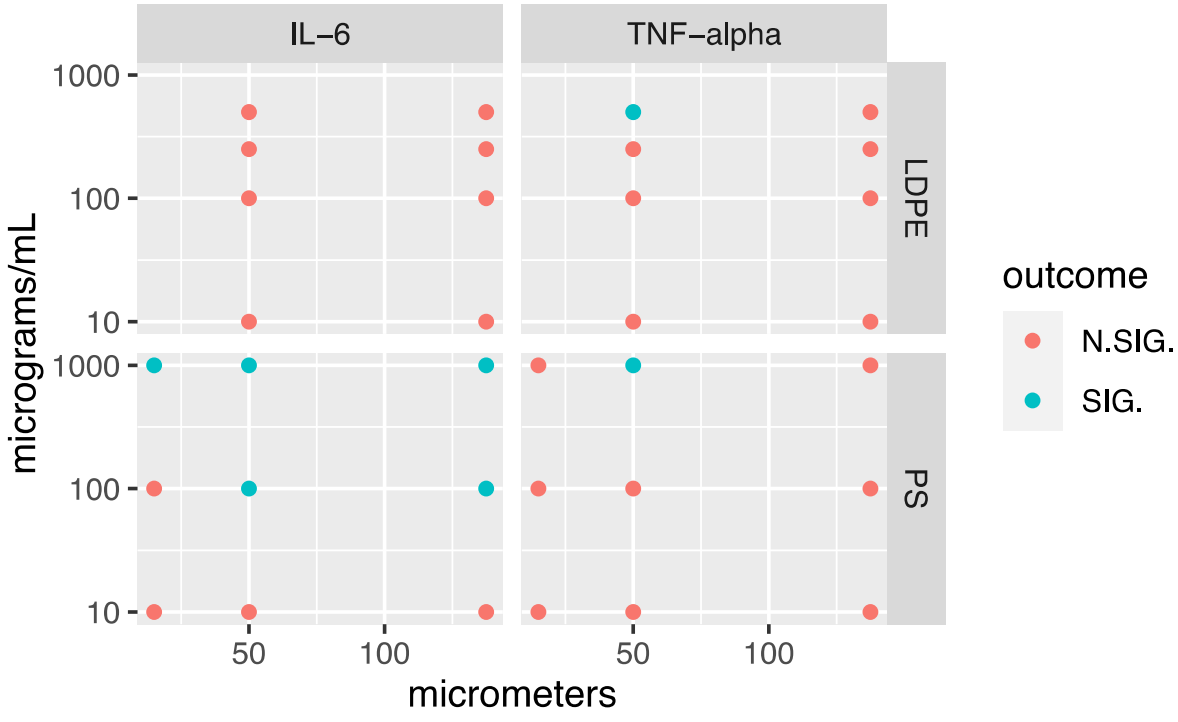


Figure 76. Thresholds for immune responses to polystyrene (PS) and low-density polyethylene (LDPE) MPs. N. SIG., not significantly different and SIG. significantly different from the control sample. Mg/mL expressed in a $\log_{10}$ scale.

Table 36. MP reference doses for human cell viability effects. Lowest applied non-spherical microplastic (MP) doses resulting in significant reduction of cell viability after exposure to irregularly shaped MPs corrected for human variability.

Cell model	Test	Polymer	Size (µm)	MP concentration		RfD	
				µg/mL	MPs/mL	µg/mL	MPs/mL
Caco-2							
	MTT	PP	67.1	10000	70241	1000	7024.1
	Caspase-8	PP	67.1	50000	351205	5000	35120.5
	MTT	PVC	136.5	75000	40228	7500	4022.8
HDF	CCK-8						
		PS					
			15	10	5630	1	563
			50	10	152	1	15.2
			137.5	10	7	1	0.7
		LDPE					
			50	1000	16643	100	1664.3
			137.5	1000	800	100	80
HeLa	CCK-8	PS					
			15	10	5630	1	563
			50	10	152	1	15.2
			137.5	10	7	1	0.7
HepaRG	MTT	PVC	136.5	100000	53638	10000	5363.8
HepG2	MTT	PE	90.1	50000	138889	5000	13888.9
KATO III	CCK-8	PS					
			15	100	56306	10	5630.6
			50	100	1520	10	152
PBMC	LIVE/ DEAD kit						
		PS					
			15	100	56306	10	5630.6
			50	100	1520	10	152
			137.5	1000	727	100	72.7
		LDPE					
			50	500	8321	50	832.1
			137.5	250	200	25	20

Note: Caco-2, human adenocarcinoma cell line; CCK-8, cell counting kit 8; HDFs, human dermal fibroblasts; HeLa, cervical cancer cells; HepaRG, human hepatic cells; HepG2, Human Caucasian hepatocyte carcinoma cells; KATO III, gastric cancer stem cells; LDPE, Low-density polyethylene; LIVE/DEAD kit, viability/cytotoxicity test; MTT assay, cellular metabolic activity colorimetric assay; PBMCs, peripheral blood mononuclear cells; PE, polyethylene; PP, polypropylene; PS, polystyrene; PVC, polyvinyl chloride

Table 37. MP reference doses for human cellular immune responses. Lowest applied MP doses resulting in significantly altered cytokine responses after exposure to irregularly shaped MPs, corrected for human variability and to extrapolate to NOAELs.

Cell model	Cytokines	Polymer	Size (µm)	MP concentration		NOAELs		RfD	
				µg/mL	MPs/mL	µg/mL	MPs/mL	µg/mL	MPs/mL
Caco-2	IL-8								
	MCP-1								
	IL-8 mRNA[a]								
	MCP-1 mRNA[a]								
		PS	0.4402	20	290197	2	29019.7	0.2	2901.97
PBMC		PS							
	IL-6								
			15	1000	563068	100	56306.8	10	5630.68
			50	100	1520	10	152	1	15.2
			137.5	100	73	10	7.3	1	0.73
	TNF-alpha								
		LDPE	50	500	8321	50	832.1	5	83.21
		PS	50	1000	15202	100	1520.2	10	152.02

Note: Caco-2, human adenocarcinoma cell line; IL-, interleukin; LDPE, Low-density polyethylene; MCP-1, Monocyte chemoattractant protein-1; PBMCs, peripheral blood mononuclear cells; PS, polystyrene; TNF-α, Tumour Necrosis Factor alpha

8.2.1. Margin of exposure approach (MOE)

This approach has been proposed by EFSA (2012b) to address primarily the specific issues in the dose-response assessment specifically for substances that have the potential to exert genotoxic and carcinogenic effects. According to the findings of the rapid review, although genotoxicity was examined, there were no observed effects on human cells in the *in vitro* studies (chapter 7). This approach can also be used for threshold effects (FAO and WHO, 2009) but data from a BMD analysis are still required. Unfortunately, it has already been established that the BMD approach cannot be implemented for MPs at this point (section 8.2), therefore the MOE approach is similarly incompatible with the existing evidence.

8.2.2. Threshold of toxicological concern (TTC) approach

The implementation of the TTC approach was also examined. TTC can be applied for the assessment of the toxicity of chemicals found in food for which human exposure levels are estimated to be low and chemical structure is known (EFSA (SC), 2019). At first glance, MPs fulfil both of these conditions. Before the implementation of the generic scheme-TTC decision tree (see section 3.7.3), there are three initial considerations:

1. Examine the scientific literature to establish that MPs are not part of a group of substances for which toxicity data are well-established.

This condition is partly fulfilled since although toxicity data are well-established for polymers (Lithner et al., 2011) the toxicity profile of MPs in still under examination.

2. Check if MPs are not regulated by food/feed legislation.

This condition is met, since MPs are not currently regulated as food or feed contaminants/pollutants.

3.
    a. Check if MPs are part of substances that are either not represented in the database of the chemical classification or do not fall within the domain of applicability: inorganic substances, proteins, nanomaterials, radioactive substances, organosilicon substances, metals in elemental, ionic or organic form.

MPs partly fall into one of the exclusion categories since not all of them are organic substances.

b. Check if MPs have special properties: carcinogens, steroids, substances with a potential for bioaccumulation.

MPs have been shown to bioaccumulate in marine organisms and specifically within each trophic level (Miller et al., 2020).

After these initial considerations, the decision was made that it would not be appropriate to apply the TTC approach for MPs.

8.3. Exposure assessment

The results of the meta-analysis and statistical synthesis in the systematic reviews (Chapters 4 - 6) were the basis for the exposure assessment for the three food categories. The estimated levels of MP contamination are used in combination with data on food consumption patterns to produce the first step of the exposure modelling.

8.3.1. Human MP exposure via the consumption of salt

According to the WHO (2012), the daily consumption of sodium should be less than 2000 mg of sodium, which is roughly equivalent to ≈ 5 g of salt for adults (> 16 years old) and adjusted downwards for children according to their energy needs. The European Food Safety Authority (EFSA NDA, 2019) recently agreed that an intake of 2000 mg should be the daily intake limit for adults. In Europe, 95% of sodium is consumed in the form of salt while actual consumption of salt in the majority of European countries is estimated between 7 and 12 g (≈9.5 g) per day (EC, 2020). Similarly, the Australian and New Zealand governments (Food Standards Australia and New Zealand, 2016) as well as the U.S. Department of Health & Human Services (HHS) adopting the 2015–2020 Dietary Guidelines for Americans published by the U.S. Department of Health and Human Services and U.S. Department of Agriculture (2015) recommend that adults should consume less than 2300 mg of sodium daily (≈ 5.75 g salt). Actual consumption in the U.S. is on average 3400 mg per day (≈ 8.5 g of salt) and has been reported to be as high as 4583 mg in males (aged 30-39) and 3309 mg in females (aged 30-39) (U.S. Department of Agriculture, 2017). The UK NHS (2018) recommends a similar daily sodium intake of 2400 mg (≈ 6 g of salt) for adults, while actual consumption is 8.1 - 8.8 g of salt/day (FSA, 2018), 8.4 g of salt/day in England (PHE, 2020b). The Chinese Dietary Guidelines also propose consumption of < 6 g of salt per day but the actual consumption is 10.5 g per day (Chinese Nutrition Society, 2016, WHO, 2020a). These values refer to sodium consumption coming from all sources including sodium that is found naturally in food, salt and other forms of sodium added in processed

food (off the shelf, restaurants) and salt that is added by individuals while cooking and eating. The source of dietary sodium intake varies between countries according to dietary patterns. In the US, around 15% comes from sodium found naturally in food, 70% from processed food and around 11% from the salt individuals add during cooking and eating (American Heart Association, 2018). Almost the same estimations are reported for sodium in the European Union: 10-15% naturally occurring in food, 70-75% in processed food and 10-15% from sodium added during cooking and eating (EC, 2020). On the other hand, in the People's Republic of China, 76% of sodium dietary intake has been attributed to salt added in home cooking (Anderson et al., 2010). The dietary intake of sodium/salt that is of interest for the MP exposure assessment is only the amount that is artificially added in food. Using the data presented above as a guide, a 15% reduction was implemented across all the recommended intake values to account for sodium that is found naturally in food and is not entirely or partially attributed to the consumption of salt.

According to the WHO (2020b), actual worldwide salt consumption is estimated to be 9-12 g per day, which is twice the level of the recommended sodium uptake. The exposure uptake assessment for MPs via the consumption of salt for the potential/applied dose was calculated based on both the recommended sodium uptake values (Tables 38-43) and actual salt consumption data (Table 44). The recommended sodium values are more detailed in terms of age group and sex. Computation for estimations across countries according to age is difficult due to the different age ranges used by the organizations. In addition, a blanket estimation of 10 g of salt was used as the daily consumption to represent the worldwide salt consumption. These results should be seen only as indicative given that adult salt consumption likely varies widely from country to country

According to the recommended sodium uptake values, as shown in Tables 38-43, the yearly MP potential dose via the consumption of salt of all types ranges for infants from 7.9 ± 1.4 (CI 95%) to 15.8 ± 2.8 (CI 95%) MPs, for children of all ages from 31.7 ± 5.6 (CI 95%) to 95 ± 16.8 (CI 95%) MPs and for adults from 79.2 ± 14 (CI 95%) MPs to 95 ± 16.8 (CI 95%) MPs. Using an overall estimation of 10 g daily salt consumption, the worldwide human potential dose from the consumption of salt intended for human consumption is estimated to be 186 ± 33 (95% CI) MPs and a high-end exposure at 371 MPs (Table 44). For the high-end exposure estimate the upper limit of the prediction interval (PI 95%) of the mixed effects model was used (Figure 77). The PI was used instead of the CI because of the high heterogeneity (I^2) detected in the meta-analysis (IntHout et al., 2016).

Table 38. Daily MP potential dose through salt consumption per age group for Europe, according to EFSA NDA (2019) sodium dietary daily reference values.

Daily uptake	Infants 7–11 months			Children 1–3 years			Children 4–6 years			Children 7–10 years			Children 11–17 years and Adults 18+		
Meta-analysis	MPs	95% CI		MPs	95% CI		MPs	95% CI		MPs	95% CI		MPs	95% CI	
Sea salt	0.0	0.0	0.0	0.1	0.0	0.2	0.2	0.0	0.3	0.2	0.1	0.4	0.2	0.1	0.4
Lake salt	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.1	0.2
Rock salt	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.1	0.1	0.0	0.1	0.1	0.0	0.2
Well salt	0.1	n/a	n/a	0.3	n/a	n/a	0.4	n/a	n/a	0.5	n/a	n/a	0.6	n/a	n/a
All types	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.3
Statistical summary		MPs from to			MPs from to			MPs from to			MPs from to			MPs from to	
Sea salt		0.0	0.7		0.0	3.9		0.0	4.6		0.0	6.0		0.0	7.1
Lake salt		0.0	0.2		0.0	1.1		0.0	1.3		0.0	1.7		0.0	2.0
Rock/well salt		0.0	0.1		0.0	0.5		0.0	0.6		0.0	0.7		0.0	0.9
All types		0.0	0.7		0.0	3.9		0.0	4.6		0.0	6.0		0.0	7.1

Note: CI, confidence internal

Table 39. Yearly MP potential dose through salt consumption per age group for Europe, according to EFSA NDA (2019) sodium dietary daily reference values.

Yearly uptake	Infants 7–11 months			Children 1–3 years			Children 4–6 years			Children 7–10 years			Children 11–17 years and Adults 18+		
meta-analysis	MPs	95% CI		MPs	95% CI		MPs	95% CI		MPs	95% CI		MPs	95% CI	
Sea salt	9.1	2.2	16.0	50.1	12.0	88.2	59.2	14.2	104.2	77.4	18.6	136.2	91.1	21.8	160.3
Lake salt	5.8	4.1	7.6	32.1	22.5	41.7	38.0	26.6	49.3	49.6	34.8	64.5	58.4	40.9	75.9
Rock salt	2.9	-0.6	6.3	15.8	-3.3	34.9	18.6	-3.9	41.2	24.4	-5.1	53.9	28.7	-6.0	63.4
Well salt	21.6	n/a	n/a	118.6	n/a	n/a	140.2	n/a	n/a	183.3	n/a	n/a	215.6	n/a	n/a
all types	7.9	6.5	9.3	43.5	35.8	51.3	51.5	42.3	60.6	67.3	55.4	79.2	79.2	65.2	93.2
Statistical summary		MPs from to			MPs from to			MPs from to			MPs from to			MPs from to	
Sea salt		0.0	259.7		0.0	1428.2		0.0	1687.9		0.0	2207.3		0.0	2596.8
Lake salt		1.2	71.7		6.8	394.2		8.1	465.8		10.5	609.2		12.4	716.7
Rock/well salt		0.0	31.6		0.0	174.1		0.0	205.7		0.0	269.0		0.0	316.5
all types		0.0	259.7		0.0	1428.2		0.0	1687.9		0.0	2207.3		0.0	2596.8

Note: CI, confidence internal

Table 40. Daily MP potential dose through salt consumption per age group for USA, according to tolerable upper intake level recommended by the U.S. Department of Agriculture (2015).

Daily uptake	Child 1-3			Child 4-8			Child 9-13			Child 14-17 and Adult 18 - 51+		
meta-analysis	MPs	95% CI		MPs	95% CI		MPs	95%		MPs	95%	
Sea salt	0.2	0.0	0.3	0.2	0.1	0.4	0.3	0.1	0.5	0.3	0.1	0.5
Lake salt	0.1	0.1	0.2	0.2	0.1	0.2	0.2	0.1	0.2	0.2	0.1	0.2
Rock salt	0.1	0.0	0.1	0.1	0.0	0.2	0.1	0.0	0.2	0.1	0.0	0.2
Well salt	0.4	n/a	n/a	0.6	n/a	n/a	0.6	n/a	n/a	0.7	n/a	n/a
all types	0.2	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.2	0.2	0.3
Statistical summary		MPs from to			MPs from to			MPs from to			MPs from to	
Sea salt		0.0	5.3		0.0	6.8		0.0	7.8		0.0	8.2
Lake salt		0.0	1.5		0.0	1.9		0.0	2.2		0.0	2.3
Rock/well salt		0.0	0.7		0.0	0.8		0.0	1.0		0.0	1.0
all types		0.0	5.3		0.0	6.8		0.0	7.8		0.0	8.2

Note: CI, confidence internal

Table 41. Yearly MP potential dose through salt consumption per age group for USA, according to tolerable upper intake level recommended by the U.S. Department of Agriculture (2015).

Yearly uptake	Child 1-3			Child 4-8			Child 9-13			Child 14-17 and Adult 18 - 51+		
meta-analysis	MPs	95% CI		MPs	95% CI		MPs	95% CI		MPs	95% CI	
Sea salt	68.3	16.4	120.2	86.5	20.7	152.3	100.2	24.0	176.3	104.7	25.1	184.3
Lake salt	43.8	30.7	56.9	55.5	38.9	72.1	64.2	45.0	83.5	67.2	47.0	87.3
Rock salt	21.5	-4.5	47.6	27.2	-5.7	60.2	31.6	-6.7	69.8	33.0	-7.0	72.9
Well salt	161.7	n/a	n/a	204.8	n/a	n/a	237.2	n/a	n/a	248.0	n/a	n/a
all types	59.4	48.9	69.9	75.2	61.9	88.5	87.1	71.7	102.5	91.0	74.9	107.2
Statistical summary		MPs from to			MPs from to			MPs from to			MPs from to	
Sea salt		0.0	1947.6		0.0	2467.0		0.0	2856.5		0.0	2986.3
Lake salt		9.3	537.5		11.8	680.8		13.7	788.3		14.3	824.2
Rock/well salt		0.0	237.3		0.0	300.6		0.0	348.1		0.0	363.9
all types		0.0	1947.6		0.0	2467.0		0.0	2856.5		0.0	2986.3

Note: CI, confidence internal

Table 42. Daily MP potential dose through salt consumption per age group for UK, according to NHS daily sodium consumption recommendations (NHS, 2018)

Daily	Babies <1			Child 1-3			Child 4-6			Child 7-10			Child 11-17 and Adult 18+		
Meta-analysis	MPs	95% CI from to		MPs	95% CI from to		MPs	95% CI from to		MPs	95% CI from to		MPs	95% CI from to	
Sea salt	0.0	0.0	0.1	0.1	0.0	0.2	0.1	0.0	0.3	0.2	0.1	0.4	0.3	0.1	0.5
Lake salt	0.0	0.0	0.0	0.1	0.0	0.1	0.1	0.1	0.1	0.2	0.1	0.2	0.2	0.1	0.2
Rock salt	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.1	0.0	0.2	0.1	0.0	0.2
Well salt	0.1	n/a	n/a	0.2	n/a	n/a	0.4	n/a	n/a	0.6	n/a	n/a	0.7	n/a	n/a
All types	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.3	0.3	0.2	0.3
Statistical summary		MPs from to			MPs from to			MPs from to			MPs from to			MPs from to	
Sea salt		0.0	1.4		0.0	2.8		0.0	4.3		0.0	7.1		0.0	8.5
Lake salt		0.0	0.4		0.0	0.8		0.0	1.2		0.0	2.0		0.0	2.4
Rock/well salt		0.0	0.2		0.0	0.3		0.0	0.5		0.0	0.9		0.0	1.0
All types		0.0	1.4		0.0	2.8		0.0	4.3		0.0	7.1		0.0	8.5

Note: CI, confidence internal

Table 43. Yearly MP potential dose through salt consumption per age group for UK, according to NHS daily sodium consumption recommendations (NHS, 2018)

Yearly uptake	Babies < 1			Child 1-3			Child 4-6			Child 7-10			Child 11-17 and Adult 18+		
meta-analysis	MPs	95% CI from to		MPs	95% CI from to		MPs	95% CI from to		MPs	95% CI from to		MPs	95% CI from to	
Sea salt	18.2	4.4	32.1	36.4	8.7	64.1	54.6	13.1	96.2	91.1	21.8	160.3	109.3	26.2	192.3
Lake salt	11.7	8.2	15.2	23.4	16.4	30.4	35.0	24.5	45.5	58.4	40.9	75.9	70.1	49.1	91.1
Rock salt	5.7	-1.2	12.7	11.5	-2.4	25.4	17.2	-3.6	38.0	28.7	-6.0	63.4	34.4	-7.3	76.1
Well salt	43.1	n/a	n/a	86.2	n/a	n/a	129.4	n/a	n/a	215.6	n/a	n/a	258.7	n/a	n/a
all types	15.8	13.0	18.6	31.7	26.1	37.3	47.5	39.1	55.9	79.2	65.2	93.2	95.0	78.2	111.8
Statistical summary		MPs from to			MPs from to			MPs from to			MPs from to			MPs from to	
Sea salt		0.0	519.4		0.0	1038.7		0.0	1558.1		0.0	2596.8		0.0	3116.2
Lake salt		2.5	143.3		5.0	286.7		7.4	430.0		12.4	716.7		14.9	860.0
Rock/well salt		0.0	63.3		0.0	126.6		0.0	189.9		0.0	316.5		0.0	379.7
all types		0.0	519.4		0.0	1038.7		0.0	1558.1		0.0	2596.8		0.0	3116.2

Note: CI, confidence internal

According to the statistical summary based on a 10g daily salt consumption, human exposures are in the range 0 - 6110 MPs per year (Table 44). Note that in the statistical summary, the well samples have been consolidated with the rock samples. Modelling was based on analysis of salt samples that were commercially available for human consumption and assumes no losses of MP contamination during cooking. It is expected that some portion of MP might be extracted during food preparation and cooking, thus reducing the exposure levels, but there are no available data at this point to account for such an effect; it is recognized that this is a limitation of the calculated exposure levels and in the future, they could be adjusted downwards.

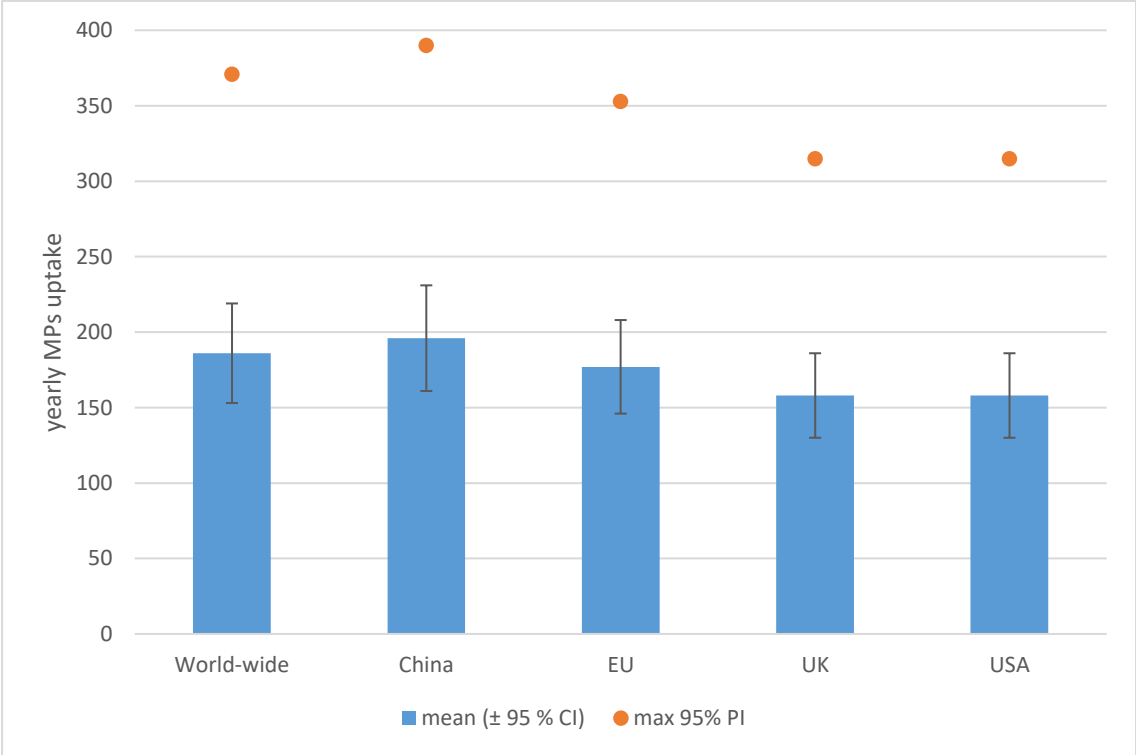


Figure 77. Yearly MPs uptake via the consumption of salt per country and worldwide. The error bars represent the ± 95% confidence interval (CI). The points represent the max exposures based on the upper limit of the 95% prediction interval (PI).

Table 44. Yearly world-wide MP uptake through salt consumption estimates for adults.

	World-wide			China			EU			UK			USA		
Salt type	Average MPs uptake [a]	95% CI		Average MPs uptake	95% CI		Average MPs uptake	95% CI		Average MPs uptake	95% CI		Average MPs uptake	95% CI	
Sea salt	214	51	377	225	54	396	204	49	358	182	44	321	182	44	321
Lake salt	137	96	179	144	101	187	131	91	170	117	82	152	117	82	152
Rock salt	67	-14	149	71	-15	157	64	-14	142	57	-12	127	57	-12	127
Well salt	507			533			482			431			431		
All types	186	153	219	196	161	230	177	146	208	158	130	186	158	130	186
95% PI		14	371		14	390		13	353		12	315		12	315
		Range of MPs uptake [b]			Range of MPs uptake						Range of MPs uptake			Range of MPs uptake	
Sea salt		0	6110		0	6416		0	5805		0	5194		0	5194
Lake salt		29	1686		31	1771		28	1602		25	1433		25	1433
Rock/well salt		0	745		0	782		0	707		0	633		0	633
All types		0	6110		0	6416		0	5805		0	5194		0	5194

[a] meta-analysis, [b] statistical summary. Note: CI, confidence internal; PI, prediction interval

More detailed salt-intake data exist for England as reported in the National Diet and Nutrition Survey (NDNS) by Public Health England (PHE, 2020b). These estimated salt-intake (g/day) values provided for three adult age groups and by sex are used to produce more detailed and sophisticated MP exposure estimations as presented in Appendix 37 for daily exposures and in Appendix 38 for annual estimated MP exposures. These values will also be used to illustrate the sensitivity analysis process for the uncertainty introduced by both the salt uptake estimations and the MP salt contamination estimation levels (section 8.3.1). Table 45 presents the studies that were included in the salt SR meta-analysis and the corresponding size distribution of MPs (section 4.2). For the calculation of internal doses, the dose estimation model could not be fitted as the details of the size range of interest (< 150 μm) were not reported by the studies. Iniguez et al. (2017) did not report size ranges at all. Gundogdu (2018) reported the highest percentage in lake salt samples at 9.8% in the size range < 500 μm, and Kim et al. (2018) reported the highest percentage of 61% in rock samples for the same size range. Lee et al. (2019) reported that 81% of MPs were < 500 μm in size across all samples. The results from the studies are inconsistent and do not allow for extrapolation.

Table 45. Salt studies used in meta-analysis MP concentrations.

Study (year)	Sample type	n	Mean MPs/kg	SD	Size below 500 μm
Gundogdu (2018)	sea	5	46	12.6	500 μm – 200 μm: 9.1%, 200 -100 μm: 1.3%, < 100 μm: 3%
	lake	6	37.5	14.1	500 μm – 200 μm: 9.8%, 200 -100 μm: 4%, < 100 μm: 3.6%
	rock	5	11.8	1.2	500 μm – 200 μm: 6.8%, < 200 μm: 0
Iniguez et al. (2017)	sea	16	124.06	56.43	Not reported
	well	5	139	26.24	
Kim et al. (2018)	sea	28	675	2560	500 μm – 100 μm: 47%
	rock	9	38	55	500 μm – 100 μm: 61%
	lake	2	245	307	500 μm – 100 μm: 55%
Lee et al. (2019)	sea	10	9.5	6.1	90 μm – 500 μm: 81%

Note: The size distribution % below 500 μm is presented in the last column on the right; n, number of samples; SD, standard deviation

8.3.2. Human MP exposure via the consumption of drinking water

Water intake in adults varies depending on gender, climate, diet and physical activity. The WHO guideline value for daily water consumption is 2 L for adults (with a default body weight of 60 kg), 1 L for children (default body weight of 10 kg) and 0.75 L for infants (default body weight of 5 kg) (WHO, 2017). Maximum daily human exposures were calculated by using the highest MP content evidence that have been rated of low and unclear RoB for the three continents, and the WHO values for daily water consumption and use (WHO, 2017). The highest possible daily exposures were calculated for Europe at 1260 MPs for TW and 9800 MPs for BW (Table 46). These exposures are significant underestimations since they assume that all populations have access to treated drinking water which is not the case. These high exposure levels are driven more by the amount of drinking water that is consumed and less the absolute MP content of water compared to other food categories. A further possible exposure pathway that has not yet been investigated may occur from the use of MP contaminated water for incorporation into food. According to WHO estimations, 7.5 L of water per capita per day (WHO, 2017) is used by most people in most situations around the world for hydration and incorporation into food. This is a complex issue since it is not clear to what extent MPs in the water would be taken up into the foodstuffs. This would depend on how the food is prepared and would have geographic and cultural variation. Nevertheless, further research into this issue is clearly warranted as it is another potential pathway for MPs in water to enter the human body. The particle size limitations for calculating internal doses are discussed in detail in section 5.5.3 for TW and 5.6.3 for BW and a tabular comparison is provided in Table 47. In the U.S.A., EPA has also historically assumed a similar drinking water ingestion rate to WHO, of 2 L/day for adults and 1 L/day for infants and children under 10 years old; while these rates also include beverages including TW. More detailed and specialized intake rates have been estimated, in order to be used in risk assessments, based on the National Health and Nutrition Examination Survey (NHANES) data for 2005−2010, and reported in the Exposures Factors Handbook (EPA, 2019a). The recommended values to be used in exposure assessments include both direct and indirect ingestion; direct when drinking water is consumed as a beverage and indirect when it is added during beverage or food preparation. EPA proposes the use of per capita intake rates (2-day average) for the executing exposure assessments, which is the intake that has been averaged across the entire population. The results of the exposure assessment for the U.S.A. are presented in Appendix 39 for TW and in Appendix 40 for BW. The combined estimates for both TW and BW consumption, derived from the findings of Chapter 5, are provided in Table 48. It should be highlighted again that exposures include both direct and indirect water consumption.

Table 46. Maximum daily and yearly MP uptake via water direct and indirect consumption per capita.

			Adults [a]		Children [b]		Infants [c]	
Continent	TW/BW	Max MPs/L (study)	Daily MP uptake	Yearly MP uptake	Daily MP uptake	Yearly MP uptake	Daily MP uptake	Yearly MP uptake
Europe	TW	628 (Pivokonsky et al., 2018)	1256	458440	628	229220	471	171915
	BW	4889 (Oßmann et al., 2018)	9778	3568970	4889	1784485	3667	1338364
Asia	TW	440 (Tong et al., 2020)	880	321200	440	160600	330	120450
	BW	140 (Kankanige and Babel, 2020)	280	102200	140	51100	105	38325
North America	TW	18 (Shruti et al., 2020)	36	13140	18	6570	14	4928
	BW	10.4 (Mason et al., 2018)[d]	21	7592	10	3796	8	2847

[a] Adults: 2 L water/day, default body weight 60 kg

[b] Children: 1 L water/day, default body weight 10 kg

[c] Infants: 0.75 L water/day, default body weight 5 kg (WHO, 2017)

[d] The results of the Mason et al. (2018) study were used since it was the only that sampled brands of BW from multiple continents including America (n=3)

Note: The highlighted columns represent the potential maximum MP human exposures. BW, bottled water; MP, microplastic; TW, tap water.

Table 47. Relationship between MP size range and MPs content in drinking water.

