POTENTIAL HEALTH EFFECTS OF INGESTED MICRO AND NANOPLASTICS ON HUMANS. Lessons learned from *in vivo* and *in vitro* mammalian systems.

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Running Title: Potential health effects of ingested micro and nanoplastics.

Abstract

In recent years, the terms "microplastics" (MPLs) and "nanoplastics" (NPLs) have caught increasing attention and have become the focus of many studies in ecological and environmental research areas. These tiny particles, mainly derived from the degradation of plastics, pollute the marine and terrestrial ecosystems having the ability to enter into the food web. In this way, the human consumption of food contaminated with MPLSs and NPLs is unavoidable and its related effects are still unclear. In the presented work, with the aim of complementing previous reviews on this topic, new studies related to exposure, absorption, and toxicity in mammalian *in vivo* and *in vitro* studies are presented. As novel information extracted from this work, gaps, and limitations hindering the drawing of firm conclusions and the preparation of a reliable risk assessment are identified. Subsequently, recommendations for *in vivo* and *in vitro* testing methods are given in order to perform further relevant and targeted research.

Keywords

Nanoplastics, microplastics, toxicity, mammalian models, adverse effects, human health.

1. Introduction

Since plastics started its exploitation in the fifties of the last century, they have presented uncountable applications in industry due to the wide range of controlled physico-chemical properties that can be achieved, and its rapid and low-cost production. In this context, the synthesis of plastics increased from 16.5 million tons in the fifties to 364 million tons at present with a forecast of tripling this number by 2050 (Guglielmi, 2017). These numbers have been accompanied by a corresponding increase, also exponential, in the plastic waste produced. Inevitably, the existent recycling processes cannot avoid that millions of tons of plastic waste end up in the environment being aquatic media the main dump of this waste.

Once the plastics accumulate in the different ecosystems, the environmental conditions favor physico-chemical processes that lead to a continuous degradation, generating the so-called microplastics (MPLs) and nanoplastics (NPLs) based on their “micro” (< 5000 μm) or “nano” (< 100 nm) sizes (EFSA, 2016; Bouwmeester et al., 2015) These particles, being the topic of this review, have raised special concern among the scientific community during the last decade since they are increasingly emerging and accumulating in all the environments with not enough relevant information about their potential effects on humans. Thus, the interaction of MPLs and NPLs with both the ecosystems and human populations are not completely characterized, neither its potential adverse effect on human health.

Humans can incorporate MPLs and NPLs mainly through two routes of entry, namely inhalation, and ingestion. Inhalation of these particles can occur in outdoor and indoor environments as a result of microfiber release from synthetic textiles, among many other sources (Prata, 2018). However, more attention is paid to oral ingestion of MPLs and NPLs after recent data showing their abundant presence in different food and beverage sources, having also detected in human stools (Liebmann et al., 2018). Although high toxicities have not been found in first but scarce mammalian studies, some are the reasons to prioritize the research on the biological effects of these particles. Firstly,

differing from macroscale particles, following human exposure of MPLs and NPLs their small size facilitates epithelial absorption and systemic biodistribution to organs (Wright and Kelly, 2017). Secondly, the biopersistence of these materials in biological environments on one hand, and the continuous human exposure, on the other hand, may lead to the bioaccumulation of MPLs and NPLs in tissues and organs, leading to long-term effects, an urgent and important unexplored parameter to study. In addition, apart from their polymeric portion, MPLs, and NPLs also present different types of well-known toxic additives, potentially producing adverse related effects (Revel et al., 2018). Last but not least, the high surface per volume and the hydrophobicity presented by these particles promote the adsorption of other chemical compounds making them perfect carriers for persistent organic pollutants (POPs) or heavy metals (UN Environment Programme).

Considering all the above, and in order to complement the information gathered in previous works, this review aims to contribute to the field with the more recent data available about *in vivo* and *in vitro* mammal toxicity effects of MPLs and NPLs considering oral exposure, as the main exposure route. More importantly, experimental limitations representing realistic scenarios are identified and recommendations regarding *in vitro* and *in vivo* experiments are given for a future, guided and useful research.

2. Nature and occurrence

Different synthetic polymers are used in plastics depending on their intended use. Among them, low-density polyethylene (LDPE) is used in packaging materials, and by far is the major component of terrestrial plastic litter in form of plastic bags and sheets (Barnes et al., 2009). High-density polyethylene (HDPE) is used among other applications for corrosion-resistant piping, geomembranes, and plastic lumber, while polyethylene terephthalate (PET) is used overall in plastic bottles (Bouwmeester et al., 2015). Altogether, polyethylene is the most produced polymer comprising more than 40% of the total plastics produced (U.S. Environmental Protection Agency, 2012). Other polymers

commonly used include polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC) and polyactide (PLA).

Once these polymers become plastic waste, many tones scape from the recycling chain and are incorporated into the environment, starting a long process of polymer degradation. This process is the first source of MPLs and NPLs generation. However, as technologies are producing nanoenabled products for improved industrial properties, nanoparticles and microparticles are also directly produced at the micro/nanoscale, In this way MPLs and NPLs can also be released into the environment as “engineered produced” in paints, adhesives, and cosmetics, among many other sources (Lehner et al., 2019).

Regarding degradation, after long exposure mainly to UV light, physical abrasion and/or to microorganisms, macropolymers eventually break apart. Chemical bond breaks form smaller and smaller fragments comprised in the micro and nanoscale (Thompson et al., 2004; Cole et al., 2011; Lambert et al. 2014). Due to the exacerbated number of plastic litter already polluting the planet, this fragmentation process produces a heterogeneous group of sizes, shapes and chemical compositions difficult to characterize. Despite the formation of these complex mixtures, researchers have identified PE, PP, and PS in the first three positions of the ranking for MPLs occurrence in marine ecosystems, naturally reflecting its abundance in form of plastic waste (Hidalgo-Ruz et al., 2012).

The global concern that has emerged around the potential health effects of MPLs and NPLs, has led to an increase of scientific reports showing the presence of MPLs in practically all the environments and matrices all over the world, including marine and freshwater systems, outdoor and indoor atmospheres, soils and sediments (Schneider et al., 1996; van Sebille et al., 2015; Li et al., 2016; Dris et al., 2016). However, although analysis from different environmental samples is increasing rapidly, methodology standardization is still lacking, hampering quantification and comparison analysis (Renner et al., 2018).

In addition to synthetic polymers, about 4% of the total weight of plastics is formed by additives giving specific physicochemical properties to the desired final product (Andrady and Neal, 2009). From the diversity among tens of thousands of additives, phthalates used as plasticizers are the most generally used (Leslie et al., 2011) and their potential health effects have been widely evaluated as recently reviewed (Radke et al., 2019; Ma et al., 2019). Nonetheless, a long list of many other additives are used such as brominated flame retardants, bisphenol analogs, surfactants, lubricants, fragrances, pigments, biocides, etc., many of them also linked to potential toxic effects (Andrady and Neal, 2009; Lambert et al.,2014).

