Systematic Review

Effects of Nano and Microplastics on the Inflammatory Process: *In Vitro* and *In Vivo* Studies Systematic Review

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Abstract

Background: Microplastics (MPs) and Nanoplastics (NPs) are plastic fragments that spread in the environment and accumulate in the human body, so they have been becoming a worldwide environmental concern because of their potential human health effects. The aim of this systematic review was to investigate the prospective impact of MPs and NPs on the inflammatory process. **Methods**: Electronic article search was performed on PubMed, Scopus and Web of Science international databases from 1 Jan 2012 to 31 Dec 2021. Screenings of titles, abstracts and full texts were performed according to the Preferred Reporting Item for Systematic Review and Meta-analyses (PRISMA). The methodological quality of the studies was checked by the Toxicological data Reliability Assessment Tool. **Results**: Electronic article search identified 125 records, from which 6 *in vitro*, 11 *in vivo* and 2 both *in vivo* and *in vitro* studies were included. Both *in vivo* and *in vitro* studies have showed an increase of different inflammatory outcomes (Interleukines, Tumor necrosis factor, Chemokines, Interferones, Transcription factors, Growth factors, Oxydoreductase, Proteins and others), thus it seems to confirm the association with the exposure to microplastics of different types, sizes, exposure times and exposed species. **Conclusions**: This systematic review seems to support the relationship between the exposure to MPs and the inflammatory process both *in vivo* and *in vitro*. Greater caution is needed about the role of NPs because of a very small number of studies. Additional high-quality studies are warranted to confirm these results, especially the research should be focused on NPs being lacking literature.

Keywords: microplastics; nanoplastics; inflammation; health; *in vivo* studies; *in vitro* studies

1. Introduction

Microplastics are the consequence of the high production of plastics and of its unmanaged release in the environment. Extreme solidity at room temperature, excellent electrical, thermal, and acoustic insulation, portability, ease of use thanks to the lightweight and cheapness are the main properties of plastic. These properties made it the most widespread and the most used synthetic material worldwide, causing a global pollution [1–3]. It was found in soil, sea, air, and also in the Arctic sea ice [4,5].

Plastic is made up of polymers set such as polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polyethylene terephthalate (PET), polycarbonate (PC), polymethyl methacrylate (PMMA), polyurethane (PU), etc. and several classes of additives (plasticizers) are added in formula for improving their performance [1,6].

The plastic degradation, due to thermo-oxidative degradation processes and/or mechanical fragmentation and/or biodegradation, produces smaller particles classified according to the size as: nanoplastics ≤0.1 μm (NPs), 0.1 μm < microplastics <5 mm (MPs), 0.5< mesoplastics <5 cm, 5< macroplastics <50 cm, and megaplastics >50 cm [7,8]. Moreover, MPs has been categorized as: primary (particles resulting from the commodity production such as exfoliating, toothpastes or cosmetics, and personal hygiene products) and secondary (particles resulting from the degradation of products such as packaging or clothing) [9–11].

MPs and NPs size seems to favor their penetration into animal and vegetal tissues and cells besides accumulation in organs [11–13], causing alterations in physiological process [3]. Some studies highlighted the capable of MPs and NPs to cause toxicity, chronic inflammation and increase risk of neoplasm [7,14–17]. The *in vivo* studies show that both MPs and NPs are absorbed and accumulated in the tissues altering the correct functioning of organs and systems [18–20].

The human body is continuously exposed to microplastics through digestion, inhalation or dermal contact. Human's ingestion is considered the simplest form of exposure to MPs and NPs [12,13,21]; which could cause an inflammatory response. *In vitro* test showed that NPs alter the cell membrane surface and trigger the inflammatory process [18]. Also, the exposures to MPs through inhalation and dermal contact were identified as triggers of lung and skin pro-inflammatory responses [17,22,23] which can lead to an increased risk of developing cancer [24].

The aim of the present study was to review the *in vitro* and *in vivo* studies that evaluated the impacts of exposure
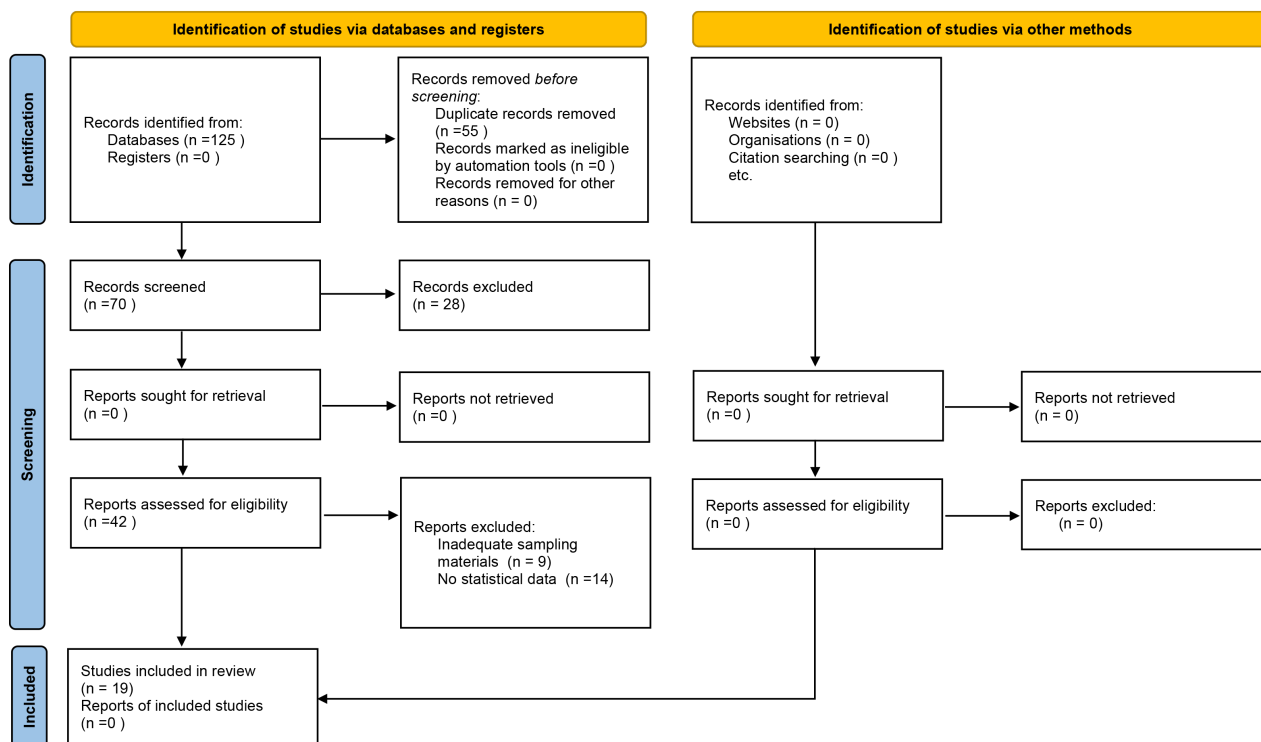
Fig. 1. Flow chart of studies identification and selection [25].

to MPs and NPs on inflammatory process.

2. Materials and Methods

2.1 Search Strategy and Study Selection

We conducted this systematic review according to the protocol registered in PROSPERO (CRD348887). The protocol was not published in any peer-reviewed journal. The development of the protocol was guided by the Preferred Reporting Item for Systematic Review and Meta-analyses (PRISMA) Statement [25].

