

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

Present Knowledge in Food Safety

Brouwer, Hugo; van Oijen, Femke L.N.; Bouwmeester, Hans

<https://doi.org/10.1016/B978-0-12-819470-6.00014-7>

This publication is made publicly available in the institutional repository of Wageningen University and Research, under the terms of article 25fa of the Dutch Copyright Act, also known as the Amendment Taverne.

Article 25fa states that the author of a short scientific work funded either wholly or partially by Dutch public funds is entitled to make that work publicly available for no consideration following a reasonable period of time after the work was first published, provided that clear reference is made to the source of the first publication of the work.

This publication is distributed using the principles as determined in the Association of Universities in the Netherlands (VSNU) 'Article 25fa implementation' project. According to these principles research outputs of researchers employed by Dutch Universities that comply with the legal requirements of Article 25fa of the Dutch Copyright Act are distributed online and free of cost or other barriers in institutional repositories. Research outputs are distributed six months after their first online publication in the original published version and with proper attribution to the source of the original publication.

You are permitted to download and use the publication for personal purposes. All rights remain with the author(s) and / or copyright owner(s) of this work. Any use of the publication or parts of it other than authorised under article 25fa of the Dutch Copyright act is prohibited. Wageningen University & Research and the author(s) of this publication shall not be held responsible or liable for any damages resulting from your (re)use of this publication.

For questions regarding the public availability of this publication please contact openaccess.library@wur.nl

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

Hugo Brouwer, Femke L.N. Van Oijen and Hans Bouwmeester

Division of Toxicology, Wageningen University, Wageningen, The Netherlands

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 terephthalate (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.

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 (SiO₂), titanium dioxide (TiO₂) 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 general⁵¹ 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 nanomaterials⁵⁵ as, also there, the interactions with the intestinal microbiome have not been studied in detail (as recently reviewed⁵⁶). 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 cells⁵⁹ 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 CeO₂, TiO₂, ZnO nanomaterials in a model colon reactor resulted in a decreased butyrate production upon incubation with CeO₂ 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 transcytotic 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 variations 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 TiO₂ 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 TiO₂ nanoparticles (mean size 260 nm), TiO₂ 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 TiO₂ after ingestion of 160 and 380 nm TiO₂ nanoparticles.⁹⁶ The presence of reflective particles in blood was interpreted as evidence of the presence of TiO₂ 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/mm³ 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 ($0.63\text{--}2.42 \mu\text{g}/\text{m}^3$).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 calculated⁹⁸ 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 Authority¹⁰⁰ 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 microplastics 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 µg/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 µg/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 µg/cm ² in HT29MTX-E12: WST-1 and LDH							
50 µg/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 h 10–20 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 5 µm no effects	LY transport	CaCO-2 cells (exposure with and without in vitro digestion)	104
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 500 nm 120 µg/mL 1 µm 120 µg/mL 3 µm 120 µg/mL	DCFH-DA	CaCO-2	102
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
<i>(Continued)</i>						

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	IL-8 and MCP-1	CaCO-2 cells (exposure with and without in vitro digestion)	104
			5 µm no effect			
PS	50 nm	0, 10, and 50 µg/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 µg/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 µg/cm ² for 24 h	50 µg/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 µg/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 µg/cm ² for 24 h	50 µg/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 µg/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 µg/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	Q-RT-PCR	CaCO-2 cells (exposure with and without in vitro digestion)	104
			5 µm no effect			

PS	5 μ m	0, 12.5, and 50 mg/L for 24 h	NF- κ B, 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 μ g/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 μ g/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 μ g/mL for 24 h	Increased mitochondrial activity at concentrations \geq 25 μ g/mL	Mitoprobe TMRM assay	CaCO-2 cells	106
PS	300 nm; 500 nm; 1 μ m; 3 μ m; 6 μ m	120 μ g/mL for 24 h	500 nm 120 μ g/mL	JC-1 assay kit	CaCO-2	102
			1 μ m 120 μ g/mL			
			3 μ m 120 μ g/mL			
			6 μ m 120 μ g/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 ones¹⁰² (Table 40.1). Interestingly also an effect on the mitochondrial membrane has been shown¹⁰² (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., Raji-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 insensitive 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 algorithm¹²¹ 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 TiO₂ 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 thyroid¹⁴⁰ 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 nanotoxicology³⁶ should be applied also in studying and reporting effects of nano- and microplastics. Most of these recent rodent 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 heterogeneous 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.

Acknowledgments

Funding for our research in this area has been provided by ZonMW (Project number: 458001001) and EU-H2020 Plasticheal (Grant agreement ID: 965196).

References

1. Geyer R, Jambeck JR, Law KL. Production, use, and fate of all plastics ever made. *Sci Adv*. 2017;3(7):e1700782. Available from: <https://doi.org/10.1126/sciadv.1700782>.
2. Rubio L, Marcos R, Hernandez A. Potential adverse health effects of ingested micro- and nanoplastics on humans. Lessons learned vivo vitro mammalian model. *J Toxicol Environ Health B Crit Rev*. 2020;23(2):51–68. Available from: <https://doi.org/10.1080/10937404.2019.1700598>.
3. PlasticsEurope. *Plastics—the Facts 2020*. Brussels: Association of Plastics Manufacturers; 2020.
4. Cole M, Lindeque P, Halsband C, Galloway TS. Microplastics as contaminants in the marine environment: a review. *Mar Pollut Bull*. 2011;62(12):2588–2597. Available from: <https://doi.org/10.1016/j.marpolbul.2011.09.025>.
5. Jambeck JR, Geyer R, Wilcox C, et al. Marine pollution. Plastic waste inputs from land into the ocean. *Science*. 2015;347(6223):768–771. Available from: <https://doi.org/10.1126/science.1260352>.
6. Fendall LS, Sewell MA. Contributing to marine pollution by washing your face: microplastics in facial cleansers. *Mar Pollut Bull*. 2009;58(8):1225–1228. Available from: <https://doi.org/10.1016/j.marpolbul.2009.04.025>.
7. Alexander J, Barregard L, Bignami M, et al. Presence of microplastics and nanoplastics in food, with particular focus on seafood. *EFSA J*. 2016;14(6). Available from: <https://doi.org/10.2903/j.efsa.2016.4501>.
8. Bouwmeester H, Hollman PC, Peters RJ. Potential health impact of environmentally released micro- and nanoplastics in the human food production chain: experiences from nanotoxicology. *Environ Sci Technol*. 2015;49(15):8932–8947. Available from: <https://doi.org/10.1021/acs.est.5b01090>.

