

# Chapter 12

## Cellular and Animal Toxicities of Micro- and Nanoplastics



Manikantha D, C. D. S. L. N. Tulasi, and Kalyani Chepuri

**Abstract** Plastic-based items are abundantly found on the globe because of their immense utility in daily lives. The poor biodegradability of plastics, particularly micro- and nanoplastics, has recently sparked environmental concerns around the world. These anthropogenic pollutants are either generated, particularly in the tiny size range, for diverse commercial applications or result from the environmental fragmentation of macropolymers. Micro- and nanoplastics are now found in large quantities in the oceans, freshwater bodies, and on land, as well as in food. Micro- and nanoplastics' biological effects on aquatic creatures are extensively known, but their effects on human systems have not been thoroughly examined. The potential pathways of exposure to micro- and nanoplastics, the biological consequences of these particles in human cells, factors influencing toxicity, and the likely mechanisms of cytotoxicity are all discussed in this chapter. In general, cellular toxicity appears to be induced by oxidative stress, membrane damage, immunological response, and genotoxicity in micro/nanoplastics due to their tiny size, positive charge, high dose, and inclusion of hazardous chemicals or contaminants. A thorough understanding of these chemicals' cellular destiny and toxicity may aid in extrapolating dangers to mammals.

**Keywords** Microplastics · Environmental concerns · Biological effects · Mechanism of toxicity

### 12.1 Introduction

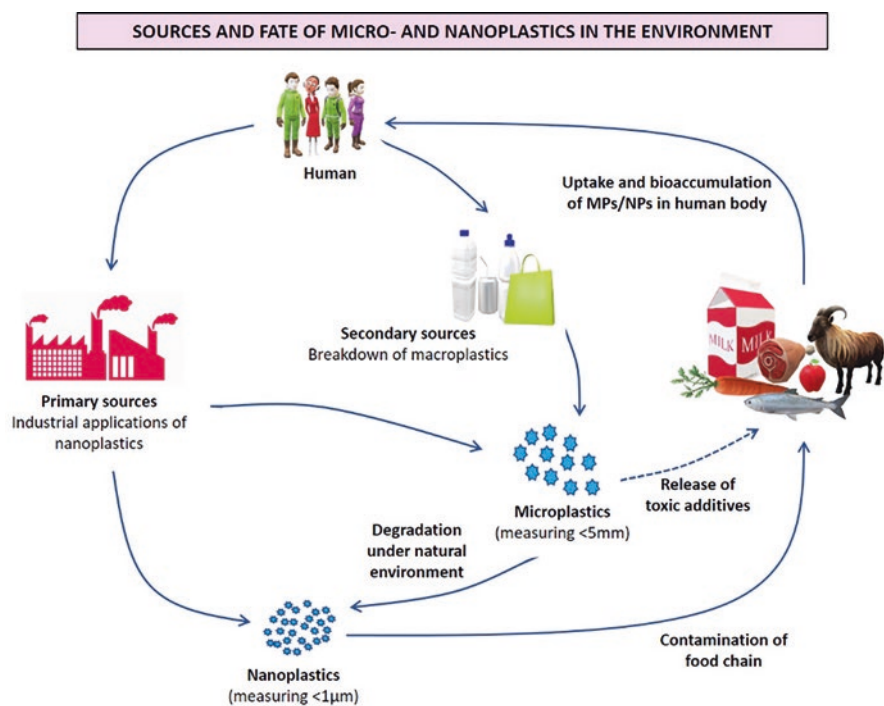
In our daily lives, plastic and plastic-based goods are extremely useful. From 15 million tonnes in 1964 to 359 million tonnes in 2018, global plastic manufacturing surged by around 24 times (Vidal, 2020; World Economic Forum, 2016). Plastic is now mostly used in packaging (26% of total Vidal, 2020; World Economic Forum,

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M. D · C. D. S. L. N. Tulasi · K. Chepuri (✉)  
Centre for Biotechnology, Jawaharlal Nehru Technological University Hyderabad, Institute of  
Science & Technology, Hyderabad, Telangana, India

2016 production), but it is also used in other industries such as electronics, construction, transportation, healthcare, and agriculture. The poor biodegradability of these polymers, on the other hand, poses a significant environmental risk. Every year, an estimated eight million tonnes of plastic waste enters the oceans, with 269,000 tonnes of plastic floating on the surface (National Oceanic and Atmospheric Administration, 2019). Plastics can break down into microplastics (0.1 m–5 mm diameter) or nanoplastics (0.1 m diameter) (NJDEP-Science Advisory Board, 2015), over time due to microbial degradation, extended ultraviolet radiation exposure, or physical wear. Microplastics (MPs)/Nanoplastics (NPs) are also produced for use in air blasting technologies, cleansers, cosmetics, medicine delivery formulations, paints, and toothpaste, adding to the MPs/NPs pool already present in the environment (Cole et al., 2011; Sharma & Chatterjee, 2017). Clothing, cigarette filters, automobile tires, and fishing equipment are also potential sources (Toussaint et al., 2019) (Fig. 12.1).

Plastics in the environment interact with terrestrial and marine biota, causing considerable worry about severe ecological consequences. Plastics consumed by



**Fig. 12.1** Sources and fate of micro and nanoplastics in the environment. Consumers and industries produce MPs/NPs from primary and secondary sources. Degradation of macroplastic materials that dissolve into micron-sized particles into nanoplastics. MPs/NPs found in both the aquatic and terrestrial environment, eventually entering the food chain and water supplies, resulting in their uptake and bioaccumulation in the human body

organisms can bioaccumulate and make their way up the food chain to humans via trophic transfer (Carbery et al., 2018; Chae et al., 2018; Nelms et al., 2018). In addition, individuals can be exposed to plastic through the eating of plastic-contaminated food or the inhalation of plastic-polluted air (Prata et al., 2020). Seafood (fish, shrimp, mussels), home products (sea salt, honey, sugar, plastic tea bags), tap water, bottled water, beer, construction sites, factories, and agriculture have all been found to contain micronized plastics (Barbosa et al., 2020; de Souza Machado et al., 2018; EFSA Panel on Contaminants in the Food Chain, 2016; Hesler et al., 2019; Karami et al., 2017; Mason et al., 2018). The body's excretory system is estimated to discharge >90% of micro-and nanoplastics in feces once consumed (Smith et al., 2018). The human stool has been found to contain 50–500 m sized PP and PET microplastics (20 particles/10 g stool) (Schwabl et al., 2019).

Due to ethical considerations, no meta-analysis clinical trial has been/can be undertaken to evaluate health risks in humans except from risk assessment data extracted from *in vivo* experiments (Yang et al., 2019a, b). As a result, the health effects of MPs/NPs on humans are unknown. It is unknown whether MPs/NPs can be absorbed and bioaccumulated by humans through ingestion/inhalation or other modes of exposure. The pharmacokinetics, pharmacodynamics, and variables that determine the pharmacological response to MPs/NPs are still unknown. In the absence of clinical evidence, *in vitro* investigations in human or other mammalian cells may be able to shed light on these issues. This chapter looks at recent research that looked at the biological effects of MPs/NPs in mammalian cells using various exposure methods. The need of addressing MPs/NPs toxicity about particle size, dose, charge, exposure period, additives/leachates, and/or other co-contaminants has been emphasized.

