Microplastics and Nanoplastics

REVIEW Open Access

Paradigms to assess the human health risks of nano- and microplastics

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Abstract

Human exposure to nano- and microplastics (NMPs) has raised major societal concerns, yet no framework to assess the risks of NMPs for human health exists. A substantial proportion of plastic produced worldwide is not properly disposed and persists in the environment for decades while degrading. Plastic degradation generates a size continuum of fragments, including nano- and microplastic particles, with numerous associated environmental pollutants and plastic additives, and microbial communities colonising their surfaces. The ubiquitous presence of NMPs, their availability for uptake by organisms and their potential to act as vectors for toxicants and pathogens render risk assessment a priority on the political agenda at the global level. We provide a new, fully integrated risk assessment framework tailored to the specificities of NMPs, enabling an assessment of current and future human health risks from NMPs. The framework consists of four novel paradigms to the traditional risk assessment methodology. These paradigms deal with techniques in NMP analysis, gaps in empirical data, theoretical and modelling approaches and stakeholder engagement. Within the proposed framework, we propose how we can use research experiences gained so far to carry out the different steps of the assessment process, and we define priorities for further research.

Keywords: Microplastics, nanoplastics, risk assessment, human health, hazard identification, exposure assessment, hazard characterization, stakeholder engagement, food, air

Introduction

Studies conducted in recent years have shown strong evidence that humans are exposed to nanoplastics (NPs; size range <1 μm [1]) and microplastics (MPs; size range 1 μm - 5 mm) dispersed ubiquitously in the environment. Currently, there are insufficient hazard and exposure data, as well as insufficient conceptual approaches, to perform a meaningful human health risk assessment of nano- and microplastics (NMPs) [2–4]. MPs originate from consumer products intentionally containing micron-sized plastic particles and fibres (*primary MPs*; e.g. cosmetic products, cleaning products, paints, textiles, etc.) and from gradual degradation and fragmentation of larger plastic items (*secondary MPs*) [5–10]. It is likely that a significant source of NPs derives from the further fragmentation of MPs, as demonstrated by plastic degradation studies, and the fact that environmental concentrations of NPs are increasing [11, 12]. While MPs have been studied mostly in the context of the marine environment, there is growing evidence of their presence and accumulation in terrestrial, freshwater, and atmospheric compartments [6]. However, accurate

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qualitative or quantitative data regarding NP concentrations in the environment are lacking due to the current analytical challenges in their isolation and detection in complex matrices. Widespread NMP pollution makes humans vulnerable to daily exposure *via* several routes, in particular oral and respiratory. Hence, there is an urgent need to assess the potential detrimental impacts on human health.

The potential impact of NMPs on human health has only recently emerged as a concern, despite a growing body of evidence showing intake and adverse effects on other organisms. Accumulation of plastic debris from different natural environments has been demonstrated for many species, and *in vivo* and *in vitro* ecotoxicological studies have demonstrated the potential of NMPs to elicit toxicological activity (e.g. oxidative stress *via* free radical generation, immunological responses, alteration of gene expression, genotoxicity, endocrine disruption, neurotoxicity, reproductive abnormalities, transgenerational effects, and behavioural abnormalities) [13]. In contrast, many aspects related to the behaviour, fate and effects of NMPs in the human body (e.g. adsorption across membranes, translocation to secondary tissues and organs, accumulation, acute and long-term effects, and elimination) remain largely unknown [6].

The scarcity of information regarding the potential human health risks associated with NMP exposure is currently a limitation to establish whether extensive regulatory actions are required for safeguarding public health and wellbeing worldwide in synergy with food safety and ecosystem integrity. International scientific organisations have emphasized the need for data on the human health impact of NMPs that provides the necessary evidence base for effectively supporting policy-making. Prompted by growing public concerns over this issue, as well as requests from national health authorities, several organisations (i.e., World Health Organization (WHO) [14], European Food Safety Authority (EFSA) [15], Science Advice for Policy by European Academies (SAPEA) [6]) have carried out their first expert evaluations of the overall state-of-the-art knowledge and risk characterization in relation to single exposure sources (i.e., drinking water, seafood). According to their reports, it seems that the available evidence does not highlight specific issues or concerns with respect to the existence of a widespread risk to human health. However, they make us aware that quantitative information regarding NMP exposure and toxicity is too scarce to allow for definitive conclusions on NMP risks for human health, both at present and in the future, where NMP pollution is expected to rise [16, 17].

To provide science-driven guidance to regulators in order to inform potential actions related to plastic pollution, there is an urgent need for a human health risk assessment - that i) looks at the full-size continuum of plastic particles, ii) uses comprehensive toxicological data from human-relevant models, including toxicokinetics-toxicodynamics and long-term impacts, and iii) uses reliable data sets that account for multiple exposure routes. It is expected that key exposure routes to be considered are inhalation, especially in indoor environments, and ingestion of contaminated food, beverages and drinking water [6, 14, 18]. In fact, the atmospheric compartment contains NMPs that become airborne *via* resuspension of terrestrial/indoor dust, as well as inputs from direct sources (e.g., particles released from traffic, incineration, industrial emissions); food and beverages can be contaminated through several pathways, including direct exposure, trophic transfer and during industrial/domestic processing. In the specific case of drinking water, NMPs can come directly from the freshwater supply source (i.e., NMPs entering freshwater systems *via* surface run-off, treated/untreated wastewater and industrial effluents, sewer overflows, degraded plastic waste, atmospheric deposition), from treatment and distribution systems (for tap water), from the bottling processes and the bottle itself (for bottled water) [19–21].

To be able to assess the current and future risks of NMPs to human health, we argue that a new and fully integrated risk assessment framework tailored to NMP-specific features is required, as well as more data on NMP exposure and toxicity. In fact, as already happened for other particulate chemicals (e.g. engineered nanomaterials) and highly diverse classes of contaminants (e.g. per- and polyfluoroalkyl substances), NMPs do not easily fit within traditional risk assessment frameworks because their extreme diversity (of size, shape, chemical properties associated with adsorbed/absorbed chemicals, biofilms) results in high levels of uncertainty in hazard and exposure. Furthermore, such an assessment for NMPs is not at all '*business as usual*'. The specific features of plastic particles require a major rethinking with respect to the tools used within each component of the risk assessment, implying a considerable suite of crucial innovations will need to be developed 'from scratch' to address key knowledge gaps [4, 22, 23].

Scientifically, NMP research has benefitted significantly over the last decade from the considerable effort spent to understand and tackle plastic pollution. Furthermore, NMP research has been informed by the transfer of knowledge from research on engineered nanomaterials (nanotoxicology) [24–26], particle and fibre toxicology [27] and particulate air pollution studies [28–30]. Although the quantitative assessment of the human health risk associated with NMP exposure remains an ambitious research objective at present, important milestones have been reached in the last decade that

have driven progress and made the achievement of this goal more feasible. For instance, there has been rapid development and optimisation of analytical techniques for identifying and quantifying NMPs in complex matrices, as well as a focus on identifying existing weaknesses that are being used as a basis for further innovation in this area. While the larger size of MPs has resulted in faster development of analysis techniques for this group of particles, the development of techniques specifically for NPs has gained much more attention in recent years [31–34]. Methods for standardization and harmonization have been defined, based on quality assurance/quality control (QA/QC) criteria, which can be used in NMP research [14, 35–37]. Furthermore, computer modelling tools and approaches for predicting specific aspects of the behaviour and fate of NMPs [38] and methods for handling (submicron-)particulate contaminants for exposure and hazard assessment [39] have been developed.

Earlier reviews have been published addressing the hazard and toxicity of NMPs, analytical challenges for assessing NMPs in matrices relevant for human exposure (water, food and air), and provisional exposure assessments [18, 33, 40–44]. To our knowledge, no review has specifically addressed the integral question of NMP risk assessment for human health and suggested how to use the state-of-the-art knowledge to bring this research and policy goal within reach. Here, we review the literature and provide guidance on achieving human health risk assessment for NMPs. We propose an innovative and holistic framework for human health risk assessment for NMPs (hereafter referred to as HRA-NMP, i.e. holistic human health risk assessment framework for NMPs) and offer a path forward on how to use the best available science to tackle the remaining knowledge and data gaps in agreement with the recommendations of leading research and regulatory bodies [6].

Holistic human health risk assessment for NMPs

As for any chemical, the HRA-NMP builds on the classical four pillars of risk assessment: *hazard identification*, *hazard characterization*, *exposure assessment* and *risk characterization* (Fig. 1, and Supplementary Table 1). In addition, it takes into account some NMP peculiarities that enhance the complexity of the overall assessment, i.e. i) the coexistence of three classes of hazards (physical, chemical and microbiological), which all have to be identified and characterized to inform the cumulative *risk characterization*, ii) the particulate nature and nano-/micro-dimensions, which call for the use of specific analytical methodologies, in particular for assessing exposure, and iii) the general awareness and concern regarding NMP pollution currently existing at both public and political levels, which calls for the identification of effective mitigation strategies in dialogue with all stakeholder groups.

To address these needs, the HRA-NMP integrates four additional NMP-specific paradigms into the standard human health risk assessment framework (Fig. 1):

1) sampling, sample processing and analysis techniques that specifically target the smallest size fractions down to the (sub-)micron scale;

2) empirical data on the actual occurrence and effects of NMPs regarding all relevant exposure pathways, with the novel aspect of including chemical and microbiological hazards associated with NMPs in the assessment;

3) models based on new concepts, theories and algorithms that extend beyond the analytical detection limits (where necessary), to align dissimilar exposure and effect data *via* novel re-scaling methods, to probabilistically address uncertainty in the assessment, to identify which factors the calculated risk is most sensitive to, and to enable prospective risk assessments across age and cultural groups based on future emission scenarios;

4) two-way engagement with end-users in the process, specifically stakeholders from governmental bodies and regulatory agencies, society and industry, and use of mechanistic insights from the social sciences (risk perception and risk communication science) where possible.

Below, we review recent literature related to each of the aforementioned paradigms, and provide practical recommendations to overcome the main knowledge gaps that still challenge a prompt assessment of human health risks associated with NMP exposure.

Paradigm 1: Analytical techniques that cover the full NMP size range

To implement the HRA-NMP, more reliable data on NMP occurrence in food, beverages, drinking water and airborne matrices are needed. While different extraction and analysis methods targeting the micron range are now available for routine application, those targeting the sub-micron range are still under development. Irrespective of the technique used, it is paramount to highlight the importance and difficulty of sampling and sample pre-processing prior to instrumental measurements owing to multiple steps needing to be optimized: bulk sample collection, separation, digestion among other steps leading to the final identification and quantification of NMPs [45]. Moreover, due to the widespread MP contamination, the application of strict QA/QC measures during the whole procedure is of great importance to avoid and mitigate cross-contamination that would lead to inaccurate results [46].