9. Cozar A, Echevarria F, Gonzalez-Gordillo JI, et al. Plastic debris in the open ocean. *Proc Natl Acad Sci U S A*. 2014;111(28):10239–10244. Available from: <https://doi.org/10.1073/pnas.1314705111>.
10. Kuhn S, van Oyen A, Bravo Rebolledo EL, Ask AV, van Franeker JA. Polymer types ingested by northern fulmars (*Fulmarus glacialis*) and southern hemisphere relatives. Article. *Environ Sci Pollut Res Int*. 2021;28(2):1643–1655. Available from: <https://doi.org/10.1007/s11356-020-10540-6>.
11. Kuhn S, van Oyen A, Booth AM, Meijboom A, van Franeker JA. Marine microplastic: preparation of relevant test materials for laboratory assessment of ecosystem impacts. *Artic Chemosphere*. 2018;213:103–113. Available from: <https://doi.org/10.1016/j.chemosphere.2018.09.032>.
12. Abidli S, Antunes JC, Ferreira JL, Lahbib Y, Sobral P, El Menif NT. Microplastics in sediments from the littoral zone of the north Tunisian coast (Mediterranean Sea). *Estuar Coast Shelf S*. 2018;205:1–9. Available from: <https://doi.org/10.1016/j.ecss.2018.03.006>.
13. Obbard RW, Sadri S, Wong YQ, Khitun AA, Baker I, Thompson RC. Global warming releases microplastic legacy frozen in Arctic Sea ice. *Earths Future*. 2014;2(6):315–320. Available from: <https://doi.org/10.1002/2014ef000240>.
14. Kanhai DK, Officer R, Lyashevskaya O, Thompson RC, O'Connor I. Microplastic abundance, distribution and composition along a latitudinal gradient in the Atlantic Ocean. *Mar Pollut Bull*. 2017;115(1–2):307–314. Available from: <https://doi.org/10.1016/j.marpolbul.2016.12.025>.
15. Horton AA, Svendsen C, Williams RJ, Spurgeon DJ, Lahive E. Large microplastic particles in sediments of tributaries of the River Thames, UK—abundance, sources and methods for effective quantification. *Mar Pollut Bull*. 2017;114(1):218–226. Available from: <https://doi.org/10.1016/j.marpolbul.2016.09.004>.
16. Zimmermann L, Dierkes G, Ternes TA, Volker C, Wagner M. Benchmarking the in vitro toxicity and chemical composition of plastic consumer products. *Environ Sci Technol*. 2019;53(19):11467–11477. Available from: <https://doi.org/10.1021/acs.est.9b02293>.
17. Hahladakis JN, Velis CA, Weber R, Iacovidou E, Purnell P. An overview of chemical additives present in plastics: migration, release, fate and environmental impact during their use, disposal and recycling. *J Hazard Mater*. 2018;344:179–199. Available from: <https://doi.org/10.1016/j.jhazmat.2017.10.014>.
18. Brennecke D, Duarte B, Paiva F, Cacador I, Canning-Clode J. Microplastics as vector for heavy metal contamination from the marine environment. *Estuar Coast Shelf Sci*. 2016;178:189–195. Available from: <https://doi.org/10.1016/j.ecss.2015.12.003>.
19. Koelmans AA, Bakir A, Burton GA, Janssen CR. Microplastic as a vector for chemicals in the aquatic environment: critical review and model-supported reinterpretation of empirical studies. *Environ Sci Technol*. 2016;50(7):3315–3326. Available from: <https://doi.org/10.1021/acs.est.5b06069>.
20. Li J, Zhang K, Zhang H. Adsorption of antibiotics on microplastics. *Environ Pollut*. 2018;237:460–467. Available from: <https://doi.org/10.1016/j.envpol.2018.02.050>.
21. Mato Y, Isobe T, Takada H, Kanehiro H, Ohtake C, Kaminuma T. Plastic resin pellets as a transport medium for toxic chemicals in the marine environment. *Environ Sci Technol*. 2001;35(2):318–324. Available from: <https://doi.org/10.1021/es0010498>.
22. Mendoza LMR, Jones PR. Characterisation of microplastics and toxic chemicals extracted from microplastic samples from the North Pacific Gyre. *Environ Chem*. 2015;12(5):611–617. Available from: <https://doi.org/10.1071/En14236>.
23. Rochman CM, Kurobe T, Flores I, Teh SJ. Early warning signs of endocrine disruption in adult fish from the ingestion of polyethylene with and without sorbed chemical pollutants from the marine environment. *Sci Total Environ*. 2014;493:656–661. Available from: <https://doi.org/10.1016/j.scitotenv.2014.06.051>.
24. Rodrigues JP, Duarte AC, Santos-Echeandia J, Rocha-Santos T. Significance of interactions between microplastics and POPs in the marine environment: a critical overview. *Trac-Trend Anal Chem*. 2019;111:252–260. Available from: <https://doi.org/10.1016/j.trac.2018.11.038>.
25. Wang W, Wang J. Different partition of polycyclic aromatic hydrocarbon on environmental particulates in freshwater: microplastics in comparison to natural sediment. *Ecotoxicol Environ Saf*. 2018;147:648–655. Available from: <https://doi.org/10.1016/j.ecoenv.2017.09.029>.
26. Wang Z, Chen M, Zhang L, et al. Sorption behaviors of phenanthrene on the microplastics identified in a mariculture farm in Xiangshan Bay, southeastern China. *Sci Total Environ*. 2018;628–629:1617–1626. Available from: <https://doi.org/10.1016/j.scitotenv.2018.02.146>.
27. Wu P, Huang J, Zheng Y, et al. Environmental occurrences, fate, and impacts of microplastics. *Ecotoxicol Environ Saf*. 2019;184:109612. Available from: <https://doi.org/10.1016/j.ecoenv.2019.109612>.
28. Wang Y, Huang J, Zhu F, Zhou S. Airborne microplastics: a review on the occurrence, migration and risks to humans. *Bull Environ Contam Toxicol*. 2021;. Available from: <https://doi.org/10.1007/s00128-021-03180-0>.
29. Rahman A, Sarkar A, Yadav OP, Achari G, Slobodnik J. Potential human health risks due to environmental exposure to nano- and microplastics and knowledge gaps: a scoping review. *Sci Total Environ*. 2021;757:143872. Available from: <https://doi.org/10.1016/j.scitotenv.2020.143872>.
30. Bouwmeester H, Brandhoff P, Marvin HJP, Weigel S, Peters RJB. State of the safety assessment and current use of nanomaterials in food and food production. *Trends Food Sci Tech*. 2014;40(2):200–210. Available from: <https://doi.org/10.1016/j.tifs.2014.08.009>.
31. Van Raamsdonk LWD, van der Zande M, Koelmans AA, et al. Current insights into monitoring, bioaccumulation, and potential health effects of microplastics present in the food chain. *Foods*. 2020;9(1):72. Available from: <https://doi.org/10.3390/foods9010072>.
32. Vethaak AD, Legler J. Microplastics and human health. *Science*. 2021;371(6530):672–674. Available from: <https://doi.org/10.1126/science.abe5041>.
33. Kelly FJ, Fussell JC. Toxicity of airborne particles—established evidence, knowledge gaps and emerging areas of importance. *Philos Trans A Math Phys Eng Sci*. 2020;378(2183):20190322. Available from: <https://doi.org/10.1098/rsta.2019.0322>.
34. Riediker M, Zink D, Kreyling W, et al. Particle toxicology and health—where are we? *Part Fibre Toxicol*. 2019;16(1):19. Available from: <https://doi.org/10.1186/s12989-019-0302-8>.
35. Bouwmeester H, Dekkers S, Noordam MY, et al. Review of health safety aspects of nanotechnologies in food production. *Regul*