The cellular pathways that contribute to toxicity after MPs/NPs internalization are also discussed, as well as recommendations for future research. The information offered in this research will aid in a better understanding of the potential implications of human plastic exposure. Because *in vitro* research using weathered particles is essentially missing, the focus is mostly on studies utilizing MPs/NPs purposefully generated in the micro/nano-size range. Particle preparation techniques were notably noted in the review for experiments involving particles generated from technologies that mimicked the environmental degradation of bigger polymers.

## 12.2 Polymer Types of MPs/NPs

A variety of pathways could lead to breaking down plastics into macroplastics (>25 mm), mesoplastics (5–25 mm), microplastics (5 mm), and nanoplastics (0.1  $\mu\text{m}$ ). Microplastics (MPs) are plastic particles with a diameter of less than 5 mm that can be found in the environment in sizes ranging from a few microns to a few millimeters, and even nano-sized particles, which often have an unevenly mixed state (Boyle & Örmeci, 2020).

### ***12.2.1 Primary Type***

Primary (initially and consciously created for industrial and domestic uses within a microscopic size) and secondary (originally and purposefully manufactured for industrial and domestic applications within a microscopic size) origins are both present in microplastics (resulting from the continuous breakdown of large plastic debris). Primary microplastics (microbeads) are widely used in cosmetics formulations such as makeup, sunscreen, nail polish, hair coloring, eye shadow, shower gels, and personal care products containing scrubs and toothpaste, facial cleansers, and air-blasting.

### ***12.2.2 Secondary Type***

Secondary microplastics are formed by the breakdown and degradation of large plastic debris into small fragments when exposed to high solar UV radiation and mechanical abrasion as a result of a combination of physical (mechanical), chemical (photolytic), and biological processes and can be transported directly into marine environments from coastlines, rivers, and sewage pipes.

There are numerous different varieties of polymers, each of which can be classified as either natural or synthetic (Koelmans et al., 2015). PET (polyethylene terephthalate), HDPE (high-density polyethylene), PVC (polyvinyl chloride), LDPE (low-density polyethylene), PP (polypropylene), PS (polystyrene), and PU (polyurethane) are examples of synthetic polymers (PUR). Another important factor that affects the floating and sinking of MPs/NPs, as well as the removal rate, is chemical composition. There are currently around 30 different MPs/NPs types available. More than 30 different types of MPs/NPs polymers have indeed been discovered so far (Sun et al., 2019).

## **12.3 Detection of MPs/NPs**

Understanding the behavior and bioavailability of microplastics requires precise knowledge of physical and chemical properties (i.e., form, size, polymer compositions, and functional groups) (Fu et al., 2020). Separation, identification, quantification, and characterization of plastics in terms of physicochemical attributes are all part of the detection process. MPs/NPs can be characterized in a variety of ways, including microscopic, chromatographic, and sophisticated spectroscopic techniques (Mintenig et al., 2018; Shim et al., 2017).

### 12.3.1 Separation

The initial and most important stage in the separation of MPs/NPs is usually accomplished using sieves with various mesh sizes. For MPs/NPs separation, these can be employed alone or in a sequence (Hollman et al., 2013). Filters with a fine mesh are commonly used to separate small MPs of size 5  $\mu\text{m}$  (Löder & Gerdt, 2015). Furthermore, chromatographic techniques, both active and passive separation, are used to separate the majority of MPs/NPs with a size range of 1  $\mu\text{m}$  that is of plastic origin (Mintenig et al., 2018). Field-Flow Fractionation (FFF) is used in active separation, while Hydrodynamic Chromatography (HDC) is used in passive separation (Mendoza & Jones, 2015). Both methods, when combined with sophisticated techniques such as GC-MS, size-exclusion chromatography, and plasma mass spectroscopy, have been demonstrated to be useful in quantifying and characterizing MPs/NPs of various chemical forms of PS, PE, and PACR with sizes ranging from 50 to 9900 nm and 90 to 106 m (Gigault et al., 2017; Correia & Loeschner, 2018; Philippe et al., 2014; Pirok et al., 2017). In the described study, MPs/NPs were extracted from tap water, surface water, and fish samples.

### 12.3.2 Visualization

A second phase in the identifying process is visualization. Large MPs are often recognized with a standard microscope, and their shape, color, and light transmittance can later be used to separate them from a combination of non-plastics (Hidalgo-Ruz et al., 2012). The hue of plastic litter may reflect its state of degradation and could be used as a proxy for environmental exposure duration (Marti et al., 2020). Polypropylene fibers, for example, were discovered to be typically hazy or red, whereas milky white color forms PS, and yellow and brown color generates PE, PP, PVC, PS, and PET (Eriksen et al., 2013; Brandon et al., 2016; Vianello et al., 2013). Though large microplastics with distinguishable colors or morphologies can be visually sorted and identified, particles without distinguishable color or form are difficult to sort with the naked eye. To identify confusing plastic-like particles, electron microscopy with magnified pictures is required (Song et al., 2015). SEM-EDS is a technique that combines scanning electron microscopy with energy-dispersive X-ray spectroscopy to characterize the shape of amazingly small materials and estimate their chemical constituents (Goldstein et al., 2017). SEM could produce high-resolution topographical images of objects, allowing microplastics to be distinguished from those other plastic-like particles more easily (Cooper & Corcoran, 2010). By identifying the characteristic X-rays released from the elements well within the specimen by the electron beam, EDS offers elemental information about the samples, allowing for certain characteristics of micro-plastic recognition in sample composites. The identification of several MPs/NPs (PP, PS, PE, PA) in seawater, shallow waterways, and beaches with sizes ranging from 1 to

5 mm was aided by these forms of microscope visualization (Fries et al., 2013). Fluorescence Microscopy has also proven to be a promising method for identifying plastic particles in seawater and studying their effects on marine assemblages and settling rates. For the first time, Qiu et al. (2015) used FCM to examine the presence and prevalence of microplastics (PET, PE, HDPE, and PS) with a size range smaller than 5 mm in China (Qiu et al., 2015). The tendency of zooplankton to consume microplastics was studied using fluorescence microscopy. This study found that marine microplastic debris (especially PS) with sizes ranging from 7.3 to 30.6  $\mu\text{m}$ ) can have a deleterious influence on zooplankton physiology and overall health (Cole et al., 2013). Similarly, fluorescence microscopy was employed in another work to discover the existence of fluorescent microplastic beads in copepods (PS with sizes ranging from 0.05 to 6  $\mu\text{m}$ ). The findings revealed that micro or nanoscale PS beads may reduce the survival rate and fertility of marine copepods. The colorful plastic fibers in the sample were observed using fluorescence microscopy, and this study successfully quantified the prevalence of MPs of uncertain origin with sizes ranging from 0.5 to 1 mm in Swedish west coast waters (Sweden, 2007). Fluorescence microscopy was utilized to confirm the integration of microspheres (Fluoresbrite carboxylate) with sizes ranging from 3.6 to 11  $\mu\text{m}$  into planktons and to assist in microsphere quantification (Okubo et al., 2018).

