A rapid review and meta-regression analyses of the toxicological impacts of microplastic exposure in human cells.

Evangelos Danopoulos[a]*, Maureen Twiddy[a], Robert West[b], Jeanette M. Rotchell[c]

[a]Hull York Medical School, University of Hull, Hull, HU6 7RX, United Kingdom

[b]Institute of Health Science, School of Medicine, University of Leeds, Leeds, LS2 9LU, United Kingdom

[c]Department of Biological and Marine Sciences, University of Hull, Hull, HU6 7RX, United Kingdom

*corresponding author: Allam Medical Building, University of Hull, Hull, HU6 7RX. Email: hyen7@hyms.ac.uk, telephone: +44 1482 463279

Abstract

Humans are exposed to microplastics (MPs) daily via ingestion and inhalation. It is not known whether this results in adverse health effects and, if so, at what levels of exposure. Without epidemiological studies, human cell *in vitro* MP toxicological studies provide an alternative approach to this question. This review systematically synthesised all evidence and estimated thresholds of dose–response relationships. MEDLINE and Web of Science were searched from inception to March 2021 and study quality was rated using a novel risk of bias assessment tool. Seventeen studies were included in the rapid review and eight in the meta-regression. Four biological endpoints displayed MP-associated effects: cytotoxicity, immune response, oxidative stress, barrier attributes, and one did not (genotoxicity). Irregular shape was found to be the only MP characteristic predicting cell death, along with the duration of exposure and MP concentration (μg/mL). Cells showed varying cytotoxic sensitivity to MPs, with Caco-2 cells (human adenocarcinoma cell line) being the most susceptible. Minimum, environmentally-relevant, concentrations of 10 μg/mL (5-200 μm), had an adverse effect on cell viability, and 20 μg/mL (0.4 μm) on cytokine release. This work is the first to quantify thresholds of MPs effects on human cells in the context of risk assessment.

Abbreviations

ABCC2 and ABCG2, ATP-binding cassette (ABC) transporters; ABS, acrylonitrile butadiene styrene; A549 adenocarcinomic human alveolar basal epithelial cells, BEAS-2B, human lung epithelial cells; BeWo b30, human placental choriocarcinoma cell line; Caco-2, human adenocarcinoma cell line; CCK-8, cell counting kit 8; COOH, carboxy-modified surface; COPD, chronic obstructive pulmonary disease; CPS, Carboxylated polystyrene; ELISA, Enzyme-Linked Immunosorbent Assay; HCA, high content analysis; HDPE, high-density polyethylene; HDFs, human dermal fibroblasts; HeLa, cervical cancer cells; HepaRG, human hepatic cells; HepG2, Human Caucasian hepatocyte carcinoma cells; HMC-1, the human mast cell line-1; HPEC- A2 cells, SV40-transformed microvascular human placental venous endothelial cells; HT29-MTX-E12, a mucus-secreting subclone from colon adenocarcinoma HT29 cells differentiated into mature goblet cells; IL-, interleukin; KATO III, gastric cancer stem cells; LDH, lactate dehydrogenase; LDPE, low-density polyethylene; LIVE/DEAD kit, viability/cytotoxicity test; MCP-1, Monocyte chemoattractant protein-1; LOAEL, lowest-observed-adverse-effect level; MDM, human blood monocyte-derived macrophages; MDDC, dendritic cells; M-cell, Microfold cells; MTS assay, colorimetric cell proliferation assay kit; MTT assay, cellular metabolic activity colorimetric assay; NIH/ 3 T3, murine fibroblast cell line; NOAEL, no-observed-adverse-effect-level, NP, nanoplastics; PBMCs, peripheral blood mononuclear cells; PAN, polyacrylonitriles; PA6, polyamide; PCR, polymerase chain reaction; PE, polyethylene; PET, Polyethylene terephthalate; PP, polypropylene; PS, polystyrene; PU, polyurethane; PUR, polyurethanes; PVC, polyvinyl chloride; p53, sensitive reporter cell line based on the human liver carcinoma cell line; Raji B, human lymphocytes cells; RT-PCR, Reverse transcription polymerase chain reaction; T98G, human glioblastoma multiforme cells; TEER, transepithelial electrical resistance; THP-diff., THP-1 cells differentiated into macrophages; THP-1, human monocytic cell line; TNF-α, Tumour Necrosis Factor alpha; t-PS, digestive tract transformed PS-MPs; TPU,

polyurethane; U937, human histocytic lymphoma cells; WST-1 assay, cell proliferation assay; ZO-1, Zonula occludens-1

Keywords

Dose-response, risk assessment, human health, toxicity, immune response

1. Introduction

The prevalence of microplastics (MPs) is ubiquitous, found in almost every compartment of the environment; in the air (Wright et al., 2020), food (Teng et al., 2019) and drinking water (Zhang et al., 2020). MP contamination will continue to rise as plastic production and use around the world increases (Lebreton and Andrady, 2019). If plastic waste mismanagement continues as it is or increases, it is predicted that within a century, MP ecological risks will be widespread in ecosystems across the world (SAM, 2019; SAPEA, 2019). Two environmental routes of exposure are proposed for humans: ingestion (dietary and non-dietary) and inhalation, as established by numerous studies and reviews and reported widely (EFSA, 2016; Gallo et al., 2018; GESAMP, 2016; Karbalaei et al., 2018; Lusher et al., 2017; Prata, 2018). The presence of MPs has been verified in human colectomy samples (Ibrahim et al., 2021), human placenta (Ragusa et al., 2021) and in human lung tissue (Amato-Lourenço et al., 2021; Pauly et al., 1998). Furthermore, when human stool samples were collected from eight volunteers, as part of a prospective case series study, all of them were found positive for MP contamination (Schwabl et al., 2019). A third environmental exposure route has also been proposed via dermal absorption but currently there is no evidence to support it (BfR, 2014). Another recognized exposure route (not environmental) for MPs is via the degradation of medical prosthetics that are entirely made of or contain plastic and present an entirely different paradigm for MP human exposures and effects (Doorn et al., 1996; Minoda et al., 2003; Urban et al., 2000; Willert et al., 1996).

A wide range of MP whole-organism (apical) and mechanistic toxic effects have been discovered in a range of biota, most of which come from the marine ecosystem. The toxic effects concern multiple life stages, including developmental, behavioural, genotoxic and metabolic as well as increased mortality, immune responses and intestinal barrier dysfunction (Chang et al., 2020; Hale et al., 2020; Huang, Z. et al., 2021; Prüst et al., 2020).

Risk assessment (RA) is the first and key part of an integrated risk analysis and its outcomes are a qualitative or quantitative expression of the likelihood of a hazard, in this case MPs, to cause harm (FAO and WHO, 2009). The aims of a human health RA are to estimate the risk to a specific population (general or sub-population) that has been exposed to an agent, taking into consideration the characteristics of both the agent and the population (IPCS, 2004). Human risk assessments usually include epidemiological studies but in the case of MPs, the only currently available scientific toxicological data come from *in vitro* studies (animal and human cells) and *in vivo* animal studies, most of which focus on marine organisms and to a lesser extent, on rodents (e.g. Devriese et al., 2017; Li et al., 2020; Santana et al., 2018). There are four interconnected processes in a RA: hazard identification, hazard characterisation/ dose-response, exposure assessment and risk characterization (WHO & IPCS, 2010). The toxicity biological endpoints considered in a risk assessment can include early mechanistic responses, but also extend to apical biological endpoints (IPCS, 2009) which are beyond the focus of this review.

The aim of this rapid review and meta-regression was to identify all currently available scientific data on MP toxicity on human cells, assess their quality and collate data to define thresholds of dose–response relationships, in order to inform a human RA. Such thresholds are health-based guidance values based on available toxicological evidence which provide an estimate of the safe levels of human exposure for different biological endpoints and health outcomes (EPA, 2014). A further objective was to detect whether there was an association between specific characteristics of the experimental conditions and the resulting toxicity in human cell lines. In the absence of epidemiological evidence, human cell lines are one of the currently available sources of scientific evidence for human health effects, the other being animal *in vivo* and *in vitro* studies, which are beyond the scope of this review.

2. Methods

The methodology used for the rapid review (Garritty et al., 2020; Hamel et al., 2021) was based on a simplified version of the systematic review guidelines (Higgins et al., 2021), and used a protocol based

on the guidelines set by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses protocols (PRISMA-P) (Moher et al., 2015; Shamseer et al., 2015). The eligibility criteria stated that only experimental study designs were eligible for inclusion. No publication date limits were set. Only studies that used human-cell models to test any toxicity effects from MPs were included. When a study also used animal cells, the outcomes were not included in the review. Studies that focused only on NPs (<100nm) were not included. MPs were defined to have a size range from 100 nm to 5 mm (Lusher et al., 2017). When a study tested both MPs and NPs, only the results for the former were included.

The following online databases/sources were searched from launch date using the Web of Science interface: Web of Science core collection (1900 onwards) and MEDLINE (1950 onwards). In addition, the reference lists of any relevant reviews discovered, were searched. The last search was executed on the 19th of March 2021. Search terms included: microplastic, human cell (see SM1, part 2). Study screening was executed at two levels and the screening questions were developed according to the eligibility criteria. In the first level, only titles and abstracts were reviewed. For studies that met the inclusion criteria, full papers were downloaded for the second-level screening. The reasons for excluding any studies at the second level of screening were recorded and reported in the results. Data extracted were: test MP characteristics (size, origin, shape, polymer, density), test cell model characteristics (origin, cell density), MP concentration of applied dose (in any quantified unit), duration of exposure, biological endpoint, test, biological marker and outcomes.

2.1. Synthesis of the results

The primary outcomes of interest were toxicity descriptors concerning all possible biological endpoints, expressed either quantitatively or qualitatively. Each study included multiple outcomes testing a range of experimental conditions. Different methodologies and methods were used across studies. Similar biological endpoints, tests and biological markers were grouped to achieve the best possible relevance and comparability. All outcomes were synthesized and explored in a narrative analysis following the guidelines set down by the Centre for Reviews and Dissemination (2009) and

the Cochrane collaboration (Higgins et al., 2021) and the results were reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement (Liberati et al., 2009; Moher et al., 2009). Quantitative results were explored via meta-regression, modifying the approach of Borenstein (2009) and dose–response thresholds were reported in a statistical summary. The initial protocol for the rapid review included a traditional meta-analysis design using mixed-effects models (random and fixed-effects) to collate scientific data. Unfortunately, a meta-analysis was not possible as effect sizes were not reported, only the statistical significance of the effect at certain probability thresholds (for further information see 3.4).

A novel meta-regression analysis was used instead to explore and assess the relationship between certain predictors, namely, the experimental characteristics (from now on termed covariates) and the dependent variable (effect size) which in this case was the binary outcome of whether a statistically significant difference from the results of the negative control samples (using probabilistic analysis) was detected or not, from now on denoted as SIG. and N. SIG. The relationship between covariates and outcome is measured by estimating the probability of class, where class is the binary outcome, 0 or 1 (Osborne, 2015). One limitation of the analysis was that unit weights were assigned to the studies as the precision of their respective effect estimate was not known. In order to achieve meaningful analysis grouping and comparison, results were collated, in the first instance, by biological endpoints and then by the reported outcome, where it was possible and appropriate. A series of simplifications were applied on the covariates for coherence and to allow meaningful analysis (see Supplementary Material, SM 1, part 1). The main outcomes of the logit model were the intercept and the regression coefficient estimates (β) which accompanied by a p value informed us as to the effect of the covariate on the outcome. All analysis was performed in R (version 4.1.1) (R Core Team, 2019) using RStudio (version 1.2.1335). A series of diagnostic tests were used to evaluate the logit models. Multi-collinearity was assessed by calculating the Variance Inflation Factor (VIF) value (Craney and Surles, 2002; Thompson et al., 2017). The overall performance of the models was judged by the prediction

error of the coefficients in the model, which was calculated using the MASS package in R (Venables and Ripley, 2002). Predictions of both outcomes were also reported in a contingency table. Linearity between the covariates and the logit of the outcome were explored graphically. Extreme values and influential values were detected by visualizing the Cook's distance values (Osborne, 2015) and examining the standard residual errors (Menard, 2002). All-subset logistic regression was also used to detect the best possible combination of covariates to predict the outcome. The criterion to determine the best-subset model was the Akaike Information Criterion (AIC).

Furthermore, multilevel logistic modelling was used to account for the heterogeneity caused by the data clustered within different studies (Sommet and Morselli, 2017). The multilevel models used a random intercept representing the nesting of the data in the studies. Three steps were used: first, a null (empty) model was created which did not include any of the level-1 predictors but allowed intercepts to vary across clusters and calculated the intraclass correlation coefficient (ICC), which quantifies the proportion of the variation between the clusters in the total variation. Second, a model was fitted that included a random intercept and a fixed slope, to examine the variation of the level-1 effects between clusters and third, random intercept and random slope/s models were fitted to understand the variance of slopes across clusters (Aguinis et al., 2013). Analysis was performed in R (R Core Team, 2019) using the additional package of lme4 (Bates et al., 2015). The overall assessment of the certainty of the evidence for each study was guided by the five domains of the GRADE framework (Higgins et al., 2021) and classified into four certainty ratings: high, moderate, low and very low.

2.2. Risk of Bias (RoB) assessment

An integral part of any systematic review is the assessment of each studies' validity (reporting, internal and external). This process is termed a risk of bias (RoB) assessment and uses a checklist approach to promote an objective assessment, based on the published or readily available material. A number of RoB tools exist (Hooijmans et al., 2014; Schaefer and Myers, 2017; Whaley et al., 2020; Woodruff

and Sutton, 2014). A tool was needed for application in the field of MP toxicological studies to address the specific issues arising in this particular field.

The development of the MP toxicological RoB tool (MP-tox-RoB) has been informed by the US National Toxicology Program's Office of Health Assessment and Translation (OHAT) (OHAT, 2019) RoB tool, guidelines by US EPA (2018) under the Toxic Substances Control Act (TSCA) risk evaluations and our previously developed RoB tool for MP environmental research (Danopoulos et al., 2020a; 2020b; 2020c). The principles underpinning its development are those that govern the Cochrane systematic reviews of interventions (Higgins et al., 2021; Sterne et al., 2016). There are eight domains tailored to MPs research with 31 signalling questions: test MP and model information, test design, MP exposure characteristics, quality assurance/control and confounding, outcome assessment, analysis, result reporting and other sources of bias followed by an overall rating. The check list can be found in SM 1, (Table S1). The MP-tox-RoB tool is intended for the appraisal of studies employing experimental study designs. The overall rating of each study could be low, moderate, serious or critical (SM1, Table S2) and it was used to judge the inclusion of the study's evidence in the rapid review and the meta-regression. More information on the tool's assessment process is provided in the explanation/elaboration section (SM1, part 4). MP-tox-RoB is not based on static scales but scientific judgement and the currently available body of evidence. In this sense, the tool will be continuously evolving since the standard of each study is measured against other similar studies and not a 'gold standard'. As new studies become available the standard will inevitably shift, aiming to become increasingly higher as studies' quality enhance. It is essentially a state-of-the-science approach not a gold-standard approach.

3. Results

3.1. Study selection

Database searches identified 166 publications, and a further two were identified from searching the reference lists of relevant reviews. During the first level screening 144 studies were excluded based on their title and abstract. The full text of 24 studies was then assessed and 17 met the eligibility criteria set for this rapid review. Eight of those studies were included in a quantitative meta-regression (Figure 1). The reasons for the exclusion of the studies in the second-level screening are provided in SM 1, part 3.

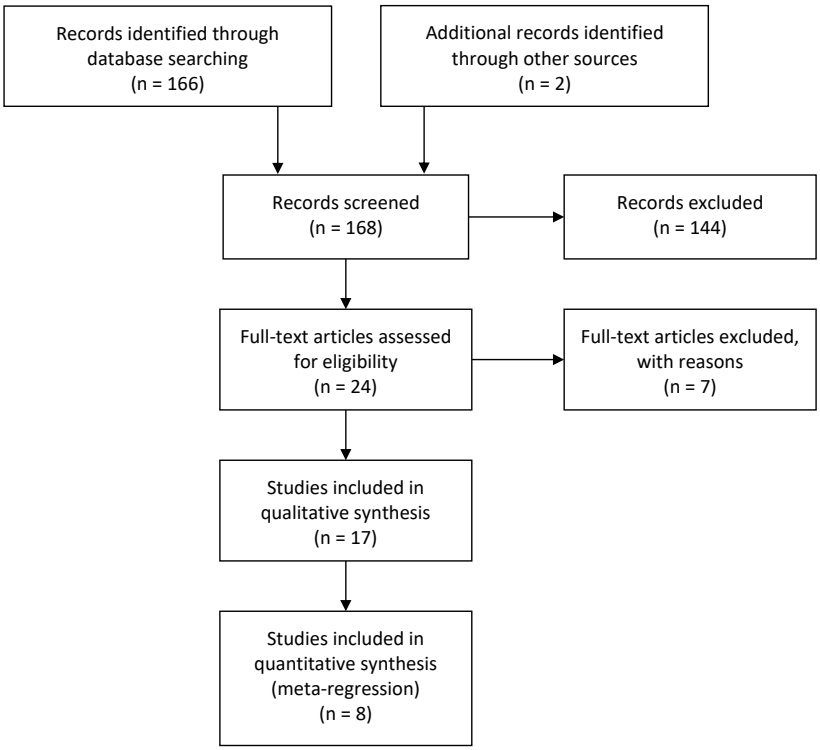

Figure 1. Prisma flow diagram. The chart illustrates the flow of information in the initial parts of the rapid review starting from the identification of records and through the first and second-level screening. The reasons for any exclusion of papers in the full-text assessment are provided in Supplementary material 1, part 2.

3.2. Study characteristics

The characteristics of the studies are presented in Table 1. In order to facilitate the presentation of this versatile data frame, the biological endpoints have been grouped in five categories: cytotoxicity, immune response, oxidative stress, barrier attributes and genotoxicity, as illustrated in Figure 2. The studies used 15 different cell models and co-cultures, testing 10 different polymers, using more that 30 different tests/biological markers. Full test conditions and results are presented in a spreadsheet in Supplementary material 2.

Table 1. Study characteristics for microplastic (MP) toxicological human cell studies.