3. Main exposure routes in humans

Human exposure to MPLs and NPLs is growing inevitably due to the degradation and fragmentation of the millions of tons of plastics produced over the last 6 decades. The main portals of entry by which these materials could a priori entail a potential risk for the human population are the pulmonary and the gastrointestinal tract (GIT). MPLs and NPLs could potentially become airborne from many different sources, such as detachment of fibers from synthetic textiles or from the action of the wind over the wastewater treatment sludge (among many others) (Revel et al., 2018; Prata, 2018). From the number of airborne particles potentially inhaled, only a fraction would reach the lungs. Thus, due to their hydrophobicity, inhaled MPLs and NPLs could be repelled by lung lining so being eliminated via mucociliary clearance through the GIT. However, in those individuals whose clearance mechanisms are impaired, some fibers may avoid this protective mechanism of the lung and, consequently, reach the lungs (Wright and Kelly, 2017). Nevertheless, and despite the emerging data about the increasing levels of airborne MPLs, experts predict that the greater exposure to these particles will be via the GIT (Lehner et al., 2019).

Ingestion of contaminated aquatic organisms was the first identified concern related to human exposure of MPLs and NPLs (Bouwmeester et al., 2015; EFSA, 2016). Actually,

MPLs have been already found in hundreds of aquatic species, from zooplanktonic organisms in the base of the web food chain to higher trophic levels, in both invertebrate and vertebrate species (Thompson et al., 2004; Moore 2008; Leslie et al., 2011; Cole et al., 2011; Wright et al., 2011; Waring et al., 2018). The presence of MPLs in these organisms is known to come directly from the ingestion of MPLs, as well as by the consumption of lower trophic organisms (von Moos et al., 2012; Santana et al 2017). Regarding human exposure to MPLs and NPLs via ingestion, some considerations have to be taken. On one side, the ingestion of vertebrate marine organisms containing ingested MPLs does not mean direct exposure since normally; humans do not eat the GIT of these species. In this case, direct exposure would only happen if MPLs and NPLs could translocate through fish GIT or gills, and distribute by the different organs and tissues through its circulatory system (Wright and Kelly, 2017). Although MPLs of sizes >20 μm are not usually presented in the internal organs of fishes, lower sizes can cross GIT barriers and gills, and be present in internal organs (Su et al., 2019). In a freshwater ecosystem model, with a food chain containing four species, it was confirmed that the nanoplastics adhered to the surface of the primary producer, were present in the digestive organs of the higher trophic level species. In such model, NPLs induced histopathological changes in the livers and, additionally, they penetrated the embryo walls and were present in the yolk sac of hatched juveniles (Chae et al., 2018). In spite of the results showing the potential bioaccumulation/biomagnification along the marine food chains, the lack of standardized methodologies for nanoplastics detection makes NPLs fate into marine organisms a real environmental and health challenge (Ferreira et al., 2019). Conversely, mollusks and other organisms entirely consumed (including GIT) entail higher human ingestion of particles, representing the first source of dietary exposure to MPL and NPLs coming from aquatic environments (Lenher et al., 2019).

Other than aquatic organisms, recent studies have identified alternative sources of MPLs and NPLs likely involving higher levels of exposure (Rist et al. 2018; Catarino et al., 2018). Thus, until now, MPLs have been found in several processed foods and drinks.

Some of these identified products include sugar, honey, salt and canned sardines (Liebezeit and Liebezeit, 2013; Yang et al., 2015; Karami et al., 2017), as well beer and even tap and bottled water (Liebezeit and Liebezeit, 2014; Kosuth et al., 2017; Schymanski et al., 2017). The number of studies analyzing the content of MPLs in different edible products is growing quickly and its presence is likely. The reason: this ubiquitous environmental contamination, coming from surrounding plastic polymer release, ends up suspended in the air and eventually precipitating on different surfaces including edible products (Rist et al., 2018). In addition, plastic packaging release has also been identified as a possible source of food and beverage contamination, as shown in different studies (Castle, 2007; Cooper, 2007; Cox et al., 2019; Toussaint et al., 2019). Despite the big number of variables and the uncertainties identified, some are the studies focused on estimating human exposure levels to MPLs under different considerations. Specifically, considering atmospheric MPLs fallout on edible products a study has shown how MPLs could end up in a dinner plate during a meal entailing a significant oral exposure. Thus, the scientists detected an average of 7 particles per meal, representing 70,000 particles ingested per year (Catarino et al., 2018). In other recent studies, scientists created a database of human exposure estimates based on a thorough review of the literature of commonly contaminated consumed foods, in combination with U.S. dietary data. In that study, the potential consumption of MPLs through inhalation was also taken into consideration as well as the American consumption of tap and bottled water. Thus, Cox and collaborators estimated an annual MPLs consumption in the US of 39,000-52,000 particles considering only food intake, increasing to 74,000-121,000 when inhalation was also considered. An addition of 90,000 MPLs of ingestion was calculated when individuals meet their recommended water intake through only bottled sources, compared to the additional 4,000 MPLs if alternatively tap water is consumed (Cox et al., 2019). In conclusion, recent studies and growing evidence suggest main human ingestion of MPLs and NPLs coming from consumed contaminated organisms (mainly aquatic), atmospheric fallout during food and beverage production, and plastic

packaging release as seen in bottled water (Schymanski et al., 2017; Mason et al., 2018; Rist, 2018).

4. Absorption, biodistribution, and elimination

Human ingestion of MPLs and NPLs would involve a systemic condition only if these particles are able to be absorbed through the intestinal barrier and distributed to organs and tissues via lymphatic and/or blood system. In order to address this issue, in this review absorption, biodistribution, and bioaccumulation studies of MPLs and NPLs in *in vivo* and *in vitro* mammal models are considered.

4.1. Absorption

Absorption of nutrients and chemicals is a process carried out similarly among mammalian species. Therefore, mice, rats, hamsters, and guinea pigs have been historically used for absorption and toxico-kinetic studies (OCDE, 2010). However, the vast combination of possible physico-chemical properties seen in this heterogeneous complex of MPLs and NPLs makes difficult the use of *in vivo* mammalian models to specifically assess absorption, among other parameters. In this context, *In vitro* models are commonly used and recommended for practical, cost-effective and ethical reasons. Among the different cell lines available for representing human small intestine, differentiated Caco-2 cells are the most widely used (Neutra and Louvard, 1989; Zweibaum et al., 1991). These cells, representing enterocytes (the most abundant epithelial cell type in the intestine) growing on a semi-permeable membrane, can be used to assess the absorption of the compound from the apical to the basolateral chamber (Lefebvre et al., 2015). In addition, the establishment of co-cultures of Caco-2 along with HT-29, for mucus secretion, and with the lymphocytic Raji-B cells, for inducing the “M” cells of the Payer’s Patches, properly represents the physiology of the intestinal barrier (as indicated in Figure 1). This model has been successfully applied to pharmacological and toxicological studies, and commonly used for assessing the effective translocation of nanomaterials (Vila et al., 2018; Garcia-Rodríguez et al., 2018). Extensively, this

model can be proposed for the study of MPLs and NPLs absorption, including cell uptake and translocation through the barrier.