Study, Year	Sample type	MPs/L	±SD	Range MPs/L	Dominant MP size range (μm)	MPs/dominant size range	Polymers
Mintenig et al. (2019)	TW	0.0007		0 – 0.0007	50-150	100%	Polyester 62%, PVC 14%, PA and epoxy resin 9%, PE 6%
Pivokonsky et al. (2018)	TW	443	10		1-5	25-60%	PET 41%, PP 33%
		338	76				PET 62%, PP
		628	28		5-10	30-50%	PE 35%, PET 26%, PP 16%
					< 10	up to 95% across	
Shruti et al. (2020)	TW	18	7	5 ± 2 to 91 ± 14	100 – 1000	75%	PTT, epoxy resin
Strand et al. (2018)	TW	< 0.58			≥ 100	majority of the results	PP 50%, PS 25%, PET 25%
Tong et al. (2020)	TW	440	275	0 to 1247	< 50	dominant	PE 26.8%, PP 24.4%, co PE-PP 22.0%, PPS 7.3%, PS 6.5%, PET 3.3%
M. Zhang et al. (2020)	TW	0.7	0.6	0.3 to 1.6	500 - 1000	46%	Rayon, PET, PE, PS, Polyester, PAA, PMPS, PI
Kankanige and Babel (2020)	BW	140	19		6.5 – 20	dominant	PET 28.4%, PE 24.2%, PP 18.1%, PA 7.2%, PVC 4.4%
		52	4				

Mason et al. (2018)	BW	10.4 (≥ 100 µm) 315 (6.5-100 µm)		0-14	6.5 - 100	95%	PP 54%, Nylon 16%
Oßmann et al. (2018)	BW	4889	5432		< 5	95%	PET, PP, PET and olefin, PE
		2649	2857		< 1.5	50%	PET, PET and olefin, PP, PE
		6292 3074[d]	10521 2531[d]				PE, PP, Styrene-Butadiene, PET
Schymanski et al. (2018)	BW	118	88	28-241	5 -20	80%	PET 84%, PP 7%, PE 5%, PA 2%
		14	14	2-44			
		11	8	5-20			
		50	52	4-156			
Wiesheu et al. (2016)	BW	1 in the sample			Inconclusive results		PET
Zuccarello et al. (2019a)	BW	5.42 X 10^7	1.95 X 10^7	3.16 X 10^7 to 1.1 X 10^8	0.5-10		Not specified

Note: Table 47 brings together data from Table 15 and the narrative analysis from sections 5.5.3 and 5.6.3. The highlighted cells represent the lowest and highest levels of MPs for TW and BW. BW, bottled water; PP polypropylene, PVC polyvinyl chloride, PA polyamide (nylon), PE polyethylene, PET polyethylene terephthalate, PS polystyrene, PTT poly trimethylene terephthalate, PPS polyphenylene sulphite, PAA polyacrylic acid, PMPS poly (methyl phenyl siloxane), PI poly (isoprene); TW, tap water

Table 48. MPs exposure assessment via the consumption for combined bottled (BW) and tap water (TW), for the U.S.A.

	TW and BW ingestion rates		MPs daily uptake		MPs yearly uptake	
	mean	95th Percentile	mean	95th Percentile	mean	95th Percentile
age group	mL/day	mL/day	max[a]	max[a]	max[a]	max[a]
1 to < 2 years	217	921	439	2095	160165	764786
2 to < 3 years	310	1311	642	3094	234361	1129464
3 to < 6 years	329	1319	722	3291	263600	1201284
6 to < 11 years	450	1802	947	4246	345770	1549953
11 to < 16 years	550	2490	1347	6230	491558	2273773
16 to < 21 years	816	3400	2132	8527	778044	3112246
21 to < 30 years	1240	4736	2735	11019	998099	4021926
30 to < 40 years	1370	4932	2855	11470	1041895	4186609
40 to < 50 years	1307	4860	2640	11131	963689	4062792
50 to < 60 years	1298	4727	2272	10430	829428	3806792
60 to < 70 years	1219	4433	1950	9009	711783	3288374
70 to < 80 years	962	3366	1414	6771	516010	2471459
80+ years	892	2859	1020	4936	372433	1801570
21 to < 50 years	1309	4863	2744	11256	1001473	4108582
50+ years	1175	4289	1901	8923	693921	3256922
all ages	1037	4211	2040	9334	744718	3407011

[a] maximum MP bottled water (BW) contamination. Note: the highlighted results are used in the uncertainty/sensitivity analysis in section 8.4.1.

Note: the exposure estimates are based on the intake rates proposed by the National Health and Nutrition Examination Survey (NHANES) data for 2005−2010, and reported in the Exposures Factors Handbook (EPA, 2019a).

8.3.3. Human MP exposure via the consumption of seafood

According to the Food and Agriculture Organization of the United Nations (FAO, 2020), global human consumption of fish and seafood in 2017 was 20.38 kg/capita/year; breaking down as: fish at 15.21 kg/capita/year, molluscs at 2.65 kg/capita/year, crustaceans at 2.06 kg/capita/year and cephalopods at 0.47 kg/capita/year (live-weight equivalent). The data from the FAO cover 173 countries around the world (FAO, 2020), and indicate significant variability in fish and seafood consumption by country ranging from 0.25 kg/capita/year in Afghanistan to 90.71 kg/capita/year in Iceland.

Combining the data for global human consumption of seafood with the outcomes of the statistical summary in the systematic review (Chapter 6), extrapolates to a yearly MPs uptake of 0 to 27,825 MPs for molluscs, 206 to 17,716 MPs for crustaceans and 31 to 8,323 MPs

for fish (Table 49). The total maximum yearly MP uptake from all seafood categories, based on FAO (2020) data could be as high as 53,864 MPs. Seafood consumption between countries varies greatly and is predominantly connected to geography and culture. For example, it is estimated that people in Angola consume 0.01 kg of molluscs per year, whereas in Hong Kong this rises to 15.32 kg per year (FAO, 2020). The variations of projected maximum yearly MP uptake from global consumption of molluscs is illustrated in Figure 78, for crustaceans in Figure 79 and for fish in Figure 80. The numerical data for the maps can be found in Appendix 35.

Table 49. MP yearly uptake from the consumption of seafood.

Mean yearly uptake [a]	**MPs**	**95% CI**
Molluscs		
Clams	3312	±1431
Mussels	1881	±557
Oysters	1113	±610
Scallops	1272	±769
Overall	2067	±503
Range of yearly uptake [b]		
Invertebrates		
Mollusc	0 to 27825	
Crustacean	206 to 17716	
Fish		
Anchovy	31 to 279	
Sardine	62 to 2387	
Lance	230	
Comber	8323	
Overall	31 to 8323	

[a] based on the meta-analysis results

[b] based on the statistical summary results

Note: The consumption has been calculated for each family and then pooled for each of the three phyla; molluscs, crustacean and fish corresponding to the yearly global seafood consumption data (FAO, 2020). MPs, microplastics; 95% CI, confidence interval.

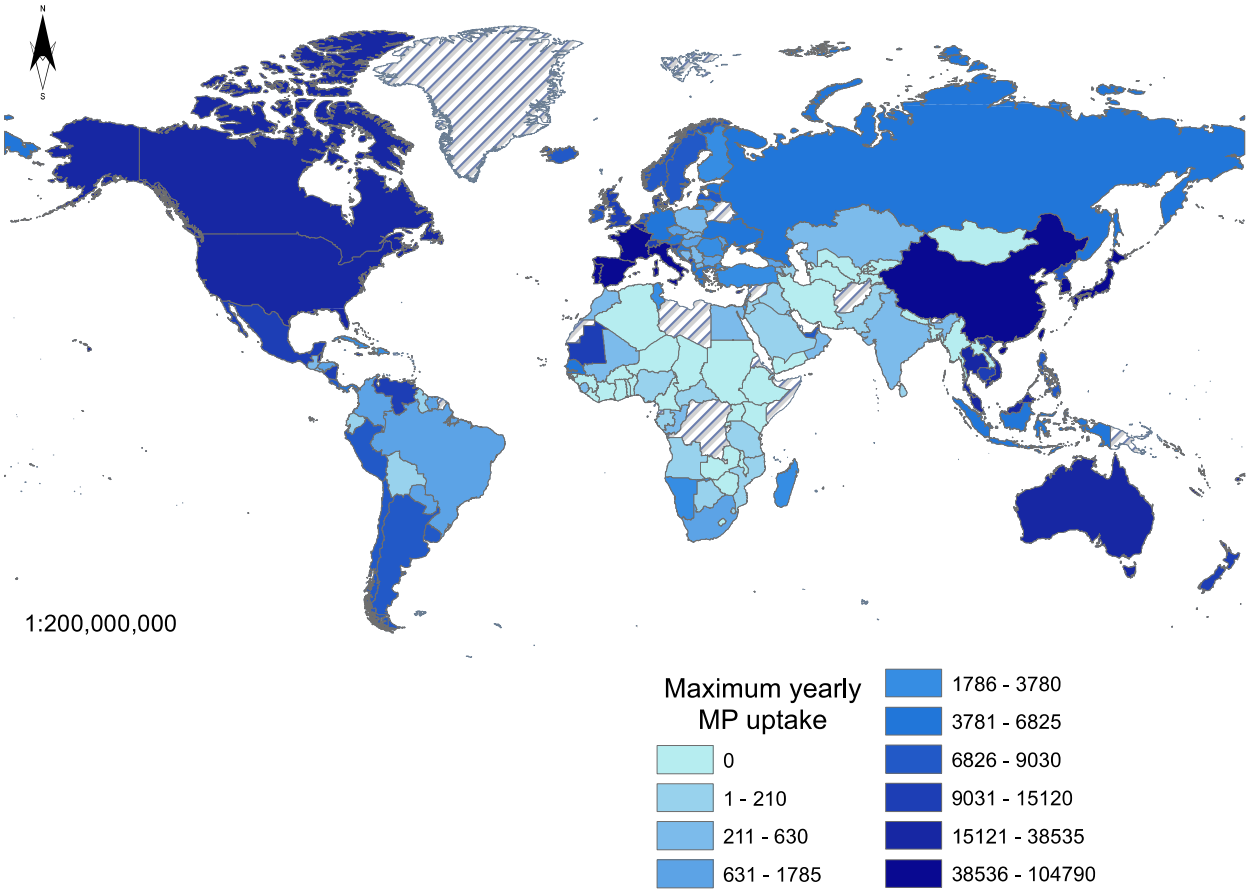


Figure 78. Predicted global yearly maximum microplastic (MP) particles uptake through mollusc consumption. The data have been calculated using the FAO (2020a) consumption data for the different mollusc families per country and the maximum MPs/g content of molluscs derived from the statistical summary results herein. The numerical data is shown in Appendix 35. MP data were classified in ten categories using quantile classification for illustration purposes. The hatched areas illustrate countries for which data on mollusc consumption were not available. (ArcGIS basemap: World Light Gray Base and Reference; Sources: Esri, HERE, Garmin, OpenStreetMap contributors, and the GIS User Community; created 26/09/2011)

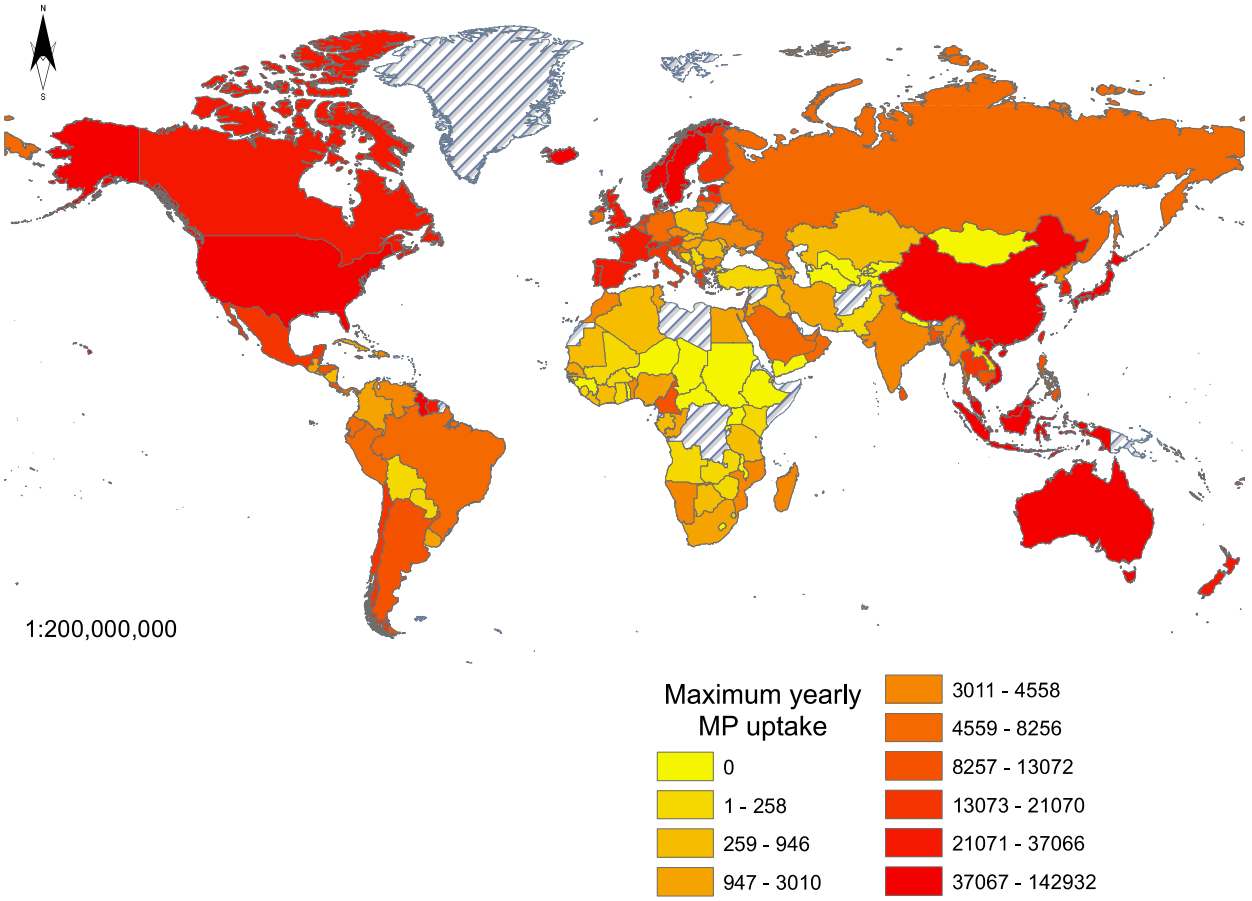


Figure 79. Predicted global yearly maximum microplastic (MP) particles uptake through crustacean consumption. The data were calculated using the FAO (2020) consumption data for crustacean per country and the maximum MPs/g content of crustacean derived from the statistical summary results herein. The numerical data is shown in Appendix 35. MP data have been classified in ten categories using quantile classification for illustration purposes. The hatched areas illustrate countries for which data on mollusc consumption were not available. (ArcGIS basemap: World Light Gray Base and Reference; Sources: Esri, HERE, Garmin, OpenStreetMap contributors, and the GIS User Community; created 26/09/2011)

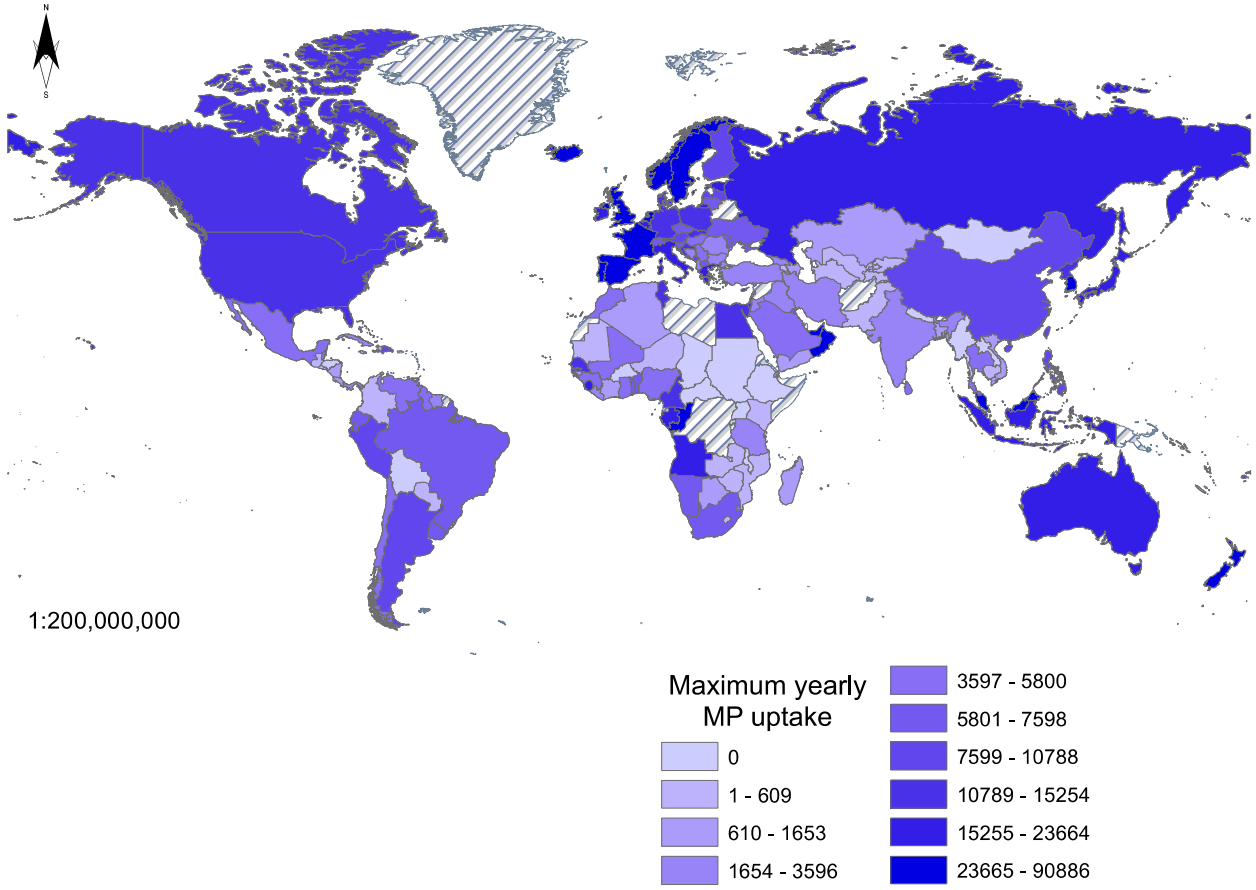


Figure 80. Predicted global yearly maximum microplastic (MP) particles uptake through fish consumption. The data were calculated using the FAO (2020) consumption data for fish per country and the maximum MPs/g content of fish derived from the statistical summary results herein. The numerical data is shown in Appendix 35. MP data have been classified in ten categories using quantile classification for illustration purposes. The hatched areas illustrate countries for which data on fish consumption were not available. (ArcGIS basemap: World Light Gray Base and Reference; Sources: Esri, HERE, Garmin, OpenStreetMap contributors, and the GIS User Community; created 26/09/2011)

In the UK, detailed data on seafood consumption are available from the NDNS (PHE, 2020a). The results of the exposure assessment using these consumption data and the results of the statistical analysis across all seafood categories (Table 20) are presented in Appendix 41. For both women and men, the highest estimates of exposure are observed in the 65-74 age group reaching maximum uptakes of 242,000 and 252,000 MPs/year, respectively (based on the mean values of seafood consumption).

8.3.4. Aggregate exposures and limitations in exposure assessment

The aggregate dietary exposures for all three food themes can be achieved by using the results of the statistical summaries (Figure 81), since meta-analysis could not be executed for drinking water (see sections 5.5 and 5.6). Taking into consideration the TW results which is the most relatable water consumption source for the majority of the population, the highest potential annual MPs exposures are up to half a million MPs (518,414 MPs) (Table 50). If BW is added in the calculations, the number increases to three and a half million MPs (3,628,944 MPs). These exposures have been calculated based on the world-wide average consumption data for adults and they refer to applied doses. Exposures can also be calculated for more specific populations. Consumption patterns are affected by socioeconomic parameters such as culture, geography, age group, gender as well as health conditions e.g. food allergy/intolerance and food consumption choices (vegetarians, vegan etc.).

Table 50. Aggregate yearly dietary exposures to MPs from three media: salt, seafood and drinking water.

Medium of exposure	Meta-analysis			Statistical synthesis	
	Average	CI 95%			
		from	to	from	to
salt	186	153	219	0	6110
molluscs	2067	1537	2571	0	27825
crustacean	-	-	-	206	17716
fish	-	-	-	31	8323
TW	-	-	-	0	458440
BW	-	-	-	0	3568970
Aggregate[a]	2253	1690	2790	237	518414
Aggregate[b]	-	-	-	-	3628944

[a] tap water (TW) consumption only

[b] bottled water (BW) consumption only

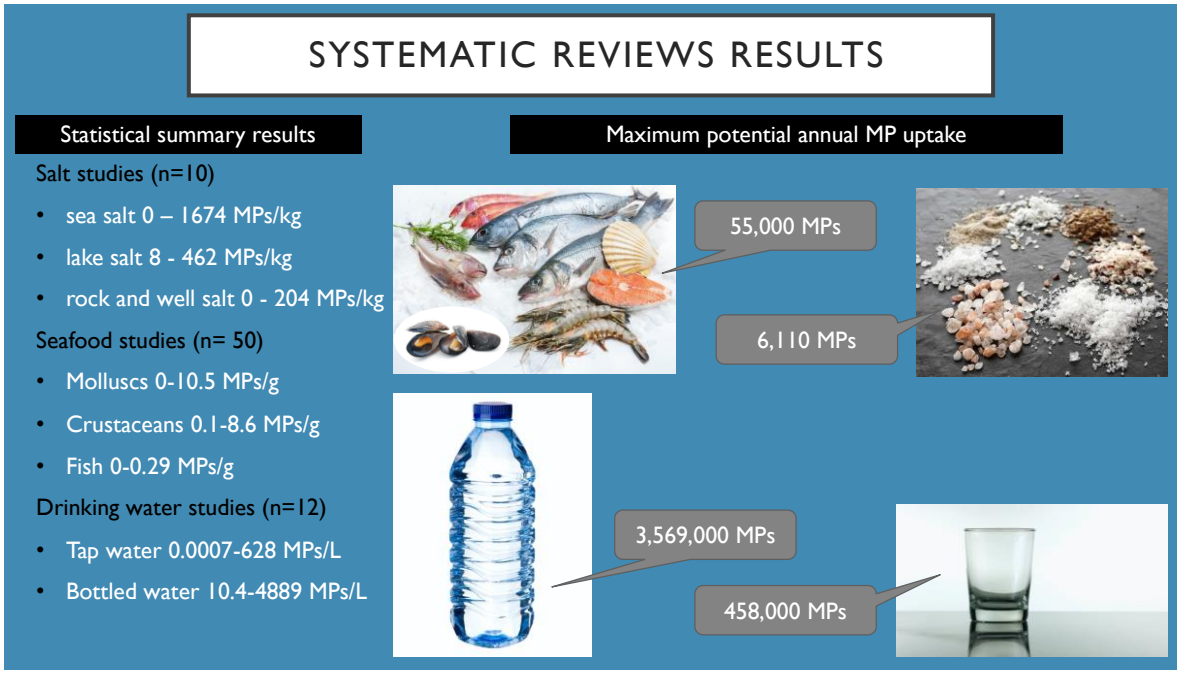


Figure 81. Combined results across the three food themes explored in systematic reviews.

Limitations in the food consumption data drastically affect exposure modelling. In the UK, there is detailed published data on salt intake (g/day), by sex and age group (adults only), for each country: for England by Public Health England (PHE, 2020b), for Scotland by Food Standards Scotland (FSS, 2014), for Wales by the National Centre for Social Research (NCSR, 2007) and for Northern Ireland by the FSA (2015). The reports cited are the latest published for each country of the UK. It is evident that reports are for different time periods and as such their combination is not possible.

A further limitation is that although all the reports use the same three age groups (19-34, 35-49 and 50-64), there is no data on children and adults over 65. When examining seafood consumption data in the UK, there is data available from the NDNS (PHE, 2020a). Unfortunately, the age groups used in this report are: 1.5-3, 4-10, 11-18, 19-64, 65+, 65-74 and 75+, which are not the same age groups used for the salt intake NDNS (PHE, 2020b), therefore hindering calculations for aggregate exposures. Another source of information for seafood consumption in the UK is the Family Food report which is published annually by DEFRA (2020). Family Food reports the results of the Living Costs and Food Survey, which provides data on average seafood purchased (g per person/week) for household purchases (including takeaways) and eating out purchases. The report does not differentiate between age and gender groups. Finally, despite a careful search, details of the average intake of drinking water for the UK are not apparently available. Therefore, combination of all three food themes for aggregate exposure assessments for specific sub-populations is not always possible.

Similar limitations exist in other countries. For example, in the EPA in the US has produced an Exposure Factors Handbook (EPA, 2011) proposing specific values to be used in exposure assessments but uses different metrics for food groups. For drinking water consumption the per capita intake rates expressed in both mL/day and mL/kg-day (EPA, 2019a) but for seafood consumption they propose the use of per capita intake rates expressed only in g/kg-day, which hinders the calculation of aggregate exposures.

According to the results of the world-wide exposure modelling, drinking water is the medium that drives human exposures. The order of magnitude, even when only TW intake is assumed, is one time larger than seafood. In addition, water consumption provides the best estimation for the general population. Furthermore, the MP drinking water studies were the only ones to provide a comprehensive description of the MP size ranges detected in their samples (Table 47) making it possible to use Equation 7 and/or Equation 8 (section 3.7.4.5) to model possible internal doses according to the predefined absorption factors. The results of the drinking water SR are based on a statistical summary. For TW, the maximum level of contamination was reported by Pivokonsky et al. (2018) at 628 MPs/L (± 28 SD). For this study, the upper size limit of detected MPs was 100 μm, with a minimum content of MPs detected in the size range between 50-100 μm, 25–60% between 1-5 μm and 30-50% between 5-10 μm, overall, up to 95% of MPs were < 10 μm.

According to the reported size ranges, Equation 7 can be used for the 150 μm upper size of the absorption factor leading to 100% of the detected MPs qualifying for the potential internal dose for MPs that have the potential to cross the gut barrier. The average size of 5 μm will be taken as the reference MP size to calculate the contamination expressed in μg in order to use it in the hazard characterization processes using the equations proposed by Connors et al. (2017) and used in ecological risk assessment by Besseling et al. (2019) and Burns and Boxall (2018). A limitation of this equation is that it assumes that all MPs are spherical, which is not the case for the MPs found in the environmental studies (see section 7.11.3). Nevertheless, the difference in the order of magnitude is assumed to be no more than two, thus achieving adequately comparable results. The densities of the three predominant polymers detected in the samples by Pivokonsky et al. (2018) (PE, PET and PE) (see Table 47) are used for the calculations.

Tap water MP contamination results were used, in the first instance, over bottled water as they provide a baseline for human exposures for the general population, using a conservative modelling approach and thus avoiding overestimations. Modelling was based on the WHO (2017) assumption for drinking water intake: 2 L/day for adults (60 kg body weight), 1 L/day

for children (10 kg body weight) and 0.75 L/day for infants (5 kg body weight). Applying Equation 6 (section 3.7.4.4) for tap drinking water results in $E_{ing(max)}$=0.09 μg/day and $E_{ing(max)}$=32.14 μg/year for adults, $E_{ing(max)}$=0.04 μg/day and $E_{ing(max)}$=16.07 μg/year for children and $E_{ing(max)}$=0.03 μg/day and 12.05 μg/year for infants (Table 51). The use of the $AF_{ing.a}$ (Equation 7) was not required since all MPs were < 150μm. The use of $AF_{ing.b}$ (Equation 8) was not possible due the level of detail in the reported data. The exposures based on bottled water consumption are also presented in Table 52.

Table 51. Maximum daily and yearly MP intake via the consumption of drinking tap water (TW) expressed in both MPs number and μg.

		Daily		Yearly	
	Polymer	MPs	μg	MPs	μg
Adults	PE	570	0.035	208132	12.805
	PET	425	0.038	154953	13.691
	PP	261	0.015	95356	5.648
	Max	1256	0.09	458440	32.14
Children	PE	285	0.018	104066	6.402
	PET	212	0.019	77476	6.846
	PP	131	0.008	47678	2.824
	Max	628	0.04	229220	16.07
Infants	PE	214	0.013	78049	4.802
	PET	159	0.014	58107	5.134
	PP	98	0.006	35758	2.118
	Max	471	0.03	171915	12.05

Note: Intake expressed in μg is based on an average 5 μm MP size. PE, polyethylene; PET, polyethylene terephthalate; PP, polypropylene.

Table 52. Maximum daily and yearly MP intake via the consumption of drinking bottled water (BW) expressed in both MPs number and μg.

		Daily		Yearly	
	Polymer	MPs	μg	MPs	μg
Adults	PE	4439	0.273	1620312	99.686
	PET	3305	0.292	1206312	106.586
	PP	2034	0.120	742346	43.971
	Max	9778	0.69	3568970	250.24
Children	PE	2220	0.137	810156	49.843
	PET	1652	0.146	603156	53.293
	PP	1017	0.060	371173	21.985
	Max	4889	0.34	1784485	125.12
Infants	PE	1665	0.102	607617	37.382
	PET	1239	0.110	452367	39.970
	PP	763	0.045	278380	16.489
	Max	3667	0.26	1338364	93.84

Note: Intake expressed in μg is based on an average 5 μm MP size. PE, polyethylene; PET, polyethylene terephthalate; PP, polypropylene.

8.4. Risk characterization

Risk characterization involves the comparison of the health-based values and the exposure levels in different populations in order to examine whether they are exceeded and in which circumstances. The scope of this risk assessment was to first identify whether human MP dietary exposures could be established and then examine the potential risk brought about by this hazard. Within this thesis, the derivation of health-based values for apical endpoints was not feasible, since only the results of *in vitro* studies were used in the dose-response assessment. Another limitation of MP research is that toxicokinetic and toxicodynamic evidence is sparse; therefore, there is very little information on how MPs behave after their uptake in the human body.

According to the findings of the systematic reviews (Chapters 4-6) and the exposure assessment (section 8.3), human dietary exposures to MPs are established and are ubiquitous across the world and across different demographic groups. The exposures are a function of the level of contamination of food and the particular consumption patterns. Although MP exposures for specific populations, in terms of age groups, geographic locations and media (water, seafood, salt) were able to be modelled, the estimation of MP exposures coming from

the direct consumption of drinking water was found to be the best model to base the risk assessment.

An initial comparison between the estimated exposure levels and the RfDs, defined as the CrEf on the cellular level, for the endpoints of cytotoxicity (Table 36) and immune responses (Table 37) was attempted. For both biological endpoints the estimated MP exposure levels coming just from one medium (TW) exceed the RfDs for the yearly but not for the daily exposures (Table 51) for all three age groups. The comparison for adults is illustrated in Figure 82 for cell viability and in Figure 83 for immune responses. On the other hand, for exposures from the consumption of BW, based solely on daily consumptions, thresholds are very close to exceed RfDs for cytotoxicity while they do exceed RfDs for immune responses for adults (Table 52), as illustrated in Figure 84 and Figure 85, respectively.

In other words, the assessment results show that estimated current dietary exposures to MPs, even when only one medium is considered (drinking water), are at a level that could have adverse mechanistical effects, thus posing a risk to health. The thresholds are exceeded for certain cell models, while it should also be noted that there is a discrepancy between the test MP sizes and the environmental MP sizes in the water samples. These comparisons assume no loss of MPs within the ADME processes (section 3.7.2), thus introducing an important limitation. Another significant finding is that the results of the *in vitro* toxicological experiments have so far showed that MPs do not exert genotoxic and/or carcinogenic effects.

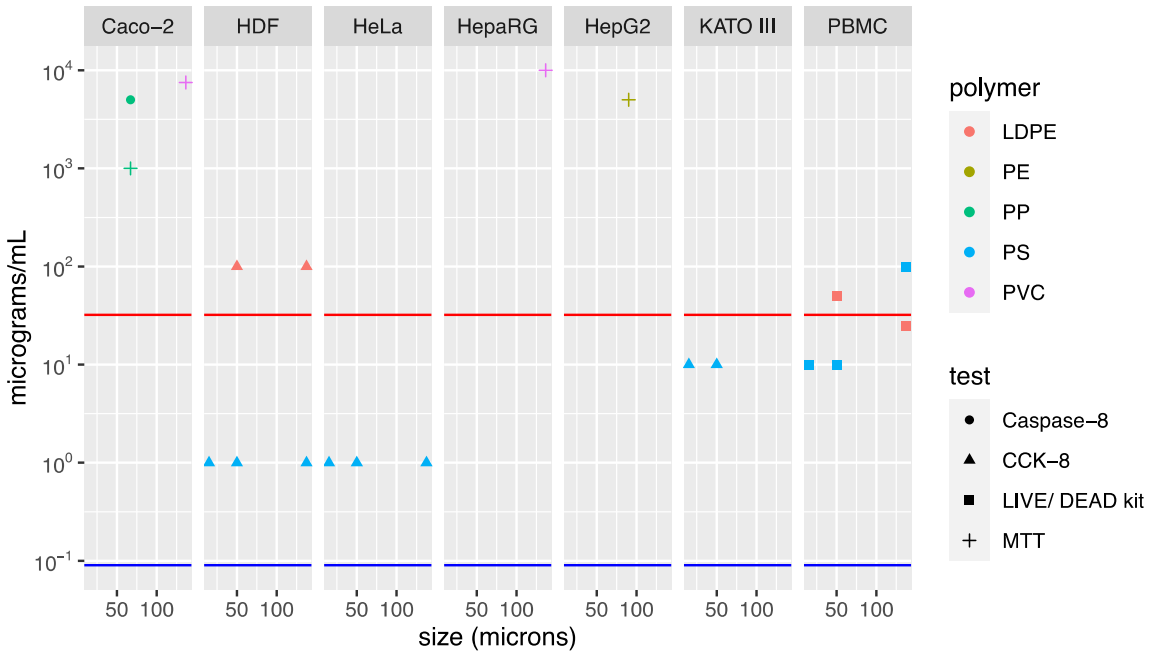


Figure 82. Risk characterization for cell viability: reference doses (RfDs) (Table 36) compared to daily (blue line) and yearly (red line) MPs exposures from tap water (TW) expressed in μg/mL (Table 51) in $\log_{10}$ scale.