Study	Polymer	Origin	Particle size (µm)	Shape	Cell model	Biological endpoint
Brown et al. (2001)	PS	primary	0.202 and 0.535	Spherical	A549	Immune response
Choi et al. (2020)	PS	secondary	5−25, 25−75 and 75−200	Randomly shaped	PBMCs	Cytotoxicity [a]
					RBC-removed PBMCs	Immune response
					KATO III cells	Cytotoxicity
					HeLa cells	Cytotoxicity
					HDFs	Cytotoxicity, Oxidative stress
Choi et al. (2021)	HDPE	primary	1–10, 50 (45-53), and 100 (90-106)	Spherical	PBMCs	Cytotoxicity, Immune response
					HMC-1 cell line	Immune response
	LDPE	secondary	25-75 and 75-200	Randomly shaped	HeLa	Cytotoxicity
					HDFs	Cytotoxicity, Oxidative stress
(Dong et al., 2020)	PS	primary	1.72 ± 0.26	Spherical	BEAS-2B cells	Cytotoxicity, Oxidative stress, Immune response, Barrier integrity, Predictive biomarker for COPD

Goodman et al. (2021)	PS	primary	1 and 10	Spherical	A549	Cytotoxicity, Cell proliferation, Internalization
Han et al. (2020)	PVC	secondary	25-75 and 75-200	Irregular	PBMCs	Cytotoxicity, Immune response
	ABS				HMC-1 cell line	Immune response
					HDFs	Cytotoxicity
					HeLa cells	Cytotoxicity
Hesler et al. (2019)	COOH - PS	primary	0.5, (0.4658 ± 0.0102)	Spherical	Co-culture: Caco-2 and HT29-MTX-E12	Cytotoxicity, Barrier integrity, Translocation, Uptake
					BeWo b30 cell line	Cytotoxicity
					Co-culture: BeWo and HPEC- A2 cells	Barrier integrity, Translocation, Uptake
					p53-sensitive reporter cell line	Genotoxicity
Hwang et al. (2019)	PP	secondary	~20 and ~200 (25–200)	Various shapes	PBMCs	Immune response
					HDFs	Cytotoxicity, Oxidative stress
					HMC-1 cell line	Immune response
Hwang et al. (2020)	PS	primary	0.460, 1, 3, 10, 40 and 100	Spherical	HDFs	Cytotoxicity, Uptake
					PBMCs	Cytotoxicity, Immune response, Uptake
					HMC-1 cell line	Immune response
Lehner et al. (2020)	PA6	secondary	72 [b]	Fragments	Co-culture: Caco-2/HT29-MTX/ MDM/MDDC	Cytotoxicity, Immune response, Barrier integrity
	PU (hardened)		253 [b]			
	TPU (ester)		264 [b]			
	PP (Sun)		282 [b]			
Liu et al. (2020)	PS	primary	0.1 and 5	Spherical	Caco-2 monolayer model	Barrier integrity, Permeability, Oxidative stress,

	t-PS [c]	secondary	0.4402 [d]			Paracellular and trans-membrane transport, Immune response
(Schirinzi et al., 2017)	PE	primary	3–16 (with NPs 0.1 – 0.6)	Spherical	T98G cells	Cytotoxicity, Oxidative stress
	PS	primary	10 (with NP 0.04 – 0.25)	Spherical	HeLa cells	Cytotoxicity, Oxidative stress
Stock et al. (2019)	PS	primary	1, 4, 10	Spherical	Caco-2 cell line	Cytotoxicity, Uptake
					Co-culture: (mucus) model: Caco-2 cells and HT29-MTX-E12 cells	Uptake
					Co-culture: (M-cell) model: Caco-2 cells and Raji B	Uptake
					M0 macrophages (from THP-1 cell line), M1 and M2	Uptake
					M1, M2 [e]	Macrophage polarization
Stock et al. (2021)	PE	primary	2.2 (1-4), 16.5 (10-20)	Spherical	Caco-2 cells	Cytotoxicity
					HepaRG	Cytotoxicity
	PE	primary	90.1 [f]	Powder		
	PP	secondary	67.1 [f]	Powder	HepG2	Cytotoxicity
	PET	primary	60 [f]	Powder		
	PVC	primary	136.5 [f]	Powder	Caco-2 model	Uptake
Wang et al. (2020)	PS	primary	0.3, 0.5, 1, 3, 6	Spherical	Caco-2	Cytotoxicity, Oxidative stress, Uptake
Wu et al. (2019)	PS	primary	0.1 and 5	Spherical	Caco-2 cells	Uptake, Cytotoxicity, Oxidative stress, Barrier integrity
Wu et al. (2020)	PS	n/r	5	n/r	Caco-2 cells	Cytotoxicity, Oxidative stress, Gene expression alteration

222 [a] cytotoxicity was accessed via cell viability unless stated otherwise, [b] median size, [c] original and
223 transformed via a digestive process to mimic human digestive processes, [d] 100 nm transformed size:

440.2 nm, 5µm transformed size: not reported (n/r), [e] M0 macrophages differentiated from THP-1 cell line, exposed to MPs, and then polarized to M1 and M2, [f] polydisperse, mean diameter provided in the source, [g] spherical according to the manufacturer Microparticles GmbH. Note: ABS, acrylonitrile butadiene styrene; A549 adenocarcinomic human alveolar basal epithelial cells, BEAS-2B, human lung epithelial cells; BeWo b30, human placental choriocarcinoma cell line; Caco-2, human adenocarcinoma cell line; COOH, carboxy-modified surface; COPD, chronic obstructive pulmonary disease; CPS, Carboxylated polystyrene; HDFs, human dermal fibroblasts; HeLa, cervical cancer cells; n/r, not reported; HepaRG, human hepatic cells; HepG2, Human Caucasian hepatocyte carcinoma cells; HMC-1, the human mast cell line-1; HPEC- A2 cells, SV40-transformed microvascular human placental venous endothelial cells; HT29-MTX-E12, a mucus-secreting subclone from colon adenocarcinoma HT29 cells differentiated into mature goblet cells; KATO III, gastric cancer stem cells; MDM, human blood monocyte-derived macrophages; MDDC, dendritic cells; M-cell, Microfold cells; M0,1,2, macrophages; NIH/ 3 T3, murine fibroblast cell line; NP, nanoplastics; PBMCs, peripheral blood mononuclear cells; PA6, polyamide; PE, polyethylene; PP, polypropylene; PS, polystyrene; PU, polyurethane; p53, sensitive reporter cell line based on the human liver carcinoma cell line; Raji B, human lymphocytes cells; RBC, red blood cells; T98G, human glioblastoma multiforme cells; THP-diff., THP-1 cells differentiated into macrophages; THP-1, human monocytic cell line; t-PS, digestive tract transformed PS-MPs; TPU, polyurethane ; U937, human histocytic lymphoma cells

The studies used 28 test MPs: 16 primary and 11 secondary, while the origin of one test MPs was not defined (Wu et al., 2020). The primary test MPs were spherical (13 out of 16) and powders (three out of 16); the secondary MPs (11) were all consisting of irregular shapes. Seven out of the 17 studies did not use spherical MPs. Choi et al. (2020), Han et al. (2020), Hwang et al. (2019) and Lehner et al. (2020) used secondary, randomly-shaped, in-house produced MPs. Choi et al. (2021) used both spherical, primary MPs (HDPE) and randomly-shaped, secondary MPs (LDPE). Stock et al. (2021) also used a combination of primary, commercially sourced microspheres (PE) and powders (PE, PT, PVC) as well as secondary, grounded powders (PP). Liu et al. (2020) used both primary, spherical PS MPs and secondary, irregularly shaped MPs. All the studies, apart from Lehner et al. (2020) and Liu et al. (2020) used a variation of a ball-mill method to create their secondary MPs. Lehner et al. (2020)

253 used a combination of methods applying cryogenic temperatures followed by milling, while Liu et al.
254 (2020) used a digestion process to mimic the digestive tract. Wu et al. (2020) did not report the origin
255 nor the shape of the MPs they used.

256 Four studies (Choi et al., 2020; Choi et al., 2021; Han et al., 2020; Hwang et al., 2019) reported only
257 the size ranges used in the experiments, while 10 studies provided the exact sizes (Brown et al., 2001;
258 Dong et al., 2020; Goodman et al., 2021; Hesler et al., 2019; Hwang et al., 2020; Liu et al., 2020; Stock
259 et al., 2019; Wang et al., 2020; Wu et al., 2019; Wu et al., 2020), one study (Lehner et al., 2020)
260 provided the MP size distributions (D10, D50 and D90). One study (Schirinzi et al., 2017) provided a
261 range value for one of the test MPs (PE) and a specific size for the other (PS). One study (Stock et al.,
262 2021) provided ranges for two test MPs (PE 1-4, 10-20 μm) accompanied by the mean diameter, as
263 measured in the laboratory via SEM, for those and the remaining test MPs (PP, PET, PVC and PE 90
264 μm). The overall size range was 0.1 to 282 μm.

265

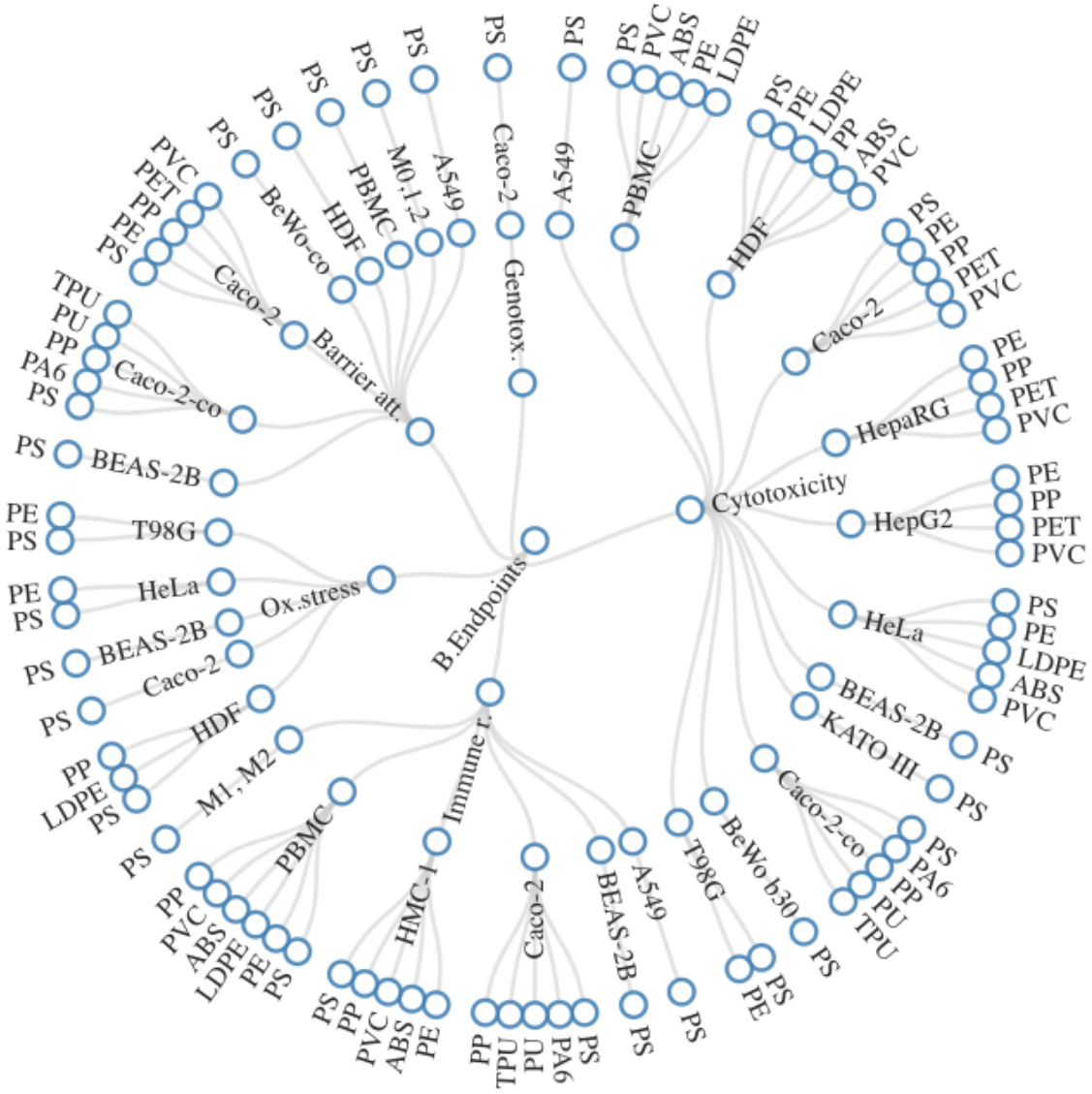

Figure 2. Biological endpoints, cell models and test MPs polymers used in the cumulative experiments reported by all studies. Note: ABS, acrylonitrile butadiene styrene; A549, adenocarcinomic human alveolar basal epithelial cells; Barrier att., Barrier attributes; BEAS-2B, human lung epithelial cells; BeWo b30, human placental choriocarcinoma cell line; Caco-2, human adenocarcinoma cell line; co, coculture; Genotox., Genotoxicity; HDFs, human dermal fibroblasts; HeLa, cervical cancer cells; HepaRG, human hepatic cells; HepG2, Human Caucasian hepatocyte carcinoma cells; HMC-1, the human mast cell line-1; Immune r., Immune response; KATO III, gastric cancer stem cells; LDPE, low-density polyethylene; M0,1,2, macrophages; Ox. Stress, Oxidative stress; PBMCs, peripheral blood mononuclear cells; PA6, polyamide; PE, polyethylene; PP, polypropylene; PS, polystyrene; PU, polyurethane; T98G, human glioblastoma multiforme cells; TPU, polyurethane

3.2.1. Conversion of MPs mass to particle number

All the studies apart from one (Stock et al., 2019) used the mass of the particles to denote the MP concentrations of the dose used in the experiments. Of the 17 studies included in the analysis, eight attempted to convert the concentrations to another metric. Brown et al. (2001) and Goodman et al. (2021) reported concentrations in both mg/mL and MPs/mL, while Stock et al. (2019) expressed the concentrations in MPs/mL, pg/mL, μm^2/mL and μm^3/mL. None of the three studies reported their method for the conversions. Choi et al. (2020) and Choi et al. (2021) used the basic volume to mass conversion assuming that the particles were cubes, although they used spherical and randomly shaped MPs. Dong et al. (2020) is one of the two studies that reported the concentration by surface area (cm^2) and stated that the mass concentration can be converted to particle concentration by multiplying by 5.12×10^3, but did not provide any rationale for this conversion. Han et al. (2020) proposed the averaging of volumes and densities across MPs to calculate exposures in MPs/mL. Hwang et al. (2020) used the more specialized equations proposed by Connors et al. (2017).

For the purposes of this review, a conversion was used for any concentrations reported in the toxicity studies (µg/mL) where studies did not supply both metrics (of either the amount or the mass), to the metrics commonly used within the environmental studies (MPs/mL). The rationale for this approach was that more details were available for the substances, as they have been handled in a controlled environment. This conversion is therefore an estimation of what is used, primarily, to detect whether the order of magnitude used in toxicity studies is relevant to the results reported by environmental studies. It must also be noted that the concentrations expressed by surface area (cm^2) could not be converted nor directly compared to the rest of the units. To our knowledge, an available method does not exist for the conversion of the concentration of irregularly shaped MP from µg/mL to MPs/mL or vice versa. Therefore, the equation by Connors et al. (2017) for converting MP mass concentration to abundance concentration was used for both spherical and irregularly shaped MPs. The equation is an extension to the basic relationship between size, weight and density. When the conversions were

reported by the studies, those concentrations were used. When the studies did not report the density of the polymer, the standard density reported in literature was used: PE ≈ 0.940 g/cm^3, PP ≈ 0.905 g/cm^3 (Plastics Europe, 2021), and PS ≈ 1.053 g/cm^3 (Mark, 1999).

3.3. Risk of bias

The results of the RoB assessment are presented in SM1, Table S3 and in Figure 3. Five of the studies were found to be of critical RoB and their results were omitted from the narrative and the meta-regression analysis. All of the studies were assessed to have a RoB above the rating of low, implying that they all suffered from deficiencies in some aspect. The only domain where critical RoB rating was assigned was the test MPs and test model. Four studies (Han et al., 2020; Hwang et al., 2019; Wang et al., 2020; Wu et al., 2020) did not provide information on the origin or identification of the basic test material, whether MPs or cells.

The domain with the highest serious RoB rating was results reporting, where a series of issues were noted. For example, Choi et al. (2020) stated that cell death was not affected following a 1-day exposure to PS particles, but in a results figure, a significant difference ($p<0.01$) is reported for the dose with MP concentration of 1000 µg/mL for the 5-25 µm size. Hwang et al. (2020) reported, in the methods section, the use of four sizes of PS particles (460 nm, 1 µm, 3 µm, 10 µm) and six concentrations of PS MPs (1, 10, 100, 500, and 1,000 µg/mL) for the cytotoxicity tests. However, in the results section for the PBMCs, only three sizes (460 nm, 3 µm, 10 µm) were reported and an additional concentration of 0.5 µg/mL is reported. Stock et al. (2019) did not report all the doses used for the cytotoxicity assays. In the supporting information (Figure S4), four doses for each of the three particle sizes are reported but not all of them. From the figures included in the results (Fig. 3, S1, S2, and S3), it appears that for the sizes of 1 and 4 µm, more than four doses were used but not all reported. In addition, the conclusion states that the sizes of 4 and 10 µm particles were non-toxic, but the corresponding figures suggest that only the 10 µm size appears to have no significant impact.

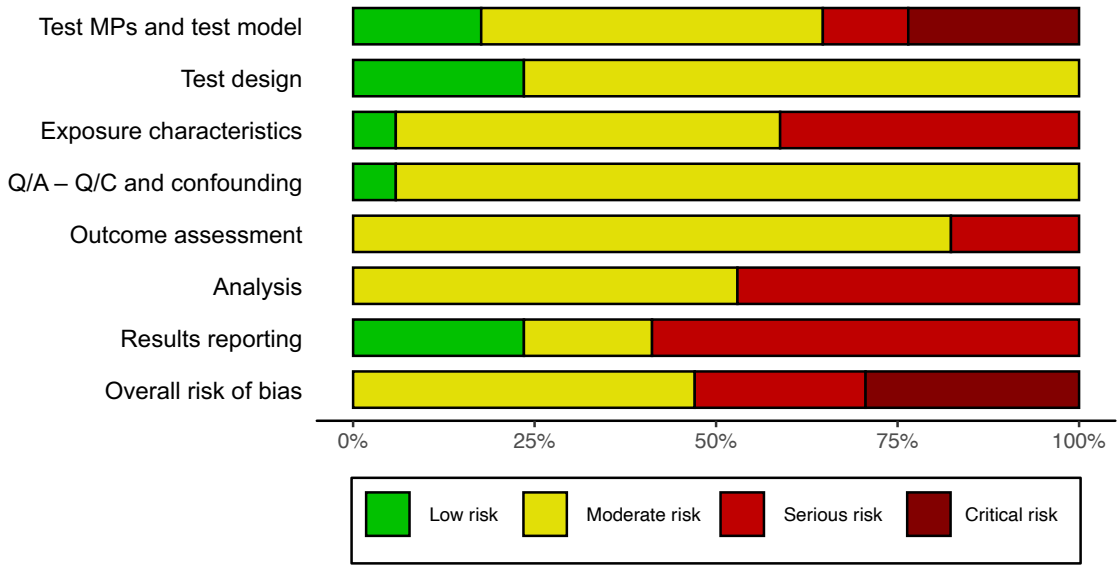

Figure 3. Risk of Bias (RoB) assessment rating results. The four ratings are illustrated by percentage. Individual rating per study and per domain is provided in Table S3 (SM2). Rating was executed according to the RoB tool. Note: MPs, microplastics; Q/A, quality assurance; Q/C, quality control.

3.4. Synthesis

In accordance with the aims and objectives of this rapid review, the results of the studies are presented by the biological endpoint that was under examination (Figure 2). When studies examined more than one biological endpoint, the outcomes are discussed separately. The majority of the studies reported their results only graphically. Therefore, the only "quantitative" results that could be extracted for all the experimental conditions was the binary outcome SIG. and N. SIG. It should be noted that some of the studies also reported in the figures the level of the detected significance ($p<0.05$, 0.01 or 0.001) and these results are also reported in SM2. Certain outcomes, especially those related to cell barrier behaviour (e.g. MP uptake), were only discussed qualitatively and are explored in a narrative analysis. None of the studies provided the raw results, hindering traditional meta-analysis approaches. In addition, the majority of the studies did not report the exact number of repeated tests and replicates for each experimental condition, while there was also ambiguity as to the density of the cells. All these pieces of information are vital for the execution of more in-depth analysis. It should also be noted that

seven studies did not report the use of positive control samples (Goodman et al., 2021; Hesler et al., 2019; Liu et al., 2020; Schirinzi et al., 2017; Stock et al., 2019; Wang et al., 2020; Wu et al., 2020). Positive control samples are commonly used as an additional step to test the efficiency of the experimental process. There was a complete absence of quality assessment and quality control (QA/QC) reporting for cross contamination of test material and test models by airborne MPs. Only one study (Prietl et al., 2014) reported that they examined the test material for contamination with substances that could interfere with the experiments such as endotoxins. Stock et al. (2021) was the only study to include a limit of detection (LOD) method for each particle type, thus incorporating a quality assurance into their experiments.

Only about a quarter of the studies (Choi et al., 2020; Choi et al., 2021; Han et al., 2020; Hwang et al., 2020) used data from environmental studies to provide a rationale for the concentrations of MPs used in their experiments. The exposure to MPs on a weekly basis was largely the starting point for calculating exposures for longer period of times. Choi et al. (2020) applied estimated exposures for life-long exposures and used data from drinking water MPs contamination (Mason et al., 2018), while Choi et al. (2021) and Han et al. (2020) used data for various food categories (Cox et al., 2019). Apart from using data on food and water contamination, Hwang et al. (2020) also included data for personal care products and assumed that using a facial scrub product which contains MPs can lead to MPs intake, which has no scientific basis. They state that intake of PS MPs from personal care or biomedical products is 4,594 – 94,500 per 5 mL of product per day. The study by Napper et al. (2015) is cited, which provides these data but refers to the quantities of MPs released by a product to the environment and not the intake of MPs by humans. Dermal absorption of MPs has been proposed as a possible route for MPs exposure, but it has yet to be proven. According to the current practice in toxicology studies in the field of MPs, 1 mg/mL was used as the maximum acceptable MP concentration of the applied dose referring to life-long dietary exposures.