From the existing mechanisms of particle uptake in the intestine, internalization via “M” cells and persorption have been described as the most likely mechanisms for MPLs uptake due to the extensive range of sizes able to be absorbed (Wright and Kelly, 2017). “M” cells, forming 5 to 10% of cells in Peyer’s patches in humans, are able to take up by endocytosis big amounts of material up to 10 μm from the lumen, and transport it to the “lamina propia” linked to lymph nodes and, eventually, to the blood system (Eldridge, 1989¸ Kucharzik et al., 2000). An alternative to the internalization of large particles through the small intestine is persorption. By this mechanism, particles up to 150 μm can cross the cellular barrier through gaps in the epithelium, known as desquamation zones (Steffens, 1995). Several *in vivo* studies using different mammalian organisms have been conducted to assess the permeability of the epithelial membrane to MPLs. In general, low percentages of absorption of polymeric particles up to 150 μm have been observed, constituting the intestinal epithelium an important and robust barrier against these types of materials, as detected using latex microspheres and nanospheres (Jani et al., 1989; LeFevre et al., 1989). Among these types of studies, Volkheimer and colleagues observed PVC MPLs (5 -110 μm) in the lymphatic nodes, and portal veins uptaken through the epithelial barrier (Volkheimer, 1975). Hussain and collaborators also detected various types of MPLs (0.1 -150 μm) in the lymphatic system of different species from rodents to humans, throughout dogs and rabbits (Hussain et al., 2001). More recently, researchers observed significant epithelial barrier translocation of PS MPLs (from 1 to 20 μm) in mouse models (Deng et al., 2017; Stock et al., 2019). In these studies, MPLs accumulated in liver, kidney, and gut, and the tissue-accumulation kinetics was strongly depending on the MPLs size.

Despite the well-established *in vitro* absorption model previously mentioned, scarce studies can be found regarding absorption of MPLs and NPLs using this approximation. One study was found where Stock and collaborators found limited absorption of PS

MPLs (1, 4 and 10 μm) using a Caco-2 co-culture model (Stock et al., 2019). In addition, the use of the monoculture model of Caco-2 cells has been useful to demonstrate the transcytosis mechanisms involved in the uptake of polystyrene NPLs and the role of lysosomes as a dead-end for NPLs (Reinholz et al., 2018). An interesting approach used laser ablation of polymers to form polyethylene terephthalate nanoplastics, to better mimicry real environmental nanopollutants, Using this approach authors obtained NPLs of about 100 nm average size, although with an important heterogeneity in size and shape. The obtained NPLs were largely internalized in endolysosomes, showing intracellular biopersistence and long-term stability. Furthermore, the obtained NPLs showed a high ability to cross the simple model of intestinal barrier (Magri et al., 2018). In the case of NPLs, smaller size facilitates gut translocation and systemic distribution of particles. Bigger rates of absorption have been observed in the nanoscale when compared to micro range sizes (Jani, 1989; Doyle-McCullough et al., 2007). Therefore, besides internalization through the above-mentioned mechanisms, uptake through enterocytes has been observed for polymeric particles smaller than 100 nm (Jani et al., 1992). Considering the uptake of nanoparticles, information can be extracted from the fields of nanomedicine and nanotoxicology, where a wide variety of engineered nanoparticles has been observed *in vivo* and *in vitro* crossing the epithelial barrier. Gathering information about polymeric PS NPLs, widely used in last decades, using *in vivo* models, differences in uptake were observed reaching as high as 7% of bioavailability after treatments (Jani, 1989; Hillery et al., 1994; Hillery and Florence, 1996). Regarding *in vitro* studies, variations in uptake have been detected ranging from 0.2 to 10% depending on nanoparticle physicochemical properties (des Rieux et al., 2007; Kulkarni and Feng, 2013; Walczak et al., 2014; Walczak et al., 2015; Walczak et al., 2015) In general, the intestinal epithelium constitutes an important barrier to MPLs and NPLs and low rates of absorption have been observed. However, smaller sizes enhance the ability of the particle to translocate and to be biodistributed, entailing a risk for human health (EFSA, 2016).

Biodistribution

Following uptake, MPLs and NPLs can be distributed systemically to organs and tissues (Jani et al., 1989; Volkheimer, 2001). Again, physico-chemical properties will determine the kinetics and biodistribution of these particles. Size has been shown as an important modulator determining how deeply particles penetrate into organs. Thus, small particles (<1.5 μm) have been observed disseminated in different organs (EFSA, 2016). However, size is not the only factor modulating organ biodistribution since bigger particles have also been observed accumulated in organs. In this context, Deng et al. published a relevant study on the effect of oral ingestion of 5 μm and 20 μm PS MPLs, regarding their accumulation in different tissues using a mouse *in vivo* model. Specifically, using fluorescent PS MPLs, it was observed that such particles were able to cross the intestinal membrane and accumulate in different organs such as liver and kidney after 28 days of exposure. Interestingly, this distribution followed kinetics and accumulation dependent on the size of the particles (Deng et al., 2017). However, controversy exists, since negative results have also been reported. Thus, Stock and collaborators showed low *in vivo* uptake and no biodistribution in any organ after mice were exposed to 1, 4 and 10 μm PS MPLs for 28 days (Stock et al., 2019).

These contradictory results point out the urgent need for accurate methods to detect and quantify the presence of MPLs and NPLs in biological matrices. Although the detection and quantification of MPLs seem to be more or less established (Cole et al., 2014; Prata et al., 2019) most problematic is the identification of NPLs (Correia and Loeschner, 2018; Zhou et al., 2019).

Bioaccumulation

Although there are many studies showing the accumulation of MPLs and NPLs, mainly in marine organisms (Ferreira et al., 2019), this type of study is very scarce in mammals. In marine organisms the internal accumulation seems to be species-specific and

influenced by particle size. Thus, 10 µm MPLs are present into the circulatory system of mussels (Browne et al., 2008) while MPLs sized 5 µm are accumulated in the liver of zebrafish (Yifeng et al., 2016). Nevertheless, all these studies do not study the changes in the presence of MPLs/NPLs in time-course experiments to measure absorption, distribution, biotransformation, and elimination kinetics.

Accumulation studies in mammals are lacking with the exception of the pioneering study of Deng et al. (2017). In that study bioaccumulation was reported in mice using fluorescent PS MPLs sized 5 and 20 µm. Mice were daily exposed and accumulations of both sizes of MPLs were detected in the liver, kidney, and gut. Interestingly, this study evaluated the bioaccumulation over time. To this end, animals were sacrificed at 1, 2, 4, 7, 14, 21, and 28 days after exposure and tissue samples from liver, kidney, and gut were obtained. Results clearly indicated accumulation over time although with differences between tissues. Gut and kidney accumulated more 5 µm sizes while liver accumulated more 20 µm sizes.

The removal of MPLs from the systemic fluids has been reported through different routes. Splenic filtration, urine excretion, and bile excretion through feces have been described (Volkheimer, 2001; EFSA 2016). Other fluids such as cerebrospinal fluid or milk in animals and lactating women have also been reported (Volkheimer, 1975; Wright and Kelly, 2017). However, the small sizes characterizing these particles, as well as other physico-chemical properties such as hydrophobicity, could hinder the mechanical clearance leading to biopersistence. This fact was observed and highlighted by Magri et al. showing a great ability of PET NPLs to cross the epithelial barrier, showing strong stability and long-term biopersistence using a Caco-2 *in vitro* co-culture model of intestinal barrier (Magrì et al., 2018). This property is a red flag in terms of potential adverse effects since could lead to their accumulation and potentiate associated long-term toxic effects.

5. Health effects

Although many studies have focused on MPLs presence and their toxicological effects in aquatic organisms, information about the effects of MPLs and NPLs on human health is still very scarce and preliminary (Galloway, 2015; Rist et al., 2018; Lenher et al., 2019). In line with the purpose of this review and in order to better extrapolate effects to humans, in this section only toxicity studies considering mammalian model organisms *in vivo* or *in vitro* have been included.