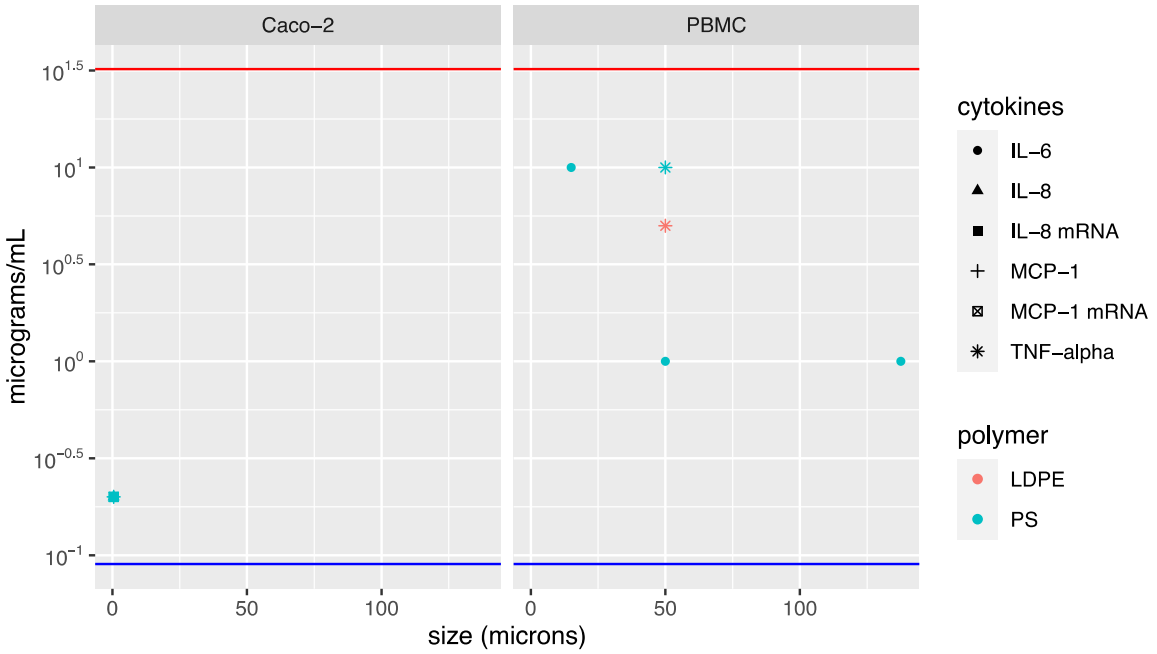


Figure 83. Risk characterization for immune responses: reference doses (RfDs) (Table 37) compared to daily (blue line) and yearly (red line) MPs exposures from tap water (TW) expressed in µg/mL (Table 51) in $\log_{10}$ scale.

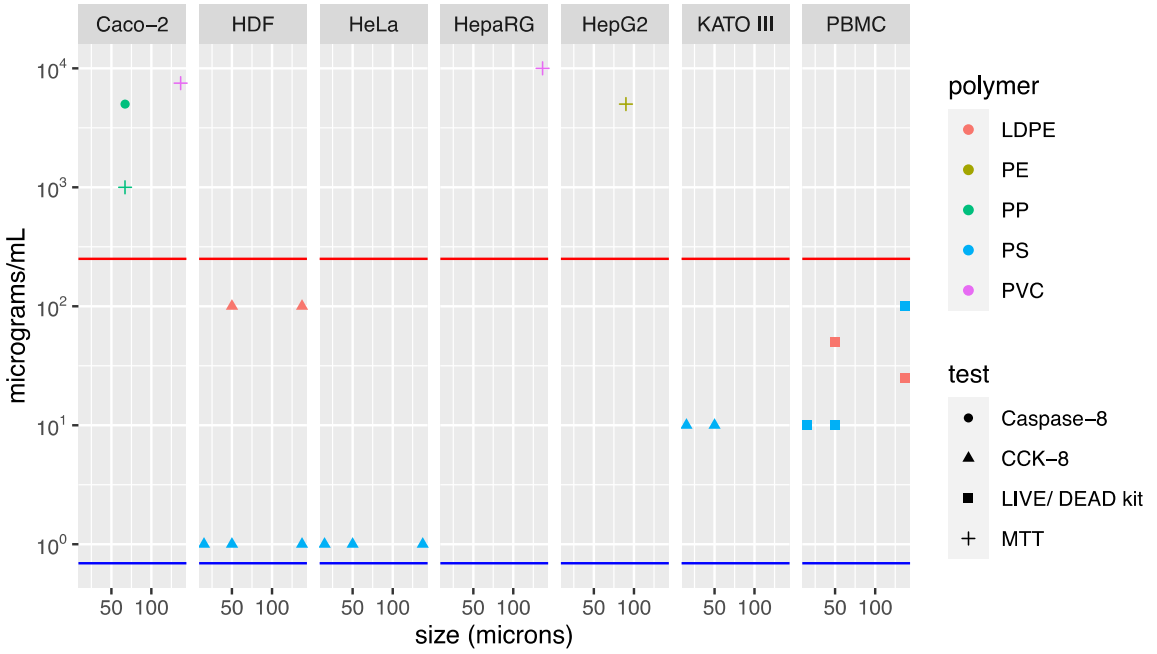


Figure 84. Risk characterization for cell viability: reference doses (RfDs) (Table 36) compared to daily (blue line) and yearly (red line) MPs exposures from bottled water (BW) expressed in µg/mL (Table 52) in $\log_{10}$ scale.

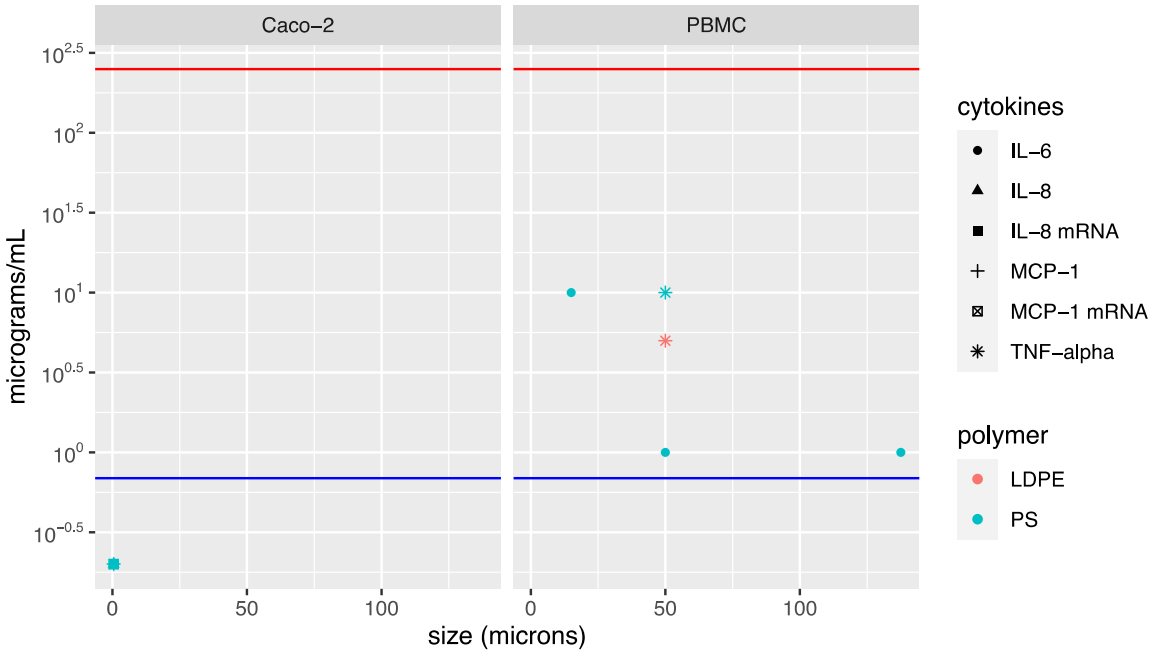


Figure 85. Risk characterization for immune responses: reference doses (RfDs) (Table 37) compared to daily (blue line) and yearly (red line) MPs exposures from bottled water (BW) expressed in μg/mL (Table 52) in $\log_{10}$ scale.

8.4.1. Uncertainty/ sensitivity analysis

A formal uncertainty analysis for the exposure modelling has not been executed since the present risk characterization refers to a worst case scenario (FAO and WHO, 2009). The uncertainty analysis for the levels of contamination is provided in the confidence and prediction intervals of the meta-analysis results. Uncertainty was also introduced from the consumption data since only guidance values were available for the consumption of drinking water and only on a worldwide basis. A sensitivity analysis to identify which component of the risk characterization is likely to introduce the most substantial uncertainties was also considered. Due to the lack of detailed information on water consumption patterns around the world, the sensitivity analysis could not be completed. Nevertheless, the input of the introduced uncertainty is evident in the exposure assessment for TW and BW in the U.S.A as presented in Appendix 39, Appendix 40 and Table 48, where the use of the mean or the 95th Percentile of the consumption values, materially affected the modelling results. The same trend in also evident in Appendix 41 where the MP uptake via seafood consumption is presented for the UK. In order to illustrate further the implementation of a sensitivity analysis, the dataset produced by the estimated salt intake for England is used (section 8.3.1, Appendix 38). According to the results of the salt intake for both sexes and for the entirety of the age groups (19-64) the estimated salt intake is 8.4 (±4.1 SD) g/day or 3060 (±1507 SD) g/year, combined with MP contamination levels modelled via meta-analysis, the levels

of exposure are presented in Table 53. The uncertainty introduced by each input can be examined simply by keeping the average value for the calculation of the model for one factor and using the range (SD, CI or PI) for the other. In the results, the highest uncertainty is introduced when using the PI 95% to express the MP level of contamination in salt.

Table 53. Sensitivity analysis for MP exposure estimates via the consumption of salt.

		MP annual exposure estimates				
			CI 95%[a]		PI 95%[a]	
Estimated salt intake (g/year)		average	from	to	from	to
		0.051	0.042	0.060	0.004	0.102
average	3060.2	156.2	128.5	183.8	11.4	311.1
SD	1507.5	76.9				
from	1552.7	79.2	65.2	93.3	5.8	157.9
to	4567.7	233.1	191.8	274.4	16.9	464.4

[a] the exposures are based on the meta-analysis results (Table 13). Note: The highlighted cells illustrate the highest level of uncertainty. CI, confidence interval; PI, prediction interval.

Uncertainty expressed as the limitations of the risk assessment can be improved in the future when more and better-quality data are available. Variability expressed as the heterogeneity in exposures and biological responses can also be improved by using better data but cannot be completely removed, since it is inherent in the population characteristics.

8.5. Further routes of human MP exposures

In addition to food ingestion, atmospheric MP contamination presents an additional pathway for MP human exposures (Chen et al., 2020), related to direct exposures via inhalation (Wright et al., 2020) and indirect exposures via non-dietary ingestion routes of hand-to-mouth behaviour (Gasperi et al., 2018), inadvertent ingestion (Abbasi et al., 2018) and occupational exposures (Gallagher et al., 2015). Recent studies have started to quantify indoor and outdoor air MP levels, for example, Dris et al. (2017) reported concentrations of 1.0-60 MPs/m^3 (indoor) and 0.3-1.5 MPs/m^3 (outdoor) in air, while in our recent study the household levels of MPs averaged at 1414 MP m^{-2} day^{-1} ± 1022 (mean ± SD) (Jenner et al., 2021). Regarding outdoor air, Liu et al. (2019) measured levels of 0–4.18 MPs/m^3.

A recent review has attempted to extrapolate to human exposures reporting annual inhalation of 1.9×10^3 –1.0×10^5 MPs (indoors) and 0–3.0×10^7 MPs (outdoors) (Qun Zhang et al., 2020). These additional pathways must be included in an aggregate human exposure scenario

to account for multiple pathways, routes and media (EPA, 2019b, FAO and WHO, 2009). A direct comparison between the magnitude of exposure via different pathways is not advisable at this point since the endpoint of the exposures might be different and the internal doses of MPs are likely to vary and depend on the physicochemical MP characteristics (e.g. size, hydrophilicity) (Galloway, 2015) and the responses of the barrier organ i.e. the GI tract (Keshav et al., 2013, Vancamelbeke and Vermeire, 2017) and the lower regions of the respiratory tract (Timbrell, 2009). The presence of MPs was confirmed in both human lung tissue (Pauly et al., 1998) and the GI tract (Schwabl et al., 2019). There is evidence that occupational exposure to high levels of airborne MPs can impact upon human health (Donaldson and Tran, 2004, Gallagher et al., 2015, Pauly et al., 1998) but further research is needed to understand whether dietary MPs exposures can have a detrimental effect on the human GI system.

8.6. Existing risk assessments

In recent years, a few attempts have been made to assess the risk posed by MPs in different environmental compartments using different methodological approaches (see Table 54). The majority of the studies focus on the marine/aquatic environment, executing ecological risk assessments and only a few have focused on human health (Figure 86). In addition, a series of papers use the term risk assessment erroneously. For example, the study by Ustabasi and Baysal (2019) comprised a risk assessment for the MPs released in the environment via toothpaste. In fact, the study analysed toothpaste samples for the presence of MPs and then extrapolated results to potential environmental emissions. Regarding human-related risk assessment, although some papers state that this has been executed, the methodology and methods currently being used in formal risk assessments (see section 3.5 and 3.7), as endorsed by major organizations around the world (e.g. WHO, EPA), have not been followed. These issues highlight the need for careful considerations and robust research in the field of risk assessment.

Table 54. Microplastic risk assessment studies.

		Study design					
		Reviews	Risk assessments		Opinion/ meeting	Experimental	Methods
topic			primary	secondary			
Marine/ aquatic	18	1	11	5			1
Soil	2	1	1				
Toothpaste	1					1	
Food	4		1	1	1	1	

Human health	3	1			1		1
Atmosphere	1		1				
Environmental[a]	4						4
MP and other substances	5		2			3	

[a] other compartments besides marine/aquatic and soil, or multiple compartments. Note: Results of a search using two databases (MEDLINE and Web of Science core collection) on the 24th of November 2021.

8.6.1. Ecological MP risk assessments

The ecological risk assessment studies can be divided into two broad categories according to the choice of methods, aims and objectives. Liu et al. (2019) executed environmental sampling to define atmospheric MP level and used the findings in an ecological risk assessment model, based on the chemical toxicity of polymers as defined by Lithner et al. (2011). The authors used the ecological risk index (ERIn) for calculations, which is a method developed for aquatic pollution by Hakanson (1980). Although the authors conclude that suspended MPs pose only a minor ecological risk, they recognize that significant limitations exist in their analysis. One of the limitations was the use of MPs levels measured in Paris (Dris et al., 2017), as their background contamination levels, potentially introducing serious systematic error. They also note that the purpose of their risk assessment was to provide a preliminary discussion and that they did not focus on the "exact data values"(Liu et al., 2019: 464). ERIn was also used by Li et al. (2021) for evaluating MP pollution of pond sediments. Li et al. (2021) also recognize that the use of this method has serious limitations as the background values are unknown which hinders the calculation of the exact risk index. Wang et al. (2021) also used this method but additionally used the similar method of pollution index (PLI) (Tomlinson et al., 1980) for the risk assessment of surface waters. For their calculations they used the minimum concentration of MP identified across all sampling sites but did not provide a justification for this alteration in the methods. The same two methods were also implemented by Xu et al. (2018) for the risk assessment of surface waters, by Pan et al. (2021) for an estuarine environment, by Peng et al. (2018) for river sediments, by Ranjani et al. (2021) for coastal sediments and by Yin et al. (2021) for surface water in nature reserves. Pico et al. (2021) only used the PLI method and focused on treated wastewater.

Although several studies have been using the ERIn and PLI methods for MP ecological risk assessments, the majority did not recognize the important inherent limitations. The ERIn method was developed as a diagnostic tool to help define which substances and which locations/environmental compartments should be prioritised for water pollution control

measures. Hakanson (1980) stresses that the tool can only be applied to limnic systems, while sediment data were used exclusively in its development. A further limitation that is not recognized by any of the studies is that they used the Lithner et al. (2011) indexes which is a polymer hazard ranking and does not take into consideration the specific physicochemical characteristics of MPs. Although the studies provide a new perspective to environmental MP contamination, the confidence in their results is limited.

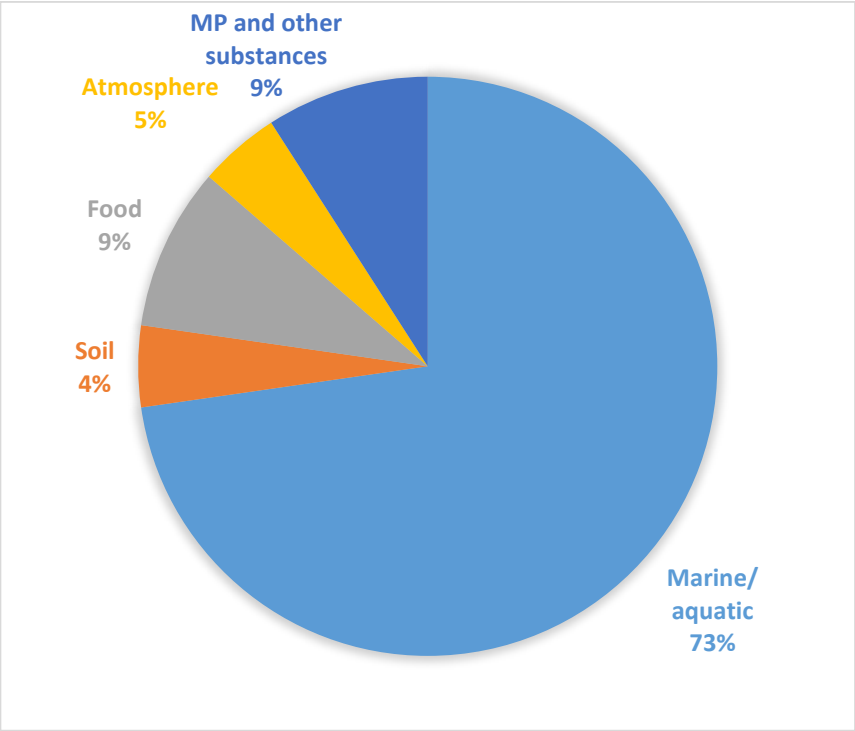


Figure 86. Risk assessment studies categorized by focus

A different approach was adopted by the following studies largely based on species sensitivity distribution (SSD) models. Everaert et al. (2018) attempted a risk assessment of MPs in the oceans. For their risk assessment they used inputs for the MP levels of contamination in marine waters, MP levels in marine bivalves, and results from animal MP toxicological studies to calculate the predicted environmental concentrations (PEC) and the predicted no effect concentrations (PNEC). They calculated a no-harm threshold concentration for MPs (6650 particles m^{-3}) and predicted that no adverse effects will occur until the year 2100. On the other hand, Everaert et al. (2018) did expect adverse effects along the coasts, where this threshold was currently exceeded, in the second half of the 21st century. They note that more ecotoxicological data for environmentally relevant concentrations of MPs were needed to verify their modelling outcomes. Burns and Boxall (2018) stated that they undertook a systematic review to assess the adverse effects of MPs in the aquatic environment (freshwater and marine). Unfortunately, the methods for the systematic review

were not reported and therefore it cannot be accessed. They concluded that current data did not suggest that MPs can cause harm to the environment. They note that the available data were not always environmentally relevant, that a lot of locations around the world have not been examined and that more extensive research is needed for the risk characterization. The study by Besseling et al. (2019) focused on both NPs and MPs in the aquatic environment. Regarding MPs in water, they concluded that MP concentrations might indeed pose a threat, but only to sensitive species that live in MP hotspot locations, situated in near-shore regions. They based their results on worst case scenarios, taking into consideration possible underestimations of MP concentrations, and stressed that their results were mainly presented to illustrate their recommended approaches and do not substantiate strong conclusions.

Two studies focused on freshwater environments. Adam et al. (2019) concluded that although risk cannot be ruled out, there is no immediate risk to the environment posed by MPs. Xin Zhang et al. (2020) on the other hand, used primary data from sampling to calculate the PNEC values. They stated that due to insufficient data they were only able to produce PNEC values for surface waters and not for sediments. They concluded that the risk to freshwater species was high for areas near tributaries and low in all the other areas they sampled. They did not compare their results with other studies in the field.

Jung et al. (2021) combined primary sampling data from sea waters (coastal, continental shelf and deep sea), and toxicology *in vivo* experiments using a fish species (*Cyprinodon variegatus*) and secondary data. They focused on a narrower size range of 20 – 300 μm of only non-spherical MPs, aligned to their primary data results, concluding that current levels of MPs contamination do not pose a risk to the aquatic ecosystem. The PNEC values were far lower than those reported by previous studies (hazardous concentration for protecting 95% of the species (HC_5): 58.7 MPs/L compared to 1016-64,000 MPs/L) and were attributed to the use of lower toxicity values which were in turn affected by the focus on non-spherical MPs. Similar to the previous studies they also note that the predicted future rise of MPs environmental contamination is likely to shift the risk assessment result.

Chen et al. (2021) is the only aquatic risk assessment to have used a toxicokinetic and toxicodynamic model. They based their models on data coming from *in vivo* toxicology studies using zebrafish (*Danio rerio*) and red tilapia (*Oreochromis niloticus*). They also reported using systematic reviews to collect the data used for estimating environmental MP levels. Unfortunately, they did not provide any details on the systematic review process and therefore it cannot be appraised. They concluded that MPs do pose a risk to fish health and associated the health effects to metabolic disturbances. There is a major difference in their

findings compared to the Burns and Boxall (2018) and the Adam et al. (2019) studies. Chen et al. (2021) attribute this shift to the fact that they used toxicokinetic/toxicodynamic modelling thus addressing in more detail the potential biological effects; taking into consideration the size of MPs and the use of updated environmental concentration predictions.

8.6.2. Food and human MP risk assessments

The FAO report by Amy Lusher et al. (2017) on MPs in fisheries and aquaculture includes a risk characterization case study for bivalves consumption. The focus was only on the MP additives and the contaminants that MPs might carry as vectors. The report stated that a risk assessment of the MPs themselves was not possible due to lack of scientific data. Amy Lusher et al. (2017) concluded that the effects caused by the uptake of MPs' contaminants and additives via the consumption of contaminated seafood was very likely to be negligible.

Welle and Franz (2018) executed a literature review to identify the levels of MP contamination in bottled natural mineral water. They reported MP concentrations in a range between 0.1-10 μg/L and used these results in a human exposure assessment reporting exposures of 0.17, 1.0 and 2.0 μg/kg b.w. per day for adults, toddlers and infants, respectively. They based their exposure assessment on a 1 L bottled water consumption per day, but did not provide evidence to support this consumption rate. It should be noted that they use this consumption estimation for adults, toddlers and infants alike. Furthermore, they used the results coming from one *in vivo* animal study, which exposed rats to PE and PET ground fabric (Merski et al., 2008), to set human MPs TDI values for humans of 5 mg/kg (body weight). They adjusted their exposure assessment to take into consideration a correction factor of 1% for potential absorption based on toxicokinetic hypothesis and used the margin of safety approach (EFSA, 2010). The 1% correction was not justified by the authors other than it was a more conservative assumption than the 0.3% which was mentioned in the report by EFSA (2016) (see section 3.7.4.5). They concluded that only a small fraction could be absorbed and that the MP level of contamination in bottled water did not pose a risk to human health. This study used a similar approach to the present risk assessment, recognizing the need for corrections to estimate internal doses (see section 3.7.4.5, Equation 7 and Equation 8).

The Ferrante et al. (2021) study states that a risk assessment was executed, but the content of their research was to analyse commercially relevant fish samples (6 species) to define MP levels and then used their results for a human exposure assessment. They concluded that the

exposure to MPs via the consumption of seafood would be negligible when compared to exposures via bottled water consumption. Yozukmaz (2021) examined MP contamination in one mussel species (*M. galloprovincialis* and *R. decussatus*). The authors maintain that they executed a risk assessment and incorrectly claim that there is a maximum acceptable limit of 1000 MPs per 250 g of mussel tissue, citing a FAO (2017) newsletter which actually states that a TDI has not been established. They then compare the findings of their experiments to this limit. They conclude that MPs may pose a risk to human health since this limit was almost exceeded in their analysis.

Pastorino et al. (2021) collected primary data from the edible freshwater *Sinotaia quadrata* (Gasteropoda) and attempted a risk assessment for a range of potential toxic substances, including MPs. They concluded that the potential human health risk for MPs could not be assessed due to lack of data on adverse effects, coinciding with the data issues around health effects recognized in this thesis.

The European Chemicals agency recently published a (non-peer reviewed) proposal for restrictions to be imposed on intentionally added MPs in commercial products (ECHA, 2019). The proposal was based on an extensive risk assessment which took into consideration the known data from the literature concerning the release of MPs into the environment and their effects to biota including humans. For the quantitative ecological risk characterization, the results of the aforementioned risk assessments by Everaert et al. (2018), Burns and Boxall (2018) and Besseling et al. (2019) (section 8.6.1) were used. For the human health the FAO report by Amy Lusher et al. (2017) and the EFSA (2016) report on MP in seafood were used. ECHA concluded that MPs should be characterized “non-threshold substances and that releases to the environment are considered as a proxy for risk” (ECHA, 2019: 73). This means that a threshold below which effects to biota are not detected could not at that point be set. They concluded that MPs can cause eco-toxicological effects and that the effects will be hard to reverse in the future and propose a restriction on the use of MPs in consumer and professional products. They referred to a wide range of products: cosmetics, fertilizers, detergents and maintenance products, biocides, medical devices etc. A direct comparison with the present risk assessment cannot be made due to the context differences in the risk assessment focus.

8.7. Chapter conclusions

To summarize, taking into consideration that:

- only three food categories have been examined for MP contamination,

- the threshold for mechanistic toxic effects are likely to be exceeded,
- environmentally relevant levels of MP exposure have been associated with adverse effects on the cellular level,
- MP are proven to be persistent and bioaccumulate in marine organisms, creating implications for other species and potentially for humans,
- there is an increasing input of plastic waste disposal into the environment that will inevitably increase MP contamination and MP exposures (sections 1.1.1 and 8.6.1),
- there is limited remediation potential for MP after they are released in the environment, (section 8.6.1)

there is a high likelihood that human MP dietary exposures, along with other environmental exposures, can pose a risk to human health.

In this chapter the individual components of the scoping, systematic and rapid reviews, along with their meta-analysis and meta-regression components, were brought together to inform the four steps of the risk assessment. This analysis is the first attempt, to my knowledge, to execute a formal MP human health risk assessment incorporating up-to-date, well-established methodology along with novel methods. The limitations of the analysis are inextricably connected with the limitations of the currently available data and their quality. Nevertheless, the results provide the evidence base and establish the dietary MP exposure route via ingestion of contaminated food, propose possible health effects, both mechanistic and apical, and set thresholds for critical effects on the cellular level. Furthermore, the analysis highlights that the levels of environmental exposures exceed, in some cases, the thresholds of effect; with the caveat of lack of knowledge on MP toxicokinetics/ toxicodynamics in the human body.

Chapter 9. Discussion

9.1. Findings

Although the need for a risk assessment of MPs in terms of environmental exposures to humans has been recognized (SAM, 2019, SAPEA, 2019), it had yet to be attempted. The uncertainties surrounding this emerging contaminant present a number of difficulties related to the absence of standardization of experimental methods as well as the lack of human toxicity data (Gouin et al., 2019). These issues could be overcome by using methodology coming from other disciplines, such as those used in health sciences to collate data coming from various studies. Throughout this thesis, the steps of an evidence-based human health risk assessment have been illustrated.

The data for this thesis have been identified, collected, appraised, synthesised, and reported by using extensive, state-of-the-art reviewing and meta-analysis / meta-regression methods (sections 3.1 and 3.2). The planning and execution of these processes have been documented from the onset of the thesis and throughout. Thus, the claim can be made that the thesis has achieved reproducibility and transparency. The project was highly exploratory from the start, as such, the first step was to execute a set of ScRs in order to map the existing evidence and make informed decisions on what the realistic aims and objectives could be. In the first instance, the decision was made to focus on MPs which was one of the three EECs that were initially under consideration. Second, by mapping the existing evidence on potential human exposures, it became obvious that the majority of the data, and, indeed, the best quality data, were on the levels of contamination on what could be considered as media for dietary exposure pathway via the ingestion uptake route. Therefore, the next major decision was to effectively focus the thesis towards a food safety direction. Food safety on an evidence-based policy and legislative level aims to reduce, eliminate or avoid a risk to health. The determination of measures and actions that can protect health is achieved via the systematic methodology of risk analysis. Risk assessment is the first component of the risk analysis which was ultimately the framework and fabric of this thesis (Figure 9).

All the individual components of this thesis feed into the different steps and processes of risk assessment. Although the steps of risk assessment are not necessarily linear, traditionally the order followed is: hazard identification, hazard characterization, exposure assessment and risk characterization. In this thesis, the order that the individual steps were executed was somewhat different.

The results of the three systematic reviews on the MP contamination of food for human consumption built a robust data base, establishing the MP dietary pathway for the ingestion route of exposure (section 8.3). The systematic reviews fed into the hazard identification, in terms of defining MP physicochemical characteristics, and the exposure assessment. The vast majority of the samples analysed by the 72 studies that were reviewed in total, were indeed contaminated by MP at different levels. Apart from establishing the MP dietary pathway, the systematic reviews had further significant outcomes: levels of food contamination were identified in different media and aligned to further characteristics, dominant MP polymeric composition was discovered while strengths and weaknesses in MP environmental research were brought to view.

The level of contamination varied across the different media, while there was also a lot of variation within the categories of each food theme (section 8.3.4, Table 50). The quantitative results of the statistical summaries, along with the results of the meta-analyses (sections 4.5 and 6.5.2) were the basis of the exposure assessment modelling (section 8.3). The level of contamination can be attributed to the inherent characteristics of each medium and the geographical source (sections 4.9, 5.7 and 6.9). For salt, the individual origin in terms of sea, lake and rock/well, seemed to be connected with the resulting levels of MPs. Sea salt exhibited the highest contamination, followed by lake and rock/well. Similarly, tap water samples were far less contaminated than the bottled water samples. An association between geographical origin and level of contamination was not detected for either medium. This might be due to limitations in the available data. For both media of salt and water, MP contamination can be divided to environmental or primary i.e. how much is originally in the medium, and secondary, whereby MPs are introduced during processing (including transportation and packaging). This thesis has demonstrated that both categories of contamination can be affected by geographical location. Levels of environmental MP contamination vary across the world. Likewise, processing and food safety standards also vary and are guided by local food legislation and policy. On the other hand, in seafood, the third medium that was reviewed, MP levels were found to be affected by both the organisms' characteristics i.e. habitat and feeding, and the geographical origin. Filter and bottom feeder organisms (molluscs) were found to be more highly contaminated, associating exposure and retention of MP to feeding habits and physiology. Furthermore, the highest levels of MP molluscs' contamination were associated with the habitat off the coasts of Asia. This finding could be further hypothesised to be connected with the level of environmental contamination in those areas (Jambeck et al., 2015). MP origin and fate in the environment is a special field in MP research in its own right, and has not been examined within this thesis. In terms of polymeric composition of MP food contamination, PP and PE were dominant in both salt

and seafood highlighting the link between plastic production, waste mismanagement and MP environmental contamination.

The available scientific data and their quality introduced important limitations. The development of a bespoke RoB assessment tool within this thesis enabled the systematic and transparent quality assessment of every study included in the reviews (section 3.2.7). The overall quality of the evidence as assessed using standardised methods was found to be low to moderate. The process and the results of the RoB assessment informed the inclusion/exclusion of evidence in the final results but also highlighted specific areas where MP research design, execution and reporting needs further development and standardization (sections 5.9 and 6.10). These areas can be summarized across all food themes as follows:

- Study design
    - Setting clear and realistic aims and objectives, taking into consideration current scientific methods, available technology, and time restrictions.
    - Communicating the overall rationale of the study design.
- Sampling methodology
    - Defining number of samples and replicates. Report size, location, frequency, instruments, and connecting all the above to the study design and rationale.
    - Detailed registration of sample characteristics and reporting.
- Laboratory analysis
    - Particle extraction process specifications: sample volumes, chemicals used for digestion and density separation, type and pore size of filters.
    - Spectral analysis:
        - Use of one of the currently validated methods: FT-IR, RM, SEM, Pyr-GC-MS and SEM/EDS.
        - Proportion of extracted particles for analysis.
        - Spectral similarity index and choice of spectral libraries
        - Report MP concentrations in as many metrics (e.g. MPs/mL, μg/mL) as possible to aid comparison and use in toxicological studies.
- Statistical analysis
    - Justification for use of statistical methods and detailed reporting of results not only in figures.
- Quality control/ Quality assurance
    - Use of procedural blank samples throughout the execution of the study.