Until routinely applicable technologies that overcome the current main analytical limitations [22] are available in the market, a combination of approaches is

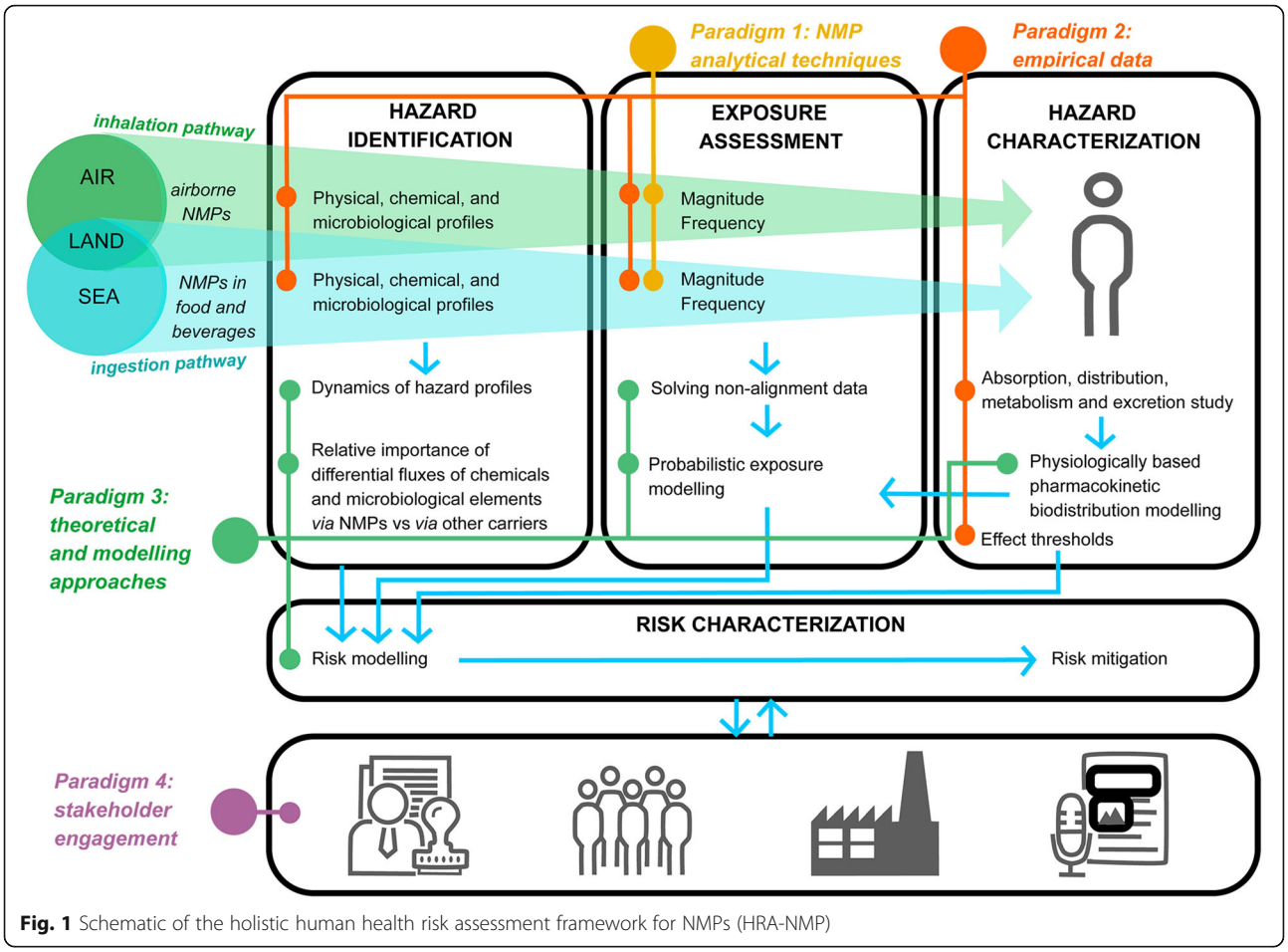

Fig. 1 Schematic of the holistic human health risk assessment framework for NMPs (HRA-NMP)

recommended. This includes leveraging the unique advantages of each individual technique and the use of re-scaling modelling to fill any possible remaining gaps in the targeted particle size continuum, in particular at the low nano-size range (more details in Section 5.2.1). Below, we provide a brief overview of the possible approaches and analytical instrumentation for application in NMP identification and quantification. These can be purposely combined to cover the full NMP size continuum, as suggested in Section 3.3. A detailed presentation of NMP analytical methods is not provided here as this is outside the scope of the present paper. For a more comprehensive summary on this topic, the reader is referred to specialized recent reviews [31, 32, 47–52].

State of the art technologies

The analysis of small MPs in real samples of relevance for assessing human exposure strongly relies on their chemical identification. The visual-based approaches, often used for pre-sorting or even identification of larger MPs in other research areas (e.g., monitoring of MP pollution in marine environments), are not recommended for the size range of HRA-NMP, as they do not allow the discrimination of MPs from biomaterials or other inorganic particles nor the visualization of smaller-sized particles of primary interest. Fluorescent staining techniques have been shown to offer advantages over purely visual identification by achieving faster selection of particles and reducing researcher bias [53]. Furthermore, they allow to extend the lower size identifiable into the low micron range [54] and achieve a basic polymer identification [55]. It is also widely acknowledged that the approach has some significant limitations. These include i) co-staining of residual organic and inorganic materials, ii) precipitation of the fluorescent dye (e.g. Nile Red) leading to the formation of false-positive particles, iii) unsuitability for some particle types (e.g., black, fibres or comprised of rubber), and iv) lack of harmonised methods [54]. Fourier transform infrared (FTIR) spectroscopy and Raman spectroscopy are the most commonly used methods for chemical identification of MPs in studies [56–58]. Both spectroscopy techniques are non-destructive and can be combined with microscopy and imaging techniques [59] for the identification and

number-based quantification of very small MPs down to approximately 10 μm (micro-FTIR) and 1 μm (micro-Raman). A range of FTIR-based techniques is available and routinely used to analyse MPs. They do not require extensive preparation of MPs beyond removal of organic matter via digestion with acids, hydroxides, enzymes or oxidizing agents as Fenton's reagent. By using FTIR in the attenuated total reflectance mode (ATR-FTIR), plastic particles down to 10 μm can be accurately detected and measured even directly [46] on filters without any visual preselection. The accurate detection of MPs in environmental samples with high levels of biotic material has been achieved with this approach [60]. The direct contact of contaminated filters to the ATR crystal is a crucial and susceptible preparation step. For this reason, it is recommended applying FTIR directly on filters by True Specular Reflectance/Reflection-Absorption. Depending on filter type, there might be some chances to additionally work in transmission mode, supposed sufficient light can pass the filter for attaining an appropriate signal-to-noise ratio. More advanced techniques, such as micro-FTIR, combine microscopic imaging and particle-size determination with FTIR, where individual particles down to sizes of ca. 10 μm can be detected. The coupling of microscopes with an ATR unit allows for the selective analysis of either small particles or areas on larger particles [52]. Chemical imaging (micro-FTIR) allows all particles on a filter to be analyzed, even if they form particle clusters. Hyperspectral imaging via focal plane array (FPA) detectors, which currently can collect up to 128 x 128 spectra/pixels within a single scan [61] currently represent the state of the art in MP analysis because they allow fast, effective identification and quantification of MP [52]. The polymeric composition of MPs is determined by FTIR spectroscopy based on matching the characteristic IR absorption spectra of each polymer type to library spectra [62]. Raman spectroscopy is often considered a complementary spectroscopic technique to FTIR. It allows for the identification of MPs directly on filters, for example from air sampling campaigns [63], without extensive visual pre-sorting process. However, any remaining biotic material needs to be removed to avoid fluorescence [64] even though this drawback can be overcome by selecting the proper excitation laser wavelength and suitable data pre-processing methods. Raman spectroscopy combined with microscopy and imaging techniques (i.e. micro-Raman) has been used for the identification of very small MPs <10 μm in size [59, 65–67]. This approach is most suitable for simple matrices like drinking water, whereas in more complex sample matrices, only particles >5 μm have been identified. It has been proposed that the technique might be able to measure particles down to the upper limit of the NP size range (<1 μm) [65]. Although Raman spectroscopy is typically considered to be non-destructive, it is worth noting that the method uses a focused laser beam that may cause damage to the analysed particles, especially in the case of very small plastic particles.

In addition to IR and Raman spectroscopy techniques, pyrolysis gas chromatography - mass spectrometry (Py-GC-MS) is increasingly applied for the identification of NMPs in environmental samples. Py-GC-MS is a destructive technique that does not allow for assessing the number and morphologies of MPs present in samples but provides a mass-based quantification and accurate identification of different plastic types [57]. This technique also has the advantage of providing information on plastic-associated chemicals (e.g., additives), which might be toxic [68]. The technique is most suited for determining the relative contribution and quantity of individual polymer types in environmental or biological samples, with more information achieved if the sample has been fractionated into specific size classes prior to analysis. The primary challenge with Py-GC-MS is the accurate transfer and drying of particle suspensions generated in sample processing to the small sample holders used in the analysis. While the technique can be used for polymer identification of single particles, it is not efficient for this purpose and it has been estimated that the minimum particle size required for Py-GC-MS analysis is around 100 μm [69], primarily due to the difficulty of handling smaller particles. However, Py-GC-MS has shown a greater sensitivity for the mass-based detection of very small MPs and NPs that are below the limits of FTIR and Raman detection. Indeed, particles <1 μm have been identified as plastics based on a combination of Py-GC-MS and statistical approaches applied to samples taken from the North Atlantic Subtropical Gyre [70]. Added advantages of Py-GC-MS are its broad compatibility with a range of extraction and purification processes and the fact that the detection is almost unaffected by insufficiently removed organic matrix [71].

A recent study comparing Py-GC-MS and hyperspectral FTIR imaging spectroscopy for the analysis of MPs in environmental samples found the overall trends in MP contamination were very similar, but that there were differences in the observed polymer compositions [72]. Importantly, the authors highlight the importance of selecting the identification and quantification technique, or combination of techniques, that is most suited to the research or monitoring question be asked.

For nano-sized particles, other spectroscopic approaches, such as surface-enhanced Raman spectroscopy (SERS) and fluorescence have proven capabilities for identifying different particle sizes at very low concentrations [73, 74], with promising results demonstrated particularly for SERS [73, 75]. In addition, recent advances

in nano-FTIR, IR-Enhanced Atomic Force Microscopy, Tip-Enhanced Raman Spectroscopy and Raman tweezers may complement SERS in the analysis of plastic particles as small as 20 nm [40, 76, 77]. However, limited research has been done with these methods to this specific research field of NMPs so far, most likely because these methods are generally located in laboratories with research focus on fundamental topics in materials science and on method itself.

Pioneering technologies

With regards to innovative technologies addressing the isolation, identification and quantification of NMPs that cause risks for human health, different combinations of methods are currently under research for targeting different size ranges. For MP particles down to 1 μm, techniques employing a correlative combination of light microscopy or hyperspectral imaging with identification by laser confocal Raman and TOF-SIMS are under development [78–80]. For plastic particles in the nanoscale, correlative scanning electron microscopy (SEM) -confocal Raman spectroscopy has being explored [81, 82]. Critical for the analysis of particles <1 μm in simple and complex matrices is the need of employing appropriate isolation and fractionation techniques for the particles (i.e., sub-fractions in the nanoscale). This has to be balanced against the probable need for multiple detection and analytical approaches that generate the required data of physico-chemical properties (size, shape, polymer type) and to achieve mass-based or number-based quantification. Therefore, research is moving toward hyphenated fractionation approaches to obtain continuous size distributions of NMPs followed by multi-instrument identification and quantification. Among these approaches, continuous fractionation techniques based on centrifugal field flow fractionation, asymmetric field flow fractionation and differential mobility analysis (DMA) enable isolation of targeted particle fractions <1 μm [83, 84]. Then, collected particle fractions are subjected to a range of imaging techniques for determining NP size, shape and surface morphology (e.g., dynamic light scattering, nanoparticle tracking analysis, UV spectroscopy, laser induced breakdown detection, SEM, transmission electron microscopy (TEM)) and methods for identifying and quantifying NPs (e.g., thermal extraction and desorption (TED) GC-MS, Py-GC-MS). Again, many of these techniques present challenges, including sample preparation, changing particle properties (SEM and TEM) and high limits of detection (GC-MS techniques). For field flow fractionation (FFF) approaches, problems with particle-membrane interaction and limited concentration ranges have not been solved yet [85], as well as the need for representative calibration materials for the quantification of plastic particles <1 μm. It is also worth highlighting that sample digestion methods, typically applied for isolating MPs from complex matrices (sediment, soil, biota), need further evaluation for NPs to ensure these processes do not compromise the targeted particles.