MPLs and NPLs are raising an increasing concern as potential toxicants mainly due to three different factors. Thus, the intrinsic nature of polymers, the leaching of plastic additives, and the potential adhesion of contaminants have been highlighted as the leading modulators for adverse effects (Bouwmeester et al., 2015). Extended toxicity literature has been dedicated to both plastic additives and adhered contaminants (Meeker et al., 2009; Talsness et al., 2009; EFSA, 2016, Magara et al., 2019). Consequently, although considering the relevance of these factors inherent to the toxicity of any size of plastic, these elements are out of the scope for the approach of this review.

Nature of MPLs and NPLs as toxicity modulators

Regarding the intrinsic chemical composition of MPLs and NPLs, Lithner et al. conducted a comprehensive hazard ranking of plastic polymers based on internationally agreed criteria for identifying physical, environmental and health risks (Lithner et al., 2011). According to its classification as carcinogenic, mutagenic, or both, polyurethanes, polyvinylchloride, epoxy resins, and styrenic polymers were placed in the highest positions of the ranking, whereas PE, polyvinyl acetate (PVA) and PP were classified as potentially less hazardous (Lithner et al., 2011).

Apart from the chemical nature, there are some other factors inherent to the polymer which could be influencing its toxicity. In the first place, going down in size facilitates intestinal absorption and systemic distribution, affecting the cellular fate of particles. Therefore, as derived from the last section, the toxicity target of MPLs and NPLs will depend in part on their size. Particles higher than 150 μm will cause a local effect on the

gut, whilst smaller particles (<150 μm) could cause toxicity to several secondary organs and tissues (EFSA, 2016).

Also considering the nature of the polymers, polymerization, and processing of plastics during synthesis generate reactive oxygen species (ROS), which are a very common factor causing cellular damage (Wright and Kelly, 2017). The concentration of these free radicals presented in the particles can easily increase due to dissociation of the C−H bonds caused by light exposure or interaction with transition metals during the weathering process (White et al., 1994; Gewert et al., 2015). In relation with this, it should be indicated that environmental photodegradation and biodegradation produces surface changes affecting their functional groups (e.g., -COOH, -NH_2) (Andrady, 2011), what can alter the toxicological profiles. Other particle properties such as shape or surface charge, among others, have been also identified as potential toxicity factors of MPLs and NPLs (Kim et al., 2016).

Potential biological effects of MPLs and NPLs

Cytotoxicity

Some studies have evaluated *in vitro* the effects of MPLs and NPLs on cell viability, generally showing mild effects. In 2018, a study published by Magrì et al., did not detect any cytotoxic effect of fluorescent PET NPLs in Caco-2 intestinal cells (Magrì et al., 2018). Also using Caco-2 cell line, Wu and collaborators found PS NPLs (0.1 μm) and PS MPLs (5 μm) causing little cytotoxicity, mild changes in membrane integrity and negligible changes in its fluidity. However, disruptions of the mitochondrial membrane potential were observed as induced by both sizes of MPLs. Interestingly, the effects induced by the larger size (5 μm) were higher than those induced by 0.1 μm sizes (Wu et al., 2019). Stock et al. in a recent publication showed the effects on cytotoxicity of fluorescent PS MPLs (1, 4 and 10 μm) using Caco-2 cells and monocyte-like THP-1 cells. In that study, particles only affected Caco-2 cell line viability when high and non-environmentally relevant concentrations of 1 μm PS were used (Stock et al., 2019). That

lack of toxic effects agrees with those reported in both cerebral (T98G) and epithelial (HeLa) human cells. In both cases the use of PE MPLs (3-16 μm) and PS MPLs (10 μm) in a range of 0.05 -10 μg/mL did not produce significant effects on cell viability (Schirinzi et al., 2017). This lack of toxic effects on Caco-2 cells was confirmed in an intestinal co-culture model (Caco-2/HT29-MTX-E12) where the use of 50 nm and 0.5 μm COOH-modified PS particles did not induce toxic effects in the range of 0.01-100 μg/mL, although some changes in the metabolic activity were detected at the highest doses. Authors also used placental trophoblast cells (BeWo b30) and, in this case, toxic effects were observed with slight but significant increases in the metabolic activity at concentrations higher than 5 μg/mL (for 50 nm) and changes in the mitochondrial activity only at concentrations from 0.01-10 μg/mL (for 0.5 μm) (Hesler et al., 2019).

These results differ from those reported in the human lung epithelial BEAS-2B cells were the MTS assay showed a dose-dependent significant decreased in cell viability after exposures to PS NPLs. Results attained statistical significance up 10 μg/mL. In addition, NPLs exposure showed autophagic- and endoplasmic reticulum (ER) stress-related metabolic changes (Lim et al., 2019). Toxicity of MPLs/NPLs can be modified by the surface area, as demonstrated using negatively charged carboxylated PS and positively charged amino-modified PS nanoparticles. The positively charged NPLs displayed higher cellular toxicity, disturbing the integrity of the cell membrane, on both normal (NIH 3T3) and cancer (HeLa) cells, although the effects were more pronounced in normal cells (Liu et al., 2011).

Oxidative stress

Following the interaction between particles and cellular environments, the generated ROS settling in the surface of the particles makes them able to cause cellular oxidative stress. If these reactive particles are not small enough to be absorbed through the epithelium, they could still have the ability to induce local inflammation of the gut which in turn could disrupt the membrane increasing the crossing of the particles (Powell et al.,

2007). On the other side, if particles are small enough to cross the intestinal epithelium, the ROS located on the surface of particles would potentially increase the toxicity outcome mediating cellular responses in different cell targets.

Regarding the *in vitro* studies carried out specifically with the intention to evaluate the oxidative potential of MPLs and NPLs derived from environmental exposures, only a few studies are found in the literature. Specifically, Schirinzi and colleagues showed a slight potential of PE and PS MPLs and NPLs to disturb homeostasis at oxidative cellular level, using cerebral and epithelial cell lines. Specifically, they identified oxidative stress as possible mechanism responsible for cellular adverse effects, although non-significant responses were found among some polymers, concentrations, and cell lines (Schirinzi et al., 2017). In another study using Caco-2 cell line, PS NPLs (0.1 μm) and PS MPLs (5 μm) caused mild molecular effects including oxidative damage (Wu et al., 2019). Furthermore, using human dermal fibroblasts and murine macrophages it was observed that MPLs in the range of 25 μm, at the concentration of 1000 μg/mL were able to increase ROS levels by approximately 30%. Nevertheless, larger sizes did not cause any change in the basal levels of ROS (Huang et al., 2019). With regard to *in vivo* studies, Deng et al. published a study using a mouse *in vivo* model where several biomarkers of toxicity were analyzed. Among them, fluorescent PS MPLs of 5 μm and 20 μm, were linked to disturbance in oxidative stress levels (Deng et al., 2017).

