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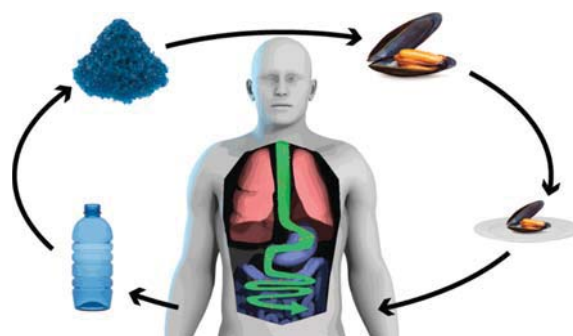
Emergence of Nanoplastic in the Environment and Possible Impact on Human Health

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ABSTRACT: On account of environmental concerns, the fate and adverse effects of plastics have attracted considerable interest in the past few years. Recent studies have indicated the potential for fragmentation of plastic materials into nanoparticles, i.e., “nanoplastics,” and their possible accumulation in the environment. Nanoplastics can show markedly different chemical and physical properties than their bulk material form. Therefore possible risks and hazards to the environment need to be considered and addressed. However, the fate and effect of nanoplastics in the (aquatic) environment has so far been little explored. In this review, we aim to provide an overview of the literature on this emerging topic, with an emphasis on the reported impacts of nanoplastics on human health, including the challenges involved in detecting plastics in a biological environment. We first discuss the possible sources of nanoplastics and their fates and effects in the environment and then describe the possible entry routes of these particles into the human body, as well as their uptake mechanisms at the cellular level. Since the potential risks of environmental nanoplastics to humans have not yet been extensively studied, we focus on studies demonstrating cell responses induced by polystyrene nanoparticles. In particular, the influence of particle size and surface chemistry are discussed, in order to understand the possible risks of nanoplastics for humans and provide recommendations for future studies.



1. INTRODUCTION

Synthetic polymers are one of the most important classes of materials of the 21st century and impact our society and daily life in ways that cannot be overestimated.¹ The properties of polymers are directly linked to their macromolecular architectures, which can readily be varied in a broad parameter space. As a result, a plethora of synthetic polymers have been developed that cover a broad range of attractive mechanical and other characteristics, including many property combinations that cannot be accessed with naturally occurring polymers such as proteins and cellulose.² The large family of synthetic polymers has traditionally been classified into four groups, namely, (i) thermoplastic polymers or “plastics”, i.e., polymers that melt above a specific temperature and can be shaped before solidification upon cooling; (ii) thermosetting polymers or “thermosets”, which are provided as (normally liquid) precursors and are irreversibly cured after shaping through a chemical reaction into an infusible, insoluble polymer network; (iii) elastomers; and (iv) synthetic fibers.³ Strictly speaking, only group (i) and subsets of groups (iii) and (iv) display thermoplastic character and should thus be referred to as “plastics”. However, as an overwhelming majority of polymer particles in the environment stem indeed from thermoplastic materials, the environmental science community has adopted the practice of referring to all polymers as “plastics”; we follow this convention herein as well. Polymers are still mainly derived from petroleum-based raw materials

(although there is an increasing trend toward using biobased feedstocks),⁴ and their immense utility is based on their vast range of chemical and physical properties that can be accessed and their low production and processing costs. This makes polymeric materials useful for manifold applications that range from packaging materials to toys and sporting goods to medical devices and construction materials.^{5–7} Commercially used polymers are always formulated with additives such as stabilizers, flame retardants, plasticizers, fillers, and pigments, which contribute to the overall materials properties.⁸ Although additives are typically present in much smaller amounts than the polymer, they are not necessarily benign and can have an adverse effect on the environment.⁹ The production of polymers, including thermoplastics, thermosets, and elastomers, as well as biodegradable and biobased plastics, has increased significantly over the past 60 years (Figure 1). In 2016, around 335 million metric tons of polymers used as plastics were produced worldwide, representing trillions of dollars in terms of global economic returns, with China being the leading producer.¹⁰

Growing production also leads to an increase of plastic waste and, unfortunately, also promotes widespread accumulation in

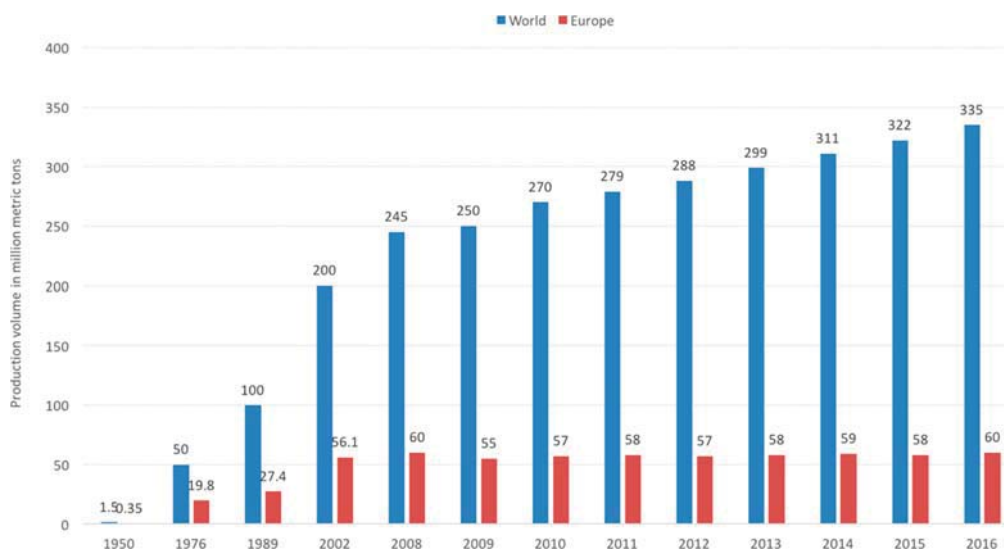


Figure 1. Polymer production worldwide and in Europe, including thermoplastics, polyurethanes, and other polymers (thermosets, adhesives, coatings, and sealants) but not polyethylene terephthalate (PET), polyamide (PA), polypropylene (PP), or polyacrylic fibers. The growth rate of polymer production in Europe has stabilized in the past decade, which is related to the effects of the economic crisis of 2007–2008, as well as higher energy and raw material prices leading to higher product prices and decreasing competitiveness with the flourishing Middle Eastern plastics industry. Numbers adopted from PlasticEurope.¹¹

the environment, where the material can become brittle and start to fragment. In the environment the degradation of macroscopic plastic objects (>5 mm) into smaller pieces, often referred to as microplastics, typically occurs by a combination of chemical and physical processes that notably can involve photodegradation, oxidation, hydrolytic degradation, and mechanical disintegration. These processes are collectively referred to as weathering and, depending on the polymer type and morphology, can differ significantly.^{12,13} Photodegradation caused by the ultraviolet (UV) portion of sunlight generally initiates the weathering process; this radiation is capable of breaking chemical bonds in synthetic polymers.¹⁴ The process affects not only the polymers themselves but also additives implemented within the materials, resulting in changes in their chemical structure and physical properties. Oxygen can increase the absorption of UV radiation by the formation of a complex with conjugated unsaturated hydrocarbons, thereby accelerating the degradation process.¹⁵ In addition, polymers such as polyesters or polyamides can also be degraded by hydrolysis, i.e., the cleavage of ester or amide bonds by reaction with water.^{16,17} These chemical degradation processes cause a reduction of the polymer molecules' molecular weight. Eventually this leads to a reduction of mechanical strength and in many cases an increased absorption of water, which causes a further reduction of the mechanical properties and creates bulk and surface stress gradients, which eventually lead to cracking of the material. External mechanical forces also contribute to the fragmentation into smaller pieces, whose dimensions gradually diminish over time. The degraded plastic pieces form a highly heterogeneous collection, varying in size, shape, and density as well as the chemical composition of the specific material.

Newest observations studying the degradation of polyethylene microplastic pellets have shown that already an exposure time of 8 weeks in artificial seawater induces severe microcracking of the pellets surfaces, which, in combination with mechanical forces, can break down the material into smaller sized particles.¹⁸ Further studies also revealed the

formation of nanosized polystyrene particles after 4 weeks of exposure in a weathering chamber.¹⁹

Several studies have been published in the past few years estimating the mass of the overall global plastic waste as well as the mass of plastic waste entering our oceans (Figure 2), which are considered to be the global ecosystem that is mostly affected by plastic waste. In 2009, Barnes et al.²⁰ estimated that plastic materials constitute 10% of the total discarded waste. Jambeck et al.²¹ presented numbers in the range of



Figure 2. Plastic sample taken from an expedition in the Ligurian Sea in May 2018 by the “Sail and Explore Association” using a manta trawl during 30 min with a mesh size of 0.3 mm. The Mediterranean Sea represents one of the most polluted seas worldwide with nearly four times higher concentrations of microplastic fragments compared to the North Pacific Gyre and a record-breaking number of 1.25 million microplastic fragments per square kilometer floating.⁵³ Reprinted with permission from Sail and Explore Association, Copyright 2018.