The application of chemometric approaches is considered to have high potential for assisting and strengthening the chemical identification of plastics at the nano range. In fact, it has been demonstrated that combining the signals of spectroscopy methods increases the accuracy and efficiency of MP detection [86, 87]. Chemometric algorithms, which integrate the signals of FTIR and Raman spectroscopy with those of SERS and fluorescence-based methods, are considered to substantially increase the capability to reveal differences in particle size down to the submicron scale and to detect NMPs at very low concentrations. However, research in this area is limited. Multiparametric machine learning algorithms, such as PLS-DA (Partial Least Squares Discriminant Analysis), SVM (Support Vector Machines), Random Forest, Boosting algorithms and others, or methods based on Artificial Neural Networks, have become more and more important, particularly in spectroscopy, where many variables play a significant role. In Raman spectroscopy, machine learning is widely used, and multivariate analysis for FTIR data on MPs has also been used with success [88]. It is evident that learning algorithms deliver more reliable results when more significant features are used as input. As the aforementioned methods (Raman, SERS, FTIR, and fluorescence) all contain complementary specific and significant NMP fingerprints regarding polymer type, size and concentration, the mathematical combination of all of them in machine-learning algorithms has potential for leading to a much more powerful technique. Instead of analysing the spectroscopic data of a single method, the entirety of measured and individually pre-processed information from all methods can be combined in large matrices as input for supervised machine learning algorithms for the development of predictive models, classification and statistical analysis of data. The extended information combining several analytical methods can be used for the prediction and quantification of concentrations and particle size classes and for multinomial classification of material type. However, this is still not developed for such multispectroscopic approaches, remaining an interesting frontier to explore towards pushing instrumental detection limits to much lower values in terms of particle size and concentration.

The above techniques represent examples of cutting edge concepts approaches for the identification and quantification of plastic particles <10 μm and down into the nanoscale. While some of them may prove to be technically suitable for achieving this goal, their

widespread uptake and application for NMP analysis will ultimately be influenced by a range of factors, including sample throughput, the quantity of particles in a filter/sample, availability of equipment in most laboratories and the purchase and operating costs [52]. Furthermore, sample processing techniques may need optimization for specific combinations of approaches and dedicated data processing platforms will need to be developed.

Practical recommendations

Multimethod approach

In general, to develop methods targeting MPs >10 μm, relatively well-developed techniques, such as micro-FTIR and micro-Raman spectroscopy, offer very strong options for combining particle imaging, size determination, particle identification and number-based quantification. Such instruments are commercially available, including options that allow for a high degree of automation and high-throughput, as FPA micro-FTIR [54], and work towards harmonisation of methods is already ongoing. The remaining limitations with such approaches might be overcome by combining multiple spectroscopy techniques with machine learning algorithms and innovative probabilistic rescaling modelling (see Section 5.2.1). Given expectations on the joint performance of the four aforementioned spectroscopy methods (Raman, SERS, FTIR, and fluorescence), mathematically combining them in machine learning algorithms could be worth investigating within the scope of implementing HRA-NMP. With regards to innovative technologies for addressing particles <1 μm, promising performance is expected for technologies that have been developed under several national and international projects, as, for example JPI Oceans project ANDROMEDA (https://www.andromedaproject.net/), TRAMP (https://www.microplasticlab.com/projects) and REVEAL (https://www.sintef.no/en/projects/2020/reveal/), which combine multiple separation (e.g., FFF, DMA), imaging (e.g., SEM, TEM), sizing (e.g., DLS, NTA), identification (laser confocal Raman, Py-GC-MS) and quantification techniques in a stepwise approach. Again, combining spectroscopy techniques with chemometric approaches may help to extend the application of such methods. In this context, it is of fundamental importance that the research community working on NMP exposure and effect assessment closely follows the developments with respect to NMP analysis in order to apply the latest approaches when they become available.

Method harmonisation and QA/QC

Standardized methods for analysis, as well as sampling and data reporting, do not exist yet. Progress is further complicated due to the lack of relevant standards and reference materials that reflect the irregular-shaped, partially degraded NMPs found in the environment [89, 90]. However, multiple efforts are currently underway toward standardization (e.g., EU H2020 project EURO-qCHARM) and some important achievements have already been obtained. For instance, in support to the NMP research community, interlaboratory studies are launched by several organizations (e.g., Joint Research Centre (JRC), Quasimeme), and specific recommendations towards harmonisation have been agreed upon the frame of multiple international research consortia and expert groups [35–37, 42, 91–95]. These provide guidance on the nomenclature and definitions to be applied, sampling and analytical methodologies to be preferred on a case-by-case basis, data formatting, metadata to be reported and risk profiling in various targets. Furthermore, they highlight key QA/QC measures that need to be implemented during all steps of NMP analysis, i.e. sampling, sample preparation, polymer identification and quantification [35–37, 84]. For instance, procedures regarding method validation (e.g. use of spiked samples to overcome the current lack of certified reference materials for NMPs) and the control of sampling and post-sampling contamination (e.g. procedural blanks, clean room, lab and equipment cleaning, labware and lab clothes, etc.) have already been established for MPs in matrices such as biota and water [36, 37] based on common QA/QC criteria in analytical chemistry. As a next step, these procedures can be adapted and optimized for other matrices, including foodstuffs and beverages in the human diet, which represent a very broad group of matrices that may require dedicated approaches for isolating NMPs. It is recommended that new methods and protocols for sampling and analysing NMPs in the HRA-NMP build on these efforts in accordance with agreed harmonisation principles in NMP research and international organizations (e.g. JRC, Organization for Economic Co-operation and Development (OECD), ISO) [14, 96, 97]. The same criteria should be applied when reviewing existing data reported in the literature to ensure consistency within the datasets to be used in the HRA-NMP.

Paradigm 2: Empirical data

Owing to the limitless number of physical, chemical and microbiological profile combinations, it is practically impossible to empirically describe the physical, chemical and microbiological hazards of real life ingested and inhaled NMPs as a combined unit. Therefore, the HRA-NMP performs the assessment of each of these risk components separately within the *hazard identification* and *hazard characterization* pillars, merging the obtained insights with the outcomes of the *exposure assessment* in the *risk characterization* (Fig. 1). It is recognized that new empirical data are needed to improve the

identification of the real hazards of ingested and inhaled NMPs, exposure pathways and mechanisms of toxicity. As detailed below, the HRA-NMP adopts a tiered approach (e.g., in the *hazard characterization*) and hypothesis-driven approaches combined with modelling, enabling extrapolations of hazard metrics in time and space (e.g., in the *hazard identification*) to rationalise measurements.

Hazard identification

As previously mentioned, the potential hazards of NMPs for human health come in three forms: 1) the particles themselves (i.e., physical hazard), 2) the chemicals that can leach from the polymeric core and surface (unbound monomers and additives, adsorbed and absorbed environmental pollutants; i.e., chemical hazard), and 3) microbiological elements, including associated microorganisms and pathogens colonising NMP surfaces, and their mobile genetic materials (MGMs; i.e., microbiological hazard). The HRA-NMP characterizes these three hazards for all NMP classes with potential for exposure (primarily ingestible and inhalable NMPs) and give special attention to this assessment in consideration of the fact that the hypothesis that NMPs may transfer hazardous chemicals and pathogens to organisms, including humans, has been central to the perceived risk of NMP pollution [6]. Although we have provided a distinct categorisation here, it should be noted that these parameters are not isolated, and can impact upon each other. For example, the size of a particle when moving from micro-scale to nanoscale will impact upon these other hazard considerations; the leaching of chemical substances may increase with the increase in relative surface area, or the adsorption of substances to particle surfaces may increase, again with the increase in relative surface area. Likewise nanoscale particles may be more likely to translocate than micron-sized particles and therefore present a more varied biodistribution, these are considerations discussed in later sections.

The HRA-NMP aims at grounding the whole risk assessment process in the actual hazards of NMPs at the moment of exposure and under realistic (environmental and real-life) scenarios. In this context, it takes into account the primary consideration that physical, chemical and microbiological hazards can change during the life cycle of a plastic particle as they are shaped by a combination of intrinsic particle properties [22, 23], features of the dispersive media [98] and extrinsic processes occurring prior to exposure (e.g. environmental degradation and food processing). Depending on the specific exposure routes, some inherent properties and hazards may remain unaltered during NMP transfer from the environment to the human body, whereas others can be substantially affected by transformations occurring prior to exposure. Furthermore, the HRA-NMP acknowledges that humans are exposed to chemicals and pathogens *via* several pathways and that NMPs represent just one of the possible carriers. Considering this, an understanding of the dynamics of chemical and microbiological properties with the potential for threatening human health, together with the relative role of NMPs as a vector of these hazards to humans, are the key objectives of the HRA-NMP's hazard identification. In line with a view largely held by experts, the questions specifically addressed here are: Do NMPs present in food and air release harmful toxicants, as well as pathogens and hazardous MGMs once inside the human body? How do degradation processes that occur before exposure (e.g., UV degradation under environmental conditions, thermal degradation during food processing) influence the hazard profiles predicted from the bulk properties of commercial plastics? Are these chemical and microbiological exposures relevant compared to those vectored by all the other (natural) carriers?

While specific modelling tools are currently available to support tasks in this part of the HRA-NMP (see Section 5.1), empirical data feature important gaps, impairing the complete and correct identification of potential health hazards. As described in detail in Section 4.2, the current knowledge regarding NMP uptake by humans *via* inhalation and *via* consumption of food, beverages and drinking water is generally scarce, with NMPs in air and in edible samples being relatively recent and underrepresented topics in the literature, respectively. In addition, most of the available studies describe NMPs by their physical properties (mainly size and shape) and polymeric composition, whereas data concerning their chemical and biological properties are scarce.

