Mitochondria as a Target of Micro- and Nanoplastic Toxicity

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Abstract

Mitochondria are unique organelles to perform critical functions such as energy production, lipid oxidation, calcium homeostasis, steroid hormone synthesis in eukaryotic cells. The proper functioning of mitochondria is crucial for cellular survival, homeostasis, and bioenergetics. Mitochondrial structure and function are maintained by the mitochondrial quality control system, which consists of the processes of mitochondrial biogenesis, mitochondrial dynamics (fusion/fission), mitophagy, and mitochondrial unfolded protein response UPR\(^{MT}\). Mitochondrial dysfunction and/or damage is associated with the initiation and progression of several human diseases, including neurodegenerative, cardiovascular, age-related diseases, diabetes, and cancer. Environmental stress and contaminants may exacerbate the sensitivity of mitochondria to damage which cause mitochondrial dysfunction. There is growing evidence about the impact of nano-(NPs) and microplastics (MPs) on mitochondrial health and function. MPs/NPs were reported to trigger oxidative stress and reactive oxygen species production, the eventually change mitochondrial membrane potential. MPs/NPs can cross through the biological barriers in the human body and internalize by the cells, potentially altering mitochondrial dynamics, bioenergetics, and signaling pathways, thus impacting cellular metabolism and function. This review states the effects of MPs/NPs on mitochondrial homeostasis and function as well as on mitochondrial membrane dynamics, mitophagy, and mitochondrial apoptosis are discussed.

Keywords: Microplastics; nanoparticles; mitochondria; mitophagy; mitochondrial membrane dynamics.

Impact Statement

Given the critical role of mitochondria in cellular and organismal health, MPs/NPs pose a significant threat to mitochondrial health and function. The current evidence underscores the urgency of addressing the pervasive problem of MP/NP pollution, not only for the protection of the environment but also for human health. The information provided here should inspire and guide further research in several directions. The specific molecular mechanisms by which MPs/NPs affect mitochondrial health need to be elucidated. A deeper understanding of these processes could inform the development of strategies to mitigate these effects or be used as biomarkers of exposure or toxicity. In addition, this information should motivate regulators to reassess the environmental and health risks associated with MP/NP pollution, incorporating new knowledge on mitochondrial effects into these assessments. This could help to shape more comprehensive and effective strategies for dealing with plastic pollution, ranging from policies to reduce plastic waste and promote more sustainable materials, to remediation of existing pollution.
Introduction

Background on Microplastics (MPs) and Nanoplastics (NPs)
Over the past 70 years, the use of plastics has increased more than many other products, but the waste of plastics has spread throughout the environment as well (Kayan 2020). This has given rise to the term 'plastic debris', which is defined as "human-generated solid polymeric material waste that is intentionally or accidentally released into the environment". The production of plastic products reached approximately 390.7 million tons in 2021, and the negative impacts of persistent plastic waste on aquatic and terrestrial environmental health are of serious concern (EuropeanCommission 2019; PlasticsEurope 2022; Thompson et al. 2009). Polyethylene (PE), polypropylene, polystyrene (PS), polyvinyl chloride (PVC), polyethylene terephthalate (PET) and polyurethane used in the production of plastics are attracting more attention because they are produced in large quantities and are widespread in the environment (EuropeanCommission 2019; Pinto Da Costa 2020; PlasticsEurope 2021; Revel et al. 2018; Science Advice for Policy by European 2019; Vert et al. 2012). Although studies to determine the potential effects of plastic debris on human health have increased exponentially over the past decade, current knowledge is still insufficient to determine the risk. Of all the plastic produced, ~33% is not suitable for recycling and is thrown into the environment within one year of production (Koelmans et al. 2014). During the incineration process, which is one of the ways of dealing with plastic waste, toxic chemicals (such as furan, dioxin) that are harmful to human health and the environment are released. Furthermore, many countries have yet to introduce legislation to regulate the recycling of plastic waste, often opting instead for the cheaper and easier route of landfilling (Crawford and Quinn 2017).

