Investigations of Microplastic in Human Blood and It's Impact On Mammalian System

[1]Ayaz.A.Shaikh, [2]Naved.Y.Shaikh, [3]Ashik.S.Khatik

Department of Pharmacy
Shastry institute of pharmacy Erandol
District Jalgaon.

Abstract- **Plastics have enormous impacts to every aspect of daily life including technology, medicine and treatments, and domestic appliances. Most of the used plastics are thrown away by consumers after a single use, which has become a huge environmental problem as they will end up in landfill, oceans and other waterways. These plastics are discarded in vast numbers each day, and the breaking down of the plastics from micro- to nano-sizes has led to worries about how toxic these plastics are to the environment and humans.**

The global increase in plastic production has been a matter of debate and a growing environmental concern the past decades. As a result, microplastic have been discovered in several environmental media and detected in the food chain. Evidence suggests that humans are ingesting microplastic through contaminated food and drink and microplastic has been discovered in human feces albeit in a limited number of individuals. Thus, a proportion number of ingested MP may be absorbed and translocated into the human blood stream. A limited number of studies have identified nanoplastic can interact with platelets and thus increase the risk of thrombosis.

The new research was funded by the Dutch National Organisation for Health Research and Development and Common Seas, a social enterprise working to reduce plastic pollution.

"Plastic production is set to double by 2040," said Jo Royle, founder of the charity Common Seas. "We have a right to know what all this plastic is doing to our bodies." Common Seas, along with more than 80 NGOs, scientists and MPs, are asking the UK government to allocate £15m to research on the human health impacts of plastic. The EU is already funding research on the impact of microplastic on foetuses and babies, and on the immune system.

Keywords: **microplastic's, Nanoplastic's, blood, effect on mammalian system, toxicity, Microplastics exposure.**

INTRODUCTION:

Marine debris refers to any human-generated solid material that enters and pollutes our oceans, including plastics, metals, glass, and other waste products. Among these, plastic waste has become a major concern due to its durability, widespread use, and inadequate disposal practices. It is estimated that more than 8 million metric tons of plastic waste enters oceans yearly. This waste often takes centuries to decompose, leading to long-lasting pollution and negative impacts on marine ecosystems. About 7800 metric ton of plastic is been produced until 2015. Research states that marine debris primarily consists of thrown out and scrap plastics. A nearly 60 million tonne increase in plastic production occurred from 2014 to 2019, producing approximately 370 million tonne of plastic in 2019. Due to the relatively low cost and convenience of manufacturing plastic products The soil and oceans are polluted with hundreds of tons of plastic. Plastic debris accounts for 60-80% of marine litter.[1]

Plastic is a ubiquitous material in our daily lives that has become unavoidable. Due to its exceptional physical and chemical properties including exibility, infrangibility, low density, low electrical conductivity, etc. as well as its low cost, plastic has essentially supplanted the usage of wood and metal in many applications.1 The most common types of plastics used globally in medical, industrial, and consumer products are polypropylene (PP; medical and electronic equipment, straws, furniture), polyethylene (PE), which is mainly used in its low density form (LDPE; bin bags, plastic wrap, shopping bags) and high-density form (HDPE; irrigation and drainage pipes, shampoo bottles, detergent bottles),polyvinyl chloride (PVC; electrical cable insulation, doorframes, toys, pipes), polystyrene (PS; foam food containers, plastic containers, rigid trays, audio and video cassettes, lids, and tumblers)and polyethylene terephthalate (PET; bottles, vehicle tires, conveyers, drive or seat belts, food trays).Plastic is beneficial; however, it takes hundreds of years to decompose and is not biodegradable.

The natural environment is gradually becoming contaminated with plastic debris, including used plastic bags, bottles, and containers; this contamination has emerged as a critical global issue causing environmental stress and uncontrollable harm to living systems.

A prognosis for 2050 predicts that the amount of plastic garbage in land and/or the environment will reach close to 12 000 Mt globally, and in seas and oceans, it will surpass. Plastic fragments degrade when exposed to ultraviolet light, weathering, and biodegradation, resulting in the formation of a heterogeneous mixture of microplastics (MPs) and nanoplastics (NPs). These plastic residues are classified as large microplastics (5 mm to 1 mm), small microplastics (1 mm to 1 mm), and nanoplastics (<1 mm).

The ocean and other bodies of water are significantly contaminated by these microplastics. Thompson et al. formally coined the term "microplastic" (MP) in 2004, in response to the growing problem of plastic pollution in the seas, and stated that these lightweight fragments follow atmospheric currents and are dispersed globally. Microplastics are therefore present in every environmental compartment (air, soil, and bodies of water) and their presence is increasing at an alarming rate. MPs can be classified into two groups: primary and secondary. Primary MPs are directly released into the environment as microscale plastics (<5000 mm), such as in cosmetic products, toothpaste, pharmaceutical vectors (nanovehicles), etc.[2]

A 2016 UN report documented over 800 animal species contaminated with plastic via ingestion orentanglement—a figure 69% greater than that reported in a 1977 review, which estimated only 247 contaminated species Of these 800 species, 220 have been found to ingest microplastic debris in natura
Plastic ingestion occurs across taxa within different trophic levels, including marine mammals, fish, invertebrates, and fish-eating birds Plastic particles are often found concentrated in an organism's digestive tract during carcass dissection and laboratory research. With preference to smaller particles, micro- and nanoplastics can persist in the animal's body and translocate from the intestinal tract to the circulatory system or surrounding tissue.[7]

Marine anthropogenic litter causes harm to a wide range of marine biota. Seabirds, fish, turtles and marine mammals suffer from entanglement with and ingestion of marine litter items as illustrated by countless pictures of animals injured and strangled by discarded fishing gear in the public media. However, we have only limited knowledge about the implications of marine litter for the many less charismatic invertebrate species that easily escape public perception but play important roles in marine ecosystems. Although already mentioned in the late 1980s (Ryan 1988), it took Thompson's time series (Thompson et al. 2004) to raise public awareness of the widespread presence of microplastics, which are used in industrial production processes, cosmetics and toothpaste or generated through degradation of larger items. Indeed, substantial concentrations of microplastics were recently reported from remote and presumably unspoiled environments such as the deep seafloor (Woodall et al. 2014) and Arctic sea ice, which is considered a historic global sink at least until its plastic load is released into the ocean during the projected increase of ice melts (Obbard et al. 2014). Microplastics are available for ingestion by a wide range of organisms, and there are indications that microplastics are propagated over trophic levels of the marine food web (Farrell and Nelson 2013; Setälä et al. 2014). However, scientists have only recently started to investigate whether the contamination of marine organisms with plastics and associated chemicals is causing harm to ecosystems and human health (Browne et al. 2013; Bakir et al. 2014; De Witte et al. 2014; Van Cauwenberghe and Janssen 2014).[8] MPs have been found to present in the soft tissues of two common bivalves that humans consume: Mytilus edulis and Crassostrea gigas Based on the abundance of MPs in the bodies of these two commercial bivalves, European consumers of shellfish are estimated to intake 11,000 kinds of MPs in their diet each year, indicating that the MPs accumulated in bivalves could be an important exposure route for people who consume seafood.[14]

DISCUSSION & RESULT:

MNPs Distribution:

MNPs entered into tissues are taken care of by tissue macrophages . MNPs also interact with immune cells leading to transport to the lymphatic system and also to different organs From the lymphatic system, MNPs can enter the bloodstream and also from primarily exposed organs into the bloodstream Once MNPs reach the bloodstream via blood, they distribute to the whole body. MNPs have been found in various organs, including the liver spleen kidneys and urine bone marrow brain and cerebrospinal fluid and the placenta MNPs-associated inflammatory responses decrease the integrity of epithelial barriers and activate macrophages leading to increased internalization of MNPs Irreversible accumulation of MNPs in human tissues for a prolonged period of time contributes to severe health issues.

Liver:

MNPs can cause hepatotoxicity and also affect lipid metabolism in the liver. MNPs can induce DNA damage and release in the nucleus and mitochondria. Potential risks of MNPs on the onset of liver steatosis, fibrosis, and cancer were also reported. MNPs are permeable to liver cells and accumulate in the liver.