4.8–12.7 million tons of mismanaged plastic waste that was generated in 192 coastal countries and discarded into the oceans in the year 2010. In addition, based on data collected in 24 expeditions conducted during 2007–2013, Eriksen et al.²² reported that a minimum of 5.25 trillion plastic particles with a weight of up to 270,000 tons are floating in the world's oceans. Another study reported the number of small plastic particles (nominally <200 μm) to be in the range of 15–51 trillion, with a collective weight of 93,000 to 236,000 tons, which corresponds to approximately 1% of the global plastic waste entering the oceans in the year 2010.²³ A recent study from 2018 predicted, using data received from multivessel and aircraft surveys in the so-called “Great Pacific Garbage Patch”, that at least 79 thousand metric tons of ocean plastic are floating inside an area of 1.6 million km^2 , which correlates to numbers 4–16 times higher than previously reported.²⁴ Other studies have also proven the occurrence and accumulation of large plastic items in the deep sea,^{25–27} and recently published data show that plastic objects have been ingested by animals of the benthic zone, assuming a sedimentation behavior of the plastic particles.^{28,29} Sedimentation of plastic particles is only possible if the density of plastics is higher than that of seawater (which is the case for roughly a third of all polymers produced) or if they become negatively buoyant by the formation of biofilms and adherence to other particles.^{30–33} The smaller the particles are, the faster they can reach their critical sinking density.³⁴ Plastic particles are also deliberately added to personal care products.³⁵ A threat that has thus far been underestimated is the impact of microplastics in soils, sediments, and freshwater on the terrestrial ecosystem.³⁶ Research has only recently begun in this direction, as an estimated 80% of microplastic pollution in the oceans originates from land.^{37,38}

In the past decade, much attention has been paid to the formation of microplastics. The exact definition of these particles differs but generally includes particles with dimensions between 1 μm and 5 mm.³⁹ The first small plastic particles (<5 mm) were detected in open waters in the 1970s.^{40,41} Recent studies confirmed the expectation that the degradation process of plastic particles does not stop at the micrometer level and that plastic microparticles continue to disintegrate to form plastic nanoparticles.^{19,42,43} Such nanoparticles usually exhibit different chemical and physical properties than macroscopic objects based on the same material.⁴⁴ In addition, their interactions with living organisms can also greatly differ.⁴⁵ Thus, the differences between micro- and nanoplastics are not trivial, and the interactions of nanoplastics with the environment and organisms are a specific concern.⁴⁶ Like plastic microparticles, nanoplastics can adsorb and carry hydrophobic chemicals that have a potential biological and toxicological impact on the environment, such as polychlorinated biphenyls (PCBs) released from objects like electrical devices, inks, paints, or the pesticide dichlorodiphenyltrichloroethane (DDT).^{47–49} A clear understanding of the interaction of nanoplastics with the environment, especially with living organisms, is important to assess possible health hazards, because nanoplastic particles can react differently than their micron-sized counterparts. However, current research aimed at addressing the effects of plastic is focusing mainly on aquatic systems and only limited data are available regarding the impact of nanosized plastic particles on human health,^{50,51} although their formation in the environment is increasing and thus also the possible transfer to humans via the food chain.⁵²

It is apparent that the pollution of oceans with plastic has become a major environmental issue. Although the long-term impact of micro- and nanoplastics in the (aquatic) environment is still difficult to predict, this aspect might prove to be a significant challenge to our society.⁵⁴ The purpose of this review is to provide an overview of current knowledge specific to nanoplastics and their impact on the environment and human health. We briefly discuss sources and nanoplastic formation in the aquatic environment and the impact of nanoplastics on aquatic species and human exposure which is most likely via seafood. Furthermore, we will focus on possible entry routes of nanoplastics into the human body focusing on the cellular level.

2. SOURCES AND FATE OF PLASTIC IN THE ENVIRONMENT

The accumulation of plastics and their degradation products in the environment has continuously increased in recent decades, which is unsurprising given that 50% of plastic products are produced for single-use applications and are soon discarded.⁵⁵ Discarded packaging plastics account for a significant part of the total solid waste that ends up in landfills, where, depending on their composition, they remain unchanged or may degrade into fragments upon microbial heat production and further biodegrade to carbon dioxide and water.⁵⁶ Macroplastics represent the main source of plastic litter. Once in the environment they can lead to entanglement and, despite their size, being ingested and retained by various species, including seabirds, fish, and cetaceans, which then often die from related causes such as starvation.^{57,58} Microplastic particles affect an even greater number of organisms, including primary consumers of the food chain such as zooplankton, bivalves, and small fish.^{59–61} It has been shown that (fragments of) textile fibers with dimensions in the micrometer range, which are disengaged from clothes during washing, are not removed by the filter systems of wastewater treatment plants and therefore end up in the environment.⁶² In addition, personal care products such as toothpastes and facial scrubs often contain polyethylene-based microplastic particles which then end up in the wastewater.⁶³ The presence of microplastics has been reported all around the globe, from polar regions frozen in arctic ice to the open water around the equator, and from coastal areas down to the deep sea.^{30,64} The process of plastic degradation progresses with increased time in the environment and may eventually lead to the formation of nanoparticles.⁶⁵ For example, a recent study by Lambert et al. demonstrated the formation of plastic nanoparticles under laboratory conditions when disposable polystyrene coffee cup lids were exposed to UV light.¹⁹ This observation supports the notion that wherever polymeric objects are released in the environment, the degradation to nanoplastic should a priori be considered. At the same time, it is clear that the degradation pathways of different polymer types can vary significantly—while some polymers degrade into nanoparticles, others are known or can be expected to form water-soluble fragments. From experimental observations made so far, it can be estimated that plastic nanoparticles can be released into the environment as “primary”, i.e., intentionally manufactured particles for industrial purposes, e.g., paints, adhesives, electronics, and cosmetics, and/or emerge as “secondary particles” as a result of the degradation of larger plastic objects.^{66–68} Primary nanoplastics used in households and industry are most likely

not being collected by the wastewater treatment facilities and are thus discharged as sewage into the aquatic environment.

However, our current knowledge of nanoplastics in the environment is still very limited and only a few scientific publications are available. In addition, the literature remains inconsistent about the categorization of the particle sizes.³⁹ A number of studies have set the upper size limit of nanoplastics at either 100 or 1000 nm. A recent publication by Gigault et al. proposes an appropriate definition of nanoplastics on the basis of physical and chemical properties. According to the authors, a nanoplastic is defined as “particles within a size ranging from 1 nm up to 1000 nm resulting from the degradation of industrial plastic objects and can exhibit a colloidal behaviour”.⁶⁹ One could, however, recommend the use of the official EU recommendation on the definition for nanomaterials, i.e., “A natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range of 1 nm–100 nm”.⁷⁰ Another difficulty is that currently no detection method can confirm the presence of nanoplastic components in the environment.⁷¹

2.1. Effects of Nanoplastics on the Aquatic Environment. Numerous studies have shown that macro- and microplastics can have a significant adverse impact on the aquatic environment and have been reported elsewhere in detail.^{72–74} But what are the effects of nanoplastic particles on the aquatic environment?

