MAPPING INTERNAL EXPOSURE AND BIOKINETICS OF MICRO-NANOPLASTICS IN HUMANS: A SCOPING REVIEW OF KNOWLEDGE AND GAPS

General research profile – Internship report

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Abstract

Humans are inevitably exposed to micro-nanoplastics (MNPs) via inhalation and ingestion. While there is an increasing amount of evidence underlying the risk of MNPs on human health, a robust risk assessment of MNPs is currently not available. Biokinetic knowledge of MNPs is limited in availability and needs a comprehensive summary of what is known and unknown. Therefore, this scoping review provides an overview of the reported state of knowledge and gaps in biokinetics – absorption, distribution, metabolism, and excretion (ADME) of MNPs in humans. The literature review was combined with expert interviews to gain deeper insights into the latest research findings and kinetic hypotheses. The combined data was disproportionate between four aspects of ADME. The absorption of MNPs was found dependent on plastic particle size and dosage. Endocytosis and phagocytosis by macrophages were introduced as probable absorption mechanisms. After respiratory or intestinal absorption, MNPs can be distributed to various organs and tissues in the body via the blood circulatory system. The literature and experts considered the metabolism of MNPs in humans unlikely, but there is a limited amount of data to conclude. *In vitro* assessments reported a potential metabolism of plastic particles. Currently, biokinetic data still lack quantitative absorption and excretion rate, and plastic polymers other than polystyrene are left unexplored. Most importantly, realistic biokinetic data are scarce. Therefore, future MNPs research work should consider conducting experiments to assess a realistic uptake and excretion rate. Researchers should also involve various polymers such as polypropylene, and work to discover probable distribution mechanisms of MNPs.

Layman's summary

Plastic waste in the environment can break down into smaller particles in micro and nano sizes, known as micro-nanoplastics (MNPs). Humans can contact them by ingesting products contaminated with MNPs, inhaling dust, and skin contact with products containing MNPs. Currently, there is a lack of understanding of the health risks of MNPs. Specifically, there is little research on how MNPs behave in the human body, a process called biokinetics. There are four components of biokinetics: absorption, distribution, metabolism, and excretion. To gain a better understanding of the risk of MNPs, their biokinetics data is required.

By conducting a literature review and expert interviews, this scoping review worked to identify what is known and unknown about MNPs in humans in terms of biokinetics. This study is valuable for identifying evidence available to explain MNPs' biokinetics and related knowledge gaps. Also, it helps to navigate future research in need of prioritization. The literature review included 21 publications, including original (*in vivo, in vitro, and ex vivo)* experiments, reviews, and reports. 5 experts participated in interviews, which included physiologically based kinetic (PBK) modelers, inhalation experts, and a blood-brain barrier expert.

Absorption of MNPs is the process where plastic particles are taken up from the site of administration and internalized in the human body. For inhaled MNPs, small particles sized between 0.01 - 1μm (aerodynamics diameter) can reach the alveolar region. There, MNPs are likely to be taken up through epithelial layers (protective lining airways) through alveolar gas exchange where oxygen comes in and carbon dioxide leaves the blood. Alternatively, inhaled MNPs can be taken up by alveolar macrophages which engulf them. Ingested particles smaller than 500 nm are likely endocytosed, where a bubble-like outer layer wraps MNPs and moves them into the cell. Particles larger than 500 nm are likely to be absorbed by intestinal macrophages. Particle size is influential for absorption, and absorption was also found dose-dependent for multiple *in vitro* assessments.

MNPs can be distributed to the brain, respiratory organs, digestive organs, and reproductive organs (including the blood circulatory system). Contradicting results were reported from the literature about the detection site. The discrepancies are attributed to the lack of standardized testing methods and different detection methods involved. The metabolism of MNPs focuses on the potential change of MNPs in humans after absorption. There is very

limited evidence available to evaluate MNPs metabolism, which the literature and experts hypothesize is unlikely to happen. Similarly, the excretion of MNPs also suffers a lack of knowledge. The literature often doesn't distinguish the excretion of MNPs that pass the gastrointestinal tract and are excreted (no absorption) and those eliminated after uptake.

The lack of realistic and quantitative biokinetic data is the most critical knowledge gap, which needs to be prioritized in the future. Also, the actual absorption mechanism could be more complex than what was outlined. The distribution mechanism between organs, tissues, and internal barriers is unclear. Furthermore, the biokinetics of various plastic polymers requires future research because polystyrene is predominantly utilized in experiments.

(Word count: 499)

1. Introduction

Plastic, an abundantly used material in global society, holds a different reputation now than when it was introduced in the 1950s. What once was associated with innovation is now frequently paired with pollution. Irresponsible use and inappropriate waste management are accountable for plastic pollution. The global production of plastic is expected to reach 1,100 million tons by 2050. Approximately 85 percent will become unregulated waste (UN Environment Programme, 2022).

Plastic pollution is an urgent and complex environmental challenge in the 21st century. The degradation of plastic products introduces plastics in various sizes and shapes difficult to monitor (Domenech et al., 2020). Fragmentation, the process of plastic degradation is triggered by weathering and abrasion of plastic over time (Gerritse et al., 2020). Once released into the environment, plastic can degrade into micro- and nano-sized particles until it becomes no longer visible to the naked eye (Plastic Soup Foundation, 2022). A plastic particle less than 5 mm in size is commonly denoted as microplastics (Thompson, 2004). The size of nanoplastics was debated whether it should be 1 – 1000 nm suggested by Guigault et al., (2018), or less than 100 nm following the recommended definition of nanomaterials by the European Commission for Nanomaterials (European Union, 2011). Given the inconsistent terminology, the World Health Organization reports nanoplastics as ≤ 1μm, and micro-nanoplastics (MNPs) size ranging from 1 nm to 5000 μm. This review adheres to the suggestion of WHO (2022).

As an environmental pollutant, MNPs are distinguished between primary and secondary plastics. Primary microplastics are intentionally manufactured to be small in size, such as plastic pellets, particles present in cosmetics (European Parliament, 2018). They can directly enter the environment in their original small sizes (Gonçalves & Bebianno, 2021). Secondary microplastics are derived from the fragmentation of plastic waste via physical, chemical, or biological degradation (Andrady, 2011; Browne et al., 2007). Similarly, nanoplastics are generated by the fragmentation or deformation of microplastics from the environment (Lee et al., 2023). Plastic pollution in the environment is documented in the hydrosphere, atmosphere, lithosphere, and biosphere (Wright & Kelly, 2017).

MNPs accumulating in the natural environment can trigger ecological and human health risks (Aardema et al., 2024). Inevitably, humans are exposed to MNPs. Relevant human exposure routes include ingestion (food or drinking water contaminated with microplastics), inhalation (aerosols and dust), and dermal contact (microplastics in textiles and personal care products) (Prata et al., 2020). Many studies demonstrated that MNPs can cause toxicity in human-relevant models (WHO 2022; Liu et al., 2023). However, the magnitude of harm imposed by MNPs on human health remains unclear and largely not known.

The assessment of the human health risks of MNPs is a challenging task. It requires careful analyses and high-quality (reliable and relevant) research data. Generally, the steps of risk assessment include problem formulation, exposure assessment, hazard identification & characterization, and risk characterization (including uncertainty analysis) (More et al., 2019). Thus, the risk assessment of MNPs requires data on hazards, exposures, (physio-chemical) particle characteristics, and toxicokinetics of MNPs. Unfortunately, a proper and robust human risk assessment of MNPs is currently not possible (Gouin et al., 2022). There is insufficient physical, biological, or chemical data in quantity and sufficient quality. Also, the exposure assessment of MNPs faces the lack of standardized testing methods (Barbosa et al., 2020). Commonly used analytical methods such as (conventional) Raman spectroscopy can typically detect plastic particles greater than 10 μm in size (Mariano et al., 2021). Moreover, spherical and pristine polystyrene (PS) particles are abundantly utilized in experiments whereas in reality, humans are exposed to a mixture of sizes, shapes, and types of MNPs.

Understanding the extent of the health effects of MNPs in humans requires data on internal exposure and biokinetics, in addition to hazard data. In the gut, particles smaller than 10 μm may be absorbed via endocytosis and phagocytosis in the Peyer's patches of the ileum (SAPEA, 2019). Also, persorption, the passive absorption of particles measuring up to 150 μm from the intestinal lumen through gaps in the mucosa was reported (WHO, 2022). It appears that MNPs can translocate through the body via blood and its circulatory system (Leslie et al., 2022). Whether MNPs are ingested or inhaled, their biokinetics are expected to be influenced by their size, shape, density, and surface chemistry (WHO, 2022). According to the report from WHO (2022), there is sufficient data to conclude that MNPs > 150 μm are unlikely to be absorbed and that absorption increases with the decreasing particle size via oral ingestion.

Biokinetics data (i.e. data on the absorption, distribution, metabolism, and excretion of MNPs in humans) of MNPs requires a comprehensive summary. As WHO (2022) identified, there is insufficient information to address biodistribution (uptake, retention, clearance, translocation, and its rate) among organs, internal barriers, and blood. Currently, limited biokinetic information is scattered among various sources making it challenging to grasp the overview of what is known and unknown. Therefore, the research question asks: "*What is the current state of knowledge and gaps on internal exposure and kinetics of micro-nanoplastics (MNPs) in humans?"* To answer this question, a scoping review was performed in which a literature review was combined with expert interviews.

2. Methodology

2.1 Scoping Review

Prior to expert interviews, a scoping review was performed to examine the currently available knowledge and the latest findings on the biokinetics of MNP exposure in humans. A scoping review was specifically chosen over a systematic review considering the aim and the nature of the research. As stated in the introduction, this review's core elements are identifying and analyzing knowledge and its gaps, which is a distinct feature of a scoping review (Munn et al., 2018). Also, a distinctive feature of a scoping review is that an assessment of methodological limitations/risk of bias in the evidence is generally not performed (Munn et al., 2018). Simply, this paper aims to produce an overview of the evidence (biokinetics of MNPs in humans) rather than to assess the quality of available literature.