### 12.3.3 Characterization

Physiological features of MPs/NPs can be examined at the third level of characterization. The hydrodynamic size as well as the surface charge of particles has been studied extensively using modern technologies such as Dynamic light scattering (DLS) (Bhattacharjee, 2016). In addition, the DLS approach can be integrated into other systems to provide quick and easy identification of microplastic deterioration. Gigault et al. used a photo-reactor in conjunction with DLS to study the photocatalytic degradation of ocean microplastic particles under various conditions without the need for sample or manipulation (15). In addition to hydrodynamic size measurement, nanoparticles tracking analysis (NTA) is an improved approach for measuring concentrations of poly-dispersed substances. With monochrome photography, NTA illuminates free diffusing particles with strong laser light to trace their Brownian motion (Gigault & Budzinski, 2016). The other sophisticated approach for testing the presence of organic substances or carbon in surface and ground waters is fluorescence spectrophotometry. The use of fluorescence spectroscopy to evaluate toxicity and explore the detrimental impacts of microplastics on microorganisms in soil and water, such as suppression of enzyme activity and energy metabolism, has been demonstrated (Henderson et al., 2009). Chen et al. (2018) mapped and described the microstructure of MPs (PS) with diameters of 20 mm, 6 mm, 500 nm, and 80 nm to analyze MPs' environmental behavior. Similarly, detection of accumulated microplastics, particularly (PS) of size 5  $\mu\text{m}$  in the gills, liver, and gut of crabs, detection of cadmium, lead, and bromine in MPs of uncertain

origin with sizes 5–10  $\mu\text{m}$  from beach waters, and measurement of NPs concentration (PS) of sizes 45  $\mu\text{m}$  and 50 nm in zebrafish larvae and nematodes (Yu et al., 2018; Massos & Turner, 2017; Chen et al., 2017, 2018; Zhao et al., 2017).

Finally, advanced technology like Fourier transform infrared (FTIR) spectroscopy, can be used to analyze the chemical/functional group composition of MPs/NPs. It collects chemical data by sensing the modes of vibration of analyte at various infrared frequencies throughout a broad-spectrum range (Stuart, 2005). From sand samples collected in Sishili Bay, North Yellow Sea, FTIR was used to detect eight polymer kinds of MPs (Rayon, PE, PP, PA, PET, PS, PMMA, and PU) with diameters ranging from 34.97 to 4983.73  $\mu\text{m}$ . These findings show that river and sewage discharge, as well as maritime activities, were the main sources of MP pollution (Zhang et al., 2019). Yang et al. (2019a, b) used FTIR to demonstrate the presence of MPs (PET, PS, PP) of varied sizes in municipal wastewater from China's biggest water reclamation plant (Yang et al., 2019a, b). FTIR was also used to determine the existence of airborne MPs (PET, PES, PAN, RY, EVA, PAA, EP, ALK) in China's atmosphere, as well as the polymer kinds of MPs (PET, PP, PS, Nylon) deposits in the Pacific Ocean (Liu et al., 2018a, b; Peng et al., 2020). Raman spectroscopy is another prominent biochemical characterization mapping technique that uses the Raman effect to extract the vibrational modes and identify analytes of samples by using the frequency response of inelastically scattered light from the samples (Araujo et al., 2018). Micro-Raman spectroscopy can detect MPs with a resolution of up to 10  $\mu\text{m}$ , whereas Raman spectroscopy can detect MPs greater than roughly 1  $\mu\text{m}$  (Imhof et al., 2016). Several other methods, such as matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) (Karas & Krüger, 2003), high-performance liquid chromatography (HPLC) (Siddiqui et al., 2017), and atomic force microscopy and its combinations with IR and Raman as a unique scanning probe technique, open up new opportunities in microplastics and nanoplastics characterization. AFM may be used to detect and quantify a variety of material physical and mechanical properties like elasticity, surface electric properties, and chemical properties (Akhatova et al., 2022). Several studies have also shown the presence of different micro and nanoplastics present in various food stuffs (Table 12.1).

## 12.4 Exposure of MPs/NPs to the Biological System

MPs/NPs can be ingested, inhaled, absorbed through the skin, or administered intravenously (NJDEP-Science Advisory Board, 2015). When particles are swallowed, they first come into contact with the gut mucosa, followed by the epithelia, which together provide a formidable barrier to xenobiotic uptake. Several investigations have revealed, however, that micro/nanoparticles can pass through the intestinal barrier and enter the bloodstream (Jenkins et al., 1994; Reineke et al., 2013; Walczak et al., 2015). Airborne MPs/NPs would come into direct contact with the mucus layer, periciliary layer, ciliated cells, non-ciliated secretory cells, and basal cells of



**Table 12.1** Studies assessing the micro- and nanoplastics present in food stuffs

S. No.	Food stuffs	Polymer	Particle size	References
1.	Fish	PE, PP, PA, PS, PET, PVC, PAN	>20 $\mu\text{m}$	Su et al. (2019)
2.	Clam, oyster, mud snails	PE, PET, PA	10–5000 nm in diameter	Naji et al. (2018)
3.	Mussel	PE CP, PET, PA, PS, PP	150–6000 $\mu\text{m}$	Nalbone et al. (2021)
4.	Crab, prawn	PE, PP, PET	–	Akhbarizadeh et al. (2019)
5.	Oyster	PET, PA, PE, PS, PP, PPS	4–2100 $\mu\text{m}$	Teng et al. (2019)
6.	Poultry meat (packed)	Epoxy resin, rayon, PET, PS, PMPS	130–250 $\mu\text{m}$	Kedzierski et al. (2020)
7.	Apple	PET, poly (ether-urethane) PET, PVCA, PVC, PES, PEVA, PVK	1.99 $\mu\text{m}$	Conti et al. (2020)
8.	Pear		2.10 $\mu\text{m}$	
9.	Broccoli		1.51 $\mu\text{m}$	
10.	Potato		20 $\mu\text{m}$	
11.	Lettuce		20 $\mu\text{m}$	
12.	Sea salt	PP, PET, PS, PP	40–170 $\mu\text{m}$	Kosuth et al. (2018)
13.	Tap water	MP fragments	50–500 $\mu\text{m}$	Mintenig et al. (2019)
14.	Bottled water	MP fragments, fibers	–	Schymanski et al. (2018)

Modified from Llorca and Farré (2021)

CP Cellophane, HDPE high-density polyethylene, PA polyamide, PAA polyacrylic acid, PAN polyacrylonitrile, PE polyethylene, PES poly {p-phenylene ether sulfone}, PET polyethylene terephthalate, PEVA polyethylene-vinyl-acetate, PMPS polymethyl pentene, PP polypropylene, MP microplastics, PPS polyphenylene sulphide, PS polystyrene, PVC polyvinyl chloride, PVCA vinyl chloride/vinyl acetate copolymer, PVK poly {N-vinyl carbazole}

the respiratory tract (Ganesan et al., 2013; Gasperi et al., 2018). When inhaled repeatedly, plastic fibers can infiltrate lung tissues, causing inflammation and subsequent genotoxicity (Gasperi et al., 2018). In the lungs of textile workers, granulomatous lesions harboring foreign substances (perhaps polyester, nylon, or acrylic dust) were discovered (Pimentel et al., 1975). Because the stratum corneum impedes the passage of molecules larger than 500 Da (1 nm) across the skin layers, transdermal absorption of MPs/NPs via intact skin is unlikely (Bos & Meinardi, 2000). MPs/NPs can also enter the bloodstream through plastic-based intravenous catheters, syringes, and other medication delivery methods (Stapleton, 2019). Because they are “inert and biocompatible,” polystyrene micro and nanoparticles are frequently utilized as vectors for medication delivery or to study bio-interactions (Loos et al., 2014a; Poon et al., 2016). Plastic absorption by cells, as well as the release of plastic additives or surface-adsorbed pollutants, can have a deleterious impact on cell function (Bouwmeester et al., 2015).