In recent years several experimental studies using model polystyrene nanoparticles have emphasized that various organisms such as daphnia, mussels, zooplankton, and algae can actively ingest nanoplastic particles or adsorb them to their surfaces.^{75–78}

In a laboratory setting, the effects of amino-functionalized polystyrene nanoparticles with a diameter of 50 nm and concentrations between 1–50 $\mu\text{g}/\text{mL}$ have been investigated in the hemocytes of the marine bivalve *Mytilus galloprovincialis* by Canesi et al.⁷⁹ The hemocytes were exposed to different concentrations of amino-functionalized polystyrene particles, which stimulated an increase in extracellular reactive oxygen species and lead to apoptosis within 1 h of exposure. This rapid response is in line with the physiological role of hemocytes in cell-mediated immunity. Sun et al. showed that nanoplastic particles at a size of 50 nm and a concentration of 80 mg/L induce toxicity related to oxidative stress toward marine bacterium *Halomonas alkaliphila* compared to microplastic particles at a size of 1 μm . Furthermore, amine-modified 50 nm polystyrene nanoparticles induced higher oxidative stress toward bacteria than that induced by nonmodified particles.⁸⁰ The toxicity of amine-modified polystyrene particles was further proven by Tallec et al. Exposure of 50 nm polystyrene nanoparticles at concentrations between 0.1 and 25 $\mu\text{g}/\text{mL}$ to Pacific oysters (*Crassostrea gigas*) showed an increase in toxicity for both gametes and embryos in comparison to nonmodified particles. Overall, their data highlight that exposures to polystyrene nanoparticles may have deleterious effects on planktonic stages of oysters.⁸¹ Brandts et al. investigated the effects of polystyrene nanoparticles, individually or combined with carbamazepine, on the mediterranean mussel *Mytilus galloprovincialis*. Carbamazepine is among the most commonly detected drugs in the environment and may adsorb onto nanoplastics. For this purpose, mussels were

exposed for 96 h to a range of concentrations of 110 nm polystyrene particles (from 0.05 up to 50 mg/L), to carbamazepine (6.3 $\mu\text{g}/\text{L}$) alone and to a mixture of polystyrene + carbamazepine (0.05 mg/L + 6.3 $\mu\text{g}/\text{L}$). Observations of different biomarkers from the digestive glands, gills, and hemolymph were conducted. After exposure to polystyrene, carbamazepine, and their mixture, clear evidence for genotoxicity was found in hemocytes as well as significant downregulation in gene expression after combined exposure of polystyrene and carbamazepine. Moreover, it could be shown that even at the lowest concentrations tested, polystyrene nanoparticles can induce oxidative damage.⁸² Della Torre et al. demonstrated that the accumulation of 50 nm amino-modified polystyrene nanoparticles at tested concentrations of 1–50 $\mu\text{g}/\text{mL}$ in sea urchin embryos induced changes in gene expression and embryotoxicity.⁸³ A recent study investigated the effects of 75 nm polystyrene nanoparticles at concentrations between 10 and 400 mg/L on the physiological changes (i.e., survival) and expression levels of stress defense genes of *Daphnia pulex*. The expression of the gene encoding the energy-sensing enzyme AMPK (adenosine monophosphate-activated protein kinase) was influenced by the nanoplastic in different age groups. Thus, age must be considered as a factor of great relevance when predicting toxic effects.⁸⁴

Effects of nanoplastic on the innate immune system of fish have been reported by Greven et al., indicating that the stress response to polystyrene and polycarbonate nanoparticles could stimulate the degranulation of primary granules, oxidative burst activity, and neutrophil extracellular trap release.⁸⁵ Experiments with zebrafish revealed that after 7 days of exposure to fluorescent polystyrene nanoparticles with a diameter of 70 nm and at concentrations between 0.025 and 0.2 $\mu\text{g}/\mu\text{L}$, inflammation and lipid accumulation in the liver occurred.⁸⁶ Marques-Santos et al. investigated the effect of 50 nm amino-modified polystyrene particles at concentrations of 1–25 $\mu\text{g}/\text{mL}$ on sea urchin *Paracentrotus lividus* immune system cells (coelomocytes) in the presence of celomic fluid and at different particle concentrations. Amino-modified polystyrene particles acquired a protein corona once incubated with celomic fluid, dominated by the toposome precursor protein, which triggers particle–coelomocytes interactions and toxicity.⁸⁷

Wegner et al. showed that 30 nm polystyrene nanoparticles, that were tested at concentrations of 0.1–0.3 g/L, have an adverse effect on the feeding behavior of the blue mussel *Mytilus edulis* because of a reduced filtering activity in the presence of the particles.⁸⁸ Pitt et al. investigated the potential toxicity of polystyrene nanoparticles in developing zebrafish (*Danio rerio*) and characterized the uptake and distribution of the particles within embryos and larvae. Embryos at 6 h postfertilization were exposed to 51 nm polystyrene nanoparticles (approximately 1 mg/(g of fish)) up to 120 h postfertilization. At 24 h postfertilization the particles could be found in the yolk sac and had migrated to the gastrointestinal tract, gallbladder, liver, pancreas, heart, and brain between 48 and 120 h postfertilization. In addition, it could be seen that the particles altered larval behavior as evidenced by swimming hypoactivity in exposed larvae.⁸⁹ An initial study of the effect of nanoplastic particles delivered through the food chain (algae–zooplankton–fish) on the brain tissue of fish was conducted by Mattsson et al. They showed that 52 nm positively charged amino-modified polystyrene nanoparticles above 0.075 g/L are toxic to *Daphnia magna* and that fish eating nanoplastic

contaminated *Daphnia* showed behavioral disorders. Furthermore, polystyrene nanoparticles were detected in the brain tissue of all fed fish using hyperspectral imaging. This finding proves for the first time that plastics nanoparticles can be transported across the blood–brain barrier in fish.⁹⁰

The majority of the mentioned studies showing an adverse effect on different organisms were conducted using amino-modified polystyrene nanoparticles and concentrations which are not environmentally relevant as they are all too high. The amino modification induces a positive surface charge to the particles, which is experimentally interesting to test charge effects. Nevertheless, it is well-known from the field of nanomedicine that aminated surfaces are mainly used for coupling to, e.g., proteins or antibodies and that the positive charge of cationic polymers can facilitate cellular uptake and endolysosomal escape, thereby overcoming major cellular barriers and consequently increasing the possibility for further intracellular reactions.⁹¹ The most important class of plastic materials containing nitrogen in the form of amides is polyamides. These can be hydrolyzed to afford amines, but it is not entirely clear to what extent the amines remain intact (depending on the type, they may be protonated and oxidized). However, polyamides are not widely used as packaging materials, and therefore amine-containing nanoplastic particles stemming from polyamides are not expected to be a major environmental factor.

Additionally, nanoplastic particles in the environment will most probably interact with their surroundings, leading to homo- or heteroagglomerates composed of different constituents, due to the size and hydrophobic properties of the materials.^{92,93} This might lead to the formation of a surface corona of various adsorbed molecules evoking a change of the physical and chemical parameters as well as a reduced surface-area-to-volume ratio, thus changing their biological activity and maybe affecting their sedimentation properties.⁹⁴ These facts have to be considered for experimental approaches regarding influences on aquatic systems, in order to mimic natural conditions as closely as possible.

For nanoplastics, uptake by several aquatic species, the adsorption of hydrophobic persistent organic pollutants (POPs), and the leaching of chemicals and POPs have been demonstrated, highlighting their potential biological and toxicological impacts on the environment.⁹⁵

However, a number of studies showing adverse effects such as reactive oxygen species production and reproductive malfunctioning upon exposing aquatic organisms to nanoplastics have used concentrations several orders of magnitude higher than concentrations predicted to be environmentally relevant such as 1 pg L^{-1} to $15 \text{ } \mu\text{g L}^{-1}$ for nanoplastics at sizes of about 50 nm.⁹⁶ Reflecting this fact will help to understand the impact of environmentally relevant nanoplastic concentrations. In addition, there is a lack of knowledge regarding how nanoplastics (and hitchhikers) are transferred up the food chain and how they accumulate and interact with the environment, especially with living organisms. Thus, the impact of a contaminated food chain on humans and the resulting health hazards are not at all clear.

3. IMPACT OF NANOPLASTICS ON HUMAN HEALTH

Exposure to nanoplastic might occur via oral inhalation, ingestion, or absorption by the skin in connection with the use of plastic products or unintentional means (Figure 3). Inhalation is likely to be relevant in occupational exposure

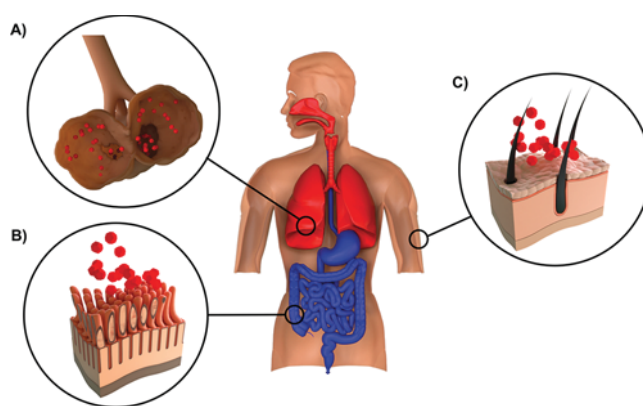


Figure 3. Schematic illustration showing the three major pathways of human exposure to nanoplastics, i.e. via (A) the lung, (B) the gastrointestinal (GI) tract, and (C) the skin.