Plastic waste accumulates in large quantities in terrestrial, marine and freshwater ecosystems. Today, plastic debris and the pollution it causes are recognized as one of the most important global environmental threats (EuropeanCommission 2019; Hale et al. 2020; PlasticsEurope 2021; Ryan et al. 2009; Science Advice for Policy by European 2019; Villarrubia-Gómez et al. 2018). It is estimated that ~10% of all plastics produced to date end up as litter in the oceans (Laglbauer et al. 2014). It has been reported that 61-87% of litter ≥5 mm in size and 98-99% of litter <5 mm in size is plastic (plastic film, plastic fibers, polystyrene, plastic pellets) (Tekman 2021). Recently, in addition to the visible macro form of plastic waste, microplastic (MP) and nanoplastic (NP) particles have also raised ecotoxicological concerns (Mattsson et al. 2018). MPs and NPs can be found as primary and secondary plastic particles, depending on the way they are formed. Primary particles are produced in a fixed size for the purpose, usually in the form of beads, while secondary particles are formed by the degradation of larger plastic materials (McDevitt et al. 2017). Secondary MPs/NPs are much more abundant in the environment than primary ones (Hale et al. 2020). Anthropogenic impacts, environmental factors such as solar radiation (UV photooxidation reactions), wind and waves, and abrasion from car tires are effective in the formation of secondary MPs/NPs pollution (Andrady 2015; Wagner et al. 2018). Although the rate and amount of nanofragmentation in nature is unknown, it is predicted that the fragmentation of MP particles with a size >100 nm-5 mm into NP particles with a size of 100 nm would lead to an NP particle concentration >10^14 times higher than the current MP particle concentration (Besseling et al. 2019). Ter Halle et al. (2017) detected PVC, PET, PS and PE polymers with a size of 1-999 nm in ocean surface samples for the first time. Although MP/NP pollution is considered a global problem, their potential risks to human health are far from known with the available data (Thompson et al. 2005; Thompson et al. 2004). Field studies have shown the presence of MP in a large proportion of living
organisms in the food chain (Hermsen et al. 2018; Lusher et al. 2017; Lusher et al. 2013). MPs has also been detected in bottled water and tap water (Kosuth et al. 2018; Mason et al. 2018; Mintenig et al. 2019; Mintenig et al. 2017). Studies have started to reveal that MPs/NPs can trigger physical and chemical toxicity in organisms (Bergmann et al. 2015; Klages et al. 2015; Wagner and Lambert 2018). In aquatic organisms, MPs have been shown to cause oxidative stress, genotoxicity, neurotoxicity, developmental delay, reduced reproductive success and death (De Sá et al. 2018). In addition, in vivo studies have shown that primary MPs/NPs accumulate in tissues after oral or respiratory exposure (Deng et al. 2021; Fan et al. 2022; Jeong et al. 2022a; Meng et al. 2022; Xu et al. 2021; Yang et al. 2022).

**Exposure and toxicity of MPs/NPs**

In humans, to determine whether exposure to plastic particles poses a public health risk, it is first necessary to understand the exposure to these substances and the hazard associated with exposure. Humans are exposed to MPs/NPs primarily through oral and dermal routes, inhalation, and during medical procedures (Prata et al. 2020). The fact that MPs have been detected in human feces and tissues (placenta, lung, and whole blood) in clinical studies and are beginning to be associated with disease suggests that the potential effects of MP/NP exposure should be taken seriously (Amato-Lourenço et al. 2021; Jenner et al. 2022; Leslie et al. 2022; Ragusa et al. 2021; Schwabl et al. 2019; Yan et al. 2022). While the detection of human MP exposure is still very new, the extent of NP exposure is unfortunately not yet known due to the lack of methods to detect particles of this size (EuropeanCommission 2019; Science Advice for Policy by European 2019).

Plastic particles smaller than 150 µm can enter the system by crossing the intestinal epithelium; NPs smaller than 100 nm can easily be taken into the cell and pose a threat to humans (Celebi Sözener et al. 2020; EFSA 2016). In vitro studies have shown that primary PS MPs/NPs are taken into cells, reduce cell viability, trigger apoptosis, alter reactive oxygen species (ROS) production, mitochondrial membrane potential (MMP) and function (Forte et al. 2016; Li et al. 2022; Prietl et al. 2014; Sun et al. 2023b; Wang et al. 2022a; Wu et al. 2019; Xu et al. 2019a).

When considering the routes of human exposure to NPs and MPs, the respiratory and digestive systems are the first areas of concern. However, it clearly demonstrates that MPs/NPs, which are small in size, can overcome biological barriers in humans and circulate through the blood system to access other tissues (Leslie et al. 2022). It has also been reported that NPs can cross the air-blood barrier in the lung and enter the bloodstream (Prata et al. 2020), while primary exposure to PS NPs can cause lung damage (Wu et al. 2023). In addition, primary PS NPs have been shown to accumulate in the brain by crossing the blood-brain barrier after intravascular injection (Yang et al. 2004). Mice exposed to primary PS NPs had a significant increase in blood glucose, glucose intolerance and insulin resistance. PS NPs exacerbated STZ-induced type 2 diabetes (Wang et al. 2023). PS NPs induced Parkinson's disease-like neurodegeneration in mice, so NP exposure should be carefully considered as a neurological health risk (Liang et al. 2022). Mitochondrial dysfunction, endoplasmic reticulum (ER) stress, oxidative stress, and lysosomal membrane damage are observed in diseases such as neurodegenerative diseases, inflammation, metabolic stress, oxidative stress, diabetes, cardiovascular diseases, gastrointestinal diseases, kidney and lung diseases, skin diseases, aging, and cancer (Burgos-Morón et al. 2019; Calì et al. 2011; Herst et al. 2017; Lee et al. 2022; Rana 2020; Ryter et al. 2018; Shacham et al. 2019; Xu et al. 2019b).

The organelles mitochondria, endoplasmic reticulum and lysosome, which play an important role in the pathophysiology of these diseases, are also targets of MP/NP toxicity (Halimu et al. 2022;
In this review, we will discuss studies revealing the effects of MPs/NPs on mitochondria.

