Chapter 40

Potential human health effects following exposure to nano- and microplastics, lessons learned from nanomaterials

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Abstract
A substantial part of the plastic produced worldwide ends up in the environment and degrades into nano- and microplastics. The particles are ubiquitously present in the air and enter the food production chain as contaminants. Ingestion of nano- and microplastics present in food and drinking water, or those present in swallowed lung mucus that contain trapped particles, represent the main route of human exposure. Yet much remains to be studied on the intestinal uptake by humans and the potential this exposure has to result in adverse health effects. Here we review the current knowledge and relate this to lessons learned from the field of nanotoxicology. We discuss how in vitro and in silico approaches can be used to support the risk assessment of nano- and microplastics.

Keywords: Nanoplastics; microplastics; toxicity; uptake; in vitro models; PBK models

40.1 Introduction

Worldwide the production and use of plastic is on the rise. The yearly production has now exceeded 360 million metric tons, of which around 40% is used for packaging.[1–3] A substantial part of the produced plastic ends up in the environment. The combined exposure to ultraviolet light and mechanical action in the environment causes the plastic material to become brittle and degrade into smaller-sized fragments.[4,5] Apart from these plastic fragments (often referred to as secondary particles), also primary nano- and microplastics can be found in the environment and human food products. Primary nano- and microplastics are intentionally manufactured for industrial purposes like pelleted precursors for plastic products, or have been used as abrasives in cleaning products.[4,6] Collectively these materials are called nano- and microplastics, where plastic fragments with a size range between 100 and 5 mm are commonly defined as microplastics, whereas particles with a size <100 nm are defined as nanoplastics.[7–9]

Micro and nanoplastics represent a highly diverse class of contaminants which can be found in a broad range of shapes and sizes.[7] Importantly the polymer composition of nano- and microplastics in environmental or food samples is very heterogenous with polyethylene (PE), polypropylene (PP), and polystyrene (PS) being the most abundant polymer types[10–12] though polyethylene terephtelate (PET) and poly-vinyl-chloride (PVC) are also frequently detected.[13–15] Additional complexity is introduced as micro and nanoplastics can contain a diversity of chemical mixtures comprised of compounds such as plasticizers, flame retardants, stabilizers, fillers and pigments to improve the functionality of the product.[16,17] Due to the relatively high surface area to volume ratio and hydrophobicity, micro and nanoplastics can adsorb chemicals from the environment. Well known examples of adsorbed chemicals are polychlorinated biphenyls, dioxins, polycyclic aromatic hydrocarbons, and polybrominated diphenyl ethers and pharmaceuticals.[18–27]

Humans can be exposed to micro and nanoplastics mainly via inhalatory or oral routes of exposure. Several studies have shown that micro and nanoplastics are present in the outdoor and indoor air.[28,29] From the inhalable (or respiratory) fraction of micro and nanoplastics at least a fraction will be trapped in the lung mucus and cleared via the mucociliary escalator. These trapped micro and nanoplastics are subsequently swallowed and enter the gastrointestinal tract. Direct ingestion of micro and nanoplastics, for instance via drinking water or consumed food also is an important source of human exposure.

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The presence of micro and nanoplastics in a great diversity of food items, ranging from bottled water, shell fish, honey and packaged food has been shown (and reviewed before[30,31]). Upon passage through the intestinal (or lung) epithelial barrier micro and nanoplastics may have the potential to cause adverse human health effects.[32] At this point parallels can be drawn with health effects observed after prolonged exposure to (ultra) fine dust that has been shown to trigger oxidative stress and inflammation ultimately resulting in cardiovascular and respiratory diseases.[33] In studying the hazards and risks of nano- and microplastics important lessons can also be learned from experiences with engineered nanomaterials.[34–36] The exposure to nanomaterials is similar to that of nano- and microplastics, since intestinal uptake represents an important potential route of entry of these materials.[37] Human exposure to nanomaterials can be both unintentional and intentional because of their deliberate addition to food, their widespread use in food packaging and other domestic products and the potential for their inadvertent ingestion from environmental contamination.[30] Engineered (nano)materials, including silicon dioxide (SiO_2), titanium dioxide (TiO_2) and silver (Ag) nanoparticles have been detected in food[38–41] which, together with nanomaterials present in products such as toothpaste, cosmetics and sun cream, have a clear potential for ingestion by humans.

Here we will review the potential mechanism by which nano- and microplastics can pass the intestinal epithelium, as well as available evidence for potential adverse health outcomes. The study of potential health effects of nano- and microplastics is in its infancy, although there is a surge in the number of recently published papers. We will discuss which parallels can be drawn between the studies of micro and nanoplastics and the development of the field of nanosafety.[34]

40.1.1 Effects of conditions in the gastrointestinal tract on nano- and microplastics

Nano- and microplastics that are swallowed are subjected to physical and biochemical conditions that are very different from those encountered via other exposure routes.[42] Upon entry into the stomach the materials encounter an environment that has an extremely low pH and a high ionic strength. Further pH changes in the small intestine, the presence of mucus and the resident microbiota in the GIT lumen add additional complexity to the physicochemical properties of the ingested nano- and microplastics[43,44] potentially resulting in differences in toxicokinetics and toxicodynamics of the micro and nanoplastics.