- Use of standard quality control measures in MP research, such as avoiding the use of plastic material whenever possible, thorough cleaning of all instruments etc.
- Use of LOD/LOQ methods when is it feasible and realistic to do so.

The lack of detailed reporting of sample sizes and outcomes reduces confidence in study findings, and hinders the use of scientific data in consequent meta-analysis attempts. Meta-analysis studies are essential, especially in the field of MP research, where there are no environmental monitoring systems in place. Another key benefit of meta-analysis is that in many cases individual studies may be very small, so pooling the results provides much more robust results. Therefore, in order to achieve a large scale understanding of the issues, collating scientific data from individual studies is not only insightful but, in reality, the only way forward.

The results of the rapid review of MP toxicity on human cells informed two parts of the risk assessment: hazard identification (section 8.1) and hazard characterization (section 8.2). Four biological endpoints were found to be affected by MP exposure. A similar level of heterogeneity in the experimental designs of the toxicological studies was identified as in the environmental studies. This finding coincides with the relatively short period of time that MPs have been researched in toxicological studies, making designs more exploratory than standardized. Issues with the minimum, maximum and the range of the applied concentrations of the test MPs, and further MP characteristics, such as polymer type and shape hinder in-depth comparison and synthesis of scientific data. A series of recommendations (section 7.12) could help focus the study designs in future studies. During the execution of this rapid review, a novel RoB tool (MP-tox-RoB) was developed for the systematic and transparent appraisal of MP toxicology studies (section 3.4.2). MP-tox-RoB has a dual role as it can also be used a matrix for the design of future toxicological MP studies, addressing every part of research stages.

Regarding the risk assessment process, there at least four significant outcomes coming from the rapid review. First, a wide range of human mechanistic (cellular level) biological endpoints have been shown to be affected by exposure to MPs (Table 21 and Figure 51). Second, apart from the conventionally expected experimental characteristics of MP concentration and duration of exposure, MP shape was also found to significantly affect cell viability (section 7.11.3). In fact, it was the only MP characteristic that was found to predict cell death. Third, non-monotonic dose-response relationships were observed in cytotoxicity results (section 7.10.1). Fourth, threshold levels of effects were identified and found to be

environmentally relevant (7.11.4). All four outcomes were explored in both the rapid review and the risk assessment process.

The results of the MP human health ScR (sections, 2.2.1 and 8.1.1) was the other source of information feeding into the hazard identification step, providing 'big picture' insights. This review had a wider scope thus capturing more versatile human health MP outcomes and routes, including non-dietary ingestion and inhalation and occupational settings, which were outside the primary focus of the risk assessment. The findings of the ScR helped frame the aims and objectives of this thesis but also identified possible routes for future research (see section 9.2).

The final analysis of the risk assessment process is executed in the risk characterization step. The fundamentals of this step consist of comparing the levels of exposure to the hazard (exposure assessment) to the threshold levels (hazard characterization) of health effects (hazard identification) and determining whether they are exceeded, and under what circumstances (risk characterization). If the thresholds are exceeded, risk to health is present. Lack of scientific evidence did not allow for the completion of the risk characterization. Nevertheless, preliminary results suggest that estimations of current exposures via dietary ingestion are indeed exceeding the levels of thresholds of effects (sections 8.3.4 and 8.4). Overall, the findings of the risk assessment support the implementation of the precautionary principle for the protection of the environment (section 3.5.5), and more specifically, for managing food safety against the physical and/or chemical hazard of MPs (sections 5.9, 6.9 and 9.3.1). The study by Welle and Franz (2018) is the only study to have attempted a similar approach to the present thesis, but on a much smaller scale and using less robust methodology (section 8.6.2). Focusing only on the consumption of MP contaminated bottled water they concluded that there would be no risk posed to human health based on the assumption that only a small fraction of the MPs would be able to constitute internal and therefore hazardous doses. Their analysis is aligned with the analysis and the findings of this risk assessment in recognizing the need for further MP toxicokinetics and toxicodynamic research.

Looking at the body of the existing environmental risk assessments (see section 8.6.1), a convergence on three topics is observed, which coincide with the findings of the present risk assessment:

- The need for better and more focused data on MP environmental contamination and MP health effects incorporating toxicodynamic and toxicokinetic data.

- The distinction between the risk posed by MPs to species of varying sensitivity and habitat.
- The assumption that MP contamination will inevitably increase in the future, pushing the risk characterization results in regions of higher concern.

A recent EU evidence review report came from the Science Advice for Policy by European Academics (SAPEA, 2019). They examined the issue of MPs from two perspectives: natural sciences and society, and behavioural sciences. The report pointed out that although the number of studies is growing rapidly, knowledge around MPs is not growing at the same pace, which can be attributed to the inherent complexity of the issue at hand. The report acknowledged the need for standardization in all aspects of research and testing at environmentally relevant doses; which is aligned with the findings and the recommendations of the present risk assessment. It also took account of the social dimension of the issue, especially in terms of policies, interventions and public engagement as tools of reducing MPs pollution. The report concluded that the degree of MPs and NPs toxicity as well as their impacts on the environment were uncertain. The European Commission has also recently published an independent expert report on the environmental and health risks of MPs (SAM, 2019). The report had been largely based on the aforementioned SAPEA (2019) report. Their recommendations also coincided with the ECHA (2019) proposal. The report made a case for new policy to restrict MPs pollution, addressing the MPs issue in a socio-economic context, and global cooperation for research and policies.

In the UK the FSA is advised on food safety issues by science advisory committees. The committee on toxicity (COT) has recently published an overarching statement on the potential risks from exposure to MPs (COT, 2021). They concluded that a risk assessment could not be executed at the moment due to significant data gaps around MP toxicity, low confidence in the data of food MP contamination, and lack of standardised MP related methodologies. They propose a set of future research priorities to tackle these issues. Their findings and recommendations highlight the significance and the contribution to knowledge of the thesis.

9.1.1. Strengths and limitations

Data gaps in the specific areas of MP toxicokinetic and toxicodynamic characteristics and standardized dietary patterns, have hindered the execution of a more detailed and complete risk characterization. In addition, the quality of the existing scientific data and the highlighted heterogeneity of analytical methods and reporting standards have, to an extent,

affected the external validity of the findings. The risk assessment is based only of the three food categories for which data were currently available and does not provide the full picture of MP human exposures via dietary ingestion.

Nevertheless, this thesis represents one of the first (if not the first) attempts of a formal food safety risk assessment concerning the emerging contaminant of MPs; providing contributions to knowledge on both methods and food safety outcomes. Up-to-date, robust methodology and methods have been used throughout the thesis. Transparency and reproducibility was, by design, build in the fabric of the thesis. Two novel RoB tools have been developed to assess the quality of existing environmental and toxicological MP studies and guide the design of future studies.

9.2. Future work

In the course of this thesis, significant data gaps in human MP risk assessment have started to be filled in. Nevertheless, further research is needed. The most important data gap identified in the present work is in the MP toxicokinetics area, where evidence is scarce. There are two fields that can provide data to this end: MP *in vivo* and *in vitro* animal studies (section 7.11) and medical prosthetics wear and effects studies (sections 2.2.1.2 and 3.7.1). Both these areas have an increasing number of studies. Comprehensive systematic or rapid reviews could be executed to identify this scientific data and attempt to synthesise them. Separate reviews are needed targeting animal data including studies on rats (Rafiee et al., 2018, Li et al., 2020), mice (Deng et al., 2020, Jin et al., 2020) and zebrafish (Malafaia et al., 2020, Santos et al., 2020) species. Data coming from animal studies can help define the dose-response relationship using a BMD or a NOAEL approach (see section 3.7.3) and better describe the toxicological profile of MPs.

The use of medical prosthetic implants has been widespread in the past decades; many are partially or entirely made of plastic material. Issues with wear and tear of these implants, and the production (degradation) of plastic particles, have been investigated for several years concerning mainly orthopaedic implants (Alias et al., 2012, Catelas et al., 1999, Gajski et al., 2014, Holding et al., 2006, Witkiewicz et al., 1993). Data from these studies could be a valuable source of additional information towards toxicokinetics and toxicodynamics of MPs. The focus of these studies is somewhat different to the MP toxicological studies so care should be taken to consider whether their results can be extrapolated to MPs. Nevertheless, many studies focus on PE wear particles which is one of the dominant polymers identified in food, which further highlights their potential use.

9.2.1. MP risk analysis

Risk assessment sits within the overarching processes of risk analysis. It is the first and integral step, followed by risk management and risk communication (sections 1.2, 1.3). Regarding dietary exposures to MPs, in the future, risk management, novel identification and mitigation processes and strategies must be identified and/or developed to inform a risk management plan. The focus should be on cutting-edge technology that can readily identify MP contamination at the food industry level and techniques that can extract MPs both at the industry and at the consumer level. For example, most data collection on MPs is done with well-established spectroscopy such as FTIR and RM methods in the laboratory. However, there is a clear need to think about novel, user-friendly technology which can be deployed directly where food processing is taking place so that the level of MP contamination can be assessed on a real-time basis. The other aspect is how to remove MPs from foodstuffs and how to prevent MP contamination in the first place. For example, as highlighted in the seafood systematic review (section 6.5.1), depuration of molluscs, i.e. placing them in clean water tanks before they are released to the food market, has potentially shown impact on the level of MP contamination (Birnstiel et al., 2019).

The final element is risk communication where a risk perception and communication strategy for stakeholders is constructed. A strategic partnership with government agencies and food industry drivers is needed. Risk communication requires identifying key stakeholders and understanding the form which would be the most useful for them in terms of understanding the results of the risk assessment and risk management steps. This will differ by audience, from government to industry, environmental professionals to general public. In a wider perspective, MP contamination of food has an asymmetrical impact on developing countries where MP contamination of e.g. fresh water is much higher. There is therefore very interesting scope to build projects together with stakeholders in ODA countries.

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Appendices

Appendix 1

a) Term search strategy for the microplastics and health effects Scoping Review:

1. **TOPIC:** (microplastic*) *AND* **TOPIC:** (human AND health) (307)
2. **TOPIC:** (polyethylene AND plastic*) *AND* **TOPIC:** (human AND health) (339)
3. **TOPIC:** (polypropylene) *AND* **TOPIC:** (human AND health) *AND* **TOPIC:** (plastic*) (140)
4. **TOPIC:** (poly AND vinyl OR polyvinyl) *AND* **TOPIC:** (human AND health) *AND* **TOPIC:** (plastic*) (216)
5. **TOPIC:** (polystyrene OR polyurethane OR styrene) *AND* **TOPIC:** (human AND health) *AND* **TOPIC:** (plastic*) (279)
6. **TOPIC:** (fibre* OR fiber* OR flock) *AND* **TOPIC:** (human AND health) *AND* **TOPIC:** (synthetic OR *plastic* OR nylon) *NOT* **TOPIC:** (optical) *NOT* **TOPIC:** (mineral) *NOT***TOPIC:** (asbestos) *N OT* **TOPIC:** (feed OR food) *NOT* **TOPIC:** (glass) *NOT* **TOPIC:** (diet OR dietary) *NOT* **TOPIC:** (muscle) (446)
7. **TOPIC:** (*plastic*) *AND* **TOPIC:** (wear OR debris OR particle*) *AND* **TOPIC:** (health) *AND* **TOPIC:** (human) *NOT* **TOPIC:** (mouse OR mice OR rat* OR fish*) (1,340)
8. **TOPIC:** (*plastic*) *AND* **TOPIC:** (wear OR debris OR particle*) *AND* **TOPIC:** (toxic) *NOT* **TOPIC:** (mouse OR mice OR rat* OR fish*) (583)

At this point duplicates were removed. A combination of the searches from 1-8 brought the following results:

9. #8 OR #7 OR #6 OR #5 OR #4 OR #3 OR #2 OR #1 (2,973).
   Two additional term searches were executed when additional topics when additional topics were identified:

10. **TOPIC:** (plasticiser*) *AND* **TOPIC:** (health AND human) (74)
11. **TOPIC:** (tire* Or tyre*) *AND* **TOPIC:** (wear OR debris OR particle*) *AND* **TOPIC:** (health AND human) *AND* **TOPIC:** (*plastic*) (20)

At this point duplicates were removed again. The last combination off all the previous results brought:

12. #11 OR #10 OR #9 (3,026)

b) Term search strategy for the 3-D printer dust and health effects Scoping Review:

1. **TOPIC:** (3 or three)
   AND **TOPIC:** (d or dimensio*)
   AND **TOPIC:** (print*) *AND* **TOPIC:** (health) (692)
2. **TOPIC:** (3d or 3-d or 3?d) *AND* **TOPIC:** (print*) *AND* **TOPIC:** (health) (713)

Searches were pointed-duped. The final combined search identified 994 possible hits:

3. #1 OR #2 (994)

c) Term search strategy for the estrogens and health effects Scoping Review:

TOPIC: (estrogen* OR oestrogen* or *estrone* or *estradiol* or *estriol* or phthalate or bisphenol or "flame retardant*" or xenoestrogen* or xeno-estrogen* or "xeno estrogen*" or "xeno-oestrogen*")
AND **TOPIC:** (human)
AND **TOPIC:** (health OR effect)
AND **TOPIC:** (contamin* OR pollut*)
NOT **TOPIC:** (mouse OR mice OR rat OR rats or fish* or phyto* or plant*) (4,149)

d) Term search strategy for the microplastics' distribution Scoping Review:

1.1 **TOPIC**: (microplastic*) (2,218)
1.2 **TOPIC**: (micro-plastic*) (272)
At this point search 1 and 2 where combined using OR and returned:
1.3 #2 OR #1 (2,312)
1.4 **TOPIC**: (nano-plastic*) (37)
1.5 **TOPIC**: (nanoplastic*) (196)
At this point search 4 and 5 were combined using OR and returned:
1.6 #5 OR #4 (204)
At this point search 3 and 6 were combined using OR and returned:
1.7 #6 OR #3 (2,377)
Another search strategy was then build in parallel:
2.1 **TOPIC**: (plastic*) (612,585)
2.2 **TOPIC**: (microfibre*) (465)
2.3 **TOPIC**: (micro-fibre*) (100)
2.4 **TOPIC**: (microfiber*) (4,690)
2.5 **TOPIC**: (micro-fiber*) (506)
2.6 #5 OR #4 OR #3 OR #2 (5,401)
2.7 #6 AND #1 (964)

At this point searches 1.7 and 2.7 were combined using OR and returned:
#1.7 OR #2.7 (3,280)
which is the final number of the studies produced by the search strategies.

Appendix 2

MPs extraction protocol for drinking water

Mintenig et al. (2019)	**Panno et al. (2019)**	**Pivokonsky et al. (2018)**
Filtered through 3μm stainless steel cartridge filters		
Residual water and air removed from filter units using filtered compressed air		
Filter units filled with diluted hydrochloric acid to dissolve calcium carbonate and iron precipitates (24h)		
Filters rinsed with Milli-Qand and ethanol		
Retentate was collected on 3μm stainless steel filters		
Filters covered with 30 mL hydrogen peroxide		Wet peroxide oxidation on a 75 °C stirring hotplate for 30 min*
Incubated for 24 h at 40 °C		Digest for 24 h
Samples was enriched onto a 0.2 μm aluminium oxide filter	Filtered through a 0.45 μm filter	Membrane filters (PTFE) of 5 μm and then 0.2 μm.
Filters were dried at 40 °C	Filters dried at 75 ◦C for 24 h	Filters were dried at 30 °C for 30 min
Additional step for raw water samples: density separation using a zinc chloride solution to remove iron oxide particles		
settling time of 24 h		

*to remove organic material

Appendix 3

Authors who were contacted for further data or explanation of their published material on salt studies ("No" means that the authors were sent a maximum of three emails requesting additional information to which they did not respond)

Authors	**Provided extra data**
Birnstiel et al. (2019)	No
Bour et al. (2018)	No
Collard et al. (2017a)	Yes
Collard et al. (2017b)	Yes
Fang et al. (2018)	Yes
Karami et al. (2017a)	No
Karami et al. (2017b)	No
Karami et al. (2018)	No
Li H. X. et al. (2018)	No
Li J. et al. (2018)	Yes
Li J. et al. (2015)	Yes
Naji et al. (2018)	No
Qu et al. (2018)	No
Seth and Shriwastav (2018)	No
Su et al. (2018)	Yes
Thushari et al. (2017)	No
Yang et al. (2015)	No
Zhang et al. (2019)	No

Seafood studies:

Authors	**Provided extra data**
Birnstiel et al. (2019)	No
Collard et al. (2017a)	Yes
Collard et al. (2017b)	Yes
Fang et al. (2018)	Yes
Karami et al. (2017c)	No
Karami et al. (2018)	No
Li J. et al. (2016)	Yes
Li H. X. et al. (2018)	Yes
Qu et al. (2018)	No
Su et al. (2018)	Yes
F. Zhang et al. (2019)	No

Appendix 4

a) Search strategy for MEDLINE and EMBASE (OVID) with Medical Subject Headings (MeSH) searching.

	Search terms
1	microplastic*
2	micro-plastic*
3	nanoplastic*
4	nano-plastic*
5	plastic/
6	micro
7	fiber*
8	fibre
9	microfiber*
10	microfibre*
11	micro-fiber*
12	micro-fibre*
13	particle*
14	particle size/
15	pellet*
16	fragment*
17	film*
18	filament*
19	rubber/
20	5 and 6
21	5 and 7
22	5 and 8
23	5 and 9
24	5 and 10
25	5 and 11
26	5 and 12
27	5 and 13
28	5 and 14
29	5 and 15
30	5 and 16
31	5 and 17
32	5 and 18
33	5 and 19
34	1 or 2 or 3 or 4 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33

35	food quality/ or food dye/ or food ingredient/ or canned food/ or food packaging/ or food contamination/ or food industry/ or food insecurity/ or cooked food/ or food safety/ or food analysis/ or food chain/ or fast food/ or dried food/ or sea food/ or food handling/ or food security/ or food/
36	water table/ or drinking water/ or water quality/ or tap water/ or water pollutant/ or water contamination/ or water pollution/
37	sea food/
38	fish/
39	bivalve disease/ or bivalve/
40	Crustacea/
41	35 or 36 or 37 or 38 or 39 or 40
42	34 and 41
43	nanoparticle/ or nano
44	5 and 43
45	41 and 44
46	42 or 45

b) Search strategy for Web of Science

	Search terms
1	**TOPIC:** (microplastic*) *OR* **TOPIC:** (micro-plastic*)
2	**TOPIC:** (nanoplastic*) *OR* **TOPIC:** (nano-plastic*)
3	**TOPIC:** (microfibre*) *OR* **TOPIC:** (micro-fibre*) *AND* **TOPIC:** (plastic*)
4	**TOPIC:** (microfiber*) *OR* **TOPIC:** (micro-fiber*) *AND* **TOPIC:** (plastic*)
5	**TOPIC:** (rubber*) *AND* **TOPIC:** (micro*) *AND* **TOPIC:** (plastic*)
6	#5 OR #4 OR #3 OR #2 OR #1
7	**TOPIC:** (food)
8	**TOPIC:** (water) *AND* **TOPIC:** (drinking)
9	**TOPIC:** (seafood)
10	**TOPIC:** (fish*)
11	**TOPIC:** (bivalve*) *OR* **TOPIC:** (bivalvia*)
12	**TOPIC:** (crustacean*)
13	#12 OR #11 OR #10 OR #9 OR #8 OR #7
14	#13 AND #6
15	**TOPIC:** (nano) *AND* **TOPIC:** (plastic*)
16	#15 OR #6
17	#16 AND #13
18	**TOPIC:** (micro) *AND* **TOPIC:** (plastic*)
19	#18 OR #16
20	#19 AND #13

Appendix 5

RoB tool explanation/elaboration

Study design

1. Is the design appropriate for the questions of the study? Yes/No

Explanation: The design must be observational (non-analytic) which is the appropriate design for an environmental study in this field.

Sampling

Sampling method

2. Has the method been used in other studies? Yes/No and what they are

3. Is the method validated? Yes/No

Explanation: Depending on the sample under examination (salt, seafood species, water) the sampling method should be the one usually used in the field or state why they used a different one.

4. Are there precautions in place to protect further contamination of the sample? Yes/No and what they are.

Explanation: These precautions begin from the collection of the sample and continue until the end of the analysis. They include: putting the sample in a container that is free from MPs immediately after sampling, executing all procedures in a laminar flow chamber, wearing plastic-free laboratory coats and gloves, thoroughly cleaning all surfaces and equipment, filtering all reagents to remove MPs before use, and using procedural blank samples throughout the experimental protocol.

Sample location

5. Is there a rationale available? Yes/No

Explanation: The study should provide a rationale on how they chose the sampling location according to the objectives of their study.

6. Is the location appropriate? Yes/No

Explanation: The location should be appropriate to the objectives of the study.

Sample randomization

7. Is the sampling method guarantying randomization of the sample? Yes/No

Explanation: According to sampling methodology guidelines.

Use of procedural blank samples

8. Are the results of the procedural blank samples reported? Yes/No

Explanation: Reporting of the results is vital for the validity of the study.

Use of replicate samples

9. Is the study using replicate samples? Yes/No

10. How many? Number

Explanation: According to common experimental practice, replicates must be used. In any case they should be not less than three.

Analysis

Particles' extraction method

11. Is the method used by other studies? Yes/No

12. Is the method validated? Yes/No

Explanation: The method is considered validated when it has been used by other peer-reviewed studies or if there is a validation protocol embedded in the process.

Particles' identification method

13. Is the method one of the four validated methods? Yes/NO

Explanation: The four validated methods are Fourier-transform infrared spectroscopy (FT-IR), Raman spectroscopy (RM), pyrolysis gas chromatography/ mass spectrometry (Pyr-GC-MS) and scanning electron microscopy plus energy-dispersive X-ray spectroscopy (SEM/EDS).

Amount of sample analysed for composition.

14. How much of the sample has been analysed? Numerical value

Explanation: The higher the proportion of the sample the higher the confidence in their findings. A specific number is not used for this rating as these will vary between different sample categories and as such they are assessed within their category.

Particle composition match to the library of choice

15. Is the match > or < 60% match? Yes/No and numerical value

Explanation: According to current scientific practices a spectral match lower than 60% is not considered reliable.

Library of choice (type, kind)

16. Is the library made by the lab or is it a commercial library?

Explanation: Spectra and Pyrograms can be compared to either bespoke or commercial libraries. The latter are considered more reliable as they are available for validation/verification.

17. Is one library or more being used? Yes/No

It is common practice that more than one library is used to improve the match quality and strength.

Statistical analysis

18. Is the statistical analysis appropriate for the sample?

Explanation: Appropriate means the statistical analysis that is commonly used according to the objectives of the study.

Interpretation

19. Has the interpretation of the results been based on the outcomes of the analysis?

Explanation: The conclusions should be based on the reported results from the experimental analysis and statistical analysis.

Quality of reporting

Methodology

20. Have the methods used in the study been reported in detail?

Explanation: All methods throughout the study protocol should be reported in detail in the main paper or in supporting material so that the study can duplicated and verified. The answer to this question also draws from the previous answers given in the tool in the domains of sampling and analysis.

Limitations

21. Has the study recognized limitations?

Explanation: Authors should report how their results relate to the wider picture of their field and whether have identified important limitations. Understanding and reporting limitations relates to both internal and external validity of the study.

Appendix 6

Search term strategy for MP *in vitro* toxicology rapid review

The terms were searched using the Web of Science interface, searching simultaneously two databases: Web of Science Core Collection and MEDLINE.

1. **TOPIC:** microplastic
2. **TOPIC:** microplastic*
3. **TOPIC:** micro-plastic
4. **TOPIC:** micro*
5. **TOPIC:** plastic*
6. 4 AND 5
7. **TOPIC:** human
8. **TOPIC:** cell
9. 7 AND 8
10. 1 OR 2 OR 3 OR 6
11. 10 AND 9

Appendix 7

Simplifications for logistic regression modelling

Density: Choi et al. (2021) used both HDPE and LDPE, HDPE-MPs (density, 0.96 g/cm^3) were included in the same group as the rest of the PE-MPs used by other studies (density range: 0.94 to 1.070 g/cm^3) while the LDPE-MPs were left separately (density; 0.918 g/cm^3). Similarly, the COOH-PS-MPs were grouped with the rest of the PS-MPs and the PP(Sun)-MPs with the PP-MPs.

Cell models: The two Caco-2 co-cultures (Caco-2/HT29-MTX-E12; Caco-2/HT29-MTX/MDM/MDDC) were grouped as one for the cytotoxicity outcome.

Shape: For the shape covariate, all the shapes that were not defined as spherical by the authors or the commercial manufacturers were put in a category termed irregular, except for the study by (Wu S. et al., 2020) which did not provide any information on the shape nor origin of the test MP.

Appendix 8

Reasons for the exclusion against the inclusion/exclusion criteria for all food categories

Full-text articles excluded in the second level screening n =153

30: focused only on the GI tract of the seafood, for seafood that is not eaten whole

26: MPs identification method was not one of the four accepted in this SR

15: did not use procedural blanks samples

12: the sample was not food or drinking water

9: studies were not available

8: studies were not environmental study

4: the studies did not mention any MPs content data

4: the results were not specific to MPs

3: papers reporting conferences

1: was a duplicate publication

1: was a corrigendum to a study that is already included in the review

41: focused on other food categories (34 seafood, 7 drinking water)

Reasons for the exclusion only of salt studies

1: the results were not specific to MPs

1: not available in the English language

2: MPs identification method was not one of the four accepted in this SR

R**easons for the exclusion only of salt studies (re-run of searches)**

3: the sample was not food

3: review papers

2: sample collected directly from the environment

1: did not use procedural blanks samples

Reasons for the exclusion of drinking water studies

3: the sample was not drinking water

2: studies were not available

2: studies were not environmental study

1: MPs identification method was not one of the four accepted in this SR

Reasons for the exclusion of drinking water studies (re-run of searches)

2: not drinking water

1: study was not environmental study

1: MPs identification method was not one of the four accepted in this SR

1: not primary research

Reasons for the exclusion of seafood studies (original searches, including all food themes)

30: focused only on the GI tract of the seafood, for seafood that is not eaten whole

26: MPs identification method was not one of the four accepted in this SR

15: did not report the use of procedural blanks samples

14: focused on other food categories (salt and drinking water)

11: the sample was not food or drinking water

9: studies were not available

8: studies were not environmental study

4: the studies did not mention any MPs content data

4: the results were not specific to MPs

3: papers reporting conferences

1: was a duplicate publication

1: was a corrigendum to a study that is already included in the review

Reasons for the exclusion of seafood studies (re-run of searches)

15: focused only on the GI tract of the seafood, for seafood that is not eaten whole

12: did not report the use of procedural blanks samples

9: MPs identification method was not one of the four accepted in this SR

4: the results were not specific to MPs

3: the sample was not seafood

3: data not available

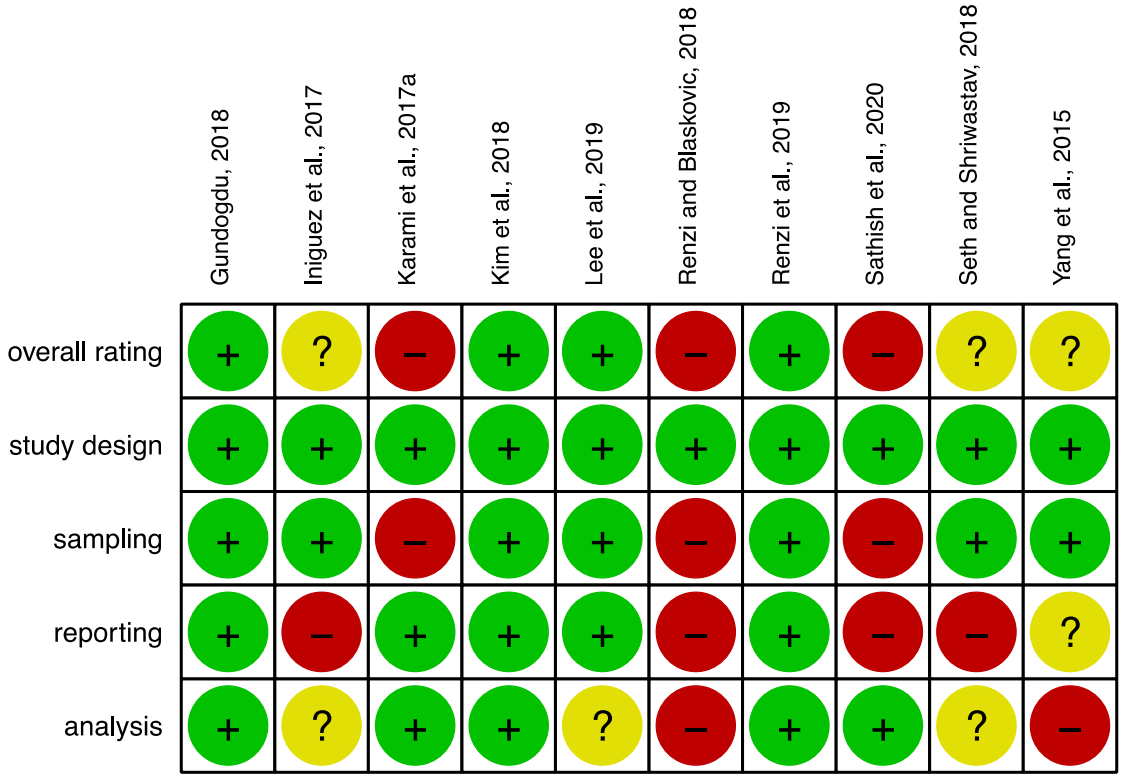
2: not environmental study

1: not available

1: was a duplicate publication

Appendix 9

Salt studies individual Risk of Bias (RoB) assessment. Red (-) indicates high RoB, green (+) indicates low RoB and yellow (?) indicates unclear RoB



Appendix 10

GRADE certainty framework assessment for salt studies.

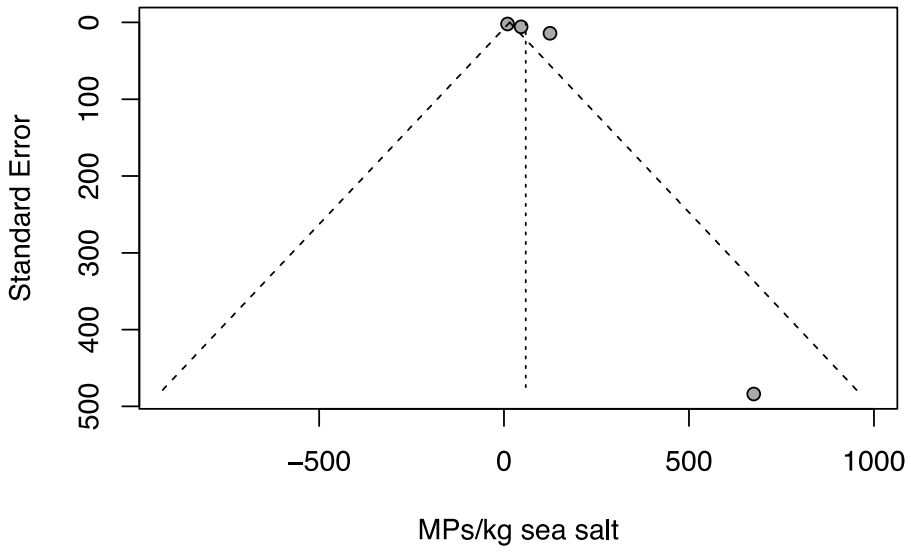
Domains for assessing certainty of evidence by outcome	**Results section**	**Reasons for lowering or increasing the certainty of evidence**
Risk of bias (downgrading)	Three studies were rated as of high Rob, three of unclear and the rest four of low RoB. Rating was accessed over four domains. **Meta-analysis** Lake: two low RoB studies Rock: two low RoB studies Sea: three low RoB studies and one of uncertain RoB Well: no meta-analysis. **Statistical summary/ narrative analysis** All high RoB studies were excluded from the synthesis.	For the meta-analysis, the evidence will not be downgraded as the one study that was rated as of high risk of bias was not included in the analyses. For the statistical summary the evidence will not be downgraded as the three high RoB studies were excluded from the synthesis.
Inconsistency (heterogeneity) (downgrading)	**Meta-analysis** Heterogeneity was high in the analysis of sea salt. **Statistical summary/ narrative analysis** The range of MP content was higher in the sea salt results, but in the same order of magnitude as the rest of the salt origins.	Downgrade the meta-analysis evidence only for the sea salt outcomes due to high heterogeneity as measured by I^2, Chi^2, and corresponding p value.
Indirectness (downgrading)	All studies measured the outcome addressed by the question of the review.	Will not downgrade evidence.
Imprecision (downgrading)	The sample size n for the studies varied from 8 to 39 brands/products. The 95% CIs for the studies in the meta-analysis are acceptable.	Will not downgrade evidence.
Publication bias (downgrading)	Publication bias was substantial but addressed in synthesis.	For the meta-analysis the funnel plot detected bias coming from one study. This study has already been observed to disproportionally affect the meta-analysis due to extreme size effects, which has been

		taken into consideration in the synthesis of the evidence. For the statistical summary the country of origin did not point to publication bias.
Large effects (upgrading)	The estimates are not large, consistent and confident enough to allowing an upgrade of the evidence.	Will not upgrade the evidence.
Dose response (upgrading)	Dose response cannot, at this point, be accessed for these studies.	Not applicable.
Opposing plausible residual bias and confounding (upgrading)	All confounders have been considered.	The estimate of effect was controlled for possible confounders by measures to protect/measure post-sampling contamination and high accuracy identification of the chemical composition of particles. The certainty of the evidence will be increased for all studies.