scenarios that involve nanoplastic-containing aerosols,⁹⁷ whereas potential contact with the skin can occur through the use of personal care products such as nanoplastic-containing skin care and cleansing products, or contaminated water or air. According to the current knowledge, ingestion of nanoplastic particles is likely to represent the main route of entry, since nanoplastic particles can be ingested by eating seafood or drinking contaminated water. In addition, nanoplastic uptake and accumulation, as well as trophic transfer of nanoplastic within aquatic organisms has been demonstrated under experimental conditions, thus fortifying the possibility that nanoplastics might accumulate in the food chain, and thus result in human exposure.^{98,99} Microplastic particles have already been found in several seafood species, such as fish, shrimps, and bivalves, but also in other foods, such as honey, beer, salt, and sugar.^{60,100–104} Recent studies using FTIR spectroscopy have also shown the existence of microplastics in tap water and bottled water as well as drinking water from groundwater sources. Out of 159 samples of globally sourced tap water, 81% were found to contain microplastic particles, mainly fibers smaller than 5 mm with an overall mean of 5.45 particles/L.¹⁰⁵ From a total of 259 individual bottles of water from 11 different brands and 27 different lots, 93% showed signs of microplastic contamination with an average of 10.4 particles/L.¹⁰⁶ Analysis of groundwater from the northwestern part of Germany revealed that an overall mean of 0.7 microplastics/m³ can be found.¹⁰⁷ These studies reiterate that the occurrence of nanoplastics in various food products cannot be ruled out. Unfortunately, there are currently no routine methods available that permit detection of nanoplastics in foods, and as a result, no data are available that go beyond the above-mentioned research works.

Human health might also be affected due to the transfer or leaching of chemical additives from the plastic material itself. Within the plastic manufacturing process, chemicals such as plasticizers, pigments, or stabilizers are added to give the desired properties of the final product, e.g., their flexibility, color, and stability. Nowadays, thousands of different chemicals are currently used for these purposes, and it is known that some of these chemicals can leach out during the product life cycle into the environment, leading to endocrine disruption or acute toxicity when exposure to organisms occur.¹⁰⁸ The same considerations apply for the monomers (i.e., the chemical building blocks) used to manufacture the polymers in the first place (of which small amounts may

remain in the polymers) and the products formed by the chemical degradation of polymers. The most prominent example of a leaching monomer is bisphenol A (BPA), which is used for the preparation of polycarbonate and certain epoxy resins. It has been shown that BPA causes adverse effects in humans due to its estrogenic activity, including several metabolic diseases as well as reproductive and developmental effects.^{109–111} In particular, polycarbonate drinking bottles used for newborns showed high leaching of BPA. Newborns have a much higher risk than adults since a higher internal body burden is expected, expressed as concentration in blood/plasma, due to increased absorption or decreased elimination compared to the internal body burden of adults.¹¹²

3.1. Entry Routes of Nanoplastics into the Human Body. Three major exposure pathways to nanoplastics are possible, i.e., via the lung, the gastrointestinal (GI) tract, and the skin. The lung has a very large alveolar surface area of ca. 150 m², with a very thin tissue barrier of less than 1 μm,¹¹³ allowing nanosized particles to penetrate into the capillary blood system and to distribute throughout the human body.^{114,115} Several studies have shown that exposure to synthetic polymers can have an adverse effect on the health if absorbed by the respiratory system.^{116–118} Studies applying polystyrene particles to alveolar epithelial cells *in vitro* have shown that nanoplastic particles are taken up and that the uptake rate is size-dependent (Figure 4).^{119–122} Recently,

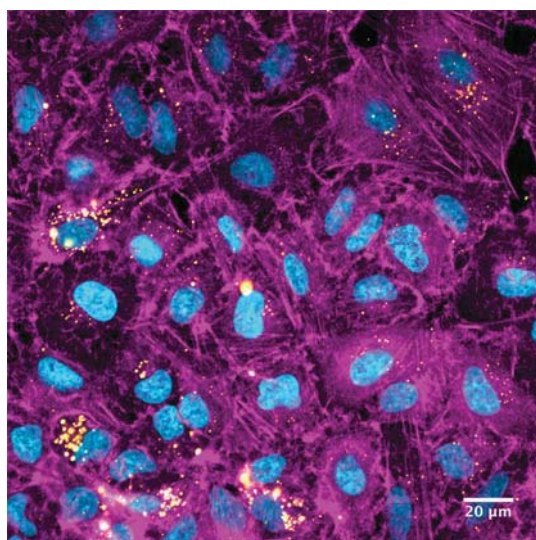


Figure 4. Human lung epithelial cancer cells (A549) labeled for F-actin (purple) and the nuclei (blue) showing uptake of 200 nm amino-modified polystyrene particles labeled with FITC (yellow) after exposure for 24 h.

synthetic fibers in atmospheric fallout in urban areas have been highlighted as a possible source for human exposure to microplastics by inhalation.¹²³ Atmospheric fallout of microplastics, collected with a stainless steel funnel, was investigated in two different urban and suburban sites in Paris. About 30% of particles observed were synthetic fibers with diameters between 7 and 15 μm and predominant sizes of 200–600 μm. Between 2 and 355 particles/m²/day, with an average of 110 ± 96 particles/m²/day were observed. Fluxes were found to be systemically higher at the urban site than at the suburban site. Rainfall was also shown to have a clear effect on the concentrations observed.¹²³ Dris et al. investigated micro-

plastic fibers in indoor and outdoor air. Two private apartments and one office were chosen as indoor sites, where a concentration between 1.0 and 60.0 fibers/m³ was measured, which was significantly higher than the outdoor concentration (between 0.3 and 1.5 fibers/m³). Around 33% of the fibers they collected indoors were of petrochemicals origin with polypropylene being predominant, while the resting 67% consisted mainly of cellulose.¹²⁴ However, there are thus far no data available concerning the number or concentration of aerosolized nanoplastics. The numbers presented by the studies of Dris et al. are more than 1000 times lower than the permissible exposure maximum of 5 mg/m³ respirable nuisance dust/(8 h working day) established by the U.S. Occupational Safety and Health Administration (OSHA).¹²⁵ This suggests that the concentrations of microplastics found in the air in these studies might be too low to have an adverse effect on human health. Nevertheless, more data are needed, in particular taking into account the physicochemical characteristics of the material as well as their sizes.

Furthermore, Wright and Kelly suggest that human exposure to micro- and nanoplastics through inhalation might occur by the transportation of sea salt aerosols. These aerosols can arise from wave action containing polymer particles of appropriate sizes, which then can be transported by the wind to coastal environments. Another scenario involves the application of wastewater treatment sludge containing plastic particles to agricultural land as a fertilizer. Once the sludge is dried, wind can further transport the plastic particles and distribute them. For further information regarding inhalation exposure of microplastics, we refer readers to Wright and Kelly,⁵⁰ Gasperi et al.,¹²⁶ and Prata,⁹⁷ who provide detailed overviews of the topic.

Although the concentration of synthetic polymer particles in the air is usually very low, the GI tract, with its large surface area of ca. 200 m², represents the primary exposure site for plastic particle uptake. Ingestion and accumulation of nanoplastic particles in the GI tract has been demonstrated in a wide range of aquatic organisms, such as fish, mussels, and also birds.^{89,98,127–129} Human uptake of plastics might occur by intentional swallowing, leading in the worst case to a plastic bezoar,¹³⁰ a rare cause of GI obstruction occurring mainly in people with psychiatric ailments or, more likely, unintentionally via the food chain by the consumption of plastic-contaminated food and drinks or possibly through migration of nanoplastic particles from the packaging materials into food products.