- Toxicol Pharmacol.* 2009;53(1):52–62. Available from: <https://doi.org/10.1016/j.yrtph.2008.10.008>.
36. Bouwmeester H, Lynch I, Marvin HJ, et al. Minimal analytical characterization of engineered nanomaterials needed for hazard assessment in biological matrices. *Nanotoxicology.* 2011;5(1):1–11. Available from: <https://doi.org/10.3109/17435391003775266>.
 37. Harisa GI, Badran MM, Alanazi FK, Attia SM. An overview of nanosomes delivery mechanisms: trafficking, orders, barriers and cellular effects. *Artif Cell Nanomed Biotechnol.* 2018;46(4):669–679. Available from: <https://doi.org/10.1080/21691401.2017.1354301>.
 38. Weir A, Westerhoff P, Fabricius L, Hristovski K, Von Goetz N. Titanium dioxide nanoparticles in food and personal care products. *Environ Sci Technol.* 2012;46(4):2242–2250. Available from: <https://doi.org/10.1021/es204168d>.
 39. Peters RJ, van Bemmel G, Herrera-Rivera Z, et al. Characterization of titanium dioxide nanoparticles in food products: analytical methods to define nanoparticles. *J Agric Food Chem.* 2014;62(27):6285–6293. Available from: <https://doi.org/10.1021/jf5011885>.
 40. Peters R, Kramer E, Oomen AG, et al. Presence of nano-sized silica during in vitro digestion of foods containing silica as a food additive. *ACS Nano.* 2012;6(3):2441–2451. Available from: <https://doi.org/10.1021/nn204728k>.
 41. Taboada-López MV, Alonso-Sejío N, Herbello-Hermelo P, Bermejo-Barrera P, Moreda-Piñeiro A. Determination and characterization of silver nanoparticles in bivalve molluscs by ultrasound assisted enzymatic hydrolysis and sp-ICP-MS. *Microchem J.* 2019;148:652–660. Available from: <https://doi.org/10.1016/j.microc.2019.05.023>.
 42. Bellmann S, Carlander D, Fasano A, et al. Mammalian gastrointestinal tract parameters modulating the integrity, surface properties, and absorption of food-relevant nanomaterials. *Wiley Interdiscip Rev Nanomed Nanobiotechnol.* 2015;7(5):609–622. Available from: <https://doi.org/10.1002/wnan.1333>.
 43. Walczak AP, Fokkink R, Peters R, et al. Behaviour of silver nanoparticles and silver ions in an in vitro human gastrointestinal digestion model. *Nanotoxicology.* 2013;7(7):1198–1210. Available from: <https://doi.org/10.3109/17435390.2012.726382>.
 44. Abdelkhalik A, van der Zande M, Punt A, et al. Impact of nanoparticle surface functionalization on the protein corona and cellular adhesion, uptake and transport. *J Nanobiotechnol.* 2018;16(1):70. Available from: <https://doi.org/10.1186/s12951-018-0394-6>.
 45. Van Der Zande M, Vandebriel RJ, Groot MJ, et al. Sub-chronic toxicity study in rats orally exposed to nanostructured silica. *Part Fibre Toxicol.* 2014;11(1):8. Available from: <https://doi.org/10.1186/1743-8977-11-8>.
 46. Lundqvist M, Stigler J, Elia G, Lynch I, Cedervall T, Dawson KA. Nanoparticle size and surface properties determine the protein corona with possible implications for biological impacts. *Proc Natl Acad Sci U S A.* 2008;105(38):14265–14270. Available from: <https://doi.org/10.1073/pnas.0805135105>.
 47. Lesniak A, Fenaroli F, Monopoli MP, Aberg C, Dawson KA, Salvati A. Effects of the presence or absence of a protein corona on silica nanoparticle uptake and impact on cells. *ACS Nano.* 2012;6(7):5845–5857. Available from: <https://doi.org/10.1021/nn300223w>.
 48. Docter D, Distler U, Storck W, et al. Quantitative profiling of the protein coronas that form around nanoparticles. *Nat Protoc.* 2014;9(9):2030–2044. Available from: <https://doi.org/10.1038/nprot.2014.139>.