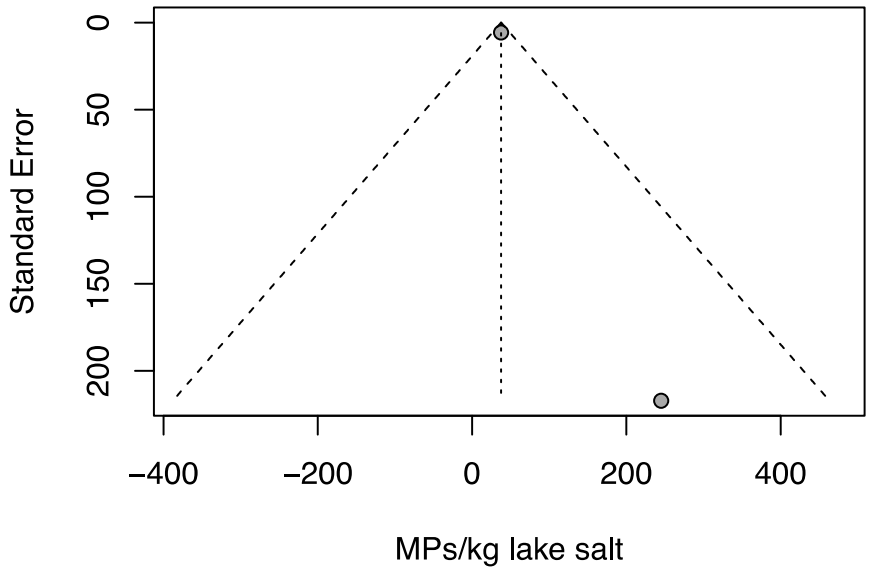
Appendix 11

Funnel plots for sea (A), lake (B) and rock (C) salt studies results. Dots represent individual studies. The vertical dotted line represents the pooled effect size. Diagonal lines represent pseudo 95% confidence limits.

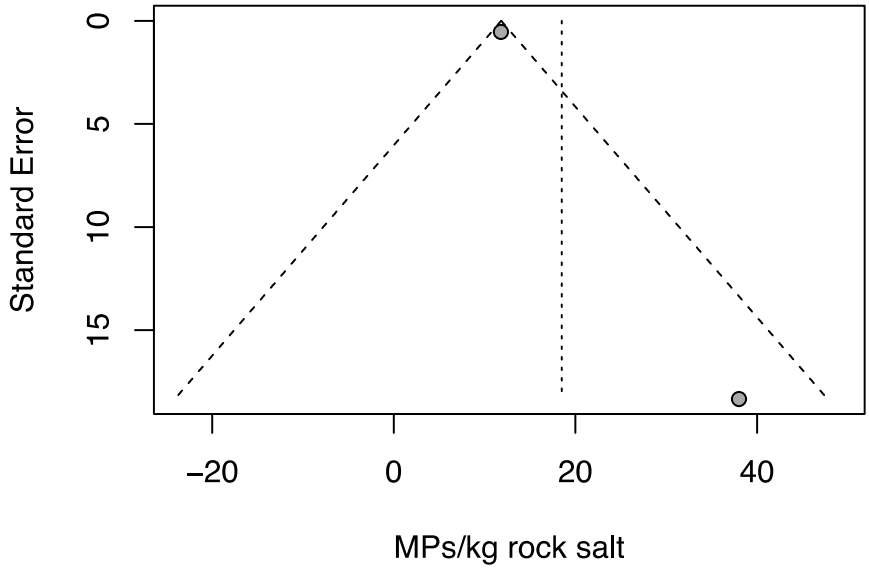
A.



B.

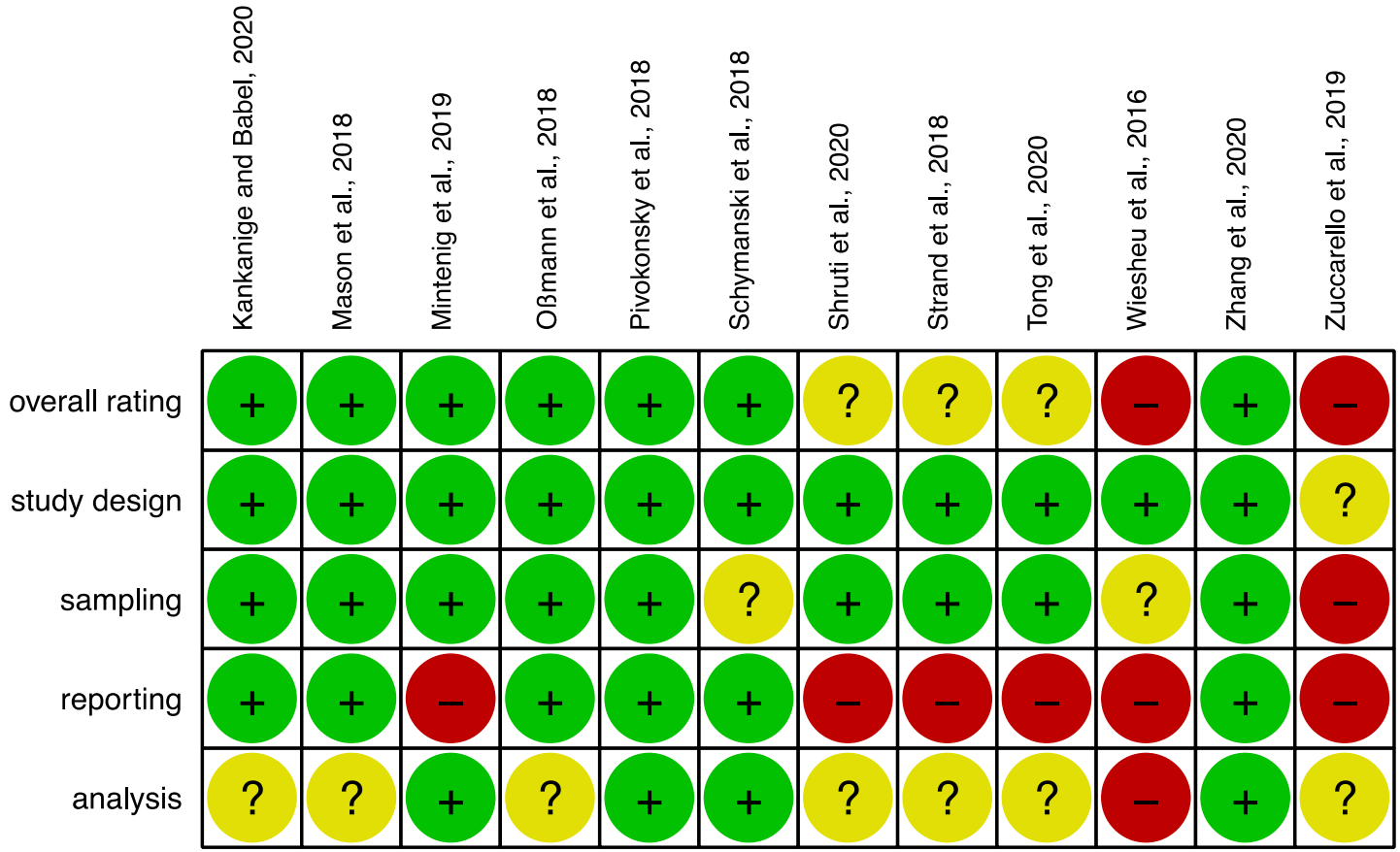


C.



Appendix 12

RoB assessment in individual water studies. The figure shows the rating for the four domains and the overall rating for each study. Red (-) indicates high RoB, green (+) indicates low RoB and yellow (?) indicates unclear RoB



Appendix 13

Particle identification specifications for tap water studies.

Study	Filter pore size	Method	Min size for spectral analysis	Particles extracted	Particles for analysis	% for analysis	Spectral similarity index	Verified MPs
Mintenig et al. (2019)	3 µm, 0.2 µm	FT-IR	≥ 20 µm	n/s[a]	n/s	100%	n/s	n/s
Pivokonsky et al. (2018)	5 µm, 0.2 µm	RM	1 µm	n/s	n/s	~25%	80%	n/s
		FT-IR	≥ 10 µm					
Shruti et al. (2020)	0.22 µm	m-RM	500 µm	n/s	n/s	n/s	n/s	n/s
Strand et al. (2018)	~12 µm[b], 0.2 µm[c]	FT-IR	≥ 10 µm	n/s	n/s	10% of 3 out of 17 samples.	n/s	3%
Tong et al. (2020)	0.2 µm	RM	n/s	n/s	n/s	n/s	n/s	n/s
M. Zhang et al. (2020)	0.45 µm	m-FT-IR		n/s	n/s	100%	70%	n/s

[a] not specified, [b] for MP content, [c] for spectral analysis

Note: FT-IR, Fourier-transform infrared spectroscopy; MPs, microplastics; n/s: not specified; RM, Raman spectroscopy

Appendix 14

Particle identification specifications for bottled water studies.

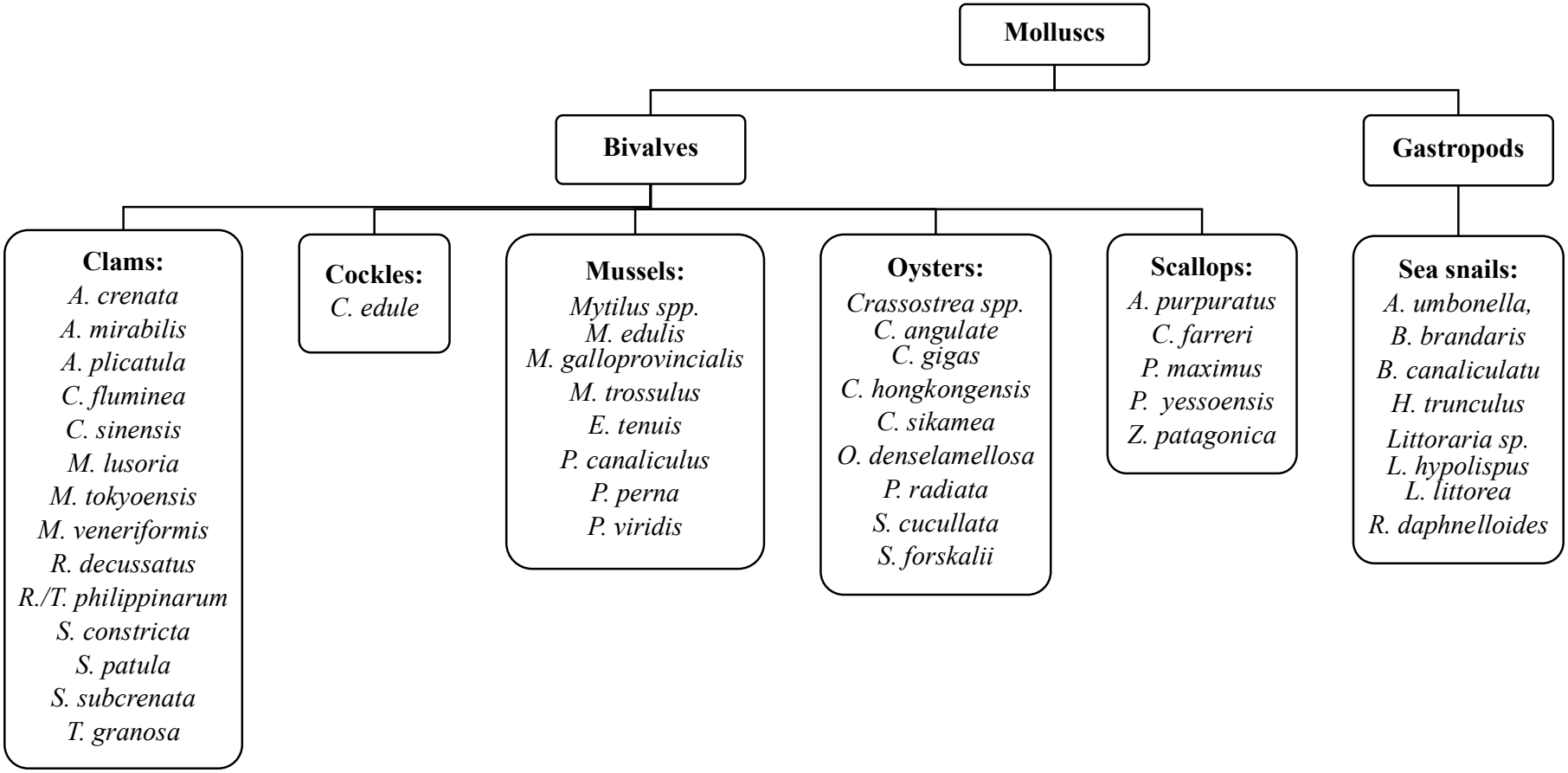
Study	Filter pore size	*Method*	Min size for spectral analysis	Particles extracted	Particles for analysis	% for analysis	Spectral similarity index	Verified MPs
Kankanige and Babel (2020)	0.45 µm	FT-IR	≥ 50 µm	839	839	100%	60%	45.8%
		RM	1-50 µm	n/s[a]	n/s	n/s	n/s	n/s
Mason et al. (2018)	1.5 µm	FT-IR	≥ 100 µm	n/s	~1000	~50%	70%	40%
Oßmann et al. (2018)	0.4 µm	RM	≥ 1 µm	n/s	n/s	4.4% of each filter area	n/s	n/s
Schymanski et al. (2018)	3 µm	RM	≥ 5 µm	n/s	~1000[b]	100%	70%	0.03 to 10.7%
Wiesheu et al. (2016)	0.45 µm	RM	≥ 1 µm	n/s	1	100%	n/s	n/s
Zuccarello et al. (2019a)	n/a[c]	SEM-EDX	0.5 µm	n/a	n/a	0.2% of each stub area	n/a	n/a

[a] not specified, [b] for each sample in the 5-10 µm size fraction, [c] not applicable

Note: FT-IR, Fourier-transform infrared spectroscopy; MPs, microplastics; n/s: not specified; RM, Raman spectroscopy; SEM-EDX, Scanning Electron Microscopy - Energy Dispersive X-Ray Spectroscopy

Appendix 15

Phylogenetic tree for the molluscan phylum. The species that are included in each study are presented in section 6.5.1.



Appendix 16

Particle extraction procedure details for seafood organisms.

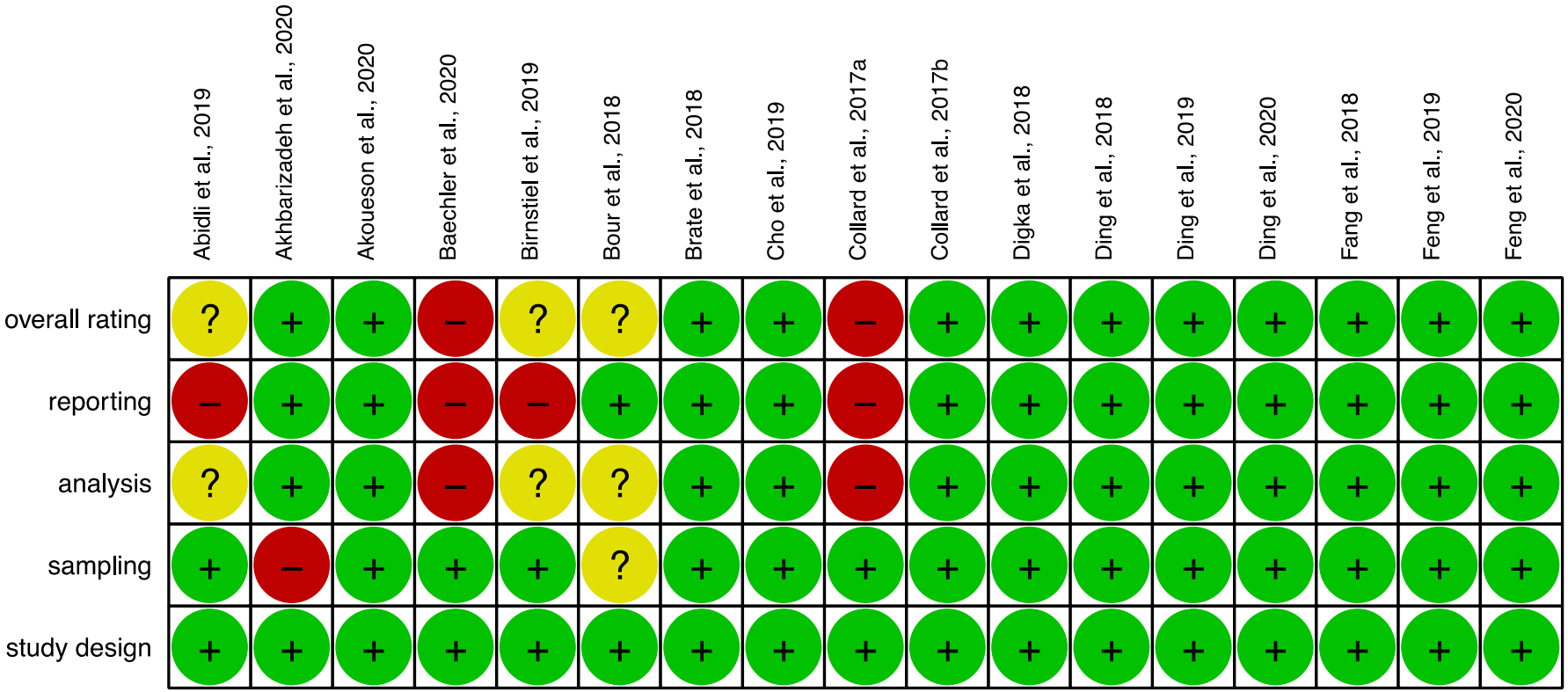
Study	Sample phylum	Extraction method	Chemical agent	Density separation
Abidli et al. (2019)	Bivalves/ gastropod molluscs	Li H. X. et al. (2018)	H_2O_2	yes
Akhbarizadeh et al. (2020)	Fish	Karami et al. (2017c)	KOH	no
Akoueson et al. (2020)	Fish, bivalve molluscs and crustacean	Li J. et al. (2018)	H_2O_2	no
Baechler et al. (2020)	Bivalve molluscs	developed their own	KOH	yes
Birnstiel et al. (2019)	Bivalve molluscs	Van Cauwenberghe et al. (2015)	H_2O_2	yes
Bour et al. (2018)	Bivalve molluscs and crustacean	Avio et al. (2015) and Dehaut et al. (2016)	KOH	yes
Brate et al. (2018)	Bivalve molluscs	Dehaut et al. (2016)	KOH	no
Cho et al. (2019)	Bivalve molluscs	Karami et al. (2017a)	KOH	no
Collard et al. (2017a)	Fish	Collard et al. (2015)	NaClO and CH_3OH	yes
Collard et al. (2017b)	Fish	Collard et al. (2015)	NaClO and CH_3OH	yes
Digka et al. 2018	Fish and bivalve molluscs	Mathalon and Hill (2014)	H_2O_2	no
Ding et al. (2018)	Bivalve molluscs	Ding et al. (2018)	KOH	no
Ding et al. (2019)	Bivalve molluscs	Ding et al. (2018)	KOH	no
Ding et al. (2020)	Bivalve/ gastropod molluscs	Ding et al. (2018) and Jinfeng Ding et al. (2019)	KOH	no
Fang et al. (2018)	Bivalve/ gastropod molluscs and crustacean	Dehaut et al. (2016) and Phuong et al. (2018a) Floatation/filtration: Li J. et al. (2015) and Li J. et al. (2016)	KOH	yes
Feng et al. (2019)	Fish	Dehaut et al. (2019), Foekema et al. (2013), Hermsen et al. (2018) and	KOH	yes

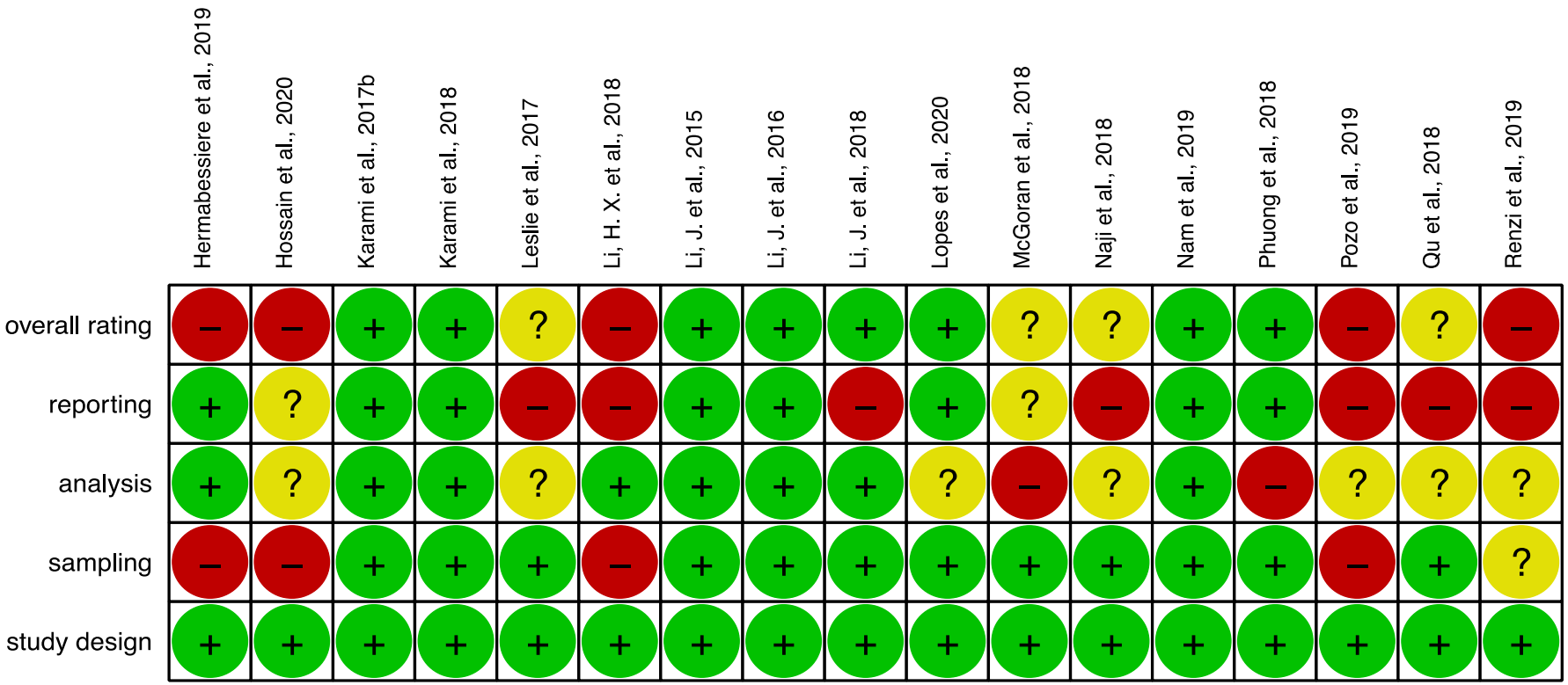
		Karami et al. (2017b)		
Feng et al. (2020)	Echinodermata	Foekema et al. (2013) and Karami et al. (2017b)	KOH	no
Hermabessiere et al. (2019)	Bivalve molluscs	Dehaut et al. (2016)	KOH	no
Hossain et al. (2020)	Crustacean	Li J. et al. (2015) and Su et al. (2016)	H_2O_2	yes
Karami et al. (2017c)	Fish	Karami et al. (2017b)	KOH	yes
Karami et al. (2018)	Fish	Karami et al. (2017b)	KOH	yes
Leslie et al. (2017)	Bivalve/ gastropod molluscs and crustacean	Van der Horst (2011), (2013)	H_2O_2	no
Li H. X. et al. (2018)	Bivalve molluscs	Li J. et al. (2015)	H_2O_2	yes
Li J. et al. (2015)	Bivalve molluscs	developed their own	H_2O_2	yes
Li J. et al. (2016)	Bivalve molluscs	Li J. et al. (2015)	H_2O_2	yes
Li J. et al. (2018)	Bivalve molluscs	Li J. et al. (2016)	H_2O_2	yes
Lopes et al. (2020)	Fish	Dehaut et al. (2016)	KOH	no
McGoran et al. (2018)	Crustacean	developed their own	No digestion	no
Naji et al. (2018)	Bivalve/ gastropod molluscs	Li J. et al. (2015)	H_2O_2	yes
Nam et al. (2019)	Bivalve molluscs	Phuong et al. (2018b)	KOH	yes
Phuong et al. (2018a)	Bivalve molluscs	Phuong et al. (2018b)	KOH	yes
Pozo et al. (2019)	Fish	Lindeque and Smerdon (2003)	Proteinase-K	no
Qu et al. (2018)	Bivalve molluscs	Li J. et al. (2015)	H_2O_2	yes
Monia Renzi et al. (2019)	Fish	Nuelle et al. (2014) and Avio et al. (2015b)	H_2O_2	yes
Su et al. (2018)	Bivalve molluscs	Li J. et al. (2015) and Su et al. (2016)	H_2O_2	no
Su et al. (2019)	Fish	Jabeen et al. (2017)	H_2O_2	no
Sun et al. (2019)	Fish	Desforges et al. (2015)	HNO_3	no
Tanaka and Takada (2016)	Fish	Foekema et al. (2013) and	KOH	yes

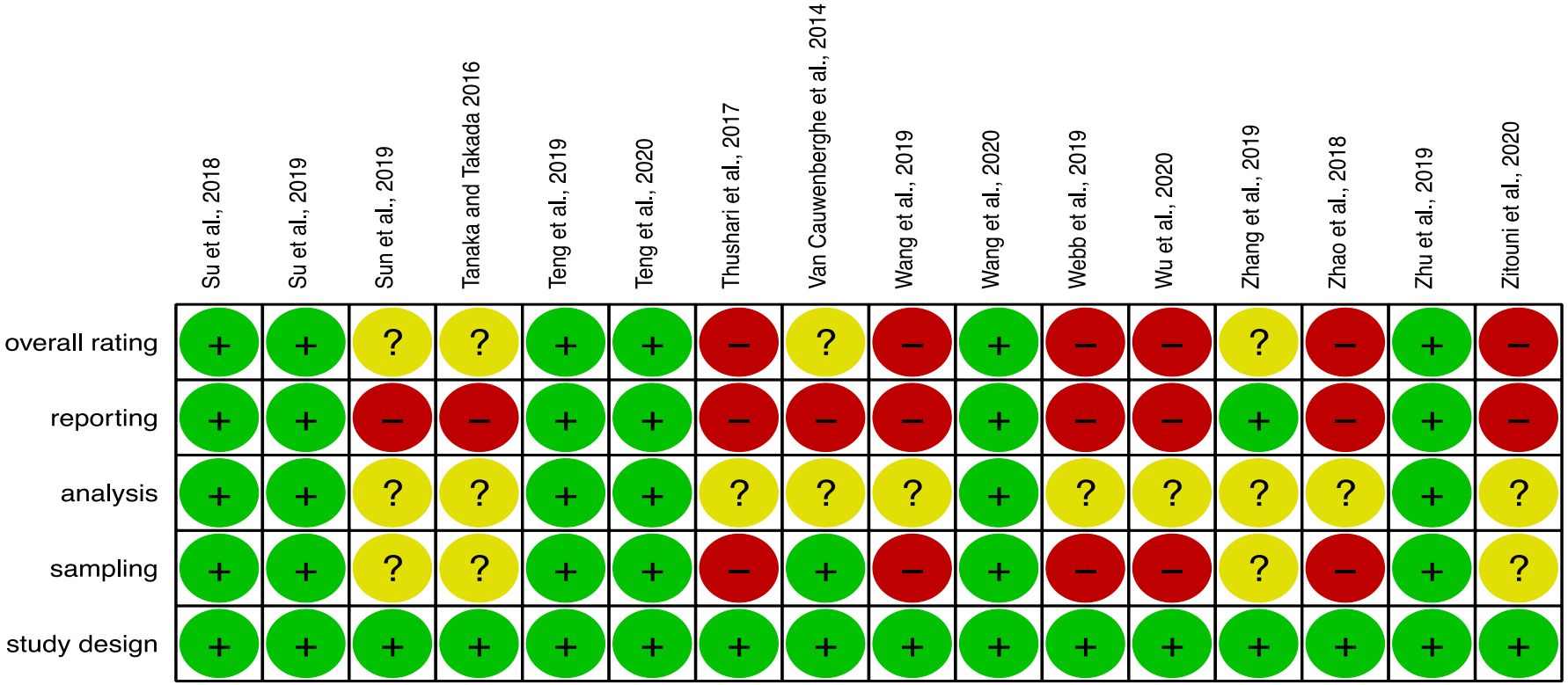
		Rochman et al. (2015)		
Teng et al. (2019)	Bivalve molluscs	Munno et al. (2018)	H_2O_2	no
Teng et al. (2020)	Fish	Munno et al. (2018)	KOH and H_2O_2	no
Thushari et al. (2017)	Bivalve/ gastropod molluscs and crustacean	Claessens et al. (2013)	HNO_3	no
Van Cauwenberghe and Janssen (2014)	Bivalve molluscs	Claessens et al. (2013)	HNO_3	no
Wang et al. (2019)	Bivalve molluscs and crustacean	Claessens et al. (2013)	HNO_3	no
Q. Wang et al. (2020)	Fish	Munno et al. (2018)	KOH and H_2O_2	no
Webb et al. (2019)	Bivalve molluscs	Claessens et al. (2013)	HNO_3	no
F. Z. Wu et al. (2020)	Fish, bivalve molluscs and crustacean	Li J. et al. (2015)	KOH and H_2O_2	no
F. Zhang et al. (2019)	Crustacean	Masura et al. (2015)	H_2O_2	yes
Zhao et al. (2018)	Bivalve molluscs	Zhao et al. (2017)	H_2O_2	yes
Zhu et al. (2019)	Bivalve molluscs	Foekema et al. (2013) and Karami et al. (2017a)	KOH	no
Zitouni et al. (2020)	Fish	Dehaut et al. (2016) and Phuong et al. (2018b)	KOH	yes

Appendix 17

RoB rating for all seafood studies. The table shows the rating for the four domains and the overall rating for each study. Red (-) indicates high RoB, green (+) indicates low RoB and yellow (?) indicates unclear RoB (Unclear RoB is given to a study when substantial information to make an informed assessment have not been reported).