The dynamics in pH and ionic shifts that nano- and microplastics encounter during stomach and intestinal transit can affect the dispersity of the material, and could result in an agglomeration of the particles, as seen earlier for (silica) nanomaterials.[40] Particle agglomeration at high concentrations might result in a lower bioavailable concentration resulting in a nonlinear dose response curve (i.e., low effects at high nominal concentration administered orally), as observed following oral administration of silica nanomaterials.[45] Additionally the rich luminal environment results in coating of the nano- and microplastics with biomolecules,[43,44,46–49] that affect the uptake of micro and nanoplastics. Unlike metal(oxide) nanomaterials of which some dissolve under acidic conditions,[50] dissolution of micro and nanoplastics particles is unlikely to occur under physiological conditions.

A relatively unexplored area is the potential interaction of nano- and microplastics with the intestinal microbiome. The intestinal microbiome is known for its crucial role on human health in general[51] and its role in the metabolism of foodborne chemicals.[52,53] However the interactions between particles and the intestinal microbiome has to be better explored.[54] Only little can be learned from engineered nanomaterials[55] as, also there, the interactions with the intestinal microbiome have not been studied in detail (as recently reviewed[56]). Some evidence from rodents is appearing that high concentrations of intestinal nano- and microplastics can affect the diversity and composition of the gut microbiota,[57] however, the functional consequences need additional investigation. Many of the effects of metal nanomaterials on bacteria have been attributed to ions dissociated from nanomaterials,[56,58] thus it might be postulated that micro and nanoplastics have less effect on bacteria directly. However, micro and nanoplastics can contain cocktails of associated chemicals,[18–27] that potentially can affect the intestinal micro-organisms. Interestingly some studies have shown that nanomaterial exposure affects not only the composition of the microbial community (in the cecum), but also increased the production of the bacterial metabolite butyrate following a 21 day exposure of mice to chitosan particles loaded with copper sulfate,[59] while oral exposure of mice for 35 days to particulate matter (PM1) at 10 mg/g/day decreased intestinal butyrate concentrations.[60] Butyrate serves as a key energy source of intestinal cells[59] and as a critical mediator in other responses and might thus represent a vector of biological action following exposure to nanomaterials (and nanoplastics).[61,62] In vitro studies using human stools also confirmed that nanoparticles can affect intestinal metabolism. A 5-day incubation with CeO_2, TiO_2, ZnO nanomaterials in a model colon reactor resulted in a decreased butyrate production upon incubation with CeO_2 nanoparticles only.[63] No related studies on the interaction of nano- and microplastics have been identified so far.

In conclusion, the physicochemical properties of micro and nanoplastics like engineered nanomaterials

can be affected by the biochemical conditions in the human gastrointestinal tract which in turn might affect the toxicokinetics and toxicodynamics of these materials. The effects micro and nanoplastics have on the intestinal microbiome and the microbial metabolism are yet largely unknown, but given the importance of the intestinal microbiome on human health this needs to be studied. It is important to note that there are differences between the human and rodent microbiome and that there are differences in microbiome diversity of communities collected from human stools and the communities in the intestine, complicating further studies. For instance, the microbial composition in the human small intestine was reported to be far less complex than that of the large intestine.[64] Thus a careful design of studies is needed.

40.1.2 Potential mechanisms of intestinal nano- and microplastics uptake

Mechanistic information on nanoparticle uptake processes across the intestinal epithelium has been almost exclusively derived from in vitro studies.[65,66] The used in vitro cell models attempt to emulate critical intestinal nano- and microplastics uptake functionality. The small intestine is mostly lined by enterocytes that bear a dense microvillus brush-border on the apical (lumen) side of the cells. The epithelium is sealed by tight junctions between the cells that prevent passage of most materials. Interspersed between enterocytes are goblet cells which secrete negatively charged mucus onto the gut epithelium. The mucus lining provides an additional barrier to the diffusion of particulates towards the epithelium, with smaller particles penetrating more easily through the mucus layer and positively charged particles getting trapped in the mucus.[67] The apical membrane of enterocytes is also covered with a complex glycocalyx which forms a size-selective barrier by the potential interaction of particulate material with surface molecules.[68,69] Lastly, so-called microfold cells (M-cells) can be found in specialized lymphoid-associated regions in the intestinal epithelium, the Peyer's patches and other gut-associated lymphoid tissues (GALT).[70] M-cells have a thinner apical glycocalyx and mucus layer and have the capacity to transport material, including inert particles, viruses and bacteria, and to deliver them to the underlying lymphoid cells.[71] Epithelial cells form a tight, dense monolayer and for that reason the paracellular route (i.e., between cells) of passage seems very unlikely for nano- and microplastics. Therefore it is often assumed that transcellular routes predominate, as also shown by in vitro experiments with epithelial cells.[72–78] It is likely that particles in the nano-size range are internalized through clathrin- and/or caveolin-dependent endocytosis, which operates in polarized epithelia,[79] while uptake of larger particles (>150 nm) occurs mainly by phagocytosis and micropinocytosis. The best characterized route for transcellular particle translocation is that through the aforementioned M-cells. However, transport through M-cells does not necessarily mean that the nanoplastics reach the bloodstream as M-cells are closely associated with immune cells. A study described that orally administered glucan and poly(lactic-*co*-glycolic acid) (PLGA) nanoparticles in mice were transported through M-cells and subsequently endocytosed by dendritic cells in the Peyer's patches and subsequently retained, thereby not reaching the bloodstream.[80] The close connection to the immune system also indicates that the intestinal immune homeostasis may be influenced by nanoplastics. This was also shown in a study in which it was observed that amorphous magnesium-substituted calcium phosphate nanoparticles enter the Peyer's patches via M-cells. These nanoparticles are spontaneously formed from calcium and phosphate ions that are naturally secreted into the lumen of the distal small intestine. These particles trap soluble macromolecules, such as bacterial peptidoglycan and orally fed protein antigens, which upon entering the Peyer's patches might result in interactions with the local immune system.[81] Despite their well-established transcystotic capacity, M-cells are scarce and other less efficient uptake routes via normal enterocytes may be quantitatively more important for nanoplastic uptake.[82] Indeed, significant uptake of particulate PLA-PEG nanomaterials (200 nm) has been reported in rat epithelial cells in vivo, with no preference for Peyer's patches compared to enterocytes in the villi.[67,83] To complicate this observation, in vitro results suggest different mechanism of uptake of these nanomaterials compared to 200 nm PS particles and 290 nm chitosan particles.[67,83] Earlier studies reported that while larger particles are preferentially taken up by rat Peyer's patches, uptake by enterocytes was significant and became more so as particle sizes of polymer nanoparticles decreased to 100 nm.[84–86] These data suggest that nano-sized particles may access additional uptake routes to those available for larger particles and support the concept that lower efficiency of nanoplastic uptake by enterocytes might be offset by its vastly larger presence in the intestinal epithelium compared with the specialized M-cells.