## 12.5 Factors That Influence Their Cytotoxicity

Plastic toxicity varies depending on the polymer type. Based on the hazard classification of monomers, polyurethane, polyacrylonitriles, PVC, epoxy resins, and styrene-based copolymers have been classified as the most dangerous (category 1A or 1B mutagen or carcinogen) (Lithner et al., 2011). Several factors can influence particle cytotoxicity within a plastic class.

### 12.5.1 Size of MPs/NPs and Dosage

Small particles are often internalized to a higher extent by cells than large particles (Florence et al., 1995). Smaller particles can be taken up via endocytic or passive absorption, but bigger particles require phagocytosis by specialized cells (Alberts et al., 2002). Internalization of 44 nm PS particles was an ATP-independent passive process in two primary mammalian cell lines – bovine oviductal epithelial cells and human colon fibroblasts (Fiorentino et al., 2015). In most cases, particle size and toxicity have an inverse relationship. Particles smaller than 10 nanometers are thought to act as a gaseous substance that can easily infiltrate tissues and cause broad damage (Bahadar et al., 2016). When evaluated using 100 L of 1 mg/mL particle solution in monomac-6 human monocytic cells, 64 nm PS particles induced a considerable rise in intracellular  $Ca^{2+}$  levels compared to bigger PS particles (202 and 535 nm) (Brown et al., 2001). The 64 nm particles produced higher IL-8 expression in human lung cancer A549 cells after 2 hours than the 202 and 535 nm particles (Brown et al., 2001).

Smaller particles can be more easily absorbed through the colon or lungs, affecting particle cellular fate and biodistribution. Particles larger than 150 nm can have local effects in the stomach, whereas smaller particles can cause toxicity in a variety of secondary organs and tissues (EFSA Panel on Contaminants in the Food Chain, 2016; Rubio et al., 2020). For particle intake and transport, however, a Goldilocks zone for size may exist. For example, 40 nm PS particles were found to have a greater absorption in 1321N1 human astrocytoma and A549 cells than 20 and 100 nm PS particles (Varela et al., 2012). The transfer of 200 nm PS particles through the cells in a microfluidic model of the blood-brain barrier (BBB) utilizing hCMEC/D3 cells was higher than that of 100 and 500 nm particles (Nowak et al., 2019).

High doses and long-term exposure can cause increased cellular absorption and toxicities. For example, at 20 and 50 g/mL doses, 20 nm plain PS particles did not cause toxicity in THP-1 monocytes in 24 hours, but at 200 g/mL, cellular viability was reduced to 12% (Mrakovcic et al., 2014). Cell number reduced considerably after 16 days of incubation with 50 g/mL of 20 nm PS particles, exhibiting dosage- and time-dependent toxicity. Similar findings were made with human umbilical vein EAhy 926 cells, where 20 nm plain PS particles displayed dose-dependent

cytotoxicity in 24 hours, with an  $IC_{50}$  value of 120 g/mL (Mrakovcic et al., 2013). Cell number dropped by 50% after a 28-day incubation period with a 20 g/mL dosage of these particles.

### 12.5.2 Charge

Surface charge influences particle absorption, translocation, and toxicity. Positively charged PS particles transported 20 and 100–120 nm aminated or carboxylated PS particles 20–40 times faster than negatively charged PS particles across rat alveolar epithelial cell monolayers (Yacobi et al., 2010). Furthermore, cationic particles exhibit higher cytotoxicity in non-phagocytic cells than their anionic counterparts, owing to plasma membrane damage (Frohlich, 2012). Aminated PS particles (110 nm) decreased THP-1 cell proliferation, although carboxylated PS particles of similar size did not affect cell division (Loos et al., 2014a). The viability of THP-1 cells was similarly dramatically reduced after 72 hours of incubation with 10–100 g/mL aminated particles but not with carboxylated PS particles (Loos et al., 2014b).

### 12.5.3 Additives

Stabilizers, plasticizers, lubricants, dyes, and flame retardants are among the plastic additives/leachates that make up an average of 4% of microplastic content and can be harmful (Bouwmeester et al., 2015; Campanale et al., 2020; EFSA Panel on Contaminants in the Food Chain, 2016). Bisphenol A, phthalates, and brominated flame-retardants, all of which alter endocrine function, are of special concern (Campanale et al., 2020; De Toni et al., 2017; Legler & Brouwer, 2003; Rubin, 2011). At temperatures above 60 °C, commercially available PET water bottles leached Sb into the water; temperatures that could be reached if bottles were left inside cars and garages during the summer (Westerhoff et al., 2008). Surfactants can lyse cell membranes or affect the structure and function of cell surface receptors, glycoproteins, proteoglycans, signaling molecules, extracellular matrix components, and lipid rafts, to mention a few (Yong et al., 2020). However, the presence and release of additives do not always constitute a health risk, as toxicity is determined by the plastic composition and the velocity of leachate migration, or the amount and solubility of leachate in the surrounding environment. In fatty foods and when stored at high temperatures or for long periods, there is a higher migration of additives from plastics (Hahladakis et al., 2018).

### **12.5.4 Adsorbed Pollutants**

MPs/NPs can absorb additional pollutants such as persistent organic pollutants (POP), heavy metals, and pathogens due to their small size and high surface to volume ratio (de Souza Machado et al., 2018; Yu et al., 2019). Persistent organic pollutants (polychlorinated biphenyls, polycyclic aromatic hydrocarbons, DDT), heavy metals (Cd, Cr, Cu, Zn, Sb, Al, Br, Hg, As, Sn, Ti, Co, Ba, Mn), and microorganisms (pathogenic vibrio spp.) can all be vectored by plastics (Brennecke et al., 2016; Campanale et al., 2020; Kirstein et al., 2016; Prinz & Korez, 2020; Rodrigues et al., 2019; Velzeboer et al., 2014). Pyrene and BDE-47 were carried by polystyrene nanoparticles (100 nm) in saturated soil (Liu et al., 2018a).

## **12.6 Toxicity Caused to Human Cells (In Vitro)/Potential Effects on Organ System**

It's crucial to look at MPs/NPs uptake and biological consequences in cells that are either immediately exposed to them or come into contact with them after systemic absorption. A list compiled of recent MPs/NPs toxicity studies was completed in mammalian cells from 2001 to 2020. MPs/NPs can be internalized by gastrointestinal, airway, immune, and other miscellaneous cell types and induce various cellular responses, the nature and extent of which may be governed by MPs/NPs size, dose, charge, exposure time, and the presence of additives/leachates/co-contaminants, as described in this section (Fig. 12.2).

### **12.6.1 Immune Cells**

Immune cells serve as the body's gatekeepers, assisting in the clearance of infections and xenobiotics. As a result, they are likely to interact with MPs/NPs found in food, water, and air, as well as those absorbed into the systemic circulation via various routes of exposure. Smaller particles can enter immune cells via clathrin/caveolae-mediated endocytosis, clathrin/caveolae-independent internalization, micropinocytosis, and phagocytosis, while microparticles can be ingested by immune cells via phagocytosis or micropinocytosis (Firdessa et al., 2014).