The scoping review adheres to the framework of the scoping review by Arksey & O'Malley (2005). Five stages of a scoping review are defined as follows: (1) Identifying the research question (2) identifying relevant studies (3) study selection (4) charting the data (5) collating, summarizing, and reporting the results. As an additional element to the data collection, expert interviews were conducted. This is an optional but recommended step in the scoping review (Peters et al., 2015).

Eligibility criteria

Eligible studies address the knowledge of biokinetics of MNPs in humans and/ or the knowledge gap of the topic. Literature assessing any of the four aspects of ADME of MNPs was considered, regarding human health. Studies only covering the toxicological effects of MNPs exposures were excluded because they do not focus on how the particles behave within humans. For animal experiments with MNPs, only studies involving rodent models were included. Rodent models are an acknowledged choice in biomedical research due to their physiological homology to humans (Domínguez-Oliva et al., 2023). Publications that used zebrafish or any other animal models were excluded. Also, studies that cover the external exposure of MNPs or environmental pollution due to plastics were excluded for their irrelevance. Original peer-reviewed articles such as reviews and experimental papers, in English (accessible online) and published after 2004 were included. The cut-off date of 2004 was because most MNP research was performed after 2004.

Search strategy

Relevant studies were identified between November and December 2023 using PubMed, Scopus, and EMBASE databases. Keywords that were used include 'microplastics', 'nanoplastics', 'human', 'health', 'rodent', 'exposure', 'internal', 'kinetics', 'uptake', and 'adme', and they were used in various combinations. Additionally, two reports were included upon recommendation by experts. The final search was conducted on the 6th of December, 2023. This search yielded 63 results in PubMed, 67 in Scopus, and 161 in EMBASE. Search syntaxes used in three databases are organized in the supplementary document (see **Figure S.1, S.2, S.3**).

Study selection

The study selection process included three stages: title screening, abstract screening, and full-text assessment. **Figure 1** illustrates the flowchart diagram of study selection. The list of selected literature is available in the supplementary document (see **Figure S.4**). After removing duplicates from three databases, 277 papers were screened for their title and abstract. There were 48 papers left for full-text assessment, and of them 27 papers were further excluded. As a result, 21 papers were finally selected for the scoping review.

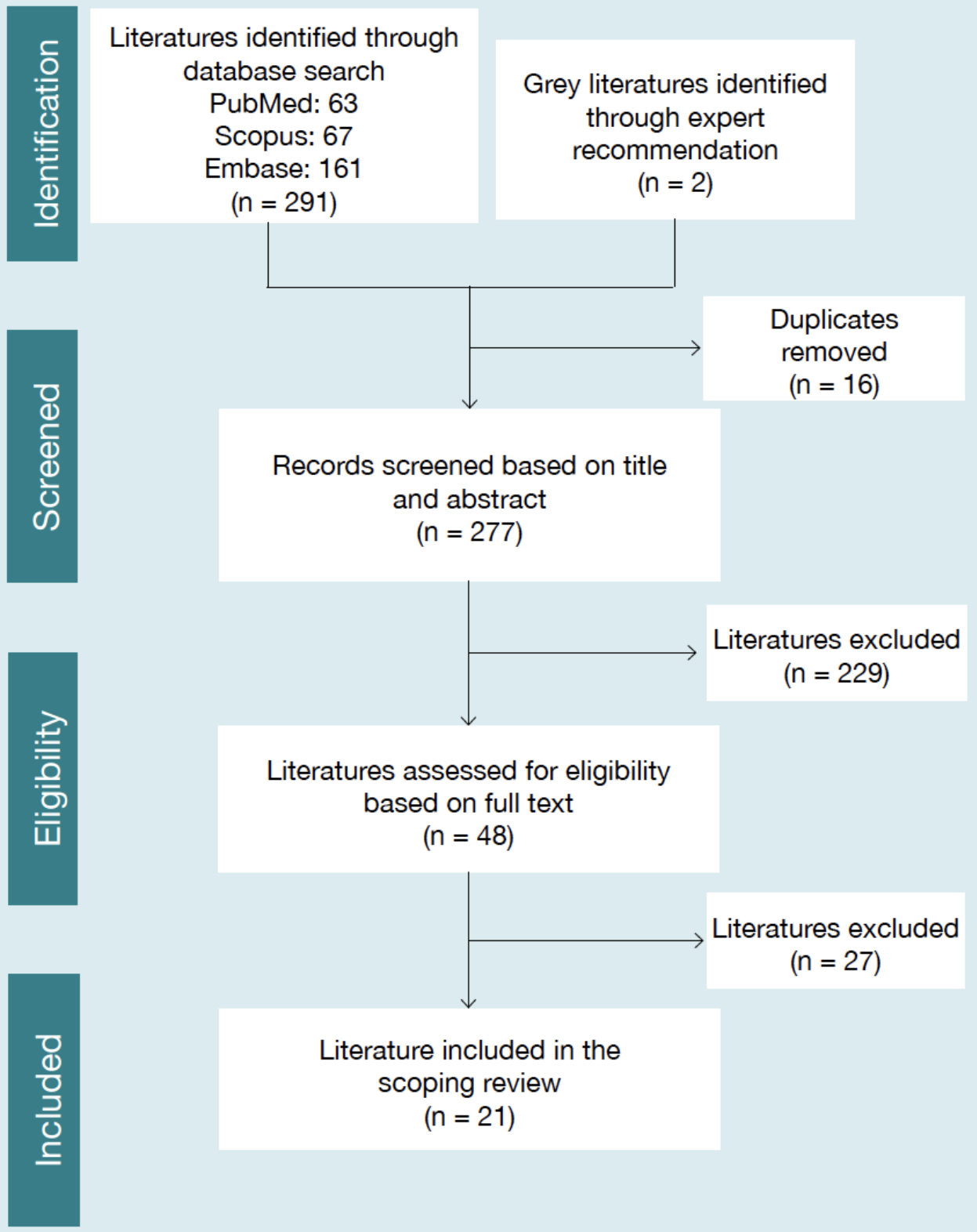

Figure 1. Study selection flowchart

Data charting – extracting data and syntheses

Selected literature were charted for their study characteristics in Microsoft (MS) Excel. Included categories were: **literature information** (*DOI, author, year, study location, title, article type, purpose*), **study design & materials** (*study model, polymer, shape, size, relevant exposure route, exposure method, test concentration/dose, exposure duration, organ, test species/ test population, detection method*), ***internal exposure*** (*checkmarks for applicable categories: internal barrier, translocation routes, uptake, accumulation, absorption, distribution, metabolism, excretion*), and **evaluations** (*major findings, knowledge gap & future direction, limitations, contradictions, key papers mentioned*). Subsequently, conventional qualitative content analysis involves a process of condensing raw data into categories (Elo & Kyngäs, 2008). This review was conducted with an inductive approach, adequate to investigate emerging and latest research findings (Elo & Kyngäs, 2008). The literature data was categorized for state of knowledge (biokinetics - ADME), and knowledge gaps.

2.2 Expert Interviews

Five expert interviews were conducted to seek expert views on the topic and facilitate iterative improvement to the scoping review. Experts are defined as professionals who have expertise in the field of MNPs research through formal training and research. For this study, experts affiliated with Dutch research institutes/universities or (governmental) public health organizations were approached. Participants were selected based on recommendations to ensure that a wide range of expertise of ADME was covered. Such selection approach for experts is also known as purposive sampling, a term in qualitative research to identify and select relevant personnel (Palinkas et al., 2015). **Table 1** summarizes the experts included in the interviews.

The interviews were semi-structured. One interview was conducted face-to-face and four by MS Teams video meeting. All participants were recruited via email invitations. Experts signed an informed consent form and read the briefing document (introducing the research and aim) before the interview. Experts agreed that the interviews would be recorded and transcribed. Informed consent and semi-structured question lists are available as supplementary documents. All personal information mentioned during the interview (name and affiliation) was handled confidentially. The in-person interview was manually transcribed using MAXQDA 2022 (VERBI Software, 2021). Interviews held in MS Teams were recorded and transcribed using the built-in

'live transcription' function in the application, then thoroughly checked and edited for correctness.

Experts	Expertise	Organization type
Expert 1	PBK modeling	Research organization
Expert 2	Human inhalation exposure & translocation of MNPs in respiratory organs	Research organization
Expert 3	Blood-brain barrier, translocation of MNPs	University
Expert 4	Inhalation exposure & detection of MNPs in blood	University, research organization
Expert 5	Operational PBK modeling & sensitivity analysis	University

Table 1. Summary of experts included in expert interviews

After transcribing all interviews, generated transcripts were analyzed using thematic analysis for qualitative data. According to Braun & Clarke, (2006), thematic analysis is a method for identifying, analyzing, and reporting patterns (themes) within data. It is particularly useful for summarizing key features of a large body of data, highlighting their similarities and differences, and generating unanticipated insights (Braun & Clarke, 2006).

Transcribed interviews were thematically coded with an inductive analytic approach. A coding tree was created as an example of a hierarchical coding frame. This is a beneficial structural tool to help categorize and efficiently interpret large amounts of expert interview data. Interview data are categorized into three levels: *theme, category, and code* where a code represents the lowest level of data, and a theme represents the highest level concept that is relevant to answering the research question. A code is a label assigned to segments of interviews that capture a specific concept or an idea shared by experts, and a theme is a collection of overarching ideas and concepts that emerged (Braun & Clarke, 2006). Between codes and themes, categories represent the grouping structure of codes that belong to a theme.

3. Results

3.1 Literature review

Description of included articles

As shown in the current scoping review, most of the studies conducted on MNPs were recently published. **Table 2** illustrates the descriptive statistics of articles included in the present scoping review, for the year of publication, location of the study, relevant exposure routes covered by studies, and their type of research. 47% of articles were published in 2023, and the majority of them (47%) took place in China. More than half of the articles (63%) covered ingestion as a relevant exposure route to MNPs, which they mainly (63%) explored in experimental studies. Notably, more articles covered both ingestion and inhalation (31%) in their work than focusing merely on inhalation (15%). Also, this scoping review is conducted on the combination of experimental studies (63%), review studies (36%), and narrative reports (10%).