Paracellular transport is likely not a major route for nanoplastic passage through the healthy intestinal epithelium unless nanoplastics are small enough or have surface properties that increase tight junction permeability. However, there will not be such a strict limitation on paracellular transport in areas where the epithelium is damaged, during normal cell turnover at villus tips and in pathological states where intestinal epithelium translocation may be enhanced. For example, it is well known that bacterial translocation is enhanced by

conditions such as trauma, inflammation, stroke, and chronic alcohol use.[87,88] This translocation is likely to be mirrored by increased uptake of particulate material. This concept is supported by in vitro studies demonstrating enhanced penetration of 2 μm PS particles across cultured CaCO-2 cells following alcohol treatment or irradiation, which enhance tight junction leakage.[89,90] Studies using in vitro models of the inflamed intestine have also reported increased cytotoxicity and inflammation after exposure to PE and PVC microplastics (but not following exposure to PS nanoplastics) compared to the healthy situation. Translocation could however not be measured since the size of the particles did not allow them to pass the transwell pores on which the intestinal cells were grown.[91,92] Nano- and microplastics come with a great diversity in size, shape, and polymer composition. How these variation contribute to different uptake profiles remains to be elucidated. Comparison of environmental plastic particles with engineered nanoplastics is challenging as differences in surface properties and shape might differentially govern uptake processes.

40.1.3 Nanomaterial uptake following ingestion by humans and rodents

Only very little human data is available on the potential systemic availability of nano- and microplastics. Recently a pilot study was published in which microplastic fragments were observed in the placentas from six patients with uneventful pregnancies.[93] Some of these fragments, with a size around 5 and 10 μm were observed on the fetal side of the placenta using Raman microspectroscopy.[93] Somewhat more information is available on particulate matter exposure in general. In a single dose study using human volunteers (n = 9) an oral exposure to 5 mg/kg body weight (315–620 mg person) of TiO_2 particles (10, 70, 1800 nm) did not result in a detectable concentration in urine 72 h postexposure. In addition, no values outside clinical ranges (whole blood erythrocytes) were observed.[94] However, in a comparable study where seven human volunteers ingested 100 mg food grade TiO_2 nanoparticles (mean size 260 nm), TiO_2 was observed in blood 2 h after administration, which peaked at 6 h following ingestion.[95] This study supported earlier findings where blood samples contained increased levels of TiO_2 after ingestion of 160 and 380 nm TiO_2 nanoparticles.[96] The presence of reflective particles in blood was interpreted as evidence of the presence of TiO_2 particles but this was not confirmed by direct analysis of particle composition (e.g., by single particle inductively coupled plasma mass spectrometry). Lastly, carbon black particles have been detected in human placentas in concentrations averaging (standard deviation between brackets) 0.95×10^4 (0.66×10^4) and 2.09×10^4 (0.9×10^4) particles/mm^3 placenta tissue for low and high exposed mothers, respectively. The authors showed that the placental carbon black load was positively associated with mothers' residential carbon black exposure during pregnancy (0.63–2.42 μg/m^3).[97]