It has been shown that microplastics ingested by fish are poorly absorbed via the GI barrier into the tissue, and gutting of the fish reduces the possibility of consuming microplastic particles. In contrast, polystyrene nanoparticles have been shown to overcome the GI barrier and to translocate into the underlying tissue, as demonstrated by several groups with filter feeders and shellfish.^{76,88,128} Data on the release of nanoplastics from packaging released into food products are currently unavailable; therefore, the exposure to humans cannot be estimated for this method of intake.¹³¹

Most of our current knowledge regarding nanoplastic and intestine interaction comes from cell culture studies using intestine cells. Several *in vitro* studies have investigated the internalization and translocation of polystyrene nanoparticles in intestine cell monocultures or even more complex human intestinal cell models. Forte et al. showed that polystyrene nanoparticles with a diameter of 44 nm accumulate faster and

more efficiently in the cytoplasm of human gastric adenocarcinoma cells than otherwise identical particles with a diameter of 100 nm. In addition, the 44 nm particles showed a strong upregulation of the interleukin (IL)-6 and IL-8 genes, which are involved in gastric pathologies.¹³² Walczak et al. determined the translocation of polystyrene particles with diameters of 50 and 100 nm, having positively and negatively charged as well as uncharged surfaces, in three in vitro intestine cell models of increasing complexity using Caco-2, HT29-MTX, and M-cells. Their results showed that both the size and the chemical composition of the particle surface impacts their translocation. The size clearly affected the translocation of the nanoparticles, ranging up to 7.8% for the 50 nm and 0.8% for the 100 nm particles. However, the surface chemistry seems more important, as the two types of negatively charged 50 nm NPs had a greater than 30-fold difference in translocation rates.¹³³ Mahler et al. suggested that acute oral exposure of positively charged polystyrene nanoparticles can disrupt intestinal iron transport and cellular uptake.¹³⁴

The cellular uptake of nanoparticles is strongly influenced by their interactions with surrounding biological components, such as proteins, phospholipids, or carbohydrates, due to their size, surface chemistry, and charge.¹³⁵ Adsorption of proteins from body fluids on nanoparticle surfaces results in the formation of a so-called “protein corona” around the particle.¹³⁶ Hence, interactions between organs and tissue generally occur with protein-coated—rather than bare—nanoparticles, likely causing changes in the characteristics and properties of the nanoparticle. Polystyrene nanoparticles have been shown to form protein corona complexes in in vitro GI studies, resulting in increased translocation,¹³⁷ the change of the protein corona composition over time depending on the local environment,¹³⁸ and increased cellular uptake and toxicity.¹³⁹ Furthermore, the protein corona caused the polystyrene nanoparticles to be retained in the gut.

In conclusion, it has been shown that, in contrast to microplastic particles, nanoplastic particles overcome the GI barrier in aquatic organisms and can translocate into the underlying tissue. In addition, polystyrene nanoparticles can translocate the human intestine barrier in vitro. However, the reported studies rely solely on model polystyrene nanoparticles and are not from environmental samples (Figure 5). Other polymers such as PP, PE, and PET are, however, the main polymer material present in the environment. Thus, extrapolations of findings from polystyrene materials to other polymers should be made with caution, and new model studies should be introduced including PP, PE, and PET.

The skin forms a protective barrier against external influences such as physical injuries, chemical agents, or bacteria. Contact of plastics with the skin might occur via the use of cosmetic products containing nanoplastic particles or with nanoplastic-contaminated water. The stratum corneum is the physical barrier of the skin, and due to the hydrophobic properties of the plastic particles, significant nanoplastic uptake through human skin in water is not to be expected. However, possible entry routes include hair follicles, exits of sweat glands, or via injured skin areas.¹⁴⁰ Skin penetration and resulting tissue distribution of plastics has been studied by Alvarez-Roman et al., who applied fluorescent polystyrene particles with diameters of 20 and 200 nm to a porcine skin tissue model.¹⁴¹ Confocal laser scanning microscopy images revealed a higher accumulation of the 20 nm polystyrene nanoparticles than with the 200 nm sized particles in the follicular openings,

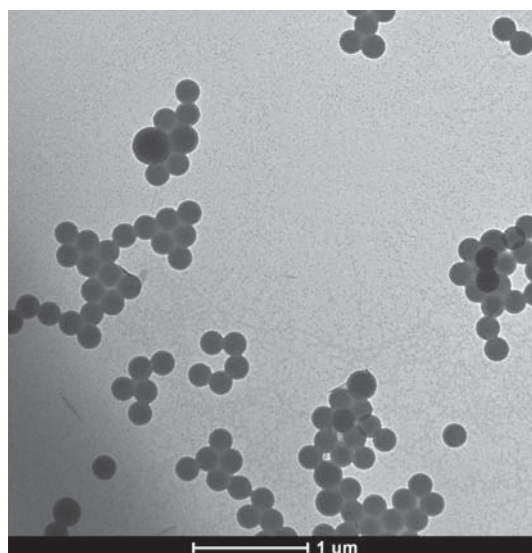


Figure 5. Transmission electron microscopy image showing commercially available spherically shaped polystyrene nanoparticles used for in vitro studies.

whereas neither particle type was found to penetrate into deeper skin tissue, possibly due to the barrier properties of the stratum corneum. These results were confirmed by an additional study conducted by Campbell et al. a few years later, who observed that polystyrene particles with diameters of 20–200 nm penetrate only into the surface layers, approximately 2–3 μm , of the stratum corneum.¹⁴² A study conducted by Vogt et al. identified fluorescent polystyrene nanoparticles of a diameter of 40 nm in the perifollicular tissue of human skin explants pretreated with cyanoacrylate follicular stripping and confirmed uptake by Langerhans cells after transcutaneous application to human skin.¹⁴³ Microbeads used in cosmetics such as scrubs and shampoos are processed by mechanical means that may lead to their fragmentation into potentially more hazardous nanoplastics. In a recent publication, Hernandez et al. tested facial scrubs containing polyethylene microbeads with diameters of 200 μm for the presence of nanoplastics. Scanning electron microscopy data revealed nanoparticles ranging in size from 24 ± 6 to 52 ± 14 nm. The material composition was tested by X-ray photoelectron spectroscopy and Fourier transform infrared spectroscopy, which confirmed that the identified nanoparticles consisted of polyethylene.⁶⁷ The further potential ability of nanoplastics to overcome the skin barrier can be extrapolated from data available from studies using nanoparticles. Skin stress evoked by UV radiation has a widespread effect on the integrity of the skin barrier. An initial in vivo study conducted by Mortensen and colleagues investigated nanoparticle skin penetration of carboxylated quantum dots (QDs) with and without UV exposure. Their results showed qualitatively higher levels of penetration of QD nanoparticles in the UV exposed mice, due to the perturbed expression of tight-junction-related proteins (Zonula occludens-1, claudin-1, and occludin), which promote intercellular adhesion.¹⁴⁴ The effect that common commercial skin care lotions may have on the penetration of QD nanoparticles into the skin was determined by Jatana et al. In their study, QDs were added to five commercial skin lotions and applied to freshly excised human skin as well as C57BL/6 hairless mice. Their findings suggest that certain ingredients

(e.g., urea, glycerol, and α -hydroxyl acids) found in common commercial skin care lotions can enhance NP penetration into the skin.¹⁴⁵ In addition, several chemical penetration enhancers have shown their potential to support skin penetration by nanoparticles.¹⁴⁶ Kuo et al. illustrated the differences between oleic acid, ethanol, and oleic acid–ethanol enhancers for the transport of zinc oxide nanoparticles. The results showed that all three were capable of enhancing the transdermal delivery of zinc oxide nanoparticles.¹⁴⁷ However, there is currently little evidence that polymeric nanoparticles larger than 100 nm can penetrate into intact skin.¹⁴⁸

Once the plastic particles have entered the human body, they may overcome the primary tissue barriers and be transported through the bloodstream to secondary organs. In vitro studies have shown that carboxylated polystyrene nanoparticles can adsorb to, and penetrate into, red blood cells (RBCs) as a result of van der Waals, electrostatic, hydrogen bonding, and hydrophobic forces between polystyrene and the RBCs.^{149,150} This so-called cellular hitchhiking mechanism allows the polystyrene nanoparticles to avoid rapid clearance by the liver and spleen and, therefore, increase their circulation time.

Secondary barriers able to be reached via the bloodstream include the placental barrier and the blood–brain barrier (BBB). Grafmueller and colleagues showed with an ex vivo human placental perfusion model that polystyrene particles with diameters of up to 240 nm can be taken up by cells of the placental barrier and transported from the fetal to the maternal blood circulation.¹⁵¹ The operating translocation mechanism seems likely to be an energy-dependent transport pathway. The BBB is a highly selective barrier regulating the uncontrolled diffusion of molecules into the brain. However, Yang et al. demonstrated BBB permeability with polystyrene nanoparticles having a diameter of 20 nm using an in vivo rat model by implantation of a microdialysis probe into the cerebral cortex of anesthetized rats injected with fluorescent polystyrene nanoparticles.¹⁵² A recent study by Rafiee et al. attempted to assess the neurobehavior of rats exposed to pristine polystyrene nanoparticles upon oral exposure. They investigated different concentrations of 25 and 50 nm polystyrene particles (1, 3, 6, and 10 mg of polystyrene nanoparticles/(kg of body weight)/day) administered orally with adult male Wistar rats for 5 weeks. No statistically significant behavioral effects were observed in any of the tests performed.¹⁵³

In conclusion, it has been shown by in vitro and in vivo studies that micro- as well as nanoplastic particles might be taken up into the human body and can overcome tissue barriers, thus also allowing interaction with single cells. However, real-world plastics sources (rather than commercially available perfectly spherical polystyrene particles) have not yet been used; therefore, further studies applying different plastic materials with significant size and/or shape polydispersity as well as environmentally relevant concentrations need to be conducted to fill the existing knowledge gaps.

