Science for Communities

HEALTH RISK ASSESSMENT: MICROPLASTICS

DECEMBER 2022

Abhishek Gautam
Peter Cressey

ESR Risk Assessment and Social Systems Group

PREPARED FOR: Ministry of Health
CLIENT REPORT No: FW22034
REVIEWED BY: Dr Jeff Fowles, Tox-Logic

Peer reviewer	Management Reviewer	Project Manager
		
Dr Jeff Fowles	**Jan Powell**	**Peter Cressey**
Tox-Logic	Service Lead, Risk Assessment and Social Systems Group	Science Leader, Risk Assessment and Social Systems Group

TABLE OF CONTENTS

ACRONYMS AND ABBREVIATIONS

AChE	Acetylcholinesterase
ADI	Acceptable Daily Intake
AUC	Area under the curve
ADME	Adsorption, Distribution, Metabolism, Excretion
BBB	Blood brain barrier
bw	Body weight
ECHA	European Chemical Agency
EFSA	European Food Safety Authority
EPA	Environmental Protection Authority
ESR	Institute of Environmental Science and Research Limited
HDL-C	High-density lipoprotein cholesterol
HDPE	High-density polyethylene
IL-1α	Interleukin-1α
IL-1β	Interleukin-1β
IL-6	Interleukin-6
IL-8	Interleukin-8
LD_{50}	Lethal dose
LDL-C	Low-density lipoprotein cholesterol
MNPCE	Micronucleated polychromatic erythrocyte
MPs	Microplastics
NOAEL	No-observed-adverse-effect level
NPs	Nanoplastics
PAN	Polyacrylonitrile

PE	Polyethylene
PET	Polyethylene terephthalate
PMMA	Poly(methyl methacrylate)
PP	Polypropylene
PS	Polystyrene
PSU	Poly aryl sulfone
PTFE	Polytetrafluoroethylene
PVA	Polyvinyl alcohol
PVC	Polyvinyl chloride
PVDC	Polyvinylidene chloride
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RfD	Reference dose
TAG	triacylglycerols
TC	Total cholesterol
TCE	Total cholesterol esters
TDI	Tolerable Daily Intake
TG	Triglycerides
TNF-α	Tumour necrosis factor-α
WHO	World Health Organization

EXECUTIVE SUMMARY

The purpose of this report is to review the evidence for adverse human health effects from inhalation, ingestion or dermal exposure to microplastics (MPs). MPs are a heterogeneous mixture of differently shaped materials referred to as fragments, fibres, spheroids, granules, pellets, flakes or beads, in the size range of 0.1–5,000 µm. Primary MPs are materials that originate directly from manufactured products, while secondary MPs may originate from degradation of plastic substances.

MPs are ubiquitous in the environment. MPs are found in air, water, food and its packaging, soil, and personal care products. Humans can be exposed to MPs through oral, dermal and inhalation routes of exposure. MP are mainly composed of polymers and frequently also contain additives and plasticisers. The most abundant polymers in MP are polyethylene (PE), polyethylene terephthalate (PET), polyamide (PA), polypropylene (PP), polystyrene (PS), polyvinyl alcohol (PVA) and polyvinyl chloride (PVC).

There are *in vitro* and *in vivo* studies which provide some information on the absorption distribution, metabolism and excretion (ADME) of MPs. However, the results of these studies are inconsistent, particularly on uptake of MPs by different organs. For example, in one study MPs (5 and 20 µm) displayed tissue accumulation over time in the liver, kidney and gut of mice. Contrary to this, in another study no MPs (1, 4 and 10 µm) were detected in kidneys, spleen and liver and only a very small number of plastic particles was detected in the jejunum and duodenum of mice. MPs can also accumulate in the brain of mice by disrupting the blood brain barrier (BBB). In humans, MPs have also been detected in placental and meconium samples. Size dependent uptake of MPs has been reported in human placental cells after 24-hour exposure. Hence, maternal exposure to MPs may result in placental uptake, transplacental transport, and foetal exposure. MPs are generally considered to be chemically inert, although they may differ in their surface charge characteristics, and there is no evidence of metabolism in humans or animals. MPs are excreted from the body through urine and faeces. Overall, the absorption of MPs is expected to be limited, although MPs smaller than 150 µm may translocate across the gut epithelium causing systemic exposure.

Toxicity studies (*in vivo* and *in vitro)* on different MPs were summarised. MPs can cause pathological changes to the gut which include reduction in mucus secretion, gut barrier dysfunction, intestinal inflammation, microbiota dysbiosis (gut, nasal and lung), impair glucose tolerance and hepatic lipid deposition. The *in vitro* studies in various human cell lines show that MPs can be cytotoxic, decrease cell viability and increase reactive oxygen species (ROS) levels. ROS then may facilitate tissue damage through inflammation with the release of proinflammatory cytokines, such as tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6) and interleukin-8 (IL-8). Confounding factors such as the presence of impurities (e.g., endotoxins) or chemicals added to plastic should be considered when assessing the effects of MPs *in vitro*. In most studies, cellular uptake of MPs was observed, but they generally had insignificant toxicity. The toxic effects were observed with the lowest MP size and at the highest concentrations tested.

The *in vivo* studies in rats and mice provide limited evidence on the toxicity of MPs. Studies conducted in the last 2-3 years provide some insights on toxicity after acute and short-term exposure (up to 90 days). MPs are not acutely toxic and the LD_{50} (oral) in mice was >2000 mg/kg. The results after sub-acute exposure were inconsistent. One study reported inflammation of the lungs and liver of mice whereas other studies reported no toxicity after

sub-acute exposure. MPs also increased the secretion of interleukin-1 alpha (IL-1α) in serum, thus inducing intestinal inflammation in mice. There was evidence that MPs might have neurotoxicity potential as they decreased acetylcholinesterase (AChE) activity in the liver of mice and exhibited cognitive and memory deficits. Maternal exposure to MPs (0.5 and 5 µm) in mice in two studies resulted in altered biochemical parameters as well as altered fatty acid and amino acid metabolism in F1 and F2 offspring and provided some evidence on long-term metabolic consequences. All these effects showed a close relationship with metabolic disorders.

Overall, there are limited conclusive studies (toxicokinetic, toxicity, epidemiology, pharmacology) related to health effects of MPs. Further studies are required to fully understand the ADME of MPs. The available *in vitro* studies in human cell lines often used extremely high concentrations and testing predominantly PS-MPs, which are not considered to be representative of environmental exposure. The *in vivo* studies provide conflicting results, often had study deficiencies and provided weak results. The properties of the particles tested were also not adequately described. Long term studies are also needed to explore the health effects after chronic exposure. Very few studies provided satisfactory information on the homogeneity of the exposure or the stability of MPs in drinking-water. A dose–response relationship cannot be established at this time from the studies available. Therefore, the health risks of MPs to humans remain unclear and further research will be required.

1 INTRODUCTION

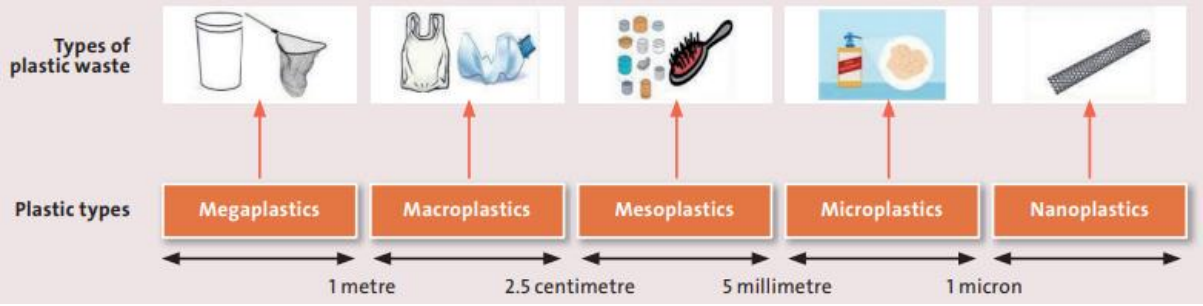
The purpose of this report is to review the evidence for adverse human health effects from inhalation, ingestion or dermal exposure to microplastics (MPs). As the current study is primarily concerned with potential effects on human health, studies conducted only in mammalian cell lines and mammalian species were considered.

There are some studies available on nanoplastics (NPs). However, for NPs, knowledge of the relationship between particle characteristics and toxicity is based on laboratory studies with fabricated particles, mainly submicrometre- sized polystyrene spheres. For environmentally realistic conditions, this relationship is difficult to address, because, apart from polymer identity toxicologically relevant characteristics of environmental NPs (such as shape, submicrometre- size range, area, volume, surface chemistry, biopersistence and zeta potential) are unknown (Koelmans *et al.*, 2022). Hence, this report will only cover toxicology and health effects limited to MPs.