Hermabessiere et al., 2019
Hossain et al., 2020
Karami et al., 2017b
Karami et al., 2018
Leslie et al., 2017
Li, H. X. et al., 2018
Li, J. et al., 2015
Li, J. et al., 2016
Li, J. et al., 2018
Lopes et al., 2020
McGoran et al., 2018
Naji et al., 2018
Nam et al., 2019
Phuong et al., 2018
Pozo et al., 2019
Qu et al., 2018
Renzi et al., 2019
overall rating
reporting
analysis
sampling
study design


Su et al., 2018
Su et al., 2019
Sun et al., 2019
Tanaka and Takada 2016
Teng et al., 2019
Teng et al., 2020
Thushari et al., 2017
Van Cauwenberghe et al., 2014
Wang et al., 2019
Wang et al., 2020
Webb et al., 2019
Wu et al., 2020
Zhang et al., 2019
Zhao et al., 2018
Zhu et al., 2019
Zitouni et al., 2020
overall rating
reporting
analysis
sampling
study design

Appendix 18

Procedural blank samples results use

Study (year)	Phylum	Procedural blanks results use
Abidli et al. (2019)	molluscs	free of contamination
Akhbarizadeh et al. (2020)	fish	absolute number subtracted
Akoueson et al. (2020)	molluscs, fish	statistical test executed to compare sample results and blank results
Baechler et al. (2020)	molluscs	blank contamination taken into account
Birnstiel et al. (2019)	molluscs	considered to be negligible (no justification)
Bour et al. (2018)	molluscs, crustaceans	free of contamination
Bråte et al. (2018)	molluscs	corrected by subtracting the daily mean value of the blank per sample
Cho et al. (2019)	molluscs	detection limit was calculated as three times the average number of particles in blank samples
Collard et al. (2017a)	fish	free of contamination
Collard et al. (2017b)	fish	free of contamination
Digka et al. (2018)	molluscs, fish	absolute number subtracted
Ding et al. (2018)	molluscs	background value was deducted in the statistical results
Jinfeng Ding et al. (2019)	molluscs	free of contamination
Ding et al. (2020)	molluscs	free of contamination
Fang et al. (2018)	molluscs, crustaceans	free of contamination
Feng et al. (2019)	fish	considered to be negligible (no justification)
Feng et al. (2020)	echinodermata	considered to be negligible (no justification)
Hermabessiere et al. (2019)	molluscs	results not reported
Hossain et al. (2020)	crustaceans	results not reported
Karami et al. (2017c)	fish	free of contamination
Karami et al. (2018)	fish	free of contamination
Leslie et al. (2017)	molluscs, crustaceans	corrected for the blanks
Li H. X. et al. (2018)	molluscs	results not reported
Li J. et al. (2015)	molluscs	not specified how the results were used
Li J. et al. (2016)	molluscs	not taken into consideration, blanks results accounting for less than 5% of the average number of microplastics in mussels
Li J. et al. (2018)	molluscs	statistical test executed to compare sample results and blank results
Lopes et al. (2020)	fish	free of contamination
McGoran et al. (2018)	crustaceans	limit of detection set by results

Naji et al. (2018)	molluscs	free of contamination
Nam et al. (2019)	molluscs	free of contamination
Phuong et al. (2018a)	molluscs	free of contamination
Pozo et al. (2019)	fish	results not reported
Qu et al. (2018)	molluscs	free of contamination in lab, considered to be negligible (no justification) in field
Monia Renzi et al. (2019)	fish	data were corrected by the subtraction of blanks
Su et al. (2018)	molluscs	contamination was not subtracted from the final results, (no justification)
Su et al. (2019)	fish	statistical test executed to compare sample results and blank results
Sun et al. (2019)	fish	free of contamination
Tanaka and Takada (2016)	fish	free of contamination
Teng et al. (2019)	molluscs	contamination was removed when the microplastic abundance was counted
Teng et al. (2020)	fish	average number of MPs in blank samples was subtracted when calculating the abundance
Thushari et al. (2017)	molluscs, crustaceans	results not reported
Van Cauwenberghe and Janssen (2014)	molluscs	free of contamination
Jun Wang et al. (2019)	molluscs, crustaceans	results not reported
Q. Wang et al. (2020)	fish	considered to be negligible (no justification)
Webb et al. (2019)	molluscs	results not reported
F. Z. Wu et al. (2020)	molluscs, crustaceans, fish	results not reported
F. Zhang et al. (2019)	crustaceans	free of contamination
S. Y. Zhao et al. (2018)	molluscs	results not reported
Zhu et al. (2019)	molluscs	reported MPs abundance was corrected by the procedural blank data
Zitouni et al. (2020)	fish	free of contamination

Appendix 19.

Composition identification process characteristics for molluscan studies.

Study	Process	Library used	% of particles for analysis	No of particles analysed	% of particles verified as MPs	Similarity to spectra library (%)
Abidli et al. (2019)	FT-IR	not specified	n/s	30	n/s	n/s
Akoueson et al. (2020) fish	FT-IR	Hummel polymer library database	16	96	17-5	> 70
Akoueson et al. (2020) scallops	FT-IR		27	101	16-60	> 70
Baechler et al. (2020)	FT-IR	not specified	0.9	26	n/s	20-95
Birnstiel et al. (2019)	FT-IR	not specified	n/s	n/s	n/s	n/s
Bour et al. (2018)	FT-IR	not specified	n/s	n/s	n/s	> 70
Brate et al. (2018)	FT-IR	not specified	25	224	n/s	n/s
Cho et al. (2019)	RM	Thermo Scientific spectral library database	100	n/s	n/s	> 70
Digka et al. (2018)	FT-IR	Made in-house	20	n/s	n/s	> 80
Ding et al. (2018)	FT-IR	OMNIC picta software library	100	n/s	n/s	n/s
Ding et al. (2019)	FT-IR, SEM	Sadtler spectra library	100	n/s	n/s	> 70
Ding et al. (2020)	FT-IR	Sadtler spectra library	100	373	n/s	> 70
Fang et al. (2018)	FT-IR	Thermo Scientific spectral library database	36	182	69	n/s
Hermabessiere et al. (2019)	RM	Made in-house	80	1312	17.3	> 70
Leslie et al. (2017)	FT-IR	Bruker library	6	n/s	100	n/s

Li H. X. et al. (2018)	FT-IR	OMNIC software polymer spectral database	n/s	139	89.2	n/s
Li J. et al. (2015)	FT-IR	e.g., Hummel Polymer and Additives and Polymer Laminate Films	n/s	n/s	n/s	n/s
Li J. et al. (2016)	FT-IR	not specified	8.5	126	84.5	> 80
Li J. et al. (2018)	FT-IR	Perkin Elmer: PolyATR, AR Polymer Introduc-tory, NDFIBS, RP, CRIME, FIBRES 3, POLY1, POLYADD 1	13	138	34	> 70
Nam et al. (2019)	FT-IR	not specified	100	n/s	n/s	> 60
Naji et al. (2018)	FT-IR	not specified	3	59	n/s	n/s
Phuong et al. (2018a)	FT-IR	Perkin Elmer Polymer database	100	3285	6	> 60
Qu et al. (2018)	FT-IR	Bruker database	n/s	306	n/s	n/s
Su et al. (2018)	FT-IR	Bruker database	11.5	150	81	> 70
Teng et al. (2019)	FT-IR	OMNIC polymer spectra library	24.7	301	94	> 70
Thushari et al. (2017)	RM	not specified	n/s	n/s	n/s	n/s
Van Cauwenberghe and Janssen (2014)	RM	not specified	n/s	n/s	n/s	n/s

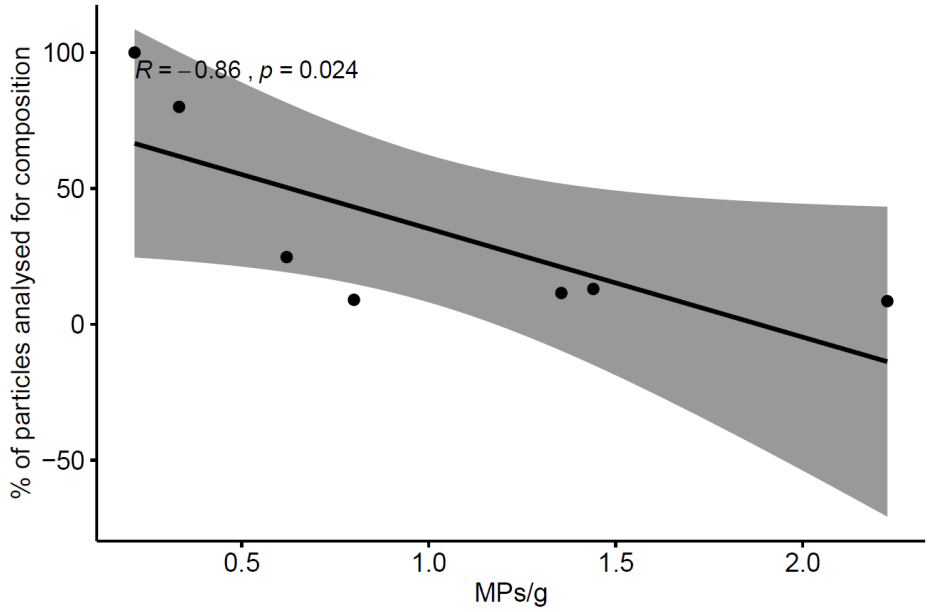
Wang et al. (2019)	FT-IR, SEM	not specified	n/s	200	n/s	n/s
Webb et al. (2019)	FT-IR	not specified	100	21	n/s	n/s
F. Z. Wu et al. (2020)	FT-IR	OMNIC polymer spectra library	100	n/s	n/s	> 70
Zhao et al. (2018)	FT-IR, RM	not specified	n/s	n/s	n/s	n/s
Zhu et al. (2019)	FT-IR	Thermo Fisher Scientific library of polymers provided by in their software (OMNIC Picta)	9	158	73	> 60

Note: FT-IR, Fourier-transform infrared spectroscopy; MPs, microplastics; n/s: not specified; RM, Raman spectroscopy; SEM, scanning electron microscope

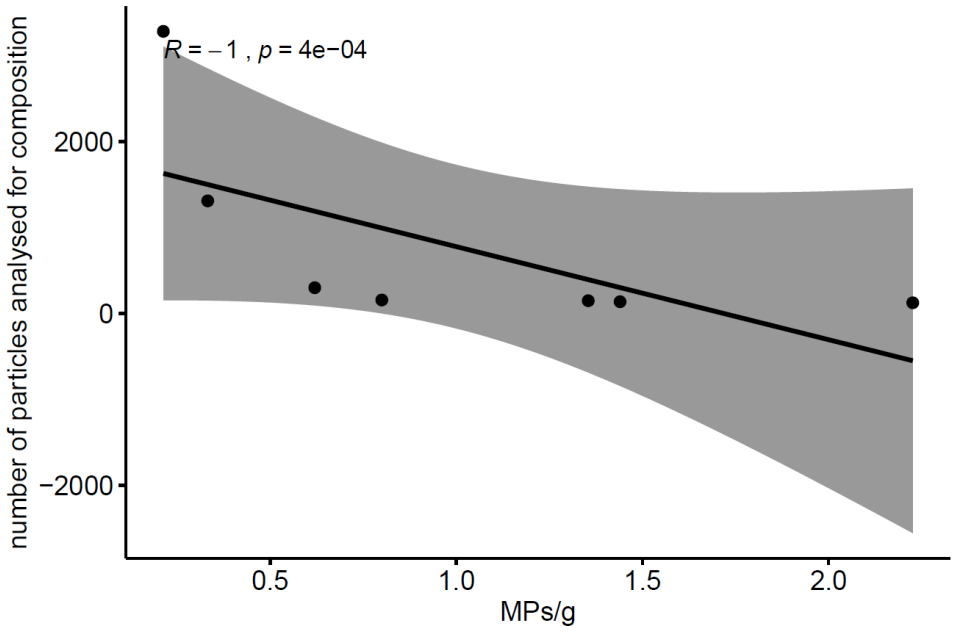
Appendix 20

Spearman correlation analysis between (A) the amount of MPs/g in mussels and the percentage of the particles analysed for composition, (B) the amount of MPs/g in mussels and the number of the particles analysed for composition. R is the correlation coefficient with the corresponding p value.

A



B



Appendix 21

Meta-analysis data pooled by family of molluscs.

Study	Class	Family	N	MPs/g	SD	Geographic location	Continent	S.A.	RoB	Source	Habitat
Baechler et al. (2020)	bivalve	clams	142	0.16	0.02	USA	Americas	FT-IR	high	Environment	Wild
Cho et al. (2019)	bivalve	clams	60	0.34	0.31	South Korea	Asia	RM	low	Market	Farmed
Ding et al. (2019)	bivalve	clams	20	0.53	0.47	China	Asia	FT-IR	low	Market	N/A
Fang et al. (2018)	bivalve	clams	57	0.07	0.10	Bering Sea and Chukchi Sea	Asia and Americas	FT-IR	low	Environment	Wild
Li J. et al. (2015)	bivalve	clams	120	4.24	2.36	China	Asia	FT-IR	low	Environment	Wild/ Farmed
Su et al. (2018)	bivalve	clams	208	1.35	1.40	China	Asia	FT-IR	low	Environment	Wild
Wang et al. (2019)	bivalve	clams	20	6.90	2.10	South Yellow Sea, Korea and China	Asia	FT-IR	high	Environment	Wild
Hermabessiere et al. (2019)	bivalve	cockles	100	0.47	0.37	France	Europe	RM	high	Environment	Wild
Brate et al. (2018)	bivalve	mussels	332	0.97	2.61	Norway	Europe	FT-IR	low	Environment	Wild
Cho et al. (2019)	bivalve	mussels	60	0.12	0.11	South Korea	Asia	RM	low	Market	Farmed

Ding et al. (2019)	bivalve	mussels	20	0.29	0.24	China	Asia	FT-IR	low	Market	N/A
Hermabessiere et al. (2019)	bivalve	mussels	100	0.20	0.13	France	Europe	RM	high	Environment	Wild
Li J. et al. (2018)	bivalve	mussels	162	1.74	0.79	U.K.	Europe	FT-IR	low	Environment	Wild
Li J. et al. (2018)	bivalve	mussels	36	0.91	0.19	U.K.	Europe	FT-IR	low	Market	Farmed
Li J. et al. (2015)	bivalve	mussels	18	2.39	1.32	China	Asia	FT-IR	low	Environment	Wild/ Farmed
Nam et al. (2019)	bivalve	mussels	5	0.29	0.14	Vietnam	Asia	FT-IR	low	Environment	Wild
Phuong et al. (2018a)	bivalve	mussels	120	0.23	0.20	French Atlantic coasts	Europe	FT-IR	low	Environment	Wild/ Farmed
Van Cauwenberghe and Janssen (2014)	bivalve	mussels	36	0.36	0.07	Germany	Europe	RM	unclear	Environment	Farmed
Webb et al. (2019)	bivalve	mussels	96	0.03	0.04	New Zealand	Australia/ Oceania	FT-IR	high	Environment	Wild
Zhao et al. (2018)	bivalve	mussels	37	0.60	1.20	Avery Point dock, U.S.A.	Americas	FT-IR, RM	high	Environment	Wild
Abidli et al. (2019)	bivalve	oysters	3	1.48	0.02	Tunisia	Africa	FT-IR	unclear	Environment	Wild
Baechler et al. (2020)	bivalve	oysters	141	0.35	0.04	USA	Americas	FT-IR	high	Environment	Farmed
Cho et al. (2019)	bivalve	oysters	60	0.07	0.06	South Korea	Asia	RM	low	Market	Farmed

Phuong et al. (2018a)	bivalve	oysters	60	0.18	0.16	French Atlantic coasts	Europe	FT-IR	low	Environment	Wild/ Farmed
Teng et al. (2019)	bivalve	oysters	306	0.62	0.88	China	Asia	FT-IR	low	Environment	Farmed
Van Cauwenberghe and Janssen (2014)	bivalve	oysters	11	0.47	0.16	Germany	Europe	RM	unclear	Market	Farmed
Zhu et al. (2019)	bivalve	oysters	20	0.80	0.20	China	Asia	FT-IR	low	Environment	Wild
Akoueson et al. (2020)	bivalve	scallops	10	0.29	0.10	Chile	Americas	FT-IR	low	Market	N/A
Cho et al. (2019)	bivalve	scallops	60	0.08	0.08	South Korea	Asia	RM	low	Market	Farmed
Li J. et al. (2015)	bivalve	scallops	6	2.34	0.78	China	Asia	FT-IR	low	Environment	Wild/ Farmed
Abidli et al. (2019)	gastropods	sea snails	9	0.70	0.11	Tunisia	Africa	FT-IR	unclear	Environment	Wild
Fang et al. (2018)	gastropods	sea snails	43	0.08	0.07	Bering Sea and Chukchi Sea	Asia and Americas	FT-IR	low	Environment	Wild

Note: The risk of bias (RoB) rating results can be found in Appendix 17. The microplastic (MP) content for species of the same family in the same study were pooled using the formulae for combining groups (Higgins and Green, 2011: Table 7.7.a). FT-IR, Fourier transform infrared, N/A, not available; RM, Raman; SA, spectrum analysis method; SD, standard deviation.

Appendix 22

Molluscan studies subgroup analysis results.

		Geographic location	**Continent**	**S.A.**	**RoB**	**Source**	**Families**	**Classes**
Bivalves								
	Q	698.52	215.69	57.43	15.42	29.33	33.73	0.82
	p	3.85×10^{-139}	1.25×10^{-44}	3.38×10^{-13}	0.0005	6.1×10^{-8}	2.7×10^{-6}	0.37
Clams								
	Q	274.41	60.26	58.16	0.45	44.96	-	-
	p	3.58×10^{-58}	8.23×10^{-14}	2.42×10^{-14}	0.50	2.02×10^{-11}	-	-
Mussels								
	Q	949.96	52.07	12.21	13.11	0.38	-	-
	p	1.06×10^{-198}	2.9×10^{-11}	0.0022	0.0014	0.54	-	-
Oysters								
	Q	10866.76	8826.32	1.33	1.77	1.78	-	-
	p	< 0.01 [a]	< 0.01 [a]	0.25	0.41	0.18	-	-

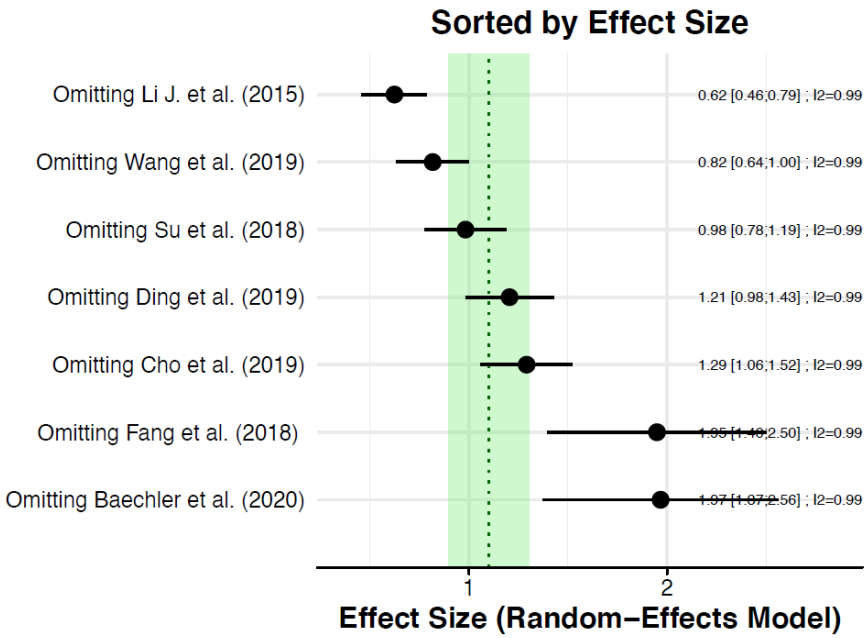
[a] beyond the precision of the code

Note: The characteristics of the studies are presented in Appendix 21, Q refers to the Chi^2 Cochran's Q Statistic results and the corresponding p value; S.A, spectrum analysis method (Fourier-transform infrared spectroscopy or Raman spectroscopy); RoB, Risk of Bias assessment rating; Source, collected from the environment or a market.

Appendix 23

Influence analysis forest plots of random-effects model for clams using the leave-one-out method, sorted by (A) effect size estimate, expressed as microplastics per g (MPs/g) and 95% confidence interval (CI) and (B) heterogeneity expressed in I^2. The pooled effect is recalculated each time leaving out one study. In both figures results are ordered from low to high.

A



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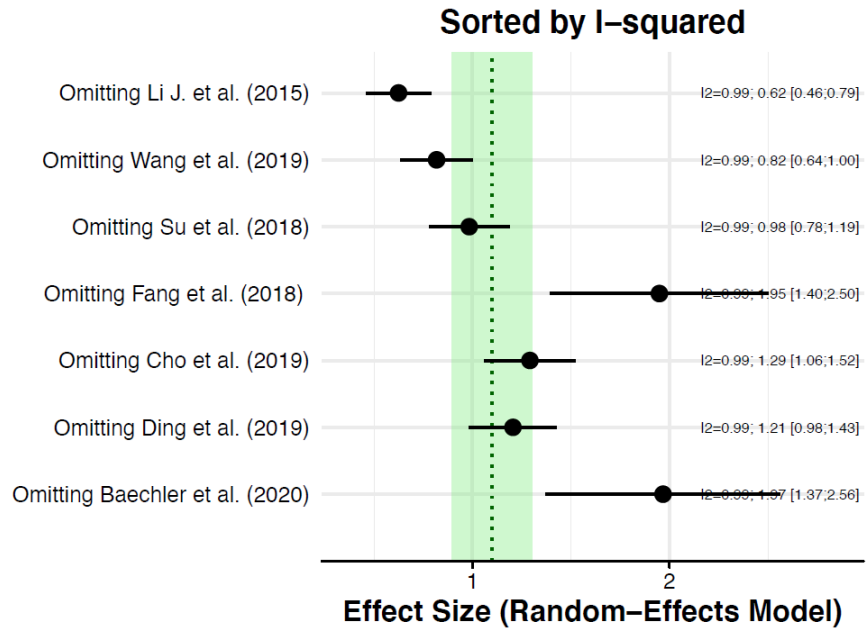
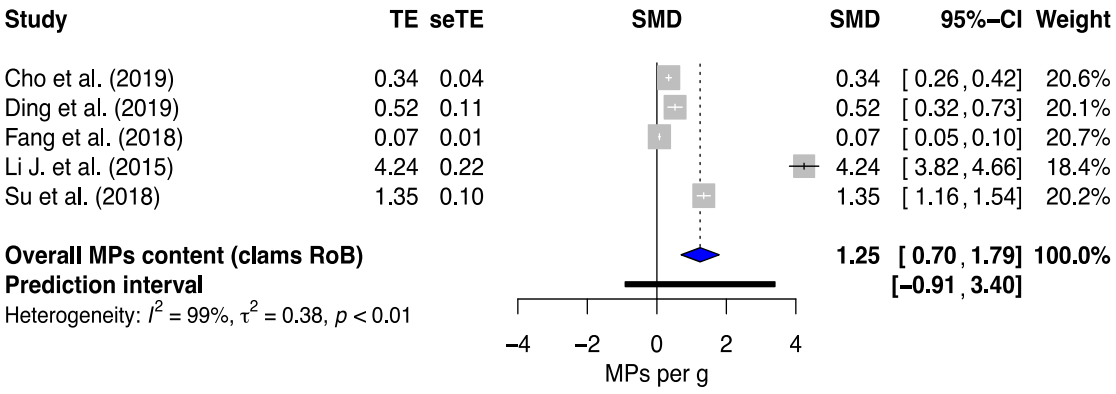


Appendix 24.

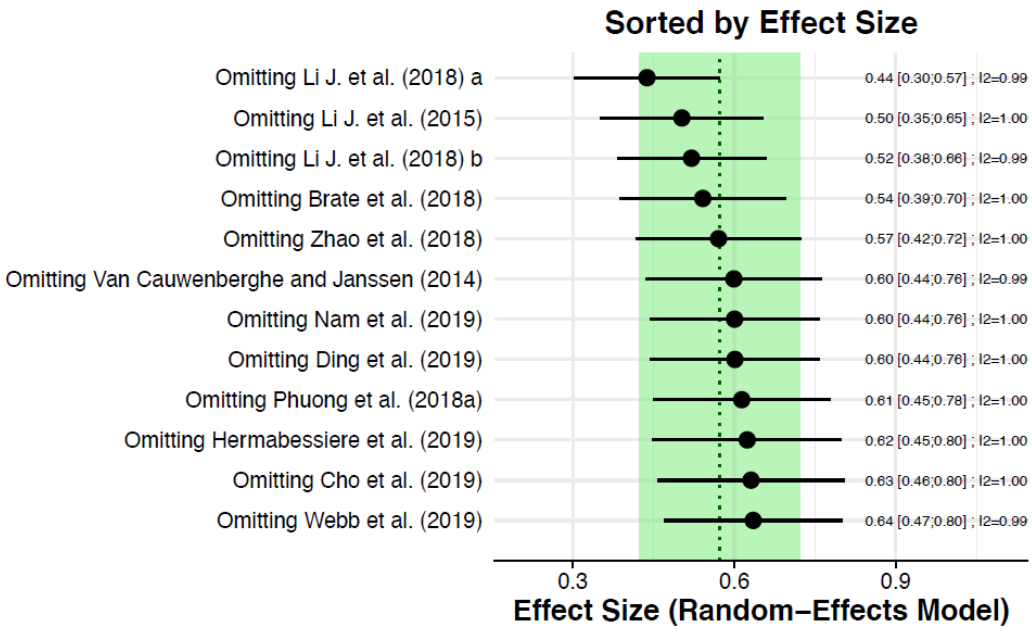
Forest plot for random-effects model results for clams excluding two high RoB studies (Baechler et al., 2020, Jun Wang et al., 2019). The x axis represents the standardized mean difference (SMD) expressed in microplastics per gram (MPs/g). TE is the MP content reported by each study and seTE is the calculated standard error (SE). The vertical line is the line of null effect where MP content is 0. The grey boxes represent the pooled effect estimate and the whiskers the CI 95%. The size of the boxes is proportional to the study weight. The diamond is the combined point estimate and CI 95%, and the dotted line is the overall pooled effect. The black box represents the 95% prediction interval.



Appendix 25

Influence analysis forest plots of random-effects model for mussels using the leave-one-out method, sorted by (A) effect size estimate, expressed as microplastics per g (MPs/g) and 95% confidence interval (CI) and (B) heterogeneity expressed in I^2. The pooled effect is recalculated each time leaving out one study. In both figures results are ordered from low to high. Li J. et al (2018) a, samples collected from environment, Li J. et al (2018) b, samples collected from market.

A



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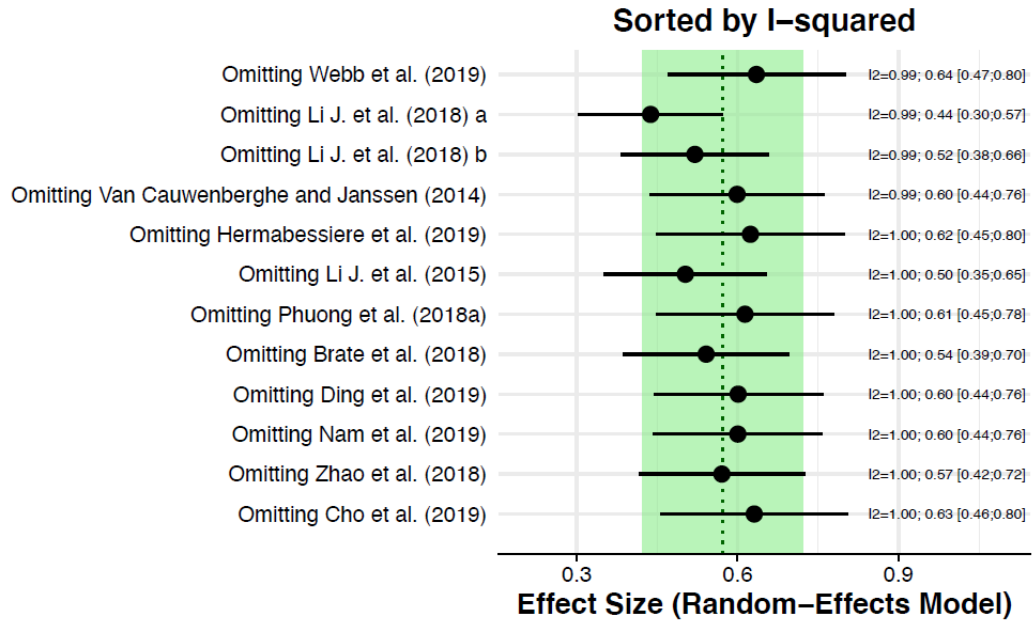
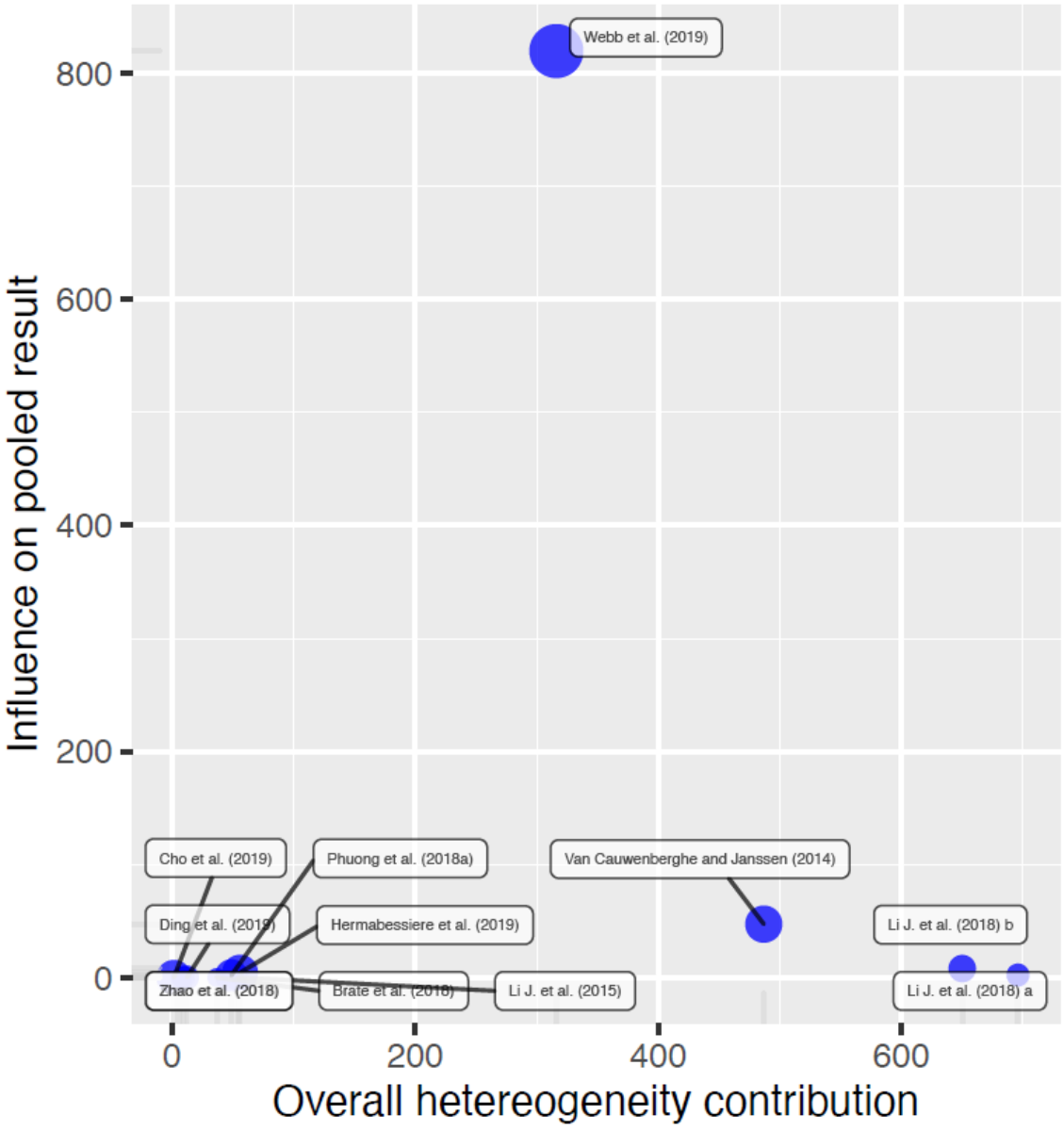


Appendix 26

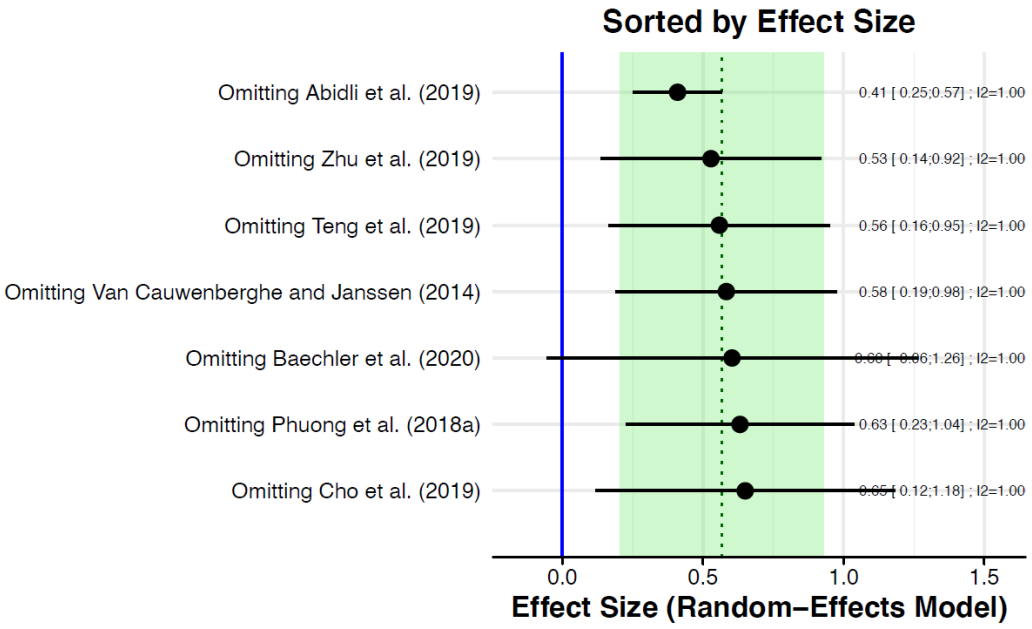
Influence analysis Baujat Plot of random-effects model for mussels. The horizontal axis illustrates statistical heterogeneity as measured by Cochran's Q statistic. The vertical axis illustrates the influence on the pooled result. Li J. et al (2018) a, samples collected from environment, Li J. et al (2018) b, samples collected from market.



Appendix 27

Influence analysis forest plots of random-effects model for oysters using the leave-one-out method, sorted by (A) effect size estimate, expressed as microplastics per g (MPs/g) and 95% confidence interval (CI) and (B) heterogeneity expressed in I^2. The pooled effect is recalculated each time leaving out one study. In both figures results are ordered from low to high.