Unfortunately, rodent studies do not provide much more data on the oral uptake of micro and nanoplastics. Earlier we estimated that only 0.2% of a single dose (administered via oral gavage) of 125 mg 50 nm PS particles was detected in the body after 6 h, which was increased to 1.7% for negatively charged PS particles of the same size.[98] Such low oral bioavailability of PS particles was recently confirmed in a study that used a single administration by gavage of desferrioxamine and radiolabeled plastic particles of various sizes (0.1 mg/animal of 20 nm, 220 nm, 1 μm, and 6 μm). For the smallest particles of 20 nm <0.001% was detected 48 h after administration.[98,99] We calculated[98] from earlier studies by Jani and colleagues (1990) that 6.6% of the administered 50 nm and 5.9% of 100 nm PS particles ended up in the body (1.25 mg/kg bw daily for 10 days).[85] Based on an extensive review of the literature the European Food Safety Authority[100] concluded that intestinal absorption of particles of 2–3 μm was not higher than 0.3%, based on rodent studies and ex vivo models using human tissues.[7] For 500 nm carboxylated particles administered by oral gavage for 5 days in a concentration of 12.5 mg/kg bw the total uptake was estimated to be 37.6%.[100] In another study rats were administered 20 nm rhodamine-labeled nanopolystyrene beads (2.64×10^{14} particles) via intratracheal instillation on gestational day (GD) 19. One day later, nanopolystyrene particles were detected in the maternal lung, heart, and spleen. PS nanoparticles were also observed in the placenta, fetal liver, lungs, heart, kidney, and brain suggesting maternal lung-to-fetal tissue nanoparticle translocation in late stage pregnancy.[101]

We conclude that there is a lack of data on uptake of micro and nanoplastics by humans, but based on the limited human data, systemic availability cannot be excluded. The limited amount of available data from animal studies is inconclusive as the reported uptake ranges from low (< 0001% for 20 nm particles) to high (37.6%) intestinal uptake depending on size and surface charge of the studied materials. Clearly more data is urgently needed on the uptake (rates) of micro and nanoplastics to which humans are exposed.

40.1.4 Effects of nano- and microplastics on gastrointestinal epithelium in vitro

The number of in vitro studies in which the potential effects of nano- and micro plastics are investigated is increasing rapidly. In Table 40.1, an overview of in vitro studies

TABLE 40.1 Results of in vitro studies using models for the human intestinal epithelium.

	Material	Size	Concentration exposure duration	Significant effects in vitro	Assay	Cell model	References
Cytotoxicity							
	PS	100 nm; 5 μm	0, 1, and 20 μg/mL for 96 h	100 nm 20 μg/mL increased LDH5 μm no effect	LDH	CaCO-2 cells (exposure with and without in vitro digestion)	104
	PS	5 μm	0, 1×10^{-1}–10^{-8} mg/mL for 24 and 48 h	No effects	MTT	CaCO-2	105
	PS	300 nm; 500 nm; 1 μm; 3 μm; 6 μm	0, 20, 50, 70, 90, and 120 μg/mL for 24 h	300 nm: all concentration	MTT	CaCO-2	102
				500 nm: 120 μg/mL			
				1 μm: 90 and 120 μg/mL			
				3 μm: 70, 90 and 120 μg/mL			
				6 μm: 50, 70, 90, and 120 μg/mL			
	PS	50 nm;	0, 1, 5, 10, and 50 $\mu g/cm^2$ for 24 h	No effects	WST-1, LDH, number of nuclei	Normal mono and normal and inflamed cocultures CaCO-2/HT29-MTX-E12/THP-1	91
	PS	50–100 nm	0, 25, 50, 100, 125, 150, 175, and 200 μg/mL for 24 and 48 h	175 and 200 μg/mL slight decrease in cell numbers after 48 h	Beckman counter method	CaCO-2 cells	106
	PS	50–100 nm	0, 25, 50, 100, 125, 150, 175, and 200 μg/mL for 24 h	No effects	Beckman counter method	Coculture of CaCO-2/HT29 and a triple culture of CaCO-2/HT29 + Raji B	107
	Carboxyl-modified PS	1, 4, and 10 μm	0, ∼10^3, ∼10^6, 10^7, ∼10^8 particle/mL for 48 h	1 μm ∼10^7 ∼10^8 particle/mL	MTT	CaCO-2	108
				4 μm ∼10^8 particle/mL			
	Amine-modified PS	50 nm	0, 1, 5, 10, and 50 $\mu g/cm^2$ for 24 h	All concentration in CaCO-2, WST-1 (LDH in highest two concentrations)	WST-1, LDH, number of nuclei	Normal and inflamed mono and cocultures CaCO-2/HT29-MTX-E12/THP-1	91
				10 and 50 $\mu g/cm^2$ in HT29MTX-E12: WST-1 and LDH			
				50 $\mu g/cm^2$ in triple cultures increased LDH			
	PET	<100 nm	0, 1, 5, 15, and 30 μg/mL for 24, 48, and 96 h	No effects	MTS and LDH	CaCO-2	109
	PET	Polydisperse	0, 1, 5, 10, 25, 50, 75, and 100 mg/mL for 24 h	No effects	MTT	CaCO-2	110
	PE	1–4 μm; 10–20 μm; polydisperse	1–4 μm: 0, 1, 5, 10, 25, and 50 mg/mL for 24 h10–20 μm; 0, 1, 5, 10, and 25 mg/mL for 24 h	No effects	MTT	CaCO-2, reversed exposure	110
			Polydisperse: 1, 5, 10, 25, 50, 75, and 100 mg/mL for 24 h				