1.1 MICROPLASTICS – OVERVIEW

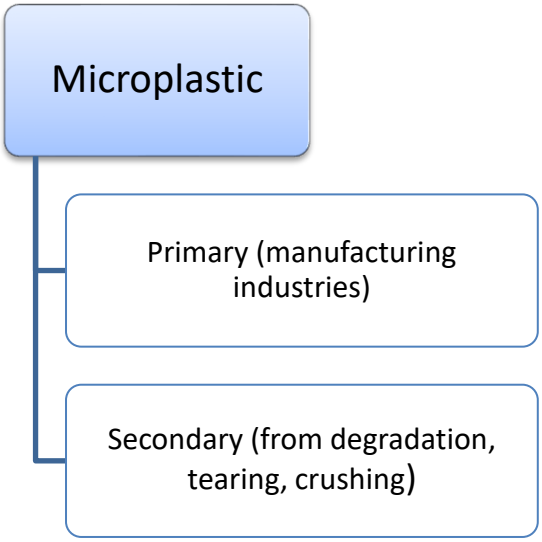
Microplastics (MPs) are considered to be emerging environmental pollutants globally. MPs are ubiquitous in human daily life and in the environment. There is no consensus on an official definition of MPs, but in general they are considered to include plastic particles in the size range 0.1–5000 µm or 0.1 µm – 5 mm. The European Food Safety Authority (EFSA) defines MPs as “a heterogeneous mixture of differently shaped materials referred to as fragments, fibres, spheroids, granules, pellets, flakes or beads, in the range of 0.1–5,000 µm” (EFSA, 2016).

Figure 1. Size range of plastics.



Source: (Chatterjee and Sharma, 2019)

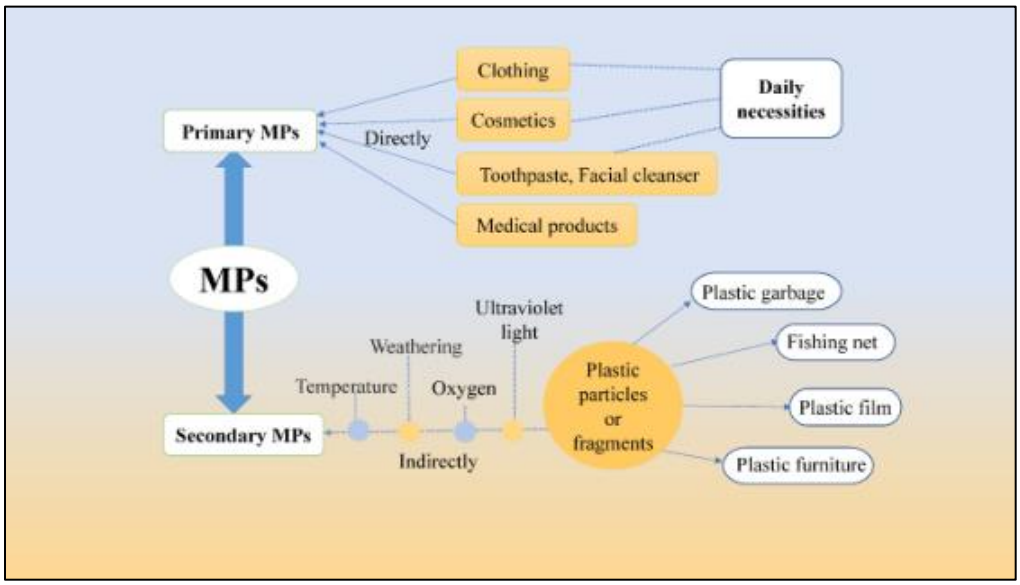
Figure 2. Subcategories of microplastics and their source of environmental release.



MPs are further classified into: Primary and Secondary MPs based on their source of environmental release. Primary MPs originate directly from manufactured products such as microbeads that are often added to personal care products, plastic powders used in moulding and spherical or cylindrical virgin resin used during production of plastic products as well as microfibers from clothing.

Secondary MPs may originate from degradation of plastic substances via light, ultraviolet radiation, embrittlement, biological factors, and sea-salt aerosol formation (Dong et al., 2020; EFSA, 2016).

Figure 3. Sources of microplastics



Source: (Sun et al., 2022)

1.1.1 CHEMICAL COMPOSITION OF (MICRO) PLASTICS

MPs are composed of polymers, which are generally chemically inert. However, MPs may differ in their surface charge characteristics and it is unknown what the implications of these differences are for their potential toxicity. It has been reported that MP samples collected from different sites were mainly composed of polymers: polypropylene (PP), polyethylene (PE), polyvinyl chloride (PVC), polystyrene (PS), polyvinylidene chloride (PVDC)(Saran), poly methyl methacrylate (PMMA), poly aryl sulfone (PSU), polyacrylonitrile (PAN), polyvinyl alcohol (PVA), polytetrafluoroethylene (PTFE, Teflon) and cellulose. (Kedzierski *et al.*, 2022; Severini *et al.*, 2020; WHO/FAO, 2022). MPs frequently also contain additives, plasticisers and residual polymers (Salthammer, 2022).

1.2 REGULATION OF MICROPLASTICS IN NEW ZEALAND

In New Zealand, MPs are regulated under the Waste Minimisation (Microbeads) Regulations 2017 or Microbeads regulation (MfE, 2017). Under this regulation, microbeads mean a water-insoluble plastic particle that is less than 5 mm at its widest point. These regulations prohibit the manufacture and sale of wash-off products (other than medical devices or medicines) that contain microbeads for one or more of the following purposes:

1) exfoliation of all or part of a person's body
2) cleaning of all or part of a person's body
3) abrasive cleaning of any area, surface, or thing
4) visual appearance of the product.

The New Zealand Environmental Protection Authority (NZEPA) list the following products containing microbeads are not banned in New Zealand (NZEPA, 2015):

1) personal hygiene products containing natural particles, such as ground nutshells, pumice or other biodegradable materials
2) medicines and medical devices for direct therapeutic purposes, as defined in New Zealand law
3) goods that are produced in or imported into Australia and are lawfully sold in Australia (these goods are recognised under a trade agreement between New Zealand and Australia, the Trans-Tasman Mutual Recognition Act 1997).
4) microbeads, including glitter, in wipe-off products, such as cosmetic makeup
5) microbeads, including glitter, sold as a craft material

Convention on Plastic Pollution: This is a global treaty targeting plastic pollution and is adopted by 175 countries at an annual conference held by the United Nations Environment Program (UNEP). As a member state of the United Nations, Aotearoa New Zealand is working with other countries on this global agreement to eliminate plastic pollution. This agreement will cover the full life cycle of plastics from production to disposal and will include MPs in its scope (EIA, 2020; MfE, 2022).

2 HAZARD IDENTIFICATION

2.1 PREVIOUS ASSESSMENTS

No previous assessments of health effects were found for MPs in New Zealand.

2.2 HEALTH EFFECTS

2.2.1 Observations in humans

There was one study identified that reported the presence of MPs in placental tissue and its association with birth outcomes, as assessed by neonatal anthropometric measurements. Another study reported associations between MPs and microbiota in placentas and meconium. Both are summarised below.

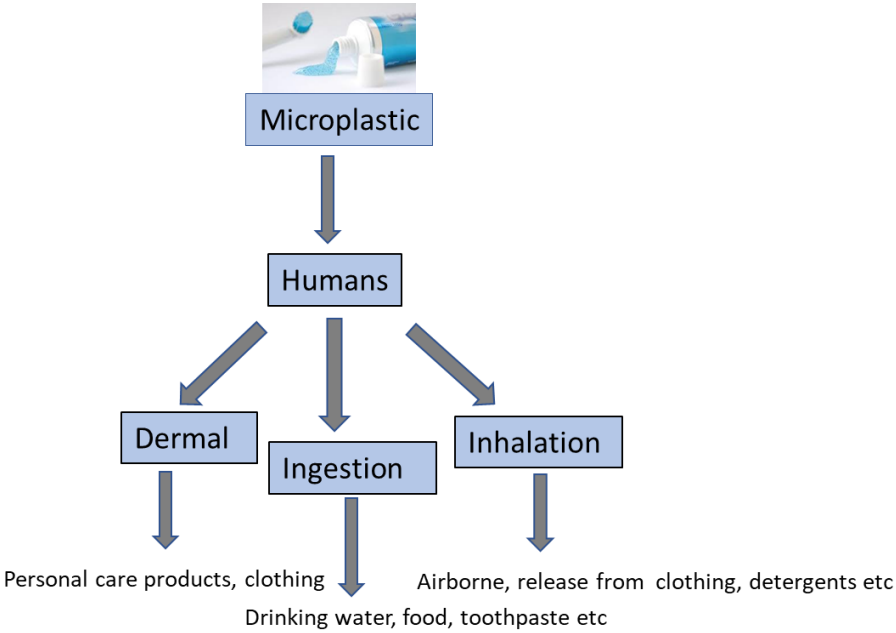
MPs (<10 µm) were found in the fresh placentas of young women (n=43) in Tehran, Iran. Out of 43 participants, 30 mothers had normal weight babies. The remaining 13 participants were intrauterine growth restriction (IUGR) pregnancies. In the normal group, four out of 30 (13%) participants had microplastic particles in their placentas above the limit of detection (LOD), whereas all placental tissues within IUGR pregnancies showed plastic particulates. In total, 6 and 302 MPs were extracted from placentas donated by participants in normal and IUGR pregnancies, respectively, demonstrating significantly higher load of plastic particulates in IUGR pregnancies (mean = 23 particles per placenta) when compared with the normal group (mean = <1 particle per placenta). Most of the MPs comprised of PE (polyethylene) and PS (polystyrene) polymers. Inverse associations between MPs exposure and birth outcomes were observed in terms of birth weight, length, and head circumference among those with IUGR as compared to normal pregnancies (Amereh *et al.*, 2022).