A



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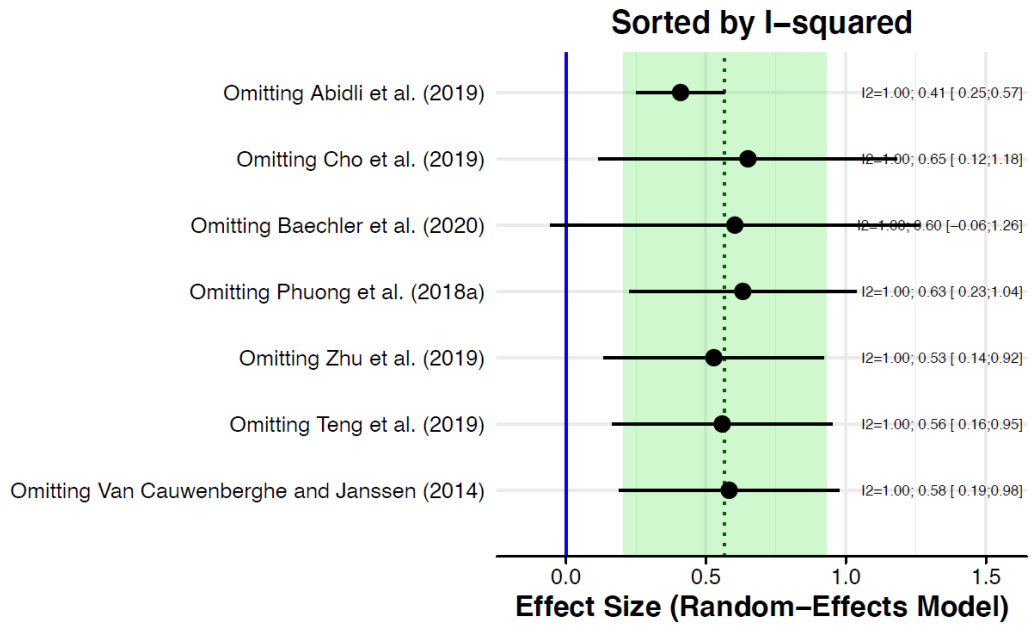
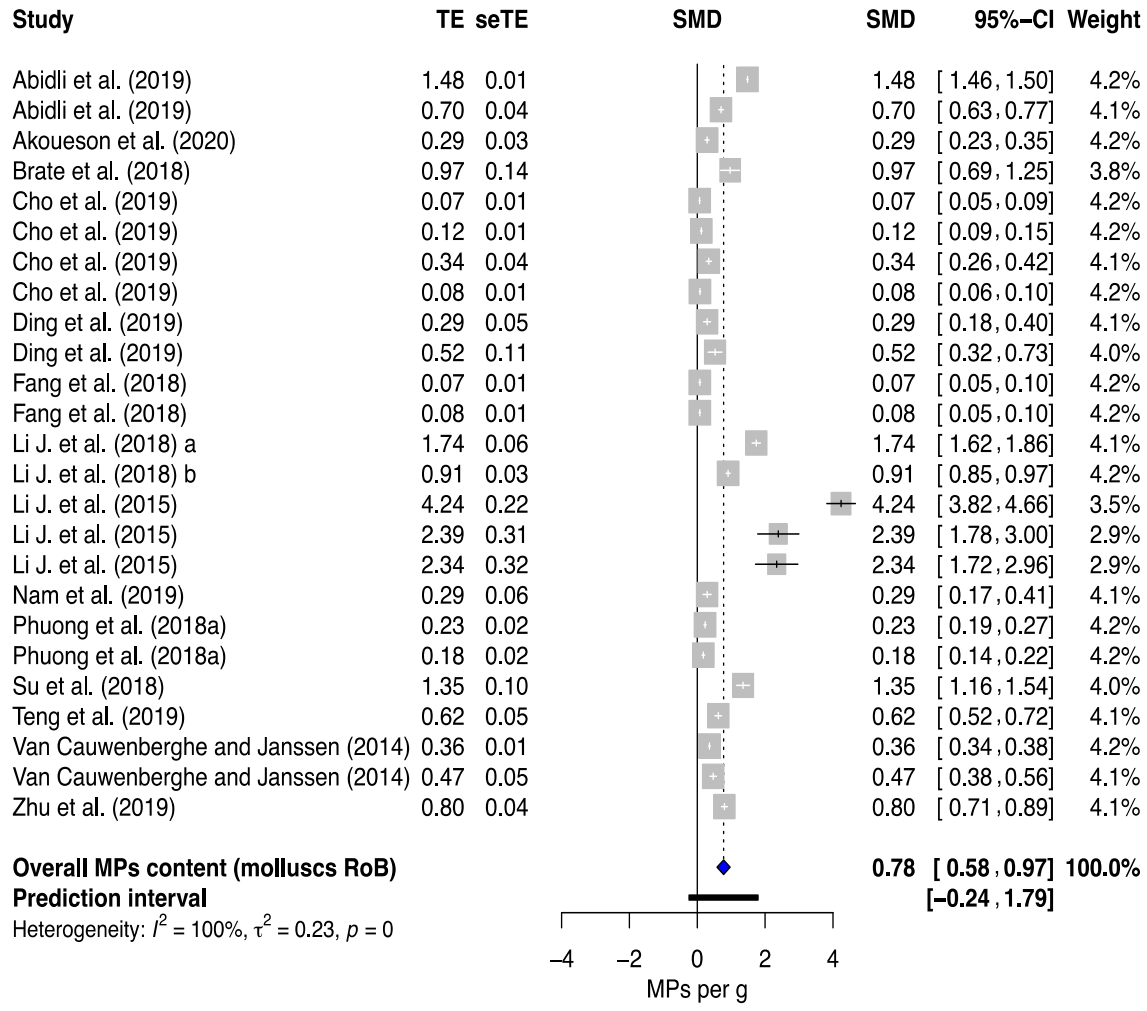


Appendix 28

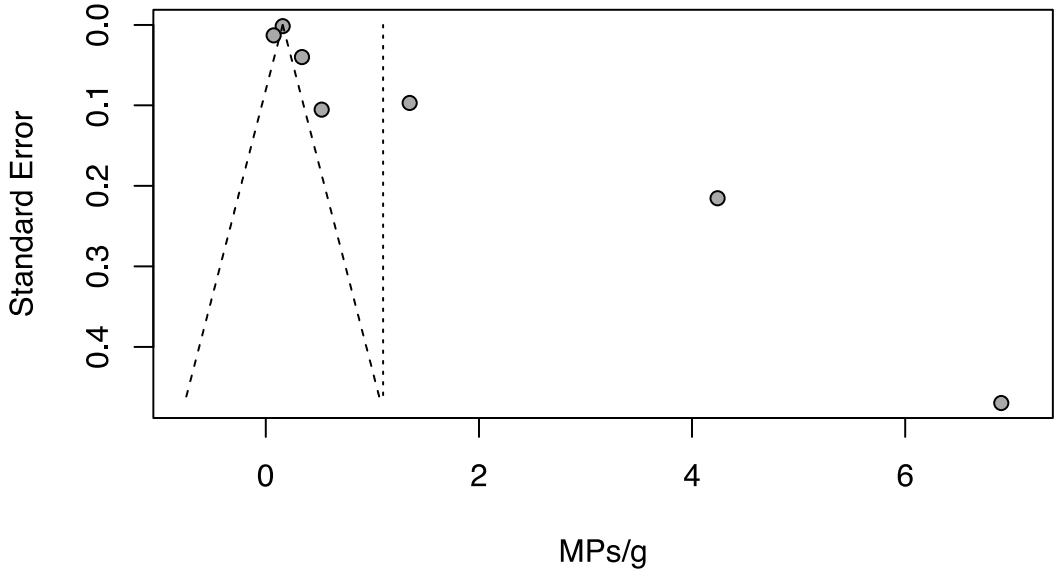
Forest plot for random-effects model results for all molluscan families excluding the five high RoB studies (Baechler et al., 2020, Hermabessiere et al., 2019, Jun Wang et al., 2019, Webb et al., 2019, S. Y. Zhao et al., 2018) . The x axis represents the standardized mean difference (SMD) expressed in microplastics per gram (MPs/g). TE is the MP content reported by each study and seTE is the calculated standard error (SE). The vertical line is the line of null effect where MP content is 0. The grey boxes represent the pooled effect estimate and the whiskers the confidence interval (CI) 95%. The size of the boxes is proportional to the study weight. The diamond is the combined point estimate and CI 95%, and the dotted line is the overall pooled effect. The black box represents the 95% prediction interval. Li J. et al (2018) a, samples collected from environment; Li J. et al (2018) b, samples collected from market.



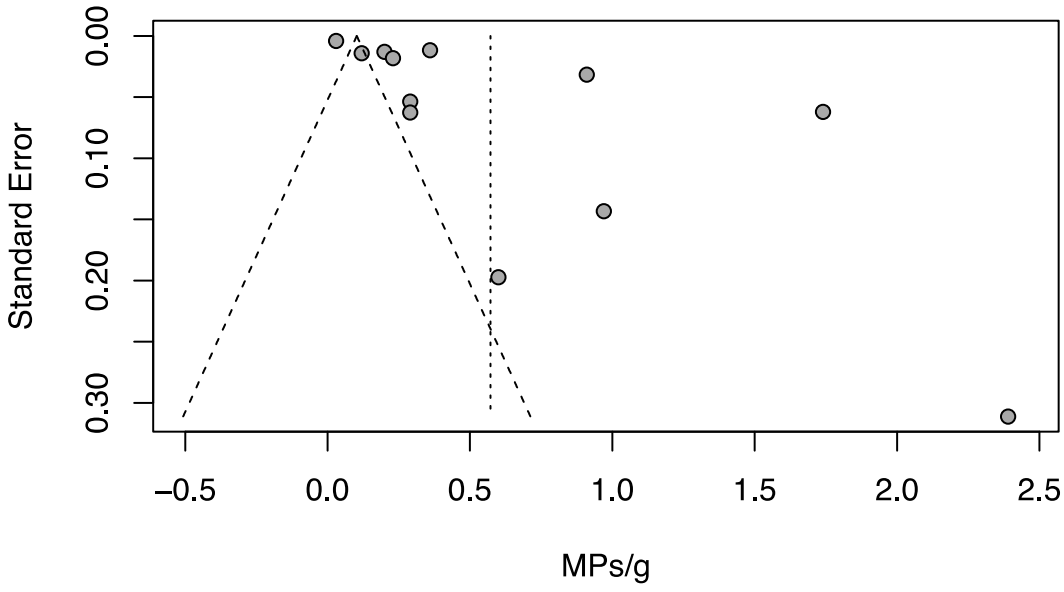
Appendix 29

Publication bias funnel plots (A) for clam studies, (B) for mussel studies, (C) for oyster studies and (D) for scallop studies. Dots represent individual studies. The vertical dotted line represents the pooled effect size. Diagonal lines represent pseudo 95% confidence limits.

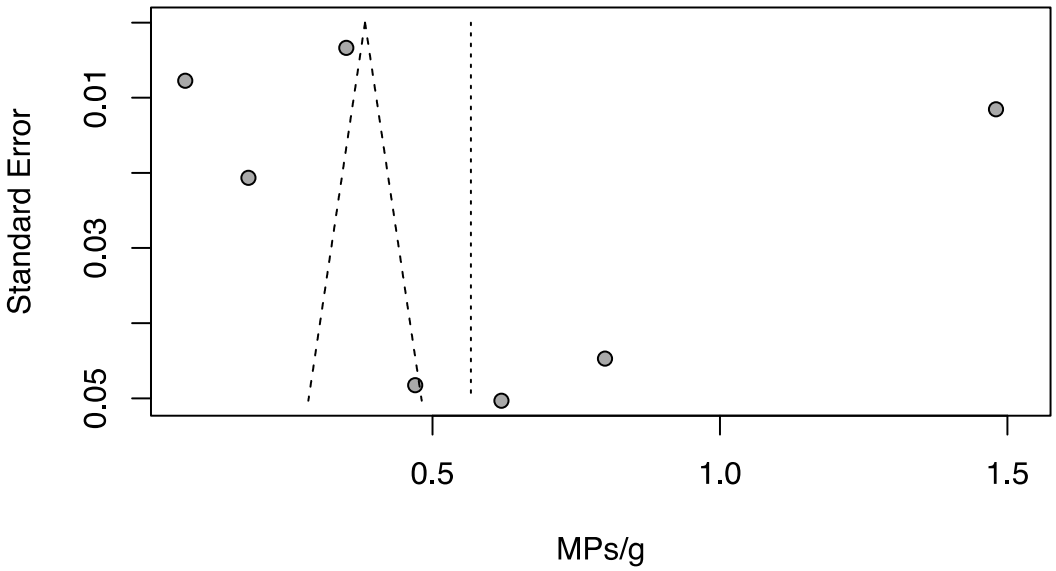
A



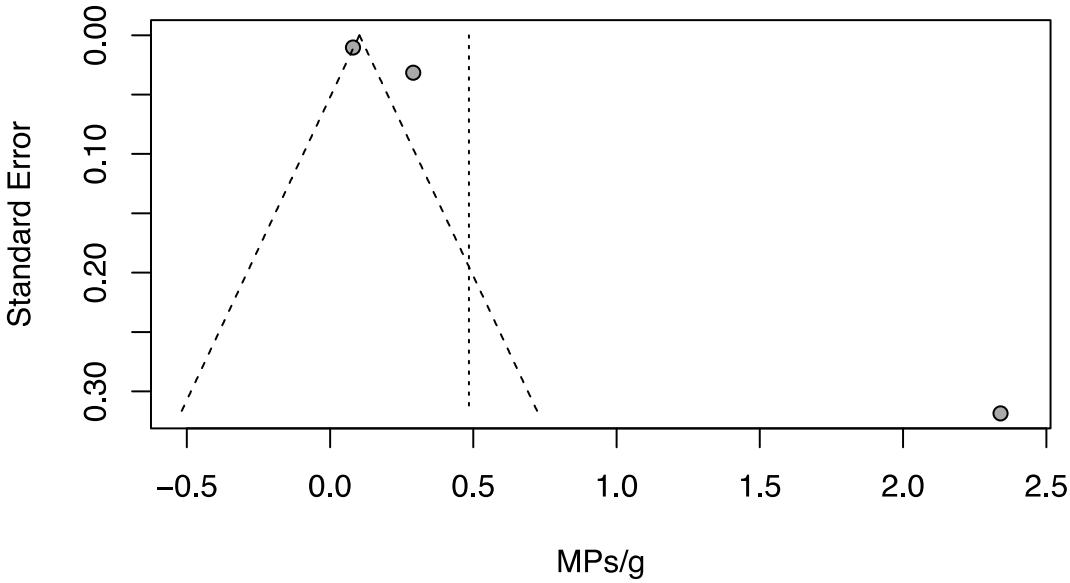
B



C

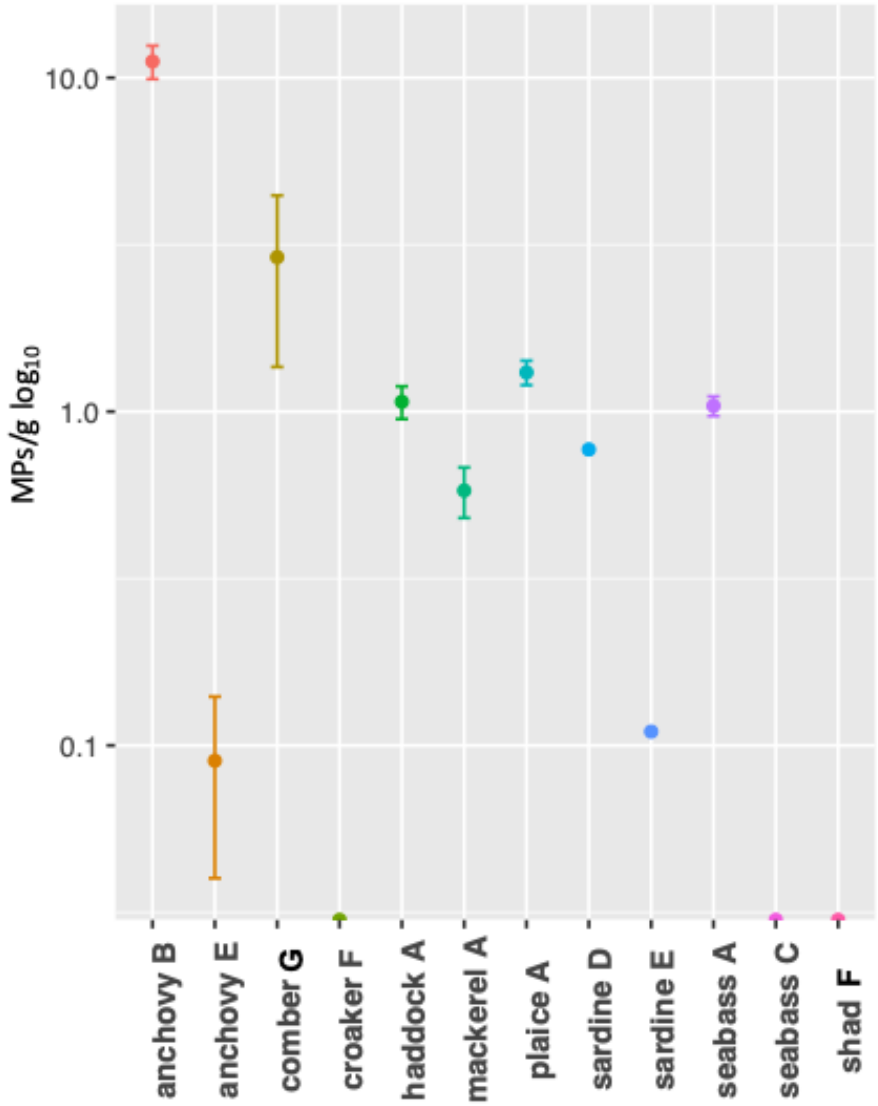

Standard Error
0.01
0.03
0.05
0.5
1.0
1.5
MPs/g

D


Standard Error
0.00
0.10
0.20
0.30
−0.5
0.0
0.5
1.0
1.5
2.0
2.5
MPs/g

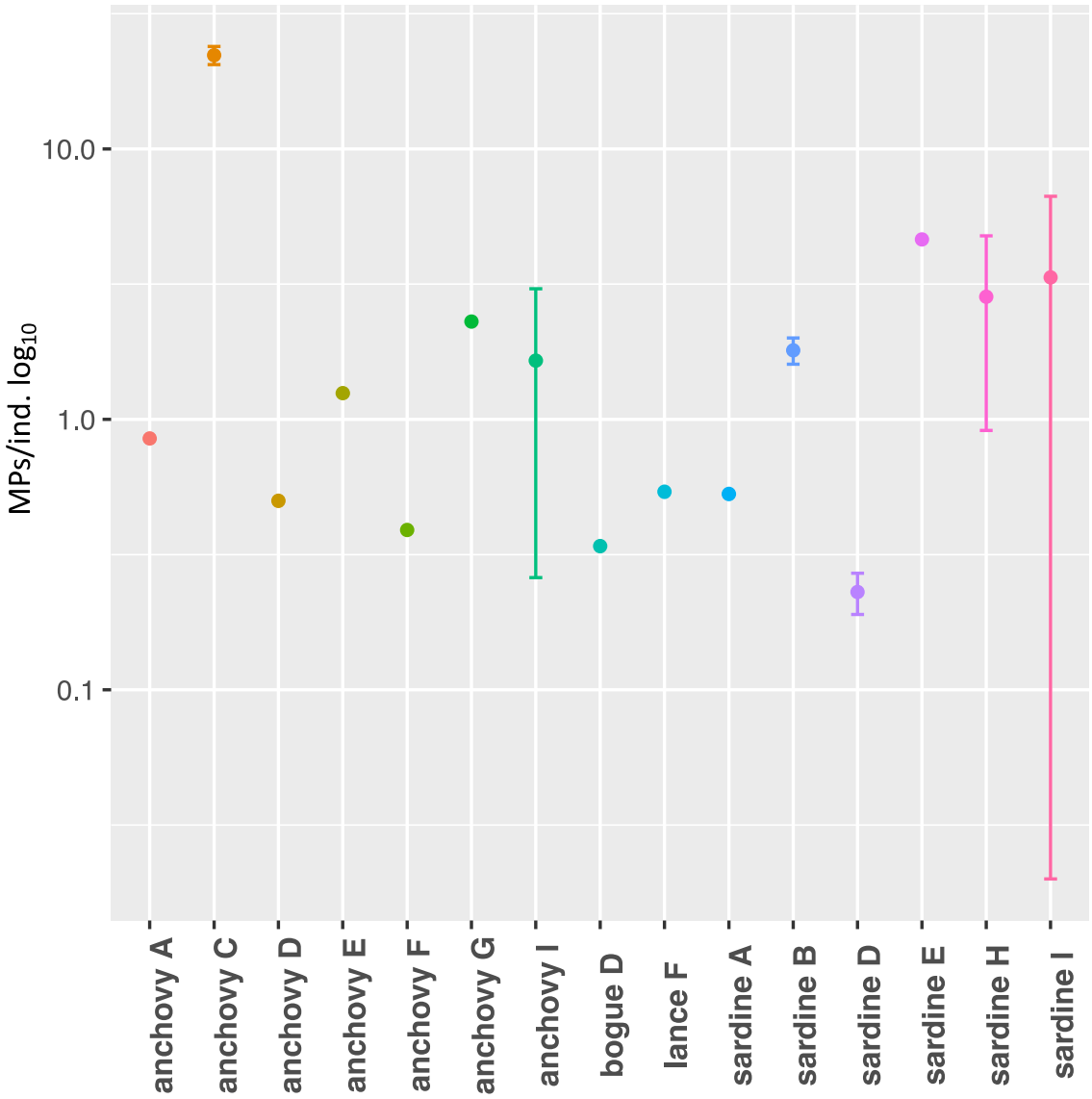
Appendix 30

The overall microplastics per gram (MPs/g) content for fish illustrated in a $\log_{10}$ scale. Points represent mean MPs/g values and whiskers represent the corresponding standard deviations (SD) for the studies that reported them. (A) Akoueson et al. (2020), (B) Feng et al. (2019), (C) Su et al. (2019), (D) Teng et al. (2020), (E) Wang et al. (2020), (F) Wu et al. (2020), (G) Zitouni et al. (2020).



Appendix 31

The overall microplastics per individual organism (MPs/ind.) content for fish illustrated in a $\log_{10}$ scale. Points represent mean MPs/g values and whiskers represent the corresponding standard deviations (SD) for the studies that reported them. (A) Collard et al. (2017b), (B) Digka et al. (2018), (C) Feng et al. (2019), (D) Lopes et al. (2020), (E) Renzi et al. (2019), (F) Sun et al. (2019), (G) Tanaka and Takada (2016), (H) Teng et al. (2020), (I) Wang et al. (2020).



Appendix 32

MP content in fish per mass.

Study	**Sample**	**N=**	**MPs/ind.**	**Weight range (g)**	**Weight mean (±SD) (g)**	**MPs/g**
Digka et al. (2018)	sardines *S. pilchardus*	36	1.8	-	9.63 ± 1.46	0.19
Renzi et al. (2019)	anchovies *E. encrasicolus*	80	1.25	10.4 - 20	16.8 ± 4	0.07
Renzi et al. (2019)	sardines *S. pilchardus*	80	4.63	15.2 - 26.9	20.22 ±4.2	0.23
Sun et al. (2019)	anchovies *Setipinna taty*	20	0.35	4.7-20.7	10.5 ± 3.6	0.03
Sun et al. (2019)	anchovies *A. commersonii*	30	0.40	16.4-39.8	29.3 ± 6.9	0.01
Sun et al. (2019)	anchovies *E. japonicus*	280	0.39	2.8-7.2	4.3 ± 1.1	0.09
Sun et al. (2019)	lances *A. personatus*	50	0.54	1.2-20.5	7.1 ± 5.9	0.08

Note: The conversion was based on the mean value of mass per sample and the mean value of MPs/individual. Weight is expressed in grams (g) of tissue per individual organism. ind., individual organism; MPs, microplastics; SD, standard deviation

Appendix 33

Composition identification process characteristics for fish studies

Study	Process	% of particles for analysis	No. of particles analysed	% of particles verified as MPs	Similarity to spectra library (%)
Akhbarizadeh et al. (2020)	RM	42	70	92	85
Akoueson et al. 2020	FT-IR	16	96	17 to 59	70
Collard et al. (2017a)	RM	n/s	n/s	n/s	n/s
Collard et al. (2017b)	RM	n/s	46	61%	n/s
Digka et al. (2018)	FT-IR	20	n/s	n/s	80
Feng et al. 2019	FT-IR	n/s	200	74	70
Karami et al. (2017b)	RM	100	61	59%	n/s
Karami et al. (2018)	RM	100	21	28.6	n/s
Lopes et al. 2020	FT-IR	20	38	n/s	70
Pozo et al. 2019	FT-IR	100	n/s	n/s	n/s
Renzi et al. (2019)	FT-IR	n/s	n/s	n/s	n/s
Su et al. 2019	FT-IR	100%	n/s	77	70
Sun et al. (2019)	FT-IR	45.7	252	n/s	n/s
Tanaka and Takada (2016)	FT-IR	100	173	87	70
Teng et al. 2020	FT-IR	33.1	300	30	70
Wang et al. 2020	FT-IR	> 40	608	> 91	70
Wu et al. 2020	FT-IR	100	n/s	n/s	70
Zitouni et al. 2020	RM	100	n/s	n/s	n/s

Note: FT-IR, Fourier-transform infrared spectroscopy; MPs, microplastics; n/s: not specified; RM, Raman spectroscopy.

Appendix 34

GRADE certainty framework assessment for seafood studies.

Domains for assessing certainty of evidence by outcome	**Results section**	**Reasons for lowering or increasing the certainty of evidence**
Risk of bias (downgrading)	Seven studies were rated as of high Rob, 11 of unclear and the rest 16 of low RoB. Rating was accessed over four domains. **Meta-analysis** Molluscs: Clams, two high RoB out of seven. Mussels, three high RoB out of twelve. Oysters one high RoB out of seven, scallops and sea snails zero high RoB. Crustacean, no meta-analysis. Fish, no meta-analysis. **Statistical summary/ narrative analysis** Molluscs, eight high RoB out of 27. Crustacean, four high RoB out of five. Fish, three high RoB studies out of twelve. Echinodermata, only one study was included, and it was of low RoB.	For the meta-analysis, the evidence will not be downgraded as the results of the studies that are rated as of high RoB will not be included in the summary of evidence. For the statistical summary, in the molluscs the evidence will not be downgraded as only 29% of the studies have been rated as of high RoB. For the crustacean the evidence will be downgraded as 80% of the studies have been rated as of high RoB. For the fish, the evidence will not be downgraded as only 25% of the studies have been rated as of high RoB. For echinodermata, the evidence will not be downgraded.
Inconsistency (heterogeneity) (downgrading)	**Meta-analysis** Heterogeneity across studies was high. **Statistical summary/ narrative analysis** The range of MP content was high for all phyla.	Downgrade the meta-analysis evidence due to high heterogeneity as measured by I^2, Chi^2, and corresponding p value.
Indirectness (downgrading)	The majority of the studies measured the outcome addressed by the question of the review.	Will not downgrade evidence.

Imprecision (downgrading)	The sample size n for the studies varied from 5 to 760 organisms. 95% CI for the studies in the meta-analysis are acceptable for the majority of the studies.	Will not downgrade evidence.
Publication bias (downgrading)	Publication bias was not substantial.	For the meta-analysis the funnel plots did not detect substantial bias. For the statistical summary the country of origin did not point to publication bias. The evidence will not be downgraded.
Large effects (upgrading)	The estimates are not large, consistent and confident enough to allowing an upgrade of the evidence.	Will not upgrade the evidence.
Dose response (upgrading)	Dose response cannot, at this point, be accessed for these studies.	Not applicable.
Opposing plausible residual bias and confounding (upgrading)	All confounders have been considered.	The estimate of effect was controlled for the following possible confounders: contamination of samples post sampling, misrepresentation of particles as MPs. The use of procedural blank samples and the use of a technique to identify the chemical composition of the particles are the built-in fail-safes of the review. The certainty of the evidence will be increased for all studies.

Note: CI, confidence interval; MPs, microplastics; RoB, risk of bias.

Appendix 35

Estimation of microplastic (MP) particles yearly uptake from the consumption of seafood across countries.

Country	Fish min (pelagic)	Fish max (demersal)	Molluscs min	Molluscs max	Crustaceans min	Crustaceans max
Albania	15.8	3132	0	3885	35	3010
Algeria	27.5	1218	0	0	11	946
Angola	125.1	17255	0	105	3	258
Antigua and Barbuda	126.8	17632	0	82005	549	47214
Argentina	13.8	8323	0	8085	143	12298
Armenia	7.6	406	0	315	35	3010
Australia	45.7	17371	0	30135	481	41366
Austria	39.8	8091	0	5460	171	14706
Azerbaijan	18.4	1073	0	105	17	1462
Bahamas	132.9	9947	0	15120	714	61404
Bangladesh	4.9	1421	0	0	121	10406
Barbados	233.7	7801	0	5985	183	15738
Belarus	75.2	9541	0	2625	17	1462
Belgium	42.2	22214	0	26460	113	9718
Belize	49.5	3770	0	30870	231	19866
Benin	13	7018	0	0	52	4472
Bolivia (Plurinational State of)	4	0	0	105	1	86
Bosnia and Herzegovina	19.3	3219	0	315	2	172

Botswana	31.7	841	0	105	7	602
Brazil	9.5	7395	0	1680	55	4730
Bulgaria	30	2900	0	1365	40	3440
Burkina Faso	38	0	0	0	2	172
Cabo Verde	55.2	9077	0	6825	49	4214
Cambodia	5.1	116	0	11760	122	10492
Cameroon	81.2	12934	0	0	145	12470
Canada	49.9	14065	0	31920	418	35948
Central African Republic	9.4	0	0	210	0	0
Chad	0.2	0	0	0	0	0
Chile	47.8	5684	0	7980	215	18490
China	3.9	9831	0	104790	457	39302
China, Hong Kong SAR	31.3	21489	0	160860	1351	116186
China, Macao SAR	5.6	2088	0	125475	1197	102942
China, mainland	3.3	9773	0	105000	454	39044
China, Taiwan Province of	31.1	10353	0	69720	344	29584
Colombia	27.6	406	0	840	16	1376
Congo	85.5	29087	0	630	12	1032
Costa Rica	82.1	1653	0	6930	128	11008
Côte d'Ivoire	107.6	1421	0	0	5	430
Croatia	88.2	12151	0	5775	87	7482
Cuba	14.5	2320	0	3780	21	1806
Cyprus	40	15109	0	32550	244	20984
Czechia	29.3	7395	0	1260	37	3182
Democratic People's Republic of Korea	2.5	12354	0	6930	52	4472

Denmark	104.1	14384	0	13230	524	45064
Djibouti	25.9	2059	0	210	7	602
Dominica	204.5	7366	0	0	60	5160
Dominican Republic	31.7	9367	0	3045	51	4386
Ecuador	31.4	7192	0	105	96	8256
Egypt	43.2	13804	0	630	24	2064
El Salvador	45.5	145	0	315	43	3698
Estonia	23	21315	0	6300	295	25370
Eswatini	9.1	4089	0	210	3	258
Ethiopia	0.2	0	0	0	0	0
Fiji	177.8	10150	0	27510	105	9030
Finland	97	9077	0	2100	162	13932
France	73.8	37207	0	56490	379	32594
French Polynesia	264	3364	0	38535	100	8600
Gabon	143.1	23664	0	105	9	774
Gambia	211	13514	0	630	41	3526
Georgia	43.3	2146	0	1575	4	344
Germany	24.9	11455	0	3990	88	7568
Ghana	179.8	4553	0	0	3	258
Greece	43.5	19546	0	6300	164	14104
Grenada	174.4	16385	0	2625	115	9890
Guatemala	7	58	0	315	26	2236
Guinea	61.5	4843	0	0	0	0
Guinea-Bissau	4.4	870	0	0	3	258
Guyana	35.2	5046	0	105	1318	113348

Haiti	22.4	551	0	210	5	430
Honduras	8.2	0	0	735	152	13072
Hungary	14.1	3712	0	1365	16	1376
Iceland	431.2	73863	0	6930	1662	142932
India	5.3	1682	0	630	43	3698
Indonesia	117.3	18357	0	4725	469	40334
Iran (Islamic Republic of)	40.3	2958	0	0	23	1978
Iraq	1.7	2494	0	105	4	344
Ireland	110	21315	0	7455	55	4730
Israel	62.9	12760	0	2940	71	6106
Italy	51.4	22011	0	69825	223	19178
Jamaica	81.6	609	0	9450	147	12642
Japan	138.7	19082	0	59010	609	52374
Jordan	35.6	1972	0	105	12	1032
Kazakhstan	12.4	725	0	315	8	688
Kenya	3	435	0	0	1	86
Kiribati	560.4	46748	0	1890	19	1634
Kuwait	38.2	7192	0	420	146	12556
Kyrgyzstan	3.8	464	0	0	0	0
Lao People's Democratic Republic	0.5	0	0	0	1	86
Latvia	105.3	7917	0	7140	163	14018
Lebanon	33.1	3944	0	1365	96	8256
Lesotho	11.5	348	0	0	0	0
Liberia	32.2	3596	0	0	6	516
Lithuania	257	5916	0	2835	78	6708

Luxembourg	45.9	25027	0	38220	492	42312
Madagascar	8.9	638	0	2205	49	4214
Malawi	0.3	0	0	0	0	0
Malaysia	184.1	38483	0	16275	463	39818
Maldives	825.4	1479	0	15435	354	30444
Mali	3.8	5568	0	315	1	86
Malta	152.2	9483	0	48930	200	17200
Mauritania	35.2	435	0	11130	11	946
Mauritius	116.7	10643	0	1785	226	19436
Mexico	41.1	4959	0	10500	245	21070
Mongolia	0	0	0	0	0	0
Montenegro	51	8816	0	10185	78	6708
Morocco	156.1	4988	0	315	42	3612
Mozambique	1.9	174	0	105	53	4558
Myanmar	4.9	0	0	0	36	3096
Namibia	60	7192	0	3465	45	3870
Nepal	0.1	0	0	0	0	0
Netherlands	57.4	28188	0	7875	237	20382
New Caledonia	130.4	1450	0	41475	103	8858
New Zealand	57.1	34278	0	9555	253	21758
Nicaragua	41.4	957	0	14910	4	344
Niger	1.2	319	0	0	0	0
Nigeria	27.4	5655	0	210	29	2494
North Macedonia	24.7	6844	0	525	3	258
Norway	59	67744	0	8820	1029	88494

Oman	102.3	38918	0	420	95	8170
Pakistan	3.1	174	0	105	2	172
Panama	74.2	2436	0	4515	67	5762
Paraguay	4.4	29	0	735	3	258
Peru	139.8	10701	0	6930	90	7740
Philippines	140.4	10788	0	8505	104	8944
Poland	43.9	12064	0	420	8	688
Portugal	77.2	90886	0	41685	362	31132
Republic of Korea	139.4	56347	0	100170	353	30358
Republic of Moldova	43.1	6322	0	1050	11	946
Romania	27.5	1827	0	1995	10	860
Russian Federation	52.2	21663	0	5145	57	4902
Rwanda	1.7	0	0	0	0	0
Saint Kitts and Nevis	35.7	19198	0	86730	259	22274
Saint Lucia	171.6	1334	0	42420	124	10664
Saint Vincent and the Grenadines	53	6409	0	10815	35	3010
Samoa	368.8	667	0	29085	246	21156
Sao Tome and Principe	181	18415	0	2415	13	1118
Saudi Arabia	38.2	5800	0	105	63	5418
Senegal	115.3	10933	0	4935	22	1892
Serbia	26.7	3857	0	315	2	172
Sierra Leone	163.9	20300	0	630	3	258
Slovakia	26.6	11252	0	525	7	602
Slovenia	32.9	7598	0	11340	56	4816
Solomon Islands	195.1	29	0	840	8	688

South Africa	26.5	7250	0	1155	27	2322
Spain	114.4	37613	0	96390	349	30014
Sri Lanka	207.6	5684	0	210	104	8944
Sudan	1	0	0	0	0	0
Suriname	60.7	2407	0	735	431	37066
Sweden	54.1	26970	0	9030	755	64930
Switzerland	27.2	7482	0	12285	260	22360
Tajikistan	0.7	319	0	0	0	0
Thailand	109.7	5075	0	22680	225	19350
Timor-Leste	42.4	29	0	2940	14	1204
Togo	33.6	7076	0	0	0	0
Trinidad and Tobago	79.4	15254	0	4620	195	16770
Tunisia	68.9	13891	0	2100	35	3010
Turkey	25.7	2175	0	2730	3	258
Turkmenistan	2.9	29	0	0	0	0
Uganda	0	0	0	0	0	0
Ukraine	57.5	7424	0	6300	37	3182
United Arab Emirates	60.7	24360	0	7035	63	5418
United Kingdom of Great Britain and Northern Ireland	32.6	24563	0	12915	358	30788
United Republic of Tanzania	5	2262	0	105	5	430
United States of America	26.6	13282	0	31290	643	55298
Uruguay	35.2	7366	0	7350	24	2064
Uzbekistan	1.4	29	0	0	0	0
Vanuatu	124.4	783	0	1995	315	27090

Venezuela (Bolivarian Republic of)	62.1	3654	0	10500	44	3784
Viet Nam	23.1	754	0	28455	489	42054
Yemen	26.5	1479	0	0	0	0
Zambia	42.6	58	0	0	1	86
Zimbabwe	13.7	174	0	0	1	86

Note: The consumption was calculated for each family and then pooled for each of the three phyla; molluscs, crustacean and fish corresponding to the yearly consumption data (FAO, 2020). The MP content of seafood is based on the statistical summary results (Table 5). Min, minimum microplastic particles uptake per year; max, maximum microplastic particles uptake per year.