 49. Tenzer S, Docter D, Kuharev J, et al. Rapid formation of plasma protein corona critically affects nanoparticle pathophysiology. *Nat Nanotechnol.* 2013;8(10):772–781. Available from: <https://doi.org/10.1038/nnano.2013.181>.
 50. Abdelkhalik A, van der Zande M, Undas AK, Peters RJB, Bouwmeester H. Impact of in vitro digestion on gastrointestinal fate and uptake of silver nanoparticles with different surface modifications. *Nanotoxicology.* 2020;14(1):111–126. Available from: <https://doi.org/10.1080/17435390.2019.1675794>.
 51. Fan Y, Pedersen O. Gut microbiota in human metabolic health and disease. *Nat Rev Microbiol.* 2021;19(1):55–71. Available from: <https://doi.org/10.1038/s41579-020-0433-9>.
 52. van Dongen KCW, van der Zande M, Bruyneel B, et al. An in vitro model for microbial fructoselysine degradation shows substantial inter individual differences in metabolic capacities of human fecal slurries. *Toxicol In Vitro.* 2021;72:105078. Available from: <https://doi.org/10.1016/j.tiv.2021.105078>.
 53. Jin J, Beekmann K, Ringo E, Rietjens IMCM, Xing FG. Interaction between food-borne mycotoxins and gut microbiota: a review. *Rev Food Control.* 2021;126:107998. Available from: <https://doi.org/10.1016/j.foodcont.2021.107998>.
 54. Bouwmeester H, Van Der Zande M, Jepson MA. Effects of food-borne nanomaterials on gastrointestinal tissues and microbiota. *Wiley Interdiscip Rev Nanomed Nanobiotechnol.* 2018;10(1):e1481. Available from: <https://doi.org/10.1002/wnan.1481>.
 55. Pietriousti A, Magrini A, Campagnolo L. New frontiers in nanotoxicology: gut microbiota/microbiome-mediated effects of engineered nanomaterials. *Toxicol Appl Pharmacol.* 2016;299:90–95. Available from: <https://doi.org/10.1016/j.taap.2015.12.017>.
 56. Lamas B, Martins Breyner N, Houdeau E. Impacts of foodborne inorganic nanoparticles on the gut microbiota-immune axis: potential consequences for host health. *Part Fibre Toxicol.* 2020;17(1):19. Available from: <https://doi.org/10.1186/s12989-020-00349-z>.
 57. Huang ZZ, Weng Y, Shen QC, Zhao Y, Jin YX. Microplastic: a potential threat to human and animal health by interfering with the intestinal barrier function and changing the intestinal microenvironment. *Sci Total Environ.* 2021;785. Available from: <https://doi.org/10.1016/j.scitotenv.2021.147365>.
 58. Frohlich EE, Frohlich E. Cytotoxicity of nanoparticles contained in food on intestinal cells and the gut microbiota. *Int J Mol Sci.* 2016;17(4):509. Available from: <https://doi.org/10.3390/ijms17040509>.
 59. Donohoe DR, Garge N, Zhang X, et al. The microbiome and butyrate regulate energy metabolism and autophagy in the mammalian colon. *Cell Metab.* 2011;13(5):517–526. Available from: <https://doi.org/10.1016/j.cmet.2011.02.018>.
 60. Kish L, Hotte N, Kaplan GG, et al. Environmental particulate matter induces murine intestinal inflammatory responses and alters the gut microbiome. *PLoS One.* 2013;8(4):e62220. Available from: <https://doi.org/10.1371/journal.pone.0062220>.
 61. Jin Y, Lu L, Tu W, Luo T, Fu Z. Impacts of polystyrene microplastic on the gut barrier, microbiota and metabolism of mice. *Sci Total Environ.* 2019;649:308–317. Available from: <https://doi.org/10.1016/j.scitotenv.2018.08.353>.
 62. Lu L, Wan Z, Luo T, Fu Z, Jin Y. Polystyrene microplastics induce gut microbiota dysbiosis and hepatic lipid metabolism disorder in mice. *Sci Total Environ.* 2018;631–632:449–458. Available from: <https://doi.org/10.1016/j.scitotenv.2018.03.051>.