MPs/NPs can be quickly internalized by immune cells such as monocytes, macrophages, fibroblasts, and mast cells (Heinlaan et al., 2020). At dosages less than 100 µg/mL, nano- and sub-micron-sized particles did not appear to cause cytotoxicity in THP-1 macrophages. In most cases, the positive charge and tiny size exacerbated toxicity (Hwang et al., 2019). The viability of cells was harmed by particle leachates and additions. However, PS particles were used in the bulk of this research to explore biological effects in immune cells. To fully comprehend the influence of

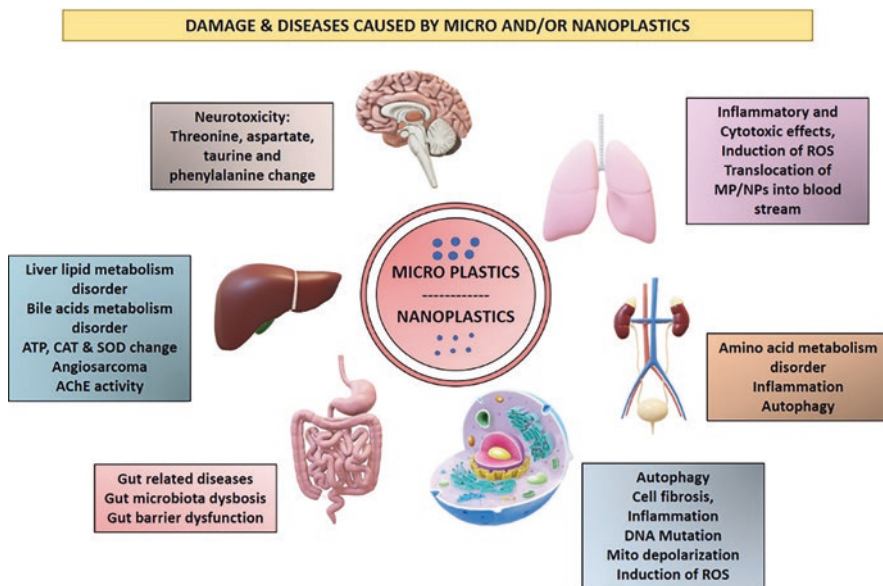


Fig. 12.2 Damage and diseases caused by micro- and/or nanoplastics

diverse plastic types on immune cells, more research employing different plastic polymers is required.

### 12.6.2 *Gastrointestinal Cells*

MPs/NPs toxicity in gastrointestinal cells has been intensively researched because ingestion of MPs/NPs contaminated food or water is the primary source of exposure to these particles. Enterocytes, mucus-producing goblet cells, and microfold or M cells make up the majority of the intestinal epithelia. Caco-2 cells, a commonly used in vitro model for enterocytes, were found to ingest 100-nm carboxylated PS particles largely by micropinocytosis-mediated uptake at the apical surface, followed by mostly storage or limited exocytosis at the basolateral membrane end (Reinholz et al., 2018). Regular epithelial cell shedding every 4–5 days could remove polystyrene particles retained in the cells (Reinholz et al., 2018). Diffusion across the cell membrane followed by basal exocytosis was a secondary, although limited, mechanism of uptake and excretion.

In vitro investigations of gastric cells show that nanoplastics can be taken up by stomach cells and that smaller particles are potentially more harmful than bigger particles. To better understand MPs/NPs toxicity in the stomach, more research is needed in other gastric cells such as SNU-1, SNU-5, and KATO III, utilizing particles of various sizes, charges, dosages, and exposure times (Liao & Yang, 2020). Numerous researches have looked into the toxicity of MPs/NPs in the intestine,

however, no consensus has been reached on size/charge-dependent transport or toxicity (Baos et al., 2012; Chen et al., 2020; Collado-Gonzalez et al., 2019). To reconcile the contradictory data, a factorial study design assessing the effects of various plastic polymers, charges, sizes, doses, and exposure times in intestinal cells is required.

### **12.6.3 Airway Cells**

Clothes, dried sludge, agriculture, tires, manufacturing operations, and sea salt aerosols are all reported sources of airborne microplastics (Wright & Kelly, 2017). Microplastics were found in air fallout in Paris, with a higher quantity in densely populated areas than in less densely populated areas (Gasperi et al., 2018; Wright & Kelly, 2017). When breathed in, plastic particles may pass through mucociliary clearance systems in the respiratory tract, especially if the particle size is more than 1  $\mu\text{m}$ , or (ii) pass through the pleura and be absorbed by lung epithelial cells. As seen among workers in nylon flock, polyester, polyolefin, and polyamide fiber plants, inhaled plastic dust can cause respiratory distress such as irritation of the respiratory tract, dyspnea, decreased lung capacity, coughing, increased phlegm production, interstitial fibrosis, and granulomatous lesions (Wright & Kelly, 2017). Human lung tissues have also been discovered to contain plastic fibers (Pauly et al., 1998). Fibers made of polypropylene, polyethylene, and polycarbonate can last up to 6 months in extracellular lung fluid (Gasperi et al., 2018). The size, type, concentration, and duration of exposure to these particulate materials all influence the health risks they pose (occupational vs occasional). To assess if the airborne MPs/NPs constitute a health risk, researchers must first determine how they interact with respiratory cells (uptake, transport, cytotoxic potential, and metabolic effect).

MPs/NPs can be absorbed by several immortalized and primary airway epithelial (bronchial, alveolar) cells, according to *in vitro* investigations. Positively charged beads, a greater dose, and the inclusion of additives are all linked to increased cytotoxicity. The influence of nano- and sub-micron-sized PS particles on respiratory cells has been the focus of research. To model chronic exposure to varied MPs/NPs, however, the toxicity of different plastic polymers and at longer time points (72, 96, or more) are required. Furthermore, investigations involving airway cells at the air-lung interface are required for a more realistic understanding of the interaction between MPs/NPs exposure and lung damage.

### **12.6.4 Mammalian Cells**

The toxicity of MPs/NPs has been investigated in a variety of mammalian cells, including blood, cerebral, endothelial, epithelial, hepatic, kidney, melanoma, ovarian, and placental cells, due to the possibility of systemic exposure or absorption.

T98G glioma cells and HeLa epithelial cells were used to test the cytotoxicity of PE microspheres (0.1, 0.6, and 3–16) and PS particles (40–250 nm and 10) (Schirinzi et al., 2017). In 24 hours, the particles (0.05–10 mg/L) did not affect cellular viability in either cell line. In Madin Darby canine kidney cell II monolayers, charge-dependent quicker trafficking of PS particles was reported, with amidine-functionalized 20 and 120 nm PS beads translocating 500 times faster than 20 and 100 nm carboxyl-functionalized beads (Fazlollahi et al., 2011). In ovarian cancer cells SK-OV-3 and NIH-OVCAR3, 50 nm amine-functionalized PS particles were quickly taken up, accumulated in lysosomes, and caused cytotoxicity within 4–8 hours, whereas 30 nm carboxyl functionalized beads did not accumulate in lysosomes and were not cytotoxic even after 24 hours of treatment (Ekkapongpisit et al., 2012). The hemolytic potential of 100 nm PS particles isolated from commercial face washes was compared to 100 nm virgin PS particles in erythrocytes (Gopinath et al., 2019). Overnight incubation with isolated particles at 5 g/mL resulted in 40% hemolysis, but only 22% with virgin particles. The inclusion of additives or other toxic polymers on the surface of isolated particles was blamed for the greater toxicity of isolated PS compared to virgin PS.

These findings show that a variety of non-phagocytic cells can internalize MPs/NPs, with size and charge being the most important factors. When compared to large, negative, or non-functionalized particles, smaller, positively charged particles are more likely to be taken in and cause cellular damage. HeLa and T98 glioblastoma cells, on the other hand, demonstrate no size-dependent toxicity. Small (200 nm) particles can be internalized by red blood cells, whereas large (1000 nm) particles cannot. The toxic effects shown by different micro- and nanoplastics are mentioned below (Table 12.2).