In terms of mode of exposure, the majority of the studies considered the ingestion route. Three studies focused on the inhalation route. Dong et al. (2020) used two doses with MP concentrations of 10 and 100 μg/cm^2: one for general public and one for occupational exposures but did not offer a rationale. The lower dose (10 μg/cm^2), however, is in line with data from environmental studies (Wright et al., 2020). Goodman et al. (2021) also stated that the MP concentrations considered for the doses (0.05 - 100 μg/mL) represented urban and industrial exposures but did not offer a justification. Brown et al. (2001), on the other hand, argued that although the MP concentration of the doses (1000 μg/mL) were larger than those found in ambient air, they were used to account for the susceptibility of the population that is ordinarily affected by ultra-fine particle inhalation.

Four rather obvious but important parameters of the test MP and the test exposure must be noted. When the size, and, therefore, the mass per particle of the test MPs remains the same, increasing the concentration of the exposure (μg/mL) also increases the number of particles in the concentration (MPs/mL). If the size of the test MPs is increased, and the concentration of the exposure (mg/mL) is kept the same (as with the previous size of the test MPs) the number of particles in the concentration (MPs/mL) will inevitably decrease. Furthermore, when comparing different polymers with varying densities, the same concentration (μg/mL) contains more MPs/mL as the density of the polymer decreases. The relationship between these three variables must be taken into consideration in any attempt to analyse the data from the toxicology studies. The key distinction is whether to hypothesise that the MP effect is related to the mass of the dose, and therefore inextricably linked to the delivered volume of the substance, or to the number of particles which might also be linked to other parameters of the substance such as the surface charge. The shape of the test MP both affects the volume - mass relationship and the number of particles, and is, moreover, connected to surface characteristics of the test substance and possible physical MP effects. Untangling the mechanistic origin of possible MP effects is necessary in order to understand the overall toxicological behaviour of MPs.

392 ### 3.5. Cytotoxicity

393 #### 3.5.1. Narrative analysis

394 Sixteen studies examined cytotoxicity effects on human cells after exposure to MPs (Table 1). Five of
395 the studies (Han et al., 2020; Hwang et al., 2019; Stock et al., 2019; Wang et al., 2020; Wu et al., 2020)
396 were rated as of critical RoB and were excluded from further analysis (Table 2). Cytotoxicity was
397 measured in terms of cell viability, cell proliferation, metabolic activity or cell barrier damage, with
398 several studies looking at more than one of these expressions (Table 1). The studies used 11 different
399 cell models, tested nine polymers of two shapes and origins, ranging from 0.1 to 282 μm. Applied
400 doses ranged from MP concentrations of 0.01 to 100000 μg/mL while 14 tests/ biological markers
401 were used. Two studies (Dong et al., 2020; Lehner et al., 2020) expressed the MP concentrations of
402 applied doses as $\mu g/cm^2$, ranging from 1 to 1305.5 and the results could not be directly compared with
403 the rest of the studies. All the details can be found in SM2. The results can be broadly grouped by the
404 reported outcome of the applied tests. Six different tests reporting cell viability rates compared with
405 negative control samples (CCK-8, HCA assay, LIVE/DEAD kit, MTS assay, MTT assay, WST-1
406 assay), were used by seven studies (SM2). Significant results were reported for exposure to MPs of
407 five different polymers (LDPE, PE, PP, PS and PVC), of spherical and irregular shape, of primary and
408 secondary origin, with a size range of 0.5 to 137.5 μm and applied doses of MP concentrations between
409 0.01 and 100000 μg/mL, exposed for 24 and 96-hour durations. Goodman et al. (2021) also used an
410 MTT assay but reported the absorbance of MTT, instead of cell viability, as a measure of cellular
411 metabolic activity (cell proliferation). Significant results were reported for every condition tested (PS
412 MPs, sizes 1 and 10 μm, concentrations 0.05 to 100 μg/mL). Goodman et al. (2021) argued that the
413 sole use of MTT assays for measuring cell proliferation and cell viability can introduce error, since,
414 when used for prolonged exposure duration, metabolic activity and cell numbers cannot be
415 disentangled and, accordingly, used further tests to verify results. Cell proliferation was examined by
416 measuring the expression of the Ki67 marker reporting reduced ability. Goodman et al. (2021) also

used Trypan Blue exclusion and Calcein-AM/FACS assays, and reported little cytotoxicity of the exposed cells, but did not report significance levels. Dong et al. (2020) used the Trypan Blue exclusion assay reporting significant results only for PS MPs (1.72 μm) at concentrations of 10, 100 and 1000 μg/cm^2. Enzymatic activity of caspase-3, 8 and 9 (reported as fold change) was measured by one study (Stock et al., 2021) as a secondary measure of cytotoxicity (for their contribution to the cell apoptosis pathway) and reported significant results only on caspase-8 activity at concentrations of 50000 μg/mL for PE MPs (2.2 μm) and PP MPs (67.1 μm) confirming the results obtained from corresponding MTT assays. Two studies (Lehner et al., 2020; Wu et al., 2019) measured the release of LDH as a measure of integrity of the cell membrane and one (Liu et al., 2020) of the monolayer as related to cytotoxicity and all reported not significant results.

3.5.2. Meta-regression: Cell viability

Logistic regression modelling and multilevel modelling was used to examine the relationship between the variables of the experimental characteristics and the outcome of the cytotoxicity tests. Seven studies (Choi et al., 2020; Choi et al., 2021; Hesler et al., 2019; Hwang et al., 2020; Schirinzi et al., 2017; Stock et al., 2021; Wu et al., 2019) expressed results in terms of cell rate viability (using six different tests: CCK-8, HCA, Live/Dead kit, MTS, MTT, WST-1) and were found to be similar enough to be grouped for a meaningful meta-regression analysis. It should also be noted that Choi et al. (2021) did not report the results of eight samples regarding the exposure of HeLa cells to LDPE and therefore, the data were not included in the synthesis. The characteristics of covariates that were explored, coming from the seven studies that reported the rate of cell viability (310 data points), are presented in Table S4. The first step in this analysis, which used such a diverse data frame with many covariates, was to present the data visually to examine distributions and detect possible relationships (Ennos and Johnson, 2018). A series of observations were made by examining Figure 4 A-D, where three of the categorical covariates (cell model, cytotoxicity test, test polymer) and one integer covariate (duration) are presented. The most-used cell model was HDFs followed by PBMCs (Figure 4.A), the most-used

test was CCK-8 followed by the MTT assay (Figure 4.B), the most-used test polymer was PS followed by PE (Figure 4.C) and the most-used exposure time was 24 hours (Figure 4.D). The exposure of 12 hours had no significant results (Figure 4.D).

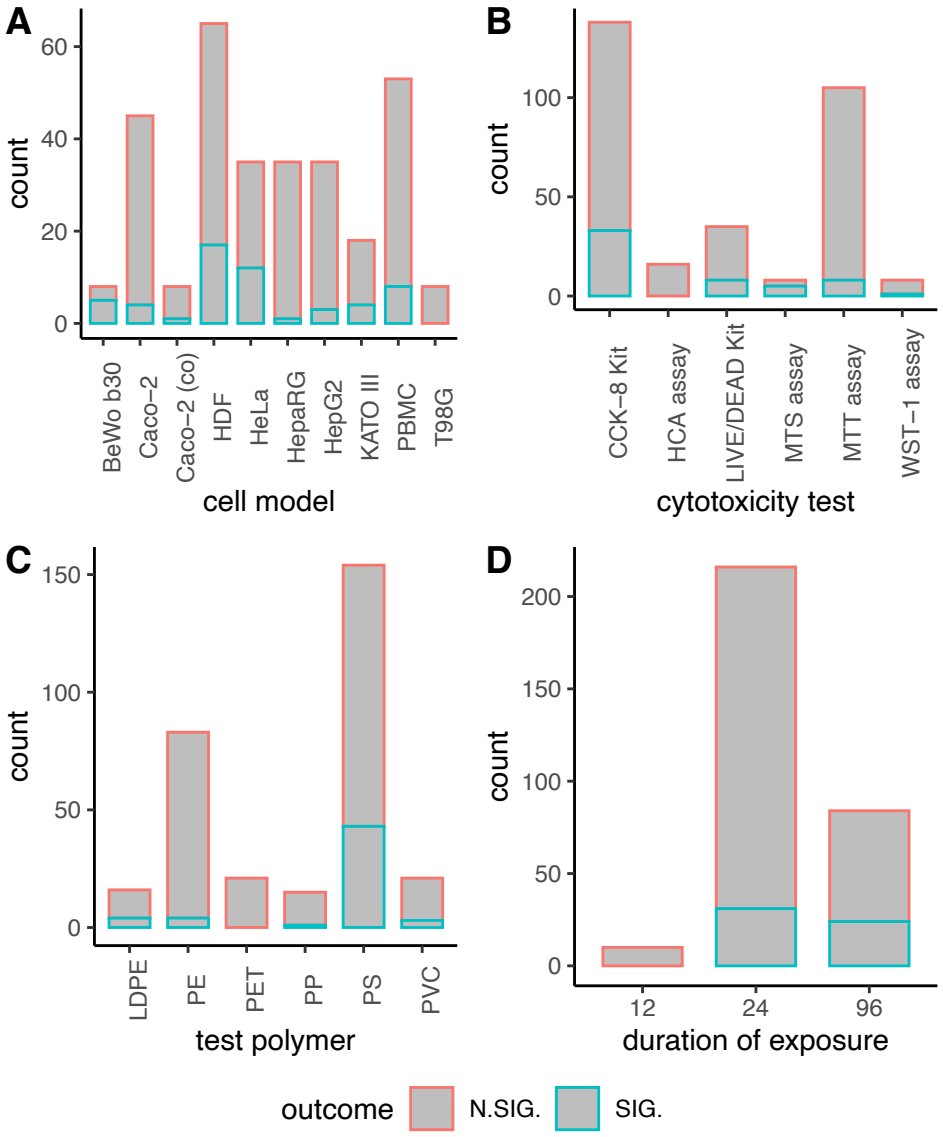

Figure 4. Distribution of the categorical covariates for the cell viability biological endpoint in the studies included in the meta-regression analysis; (A) cell model, (B) cytotoxicity test, (C) test polymer, and (D) integer covariate of duration of exposure. The outcome of significance results for the cell viability (cytotoxicity) biological outcome are highlighted in red/blue outlines. Note: BeWo b30, human placental choriocarcinoma cell line; Caco-2, human adenocarcinoma cell line; CCK-8, cell counting kit 8; co, coculture; HCA, high content analysis; HDFs, human dermal fibroblasts; HeLa, cervical cancer cells; HepaRG, human hepatic cells; HepG2, Human Caucasian hepatocyte carcinoma

cells; KATO III, gastric cancer stem cells; LDPE, low-density polyethylene; LIVE/DEAD kit, viability/cytotoxicity test; MTS assay, colorimetric cell proliferation assay kit; MTT assay, cellular metabolic activity colorimetric assay; N.SIG., not significantly different outcome as compared to the control; PBMCs, peripheral blood mononuclear cells; PE, polyethylene; PET, Polyethylene terephthalate; PP, polypropylene; PS, polystyrene; PVC, polyvinyl chloride; T98G, human glioblastoma multiforme cells; SIG,. significantly different result as compared to the control; WST-1 assay, cell proliferation assay

The relationship of the covariates of origin and shape are illustrated in Figures S1 and S2. Out of the test MPs of primary origin (207), 69.5% (144) were spherical and the remaining 30.5% (63) were of irregular shape. Unsurprisingly, 100% of the secondary test MPs were of irregular shape. All spherical MPs were of primary origin, and all irregularly shaped MPs were of secondary origin. This overlap was taken into consideration in the analysis. Regarding the significant reported outcomes for the primary MPs (14), these were spherical (57%, 8 out of 14) and irregular (43%, 6 out of 14) shaped MPs. A relationship between secondary MPs of irregular shape and toxicity was observed.

The distribution of the numerical covariates was examined statistically using the Shapiro test followed by a skewness test (Table S5). All the data were found to be not normally distributed and present moderate to high skewness, so the Spearman correlation test was used to detect correlations. Normality of the independent variables is not an assumption for logistic regression (Osborne, 2015). The numerical covariates correlation tests are presented in Figure 5. A significant positive correlation ($\rho=0.386$, $p<0.05$) was detected between the size of the MPs and the applied concentrations expressed in mass/mL, while a significant negative correlation ($\rho=-0.687$, $p<0.05$) was found between the size and the concentrations expressed in MPs/mL. Finally, a significant positive correlation ($\rho=0.316$, $p<0.05$) was also found between the doses of test MPs expressed in concentrations of mass and particle number. This trend was also identified when the binary outcome (SIG., N.SIG.) was tested separately as shown in Figure 5. These correlations were also taken into consideration in the next parts of the analysis. A basic assumption in logistic regression is that all variables must be independent and should

not be highly correlated with each other. Multicollinearity could reduce the effectiveness of the model (Stoltzfus, 2011). The existing conceptual and statistical correlations between the three numerical covariates dictate that not all three can be included in the same model.

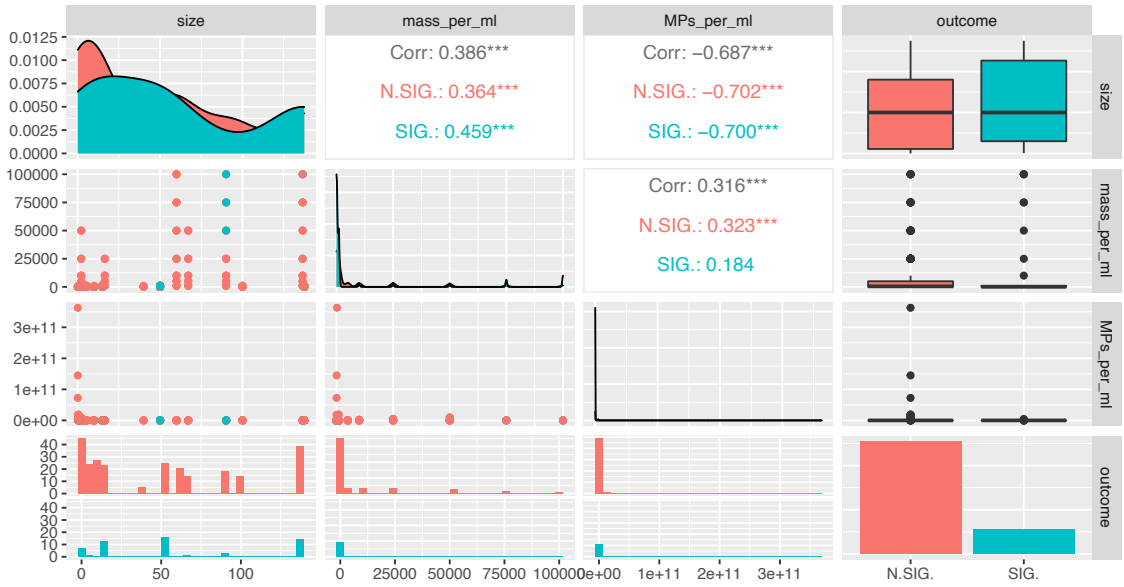

Figure 5. Correlogram between the numerical covariates and the outcome for the cell viability (cytotoxicity) biological outcome. The scatterplots for each pair of numerical covariates are displayed on the left part, Spearman correlation test results are displayed on the right, the diagonal shows the covariates' distribution. Note: N. SIG.: not significant difference as compared to the control, SIG.: significant difference as compared to the control, Corr.: Spearman rank corelation ρ. Blue: SIG, Red: N. SIG.. MP size in µm. MP concentration expressed in both µg/mL and MP/mL.

Another important parameter was the range of sizes and concentrations that have been tested. As shown in Figures 6 and S3, the majority of testing was focused on the smaller size range of MPs where many different concentrations were tested. On the other hand, when looking at the doses tested, their distribution, expressed in MPs/mg (Figure S3), was more skewed than when expressed in µg/mL (Figure 6). This under-representation in doses (sizes and concentrations) can also be detected by observing the quartiles illustrated in Figure S4, where the number of tests has been allocated in quartiles.

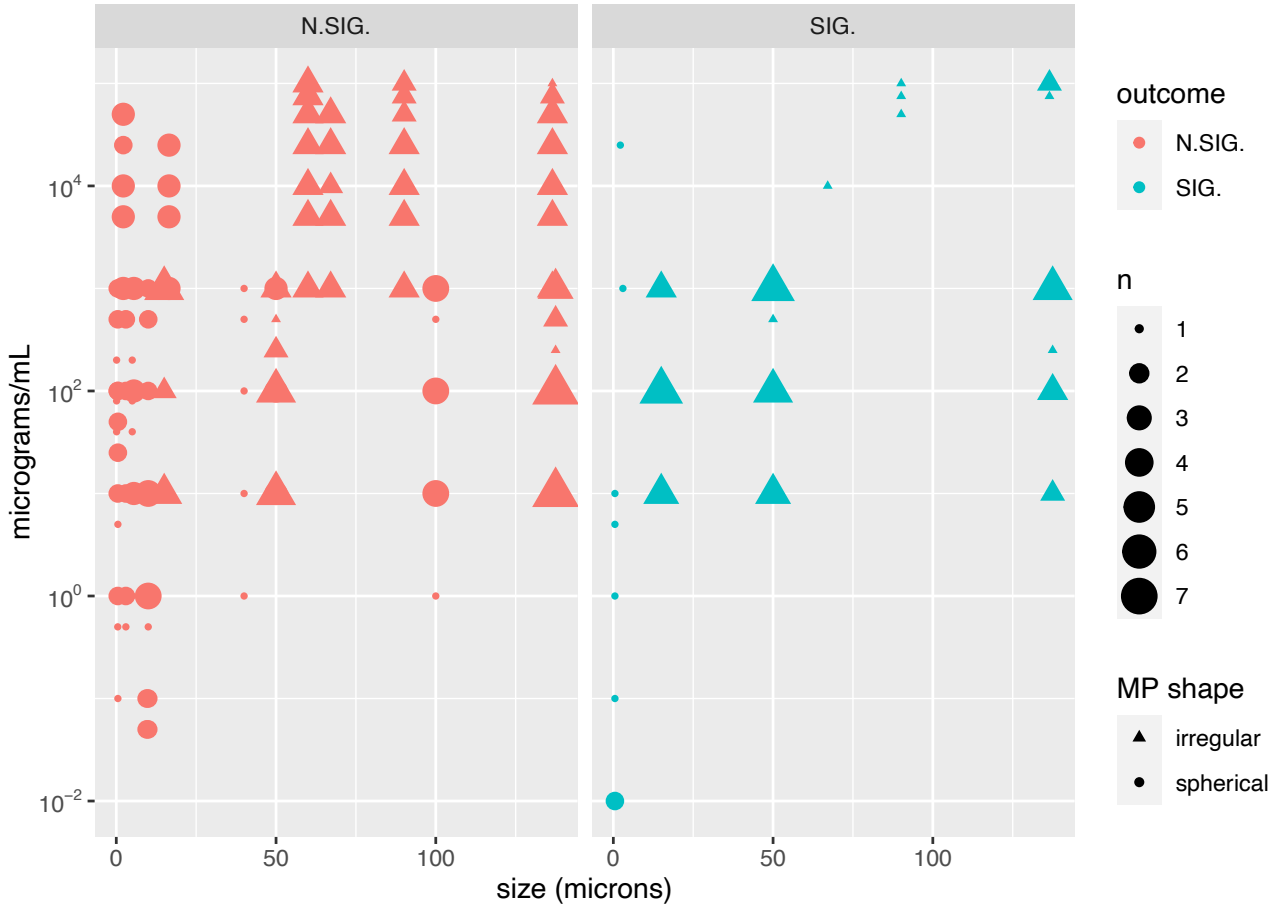

Figure 6. Distribution of test MPs characteristics of concentration (µg/mL) and size (µm) for the cell viability (cytotoxicity) biological outcome. N denotes how many times the same experimental conditions were tested by studies. SIG. statistically significant outcome as compared to the control, N.SIG. not statistically significant outcome as compared to the control.