**Mitochondrial toxicity of MPs/NPs**

*Impact of MPs/NPs on mitochondrial structure and function*

Due to their size and surface properties, MPs/NPs can physically interact with cellular structures, including mitochondria. Often referred to as the 'powerhouses of the cell', mitochondria are key organelles responsible for ATP production through oxidative phosphorylation. In addition to energy production, they regulate several cellular processes, including calcium homeostasis, apoptosis and the generation of reactive oxygen species (ROS). Exposure to these pollutants can induce mitochondrial damage and dysfunction, disrupting normal cellular operations and potentially leading to cell death. There is evidence that these particles can penetrate cell membranes and accumulate inside cells, possibly targeting mitochondria (Lee et al. 2022). This penetration appears to be facilitated by NPs small size, allowing them to cross biological barriers more easily than larger particles (Yang et al. 2021). Once inside the cell, MPs/NPs can disrupt mitochondrial structure. Studies in mice have shown that exposure to PS MPs can cause significant morphological changes in mitochondria, such as swelling and loss of cristae (Lin et al. 2022a). Building on the structural disruption mentioned above, these physical interactions may also induce functional abnormalities in mitochondria, further contributing to their toxicity. As the powerhouse of the cell, mitochondria play a critical role in maintaining cellular energy homeostasis (Lee et al. 2015). Recent evidence suggests that PS NPs can impair mitochondrial energy production capacity by disrupting the electron transport chain, leading to reduced ATP synthesis (Li et al. 2023a; Lin et al. 2022b; Trevisan et al. 2019). The impairments observed in mitochondrial function are not limited to energy production, but also extend to other vital processes such as signaling, mitochondrial dynamics, mitophagy, calcium homeostasis, and apoptosis. In *in vitro* (Table 1) and *in vivo* (Table 2) studies, decreased mitochondrial membrane potential ($\Delta \Psi_m$) was observed after PS, PVC and PET MPs/NPs (Chen et al. 2023; Chen et al. 2022; Florance et al. 2022; Halimu et al. 2022; Koner et al. 2023; Li et al. 2022; Liu et al. 2022; Salimi et al. 2022; Wang et al. 2020; Wu et al. 2019; Zhang et al. 2023; Zhang et al. 2022a). In a study by Sun et al., exposure to PS NPs (size 20 nm) resulted in collapse of $\Delta \Psi_m$, an event associated with the activation of cellular apoptosis, at TM3 mouse Leydig cells (Sun et al. 2023b). This finding was reinforced by studies showing increased expression of apoptosis-related proteins in cells exposed to MPs/NPs, providing further evidence that these particles may interfere with the role of mitochondria in the regulation of apoptosis (Li et al. 2021; Li et al. 2023b; Wang et al. 2022b). In addition, the interaction of MPs/NPs with mitochondria could also affect cellular calcium homeostasis, as calcium is critical for several mitochondrial functions, including ATP production, this disruption could have significant consequences for cellular health. Research has shown that PS nanoparticles with a size of 20 nm can increase intracellular calcium levels, possibly by disrupting mitochondrial calcium handling at SHSY-5Y human neuroblastoma cells and protozoan *Tetrahymena thermophila* which has a strong ability to ingest particles (Meindl et al. 2015; Wu et al. 2021). The impact of MPs/NPs on mitochondrial structure and function potentially links to various pathologies, highlighting the importance of elucidating the mechanistic pathways of these interactions for understanding systemic and long-term health effects.

*Induction of Mitochondrial ROS by MPs/NPs*
One of the critical consequences of the interaction of MPs/NPs with mitochondria is the induction of ROS. ROS are chemically reactive molecules that can be a by-product of normal metabolic processes, but when produced in excess they can lead to oxidative stress, causing damage to DNA, proteins and lipids in cells (Thannickal and Fanburg 2000). MPs/NPs have been reported to trigger the overproduction of ROS in mitochondria. A study by Li et al. showed that exposure to PS NPs with a diameter of 21.5 nm increased mitochondrial ROS levels in human hepatocellular carcinoma cells in a dose-dependent manner (Li et al. 2023a). Similar findings were observed in fish gills exposed to 1-5 µm sized MPs, where elevated levels of mitochondrial ROS were associated with a significant increase in lipid peroxidation, a marker of oxidative damage (Santos et al. 2022). Furthermore, this ROS-induced oxidative stress may exacerbate mitochondrial dysfunction by damaging mitochondrial proteins and disrupting ΔΨm, thereby amplifying the detrimental effects of MPs/NPs on mitochondrial function (Li et al. 2023a; Wang et al. 2021). Adding to this complexity, shape also appears to play a role in the interaction of these pollutants with cellular systems. Spherical and fiber/fragment-shaped PS MPs and NPs reduced intracellular H2O2 levels attributable to mitochondrial stress responses such as increased mitochondrial DNA content, footprint and morphology in Caco-2 cells (Saenen et al. 2023).