Liu *et al.* (2022) explored MP (20−500 µm) exposure in placentas (n = 18) and meconium samples (n = 12), and the potential correlation of MP exposure with microbiota in placentas and meconiums. MPs (16 types) were detected in all the samples. Polyamide (PA) and polyurethane (PU) accounted for greater than 78% of the total amount of MPs. More than 76% of the MP particles were in the size range 20−50 µm followed by 50−100 and 100−150 µm, with particles >150 µm only detected in meconium samples. At the phylum level, both placenta and meconium microbiota were mainly composed of Proteobacteria, Bacteroidota, and Firmicutes. There were significant differences between placenta and meconium microbiota in β-diversity and gut composition. Additionally, polystyrene was inversely related with the Chao index of meconium microbiota. PE was consistently inversely correlated with several genera of placenta microbiota. The total MPs, PA, and PU consistently impacted several genera of meconium microbiota.

2.2.2 Absorption, distribution, metabolism and excretion (ADME)

MPs are tiny pieces of synthetic polymers (plastics), found in the environment (including freshwater and seawater, sediments, biota, soils, and ambient air) as well as in drinking-water, food, and in personal care products. Recently, for the first time MPs (≥0.7 µm) were detected in human blood and placenta (Leslie *et al.*, 2022; Ragusa *et al.*, 2021). Humans can be exposed to MPs through oral, dermal and inhalation routes of exposure.

Figure 4. Exposure route of microplastics.



Data on the toxicokinetics of MPs are sparse. In general, the absorption and distribution of MPs is size dependent, with an inverse relationship between uptake and particle size. MPs do not undergo metabolism and are predominantly eliminated through faeces.

Absorption and distribution: Some *in vitro* and *in vivo* studies provide information on the absorption of MPs. MPs (>150 µm) are not absorbed, and only local effects on the immune system and inflammation of the gut are to be expected. Smaller MPs (< 150 µm) may lead to systemic exposure (EFSA, 2016).

Studies with polystyrene (PS) particles (0.05– 3 µm) showed systemic bioavailability in rats with particles detected in the liver and spleen, with an inverse correlation between absorption and particle size. The extent of absorption of 0.05 µm particles was 34% and of the 0.1 µm particles was 26% (as measured by determination of polystyrene content), of which total, about 7% (0.05 µm) and 4% (0.1 µm), was in the liver, spleen, blood and bone marrow. Particles larger than 0.1 µm did not reach the bone marrow, and those larger than 0.3 µm were absent from blood. No particles were detected in heart or lung tissue (Jani *et al.*, 1990).

Deng *et al.* (2017) investigated the tissue distribution, accumulation, and tissue-specific health risk of MPs in mice. Animals received 5 and 20 µm diameter PS particles at daily concentrations of 0.01, 0.1 and 0.5 mg/day for up to 28 days. MPs accumulated in the liver, kidney and gut. The 5 µm MPs accumulated in kidney and gut to significantly higher concentrations than 20 µm MPs. However, significantly fewer 5 µm MPs were retained in liver compared to 20 µm MPs after 4 weeks of exposure. MPs could still be observed in the three tissues one week after termination of the exposure.

Contrary to the above results, no MPs (1, 4 and 10 µm) were detected in kidneys, spleen and liver of mice in another 28-day study. Only a very small number of plastic particles was detected in the jejunum and duodenum but could not be quantified (Stock *et al.*, 2019). It should be noted that in the report of this study the administered MP doses were described in terms of the number, rather than the weight, of MP particles

Polytetrafluoroethylene (PTFE) MPs (5 µm or 10-50 µm) were not detected in the blood of male mice after administering a single oral dose of 2000 mg/kg PTFE MPs. Hence, the pharmacokinetic parameters for PTFE could not be calculated (Lee *et al.*, 2022a).

PS-MPs of various particle diameters (0.5, 4, and 10 µm) were reported to be accumulated in the brain of mice in a neurotoxicity study. The blood brain barrier (BBB) was disrupted which could lead to inflammation and neurodegenerative diseases (Jin *et al.*, 2022).

MPs have also been detected in the placenta on both the foetal and maternal sides (Amereh *et al.*, 2022). In an *in vitro* study, size-dependent placental uptake of pristine and weathered micro- and nanoplastic (PS and HDPE) was observed in two human placental cell types after 24 h exposure (Dusza *et al.*, 2022). This suggests that maternal exposure to MPs may result in placental uptake, transplacental transport, and foetal exposure.

EFSA (2016) determined an oral absorption of ≤0.3% for MPs with size <150 µm based on the limited data available at that time.

There were no studies available on dermal absorption of MPs. Dermal exposure could potentially occur while using personal care products or by wearing clothes containing MPs. The size of skin pores is in the range 40 – 80 µm, so dermal barriers could be crossed by nanoplastics (NPs, <0.1 µm), MPs, the monomers and plastic additives (Wu *et al.*, 2022).

Metabolism: Plastics are generally considered chemically inert synthetic organic polymers and cannot be metabolised. There is no evidence that MPs are metabolised either in humans or animals (Wu *et al.*, 2022).

Excretion: After ingestion, >90% of MPs are reported to be excreted in faeces, especially large particles >150 µm (Wu *et al.*, 2022). This is evident as MPs of a wide range of sizes (50 to 500 µm) were detected in human stool (Schwabl *et al.*, 2019)..

2.2.3 Toxicity studies on microplastics

There is a scarcity of toxicity data for humans *in vivo* at the moment. However, there are many studies that have explored the effects of MPs on human cell cultures. Exposure to MPs may lead to oxidative stress, cytotoxicity and translocation to other tissues leading to chronic inflammation which may increase the risk of cancer (Prata *et al.*, 2020; Vethaak and Legler, 2021). MPs can cause pathological changes to the gut including reduction in mucus secretion, gut barrier dysfunction, intestinal inflammation, gut microbiota dysbiosis and impair glucose tolerance and hepatic lipid deposition (Toto *et al.*, 2022; Wang *et al.*, 2021; Xie *et al.*, 2022). Intranasal exposure to MPs can also alter the nasal and lung microbiota which can cause respiratory illness (Zha *et al.*, 2023). MPs have also been detected in the placenta on both the foetal and maternal sides in humans (Amereh *et al.*, 2022). Hence, maternal exposure to MPs may result in placental uptake, transplacental transport, and foetal exposure.

2.2.3.1 In vitro studies

Several *in vitro* studies are available using human cell lines to assess the harmful effects of MPs. These studies in different cell lines reveal that MPs can be cytotoxic, decrease cell viability and increase concentrations of reactive oxygen species (ROS). ROS may facilitate tissue damage due to inflammation with the release of proinflammatory cytokines, such as

tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6) and interleukin-8 (IL-8). It was observed that the expression of IL-6, TNF-α and IL-8 was significantly increased after exposure to MPs (Dong *et al.*, 2020; Hwang *et al.*, 2019; Wu *et al.*, 2019). Confounding factors such as the presence of impurities (e.g., endotoxins) or chemicals added to plastic should be considered when assessing the effects of MPs *in vitro*. In most studies, cellular uptake of MPs was observed, but they generally had insignificant toxicity. The toxic effects were observed with the lowest MP size and at the highest concentrations tested (Dong *et al.*, 2020; Hwang *et al.*, 2019; Yong *et al.*, 2020). The *in vitro* studies are summarised in Table 1.