Heart:

investigated the presence of MPs in human arterial thrombi using μRaman spectroscopy In their study, 16 out of the 26 samples analyzed resulted in being positive for MPs. A total of 87 particles with a size range between 2.1 and 26 μm were found globally[21]MPs exposure has been reported to increase troponin I and creatine kinase-MB (CKMB) levels in serum, leading to structural damage to the myocardium and apoptosis, also associated with collagen proliferation in the heart. Exposure to MPs can cause cardiac fibrosis by activating the Wnt/β-catenin pathway. It was also reported that MPs-induced oxidative stress mediates cardiovascular toxicity.[9]

Air pollutants, such as particulate matter, promote cardiotoxicity, compromising vascular function, increasing blood pressure, and promoting myocardial infarction, thus highlighting that they represent a risk factor for cardiovascular disease. Therefore, investigating the potential for MP/NPs role in cardiovascular toxicity may have enormous social and economic benefits.[27]

Effect of MNPs on Immune system.

The immune system is a network of lymphoid organs, tissues, cells, humoral substances, and cytokines that work together to defend the body An important function of the immune system is to eliminate invading bacteria, foreign cells, macromolecular compounds (antigens), and extraneous particles. MNPs trigger a local or systemic immunological response when entering an organism, and some MNPs generate a protein corona on their surface that enables them to escape the immune system Experiments on animals or cells have shown that MNPs can lead to increased secretion of pro-inflammatory cytokines, disrupting immune homeostasis and ultimately leading to immune system disorders such as autoimmune diseases.[10]

It is reasonable to expect the immune system to be affected by microplastic exposure, as its main function is to provide protection against foreign particles. Inflammation is an immune response against damaged cells, irritants and pathogens that is highly coordinated by genes expressed in immune cells, particularly neutrophils. Inflammation can be acute (short-term) followed by healing or chronic (long-term) and characterized by persistent and increased expression of inflammatory cytokines.[26]

MNPs, once entered into the human body, interact with immune cells to produce an inflammatory response, it depends on the size of the plastic particles, and it was reported to be more with microplastics than nanoplastics Increased production of IL-6 and IL-8 by macrophages on exposure to polystyrene nanoplastics results in a profound increase in the inflammatory response. TNFα and IL-1 mediated inflammatory response on exposure to polyethylene MNPs has been reported Prolonged exposure-mediated increased inflammation and associated ROS production are responsible for hemolysis Increased ROS production is also associated with the onset of autoimmune diseases. MNPs-mediated production of anti-inflammatory mediators and suppression of T-cells was also reported MNPs exposure also induces cytokine production associated with inflammation, immune stimulation, stress response, and proliferation of human white blood cells It was reported that increased pro-inflammatory mediator secretion due to MNPs exposure is associated with cytotoxicity The involvement of toll-like receptors in mediating inflammatory reaction cascade in response to MNPs exposure has also been reported Disruption in microbiome composition due to exposure to MNPs has been reported to be responsible for changes in immune response.[9]

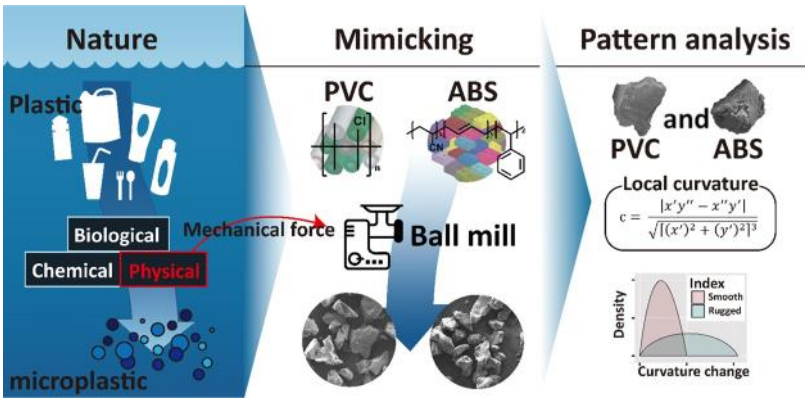

Figure 1. Schematic illustration of mimicking the environment and shape pattern analysis used in this study.

penicillin/streptomycin, and 10% FBS. All cells were cultured in the same way. Briefly, PBMCs were pretreated with the RBC lysis buffer (Roche, Germany) for 5 min before seeding. The supernatant was then removed by centrifugation at 330g for 7 min. Once isolated, all cells were cultured at appropriate seeding densities in 100 mm dishes (SPL) and grown to a confluency of 70% or greater after 2−3 days at 37 °C in a humidified atmosphere of 5% CO2.

Hemolysis. Sheep blood cells (MBcell) were centrifuged at 2000 rpm for 5 min to obtain only red blood cells. Repeated centrifugation was performed until we obtained clear serum. The RBC pellet was then suspended in phosphate-buffered saline (PBS), and the RBCs and microplastic particles were mixed in a 1:1 (v/v) ratio and seeded in a 96-well plate. The plate was shaken for 1 h at 500 rpm for physical contact between the fine plastic and the RBC, and then, they were centrifuged at 2500g for 5 min. Supernatants (100 μL) were loaded in a fresh plate and analyzed by UV absorbance at 450 nm.
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Fluorescence Microscopy. PBMCs were seeded at a density of 5 × 104 cells/0.33 cm2 in a 96-well plate and cultured at 37 °C in a 5% CO2 incubator. After 1 day, various concentrations (10, 100, and 1000 μg/mL) of microplastics were added to each well for 1 and 5 days. Cells were treated with 1% Triton X-100 (Sigma-Aldrich, St. Louis, MO, USA) as a negative control. Each plate was centrifuged at 330g for 7 min, after which we removed the supernatant. Cells were then diluted in 1× PBS according to the protocol for the Live/Dead cell viability assay kit (Thermo Fisher Scientific, Waltham, MA, USA), and the rest of the assay was then completed as per the manufacturer's instructions. Finally, the labeled cells were observed using a fluorescence microscope (OLYMPUS Tokyo, Japan).

(Cell Counting Kit) Assay. HDFs and HeLa cells were seeded at 1×10^4 cells/cm^2 in a 96-well plate and cultured at 37 °C in a 5% CO_2 incubator. After 1 day, each well was treated for 1 day with various concentrations of microplastics. Cells were treated with 20% dimethyl sulfoxide (Sigma-Aldrich) as a negative control. After 1 day of treatment, supernatants were removed and 10% CCK-8 reagent (Dojindo, Kumamoto, Japan) was added. Absorbance was then measured at 450 nm using a plate reader (VERSA Max; Molecular Devices, Union city, CA, USA). Enzyme-Linked Immunosorbent Assay. PBMCs were seeded at 1×10^6 cells/mL in a 24-well plate and cultured at 37 °C in a 5% CO_2 incubator. After 1 day, each well was then treated with various concentrations of microplastics and left for 4 days. The plate was then centrifuged at 330g for 7 min. The supernatant was collected and centrifuged at 10,000 rpm at 4 °C for 10 min. The supernatants were loaded onto an enzyme-linked immunosorbent assay (ELISA) plate, and the assay was completed using the manufacturer's protocol. Cytokines (IL-2, IL-6, and TNF-α) were quantified using an ELISA kit (BioLegend, San Diego, CA, USA). Absorbance was measured at 450 nm using a plate reader (VERSA Max; Molecular Devices, Union city, CA, USA).Histamine Assay. HMCs-1 were seeded at 5×10^5 cells/mL in a 96-well plate and cultured at 37 °C in a 5% CO_2 incubator. After 1 day, each well was treated with various concentrations of microplastics and incubated for 2 days. The supernatants were collected, and the amount of histamine released from the HMCs-1 was analyzed using a histamine assay kit (BioVision, Milpitas, CA, USA). Samples were loaded onto the plate, and the assay was completed using the manufacturer's protocol.