3.5.2.1. Regression models

The relationship between experimental conditions and the outcomes was explored through regression models. Two models were fitted in the first instance: one including the MP concentration expressed in µg/mL and one in MPs/mL. The first model showed a better fit as both the residual deviance (RD) and the AIC values were lower: RD 156.7 as against 168.04 (null 289.82), AIC 202.7 as against 214.04. Therefore, all consecutive models only included the covariate of MP concentration expressed in µg/mL, also recognizing that the MPs/mL metric is an estimation of the concentrations. The first configuration of the model included all covariates. Three estimate coefficients (secondary origin, MTS

assay and WST-1 assay) were not defined because of singularities. Using the alias(x) function (in R) revealed that all three are highly correlated and linearly dependent with a number of other covariates. Removing these covariates from the model did not affect the fit as the RD rose from 156.7 to 157.57 while AIC was reduced from 202.2 to 197.57 indicating a better fit. The difference between the two models was not significant when compared using a likelihood ratio test (ANOVA, $p>0.05$). It should also be noted that, as previously explored, there was an overlap between the covariates shape and origin, so both could be explored, to an extent, by keeping one in the model. VIF was found to be <3 for all of the six remaining covariates so the conclusion was that there was not strong multi-collinearity between the covariates (Craney and Surles, 2002; Thompson et al., 2017). Ten regression coefficient estimates were found to be statistically significant, seven coming from the cell model covariate, one from MPs characteristics and two from experimental characteristics. One coefficient was categorical (irregular shape, $\beta=5.913$, $p<0.001$), one numerical (MP concentration in μg/mL, $\beta=0.00005$, $p<0.01$) and one integer (duration, $\beta=0.02$, $p<0.01$). The powder shape exhibited a much lower effect size ($\beta=0.669$) and it was not found to be statistically significant ($p>0.05$). In order to examine the covariate of origin, a further model was fitted excluding the shape covariate which caused the multicollinearity. All the same regression coefficient estimates were found to be statistically significant (seven cell models, concentration and duration) with marginally different effect sizes, plus the secondary origin ($\beta=5.894$, $p<0.001$). The AIC was found to be reduced slightly from 197.5 to 195.75 and the fit of the model did not significantly improve (ANOVA, $p>0.05$). All the irregularly shaped MPs in the dataset were secondary and all the spherical were primary, only the powders came from both sources. In order to explore this relationship, a model was fitted where the characteristics of shape and origin were merged into four categories: primary-spherical, primary-powder, secondary-powder, secondary-irregular and only the estimation coefficient for secondary-irregular MPs was found to be statistically significant ($\beta=5.537$, $p<0.01$). In this model the polymer covariate could not be included due to

multicollinearity. Following these results, the choice was made to go forward with the model that included only shape and not origin.

Regarding the cell model covariate, seven out of the 10 cell models had statistically significant regression coefficient estimates. Ranked by effect size, Caco-2 cells exhibited the highest prediction of cell death (β=-4.6, $p<0.05$), followed by HepG2 cells (β=-4.9, $p<0.05$), HDFs (β=-5.53, $p<0.001$), HeLa cells (β=-5.88, $p<0.001$), HepaRG cells (β=-6.47, $p<0.05$), PBMCs (β=-7.2, $p<0.001$) and KATO III cells (β=-8.12, $p<0.001$), as compared to the reference class of BeWo cells (β=-0.63, $p=0.55$). To summarise, the cell model used, the MP characteristic of irregular shape (secondary origin) and the experimental characteristics of MP concentration and duration of exposure predicted the toxic outcome.

The classification prediction accuracy of the model was 89.4%, indicating the overall performance of the model. In order to examine the usefulness of the model, it is important to determine how accurately it can predict the outcomes (SIG./N. SIG.) (Ennos and Johnson, 2018). A data frame was created to show whether the model correctly assessed the outcome for each data point, these predictions are shown in a classification table (Table S6). These show the model correctly predicted the "N. SIG." outcome at a rate of 93.3% and the "SIG." outcome at a rate of 63.6%.

The linearity assumption was tested by creating a series of scatterplots to determine if there was a linear relationship between the numerical covariates and the logit of the outcome. As illustrated in Figure S5, the linearity assumption was not met, which might have caused the covariates to affect the model results disproportionally. The all-subset logistic regression method was subsequently used in an attempt to identify the subset of covariates that produced the best performing logit model. The best-subset model excluded the covariates of polymer type and size from the model, indicating that they hindered the model's performance. The residual deviance of the model was 168.02 (d.f. 296) and the

AIC 196.2, showing a slight improvement in only the AIC value. VIF was found to be <3 for all of the remaining covariates. The classification prediction accuracy was calculated at 88.1% indicating that the performance of the best-subset model was not compromised, while the model was simplified by reducing the number of the covariates. The aim of the all-subset process was to find a less complex model without compromising accuracy. The predictions of the outcomes are shown in a classification table (Table S7).

In the best-subset model (as in the previous model), the regression coefficient estimate was found to be statistically significant for a number of covariates. Seven of the types of cell models had statistically significant large effect sizes, indicating that specific cells were more vulnerable to reduced viability due to MP exposure than others. The second covariate that stood out was shape. According to the model, irregular- (randomly) shaped MPs of secondary origin displayed a larger effect size (β=5.334, $p<0.001$) than spherical MPs of primary origin, while powder MPs had a smaller effect size (β=-0.05578), but the regression coefficient estimate was not statistically significant ($p>0.05$). Two further coefficients: duration and MP concentration (µg/mL) had statistically significant results but small effect sizes β=0.0233 ($p<0.01$) and β=0.0000379 ($p<0.01$), respectively.

The best-subset model also improved the linearity between the numerical covariates and the logit of the outcome, as shown in Figure S6, but did not change it substantially. In order to compare the full and the best-subset model, a likelihood-ratio test was performed (ANOVA) which found that the fitness of the best-subset model did not significantly improve (χ^2=-10.5, Df=-6, $p>0.05$) compared to the full model, while it did improve compared to the null model (χ^2=121.8, Df=13, $p<0.001$). The Cook's distance values were used to visualise the most extreme values (Figure S7) (Osborne, 2015). Although extreme values were depicted in Figure S7, in order to examine whether the values were also influential covariates, the standard residual error was examined and was found to be at acceptable

582 levels (<3) (Figure S8) (Menard, 2002). Following this examination, the conclusion was that no
583 influential outliers were found in the data set.

584 **3.5.2.2. Sensitivity analysis**

585 In order to examine if the relationship between the covariates and the outcomes still held when the cell
586 model characteristic was removed, the logit model was fitted again only for the HDF cell model data,
587 which was the largest cell model subgroup in the data frame (65 data points). Only the covariates
588 indicated by the all-subset process (shape, duration, MP concentration) were used in this model in
589 order to achieve as direct a comparison as possible. In this data frame, only two of the three shape
590 categories are included (spherical and random). Once again, the relationship between shape and
591 outcome is statistically significant, as the spherical test MPs of primary origin were found to be less
592 likely (β=-5.514, $p<0.001$) than irregular MPs of secondary origin to have a SIG. outcome. The
593 duration covariate was also found to be marginally statistically significant (β=0.03, p=0.05). A further
594 model was fitted for the next largest data frame grouped by the cell model, which was PBMC cells (53
595 data points). A weak relationship between the concentration of MPs (µg/mL) and the outcome was
596 found to be significant (β=0.003, $p<0.05$), while the trends of duration and shape (and origin) were
597 detected but were not found to be significant: β=0.03, p=0.06 and β=-0.21, p=0.99, respectively. The
598 third largest data frame grouped by the cell model was Caco-2 cells (45 data points). Unfortunately,
599 no study tested irregularly-shaped test MPs so the relationship could not be examined. Five studies
600 were rated as of critical RoB (Table S3). The effectiveness of the RoB rating could not be assessed
601 due to missing data. The covariate of test MP shape was not reported or reported ambiguously by two
602 studies (Hwang et al., 2019; Wu et al., 2020), test MP origin was not reported by one study (Wu et al.,
603 2020) and the duration of exposure was not reported for a fraction of their experiments by one study
604 (Hwang et al., 2019).

3.5.2.3. Multilevel models

The failure of the linearity assumption could be attributed to the heterogeneity of the data frame being extracted by seven different studies, the heterogeneity of the experimental conditions across the studies and the inability to weight the studies. To account for the heterogeneity caused by the clustering of the data in studies, multilevel logistic regression models were fitted. First a null model was fitted. The ICC of the null model was 0.41, meaning that 41% of the variations in the outcome could be attributed to the clustering of the data in the seven studies. Next a random intercept and fixed slope model was fitted. The model included all the covariates that were used in the full logistic regression model: cell model, polymer, shape, duration, size (μm) and MP concentration (μg/mL), plus a random intercept to account for the clustering of the data by study. The multilevel model had the same results in terms of prediction of coefficient estimates and accompanying p values. The same results were also generated when the multilevel model used only the three covariates included in the best-subset model: cell model, shape, duration and MP concentration (μg/mL), plus a random intercept for the studies. The fact that the results remained the same in the multilevel modelling can be attributed to the results of the random-effects variance for the studies' 1-level grouping. The variance was 0, which means that the variation between the clusters could be explained by the residual variance. In addition, it could also be related to the small number of clusters.

Random-intercept and random-slope multilevel models were also fitted. The random-slope variance was tested for all the covariates, one at a time. A likelihood ratio test was executed to compare each model with the fixed-slope model, where the deviance of the models was compared as a measure of fitness. None of the random-slope models were found to improve in a statistically significant manner from the fixed-slope model. It should also be mentioned that it was not conceptually hypothesised that there would be a difference of the covariates' effects between studies.

3.6. Immune responses

3.6.1. Narrative analysis

Ten studies considered immune responses to MP exposure (Table 1), examining different outcomes broadly divided into release of histamine, release of (pro-) inflammatory cytokines and myokines (IL-1β, 2, 6, 8,10, MCP-1, TNF-α), gene expression of cytokines (*IL-8* and *MCP-1*) and differentiation of THP-1 cells into macrophages and polarization. Three studies (Han et al., 2020; Hwang et al., 2019; Stock et al., 2019) were rated of critical RoB and were excluded from analysis, two further studies expressed MP concentrations as μg/cm^2 (Dong et al., 2020; Lehner et al., 2020) and as such could not be directly compared with the rest of the studies. The release of cytokines/myokines was measured using ELISA and gene expression via RT-PCR and results were reported using quantitative measures by comparison to negative control samples. A wide range of experimental designs was used: five cell models, seven polymers, three shapes, two origins, two tests, nine biological markers, MP sizes ranging from 0.202 to 283 μm, durations from 2 to 96 hours and MP concentrations from 1 to 1000 μg/mL and from 10 to 1305.5 μg/cm^2. The full experimental details and the results can be found in SM2. Five studies reported results of significant immune response effects as follows. Although nine biological markers were tested, only four were found to be significantly affected by MPs exposure. Choi et al. (2020) found that exposure to irregularly shaped PS MPs significantly affected the release of IL-6 and TNF-a at MP concentrations as low as 100 μg/mL, while all experiments had a 24-hour duration. Choi et al. (2021) reported that the same biological markers were significantly affected by spherical PE and irregular LDPE MPs at MP concentrations of 500 – 1000 μg/mL, for 96-hour experiments. Hwang et al. (2020) reported the same markers being affected by spherical PS MPs ranging from 0.46 to 10 μm at a MP concentration of 500 μg/mL, for 4-hour and 96-hour exposures. Finally, Liu et al. (2020) reported that IL-8 and MCP-1 release were affected by irregular PS MPs (0.404 μm) at a very low MP concentration of 20 μg/mL, for 96-hour durations. It should be noted that Liu et al. (2020) was the only study examining MCP-1 but other studies measured IL-8. Dong et al.

(2020) reported that both IL-6 and IL-8 were affected by spherical PS MPs (1.72 μm) at MP concentrations of 10 and 1000 μg/cm^2, after 24-hour exposures.

3.6.2. Meta-regression: Cytokine release

Four studies (Choi et al., 2020; Choi et al., 2021; Hwang et al., 2020; Liu et al., 2020) that examined the release of cytokines using ELISA techniques were included in the analysis, comprising 136 data points. The studies expressed the results in terms of release amount (pg/mL) compared to the control samples and measured six different cytokines. The characteristics of covariates that were explored are presented in Table S8. The categorical covariates are illustrated in Figure S9 A-D. A few preliminary observations can be made from inspection of the figures. The most used cell model was PBMCs followed by Caco-2 (124 and 12 out of 136, respectively) (Figure S9.A). PS was the most used test polymer, followed by PE and LDPE (102, 18 and 16 out of 136, respectively) (Figure S9.B). The duration of exposure most frequently adopted was 96 hours (Figure S9.C), and two of the immune responses under examination have no SIG. outcomes (Figure S9.C). Figure S10 shows the relationship between the origin and shape covariates, where it is evident that all of the primary MPs that were tested were spherical, and all of the secondary MPs were of irregular shape. Thus, only one of the covariates could be included in the analysis but describe both MP characteristics.

The distribution of the numerical covariates was examined statistically using the Shapiro test followed by a skewness test (Table S9). All data were found to be not normally distributed and present moderate to high skewness. The Spearman correlation test was used to detect correlations. A not significant positive correlation (ρ=0.12, p=0.15) was detected between the size of the MPs and the applied dose expressed in MP concentration of μg/mL, while a significant negative correlation (ρ=-0.872, p<0.05) was found between the size and the concentrations in MPs/mL. Finally, a significant positive correlation (ρ=0.265, p<0.05) was also found between the doses of test MPs expressed in concentrations of mass and particle number. The same trend was also identified when the binary

outcome was tested separately as shown in Figure S11. As noted in the cytotoxicity analysis, the conceptual and statistical correlations between the three numerical covariates dictate that not all three can be included in the same model. The ranges of the sizes and MP concentrations that have been tested in this data frame are illustrated in Figures S12 and S13. Similar to the cytotoxicity data frame (see previous section), testing focused on the smaller MP size, while the range and distribution of MP concentrations was better covered in doses expressed in μg/mL than MPs/mL.

3.6.2.1. Regression models

The model was first fitted with all the covariates on Table S8, but two coefficients (secondary origin, MCP-1 test outcome) were not defined because of singularities, as they were highly correlated and linearly dependent on shape, cell model and test outcomes. Excluding the two covariates and refitting the model affected the residual deviance only marginally (55 from 49.1, null dev.= 98.5) nor did it notably change the AIC (73 from 75). It must be noted again that all primary MPs were spherical and all secondary were irregularly shaped. Only one regression-coefficient estimate was found to be statistically significant: MP concentrations expressed in μg/mL (β= 0.004, $p<0.05$), but when testing for multicollinearity by calculating the VIF value, three covariates were found to exceed 5 (cell model, duration and dose in MPs/mL) and one almost 10 (duration) indicating a problematic amount of collinearity present. As the correlation between the MP concentrations expressed in μg/mL and in MPs/mL was already conceptually (and statistically) known, two models were fitted one excluding μg/mL and one excluding MPs/mL. The outcomes of the model revealed that by excluding MPs/mL, all the covariates had VIF values below 2, while, when excluding μg/mL, VIF values continued to be above 5 for three covariates (cell model, duration and MP concentration) which indicates high multi-collinearity. Therefore, the decision was made to proceed without the covariate of dose expressed in concentrations of MPs/mL, also recognizing that this metric is an estimation of the concentrations. The model results showed two regression coefficient estimates as statistically significant, concentration (μg/mL) (β=0.005, $p<0.05$) and duration (β= -0.03, $p<0.05$). The shape and origin covariate was not

found to be statistically significant but spherical primary MPs (as opposed to irregular shape secondary MPs) did have a negative association with the outcome displaying a larger effect size of β=-1.15. The all-subset regression method was consequently applied, which indicated that the best-subset model excluded the polymer, shape and size covariates. The best-subset model found the three remaining covariates to be statistically significant estimates: duration (β=-0.03, $p<0.05$), PBMC cell model (β=-3.2, $p<0.05$) and concentration (μg/mL) (β=0.004, $p<0.05$). VIF value was <2.

Comparing the two models, the residual deviance marginally increased from 61.072 to 64.578, but the AIC decreased from 77.072 to 72.578 in the best-subset model. The overall prediction accuracy was higher for the full model at 91.2% than the best-subset model 89.7%, so the exclusion of the covariates somewhat affected the performance of the model. The predictions for each outcome for the full and the best-subset model are shown in classification tables (Tables S10-11). Both models were better in predicting the N.SIG. outcome (98.3%) than the SIG. outcome (37.5% and 25%) but the overall prediction accuracy was very high (91.2% and 89.7%).

Apart from the multicollinearity, which was tested for each model individually, further diagnostics were executed to test the basic assumptions of logistic regression. The linearity assumption was examined through a series of scatterplots to detect if there was a linear relationship between the numerical covariates and the logit of the outcome. As shown in Figures S14 and S15, the linearity is improved in the best-subset model but is still not fully linear. The most extreme values were visualized using the Cook's distance values (Figure S16) (Osborne, 2015). The standard residual error for all the covariates were at acceptable levels (<3) as illustrated in Figure S17 (Menard, 2002).

3.6.2.2. Sensitivity analysis

The biological-marker covariate was also fitted to detect if it was associated with the results. The cell-model covariate was excluded from this model as it presented singularities with the outcome. The

regression-coefficient estimates were not statistically significant for any of the six biological markers (Table S6). A further model was fitted for the largest subgroup of the data frame, categorized by biological marker. The IL-6 outcome was chosen with 44 data points and 12/32 distribution of outcomes (SM2). The model results showed that no coefficients were statistically significant, but VIF values were extremely high, pointing to strong multicollinearity. The last model to be explored was a subgroup of the data frame that included only the PBMC cell models (124 data points) which was previously found to be a statistically significant predictor. The model could not express the covariate of origin due to singularities. The model excluding origin found MP concentration as the only statistically significant covariant (β=0.005, $p<0.05$), while all VIF values were <3.

The RoB influence could be tested in this data frame (184 data points). Three RoB categories were included in the RoB covariate: moderate, serious and critical. The two covariates of origin and test outcome could not be defined due to singularities and were not included in the model. Comparing the RoB model with the full model we see that four prediction coefficients were statistically significant, two similar to the RoB constrained model: duration (β= -0.029, $p<0.05$) and MP concentration (β=0.002, $p<0.05$) and a further two: spherical shape (β= -1.548, $p<0.05$) and size (β= -0.015, $p<0.05$), with VIF values <2. The overall prediction accuracy was reduced to 88%, residual deviance 103.3 (null 138.65) and AIC 125.3. The all-subset regression method was used, which excluded the covariates of cell model and polymer, and retained the coefficients of duration (β= -0.018, $p<0.05$), MP concentration (β=0.002, $p<0.05$), spherical shape (β= -1.354, $p<0.05$) and size (β= -0.014, $p<0.05$), in the best-subset model, with marginally changed effect sizes and VIF <2. Residual deviance of the best-subset model was 110.43 and AIC 120.43. The overall prediction improved marginally at 88.5% but was still less than the restricted RoB model.

3.6.2.3. Multilevel models

Multilevel logistic regression models were subsequently fitted to account for the data clustering depended on the four studies included in the data frame. The ICC of the null model was 0.095, meaning that 9.5% of the variations in the outcome could be attributed to the clustering of the data in the four studies. The multilevel mixed model included fixed effects for the covariate and a random intercept for the four studies. The covariates used for the model were: cell model, polymer, shape, duration, size (μm) and MP concentration (μg/mL). The results were similar to the previous model. Consequently, a further model was fitted excluding the cell model covariate that was excluded by the all-subset regression process. This model also produced the same results. Random-slope, random-intercept models were also fitted testing one covariate at a time. Using the likelihood ratio test, none of the random-slope models were found to significantly improve from the fixed slope.

3.7. Histamine release, oxidative stress, genotoxicity

Histamine release was examined by four studies (Choi et al., 2021; Han et al., 2020; Hwang et al., 2019; Hwang et al., 2020) (Table1). Each used one cell model (HMC-1), tested five different polymers and used two different tests (ELISA kit, histamine assay) (Figure S18). Only two studies (Han et al., 2020; Hwang et al., 2019) reported significant outcomes, and these were rated of critical RoB, therefore the data could not be explored in a meta-regression. The rest of the studies (Choi et al., 2021; Hwang et al., 2020) tested two polymers PE and PS for sizes ranging from 5.5 to 100 μm and MP concentrations ranging from 10 to 1000 μg/mL for PE and 0.46 to 100 μm and MP concentrations of 500 μg/mL for PS, but all of the test MPs were of spherical shape.