63. Taylor AA, Marcus IM, Guysi RL, Walker SL. Metal oxide nanoparticles induce minimal phenotypic changes in a model colon gut microbiota. *Environ Eng Sci*. 2015;32(7):602–612. Available from: <https://doi.org/10.1089/ees.2014.0518>.
64. Zoetendal EG, Raes J, Van Den Bogert B, et al. The human small intestinal microbiota is driven by rapid uptake and conversion of simple carbohydrates. *ISME J*. 2012;6(7):1415–1426. Available from: <https://doi.org/10.1038/ismej.2011.212>.
65. Braakhuis HM, Kloet SK, Kezic S, et al. Progress and future of in vitro models to study translocation of nanoparticles. *Arch Toxicol*. 2015;89(9):1469–1495. Available from: <https://doi.org/10.1007/s00204-015-1518-5>.
66. Lefebvre DE, Venema K, Gombau L, et al. Utility of models of the gastrointestinal tract for assessment of the digestion and absorption of engineered nanomaterials released from food matrices. *Nanotoxicology*. 2015;9(4):523–542. Available from: <https://doi.org/10.3109/17435390.2014.948091>.
67. Behrens I, Pena AI, Alonso MJ, Kissel T. Comparative uptake studies of bioadhesive and non-bioadhesive nanoparticles in human intestinal cell lines and rats: the effect of mucus on particle adsorption and transport. *Pharm Res*. 2002;19(8):1185–1193. Available from: <https://doi.org/10.1023/a:1019854327540>.
68. Szentkuti L, Lorenz K. The thickness of the mucus layer in different segments of the rat intestine. *Histochem J*. 1995;27(6):466–472.
69. Frey A, Giannasca KT, Weltzin R, et al. Role of the glycocalyx in regulating access of microparticles to apical plasma membranes of intestinal epithelial cells: implications for microbial attachment and oral vaccine targeting. *J Exp Med*. 1996;184(3):1045–1059. Available from: <https://doi.org/10.1084/jem.184.3.1045>.
70. Clark MA, Jepson MA, Hirst BH. Exploiting M cells for drug and vaccine delivery. *Adv Drug Deliv Rev*. 2001;50(1–2):81–106. Available from: [https://doi.org/10.1016/s0169-409x\(01\)00149-1](https://doi.org/10.1016/s0169-409x(01)00149-1).
71. Neutra MR, Frey A, Kraehenbuhl JP. Epithelial M cells: gateways for mucosal infection and immunization. *Cell*. 1996;86(3):345–348. Available from: [https://doi.org/10.1016/s0092-8674\(00\)80106-3](https://doi.org/10.1016/s0092-8674(00)80106-3).
72. Mahler GJ, Shuler ML, Glahn RP. Characterization of CaCO-2 and HT29-MTX cocultures in an in vitro digestion/cell culture model used to predict iron bioavailability. *J Nutritional Biochem*. 2009;20(7):494–502. Available from: <https://doi.org/10.1016/j.jnutbio.2008.05.006>.
73. Natoli M, Leoni BD, D'Agnano I, Zucco F, Felsani A. Good CaCO-2 cell culture practices. *Toxicol In Vitro*. 2012;26(8):1243–1246. Available from: <https://doi.org/10.1016/j.tiv.2012.03.009>.
74. Nkabinde LA, Shoba-Zikhali LN, Semete-Makokotlela B, et al. Permeation of PLGA nanoparticles across different in vitro models. *Curr Drug Delivery*. 2012;9(6):617–627. Available from: <https://doi.org/10.2174/156720112803529819>.
75. Koeneman BA, Zhang Y, Westerhoff P, Chen Y, Crittenden JC, Capco DG. Toxicity and cellular responses of intestinal cells exposed to titanium dioxide. *Cell Biol Toxicol*. 2010;26(3):225–238. Available from: <https://doi.org/10.1007/s10565-009-9132-z>.
76. Pereira DI, Bruggraber SF, Faria N, et al. Nanoparticulate iron(III) oxo-hydroxide delivers safe iron that is well absorbed and utilised in humans. *Nanomedicine*. 2014;10(8):1877–1886. Available from: <https://doi.org/10.1016/j.nano.2014.06.012>.