The literature search was performed through Pubmed, Scopus and Web of Science international databases. The keywords and search terms used were ("microplastics" OR "nanoplastics"), AND ("inflammation" OR "inflammatory response") AND ("health" OR "wellness").

Articles were initially screened based on the title and abstract according to the scope of this review, the articles that do not include original data on MPs/NPs and inflammatory process and based on the publication type (i.e., reviews, comments, opinion, letters, or abstract) were excluded. We included only studies published in English, from 2012 to 2021 and that report experimental data from *in vivo* and *in vitro* controlled exposure studies. Furthermore, a hand search of the reference lists of relevant studies was also performed to check papers that met our selection criteria but missed the keyword search criteria.

Two researchers (M.Fi and E.P.) performed data selection, extraction and quality assessment independently. Any

disagreement between the two researchers was resolved through consensus session with a third researcher (M.Fe.).

In the screening phase, this systematic review was split into two main sections:

(a) The first referring exclusively to *in vitro* models and,

(b) The second referring exclusively to *in vivo* models, both for the assessment of the inflammatory capacity of NPs and MPs through the evaluation of the inflammatory biomarkers.

We excluded: (i) studies whose method of inflammatory assessment was not clear, or incompletely described or that do not evaluate, or only evaluate the inflammatory of MPs/NPs qualitatively; (ii) studies that do not evaluate inflammatory through methods specific for MPs/NPs; (iii) studies that only report other health effects (e.g., carcinogenic effect); (iv) studies other than *in vitro*, e.g., *in silico*; and (v) studies not reporting statistical data.

2.2 Data Collection and Synthesis

The following descriptive and quantitative information was extracted from each of the eligible study for both sections, i.e., *in vitro* and *in vivo* studies: authors and year of publication, type and size of MPs/NPs, dose/exposure time, inflammatory biomarkers, animals (*in vivo* studies)/cell models (*in vitro* studies), assay(s) and outcomes. Information was summarized and organized in tables and for each table studies can be identified by their listed study details

Table 1. General information of the included *in vitro* end/or *in vivo* studies.

Author, year	Particles Type	Size — MPs (5 mm–0.1 μm): / NPs (<0.1 μm):	Dose/exposure time	Biomarkers	Cell/Animal models	Assay (s)
			In vitro studies			
Dong *et al.*, 2020 [29]	PS	1.72 ± 0.26 μm	1–1000 μg/cm^2, 24 and 48 h	IL-6, IL-8, HO-1, ROS, AAT	Normal human lung epithelial BEAS-2B cells maintained in LHC-9 medium and incubated at 37 °C in a humidified atmosphere containing 5% of CO_2	Cytotoxicity assay, ELISA assay, DCFH-DA assays, Western blot assay, TEER
Visalli *et al.*, 2021 [33]	PS	3 and 10 μm	100–1600 particles/mL; 0.5, 1, 2, 3, 4, 5, 6, and 24 h; 7, 14, 21, 28 and 48 days	ROS	Human intestinal cell line HT-29 grown in RPMI 1600 medium with 2 mM L-glutamine, 1 mM sodium pyruvate, 10% (v/v) fetal bovine serum, 100 IU/mL penicillin and 100 μg/mL streptomycin at 37 °C and 5% CO_2	Viability Assays, Comet Assay, MTT assay
Jeon *et al.*, 2021 [37]	PP and PS	100 μm	200 mg/mL; 0.5, 1, 2, 3, 4, 5, 6, and 24 h	In THP-1 cells: IL-6, TNF-a, IL-1β, MIP-1β and ROS; In Caco-2 and HepG2 cells: IL-6, IL-8 and TNF-a	Caco-2 cells, HepG2 cells, THP-1 cells	Lactatedehydrogenase (LDH) assay kit, DCFH-DA assay, DCFDA/H2DCFDA-Cellular ROS Assay, Bicinchoninic acid assay
Busch *et al.*, 2021 [38]	PVC, PS-NH2, PS	PS: From 59 ± 8 to 224 ± 286 nm; PS-NH2: From 59 ± 9 to 88 ± 17 nm; PVC: From 279 ± 112 to 1148 ± 861 nm	0, 1, 5, 10, 50 μg/cm^2, 24 h	IL-1β, IL-6, IL-8, TNF-α	Caco-2 cells, HT29-MTX-E12 cells, THP-1 cells. Caco-2 cells were cultured in MEM, the second line in DMEM and the third in RPMI	Lactate dehydrogenase (LDH) assay, Alkaline comet assay, WST-1 assay, ELISA, TEER
Hwang *et al.*, 2020 [42]	PS, PP, PE	0, 46 μm, 1 μm, 3 μm, 10 μm, 40 μm, 100 μm	500 μg/mL, 16 h	IL-2, IL-6, IL-10, TNF-α	Human dermal fibroblasts (HDFs), Human peripheral blood mononuclear cells (PBMCs), Human Must cells line (HMC-1)	Hemolysis assay, Histamine assay, ELISA
Lehner *et al.*, 2020 [43]	Polymers such as tire wear and polyolefins	50–500 μm	823.5–1380.0 μg/cm^2, 21 days	IL-8, TNFα, IL-1β	Caco-2 cells, HT29-MTX-E12 cells, MDM cells, MDDC cells. The first one and second one was cultivated at 37 °C under a 5% CO_2 in DMEM	Cell viability assay (LDH), ELISA
			In vivo studies			
Hou *et al.*, 2021 [27]	PS	5 μm	100, 1000 10000 μg/L, 35 days	Nrf2/HO-1, NF-κB, TNF-α, IL-1β, IL-6	Four to 5 weeks old ICR male mice placed in a pathogen-free animal room (T 22 ± 2 °C, humidity 50–60%) and a 12/12 light/dark cycle. Testicular tissue was analyzed	Western blot analysis, qPCR
Li *et al.*, 2020 [28]	PE	10–150 μm	2, 20, 200 μg/L for 3 day in a week and for 5 consecutive weeks	TLR4, AP-1, IRF5, IL-1α, G-CSF, IL-2, IL-5, IL-6, IL-9, IP-10, RANTES	Male and 5-week-old SPF grade mice C57BL/6. Intestinal tissue was analyzed	Chao1 diversity index, Shannon index and UniFranc beta diversity metrics, Wilcoxon test, Mouse cytokine/Chemokine magnetic bead panel 96-well plate assay
Zheng *et al.*, 2021 [30]	PS	5 μm	500 μg/L, 28 days	IL-1β, TNF-α, IFN-γ, TG, PPAR-γ, MDA	Six weeks old male C57 mice maintained at a T 24 ± 0.5 °C with a photoperiod consisting of 12/12 light and dark hours. Liver mice was analyzed	ELISA, FITC-dextran intestinal permeability assay
Sun *et al.*, 2021 [31]	PE	1–10 μm	0.002, 0.2 μg/g/d, 31 days	IL-8, IL-6, IL-10, IL-1β, GPR41, GPR43, ERK1, NF-κB, TLR4, MyD88	Eight weeks old female ICR mice kept in a cage with a cycle of 12/12 light and dark hours. Mice' colon tissue and feces were analyzed	Biochemical assay
Chen *et al.*, 2020 [32]	PS	0.05, 0.50, 6.00 μm	0.1, 1×10^3, 1×10^6 particles/mL, 21 days	CYP1A1, BMP4, GATA4, NKx2.5, FGF8, JAK, IL6, CCL11, SOD, NF-κB	Four months old *Oryziasmelastigma* maintained in aerated 30‰ artificial seawater at 6.0 ± 0.2 mg O_2/L at 28 ± 2 °C in a 14 h light: 10 h dark cycle. Embryos of *Oryziasmelastigma* were analyzed in particular heart rate, hatching time and hatching rate	RT - qPCR assay
Capó *et al.*, 2021 [35]	LDPE	100–500 μm	250–690 μg of MPs, 120 days	CAT, SOD, GRd, GST, MPO, MDA	Seven months old *Sparusaurata* (length 11.8 ± 0.3 cm and weight 44.9 ± 3.1 g) exposed to a photoperiod of 16: 8 h light and dark cycle in a T maintained at 19 ± 2 °C and a O_2 concentration about 5.9–6.1 ppm. Liver of *Sparusaurata* was analyzed	Biochemical assay

Table 1. Continued.