With specific reference to the chemical hazard, recent evidence from environmental studies suggests the role of NMPs as vectors for environmental pollutants is minor compared to that occurring *via* natural carriers [99]. This has been supported by laboratory studies which demonstrated that uptake of hydrophobic pollutants adsorbed to NMPs *via* ingestion was negligible when the same pollutants were also available in the aqueous phase and/or food [100–102] . While experimental studies conducted with invertebrates are not necessarily directly relevant to human exposures, modelling studies of human exposure also suggest a similarly low level of risk, especially compared to other exposure pathways [102]. For examples, human exposure predictions by EFSA, Food and Agriculture Organization (FAO) and WHO have independently concluded that the chemical risk of ingestible MPs for human health is likely to be small [14, 15, 103]. According to their very conservative exposure scenario calculations, the transfer of specific classes of persistent organic pollutants (i.e., PCBs, PAHs), as

well as common plastic additives (e.g., bisphenol A, nonylphenol), from ingested MPs have a negligible effect on the total exposure associated with the consumption of specific food types (i.e. seafood) and drinking water. However, the available information does not currently allow for full understanding of the implications of NMP chemical proprieties for human health. Importantly, the situation may be different for the smallest particles of the plastic continuum, especially with reference to plastic additive chemicals [6]. Plastic contains a wide array of additive chemicals (e.g., plasticizers, colourings, fillers, flame retardants, antioxidants, etc.) used by industry to create the countless types of plastic materials that are on the market today. In addition, plastics also contain a range of residual monomers, catalysing agents used in chemical processing and potentially non-intentionally added substances carried over from the raw materials (usually petroleum oil), none of which are chemically bound to the polymeric chains. Residual concentrations of these chemicals may reside in NMPs, and this would apply to NMPs in foodstuffs and inhaled air. These chemicals have the potential to leach from the core/surface of the material to the surroundings [104]. Research has demonstrated that the leachates from a range of plastic and rubber materials can trigger toxicity *in vitro* [105, 106] and *in vivo* [107, 108]. There is also some evidence that environmental (UV) degradation can influence the toxicity of NMP leachates [8, 109]. This raises the question of whether degradation mechanisms associated with food processing/preparation practices can influence the chemical and physical hazard profiles of NMPs contained in food, an issue that has yet to be investigated to our knowledge. Humans mainly eat *processed* food, obtained through a variety of steps, from primary processing (e.g., grinding grain to produce raw flour), to secondary processing (i.e., food preparation, e.g., preparing bread), and to tertiary processing (e.g., generating highly transformed unhealthy foods in respect to human dietary needs, rich in sugar and salt but poor in fibre). Owing to this complexity, food processing is expected to modify the hazard profiles of NMPs originally contained in the natural/agricultural/farmed products, as well as representing a source of additional NMPs in the final product. For example, the most temperature sensitive plastic particles can be sensitive to heating practices, leading to consequences in terms of risk. In line with EFSA [110], the HRA-NMP considered it crucial to address this issue due to the potential consequences in terms of human health risk.

As regards the microbiological hazard, there is increasing evidence that NMPs provide favourable conditions for the establishment of microbial taxa that differ from those in the surrounding environment (water, natural aggregates or atmospheric particulate), thereby altering the structure and composition of environmental microbial communities [111]. However, the available data is currently insufficient to confirm whether NMPs represent a preferential environment for the growth or survival of pathogens compared to other natural particles and act as a significant vector for transfer of biofilm [112] and microbiological elements able to be embedded into human microbiota. Human microbiota (i.e. the microbial community residing in human mucosae, with particular reference to gastrointestinal tract and lungs) naturally interacts with any microbial community to which it comes into contact [113]. This implies exchange of microorganisms between microbial communities, and genes between the relative microbiomes (i.e. the whole collection of microbial genes) [114]. The latter occurs by horizontal transfer and by *ex vivo* transfer of MGMs, and it is considered an effective mechanism for microorganisms to acquire new genetic skills, including antimicrobial resistance, pathogenicity and virulence. Harmful antimicrobial resistant bacteria, protozoa, viruses and MGMs can be found in any environmental compartment [115], and in particular in air and water systems receiving inputs from waste and sewage treatment plants, hospitals, pharmaceutical production, intensive farming and aquaculture, etc. [116]. Therefore, conceptually they can contaminate air, water sources and food webs, colonize particles therein, including NMPs, and expose humans to biological hazards through ingestion. Even when viable bacteria are not directly transported, bacterial compounds [111, 117, 118] such as lipopolysaccharides, may remain on NMP surfaces and possibly trigger strong immunomodulatory effects [119]. In this context, NMPs are thought to favour an increased permissiveness towards MGMs carrying antibiotic resistance genes and eventually other genes thus facilitating the establishment of novel traits in bacterial communities by evolutionary changes at the species level [111].

In the HRA-NMP, it is considered crucial to assess how and to what extent NMPs select for unique microbial networks [120] and act as substantial vectors of pathogens and MGMs [121–123]. Furthermore, the HRA-NMP deems it fundamental to establish surveillance systems able to determine their load and significance for human health and, most importantly, quantify the relative importance of fluxes of pathogens and MGMs *via* NMPs compared to those *via* other (natural) particles and pathways. With specific reference to the ingestion route, the HRA-NMP takes into primary consideration that pathogens and MGMs accumulate and magnify along food chains, and MGMs are not affected by transformations occurring during trophic transfer, nor by thermal degradation during food cooking.

Practical recommendations

Physical hazard identification Substantial data referring to physical properties (e.g. size and shape) and polymeric composition of NMPs in many matrices of HRA-NMP relevance (e.g. key food categories such as seafood, drinking water and air in different indoor/outdoor microenvironments) are available in the literature, as many studies addressing NMP contamination report this information beside concentration [36, 40, 124]. This information can now be used to inform the *physical hazard identification* in the HRA-NMP, upon data quality evaluation.

Chemical hazard identification Key insights regarding the potential for chemical hazard of ingested and inhaled NMPs can be obtained *via* bioelution experiments using representative plastic particles and relevant simulated biological fluids (SBFs, i.e., synthetic solutions representing specific human physiological fluids of target exposure sites). The testing of pristine and partially degraded reference materials under controlled laboratory conditions [89, 90], allows for modelling the bioleaching dynamics and provide the *risk characterization* with a wide range of scenarios and longer timeframes. Degradation scenarios relevant for the inhalation and ingestion routes should include: i) UV degradation under wet and dry conditions, and ii) thermal degradation simulating food processing. Key parameters that affect chemical solubility (i.e., temperature, pH, ionic strength, the presence of precipitants and ligands) and bioelution systems (i.e., static systems, flow-through, and tangential flow) have to be selected according to the specific environment being modelled, i.e. the gastrointestinal tract (GIT) and pulmonary system.

Standard test methods for using SBFs in the characterisation of nanomaterial solubility in gastric fluid are provided by ISO (ISO/TR 19057) [125] and can be used as reference here. With regards to the inhalation route, for which there is currently no common or standardised methodology, new procedures aligning with the suggestions made in the ISO technical report (ISO/TR 19057) [125] can be set up for assessing particle and fibre solubility in simulated lung fluids. These should provide simulation of specific extracellular (lung lining fluid) and intracellular compartments (lysosomal fluid), as the two most likely environments that inhaled particles will encounter. As regard the assessment of possible changes to NMP potential for bioleaching of toxicants following heat-based food processing, references for the selection and technical implementation of test conditions can be taken from CEN ISO standards for testing migration of substances from plastic materials/articles intended to come into contact with foodstuff (i.e. CEN standard EN 13130 series, UNI EN 1186-1:2002) and reference consensus guidelines [126].

Microbiological hazard identification A better understanding of the rates of MGMs exchange within NMP-associated microbial communities is timely. This would allow the measurement of the effects of plastic pollution on the microbial ecology of eco-systems, bacterial evolution and the emerging risks to environmental and human health. The most effective approach to investigate the NMP-associated microbial communities is the metagenomics/metataxonomic one, to be performed at a convenient coverage to allow identifying low abundance species. Sampling should follow an adequate frequency to allow building robust ecological networks.

Exposure assessment

The latest rough estimate of the annual human body intake of MPs through inhalation and consumption of foodstuff is in the range of 74,000 and 113,000 particles (depending on age and sex), suggesting substantial exposure [43]. Furthermore, it has been estimated that the annual consumption of water can increase the human uptake of additional 4,000 to 90,000 particles per year, depending on the water source preferentially consumed (only tap water *vs* only bottled water, respectively) [43]. According to the authors, this evaluation is likely to be underestimated as exposure to plastic particles <5 μm have not been documented due to the limitations of analytical methods. Innovations in NMP sampling and analysis, advancements in method harmonisation (Section 3) and the application of rescaling modelling to deal with possible gaps in the lower size range of the exposure dataset (more details in Section 5.2.1) are all anticipated to make a significant contribution to the *exposure assessment*. Furthermore, the current estimation is expected to be soon updated by new studies targeting NMP inhalation within a wider variety of indoor/outdoor microenvironments and NMP ingestion *via* all main food and beverage categories in the human diet.

To assess the human exposure to NMPs in HRA-NMP, parameters defining the extent of exposure such as frequency of consumption as well as prevalence and concentration have to be defined for both the inhalation and the ingestion pathways. While the frequency and duration of food and air exposures can be derived from available databases (e.g., EFSA and WHO food consumption databases; UN Time Use Statistics database, multinational Time Use Study database reporting), the assessment of the magnitude of NMP presence bases on empirical data of NMP concentrations in food, beverages and in the inhalable and respirable aerosol fractions.

At present, NMPs in food and beverages are currently under-represented in the literature, with the exception of

MPs in seafood products for which substantial evidence exists. NMPs in air have only recently started being studied, with only a few scientific papers published so far [18, 40, 127–133]. Furthermore, for the lower size range of NPs (<100 nm), no occurrence data exist for matrices of HRA-NMP relevance neither for other kinds of samples.

Although preliminary, the available evidence prompts the exposure assessment in the HRA-NMP to give special attention to airborne NMPs as it suggests substantial inhalation by humans in indoor and outdoor environments [43, 44]. Primary and secondary NMPs can be generated by a variety of industrial and environmental processes and practices. Once in the environment, they can accumulate in dust and soils and be suspended into the atmosphere [18, 41, 133, 134]. MPs have been found in the atmosphere as fibres and fragments, the most frequently reported shapes, as well as spheres and films [40]. They are derived from domestic, vehicular and industrial sources and they can be inhaled in respect of their size and shape [18, 135]. In these regards, data show that both outdoor and indoor airborne NMPs feature a size distribution compatible with inhalation and deposition in the airway, entailing a potential for triggering inflammation and other effects due to the chemical and microbiological profiles of the NMPs in question. Furthermore, it is expected that airborne NMPs contain a substantial respirable-sized fraction (i.e., NMPs that reach the deep lung and are potentially subject to interaction with macrophages and epithelial cells; typically, particles <2-4 μm aerodynamic diameter) given the fragmentation process occurring during the aging of plastic, although not experimentally assessed yet due to analytical challenges.

With reference to the ingestion route, there is agreement that NMPs can contaminate all human sources of food and beverages, including drinking water, as a result of their ubiquity in the environment and ability to bioaccumulate in human food webs [15, 41]. In addition, contamination can reach food also during other phases of the chain, such as industrial production [33], packaging [136], and domestic food processing [137]. Thus far, MPs have been reported in limited products intended for human consumption (i.e. seafood, sugar, table salt, honey, soft drinks, energy drinks, cold tea and beer [138], beside drinking water), and data often lack in comparability and do not meet minimal quality criteria. In addition, NMP contamination associated with different food processing techniques as well as different packaging conditions is still largely unknown and needs to be addressed to allow proper assessment [15]. However, this research area is in continuous expansion as highlighted by reviews on this topic [33, 44], and the quality of data is expected to improve substantially in the next years considering the ongoing efforts toward harmonization (see Section 3.3).

Practical recommendations

Rational use of the existing literature data The absence of standardization in NMP analysis poses difficulties in comparability of NMP occurrence data present in the literature. Furthermore, not infrequently, authors report difficulties with blank samples [139], and some methodologies used in published studies have been recently questioned as results were related to background contamination and potential erroneous identification of plastic particles [140, 141]. Therefore, data from individual published case studies should be carefully evaluated before using them in the *exposure assessment* of HRA-NMP. In this context, it is recommendable to review literature data by applying the same QA/QC criteria used in NMP method development (see Section 3) in order to ensure consistency within the *exposure* dataset.