77. Song ZM, Chen N, Liu JH, et al. Biological effect of food additive titanium dioxide nanoparticles on intestine: an in vitro study. *J Appl Toxicol*. 2015;35(10):1169–1178. Available from: <https://doi.org/10.1002/jat.3171>.
78. Gaiser BK, Fernandes TF, Jepson M, Lead JR, Tyler CR, Stone V. Assessing exposure, uptake and toxicity of silver and cerium dioxide nanoparticles from contaminated environments. *Environ Health*. 2009;8(1):S2. Available from: <https://doi.org/10.1186/1476-069X-8-S1-S2>.
79. Howe SE, Lickteig DJ, Plunkett KN, Ryerse JS, Konjufca V. The uptake of soluble and particulate antigens by epithelial cells in the mouse small intestine. *PLoS One*. 2014;9(1):e86656. Available from: <https://doi.org/10.1371/journal.pone.0086656>.
80. De Jesus M, Ostroff GR, Levitz SM, Bartling TR, Mantis NJ. A population of Langerin-positive dendritic cells in murine Peyer's patches involved in sampling beta-glucan microparticles. *PLoS One*. 2014;9(3):e91002. Available from: <https://doi.org/10.1371/journal.pone.0091002>.
81. Powell JJ, Thomas-McKay E, Thoree V, et al. An endogenous nanomineral chaperones luminal antigen and peptidoglycan to intestinal immune cells. *Nat Nanotechnol*. 2015;10(4):361–369. Available from: <https://doi.org/10.1038/nnano.2015.19>.
82. Delon L, Gibson RJ, Prestidge CA, Thierry B. Mechanisms of uptake and transport of particulate formulations in the small intestine. *J Control Release*. 2022;343:584–599. Available from: <https://doi.org/10.1016/j.jconrel.2022.02.006>. 35149142.
83. McClean S, Prosser E, Meehan E, et al. Binding and uptake of biodegradable poly-DL-lactide micro- and nanoparticles in intestinal epithelia. *Eur J Pharm Sci*. 1998;6(2):153–163. Available from: [https://doi.org/10.1016/s0928-0987\(97\)10007-0](https://doi.org/10.1016/s0928-0987(97)10007-0).
84. Hillery AM, Jani PU, Florence AT. Comparative, quantitative study of lymphoid and non-lymphoid uptake of 60 nm polystyrene particles. *J Drug Target*. 1994;2(2):151–156. Available from: <https://doi.org/10.3109/10611869409015904>.
85. Jani P, Halbert GW, Langridge J, Florence AT. Nanoparticle uptake by the rat gastrointestinal mucosa: quantitation and particle size dependency. *J Pharm Pharmacol*. 1990;42(12):821–826. Available from: <https://doi.org/10.1111/j.2042-7158.1990.tb07033.x>.
86. Jani PU, McCarthy DE, Florence AT. Nanosphere and microsphere uptake via peyer patches - observation of the rate of uptake in the rat after a single oral dose. *Int J Pharm*. 1992;86(2–3):239–246. Available from: [https://doi.org/10.1016/0378-5173\(92\)90202-D](https://doi.org/10.1016/0378-5173(92)90202-D).
87. Berg RD. Bacterial translocation from the gastrointestinal tract. *J Med*. 1992;23(3–4):217–244.
88. Berg RD. Bacterial translocation from the gastrointestinal tract. *Adv Exp Med Biol*. 1999;473:11–30. Available from: https://doi.org/10.1007/978-1-4615-4143-1_2.
89. Moyes SM, Morris JF, Carr KE. Culture conditions and treatments affect CaCO-2 characteristics and particle uptake. *Int J Pharm*. 2010;387(1–2):7–18. Available from: <https://doi.org/10.1016/j.ijpharm.2009.11.027>.
90. Moyes SM, Killick EM, Morris JF, Kadhim MA, Hill MA, Carr KE. Changes produced by external radiation in parameters influencing intestinal permeability and microparticle uptake in vitro. *Int J Radiat Biol*. 2008;84(6):467–486. Available from: <https://doi.org/10.1080/09553000802078388>.
91. Busch M, Bredeck G, Kampfer AAM, Schins RPF. Investigations of acute effects of polystyrene and polyvinyl chloride micro- and