Description	Number (%)
Year of publication	
2023	9 (47)
2022	8 (42)
2021	1 (5)
2020	1 (5)
2019	2 (10)
Location of study	
China	9 (42)
South Korea	2 (9)
Other countries (Germany, Italy, Spain, Portugal, US, Serbia, Thailand, Taiwan)	8 (38)
N/A	2 (9)
Relevant exposure routes	
Ingestion	12 (63)
Inhalation	3 (15)
Both (Ingestion & Inhalation)	6 (31)
Type of research	
Experimental studies	12 (63)
Review studies	7 (36)
Narrative report	2 (10)

Table 2. Descriptive statistics of studies included in the scoping review

Detailed information on twelve experimental studies is organized in **Table 3** and **Table 4** for *in vivo* & *ex vivo* and *in vitro* studies, respectively. To note, all *in vivo* studies were conducted with rodents. For *in vivo* studies, most plastic particle diameters ranged between > 100 nm to 1 μm. The exposure duration typically lasted for three to five weeks, and fluorescent microscopy was the most common detection method. For *in vitro* studies, plastic particle diameter ranged between ≤ 100 nm to 1 μm. The exposure duration typically lasted for two days, and confocal microscopy was the most commonly employed detection method.

Biokinetics – Absorption (ADME)

Absorption of MNPs is the process where plastic particles are taken up from the site of administration and internalized in the human body. For MNPs, relevant exposure routes include ingestion and inhalation. The uptake of MNPs through dermal contact is considered of limited importance (Prata, 2023), therefore it is not of focus in this scoping review. Absorption after ingestion and inhalation are reported separately.

Table 3. Summary descriptive table of *in vivo* (rodent) and *ex vivo* studies

Polymer type	Particle diameter	Exposure	Exposure dose	Exposure duration	Detection method	Main findings	Reference
Carboxylate modified, fluorescent PS	40 nm, 200 nm	Daily, oral ingestion	Low dose: 0.01 mg/day High dose: 0.1 mg/day	5 weeks	confocal imaging / microscopy	Orally administered PS particles pass through rodent digestive system	Nikolic et al., (2022)
Fluorescent PS	2 μm	Twice a week, oral gavage	Doses based on rodent body weight: 0.008 mg/g or 0.016 mg/g	4 weeks (low dose)and 8 weeks (high dose) respectively	Raman spectroscopy, fluorescence microscopy	Fluorescent PS particles detected in liver and brain	Lee et al., (2022)
Fluorescent PS	0.5 μm, 4 μm, 10 μm	Daily, oral ingestion	1 mg/day * The authors did not disclose why they included such a high exposure dose	28 days (4 weeks)	biofluorescence imaging (BFI)	4 μm, 10 μm PS particles could accumulate in the testis of mice	Jin et al., (2021)
Fluorescent PS	5 μm,50 μm, 100 μm, 200 μm	Daily, oral ingestion	Doses based on rodent body weight: 0.08 mg/g (including 0.02 mg/g of 5 μm,50 μm, 100 μm, 200 μm respectively)	10 weeks	Fluorescence microscopy	5 μm PS particle could accumulate in the blood vessels, liver, and kidney of rodents	Huang et al., (2022)

PS particles	Small class: 40 μm – 60 μm, larger class: 40μm – 100 μm	Daily, oral ingestion	Doses based on rodent body weight. Low dose: 0.05 mg/g High dose: 0.5 mg/g Four exposure groups: low dose + small class, low dose + larger class, high dose + small class, high dose + larger class	21 weeks	Raman spectroscopy	No notable accumulation occurred in rodent gut or liver	Deng et al., (2022)
***Ex vivo* studies**							
PP, PET, PS, and PVC particles detected, 108 MNPs particles in total	89% of detected MNPs were 20 – 100 μm in diameter	**Human lung tissue samples** (parenchyma)	N/A, patients tissue samples were collected to detect and study the MNPs present in lung tissues. Ethanol 5.0 g was used as control group for contamination assessment and consistency check		Laser direct infrared imaging system, SEM	Quantified MNPs present in human lung tissue samples	Wang et al., (2023)
Fluorescent PS	300 nm	*Ex vivo* gut sacs (rodent)	12 mg kg^{-1}or 500 mg kg^{-1}	4 weeks	TEM	MNPs found in liver, kidney, spleen, intestine of mice	Meng et al., (2023)

Table 4. Summary descriptive table of *in vitro*

Polymer type	Particle diameter	Test model	Test concentration	Exposure duration	Detection method	Main findings	Reference
Fluorescent PS	50nm	Intestinal organoid (HIPSCs, derived from urinary renal epithelial cells)	10 and 100 μg/mL (dose:100 μg, 1000μg)	1 and 2 days	High-resolution 3D imaging	PS accumulation found in intestinal organoids	Hou et al., (2022)
Fluorescent PS	0.1 μm and 1 μm	Human alveolar cell line A549	12.5 to 200 μg mL^{-1} (dose: 1.25μg/well)	0.5, 1, 3, 24h	Fluorometric reading, confocal laser scanning microscopy	Uptake was dose-dependent	Lagana et al., (2023)
Fluorescent, Amine-modified PS	100 nm	Human red blood cells	100 μg/mL, 500 μg/mL	3h	Confocal microscopy, flow cytometry	PS were attached to RBCs, dose-dependent	Kim et al., (2022)
Fluorescent PS (carboxylic surface groups)	50 nm, 0.5 μm	Intestinal and placental co-cultures (caco-2 cell line, goblet cells, BeWo b30)	10 μg/mL, 100 μg/mL	24h	Confocal microscopy,	Cellular uptake and intracellular accumulation observed	Hesler et al., (2019)
Fluorescent, pristine PS	Fluorescent: 0.04 μm – 0.09 μm, Pristine: 0.05 μm – 0.1 μm	Caco-2, HT29 cell line, Raji-B	0, 1, 25, 50, 100 μg/mL	24h	TEM	50 nm particles detected, internalization followed dose-dependent pattern	Domenech et al., (2020)

Absorption – Inhalation

Not all inhaled plastics are absorbed. If MNPs in the respiratory tract are cleared by mucociliary clearance, there is no internal absorption. The review of Vattanasit et al., (2023) mentions that MNPs less than 10 μm in size are probably removed by mucociliary clearance referencing Yang et al., (2022). Prata (2018) assumes that most of the fibers could be cleared from the respiratory tract. On the contrary, Wright and Kelly (2017) reported that fibers of 15 – 20 μm (length-to-diameter) cannot be removed from macrophages in the lungs. The authors of the review state that fiber plastic particles are not easily phagocytosed. They support their view using the findings that long and thin fibers were found incompletely phagocytosed and more persistent (Donaldson et al., 1993), and the observation that where nanofibers were incompletely engulfed by macrophages (Allegri et al., (2016)).

On the other hand, when MNPs reach the alveolar region and are taken up through (alveolar) epithelial layers into the circulatory system, then it is considered a valid absorption. In their review, Wu et al., (2022) reported that MNPs can be taken up by the epithelial layers through the alveolar gas exchange into blood vessels. Fibers were the most prominent shape of inhaled particles compared to others by 49% (Rist et al., 2018). WHO (2022) underlined the role of alveolar macrophages involved in the absorption process for phagocytosis. For inhaled plastics, the aerodynamic diameter is an appropriate indicator of the size. Available evidence suggests that MNPs larger than 10 μm are not likely to pass further than the nose, and particles larger than 2 μm are likely to stay in the upper respiratory tract (WHO, 2022). Smaller particles sized between 0.01 - 1 μm are likely to reach the lower respiratory area such as the pulmonary, alveoli, and gas exchange area (WHO, 2022). Additionally, Wu et al., (2022) suggest that plastics larger than 0.2 μm in the bloodstream may be removed into the intestine via splenic filtration. Particles smaller than 0.1 μm were speculated to remain in the blood (Rist et al., 2018).

A dose-dependent relationship of inhaled MNPs was demonstrated *in vitro* by Lagana et al., (2023). The authors quantified the uptake of 0.1 and 1 μm PS plastics on the human alveolar epithelial cells (A549 cell line) using 1.25 - 2.5 μm/well and 5 – 20 μm/well for 24h using fluorometric reading and confocal laser scanning microscopy. Their results revealed a statistically significant dose-effect correlation for both sizes of plastics. Also, the amounts of

internalized PS decreased as the exposure time increased for all tested doses. A decrease between 20% (at the lower dose) and 30% (at a higher dose) was observed. For the nano-sized PS particles, this trend was observed only at the higher exposure doses (decline percentage greater than 30), while the uptake at lower doses increased by an average of 20% during the entire exposure. Lagana et al., (2023) calculated the total number of internalized particles range as ~200 for microplastics and 200,000 for nanoplastics per μg internalized. The uptake rate was estimated at 5.1-91.2 pg/cell. Consistent with their findings, WHO (2022) notes that the absorption rate for MNPs appears to decrease with greater particle size, becoming negligible for particles > 150 μm.

An *ex vivo* study of human lung tissue (parenchyma) by Wang et al.. (2023) provided strong evidence of deposited MNPs in the respiratory system. Non-smoking patients that were diagnosed with lung cancer were involved and lung tissue samples were taken from normal tissues that were more than 5 cm away from the lesion site. No explicit description of the control group was explained. The authors combined laser direct infrared imaging system and scanning electron microscope (SEM) to quantify MNPs in human lung tissue samples (parenchyma). Results revealed that there were 108 MNPs particles detected in 11 lung tissue samples with fiber being the most dominant shape by 18%. The majority (89%) of detected MNPs were sized 20 – 100 μm in diameter. Among them, PP, PET, and PS were the most abundant types of polymers, with 34%, 21%, and 8% respectively. The median concentration of lung tissues was 2.19 particles/g. Wang et al., (2023) noted that the concentration was calculated by dividing the quantity by the weight of the relevant lung tissue. WHO (2022) also reported MP detected in lung tissues by introducing the work of Amato-Lourenço et al. (2021). The authors confirmed the presence of MPs (size ranging from 3.92 ± 1.96 μm) from lung tissue collected during routine coroner autopsies of 20 non-smoking adults. PET and PP were the main polymers.