	PA	<300 μm	0, 823.5–1380.0 μg/cm^2 for 6, 24, and 48 h	No effects	LDH	CaCO-2 + HT29-MTX + MDMs + MDDCs	[111]
	PE	200–9900 nm	0, 10, and 50 μg/cm^2 for 24 h	50 μg/cm^2 in both stable and inflamed tricultures	LDH	Normal cocultures CaCO-2/HT29-MTX-E12/THP-1, normal exposure and reversed exposure. Inflamed cocultures Caco-2/HT29-MTX-E12/THP-1, reversed exposure	[92]
	PP	Polydisperse	0, 1, 5, 10, 25, and 50 mg/mL for 24 h	Only significant effects the 10 mg/kg	MTT	CaCO-2, reversed exposure	[110]
	PP	< 300 μm	0, 823.5–1380.0 μg/cm^2 for 6, 24, and 48 h	No effects	LDH	CaCO-2 + HT29-MTX + MDMs + MDDCs	[111]
	PU (hard and ester)	<300 μm	0, 823.5–1380.0 μg/cm^2 for 6, 24, and 48 h	No effects	LDH	CaCO-2 + HT29-MTX + MDMs + MDDCs	[111]
	PVC	<50 μm	0, 1, 5, 10, and 50 μg/cm^2 for 24 h	Only in inflamed triculture at 50 μg/cm^2 (reduced number of nuclei)	WST-1, LDH, number of nuclei	Normal and inflamed mono and cocultures CaCO-2/HT29-MTX-E12/THP-1	[91]
	PVC	Polydisperse	0, 1, 5, 10, 25, 50, 75, and 100 mg/mL for 24 h	Cytotoxic at 75 and 100 mg/mL	MTT	CaCO-2	[110]
Barrier integrity							
	PS	50 nm	0, 10, and 50 μg/cm^2 for 24 h	No effects	TEER	Normal and inflamed cocultures CaCO-2/HT29-MTX-E12/THP-1	[91]
	PS	50–100 nm	0, 1, 25, 50, and 100 μg/mL for 24 h	No effects	TEER, LY transport	Coculture of CaCO-2/HT29 and a triple culture of CaCO-2/HT29 + Raji B	[107]
	PS	100 nm; 5 μm	0, 1, and 20 μg/mL for 96 h	100 nm 20 μg/mL increased LY transport	LY transport	CaCO-2 cells (exposure with and without in vitro digestion)	[104]
				5 μm no effects			
	Amine-modified PS	50 nm	0, 10, and 50 μg/cm^2 for 24 h	50 μg/cm^2 in healthy and inflamed triculture models	TEER	Normal and inflamed cocultures CaCO-2/HT29-MTX-E12/THP-1	[91]
	PE	200–9900 nm	0, 10, and 50 μg/cm^2 for 24 h	No effects	TEER	Normal and inflamed cocultures CaCO-2/HT29-MTX-E12/THP-1, reversed exposure.	[92]
	PVC	< 50 μm	0, 10, and 50 μg/cm^2 for 96 h	No effects	TEER	Normal and inflamed cocultures CaCO-2/HT29-MTX-E12/THP-1	[91]
ROS generation							
	PS	300 nm; 500 nm; 1 μm; 3 μm; 6 μm	0 and 120 μg/mL for 24 h	300 nm 120 μg/mL	DCFH-DA	CaCO-2	[102]
				500 nm 120 μg/mL			
				1 μm 120 μg/mL			
				3 μm 120 μg/mL			
	PS	50–100 nm	0, 1, 25, 50, and 100 μg/mL for 24 h	No effects	DCFH-DA	CaCO-2 cells	[106]
	PS	50–100 nm		No effects			[107]

(*Continued*)

TABLE 40.1 (Continued)