Absorbance was measured at 450 nm using a plate reader (VERSA Max; Molecular Devices, Union city, CA, USA).Statistical Analysis. GraphPad Prism (GraphPad Prism Software Inc., USA) was used to conduct statistical analyses and produce graphical representations of the data. Numerical data in the graphs represent the mean value with error bars. Differences between the control (CTRL) and the test groups were compared using Student's t-test. and indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. All the experiments were completed a minimum of three times.[3] Over 80% of microplastics are produced on land, with less than 20% originating from the sea[17]Microplastics are normally produced when some plastics are exposed to the environment and degraded. As shown in Figure.1, we used a ball mill in a short time to mimic the physical degradation of the environment. In a previous study, a size of 10−200 μm of the microplastics was mainly found in the marine environment. Thus, we used PVC and ABS which are in the size range below 200 μm. The surface pattern of broken plastics was analyzed through statistical analysis. However, the microplastics used here are not naturally produced, but rather, they are plastics that were purchased and milled. In addition, the microplastics were

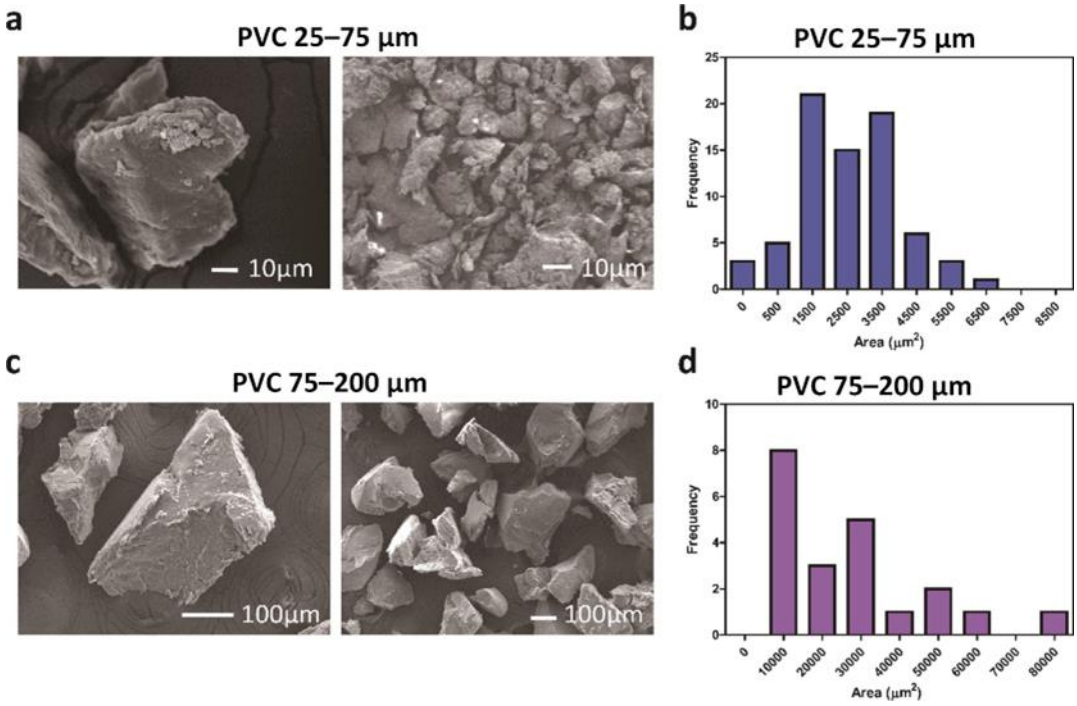

Figure 2. Morphology and size distribution of PVC microplastics. SEM image and overall size distribution of (a,b) 25−75 µm PVC and (c,d) 75− 200 µm PVC microplastics (n = 73, 21) calculated from SEM images.

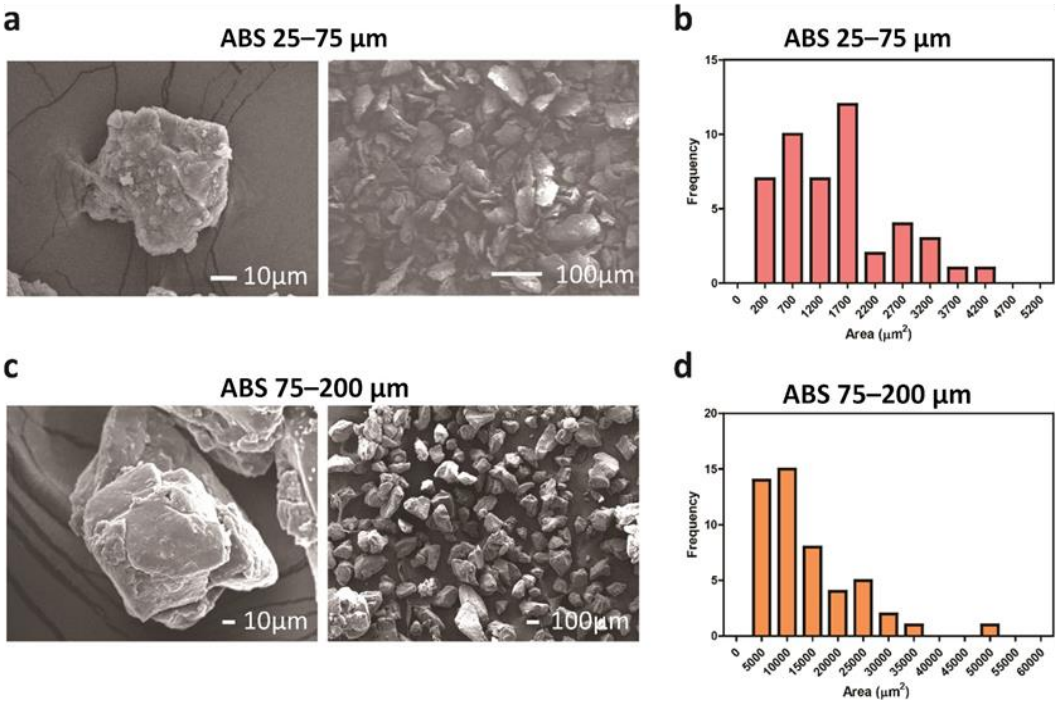

Figure3. Morphology and size distribution of ABS microplastics. SEM image and overall size distribution of (a,b) 25−75 µm ABS and (c,d) 75− 200 µm ABS microplastics (n = 47, 50) calculated from SEM images

In the marine environment. Thus, we used PVC and ABS which are in the size range below 200 µm. The surface pattern of broken plastics was analyzed through statistical analysis. However, the microplastics used here are not naturally produced, but rather, they are plastics that were purchased and milled. In addition, the microplastics were purchased online, so the exact composition of the final product is not known.

We made microplastic debris using ball mills and then used SEM and optical microscopy (OM) to evaluate the shape and size of both PVC (Figures 2a and S1) and ABS (Figures 3b and S2). We measured the area distribution of the microplastic debris using ImageJ software. The area of 25−75 µm PVC and 75− 200 µm PVC was mainly 1500−3500 μm^2 (average: 3060 μm^2) and 10000−30000 μm^2 (average: 20846 μm^2), respectively (Figure 2b,d). Furthermore, the

area of 25−75 μm ABS and 75−200 μm ABS was mainly 200−1700 μm^2(average: 1730 μm^2) and 5000−15000 μm^2 (average: 15953 μm^2), respectively (Figure 3b,d).

According to the WWF (World Wildlife Fund), people consume roughly about 2000 plastics every week, introduced through common food and beverages. We assumed that the used microplastic is in the form of a sphere and set the volume based on the average value of the area distribution (Figures 2 and 3).

The weight per microparticle (0.6 μg/microparticle) was calculated by averaging the volume (0.2 mm3) and density (1.2 g/cm3) of all sizes and types of used microplastics. Based on this calculation, 1000 μg of microplastics was treated on the assumption that microplastics were egested in small amounts or ingested in excessive amounts. Microplastics (100 and 10 μg) were treated, assuming that the microplastics consumed in small amounts were egested.Therefore, the concentration of the used microplastics was set at 0.01 mg/mL to 1 mg/mL. The easiest way to confirm the effects of the edge geometry on cytotoxicity is the hemolysis test. Hemolysis is the breakdown of RBCs. We evaluated the hemolysis by contact at various concentrations of each of the microplastics[3]

Microplastic & Nanoplastic effect on Mammalian system:

The effects of MPs/NPs on mammalian cells and tissues, particularly humans, have remained rather unclear. While plastics are generally perceived to pose minimum risk to human, several recent scientific findings, picked up by the popular press, have heightened the worry of possible tissues penetrance and adverse effects of MPs/NPs due to their small sizes. Humans could accumulate MPs/NPs from different food sources as well as drinking water. Plastic water containers and plastic teabags are, perhaps unsurprisingly, common sources for human ingested MPs/NPs. MPs/NPs could also be taken up by inhalation. MPs/NPs have also been detected in human stool samples an indication that the quantity taken in is significantly large. A recent World Health Organization's (WHO) report on "Microplastic in drinking water" indicates that there is not yet proof of harm, but it also calls for more research to be carried out.