Table 1: *In vitro* studies of effects of MP exposure on human cell lines

MPs type and size (µm)	Test system	Concentration	Exposure time (hr unless otherwise stated)	Results	Reference
Pristine and Weathered PS (0.05 – 10) and HDPE (0 - 80)	Nonsyncytialised and syncytialised BeWo b30 choriocarcinoma cells	0.1, 1, 10, and 100 µg/mL	24	a) Size-dependent placental cell uptake of pristine and weathered MPs was observed b) Cellular transport was limited and size dependent. c) Cytotoxicity not observed and plasma membrane integrity was also not affected by any of the conditions tested, except for 0.05 µm PS particles at the highest concentration	(Dusza *et al.*, 2022)
PE-MP (1 - 10)	M-ARCOL and Caco-2/HT29-MTX intestinal cell culture	2.6 mg/mL (2,600 µg/mL) of a deionised water solution containing 0.01 % (w/v) Tween 80	14 days	a) Increase in abundance of potentially harmful bacteria, (Desulfovibrionaceae and Enterobacteriaceae*)*, and a decrease in beneficial bacteria (Christensenellaceae and Akkermansiaceae*).* b) No effect on intestinal barrier function (intestinal and transcellular permeability) mucin synthesis or IL-8 production, probably due to the few changes observed in the profiles of gut microbiota metabolites	(Fournier *et al.*, 2023)
PS-MP (2)	CCD-18 Co normal human intestinal cell model	20 µg/mL	48	Internalisation of MPs and increased level of glucose oxidation via lactate, glycolytic reserve and pentose phosphate pathway.	(Bonanomi *et al.*, 2022)

		20 µg/mL	4 weeks	Gradual internalisation of MPs, ROS levels significantly increased, and significant decrease in mitochondrial function and ATP production.	
Pristine PS-MP (2)	Adenocarcinomic human alveolar basal epithelial cells (A549 cells)	Cellular uptake: 0, 5, 25, 100 µg/mL Cell Viability: 2.5, 5, 10, 25, 50, 100, 200 and 400 µg/mL Genotoxicity: 0, 50, 100 µg/ mL ROS: (100, 200 and 400 µg/mL	24 ROS: 6	a) No accumulation of MPs in the A549 cells. b) No difference on the cell viability between MPs and control, no cytotoxicity. c) Increase in micronuclei fractions by 1.4 fold. Not genotoxic d) Increased the ROS production but not dose dependent.	(Shi *et al.*, 2022)
PP-MP (20, 25 - 200)	Human dermal fibroblast cells (HDF-cells)	10, 50, 100, 500, and 1000 µg/mL	48	a) Decreased cell viability at highest concentration for smaller PP particles (~20 µm). b) Increased ROS levels for smaller PP particles (~25 µm) at highest concentration c) Low degree of induction of proinflammatory cytokines IL-6 and TNF-α from PBMCs	(Hwang *et al.*, 2019)

PS-MP (0.1, 5)	Human intestinal epithelial cell line (Caco-2)	1, 10, 40, 80, 200 µg/mL	12	a) Low toxicity on cell viability, oxidative stress, and membrane integrity and fluidity b) Increased ROS levels at 200 µg/mL	(Wu *et al.*, 2019)
PS-MP (2)	Human kidney proximal tubular epithelial cells (HK-2 cells)	25, 50, 100, 200, 400, or 800 µg/mL	5 min – 3 days	a) No cytotoxicity, b) Increased ROS levels from 200 µg/mL	(Wang *et al.*, 2021)
PS-MP (1, 4, 10)	Human intestinal epithelial cell line (Caco-2)	100,000 (1 µm), 250,000 (4 µm) or 60,000 (10 µm) particles per mL medium	24 and 48	Decreased cell viability for 1 µm MPs at highest concentrations only	(Stock *et al.*, 2019)
PS-MP (1, 4, 10)	Human lung epithelial cells (BEAS-2B)	1, 10, 100 and 1000 µg/cm^2	24 and 48	a) Decreased cell viability at 1000 µg/cm^2 after 24-hr and cytotoxic at concentrations ≥10 µg/cm^2 for 48 hr b) Increased ROS levels at 1000 µg/cm^2 c) Oxidative stress and inflammatory responses, increased IL-6 (10 and 1000 µg/cm^2) and IL-8 expression (1000 µg/cm^2) after 24-hr.[a]	(Dong *et al.*, 2020)
PP, PU, PA, tire rubber polydisperse (50–500)	Human intestinal epithelial cell line (Caco-2)	823.5–1380.0 µg/cm^2	6, 24 and 48 h	No cytotoxicity No release of inflammatory cytokines No changes in barrier integrity	(Lehner *et al.*, 2020)

MP: microplastics, PP: polypropylene, PS: polystyrene, PU: polyurethane, PA: polyamide, IL: interleukin, TNF: tumour necrosis factor, PBMC: peripheral blood mononuclear cells, ROS: reactive oxygen species

a) Inflammatory responses were evaluated at 10 and 1000 µg/cm^2 only.

2.2.3.2 In vivo studies

There have been several rodent studies in the last 5 years that have reported the toxicity of MPs, which are summarised below and in Table 2.

1. MPs exposure effects intestinal permeability and intestinal flora. The intestinal permeability was slightly (but non-significant) changed in rats when fed orally with 0.1% pristine polyamide (15–20 μm) or polyethylene (40–48 μm) particles or a 1:1 w/w mixture of both polymers for 5-weeks. No change in the expression of the pro-inflammatory proteins was observed, and plasma concentrations of C-reactive protein (CRP) were not influenced by MP administration. Therefore, MPs did not cause intestinal inflammation in rats (Toto *et al.*, 2022). Short-term MP (PE, PET, PP, PS and PVC) exposure to mice disrupted the colonic microenvironment and was accompanied by inflammation. Histopathological examination revealed colon tissue damage in all the treatment groups. PS treated mice had the highest proportion of inflammatory cells in all treatment groups. The ratio of Bacteroidetes and Firmicutes in PE, PET and PP treatment groups heightened, and the relative abundance of Ruminococcaceae and Lachnospiraceae increased significantly. At the genus level, *Alistipes* bacteria in PS treatment group significantly decreased (Xie *et al.*, 2022).

2. PS-MPs exposure through drinking water impaired glucose tolerance and hepatic lipid deposition at high dose levels in mice (Wang *et al.*, 2022). Mice received either normal drinking-water or drinking-water containing 100 μg/L or 1000 μg/L MPs for 8 weeks. There were no changes in body weight, serum triglycerides and total cholesteryl esters (TCEs). At high dose levels, mice showed higher blood glucose 30 minutes and 60 minutes after dextrose injection and impaired glucose tolerance in a glucose tolerance test (GTT). Intracytoplasmic lipid accumulation was observed in the liver at high dose. Lipidomic analysis showed significant alteration in hepatic lipid species particularly with free fatty acids (FFAs) and serum triacylglycerols (TAGs). No alteration of total lipid content was found in mouse liver after MPs exposure. However, the level of hepatic FFAs in the low dose and high-dose groups was increased by 20.2% and 26.4%, respectively. It is worth noting that there was an increase in saturated fatty acids (SFAs) subclass, while total monounsaturated or polyunsaturated fatty acids (MUFAs or PUFAs) were not affected by MPs exposure. No statistically significant difference was found in hepatic total diacylglycerols (DAGs), TAGs, CEs, ceramides, sphingomyelins, phosphatidylethanolamines, and phosphatidylethanolamines. Hence, MPs exposure could increase hepatic FFAs and some neutral lipids, glycerophospholipids, and sphingolipids species in mice. Liver transcriptional profile indicated MPs exposure-induced differentially expressed genes (DEGs) enriched in pathways of lipid metabolism and unfolded protein response. Furthermore, most altered lipid species were significantly correlated with DEGs enriched in lipid metabolic signalling.

3. PS-MPs are reported to induce insulin resistance in mice (Huang *et al.*, 2022). Animals were fed a normal chow diet (NCD) or a high-fat diet (HFD) and containing PS-MP for 10 weeks by gavage at a dose of 80 mg/kg bw/day. Fasting blood glucose and insulin levels were significantly increased in the NCD. Insulin levels also increased in the HFD group but this was not significant. Response in an oral glucose tolerance test (OGTT) was significantly increased in both groups. Response in an insulin tolerance test (ITT) was significantly higher in the NCD group. Homeostasis model assessment of insulin resistance (HOMA-IR) was also significantly increased

in both groups. These results suggest that PS exposure may induce glucose intolerance and insulin resistance.
PS treatment also caused an increase in the plasma levels of pro-inflammatory cytokines and lipopolysaccharide (LPS) in mice. TNF-α, IL-1β and LPS were significantly increased in the NCD and HFD groups. Smaller PS-MPs (5 µm) were detected in blood vessels, the liver, and kidneys, but no PS was detected in the pancreas or heart. This indicates that small particle size PS were absorbed into blood and accumulated in specific tissues following gavage administration for an extended period of time. There were histopathological changes in the kidneys, pancreas, liver and adipose tissue. NCD + PS and HFD groups had widened balloon space, tubular dilatation, vacuolar degeneration and renal interstitial inflammatory cell infiltration. Severe vacuolar degeneration was observed in the HFD group. In pancreatic sections, islets in both the groups had an irregular shape compared to controls. Hepatocyte nuclear pyknosis and sinusoidal dilatation were observed in the NCD group and pathological changes of vacuolisation were further aggravated in the HFD group. Adipocyte size had increased and varied in size in the NCD group whereas in the HFD group adipocyte size was increased with vasodilatation and congestion. The gut microbiota composition was also altered after PS-MP exposure. A decrease in the Firmicutes to Bacteroidetes ratio was observed. There was an increase in the relative abundance of Gram-negative bacteria such as Prevotellaceae and Enterobacteriaceae.