**Effects of MPs/NPs on Mitochondrial Dynamics**

MPs/NPs can also affect mitochondrial dynamics, a process that is critical for maintaining mitochondrial function and overall cellular health. Mitochondrial dynamics involves the balanced processes of mitochondrial fission and fusion, which are necessary for cell survival, adaptation to metabolic changes, and removal of damaged mitochondria (Youle and van der Bliek 2012). In general, Fis1, Mff, MiD49, MiD51 and dynamin-associated protein 1 (Drp1) are involved in mitochondrial fission (Bleazard et al. 1999; Mozdy et al. 2000; Tieu and Nunnari 2000). In mammals, Drp1 is usually distributed in the cytosol and some of it is found in the form of a dot on the outer membrane of mitochondria (Smirnova et al. 2001). During fission, dynamin homologues are transported to the Drp1 outer membrane knuckle region by intermediary proteins (Fis1, Mff, MiD49 and MiD51), where they form large homomultimetric structures that spirally envelop the mitochondria (Atkins et al. 2016). Mitochondrial fission also plays an active role in the even distribution of mitochondria to daughter cells during cell division, as well as in the transport of the organelle to energy-demanding sites in the cell, such as neuronal axons and lamellipods. Recent studies revealed that MPs/NPs may disrupt this balance and cause abnormal mitochondrial dynamics. A study in human liver cells showed that exposure to PS NPs led to increased mitochondrial fission, as evidenced by a significant increase in the expression of the fission protein Drp1 and p-Drp1 (Li et al. 2023a). Excessive fission is often associated with mitochondrial fragmentation and cell death, suggesting a potential pathway for NP-induced toxicity. Conversely, NPs may also interfere with mitochondrial fusion, a process necessary for the sharing of mitochondrial DNA and other essential components. Mitochondrial fusion is a complex process in which two neighbouring organelles are connected to each other and two independent membranes (inner and outer membranes of mitochondria) are fused in harmony without any significant loss of mitochondrial proteins (e.g. cytochrome c) that could lead to cell death. In mammals, Mfn1 and Mfn2 proteins called mitofusins are involved in outer membrane fusion, while OPA1 protein is involved in inner membrane fusion. Fu et al. found that exposure to amino-functionalized PS NPs increased the mRNA expression level of MFN2 (mitochondrial fusion related gene) in Human Umbilical Vein Endothelial Cells (HUVECs) (Fu et al. 2022). Another study conducted on human
bone marrow-derived mesenchymal stem cells (hBM-MSCs) also revealed that surfactant-free amine-functionalized PS NPs and PS NPs with decreased cross-linking density (DPS-NPs) led to upregulation of MFN2 expression and downregulation of FIS1 (mitochondrial fission related gene) expression (Im et al. 2022). Interestingly there were opposite results regarding OPA1 levels in mouse and chicken experiments when exposed to PS MP’s. It was found that after GC-2 mouse cells were exposed to PS MP’s for 24 hours, both mRNA and protein expression levels of OPA1 was increased along with Drp1 (Liu et al. 2022). However, in another study conducted on chickens it was shown that after 42 days of exposure to PS MP’s, mRNA and protein expression levels of OPA1 was decreased along with Mfn1 and Mfn2 suggesting a decrease at mitochondrial fusion. Conversely, Drp1 mRNA and protein expression levels were increased suggesting an increase at mitochondrial fission (Zhang et al. 2022b). These conflicting findings underscore the complexity of microplastic interactions within biological systems and highlight the species-specific responses to PS MP exposure, which may affect mitochondrial dynamics in diverse ways.

**Induction of Mitochondrial Unfolded Protein Response (UPR\textsuperscript{mt}) by MPs/NPs**

MPs/NPs may also exert their toxic effects by disrupting the mitochondrial unfolded protein response (UPR\textsuperscript{mt}), a protective cellular mechanism that is activated in response to the accumulation of misfolded proteins in mitochondria (Xu et al. 2022). The UPR\textsuperscript{mt} plays a critical role in maintaining mitochondrial proteostasis, thereby contributing to overall mitochondrial health and functionality. Due to their ability to induce oxidative stress and disrupt mitochondrial function, MPs/NPs may lead to protein misfolding within mitochondria. A study by Liu and Wang showed that exposure to PS NP particles with a size of 100 nm significantly increased the expression of HSP6, a marker of the UPR\textsuperscript{mt}, in *Caenorhabditis elegans* (Liu and Wang 2021). This suggests that NPs may lead to protein misfolding and subsequent activation of the UPR\textsuperscript{mt}. However, chronic activation of the UPR\textsuperscript{mt}, as may occur with continuous or repeated exposure to NPs, may become maladaptive. Prolonged activation of the UPR\textsuperscript{mt} has been associated with mitochondrial dysfunction (Lin et al. 2016). Therefore, MPs/NPs-induced activation of the UPR\textsuperscript{mt} may represent another mechanism of their cellular toxicity. In addition, disruption of the UPR\textsuperscript{mt} may have further implications for mitochondrial dynamics, as protein homeostasis is crucial for maintaining balanced fission and fusion processes. Thus, the interaction of MPs/NPs with the UPR\textsuperscript{mt} could add another layer of complexity to their impact on mitochondrial health.