Could environmental MPs/NPs gain access to cells and tissues and be harmful to humans? Although ecotoxicology data with marine invertebrate indicate that this is so, more barriers and obstacles would likely be encountered by MPs/NPs in order to gain access to cells and tissues of vertebrates compared to simpler invertebrates. Here, we review current results on how MPs/NPs might affect humans by scrutinizing studies done to date on mammalian (mouse) models and human cells. We begin with a quick survey of MP/NP feeding studies done on marine vertebrates, focusing on fishes. A meta-analysis on the effect of MP exposure on fish has been reported by Foley and colleagues in 2018 and the field has also been recently reviewed but several newer reports have now appeared. This quick look would allow some comparison of findings in more ecologically relevant settings with that of laboratory experiments with mice and human cells[4] NMPs were shown to alter the gut microbiome in mice] and larval zebrafish hence unexpected effects of NMPs via the microbiota-gut-brain axis on neurological functions are conceivable. An interaction and fast colonization of plastic particles by microbes in aquatic environments is already known and it seems plausible that such colonized particles could transfer microbes (including pathogenic species) to organisms if ingested. Hence, NMPs could be both carriers of microbes, as well as substrate for (gut) microbiomes, affecting metabolic processes or altering microbiome composition leading to dysbiosis.[20]

A special emphasis is made on experimental studies of MP effects in laboratory rodents. Mice and rats undoubtedly remain the main model organisms in the research on human diseases. Rodent models have been widely applied to understand the mechanisms of pathogenesis, to test the efficacy of candidate drugs, and to predict the side effects and individual responses. The harmful MP effects in mice and rats are closely related to the corresponding risks in humans.[29]

Mechanisms Underlying MPs/NPs' Acute or Chronic Toxicity in Mammalian Cells:

Research based on laboratory.

Given that MPs can enter terrestrial higher mammals and humans through various pathways and then accumulate to exert health hazards, we reviewed the relevant literature and summarized the toxic effects and related mechanisms of MPs on terrestrial higher mammals in Supplement, the toxic effects of MPs mainly include intestinal toxicity, metabolic disruption, reproductive toxicity, neurotoxicity, immunotoxicity, cardiotoxicity, and impaired pulmonary function In general, extremely high concentration of MPs/NPs are indeed cytotoxic.

Cell death could occur via necrotic plasma membrane rupture or some form of programmed cell death. An important point to note on the former, rather non-specific mode of death is the surfactant molecules that are typically associated with most MP/NP preparations. At high concentrations, these would be disruptive to the lipid bilayer of the plasma membrane (PM). Even at moderate levels, these could disrupt important cellular surface structures such as proteoglycans and other extracellular matrix components or hinder cellular signaling processes that require extracellular ligand and cell surface receptor interactions. Therefore, cellular physiology would be affected to varying degrees by plastic associated surfactants, and the documented changes in various transcripts in cells could also be due to this and other processes/factors described below.[6]Microplastic exposure leads to improper gut health, weakening of the

immunity status of the body, as well as an increase in the rate of development of different inflammatory conditions. Intestinal dysbiosis associated.[24] Even at low concentrations, chronic exposure to heavy metals promotes chronic inflammation and subsequently develops carcinogenesis[25]

The smaller NPs in particular could be taken up with some ease depending on the cell type via endocytosis Endocytosed NPs present a problem for several reasons. Firstly, they could, as per the plasma membrane discussed above, potentially permeabilize the endosomal membranes if present at high concentrations. If this happens, the NPs released into the cytosol could potentially interact with and affect important organelles such as the mitochondria or the nucleus, as well as cellular processes such as mitotic spindle formation and chromosomal migration during cell division.

Secondly, MPs/NPs would likely interfere with the trafficking of transport carriers in the cell along the exocytic pathway and as such would potentially inhibit the cell surface expression of important signaling receptors or membrane transporters. Thirdly, they are likely to perturb endosomal membrane traffic on which many important cellular processes are dependent, including surface protein turnover and signaling attenuation, as well as retrograde signaling from endosomal compartments. It is unclear if NPs could themselves ever be subjected to inter-compartmental transport efficiently in the endosomal pathway.
Even if the NPs could eventually end up in the lysosome, they are unlikely to be readily digested. The accumulation of NPs in late endosome or lysosomes would perturb the degradative functions of these organelles and importantly impair the critical cellular membrane turnover process of macroautophagy An impairment of autophagic clearance could potentially lead to positive feedback processes that culminate in autophagic cell death. On the other hand, internalized MPs/NPs may also stimulate autophagy. Metallic nanoparticles are known to modulate autophagy and MPs/NPs may speculatively do likewise.[4]

At the very least, these processes would constitute a form of cellular stress. Stresses at the PM and the endo-lysosomes would trigger cellular stress responses. In work done with species of the fresh water flea Daphnia, PS NPs exposure affected growth and reproduction and interestingly resulted in the elevation of AMP activated protein kinase (AMPK), which is an indication of stress response Perhaps a more general associated phenomenon with regards to cellular stress response appears to be the production of ROS, which was in fact recently identified as the molecular initiating event (MIE) by adverse outcome pathways analysis of reports in the field ROS production in cells occurs in two general ways from the mitochondrial electron transport chain (ETC) during routine aerobic respiration or via the oxidative bursts of NADPH oxidases (NOXs) An increase in ROS from the former could result from mitochondrial function impairment, while the latter is normally a consequence of bacterial invasion, as NOXs are activated by bacterial products and cytokines.

All cells are endowed with an evolutionarily conserved innate immunity mechanism, typically functioning against invasion of pathogens or exposure to xenobiotics However, the components of the innate immune system, such as the Toll-like receptors (TLRs), could also respond to a set of endogenous or secreted molecules collectively known as alarmins, or damage-associated molecular patterns (DAMP) and the outcome is what is termed sterile-inflammation, i.e. inflammatory responses without pathogenic infection In the body, pro-inflammatory cytokines released from such localized inflammations would attract circulating immune cells, and this could worsen the local inflammation, and cause cell and tissue death. NPs has indeed been shown to act as stressors to the innate immune system of fish and this is likely also the case for mammalian (including human) cells. The cellular and tissue invasion and general pathological mechanism of MPs/NPs in mammalian cells is summarized.

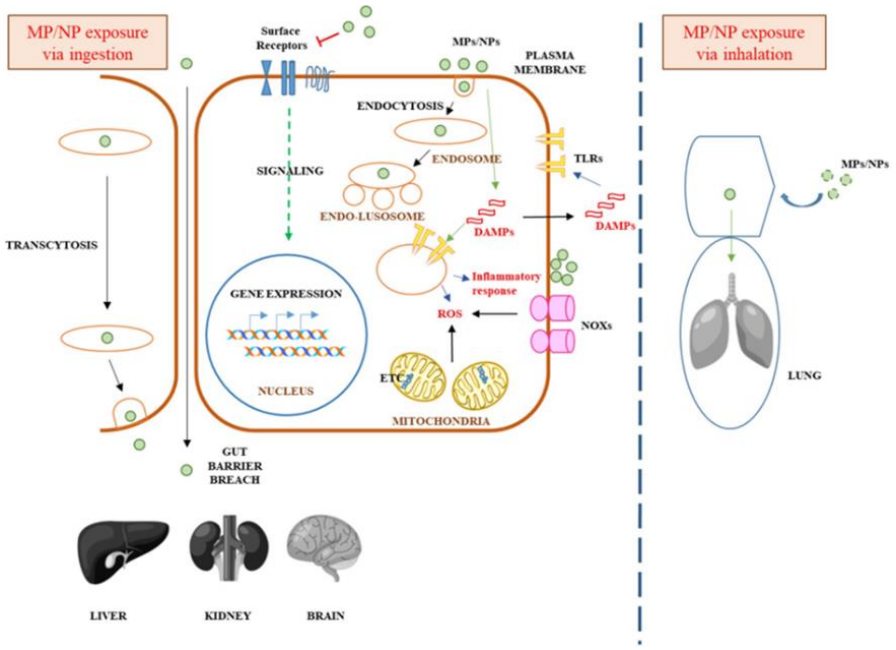

Figure 4. A schematic diagram illustrating potential (speculative at the moment) cellular mechanisms of MP/NP toxicity. MPs/NPs can be taken up through ingestion and inhalation. MPs/NPs could damage the plasma membrane and impair the gut barrier (left). These could also perturb signaling of cell surface receptors, and alter gene expression in the nucleus.