4. Intranasal administration of PS-MPs (5 µm) every other day for five weeks at 10 µg/µL (containing 100 µg MP or NP) altered nasal microbiota (*Staphylococcus, Sphingomonas and Flavobacterium*) and lung microbiota (*Roseburia, Eggerthella, Corynebacterium*) in mice (Zha *et al.*, 2023). *Staphylococcus* could colonise the upper airway and is associated with chronic airway diseases. MP had a stronger influence on the lung microbiota than NP. Haemorrhage and exudates were found in the lung tissue suggesting that MPs could induce lung injury.

5. Deng *et al.* (2017) investigated the toxicity of MPs in male mice receiving PS-MPs at daily concentrations of 0.01 (1×10^5 5 µm PS-MPs and 2×10^3 20 µm PS-MPs), 0.1 (1×10^6 5 µm PS-MPs and 2×10^4 20 µm PS-MPs) and 0.5 mg per day (5×10^6 5 µm PS-MPs and 1×10^5 20 µm PS-MPs) for up to 28 days. No mortality was observed. There were no changes in body weight and liver weights as compared to the controls. However, the relative liver weight was significantly decreased in the high dose group. Food intake was also increased significantly in the mid and high dose group of 20 µm MPs. Histopathology of liver revealed inflammation and lipid droplets in livers of MPs-treated mice. Markers of energy metabolism (ATP level) and lipid metabolism (T-CHO and TG) were also significantly decreased in MP-treated animals. The activity of acetylcholinesterase (AChE), a biomarker of potential for neurotoxicity, in liver also increased which may lead to a reduction of cholinergic neurotransmission efficiency.
Braeuning (2019) raised many concerns regarding the above study. He noted that the histological evidence of liver inflammation and hepatic lipid accumulation in treated mice does not provide unequivocal evidence of an effect owing to the quality of the histopathological analyses. Thus, he considered that the small variations in biochemical measurements might be due to the biological variance expected from five animals per group.

6. Contrary to the above study, in a 28-days *in vivo* feeding study in male mice, no statistically significant effects of MPs exposure on body and organ weights were observed. Animals were dosed three times per week by oral gavage with a mixture of

1, 4 and 10 µm PS-MPs. There were no histopathological changes (liver, spleen, kidney) or inflammatory responses observed (Stock *et al.*, 2019).

7. The toxicity of 10-50 µm PE-MPs at doses of 500, 1000 and 2000 mg/kg bw/day was investigated in a single and 28-day repeated oral dose toxicity study in ICR mice. No signs of toxicity were observed after the single exposure. The LD_{50} was estimated to be >2000 mg/kg/day (Lee *et al.*, 2022b). Histopathological evaluation after repeated exposure revealed granulomatous inflammation with mixed inflammatory cells (lymphocytes and mononuclear cells) in the alveolar space of the lungs from two females in the low-dose group (500 mg/kg), two males and two females of the middle-dose group (1000 mg/kg), and two males and two females of the high-dose group (2000 mg/kg). No other signs of toxicity were observed in either of the sexes. The no-observed-adverse-effect-level (NOAEL) was estimated to be less than 1000 mg/kg in males and 500 mg/kg in females (Lee *et al.*, 2022b). It should be noted that no statistical analyses were conducted and the inflammation observed may or may not have been MP related, although PE-MPs were detected in lung tissue.

8. The same research group also investigated toxicity of PTFE MPs (5 µm and 10–50 µm) in a single (3/sex/group) and 28-day repeated oral dose (10/sex/group) toxicity study in ICR mice. In each study, animals received PTFE MPs at doses of 500, 1000 and 2000 mg/kg bw per day. No mortality nor morbidity and signs of toxicity were observed after the single and 28-day exposure. The LD_{50} for both sizes was estimated to be >2000 mg/kg/day. The NOAELs for two sizes was estimated to be >2000 mg/kg bw/day. The human NOAEL dose was estimated using the HED converting table and was 9720 mg per 60 kg (Lee *et al.*, 2022a).

9. Kim *et al.* (2021) conducted an oral acute (single dose) and subacute (28-day repeated dose) toxicity study with weathered polypropylene microplastic (PP-MS, <150 µm) in rats. The samples did not release chemical additives in simulated body fluids, the results of this study are good for evaluating particle-oriented toxicity. Animals were dosed by oral gavage at 0, 6.25, 12.5, and 25 mg/kg bw/day. Potential genotoxicity was assessed by an *in vivo* bone marrow micronucleus test. Additionally, skin sensitisation and eye irritation assays were performed using the 3-dimensional (3-D) reconstructed human skin or corneal culture models, respectively. No mortality or clinical signs of toxicity were observed at 25 mg/kg after single or repeated exposure. PP-MPs did not show skin or eye irritation potential. PP-MP treatment did not increase the micronucleated polychromatic erythrocyte (MNPCE) frequency in either sex and was negative for genotoxicity. Absence of effects in this study was attributed to minimal or no absorption of PP-MPs from the gastrointestinal tract. The NOAEL was estimated to be 25 mg/kg bw/day, the highest dose administered.

10. A 90-day oral toxicity study was conducted in ICR mice. Animals were dosed by gavage with polyethylene microplastics (PE-MPs, 40-48 µm) at 3.75, 15, or 60 mg/kg bw/day. Body weight gain was significantly reduced only in treated male mice, although the decrease did not follow a dose-response relationship, with the greatest decrease in the lowest dose group. Pathological lesions were observed in some tissues of both sexes, although all changes were classified as minimal or slight. The relative proportion of neutrophils in the blood was significantly increased in both the sexes, with a clear dose-response relationship. The relative proportion of lymphocytes in the blood was significantly decreased in both the sexes. The NOAEL for general toxicity of PE-MPs was estimated to be lower than 60 mg/kg bw/day (Park *et al.*, 2020).

The study also included a reproduction/development screening test. Five female mice were continuously dosed during the lactation period and were sacrificed, together with their pups, on day 21 after birth. Five pups (four pups in the mid dose group and one pup in the high dose group) died at day 1 after birth, and importantly, the number of live births per dam and body weight of pups (within 6 h after birth) was significantly decreased in the high dose group compared to the control group. The immunoglobulin A (IgA) level in the blood stream was significantly elevated in the dams administered with PE-MPs compared to controls, and the subpopulation of lymphocytes within the spleen was altered. The NOAEL for reproductive and developmental toxicity of PE-MPs dosed repeatedly for 90 days was estimated to be lower than 15 mg/kg bw/day (Park *et al.*, 2020).

11. The neurotoxicity potential of PS-MP (0.4, 4 and 10 µm) was studied in mice. Animals received MP in drinking water containing 100 and 1000 µg/L for 180 days. The blood brain barrier was disrupted and MPs accumulated in the brain. Morris water maze and novel object recognition tests showed that mice exhibited cognitive and memory deficits compared with control mice. The mRNA levels of inflammatory cytokines TNF -α, IL-1β, IL-6, CXCL10, and MCP-1 were higher in the treated mice. All the effects were concentration dependent but independent of the particle size (Jin *et al.*, 2022).

12. In a developmental toxicity study in mice, females received drinking-water containing PS-MPs of 0.5 or 5 µm at concentrations of 100 or 1000 µg/L from the beginning of gestation to litter birth. No effect on offspring survival or sex ratio was observed. Biochemical parameters involving serum total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) levels and hepatic TC and TG levels were altered at different degrees in offspring (F1 generation). Free carnitine (C0) and acyl-carnitine were also changed, the contents of C0 increased significantly in the serum of female offspring; while the long chain acylcarnitines (C16, C16:1, C18, C18:1) were decreased significantly for male offspring. The levels of C0 and C0/(C16 + C18) increased, which showed a close relationship to fatty acid metabolism disorder. Effects were generally greater following exposure to the 5 µm particles than the 0.5 µm particles. This indicated that exposure to MP in utero could cause disordered fatty acid metabolism after birth, particularly with larger diameter particles. However, lack of information on dose makes it difficult to interpret the significance of these findings. (Luo *et al.*, 2019a).

13. In a two-generation reproductive and developmental toxicity study in mice, the effect of PS-MPs exposure during gestation and lactation and the potential effects on dams and the F1 (both post-natal day (PND) 42 and 280) and F2 (PND 42) generations was evaluated. Female mice received drinking-water containing PS-MPs (0.5 or 5 µm) at concentrations of 100 or 1000 µg/L during pregnancy and lactation. Maternal MPs exposure had intergenerational effects and caused long-term metabolic consequences in the F1 and F2 generations. MPs exposure during gestation and lactation indicated that MPs altered glycolipid metabolism-related physiological indexes in serum and liver of dams and their F1 and F2 generations. MPs exposure showed fewer effects (serum and hepatic indexes) on F2 offspring. In the F1 generation (PND 42), the composition of gut microbiota did not change significantly, while the hepatic transcriptome and serum metabolite changes showed a potential risk of metabolic disorder. The effects on F2 were much lower than those in dams and F1 offspring, that just a few genes were significantly altered. Potential of hepatic

lipid accumulation was observed in adult F1 mice (PND 280), especially in females (Luo *et al.*, 2019b).