**Effects of MPs/NPs on Mitophagy**

Another important aspect to consider in the interaction between MPs/NPs and mitochondria is the process of mitophagy, the selective degradation of damaged mitochondria by autophagy. This mechanism plays an important role in maintaining cellular homeostasis by removing dysfunctional mitochondria and recycling their components (Onishi et al. 2021). In the context of MP/NP-induced mitochondrial damage, the PINK1/Parkin pathway plays a pivotal role. Upon mitochondrial depolarization or damage, PINK1, a kinase, stabilizes on the outer mitochondrial membrane. This stabilization signals the recruitment of Parkin, an E3 ubiquitin ligase and once Parkin is recruited, it ubiquitinates various mitochondrial proteins (Mfn1, Mfn2, Drp1 and TOM20) marking the damaged mitochondria for degradation (Gegg and Schapira 2011; Wang et al. 2011; Yoshii et al. 2011). This selective autophagy process, crucial for cellular health, ensures the removal of dysfunctional mitochondria, thereby preventing potential cellular damage induced by MPs/NPs. A study by Xu et al. found that PS NPs with the size of 100 nm accumulated in mitochondria and induced PINK1/Parkin-mediated mitophagy in mice, likely as an effort to
eliminate mitochondria damaged by oxidative stress and mitochondrial dysfunction (Xu et al. 2023). This observation is consistent with the known role of mitophagy as a response to stressful conditions, such as ROS overproduction (Onishi et al. 2021). However, continuous or high-level activation of mitophagy could be detrimental. Prolonged stimulation of mitophagy, especially in the absence of effective biogenesis to replace degraded mitochondria, could lead to overall loss of mitochondrial mass and function, contributing to further cellular stress and even cell death (Kubli and Gustafsson 2012). Furthermore, the involvement of mitophagy highlights the interconnectedness of the different mitochondrial responses to MPs/NPs exposure. These findings also emphasize the complex and potentially detrimental effects of MPs/NPs pollution on mitochondrial health and cellular function, including disruptions in energy production, increased oxidative stress and induction of apoptotic pathways. The urgent need for targeted research to fully understand the extent of MPs/NPs toxicity, the implementation of stricter pollution controls to reduce exposure, and the development of innovative solutions to remove existing pollutants from the environment is underscored by these negative outcomes. This will help protect public health and biodiversity. Mitochondrial biogenesis, the formation of new mitochondria within the cell, is another critical cellular process that could be disrupted by exposure to MPs/NPs. Mitochondrial biogenesis is essential for replacing damaged mitochondria and adjusting the mitochondrial population within a cell to meet changing metabolic demands (Kubli and Gustafsson 2012). Disruption of this process can have a significant impact on cellular health, potentially leading to energy depletion, increased oxidative stress and increased susceptibility to cell death. Exposure to environmental stressors, such as MPs/NPs, could potentially trigger such disruptions. However, a latest study by Jeong et al. revealed that mitochondrial biogenesis was increased at PS MPs and chromium exposed fresh water flea, Daphnia magna, compared to chromium only treated group suggesting that MPs expel chromium from cells (Jeong et al. 2022b). The group exposed to chromium-only showed a decrease in PGC-1a gene expression and an increase in Drp1 gene expression, indicating that chromium may cause mitochondrial dysfunction. However, exposure to both MPs and chromium resulted in increased PGC-1a expression and decreased Drp1 expression, suggesting a potential mitigating effect on mitochondrial dysfunction compared to chromium exposure alone. While the available study provides initial insights into the potential impacts of MPs/NPs on mitochondrial biogenesis in freshwater fleas, including their intriguing role in mitigating the effects of heavy metals, it should be emphasized that this research does not extend to human data. Consequently, a comprehensive understanding and broader conclusions regarding such effects in humans necessitate further in-depth studies.

Conclusion
The toxicity of MPs/NPs is a serious environmental and public health problem that is not yet fully understood. The unique physicochemical properties of these particles, including their small size and large surface area, enable them to penetrate biological membranes and accumulate in various organs where they can induce a range of adverse effects. This review has highlighted one particular area of concern - the effects of exposure to MPs/NPs on mitochondria, a critical cellular organelle responsible for energy production and several other vital functions. Evidence suggests that MPs/NPs can induce mitochondrial dysfunction, primarily through the generation of oxidative stress, which damages mitochondrial components and impairs mitochondrial function. This can result in reduced ATP production, which can disrupt cellular processes and lead to cell death. MPs/NPs have also been found to physically interact with
mitochondria, causing structural damage and contributing to functional impairment. These effects can in turn trigger a cascade of cellular responses, from inflammation to apoptosis, contributing to the overall toxicity of MPs/NPs. In addition, exposure to MPs/NPs may disrupt the dynamic processes that maintain mitochondrial health, including mitochondrial dynamics and the UPR\textsuperscript{mt}. Also, emerging research suggests that MPs/NPs could disrupt mitochondrial biogenesis, potentially leading to a decrease in mitochondrial mass and further impairing cellular health and function. Such a chain of detrimental effects highlights the importance of understanding the impact of MPs/NPs exposure on mitochondria, not only in terms of cellular health, but also considering potential systemic effects and long-term effects on organismic health.