Endocytosed MPs/NPs could also perturb the endocytic pathway function and compromise the endosomal membranes. Stresses arising from the above could activate the cellular innate immune system, with endogenous and secreted damage-associated molecular patterns (DAMP) inducing the innate immunity-mediating toll-like receptors (TLRs). Stresses could induce ROS production from the NADP oxidases (NOXs). Mitochondrial impairment, either by MPs/NPs from endosomes or in response to stresses, could also produce more ROS through impairment in the efficiency of electron transport chain (ETC) processes. MPs/NPs gain access into the circulation if the gut–vascular barrier is compromised or it may speculatively occur by transcytosis, thus reaching other organs. The lung probably has a more direct access to airborne MPs/NPs (right).[4]

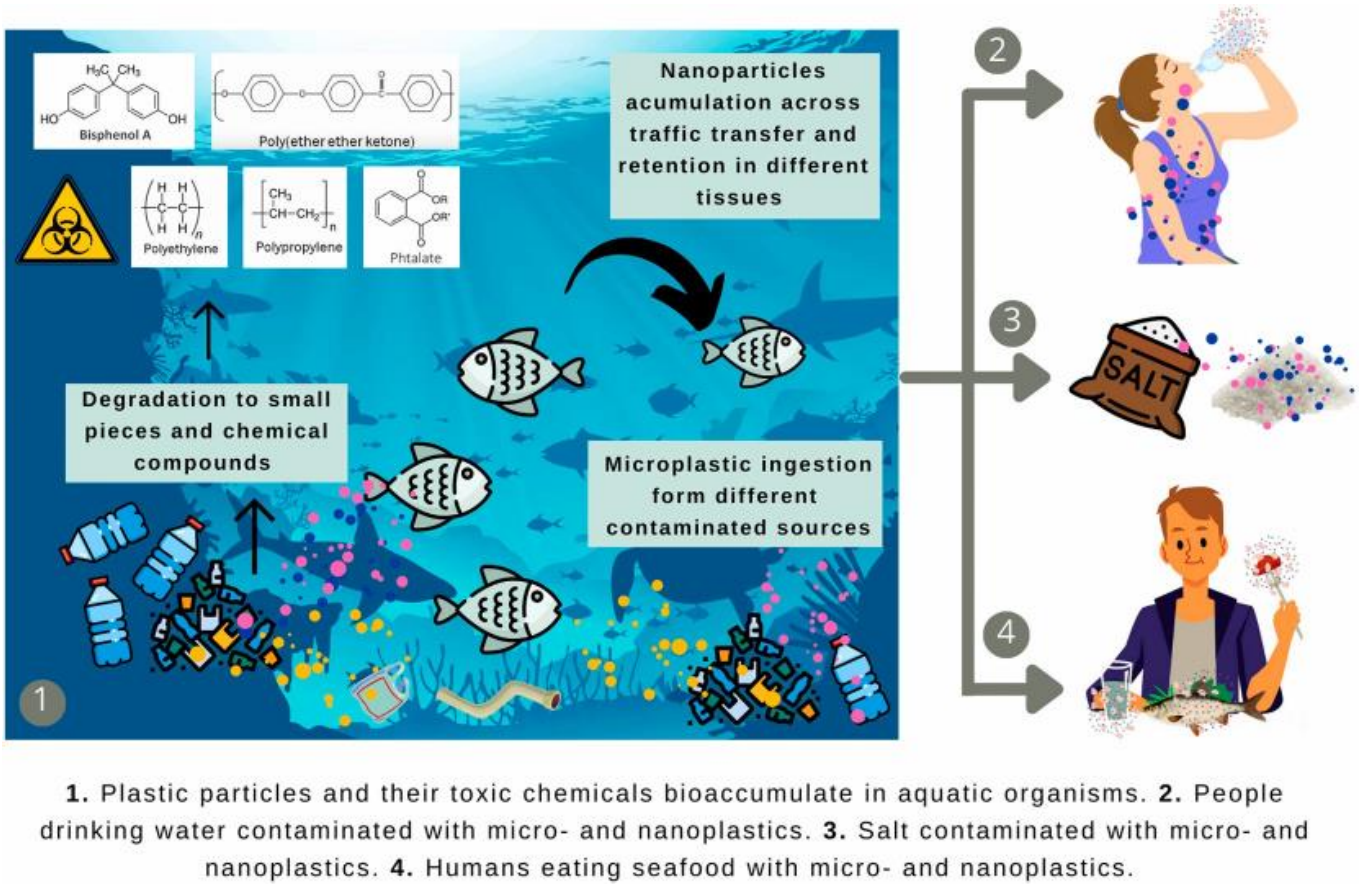

Fig. 5. Representative model microparticles and nanoparticles formation, release of toxic compounds from plastic waste, traffic transfer across alimentary chain and the main sources of oral contamination from water, salt and seafood intake. In this model, humans were used to represent the contamination of mammals with plastic particles. Only a few toxicants present in the composition of several plastic polymers were represented in this model.[5] Exposure of earthworms (Eisenia fetida) for up to 28 days to artificial soil with low-density polyethylene particles (100–200 μm; 0.1–1.5 g/kg soil) resulted in skin damage following exposure to 1.5 g/kg soil. Particle ingestion (following 14–28 days exposure to 1.5 g/kg soil) was confirmed by extraction and counting of polyethylene particles, although the distribution of the particles within the earthworms is unknown. Exposure for 28 days to polyethylene particles at 1.0 g/kg soil, but not at 1.5 g/ kg soil, resulted in increased catalase activity and malondialdehyde levels, suggesting the animals showed signs of oxidative stress. Additionally, exposure to 1.0 g/kg and 1.5 g/kg soil for respectively 21 and 28 days, increased AChE activity.[30]

Fortunately, all the studies reviewed evaluated oral exposure to plastic particles, which is the usual exposure route in mammals and the most frequent form of environmental contamination with these residues Furthermore, most studies indicated that plastic particles exerted a dose-dependent negative effect on reproductive organs However, administration by gavage or free access to drinking water indicates variable experimental control of plastic particles exposure, which may limit the characterization of dose-dependent responses due to the difficulty in ensuring the amount of particles ingested in ad libitum exposure models Unfortunately, ingestion of these plastic particles was evaluated for short periods (28–90 days), restricting the available evidence to the acute reproductive toxicity.

In general, plastic particles were detected in male and female gonads. This finding was coupled with extensive microstructural damage, especially degeneration of the seminiferous epithelium Sertoli cells death and disruption of the blood- testis barrier integrity sperm malformation or degeneration flame retardants exhibit particular toxicological interest as they act as potent endocrine disruptors, proinflammatory and prooxidant agents. The fate of MP particles after ingestion is mostly unknown. There are no data demonstrating whether MP can translocate from the gut cavity or if MP particles are entirely excreted with the feces.[19]

From a mechanistic approach, the reviewed studies reinforced the evidence linking dose-dependent gonadal damage to the inflammatory and redox imbalance attributed to chemicals present in plastic composition. Accordingly, plastic particles upregulated prooxidant pathways (e.g., ROS production, lipid and DNA oxidation) [and inhibited antioxidant defenses (e.g., Nrf2, HO-1, SDH, LDH, GSH-PX, CAT and SOD) This response is potentially associated with

intracellular accumulation of plastic particles and lysosomal instability, directly releasing ROS or ROS-catalyzing enzymes in the interstitial compartment [59]. In addition, the gonadal oxidative stress induced by this plastic products was closed correlated to NF-κB [39,45], JNK, p38 MAPK [37,44], Wnt and β-catenin activation As these pathways modulate cytokines and chemokines production [60–62], the upregulation of proinflammatory effectors (e.g., TNF-α, IL-1β, IL-6, IL- 1β, IL-18 and MCP-1 and CXCL10) was consistently implicated in plastic particles-induced gonadal damage Interestingly,