	Material	Size	Concentration exposure duration	Significant effects in vitro	Assay	Cell model	References
			0, 1, 25, 50, and 100 μg/mL for 24 h		DCFH-DA and DHE	Coculture of CaCO-2/HT29 and a triple culture of CaCO-2/HT29 + Raji B	
	PET	<100 nm	0, 1, 5, 15, and 30 μg/mL for 24 h	No effects	DCFH-DA	CaCO-2	109
Inflammatory cytokine release							
	PS	100 nm; 5 μm	0, 1, and 20 μg/mL for 96 h	Increased secretion of IL-8, MCP-1 of digested 100 nm 20 μg/mL 5 μm no effect	IL-8 and MCP-1	CaCO-2 cells (exposure with and without in vitro digestion)	104
	PS	50 nm	0, 10, and 50 $\mu g/cm^2$ for 24 h	No effects	IL-1beta, IL-6, IL-8 and TNF-alpha release	Inflamed tricultures CaCO-2/HT29-MTX-E12/THP-1 and stable tricultures CaCO-2/HT29-MTX-E12/THP-1 (only IL-1beta and IL-8)	91
	Amine-modified PS	50 nm	0, 10, and 50 $\mu g/cm^2$ for 24 h	No effects	IL-1beta, IL-6, IL-8 and TNF-alpha release	Inflamed tricultures CaCO-2/HT29-MTX-E12/THP-1 and stable tricultures Caco-2/HT29-MTX-E12/THP-1 (only IL-1beta and IL-8)	91
	PVC	<50 μm	0, 10, and 50 $\mu g/cm^2$ for 24 h	50 $\mu g/cm^2$ Significant increased IL-1beta release, inflamed triculture, not significant for IL-6, IL-8 and TNF-alpha	IL-1beta, IL-6, IL-8 and TNF-alpha release	Inflamed tricultures CaCO-2/HT29-MTX-E12/THP-1 and stable tricultures CaCO-2/HT29-MTX-E12/THP-1 (only IL-1beta and IL-8)	91
	PU (hard & ester)	<300 μm	0, 823.5−1380.0 $\mu g/cm^2$ for 6, 24, and 48 h	No effects	TNFα, IL-8, IL-1β	CaCO-2 + HT29-MTX + MDMs + MDDCs	111
	PE	200−9900 nm	0, 10, and 50 $\mu g/cm^2$ for 24 h	50 $\mu g/cm^2$ significant increase IL-8 release stable triculture, not significant for IL-1beta, IL-6, and TNF-alpha	IL-1beta, IL-6, IL-8, TNF-alpha release	Normal and inflamed cocultures CaCO-2/HT29-MTX-E12/THP-1, reversed exposure	92
	PP	<300 μm	0, 823.5−1380.0 $\mu g/cm^2$ for 6, 24, and 48 h	No effects	TNFα, IL-8, IL-1β	CaCO-2 + HT29-MTX + MDMs + MDDCs	111
	PA	<300 μm	0, 823.5−1380.0 $\mu g/cm^2$ for 6, 24, and 48 h	No effects	TNFα, IL-8, IL-1β	CaCO-2 + HT29-MTX + MDMs + MDDCs	111
	PET	<100 nm	0, 1, 5, 15, and 30 μg/mL for 24 h	No effects	IL-8 and MCP-1	CaCO-2	109
Altered gene expression							
	PS	100 nm; 5 μm	0, 1, and 20 μg/mL for 96 h	Increased gene expression and secretion of IL-8, MCP-1 of digested 100 nm 20 μg/mL 5 μm no effect	Q-RT-PCR	CaCO-2 cells (exposure with and without in vitro digestion)	104

	PS	5 μm	0, 12.5, and 50 mg/L for 24 h	NF-κB, MAPK signaling, cytokine-cytokine receptor interaction, and toll-like receptor were strongly influenced	RNA Seq	CaCO-2	[105]
	PS	50–100 nm	0, 1, 25, 50, and 100 μg/mL for 24 h	No effects	RT PCR	Coculture of CaCO-2/HT29 and a triple culture of CaCO-2/HT29 + Raji B	[107]
	PS	50–100 nm	0, 1, 25, 50, and 100 μg/mL for 24 and 48 h	No effects	RT-PCR	CaCO-2 cells	[106]
Mitochondrial membrane potential alterations							
	PS	50–100 nm	0, 1, 25, 50, and 100 μg/mL for 24 h	Increased mitochondrial activity at concentrations ≥ 25 μg/mL	Mitoprobe TMRM assay	CaCO-2 cells	[106]
	PS	300 nm; 500 nm; 1 μm; 3 μm; 6 μm	120 μg/mL for 24 h	500 nm 120 μg/mL	JC-1 assay kit	CaCO-2	[102]
				1 μm 120 μg/mL			
				3 μm 120 μg/mL			
				6 μm 120 μg/mL			
				Reduced mitochondrial membrane potential			

PA, Polyamide; *PET*, polyethylene terephthalate; *PP*, polypropylene; *PS*, polystyrene; *PU*, polyurethane; *PVC*, polyvinyl chloride.

published in 2020 and the first half of 2021 that report the results of exposure studies using human intestinal epithelial cells is presented. Of the different polymer types studied, PS is the most frequently used. For the assessment of cytotoxicity different assays have been used, the MTT and WST-1 detect mitochondrial activity, while the LDH assay screens for membrane leakage. The MTT and WST-1 are more sensitive markers for cytotoxicity as can also be observed in Table 40.1 and larger sized particles are less cytotoxic than smaller ones[102] (Table 40.1). Interestingly also an effect on the mitochondrial membrane has been shown[102] (Table 40.1). Clearly, positively charged particles displayed increased cytotoxicity,[91] which has been observed previously.[103] For PET, PE, PA, and PP less data is available, but minimal cytotoxicity has been observed (Table 40.1). Cytotoxicity to the intestinal epithelial cells could result in a disrupted barrier function. Using TEER measurements this has not been shown, however using a longer exposure time in combination with a more sensitive approach in which the translocation of dextrans (Lucifer Yellow) was studied, increased translocation of Lucifer Yellow was observed following exposure to 100 nm PS particles.[104]

Increased production of reactive oxygen species (ROS) has been proposed as a common effect of both engineered nanomaterials and micro and nanoplastics.[112–114] Yet, from Table 40.1 the effects on ROS production following exposure of different sizes of PS to CaCO-2 cells are inconclusive. Immunotoxicity also is often mentioned as a potential adverse effect following exposure to micro and nanoplastics.[115] In some studies this was considered (Table 40.1), but in these studies only PS and PVC particles have been evaluated. A tendency that micro and nanoplastic exposure leads to increased cytokine excretion and gene expression has been noted in some studies, but not all (Table 40.1).