Author, year	Particles Type	Size — MPs (5 mm–0.1 μm): / NPs (<0.1 μm):	Dose/exposure time	Biomarkers	Cell/Animal models	Assay (s)
Zhang *et al.*, 2021 [36]	PS	2, 10 and 200 μm	10, 000 $\mu g/L$, 60 days	IL-1β, IL-8, COX2, SOD, CAT, GPx, TNF, COX1	Eight months old *Oryziasmela stigma* placed in an artificial seawater (salinity 30‰) under a 14 h/10 h light and dark cycle. T was 28 ± 1 °C, pH 7.4 ± 0.2 and 6 ± 0.2 mg/L DO. Intestinal microbiota, liver, adipose and gut tissue were analyzed	Gut microbiota assay, LDA Effect Size (LEfSe) and MetaStat assay, Mrna expression with 18s Rrna
von Moos *et al.*, 2012 [40]	HDPE	0−80 μm, 6 h	2.5 g HDPE-fluff/L, 96 h	ROS	*Mytilusedulis L.* placed in an artificial seawater (31‰ salinity) at 15 °C. HDPE particles were taken up into the stomach and transported into digestive gland where they accumulated in the lysosomal system	Lysosomal Membrane Stability (LMS) assay
Zhao *et al.*, 2021 [41]	PP, SFb L-H, LFb L-H	20 μm, 50 ± 26 μm, 200 ± 90 μm	20, 000 $\mu g/L$, 24 h	ROS, SOD, IL-1α, D-Lac	18 weeks old *Daniorerio* placed in a culture water at 28 °C, 14/10 light and dark cycle, Ph 7.4 ± 0.2. The gut of zebrafish was analyzed	Enzymatic assay, Imaging assay, ELISA
Xie *et al*, 2021 [44]	MPs and NPs	8 μm, 0.08 μm	10 $\mu g/L$–1000 $\mu g/L$, 21 days	IL-6, IL-8, IL-10, IL-1β, IFNPHI1, TNFα	*Daniorerio* placed in a culture water at 28 °C, 14/10 light and dark cycle. The zebrafish' intestinal microbial community and the intestinal tissue were analyzed	RT- qPCRs assay
Lu *et al.*, 2021 [45]	Microplastics and sphere	1–5 μm	300 μg and 20 μL saline every three days, 24 days	TNF-α, IgG1, IgE, IL-2, IL-4, IL-5	6–8 weeks old female BALB/c mice with a 12/12 light and dark cycle. The respiratory system was analyzed, in particular lungs in asthmatic and not asthmatic mice	ELISA
In vitro **and** *in vivo* **studies**						
Wang *et al.*, 2021 [34]	PS	2 μm	25, 50, 100, 200, 400, 800 $\mu g/mL$, 4, 8 weeks	ROS, Bad, Bcl2, LC3, MAPK, p38, ERK1/2, JNK, AKT-mTOR, IRE1α, ATF6, p-EIF2α/EIF2α, cPLA2, COX-1, p62	HK-2 cells, C57BL/6 mice. HK-2 cells were incubated at 37 °C with 5% CO_2. 6 weeks old male C57BL/6 placed in a room with 12 h light and dark cycle, 55% ± 10% relative humidity at 22 ± 2 °C. Kidney mice cells were analyzed.	Sulforhodamine B (SRB) assay, ELISA, Western Blot assay, Immunostaining Assay
Jin *et al.*, 2021 [39]	PS	0.5 μm, 4 μm and 10 μm	100 μL of PS-MP (10, 000 $\mu g/mL$), 28 days	TNF-α, IL-6, MCP-1, CXCL10, ZO-1	6 weeks old BALB/C Mice, GC-1 cells, Leydig cells, Sertoli cells. Testicular tissue was analyzed	qRT-PCR assay, ELISA, Western blotting

Notes: r, Pearson correlation coefficient; IL-6, Interleukin 6; IL-8, Interleukin 8; HO-1, Heme oxygenase-1; ROS, Reactive oxygen species; AAT, alpha-1 antitrypsin; ZO-1, Zonula occludens-1; TEER, trans-epithelial electrical resistance; TNF-α, tumor necrosis factor; IL-1α, Interleukin 1 α; MIP-1α, Macrophage Inflammatory Protein; IL-1β, Interleukin 1 β; IL-2, Interleukin 2; IL-10, Interleukin 10; TLR4, Tool - like receptor 4; AP-1, Activator protein 1; IRF5, Interferon Regulatory Factor 5; G-CSF, Granulocyte Colony-Stimulating Factor; IL-5, Interleukin 5; IL-9, Interleukin 9; IP-10, Interferon gamma-induced protein 10; RANTES, regulated on activation, normal T cell expressed and secreted; Nrf2, Nuclear factor erythroid 2-related factor 2; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; IFN-γ, Interferon γ; TG, liver triglyceride; PPAR-γ, Peroxisome proliferator-activated receptot – γ; MDA, Malondialdehyde; GPR 41, mammalian G protein receptors 41; GPR 43, mammalian G protein receptors 43; ERK 1-2, Extracellular signal-regulated kinases 1-2; MyD88, Myeloid differentiation primary response 88; CYP1A1, Cytochrome P450 Family 1 Subfamily A Member 1; BMP4, *Bone morphogenetic protein 4*; GATA, 4GATA-binding protein 4; NKX2.5, Homeobox protein Nkx-2.5; FGF8, Fibroblast growth factor 8; JAK, Janus kinase; CCL11, C-C motif chemokine 11; SOD, Superoxide dismutase; CAT, catalase; GRd, Glutathione reductase; GST, Glutathione-s-transferase; MPO, Myeloperoxidase; COX2, cyclooxygenase-2; GPx, Glutathione peroxidase; *COX1*, cyclooxygenase-1; DO, Dissolved Oxygen; D-Lac, D-lactate; INFPHI1, Interferon phi 1; IgG1, Immunoglobulin G1; IgE, Immunoglobulin E; IL-4, Interleukin 4; Th1, Type 1 T helper lymphocytes; Th17, Type 17 T helper lymphocytes; Bad, BCL2 Associated Agonist Of Cell Death; ER, Endoplasmic reticulum; LC3, Microtubule-associated proteins 1A/1B light chain 3B; MAPK, mitogen-activated protein kinase; p-38, mitogen-activated protein kinases 38; JNK, c-Jun N-terminal kinases; AKT, RAC (Rho family)-alpha serine/threonine-protein kinase; mTOR, mechanistic target of rapamycin; IRE1α, Inositol-requiring transmembrane kinase endoribonuclease-1α; ATF6, Activating transcription factor 6; p-EIF2α/EIF2α, Polyclonal Antibody for studying EIF2S1/eIF2-alpha (Ser52) phosphate; cPLA2, Cytosolic phospholipase A2; p62, ubiquitin-binding protein 62; MCP-1, Monocyte chemoattractan protein-1; CXCL10, Chemokine ligand 10; TJs, Tight junction proteins; HDF, Human Dermal Fibroblast; PBMCs, peripheral blood mononuclear cells; RBCs, Red blood cells; CACO 2 cells, Human Cancer Colon 2; HEPG2, Human hepatoma 2; THP-1, human monocytic *cell* line derived from an acute monocytic leukemia patient; HT29-MTX-E12, cells mature goblet cells using methotrexate; MDM, cellshuman blood monocyte- derived macrophages; MDDC, cells Human blood monocyte derived dendritic cells; HK-2, cells Human kidney proximal tubular epithelial cells.