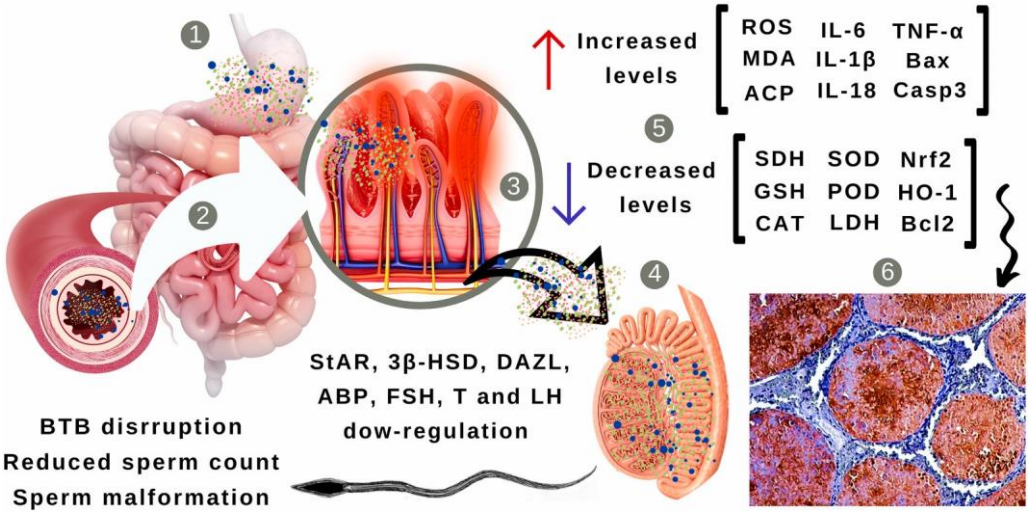

Fig. 6. Representative model of plastic particles gastrointestinal absorption, systemic distribution, testis accumulation, biochemical and microstructural damage. (1) Ingestion and plastic particles in the stomach (1) and intestine (2) of mammals. (3) Intestine inflammation and plastic particles through the lining epithelium. (4) Systemic distribution and testis accumulation of plastic particles. (5) Upregulation of prooxidant and proinflammatory molecules and down-regulation of antioxidant effectors in testis. (6) Degeneration of the seminiferous epithelium with germ cells death (apoptotic cells marked in brown color). ROS = reactive oxygen species, MDA = malondialdehyde, ACP = acid phosphatase, IL = interleukin, TNF-α = tumor necrosis factor alpha, Bax = BCL2 associated X, apoptosis regulator, Casp3 = caspase 3, SDH = succinate dehydrogenase, GSH = *glutathione*, CAT = catalase, SOD = superoxide dismutase, POD = peroxidase, LDH = lactate dehydrogenase, Nrf2=, HO-1 = heme oxygenase-1, Bcl2 = B-cell lymphoma-2 apoptosis regulator, FSH = follicle stimulating hormone, LH = luteinizing hormone, T = testosterone. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).[5]

reduced sperm count and reduced sperm motility In addition, ovarian cysts reduced number of growing follicles and apoptosis of granulosa cells were identified in female gonads. Current evidence indicates that plastic particles less than 5 to 10 µm can cross the stomach and intestinal epithelium via paracellular and transcellular pathways, allowing its systemic distribution Thus, the detection of plastic particles in testis and ovary was consistent with this gastrointestinal translocation, whose size-dependent systemic distribution was also identified in mice orally treated with 0.01, 0.1 and 0.5 mg plastic particles (5 µm and 20 µm diameter) for up to 28 days Apparently, the intestinal permeability and particles translocation can be potentiated by damage to the intestinal epithelium (Fig.6)
including the death of enterocytes, which can be mediated by inflammatory and oxidative processes triggered by plastic particles.
Experimental findings also suggested that plastic particles can translocate across living cells to the lymphatic system diffuse through blood capillaries and be captured by macrophages Thus, these particles have the potential to reach the gonads (Fig.5), disrupting the blood-testicular barrier and the follicular development Most particles larger than 10 µm are trapped and then eliminated by the digestive tract However, they cannot be considered inert, since can release oligomers or toxic molecules from chemical degradation which can also be catalyzed by oxygenase enzymes produced by some intestinal bacteria Many of these compounds, especially bisphenol A, phthalates, heavy metals and brominated flame retardants exhibit particular toxicological interest as they act as potent endocrine disruptors, proinflammatory and prooxidant agents.
From a mechanistic approach, the reviewed studies reinforced the evidence linking dose-dependent gonadal damage to the inflammatory and redox imbalance attributed to chemicals present in plastic composition. Accordingly, plastic particles upregulated prooxidant pathways (e.g., ROS production, lipid and DNA oxidation) and inhibited antioxidant defenses (e.g., Nrf2, HO-1, SDH, LDH, GSH-PX, CAT and SOD) This response is potentially associated with intracellular accumulation of plastic particles and lysosomal instability, directly releasing ROS or ROS-catalyzing

enzymes in the interstitial compartment In addition, the gonadal oxidative stress induced by this plastic products was closed correlated to NF-κB JNK, p38 MAPK Wnt and β-catenin activation As these pathways modulate cytokines and chemokines production the upregulation of proinflammatory effectors (e.g., TNF-α, IL-1β, IL-6, IL- 1β, IL-18 and MCP-1 and CXCL10) was consistently implicated in plastic particles-induced gonadal damage Interestingly, inflammatory and oxidative damage were potentiated in animals treated with higher doses of plastic particles However, these dose-dependent effects cannot be attributed to the gonadal accumulation of these particles. As the current evidence does not clarify the relationship between the administered dose and the tissue load of plastic particles, regional and systemic effects cannot be ruled out to explain this dose-dependent behavior, an issue whose understanding requires further mechanistic investigations.

As expected, the prooxidant and inflammatory responses were associated with a marked dose-dependent upregulation in cell death effectors (e.g., Bik, Bax, caspase 1 and 3) and down-regulation in genes and proteins involved in cell survival (e.g., Bcl2), spermatogenesis and postnatal folliculogenesis (e.g., PLZF, DAZL, INSL3, occludin, N-cadherin, zonula occludens 1, β-catenin, FAK, and connexin 43) Most of these molecules are not essential for mammalian fertility; however, regulates the reproductive function by controlling blood-testis-barrier integrity, germ cells structure, communication, proliferation and differentiation Thus, the depletion of connecting (e.g., FAK, claudins, occludins and cadherins) and communication (e.g., connexin) molecules breaks Sertoli cells barrier and the *adluminal* microenvironment required for a proper spermatogenesis This down-regulation also interrupts the physical- functional interactions between ovarian stroma and parenchyma, compromising oocytes-granulosa cells synchronism and postnatal folliculogenesis Conversely, depletion DAZL protein has been directly associated with infertility in male and female rodents and humans.[5]

Oxidative stress together with the age-dependent decrease in antioxidant activity and mitochondria dysfunctions are the main causes of testicular and sperm damage. In fact, reactive oxygen species (ROS) overproduction is responsible for spermatogenesis failure, the apoptotic loss of both germ and somatic cells, oxidative DNA damage, failure in gene expression and post-transcriptional gene regulation, or APT depletion.[28]

Strikingly, plastic particles toxicity has extended to endocrine disruption. Thus, animals receiving these particles manifested reduced testosterone progesterone and estrogen levels which were related to dose-dependent down-regulation of genes and/or enzymes involved in steroidogenesis, such as FSH, LH, ABP, 3β-HSD, 17β-HSD and StAR These changes indicate regional and systemic plastic-induced toxicity on hormonal regulation of reproductive function. As gonadal structure and function are dependent on adequate steroidogenesis its inhibition may be associated with down-regulation in the expression of gonadal regulatory molecules and microstructural damage identified in the reviewed studies. Thus, reproductive toxicity of plastic particles involves multiple gonadal targets associated to dose-dependent prooxidant, proinflammatory, and hormonal events that orchestrate seminiferous epithelium and blood- testis barrier disruption, sperm morphofunctional degeneration, germ cells death and follicular degeneration. Considering a critical interpretation of the evidence, the assessment of methodological quality indicated important elements of bias in the reviewed studies. Even considering the specificities of each research design, no study fulfilled all methodological criteria. The studies presented variable methodological score without a temporal influence (year of publication), indicating that elements of bias are continuously replicated despite methodological advances and availability of more sensitive and specific analytical tools. Surprisingly, 40% of the essential criteria to be reported in in vivo animal studies were neglected. Underreported aspects, such as animals' allocation sequence and concealment (allocation, treatment groups and data collection) undermine the reproducibility, internal and external validity of the reviewed studies, limiting evidence reliability Conversely, the description of animals' randomization, baseline characteristics (characterization of models and experimental conditions), outcome data adequately addressed, absence of selective outcomes, and complete characterization of plastic particles treatments represented the criteria broadly met by the reviewed studies. It is important to emphasize that these bias elements do not indicate flaws in the experimental protocols, they only point out limitations in the research report. Thus, by mapping the risk of bias in all investigated studies, this review provides objective support to delimit further studies with greater methodological rigor, providing unequivocal evidence on the reproductive impact of plastic particles in mammalians.[5]

Organic contaminants in microplastics may either be introduced during manufacture or adsorbed from the seawater (Teuten et al., 2009). Plastic can concentrate contaminants up to the order of 106 (Mato et al., 2001), thereby acting as a potential source and vector for these chemicals. In oceans and near coastal areas, concentrations of PCBs, PAHs and organochlorine pesticides (1,1-dichloro-2,2-bis (chlorophenyl)ethylene (DDE)), ranging from 1 to 200 ng/g, 4 to 10,000 ng/g and 0.1 to 250 ng/g, respectively, have been found (Bouwmeester et al., 2015). Globally, in microplastics deposited at beaches, even much higher concentrations have been detected.