3.2. Cellular Uptake Routes and the Intracellular Fate of Nanoplastic Particles. There are several pathways by which nanoparticles can be taken up by cells.¹⁵⁴ Although passive diffusion through the cell membrane (also referred to as adhesive interaction), channel- or transport-protein-mediated uptake have been described, endocytotic nanoparticle uptake is the major mechanism of cells. A range of endocytotic pathways, including phagocytosis and macropinocytosis, as well as clathrin- and caveolae-mediated

endocytosis, have been described.^{155–157} Not all of the studies we present here have been performed with intestine cells; however, our aim is to provide an overview of the current knowledge of polystyrene nanoparticles interaction with human cells.

The first interaction of nanoparticles with cells is the interaction with the outer cell membrane. Rossi et al. investigated the interaction of polystyrene particles with biological membranes by coarse-grained molecular simulations, which revealed that nanosized polystyrene particles can permeate easily into lipid bilayer membranes, alter the membrane structure, and reduce molecular diffusion, thereby affecting possible cell functions.¹⁵⁸ Fiorentino et al. investigated cellular uptake of 44 nm polystyrene particles on human colon fibroblasts and bovine oviductal epithelial cells.¹⁵⁹ By way of uptake inhibition studies, they demonstrated that the polystyrene nanoparticles were internalized mainly via a clathrin-independent uptake mechanism. dos Santos et al. studied the effects of transport inhibitors on the cellular uptake of 40 and 200 nm carboxylated polystyrene nanoparticles in human cervical HeLa cells, human glial astrocytoma 1321N1, and human lung epithelial A549 cell lines.¹⁶⁰ The results clearly indicated that in all cases polystyrene nanoparticles entered the cells via active energy-dependent processes. However, since none of the transport inhibitors could fully inhibit polystyrene uptake, the possibility remains that one cell line uses multiple uptake pathways simultaneously.

RBCs are highly specialized cells distinguished by their lack of a cell nucleus and endocytotic uptake mechanism. Studies have been conducted in order to demonstrate cellular uptake of different nanoparticles by RBCs and to better understand the operative uptake mechanism.^{161,162} Rothen-Rutishauser et al. showed uptake of nanosized polystyrene particles (diameter < 200 nm) by RBCs.¹⁶³ This result indicated neither an endocytotic nor an actin-based uptake mechanism, therefore suggesting a passive diffusion mechanism for this specific cell type.

However, it is important to mention that the uptake routes are not only size- and surface-chemistry-dependent but also cell-type-specific. The same material can show different cellular uptake pathways: for example, polystyrene nanoparticles with a diameter of 120 nm, which surface had been modified to feature amidine groups, have been shown to be taken up by rat alveolar epithelial monolayers via non-endocytic mechanisms, in contrast to MDCK-II cells, which show particle uptake in an energy-dependent manner.^{121,164} Kuhn et al. showed that a combination of several distinguishable endocytotic uptake mechanisms is involved in the uptake of 40 nm carboxylated polystyrene nanoparticles by macrophages and epithelial cells. Selected endocytotic pathway inhibitors were applied, and it was revealed in the case of J774A.1 macrophages that macropinocytosis, phagocytosis, and clathrin-mediated endocytosis pathways are involved, whereas for human alveolar epithelial cells (A549), the uptake was dependent on caveolin- and clathrin-mediated endocytosis pathways.¹⁶⁵ Nonvesicular transport through the membrane might allow the nanoplastic particles to interact directly with intracellular molecules or to release their payload, such as POPs, directly into the cytoplasm. This might lead to accumulation of POPs in the cell and potential toxicological effects on the human body.¹⁶⁶

Endocytosis of plastic particles will follow the intracellular endocytotic pathway involving early and late endosomes followed by fusion with lysosomes. Salvati et al.¹²² demon-

strated the intracellular lysosomal localization of 40–50 nm polystyrene nanoparticles in A549 cells. These findings are in agreement with other published data, which show accumulation of nanosized polystyrene particles in the lysosome, whereas no lysosomal escape or even particle degradation was observed under acidic conditions.¹⁶⁷

In another study, the importance of the physicochemical properties of mesoporous silica and polystyrene nanoparticles were compared; the results revealed clear differences in cellular uptake mechanisms using ovarian cancer cells.¹⁶⁸ It was shown that the two types of nanoparticles never showed an overlap in their endocytotic uptake routes. Mesoporous silica particles entered the cells via caveola-mediated endocytosis and, depending on their size, resided within the lysosome (50 nm) or were relocated in the cytoplasm (10 nm). Cellular uptake of polystyrene nanoparticle occurred via a caveola-independent route. Amine-modified 50 nm polystyrene particles were localized within the lysosome and showed toxicity after 4–8 h in comparison to 30 nm carboxyl-modified polystyrene particles, which did not follow the classic acidic endocytotic pathway and were not toxic. It is well-known from the literature that a positive surface charge of nanoparticles often results in increased cytotoxicity and cellular uptake by unspecific binding to negatively charged sugar moieties on the cell surface, whereas negatively charged particles impair endocytosis due to repulsive interactions.^{169,170} A variety of cellular uptake routes and intracellular localizations of polystyrene nanoparticles have been observed depending on the physicochemical properties; however, there is a lack of specific quantitative data regarding cellular entry and the fate of nanoplastics.

It is obvious that size matters and plastic nanoparticles interact differently with human cells in comparison with larger ones; however, the uptake is also dependent on the particle charge which might influence the behavior in the cell culture media. Since most of the in vitro studies described here have used polystyrene particles, it is of course difficult to extrapolate the results to other kind of plastic particles and more research with other plastics is highly recommended.

3.3. Adverse Effects of Nanoplastics. Nanosized materials in general have raised concerns regarding possible hazards and risks for the environment and especially for human health. An understanding of possible human exposure is critical to determine health hazards. Adverse effects of nanoparticles in vivo include cytotoxicity, (pro-)inflammation, or production of reactive oxygen species (ROS).¹⁷¹ A number of in vitro studies using human cell lines (Table 1) have revealed that polymer nanoparticles have the potential to activate the innate immune system, inducing inflammatory responses, or mediating oxidative stress. In in vitro experiments, Brown et al. showed pro-inflammatory responses of polystyrene particles with diameters of 202 and 535 nm upon exposure to human A549 lung cells, observing an increased IL-8 expression relative to that elucidated by polystyrene nanoparticles with a diameter of 64 nm.¹⁷² As seen by Forte et al., unmodified polystyrene nanoparticles with a diameter of 44 nm induced a strong upregulation of IL-6 and IL-8 genes in experiments using human gastric adenocarcinoma cells, indicating that the induction of pro-inflammatory responses by polystyrene does not necessarily have to be charge-driven (as suggested by many studies) but may instead be a material-based or simple particle occurrence issue.¹³² McCarthy et al. identified the activation of ion channels in human lung cells after exposure to polystyrene

nanoparticles. Application of 20 nm carboxylated polystyrene particles caused continuous short-circuit currents by the activation of basolateral K⁺ channels and the stimulation of Cl⁻ and HCO₃⁻ secretion. These findings underline that polystyrene nanoparticles may have the ability to affect the epithelial cell function and physiological processes.¹⁷³ Fuchs et al. investigated the effect of 120 nm carboxylates and amino-modified polystyrene particles on the polarization of human macrophages into M1 or M2 phenotype. Their observations revealed that the expression of the M1 markers CD86, NOS2, TNF- α , and IL-1 β was not affected, while for M2 types both nanoparticles impaired expression of scavenger receptors CD163 and CD200R, and the release of IL-10. The amino-modified particles also inhibited the phagocytosis of *Escherichia coli* by both, M1 and M2 macrophages, while the carboxylated particles did not affect the phagocytosis of the bacteria by M2, but increased protein mass in M1 and M2, TGF- β 1 release by M1, and ATP levels in M2.¹⁷⁴

A new strategy to develop efficient nanoparticle-based drug delivery systems involves the use of RBCs as a carrier system, which can increase the circulation of nanoparticles in vivo. In this context, Barshtein et al. investigated RBCs with attached polystyrene nanoparticles having diameters of 50, 108, and 243 nm, probing in particular the aggregation of RBCs and their adhesion to endothelial cells. The authors showed that the formation of RBC aggregates increased with decreasing particle size and in parallel a distinctly elevated adhesion to endothelial cells was observed.¹⁷⁵ In addition, Barbul et al. showed that polystyrene particles with sizes of 27, 45, and 100 nm adsorbed to RBCs led to shape transformations and reduced cell deformability, with the 27 nm particles showing the strongest effect.¹⁷⁶