Absorption – Ingestion

Ingested plastic particles can travel through the digestive organ and reach the gastrointestinal tract. If they are excreted via feces, there is no internal absorption. However, MNPs in the gastrointestinal tract absorbed through the intestinal epithelium are considered a valid internal uptake. Several experimental studies confirmed the uptake of MNPs in live rodents after ingestion, where fluorescent plastics were absorbed and distributed to the digestive organs.

Nikolic et al., (2022) demonstrated that orally administered fluorescent PS (40 and 200 nm) are absorbed into the rodent's digestive system (lumen). According to the authors, clarthin-mediated endocytosis was the involved mechanism. Endocytosis as an uptake mechanism is also supported in the work of Hou et al., (2022) and Meng et al., (2023) which were experimental studies included in the selected studies. Hou et al., (2022) conducted an organoid study where they exposed 50 nm fluorescent PS to intestinal organoids (HIPSCs, derived from urinary renal epithelial cells) for 1 and 2 days. The involved test concentration was 10 and 100 μg/mL and high-resolution 3D imaging was employed as detection method. Their result confirmed PS accumulation in intestinal organoids for the higher concentration group with 26% and 41% of the total uptake taking place, respectively. No accumulation was recorded for the lower concentration group. Importantly, the authors underlined endocytic uptake as critical. Additionally, clarthin-mediated endocytosis in the rodent gut sacs was also demonstrated by Meng et al., (2023), *ex vivo*. The authors used 300 nm fluorescent PS under 12mg kg^{-1}or 500 mg $kg^{-1}$for 4 weeks. TEM was the choice of particle detection. Their result indicated that 300 nm MNPs were absorbed and distributed to the liver, kidney, spleen, and intestine of mice. Moreover, it was revealed that the jejunum had the highest absorption rate of 455.68 μg g^{-1}, followed by the duodenum and ileum. Meng et al., (2023) did not disclose the exact amount for duodenum and ileum. WHO (2022) also supports the endocytic uptake of MNPs by enterocytes and mentions persorption in the small intestine epithelium as an alternative mechanism. By citing the work of Yoo et al., (2011), the WHO report suggests 500 nm as the upper size limit for endocytosis.

Particles larger than 500 nm are likely to be absorbed via intestinal macrophages during phagocytosis (WHO, 2022). The WHO report also identified the epithelium of Peyer's patches and tips of the villus as relevant physiological sites of MNPs absorption. The uptake of MNPs larger than 1 μm is assumed to frequently take place in Peyer's patches, and particles 1 – 10 μm can be absorbed by microfold cells via transcytosis (WHO, 2022).

The absorption of MNPs in the blood and systemic circulation is an important prerequisite for distribution. Kim et al., (2022) observed the *in vitro* cellular uptake and localization of 100 nm fluorescent PS particles by human Red Blood Cells (RBC) after 3h of exposure using confocal microscopy and flow cytometry. Results indicated that there was an

increase in the PS fluorescence detected in RBCs exposed at 100 or 500 μg/mL in a dose-dependent manner. Thus, the attachment of 100 nm PS on human RBCs is evident.

Evidence on the organs detected with PS particles is contrasting for organs among rodent studies, given that particle size could influence absorption. According to the work of Huang et al., (2022), 5 μm PS particles were detected in blood vessels, liver, and kidneys of rodents, *in vivo*. Specifically, four particle sizes – 5, 50, 100, and 200 μm were tested, and their accumulation in tissues (liver, kidney, blood vessels, pancreas, heart, and brain). Doses based on the rodent body weight were used – 0.08 mg/g respectively for 10 weeks, and examined with fluorescence microscopy. Notably, only 5 μm PS particles were detected, leaving the heart, brain, and pancreas undetected with PS. Lee et al., (2022) also detected fluorescent PS (2 μm) after exposing rodents for doses based on their body weight (0.008 mg/g or 0.016 mg/g) for 4 weeks and 8 weeks, respectively. Combining Raman spectroscopy and fluorescence microscopy, PS were detected in the liver and the brain of rodents (in the hippocampus and the remaining brain tissues) post ingestion. In the rodent hippocampus, the median number of particles detected was five. Lastly, Deng et al., (2022) used two size classes of PS particles (small: 40 – 60 μm, large: 40 – 100 μm) *in vivo*, under doses based on the rodent body weight: 0.05 mg/g or 0.5 mg/g. After 21 weeks of exposure and examining rodents with Raman spectroscopy, the authors concluded that there was no significant accumulation of PS in the gut and liver samples of rodents, however, some were detected in the feces sample at the end of the exposure. Deng et al., (2022) discussed that both low exposure doses and the relatively larger particle size (40 – 100 μm) could have been preventative factors for particle accumulation.

The size of plastic particles is a considerable determinant of MNPs absorption and distribution in humans. Evidence regarding MNPs size and absorption is well presented by WHO (2022). MNPs larger than 150 μm are unlikely to be absorbed. Particles that are smaller than 500 nm are likely to be absorbed with endocytosis, and particles between 500 nm and 150 μm can be taken up by intestinal macrophages. Additionally, the review by Wu et al., (2022) reports that MNPs smaller than 1.09 μm penetrate across the gut epithelium and enter the blood circulatory system, while plastics greater in size travel to the mid and hindgut.

Biokinetics – Distribution (ADME)

Absorbed MNPs can be distributed to various organs, tissues, and internal barriers by translocating through the circulatory system (blood). The site of MNPs detection provides suggestive evidence of distribution. MNPs have been detected in the brain, lung, digestive organs (stomach, liver, intestine), kidney, spleen, and reproductive organs (testis and placenta). The distribution of MNPs across several internal barriers also have been recorded. It is important to note that the underlying mechanism of MNPs distribution and translocation is not always clear (Nikiolic et al., 2022).

Several *in vivo* assessments detected fluorescent PS in rodents. Huang et al., (2022) detected 5 μm PS in blood vessels, liver, and kidney of rodents. Lee et al., (2022) also detected 2 μm PS in the liver and the brain of rodents, and Nikolic et al., (2022) detected 20 and 200 nm PS in the digestive system. Details of three studies are available in **Table 3**. Additionally, Jin et al., (2021) used 0.5, 4, and 10 μm fluorescent PS particles (1 mg/day) for 4 weeks and observed rodents with biofluorescence imaging (BFI). The authors could observe fluorescence intensity appear in testes of 4 and 10 μm PS groups, with barely any intensity in the 0.5 μm group.

As evident from the *ex vivo* assessment from Meng et al., (2023), MNPs can cross the intestinal barrier. Wu et al., (2022) point out that a substantial amount of researches indicate that MNPs ranging 10 – 250 nm in size could penetrate the blood-brain barrier and retain in the brain (Gregory et al., 2020 & McCright et al., 2022). MNPs can also cross the epithelial barriers, as demonstrated by Domenech et al., (2020). The authors selected *in vitro* barrier models (Caco-2/HT29 + Raji-B model), used PS particles of different sizes (0.04 – 0.09 μm and 0.05 – 0.1 μm) and exposed them under the following concentrations: 0, 1, 25, 50, and 100 μg/mL for 24h. Only 50 nm PS was detected with TEM, and they reached the nuclei or the monolayer's cells. The translocation from the apical to the basolateral side was observed, and its internalization followed the dose-dependent pattern.

Biokinetics – Metabolism (ADME)

Studies in this scoping review attribute the metabolism of MNPs to morphological change in the plastic particle. All experimental studies included in the current paper did not report the biodegradation of plastic polymers in humans. Also, the metabolic properties of MNPs

in humans were not discussed in the WHO report (2022). Two review studies conducted individually by Lee et al., (2023) and Prata (2023) elaborated on the few suggestive evidence on MNP morphologic alterations related to human internal exposure.

As stated in their review, Lee et al., (2023) acknowledge that studies on the morphological properties of nanoparticles aren't sufficiently available. The authors refer to the study of Stock et al., (2020) to provide evidence of morphological modifications of plastic particles during artificial in *vitro* assessment. Based on this specific research, Lee et al., (2023) state that the modification of nanoplastics properties such as size, surface chemistry, or structure can be triggered by the pH and temperature of the human body, leading to variations in the cellular uptake. Also, the authors emphasized that irregular surfaces could be created and fragmentations in humans as a part of the morphological modification of plastic particles. Lastly, by referencing Stock et al. (2020), the authors suggest that the level of cellular uptake could be affected by the *in vivo* modifications of MNPs.

However, Lee et al., (2023) only partially mentioned the result of Stock et al., (2020) leaving important details unmentioned. In the original paper, only PS particles (out of five different plastic materials used) showed slight alteration in shape, size, and the development of irregular surfaces after artificial saliva treatment. Critically, Stock et al., (2020) reported no remarkable changes in plastic particles other than PS. Their conclusion states that in an artificial *in vitro* setting, digestive fluids did not decompose particles or modify their shape and size. Prata (2023), who also cited the same work of Stock et al., (2020) mentions that common plastics are thought to be resistant to human digestive fluids. In contrast to Lee et al., (2023), WHO (2022) states that metabolism is not expected to influence the physiological fate of MNPs also citing Stock et al., (2020).