Nine studies examined oxidative stress (Table 1). Excluding the three studies rated of critical RoB (Hwang et al., 2019; Wang et al., 2020; Wu et al., 2020), two studies reported significant outcomes. Wu et al. (2019) reported a significant increase of intracellular reactive oxygen species (ROS) generation after exposure to spherical, 0.1 and 5 μm, PS MPs using Caco-2 cells at a MP concentration

of 200 µg/mL and Dong et al. (2020) after exposure to 1.72 µm spherical PS MPs using BEAS-2B cells at a MP concentration of 1000 µg/cm^2. The results of the oxidative stress tests could not be analysed in meta-regression due to the small size of the data frame (44 data points), and the use of four different measures of the outcome. Two studies examined genotoxicity (Table 1) and one was rated of critical RoB (Wu et al., 2020). The other study (Hesler et al., 2019) examined genotoxicity through testing a p53 reporter, exposing Caco-2 cells to spherical 0.5 µm PS MPs (up to 10 µg/mL), but all results were non-significant.

3.8. Cell barrier

Ten studies (Table S1) examined the cell-barrier behaviour, relating to either cell viability or a series of MP and cell-membrane or cell-model interactions: uptake (translocation, internalisation), barrier integrity, permeability and trans-membrane transport. Two studies (Liu et al., 2020; Wu et al., 2019) focused on cell barrier attributes in terms of cytotoxicity and both used the relative release of LDH as the measure. No significant change to LDH release after exposure to spherical and irregular PS MPs was reported. Barrier integrity was examined by three studies (Dong et al., 2020; Hesler et al., 2019; Lehner et al., 2020) by measuring the transepithelial electrical resistance (TEER) before and after exposure to MPs. Only Dong et al. (2020) reported a significant decrease in the barrier integrity after exposure to spherical PS MPs (1.72 µm) for 24 hours at two MP concentrations of 10 and 1000 µg/cm^2. The expression of the protein ZO-1, using an ELISA technique as a measure of disruption of the barrier, was also conducted, and a significant decrease of Z0-1 after the same exposures observed. Liu et al. (2020) examined the permeability of the cell barrier and reported significant down-regulation of the expression of transmembrane transporters (*ABCC2, ABCG2*) after exposure to irregularly shaped MPs and spherical PS MPs (5 µm) at MP concentrations of 1 and 20 µg/mL for 96 hours. Liu et al. (2020) was the only study that examined paracellular transport examining the expression of *ZO-1* and *Occludin* using qPCR, but only reported a significant down-regulation after exposure to NPs which is beyond the scope of this review. The quantitative barrier integrity / permeability results could not be

796 analysed in meta-regression due to the small size of the data frame (34 data points) and the use of six
797 different measures for the outcome.

798 MPs uptake/internalisation was examined by seven studies (Table 1) two of which were rated as of
799 critical RoB (Stock et al., 2019; Wang et al., 2020). The other five studies all used qualitative measures
800 for examining MP cellular uptake. Hesler et al. (2019) stated that spherical PS MPs (0.5 μm) were
801 internalised by both the co-cultures they used (Table 1) after a 24-hour exposure. Translocation of MPs
802 was also detected in the apical but not in the basolateral compartment of the models. Stock et al. (2021)
803 exposed MPs (PE, PP, PET, PVC) to a Caco-2 trans-well model in order to examine cell uptake via
804 microscopic examination and fluorescence quantification of the cell membranes and reported that
805 intracellular uptake was detected only for spherical, PE MPs (1-4 μm). Wu et al. (2019) reported that
806 both sizes (0.1 and 5 μm) of spherical PS MPs entered the Caco-2 cells after a 12-hour exposure.
807 Goodman et al. (2021) confirmed the internalisation of 1 μm spherical PS MPs for exposures from 24
808 to 96 hours via flow cytometry (Calcein AM and Ki67 assays) and phase-contrast microscopy, using
809 A549 cells. Hwang et al. (2020) did not report MP uptake results.

810 **3.9. Characteristics of MP toxicological profile**

811 The MP exposure characteristics that were examined in order to create a toxicological profile were
812 size, surface area, shape, surface charge, chemical composition, MP concentration and duration. Choi
813 et al. (2020) concluded that both chemical and physical effects influenced the observed toxicity.
814 Chemical effects were hypothesised to be related to the release of chemical reagents from the MPs,
815 while the physical effects came from the direct damage of cellular membranes. Choi et al. (2020) stated
816 that the effects were concentration-dependent, not MP size-dependent and noted that immune
817 responses and ROS generation were observed after short-term (i.e. 24-hour) cultures and cell death
818 after long-term cultures (i.e. after 96 hours). A subsequent study focused on the physical effects by
819 using both spherical and irregularly shaped MPs (Choi et al., 2021), concluding that the observed

toxicity was correlated with the ruggedness of the irregularly shaped MPs. In contrast, spherical MPs did not affect cell death but did induce immune responses in high MP concentrations.

Hesler et al. (2019) focused on acute toxicity and highlighted the range of toxicological effects on different cell models, noting that the sensitivity of cell models and co-cultures to MP exposure varies. Hesler et al. (2019) was one of the studies which examined whether MPs could cross biological barriers, reporting that the function of the intestinal and the placental barrier was not compromised. MPs did not cross the co-cultures, but internalization by cells was confirmed. The authors also did not exclude the possibility that long-term exposures (more than 24 hours) could have different results on uptake and detected different responses and behaviour between the two models when exposed to MPs. Furthermore, it was stated that responses were both size- and dose-dependent (MP concentration). Lehner et al. (2020) also used an intestinal model but found no cytotoxic or inflammatory responses. The size of the test MPs (50-500 μm) was proposed as a possible explanation for the absence of effects, which were much larger than the test MPs used by Hesler et al. (2019) (0.5 μm). It should also be noted that Lehner et al. (2020) was one out of two studies that did not use a dispersion of MPs but, rather, dry powder directly applied on the surface of the cells. Liu et al. (2020) used a Caco-2 monolayer and examined the effects of two MPs: one primary and one secondary, processed to mimic the conditions of the digestive tract. Differences between the measured effects on toxicity and immune responses were detected and attributed to size and shape, especially on the corona that was created on the surface of the secondary test MPs. The shape change was hypothesised to have altered the Zeta potential value (surface charge) of the test MPs. It was not reported whether the MPs affected paracellular transport but an abnormality of transmembrane transport indices were reported. Stock et al. (2021) examined MP toxic effects as a result of intra-cellular interactions but concluded that cytotoxicity could not be associated to specific polymers or shapes but only to extremely high concentrations (>10000 μg/mL) of large MPs exceeding the intracellular uptake limit of <10μm. Regarding particle uptake and transport, the only test MPs found to cross the model's barrier were in the size range between 1-4 μm

which coincides with the pore size (3 μm) of the polycarbonate membrane which was integral to the model used.

Wu et al. (2019) tested two different sizes of MPs (0.1 and 5 μm) on Caco-2 cells and found differences in mitochondrial depolarization which was attributed to the accumulation of the smaller MPs in lysosomes. The larger MPs, on the other hand, could escape lysosomes, localize in other parts of the cells and cause more damage, further triggering depletion of ATP and inhibition of ABC plasma membrane transporter activity. A different mechanism was hypothesised for the smaller MPs, which might have acted as substrates of the transporters thus causing competitive inhibition resulting in the reduction of the ABC transporters' action.

Hwang et al. (2020) stated that MPs (<1 μm) at high concentrations (>500 μg/mL) could be associated with innate rather than adaptive immune responses and suggested that cells might recognize them as pathogens. Other than that, no mechanism of toxicity has been proposed. Schirinzi et al. (2017) did not detect cytotoxic effects but did report significant effects on ROS generation which were proposed to be size-dependent, with no mechanism proposed.

Three studies focused on the inhalation route connected to the respiratory system (Brown et al., 2001; Dong et al., 2020; Goodman et al., 2021). Brown et al. (2001) initially hypothesised that inflammatory effects would be size-dependent but concluded that they were more likely connected to the MP surface area and their ability to generate oxidative activity. Dong et al. (2020) stated that the underlying mechanism for all the effects (cytotoxic and inflammatory) caused by MPs was the formation of ROS. Goodman et al. (2021) noted that there could be a difference between short-term and long-term exposures and highlighted that the effects of MPs in the lungs are likely to be cumulative for life-long exposures. These authors suggest that the observed effects (reduced proliferation,

morphological/behavioural changes) are all likely initiated by a mechanical signal caused by the MP presence.

3.10. Statistical summary of evidence

In order to use the congregated data derived from all the studies in a way that is meaningful in the context of risk assessment, threshold values must be defined. Threshold values can be expressed as no observed adverse effect level (NOAEL) or/and lowest observed adverse effect level (LOAEL), both relating to the level of exposure where no effect occurs (IPCS, 2009). The choice of the appropriate data to be included in this part of the analysis were based on conceptual justification and the results of the meta-regression. In the paradigm of dietary and atmospheric exposures of humans to MPs there is a mix of polymers as illustrated by the systematic reviews on food and drinking water contamination (Danopoulos et al., 2020a; 2020b; 2020c) and atmospheric studies (Jenner et al., 2021; Wright et al., 2020). In addition, according to the meta-regression, polymer type was not found to be a significant predictor of the outcome. The structure of the analysis, following the overarching categorization by biological outcome, must be the cell model that was used in the experiments, which was found to be a significant predictor in the meta-regression of the cytotoxicity outcome, followed by the size of MPs, since different sizes can, in theory, reach different locations of the human body, and the applied dose (MP concentration). A secondary categorization of duration can also be applied. The structure of the data synthesis follows the categorization of cell model/ polymer/ size/ concentration/ duration. The results of food-related and atmospheric MP studies also indicate that a small proportion of the MPs discovered were spherical. Consequently, only the results of non-spherical test MPs will be included, in order to achieve the best possible analogue to the MPs currently found in the environment, readily available as contaminants for human exposures. In the process of dose-response modelling, in order to ensure that the toxic responses are acknowledged across endpoints and subjects, the lowest observed levels can be used across cell models as a measure of the most sensitive cells (IPCS, 2009). Likewise, endpoints where clear dose-response is not present can be omitted. After examining the available data,

892 lowest threshold values could only be defined for the endpoints of cytotoxicity, barrier integrity and
893 immune responses. Regarding the oxidative stress biological endpoint, only non-significant values
894 were reported for irregular MPs, (summarized in Table S12). Histamine responses and genotoxicity
895 were only tested using spherical MPs.

896 **3.10.1. Cytotoxicity and barrier integrity**

897 The results for all the non-spherical shaped MPs that significantly reduced cell viability are illustrated
898 in Figure 7. The lowest doses that reduced cell viability significantly are presented in Table 6
899 categorized by cell model. The lowest MP concentration (of 10 μg/mL) was found to affect the HDF
900 and HeLa cell models both in μg/mL and MPs/mL, while the smallest MPs (15 μm) affected HDF,
901 HeLa, KATOIII and PBMC cells. One study (Liu et al., 2020) measured the effects of MP exposure
902 on the permeability of the cell barrier using a quantitative metric by evaluating transmembrane
903 transporters (*ABCC2, ABCG2*) via qPCR assay (Table 6). A series of tests/biological markers
904 investigations reported no significant results constituting a form of NOAEL, and these threshold values
905 are presented in Table S13. Full results can be found in SM2.

906 A striking finding worth highlighting, is that in a small number of studies, the highest applied MP
907 concentration per experimental condition was not the most effective, or not as effective in inducing a
908 response within one of the biological endpoints. This phenomenon has been observed in three studies
909 (Choi et al., 2020; Choi et al., 2021; Stock et al., 2021) within the results of two different cytotoxicity
910 tests. When examining the MTT assay results for Caco-2 cells exposed to PP MPs of 67.1 μm, a
911 significant result for the 10000 μg/mL dose, but not for the 25000 and the 50000 μg/mL doses, is
912 reported for the same duration of exposure (Stock et al., 2021). The authors omit this from the
913 discussion, stating that PP was non-toxic. In another study, CCK-8 assay results for the HDF cells
914 exposed to PS MPs of 15 μm, were significantly different for the 10 and 100 μg/mL doses but not the
915 1000 μg/mL dose, after a 24 hour duration (Choi et al. (2020). The same pattern was observed for the

50 µm sized MPs but not for the 137.5 µm sized MPs. Again, CCK-8 assay results for HeLa cells exposed to PS MPs (only for the two test MP sizes: 15 and 50 µm), and KATO III cells exposed to PS MPs (only for the 15 µm sized MPs) all using a 24 hour duration, show the same pattern (Choi et al. (2020). In contrast, in the same study, using the same cytotoxicity test, the same polymer but a different cell model, in this case PBMC, the highest MP concentrations were the most effective at inducing a biological response. Choi et al. (2020) attributed this non-linearity in the dose-response relationship to the physicochemical characteristics of MPs, proposing that MPs at high concentrations likely formed clusters, thus reducing their (physical) toxicity and leading to the linear toxicity pattern observed in the PBMC cells due to their greater sensitivity. This issue was also reported in a subsequent study using LIVE/DEAD assay results, when PBMC cells were exposed to 137.5 µm sized LDPE MPs for 24 hours, but no comment was made in the discussion (Choi et al., 2021). Regarding spherical MPs, the same issue was highlighted following WST-1 and MTT assays, using Caco-2 and BeWo cells exposed to 0.5 µm PS MPs (Hesler et al., 2019) and Caco-2 cells exposed to 2.2 µm PE MPs (Stock et al., 2021). Stock et al. (2021), omit these results, concluding that PE MPs were non-toxic. Hesler et al. (2019), on the other hand, recognised that lower MP concentrations exhibited higher toxicity and referenced the work by Vandenberg et al. (2012). The latter report that a non-linear dose-response relationship (nonmonotonic) and low-dose effect of endocrine disrupting chemicals (EDC) is possible. It was not clear how EDC toxic mechanisms was related to MPs or if Hesler et al. (2019) attributed MPs toxic effects to chemical, instead of physical, interactions with the cells.

Table 6. Lowest applied non-spherical microplastic (MP) doses resulting in significant reduction of cell viability after exposure to irregularly shaped MPs.

Cell model	Test	Polymer	Size (µm)	MP concentration		Duration (hours)
				µg/mL	MPs/mL	
Caco-2						
	MTT	PP	67.1	10000	70241	24
	Caspase-8	PP	67.1	50000	351205	24
	MTT	PVC	136.5	75000	40228	24
	qPCR	PS				96
			0.4402	20 [a]	290197	

			22.1	1 [b]	168	
HDF	CCK-8					
		PS				
			15	10	5630	24
			50	10	152	24
			137.5	10	7	96
		LDPE				
			50	1000	16643	24
			137.5	1000	800	24
HeLa	CCK-8	PS				
			15	10	5630	24
			50	10	152	24
			137.5	10	7	96
HepaRG	MTT	PVC	136.5	100000	53638	24
HepG2	MTT	PE	90.1	50000	138889	24
KATO III	CCK-8	PS				
			15	100	56306	24
			50	100	1520	24
PBMC	LIVE/DEAD kit					
		PS				
			15	100	56306	96
			50	100	1520	96
			137.5	1000	727	96
		LDPE				
			50	500	8321	24
			137.5	250	200	24

[a] qPCR of *ABCC2* gene expression was used to test cell membrane permeability, [b] qPCR of *ABCG2* gene expression was used to test cell membrane permeability. Note: Caco-2, human adenocarcinoma cell line; CCK-8, cell counting kit 8; HDFs, human dermal fibroblasts; HeLa, cervical cancer cells; HepaRG, human hepatic cells; HepG2, Human Caucasian hepatocyte carcinoma cells; KATO III, gastric cancer stem cells; LDPE, Low-density polyethylene; LIVE/DEAD kit, viability/cytotoxicity test; MTT assay, cellular metabolic activity colorimetric assay; PBMCs, peripheral blood mononuclear cells; PCR, polymerase chain reaction; PE, polyethylene; PP, polypropylene; PS, polystyrene; PVC, polyvinyl chloride

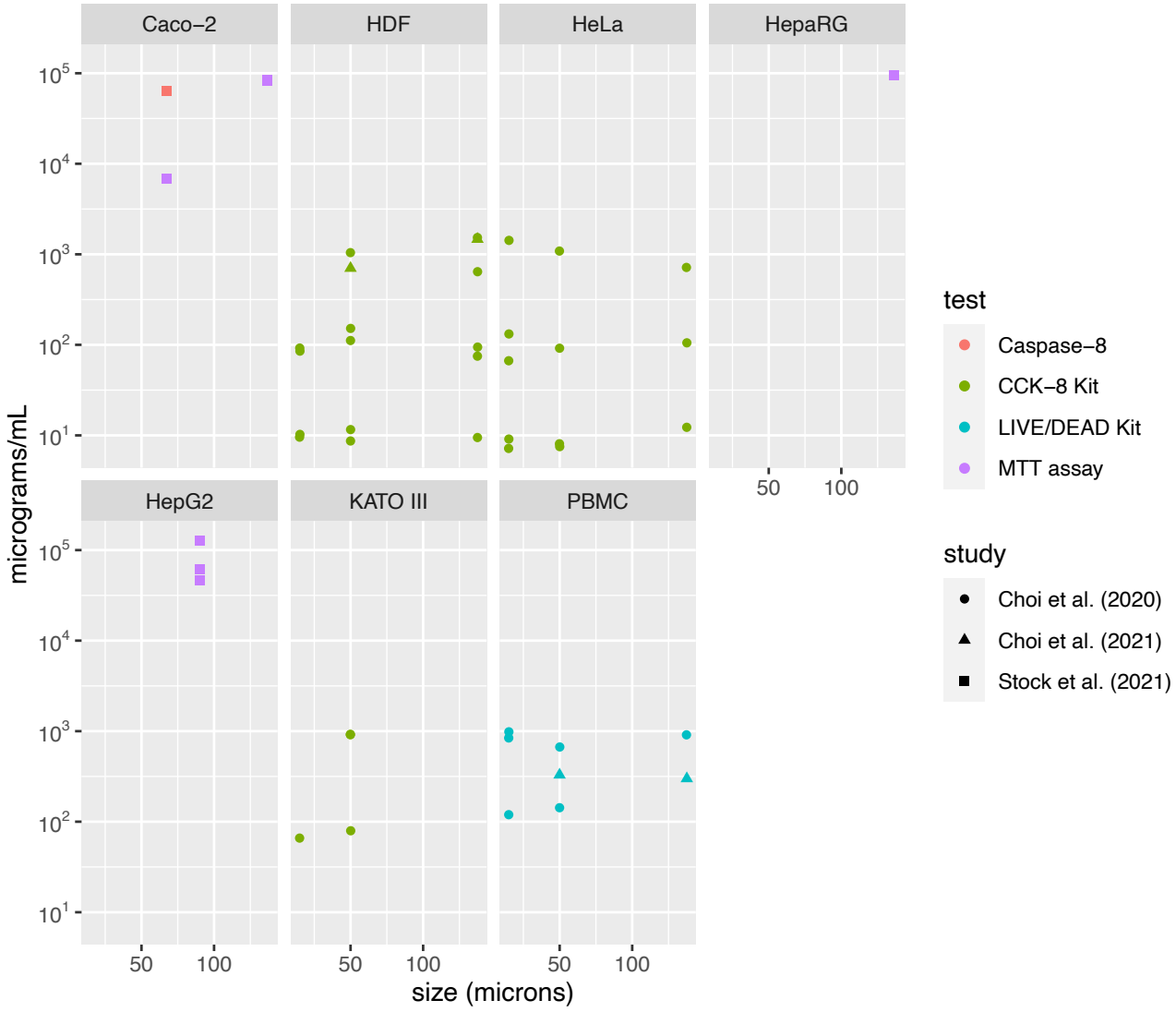

Figure 7. Applied MP doses that resulted in significant reduction of cell viability after exposure to non-spherical microplastics (MPs). Dose expressed in MP concentrations in µg/mL (log_{10} scale) and MP size in µm. Note: Caco-2, human adenocarcinoma cell line; CCK-8, cell counting kit 8; HDF, human dermal fibroblasts; HeLa, cervical cancer cells; HepaRG, human hepatic cells; HepG2, Human Caucasian hepatocyte carcinoma cells; KATO III, gastric cancer stem cells; LIVE/DEAD kit, viability/cytotoxicity test; MTT assay, cellular metabolic activity colorimetric assay; PBMCs, peripheral blood mononuclear cells

3.10.2. Immune response, cytokines

The release of four cytokines was found to be significantly affected after exposure to irregular MPs: IL-6, IL-8, MCP-1 and TNF-a (measured using an ELISA technique). In addition, gene expression of

IL-8 and *MCP-1* measured via qPCR, was found to be significantly altered (Figure S19). The lowest MP concentrations were found to affect the Caco-2 and PBMC cells (as shown in Table 7). The highest doses not to exhibit significant results are presented in Table S14.