- nanoplastics in an advanced in vitro triple culture model of the healthy and inflamed intestine. *Environ Res.* 2021;193:110536. Available from: <https://doi.org/10.1016/j.envres.2020.110536>.
92. Busch M, Kampfer AAM, Schins RPF. An inverted in vitro triple culture model of the healthy and inflamed intestine: adverse effects of polyethylene particles. *Chemosphere.* 2021;284:131345. Available from: <https://doi.org/10.1016/j.chemosphere.2021.131345>.
 93. Ragusa A, Svelato A, Santacroce C, et al. Plasticenta: first evidence of microplastics in human placenta. *Environ Int.* 2021;146:106274. Available from: <https://doi.org/10.1016/j.envint.2020.106274>.
 94. Jones K, Morton J, Smith I, Jurkschat K, Harding AH, Evans G. Human in vivo and in vitro studies on gastrointestinal absorption of titanium dioxide nanoparticles. *Toxicol Lett.* 2015;233(2):95–101. Available from: <https://doi.org/10.1016/j.toxlet.2014.12.005>.
 95. Pele LC, Thoree V, Brugger SF, et al. Pharmaceutical/food grade titanium dioxide particles are absorbed into the bloodstream of human volunteers. *Part Fibre Toxicol.* 2015;12(1):26. Available from: <https://doi.org/10.1186/s12989-015-0101-9>.
 96. Bockmann J, Lahl H, Eckhart T, Unterhalt B. Blood levels of titanium before and after oral administration of titanium dioxide. *Pharmazie.* 2000;55(2):140–143.
 97. Bove H, Bongaerts E, Slenders E, et al. Ambient black carbon particles reach the fetal side of human placenta. *Nat Commun.* 2019;10(1):3866. Available from: <https://doi.org/10.1038/s41467-019-11654-3>.
 98. Walczak AP, Hendriksen PJ, Woutersen RA, et al. Bioavailability and biodistribution of differently charged polystyrene nanoparticles upon oral exposure in rats. *J Nanopart Res.* 2015;17(5):231. Available from: <https://doi.org/10.1007/s11051-015-3029-y>.
 99. Keinanen O, Dayts EJ, Rodriguez C, et al. Harnessing PET to track micro- and nanoplastics in vivo. *Sci Rep.* 2021;11(1):11463. Available from: <https://doi.org/10.1038/s41598-021-90929-6>.
 100. Hussain N, Jani PU, Florence AT. Enhanced oral uptake of tomato lectin-conjugated nanoparticles in the rat. *Pharm Res.* 1997;14(5):613–618. Available from: <https://doi.org/10.1023/a:1012153011884>.
 101. Fournier SB, D'Errico JN, Adler DS, et al. Nanopolystyrene translocation and fetal deposition after acute lung exposure during late-stage pregnancy. *Part Fibre Toxicol.* 2020;17(1):55. Available from: <https://doi.org/10.1186/s12989-020-00385-9>.
 102. Wang Q, Bai J, Ning B, et al. Effects of bisphenol A and nanoscale and microscale polystyrene plastic exposure on particle uptake and toxicity in human CaCO-2 cells. *Chemosphere.* 2020;254:126788. Available from: <https://doi.org/10.1016/j.chemosphere.2020.126788>.
 103. Walczak AP, Kramer E, Hendriksen PJ, et al. In vitro gastrointestinal digestion increases the translocation of polystyrene nanoparticles in an in vitro intestinal co-culture model. *Nanotoxicology.* 2015;9(7):886–894. Available from: <https://doi.org/10.3109/17435390.2014.988664>.
 104. Liu S, Wu X, Gu W, Yu J, Wu B. Influence of the digestive process on intestinal toxicity of polystyrene microplastics as determined by in vitro CaCO-2 models. *Chemosphere.* 2020;256:127204. Available from: <https://doi.org/10.1016/j.chemosphere.2020.127204>.
 105. Moyan H, Dušan P. Micro- and nano-plastics activation of oxidative and inflammatory adverse outcome pathways. *Redox Biol.* 2020;37:101620. Available from: <https://doi.org/10.1016/j.redox.2020.101620>.
 106. Cortés C, Domenech J, Salazar M, Pastor S, Marcos R, Hernández A. Nanoplastics as a potential environmental health factor: effects of polystyrene nanoparticles on human intestinal epithelial CaCO-2 cells. *Environ Sci: Nano.* 2020;7(1):272–285. Available from: <https://doi.org/10.1039/c9en00523d>.
 107. Domenech J, Hernandez A, Rubio L, Marcos R, Cortes C. Interactions of polystyrene nanoplastics with in vitro models of the human intestinal barrier. *Arch Toxicol.* 2020;94(9):2997–3012. Available from: <https://doi.org/10.1007/s00204-020-02805-3>.
 108. Stock V, Bohmert L, Lisicki E, et al. Uptake and effects of orally ingested polystyrene microplastic particles in vitro and in vivo. *Arch Toxicol.* 2019;93(7):1817–1833. Available from: <https://doi.org/10.1007/s00204-019-02478-7>.
 109. Magri D, Sanchez-Moreno P, Caputo G, et al. Laser ablation as a versatile tool to mimic polyethylene terephthalate nanoplastic pollutants: characterization and toxicology assessment. *ACS Nano.* 2018;12(8):7690–7700. Available from: <https://doi.org/10.1021/acsnano.8b01331>.
 110. Stock V, Laurisch C, Franke J, et al. Uptake and cellular effects of PE, PP, PET and PVC microplastic particles. *Toxicol In Vitro.* 2021;70:105021. Available from: <https://doi.org/10.1016/j.tiv.2020.105021>.
 111. Lehner R, Wohlleben W, Septiadi D, Landsiedel R, Petri-Fink A, Rothen-Rutishauser B. A novel 3D intestine barrier model to study the immune response upon exposure to microplastics. *Arch Toxicol.* 2020;94(7):2463–2479. Available from: <https://doi.org/10.1007/s00204-020-02750-1>.
 112. Micro- and nano-plastics activation of oxidative and inflammatory adverse outcome pathways. 2020.
 113. Brand W, Peters RJB, Braakhuis HM, Maslankiewicz L, Oomen AG. Possible effects of titanium dioxide particles on human liver, intestinal tissue, spleen and kidney after oral exposure. *Nanotoxicology.* 2020;14(7):985–1007. Available from: <https://doi.org/10.1080/17435390.2020.1778809>.
 114. Boyes WK, Van Thriel C. Neurotoxicology of nanomaterials. *Chem Res Toxicol.* 2020;33(5):1121–1144. Available from: <https://doi.org/10.1021/acs.chemrestox.0c00050>.
 115. Hirt N, Body-Malapel M. Immunotoxicity and intestinal effects of nano- and microplastics: a review of the literature. *Part Fibre Toxicol.* 2020;17(1):57. Available from: <https://doi.org/10.1186/s12989-020-00387-7>.
 116. Walczak AP, Kramer E, Hendriksen PJ, et al. Translocation of differently sized and charged polystyrene nanoparticles in in vitro intestinal cell models of increasing complexity. *Nanotoxicology.* 2015;9(4):453–461. Available from: <https://doi.org/10.3109/17435390.2014.944599>.
 117. Teeguarden JG, Hinderliter PM, Orr G, Thrall BD, Pounds JG. Particokinetics in vitro: dosimetry considerations for in vitro nanoparticle toxicity assessments. *Toxicol Sci.* 2007;95(2):300–312. Available from: <https://doi.org/10.1093/toxsci/kfl165>.
 118. Stock V, Bohmert L, Donmez MH, Lampen A, Sieg H. An inverse cell culture model for floating plastic particles. *Anal Biochem.* 2020;591:113545. Available from: <https://doi.org/10.1016/j.ab.2019.113545>.
 119. Watson CY, DeLoid GM, Pal A, Demokritou P. Buoyant nanoparticles: implications for nano-biointeractions in cellular studies. *Small.* 2016;12(23):3172–3180. Available from: <https://doi.org/10.1002/smll.201600314>.