**Knowledge Gaps and Future Perspectives**
Mitochondrial damage and dysfunction are related to numerous health conditions, suggesting that exposure to MPs/NPs could have far-reaching effects on human health. Therefore, it is crucial to investigate the potential impact of MPs and NPs on human cells to raise awareness of this issue and take necessary precautions. The analytical methods used are inadequate to measure the concentration of NPs in the environment and organisms and therefore little is known about the importance of NPs for human health (Science Advice for Policy by European 2019). Therefore, it is important to first develop analytical methods that can analyze not only MPs but also NPs, which will enable a full understanding of human exposure.

The type of PS NPs that have been shown to cause adverse effects in human and animal cells in *in vitro* and *in vivo* studies are primary ones. Primary MPs/NPs have a smooth surface and uniform shape (uniform; nanobeads). Secondary MPs/NPs, on the other hand, are formed in a wide variety of shapes compared to those of primary origin (Koelmans et al. 2015; Lei et al. 2018). At the same time, when primary PS NPs are released into the environment, their structures deteriorate and their properties change after a certain period of time like secondary particles (Im et al. 2022). The shapes of secondary MP/NP particles are amorphous and it has been shown that the negative effects of particles without smooth surfaces on the cell are more than those with smooth surfaces (Qin et al. 2022; Völkl et al. 2022). Therefore, the effects of secondary MPs/NPs, which are more abundant in the environment, need to be investigated and studies need to be designed to realistically assess human exposure.

In addition, the studies in the literature were conducted with commercially available PS-type MPs/NPs. However, MPs/NPs in the environment also consist of other types of plastic polymers other than PS. Therefore, the effects of MPs/NPs composed of these types of plastic polymers on the mitochondria should be investigated as well.

Furthermore, given the wide range of plastic types, sizes, shapes and chemical compositions present in the environment, research should also focus on investigating whether and how these different factors modulate the effects of MPs/NPs on mitochondria and other cellular components. The field of MPs/NPs research, particularly in relation to their effects on mitochondria, is still evolving. Existing studies have primarily used *in vitro* models, and more *in vivo* and human epidemiological research are needed to validate these findings and gain a more nuanced understanding of these interactions and their implications for organismal health. Such studies will provide a more realistic understanding of exposure levels, uptake mechanisms and physiological consequences of MP and NP exposure. Moreover, further work is needed to clarify the molecular mechanisms underlying the effects of MPs/NPs on mitochondria and to determine the extent to which these effects contribute to the overall toxicity of these pollutants. Research in this area could
help to inform risk assessments and guide the development of strategies to mitigate the effects of NP and MP pollution.

In the production of plastics, some additives [UV stabilisers, antioxidants, plasticisers (such as phthalate diester), colourants, fillers, etc.] are added to the products along with the polymer (ECHA 2018; Murphy 2001; Ventrice et al. 2013). There are many studies revealing the effects of these chemicals added to plastics on animals and humans (Ding et al. 2021; Frederiksen et al. 2007; Gray Jr et al. 2000; Lyche et al. 2009; Svensson et al. 2011). NP/MP act as vectors for toxic chemical contaminants and pathogenic microbes by sorbing to their surfaces and cavities (Rai et al. 2022). MPs/NPs have certain properties that facilitate their ability to adsorb various environmental pollutants. In this way, they increase exposure to these chemicals along with themselves (Sun et al. 2023a). The combined effects of these chemicals need to be taken into account when elucidating the effects of MPs/NPs on mitochondria and other cell components.

This extensive body of information emphasizes the importance of increasing awareness among individuals, communities, industries, and policymakers about the potential health risks associated with MP and NP pollution. These risks include respiratory problems, endocrine disruption, and other long-term health effects. There is an urgent need for comprehensive research to better understand the impacts of plastic pollution. Effective waste management practices should be implemented to reduce pollution at the source. Policies aimed at minimizing the production and use of plastic products are necessary to protect human health and the environment. This awareness should be channeled into individual action and policy development aimed at reducing plastic waste and promoting sustainable alternatives. In addition, further research is crucial to fill gaps in our understanding of the impacts of MPs/NPs on human health, particularly the long-term effects. More comprehensive studies are needed to better characterize human MPs/NPs exposure to elucidate their mechanisms of action in our bodies, and to identify potential strategies to mitigate their impacts. The public should also be aware that these findings are based on experimental models and while they indicate potential risks, the actual human health outcomes from real-world exposure scenarios might differ, which further underscores the need for ongoing research in this field. These potential risks underscore the urgency to better understand the precise mechanisms of MP and NP toxicity and to develop effective strategies to mitigate their presence in our environment. The collective effort towards these goals will necessitate cross-disciplinary collaboration encompassing environmental science, toxicology, public health, policymaking, and more.

Author Contribution statement
Authors FDY and MAA contributed equally to the conception, design and writing of the review article. They conducted the literature search, analyzed the selected articles, and synthesized the information in the review. Both authors participated in drafting and revising the manuscript. They read and approved the final version of the manuscript.