Furthermore, Xia et al. showed the influence of 30 nm polystyrene nanoparticles on the endocytotic pathway in macrophages and human cancer cell lines A549, HePG-2, and HCT116. The particles induced the formation of large vesicle-like structures, which blocked the vesicle transport in the endocytic system and the distributions of regular proteins required in cytokinesis, which led to the formation of binucleated cells.¹⁷⁷ A recent study by Inkiewicz-Stepniak et al. investigated the role of mucin in the toxicological impact of polystyrene nanoparticles. Their findings showed that amine-modified 57 nm sized polystyrene particles have the ability to strongly interact and aggregate mucin and induced apoptosis equally on mucin- and non-mucin-secreting intestinal epithelial cells.¹⁷⁸ Further studies using cationic polystyrene nanoparticles indicated the increase in ROS production and endoplasmic reticulum stress caused by misfolded protein aggregation in macrophage (RAW 264.7) and lung epithelial (BEAS-2B) cells, leading to autophagic cell death.^{179,180} A further study confirming that polystyrene particles are able to induce ROS production was conducted by Liu et al. In their study they showed that polystyrene particles 500 nm in size could stimulate ROS generation in human liver cells.¹⁸¹

Aside from polystyrene, an in vitro study using unmodified polyethylene particles with sizes ranging from 0.3 to 10 μ m showed unequivocally that the plastic particles stimulated mice-derived macrophages to produce significant levels of cytokines such as IL-6, IL-1 β , and TNF- α .¹⁸² Furthermore, several studies of human patients have shown that normal usage of implants such as total joint replacements made of polyethylene can lead to the production of wear debris.^{183–185}

Table 1. Overview of in Vitro Studies Using Human Cell Lines Demonstrating Cellular Uptake and Effects upon Exposure to Polystyrene Nanoparticles

material, modification	size, nm	major findings	target/cell line	ref
polystyrene, unmodified	44	induced strong up-regulation of IL-6 and IL-8 genes	human gastric adenocarcinoma cells (AGS)	Forté et al. ¹³²
polystyrene, unmodified, carboxylated, amino-functionalized	50, 100	size dependency regarding particle translocation	human colon carcinoma cells (Caco-2), human colon adenocarcinoma cells (HT29-MTX), human intestine microfold cells	Walczak et al. ¹³³
polystyrene, carboxylated	44	maximum particle uptake after 30 min	human colon fibroblasts (HCF)	Fiorentino et al. ¹⁵⁹
polystyrene, carboxylated, amino-functionalized	50, 200	disruption of iron transport due to polystyrene particle exposure	human colon carcinoma cells (Caco-2), human colon adenocarcinoma cells (HT29-MTX)	Mahler et al. ¹³⁴
polystyrene, carboxylated, amino-functionalized	20, 40	higher uptake efficiency of carboxylated particles compared to amino-functionalized particles, induction of apoptosis	human colon carcinoma cells (Caco-2)	Thubagger and Reinhard ¹⁹⁰
polystyrene, amino-functionalized	57	binding of mucin and induction of apoptosis	human colon carcinoma cells (Caco-2, LS174T, HAT-29)	Inkiewicz-Stepniak et al. ¹⁷⁸
polystyrene, carboxylated	20, 40, 100	40 nm particles internalized faster than 20 or 100 nm particles in both cell lines	human lung carcinoma cells (A549), human astrocytoma 132.1N1	Varela et al. ¹¹⁹
polystyrene, carboxylated	116	cellular uptake	human lung carcinoma cells (A549)	Deville et al. ¹²⁰
polystyrene, carboxylated	40–50	cellular uptake irreversible, intracellular concentration increased linearly	human lung carcinoma cells (A549)	Salvati et al. ¹²²
polystyrene, n.d.a.	64, 202, 535	increased IL-8 expression for 202 and 535 nm particles compared to 64 nm	human lung adenocarcinoma cells (A549)	Brown et al. ¹⁷²
polystyrene, carboxylated	20	activation of ion transport	human lung adenocarcinoma (Calu-3)	McCarthy et al. ¹⁷³
polystyrene, unmodified, carboxylated, amino-functionalized	60	amino-functionalized polystyrene particles induce autophagic cell death through the induction of endoplasmic reticulum stress	human bronchial epithelium (BEAS-2B), mouse monocyte macrophage (RAW 264.7)	Chiu et al. ¹⁷⁹
polystyrene, unmodified, carboxylated, amino-functionalized	60	amino-functionalized particles induced mitochondrial changes leading to cell death	human bronchial epithelium (BEAS-2B), mouse monocyte macrophage (RAW 264.7)	Xia et al. ¹⁸⁰
polystyrene, unmodified, carboxylated, amino-functionalized	50, 100	amine-modified particle-induced oxidative stress, mitochondrial disruption and release of cytochrome C, indicating apoptotic cell death	human immortalized alveolar epithelial type 1 cells (TT1), primary human alveolar macrophages (MAC), primary human alveolar type 2 (AT2) epithelial cells	Ruenaroengsak and Tetley ⁹¹
polystyrene, unmodified, carboxylated, amino-functionalized	50	particles partly adsorbed and internalized, then released by Calu-3 cells, while THP-1 macrophages quickly incorporated all particles, aminated polystyrene nanobeads were more cytotoxic and genotoxic than unmodified and carboxylated ones	human Calu-3 epithelial cells, human monocytic leukemia cell line THP-1	Paget et al. ¹⁹²
polystyrene, carboxylated, amino-functionalized	100	concentration of internalized nanoparticles, their uptake kinetics, and mechanism may differ considerably between primary cells and a related tumor cell line	human monocytic leukemia cell line THP-1, macrophages	Lunov et al. ¹⁹³
polystyrene, carboxylated	20, 100, 200, 500, 1000	20 nm particles were taken up passively, 100–1000 nm actively and passively, 20 nm stimulated IL-8 secretion in human monocytes and induced oxidative burst, 500 and 1000 nm particles stimulated IL-6 and IL-8 secretion in monocytes and macrophages	human monocytic leukemia cell line THP-1, human histiocytic lymphoma cells U937	Priehl et al. ¹⁹⁴
polystyrene, carboxylated, amino-functionalized	120	both particle types impaired expression of scavenger receptor CD163 and CD200R, and the release of IL-10	human monocyte macrophages	Fuchs et al. ¹⁷⁴
polystyrene, unmodified n.d.a.	20, 100, 200, 500, 1000, 2000	cellular uptake pathway of polystyrene particles is cell-line-dependent	bone-marrow-derived macrophages (BMDM), fibroblast L929, kidney epithelial cells 293T	Firdessa et al. ¹⁹⁵
polystyrene, carboxylated	44	maximum particle uptake after 1 h	human renal epithelial cells (HRCE)	Monti et al. ¹⁹⁶
polystyrene, carboxylated, amino-functionalized	110	cellular uptake	human microvascular endothelial cells (ISO-HA51), human umbilical vein endothelial cells	Tenzer et al. ¹³⁸