PCBs 0.01–2,750 ng/g; PAHs 90–24,000 ng/g; 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT) and analogues (1,1-dichloro-2,2-bis(p-chlorophenyl) ethane (DDD), 1,1-dichloro-2,2-bis(chlorophenyl)ethylene (DDE)) 2–1,061

ng/g. Organic contaminants, such as PCBs, have been shown to transfer from plastic to sediment-dwelling organisms (Teuten et al., 2007) and streaked shearwater chicks (Teuten et al., 2009).[11]
Recent studies indicate that MNPLs are present in most classes of consumer products. In foodstuffs, MNPLs can be present in animals which are contaminated through their environment or food chains, as happens with seafood (Santillo et al., 2017) for example, and also they can be contaminated during their production processes or packaging (Lau and Wong, 2000; Mason et al., 2018; Du et al., 2020). In the case of drinking water, MPL contamination can come from pipes, filters, or bottles (Oßmann, 2021). In addition to a portion of MNPLs that we can ingest incidentally, such as the MPLs that are present in some toothpaste formulas. Also, dust from plastics, car tyres, paints, and textile fibres are sources of MNPLs in the Earth's atmosphere and can be inhaled or can undergo dermal interaction with humans. Moreover, MPLs are used in the formula of certain personal care products (PCPs) (Laborda et al., 2021), implying dermal interaction.[12] Given the presence of microplastics in the oceans, these particles are also detected in seafood products.[18]
Given that microplastics have been identified successively in the human placenta, newborns, and meconium, concerns about the pathways of microplastics entering the bloodstream circulatory are rising[22] Living organisms, especially humans, are exposed to M-NPLs through three main routes: ingestion, inhalation, and dermal contact. Hence, M-NPLs can enter the body by ingesting contaminated food and water, inhaling contaminated indoor and outdoor air, and cutaneous exposure to M-NPLs through dust, clothing, and personal care items One less discussed aspect of exposure to M-NPLs is the entanglement of specific marine species in plastic debris, which causes physical and biological injuries.[13]

The data indicated that the distribution of MPs in tissues is partially determined by particle size. Interestingly, the accumulations of large particles (20 μ m diameter) appeared consistently distributed among all tissues, whereas small particles (5 μ m diameter) displayed higher accumulation in the gut. Different patterns of distribution of MPs in tissues have also been demonstrated in aquatic organisms.[15] Absorption of nutrients and chemicals is a process common to all mammalian species. Therefore, mice, rats, hamsters, and guinea pigs were historically used for absorption and toxicokinetic studies (OECD 2010). However, the vast combination of possible physicochemical properties seen in this heterogeneous mixture of MPs and NPs makes the use of in vivo mammalian models difficult to specifically assess absorption distinct from other parameters. In this context, in vitro models are commonly employed and recommended for practical, cost-effective and ethical reasons. Among the different cell lines available, as representative of the human small intestine, differentiated Caco-2 cells are the most widely used·[16]
Recently, impact assessment of several MPs/NPs has been performed in various cells derived from human tissues. However, these assessments on the toxicity of MPs/NPs against human cells showed conflicting results. Most of the studies suggested that MPs/NPs induced some degree of toxicity or pathological changes in human cells, but a few studies showed that these MPs/NPs did not show any significant cellular toxicity, except at high concentrations. First, significant toxicity was detected in human cells treated with various MPs/NPs, including PS, carboxylated PS, PE, and PP. T98G and HeLa cells showed increased cytotoxicity after treatment with PE MPs (3–15 μm) or PS MPs (10 μm), and similar toxic effects were detected in Caco-2 and BEAS-2B cells treated with PS MPs (0.1–5 μm) Additionally, smaller PP particles (20 μm) induced some degree of toxicity at high concentrations in HDFs and Raw 264.7 cells, whereas larger PP particles (25–200 μm) did not induce toxicity Some small PS NPs (<100 nm) induced significant toxicity in THP-1, DMBM-2, and BEAS-2B cells at very high or low concentrations.[23]

Conclusion:
In conclusion, the literature review on marine debris highlights the findings of microplastics in human blood and plastic trash in particular. Marine debris, particularly plastic waste, poses a significant threat to marine ecosystems, organisms, and habitats. It is ubiquitous, persistent, and has detrimental ecological consequences. Plastic waste enters the marine ecosystem through various pathways, including improper waste management, stormwater runoff, and industrial activities. It is transported and distributed across the oceans through ocean currents, winds, and other natural processes. Microplastics, tiny plastic particles less than 5mm in size, have been found in various marine environments, including coastal areas, deep sea, and polar regions. They originate from both primary sources (such as microbeads and fibres) and secondary sources (as a result of the destruction and disintegration of bulkier plastic objects). The presence of microplastics in human blood has been documented in several studies, highlighting the potential for human susceptibility to microplastics through various paths, including consumption, breathing, and cutaneous contact. While the health effects of microplastics on human beings are still being studied, there is growing concern about their potential to cause physical, chemical, and biological harm. Microplastics may carry toxic chemicals, act as vectors for pathogens, and disrupt cellular functions, posing potential risks to human health. The assessment of the toxicological and carcinogenic properties of microplastics is challenging due to their diverse compositions, sizes, and shapes. Standardized methods for evaluating the health effects of microplastics are needed to ensure accurate and reliable risk assessments. The review has identified several knowledge gaps, including the need for improved understanding of the

sources, fate, and transport of plastic waste, as well as the long-term ecological and health impacts of microplastics. In light of these findings, there is an urgent need for global action to combat marine debris and microplastic pollution. This requires a multi-faceted approach that encompasses: Reduction of plastic waste at its source through measures such as plastic bags, extended producer responsibility, and sustainable product design. Improvement of waste management systems, including effective recycling infrastructure, waste collection, and proper disposal practices to prevent plastic waste from entering marine environments. Promotion of programmes for public education and awareness about the effects of marine debris and microplastics, and to foster responsible consumption and waste management behaviours. Development and implementation of policies and regulations at national and international levels to address plastic pollution and promote sustainable practices. Advancement of research and innovation to develop new materials, technologies, and solutions for plastic waste management, including alternative materials, recycling methods, and circular economy approaches. Collaboration and coordination among governments, industries, scientists, NGOs, and communities to share knowledge, resources, and best practices in tackling marine debris and microplastic pollution. It is essential that global action is taken urgently to prevent further degradation of marine ecosystems, protect biodiversity, and safeguard human health.
Some key strategies that can help combat plastic waste includes-Reducing Plastic Consumption and opting for alternatives that are reusable include cloth bags, metal straws, and refilled water bottles, to minimize single-use plastics. Proper waste management systems, including recycling facilities and awareness campaigns can promote responsible disposal and recycling of plastic waste and to promote knowledge about the impacts of plastic waste through educational initiatives, community programs, and media campaigns.

The environment is full of MPs and NPs, and people are constantly exposed to them. As a result, their cumulative intake will only rise. As of right now, it seems that there is no need to be concerned about acute toxicity or serious long-term effects that could result in noticeably higher rates of morbidity or death. But our understanding of the potential health effects of microplastics and nanoparticles (MPs/NPs) from the environment—whether from seafood or plastic bottles—remains mostly limited. It is obvious that a great deal more research is required on the long-term impacts of tissue/organ accumulation as well as on the pathogenic mechanisms at the cellular and tissue levels. It would also be required for ecologists and epidemiologists to work together to develop plans and joint investigations on the bioaccumulation of MPs and NPs in people through the food chain in different regions of the world.