(First–Author name and year of publications). For *in vitro* and *in vivo* studies, we created 10 different tables summarizing for specific outcomes (Interleukine, Tumor necrosis factors, Chemochine, Interferon, Transcriptor factors, Growth factors, Oxidoreductase, Proteins and Others).

2.3 Study Quality Appraisal

The methodological quality of the studies has been checked using the Toxicological data Reliability Assessment Tool (ToxR Tool) guidelines for reporting randomized clinical trials for *in vitro* and *in vivo* studies [26]. In particular, two researchers (E.P., M.P.) performed data selection, extraction and quality assessment independently. Any disagreement between the two researchers was resolved by consensus session with a third researcher (M.F.).

3. Results

The initial search retrieved a total of 125 studies, from which 55 were excluded because of duplicate records. A total of 70 studies were screened based on the title and abstract, from which 28 were excluded, resulting in 42 full-text studies that were considered potentially eligible for inclusion. A total of 23 studies were excluded because of carried out using a mixture of contaminants including MPs and lack of statistical data analysis. Finally, 19 studies (6 *in vitro*, 11 *in vivo* and 2 both *in vivo* and *in vitro*) were included in this review [27–45].

The full process of article collection, screening, and eligibility assessment is presented in Fig. 1.

The general/methodological information of the included *in vitro* and/or *in vivo* studies are described in Table 1 (Ref. [27–45]). In particular, 11 studies (5 *in vitro* studies [29,33,37,38,42], 4 *in vivo* [27,30,32,36] and 2 both [34,39]) investigated exposure to PS , 3 studies investigated PP exposure (2 *in vitro* [37,41,42] and 1 *in vivo* [41]), 3 studies investigated PE exposure (1 *in vitro* [42] and 2 *in vivo* [28,31]), 2 studies investigated exposure to MPs (2 *in vivo* studies [44,45]) instead of PVC exposure (1 study in seen [3]), polymers (1 *in vitro* study [43]), LDPE (1 *in vivo* study [35]), HDPE (1 *in vivo* study [40]), SFb and LFb (1 *in vivo* study [41]), sphere (1 i*n vivo* study [45]) and NPs (1 *in vivo* study [44]) was evaluated in a species-specific study.

Changes in levels of all investigated outcomes (Interleukines, Tumor necrosis factor, Chemokines, Interferons, Transcription factors, Growth factors, Oxydoreductase, Proteins and others) have been summarised in Tables 2–10.

Below we have summarized the main results for each individual study included in the review by outcomes specifying different type of plastic exposure and study design.

Table 2 (Ref. [27–32,36–39,41–45]) shows an increase in interleukins 6, 8 and 1β. In particular, after exposure to PS 6 studies (three *in vitro* studies [29,37,42], two *in vivo* studies [27,32] and one both *in vivo* and *in vitro* [39]) showed an increase in IL-6; four studies showed an increase

in IL-β1 (one *in vitro* studies [37] and three *in vivo* studies [27,30,36]) and two studies showed an increase in IL-8 (one *in vitro* study [29] and one *in vivo* study [36]). Only one *in vivo* study [45], which investigated MPs without specifying their type, showed an increase in IL-4 and IL-5. At the same time, only one *in vivo* study [31] out of three investigated exposures to PE detecting an increase in IL-8; two *in vivo* studies [28,31] out of three showed an increase in IL-6 and one *in vivo* study [31] out of three showed an increase in IL-1β. The remaining studies, that investigated the other types of micro/nanoplastics (PP, NPs, polymers, PS-NH2, PVC) or fibers (LFb), highlighted an increase in IL-1β while no significant increase was detected for other cytokines. Only one *in vivo* study [28], concerning exposure to PE, showed a decrease in IL-2 and IL-5. Instead, only one *in vitro* study [38] with exposure to PVC reported a decrease in IL-8 (Table 2).

Table 3 (Ref. [27,30,36–39,42–45]) shows the trend of TNF-a, the only member of the TNF category that have been taken into consideration in the studies. In particular, exposure to PP (evaluated in two *in vitro* studies [37,42]) did not show significant differences. Whereas 7 studies (three *in vitro* studies [37,38,42], three *in vivo* studies [27,30,36], one both [39]) assessed PS exposure, noting an increase in TNF-α in only four studies [27,30,39,42]. In addition, exposure to PVC (one *in vitro* study [38]), PS-NH2 (one *in vitro* study [38]) and PE (one *in vitro* study [42]) did not show significant differences, whereas exposure to polymers, (one *in vitro* study [43]) NPs (one *in vivo* study [44]) and MPs (two *in vivo* studies [44,45]) showed an increase. Finally, no studies reported decreases in TNF-α values (Table 3).

Table 4 (Ref. [28,32,37,39]) shows the trend of different chemokines following exposure to PP (one study *in vitro* [37]), PS (three studies respectively one *in vitro* [37], one study *in vivo* [32] and one both [39] and PE (one study *in vivo* [28]). Only one study [37] reported an increase in MIP-1β following exposure to both PP and PS. Whereas only exposure to PS (two studies [32,39]) showed an increase in both CCL11 and CXCL10. Regarding the exposure to PE only one study [28] evaluated both RANTES and IP-10 detecting an increase in the first cytokine and a decrease in the second (Table 4).

In Table 5 (Ref. [30,44,45]) we have reported the trend of INF levels following exposure to MPs (two *in vivo* studies [44,45]), NPs (one *in vivo* study [44]) and PS (one *in vivo* study [30]). Both exposure to MPs and NPs did not change the trend of IFN-y [45] and INFPHI1 [44] levels. Finally, only exposure to PS was associated with an increase in IFN-y [30] (Table 5).

Table 6 (Ref. [27–32,34]) summarizes the different transcription factors investigated in the studies included in the review that considered PS exposure (one *in vitro* study [29], three *in vivo* studies [27,30,32] and one both [34]) and PE (two *in vivo* studies [28,31]). Only ERK1 [31,34],

Table 2. Interleukine outcomes from included studies.