Appendix 36

Reasons for the exclusion of studies in the second-level screening

2: comprised method studies

1: a review

1: an opinion piece

1: an unavailable study

1: was solely testing nanoplastics, so not included as defined by this rapid review

1: not a toxicity study

Appendix 37

MPs daily exposure estimates via salt, for England by age group and sex.

Age group	19-34					35-49				
	MPs	CI 95%		PI 95%		MPs	CI 95%		PI 95%	
Estimated salt intake (g/day)	average	from	to	from	to	average	from	to	from	to
Men										
Arithmetic mean	0.4	0.4	0.5	0.0	0.9	0.5	0.4	0.6	0.0	1.1
Standard deviation	0.2	0.2	0.3	0.0	0.4	0.2	0.2	0.3	0.0	0.5
Geometric mean	0.4	0.3	0.5	0.0	0.8	0.5	0.4	0.6	0.0	1.0
Lower 95% confidence limit for the geometric mean	0.3	0.3	0.4	0.0	0.6	0.4	0.4	0.5	0.0	0.9
Upper 95% confidence limit for the geometric mean	0.5	0.4	0.5	0.0	0.9	0.5	0.4	0.6	0.0	1.1
2.5^{th} percentile	0.1	0.1	0.2	0.0	0.3	0.2	0.1	0.2	0.0	0.3
97.5^{th} percentile	1.0	0.8	1.1	0.1	1.9	1.0	0.9	1.2	0.1	2.1
Women										
Arithmetic mean	0.4	0.4	0.5	0.0	0.9	0.4	0.3	0.4	0.0	0.8
Standard deviation	0.2	0.2	0.3	0.0	0.5	0.2	0.1	0.2	0.0	0.3
Geometric mean	0.4	0.3	0.5	0.0	0.8	0.3	0.3	0.4	0.0	0.7
Lower 95% confidence limit for the geometric mean	0.3	0.3	0.4	0.0	0.6	0.3	0.3	0.4	0.0	0.6

Upper 95% confidence limit for the geometric mean	0.5	0.4	0.5	0.0	0.9	0.4	0.3	0.5	0.0	0.8
2.5th percentile	0.2	0.2	0.2	0.0	0.4	0.1	0.1	0.1	0.0	0.1
97.5th percentile	1.0	0.8	1.2	0.1	2.0	0.8	0.6	0.9	0.1	1.5
All										
Arithmetic mean	0.4	0.4	0.5	0.0	0.9	0.5	0.4	0.5	0.0	0.9
Standard deviation	0.2	0.2	0.3	0.0	0.5	0.2	0.2	0.3	0.0	0.4
Geometric mean	0.4	0.3	0.5	0.0	0.8	0.4	0.3	0.5	0.0	0.8
Lower 95% confidence limit for the geometric mean	0.3	0.3	0.4	0.0	0.7	0.4	0.3	0.4	0.0	0.7
Upper 95% confidence limit for the geometric mean	0.4	0.4	0.5	0.0	0.9	0.4	0.4	0.5	0.0	0.9
2.5th percentile	0.2	0.1	0.2	0.0	0.3	0.1	0.1	0.1	0.0	0.2
97.5th percentile	1.0	0.9	1.2	0.1	2.1	0.9	0.7	1.0	0.1	1.8
Percentage difference of sample arithmetic mean from population recommendation	2.1	1.7	2.5	0.2	4.1	2.5	2.1	3.0	0.2	5.0
Bases (unweighted)										
Men	1.8	1.5	2.1	0.1	3.6	5.7	4.7	6.7	0.4	11.3
Women	2.0	1.6	2.3	0.1	4.0	5.9	4.9	7.0	0.4	11.8
All	3.8	3.1	4.4	0.3	7.5	11.6	9.5	13.6	0.8	23.1

Age group	50-64					19-64				
	MPs	CI 95%		PI 95%		MPs	CI 95%		PI 95%	
Estimated salt intake (g/day)	average	from	to	from	to	average	from	to	from	to
Men										
Arithmetic mean	0.4	0.4	0.5	0.0	0.9	0.5	0.4	0.6	0.0	0.9
Standard deviation	0.2	0.2	0.2	0.0	0.4	0.2	0.2	0.3	0.0	0.4
Geometric mean	0.4	0.3	0.5	0.0	0.8	0.4	0.3	0.5	0.0	0.8
Lower 95% confidence limit for the geometric mean	0.4	0.3	0.4	0.0	0.7	0.4	0.3	0.5	0.0	0.8
Upper 95% confidence limit for the geometric mean	0.4	0.4	0.5	0.0	0.9	0.5	0.4	0.5	0.0	0.9
2.5^{th} percentile	0.1	0.1	0.2	0.0	0.3	0.2	0.1	0.2	0.0	0.3
97.5^{th} percentile	1.0	0.9	1.2	0.1	2.1	1.1	0.9	1.3	0.1	2.1
Women										
Arithmetic mean	0.3	0.3	0.4	0.0	0.7	0.4	0.3	0.5	0.0	0.8
Standard deviation	0.1	0.1	0.2	0.0	0.3	0.2	0.2	0.2	0.0	0.4
Geometric mean	0.3	0.3	0.4	0.0	0.6	0.3	0.3	0.4	0.0	0.7
Lower 95% confidence limit for the geometric mean	0.3	0.2	0.3	0.0	0.6	0.3	0.3	0.4	0.0	0.6
Upper 95% confidence limit for the geometric mean	0.3	0.3	0.4	0.0	0.7	0.4	0.3	0.4	0.0	0.8
2.5^{th} percentile	0.1	0.1	0.1	0.0	0.2	0.1	0.1	0.1	0.0	0.2
97.5^{th} percentile	0.7	0.6	0.9	0.1	1.5	0.8	0.6	0.9	0.1	1.5

All										
Arithmetic mean	0.4	0.3	0.5	0.0	0.8	0.4	0.4	0.5	0.0	0.9
Standard deviation	0.2	0.1	0.2	0.0	0.4	0.2	0.2	0.2	0.0	0.4
Geometric mean	0.4	0.3	0.4	0.0	0.7	0.4	0.3	0.5	0.0	0.8
Lower 95% confidence limit for the geometric mean	0.3	0.3	0.4	0.0	0.7	0.4	0.3	0.4	0.0	0.7
Upper 95% confidence limit for the geometric mean	0.4	0.3	0.4	0.0	0.8	0.4	0.3	0.5	0.0	0.8
2.5^{th} percentile	0.1	0.1	0.1	0.0	0.2	0.1	0.1	0.2	0.0	0.3
97.5^{th} percentile	0.8	0.6	0.9	0.1	1.5	0.9	0.7	1.1	0.1	1.8
Percentage difference of sample arithmetic mean from population recommendation	1.5	1.2	1.7	0.1	2.9	2.0	1.7	2.4	0.1	4.0
Bases (unweighted)										
Men	7.1	5.9	8.4	0.5	14.2	14.6	12.0	17.2	1.1	29.1
Women	7.9	6.5	9.3	0.6	15.8	15.8	13.0	18.6	1.2	31.5
All	15.1	12.4	17.7	1.1	30.0	30.4	25.0	35.8	2.2	60.6

Note: CI, confidence internal; PI, prediction interval

Appendix 38

MPs annual exposure estimates via salt, for England by age group and sex.

Age group	19-34					35-49				
	MPs	CI 95%		PI 95%		MPs	CI 95%		PI 95%	
Estimated salt intake (g/day)	average	from	to	from	to	average	from	to	from	to
Men										
Arithmetic mean	158.1	130.2	186.2	11.5	315.1	194.9	160.4	229.4	14.2	388.3
Standard deviation	79.3	65.3	93.4	5.8	158.1	83.4	68.6	98.2	6.1	166.2
Geometric mean	141.3	116.3	166.4	10.3	281.6	177.2	145.9	208.6	12.9	353.1
Lower 95% confidence limit for the geometric mean	118.8	97.7	139.8	8.6	236.6	158.9	130.8	187.0	11.6	316.5
Upper 95% confidence limit for the geometric mean	168.2	138.4	198.0	12.2	335.0	197.7	162.7	232.7	14.4	393.8
2.5^{th} percentile	51.2	42.2	60.3	3.7	102.0	62.2	51.2	73.3	4.5	124.0
97.5^{th} percentile	351.2	289.0	413.4	25.5	699.6	378.8	311.8	446.0	27.5	754.8
Women										
Arithmetic mean	156.5	128.8	184.3	11.4	311.9	139.2	114.5	163.8	10.1	277.3
Standard deviation	91.1	75.0	107.3	6.6	181.6	59.8	49.3	70.4	4.4	119.2
Geometric mean	140.0	115.2	164.8	10.2	279.0	125.3	103.2	147.5	9.1	249.7
Lower 95% confidence limit for the geometric mean	117.7	96.9	138.6	8.6	234.6	111.5	91.8	131.3	8.1	222.1

Upper 95% confidence limit for the geometric mean	166.5	137.1	196.0	12.1	331.8	140.9	116.0	165.9	10.2	280.7
2.5th percentile	68.9	56.7	81.2	5.0	137.4	26.8	22.1	31.6	2.0	53.5
97.5th percentile	366.9	302.0	431.9	26.7	731.0	274.8	226.2	323.5	20.0	547.5
All										
Arithmetic mean	157.3	129.5	185.2	11.4	313.5	166.8	137.3	196.4	12.1	332.4
Standard deviation	84.8	69.8	99.8	6.2	168.9	77.5	63.8	91.3	5.6	154.5
Geometric mean	140.7	115.8	165.6	10.2	280.3	148.8	122.5	175.2	10.8	296.5
Lower 95% confidence limit for the geometric mean	125.8	103.6	148.1	9.1	250.7	135.9	111.8	159.9	9.9	270.7
Upper 95% confidence limit for the geometric mean	157.3	129.5	185.2	11.4	313.4	163.1	134.2	191.9	11.9	324.9
2.5th percentile	57.3	47.2	67.5	4.2	114.3	40.2	33.1	47.3	2.9	80.1
97.5th percentile	382.4	314.7	450.1	27.8	761.9	321.9	264.9	378.9	23.4	641.3
Percentage difference of sample arithmetic mean from population recommendation	759.8	625.4	894.4	55.2	1513.8	917.7	755.3	1080.2	66.7	1828.3
Bases (unweighted)										
Men	651.9	536.6	767.4	47.4	1298.8	2067.5	1701.6	2433.7	150.3	4119.2
Women	726.4	597.9	855.1	52.8	1447.3	2160.6	1778.3	2543.4	157.1	4304.7
All	1378.3	1134.4	1622.5	100.2	2746.1	4228.1	3479.9	4977.1	307.4	8423.9

Age group	50-64					19-64				
	MPs	CI 95%		PI 95%		MPs	CI 95%		PI 95%	
Estimated salt intake (g/day)	average	from	to	from	to	average	from	to	from	to
Men										
Arithmetic mean	161.6	133.0	190.3	11.8	322.0	171.2	140.9	201.6	12.4	341.2
Standard deviation	71.4	58.8	84.1	5.2	142.3	79.6	65.5	93.7	5.8	158.6
Geometric mean	147.5	121.4	173.6	10.7	293.8	154.2	126.9	181.5	11.2	307.2
Lower 95% confidence limit for the geometric mean	135.1	111.2	159.0	9.8	269.1	141.6	116.6	166.7	10.3	282.1
Upper 95% confidence limit for the geometric mean	161.0	132.5	189.5	11.7	320.7	167.9	138.2	197.7	12.2	334.5
2.5^{th} percentile	54.4	44.8	64.0	4.0	108.4	58.9	48.5	69.4	4.3	117.4
97.5^{th} percentile	378.5	311.5	445.5	27.5	754.1	387.9	319.2	456.6	28.2	772.8
Women										
Arithmetic mean	126.5	104.1	148.9	9.2	252.1	141.2	116.2	166.2	10.3	281.2
Standard deviation	53.3	43.9	62.7	3.9	106.2	71.2	58.6	83.8	5.2	141.8
Geometric mean	115.6	95.2	136.1	8.4	230.3	126.9	104.5	149.4	9.2	252.9
Lower 95% confidence limit for the geometric mean	106.6	87.7	125.4	7.7	212.3	117.2	96.5	138.0	8.5	233.6
Upper 95% confidence limit for the geometric mean	125.4	103.2	147.6	9.1	249.9	137.4	113.1	161.8	10.0	273.8
2.5^{th} percentile	40.1	33.0	47.1	2.9	79.8	42.0	34.6	49.4	3.1	83.7
97.5^{th} percentile	267.0	219.7	314.2	19.4	531.9	275.6	226.8	324.4	20.0	549.1

All										
Arithmetic mean	143.8	118.4	169.3	10.5	286.5	156.2	128.5	183.8	11.4	311.1
Standard deviation	65.2	53.6	76.7	4.7	129.9	76.9	63.3	90.6	5.6	153.3
Geometric mean	130.3	107.3	153.4	9.5	259.7	139.9	115.1	164.7	10.2	278.7
Lower 95% confidence limit for the geometric mean	122.8	101.0	144.5	8.9	244.6	132.6	109.1	156.0	9.6	264.1
Upper 95% confidence limit for the geometric mean	138.4	113.9	162.9	10.1	275.7	147.6	121.5	173.7	10.7	294.1
2.5^{th} percentile	44.3	36.5	52.1	3.2	88.3	49.9	41.1	58.8	3.6	99.5
97.5^{th} percentile	279.6	230.1	329.1	20.3	557.0	332.4	273.6	391.3	24.2	662.3
Percentage difference of sample arithmetic mean from population recommendation	534.3	439.7	628.9	38.8	1064.5	740.1	609.2	871.3	53.8	1474.6
Bases (unweighted)										
Men	2607.6	2146.2	3069.6	189.6	5195.3	5327.0	4384.4	6270.7	387.3	10613.3
Women	2887.0	2376.2	3398.5	209.9	5752.0	5774.0	4752.3	6796.9	419.8	11504.0
All	5494.7	4522.4	6468.0	399.5	10947.3	11101.1	9136.7	13067.6	807.1	22117.3

Note: CI, confidence internal; PI, prediction interval

Appendix 39

MPs exposure assessment via the consumption of tap water (TW) for the U.S.A.

	TW ingestion rates		MPs daily uptake		MPs yearly uptake	
	mean	95th Percentile	mean	95th Percentile	mean	95th Percentile
age group	mL/day	mL/day	max[a]	max[a]	max[a]	max[a]
1 to < 2 years	146	565	92	355	33466	129509
2 to < 3 years	205	778	129	489	46990	178333
3 to < 6 years	208	741	131	465	47678	169852
6 to < 11 years	294	1071	185	673	67391	245495
11 to < 16 years	315	1395	198	876	72204	319762
16 to < 21 years	436	1900	274	1193	99940	435518
21 to < 30 years	781	2848	490	1789	179021	652819
30 to < 40 years	902	2967	566	1863	206756	680096
40 to < 50 years	880	2964	553	1861	201714	679408
50 to < 60 years	956	2976	600	1869	219134	682159
60 to < 70 years	941	2972	591	1866	215696	681242
70 to < 80 years	772	2273	485	1427	176958	521017
80+ years	784	2122	492	1333	179708	486405
21 to < 50 years	858	2938	539	1845	196671	673448
50+ years	902	2827	566	1775	206756	648005
all ages	711	2641	447	1659	162975	605370

[a] maximum MP tap water (TW) contamination. Note: the highlighted results are used in the uncertainty/sensitivity analysis in section 8.4.1.

Note: the exposure estimates are based on the intake rates proposed by the National Health and Nutrition Examination Survey (NHANES) data for 2005−2010, and reported in the Exposures Factors Handbook (EPA, 2019a).

Appendix 40

MPs exposure assessment via the consumption of bottled water (BW) for the U.S.A.

	BW ingestion rates		MPs daily uptake		MPs yearly uptake	
	mean	95th Percentile	mean	95th Percentile	mean	95th Percentile
age group	mL/day	mL/day	max[a]	max[a]	max[a]	max[a]
1 to < 2 years	71	356	347	1740	126698	635277
2 to < 3 years	105	533	513	2606	187371	951131
3 to < 6 years	121	578	592	2826	215923	1031432
6 to < 11 years	156	731	763	3574	278380	1304459
11 to < 16 years	235	1095	1149	5353	419354	1954011
16 to < 21 years	380	1500	1858	7334	678104	2676728
21 to < 30 years	459	1888	2244	9230	819079	3369108
30 to < 40 years	468	1965	2288	9607	835139	3506513
40 to < 50 years	427	1896	2088	9270	761975	3383384
50 to < 60 years	342	1751	1672	8561	610294	3124633
60 to < 70 years	278	1461	1359	7143	496087	2607133
70 to < 80 years	190	1093	929	5344	339052	1950442
80+ years	108	737	528	3603	192724	1315165
21 to < 50 years	451	1925	2205	9411	804803	3435134
50+ years	273	1462	1335	7148	487164	2608917
all ages	326	1570	1594	7676	581742	2801641

[a] maximum MP bottled water (BW) contamination. Note: the highlighted results are used in the uncertainty/sensitivity analysis in section 8.4.1.

Note: the exposure estimates are based on the intake rates proposed by the National Health and Nutrition Examination Survey (NHANES) data for 2005−2010, and reported in the Exposures Factors Handbook (EPA, 2019a).

Appendix 41

MPs uptake from seafood consumption in the UK. According to the National Diet and Nutrition Survey. UK Results from Years 9-11 of the Rolling Programme (2016/17-2018/19).

Age groups and descriptive statistics	Total fish g/day	MPs daily uptake		MPs yearly uptake	
		from	to	from	to
Children 1.5-3 years					
Arithmetic Mean	8	1	169	309	61801
Median	5	1	100	183	36649
SD	9	1	206	377	75354
2.5th percentile	0	0	0	0	0
97.5th percentile	28	3	610	1113	222625
Boys 4-10 years					
Arithmetic Mean	11	1	249	454	90739
Median	8	1	184	336	67227
SD	12	1	274	501	100129
2.5th percentile	0	0	0	0	0
97.5th percentile	42	5	927	1692	338472
Girls 4-10 years					
Arithmetic Mean	11	1	246	450	89957
Median	8	1	166	303	60607
SD	15	2	338	616	123233
2.5th percentile	0	0	0	0	0
97.5th percentile	51	6	1112	2029	405886
Children 4-10 years					
Arithmetic Mean	11	1	248	452	90358
Median	8	1	183	333	66669
SD	14	2	307	560	111921
2.5th percentile	0	0	0	0	0
97.5th percentile	48	5	1050	1915	383071
Boys 11-18 years					
Arithmetic Mean	13	1	279	509	101820
Median	1	0	19	34	6777
SD	19	2	427	778	155682
2.5th percentile	0	0	0	0	0
97.5th percentile	57	6	1261	2300	460085
Girls 11-18 years					
Arithmetic Mean	12	1	255	466	93112
Median	0	0	0	0	0
SD	17	2	377	688	137626
2.5th percentile	0	0	0	0	0
97.5th percentile	56	6	1236	2256	451133
Children 11-18 years					

Arithmetic Mean	12	1	267	488	97577
Median	0	0	5	9	1749
SD	18	2	403	736	147118
2.5th percentile	0	0	0	0	0
97.5th percentile	57	6	1257	2293	458651
Men 19-64 years					
Arithmetic Mean	24	3	519	948	189538
Median	11	1	245	448	89569
SD	33	4	726	1325	265041
2.5th percentile	0	0	0	0	0
97.5th percentile	113	12	2492	4548	909516
Women 19-64 years					
Arithmetic Mean	21	2	467	853	170545
Median	12	1	255	465	93030
SD	28	3	609	1111	222216
2.5th percentile	0	0	0	0	0
97.5th percentile	98	11	2158	3939	787838
Adults 19-64 years					
Arithmetic Mean	22	2	493	900	180002
Median	11	1	250	457	91377
SD	30	3	670	1223	244564
2.5th percentile	0	0	0	0	0
97.5th percentile	106	12	2331	4254	850736
Men 65 years and over					
Arithmetic Mean	28	3	621	1133	226513
Median	24	3	530	968	193606
SD	29	3	631	1152	230491
2.5th percentile	0	0	0	0	0
97.5th percentile	90	10	1975	3605	721024
Women 65 years and over					
Arithmetic Mean	28	3	611	1115	223016
Median	24	3	523	954	190850
SD	31	3	687	1253	250611
2.5th percentile	0	0	0	0	0
97.5th percentile	118	13	2588	4723	944540
Adults 65 years and over					
Arithmetic Mean	28	3	615	1123	224607
Median	24	3	523	955	191007
SD	30	3	661	1207	241407
2.5th percentile	0	0	0	0	0
97.5th percentile	104	11	2295	4189	837706
Men 65-74 years					
Arithmetic Mean	31	3	692	1264	252720
Median	26	3	578	1055	211093
SD	30	3	658	1200	240050
2.5th percentile	0	0	0	0	0

97.5th percentile	90	10	1972	3599	719777
Women 65-74 years					
Arithmetic Mean	30	3	665	1214	242754
Median	22	2	494	902	180471
SD	37	4	816	1490	297900
2.5th percentile	0	0	0	0	0
97.5th percentile	161	18	3533	6447	1289380
Adults 65-74 years					
Arithmetic Mean	31	3	678	1237	247445
Median	25	3	550	1004	200832
SD	34	4	745	1359	271747
2.5th percentile	0	0	0	0	0
97.5th percentile	119	13	2619	4779	955860
Men 75 years and over					
Arithmetic Mean	23	2	499	911	182171
Median	16	2	348	636	127197
SD	26	3	568	1037	207475
2.5th percentile	0	0	0	0	0
97.5th percentile	83	9	1823	3327	665440
Women 75 years and over					
Arithmetic Mean	24	3	533	973	194642
Median	25	3	550	1004	200750
SD	20	2	430	785	157027
2.5th percentile	0	0	0	0	0
97.5th percentile	60	7	1319	2406	481282
Adults 75 years and over					
Arithmetic Mean	24	3	519	946	189275
Median	21	2	471	860	171996
SD	22	2	493	900	180037
2.5th percentile	0	0	0	0	0
97.5th percentile	73	8	1614	2946	589146

Abbreviations / Glossary

ABCC2 and ABCG2	ATP-binding cassette (ABC) transporters
ABS	acrylonitrile butadiene styrene
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and excretion
AF	absorption factor
AGI	anogenital index
AhR	aryl hydrocarbon receptor
AIC	akaike information criterion
ATR	attenuated total reflection
ARfDs	acute reference doses
A549	adenocarcinomic human alveolar basal epithelial cells
BEAS-2B	human lung epithelial cells
BeWo b30	human placental choriocarcinoma cell line
BMD	benchmark dose
BMDL	lower confidence limit of the benchmark dose
BPA	bisphenol A
BW	bottled water
Caco-2	human adenocarcinoma cell line
CCK-8	cell counting kit 8
CH_3OH	methanol
CI	confidence interval
C_{ing}	concentration of the chemical in food or other exposure media
CL	cellulose
COOH	carboxy-modified surface
COPD	chronic obstructive pulmonary disease
CP	cellophane
CPE	chlorinated polyethylene
CPS	carboxylated polystyrene
CrEf	critical effect
DEHP	di-(2-ethylhexyl) phthalate
EDCs	endocrine disrupting chemicals
EEC	emerging environmental contaminant
EE2	17-alpha-ethinylestradiol
E_{ing}	ingestion exposure
ELISA	Enzyme-Linked Immunosorbent Assay

EPS	expanded polystyrene
ERAs	environmental risk assessments
ERI	emerging risk identification
ERIn	ecological risk index
EVA	ethylene-vinyl acetate
E1	estrone
E2	estradiol
E3	estriol
E/P	ethylene/propylene copolymer
E/P/D	ethylene/propylene/diene terpolymer
FDM	fused deposition modelling
FFF	fused filament fabrication
FT-IR	Fourier-transform infrared spectroscopy
GHS	Globally Harmonized System of classification and labelling of chemicals
GI	gastrointestinal
HACCP	Hazard Analysis and Critical Control Point
HBGVs	health-based guidance values
HCA	high content analysis
HD	high-density
HDFs	human dermal fibroblasts
HDPE	high-density polyethylene
HeLa	cervical cancer cells
HepaRG	human hepatic cells
HepG2	human caucasian hepatocyte carcinoma cells
HMC-1	the human mast cell line-1
HNO_3	nitric acid
HPEC- A2 cells	SV40-transformed microvascular human placental venous endothelial cells
HT29-MTX-E12	a mucus-secreting subclone from colon adenocarcinoma HT29 cells differentiated into mature goblet cells
H_2O_2	hydrogen peroxide
ICC	intraclass correlation coefficient
IL-	interleukin
IR	ingestion rate
KATO III	gastric cancer stem cells

KOH	potassium hydroxide
LD	low-density
LDH	lactate dehydrogenase
LDPE	low-density polyethylene
LIVE/DEAD kit	viability/cytotoxicity test
LOAEL	lowest-observed-adverse-effect level
MCP-1	Monocyte chemoattractant protein-1
MDDC	dendritic cells
MDM	human blood monocyte-derived macrophages
MeSH	medical subject heading
MFs	modifying factors
MOE	margin of exposure
MP	microplastic
MTS assay	colorimetric cell proliferation assay kit
MTT assay	cellular metabolic activity colorimetric assay
M-cell	Microfold cells
NaClO	sodium hypochlorite
NIH/ 3 T3	murine fibroblast cell line
NOAEL	no-observed-adverse-effect-level
NP	nanoplastics
NY6	nylon 6
n/r	not reported
N/A	not available
PA	Polyamide
PAA	polyacrylic acid
PAEs	phthalic acid esters
PAIS	partial androgen insensitivity syndrome
PAN	polyacrylonitrile
PA6	polyamide
PB	polybutylene
PBDEs	polybrominated diphenyl ethers
PBMA	poly (butyl methacrylate
PBMCs	peripheral blood mononuclear cells
PC	polycarbonate
PCBs	polychlorinated biphenyls
PCR	polymerase chain reaction

PE	polyethylene
PEG	polyethylene glycol
PEI	polyetherimide
PEMA	polyethylene-co-methyl acrylate
PET	polyethylene terephthalate
PEVA	polyethylene-vinyl-acetate
PGR	propylene glycol ricinoleate
PI	prediction interval
PIS	poly (isoprene)
PLA	poly-lactic acid
PLI	pollution index
PMMA	polymethyl methacrylate
PMPS	poly (methyl phenyl siloxane)
PMTDI	provisional maximum tolerable daily intake
PNMA	poly(N-methyl acrylamide)
POM	polymerized oxidized material
PP	polypropylene
PPTT	poly trimethylene terephthalate
PPS	polyphenylene sulphite
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PRISMA-P	Preferred Reporting Items for Systematic Reviews and Meta-Analyses protocols
PS	polystyrene
PTA	polyester terephthalic acid
PTFE	polytetrafluoroethylene
PTMI	provisional tolerable monthly intake
PTWI	provisional tolerable weekly intake
PU	polyurethane
PUR	polyurethanes
PVA	polyvinyl alcohol
PVAc	polyvinyl acetate
PVA-PE	poly-vinylacetate- ethylene
PVC	polyvinyl chloride
Pyr-GC-MS	pyrolysis gas chromatography/ mass spectrometry

p53	sensitive reporter cell line based on the human liver carcinoma cell line
QA	quality assurance
QC	quality control
Raji B	human lymphocytes cells
RBC	red blood cell
RfDs	Reference Doses
RM	Raman spectroscopy
RoB	Risk of bias
RT-PCR	Reverse transcription polymerase chain reaction
RY	rayon
SAN	styrene acrylonitrile
ScRs	scoping reviews
SD	standard deviation
SE	standard error
SEM-EDS	scanning electron microscopy plus energy-dispersive X-ray spectroscopy
SMD	standardized mean difference
TDI	tolerable daily intake
TEER	transepithelial electrical resistance
THP-diff.	THP-1 cells differentiated into macrophages
THP-1	human monocytic cell line
TNF-α	tumour necrosis factor alpha
TPU	polyurethane
TSCA	Toxic Substances Control Act
TTC	threshold of toxicological concern
TW	tap water
t-PS	digestive tract transformed polystyrene microplastics
T98G	human glioblastoma multiforme cells
UFP	ultrafine particles
UFs	uncertainty factors
UHMWPE	ultrahigh-molecular-weight polyethylene
U937	human histocytic lymphoma cells
VC	vinyl chloride
VIF	variance inflation factor
VOCs	volatile organic compounds

WTPs	water treatment plants
WST-1 assay	cell proliferation assay
w.w.	wet weight
ZO-1	zonula occludens-1