Exposure assessment - Inhalation route Prioritising indoor microenvironments is recommended since it is known that on average people spend most of the time indoors (i.e. house, office, shops, educational and recreational places) where the density of NMP sources is high (e.g. carpets, home textiles, furniture made by synthetic materials), and the available data suggest higher concentrations than in outdoor environments [129, 142, 143]. The sampling strategies should account for seasonal living styles and habits (e.g., open-window, heating, air conditioning), and standardized conditions (e.g., n° of adults and children living in the household, type and size of the target rooms, cleaning schedule, etc.). Both passive collectors and active pumped samplers are in use in airborne MP research [40]. To the specific scope of assessing the magnitude of inhaled and respired NMPs, the use of personal samplers should be considered (e.g., cyclones for respirable dust, IOM sampling head for inhalable dust).

Exposure assessment - Ingestion route In the HRA-NMP, the assessment of human exposure *via* ingestion covers all main human diet components (i.e., cereals, meat, fish, egg, milk, vegetable green leaf, vegetable, fruit), commonly consumed categories of commercial alcoholic and non-alcoholic drinks (e.g., soft drinks, beer, wine) and water, an important source of exposure with bottled water contributing more to human exposure. Variability is likely to be very high due to the variety of sources of NMPs in the environment and the possibility that NMPs reach food at different stages in the production chain. Existing data describe MP occurrence in some specific food categories (i.e. seafood and drinking water) and single food items (i.e., table, salt, beer, sugar, honey [21, 66, 144–160], whereas data gaps are particularly important for food other than aquatic animal

species, such as crops, vegetables and terrestrial species that can be contaminated by air, soil and water during farming [33]. The sampling strategy in the HRA-NMP has to capture the aforementioned variability and streamline the experimental effort by exploiting the existing data, avoiding duplication and identifying key sources of exposure and exposure scenarios.

Gender, age and geographic dimensions It has been acknowledged that lifestyle, habits and behaviours are influenced by gender, age, culture and geography-related factors, determining, for example, differences in diet, nutrition, behaviours and standards of living between women and men, children and adults, and among communities inhabiting different parts of the world [124]. It is expected that specific subpopulations could be differently exposed to NMPs. For instance, coastal communities heavily relying on protein from both harvested and cultured seafood could be particularly exposed by considering that the piscine food chain is very sensitive to NMP contamination [124]; crawling and hand-to-mouth contacts could particularly expose young children to NMPs contained in settled dust [127, 128]. Therefore, it is fundamental that the sampling design in the HRA-NMP accounts for all these dimensions in order to obtain sex, gender, age, subpopulation-sensitised data. In fact, the conclusions of the *risk characterisation* need to be stratified taking into account the individual variation in connection with biological, environmental, societal, and lifestyle factors that influence the magnitude and the frequency of the exposure to NMPs. The application of this approach is believed to aid in developing risk management approaches that better meet the needs of individuals and thus increasing the chances for the suggested measures to be effective.

Hazard characterisation

There is already information available that provides an indication of how certain NMP features (physical, chemical and microbiological) are implicit in toxicity, and from these studies it is possible to define a strategy to begin with the characterization of hazard posed by NMPs. As identified earlier, the vast physical and chemical combinations associated with NMPs make it difficult, or actually impossible, to test all possible iterations, making it important to select key components and likely concomitant conditions within an informed testing strategy. This strategy is likely to consist of human-based assessment, selected *in vivo* methods to address specific questions, *in vitro* tools for more explorative investigations, and *in silico* models (when data is already available). Here we provide a brief account of what is already known in regard to biodistribution and translocation of NMPs, and their associated toxicity, alongside suggestions of which features of NMPs should be investigated most urgently to implement the HRA-NMP.

To date, it has largely been engineered spherical polymer particles (which are often fluorescent) that have been a useful experimental tool in particle toxicology studies and have provided an indication of the potential toxicity of NMPs. Although ranging in composition, polystyrene (PS) particles have most commonly been used, both *in vitro* and *in vivo*. *In vivo* studies have mainly used rodent models, and studies decades old have confirmed size dependent uptake and translocation of PS particles. For example, when a dose of 1.25 mg/kg was administered to rats by oral gavage, NPs of 50 nm demonstrated the highest extent of translocation and most varied range of biodistribution, whereas micron-sized particles of 3 μm were only retained at low concentrations and not found outside the digestive system [161]. More recently, this size-dependent effect has been confirmed on numerous occasions, including observations of low translocation of micron-sized plastics following oral gavage in mice [162], transport of nano-sized PS (20, 40, 100, 200, and 500 nm) across the placenta and deposition within foetal organs following intravenous exposure to pregnant mice [163], and higher levels of toxicity and pulmonary inflammation associated with smaller nano-sized PS following intratracheal instillation of both rats [164] and mice [165]. Although these studies provide an indication that NPs may offer a wider distribution following exposure, and with it potentially greater toxicity, this is not to say that these larger (micro-sized) particles pose no hazard. For example, reduced mucus secretion and gut microbiota dysbiosis has been observed in mice exposed to PS particles of 500 nm and 50 μm in diameter *via* drinking water, which in turn caused hepatic lipid disorder; toxicity was found to similar levels for each particle [166]. Also administered by drinking water, 5 μm PS MPs were shown to disrupt the intestinal barrier of mice and cause metabolic dysregulation and again microbiota dysbiosis [167]. Translocation was not observed by Jin et al. [167], instead it was postulated that accumulation within the gut would occur.

When considering these same exposure routes *in vitro*, cell-based studies have been used to screen the toxicity of NMPs at different target sites, and to assess their interaction with intestinal and lung barriers. Investigation of the effects on the pulmonary system has focused on assessing the response of alveolar and bronchial epithelial cells, whilst assessment of toxicity to the intestine has centred on the use of intestinal epithelial models. Macrophages are part of the innate immune system and are resident in several organs that may be exposed to NMPs, and as they clear particles *via* phagocytosis following exposure of various target sites (e.g., lung, liver)

[168, 169], they provide a valuable model for assessing NMP-induced immune responses. Using these *in vitro* tools, there is a growing body of evidence, albeit contradictory at times, indicating the intrinsic toxicity of PS particles. Currently, results for NMPs collected by *in vitro* studies cannot be unconditionally used for human health risk assessment of NMPs, however, their use as screening tools to identify potential effects is invaluable; work is ongoing in developing approaches to allow unrestricted use of nanomaterials in general in risk assessment, such as quantitative *in vitro* to *in vivo* extrapolation (QIVIVE), reported within the NANORIGO framework [170].

A range of cell types have been used to investigate the response of the lung to NMPs, including A549 alveolar epithelial cells, and Calu-3 and BEAS-2B bronchial epithelial cells [171–175]. All studies used PS particles, with the exception of da Luz et al. [175], who investigated the response of lung epithelial cells to poly-lactic acid nanoparticles. All studies noted here have investigated particles with diameters <100 nm. The most commonly assessed endpoints include cytotoxicity, reactive oxygen species (ROS) production, cytokine production, antioxidant expression and cellular uptake. Barrier integrity has also been evaluated, but to a more limited extent [171]. From these studies there is evidence that NMPs can cause cell death, promote pro-inflammatory cytokine production, increase cellular ROS production, increase antioxidant expression and cause reductions in barrier integrity [164, 171, 172]. NPs of various sizes were shown to cause damage to pulmonary cells *in vitro*, however, it has been repeatedly demonstrated that particle toxicity is size-dependent, with greater toxicity typically observed as particle size decreases. Similarly, uptake of NMPs by pulmonary cells typically increases with decreasing particle size [172, 173, 175]; correlations between uptake and toxicity were also evident.

The existing *in vitro* studies evaluating an innate immune response to NMPs have used a variety of macrophage cell types, including cell lines (differentiated THP-1 human monocytes, RAW 264.7 and J774.1 mouse macrophages), and primary cells (e.g. human peripheral blood mononuclear cells (PMBCs)) [176–178]. The majority of these studies have investigated the response of macrophages to PS particles, with one study investigating the toxicity of polypropylene (PP) particles [178]. Assessment of the impact of particle size on macrophages has dominated existing studies, with the influence of other properties (e.g., charge) investigated to a more limited extent [173]. A range of particle sizes have been tested in existing studies, with most focussing on the toxicity of different sized NPs [164, 176, 177, 179] and only one example exclusively testing the toxicity of MPs [178] Assessment of cytotoxicity, inflammatory responses (e.g., cytokine production and the activation of signalling pathways that drive this response), oxidative stress (e.g., ROS production) and cellular uptake are commonly assessed, with fewer studies evaluating other endpoints, such as phagocytic function and genotoxicity.

The most common cell type used to assess intestinal responses to NMPs *in vitro* are Caco-2 human intestinal epithelial cells. Such cells can be used in an undifferentiated or differentiated form. The majority of studies have focused on assessment of cellular uptake and particle translocation [180–182]. All studies, with the exception of Magri et al. [183], tested PS particles. Furthermore, all studies, apart from Wu et al. [181] investigated particles with nano dimensions. Existing studies have demonstrated that particle uptake and translocation is size-dependent, with smaller particles being internalised by intestinal cells and translocating across the intestinal barrier to a greater extent than larger particles [180]. Furthermore, particle charge can influence NMP uptake and translocation, with neutral particles exhibiting the greatest uptake and translocation [180]. Assessment of intestinal toxicity has considered endpoints such as cytotoxicity, barrier integrity, ROS production and mitochondrial depolarisation [162, 181–183]. As discussed for other cell types, as the size of particles decreases, their toxicity to intestinal cells typically increases. Most existing studies have demonstrated that NMPs are relatively non-toxic to the intestine *in vitro,* although there are some exceptions where toxic responses have been observed [162].

As described above, the dependence on size for spherical NMP for passing biological barriers and their toxicity has been relatively well represented, and the results are quite consistent. However, the risks associated with human exposure to NMPs with parameters akin to those found with the environment, including irregular and fibrous morphologies, aging, leaching and adsorption, is less well defined, and is the more pressing research question. There is a lack of studies that have assessed the toxicity of environmental samples. This may derive from the challenges associated with obtaining sufficient quantities of particles to perform hazard testing. Only one study was identified that has investigated the toxicity of NMP samples designed to mimic those found within the environment [178], and no studies, to date, have used samples actually sourced from the environment. Hwang et al. [178] investigated the *in vitro* toxicity of irregularly shaped polypropylene microparticles, generated by ball milling, to human-derived cells. Similar approaches are encouraged; although difficulties in obtaining sufficient quantities of environmentally sourced particles to perform toxicity studies are likely to limit the extent of these, it is necessary to establish suitable methods for simulating these materials.

At systemic level, there is currently a scarcity of comprehensive ADME studies (absorption, distribution, metabolism, excretion) which can inform the HRA-NMP's *risk characterisation* (i.e., physiologically based pharmacokinetic (PBPK) modelling, Section 5.2.3) by providing parameters and threshold effect concentrations of NPs at the target sites (organs/cells) [184]. However, by today, *in vivo* tests have importantly contributed to support much of the *in vitro* evidence, demonstrating particle translocation across the lung and intestinal barriers, as well as the influence of some physicochemical properties in the biological behaviour and fate of particles [41].