### 12.6.5 *Animals*

Micro and nanoplastics have long been recognized as common contaminants in the environment. Their existence has been established in water bodies (fresh & marine), terrestrial systems, as well as the air we breathe (Rillig & Lehmann, 2020). MPs/NPs are taken up by animals, dispersed in their bodies, and deposited in several tissues, from which they were later transported widely through food chains, according to a growing body of evidence (Zhang et al., 2020) As a result, bioaccumulated micro and nanoplastics could endanger human health and the ecosystems (de Souza Machado et al., 2018) (Fig. 12.3).

MPs/NPs have an impact on the growth, development, and reproduction of organisms and can even cause mortality in individuals. These tend to generate oxidative stress reactions in organisms, disrupt pigment formation or enzyme activities, create endocrine and metabolic abnormalities, and cause various degrees of genotoxicity, cytotoxicity, and neurotoxicity due to their small size. Simultaneously, as the food chain spreads, these consequences are aggregated and magnified step by

**Table 12.2** Micro- and nanoplastics and their potential toxic effects on human health

S.No.	Characteristics of plastic particles	Particle size	Toxic effects	References
1.	Polystyrene particles	202, 535 nm	Inflammation in A549 cells	Brown et al. (2001)
2.	Carboxylated polystyrene particles	20, 44, 500, 1000 nm	Upregulation of IL-6 & 8 expression	Forté et al. (2016) Prietl et al. (2014)
3.	Carboxylated and amino modified polystyrene particles	120 nm	Altered expression of scavenger receptors, increased TGF $\beta$ 1 and energy metabolism	Fuchs et al. (2016)
4.	Unaltered polyethylene particles	0.3, 10 $\mu$ m	Increased secretion of IL-6, IL-1 $\beta$ , and TNF $\alpha$ in murine macrophages	Green et al. (1998)
5.	Polyethylene particles from plastic prosthetic implants	0.2, 10 $\mu$ m	Periprosthetic bone resorption	Nich and Goodman (2014)
6.	Polystyrene microplastics particles	5, 20 $\mu$ m	Adverse effects on neurotransmission, inflammation in the liver	Deng et al. (2017)
7.	Amine modified polystyrene nanoparticles	60 nm	Apoptosis induction in all intestinal epithelial cells	Inkielewicz-Stepniak et al. (2018)
8.	Unaltered/functionalized polystyrene	20, 40, 50, 100 nm	Apoptosis induction in several human cells	Liu et al. (2018a, b) Paget et al. (2015)
9.	PVC (poly vinyl chloride) PMMA (poly methyl methacrylate)	120, 140 nm	Reduced cell viability with a reduction of ATP and increase of ROS concentrations.	Mahadevan and Valiyaveetil (2021)
10.	Cationic polystyrene nanoparticles	50, 60, 200 nm	ROS generation, ER stress, autophagic cell death of mouse macrophages and lung epithelial cells, disrupted intestinal iron transport and cellular uptake	Xia et al. (2008) Chiu et al. (2015) Mahler et al. (2012)
11.	Pristine and fluorescent polystyrene microplastics	5 $\mu$ m	Altered amino acid and bile acid metabolism, gut microbiota dysbiosis, intestinal barrier dysfunction	Luo et al. (2019) Jin et al. (2019)
12.	Anionic carboxylated polystyrene nanoparticles	20 nm	Altered ion channel function and ionic homeostasis	McCarthy et al. (2011)

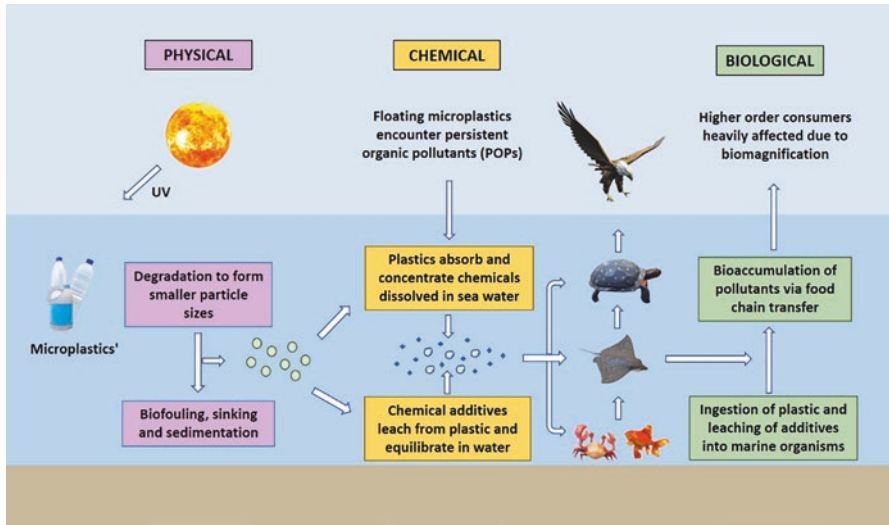
(continued)



**Table 12.2** (continued)

S.No.	Characteristics of plastic particles	Particle size	Toxic effects	References
13.	Polystyrene nanoparticles	30 nm	Blocked vesicle transport and the distribution of cytokinesis-associated proteins	Xia et al. (2016)
14.	Pristine polystyrene microparticles	5, 20 μm	Hepatic ATP level reduction and impairment of energy metabolism	Lu et al. (2018)

Modified from Yee et al. (2021)



**Fig. 12.3** The route of exposure of micro- and nanoplastics into the food chain. (Modified from Braden Wilkinson, 2019)

step from people to populations to communities and finally to ecosystems, thereby worsening the fragile natural system (Ma et al., 2020).

Smaller microplastics can significantly affect algal growth, fertility, and even disturb photosynthesis (Chen et al., 2020). It was observed that HDPE (High-Density Polyethylene) plastic beads of 10–45 μm reduced the filtering rate of *Dreissena bugensis* (quagga mussel) (Pedersen et al., 2020). Many studies have shown that MPs/NPs can pile up in the intestines of earthworms and that the growth activity was significantly lowered and the rate of mortality increased at 28, 45, and 60% w/w microplastics (Gaylor et al., 2013; Wang et al., 2020; Huerta Lwanga et al., 2016). Earthworms’ immune systems and pathological responses were also reduced by microplastics (Rodriguez-Seijo et al., 2017). In soil contaminated with microplastics, the mobility of springtails (*Lobella sokamensis*) was reduced (Kim & An, 2019). As a result, microplastics may obstruct the movement of terrestrial animals by blocking gaps.

MPs/NPs can alter the early stages of development in aquatic animals. The embryonic stage is critical for aquatic animal development, and embryonic chorion serves as an effective barrier against exogenous contaminants. It was reported that zebrafish (*Danio rerio*) embryonic chorions had an effective barrier property against micro and nanoparticles. Though embryonic chorions can effectively prevent micro- and nanoplastics, they can however have an impact on aquatic species' early development. MPs/NPs attaching to embryonic chorions may lead to an internal hypoxic microenvironment within embryos, as well as a delay in hatching. When embryos are exposed to polystyrene particles, specifically nanopolystyrene particles, the pathways of biosynthesis of unsaturated fatty acids, linoleic acid metabolism, alanine, and also glutamate, and aspartate metabolism, are significantly altered (Duan et al., 2020).