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Conflict of Interest statement
The authors declare none
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<td>hBM-MSCs human bone marrow mesenchymal stem cells</td>
<td>PS NP and amine-functionalized PS NP (400 nm)</td>
<td>0.6, 1.2, and 2.4 mg/mL        (24 h)</td>
<td>Decreased cytotoxicity and ROS scavenging effects and promoted mitochondrial fusion and inhibited mitochondrial fission after PS NP and amine-functionalized PS NP exposure in hBM-MSCs.</td>
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<td>Fu et al. (2022)</td>
<td>HUVEC human umbilical vein endothelial cell line</td>
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<tr>
<td>Study</td>
<td>Cell Line</td>
<td>Treatment</td>
<td>Concentration</td>
<td>Effect</td>
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<tr>
<td><strong>Lin et al. (2022b)</strong></td>
<td>L02 human hepatic cell line (BEAS-2B human lung epithelial cell line)</td>
<td>Nonfluorescent fluorescent PS NP (80 nm)</td>
<td>0.006, 0.0125, 0.03125, 0.0625, 0.125, and 0.25 mg/mL (48 h)</td>
<td>Mitochondrial damage evidenced by overproduction of mitochondrial ROS and alterations in ΔΨm; decreased ATP production and suppression of mitochondrial respiration.</td>
</tr>
<tr>
<td><strong>Wang et al. (2021)</strong></td>
<td>HK-2 human kidney proximal tubular epithelial cell line</td>
<td>PS MP (2 μm)</td>
<td>0.025, 0.05, 0.1, 0.2, 0.4, and 0.8 μg/mL (6 h)</td>
<td>Higher mitochondrial ROS levels at 0.2, 0.4, or 0.8 mg/mL PS MP; increased the expression of Bad after 0.8 mg/mL PS MP exposure for 5–60 min; decreased expression of Bcl2 after PS MP exposure for 20–60 min.</td>
</tr>
<tr>
<td><strong>Chen et al. (2022)</strong></td>
<td>HEK293 human embryonic kidney cells</td>
<td>PS MP (3.39 ± 0.30 μm)</td>
<td>300 ng/mL (24 h)</td>
<td>Increased ROS and oxidative stress; decreased ΔΨm.</td>
</tr>
<tr>
<td><strong>Li et al. (2022)</strong></td>
<td>Murine splenic lymphocytes</td>
<td>unmodified PS NP (20 and 50 nm), PS NP-FITC (20 and 50 nm), surface-charged PS SO3H-NPs (20 nm) and PS NH2-NPs (20 nm)</td>
<td>40 μg/mL for 20 nm, 200 μg/mL for 50 nm (6 h)</td>
<td>Increased ROS after PS SO3H-NP (20 nm), PS NP (20 and 50 nm); decreased ΔΨm after PS NH2-NPs (20 nm) after 6 h of exposure; decreased ΔΨm after PS SO3H-NP (20 nm), PS NP (20 and 50 nm) exposure with ROS accumulation; affected the basic respiratory capacity and ATP production capacity of splenocytes accompanying with the damage of mitochondrial membrane by all four PS NPs.</td>
</tr>
<tr>
<td><strong>Xu et al. (2023)</strong></td>
<td>Caco-2 human intestine epithelial cell line</td>
<td>unmodified and fluorescent-labeled PS NP, PS NP-COOH, and PS NP-NH2 (~100 nm)</td>
<td>30, 60 and 120 μg/mL (24 h)</td>
<td>Increased mitochondrial ROS; fractured, fuzzy cristae, ruptured membrane, blocked mitophagic flux, and vacuoles in mitochondria; accumulation of PS NPs in the mitochondria and the subsequent induction of</td>
</tr>
<tr>
<td>Study</td>
<td>Cell Type</td>
<td>NP Type</td>
<td>Concentration (μg/mL) (24 h)</td>
<td>Effect</td>
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<tr>
<td>Zhang et al. (2022a)</td>
<td>A549 human lung carcinoma cells</td>
<td>PET NP (122–221 nm)</td>
<td>4.92 and 49.20</td>
<td>Increased ROS, decreased tendency of ΔΨm induced by PET NP exposure</td>
</tr>
<tr>
<td>Sun et al. (2023b)</td>
<td>TM3 mouse Leydig cells</td>
<td>PS-NPs (20 nm)</td>
<td>50, 100 and 150</td>
<td>Increased ROS generation and initiated cellular oxidative stress and apoptosis; affected the mitochondrial DNA copy number and collapsed ΔΨm after PS NPs exposure accompanied by a disrupted energy metabolism</td>
</tr>
<tr>
<td>Chen et al. (2023)</td>
<td>RAW264.7 macrophage cells</td>
<td>Green fluorescein-labeled and unlabeled PS NP, PS NP-COOH, and PS NP-NH2 (100 nm)</td>
<td>10, 20, 50, 100</td>
<td>Increased intracellular ROS, depolarized ΔΨm after PS NP exposure; the most pronounced mitochondrial damage effect exhibited by PS NP-NH2</td>
</tr>
<tr>
<td>Florance et al. (2022)</td>
<td>HaCaT human keratinocytes, A549 human lung cancer cell line, RAW 264.7 murine macrophages THP-1 human monocytes Chang Liver cells</td>
<td>Yellow-green fluorescein labeled PS NP and sulfate modified PS NP (0.20 μm)</td>
<td>50 and 100</td>
<td>Increased mitochondrial ROS and decreased ΔΨm in RAW 264.7 and THP-1 cells</td>
</tr>
<tr>
<td>Liu et al. (2022)</td>
<td>GC-2 mouse spermatocyte line</td>
<td>PS MP (5 μm)</td>
<td>50, 100, 200, 400 and 800</td>
<td>Increased ROS and MDA, decreased ATP content, reduced ΔΨm; damaged the integrity of the mitochondrial genome; imbalance of homoeostasis between mitochondrial division and fusion</td>
</tr>
<tr>
<td>Study</td>
<td>Cell Line</td>
<td>NPs Size/Composition</td>
<td>Concentrations (μg/mL)</td>
<td>Effects</td>
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<td>Koner et al. (2023)</td>
<td>THP-1 macrophage cells</td>
<td>PS NP (&lt; 450 nm)</td>
<td>50, 100, 150, 200 and 500 (4/ 24 / 48 / 72 h)</td>
<td>Increased ROS at 50 μg/mL after 24 h exposure. Mitochondrial membrane damage after PS NP exposure for 4 and 24 h</td>
</tr>
<tr>
<td>Li et al. (2023a)</td>
<td>HepG2 human hepatocellular carcinoma cell line</td>
<td>PS NP (21.5 ± 2.7 nm)</td>
<td>6.25, 12.5, 25 and 50 (24 h)</td>
<td>Induced morphological changes of mitochondria; decreased ATP production and the loss of ΔΨm; increased ROS and mitochondrial fission by increased DRP1 and decreased OPA1 protein levels</td>
</tr>
<tr>
<td>Wu et al. (2019)</td>
<td>Caco-2 human colon adenocarcinoma</td>
<td>PS NP/MP (0.1 and 5 μm)</td>
<td>1, 10, 40, 80, 200 (12 / 24 h)</td>
<td>Low toxicity on cell viability, oxidative stress, and membrane integrity and fluidity; disrupted ΔΨm by both sizes of PS NP/MP; higher effects induced by 5 μm PS MP than 0.1 μm PS NPs</td>
</tr>
</tbody>
</table>
Table 2. Summary of *in vivo* studies assessing the effects of MP/NP exposure on mitochondrial function.