By assessing effects in the GIT and the liver of mice exposed to PS MP *via* drinking water, liver toxicity, gut microbiota dysbiosis, disruption of the intestinal barrier and metabolic dysregulation have been demonstrated [166, 167]. Oral exposure of rats to PS MPs has also provided evidence of translocation to the heart, resulting in cardiovascular dysfunction and fibrosis [185]. Regarding lung exposure, intratracheal instillation of PS NPs into the lungs of Sprague–Dawley rats has highlighted an influx of immune cells in a size-dependent fashion, with the smallest particles causing the greatest effect [164].

For humans, particulates that are ingested are considered systemically absorbed only when they pass both the intestinal epithelium and the liver, and when they are distributed *via* the bloodstream within the entire body. Thus far, the available experimental evidence indicates that NMPs >150 μm are unlikely to be absorbed, while limited absorption and uptake (<0.3%) into organs is estimated for NMP <10 μm. Consistent with observations by EFSA [15], FAO [103] and the previous WHO report [14], it is likely that NPs are subject to absorption. However, caution is warranted against extrapolations from the limited data available, which are restricted to either latex or PS NPs. Moreover, a recently published study [186], although not reporting direct evidence of translocation following oral gavage, the study did report pertinent observations within the reproductive system of male mice exposed to 5.0–5.9 μm diameter PS particles, including sperm deformities, decreased sperm number, motility, and testosterone, with evidence of these effects being caused through micro-PS-induced oxidative stress. Finally, although evidence obtained from pregnant mice intravenously exposed to carboxylated polystyrene NPs suggests transport across the placenta and accumulation in foetal organs (including brain, lungs, and liver) [163], the potential for mother-offspring transfer has not yet been fully addressed *in vivo*.

Practical recommendations

Continued use of *in vitro* tools It is important to consider the model used for *in vitro* testing. There have been discrepancies in the findings of different *in vitro* studies when investigating the influence of particle physicochemical properties on particle uptake by cells and translocation. For example, some studies have observed particle translocation across the intestinal barrier, whereas others have not. Differences in the findings between studies may derive from their use of different cell models (e.g., presence of mucus which can influence particle interactions with cells [180]), time point, particle concentrations and particle physicochemical properties. *In vitro* models allow a quick and cheap assessment of NMP toxicity to be performed and allow the mechanism of NMP toxicity to be probed, but are often criticised for their physiological relevance. More specifically, the response of monocultures of cells is commonly used to assess NMP toxicity, but such models do not reflect the complex structure of organs *in vivo*. Advanced 3D co-culture *in vitro* models that better mimic the *in vivo* situation are available [187, 188], and it is recommended that these models are used to investigate the toxicity of NMPs in the future. It is of benefit that such models allow for chronic, repeated exposures to test substances, which is particularly relevant to NMPs. Indeed, studies to date have focused on investigating acute responses (< 96h) following a single exposure.

Physical hazard characterisation To date, *in vitro* studies have focused on particle size, and assessment of the toxicity of PS particles, with some others to a limited extent (polyethylene, polyvinylchloride). Although size has often been shown as an important factor, other factors such as polymer composition and irregular or fibrous morphology may be implicit in potential NMP toxicity. As an indicator of any intrinsic material-dependent hazard, the hazard classifications designated for EU Classification, Labelling and Packaging (CLP) regulation (EC No 1272/2008) were used by Lithner et al. [189] to provide a range of potential polymer hazards based on the monomers they consist of. Based on monomers classified as carcinogenic and mutagenic, the following materials were ranked highest: polyurethanes, polyacrylonitriles, polyvinyl chloride (PVC), epoxy resins, and styrenic copolymers (acrylonitrile butadiene styrene, styrene acrylonitrile resin and high-impact polystyrene). As such, a greater range in particle composition is recommended. The precise composition would rely on more comprehensive characterisation, however it is worth noting that only approximately 7% of total plastic production is PS, whereas other polymer types (PET, HDPE, PVC, LDPE, PP) may each be up to 23% [190]. Due to current limitations in sampling techniques, there is little knowledge on the composition of NPs in the natural environment. Alongside these concerns are those relating to geometry, with the potential for fibrous

morphology [191] combined with perceived biopersistence of NMPs [2] identified as being of key interest to the potential toxicity of fibre-like plastic material, linking them to the fibre pathogenicity paradigm, first identified for asbestos pathogenicity. MP fragments and fibres have been identified in drinking water [14] and various food types [103, 144, 159, 160]. In fact, in water, sediment and air samples, fibres have been described as the most predominant plastic microparticle shape found [14, 127, 192].

The persistence and pathogenicity of plastic microfibres has been reflected in human hazard assessments, with interstitial fibrosis and granulomatous lesions containing acrylic, polyester, and/or nylon dust, and plastic microfibers in non-neoplastic and malignant lung tissue of patients who had worked in the synthetic fibre textile industry [193, 194]. However, *in vivo* studies on nylon fibres found that intratracheal installation of respirable fibres to rats were capable of causing an acute inflammatory response [195], whereas in others no significant toxic effect was noted and rapid clearance of fibres was observed [196]. A study by Merski *at al.* observed no significant toxic effect following oral administration of PE and PET fibres to rats [197]. These conflicting findings highlight that the relative hazard associated to plastic microfibres is still largely unclear, but would likely be governed by the relationship between fibre size and biodurability.

Possible toxicity relating to weathered NMPs From the literature available, the majority of degradation studies are from an engineering perspective. Therefore, the information relevant for toxicity assessment is limited. However, from these studies it is clear that the impact of weathering is hugely dependent on both the plastic particle composition and the environmental conditions in which they are degraded [7]. In some cases, very limited effect is noted, whereas in other cases weathering has resulted in changes to particle surfaces such as oxidation, formation of flakes, cracks and changes in crystallinity [198], all of which may impact on the toxic effect. Due to this variability, it is clear that using particles collected directly from the environment would be most relevant to accurately assess the hazard to weathered NMPs, however as discussed previously, it may become difficult to obtain enough of these samples to complete the suite of toxicity testing necessary. The impacts of NMP aging has not yet been identified in the mammalian system, but we may expect an increase in oxidative potential and therefore toxicity, with the ROS present on plastic surfaces [18] increasing during particle weathering and UV or transition metal induced dissociation of C–H bonds [199, 200]. Therefore, the use of test materials degraded *via* accelerated degradation processes under controlled laboratory conditions (i.e., UV irradiation, chemical solvents, freeze-drying processes, abrasion) could be a valuable compromise at present [8, 10, 89, 90].

Chemical hazard characterisation The chemical hazard posed by NMPs may appear obvious, as the toxicity of many of the most harmful chemical additives and adsorbed/absorbed pollutants which can leach from NMPs to body/cellular fluids following accumulation are well noted (e.g. phthalates, bisphenol A, polybrominated flame retardants, polycyclic aromatic hydrocarbons (PAHs). However, there are mechanistic concerns that will require elucidation, especially when considering NPs.

NMPs often contain potentially toxic additives, such as bisphenol A, phthalates or heavy metals, of concern for human health [142, 201–203]. Leaching of these additives is possible, and hence this must be considered with regards to human hazard, despite the relative contribution to total exposure remaining low [14, 102]. Heavy metals which readily adsorb to NMPs in the environment [204] have shown to effect responses of marine organisms, such as cadmium or mercury [205, 206]; although this has yet to be tested in mammals. Furthermore, pollution-derived compounds such as PAHs and polychlorinated biphenyls (PCBs) have been known to not only adsorb to plastic, but in the case of PAHs, to accumulate and persist in its more toxic form as transformation of the PAHs is prevented [207]. In contrast, the literature is lacking regarding the actual potential of real NMPs to leach these chemicals once inside the human body and therefore what the health implications of these substances would be when associated to NMPs. In this context, the Trojan horse toxicity mechanism, a mode-of-action for toxicity of internalised and solubilised metal particles and nanoparticles [208–210] may bear relevance to NMPs and their potential to release harmful substances. This principle has been noted for concern in regard to NMPs toxicity [211] and has, in fact, already been shown to be a driving force in mitochondrial toxicity in zebra fish exposed to PAHs adsorbed to the surface of NPs [212]. It is likely that this behaviour could be assessed using acellular bioelution methods (described in Section 4), but also using *in vitro* cell models.

In addition, with particular reference to the inhalation route of exposure, the effects induced by potentially pathogenic or inflammogenic biomolecules found in the home, which may become bound to NMPs, required further attention. Humans are exposed to airborne NMPs in indoor microenvironments, including residential environments which are rich in perennial sources of allergens, such as the common house dust mite allergen (Der p 1), found in 48 % of European homes [213, 214]. Therefore, if we are to expect residential exposure to

NMPs, we are likely to find this in co-exposure with Der p 1. Equally, the ubiquitous and persistent component of bacterial cell walls, lipopolysaccharide (LPS), which is known as a strong moderator of pro-inflammatory responses, may be expected to be present in combination with NMPs within the environment. Common allergens, such as the birch pollen major allergen Bet v 1, have been shown to exacerbate the inflammatory response of human alveolar epithelial lentiviral immortalized (hAELVi) cells when co-exposed with mesoporous silica nanoparticles [215]. Moreover, in the presence of gold nanoparticles the common house dust mite (Der p 1) allergen has been shown to have a greater protease activity and ability for disrupting the lung epithelial barrier *in vitro* [216]. The addition of LPS to the surface of normally innocuous nanomaterials such as gold, has been shown to elicit strong responses *in vitro* [119], or even act synergistically in the stimulation of pro-inflammatory responses [217]. It is highly likely that if found under the same conditions, NMPs would induce similar responses to those of the innocuous particles mentioned above, and is certainly a scenario which requires attention, as demonstrated by Inoue et al. [165]. Using a mouse model, with exposure *via* intratracheal instillation, Inoue et al. [165] were able to discern the effect of combined exposures of latex nanoparticles with either LPS or the allergen ovalbumin (OVA). It was shown that latex nanomaterials in co-exposure with LPS significantly intensified lung inflammation compared to that elicited by either latex alone or LPS alone, and this was shown for various sizes (25, 50, and 100 nm) of latex nanoparticles. Conversely, co-exposure of latex nanomaterials with OVA induced no effect greater than that already observed for OVA alone [165]. These data highlight the need for continued research within this scope of combined exposures.

ADME studies of ingested and inhaled NMPs In the HRA-NMP, the *in vivo* ADME studies are conceived to the precise scope of obtaining PBPK parameters describing NMP adsorption, distribution, metabolism and excretion, and a better understanding of fundamental aspects still largely disregarded by the literature (e.g., mother-offspring transfer) [184, 218]. Their designs are closely informed by the *hazard identification*, *in vitro hazard characterization* and *exposure assessment*. Furthermore, to keep the number of exposed animals in compliance with 3R requirements at a minimum, they prioritise the testing of i) plastic particles having biological relevant size (e.g. showing capacity to translocate across barriers and toxicity *in vitro*), ii) both realistic and worst exposure scenarios, and iii) reference materials allowing for multiple tracing approaches under single exposure settings. The latest requirement is required as adsorption and excretion assessments needs quantitative methods (e.g., ICP-MS based analysis of rare metal labels), whereas the assessment of distribution within tissues and cells needs high-throughput sensitive imaging technologies (e.g., epifluorescence microscopy of fluorochrome labels). According to the best of our knowledge, no reference materials with similar features are available in the market. However, we consider the design of innovative dual labelled PS NPs (e.g., gold and fluorochrome labels) and their synthesis at laboratory scale an achievable goal for research in nanomaterials science at present. The capability of synthesizing multilayer nanoparticles has considerably grown in the last decades, and now this expertise can be used to address the synthesis of dual-labelled core shell structures to be embedded in the polymeric matrix (e.g. PS particles containing one, and only one, Au-SiO_2 core which encases a dye specifically selected to align with optical readout requirements, e.g. rhodamine B, fluorescein or Cy5.5) [219–221].