Table 2: Toxicity studies (*in vivo)* in rodents

MPs type and size (µm)	Study	Dose	Results	Reference
Pristine PA-MP (15 - 20) and PE-MP (40 - 48)	5-week dietary exposure	0.1% w/w MP of either PE or PA or a 1:1 w/w mixture of both polymers	a) Slight but non-significant change in duodenum permeability. b) No change in plasma concentrations of CRP. No inflammation observed.	(Toto *et al.*, 2022)
PE, PET, PP, PS and PVC-MPs (150 ~ 300)	7-d oral exposure	0.2 mL/day	a) Colon tissue damage, and inflammation. The order of inflammatory cells ratio was PS>PVC>PET>PE>PP. b) Oxidative stress, concentrations of SOD, GSH, MDA and POD in the colon of mice were raised. b) Variation of intestinal flora	(Xie *et al.*, 2022)
PS-MP (1)	8-week oral exposure through drinking water- mice	100 µg/L or 1000 µg/L	a) Impaired glucose tolerance at high dose. b) Induced hepatic lipid deposition at high dose. c) Dose dependent increase in lipid metabolites particularly with FFAs and TAGs in liver.	(Wang *et al.*, 2022)
PS-MP (5)	Intranasal administration every other day for 5 weeks in mice	10 µg/µL (containing 100 µg MP or NP)	a) Altered nasal (*Staphylococcus, Sphingomonas and Flavobacterium*) and lung microbiota (*Roseburia, Eggerthella, Corynebacterium*). b) Haemorrhage and exudates were found in the lung tissue.	(Zha *et al.*, 2023)
Pristine PS-MP (< 5 mm) and Styrofoam-MP (< 5 mm)	90-day dietary exposure- Wistar rats	1, 5 and 10%	PS-MPs: no significant increase in plasma TC, TG and HDL levels in female rats. LDL was significantly increased in female rats exposed to 1% and 5% only	(Nnoruka *et al.*, 2022)

			and then decreased at 10%. No dose response Significant decrease in HDL in male rats exposed to 1%. No dose response. Plasma LDL significantly increased only for the groups receiving 5%. No dose response Higher atherogenic indices for 10% PS-MP exposed group. Styrofoam MP: LDL was significantly increased in female rats exposed to 5% and then decreased at 10%. No dose response Significant decrease in HDL in male rats at 1% and 10% only. No dose response Significant increase in TC of male rats exposed to 5% FP. Oxidative stress not observed with both MPs.	
PS-MP (5, 50, 100 and 200)	10-week dietary exposure- mice	NCD + PS and HFD + PS groups, 80 mg/kg of microplastics, including 20 mg/kg PS with 5, 50, 100 and 200 μm	a) Insulin resistance (IR) accompanied by increased plasma lipopolysaccharide and pro-inflammatory cytokines such as tumor necrosis factor-α and interleukin-1β. b) MPs (5 μm) accumulated in the liver, kidneys and blood vessels of mice. c) histopathological changes in kidneys, liver, pancreas and adipose tissue.	(Huang *et al.*, 2022)

			d) significant decrease in the richness and diversity of gut microbiota, particularly an increase in the relative abundance of Gram-negative bacteria such as Prevotellaceae and Enterobacteriaceae.	
Pristine PS-MP (5)	6-week bioaccumulation study, in drinking water- mice	100 or 1000 µg/L	a) Caused intestinal barrier dysfunction and reduced intestinal mucus secretion b) Induced gut microbiota dysbiosis c) Induced bile acid metabolism disorder	(Jin *et al.*, 2019)
Pristine PS-MP (5 and 20)	28-d oral toxicity study-mice	0.01, 0.1 and 0.5 mg/d	a) Relative liver weight significantly decreased in high dose groups. b) Signs of inflammation and lipid accumulation in liver c) Increased liver oxidative stress markers, increased AChE.	(Deng *et al.*, 2017)
PS-MP (1, 4 and 10)	28-d oral toxicity study-mice	Mixture of 1 µm (4.55 x 10^7 particles), 4 µm (4.55 x 10^7 particles) and 10 µm (1.49 x 10^6 particles)	No effects observed	(Stock *et al.*, 2019)
PE-MP (10-50)	Single and 28-d oral toxicity study-mice	Single: 2000 mg/kg bw/day 28-d: 500, 1000, and 2000 mg/kg/day	a) LD_{50} > 2000 mg/kg, no signs of toxicity. b) Inflammation in lungs c) NOAEL: <1000 (M) and <500 (F) mg/kg/day	(Lee *et al.*, 2022b)
Weathered PP-MP (85.5)	Single, 28-d oral toxicity study and Bone marrow micronucleus test-rats	Single: 25 mg/kg 28-d: 0, 6.25, 12.5, and 25 mg/kg bw/day	a) No effects observed in single and 28-day toxicity study. b) NOAEL: 25 mg/kg bw/day c) No genotoxic potential	(Kim *et al.*, 2021)
PE-MP (85.5)	5- week Immunotoxicity study, dietary exposure - mice	2, 20, and 200 µg/g	a) IL-1α level in the blood stream significantly elevated b) Intestinal inflammation at the highest dose	(Li *et al.*, 2020)

PS-MP (0.4, 4 and 10)	90-d neurotoxicity study- mice	100 and 1000 µg/L	a) Disruption of the blood–brain barrier, higher level of dendritic spine density, and an inflammatory response in the hippocampus. b) Cognitive and memory deficits	(Jin *et al.*, 2022)
PE-MP (40−48)	90-day oral toxicity and reproductive & developmental toxicity- mice	3.75, 15, or 60 mg/kg bw/day	a) Body weight gain significantly reduced in male mice b) Neutrophils in the blood stream increased in both sexes c) IgA level in the blood stream significantly elevated d) NOAEL: <60 mg/kg bw/day	(Park *et al.*, 2020)
			a) Number of live births per dam, the sex ratio of pups, and body weight of pups altered. b) IgA level in the blood stream of dams significantly elevated c) NOAEL: <15 mg/kg bw/day	
PS-MP (0.5 and 5)	Developmental toxicity study- mice	100 and 1000 µg/L	F1: biochemical parameters in the serum and liver altered, fatty acid metabolic disorder	(Luo *et al.*, 2019b)
PS-MP (5)	Two generation reproductive and developmental toxicity study- mice	100 and 1000 µg/L	a) Noticeable liver histopathology and altered serum and hepatic markers b) Metabolic disorder associated with gut microbiota dysbiosis and gut barrier dysfunction c) Intergenerational effects and caused long-term metabolic consequences in the F1 and F2 generations.	(Luo *et al.*, 2019a)

AChE: acetylcholinesterase, MP: microplastics, PP: polypropylene, PS: polystyrene, PE: Polyethylene, PET: , SOD: superoxide dismutase, GSH: glutathione, MDA: malondialdehyde , TC: total cholesterol, TG: triglyceride, HDL: high-density lipoprotein, LDL: Low-density lipoprotein ,FFA: free fatty acid, CRP: C-reactive Protein, NOAEL: no-observed-adverse-effect-level, IgA: Immunoglobulin A, IL : Interleukin 1α

3 DOSE-RESPONSE INFORMATION

No dose-response information has been established for microplastics. While some of the effects seen in the rodent studies could potentially be used for dose-response modelling, the inconsistency of findings suggests this would be premature. A conventional dose-response relationship is also not possible as the dosimetry itself is problematic as the contributions of particle number, shape, surface chemistry and size have not been elucidated. Dispersion of particles in the dosing medium is also an important factor to consider; homogeneous dispersion allows robust interpretation of results. The fate of particles in the dosing medium influences various dose metrics, including the actual delivered mass and particle number (WHO, 2022).

There are no health-based guidance values reported or derived for MPs.

Several limitations should be considered when using results from the studies summarised for risk assessment. Inadequate characterization of chemical impurities that may be associated with the monodisperse type of particles used in the studies reduces the usefulness of the results for assessing the implications for human health of exposure to the complex, heterogeneous mixture of NMP expected to occur in the environment. Thus, standard reference materials representative of environmentally relevant ingested MP should be made available. As discussed above, uncertainty in dosimetry poses challenges to interpretation and extrapolation of *in vivo* data in experimental animals to humans (WHO, 2022).

4 CONCLUSIONS

Microplastics are a heterogeneous mixture of differently shaped materials referred to as fragments, fibres, spheroids, granules, pellets, flakes or beads, in the size range of 0.1–5,000 µm (EFSA, 2016). Primary MPs are materials that originate directly from manufactured products, while secondary MPs may originate from degradation of plastic substances (Dong *et al.*, 2020).

MPs are ubiquitous in the environment. MPs are found in air, water, food and its packaging, soil, and personal care products. Humans can be exposed to MPs through oral, dermal and inhalation routes of exposure. MPs are mainly composed of polymers and frequently also contain additives and plasticisers (Salthammer, 2022). The most abundant polymers in MP are polyethylene (PE), polyethylene terephthalate (PET), polyamide (PA), polypropylene (PP), polystyrene (PS), polyvinyl alcohol (PVA) and polyvinyl chloride (PVC) (Kedzierski *et al.*, 2022).