Immune response

The activation of the immune system by inflammatory responses has been identified as one of the main effects surely associated with MPLs and NPLs exposures (Prietl et al., 2013; Lehner et al., 2019). As with the rest of toxicity parameters, the data relative to MPLs and NPLs effects on the immune system is very limited. The number of immunological studies increases if we consider other publications with different purposes such as the study of wear debris from plastic prosthetic implants, or those carried out with PS nanoparticles used as a model in pharmacological studies (Doorn et al.,1996;

Hicks et al., 1996; Xia et al., 2008; Chiu et al., 2015; Lehner et al., 2019). Thus, the results reported *in vivo* from PE (0.5 - 50 µm) and PET (0.5 - 30 µm) prosthetic wear debris indicate immune phagocyte recruitment and associated production of cytokines as an inflammatory response. The intensity of this reaction depends on the chemical composition of the plastic, with PET being more harmful than PE (Wright and Kelly, 2017). Among the *in vitro* studies carried out using PS NPLs in pharmacological studies, interleukin secretion, impaired expression of scavenger receptors (CD163 and CD200R), and even immune cell death were reported (Brown et al., 2001; Prietl et al., 2013; Fuchs et al., 2016). In a recent publication, Stock et al. showed *in vitro* immune effects of fluorescent PS MPLs (1, 4 and 10 µm) using the human monocytic THP-1 cell line. In this case, MPL exposure did not interfere with differentiation nor activation processes of the human macrophage *in vitro* model. In the same publication, the authors also reported absence of any sign of inflammatory responses when fluorescent PS MPLs were assessed after oral ingestion in mice (Stock et al. 2019). Furthermore, high concentrations of PP MPLs have shown to stimulate the immune system enhancing the potential hypersensitivity in peripheral blood mononuclear cells, murine macrophages (Raw 264.7), and human dermal fibroblast (HMC-1) cells increasing the levels of cytokines and histamines. Enhanced expression of pro-inflammatory cytokines such as IL-6, TNF alpha, and histamine was observed. Interestingly, these effects were size-dependent in a concentration manner with increases levels at high concentration and sized of 20 µm (Hwang et al., 2019).

Genotoxicity

An important and potential toxic effect linked to MPLs and NPLs is DNA damage. There are different mechanisms by which these particles could induce genotoxicity. The DNA surrounded by the nuclear membrane could be damaged directly through particle contact (only when particles are small enough to cross the nuclear membrane); indirectly, by the generation of cytoplasmic ROS reaching the nucleus; or by the impairment of the

replication and/or the repair machinery (Fadeel et al., 2012). The unrepaired DNA lesions lead to mutagenesis. If these mutations imply key genes involved in genomic integrity maintenance or cell cycle control, then carcinogenesis could initiate. Therefore, it should be considered that MPLs and NPLs able to induce genotoxicity could have the capacity to initiate carcinogenesis due to the alterations imposed on the genetic material (Fadeel et al., 2012). Surprisingly, only three studies using MPLs/NPLs in mammalian cells have been detected, evaluating DNA damage induction. The genotoxicity of fluorescent 50 nm PS nanoparticles, with different functionalized surfaces (non-functionalized, carboxylated and aminated) was assessed using the γ-H2Ax foci assay. This assay permits to detect DNA double-strand breaks. The study was performed using pulmonary epithelial cells (Calu-3) and human macrophages (THP-1). Results indicated that only aminated NPLs were able to significantly increase the levels of DNA damage. For similar NPLs concentrations and sizes, aminated PS nanobeads were more genotoxic than unmodified and carboxylated ones, as demonstrated in both cell lines (Paget et al., 2015).

The potential genotoxicity of two sized (50 nm and 0.5 μm) COOH-modified PS NPLs were analyzed using two different assays, the p53 reporter gene assay and the micronucleus assay (Hesler et al., 2019). The first assay detects changes in the expression of p53 protein using the HepG2CDKN1A-DsRed biosensor cell line. The micronucleus assay detects the induction of chromosome breakage and/or alterations in the chromosome segregation machinery in CHO-K1 cells. In spite of the strong capability of internalization, no genotoxic effects were detected in neither of the used assays. These results would agree with those previously reported using negatively charged carboxylated polystyrene and positively charged amino-modified PS NPLs and MPLs (NH2ePS) of three different diameters (50, 100 and 500 nm) on cancer HeLa cells and in normal NIH 3T3 cells. In such study, although important internalization was observed, the NPLs used were not observed to be associated with any of the components of the mitotic apparatus. In addition, no alterations in the normal cell division process were

detected (Liu et al., 2011). However, extension in the G0/G1 phase of the cells cycle with decreased expression level of cyclin D and cyclin E were observed after H2ePS exposure. These changes are usually associated with DNA damage and the activation of checkpoint controls.

6. Limitations and future perspectives

The appearance of MPL and NPLs is progressively increasing in all the environments being introduced and accumulated in the food web. However, some gaps need to be filled to draw any worthwhile conclusion regarding health effects and, subsequently, the preparation and implementation of a reliable risk assessment. Among the limitations, the inaccurate human exposure levels measured until now entails an important constraint. While MPLs have been detected in a variety of edible products, its quantification is not eased comparable. Apart from the heterogeneity of the samples, different detection technologies using different units, detection principles and detection thresholds make comparisons difficult to establish and impracticable many times (Renner et al., 2018; Rainieri and Barranco, 2019). Based on that, for the sake of making studies valuable and comparable, standardization processes are urgently required in the field. Regarding NPLs, no data is available in literature about environmental qualitative and quantitative measurements. Nowadays, technology is not sufficiently developed to sample, detect and quantify environmental particles in the nano-range. Therefore, although continuous plastic degradation from MPLs to NPLs is expected (as shown by Lambert et al., 2014 in controlled situations), real exposure scenarios are predictive and inaccurate. Therefore, efforts in this field developing new methods are extremely required to achieve accurate exposure data.

Human biomonitoring studies are a fundamental requirement to perform an occupational and environmental health risk assessment of MPLs and NPLs, nowadays missing. These studies are aimed to quantify the number of chemical compounds absorbed, transformed or accumulated in target organs, tissues or cells as consequence of occupational or

environmental exposure to chemicals (Fadeel et al., 2012). Among the capabilities, these studies allow the identification of potential hazards and the understanding of the biological mechanisms of new and emerging chemicals. For this purpose, different biomarkers are used as surrogates of designated biological events. Thus, by measuring them, information about interactions between the xenobiotic and the individual is obtained (see Figure 2). Although the implementation of biomonitoring studies to MPLs and NPLs is highly required, some issues should be addressed for its applicability. First, there is still a lack of information about appropriate and reliable biomarkers of exposure preventing the detection and tracking of the particles during time in biological matrices such as blood or urine. In the same manner, more research is needed for the identification of suitable biomarkers of effect, able to reflect early biological events preceding functional damage. Development of susceptibility biomarkers is also required allowing the identification of groups at higher risk of suffering adverse effects. With regard to the development of all biomarkers, it is crucial the establishment of reference values and limits for the measurements obtained, enabling the interpretation of the results. Nowadays, biomonitoring studies of MPLs and NPLs are still inexistent, so this point has been identified as priority for the research community.

In order to find biomarkers related to potential health effects, more information about *in vivo* and *in vitro* toxicity of ingested MPLs and NPLs is required. Until now, very few studies have investigated this issue focusing the majority of them in the study of only one specific polymer, as it is the case of PS (Mahler et al., 2012; Walczak et al., 2015); so, the conclusions cannot be extrapolated to other polymers. Therefore, scientists are encouraged to perform more experiments with a variety of polymers.