With reference to the *in vivo* exposure settings, established testing approaches in the field of pharmaceuticals can be considered suitable references to be applied here. Therefore, ADME studies of NMPs can make use of 5-week-old female C57BL6 mice as test animals housed in cages, in accordance with the Directive 2010/63/EU (5 animals/cage); NPs dosing to mice *via* drinkable water (ingestion route) and by aerial exposure using a spray device (inhalation route), separately; chronic exposure (e.g., daily administration over 12 months covers ~75% of the expected mouse lifespan).

Paradigm 3: Theoretical and modelling approaches enable extrapolation and prospective assessment

Besides the *risk characterisation*, in the HRA-NMP specific modelling tools support the *hazard identification* and the *exposure assessment*. Modelling tools are designed to be modular and flexible, allowing easy integration of new knowledge and insights, which are expected to be gained in the years ahead.

Hazard modelling

As already described, the HRA-NMP accounts for the three potential hazards linked to NMPs, i.e. physical, chemical and microbiological hazards. Furthermore, it considers relevant NMP dynamics (i.e., fate-transformation) which can modify the hazard profiles before human exposure occurs (e.g., changes in NMP physical, chemical, and microbiological profiles during degradation under real environmental and food processing conditions), while also fully accounting for the diversity and complexity of the material. The specific objectives of hazard modelling in the HRA-NMP are to

inform the *risk characterisation* regarding: 1) the dynamics of the chemical and microbiological profiles, and 2) the relative contribution to fluxes of harmful chemicals, pathogens and MGMs, carried by NMPs compared to other pathways (e.g., food items, beverages and inhalation). Examples of such modelling tools are plastic-inclusive exposure and bioaccumulation models based on mass-balance concepts and chemical kinetics, part of which rely on probabilistic approaches to capture the essentially continuous nature of NMP in the human diet and the environment [102, 207, 222].

Exposure modelling

The exposure modelling in the HRA-NMP has multiple scopes, which are i) to deal with possible gaps at the smallest particle scale of experimental datasets (e.g. NMPs << 10 μm), ii) to predict present and future exposure scenarios across different groups (e.g. different age, gender, culture, geographical areas, etc.), and iii) to translate exposure on the level of the whole body to exposure on the level of tissues and organs, for which the threshold effect doses are assessed within *hazard characterisation*. Therefore, the HRA-NMP implies the use of specific modelling tools to address each single task, as described below.

Solving the non-alignment of data, methods and approaches used to assess exposure of humans to NMPs

MP is a highly complex and diverse material [223], where the largely unknown low micron (<10 μm) and submicron fraction only adds further to this diversity and uncertainty. Although advancements in detection methods and sample pre-treatment procedures are expected to substantially extend our capabilities to measure NMPs across matrices relevant for human health (see Section 3), it is possible that size detection limits still remain higher in the near future. However, it has been demonstrated that the multidimensionality of NMPs can be addressed by considering the material as a continuum, the properties of which are described by continuous probability density functions (PDFs) for e.g. particle shape, size and density [22, 23, 102]. This has huge advantages for NMP exposure and risk assessment as, once these functions are parameterised, data gaps can be filled. As an example, it can be considered that NMP size distributions often are log-linear, leading to opportunities to extrapolate number concentrations measured for instance down to 20 μm, to number concentrations down to smaller size, using the following equation (Fig. 2 [22];):

$$CF = \frac{\int_{x_{1D}}^{x_{2D}} bx^{-\alpha}}{\int_{x_{1M}}^{x_{2M}} bx^{-\alpha}} = \frac{x_{2D}^{1-\alpha} - x_{1D}^{1-\alpha}}{x_{2M}^{1-\alpha} - x_{1M}^{1-\alpha}}$$

where CF is the correction factor needed to convert number concentrations measured within a size range to the number concentration for any other size range, subscripts 2 and 1 relate to the maximum and minimum values of the size range (μm), D and M denote desired and originally measured size ranges, and α is the slope of the particle size distribution, respectively. It is well-accepted that specific toxicological profiles exist for MPs of particular sizes and shapes (i.e., aspect ratios). For example, there could be a toxic mode-of-action where membrane translocation of particles <3 μm is followed by further uptake and distribution in body tissues and subsequent inflammatory responses. In such a case, the <3 μm (or any other cut off value) bioavailable fraction of the total exposure to the full NMP continuum can easily be estimated once the particle size distribution is known. For translocation followed by inflammation, one could, for example, sample the 10 nm to 3 μm size fraction, while further only selecting those particles from the shape distribution with an aspect ratio deemed relevant for the specific toxicological response.

Probabilistic NMP exposure modelling

HRA-NMP must provide predictions about the human health impacts of NMPs at both current and future emission levels *via* inhalation and ingestion [102]. After all, NMP environmental pollution is expected to increase in the future, and inhalation and ingestion are the dominant exposure pathways. Therefore, a probabilistic (Monte Carlo) NMP exposure model including all exposure pathways [102] has to be included in the HRA-NMP in order to account for what is known and what is likely within appropriate ranges of uncertainty across timescales, to identify the factors the calculated risk is most sensitive to, and to enable prospective risk assessments across age and cultural groups based on future emission scenarios. The model would account for i) all exposure pathways relevant for humans, with NMP concentrations in diet components and inhaled air as inputs, corrected for incompleteness of the size range as explained in Section 5.2.1, ii) consumption data for different gender, age, and/or cultural groups, iii) ingestion and egestion rates, iv) absorption rates for the bioavailable fraction of the NMP continuum from the gastrointestinal tract, v) simulated NMP concentrations in organs, whole body and stool, in order to allow validation and evaluation against measured data. All simulations would be done probabilistically in order to cover uncertainty in data input as well as the diversity of the NMP

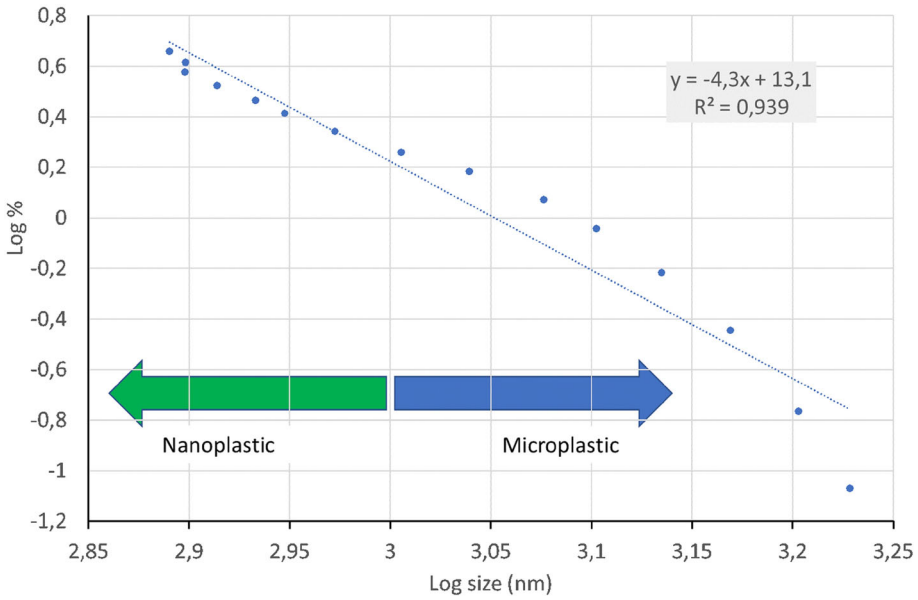

Fig. 2 Estimating <1 μm NP concentrations by rescaling >1 μm MP concentration data to the nanoscale, using empirical particle size distributions spanning both nano- and microplastic size ranges. Error propagation (not shown) is accounted for *via* Monte Carlo simulations. For this proof of principle graph, data were taken from Lambert and Wagner [224]

captured *via* PDFs for NMP size, shape and particle density [102].

With the NMP uptake fluxes modelled, quantifying the bioaccumulation of plastic-associated chemicals (also referred to as the NMP 'vector effect' or Trojan horse effect) is relatively straightforward [102, 225].

PBPK biodistribution modelling

A fundamental step in the human health risk assessment pathway is the estimation of exposure on the level of tissues and organs, for which the threshold effect doses are assessed within *hazard characterization*. For chemicals, this is usually carried out through PBPK biodistribution models, which are commonly developed and validated using *in vivo* data. PBPK models for engineered nanoparticles exist, but still need to be updated, implemented and validated for NMPs.

Beside simulating NMP distribution in the body, PBPK modelling can be used to predict uptake and biodistribution of associated chemicals. For instance, dietary chemical exposure models are available, which can be used to assess body, organ and tissue concentrations for humans consuming contaminated food. Subsequently, this can be combined with models that simulate chemical transfer from NMPs to gut fluids and gut wall adipose tissue [226] in order to quantify the percentage change of exposure due to the ingestion of NMPs. Chemical uptake then is modelled dynamically as a function of exchange rate constants, concentration gradients and gut retention time [102]. Like for analytical and effect studies [95], strict QA/QC criteria would apply to such 'vector effect' modelling studies, criteria which have been published recently [222].

Risk modelling

Risk characterization models are analytical instruments that allow for a structured effort towards identifying and characterising potential (present and future) events that may negatively impact individuals and determining the tolerability of such risk while considering other possible influencing factors.

Health risk characterization is meant to estimate the probability an adverse health outcome to occur as a result of exposure to NMPs *via* inhalation and ingestion. The *health loss* has to be quantified by means of convenient 'utility' functions, which assign a quantitative measure of loss to each possible outcome [98],.

The HRA-NMP risk characterization model is strictly informed by the outcomes of the *hazard identification*, *hazard characterization* and *exposure assessment*, and therefore it combines, i.e. jointly analyses, the following components of risk: i) data on the physical, chemical, and microbiological profiles, including possible changes occurring before human exposure (i.e. degradation in different real-world environments and during cooking and/or processing of food; e.g. size/shape modifications, persistent additives and/or adsorbed environmental contaminants, formation of biofilms possibly containing pathogens, etc.); ii) probability of exposure, i.e. how likely is it for a person and for specific vulnerable groups

of the population (e.g. children, pregnant women, people with comorbidities, etc.) to be exposed to NMPs *via* inhalation and ingestion?; iii) magnitude of exposure, i.e. when exposure does occur, to which extent are these persons exposed to NMPs *via* inhalation and ingestion?; iv) probability of adverse health outcomes, i.e. how likely are all possible adverse health outcomes that may occur upon exposure to NMPs *via* inhalation and ingestion?; v) severity of health outcomes, i.e. what are the potential health losses associated with any of the possible exposures to NMPs *via* inhalation and ingestion? This is where risk characterisation will be completed; vi) degree of control, i.e. to what extent can any outcome be prevented from happening or can the severity of the consequences be mitigated? Are there any alternatives to choose from? What are the options?; vii) sources of uncertainty of the modelling approach, dealt with through probabilistic modelling; viii) decision, i.e. how can knowledge of the risk from exposures to NMPs inform risk management for intervention and/or prevention (i.e., risk mitigation)?