120. Navabi N, McGuckin MA, Linden SK. Gastrointestinal cell lines form polarized epithelia with an adherent mucus layer when cultured in semi-wet interfaces with mechanical stimulation. *PLoS One*. 2013;8(7):e68761. Available from: <https://doi.org/10.1371/journal.pone.0068761>.
121. Hinderliter PM, Minard KR, Orr G, et al. ISDD: a computational model of particle sedimentation, diffusion and target cell dosimetry for in vitro toxicity studies. *Part Fibre Toxicol*. 2010;7(1):36. Available from: <https://doi.org/10.1186/1743-8977-7-36>.
122. DeLoid G, Cohen JM, Darrah T, et al. Estimating the effective density of engineered nanomaterials for in vitro dosimetry. *Nat Commun*. 2014;5:3514. Available from: <https://doi.org/10.1038/ncomms4514>.
123. DeLoid GM, Cohen JM, Pyrgiotakis G, et al. Advanced computational modeling for in vitro nanomaterial dosimetry. *Part Fibre Toxicol*. 2015;12:32. Available from: <https://doi.org/10.1186/s12989-015-0109-1>.
124. Thomas DG, Smith JN, Thrall BD, et al. ISD3: a particokinetic model for predicting the combined effects of particle sedimentation, diffusion and dissolution on cellular dosimetry for in vitro systems. *Part Fibre Toxicol*. 2018;15(1):6. Available from: <https://doi.org/10.1186/s12989-018-0243-7>.
125. Lee HA, Leavens TL, Mason SE, Monteiro-Riviere NA, Riviere JE. Comparison of quantum dot biodistribution with a blood-flow-limited physiologically based pharmacokinetic model. *Nano Lett*. 2009;9(2):794–799. Available from: <https://doi.org/10.1021/nl803481q>.
126. Deng L, Liu H, Ma Y, Miao Y, Fu X, Deng Q. Endocytosis mechanism in physiologically-based pharmacokinetic modeling of nanoparticles. *Toxicol Appl Pharmacol*. 2019;384:114765. Available from: <https://doi.org/10.1016/j.taap.2019.114765>.
127. Praetorius A, Tufenkji N, Goss KU, Scheringer M, Von Der Kammer F, Elimelech M. The road to nowhere: equilibrium partition coefficients for nanoparticles. *Environ Sci: Nano*. 2014;. Available from: <https://doi.org/10.1039/c4en00043a>.
128. Li M, Al-Jamal KT, Kostarelos K, Reineke J. Physiologically based pharmacokinetic modeling of nanoparticles. *ACS Nano*. 2010;. Available from: <https://doi.org/10.1021/nn1018818>.
129. Lin P, Chen JW, Chang LW, et al. Computational and ultrastructural toxicology of a nanoparticle, Quantum Dot 705, in mice. *Environ Sci Technol*. 2008;42(16):6264–6270. Available from: <https://doi.org/10.1021/es800254a>.
130. Bachler G, von Goetz N, Hungerbuhler K. A physiologically based pharmacokinetic model for ionic silver and silver nanoparticles. *Int J Nanomed*. 2013;8:3365–3382. Available from: <https://doi.org/10.2147/IJN.S46624>.
131. Bachler G, von Goetz N, Hungerbuhler K. Using physiologically based pharmacokinetic (PBPK) modeling for dietary risk assessment of titanium dioxide (TiO₂) nanoparticles. *Nanotoxicology*. 2015;9(3):373–380. Available from: <https://doi.org/10.3109/17435390.2014.940404>.
132. Carlander U, Li D, Jolliet O, Emond C, Johanson G. Toward a general physiologically-based pharmacokinetic model for intravenously injected nanoparticles. *Int J Nanomed*. 2016;11:625–640. Available from: <https://doi.org/10.2147/IJN.S94370>.
133. Li M, Panagi Z, Avgoustakis K, Reineke J. Physiologically based pharmacokinetic modeling of PLGA nanoparticles with varied mPEG content. *Int J Nanomed*. 2012;7:1345–1356. Available from: <https://doi.org/10.2147/IJN.S23758>.
134. Lin Z, Monteiro-Riviere NA, Kannan R, Riviere JE. A computational framework for interspecies pharmacokinetics, exposure and toxicity assessment of gold nanoparticles. *Nanomed (Lond)*. 2016;11(2):107–119. Available from: <https://doi.org/10.2217/nnm.15.177>.
135. Li S, Wang Q, Yu H, et al. Polystyrene microplastics induce blood-testis barrier disruption regulated by the MAPK-Nrf2 signaling pathway in rats. *Environ Sci Pollut Res Int*. 2021;324:75–85. Available from: <https://doi.org/10.1007/s11356-021-13911-9>.
136. Park EJ, Han JS, Park EJ, et al. Repeated-oral dose toxicity of polyethylene microplastics and the possible implications on reproduction and development of the next generation. *Toxicol Lett*. 2020;324:75–85. Available from: <https://doi.org/10.1016/j.toxlet.2020.01.008>.
137. Xie X, Deng T, Duan J, Xie J, Yuan J, Chen M. Exposure to polystyrene microplastics causes reproductive toxicity through oxidative stress and activation of the p38 MAPK signaling pathway. *Ecotoxicol Environ Saf*. 2020;190:110133. Available from: <https://doi.org/10.1016/j.ecoenv.2019.110133>.
138. Hou B, Wang F, Liu T, Wang Z. Reproductive toxicity of polystyrene microplastics: in vivo experimental study on testicular toxicity in mice. *J Hazard Mater*. 2021;405:124028. Available from: <https://doi.org/10.1016/j.jhazmat.2020.124028>.
139. An R, Wang X, Yang L, et al. Polystyrene microplastics cause granulosa cells apoptosis and fibrosis in ovary through oxidative stress in rats. *Toxicology*. 2021;449:152665. Available from: <https://doi.org/10.1016/j.tox.2020.152665>.
140. Amerah F, Eslami A, Fazelpour S, Rafiee M, Zibaii MI, Babaei M. Thyroid endocrine status and biochemical stress responses in adult male Wistar rats chronically exposed to pristine polystyrene nanoplastics. *Toxicol Res (Camb)*. 2019;8(6):953–963. Available from: <https://doi.org/10.1039/c9tx00147f>.
141. Wei J, Wang X, Liu Q, et al. The impact of polystyrene microplastics on cardiomyocytes pyroptosis through NLRP3/Caspase-1 signaling pathway and oxidative stress in Wistar rats. *Environ Toxicol*. 2021;36(5):935–944. Available from: <https://doi.org/10.1002/tox.23095>.
142. Li B, Ding Y, Cheng X, et al. Polyethylene microplastics affect the distribution of gut microbiota and inflammation development in mice. *Chemosphere*. 2020;244:125492. Available from: <https://doi.org/10.1016/j.chemosphere.2019.125492>.
143. Koelmans AA, Redondo-Hasselerharm PE, Mohamed Nor NH, Kooi M. Solving the nonalignment of methods and approaches used in microplastic research to consistently characterize risk. *Environ Sci Technol*. 2020;54(19):12307–12315. Available from: <https://doi.org/10.1021/acs.est.0c02982>.