There are *in vitro* and *in vivo* studies which provide some information on the absorption distribution, metabolism and excretion (ADME) of MPs. However, the results of these studies are inconsistent, particularly on uptake of MPs by different organs. For example, in one study MPs (5 and 20 µm) displayed tissue accumulation over time in the liver, kidney and gut of mice (Deng *et al.*, 2017). Contrary to this, in another study no MPs (1, 4 and 10 µm) were detected in kidneys, spleen and liver and only a very small number of plastic particles were detected in the jejunum and duodenum of mice (Stock *et al.*, 2019). MPs can also accumulate in the brain of mice by disrupting the blood brain barrier (BBB) (Jin *et al.*, 2019). MPs have also been detected in placental and meconium samples. Size dependent uptake of MPs has been reported in human placental cells after 24-hour exposure. Hence, maternal exposure to MPs may result in placental uptake, transplacental transport, and foetal exposure (Dusza *et al.*, 2022). MPs are generally considered to be chemically inert, although they may differ in their surface charge characteristics, and there is no evidence of metabolism in humans or animals to date. MPs are excreted from the body through urine and faeces. Overall, the absorption of MPs is expected to be limited, although MPs smaller than 150 µm may translocate across the gut epithelium causing systemic exposure.

Toxicity studies (*in vivo* and *in vitro)* on different MPs were summarised. MPs can cause pathological changes to the gut which include reduction in mucus secretion, gut barrier dysfunction, intestinal inflammation, microbiota dysbiosis (gut, nasal and lung) and impair glucose tolerance and hepatic lipid deposition (Fournier *et al.*, 2023; Xie *et al.*, 2022; Zha *et al.*, 2023). The *in vitro* studies in various human cell lines show that MPs can be cytotoxic, decrease cell viability and increase reactive oxygen species (ROS) levels (Bonanomi *et al.*, 2022; Huang *et al.*, 2022; Wu *et al.*, 2019). ROS then may facilitate tissue damage through inflammation with the release of proinflammatory cytokines, such as tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6) and interleukin-8 (IL-8) (Dong *et al.*, 2020; Hwang *et al.*, 2019). However, these effects were observed with the smallest MPs and at the highest concentrations tested.

The *in vivo* studies in rats and mice provide limited evidence on the toxicity of MPs. Studies conducted in the last 2-3 years provide some insights on toxicity after acute and short-term exposure (up to 90 days). MPs are not acutely toxic and the LD_{50} (oral) in mice was >2000 mg/kg (Lee *et al.*, 2022b). The results after sub-acute exposure were inconsistent. One study reported inflammation of the lungs and liver of mice whereas other studies reported no toxicity after sub-acute exposure (Deng *et al.*, 2017; Stock *et al.*, 2019). MPs also increased the secretion of interleukin-1 alpha (IL-1α) in serum, thus inducing intestinal inflammation in

mice (Li *et al.*, 2020). There was evidence that MPs might have neurotoxicity potential as they decreased acetylcholinesterase (AChE) activity in the liver of mice and exhibited cognitive and memory deficits (Deng *et al.*, 2017). Maternal exposure to MPs (0.5 and 5 µm) in mice in two studies resulted in altered biochemical parameters as well as altered fatty acid and amino acid metabolism in F1 and F2 offspring and provided some evidences on long-term metabolic consequences. All these effects showed a close relationship with metabolic disorders (Luo *et al.*, 2019a; Luo *et al.*, 2019b).

Overall, there are limited conclusive studies (toxicokinetic, toxicity, epidemiology, pharmacology) related to potential health effects from exposure to MPs. Further studies are required to fully understand the ADME of MPs. The available *in vitro* studies in human cell lines often used extremely high concentrations and have tested predominantly PS-MPs, which are not considered to be representative of MPs associated with environmental exposure. The *in vivo* studies provide conflicting results, often had study deficiencies and provided weak results. The chemical and other properties of the particles tested were also not adequately described. Long term studies are needed to explore the health effects after chronic exposure. Very few studies provided satisfactory information on the homogeneity of the exposure or the stability of MPs in drinking-water. A dose–response relationship cannot be established at this time from the studies available at this time. Therefore, the health risks of MPs to humans remain unclear and further research will be required.

5 REFERENCES

Amereh F, Amjadi N, Mohseni-Bandpei A, Isazadeh S, Mehrabi Y, Eslami A, Naeiji Z, Rafiee M. (2022) Placental plastics in young women from general population correlate with reduced foetal growth in IUGR pregnancies. Environmental Pollution; 314: 120174.

Bonanomi M, Salmistraro N, Porro D, Pinsino A, Colangelo AM, Gaglio D. (2022) Polystyrene micro and nano-particles induce metabolic rewiring in normal human colon cells: A risk factor for human health. Chemosphere: 134947.

Braeuning A. (2019) Uptake of microplastics and related health effects: a critical discussion of Deng et al., Scientific reports 7: 46687, 2017. Archives of Toxicology; 93(1): 219-220.

Chatterjee S, Sharma S. (2019) Microplastics in our oceans and marine health. Field Actions Science Reports. The Journal of Field Actions; (Special Issue 19): 54-61.

Deng Y, Zhang Y, Lemos B, Ren H. (2017) Tissue accumulation of microplastics in mice and biomarker responses suggest widespread health risks of exposure. Scientific Reports; 7(1): 1-10.

Dong C-D, Chen C-W, Chen Y-C, Chen H-H, Lee J-S, Lin C-H. (2020) Polystyrene microplastic particles: In vitro pulmonary toxicity assessment. Journal of Hazardous Materials; 385: 121575.

Dusza HM, Katrukha EA, Nijmeijer SM, Akhmanova A, Vethaak AD, Walker DI, Legler J. (2022) Uptake, transport, and toxicity of pristine and weathered micro-and nanoplastics in human placenta cells. Environmental Health Perspectives; 130(9): 097006.

EFSA. (2016) Presence of microplastics and nanoplastics in food, with particular focus on seafood. EFSA journal; 14(6): e04501.

EIA. (2020) Convention on plastic pollution toward a new global agreement to address plastic pollution. Accessed at: https://www.ciel.org/wp-content/uploads/2020/06/Convention-on-Plastic-Pollution-June-2020-Single-Pages.pdf. Accessed: 22-Dec-2022.

Fournier E, Leveque M, Ruiz P, Ratel J, Durif C, Chalancon S, Amiard F, Edely M, Bezirard V, Gaultier E. (2023) Microplastics: What happens in the human digestive tract? First evidences in adults using in vitro gut models. Journal of Hazardous Materials; 442: 130010.

Huang D, Zhang Y, Long J, Yang X, Bao L, Yang Z, Wu B, Si R, Zhao W, Peng C. (2022) Polystyrene microplastic exposure induces insulin resistance in mice via dysbacteriosis and pro-inflammation. Science of the Total Environment: 155937.

Hwang J, Choi D, Han S, Choi J, Hong J. (2019) An assessment of the toxicity of polypropylene microplastics in human derived cells. Science of the Total Environment; 684: 657-669.

Jani P, Halbert GW, Langridge J, Florence AT. (1990) Nanoparticle uptake by the rat gastrointestinal mucosa: quantitation and particle size dependency. Journal of Pharmacy and Pharmacology; 42(12): 821-826.

Jin H, Yang C, Jiang C, Li L, Pan M, Li D, Han X, Ding J. (2022) Evaluation of neurotoxicity in BALB/c Mice following chronic exposure to polystyrene microplastics. Environmental Health Perspectives; 130(10): 107002.

Jin Y, Lu L, Tu W, Luo T, Fu Z. (2019) Impacts of polystyrene microplastic on the gut barrier, microbiota and metabolism of mice. Science of the Total Environment; 649: 308-317.

Kedzierski M, Palazot M, Soccalingame L, Falcou-Préfol M, Gorsky G, Galgani F, Bruzaud S, Pedrotti ML. (2022) Chemical composition of microplastics floating on the surface of the Mediterranean Sea. Marine Pollution Bulletin; 174: 113284.

Kim J, Maruthupandy M, An KS, Lee KH, Jeon S, Kim J-S, Cho W-S. (2021) Acute and subacute repeated oral toxicity study of fragmented microplastics in Sprague-Dawley rats. Ecotoxicology and Environmental Safety; 228: 112964.

Koelmans AA, Redondo-Hasselerharm PE, Nor NHM, de Ruijter VN, Mintenig SM, Kooi M. (2022) Risk assessment of microplastic particles. Nature Reviews Materials; 7(2): 138-152.

Lee S, Kang K-K, Sung S-E, Choi J-H, Sung M, Seong K-Y, Lee J, Kang S, Yang SY, Lee S. (2022a) In vivo toxicity and pharmacokinetics of polytetrafluoroethylene microplastics in ICR Mice. Polymers; 14(11): 2220.