Prata (2023) suggests a narrative that plastic particles may result in biodegradation as a result of physiological fluids, microbiota, and inflammatory reactions for metabolism. The authors cited the work of Horvatits et al., (2022) where microplastics found in cirrhotic livers showed surface alterations. In detail, plastic particles found in human tissues included PS, PVC, PET, and PP. All tissue samples from patients without liver diseases tested negative for MPs. Additionally, Prata (2023) mentions the work of Tamargo et al., (2022) by stating that in the presence of human colonic microbiota, PET microplastics suffered significant surface changes

and structural degradation to support their narrative. What Prata (2023) didn't mention from the original study was that PET was exposed to a (computer-controlled) dynamic gastrointestinal model for an *in vitro* colonic fermentation.

Biokinetics – Excretion (ADME)

There are limited amount of information regarding the excretion of MNPs post-absorption. Prata (2023) concluded in their review that the excretion of MPs mainly occurs through the feces after removal by the liver and spleen. The excretion route involving the liver to feces was also stated by the WHO (2022) report. There, it has been explained that ingested particles (polymeric nanoparticles) were transported to the liver through the circulatory system, recirculated through the bile to the small intestine, and then excreted in feces. Wu et al., (2022) provided elaborated hypotheses on the excretion routes and probable clearance ways of MNPs in humans, where they also supported MNPs excretion through urine and feces. The authors hypothesize that some digestive enzyme-degraded MNPs may become hydrophilic and be eliminated in urine and feces (Wu et al, 2022).

Clearance, though not strictly excretion by definition, is relevant to mention for its valuable insights into the overall removal of MNPs and biokinetics in humans. Particle size was underlined as a determinant of clearance properties (Dawson et al., 2018). Their evidence suggests that the plastics greater than 144 nm in size seemed to pass the gut blood barrier and enter the liver, then filtered and transported to the digestive tract through bile and the intestinal wall. On the other hand, Du et al., (2018) explained that particles less than 10nm in size could penetrate the kidney and be eliminated via the renal pathway. In their conclusion, Wu et al., (2022) list tears, saliva, sweat, and breast milk as possible means of plastic excretion in their review, but they are not yet validated.

Knowledge gaps

Knowledge gaps in the literature review were apparent in the type of mechanism gap, chronic exposure, various plastic particles, and plastic particle fate. Hou et al., (2022) noted that the accumulation and uptake mechanisms of plastic particles with different sizes, shapes, and compositions need further investigation. The knowledge gap on the kinetic mechanism was also underlined by Zhu et al., (2023) where the mechanism of intestinal barrier function under chronic

MPs exposure remains unclear. In a similar view, Hesler et al., (2019) emphasized that quantitative research on the uptake and accumulation of plastics by organisms is scarce. Also, a better understanding of the relationship between translocation mechanisms and particle sizes would contribute to the risk evaluation of MNPs. Medley et al., (2023) stated that the mechanism of plastic particles translocating the placenta remains unclear, and diet and genetics should be investigated as maternal susceptibility factors toward MNPs.

Several authors link the kinetic mechanism gap to chronic MNP exposure. For instance, Lee et al., (2022) want to confirm the pathway of translocation of MNPs in mice, possibly by *in vivo* tracking over time. Kim et al., (2022) touched upon the realistic (long and chronic) estimation of human internal dosage of MNPs. He & Yin (2023) remarked on the lack of direct research on the reproductive organs (other than the placenta) associated with chronic exposure to MNPs. They acknowledge the difficulty of systematically addressing the accumulation of MNPs in the human reproductive system. The authors also highlighted the importance of epidemiological studies on the accumulation of MPs in the tissues and organs of mammals in the natural environment. Nikolic et al., (2023) shared a similar view where they urged for an in-depth individual and epidemiological study on acute and chronic exposure of humans to different plastic particles.

The literature acknowledge the abundant use of fluorescent polystyrene in experiments, and the knowledge gap attributed to the behavior of various plastic materials. Deng et al., (2022) stated that they only PS for exposure treatment, therefore future studies should consider other plastics. They particularly suggest polypropylene for its highest abundance in human samples. Also, they proposed to explore nanosized particles for the placental and intestinal transfer of MNPs. Lee et al., (2023) mention the lack of studies involving cells other than macrophages and caco-2 cells and the absence of research on the cellular uptake of nanofibers. Limitations of *in vitro* studies often involve extremely high test concentrations in a short time, and the frequent use of polystyrene particles in experiments rather than plastics from daily-use products has been reported. Therefore, they voice the necessity to use various types of plastics including the mixture of the different types of plastics.

The particle fate of MNPs in humans is a prominent knowledge gap. Vattanasit et al., (2023) stated in their review of MNPs that their biological fate in the human body is absent and

needs exploration. According to the authors, the data on the physical and chemical characteristics of MNPs are not readily comparable. They underline the need to expand research on indoor MNPs – particularly microfibers. Prata et al., (2023) also mention the lack of knowledge regarding the fate of MNPs in humans. They particularly emphasize the evidence of elimination and biodegradation as crucial knowledge gaps. Furthermore, the authors suggest using histopathology to identify the exact location of particles in tissues. Wu et al., (2022) shared the same view on the knowledge gap according to the particle fate. The authors point to the absence of international standards for MNPs detection and quantification and link it with the limitation of the detection method for plastics smaller than 1μm. They explain that there is a need for an accurate estimation of the absorption of MNPs in individuals, and the uptake data warrants evaluation.

The mentioned knowledge gaps are closely related to challenges in research. Most experimental studies mentioned the technological limitation in plastic particle detection. Wang et al., (2023) used Laser Direct Infrared Imaging System to study the human lung tissue (parenchyma) which had a detection limit of 20 – 500 μm. Also, the imaging system could not distinguish polyamide and protein apart. On the other hand, Nikolic et al., (2022) remarked on the downside of using fluorescent polystyrene particles combined with confocal imaging and microscopy, where polystyrene particles were not detected in the spleen due to the organ's high autofluorescence. Similarly, Jin et al., (2021) also had trouble detecting the distribution of polystyrene microplastics in testicular tissues while working with biofluorescence imaging (BFI) and *ex vivo* fluorescence images of major organs.

3.2 Expert interviews

Five interviews were conducted in total, of which two experts were PBK modelers, two were inhalation exposure experts, and one was a blood-brain barrier expert. Transcribed expert interviews were analyzed with thematic analysis, and a coding tree was formed as illustrated in **Figure 2.** In total, 22 codes, 7 categories, and 2 themes emerged. Detailed information about each code for their definition and examples (from interviews) are organized in the supplementary document as a '*coding book of expert interviews'***.** The coding tree and the coding book are meant to be read together to gain a clear understanding of their representation. Efforts were taken to disclose all knowledge shared by experts in the best manner, with respect.