It is of interest to note that in vitro models of different complexity are being used, that is, monocultures of differentiated CaCO-2 cells, cocultures with mucus producing HT29-MTX cells and representative immune cells (i.e., Raij-B or THP-1 cells). From the noted effects (Table 40.1) it is difficult to recommend which cell model to use for which type of study. In previous work on silver nanoparticles we arrived at a similar conclusion.[116] As discussed above, pH dynamics, differences in ionic strengths and the dynamic biochemical conditions that nano- and micro particles encounter during gastrointestinal digestion could influence the uptake and local toxicity. Perhaps this is caused not so much by direct particle toxicity, but more dominantly as a consequence of the particle associated protein corona. This is exemplified by the increased secretion of IL-8 and MCP-1 of CaCO-2 cells exposed to 100 nm PS particles that have been incubated in different matrices.[104]

In most studies (Table 40.1) intestinal cells have only been exposed relatively short, while a realistic human exposure is chronic. It also appears that CaCO-2 cells are relatively unsensitive and have a poor endocytotic capacity compared to other cells, (i.e., THP-1 cells). In part this can be explained by the physiological role of these different cells, but also indicates the need to include cells that better emulate the human intestinal cell function. Potential models to consider in the future are human stem cell derived intestinal epithelial models.

40.1.5 Dosimetry in vitro and physiologically based kinetic models for nano- and microplastics

Extrapolation of results from in vitro studies using intestinal cells to the in vivo intestinal epithelium must be approached with caution. Apart from the caution in extrapolating the observations from cell-line-based models to the complex in vivo situation, concerns have also been raised about the concentration used in vitro and dosimetry of the nano- and microplastics (i.e., sedimentation of nano- and microplastics) in vitro. Meaningful interpretation and comparison of the results obtained using different in vitro experiments and extrapolation to in vivo data require reliable characterization of the nano- and microplastics and their agglomerates, as well as matrix-based influences on nano- and microplastics. For soluble chemicals it is reasonable to assume that the administered concentration (or nominal media concentration) is proportional to the cellular dose, and thus is a good measure of the concentration (or dose) at the target site.[117] However, micro and nanoparticles behave as colloid particles and the definition of a nano- and microplastics concentration in an in vitro system is far more complicated. From metal(oxide) nanomaterials we know that these materials can settle, diffuse, and agglomerate differentially which is determined by the properties of the nanomaterial itself (e.g., size, density, and surface chemistry) as well as by the solution matrix (e.g., viscosity, density, presence of proteins). Thus, nanoplastic dosimetry is affected not only by the concentration and used exposure time, but also by the nanoplastic characteristics and the environment.[117] Additionally in the case of nano- and microplastics and their aggregates, buoyancy of the materials needs to be considered. Buoyant nanoplastics rapidly move away from the cell surface in unagitated systems as is common in cell culture. As such the delivered dose of such particles will be exceedingly low, potentially underestimating true toxicity which has to be compensated by utilizing advanced in vitro models such as inverted cell culture,[92,118,119] semiwet culturing[111,120] or potentially the use of dynamically flowing systems such as cell-on-a-chip models.

To estimate the in vitro dosimetry computational dosimetry models such as the ISDD, ISD3 and DG

models have been developed and refined.[121−124] These models apply to buoyant and nonbuoyant plastics (and nanomaterials) as in both cases only a fraction of the particles will reach the cell surface and robust methods for calculating the cellular dose are needed. Dosimetry models use a set of particle and matrix parameters to model particle sedimentation, aggregation, and also dissolution depending on the particles. Information which is commonly required includes information on the in vitro system, for example, the height of the liquid column, density and temperature of the medium as well as particle properties such as the average size, density and applied concentration. Information on particle aggregation can either be predicted in silico as is done in the ISDD algorithm[121] or determined experimentally by using dynamic light scattering measurements (DLS) to derive the hydrodynamic or aggregate sizes and volumetric centrifugal methods (to derive particle density) as is commonly done when working with the distorted grid and ISD3 algorithms.[123,124] The particle and aggregate properties are used to solve equations for the gravitational settling, drag force and Brownian diffusion which ultimately yields the time-dependent particle and aggregate concentrations at any given height in the liquid column.

Besides the quantitative description of the time-dependent nanoparticle concentration in vitro, efforts have also been made to predict in vivo nanomaterial concentrations based on results from in vitro studies using physiologically based kinetic (PBK) models. The generation of PBK models for nano- and microplastics is challenging due to the colloidal nature of nano- and microplastics and the lack of fundamental knowledge on nano- and microplastics ADME mechanisms (as discussed earlier in this chapter for intestinal uptake). Unlike small molecule compounds, nanoplastics are subjected to limited membrane permeability and PBK models assuming blood-flow limited transport yield less accurate nanoplastic concentrations.[125] The organ partitioning of nanoplastics is not dictated by the hydrophobicity of the compound and instead is largely governed by the phagocytotic capacity of organ-resident immune cells.[126,127] For this reason nanoparticle or micro and nanoplastic specific PBK-models commonly incorporate parameters that describe phagocytotic compartments that sequester nanoparticles within organs.[128] Despite the technical complexity, PBK models have been developed for quantum dots (20 nm),[129] metallic NPs such as silver (15−150 nm)[130] and titanium dioxide (15−150 nm),[131] nanocrystals and a handful of nanopolymers such as PLGA (50−135 nm).[132,133] While most PBK models only consider direct intravenous injection of nanoparticles, Bachler et. al. have included oral exposure of food-grade TiO_2 nanoparticles in their model to predict its ADME after oral ingestion.[131]