REFERENCES:

1. Prajapati. March 2023- Marine Debris With Special Reference To
Plastic Waste And It's Relation To Discovery Of Microplastics (Nano Particles) Found In Human Blood A Comprehensive Review.Volume No.5 2-3.
2.Rajendran And Chandrasekaran Chandrasekaran, October 2023-Journey Of Microplastics With Blood Component 2-5.
3. Seora Han, Junah Bang, Daheui Choi, Jangsun Hwang, Taeho Kim, Yoogyeong Oh, Youngdeok Hwang, Jonghoon Choi, And Jinkee Hong, February 2024- Surface Pattern Analysis Of Microplastics And Their Impact On Human-Derived Cells 4-6.
4. Cheryl Qian Ying Yong , Suresh Valiyaveettil 2 And Bor Luen Tang, February 2020- Toxicity Of Microplastics And Nanoplastics In Mammalian Systems 17-19.
5. Rodolfo C. Marcelino , Ronan M. Cardoso , Elisa L.B.C. Domingues , Reggiani V. Gonçalves , Graziela D.A. Lima , Romulo D. Novaes February 2022- The emerging risk of microplastics and nanoplastics on the microstructure and function of reproductive organs in mammals: A systematic review of preclinical evidence
5-7http://www.elsevier.com/open-access/userlicense/1.0/.
6. Liu · Liu ·Xiong · K.Xu · Y. Pu · J. Huang · J.Zhang · Yuepu Pu · Rongli Sun · Keping Cheng, January 2023-Research advances of microplastics and potential health risks of microplastics on terrestrial higher mammals: a bibliometric analysis and literature review 45:2803–2838.2828 https://doi.org/10.1007/s10653-022-01458-8.
7. M. Smith & David C. Love & Chelsea M. Rochman & Roni A. Neff , August 2018- Microplastics in Seafood and the Implications for Human Health 5:375–386 378.
8. Melanie Bergmann · Lars Gutow ,Michael Klages 2015- Marine Anthropogenic Litter page no.10.
9. Kannabiran Krishnan volum.13, 31.10.2022- A Systematic Review on the Impact of Micro-Nanoplastics
Exposure on Human Health and Disease 5-6.
10. Yudong Fenga, Chen Tua , Ruijie Li, Di Wuc, Jie Yanga,Yankai Xiac,d, Willie J.G.M. Peijnenburg e,f, Yongming Luoa, 21 August 2023- A systematic review of the impacts of exposure to micro- and nano-plastics on human tissue accumulation and health.195–207-Page no.204.
11. Jan Alexander, Lars Barregard, Margherita Bignami, Sandra Ceccatelli, Bruce Cottrill, Michael Dinovi, Lutz Edler, Bettina Grasl-Kraupp, Christer Hogstrand, Laurentius (Ron) Hoogenboom, Helle Katrine Knutsen, Carlo Stefano Nebbia, Isabelle Oswald, Annette Petersen, Vera Maria Rogiers , Martin Rose, Alain-Claude Roudot, Tanja

Schwerdtle, Christiane Vleminckx, Gunter€ Vollmer, Heather Wallace ,11 May 2016- Presence of microplastics and nanoplastics in food, with particular focus on seafood 14(6):4501.page no.17
12. Marta Llorca and Marinella Farre, September 2021-Current Insights into Potential Effects of Micro-Nanoplastics on Human Health by in-vitro Tests. volume no.3 page no.2.
13. Ajmal Khan and Zhenquan Jia February 2023- Recent insights into uptake, toxicity, and molecular targets of microplastics and nanoplastics relevant to human health impacts.pageno.3(http://creativecommons.org/licenses/by/4.0/).
14. Baorong Jiang, Alexandra E Kauffman1, Lei Li, Wayne McFee, Bo Cai, John Weinstein, Jamie R Lead,Saurabh Chatterjee, Geoffrey I Scott1, and Shuo Xiao, 2020- Health impacts of environmental contamination of micro- and nanoplastics: a review. 25:29.page.no.8.
15. Yongfeng Deng , Yan Zhang, Bernardo Lemos & Hongqiang Ren, April 2017- Tissue accumulation of microplastics in mice.page.no.06.
16. Laura Rubio, Ricard Marcos & Alba Hernandez. Dec 2019- Potential adverse health effects of ingested microand nanoplastics on humans. Lessons learned from in vivo and in vitro mammalian models. page.no.04.
17. Maxine Swee-Li Yee , Ling-Wei Hii, Chin King Looi, Wei-Meng Lim, Shew-Fung Wong, Yih-Yih Kok, Boon-Keat Tan, Chiew-Yen Wong and Chee-Onn Leong, February 2021- Impact of Microplastics and Nanoplastics on Human Health.496.page.no.02.
18. Nell Hirt and Mathilde Body-Malapel ,2020- Immunotoxicity and intestinal effects of nano- and microplastics: a review of the literature. 17:57.page.no.05
19. Jutta Dierkes and Alice Refosc, June 2022- Microplastic studies in humans: evidence from feces and effects in blood.page.no.18
20. Andreas Brachner, Despina Fragouli, Iola F. Duarte, Patricia M. A. Farias, Sofia Dembski, Manosij Ghosh , Ivan Barisic, Daniela Zdzieblo , Jeroen Vanoirbeek , Philipp Schwabl and Winfried Neuhaus, November 2020- Assessment of Human Health Risks Posed by Nano-and Microplastics Is Currently Not Feasible. , 8832.page.no.05
21. Edoardo La Porta , Ottavia Exacoustos , Francesca Lugani, Andrea Angeletti, Decimo Silvio Chiarenza , Carolina Bigatti, Sonia Spinelli, Xhuliana Kajana, Andrea Garbarino, Maurizio Bruschi, Giovanni Candiano Gianluca Caridi, Nicoletta Mancianti, Marta Calatroni, Daniela Verzola, Pasquale Esposito,Francesca Viazzi, Enrico Verrina ,and Gian Marco Ghiggeri, September 2023- Microplastics and Kidneys: An Update on the Evidence for Deposition of Plastic Microparticles in Human Organs, Tissues and Fluids and Renal Toxicity Concern. 14391.page.no.09
22.Long Zhu,Yulin Kang,Li Hui An, January 2024- Microplastics Entry into the Blood by Infusion Therapy Few but a Direct Pathway.page.no.04 https://doi.org/10.1021/acs.estlett.3c00905.
23. Jun Woo Park, Su Jin Lee, Dae Youn Hwang and Sungbaek Seo, November 2020 Recent Purification Technologies and Human Health Risk Assessment of Microplastics. 5196.page.no.12.
24. Shibabrata Pattanayak Vol.12, Issue - 2, 2022- Plastics And Their Additives Reached The Blood And Tissue Spaces What Are The Possible Consequences.page.no.130.
25. Ghulam Nabi, Shahid Ahmad ,Sana Ullah, Sahib Zada, Maliha Sarfraz, Xinle Guo,Muhammad Ismail, Kunyuan Wanghe, 31March2022- The adverse health effects of increasing microplastic pollution on aquatic mammals. 102006.page.no.05
26. Laura Janiaski, Bernadette Ardelli, 21/10/2021- An examination of the short- and long-term effects of microplastics in mammals using a mouse model.page.no.09
27. Elisa Persiani , Antonella Cecchettini , Elisa Ceccherini , Ilaria Gisone , Maria Aurora Morales, January 2023- Microplastics: A Matter of the Heart (and Vascular System),264 page no.09
28. Stefania D'Angelo and Rosaria Meccariello, March 2021- Microplastics: A Threat for Male Fertility. 2392.page.no.04
29. Natalia Zolotova1, Anna Kosyreva, Dzhuliia Dzhalilova, Nikolai Fokichev and Olga Makarova, DOI 10.7717/peerj.13503, June 2022- Harmful effects of the microplastic pollution on animal health: a literature review.page.no.03
30. Minne Prüst, Jonelle Meijer and Remco H. S. Westerink , (2020) 17:24- The plastic brain: neurotoxicity of micro- and nanoplastics.page.no.03