Lee S, Kang K-K, Sung S-E, Choi J-H, Sung M, Seong K-Y, Lee S, Yang SY, Seo M-S, Kim K. (2022b) Toxicity study and quantitative evaluation of polyethylene microplastics in ICR mice. Polymers; 14(3): 402.

Lehner R, Wohlleben W, Septiadi D, Landsiedel R, Petri-Fink A, Rothen-Rutishauser B. (2020) A novel 3D intestine barrier model to study the immune response upon exposure to microplastics. Archives of Toxicology; 94(7): 2463-2479.

Leslie HA, Van Velzen MJ, Brandsma SH, Vethaak AD, Garcia-Vallejo JJ, Lamoree MH. (2022) Discovery and quantification of plastic particle pollution in human blood. Environment International; 163: 107199.

Li B, Ding Y, Cheng X, Sheng D, Xu Z, Rong Q, Wu Y, Zhao H, Ji X, Zhang Y. (2020) Polyethylene microplastics affect the distribution of gut microbiota and inflammation development in mice. Chemosphere; 244: 125492.

Liu S, Liu X, Guo J, Yang R, Wang H, Sun Y, Chen B, Dong R. (2022) The association between microplastics and microbiota in placentas and meconium: the first evidence in humans. Environmental Science & Technology: A-L.

Luo T, Wang C, Pan Z, Jin C, Fu Z, Jin Y. (2019a) Maternal polystyrene microplastic exposure during gestation and lactation altered metabolic homeostasis in the dams and their F1 and F2 offspring. Environmental Science & Technology; 53(18): 10978-10992.

Luo T, Zhang Y, Wang C, Wang X, Zhou J, Shen M, Zhao Y, Fu Z, Jin Y. (2019b) Maternal exposure to different sizes of polystyrene microplastics during gestation causes metabolic disorders in their offspring. Environmental Pollution; 255: 113122.

MfE. (2017) Waste Minimisation (Microbeads) Regulations 2017. Accessed at: https://environment.govt.nz/acts-and-regulations/regulations/microbeads-regulations/. Accessed: 7-October-2022.

MfE. (2022) Towards a global agreement to combat plastic pollution. Accessed at: https://environment.govt.nz/what-government-is-doing/international-action/towards-a-global-agreement-to-combat-plastic-pollution/. Accessed: 22 Dec-2022.

Nnoruka UC, Okonkwo CJ, Ilechukwu I, Okonkwo CJ, Belonwu DC. (2022) Impact of polystyrene microplastic exposure on lipid profile and oxidative stress status of male and female Wistar rats. Environmental Analysis Health and Toxicology; 37(3): e2022024.

NZEPA. (2015) Microbeads ban: is your product affected? Accessed at: https://www.epa.govt.nz/news-and-alerts/alerts/microbeads-ban-is-your-product-affected/#:~:text=It%20is%20illegal%20to%20manufacture%20or%20supply%20banned%20products%20containing,in%20doubt%2C%20please%20contact%20us. Accessed: 7-October-2022.

Park E-J, Han J-S, Park E-J, Seong E, Lee G-H, Kim D-W, Son H-Y, Han H-Y, Lee B-S. (2020) Repeated-oral dose toxicity of polyethylene microplastics and the possible implications on reproduction and development of the next generation. Toxicology Letters; 324: 75-85.

Prata JC, da Costa JP, Lopes I, Duarte AC, Rocha-Santos T. (2020) Environmental exposure to microplastics: An overview on possible human health effects. Science of the Total Environment; 702: 134455.

Ragusa A, Svelato A, Santacroce C, Catalano P, Notarstefano V, Carnevali O, Papa F, Rongioletti MCA, Baiocco F, Draghi S. (2021) Plasticenta: First evidence of microplastics in human placenta. Environment International; 146: 106274.

Salthammer T. (2022) Microplastics and their additives in the indoor environment. Angewandte Chemie International Edition; 61(32): e202205713.

Schwabl P, Köppel S, Königshofer P, Bucsics T, Trauner M, Reiberger T, Liebmann B. (2019) Detection of various microplastics in human stool: a prospective case series. Annals of Internal Medicine; 171(7): 453-457.

Severini MF, Buzzi NS, López AF, Colombo CV, Sartor GC, Rimondino GN, Truchet DM. (2020) Chemical composition and abundance of microplastics in the muscle of commercial shrimp *Pleoticus muelleri* at an impacted coastal environment (Southwestern Atlantic). Marine Pollution Bulletin; 161: 111700.

Shi X, Wang X, Huang R, Tang C, Hu C, Ning P, Wang F. (2022) Cytotoxicity and genotoxicity of polystyrene micro-and nanoplastics with different size and surface modification in A549 cells. International Journal of Nanomedicine: 4509-4523.

Stock V, Böhmert L, Lisicki E, Block R, Cara-Carmona J, Pack LK, Selb R, Lichtenstein D, Voss L, Henderson CJ. (2019) Uptake and effects of orally ingested polystyrene microplastic particles in vitro and in vivo. Archives of Toxicology; 93(7): 1817-1833.

Sun Q, Li J, Wang C, Chen A, You Y, Yang S, Liu H, Jiang G, Wu Y, Li Y. (2022) Research progress on distribution, sources, identification, toxicity, and biodegradation of microplastics in the ocean, freshwater, and soil environment. Frontiers of Environmental Science & Engineering; 16(1): 1-14.

Toto B, Refosco A, O'Keeffe M, Barkhald ØH, Brønstad A, Lied GA, Yadetie F, Goksøyr A, Kögel T, Dierkes J. (2022) Intestinal permeability and gene expression after polyethylene and polyamide microplastic ingestion in Wistar rats. Toxicology Letters; 370: 35-41.

Vethaak AD, Legler J. (2021) Microplastics and human health. Science; 371(6530): 672-674.

Wang Q, Wu Y, Zhang W, Shen T, Li H, Wu J, Zhang L, Qin L, Chen R, Gu W. (2022) Lipidomics and transcriptomics insight into impacts of microplastics exposure on hepatic lipid metabolism in mice. Chemosphere; 308: 136591.

Wang Y-L, Lee Y-H, Hsu Y-H, Chiu I-J, Huang CC-Y, Huang C-C, Chia Z-C, Lee C-P, Lin Y-F, Chiu H-W. (2021) The kidney-related effects of polystyrene microplastics on human kidney proximal tubular epithelial cells HK-2 and male C57BL/6 mice. Environmental Health Perspectives; 129(5): 057003.

WHO. (2022) Dietary and inhalation exposure to nano-and microplastic particles and potential implications for human health. 924005460X. World Health Organization.

WHO/FAO. (2022) Microplastics in food commodities- a food safety review on human exposure through dietary sources. Rome: Food and Agriculture Organization of the United Nations.

Wu B, Wu X, Liu S, Wang Z, Chen L. (2019) Size-dependent effects of polystyrene microplastics on cytotoxicity and efflux pump inhibition in human Caco-2 cells. Chemosphere; 221: 333-341.

Wu P, Lin S, Cao G, Wu J, Jin H, Wang C, Wong MH, Yang Z, Cai Z. (2022) Absorption, distribution, metabolism, excretion and toxicity of microplastics in the human body and health implications. Journal of Hazardous Materials: 129361.

Xie L, Chen T, Liu J, Hou Y, Tan Q, Zhang X, Li Z, Farooq TH, Yan W, Li Y. (2022) Intestinal flora variation reflects the short-term damage of microplastic to the intestinal tract in mice. Ecotoxicology and Environmental Safety; 246: 114194.

Yong CQY, Valiyaveettil S, Tang BL. (2020) Toxicity of microplastics and nanoplastics in mammalian systems. International Journal of Environmental Research and Public Health; 17(05): 1509.

Zha H, Xia J, Li S, Lv J, Zhuge A, Tang R, Wang S, Wang K, Chang K, Li L. (2023) Airborne polystyrene microplastics and nanoplastics induce nasal and lung microbial dysbiosis in mice. Chemosphere; 310: 136764.

Science for Communities

INSTITUTE OF ENVIRONMENTAL
SCIENCE AND RESEARCH LIMITED

Kenepuru Science Centre
34 Kenepuru Drive, Kenepuru, Porirua 5022
PO Box 50348, Porirua 5240
New Zealand
T: +64 4 914 0700 **F:** +64 4 914 0770

Mt Albert Science Centre
120 Mt Albert Road, Sandringham, Auckland 1025
Private Bag 92021, Auckland 1142
New Zealand
T: +64 9 815 3670 **F:** +64 9 849 6046

NCBID – Wallaceville
66 Ward Street, Wallaceville, Upper Hutt 5018
PO Box 40158, Upper Hutt 5140
New Zealand
T: +64 4 529 0600 **F:** +64 4 529 0601

Christchurch Science Centre
27 Creyke Road, Ilam, Christchurch 8041
PO Box 29181, Christchurch 8540
New Zealand
T: +64 3 351 6019 **F:** +64 3 351 0010

www.esr.cri.nz