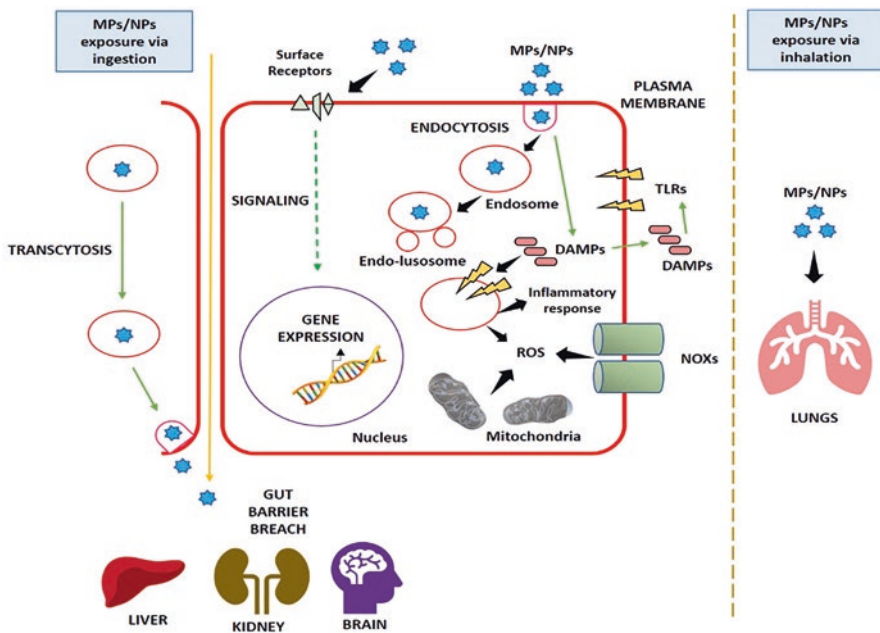
The absence of tools for characterization and measurement of these particles in complicated biological matrices has impeded research into micro and nanoplastic buildup in animal bodies. In microplastic research, ocular inspection (Sobhani et al., 2020), Fourier-transform infrared spectroscopy (González-Pleiter et al., 2019), and Raman spectroscopy (Gillibert et al., 2019) are being employed. However, these methods are often limited to particles with a diameter of 5 nm to 20  $\mu\text{m}$ , with only a few studies focused on the sub-20  $\mu\text{m}$  portion (Cole et al., 2013). Furthermore, organic debris on the micro and nanoplastics surfaces makes spectroscopic identification and quantification difficult. Pyrolysis combined with gas chromatography-mass spectrometry (Py-GC/MS) is a promising method for quantifying nanoplastics (Fischer & Scholz-Böttcher, 2017; Zhou et al., 2018). Even though this technique may be used to reliably identify and quantify micro and nanoplastics without regard to particle size, extracting and determining micro and nanoplastics from complicated biological matrices is a simple task (Mitrano et al., 2019).

## 12.7 Cellular and Molecular Interactions Caused by MPs and NPs

MPs/NPs toxicity is thought to be caused by membrane damage, oxidative stress, immunological response, and genotoxicity. Among these, MP/NP's cytotoxicity has been attributed mostly to membrane damage and oxidative stress (Bhattacharjee et al., 2014). Cationic particles, for example, have been known to damage the plasma membrane (Feng et al., 2019; Frohlich, 2012). Polyethylene nanoparticles were discovered to enter the plasma membrane bilayer's hydrophobic milieu and cause structural alterations (Holloczki & Gehrke, 2020). Endocytosed particles can permeabilize the endosomal-lysosomal membrane, allowing them to interact with intracellular organelles (Wang et al., 2018a, b; Yong et al., 2020). ROS are produced during the polymerization and processing of plastic particles, and when they come into contact with the biological environment, they cause cellular stress (Rubio et al., 2020). While big particles can cause inflammation in the gastrointestinal and

respiratory tracts, smaller particles can pass through the gut/lung barrier, causing intracellular oxidative stress and cytotoxicity in the organs where they collect (Rubio et al., 2020). Direct or indirect DNA damage caused by particle or ROS translocation into the nucleus, as well as disruption to the DNA replication/repair machinery, can all contribute to particle genotoxicity (Rubio et al., 2020) (Fig. 12.4).

MPs/NPs can disrupt nuclear membranes, generate oxidative stress, release damage-associated molecular patterns, and activate inflammatory and apoptotic/necrotic pathways in mammalian cells (Hwang et al., 2020; Yong et al., 2020). Hepatocytes from 3-month-old mice have been treated with 50 nm PS particles for 24 hours, causing an increase in ROS (superoxide dismutase and malondialdehyde concentration) and DNA damage (Zheng et al., 2019). PS beads caused superoxide radical anion ( $O_2^{\cdot-}$ ) production in human hepatocyte-derived cancer Huh-7 cells,



**Fig. 12.4** A schematic illustration depicting possible cellular processes of MP/NP toxicity. Ingestion and inhalation are two ways to absorb MPs/NPs. These have the potential to disrupt the plasma membrane and compromise the gut barrier (left). These could also disrupt cell surface receptor signaling and change gene expression in the nucleus. Endocytosed MPs/NPs have the potential to disrupt the endocytic process and impair endosomal membranes. Endogenous and secreted damage-associated molecular patterns (DAMP) triggering the innate immunity-mediating toll-like receptors (TLRs) could activate the cellular innate immune system as a result of the aforesaid stresses. Stress may cause the NADP oxidases to produce reactive oxygen species (ROS) (NOXs). Mitochondrial dysfunction, whether caused by MPs/NPs from endosomes or as a result of stress, could result in an increase in ROS due to a decrease in the efficiency of electron transport chain (ETC) operations. If the gut-vascular barrier is breached, MPs/NPs gain access to the circulation, or transcytosis may occur, allowing them to reach other organs. The lung is more likely to have direct contact with airborne MPs/NPs (right). (Modified from Yong et al., 2020)

according to another study (Liu et al., 2018b). The toxicity of positively charged PS beads in RAW 264.7 and BEAS-2B cells, on the other hand, was found to be attributable to autophagy via the Akt/mTOR and AMPK pathways (Chiu et al., 2015). The majority of research points to an oxidative stress-mediated cellular response to MPs/NPs when taken together. Many of the aforementioned toxicity processes, however, are closely interrelated, and induction of one process might trigger a cascade of toxicological responses.