Another consideration of interest when studying the potential health effects of MPLs and NPLs is the altered condition of the gut mucosa. Intestinal related diseases could be a modulator factor of internalization and related effects. Thus, patients suffering from inflammatory bowel disease already showed greater uptake of particles as compared to healthy controls, which could also lead to different adverse situations (Schmidt et al.,

2013). This is an important parameter to consider in future investigations since alterations in the gut are increasing in developed societies due to inadequate nutritional and day life habits (M'Koma, 2013). Related to this point it should be discussed the potential effects of MPLs/NPLs on gut microbioma and their consequent effects on human health. A recent study feeding mice with PS MPLs for 5 weeks detected that oral exposure to 1000 µg/L of 0.5 and 50 µm PSMPLs decreased body and liver weights. In addition, mucus secretion in the gut was significantly decreased and gut microbiota was affected, decreasing the relative abundances of *Firmicutes* and *α-Proteobacteria filum* in the feces (Lu et al., 2018). These results agree with those recently reported in male mice exposed to 5 µm pristine and fluorescent PS MPLs for six weeks. In that study, a reduction in the intestinal mucus secretion and damage in the intestinal barrier function was observed. In addition, the diversity of the gut microbiota was significantly altered, and the content of Actinobacteria decreased significantly at phylum level (Jin et al., 2019). Although the health consequences of MPLs ingestion in humans remain to be solved, the above animal studies showing gut inflammation, oxidative stress, and alterations in the gut microbiome must be an alarm signal to further explore this research way. Due to the relationship between microbiota dysbiosis and human health a recent paper point out an attractive hypothesis linking MNPLs exposure, gut microbiota alterations and mental disorders (Moulin and van Egmond, 2019). According to that the authors propose to considerer behavioral and cognitive alterations associated with exposure to MNPLs in future researches.

Long-term health effects of chronic exposure to MPLs and NPLs are a very relevant parameter, unexplored until now. Once particles get into tissues and organs, their physico-chemical properties make them prone to accumulate. This particle bioaccumulation extended in time, could affect the proper cell functionality leading to molecular adverse effects such as mutagenesis and carcinogenesis (Wright and Kelly, 2017). As previously mentioned, the massive combination of different physico-chemical properties seen in MPLs and NPLs makes impractical the use of *in vivo* mammalian

models to assess long-term effects. Therefore, in this scenario, alternative methods based on 3-R (replacement, reduction, and refinement) recommend the use of *in vitro* systems (Hartung y Sabbioni, 2011). Specifically, environmental low-dose cellular treatments extended during long exposure periods, allow studying cellular transformation processes through different well-established end-points (see Figure 3). In turn, these studies can give important information related to tumorigenesis, transferable to *in vivo* situations. As seen in nanotoxicology studies, long-term exposures to environmental (low-dose) concentrations could induce cellular carcinogenic mechanisms. This has been observed in the case of different cell types such as epithelial or fibroblastic cells exposed to different nanoparticles (Annangi et al., 2015; Vales et al., 2016; Rubio et al., 2017). As derived from this, research about long and accumulative effects of these type of particles are urgently required, presenting *in vitro* studies several advantages justifying its implementation.

As a conclusion, although technology is not sufficiently developed to properly calculate human exposure levels, the degradation of the plastic waste already accumulated everywhere in the planet, anticipate a continuous and growing human exposure to MPLs and NPLs. Oral ingestion is the first route of exposure, constituting the intestinal barrier a robust impediment for particle translocation. However, more information about the effectiveness of the intestinal barrier on the translocation of different nature of particles is needed. Until now, only a few studies have been designed to assess the health effects of environmental exposures to MPL and NPLs. Among these few studies, mild effects have generally been linked to these particles. Notwithstanding, there is still an urgent need for experimental data to investigate and better represent different biological parameters, such as the potential cellular mechanisms of different nature of particles, the effects on altered condition of the gut mucosa or the long-term effects.

REFERENCES

Andrady, A. L., and M. A. Neal. 2009. Applications and societal benefits of plastics. *Philosophical Transactions of the Royal Society B: Biological Sciences*. 364: 1977–1984. https://doi.org/10.1098/rstb.2008.0304

Andrady, A. L. 2011. Microplastics in the marine environment. *Mar. Pollut. Bull*. 62:1596-1605.

Annangi, B., J. Bach, G. Vales, L. Rubio, R. Marcos, and A. Hernández. 2015. Long-term exposures to low doses of cobalt nanoparticles induce cell transformation enhanced by oxidative damage. *Nanotoxicology*. 9:138–147. https://doi.org/10.3109/17435390.2014.900582

Barnes, D. K. A., F. Galgani, R. C. Thompson, and M. Barlaz. 2009. Accumulation and fragmentation of plastic debris in global environments. *Philosophical Transactions of the Royal Society B: Biological Sciences*. 364:1985–1998. https://doi.org/10.1098/rstb.2008.0205

Barnes, K. A., C. R. Sinclair, and D.H. Watson. 2007. *Chemical migration and food contact materials*. CRC.

Bouwmeester, H., P. C. H. Hollman, and R. J. B. Peters. 2015. Potential Health Impact of Environmentally Released Micro- and Nanoplastics in the Human Food Production Chain: Experiences from Nanotoxicology. *Environmental Science and Technology*. 49:8932–8947. https://doi.org/10.1021/acs.est.5b01090

Brown, D. M., M. R. Wilson, W. MacNee, V. Stone, and K. Donaldson. 2001. Size-Dependent Proinflammatory Effects of Ultrafine Polystyrene Particles: A Role for Surface Area and Oxidative Stress in the Enhanced Activity of Ultrafines. *Toxicology and Applied Pharmacology*. 175:191–199. https://doi.org/10.1006/taap.2001.9240

Browne M. A., A. Dissanayake, T. S. Galloway, D. M. Lowe and R. C. Thompson. 2008. Ingested microscopic plastic translocates to the circulatory system of the mussel, Mytilus edulis (L.). *Environ Sci Technol*. 42:5026–5031.

Castle L. 2007. Chemical migration into food: an overview. In: Barnes K, Sinclair R, Watson D, editors. Chemical migration and food contact materials. Sawston (Cambridge): Woodhead Publishing. 1–13.

Catarino, A. I., V. Macchia, W. G. Sanderson, R. C. Thompson, and T. B. Henry. 2018. Low levels of microplastics (MP) in wild mussels indicate that MP ingestion by humans is minimal compared to exposure via household fibres fallout during a meal. *Environmental Pollution*. 237:675–684. https://doi.org/10.1016/j.envpol.2018.02.069

Chae Y., D. Kim, S. W. Kim, Y. J. An. 2018. Trophic transfer and individual impact of nano-sized polystyrene in a four-species freshwater food chain. *Sci Rep*. 8:284.

Chiu, H.-W., T. Xia, Y. H. Lee, C. W. Chen, J. C. Tsai, and Y. J. Wang. 2015. Cationic polystyrene nanospheres induce autophagic cell death through the induction of endoplasmic reticulum stress. *Nanoscale*. 7:736–746. https://doi.org/10.1039/c4nr05509h

Cole, M., P. Lindeque, C. Halsband, and T. S. Galloway. 2011. Microplastics as contaminants in the marine environment: A review. *Marine Pollution Bulletin*. 62: 2588–2597. https://doi.org/10.1016/j.marpolbul.2011.09.025

Cole, M., H. Webb, P. K. Lindeque, E. S. Fileman, C. Halsband, T. S. Galloway. 2014. Isolation of microplastics in biota-rich seawater samples and marine organisms. *Sci Rep*. 4: 4528.

Cooper I. 2007. Plastics and chemical migration into food. In: Barnes K, Sinclair R, Watson D, editors. Chemical migration and food contact materials. Sawston (Cambridge): Woodhead Publishing; p. 228–250.

Correia M. and K. Loeschner. 2018. Detection of nanoplastics in food by asymmetric flow field-flow fractionation coupled to multi-angle light scattering: possibilities, challenges and analytical limitations. *Anal Bioanal Chem*. 410: 5603-5615.