Author, year	Particles type	Outcomes								
		IL-2	IL-4	IL-5	IL-6	IL-8	IL-9	IL-10	IL-1α	IL-1β
In vitro studies										
Dong *et al.*, 2020 [29]	PS				↑*	↑*				
Jeon *et al.*, 2021 [37]	PP				↑*a	≏				↑*b
	PS				↑*a	≏				↑*b
Busch *et al.*, 2021 [38]	PVC				≏	↓e				↑e
	PS-NH2				≏	≏				↑*
	PS				≏	≏				≏
Hwang *et al.*, 2020 [42]	PS	≏			↑*			≏		
	PP	≏			≏			≏		
	PE	≏			≏			≏		
Lehner *et al.*, 2020 [43]	Polymers					↑*				↑*
In vivo studies										
Hou *et al.*, 2021 [27]	PS				↑*					↑*
Li *et al.*, 2020 [28]	PE	↓**		↓**	↑*		↑*		↑**	
Zheng *et al.*, 2021 [30]	PS									↑*
Sun *et al.*, 2021 [31]	PE				↑	↑**		↑***		↑*
Chen *et al.*, 2020 [32]	PS				↑*					
Zhang *et al.*, 2021 [36]	PS					↑*c				↑*c
Zhao *et al.*, 2021 [41]	PP									≏
	SFb									≏
	LFb									↑*
Xie *et al*, 2021 [44]	MPs				≏	≏		≏		≏
	NPs				≏	↑**		↑*		↑***d
Lu *et al.*, 2021 [45]	MPs		↑***	↑**				≏		
In vitro and *in vivo* study										
Jin *et al.*, 2021 [39]	PS				↑*					
TOTAL		1 ↓ 3 ≏ 17 N.A.	1 ↑ 18 N.A.	1 ↑ 1 ↓ 17 N.A.	9 ↑ 7 ≏ 9 N.A.	5 ↑ 1 ≏ 13 N.A.	1 ↑ 18 N.A.	2 ↑ 5 ≏ 15 N.A.	1 ↑ 18 N.A.	11 ↑ 4 ≏ 10 N.A.

We escluded Visalli *et al.*, 2021 [33], Capó *et al.*, 2021 [35], von Moos *et al.*, 2012 [40], Wang *et al.*, 2021 [34], from Table 2 due to none of these outcomes studied.

Notes: IL-2, Interleukin 2; IL-4, Interleukin 4; IL-5, Interleukin 5; IL-6, Interleukin 6; IL-8, Interleukin 8; IL-9, Interleukin 9; IL-10, Interleukin 10; IL-1α, Interleukin 1α; IL-1β, Interleukin 1β; PS, Polystyrene; PP, Polypropylene; PVC, Polyvinylchloride; SFb, Short microplasticfibers; LFb, Long microplastic-fibers; MPs, Microplastics; NPs, Nanoplastics.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

≏ , about equal; N.A., Not applicable.

a, only for HepG2 cells statistical significance; b, only for THP-1 cells statistical significance; c, only for 2 and 200 um; d, only for 1000 μg/L NPs group; e, triple culture.

TLR 4 [28,31], NFKb [31,32] and Nrf2-HO1 [27,29] were investigated in two studies whereas for all other transcription factors we found one study only (Table 6). In particular, exposure to PS showed an increase in IRE1a, MAPK, ERK 1-2, p-INK [34], JAK [32], and PPAR-γ [30]. There were no changes in ATF6 [34] and NFKb [32], conversely one study reported decreased AKT m-TOR [34]. On the other hand, two studies obtained discordant results regarding Nrf2-HO1 [27,29]. Exposure to PE reported conflicting results for TLR4 [28,31], an increase for AP-1 and IRF-5 [28] and a decrease for ERK1 and NF-Kb [31] (Table 6).

Table 7 (Ref. [28,32]) shows the trend of growth factors after exposure to PE (one study *in vivo* [28]) and PS (one study *in vivo* [32]). There was an increase in GCSF [28] after exposure to PE whereas exposure to PS did not modify the trend of FGF8 [32] (Table 7).

Table 8 (Ref. [32,35,36,41]) shows the enzymes with antioxidant action investigated by the studies included in the review. In general, exposure to PS (two *in vivo* studies [32,36], PP (one *in vivo* study [41], LDPE (one *in vivo* study [35] and SFB, LFB (one *in vivo* study [41] showed an increase in all the enzymes investigated. In particular, after exposure to PS the values of CAT [36], GPx [36] were increased, whereas the trend of SOD (two studies [32,36]) was detected in increase in only one [36] of the two studies. Finally, exposure to LDPE [35] reported an increase in CAT, GRd and GST and no change in values for GPx and SOD, whereas the study by Zhao which investigated the exposure to PP, SFb and LFb showed an increase in SOD only following exposure to LFB [41] (Table 8).

Table 9 (Ref. [29,31,32,34–36,39,41]) shows the proteins and enzymes investigated following exposure to PS (one *in vitro* study [29], two *in vivo* studies [32,36], two both [34,39]), PE (one study *in vivo* [31]), LDPE (one *in vivo* study [35]), PP, SFB and LFB (one *in vivo* study [41]). As for the exposure to PS, an increase in BMP [32], COX1-2 [36], D-lac, Bad, LC3, p38, cPLA2 [35], MCP-1 [39] was highlighted, conversely no differences were found in CIP1A1, NRX2.5 [32] and p62 [34]. In addition, a decrease in AAT [29] and ZO-1 [29,39] levels was noted. Exposure to PE did not change the performance of GPR41, GPR43 and MyD88 [31]. Finally, an increase in MPO [35] was highlighted for exposure to LDPE, whereas following exposure to PP, SFb and LFb there was a decrease in D-Lac [41] (Table 9).

In Table 10 (Ref. [29,30,33–35,37,40,41,45]) we have reported the trend of the remaining outcomes, which we named "others" (ROS, TG, MDA, IgG1, IgE, P-

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Table 3. Tumor necrosis factor outcome from included studies.

Author, year	Particles type	Outcome (TNF-α)
In vitro **studies**		
Jeon *et al*., 2021 [37]	PP	≃
	PS	≃
	PVC	≃
Busch *et al*., 2021 [38]	PS-NH2	≃
	PS	≃
	PS	↑*
Hwang *et al*., 2020 [42]	PP	≃
	PE	≃
Lehner *et al*., 2020 [43]	polymers	↑***
In vivo **studies**		
Hou *et al*., 2021 [27]	PS	↑*[a]
Zheng *et al*., 2021 [30]	PS	↑*
Zhang *et al*., 2021 [36]	PS	≃
Xie *et al*., 2021 [44]	MPs	≃
	NPs	↑***[b]
Lu *et al*., 2021 [45]	MPs	↑*[c]
In vitro **and** *in vivo* **study**		
Jin *et al*., 2021 [39]	PS	↑**
TOTAL		7 ↑ 9 ≃ 9 N.A.

We escluded Dong *et al*., 2020 [29], Visalli *et al*., 2021 [33], Li *et al*., 2020 [28], Sun *et al*., 2021 [31], Chen *et al*., 2020 [32], Capó *et al*., 2021 [35], von Moos *et al*., 2012 [40], Zhao *et al*., 2021 [41], Wang *et al*., 2021 [34], from Table 3 due to none of these outcomes studied.
Notes: TNF-α, Tumor necrosis factor; PP, Polypropylene; PS, Polystyrene; PVC, Polyvinyl chloride; PE, Polyethylene; MPs, Microplastics; NPs, Nanoplastics.
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.
≃ , about equal; N.A., Not applicable.
a, only at high dose; b, only for 1000 μg/L; c, only for group treated with MP.

Table 4. Chemokines outcomes from included studies.