## 12.8 Regulatory Policies/International, National, and Regional Instruments

“Microplastics” (MPs) are a hot topic in the media and one of the most fiercely debated environmental issues among the general population. As a result, the public wants policymakers to address and handle the issue as quickly as possible (Sharma et al., 2021). In reality, policymakers are becoming more conscious of the problem. Some of the most powerful and influential international and intergovernmental organizations are debating the global effect of environmental plastics (e.g., G7, World Bank, United Nations, World Economic Forum, etc.) (Brennholt et al., 2018). Aside from that, the (micro)plastic/nanoplastics issue has been addressed in a few international and national rules and policy instruments. Because the majority of environmental MPs are caused by improper disposal and fragmented plastic litter, MP management is directly linked to a variety of policy areas (Coffin et al., 2021). Furthermore, regulatory responsibilities can shift throughout a single plastic product’s life cycle and include plastic production process layout, trade and consumer behavior, recycling, and waste management (Deme et al., 2022). It can also be called land-based policies, as well as sewage management and water protection, also called water-based policy (Freeman et al., 2020). As a result, plastics regulation is already addressed in several directives, recommendations, agreements, and other documents addressing the use of plastic products, beginning with restrictions on plastic monomer compositions and the addition of additives. Policy and regulatory instruments are now being established all over the globe at international/ regional/ and national levels to handle the problem of (micro) plastics in the environment (Mitrano & Wohlleben, 2020). National policy instruments are limited to a single country, whereas regional policy instruments address specific issues within a geographical region, such as Europe. International accords and regional treaties, for example, are incorporated into national legislation. For the first time in January 2018, Europe enacted ESPCE (European Strategy for Plastics in a Circular Economy), which altered the way the European Union designs, manufactures, uses, and recycles plastic items (Campanale et al., 2020). Many instruments, such as the G7 Summit (2014), the G20 Summit (2017), and the United Nations Environment Assembly (UNEA) I, II, III in 2014, 2016, and 2017, have been vital in preventing marine litter from land-based sources and in reducing marine plastic litter and

microplastics, as well as combating their spread. National instruments in countries such as the United States, the United Kingdom, Sweden, Canada, Italy, New Zealand, South Korea, Australia, Taiwan Province, and China primarily impose laws prohibiting the use of microbeads in rinse-off cosmetics, microbeads in toiletries, plastic cotton buds, microbead scrub particles in cosmetics, and the sale of microbead-containing products (Xu et al., 2021; Deng et al., 2020). Better plastic product design, increased waste plastic recycling rates, etc., and high-quality recycles will all assist to improve the demand for recycled plastics, protecting the environment, reducing marine debris, greenhouse gas emissions, and our dependence on external fossil fuels (Gago et al., 2020).

The Ministry of Ecology and Environment issued “Opinions on Further Strengthening the Control of Plastic Pollution” in January 2020, outlining three phases of action. Prohibiting and regulating the manufacture, sale, and use of certain plastic products, for example, shopping bags that are ultra-thin with 0.025 mm thickness. Simultaneously, we should encourage the use of non-plastic goods (such as paper bags and biodegradable shopping bags) and standardize plastic trash recycling and disposal. In addition, Japan, South Korea, Thailand, and other countries have also implemented harsher rules in recent years. The European Commission adopted new guidelines in May of the same year for ten typical throwaway plastics goods and fishing gear using plastics to minimize or limit the environmental effect of individual plastic products. In addition, Plastics Europe proposed the “Plastic 2030” voluntary commitment, which included “Zero Plastics to Landfill,” “Zero Pellet Loss,” and other initiatives aimed at preventing plastic leakage into the surroundings and trying to improve the resource productivity of plastic items and concentricity of plastic packaging. Some regions have seen positive results and increased public awareness of environmental preservation after China enacted the “Plastics Restriction Order” in 2008, however, this is far from enough (Wang et al., 2020). These policies, in contrast to previous rules and regulations, focus on the entire life cycle of plastic products, including the overall process and each link of manufacturing, circulation, use, reprocessing (such as mechanical and chemical recycling and energy recovery), and disposal, making it easier to establish a long-term mechanism for controlling plastic pollution. Preventing the flow of low-end plastic items from developed to developing countries, from places with high supervision and utilization competency to those with inadequate supervision and use competence, is critical. This will not only result in a circular economy but will also protect our planet and help us accomplish our sustainable development goals.

## 12.9 Conclusion/Future Directions

Although their cytotoxicity is mostly determined by their size, surface functionalization, dose, exposure period, and presence of co-contaminants, studies have revealed that micro/nanoplastics are not ‘inert’ materials. These pollutants have been found in our food, drinking water, and air, implying that we are constantly

exposed to them. As has been found with occupational PVC dust exposure, chronic exposure to plastics can lead to bioaccumulation and subsequent biological consequences. Organic contaminants and heavy metals, for example, are plastic additives or compounds that hitchhike on plastic surfaces, posing additional toxicity concerns that require further exploration. Studies on the cytotoxicity of MPs/NPs in mammalian cells revealed that MPs/NPs with small size and positive charge, when delivered at high doses for long periods and including surfactants or other adsorbed contaminants, have higher toxicity. These conclusions, however, may not apply to all cells and particle kinds. Furthermore, the influence of certain physicochemical characteristics of particles in particular cell types has yet to be thoroughly understood. A multi-end-point toxicological investigation utilizing MPs/NPs of varied types and physicochemical features at environmentally realistic concentrations in human cells is required for a complete knowledge of the health impact of MPs/NPs.

Various physicochemical parameters of MPs/NPs have different toxicological implications in different cell types, and even within the same cell type, there are discrepancies. Several of these discrepancies can be attributed to changes in experimental settings, particle type/synthesis/source/extraction method, end-points studied, and the difficulties of regulating other parameters during a study. A multi-factorial study design that uses specific cell types, uniform experimental circumstances, and end-points could help to resolve the inconsistencies in cellular responses.

In many cell types, size has been demonstrated to govern cellular uptake and viability among the other particle properties. Gastric cells, hepatocytes, immune cells, RBCs, squamous carcinoma, melanoma, and umbilical cells, for example, have higher bioreactivity with small particles, but there is no clear consensus with airway, intestinal, or ovarian cancer cells. This could be because various cell types have an optimal size range for enthusiastic internalization. Positive charges on MPs/NPs increased toxicity in the airway, immune, ovarian cancer, MDCK-II kidney cells, adrenal medulla, mammary epithelial cells, and HEPA-1 hepatocytes at particular concentrations.

Positively charged particles, in general, can interact electrostatically with the negatively charged phospholipid cell membrane, resulting in greater internalization than negatively or neutrally charged particles (Foroozandeh & Aziz, 2018). High binding of positively charged particles to the plasma membrane, on the other hand, might raise surface tension and cause membrane portion or deformation (Li & Malmstadt, 2013). High doses boosted cellular responses in the airway, adrenal medulla, RBCs, immunological, intestinal, HEPA-1, and EAhy926 umbilical cells, among other characteristics. The cytotoxicity of BEAS-2B airway cells, on the other hand, increased with a longer incubation period. Internalization of particles can be increased by a high dose and a long exposure duration, resulting in greater toxicity. It should be emphasized that spherical polystyrene has been used as the model MPs/NPs in many of these cytotoxicity experiments, owing to the commercial availability of polystyrene micro/nanobeads. It's also critical to assess the toxicity of various plastic polymers, particularly category 1 carcinogens and mutagens. Toxicity of fibrous or other shaped polymers at sizes that more closely resemble the majority of the MPs/NPs population in the natural environment is also required.



Furthermore, several investigations have been conducted at extremely high particle doses, which may or may not be environmentally relevant.

The toxicity of these particles is not conclusive due to a mismatch between the concentration, size, shape, and type of microplastics examined in the laboratory and those found in nature (Burns & Boxall, 2018). The lack of standardized analytical techniques for detecting and quantifying plastics in various matrices, as well as the establishment of plastic contamination control protocols during the analysis of collected samples, are important hurdles to determining MPs/NPs toxicity (Barbosa et al., 2020). Plastics with a diameter of fewer than 20  $\mu\text{m}$  are particularly difficult to identify and separate (Hale et al., 2020). The development of analytical procedures for MPs/NPs identification and quantitation would pave the way for the use of environmentally realistic dosages, sizes, forms, and kinds to better understand the health effects of these particles.

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