Cox, K. D., G. A. Covernton, H. L. Davies, J. F. Dower, F. Juanes, and S. E. Dudas. 2019. Human Consumption of Microplastics. *Environmental Science & Technology*. 53: 7068–7074. https://doi.org/10.1021/acs.est.9b01517

Deng, Y., Y. Zhang, B. Lemos, and H. Ren. 2017. Tissue accumulation of microplastics in mice and biomarker responses suggest widespread health risks of exposure. *Scientific Reports*. 7. https://doi.org/10.1038/srep46687

des Rieux, A., V. Fievez, I. Théate, J. Mast, V. Préat, and Y. J. Schneider. 2007. An improved in vitro model of human intestinal follicle-associated epithelium to study nanoparticle transport by M cells. *European Journal of Pharmaceutical Sciences*. 30:380–391. https://doi.org/10.1016/j.ejps.2006.12.006

Doorn, P. F., P. A. Campbell, and H. C. Amstutz. 1996. Metal Versus Polyethylene Wear Particles in Total Hip Replacements. *Clinical Orthopaedics and Related Research*. 329:S206–S216. https://doi.org/10.1097/00003086-199608001-00018

Doyle-McCullough, M., S. H. Smyth, S. M. Moyes, and K. E. Carr. 2007. Factors influencing intestinal microparticle uptake in vivo. *International Journal of Pharmaceutics*. 335:79–89. https://doi.org/10.1016/j.ijpharm.2006.10.043

Dris, R., J. Gasperi, M. Saad, C. Mirande, and B. Tassin. 2016. Synthetic fibers in atmospheric fallout: A source of microplastics in the environment? *Marine Pollution Bulletin*. 104:290–293. https://doi.org/10.1016/J.MARPOLBUL.2016.01.006

EFSA Panel on Contaminants in the Food Chain (CONTAM). 2016. Presence of microplastics and nanoplastics in food, with particular focus on seafood. EFSA J. 14: e04501. doi: 10.2903/j.efsa.2016.4501.

Eldridge, J. H., J. A. Meulbroek, J. K. Staas, T. R. Tice, and R. M. Gilley. 1989. Vaccine-Containing Biodegradable Microspheres Specifically Enter the Gut-Associated Lymphoid Tissue Following Oral Administration and Induce a Disseminated Mucosal Immune Response. In *Immunobiology of Proteins and Peptides V.* 251:191–202. Boston, MA: Springer US. https://doi.org/10.1007/978-1-4757-2046-4_18

Fadeel, B., A. Pietroiusti and A. Shvedova. A. 2012. Adverse Effects of Engineered Nanomaterials: Exposure, Toxicology, and Impact on Human Health. Boston: Academic Press.

Ferreira I., C. Venâncio, I. Lopes, M. Oliveira. 2019. Nanoplastics and marine organisms: What has been studied? *Environ Toxicol Pharmacol*. 67: 1-7.

Fuchs, A.-K., T. Syrovets, K. A. Haas, C. Loos, A. Musyanovych, V. Mailänder, K. Landfester, T. Simmet. 2016. Carboxyl- and amino-functionalized polystyrene nanoparticles differentially affect the polarization profile of M1 and M2 macrophage subsets. *Biomaterials*. 85:78–87. https://doi.org/10.1016/j.biomaterials.2016.01.064

García-Rodríguez, A., L. Vila, C. Cortés, R. Marcos, and A. Hernández. 2018. Effects of differently shaped TiO_2NPs (nanospheres, nanorods and nanowires) on the in vitro model (Caco-2/HT29) of the intestinal barrier. *Particle and Fibre Toxicology*. 15:1–16. https://doi.org/10.1186/s12989-018-0269-x

Gewert, B., M. M. Plassmann, and M. MacLeod. 2015. Pathways for degradation of plastic polymers floating in the marine environment. *Environmental Science. Processes & Impacts*. 17:1513–1521. https://doi.org/10.1039/c5em00207a

Guglielmi, G. 2017. In the next 30 years, we'll make four times more plastic waste than we ever have. *Science*. https://doi.org/10.1126/science.aan7121

Hartung, T., and E. Sabbioni. 2011. Alternative in vitro assays in nanomaterial toxicology. *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology*. 3:545–573. https://doi.org/10.1002/wnan.153

Hesler, M., L. Aengenheister, B. Ellinger, R. Drexel, S. Straskraba, C. Jost, S. Wagner, F. Meier, H. von Briesen, C. Büchel, P. Wick, T. Buerki-Thurnherr, Y. Kohl. 2019. Multi-endpoint toxicological assessment of polystyrene nano- and microparticles in different biological models in vitro. *Toxicol In Vitro*. 61:104610.

Hicks, D. G., A. R. Judkins, J. Z. Sickel, R. N. Rosier, J. E. Puzas, and R. J. O'Keefe. 1996. Granular Histiocytosis of Pelvic Lymph Nodes following Total Hip Arthroplasty. The Presence of Wear Debris, Cytokine Production, and Immunologically Activated Macrophages*. *The Journal of Bone & Joint Surgery*. 78:482–496. https://doi.org/10.2106/00004623-199604000-00002

Hidalgo-Ruz, V., L. Gutow, R. C. Thompson, and M. Thiel. 2012. Microplastics in the Marine Environment: A Review of the Methods Used for Identification and Quantification. *Environmental Science & Technology*. 46:3060–3075. https://doi.org/10.1021/es2031505

Hillery, A M, P. U. Jani, and A. T. Florence. 1994. Comparative, quantitative study of lymphoid and non-lymphoid uptake of 60 nm polystyrene particles. *Journal of Drug Targeting*. 2:151–156. https://doi.org/10.3109/10611869409015904

Hillery, A. M., and A. T. Florence. 1996. The effect of adsorbed poloxamer 188 and 407 surfactants on the intestinal uptake of 60-nm polystyrene particles after oral administration in the rat. *International Journal of Pharmaceutics*. 132:123–130. https://doi.org/10.1016/0378-5173(95)04353-5

Holden, P. A., F. Klaessig, R. F. Turco, J. H. Priester, C. M. Rico, H. Avila-Arias, J. L. Gardea-Torresdey. 2014. Evaluation of Exposure Concentrations Used in Assessing Manufactured Nanomaterial Environmental Hazards: Are They Relevant? *Environmental Science & Technology*. 48:10541–10551. https://doi.org/10.1021/es502440s

Hussain, N., V. Jaitley, and A. T. Florence. 2001. Recent advances in the understanding of uptake of microparticulates across the gastrointestinal lymphatics. *Advanced Drug Delivery Reviews*. 50:107–142.

Hwang, J., D. Choi, S. Han, J. Choi, J. Hong. 2019. An assessment of the toxicity of polypropylene microplastics in human derived cells. *Sci Total Environ.* 684:657-669.

Jani, P., G. W. Halbert, J. Langridge, and A. T. Florence. 1989. The Uptake and Translocation of Latex Nanospheres and Microspheres after Oral Administration to Rats. *Journal of Pharmacy and Pharmacology*. 41:809–812. https://doi.org/10.1111/j.2042-7158.1989.tb06377.x

Jani, P., G. W. Halbert, J. Langridge, and A. T. Florence. 1990. Nanoparticle Uptake by

the Rat Gastrointestinal Mucosa: Quantitation and Particle Size Dependency. *Journal of Pharmacy and Pharmacology*. 42:821–826. https://doi.org/10.1111/j.2042-7158.1990.tb07033.x

Jani, P. U., A. T. Florence, and D. E. McCarthy. 1992. Further histological evidence of the gastrointestinal absorption of polystyrene nanospheres in the rat. *International Journal of Pharmaceutics*. 84:245–252. https://doi.org/10.1016/0378-5173(92)90162-U

Jin, Y., L. Lu, W. Tu, T. Luo, Z. Fu. 2019. Impacts of polystyrene microplastic on the gut barrier, microbiota and metabolism of mice. Sci Total Environ. 2019, 649: 308-317.