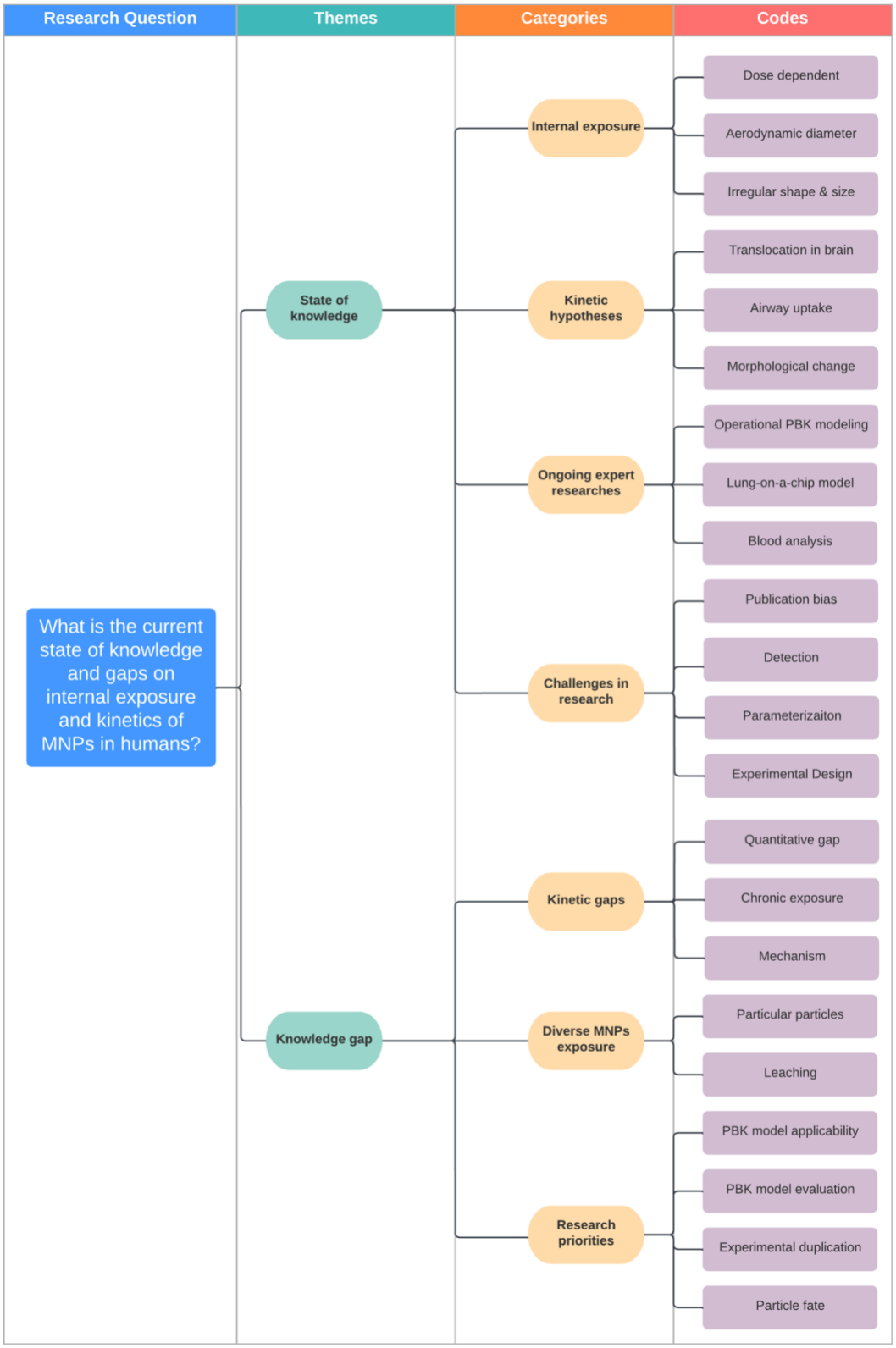

Figure 2. Coding Tree of Expert Interviews

State of knowledge – Internal exposure

Experts shared their insights on MNPs regarding particle characteristics, kinetics, and internal exposure in humans. In the context of airborne MNPs, Expert 4 observed that from collected air samples, rubber microplastics were composed of irregular shapes and sizes of particles according to SEM images. Humans are exposed to airborne MNPs via inhalation, and Expert 2 underlined aerodynamic diameter as a determinant of plastic particle fate in the respiratory tract. Expert 2 explained that light particles behave as small particles (because they are so light) and this characteristic determines where they can end up. Expert 3 researched the internal exposure of MNPs in the brain and the blood-brain barrier through *in vitro* and *in vivo* assessments, separately. In their *in vitro* assessment of the blood-brain barrier, the presence of (50 and 200 nm) fluorescent PS particles was proportional to all given doses (1, 10, and 100 μg/ml). Microscopy imaging confirmed that PS particles could cross the blood-brain barrier. Additionally, an *in vivo* assessment using rodent models and fluorescent PS particles (1 and 10 μm) was conducted via oral gavage. Rodent brain tissue slices were produced after exposure, where researchers also confirmed the presence of PS in the brain tissues of rodents. 10 μm plastics were detected more than 1 μm plastics. Expert 3 noted that they tried co-localizing plastic particles with staining specific for endothelial cells of the blood vessels that make up the blood-brain barrier. However, they faced difficulty due to unspecific and poor labeling.

In regard to the physiologically based kinetic (PBK) modeling work of MNPs in humans, Expert 5 shared that there are influential parameters (factors that significantly affect the behavior or the outcome of the model). Based on their current analyses, Expert 5 discovered that phagocytosis cells play an important role in determining the accumulation of plastics in humans. Moreover, sensitivity analysis reported the excretion rate of particles (via feces) as a very influential parameter in determining the amount of MNPs present in a body.

State of knowledge – Kinetic hypothesis

Next to insights gained from their research, experts suggested kinetic hypotheses on MNPs. These hypotheses serve as a plausible explanation for the movement and the behavior of plastic particles in humans. Expert 3 considered that MNPs can translocate in and out of the brain unless they are caught in a binding site. For instance, those binding sites could be a specific

cell type such as astrocytes or an extracellular matrix. Furthermore, Expert 3 considered the possibility that the brain is not the primary depot where MNPs end up as a longer (*in vivo*) exposure did not result in more particles in the brain. Expert 2 shared hypotheses regarding the uptake and translocation mechanism of inhaled plastic particles. Expert 2 expects that macrophages are most likely involved in the translocation process of MNPs in the airways to other organs. Alternatively, uptake by epithelial cells and lysosomes of various cell types was suggested. Expert 2 also added that the translocation of MNPs is likely to be dependent on their corona – proteins and microorganisms on the particle surface. When asked about the possibility of MNPs going through morphological changes in humans, Expert 2 thought it was unlikely. Based on their observations, macrophages, and related cells did not degrade inhaled plastic particles. Expert 2 further explained that as plastic particles in the environment take a long time to degrade with UV and temperature, it is reasonable to suppose that they are not easily degradable in the human body.

State of knowledge – Ongoing expert research

Experts shared in the interview the scope of various projects they are engaged in that cover MNPs' internal exposure to humans. These projects are currently under development and a definitive conclusion is yet to be drawn. Descriptions of these projects are meant to depict the latest developments and support the decision-making process for further MNP research directions. Expert 5 is involved in operational physiologically based kinetic (PBK) modeling work, where the goal is to understand how the microplastic properties would affect the fate of MNPs in the human body. An example of microplastic properties is particle size, so the PBK model can investigate how size would play a role in the fate of MNPs in humans. Expert 2 is involved in a collaborative project that develops a lung-on-a-chip model to assess the translocation process in the epithelial barrier layer. More importantly, in the translocation assessment of translocation and toxicity of MNPs via the inhalation route. Expert 4 is involved in an inhalation exposure study where researchers selected three locations – highway, traffic stop-and-go site, and park, and had participants cycle through these areas. Expert 4 wants to know the amount of plastic particles in the blood samples of participants, and the blood analysis work is planned.

State of knowledge – Challenges in research

In their conversation about ongoing work and research findings, experts shared the challenges they faced. Expert 3 pointed out publication bias in the context of MNPs literature, that papers with no effects are not often reported, leaving results with positive effects shared more often. Also, some papers are questioned for their transparency and reproducibility of results. Expert 1 and Expert 5 mentioned the difficulty of parameterization to obtain a specific value range for parameters in a PBK model. Their view aligned that chemical-specific parameter values are much more difficult to obtain compared to human physiological parameter values (which are already available in the literature). Experts also faced the difficulty of working with limited data about MNPs. When working with literature, Expert 5 had to work with pristine plastics as it was the only available data. Expert 5 commented that the literature limited itself to a specific set of plastics, and not a lot of data was available for microplastics. Most were related to polystyrene and its size range differed between sources. Expert 1 specifically points out the difficulty involved in translating *in vitro* test results to *in vivo* kinetic parameters. Expert 4 expressed the difficulty of accurately detecting plastic particles in human blood samples, where the purification process may induce particle loss, and requires a strictly controlled lab environment for a plastic-free work setting. As Expert 2 is involved in lung-on-a-chip model development, they face the challenge of developing a good matrix. The matrix should be rigid enough to stay in shape, but flexible enough to allow cell growth and the medium to reach cells. Expert 2 also added that realistic data on the corona of MNPs are important for assessment and evaluation, which is challenging data to obtain. Expert 3 also touched upon experimental design as a challenge in their *in vivo* study, where co-localizing particles with staining for the blood-brain barrier didn't work out well due to a lack of optimization (that couldn't be done within the time frame of the study).

Knowledge gaps – kinetic gaps

All experts emphasized the significant knowledge gap in the kinetics of MNPs in humans. Importantly, the lack of quantitative data on ADME aspects was consistently mentioned by experts. Expert 2 wished to quantify the uptake upon exposure. Expert 5 also mentioned uptake and elimination data, in the context that it is hard to obtain a good value for these parameters. They want to know the real range of parameters. Expert 1 identified the kinetic gap

for nanoplastics, as he explained that it cannot be assumed that the kinetics of nanoplastics are the same as for other nanoparticles. Expert 5 also mentioned qualitative kinetic data. In line with the quantification of particles, Expert 4 indicated the detection of particles as a knowledge gap. They explained that we need to have a good method to confirm MNPs exposure, but the method to conduct a blood analysis is a knowledge gap itself.

A knowledge gap on chronic human exposure to MNPs was brought up by Expert 4. Currently, it is only possible to study acute exposure in an *in vitro* setting that is conducted between 24 hours to 72 hours. Expert 4 raised the question of chronic effects that MNPs can pose to humans after accumulating in various organs and tissues given one year (for instance). Expert 3 had a very similar response to Expert 4, in a way that it is currently not feasible to study long-term exposure (*in vitro*). Expert 3 agreed that in an *in vitro* setting it is challenging to get a grip on long-term exposures since *in vitro* exposure is often limited to one month at most. Lastly, Expert 3 remarked that in an *in vivo* setting, prolonged exposure could be designed for a year or two for an investigation, but it is not feasible for currently ongoing projects, also budget-wise.

The knowledge gap in the uptake mechanism of plastic particles appeared to be apparent. When talking about the uptake of plastic in airways, Expert 2 speculates that if nanoparticles are taken up, macrophages would be involved. Expert 5 acknowledges the gap in mechanistic understanding related to how microplastic properties can change the absorption rate or the elimination rate.

Knowledge gaps – Diverse MNPs exposure

Experts also covered the aspect of the diversity of MNPs as a part of the knowledge gap. Expert 1 noted that MNPs are composed of various types of plastics – ranging in materials and sizes. MNPs are composed of various plastic polymers, which means that each plastic particle possess its own chemical-specific parameter in PBK modeling. As noted by Expert 5, most of the available data utilizes fluorescent PS particles, leaving other polymer and their chemical-specific parameter as a knowledge gap. Expert 3 attributed a knowledge gap in the mixture effects of plastics and their internal exposure, as in we don’t know that the presence of PET (as an example) could influence the possibility of PVC crossing an internal barrier.

Chemical leaching of plastic additives from MNPs and their influence on biokinetics is another knowledge gap. Expert 2 was concerned with the chemical leaching of plastic particles during prolonged exposure, where unidentified leached-out materials could pose a risk. It is something that we don't have much information about. Expert 4 shared a similar view about leaching and connected it to chronic effects. While there are a great number of additives used in the plastic industry, we don't know about their impact on chronic effects. As an extended aspect of diverse MNP exposure, Expert 3 briefly mentioned biocorona as a part of the knowledge gap. Expert 3 elaborated that the corona formed on plastics may or may not change when they enter and leave a cell, as a plastic particle may have a different corona than before it entered the cell.

Knowledge gaps – Research priorities

Not all shared knowledge gaps have a high priority in research. Some were prioritized over others. For PBK modeling, Expert 5 emphasized the need to develop a model that is flexible enough to handle ranges of MNPs, rather than being applicable to a specific plastic. Expert 1 had a similar view. According to Expert 5, PBK model evaluation is also required to confirm the credibility of the PBK model, which requires more experimental studies. As mentioned in the kinetic knowledge gap, experts shared a united view towards needing more experimental data. Expert 3 explained that they need more duplication studies as their previous studies were mostly preliminary pilot studies and contradictory results from the literature are numerous. Also, Expert 3 implied future work involving different types of fluorescent particles (as soon as they have them) to investigate their particle fate, if one particle type is more prone to cross the blood-brain barrier than others.