In conclusion, an estimation of dosimetry should be included in well-designed in vitro studies. Interesting developments are the use of semi−wet culturing protocols[111,120] or reversed exposure models aiming to increase the cellular contacts with the low density nano- and microplastics.[92,118,119] Reliable data on intestinal uptake are important to be able to predict in vivo uptake kinetics. Some studies have demonstrated the feasibility of nanoparticle (or plastic)-specific PBK models. Clearly, mechanistic knowledge on nanoplastic transport is very limited and current PBK models are still heavily depending on in vivo data for parameterization. The lack of in vitro parameterization of nanoplastic-PBK models hinders extrapolation to nanoplastic that lack in vivo organ concentrations and complicates extrapolation to humans where such data is exceedingly scarce. Nevertheless one promising study successfully showed interspecies extrapolation of a rodent and pig PBK models to the human situation.[134]

40.1.6 Effects of nano - and microplastics in vivo

The number of published oral exposure rodent in vivo studies increased rapidly in the past few years. Several studies focused on potential effects on the reproductive system of male[135−137] or female rodents,[138−140] potential cardiotoxicity,[141,142] and effects on the thyroid[140] and intestine.[142] Yet the results of these studies need to be interpreted with caution as mostly PS materials have been studied, and limited data on the characterization of the used microplastics is provided. Minimal characterization data needs from the field of engineered nanotoxicology[36] should be applied also in studying and reporting effects of nano- and microplastics. Most of these recent rodents studies have methodological issues in terms of the quality of the methods and approaches used in the histopathology, where essential controls are missing or blind scoring schemes appear not to be used by the authors. Yet data on increased cytokine production as observed in some of these studies might point toward adverse effects. Limited experimental data is available on the concentrations of the nano- and micro particles in tissues (as discussed above), which is needed to better relate the observed effects to the concentration of the microparticles in vivo.

Micro and nanoplastic toxicity studies thus far have used engineered nanospheres which have a monodisperse size, shape and single polymer composition. It is challenging to extrapolate these results to the real-life situation where humans are exposed to a highly heterogenous mixture of micro and nanoplastics' sizes, shapes, and composition. Interesting approaches have been proposed in which the reported environmental concentration of any given microplastic size range can be scaled to other size ranges by assuming that microplastic concentration

follows a power-law function in relation to their size.[143] This method could be used to extrapolate microplastic concentrations used in toxicological studies to true environmental concentrations, that are more relevant for human exposure. Further research is needed to define methods to reliably relate single microplastic type toxicodynamic observations to the complex environmentally realistic exposure scenarios.

40.1.7 Conclusions and future outlook

Direct exposure to nano- and microplastics via ingestion (and indirectly via swallowed particles trapped in lung mucus) is an inevitable consequence of widespread occurrence of nano- and microplastics in the environment. However, the extent of ingestion and the potential risks this exposure affects humans remains poorly defined. The recently published rodent studies might be helpful to gain more insights into this, but studies of higher quality are needed. This mainly relates to an adequate characterization of the nano- and microplastics used, and the assessment of particle concentrations at the target organ.

To understand the mechanism of uptake of nano- and microplastics in vitro studies are useful. For this several in vitro models of the gastrointestinal epithelium have been developed, ranging from layers of a single cell type (often CaCO-2 cells) to more complex cocultures that for example incorporate M-cells and mucus secreting cells. While these models aim to reproduce the complex biology of the intestinal epithelium, the design and dosimetry of the nano- and microplastics exposure conditions needs careful attention.

We have observed interesting developments in novel designs of in vitro experiments, such as semiwet or reversed exposure study designs which can solve the issues related to limited nano- and microplastic cell contact due to low density and buoyancy of some nano- and microplastics. Recent innovations toward microfluidic experimental models might further improve the relevance of the exposure conditions. These experimental innovations need to be embedded in the design and data needs for particle kinetic and dynamic modeling of nano- and microplastics to extrapolate data from in vitro to in vivo.

Currently, reported in vivo rodent and human in vivo data suggests limited oral bioavailability of nano- and microplastics. However caution is needed here as only limited types of nano- and microplastics have been studied in vivo and reported intestinal uptake is highly variable. The limited human studies point towards the presence of nano- and microplastics in tissues, suggesting that uptake is possible. Also data humans exposed to (ultra) fine dust indicate that systemic uptake of particulate matter is possible especially following lifelong exposure.

The number of toxicological studies in which rodents have been exposed to nano- and microplastics is increasing rapidly. Potential effects on reproductive system, heart, thyroid and intestine have been reported. Most of these studies only used PS microplastics and often limited data on the characterization of the microplastics used is provided. Yet data on increased cytokine production as observed in some of the studies might point towards effects related to immunotoxicity. Clearly further toxicological studies are warranted and on a wider range of materials.

Lastly methodology needs to be developed in order to be able to extrapolate the observations from single nano- and microplastic types in vitro and in vivo studies to the complex environmental exposure conditions as seen in real life.

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