Table 7. Lowest applied MP doses resulting in significantly altered cytokine responses after exposure to irregularly shaped MPs. ELISA technique used unless otherwise specified.

Cell model	Cytokines	Polymer	Size (µm)	MP concentration		Duration (hours)
				µg/mL	MPs/mL	
Caco-2	IL-8					
	MCP-1					
	IL-8 mRNA[a]					
	MCP-1 mRNA[a]					
		PS	0.4402	20	290197	96
PBMC		PS				24
	IL-6					
			15	1000	563068	
			50	100	1520	
			137.5	100	73	
	TNF-α					
		LDPE	50	500	8321	96
		PS	50	1000	15202	24

[a] polymerase chain reaction (PCR) analysis used. Note: Caco-2, human adenocarcinoma cell line; IL-, interleukin; LDPE, Low-density polyethylene; MCP-1, Monocyte chemoattractant protein-1; PBMCs, peripheral blood mononuclear cells; PS, polystyrene; TNF-α, Tumour Necrosis Factor alpha

4. Discussion

This is the first rapid review, to our knowledge, focusing on MP toxicity on human cells and attempting a meta-regression approach to determine whether MPs are toxic to humans. A large number of recent reviews have examined the topic of MP toxicity with a broader scope, including animal *in vitro* and *in vivo* studies (Chang et al., 2020; Jacob et al., 2020; Jeong and Choi, 2019; Kogel et al., 2020; Rubio et al., 2020; Shi et al., 2021). Nevertheless, the scope of this review and meta-regression is unique as the

aim was to combine quantitative and qualitative data to inform the steps of hazard identification and dose-response within a risk assessment framework. Seventeen studies were included in the rapid review reporting on five biological endpoints: cytotoxicity, immune response, oxidative stress, barrier attributes and genotoxicity. Furthermore, seven studies were included in a meta-regression concerning cell viability (cytotoxicity) and four concerning cytokine release (immune response). The findings of this rapid review and meta-regression highlight that shape, origin, concentration and duration were the main drivers in cytotoxicity as measured by cell viability tests, while cells exhibited varying sensitivity to MP exposure. MP toxicity was linked to both physical and chemical effects across the different biological endpoints, but physical toxicity was prevalent.

4.1. Risk of Bias tool and overall quality of evidence

The bespoke MP-tox-RoB played a key function in the review process and meta-regression. Five out of the 17 studies were found to be of critical RoB and their findings have been excluded from the analysis, thus elevating the overall confidence in our findings. The tool can also be used in the wider setting of MP risk assessment in the stages of hazard identification and dose-response assessment. It is not a static but an intuitive grading tool that can adapt and follow the scientific evolution of MPs research. There was a great degree of heterogeneity observed in every aspect of the experimental design among the included studies. MP-tox-RoB can also be used by researchers as a guide for the design, execution and reporting of their project, thereby encouraging much-needed harmonization and standardization which is presently lacking and is greatly needed in all aspects of MPs research (Hartmann et al., 2019).

The overall certainty of the body of evidence was assessed guided by the GRADE framework (Higgins et al., 2021). The evidence was downgraded in the domain of RoB rating and was not downgraded regarding the four domains of heterogeneity/inconsistency of results, indirectness, imprecision and publication bias. In addition, the body of evidence was not found to meet the criteria for an upgrade

998 according to the domains of large effects, dose-response or plausible confounding. Therefore, the
999 overall certainty of the body of evidence was graded as low.

000 **4.2. Polymer**

001 PS was the most tested polymer, used by 12 studies, followed by PE and PP, each used in three studies.
002 PVC was tested by two studies and all the remaining polymers (ABS, PA6, PET, PU and TPU) were
003 only tested by one study. Indeed, PS MPs have been found in abundance in the environment, especially
004 in some atmospheric studies (Allen et al., 2019), but their popularity amongst toxicologists is not fully
005 backed up by data. The polymers with the highest demand and distribution in the last decades (in
006 Europe) have been PE, PP, PVC, PU, PET followed by PS (Plastics Europe, 2008, 2017, 2019, 2020).
007 In the interest of examining more aspects of MPs contamination and targeting evidenced
008 environmental exposures, more targeted polymer types must be examined. In our recent systematic
009 reviews on MP contamination of food (Danopoulos et al., 2020a; 2020b) and drinking water
010 (Danopoulos et al., 2020c), the most abundant MP polymers as reported by 72 studies were PE, PP,
011 PET and PA, the latter missing from the most popular list. On the other hand, Lithner et al. (2011)
012 attempted to rank the hazard of polymers based on the chemical composition of their monomers,
013 ranking those exhibiting carcinogenic and mutagenic properties as the most hazardous. According to
014 their findings the polymeric families of PUR, PAN, PVC, epoxy resins, and styrenic copolymers were
015 the most hazardous. Since, possible chemical effects from MPs are still under examination, testing of
016 these specific polymers could inform us whether the effects of the monomers are still present in their
017 descendent polymeric MPs.

018 It should also be noted that only five studies used a composition-identification method to either verify
019 or identify the chemical composition of their test MPs. Two studies used Raman spectroscopy (Choi
020 et al., 2020; 2021) and three used Fourier Transform Infrared spectroscopy (FT-IR) (Dong et al., 2020;
021 Liu et al., 2020; Wu et al., 2019). Along with pyrolysis, these are the three methods that are currently

used by environmental MP studies as best practice to identify the chemical composition of particles that have been extracted from samples. There is currently an ongoing effort to create reference material for MP research in order to promote standardization between labs across the world. The use of these methods by toxicology studies (and report of the results) would assist in this process as well as promote transparency and reproducibility of their experiments.

The use of QA/QC measures are increasingly common practice in environmental MP studies but was completely absent in the toxicological studies. The combination of negative and positive control samples could be considered as a QA/QC measure to account for MP cross-contamination, regarding the outcome, but would not provide information on the possible distortion of the dose-response effect. The MP concentrations that have so far been used in the experiments are so large that additional cross contamination could be considered negligible. In the future, as MP concentrations become lower, to better represent environmental exposures, the use of QA/QC will become increasingly important.

4.3. Morphological characteristics

The majority of MP found in nature are secondary MPs of irregular shapes, as evidenced by numerous studies in various environmental compartments (Burns and Boxall, 2018) as well as biota (Akoueson et al., 2020; Li J. et al., 2018). Spherical shapes are not absent, but they are the minority. In the interest of aligning actual environmental exposures and laboratory experiments, it is our view that future MP toxicological research should be targeting secondary and irregularly shaped MPs rather that primary spheres. In addition, none of the studies tested MP fibers which is one of the most prevalent MP shapes found in the environment (Huang, Y. et al., 2021; Jenner et al., 2021). A further crucial aspect in using irregular MPs is that more and more studies hypothesise and have begun to verify, that the toxicological effects of MPs on cells are more physical than chemical. Shape is one the pivotal characteristics as highlighted by three studies in this review (Choi et al., 2020; Choi et al., 2021; Liu

et al., 2020). Liu et al. (2020) further connected origin (secondary), shape and size with surface area and charge and the creation of a corona.

The only available characteristic connected to the origin of MPs was shape. Different weathering processes in nature and in the laboratory can affect MP characteristics such as porosity, shape, size, crystallinity, leaching and chemical properties (Sun et al., 2020), which may in turn affect their potential toxicity, unfortunately this level of detail was not available in the papers under review. All the secondary test MPs used by the studies were of irregular shape and produced in-house by either a variation of the ball milling method or digestion. Overall comparison between the methods was not possible in meta-regression, since the three included studies (Choi et al., 2020; Choi et al., 2021; Stock et al., 2021) that used secondary, non-spherical MPs, all produced them via ball milling. Furthermore, the level of detail that would be needed to review the methods' specification and to compare the physicochemical characteristics of the produced secondary MPs was not available by all studies. This is an important area that must be explored as more data become available.

The relationship between the origin and the shape of the test MPs was evident in every part of the synthesis and analysis. Including both covariates of origin and shape in the same regression model for cell viability was not possible due to multicollinearity. A series of models fitting the covariates consecutively revealed that shape was a better predictor that origin. Out of the two shapes of secondary origin, only one produced significant results. The meta-regression findings on the cell viability results support the hypothesis that shape is one of the drivers of the exerted toxicity. The regression coefficient estimates of only one out of the three MP characteristics that were explored (polymer, size, shape) was found to be statistically significant. Irregular shape, as compared to spherical shape had the largest effect size ($\beta=5.913$) with the highest significance ($p<0.001$), followed by two experimental conditions of duration ($\beta=0.02$, $p<0.01$) and MP concentration expressed in μg/mL ($\beta=0.00005$, $p<0.01$) and then the type of cell model (seven out of ten, see section 3.5.2.1). This trend was also discovered in all-

subset and in multilevel modelling. The toxicity mechanism related to shape is discussed in section 4.5. On the other hand, cytokine release meta-regression modelling found that only MP concentration (μg/mL) and duration were the significant experimental characteristics as predictors of the outcome. The trend of the association between irregular shaped MPs of secondary origin and the outcome was still detected but it was not significant. In the cytokine release model experiments, the masking between origin and shape was complete and the disentanglement of the covariates was not possible.

The other striking finding of the meta-regression models was that the size of the test MPs was not a significant predictor of the outcome for both biological endpoints of cytotoxicity (cell viability) and immune response (cytokines release). Contrary to these results, four studies included in the review argued that the toxicological effects were somehow size-dependent (Hesler et al., 2019; Hwang et al., 2020; Schirinzi et al., 2017), while one study further connected MPs size with surface area (Brown et al., 2001). Nevertheless, it should be noted that all of these studies tested only primary spherical MPs, further highlighting the need for testing secondary, irregularly shaped MPs to produce more representative, and environmentally relevant results.

Regarding MP size, there is scientific evidence, beyond human studies, that MPs <20 μm could enter and translocate in the tissue of a wide range of biota (Hale et al., 2020), while others argue that particles of sizes <150 μm are expected to be able to pass the human gut barrier and cause systemic exposure with limited absorption (≤ 0.3 %) and only even smaller particles <1.5 μm to have the ability to translocate to other organs (EFSA, 2016). Recent studies analysing human sample tissue reported the discovery of MPs in ranging sizes. In human colectomy samples, the size of identified MPs ranged from 800 to 1600 μm (Ibrahim et al., 2021), in human placenta from 5 to 10 μm (Ragusa et al., 2021) and in human lung tissue from 1.6 to 5.58 μm (Amato-Lourenço et al., 2021). The differences in sizes could be attributed to the physiology of the tissues. This initial data on the size of MPs could guide the MP size ranges tested for toxicity.

4.4. Doses and relevance of environmental exposures

Only four out of the 17 studies referenced data produced by MP environmental studies to estimate the MP concentrations used in their experiments. There is currently an abundance of scientific data on the level of MP contamination on a wide range of environmental mediums, to which humans can be indirectly and directly exposed to, coming from primary studies, reviews, systematic reviews, meta-analyses and modelling. There is no reason for study designs to be based on speculations. The profile of hazard exposure can be described as a journey in the human body dependent on four processes: absorption, distribution, metabolism and elimination (or excretion) (ADME) (EPA, 2019). The final MPs uptake by the human body would be less than the MP intake through ingestion and inhalation. A large amount of MPs are expected to 'pass through' the gastrointestinal system and be expelled, thus reducing the final intake dose. Similarly, MPs could be expelled from the respiratory system by one of the available defence mechanisms (structural, secretory, cellular etc.) (Canto et al., 1994). Two parameters must be examined here: the amount of MPs that could remain in the human body, and whether the duration of time that the MPs remain in the body is enough for them to cause an effect. Exposure doses can be demarcated to applied, potential, internal (or absorbed)/delivered. Potential is the dose that is taken into the body via ingestion and inhalation, applied is the dose that is available for absorption and internal/ delivered are the doses that finally remain in the body (EPA, 2019). The endpoint of exposure science is the dose that is delivered at the location where the toxicity pathway is initiated thus triggering the health effect. WHO proposes a narrower separation to external (or administered) and internal doses (FAO and WHO, 2009). Regarding dietary exposures, the intake refers to the external dose, the amount that is systemically available would be the internal dose and the target or tissue dose is the amount that is present in the tissue of interest (IPCS, 2009).

Since all the experimental doses used in the studies included were administered directly on cells or cell models, the doses refer to internal or even target doses. Six studies applied doses of MP concentrations in the range of 1000 and 100000 μg/mL which practically correspond to doses of

118 several hundreds or even several millions of MPs particles, depending on the particle size. There is no
119 scientific evidence to support such kinds of exposures, unless examining life-long exposures, which
120 would then fundamentally alter the study designs in terms of durations. According to our previous
121 work, maximum annual MP exposures from consuming only two food categories (seafood and salt)
122 and drinking water (Danopoulos et al., 2020a; 2020b; 2020c) can reach up to 3.6 million particles,
123 which are potential doses. Applying the average density of the test MPs (1.1 g/cm^3), used by studies
124 herein, and assuming spherical shape, that level of annual exposures can be transformed to a dose of
125 around 250 μg/mL of 5 μm sized MPs, or 250000 μg/mL of 50 μm MPs, which was the size of the test
126 MPs averaged across all studies (48.5 μm). The level of these doses must be modified to represent not
127 potential but internal doses. Scientific evidence is not available at this time on MP toxicokinetics in
128 the human body but paradigms from other contaminants could potentially be applied (Dixit et al.,
129 2003). Internal doses are unlikely to be greater than such potential doses, and the latter can be used,
130 provided this caveat is made clear, as a starting point for determining the MP concentrations used in
131 toxicological experiments.

132 The range of doses tested for the cell viability and cytokines release (Figures 6, S3 and Figures S12-
133 13, respectively) reveal further limitations of the currently available data. Disregarding polymer type,
134 the cell viability doses (included in meta-regression modelling) ranged in size from 0.1 μm to 137.5,
135 but the majority of tests used the smaller sized MPs. One third of the tests (34%, 104 out of the 310
136 data points) involved test MPs in the range between 0.1 and 10 μm and although they used MP
137 concentrations of 0.01 to 50000 μg/mL, 73% of the tests applied doses up to 100 μg/mL. Similarly, in
138 the cytokine release tests although test MPs ranged from 0.4402 to 137.5 μm in size, almost half of
139 them (46%, 62 out of 136 data points) used MPs up to 10 μm, and 71% of this fraction (44 of 62 data
140 points) used doses up to 100 μg/mL. It is understandable that there a limit to the number of tests each
141 study can execute and analyse connected to timeframes and available resources, nevertheless, in the
142 future it would be useful that studies would target doses (MP sizes and concentrations) that have not

been already tested by other studies in order to have a fuller picture of potential exposures. These data might also help us understand if indeed there is a break in the linear relationship between concentrations and outcomes that has been identified in a few studies regarding the cytotoxicity results, or if it is an artefact.

The conversion of the concentrations to MPs/volume or mass is necessary in order to establish two key parameters. Firstly, whether the concentrations used in the experiments were environmentally relevant in terms of the level of exposure (for a specific duration of exposure) and secondly whether these exposures are exceeded and under what circumstances. The reason that the conversion is necessary is that the majority of environmental studies that provide evidence of MP concentrations in various mediums use the MPs per volume or mass metric (Burns and Boxall, 2018; Connors et al., 2017). Attempting the conversion of the data coming from environmental studies is not feasible as the MPs extracted from the environment are a mixture of polymers with different chemical characteristics varying in size and shape. Details at that level are not available in environmental studies. This is a shortcoming that has been widely recognized and will be hopefully tackled in future research (Burns and Boxall, 2018; Koelmans et al., 2019; Miller et al., 2021).

4.5. MP mechanisms of toxicity and thresholds of adverse effects

Little information is available on the underlying toxicity mechanisms and the experimental conditions that drive MP toxic effects. Two recent reviews (Banerjee and Shelver, 2021; Yong et al., 2020) that focused on MPs (and NPs) using human and animal *in vitro* and *in vivo* studies concluded that size, MP concentration, surface charge and duration were related to MP uptake and cell toxicity with varying effects amongst different mammalian cell models. Banerjee and Shelver (2021) also reported that cell death mechanisms could be attributed to ROS generation, DNA damage and autophagy but pointed out that these mechanisms are interrelated and might trigger each other. Prüst et al. (2020), focusing on neurotoxicity, proposed that factors that could affect the potential toxicity (besides MP

concentration and duration) was the temperature at which the exposure takes place, as well as the MP characteristics of size, hydrodynamic diameter and shape, affecting uptake, particle aggregation and surface area/internalization capacity, respectively. Different mechanisms have been proposed by the studies included in the current review. The heterogeneity of the test MPs, cell models and other experimental conditions do not allow a direct comparison. Nevertheless, MP shape is highlighted as an important MP characteristic in exerting toxicity (cell viability) by both narrative analysis and meta-regression. The shape of MPs has been hypothesised to affect cell behaviour and viability either directly or indirectly. There are different mechanistic level biochemical and physicochemical effects proposed. Rugged or even sharp shaped MPs can directly damage cell membranes upon contact, elucidating adverse effects (Choi et al., 2021). Shape, also related to surface area and surface charge, can affect MP movement, the relationship between MPs and between MPs and biological barriers, thus indirectly affecting cells. Surface charge can cause the MPs to aggregate resulting in particle agglomeration, effectively increasing their size and surface areas which in turn could affect cell uptake directly or indirectly by altering the electrostatic forces between MPs and cell membranes (Liu et al., 2020). Agglomeration, which is more related to smaller sized MPs (<0.5 μm), and movement are also affected by Brownian motion which is, in turn, depended on MP shape and size (Rist and Hartmann, 2018).

Wright et al. (2013) highlighted that the potential MP-induced adverse effects on the cellular and tissue level would vary according to MP shape; while also affecting MP uptake by marine organisms. Cellular shape-related effects were attributed to increased cellular uptake and the consequent apoptosis (Huang et al., 2010). The contribution of MP shape to toxicity has also been explored in animal *in vivo* studies. Au et al. (2015) found that PE MPs (powder) were significantly less toxic to *Hyalella azteca* than PP fibers following acute exposures. Xia et al. (2021) reported that irregularly shaped secondary PVC MPs were more toxic to *Oryzias melastigma* embryos than primary PVC MPs in powder form. The importance of shape has also been highlighted by an ecological risk assessment study as follows. Jung

et al. (2021), synthesised data from 32 *in vivo* animal studies, examining apical endpoints of toxicity on aquatic organisms, reporting that small (<20 μm) non-spherical MPs may exert higher chronic ecotoxicity impacts than spherical MPs.

The paradigm of asbestos could offer some additional insight regarding the MP mechanisms of toxicity with respect to shape. Although the chemical composition of asbestos and MP particles is not similar, there is an overlap in the size ranges, they are both highly bio-persistent compounds, and a notable proportion of MPs are fibers. The size of the biologically critical asbestos fibers is considered as ≥ 5 μm, with a diameter ≤ 3 μm (WHO, 2000). MPs have recently identified in the human lung tissue of 13 of the 20 cadavers that were autopsied (Amato-Lourenço et al., 2021). The mean particle size was 3.92 μm (±0.67) and the mean fibre length 11.23 (±1.96) μm. The majority of the MPs identified in the lung samples were fragments (87.5%) and the remainder, fibers (12.5%). While the underlying mechanisms of asbestos induced toxicity has been researched for decades, there are still significant knowledge gaps (Kuroda, 2021). Asbestos has been linked to various diseases of the lung, with cellular injury (and the consequent generation of oxidative stress) and inflammation response to exposure cited as the two initiating toxic mechanisms (Manning et al., 2002) (Brown et al., 2001; Dong et al., 2020; Goodman et al., 2021). On finding MPs in human lung tissues, Amato-Lourenço et al. (2021) proposed that MPs interaction with epithelial cell or macrophages could trigger pro-inflammatory effects. Relevantly for this review, the complex interaction between asbestos and cells/tissue is affected not only by dose and exposure duration, but also size, shape, chemical composition, the presence of metals, surface reactivity and crystallinity as well as bio persistence (Sanchez et al., 2009). The shape of fibers affect not only their potential to be inhaled, reach and remain in the lower parts of the lungs, but also their interaction and detrimental effects on macrophages, leading to long-term sustained inflammation (Manning et al., 2002). While MPs do not share the same toxicological profile as asbestos, lessons learned can be used to examine the findings herein that shape is an important component of MP toxicity.