4. Discussion

Understanding the biokinetics of MNPs is necessary for assessing their risk to human health. There is a need to understand the knowns and unknowns better to guide future research. This scoping review seeks to map the current state of biokinetics knowledge and identify gaps in MNPs research. The following discussion section delves into the evidence from the literature review and expert interviews, highlighting both similarities and differences between the two. Attention is also given to certain points/information shared in the interview but not in the literature, and vice versa. Lastly, the current main knowledge gaps are discussed.

Absorption

Based on the literature review and expert interviews, one can identify absorption as a crucial biokinetic parameter for internal exposure to plastics. The literature and experts shared some similarities regarding the information about absorption. First, the uptake mechanism of MNPs via macrophages and epithelial cells was underlined. The uptake of ingested MNPs (particles > 500 nm) via intestinal macrophages and particles > 1 μm absorption by the epithelium of Peyer's patches was underlined by WHO (2022). Expert 2 suggested that inhaled MNPs are taken up through macrophages and epithelial cells. The uptake mechanism via macrophages involves phagocytosis, a cellular process also explained in the literature and an interview. Meng et al., (2023) suggested that MNPs absorption in the gut – specifically the ileum - would involve phagocytosis. In their work on the operational PBK modeling, Expert 5 found phagocytosis cells as an influential parameter to the amount of plastics taken up and present in the human body. The result shared by Expert 5 strengthens the hypothesis of Meng et al., (2023).

Additionally, the dose-dependent relationship of MNPs absorption is another commonality mentioned by the literature and experts. The uptake of 0.1 and 1 μm plastics on the human alveolar epithelial cells (A549 cell line) was found significantly dose-dependent by Lagana et al., (2023). Also, Kim et al., (2022) observed that MNPs can be taken up or attached to human red blood cells, based on the increased MNPs fluorescence in red blood cells exposed at 100 or 500 μg/mL in a dose-dependent manner. Expert 3 added additional information that the dose-dependent nature of 1 μm and 10 μm MNPs was evident in the transfer of particles across the brain-blood barrier, assessed *in vitro*. Combining these results seems to indicate that the uptake of MNPs is dose-dependent.

Two experts suggested that lysosomes are involved in the absorption of MNPs. This phenomenon was a hypothetical suggestion. Expert 3 briefly mentioned the possibility of MNPs interacting with lysosomes in their study of the blood-brain barrier but noted that there needs further research work to confirm it. Similarly, Expert 2 mentioned that some plastic particles can interact with lysosomes during the uptake from the lungs to the circulatory system. It appears that what they underlined for the relationship between MNPs and lysosomes is that the internalized MNPs can accumulate in cellular organelles such as lysosomes. This could be explained by a study, where z-stack imaging of RBL-2H3 cells (a type of rat basophilic leukemia

cells) treated with 50 and 500 nm MNPs for 6 hours (Liu et al., 2021). The authors showed that MNPs enter with endocytosis and are mainly distributed in lysosomes. It is important to acknowledge that there could be various ways MNPs are absorbed and the actual processes may be more complicated and diverse than what was proposed.

Distribution

Internalized MNPs can translocate and distribute to various organs and tissues. The literature and experts shared similar views toward the aerodynamic diameter of inhaled plastics as a factor in particle distribution. According to literature evidence, particles > 30 μm in aerodynamic diameter are less likely to enter the nasal passages (WHO, 2022). However, particles ≤ 10 μm in aerodynamic diameter were mainly found in the nasopharyngeal region, and particles of nanometers in aerodynamic diameters are likely to deposit in the alveolar pulmonary region (WHO, 2022). Expert 2 explained that particles with smaller aerodynamics can penetrate deeper into the respiratory tract around bronchioles and alveoli. Both sources support the idea that aerodynamic diameter is a representable size indicator for inhaled MNPs and that smaller particles can penetrate deeper into the respiratory tract.

There are some discrepancies regarding the site of MNPs deposition based on literature evidence, but both sources agree that they can cross the brain-blood barrier and distribute to the brain. In their *in vivo* experiment, Expert 3 shared their findings that fluorescent MNPs can cross the blood-brain barrier. Their result aligned closely with the literature data where MNPs (2 μm, and 10 – 250 nm in size) penetrated the blood-brain barrier and were detected in the brain (Lee et al., 2022; Gregory et al., 2020 & McCright et al., 2022). Furthermore, Expert 3 provided valuable insight that was not covered in the literature. Expert 3 suspects that MNPs can translocate in and out of the brain, unless they are caught in a binding site, such as a specific cell type astrocytes or extracellular matrix. Additionally, with a disclaimer that it is highly speculative, Expert 3 added that the brain is maybe not the primary depot of MNPs. Following the line of expert hypotheses, it could be inferred that there are some specific organs/tissues where plastics can deposit, and lead to accumulation if they are bound inside.

The existing literature data about MNPs provide evidence for internal distribution and the translocation pathway, although the site of detection is contradictory. According to the literature,

ingested particles can be distributed to the esophagus, stomach, epithelium, and gut (Wu et al., 2022). MNPs that are up to 1.09 μm in diameter can pass the gut epithelial barrier and enter the circulatory system, while particles larger in diameter can pass through the GI tract without being absorbed (Wu et al., 2022). Also, plastic particles up to 130 μm can translocate to various human tissues (Cox et al., 2019). Inhaled particles pass through the nasal cavity, to the lung (trachea and bronchioles). MNPs can enter the epithelial layers from the alveolar region to the circulatory system, and particles larger than 0.2 μm (aerodynamic diameter) in the bloodstream may be removed via splenic filtration. However, particles smaller than 0.1 μm are speculated to remain in the blood (Wu et al., 2022; Rist et al., 2018). Guided by the available evidence, the plastic particle diameter as size is an influential factor in its internal distribution. The small size of plastics (< 150 μm) seems to be advantageous for distribution. Various biochemical factors and their combined influence should be considered when addressing their internal distribution.

Relying on the current data, absorbed MNPs were detected in various organs of rodents such as the liver, kidney, gut, brain, blood vessels (vascular system), spleen, lung, and reproductive organs (testis and placenta). Additionally, particles were also present in excreted feces. The discrepancy among experimental studies of MNPs detection could be attributed to various factors including the size and material of plastic particles included in the study, the employed detection method (and their limit of detection), and many more. Comparing the results of experimental studies requires caution when integrating their findings. For instance, MNPs were detected in rodents (*in vivo*, oral ingestion) according to Lee et al., (2022) while Deng et al., (2022) found no significant presence in the liver. While both research groups worked with rodents, in an *in vivo* setting, and used Raman spectroscopy, their particle size differed. Hypothetically, because Deng et al., (2022) used a bigger particle for a longer duration, plastics could have been excreted via feces before detection. However, it could also be attributed to their choice of study design. The study material and preparation by Deng et al., (2022) require evaluation. It is a common practice to use fluorescent PS particles and related microscopy imaging for *in vivo* assessment, but they did not use fluorescent particles and only used Raman spectroscopy. While Raman spectroscopy is a valid detection method, it is beneficial to combine, or include fluorescence imaging like Lee et al., (2022). Also, the authors did not thoroughly explain why they chose two size classes that are similar to each other.

Metabolism

The metabolic data of internalized MNPs exist for their potential morphologic change in the human body, and there is a noticeable disparity of evidence between sources. Currently, there are no sufficient amount of data to conclude the morphological change of MNPs in humans. When asked about this matter, Expert 2 shared the view that plastic particles are not likely to degrade based on their experimental observation. Expert 2 reasoned on the fact that plastic particles in the environment take a long time to degrade, and it requires conditions such as UV and temperature to initiate alterations. However, the human body maintains rather a stable temperature, so plastic degradation is not expected.

There is little evidence about the potential particle surface change. In their work assessing the impact of artificial digestion on the size and shape of various MNPs, Stock et al., (2020), observed that PS developed irregular surfaces after artificial saliva treatment. However, artificial gastric and intestinal fluid showed no alteration of the tested plastics (PS, PE, PP, PVC, and PET). Therefore, the authors concluded that (artificial) digestive fluids didn't decompose the particles or modify their shapes and sizes in the GIT before translocation. Horvatitis et al., (2022) detected MNPs with signs of surface alterations from cirrhotic livers (*ex vivo*). However, their result cannot be directly taken as evidence for plastics metabolism. Possibly, patients were exposed to plastic particles that already suffered surface changes, and upon internal exposure, a portion of the particles were deposited in the liver.

Despite the limited evidence, the (potential) morphological transformation of MNPs may occur after ingestion in the GI tract and after crossing the epithelial barrier, after the internalization. The work of Tamargo et al., (2022) was mentioned as evidence of PET particle surface change upon the interaction with human colonic microbiota. In this particular research, PET MNPs (size of 160 μm ± 110 μm) were observed with surface alterations after exposure to a (computer-controlled) dynamic gastrointestinal model for an *in vitro* colonic fermentation. Despite the reported evidence, it is noteworthy that PET without polymer processing additives was used in the simulation, which does not consider the realistic exposure scenarios. Furthermore, their result is insufficient to assume that different plastic materials other than PET would have the same outcome. Therefore, it is reasonable to acknowledge that there exists

limited evidence of surface alteration for PS and PET particles, but these results cannot be extrapolated to *in vivo* results.

Excretion

There is little information about the excretion of plastic particles post-uptake. Often, the literature does not distinguish the elimination of particles that pass through the gastrointestinal tract and those excreted after the uptake. Considering the research aim, the focus is on the excreted MNPs post-uptake. The most probable excretion route is that after MNPs are absorbed into the circulatory system, they can translocate to the liver, recirculate through the bile to the small intestine, and then be eliminated via feces (WHO, 2022). Hypothetically, MNPs can excrete the body via urine and sweat alternative to feces but this requires validation. The excretion pathway outlined by WHO (2022) aligned with clearance pathways from the gastrointestinal barrier to the liver, then transported to the intestinal wall through bile (Dawson et al., 2018).