Author, year	Particles type	MIP-1β	IP-10	RANTES	CCL11	CXCL10
				Outcomes		
In vitro **study**						
Jeon *et al*., 2021 [37]	PP	↑*				
	PS	↑*				
In vivo **studies**						
Li *et al*., 2020 [28]	PE		↓**	↑*		
Chen *et al*., 2020 [32]	PS				↑	
In vitro **and** *in vivo* **study**						
Jin *et al*., 2021 [39]	PS					↑**
TOTAL		1 ↑ 18 N.A.	1 ↓ 18 N.A.	1 ↑ 18 N.A.	1 ↑ 18 N.A.	1 ↑ 18 N.A.

We escluded Dong *et al*., 2020 [29], Visalli *et al*., 2021 [33], Busch *et al*., 2021 [38], Hwang *et al*., 2020 [42], Lehner *et al*., 2020 [43], Hou *et al*., 2021 [27], Zheng *et al*., 2021 [30], Sun *et al*., 2021 [31], Capó *et al*., 2021 [35], Zhang *et al*., 2021 [36], von Moos *et al*., 2012 [40], Zhao *et al*., 2021 [41], Xie *et al*., 2021 [44], Lu *et al*., 2021 [45], Wang *et al*., 2021 [34] from Table 4 due to none of these outcomes studied.
Notes: MIP-1β, Macrophage Inflammatory Protein; IP-10, Interferon gamma-induced protein 10; RANTES, regulated on activation, normal T cell expressed and secreted; CCL11, C-C motif chemokine 11; CXCL10, Chemokine ligand10; PP, Polypropylene; PS, Polystyrene.
* $p < 0.05$; ** $p < 0.01$.
N.A., Not applicable.

EIF2α/EIF2α), following exposure to PS (three *in vitro* studies [29,33,37], one *in vivo* study [30] and one both [34]), PP (one *in vitro* study [37], one *in vivo* study [41]), LDPE (one *in vivo* study [35]), HDPE (one *in vivo* study [40]), SFb and LFb (one *in vivo* study [41]) and MPs (one *in vivo* study [45]). Six studies evaluated ROS production

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Table 5. Interferon outcomes from included studies.

Author, year	Particles type	IFN-γ	IFNPHI1
		Outcomes	
In vivo **studies**			
Zheng *et al*., 2021 [30]	PS	↑*	
Xie *et al*., 2021 [44]	MPs		≃
	NPs		≃
Lu *et al*., 2021 [45]	MPs	≃	
TOTAL		1 ↑ 1 ≃ 17 N.A.	1 ≃ 18 N.A.

We escluded Dong *et al*., 2020 [29], Visalli *et al*., 2021 [33], Jeon *et al*., 2021 [37], Busch *et al*., 2021 [38], Hwang *et al*., 2020 [42], Lehner *et al*., 2020 [43], Hou *et al*., 2021 [27], Li *et al*., 2020 [28], Sun *et al*., 2021 [31], Chen *et al*., 2020 [32], Capó *et al*., 2021 [35], Zhang *et al*., 2021 [36], von Moos *et al*., 2012 [40], Zhao *et al*., 2021 [41], Wang *et al*., 2021 [34], Jin *et al*., 2021 [39], fromtable 5 due to none of these outcomes studied.
Notes: IFN-γ, Interferon–gamma; IFNPHI1, Interferon-phi 1; PS, Polystyrene; MPs, Microplastics; NPs, Nanoplastics.
* $p < 0.05$.
N.A., Not applicable.

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and all found increased ROS regardless of the exposure type [29,33,34,37,40,41]. Exposure to PS was associated with higher levels of TG and MDA [30], conversely no changes were detected regarding P- EIF2α/EIF2α [34]. Furthermore, exposure to LDPE did not show changes in MDA levels [35]. Finally, only one study showed increased levels of IgG1 and IgE [45] (Table 10).

Risk of Bias

The results of the quality assessment of the studies are reported in Figs. 2,3 and **Supplementary Tables 1, 2**. In particular, only two *in vitro* studies didn't report information on the source/origin of the test system [29,39]. Only one author did not report the necessary information on test system properties, and on conditions of cultivation and maintenance. Only one study reported the number of replicates [34].
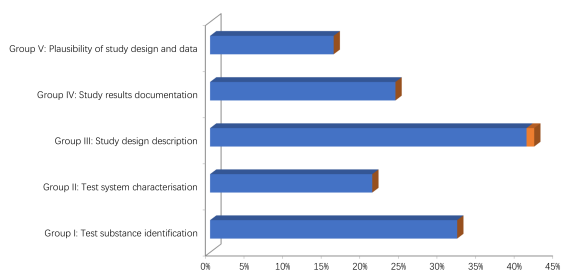

Fig. 2. Methodological quality assessment of *in vitro* studies.

Concerning in the vivo studies, only one study didn't report information on the source/origin of the test system [41]. Four studies did not give the sex of the test organism [32,35,40,44]. One study did not give age or body weight of the test organisms at the start of the study [40]. One study did not give information on the housing or feeding conditions in case of repeated dose toxicity studies [39].

Table 6. Transcription factors outcomes from included studies.

Author, year	Particles type	IRE1α	ATF6	JAK	MAPK	ERK2	TLR4	AP-1	IRF-5	NFKB	PPAR-γ	ERK1	GATA4	p-JNK	AKT-MTOR	Nrf2-HO1
In vitro study																
Dong et al., 2020 [29]	PS															↑*
In vivo studies																
Hou et al., 2021 [27]	PS															↓*
Li et al., 2020 [28]	PE						↑*	↑**	↑*							
Zheng et al., 2021 [30]	PS										↑*					
Sun et al., 2021 [31]	PE						≂			↓***		↓***				
Chen et al., 2020 [32]	PS			↑						≂			↓			
In vitro and *in vivo* study																
Wang et al., 2021 [34]	PS	↑	≂		↑	↑						↑		↑	↓	
TOTAL		1↑ 18 N.A.	1≂ 18 N.A.	1↑ 18 N.A.	1↑ 18 N.A.	1↑ 18 N.A.	1↑ 1↓ 17 N.A.	1↑ 18 N.A.	1↑ 18 N.A.	1↓ 1≂ 17 N.A.	1↑ 18 N.A.	1↑ 1↓ 17 N.A.	1↓ 18 N.A.	1↑ 18 N.A.	1↓ 18 N.A.	1↑ 1↓ 17 N.A.

We escluded Visalli et al., 2021 [33], Jeon et al., 2021 [37], Busch et al., 2021 [38], Hwang et al., 2020 [42], Lehner et al., 2020 [43], Capó et al., 2021 [35], Zhang et al., 2021 [36], von Moos et al., 2012 [40], Zhao et al., 2021 [41], Xie et al., 2021 [44], Lu et al., 2021 [45], Jin et al., 2021 [39], from table 6 due to none of these outcomes studied.
Notes: IRE1α, Inositol-requiring transmembrane kinase endoribonuclease-1α; ATF6, Activating transcription factor 6; JAK, Janus kinase; MAPK, mitogen-activated protein kinase; ERK 1-2, Extracellular signal-regulated kinases 1-2; TLR4, Tool - like receptor 4; AP-1, Activator protein 1; IRF5, Interferon Regulatory Factor 5; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; PPAR-γ, Peroxisome proliferator-activated receptorγ; GATA4, GATA-binding protein 4; p-JNK, p-Jun N-terminal kinases; AKT, RAC(Rho family)-alpha serine/threonine-protein kinase; mTOR, mechanistic target of rapamycin; Nrf2-HO1, Nuclear factor erythroid 2-related factor 2 linked to Hemeoxygenase 1; PS, Polystyrene; PE, Polyethylene.
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.
N.A., Not applicable.