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Organism</th>
<th>MP/NP type (size)</th>
<th>Concentrations (exposure time)</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wu et al. (2021)</td>
<td><em>Tetrahymena thermophila</em></td>
<td>PS NP (20 nm)</td>
<td>0.3, 1, 3, 10, and 30 mg/L (24 h)</td>
<td>Ca accumulation in mitochondria, which increased mitochondrial permeability and the generation of ROS</td>
</tr>
<tr>
<td>Jeong et al. (2022b)</td>
<td><em>Daphnia magna</em></td>
<td>PS MP / 6 μm (Nonfunctionalized)</td>
<td>0, 2.5, 5, 10, 20, and 30 mg/L (24/48 h)</td>
<td>Increased oxidative stress; inhibiting the adverse effects of chromium by increasing mitochondrial biogenesis</td>
</tr>
<tr>
<td>Zhang et al. (2023)</td>
<td><em>Danio rerio</em></td>
<td>Freen fluorescent PS NP (100 nm)</td>
<td>1 mg/L (30 days)</td>
<td>Increased ROS; damaged the mitochondrial membrane and mtDNA in brain tissue</td>
</tr>
<tr>
<td>Trevisan et al. (2019)</td>
<td><em>Danio rerio</em> wild-type zebrafish, transgenic lines Tg(Flk1:EGFP) and Tg(MLS-EGFP)</td>
<td>PS NP (44 nm)</td>
<td>0.1, 1 or 10 ppm (24 or 96 hours)</td>
<td>Decreased the mitochondrial coupling efficiency and increased NADH production, suggesting and impairment on ATP production</td>
</tr>
<tr>
<td>Zhang et al. (2022b)</td>
<td>One-day-old chicks</td>
<td>PS MP (5 μm)</td>
<td>1, 10, 100 mg/L (42 days)</td>
<td>Increased ROS; induced mitochondrial damage (TFAM, OPA1, MFN1 and MFN2 down-expression, DRP1 and Fis1 overexpression) and energy metabolism disorders (HK2, PKM2, PDHX and LDH up-regulation) by inhibiting AMPK-PGC-1α pathway in cardiomyocytes.</td>
</tr>
<tr>
<td>Xu et al. (2023)</td>
<td>Male specific pathogen-free BALB/c mice (6 weeks-old)</td>
<td>unmodified and fluorescent-labeled PS NP, PS NP-COOH, and PS NP-NH2 (~100 nm)</td>
<td>1 mg/day, gavage (28 days)</td>
<td>Increased PINK1 and Parkin expression and mitophagy in ileum tissues</td>
</tr>
<tr>
<td>Lin et al. (2022a)</td>
<td>Male C57BL/6 mice (8-week-old)</td>
<td>PS NP (94.09 ± 8.07 nm)</td>
<td>5 μg/g body weight, intraperitoneal (once every other day for two weeks)</td>
<td>Increased ROS, MDA; decreased T-SOD, GSH and CAT; observed swollen and vacuolized mitochondria in myocardial cells</td>
</tr>
</tbody>
</table>