Expert 5 shed light on the importance of excretion data, by underlining that the excretion rate (via feces) of MNPs was revealed as an influential parameter for determining the amount of MNPs present in the body. Although this was a preliminary result from an ongoing operational PBK modeling, it suggests that MNPs might accumulate in the body if not excreted. Also, it underlines that the efficiency of the clearance pathway via feces may impact the fate of MNPs.

Knowledge gaps

Scientific progress includes hypothesis testing and generating empirical knowledge. The identification of the knowledge gap is as important as the knowledge itself because it helps to set the course of future research considering risk assessment. Biokinetics research of MNPs is in its early phase, and there are more knowledge gaps to uncover than what is known. Various knowledge gaps emerged from the literature and interviews. Their implication in MNPs research and associated challenges are discussed.

The lack of quantitative biokinetic data is a critical knowledge gap that has been outlined by the literature and experts. Overall, there is a need for more quantified data for all four aspects of biokinetics, including the amount of accumulated MNPs. As mentioned by Expert 5, the quantitative uptake and elimination rate of MNPs are two important data for the operational PBK

model. Estimations of absorption rate and elimination rate for fluorescent PS particles were reported from *in vivo* experiments, but the quantitative data is still too scarce to compare between publications. Thus, more quantitative data on uptake and elimination rates are required, and they are essential for validating current estimations for realistic and accurate data. In particular, intestinal epithelial cells (caco-2, goblet cells, and enterocytes) and alveolar epithelial cells (A549 cell line as epithelial tissue, and alveolar macrophages) are relevant subjects for studying absorption rate. Additionally, the quantification of MNPs detected in organs and their tissues after absorption can provide insight into where and how much MNPs can internally distribute. Altogether, the quantified absorption rate, the amount of MNPs detected, and the elimination rate can contribute to comprehensive biokinetics data that are needed for a sound risk assessment.

Beyond the quantitative data, another significant knowledge gap lies in the biokinetic mechanism of MNPs in humans. Although the probable absorption mechanism was outlined for ingested and inhaled MNPs, the actual absorption could be more complex. There seem to be multiple ways of MNPs absorption including phagocytosis, but researchers remained speculative about the detailed mechanism of how it can happen. Moreover, it would be beneficial to understand what cellular organelles can interact with absorbed MNPs, and how their interaction can influence MNPs' particle fate. Knowledge about MNPs' interaction at the cellular level helps with understanding how MNPs can translocate internally. Specifically, it would help to test Expert 3's hypothesis about the binding of some MNPs to a specific site when they are translocating in and out of the brain. The knowledge gap exists for particular binding sites (where), and to what extent they interact with MNPs. This knowledge gap also applies to other internal barriers. Additionally, the potential formation of corona on MNPs and their influence on translocation is a relevant knowledge gap.

Currently, available studies provide no clear evidence that MNPs are metabolized in the human body. However, a few studies have assessed the biodegradation of MNPs in a thorough manner that ensures plastic metabolization after absorption, and more studies are needed. Particular attention should be given to the potential surface change of a plastic particle as it was demonstrated in an artificial setting (Tamargo, 2022) but their result holds a limited significance due to the reliability of paper. It is very difficult to conduct a robust assessment of internalized

MNPs for any signs of metabolism, as humans are prone to be exposed to MNPs that already suffered minor degradation or fragmentations.

Available studies have used relatively short exposure periods. Therefore, chronic and realistic exposure to MNPs remains an important knowledge gap. This knowledge gap encompasses the realistic scenario where humans are exposed to MNPs from a year to a lifetime. The human internal dosage and (realistic) accumulation amount in organs are currently absent. However, chronic exposure knowledge holds a significant value in assessing the exposure dynamics in a population. For instance, cumulative dose and (exposure) variability within a population can help with toxicological and epidemiologic evaluations for risk assessment.

The fact that MNPs are composed of various polymer types adds to the complexity of MNPs knowledge gaps. Now, there is only limited biokinetic data available, which mostly covers spherical, pristine PS particles often in nanoparticle size. One can assume that PP, PET, and PVC could exhibit similar particle fate, but it cannot be concluded without proper validation. Additionally, the leaching of plastic additives upon chronic exposure is also not much explored. More than 2400 different types of substances are used in plastic production, which were identified for their potential health concern to users (Wiesinger et al., 2021). There is a knowledge gap in how such limited PS biokinetics data are relevant for the whole biokinetics of MNPs that humans are exposed to. In other words, humans are exposed to a mixture of various MNPs, yet realistic biokinetics data remains scarce.

Identified knowledge gaps in MNPs research are closely tied to technical challenges. One such challenge is the accurate detection of fluorescent and nano-sized particles, which can easily overlooked. The detection limit varies with the equipment used, making the precise identification of nanoplastics challenging. This contributes to the knowledge gap in both the quantification and understanding of biokinetic mechanisms. Additionally, preparing a robust experimental design presents a hurdle; maintaining a plastic-contamination free environment and ensuring proper assessments are difficult. To overcome these challenges and gain new insights into the biokinetics of MNPs, more targeted research is needed.

5. Conclusion

By combining the literature review and five expert interviews, this scoping review provides a comprehensive summary of the internal exposure and biokinetics of MNPs in humans. For the state of knowledge, preliminary evidence exists for probable uptake mechanism and MNPs distribution sites. Not all aspects of ADME were given equal attention in the previous studies so far, leaving metabolism and excretion of biokinetics largely unexplored and scarce in evidence. As for the most important biokinetic knowledge, there are several uptake mechanisms of MNPs involving phagocytosis by macrophages and clarthin-mediated endocytosis. The size and doses of given MNPs were influential for their absorption and distribution. Generally speaking, relatively smaller MNPs (in the nanometer range) in sufficiently high doses can be absorbed and distributed more easily than bigger plastics. Results of *in vivo, in vitro*, and *ex vivo* assessments were discussed in combination, but the extrapolation of *in vitro* results (from MNPs experiments) to *in vivo* remains difficult. *In vitro* data is still relevant and important, but it requires several follow-up experiments to validate their findings and evaluate them.

There are more knowledge gaps to uncover than what is known for the biokinetics of MNPs. Quantitative data are missing for all aspects of ADME, and a lot of plastic polymers, sizes, and shapes are left unexplored for their combined exposure. Critically, the absorption rate, the accumulation of MNPs detected in organs, and the elimination rate via feces are necessary. The fact that additional (unexplored) mechanisms could be involved shouldn't be neglected. Additionally, the cumulative dose and exposure variability of a population is unknown. Lastly, the particle fate of MNPs other than PS is also largely unknown. They could exhibit similar particle fate, but it requires further validation for abundantly found polymers in humans, such as PP. Many of the outlined knowledge gaps are tied to research challenges such as the difficulty of accurately detecting fluorescent MNPs in tissues and blood samples. Moreover, preparing a robust experiment design to ensure an exposure assessment demands meticulous efforts to prepare relevant materials and prevent plastic contamination.

Limitations of the current study

Having discussed the review's findings, an evaluation of the study's advantages and limitations is necessary. As for study advantage, the expert interview was a valuable data collection method. By adding the experts' opinions on kinetic hypotheses and knowledge gaps,

this scoping review captures the complex nature and dynamics of MNPs research. Also, it can act as a supporting material for risk assessors who are working to map the exposure of MNPs in humans and help navigate future directions.

On the other hand, the semi-structured interviews (in terms of time constraints) could have limited the level of detail in shared information. Specifically, providing details for *in vitro* and *in vivo* assessments (such as test model, dose, duration, exposure route, and detection method) was found difficult during the conversation. Follow-up questions were asked for clarification when needed, but some details were inevitably overlooked. Moreover, only five experts were interviewed, meaning that not all relevant expertise was covered. Experts who have expertise in MNPs detection in organs/protein corona formation on MNPs couldn't participate due to their schedule conflict. Additionally, participant bias also could have occurred during the interview where interviewees tend to provide what is considered 'right' and socially acceptable. Given the nature of the topic and its risk to human health, participant bias could have taken the form of providing kinetic hypotheses that already have (somewhat) publications to support their theories. Consequently, the information provided from expert interviews may overlook some other probable biokinetic theories. Although experts were ensured anonymity to provide security, it is important to acknowledge that eliminating bias is challenging.

As of the literature review, it was observed that there are generally not a lot of quantities of biokinetic data available for MNPs in humans. Some experimental papers tend to not report the exact reason behind choosing a particular test dose/concentration. For review papers, some suffered publication bias where they pushed the narrative that a certain mechanism is possible while their sources were not directly related to MNPs. To prevent getting lost in the narration, a great effort was made to review the sources that papers cited to support their claim. Only when the source had an adequate title and abstract related to the review, they were included as relevant references.

This scoping review did not include a thorough assessment of the reliability of experimental studies, as most scoping review doesn't. However, the lack of standardized quality assessment and quality control of MNPs publications for risk assessment is a known problem (WHO, 2022; Gouin et al., 2022). These sources indicate that a lot of MNPs publications are not fit for risk assessment. There needs to be a distinction between available evidence and evaluated

evidence for biokinetic knowledge of MNPs. Two groups can share a common ground but need separation. The presented state of knowledge should not be used as proof that kinetics are limited to only what was reported. Rather, it should be taken as a guide to check for a probable kinetic explanation and a navigating tool for testing and confirming hypotheses in the future. In the presence of novel findings, our understanding of the biokinetics of MNPs in humans is subject to falsification and updates.

Future research

There are some future researches that are urgently necessary and should be prioritized over others. First, experiments to assess uptake rate and excretion rate (via feces) should be held with attention to MNPs after absorption and internalization. Ideally, the uptake and excretion rate via feces should be investigated in the same experiment. Secondly, both *in vivo* and *in vitro* assessments should involve plastics other than PS that are more abundantly found in humans, such as PP. Lastly, the translocation and distribution mechanism of MNPs require more attention for their particle fate in humans.

6. References

(removed for plagiarism check)