Table 7. Growth factors outcomes from included studies.

Author, year	Particles type	GCSF	FGF8
In vivo studies			
Li et al., 2020 [27]	PE	↑**	
Chen et al., 2020 [31]	PS		≂
TOTAL		1↑ 18 N.A.	1≂ 18 N.A.

We escluded Dong et al., 2020 [29], Visalli et al., 2021 [33], Jeon et al., 2021 [37], Busch et al., 2021 [38], Hwang et al., 2020 [42], Lehner et al., 2020 [43], Hou et al., 2021 [27], Zheng et al., 2021 [30], Sun et al., 2021 [31], Capó et al., 2021 [35], Zhang et al., 2021 [36], von Moos et al., 2012 [40], Zhao et al., 2021 [41], Xie et al., 2021 [44], Lu et al., 2021 [45], Wang et al., 2021 [34], Jin et al., 2021 [39] from table 7 due to none of these outcomes studied.
Notes: GCSF, Granulocyte - Colony Stimulating Factor; FGF8, Fibroblast growth factor 8; PE, polyethylene; PS, Polystyrene.
** $p < 0.01$.
N.A., Notapplicable.

Table 8. Oxydoreductase outcomes from included studies.

Author, year	Particles type	SOD	CAT	GRD	GST	GPX
In vivo studies						
Chen et al., 2020 [32]	PS	≂				
Capó et al., 2021 [35]	LDPE	≂	↑	↑*	↑	≂
Zhang et al., 2021 [36]	PS	↑**	↑**			↑**
Zhao et al., 2021 [41]	PP	≂				
	SFb	≂				
	LFb	↑**				
TOTAL		2↑ 2≂ 15 N.A.	2↑ 17 N.A.	1↑ 18 N.A.	1↑ 18 N.A.	1↑ 1≂ 17 N.A.

We escluded Dong et al., 2020 [29], Visalli et al., 2021 [33], Jeon et al., 2021 [37], Busch et al., 2021 [38], Hwang et al., 2020 [42], Lehner et al., 2020 [43], Hou et al., 2021 [27], Li et al., 2020 [28], Zheng et al., 2021 [30], Sun et al., 2021 [31], von Moos et al., 2012 [40], Xie et al., 2021 [44], Lu et al., 2021 [45], Wang et al., 2021 [34], Jin et al., 2021 [39], from table 8 due to none of these outcomes studied.
Notes: SOD, Superoxide dismutase; CAT, catalase; GRd, Glutathione reductase; GST, Glutathione-s-transferase; GPx, Glutathione peroxidase; PS, Polystyrene; LDPE, Low density Polyethylene; PP, Polyethylene; SFb, Shortmicroplastic fibers; LFb, Long microplastic fibers.
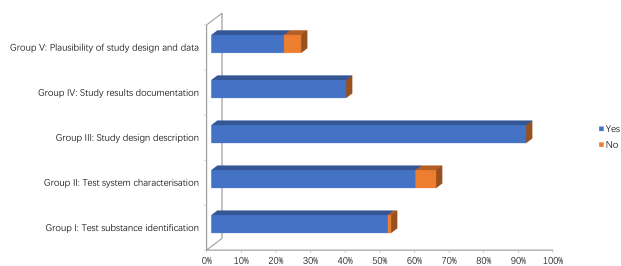* $p < 0.05$; ** $p < 0.01$.
N.A., Not applicable.

Fig. 3. Methodological quality assessment of *in vivo* studies.

4. Discussion

The *in vitro* [29,37,38,42,43] and *in vivo* [27,28,36] studies included in this review seem to confirm an association between the increase in pro-inflammatory interleukins Il-6, IL-8 and IL-1b and the exposure to microplastics of different types, sizes, exposure times and exposed species, whereas the interpretation of the results, relating to the other interleukins investigated, requires more caution because there are only few heterogeneous studies (42 *in vitro*, 28 and 45 *in vivo*) in literature to date. In particular, it is already known that these pro-inflammatory interleukins take part in the acute inflammatory response [46], whereas IL-6 acts as an anti-inflammatory myokine too [47]. Furthermore, the results of the studies seem to confirm that the persistence of acute inflammation can become chronic up to result in a further systemic inflammatory action, inducing COPD (Chronic Obstructive Pulmonary Disease), asthma [48] and Inflammatory Bowel Diseases (IBD) [31,38]. This process could be caused by the reduction of TEER and by

Table 9. Protein outcomes from included studies.

Author, year	Particles type	AAT	GPR41 / GPR43	MyD88	CYP1A1	BMP	NKX2.5	MPO	COX1 / COX2	D-lac	Bad	LC3	p38 / p62	cPLA2	MCP-1	ZO-1
In vitro study																
Dong *et al.*, 2020 [29]	PS	↓*														↓*
In vivo studies																
Sun *et al.*, 2021 [31]	PE		≈ / ≈	≈												
Chen *et al.*, 2020 [32]	PS				≈	↑*	≈									
Capó *et al.*, 2021 [35]	LDPE							↑								
Zhang *et al.*, 2021 [36]	PS								↑^a							
Zhao *et al.*, 2021 [41]	PP / SFb / LFb									↓*						
In vivo and in vitro studies																
Wang *et al.*, 2021 [34]	PS									↑*	↑	↑*	↑ / ≈	↑*		
Jin *et al.*, 2021 [39]	PS														↑*	↓
TOTAL		1↓ 18 N.A.	2≈ 36 N.A.	1≈ 18 N.A.	1≈ 18 N.A.	1↑ 18 N.A.	1≈ 18 N.A.	1↑ 18 N.A.	1↑ 18 N.A.	1↑ 1↓ 17 N.A.	1↑ 18 N.A.	1↑ 18 N.A.	1↑ 1≈ 36 N.A.	1↑ 18 N.A.	1↑ 18 N.A.	2↓ 17 N.A.

We escluded Visalli *et al.*, 2021 [33], Jeon *et al.*, 2021 [37], Busch *et al.*, 2021 [38], Hwang *et al.*, 2020 [42], Lehner *et al.*, 2020 [43], Hou *et al.*, 2021 [27], Li *et al.*, 2020 [28], Zheng *et al.*, 2021 [30], von Moos *et al.*, 2012 [40], Xie *et al.*, 2021 [44], Lu *et al.*, 2021 [45], from table 9 due to none of these outcomes studied.
Notes: AAT, alpha-1 antitrypsin; ZO-1, Zonula occludens-1; GPR 41, mammalian G protein receptors 41; GPR 43, mammalian G protein receptors 43; MyD88, Myeloid differentiation primary response 88; CYP1A1, Cytochrome P450 Family 1 Subfamily A Member 1; BMP4, Bone morphogenetic protein 4; NKX2.5, Homeobox protein Nkx-2.5; MPO, Myeloperoxidase; COX2, Cyclooxygenase-2; COX1, cyclooxygenase-1; D-Lac, D-lactate; Bad, BCL2 Associated Agonist Of Cell Death; LC3, Microtubule-associated proteins 1A/1B light chain 3B; p-38, mitogen-activated *protein* kinases 38; cPLA2, Cytosolic phospholipase A2; p62, ubiquitin-binding protein 62; MCP-1, Monocyte chemoattractan protein-1; PS, Polystyrene; PE, Polyethylene; LDPE, Low-density Polyethylene; SFb, Short microplastic fibers; LFb, Long microplastic fibers.
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.
N.A., Not applicable.
a, only for COX2.