In terms of LOAELs and NOAELs, different concentrations were effective for different biological endpoints and different cell models as summarised in Tables 6-7 and S8-10. Regarding quantitively assessed tests, doses using MP concentrations as low as 10 μg/mL had an adverse effect on cell viability and as low as 20 μg/mL on cytokine release, for irregularly shaped MPs. Oxidative stress effects were identified at doses of MP concentrations of 200 μg/mL and 1000 μg/cm^2 of spherical PS MPs. The highest MP concentration tested for histamine release with no observed effect was 1000 μg/mL of spherical PE MPs and the highest MP concentration for the genotoxicity biological endpoint with no observed effect was 10 μg/mL of spherical PS MPs. MPs uptake, examined qualitatively, was found to occur for only spherical MPs up to 5 μm in size. It should be noted that only one study (Stock et al., 2021) also analysed cellular uptake using non-spherical MPs, but used a different size range (>60 μm). Barrier integrity was reported to be affected after exposure to spherical PS MPs at MP concentrations as low as 10 μg/cm^2.

4.6. MP and human health effects; future risk assessment

The present and, arguably, the future of applied risk assessment and risk analysis is combining the best available scientific data coming from multiple studies, since commissioned, targeted studies are not always feasible or appropriate. Systematic reviews, rapid reviews and meta-analysis methodology is a very powerful and reliable tool which can be used to that end (NASEM, 2021). Nevertheless, the reliability and applicability of a systematic review is only as good as the studies it includes (Higgins et al., 2021). Unfortunately, in the present work, the overall certainty of the body of evidence was graded as low. In addition, none of the studies included in this review made their full data available. This omission has prohibited the execution of a meta-analysis and has limited the power of the meta-regression.

The outcome data that were used in the analysis were quantal (binary), therefore, information was only available on one degree of effect regarding the chance of incidence for each experimental exposure,

thus limiting our understanding of effects (IPCS, 2009). On the other hand, if raw data were made available, it could provide vital information on how the degree of effect changes when exposure characteristics change, providing a more comprehensive picture of the relationship. It is possible that the variability of the tests used for cell viability may have affected the summary of evidence, since there is no inter-comparability mechanism that can evaluate differences in the tests' sensitivity.

All the toxicological studies have been carried out under controlled conditions, in order to extrapolate from laboratory experiments to real-life environmental conditions, and from cell-based effects to system-based or whole organism effects. A series of adjustments must therefore be made within the risk assessment process. The intrinsic characteristics of MPs cause a further limitation of laboratory-based toxicological experiments as follows. MPs are detected in the environment/foodstuffs as a mix of polymers, so single-polymer exposures are not environmentally-relevant. It also is known that MPs can absorb and later sorb various toxic substances (such as hydrophobic organic chemicals) (Hartmann et al., 2017) as well as additives (plasticisers) that have been added during production (e.g. bisphenol A) (Chang et al., 2020) thus exerting synergistic toxicological effects, that are at this moment under examination (Hale et al., 2020).

5. Conclusions

MP contamination is on the verge of being established as MP pollution. A risk analysis is essential in understanding the extend of the issue in terms of adverse effects posed to humans. In the absence of epidemiological data, *in vitro* toxicology studies can be used to delineate the molecular initiating event and the consecutive key events that lead to adverse effects in an adverse outcome pathways framework. This first rapid review has synthesised and appraised currently available data using a novel RoB tool. MP adverse effects in human cells have been confirmed by the majority of the studies regarding four out of the five biological endpoints included in this review. Specifically, effects were reported concerning cytotoxicity, immune responses, barrier attributes and oxidative stress, although not always

corresponding to environmentally-relevant MPs regarding origin, shape and concentrations. Of the various MP characteristics explored, shape was found to be the single characteristic that significantly affects the cytotoxicity outcome. Out of the 10 different cell models used in the cell viability experiments, Caco-2 cells exhibited the highest association to MP effects. Furthermore, the experimental conditions that significantly affected both cytotoxicity and the induction of immune responses were MP concentration (μg/mL) and duration of exposure. Further physicochemical properties of the MPs under examination are needed to produce a fuller and more robust toxicological profile.

A series of recommendations on the design and conduct of future research will benefit upcoming risk assessments and the understanding of MP-related health effects in humans. Recommendations for future MP toxicological studies:

- Use of environmentally relevant doses based on data coming from MP environmental studies, e.g. below 250 μg/mL of 5 μm sized MPs, or 250000 μg/mL of 50 μm MPs corresponding to annual potential doses.
- Target doses (size and concentrations) that have not been the focus of testing to date (e.g. doses > 100 μg/mL for MPs < 10 μm and all environmentally relevant doses for MPs > 10 μm).
- Include secondary and irregularly shaped MP (not simply primary MP spheres for convenience of procurement)
- Test polymers that have been found to be prevalent in environmental samples/foodstuffs
- Use of FT-IR, Raman or other verified method to identify the chemical composition of the test MPs
- Use of QA/QC measures during and after experiments to verify results
- Use of the MP-tox-RoB as a set of guidelines for study design and reporting results

- Report the origin and characteristics of test MPs and cell models
- Report full data results (perhaps also lodged in a shared international repository) including
    - Number of repeated tests per experimental condition
    - Number of replicates
    - Cell density per experimental condition

More research is always needed to confirm existing results and complete the evidence gaps and the results of this rapid review and meta-regression can be used to guide future efforts. For instance, from the key findings herein, irregular shapes have biological impact, size is critical, and minimum doses of 10 µg/mL (5-200 µm) and 20 µg/mL (0.4 µm) resulted in cytotoxicity and caused immune responses, respectively, indicating that thresholds of effects are much lower than previously expected.

Acknowledgements

Funding: This work was supported by a PhD scholarship within the "Health inequalities and emerging environmental contaminants – Places and People" cluster funded by the University of Hull.

References

Aguinis, H., Gottfredson, R.K., Culpepper, S.A., 2013. Best-practice recommendations for estimating cross-level interaction effects using multilevel modeling. J Manage 39(6), 1490-1528. https://doi.org/10.1177/0149206313478188.

Akoueson, F., Sheldon, L.M., Danopoulos, E., Morris, S., Hotten, J., Chapman, E., Li, J.N., Rotchell, J.M., 2020. A preliminary analysis of microplastics in edible versus non-edible tissues from seafood samples. Environ. Pollut. 263, 114452. https://doi.org/10.1016/j.envpol.2020.114452.

Allen, S., Allen, D., Phoenix, V.R., Le Roux, G., Durántez Jiménez, P., Simonneau, A., Binet, S., Galop, D., 2019. Atmospheric transport and deposition of microplastics in a remote mountain catchment. Nat. Geosci. 12(5), 339-344. https://doi.org/10.1038/s41561-019-0335-5.

Amato-Lourenço, L.F., Carvalho-Oliveira, R., Júnior, G.R., dos Santos Galvão, L., Ando, R.A., Mauad, T., 2021. Presence of airborne microplastics in human lung tissue. J. Hazard. Mater. 416, 126124. https://doi.org/10.1016/j.jhazmat.2021.126124.

Au, S.Y., Bruce, T.F., Bridges, W.C., Klaine, S.J., 2015. Responses of Hyalella azteca to acute and chronic microplastic exposures. Environ. Toxicol. Chem. 34(11), 2564-2572. https://doi.org/10.1002/etc.3093.

Banerjee, A., Shelver, W.L., 2021. Micro- and nanoplastic induced cellular toxicity in mammals: A review. Sci. Total Environ. 755, 142518. https://doi.org/10.1016/j.scitotenv.2020.142518.

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting Linear Mixed-Effects Models Using lme4. 2015 67(1), 48. https://doi.org/10.18637/jss.v067.i01.

BfR, 2014. Polyethylene-containing microplastic particles: health risk resulting from the use of skin cleansing and dental care products is unlikely Bundesinstitut für Risikobewertung.

Borenstein, M., 2009. Introduction to meta-analysis. John Wiley & Sons, Chichester : Hoboken.

Brown, D.M., Wilson, M.R., MacNee, W., Stone, V., Donaldson, K., 2001. Size-dependent proinflammatory effects of ultrafine polystyrene particles: A role for surface area and oxidative stress

in the enhanced activity of ultrafines. Toxicol. Appl. Pharmacol. 175(3), 191-199. https://doi.org/10.1006/taap.2001.9240.

Burns, E.E., Boxall, A.B.A., 2018. Microplastics in the aquatic environment: Evidence for or against adverse impacts and major knowledge gaps. Environ. Toxicol. Chem. 37(11), 2776-2796. https://doi.org/10.1002/etc.4268.

Canto, R.G., Robinson, G.R., Reynolds, H.Y., 1994. Defense Mechanisms of the Respiratory Tract, in: Chmel, H., Bendinelli, M., Friedman, H. (Eds.), Pulmonary Infections and Immunity. Springer US, Boston, MA, pp. 1-27. https://doi.org/10.1007/978-1-4899-1063-9_1.

Chang, X., Xue, Y., Li, J., Zou, L., Tang, M., 2020. Potential health impact of environmental micro- and nanoplastics pollution. J. Appl. Toxicol. 40(1), 4-15. https://doi.org/10.1002/jat.3915.

Choi, D., Bang, J., Kim, T., Oh, Y., Hwang, Y., Hong, J., 2020. In vitro chemical and physical toxicities of polystyrene microfragments in human-derived cells. J. Hazard. Mater. 400, 123308-123308. https://doi.org/10.1016/j.jhazmat.2020.123308.

Choi, D., Hwang, J., Bang, J., Han, S., Kim, T., Oh, Y., Hwang, Y., Choi, J., Hong, J., 2021. In vitro toxicity from a physical perspective of polyethylene microplastics based on statistical curvature change analysis. Sci. Total Environ. 752. https://doi.org/10.1016/j.scitotenv.2020.142242.

Connors, K.A., Dyer, S.D., Belanger, S.E., 2017. Advancing the quality of environmental microplastic research. Environ. Toxicol. Chem. 36(7), 1697-1703. https://doi.org/10.1002/etc.3829.

Cox, K.D., Covernton, G.A., Davies, H.L., Dower, J.F., Juanes, F., Dudas, S.E., 2019. Human consumption of microplastics. Environ. Sci. Technol. 53(12), 7068-7074. https://doi.org/10.1021/acs.est.9b01517.

Craney, T.A., Surles, J.G., 2002. Model-dependent variance inflation factor cutoff values. Qual. Eng. 14(3), 391-403. https://doi.org/10.1081/QEN-120001878.

CRD, 2009. Systematic reviews : CRD's guidance for undertaking reviews in health care. Centre for Reviews and Dissemination, University of York, York, United Kingdom.

352 Danopoulos, E., Jenner, L., Twiddy, M., Rotchell, J.M., 2020a. Microplastic contamination of salt
353 intended for human consumption: A systematic review and meta-analysis. SN Appl. Sci. 2(12), 1950.
354 https://doi.org/10.1007/s42452-020-03749-0.
355 Danopoulos, E., Jenner, L.C., Twiddy, M., Rotchell, J.M., 2020b. Microplastic contamination of
356 seafood intended for human consumption: A systematic review and meta-analysis. Environ. Health
357 Perspect. 128(12), 126002. https://doi.org/10.1289/EHP7171.
358 Danopoulos, E., Twiddy, M., Rotchell, J.M., 2020c. Microplastic contamination of drinking water: A
359 systematic review. PLoS One 15(7), e0236838. https://doi.org/10.1371/journal.pone.0236838.
360 Devriese, L.I., De Witte, B., Vethaak, A.D., Hostens, K., Leslie, H.A., 2017. Bioaccumulation of PCBs
361 from microplastics in Norway lobster (Nephrops norvegicus): An experimental study. Chemosphere
362 186, 10-16. https://doi.org/10.1016/j.chemosphere.2017.07.121.
363 Dixit, R., Riviere, J., Krishnan, K., Andersen, M., 2003. Toxicokinetics and physiologically based
364 toxicokinetics in toxicology and risk assessment. J. Toxicol. Environ. Health, Pt. B Crit. Rev. 6(1), 1-
365 40. https://doi.org/10.1080/10937400306479.
366 Dong, C.-D., Chen, C.-W., Chen, Y.-C., Chen, H.-H., Lee, J.-S., Lin, C.-H., 2020. Polystyrene
367 microplastic particles: In vitro pulmonary toxicity assessment. J. Hazard. Mater. 385, 121575.
368 https://doi.org/10.1016/j.jhazmat.2019.121575.
369 Doorn, P.F., Campbell, P.A., Amstutz, H.C.J.C.O., Research, R., 1996. Metal versus polyethylene
370 wear particles in total hip replacements: A review. Clin Orthop Relat Res 329, S206-S216.
371 https://doi.org/10.1097/00003086-199608001-00018.
372 EFSA, 2016. Presence of microplastics and nanoplastics in food, with particular focus on seafood.
373 European Food Safety Authority (EFSA) Journal 14(6), 4501.
374 https://doi.org/10.2903/j.efsa.2016.4501.
375 Ennos, A.R., Johnson, M., 2018. Statistical and data handling skills in biology, Fourth edition. ed.
376 Pearson, Harlow, United Kingdom.

EPA, 2014. Framework for Human Health Risk Assessment to Inform Decision Making. U.S. Environmental Protection Agency. Office of the Science Advisor. Risk Assessment Forum.

EPA, 2018. Application of systematic review in Toxic Substances Control Act (TSCA) evaluations. United States Environmental Protection Agency.

EPA, 2019. Guidelines for human exposure assessment. United States Environmental Protection Agency.

FAO, WHO, 2009. Principles and methods for the risk assessment of chemicals in food: Environmental Health Criteria 240. Food and Agriculture Organization of the United Nations and the World Health Organization, Stuttgart, Germany.

Gallo, F., Fossi, C., Weber, R., Santillo, D., Sousa, J., Ingram, I., Nadal, A., Romano, D., 2018. Marine litter plastics and microplastics and their toxic chemicals components: The need for urgent preventive measures. Environ. Sci. Eur. 30, 1-14. https://doi.org/10.1186/s12302-018-0139-z.

Garritty, C., Gartlehner, G., Kamel, C., King, V.J., Nussbaumer-Streit, B., Stevens, A., Hamel, C., Affengruber, L., 2020. Cochrane Rapid Reviews. Interim Guidance from the Cochrane Rapid Reviews Methods Group.

GESAMP, 2016. Sources, fate and effects of microplastics in the marine environment: part two of a global assessment. The Joint Group of Experts on Scientific Aspects of Marine Environmental Protection, Working Group 40, London, UK.

Goodman, K.E., Hare, J.T., Khamis, Z.I., Hua, T., Sang, Q.-X.A., 2021. Exposure of human lung cells to polystyrene microplastics significantly retards cell proliferation and triggers morphological changes. Chem. Res. Toxicol. https://doi.org/10.1021/acs.chemrestox.0c00486.

Hale, R.C., Seeley, M.E., La Guardia, M.J., Mai, L., Zeng, E.Y., 2020. A global perspective on microplastics. J. Geophys. Res. (C Oceans) 125(1), e2018JC014719. https://doi.org/10.1029/2018jc014719.

Hamel, C., Michaud, A., Thuku, M., Skidmore, B., Stevens, A., Nussbaumer-Streit, B., Garritty, C., 2021. Defining rapid reviews: a systematic scoping review and thematic analysis of definitions and defining characteristics of rapid reviews. J. Clin. Epidemiol. 129, 74-85. https://doi.org/10.1016/j.jclinepi.2020.09.041.

Han, S., Bang, J., Choi, D., Hwang, J., Kim, T., Oh, Y., Hwang, Y., Choi, J., Hong, J., 2020. Surface pattern analysis of microplastics and their impact on human-derived cells. Acs Appl. Polym 2(11), 4541-4550. https://doi.org/10.1021/acsapm.0c00645.

Hartmann, N.B., Huffer, T., Thompson, R.C., Hassellov, M., Verschoor, A., Daugaard, A.E., Rist, S., Karlsson, T., Brennholt, N., Cole, M., Herrling, M.P., Hess, M.C., Ivleva, N.P., Lusher, A.L., Wagner, M., 2019. Are we speaking the same language? Recommendations for a definition and categorization framework for plastic debris. Environ. Sci. Technol. 53(3), 1039-1047. https://doi.org/10.1021/acs.est.8b05297.

Hartmann, N.B., Rist, S., Bodin, J., Jensen, L.H.S., Schmidt, S.N., Mayer, P., Meibom, A., Baun, A., 2017. Microplastics as vectors for environmental contaminants: Exploring sorption, desorption, and transfer to biota. Integr. Environ. Assess. Manag. 13(3), 488-493. https://doi.org/10.1002/ieam.1904.

Hesler, M., Aengenheister, L., Ellinger, B., Drexel, R., Straskraba, S., Jost, C., Wagner, S., Meier, F., von Briesen, H., Buechel, C., Wick, P., Buerki-Thurnherr, T., Kohl, Y., 2019. Multi-endpoint toxicological assessment of polystyrene nano- and microparticles in different biological models in vitro. Toxicol in Vitro 61. https://doi.org/10.1016/j.tiv.2019.104610.

Higgins, J.P.T., Thomas, J., Chandler, J., Cumpston, M., Li, T., Page, M.J., Welch, V.A., 2021. Cochrane Handbook for Systematic Reviews of Interventions version 6.2 (updated February 2021). Cochrane, 2021.

Hooijmans, C.R., Rovers, M.M., de Vries, R.B.M., Leenaars, M., Ritskes-Hoitinga, M., Langendam, M.W., 2014. SYRCLE's risk of bias tool for animal studies. BMC Med. Res. Methodol. 14(1), 43. https://doi.org/10.1186/1471-2288-14-43.

426 Huang, X., Teng, X., Chen, D., Tang, F., He, J., 2010. The effect of the shape of mesoporous silica
427 nanoparticles on cellular uptake and cell function. Biomaterials 31(3), 438-448.
428 https://doi.org/10.1016/j.biomaterials.2009.09.060.
429 Huang, Y., He, T., Yan, M., Yang, L., Gong, H., Wang, W., Qing, X., Wang, J., 2021. Atmospheric
430 transport and deposition of microplastics in a subtropical urban environment. J. Hazard. Mater. 416,
431 126168. https://doi.org/10.1016/j.jhazmat.2021.126168.
432 Huang, Z., Weng, Y., Shen, Q., Zhao, Y., Jin, Y., 2021. Microplastic: A potential threat to human and
433 animal health by interfering with the intestinal barrier function and changing the intestinal
434 microenvironment. Sci. Total Environ. 785, 147365. https://doi.org/10.1016/j.scitotenv.2021.147365.
435 Hwang, J., Choi, D., Han, S., Choi, J., Hong, J., 2019. An assessment of the toxicity of polypropylene
436 microplastics in human derived cells. Sci. Total Environ. 684, 657-669.
437 https://doi.org/10.1016/j.scitotenv.2019.05.071.
438 Hwang, J., Choi, D., Han, S., Jung, S.Y., Choi, J., Hong, J., 2020. Potential toxicity of polystyrene
439 microplastic particles. Scientific Reports 10(1). https://doi.org/10.1038/s41598-020-64464-9.
440 Ibrahim, Y.S., Tuan Anuar, S., Azmi, A.A., Wan Mohd Khalik, W.M.A., Lehata, S., Hamzah, S.R.,
441 Ismail, D., Ma, Z.F., Dzulkarnaen, A., Zakaria, Z., Mustaffa, N., Tuan Sharif, S.E., Lee, Y.Y., 2021.
442 Detection of microplastics in human colectomy specimens. JGH Open 5(1), 116-121.
443 https://doi.org/10.1002/jgh3.12457.
444 IPCS, 2004. International Programme on Chemical Safety (IPCS) risk assessment terminology. World
445 Health Organization, Geneva.
446 IPCS, 2009. Principles for modelling dose-response for the risk assessment of chemicals. World Health
447 Organization, International Programme on Chemical Safety Geneva.
448 Jacob, H., Besson, M., Swarzenski, P.W., Lecchini, D., Metian, M., 2020. Effects of virgin micro- and
449 nanoplastics on fish: Trends, meta-Analysis, and perspectives. Environ. Sci. Technol. 54(8), 4733-
450 4745. https://doi.org/10.1021/acs.est.9b05995.