Table 1. continued

material, modification	size, nm	major findings	target/cell line	ref
polystyrene, carboxylated, amino-functionalized	30, 50, 1000	50 nm positively charged PS particles accumulated within lysosome in comparison to 30 nm negatively charged particles, cellular uptake caveolae-independent	human ovarian carcinoma cells (SKOV-3), human ovarian carcinoma cells (NIH-OVCA9-3)	Maneerat et al. ¹⁶⁸
polystyrene n.d.a.	20, 200	uptake of particles by hepatocytes was size-, time-, and serum-dependent, uptake of 200 nm particles was limited, but 20 nm NPs were internalized by both cell types	human hepatocyte carcinoma cells (HepG2), human hepatoblastoma cells (C3A)	Johnston et al. ¹⁹⁷
polystyrene, carboxylated	500	polystyrene particles could stimulate O ₂ generation	human liver cells (Huh 7)	Liu et al. ¹⁸¹
polystyrene, unmodified	20, 93, 220, 560, 1010	20 nm particles taken up avidly by all cell lines; HUVEC, ECV 304, and H1975 show particle uptake up to 1010 nm; HepG2 did not take up particles larger than 93 nm	human bladder carcinoma cells (ECV 304), human hepatocyte carcinoma cells (HepG2), human head and neck squamous cancer cells (H1975), human vein endothelial cells (HUVEC)	Zauner et al. ¹⁹⁸
polystyrene, carboxylated	110	carboxylated particles internalized more rapidly and showed higher accumulation	mesenchymal stem cells	Jiang et al. ¹⁹⁹
polystyrene, amino-functionalized	100	increased cellular uptake of particles under fluidic shear stress	human embryonic kidney epithelial cells (HEK293T), human pancreatic adenocarcinoma cells (PANC-1), human colorectal adenocarcinoma cells (HT29), human lung adenocarcinoma cells (A549)	Kang et al. ²⁰⁰

n.d.a. = no data available

Wear debris polyethylene particles seem to trigger the release of pro-inflammatory factors, such as TNF- α , IL-1, and pro-osteoclastic factors such as receptor activator of NF- κ B ligand (RANKL), resulting in periprosthetic bone resorption (osteolysis) and, in the worst case, eventual loss of the implant.¹⁸⁶ As illustrated by Devane et al., the tissue around ultrahigh molecular weight polyethylene-based prostheses often contains large numbers of small particles with sizes ranging from 0.2 to 10 μ m, and macrophages have been frequently observed, confirming the recruitment of the innate immune system.¹⁸⁷ Shanbhag et al. analyzed the composition and morphology of wear debris from interfacial membranes of failed total hip replacements. Their findings revealed that most of the particles found were polyethylene and had a mean size of around 530 nm.¹⁸⁸ To overcome the observed negative effects, surgeons are now increasingly using metal-on-metal joint replacements.

Furthermore, the production of fluorescein labeled polyethylene terephthalate (PET) nanoparticles was recently shown by Magri et al.¹⁸⁹ In their study, they present a top-down approach based on laser ablation of polymers to form PET nanoparticles with weak acid groups on their surface, which should mimic real environmental nanopollutants. In vitro studies using human intestinal epithelial cells (Caco-2) showed internalization and biopersistence as well as long-term stability in a simulated lysosomal environment. In addition, cells treated with nano-PET particles did not increase ROS generation over 96 h, and no cytotoxicity was detectable. However, as suggested by the authors, possible long-term effects in cells or tissue cannot be excluded and need to be evaluated further.

In conclusion, it has been shown by in vitro and in vivo data that nanosized plastic particles can induce the activation of cell responses, especially effects on the immune system. However, long-term studies with repeated exposures are absent, as well as thorough material testing, including plastics such as PE, PET, and PP, since most of the current studies are performed with polystyrene particles. Furthermore, it is important to fill the knowledge gap between environmental relevant concentrations and the concentrations found in the organisms/cells to carry out relevant studies on the impact on human health.

4. DETECTION OF NANOPLASTICS

The reliable detection of polymer-based nanoparticles in environmental samples or complex biological matrices is highly challenging, due to their small size and the fact that their overall chemical composition is not all too different from that of organic matter. The detection of nanoplastic particles according to their size is not sufficient, as additional chemical characterization is needed for identification of the particles. To the best of our knowledge, only one study has so far been published showing the detection and chemical analysis of nanoplastics in an environmental sample. Ter Halle et al. collected water samples from the North Atlantic Subtropical Gyre, and after ultrafiltration of the water, they used dynamic light scattering to prove the presence of nanoparticles. To evaluate the chemical identity of the particles, they conducted pyrolysis coupled with gas chromatography–mass spectrometry. They estimated that the colloidal fraction signal of seawater could be attributed on average to a mixture of 73% PVC (\pm 18%), 18% PET (\pm 16%), and 9% PS (\pm 10%). In addition, they observed changes in the pyrolytic signals of polyethylene with decreasing debris size, concluding that this

could be related to the structural modification of this plastic as a consequence of weathering processes.²⁰¹

A promising possible method for the detection and chemical characterization of nanoplastic particles in complex matrices is hyperspectral imaging technology. Hyperspectral imaging is a recently established technique that quantifies the reflectance intensity of microscopic samples in the UV–vis range (400–1000 nm) to generate spectra specific to a material at pixel resolution.^{202,203} Hyperspectral imaging has proven to be suitable for the detection and characterization of microplastic samples from ocean samples as well as from soil.^{204,205} Shan et al. investigated the effects of particle size and color of microplastics found in soil. The results showed that white PE could be detected with the precisions of 84% and 77% for PE particles in size ranges of 1–5 mm and 0.5–1 mm, respectively, while the precision of black PE in the same size ranges were found to be 58% and 76%, respectively. Furthermore, Mattsson et al. demonstrated the use of hyperspectral imaging for the detection of polystyrene nanoparticles with a diameter of 52 nm within the brain tissue of fish. The brains were homogenized and polystyrene was spectrally mapped and identified in the exposed brain images using the Spectral Angle Mapper algorithm.⁹⁰

Methods used to characterize nanoparticles in general such as UV–vis spectrometry, electron microscopy, field flow fractionation (FFF), or dynamic light scattering (DLS) techniques have also been suggested to be suitable for nanoplastics. However, these methods need to be combined to achieve the chemical confirmation of the identity of the material, as this is often lacking as seen by Correia et al.²⁰⁶ New approaches might involve the introduction of nano-FTIR absorption spectroscopy and Raman spectroscopy as well as combined atomic force microscopy and infrared spectroscopy (AFM-IR), which all show the capabilities in the area of nanoscale chemical characterization.^{207–209}

In conclusion, there is still a lack of reliable methods to detect nanoplastic particles in combination with reliable chemical characterization in environmental samples and/or complex biological matrices.

5. CONCLUSIONS AND FUTURE PERSPECTIVES

Polymers represent one of the most important materials families of the 21st century, as they are used practically everywhere and strongly influence our daily lives in many different ways. At the same time, these materials as a whole are also one of the largest sources of environmental pollution that mankind is exposed to. Recent studies have presented evidence that the process of plastic degradation also results in the formation of nanoplastic with physicochemical characteristics different from the corresponding bulk materials. An increasing body of knowledge has shown that nanoplastics have adverse effects on aquatic species, but in contrast to microplastics, nanoplastics can overcome the intestine tissue in aquatic systems and therefore possibly end up in the human food chain. Thus, it appears important to accelerate the investigation of the presence and fate of nanoplastics in the (aquatic) environment. In addition to studies addressing the effects of nanoplastics on aquatic organisms, exposure risks for humans via the food chain should be explored in more detail as in vitro studies using human cell lines showed evidence that polystyrene nanoparticles are taken up and induce oxidative stress or pro-inflammatory responses. Furthermore, information on the long-term fate of ingested nanoplastics in aquatic

organisms and humans is limited, and several important questions for future studies remain such as the following: What is the concentration of nanoplastics in the water? Can this concentration impact the aquatic system and, thus the food chain, resulting in potential hazardous effects for humans? Can we confirm the occurrence of nanoplastic in the human food chain to determine the potential exposure to humans? Given these unsolved questions, new technological approaches and methods are needed for the detection of nanoplastic particles in the environment and in organisms.

In addition, it is not known to what extent nanoplastic can be further degraded after ingestion, for example under the acidic conditions found in the gut or inside the cells in the digestive organelles, i.e., the lysosomes. It is also unclear whether nanoplastic can overcome the gut–intestine epithelial barrier or if they are restricted to the gut lumen. Another interesting question is to what extent the ingestion of nanoplastic can promote the uptake of chemical hitchhikers and the possible leaching of chemicals from the plastic material that could cause harm to the human body. In addition, plastic particles appear in numerous types, shapes, and sizes, with or without associated chemicals. Inconsistent use of units, exposure media, and habitats makes it even more difficult to combine exposure and effect data in a significant characterization of the risks. This turns the development of a rational risk assessment framework for plastic into a very challenging task.⁶⁸ Unfortunately, it stands to reason that many aspects in the context of environmental polymer nanoparticles cannot be generalized. There is therefore a need to establish data for the different plastic materials found in the environment. While the most commercially most relevant polymers are polyolefins (notably different types of polyethylenes and polypropylene), polyesters, and polyurethanes, most of the studies conducted with polymer nanoparticles were carried out with polystyrene, as this material is commercially available and inexpensive and can also be easily synthesized and processed into nanoparticles. Therefore, we propose that focus should be placed on comprehensive studies using diverse plastic materials at relevant concentrations, as well as modeling chronic exposure, for a more realistic hazard and risk assessment. Future research should also move toward the terrestrial environment, about which even less is known. Studies involving terrestrial, but also freshwater eco-systems will be very supportive for the overall understanding of the micro- and nanoplastic pollution in the environment and its possible impact on human health.³⁷

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