As further explained in Section 6, gaps in knowledge needed as input for the HRA-NMP models are gathered by engaging key stakeholders and experts to assess the assumptions and quality of data. In fact, some necessary data can be hardly accessible and often not freely available, therefore stakeholder participation (industry, companies, experts) is considered an essential way to obtain such information or support verification of assumptions for construction of models.

Practical recommendations

Hazard modelling

The hazard modelling has to estimate i) bioleaching scenarios over a longer timeframe accounting for degradation processes occurring along the inhalation and ingestion routes, ii) the relative importance of the differential fluxes of chemicals (additives and environmental pollutants) and microbiological elements (pathogens and MGMs) *via* NMPs and *via* other carriers. Several existing probabilistic models, developed within earlier research projects and now available in the literature [85, 191, 206, 207], can be used as the starting point for this scope.

Exposure modelling

Rescaling model: the re-scaling and NMP data-alignment methods developed by Koelmans et al. [22], can be considered a valuable base to rescale exposure dataset in the HRA-NMP. It is advisable to further statistically explore them, in particular at the nanoscale. The uncertainty in the extrapolations is quantified using probabilistic modelling, which in turn form input for overall NMP exposure modelling. *Probabilistic NMP exposure model:* a generic probabilistic (Monte Carlo) NMP exposure model assessing total exposure of NMPs to humans *via* inhalation and ingestion can be constructed, with the rescaled NMP exposure data in relevant matrices, and the exposure frequencies and durations for these matrices as inputs [102]. To define frequency and duration of food exposure, food consumption databases (e.g., EFSA, WHO) can be used at the appropriate level of detail following the categorization by system [227] and according to available concentration data. By providing data regarding the consumption of different food items at different levels of categorization (e.g., per country, age group, gender), they allow us to investigate a wide suite of scenarios of consumption habits, including excess intake, and cover different categories with a higher risk exposure. As for the inhalation route, it is recommendable to give particular attention to exposure within indoor microenvironments where people spend most of their time and which are particularly rich in NMP sources (e.g., home textiles) [228]. The frequency and duration of exposure to airborne NMPs can be derived from the time spent at those microenvironments as defined in suitable time activity databases (e.g., UN Time Use Statistics database, the Multinational Time Use Study database). Exposure in the future can be based on projections of plastic production and fragmentation in the environment. Outputs, in the form of probability distributions of annual exposure for different global dietary patterns and age groups under both present and future exposure scenarios, can be obtained to inform *risk characterisation*. *PBPK model:* PBPK biodistribution models developed for MPs and nanoparticles [184, 229] can be used as starting point and purposely modified to account for NMP specific proprieties and behaviour. We recommend such models can be developed already, and further validated and refined as soon as empirical data, e.g., *post mortem* data become available.

Risk modelling

Variability and uncertainty have to be explicitly included in the assumptions of the models and in the input data. Monte Carlo simulation methods can be used to quantify propagation of variability and uncertainty in the models, and depending on the available data/information and conceptual model developed, the analysis of uncertainty and variability can be addressed using Bayesian hierarchical models, considering the whole risk chain (network), from sources of the NMPs to human exposure and health effects.

As previously mentioned (Section 4.2), the conclusions of the *risk characterisation* need to be stratified taking into account the individual variation in connection with biological, environmental, societal, and lifestyle factors that influence the magnitude and the frequency of the

exposure to environmental pollutants, including NMPs. This would support the following risk mitigation assessment and increase the chances for the suggested measures to be effective and to better meet the needs of the most vulnerable groups.

Paradigm 4: Stakeholder engagement

The growing body of evidence regarding the ubiquitous presence of MPs in the environment and consequent human exposure [218, 230] has cemented public anxiety about the potential impact on human health, thereby boosting political resolve to deal with plastic pollution at large. According to the latest insights from behavioural and social sciences, there is an increasing feeling of co-responsibility in the public regarding NMPs and, more generally, plastic litter-related issues [6]. Many citizen groups and stakeholders are actively engaged in campaigns and projects promoting long-lasting behavioural changes regarding plastics production, consumption and waste handling. While there is no doubt that this strong emotional involvement of the public is a good premise for the successful implementation of risk mitigation strategies [6, 36], it is also true that this can lead to overreaction and possibly bias the political prioritization of resources to control contamination and protect environment and human health. Therefore, the HRA-NMP should have a focus on keeping risk assessors, stakeholders, decision-makers and society informed about the best available/achieved information along the whole process, assimilating their needs and views, and engaging them in the scientific process, with a multiactor approach, leading to qualitative and quantitative estimates of the human health risks associated with NMP exposure. Plastics have thousands of uses in our economy and every person contributes to NMP production and dispersion in the environment. Therefore, every actor in the HRA-NMP process must be aware that regulatory actions tackling plastic pollution likely imply a complex rethink of the way our society and economic system uses synthetic polymers [231], and thus they can be warranted only by a high threshold of risk evidence.

The involvement of stakeholders in the proposed HRA-NMP process addresses two specific purposes. Firstly, it is used to deal with uncertainties connected to knowledge gaps. In fact, stakeholder knowledge and public perception are crucial for the early phase of model development that is based on knowledge and evidence, and they can make a well-founded contribution to allow risk assessors to identify possible alternative risk scenarios. Secondly, it is expected to facilitate the prompt use of the HRA-NMP's scientific outcomes in mitigation strategies development. The HRA-NMP is designed to specifically deliver "Science-for-policy" and thus, the involvement of representatives of regulatory stakeholders can maximise its impact on risk mitigation.

Practical recommendations

Social science methodologies

Methodologies used in social and behavioural sciences can be applied to effectively engage stakeholders toward the achievement of specific goals within the HRA-NMP:

i) *Scientific consensus methodology (i.e., co-creation workshops) to deal with uncertainty.* Expert engagement processes can be put in place to foster the co-creation of mitigation strategies with a multiactor approach. Engagement of target groups can be effectively obtained through the organisation of specific events.

ii) *Formative research methodology to catch the societal perception of the risk associated with NMP pollution and identify possible constraints to the definition of mitigation actions.* Formative research can be used to assess major target audience's perception of NMP risks and possible constraints from key actors in the plastic value chain. The obtained insights can inform risk mitigation strategies and recommendations for the risk analysts to appropriately perform risk communication.

iii) *Participative target setting to include a broad spectrum of options and maximize impact on policies and actions on all the issues related to plastic pollution (e.g., the European Strategy for Plastics in a Circular Economy and of the Bioeconomy Strategy).*

Target stakeholders

It is recommended that stakeholders to be engaged in the HRA-NMP are: i) regulatory bodies and associated consulting authorities and advisory groups (e.g. European Commission, EFSA, European Chemicals Agency, JRC, SAPEA in EU), ii) health bodies, food organisations and environmental authorities at national and international levels (e.g. WHO, World Organisation for Animal Health, FAO, OECD), iii) the scientific community, iv) representatives of the industry and of other key actors in the plastics value chain at national and international levels, v) civil society, operators from different value chains (e.g., food chain), the associations and organisations of the aforementioned parties, and vi) the media. The involvement of the media is due to their significant contribution in the generation of the current disparity between the scientific findings and magnitude of the public discussion, resulting in the elevated risk perception of society and policy makers [232, 233]. Key representatives of these stakeholder groups can be selected and gathered in different multi-actor panels tailored to the scopes mentioned above. It is advisable that stakeholders equally cover different geographical areas in order to take into consideration the widest societal context.

Conclusion

The NMP research community has gained experience and actively developed tools to overcome some of the identified challenges in NMP exposure and hazard assessments and perform an assessment of the risks of NMPs for human health. Here, we have provided and detailed a conceptual framework, the HRA-NMP, that demonstrates that all the necessary building blocks of the framework are available, while it is evident that actually parameterising and integrating them still requires considerable effort. Hence, given the wide variety of disciplines and stakeholders involved, it is fundamental to maximise synergies within interdisciplinary-intersectoral communities at all stages of the risk assessment pathway. In fact, to face the expected research challenges, it is fundamental to combine the expertise and skills of several disciplines (i.e. materials and nanomaterials sciences, nanoengineering, environmental and analytical chemistry, microbiology, toxicology, risk assessment, computer-based modelling, regulatory processes, behavioural sciences, media and communication) and approaches used within different sectors (i.e. public bodies in charge for food safety, human health, and environmental protection; research institutes). Furthermore, collaboration with international institutions (e.g., JRC, OECD) and networking within the research community is essential for maximizing the value and impact of the experience and knowledge gained during past and ongoing national/international projects.

Abbreviations

ADME: Absorption, distribution, metabolism, excretion; ATR-FTIR: Attenuated total reflectance - Fourier transform infrared; DMA: Differential mobility analysis; EFSA: European Food Safety Authority; FAO: Food and Agriculture Organization; FPA: Focal plane array; FTIR: Fourier transform infrared; IR: Infrared; GC-MS: Gas chromatography - mass spectrometry; GIT: Gastrointestinal tract; HRA-NMP: Holistic human health risk assessment framework for NMPs; JRC: Joint Research Centre; LPS: Lipopolysaccharide; MGMs: Mobile genetic materials; MPs: Microplastics; NMPs: Nano- and microplastics; NPs: Nanoplastics; OECD: Organization for Economic Co-operation and Development; PAHs: Polycyclic aromatic hydrocarbons; PBPK: Physiologically Based PharmacoKinetic; PCBs: Polychlorinated biphenyls; PE: Polyethylene; PDFs: Probability density functions; PET: Polyethylene terephthalate; PP: Polypropylene; ROS: Reactive oxygen species; PS: Polystyrene; PVC: Polyvinyl chloride; Py-GC-MS: Pyrolysis gas chromatography - mass spectrometry; QA/QC: Quality Assurance/Quality Control; SAPEA: Science Advice for Policy by European Academies; SBFs: Simulated biological fluids; SEM: Scanning electron microscopy; SERS: Surface-enhanced Raman Spectroscopy; TEM: Transmission electron microscopy; WHO: World Health Organization

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43591-021-00011-1.

Additional file 1. Suplementary table 1: content of chemical risk assessment steps according to WHO (1. WHO. Harmonization Project Document No. 8. WHO Human Health Risk Assessment TOOLKIT: chemical hazards. IPCS Harmon Process. 2010;1–644. Available from: https://apps.who.int/iris/handle/10665/44458).

Acknowledgements

Not applicable

Authors' contributions

Introduction: SN, CL, AK, AB; Paradigm 1: AS, KC, FC, AB; Paradigm 2: CL, SN, AB, AK, MB, HJ, TF, LT, AS, PC; SB, FC, MO, FM, MB, VP; Paradigm 3: LMG, AK; Paradigm 4: MS, GM, BT, FR. All the authors commented on previous versions of the manuscript. All the authors contributed to review and editing according to reviewer's comments. All the authors read and approved the final manuscript.

Funding

CL acknowledges funding from the Italian Ministry of Health, RC IZSVe 02/2020. AK acknowledges funding from the Dutch Research Council, NWO-TT project 13940'.

Availability of data and materials

Not applicable

Declarations

Competing interests

The authors declare the following financial/personal relationships which may be considered as potential competing interests.

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Received: 1 February 2021 Accepted: 7 June 2021
Published online: 29 June 2021

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