Table 10. Other outcomes from included studies.

Author, year	Particles type	ROS	TG	MDA	IgG1	IgE	P-EIF2α / EIF2α
In vitro studies							
Dong *et al.*, 2020 [29]	PS	↑*^a					
Visalli *et al.*, 2021 [33]	PS	↑*					
Jeon *et al.*, 2021 [37]	PP	↑*					
	PS	↑*					
In vivo studies							
Zheng *et al.*, 2021 [30]	PS		↑*	↑*			
Capó *et al.*, 2021 [35]	LDPE			≈			
von Moos *et al.*, 2012 [40]	HDPE	↑*					
Zhao *et al.*, 2021 [41]	PP / SFb / LFb	↑** / ↑**					
Lu *et al.*, 2021 [45]	MPs				↑****	↑***	
In vivo and in vitro study							
Wang *et al.*, 2021 [34]	PS	↑***					≈
TOTAL		8↑ 11 N.A	1↑ 18 N.A	1↑ 1≈ 17 N.A	1↑ 18 N.A	1↑ 18 N.A.	1≈ 18 N.A.

We escluded Busch *et al.*, 2021 [38], Hwang *et al.*, 2020 [42], Lehner *et al.*, 2020 [43], Hou *et al.*, 2021 [27], Li *et al.*, 2020 [28], Sun *et al.*, 2021 [31], Chen *et al.*, 2020 [32], Zhang *et al.*, 2021 [36], Xie *et al.*, 2021 [44], Jin *et al.*, 2021 [39] from table 10 due to none of these outcomes studied.
Notes: ROS, Reactive oxygen species; TG, liver triglyceride; MDA, Malondialdehyde; IgG1, Immunoglobulin G1; IgE, Immunoglobulin E; p-EIF2α/EIF2α, Polyclonal Antibody for studying EIF2S1/eIF2-alpha (Ser52) phosphate; PS, Polystyrene; PP, Polypropylene; LDPE, Low-density Polyethylene; HDPE, High-density Polyethylene; SFb, Short microplastic fibers; LFb, Long microplastic fibers; MPs, Microplastics.
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.
N.A., Not applicable.
a, only for 1000 ug/cm^2 concentration.

the expression of the ZO-1 protein, this leads to a loss of epithelial integrity of the barrier cells [29,39,49]. This reduction in the integrity of the barrier cells has been confirmed for microplastics of 3 μm size, through vesicles of the plasma membrane the microplastics of 60 nm size can be internalized [50].

The proinflammatory action of TNF-α following exposure to PS, in a size and concentration dependent manner, seems to be confirmed both *in vitro* [39,42] and *in vivo* studies [27,30,42]. It is already considered as an immune mediator for cell adhesion, migration, angiogenesis and apoptosis; therefore, its up regulation is a potential indicator of an

immune response and inflammation [51]. However, it must be pointed out that the studies in the literature have only investigated TNF-α and always in a heterogeneous way in terms of type of plastic, size, species exposed, dose and exposure time that do not allow us a generalization of the results [27,30,36–39,42–45].

Levels related to the chemokine MIP1β, both following exposure to PP and PS, would appear to increase but it is related to a single *in vitro* study [37], no one *in vivo* study was found in literature. This result would seem interesting because it is one of the major factors produced by macrophages and monocytes after exposure to bacterial toxins or proinflammatory cytokines [52,53]. On the other hand, it is not possible to hypothesize the same result for the other chemokines investigated as they have been included in heterogeneous studies for type of plastic, size, species exposed, dose and exposure time [28,32,37,39].

A few *in vivo* studies quantifying the levels of IFNPHI-1, IFN-γ and growth factors (GCSF and FGF8) did not allow us to draw conclusions also because they tested exposure to MPs without specifying the type. Only 1 *in vivo* study claims to have tested the PS [30]. The study of IFNPHI-1 and IFN-γ, also considered as cytokines, would be of interest due to their crucial role both in innate and acquired immunity but also as activators of macrophages which is involved in the inflammatory process [54]. In addition, GCSF is a glycoprotein that stimulates the bone marrow to produce granulocytes responsible for the acute phase of inflammation whereas FGF8 is responsible for fibroblasts growth that can cause chronic inflammation [55].

The results concerning transcription factors, investigated both through *in vitro* [29,34] and *in vivo* studies [28,30,32,34] do not allow us to reach a conclusion because, like other studies already mentioned, they are very heterogeneous. In particular, the results of NFκB, Erk1, PPAR-γ, NRF2-HO1, which are strongly involved in the inflammatory pathway, are contradictory [27,29,31,32,34].The results concerning different proteins (AAT, GPR41-43, MyD88, CYP1A1, BMP, NKX2.5, MPO, COX1 and 2, D-LAC, BAD, LC3, p38, p62, cPLA2, MCP1) [29,31,32,34–36,39]and the oxidoreductases (SOD, CAT, GRD, GST, GPX) investigated by the other studies included in the review are heterogeneous and contradictory too [32,35,37,41].

Finally, as regards the other outcomes investigated, not classifiable in the aforementioned categories, a potential association emerged between exposure to microplastics of different type, size and exposure time and ROS both *in vitro* [29,33,37] and *in vivo* studies [30,35,40,41,45]. Although ROS are known to cause chronic oxidative stress including inflammation, alteration of permeability and histopathological damage [56–58], it would underline that their formation may also depend on the surface of microplastics. This is supported by the fact that the experiment carried out with NAC-coated (N-acetylcysteine) nanoparticles reduce toxi-

city and oxidants, subsequently reducing the toxic effect on THP-1 macrophages [37].

The studies included in the review have various limitations regarding, for example, the use of different animal and cell models, size and type of particles investigated, doses and exposure time or conditions, quantified outcomes and tests used for their quantification. Moreover, it should be noted that most of the authors summarize the results through graphs in which is difficult to obtain numerical data comparable to each other and to estimate the quality. Furthermore, none of the *in vivo* studies included in the review exposed male and female mice to microplastics at the same time, this may be a limitation as the influence of sex has already been demonstrated for pollutants exposure as for example to metals [59,60]. Another limitation of the studies concerns the lack of studies on NPs, in fact, only 2 studies included in this review investigated NPs. Moreover, only one study took into consideration the limits related to methodology [43]. In particular, it has been discussed the difficulties of the particles to translocate across the epithelium to the basolateral side in the membrane of 12-well insert, whereas only one study focused on methods to detect MPs in tissues are not appropriate [39]. Surprisingly six authors showed no limitation in their studies [30,32,34,37,41,44].

Finally, we have included only English language articles in this review, and it was not possible to compare the results with those of other reviews as to the authors' knowledge there are no reviews like this one in the literature to date. Although the limitations, the results of this review may be useful for the organization of future studies. In particular, they provide information on potential outcomes that could help confirm the hypothesis of an association between exposure to various types of microplastics and the inflammatory process.

5. Conclusions

In conclusion, this review seems to support the association between the MPs exposure and the inflammation response both *in vivo* and *in vitro*. Conversely greater caution is needed regarding the role of NPs due to the very small number of studies in literature. Additional high-quality studies are warranted to confirm these results, especially the research should be focused on NPs being lacking literature.

Author Contributions

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest. GOC is serving as one of the Editorial Board members of this journal. We declare that GOC had no involvement in the peer review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to ESH.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/j.fbl2710287.

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