Jenner, L.C., Sadofsky, L.R., Danopoulos, E., Rotchell, J.M., 2021. Household indoor microplastics within the Humber region (United Kingdom): Quantification and chemical characterisation of particles present. Atmos. Environ. 259, 118512. https://doi.org/10.1016/j.atmosenv.2021.118512.

Jeong, J., Choi, J., 2019. Adverse outcome pathways potentially related to hazard identification of microplastics based on toxicity mechanisms. Chemosphere 231, 249-255. https://doi.org/10.1016/j.chemosphere.2019.05.003.

Jung, J.-W., Park, J.-W., Eo, S., Choi, J., Song, Y.K., Cho, Y., Hong, S.H., Shim, W.J., 2021. Ecological risk assessment of microplastics in coastal, shelf, and deep sea waters with a consideration of environmentally relevant size and shape. Environ. Pollut. 270, 116217. https://doi.org/10.1016/j.envpol.2020.116217.

Karbalaei, S., Hanachi, P., Walker, T.R., Cole, M., 2018. Occurrence, sources, human health impacts and mitigation of microplastic pollution. Environ. Sci. Pollut. Res.(25), 36046–36063. https://doi.org/10.1007/s11356-018-3508-7.

Koelmans, A.A., Mohamed Nor, N.H., Hermsen, E., Kooi, M., Mintenig, S.M., De France, J., 2019. Microplastics in freshwaters and drinking water: Critical review and assessment of data quality. Water Res. 155, 410-422. https://doi.org/10.1016/j.watres.2019.02.054.

Kogel, T., Bjoroy, O., Toto, B., Bienfait, A.M., Sanden, M., 2020. Micro- and nanoplastic toxicity on aquatic life: Determining factors. Sci. Total Environ. 709. https://doi.org/10.1016/j.scitotenv.2019.136050.

Kuroda, A., 2021. Recent progress and perspectives on the mechanisms underlying Asbestos toxicity. Genes and Environ 43(1), 46. https://doi.org/10.1186/s41021-021-00215-0.

Lebreton, L., Andrady, A., 2019. Future scenarios of global plastic waste generation and disposal. Palgrave Commun. 5(6). https://doi.org/10.1057/s41599-018-0212-7.

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. Arch. Toxicol. 94(7), 2463-2479. https://doi.org/10.1007/s00204-020-02750-1.

Li J., Green, C., Reynolds, A., Shi, H., Rotchell, J.M., 2018. Microplastics in mussels sampled from coastal waters and supermarkets in the United Kingdom. Environ. Pollut. 241, 35-44. https://doi.org/10.1016/j.envpol.2018.05.038.

Li, Z., Zhu, S., Liu, Q., Wei, J., Jin, Y., Wang, X., Zhang, L., 2020. Polystyrene microplastics cause cardiac fibrosis by activating Wnt/beta-catenin signaling pathway and promoting cardiomyocyte apoptosis in rats. Environ. Pollut. 265(Pt A), 115025-115025. https://doi.org/10.1016/j.envpol.2020.115025.

Liberati, A., Altman, D.G., Tetzlaff, J., Mulrow, C., Gøtzsche, P.C., Ioannidis, J.P.A., Clarke, M., Devereaux, P.J., Kleijnen, J., Moher, D., 2009. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. BMJ 339:b2700. https://doi.org/10.1136/bmj.b2700.

Lithner, D., Larsson, Å., Dave, G., 2011. Environmental and health hazard ranking and assessment of plastic polymers based on chemical composition. Sci. Total Environ. 409(18), 3309-3324. https://doi.org/10.1016/j.scitotenv.2011.04.038.

Liu, S., Wu, X., Gu, W., Yu, J., Wu, B., 2020. Influence of the digestive process on intestinal toxicity of polystyrene microplastics as determined by in vitro Caco-2 models. Chemosphere 256. https://doi.org/10.1016/j.chemosphere.2020.127204.

Lusher, A., Hollman, P., Mendoza-Hill, J., 2017. Microplastics in fisheries and aquaculture: status of knowledge on their occurrence and implications for aquatic organisms and food safety. FAO

Manning, C.B., Vallyathan, V., Mossman, B.T., 2002. Diseases caused by asbestos: mechanisms of injury and disease development. Int. Immunopharmacol. 2(2), 191-200. https://doi.org/10.1016/S1567-5769(01)00172-2.

499 Mark, J.E., 1999. Polymer data handbook. Oxford University Press, Oxford, UK.

500 Mason, S.A., Welch, V.G., Neratko, J., 2018. Synthetic polymer contamination in bottled water. Front.
501 Chem. 6(2018: 407). https://doi.org/10.3389/fchem.2018.00407.

502 Menard, S., 2002. Applied Logistic Regression Analysis. SAGE Publications, Inc., Thousand Oaks,
503 California.

504 Miller, E., Sedlak, M., Lin, D., Box, C., Holleman, C., Rochman, C.M., Sutton, R., 2021.
505 Recommended best practices for collecting, analyzing, and reporting microplastics in environmental
506 media: Lessons learned from comprehensive monitoring of San Francisco Bay. J. Hazard. Mater. 409,
507 124770. https://doi.org/10.1016/j.jhazmat.2020.124770.

508 Minoda, Y., Kobayashi, A., Iwaki, H., Miyaguchi, M., Kadoya, Y., Ohashi, H., Yamano, Y., Takaoka,
509 K., 2003. Polyethylene wear particles in synovial fluid after total knee arthroplasty. Clin. Orthop.
510 Relat. Res. 410, 165-172. https://doi.org/10.1097/01.blo.0000063122.39522.c2.

511 Moher, D., Liberati, A., Tetzlaff, J., Altman, D.G., The, P.G., 2009. Preferred reporting items for
512 systematic reviews and meta-analyses: The PRISMA statement. PLoS Med. 6(7), e1000097.
513 https://doi.org/10.1371/journal.pmed.1000097.

514 Moher, D., Shamseer, L., Clarke, M., Ghersi, D., Liberati, A., Petticrew, M., Shekelle, P., Stewart,
515 L.A., Group, P.-P., 2015. Preferred reporting items for systematic review and meta-analysis protocols
516 (PRISMA-P) 2015 statement. Syst. Rev. 4(1), 1. https://doi.org/10.1186/2046-4053-4-1.

517 Napper, I.E., Bakir, A., Rowland, S.J., Thompson, R.C., 2015. Characterisation, quantity and sorptive
518 properties of microplastics extracted from cosmetics. Mar. Pollut. Bull. 99(1), 178-185.
519 https://doi.org/10.1016/j.marpolbul.2015.07.029.

520 NASEM, 2021. The Use of Systematic Review in EPA's Toxic Substances Control Act Risk
521 Evaluations. National Academies of Sciences, Engineering, and Medicine, Washington, DC.

OHAT, 2019. Handbook for Conducting a Literature-Based Health Assessment Using OHAT Approach for Systematic Review and Evidence Integration. Office of Health Assessment and Translation (OHAT), US Department of Health and Human Services.

Osborne, J.W.a., 2015. Best practices in logistic regression. SAGE.

Pauly, J.L., Stegmeier, S.J., Allaart, H.A., Cheney, R.T., Zhang, P.J., Mayer, A.G., Streck, R.J., 1998. Inhaled cellulosic and plastic fibers found in human lung tissue. Cancer Epidemiol. Biomarkers Prev. 7(5), 419-428.

Plastics Europe, 2008. The compelling facts about plastics. An analysis of plastics production, demand and recovery for 2006 in Europe. Plastics Europe.

Plastics Europe, 2017. Plastics: the Facts 2017: An analysis of European plastics production, demand and waste data.

Plastics Europe, 2019. Plastics – the Facts 2019; An analysis of European plastics production, demand and waste data.

Plastics Europe, 2020. Plastics – the Facts 2020; An analysis of European plastics production, demand and waste data.

Plastics Europe, 2021. About plastics, Polyolefins. https://www.plasticseurope.org/en/about-plastics/what-are-plastics/large-family/polyolefins#:~:text=PP%20(polypropylene)%3A%20The%20density,resistance%2C%20but%20less%20chemical%20resistance. (accessed 01-03-2021).

Prata, J.C., 2018. Airborne microplastics: Consequences to human health? Environ. Pollut. 234, 115-126. https://doi.org/10.1016/j.envpol.2017.11.043.

Prietl, B., Meindl, C., Roblegg, E., Pieber, T.R., Lanzer, G., Fröhlich, E., 2014. Nano-sized and micro-sized polystyrene particles affect phagocyte function. Cell Biology and Toxicology 30(1), 1-16. https://doi.org/10.1007/s10565-013-9265-y.

Prüst, M., Meijer, J., Westerink, R.H.S., 2020. The plastic brain: neurotoxicity of micro- and nanoplastics. Part. Fibre Toxicol. 17(1), 24. https://doi.org/10.1186/s12989-020-00358-y.

R Core Team, 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing. https://www.R-project.org/.

Ragusa, A., Svelato, A., Santacroce, C., Catalano, P., Notarstefano, V., Carnevali, O., Papa, F., Rongioletti, M.C.A., Baiocco, F., Draghi, S., D'Amore, E., Rinaldo, D., Matta, M., Giorgini, E., 2021. Plasticenta: First evidence of microplastics in human placenta. Environ. Int. 146, 106274. https://doi.org/10.1016/j.envint.2020.106274.

Rist, S., Hartmann, N.B., 2018. Aquatic ecotoxicity of microplastics and nanoplastics: lessons learned from engineered nanomaterials. Freshwater microplastics. Emerging Environmental Contaminants? The Handbook of Environmental Chemistry 58. Springer, Cham, pp. 25-49.

Rubio, L., Marcos, R., Hernández, A., 2020. Potential adverse health effects of ingested micro- and nanoplastics on humans. Lessons learned from in vivo and in vitro mammalian models. J. Toxicol. Environ. Health, Pt. B Crit. Rev. 23(2), 51-68. https://doi.org/10.1080/10937404.2019.1700598.

SAM, 2019. Environmental and health risks of microplastic pollution; Scientific Advice Mechanism. Group of Chief Scientific Advisors. European Commission, Luxembourg.

Sanchez, V.C., Pietruska, J.R., Miselis, N.R., Hurt, R.H., Kane, A.B., 2009. Biopersistence and potential adverse health impacts of fibrous nanomaterials: what have we learned from asbestos? WIREs Nanomedicine and Nanobiotechnology 1(5), 511-529. https://doi.org/10.1002/wnan.41.

Santana, M.F.M., Moreira, F.T., Pereira, C.D.S., Abessa, D.M.S., Turra, A., 2018. Continuous exposure to microplastics does not cause physiological effects in the cultivated mussel Perna perna. Arch. Environ. Contam. Toxicol. 74(4), 594-604. https://doi.org/10.1007/s00244-018-0504-3.

SAPEA, 2019. A scientific perspective on microplastics in nature and society. Science Advice for Policy by European Academies, Berlin.

570 Schaefer, H.R., Myers, J.L., 2017. Guidelines for performing systematic reviews in the development
571 of toxicity factors. Regul. Toxicol. Pharmacol. 91, 124-141.
572 https://doi.org/10.1016/j.yrtph.2017.10.008.
573 Schirinzi, G.F., Perez-Pomeda, I., Sanchis, J., Rossini, C., Farre, M., Barcelo, D., 2017. Cytotoxic
574 effects of commonly used nanomaterials and microplastics on cerebral and epithelial human cells.
575 Environ. Res. 159, 579-587. https://doi.org/10.1016/j.envres.2017.08.043.
576 Schwabl, P., Köppel, S., Königshofer, P., Bucsics, T., Trauner, M., Reiberger, T., Liebmann, B., 2019.
577 Detection of various microplastics in human stool: a prospective case series. Ann. Intern. Med. 171(7),
578 453-457. https://doi.org/10.7326/M19-0618.
579 Shamseer, L., Moher, D., Clarke, M., Ghersi, D., Liberati, A., Petticrew, M., Shekelle, P., Stewart,
580 L.A., 2015. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P)
581 2015: elaboration and explanation. BMJ 349, 1-25. https://doi.org/10.1136/bmj.g7647.
582 Shi, Q., Tang, J., Liu, R., Wang, L., 2021. Toxicity in vitro reveals potential impacts of microplastics
583 and nanoplastics on human health: A review. Crit. Rev. Environ. Sci. Technol., 1-33.
584 https://doi.org/10.1080/10643389.2021.1951528.
585 Sommet, N., Morselli, D., 2017. Keep calm and learn multilevel logistic modeling: A simplified three-
586 step procedure using stata, R, Mplus, and SPSS. Int. Rev. Soc. Psychol. 30, 203-218.
587 Sterne, J.A.C., Hernán, M.A., Reeves, B.C., Savović, J., Berkman, N.D., Viswanathan, M., Henry, D.,
588 Altman, D.G., Ansari, M.T., Boutron, I., Carpenter, J.R., Chan, A.-W., Churchill, R., Deeks, J.J.,
589 Hróbjartsson, A., Kirkham, J., Jüni, P., Loke, Y.K., Pigott, T.D., Ramsay, C.R., Regidor, D., Rothstein,
590 H.R., Sandhu, L., Santaguida, P.L., Schünemann, H.J., Shea, B., Shrier, I., Tugwell, P., Turner, L.,
591 Valentine, J.C., Waddington, H., Waters, E., Wells, G.A., Whiting, P.F., Higgins, J.P.T., 2016.
592 ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions. BMJ 355,
593 i4919. https://doi.org/10.1136/bmj.i4919.

Stock, V., Boehmert, L., Lisicki, E., Block, R., Cara-Carmona, J., Pack, L.K., Selb, R., Lichtenstein, D., Voss, L., Henderson, C.J., Zabinsky, E., Sieg, H., Braeuning, A., Lampen, A., 2019. Uptake and effects of orally ingested polystyrene microplastic particles in vitro and in vivo. Arch. Toxicol 93(7), 1817-1833. https://doi.org/10.1007/s00204-019-02478-7.

Stock, V., Laurisch, C., Franke, J., Doenmez, M.H., Voss, L., Boehmert, L., Braeuning, A., Sieg, H., 2021. Uptake and cellular effects of PE, PP, PET and PVC microplastic particles. Toxicol in Vitro 70. https://doi.org/10.1016/j.tiv.2020.105021.

Stoltzfus, J.C., 2011. Logistic Regression: A Brief Primer. Acad. Emerg. Med. 18(10), 1099-1104. https://doi.org/10.1111/j.1553-2712.2011.01185.x.

Sun, Y., Yuan, J., Zhou, T., Zhao, Y., Yu, F., Ma, J., 2020. Laboratory simulation of microplastics weathering and its adsorption behaviors in an aqueous environment: A systematic review. Environ. Pollut. 265, 114864. https://doi.org/10.1016/j.envpol.2020.114864.

Teng, J., Wang, Q., Ran, W., Wu, D., Liu, Y., Sun, S., Liu, H., Cao, R., Zhao, J., 2019. Microplastic in cultured oysters from different coastal areas of China. Sci. Total Environ. 653, 1282-1292. https://doi.org/10.1016/j.scitotenv.2018.11.057.

Thompson, C.G., Kim, R.S., Aloe, A.M., Becker, B.J., 2017. Extracting the variance inflation factor and other multicollinearity diagnostics from typical regression results. Basic Appl. Soc. Psych. 39(2), 81-90. https://doi.org/10.1080/01973533.2016.1277529.

Urban, R.M., Jacobs, J.J., Tomlinson, M.J., Gavrilovic, J., Black, J., Peoc'h, M., 2000. Dissemination of wear particles to the liver, spleen, and abdominal lymph nodes of patients with hip or knee replacement. JBJS 82(4), 457. https://doi.org/10.2106/00004623-200004000-00002.

Vandenberg, L.N., Colborn, T., Hayes, T.B., Heindel, J.J., Jacobs, D.R., Jr., Lee, D.-H., Shioda, T., Soto, A.M., vom Saal, F.S., Welshons, W.V., Zoeller, R.T., Myers, J.P., 2012. Hormones and endocrine-disrupting chemicals: Low-dose effects and nonmonotonic dose responses. Endocr. Rev. 33(3), 378-455. https://doi.org/10.1210/er.2011-1050.

Venables, W.N., Ripley, B.D., 2002. Modern Applied Statistics with S, Fourth edition ed. Springer, New York.

Wang, Q., Bai, J., Ning, B., Fan, L., Sun, T., Fang, Y., Wu, J., Li, S., Duan, C., Zhang, Y., Liang, J., Gao, Z., 2020. Effects of bisphenol A and nanoscale and microscale polystyrene plastic exposure on particle uptake and toxicity in human Caco-2 cells. Chemosphere 254. https://doi.org/10.1016/j.chemosphere.2020.126788.

Whaley, P., Aiassa, E., Beausoleil, C., Beronius, A., Bilotta, G., Boobis, A., de Vries, R., Hanberg, A., Hoffmann, S., Hunt, N., Kwiatkowski, C.F., Lam, J., Lipworth, S., Martin, O., Randall, N., Rhomberg, L., Rooney, A.A., Schünemann, H.J., Wikoff, D., Wolffe, T., Halsall, C., 2020. Recommendations for the conduct of systematic reviews in toxicology and environmental health research (COSTER). Environ. Int. 143, 105926. https://doi.org/10.1016/j.envint.2020.105926.

WHO, 2000. Air quality guidelines for Europe, 2nd ed. ed. World Health Organization. Regional Office for Europe, Copenhagen.

WHO & IPCS, 2010. WHO human health risk assessment toolkit: chemical hazards. World Health Organization & International Programme on Chemical Safety, Geneva.

Willert, H.G., Semlitsch, M., Peltier, L.F.J.C.O., Research®, R., 1996. Tissue reactions to plastic and metallic wear products of joint endoprostheses. 333, 4-14.

Woodruff, T., J., Sutton, P., 2014. The navigation guide systematic review methodology: A rigorous and transparent method for translating environmental health science into better health outcomes. Environ. Health Perspect. 122(10), 1007-1014. https://doi.org/10.1289/ehp.1307175.

Wright, S.L., Thompson, R.C., Galloway, T.S., 2013. The physical impacts of microplastics on marine organisms: A review. Environ. Pollut. 178, 483-492. https://doi.org/10.1016/j.envpol.2013.02.031.

Wright, S.L., Ulke, J., Font, A., Chan, K.L.A., Kelly, F.J., 2020. Atmospheric microplastic deposition in an urban environment and an evaluation of transport. Environ. Int. 136, 105411. https://doi.org/10.1016/j.envint.2019.105411.

644 Wu, B., Wu, X., Liu, S., Wang, Z., Chen, L., 2019. Size-dependent effects of polystyrene microplastics
645 on cytotoxicity and efflux pump inhibition in human Caco-2 cells. Chemosphere 221, 333-341.
646 https://doi.org/10.1016/j.chemosphere.2019.01.056.
647 Wu, S., Wu, M., Tian, D., Qiu, L., Li, T., 2020. Effects of polystyrene microbeads on cytotoxicity and
648 transcriptomic profiles in human Caco-2 cells. Environ. Toxicol. 35(4), 495-506.
649 https://doi.org/10.1002/tox.22885.
650 Xia, B., Sui, Q., Du, Y., Wang, L., Jing, J., Zhu, L., Zhao, X., Sun, X., Booth, A.M., Chen, B., Qu, K.,
651 Xing, B., 2021. Secondary PVC microplastics are more toxic than primary PVC microplastics to
652 Oryzias melastigma embryos. J. Hazard. Mater. 424, 127421.
653 https://doi.org/10.1016/j.jhazmat.2021.127421.
654 Yong, C.Q.Y., Valiyaveettil, S., Tang, B.L., 2020. Toxicity of microplastics and nanoplastics in
655 mammalian systems. Int. J. Environ. Res. Public Health 17(5), 1509.
656 Zhang, M., Li, J.X., Ding, H.B., Ding, J.F., Jiang, F.H., Ding, N.X., Sun, C.J., 2020. Distribution
657 Characteristics and Influencing Factors of Microplastics in Urban Tap Water and Water Sources in
658 Qingdao, China. Anal. Lett. 53(8), 1312-1327. https://doi.org/10.1080/00032719.2019.1705476.

659