Karami, A., A. Golieskardi, C. Keong Choo, V. Larat, T. S. Galloway, and B. Salamatinia. 2017. The presence of microplastics in commercial salts from different countries. *Scientific Reports*. 7:46173. https://doi.org/10.1038/srep46173

Kim, J., S. V. Chankeshwara, F. Thielbeer, J. Jeong, K. Donaldson, M. Bradley, and W. S. Cho. 2015. Surface charge determines the lung inflammogenicity: A study with polystyrene nanoparticles. *Nanotoxicology*. 10:1–8. https://doi.org/10.3109/17435390.2015.1022887

Kosuth, M., E. V. Wattenberg, S.A. Mason, C. Tyree and D. Morrison. 2017. Synthetic Polymer Contaminating Global Drinking Water [www Document]. URL. https://orbmedia.org/stories/Invisibles_final_report.

Kucharzik, T., N. Lügering, K. Rautenberg, A. Lügering, M. A. Schmidt, R. Stoll, and W. Domschke. 2006. Role of M Cells in Intestinal Barrier Function. *Annals of the New York Academy of Sciences*. 915:171–183. https://doi.org/10.1111/j.1749-6632.2000.tb05240.x

Kulkarni, S. A., and S. S. Feng. 2013. Effects of Particle Size and Surface Modification on Cellular Uptake and Biodistribution of Polymeric Nanoparticles for Drug Delivery. *Pharmaceutical Research*. 30:2512–2522. https://doi.org/10.1007/s11095-012-0958-3

Lambert, S., C. Sinclair, and A. Boxall. 2014. Occurrence, Degradation, and Effect of Polymer-Based Materials in the Environment. In *Reviews of environmental contamination and toxicology.* 227:1–53. https://doi.org/10.1007/978-3-319-01327-5_1

LeFevre, M. E., A. M. Boccio, and D. D. Joel. 1989. Intestinal Uptake of Fluorescent Microspheres in Young and Aged Mice. *Experimental Biology and Medicine*. 190:23–27. https://doi.org/10.3181/00379727-190-42825

Lehner, R., C. Weder, A. Petri-Fink, and B. Rothen-Rutishauser. 2019. Emergence of Nanoplastic in the Environment and Possible Impact on Human Health. *Environmental Science & Technology*. https://doi.org/10.1021/acs.est.8b05512

Leslie, H. A. L. H. M. Van der Meulen, F. Kleisen, D. Vethaak. 2011. Microplastic litter in the Dutch marine environment. *Deltares*. 1-97.

Li, W. C., H. F. Tse, and L. Fok. 2016. Plastic waste in the marine environment: A review of sources, occurrence and effects. *Science of The Total Environment*. 566–567, 333–349. https://doi.org/10.1016/J.SCITOTENV.2016.05.084

Liebezeit, G., and E. Liebezeit. 2013. Non-pollen particulates in honey and sugar. *Food Additives & Contaminants: Part A*. 30:2136–2140. https://doi.org/10.1080/19440049.2013.843025

Liebezeit, G., and E. Liebezeit. 2014. Synthetic particles as contaminants in German

beers. *Food Additives & Contaminants: Part A*. 31:1574–1578. https://doi.org/10.1080/19440049.2014.945099

Lim, S.L., C. T. Ng, L. Zou, Y. Lu, J. Chen, B. H. Bay, H. M. Shen, C. N. Ong. 2019. Targeted metabolomics reveals differential biological effects of nanoplastics and nanoZnO in human lung cells. *Nanotoxicology*. 13:1117-1132.

Lithner, D., A. Larsson, and G. Dave. 2011. Environmental and health hazard ranking and assessment of plastic polymers based on chemical composition. *Science of The Total Environment*. 409:3309–3324. https://doi.org/10.1016/j.scitotenv.2011.04.038

Liu, Y., W. Li, F. Lao, Y. Liu, L. Wang, R. Bai, Y. Zhao, C. Chen. 2011. Intracellular dynamics of cationic and anionic polystyrene nanoparticles without direct interaction with mitotic spindle and chromosomes. *Biomaterials*. 32:8291-8303

M'Koma, A. E. 2013. Inflammatory bowel disease: an expanding global health problem. *Clinical Medicine Insights. Gastroenterology.* 6:33–47. https://doi.org/10.4137/CGast.S12731

Ma, Y., H. Liu, J. Wu, L. Yuan, Y. Wang, X. Du, R. Wang, P. W. Marwa, P. Petlulu, X. Chen and H. Zhang. 2019. The adverse health effects of bisphenol A and related toxicity mechanisms. *Environ Res*. 176:108575.

Magara, G., F. R. Khan, M. Pinti, K. Syberg, A. Inzirillo, and A. C. Elia. 2019. Effects of combined exposures of fluoranthene and polyethylene or polyhydroxybutyrate microplastics on oxidative stress biomarkers in the blue mussel (*Mytilus edulis*). J. Toxicol. Environ. Health, A. 82(10):616-625. http://doi: 10.1080/15287394.2019.1633451.

Magrì, D., P. Sánchez-Moreno, G. Caputo, F. Gatto, M. Veronesi, G. Bardi, G., T. Catelani, D. Guarnieri, A. Athanassiou, P. P. Pompa, D. Fragouli. 2018. Laser Ablation as a Versatile Tool To Mimic Polyethylene Terephthalate Nanoplastic Pollutants: Characterization and Toxicology Assessment. *ACS Nano*. 12:7690–7700. https://doi.org/10.1021/acsnano.8b01331

Mahler, G. J., M. B. Esch, E. Tako, T. L. Southard, S. D. Archer, R. P. Glahn, and M. L. Shuler. 2012. Oral exposure to polystyrene nanoparticles affects iron absorption. *Nature Nanotechnology*. 7:264–271. https://doi.org/10.1038/nnano.2012.3

Mason, S. A., W. Vg, and J. Neratko. 2018. Synthetic Polymer Contamination in Bottled Water. *Front. Chem*. 6:407. https://doi.org/10.3389/fchem.2018.00407

Meeker, J. D., S. Sathyanarayana, and S. H. Swan. 2009. Phthalates and other additives in plastics: human exposure and associated health outcomes. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*. 364:2097–2113. https://doi.org/10.1098/rstb.2008.0268

Moore, C. J. 2008. Synthetic polymers in the marine environment: A rapidly increasing, long-term threat. *Environmental Research*. 108:131–139. https://doi.org/10.1016/J.ENVRES.2008.07.025

Moulin, T. C., and L. T. van Egmond. 2019. A possible role for pollutants in mental disorders via gut microbiota. Sci.Total Environ. 693:133639 https://doi: 10.1016/j.scitotenv.2019.133639

Neutra, M., and D. Louvard. 1989. Differentiation of intestinal cells in vitro. M*od. Cell Biol*. 8:363-398.

OECD. 2010. Toxicokinetics. OECD Guideline for the testing of chemicals Section 4:417.

Paget, V., S. Dekali, T. Kortulewski, R. Grall, C. Gamez, K. Blazy, O. Aguerre-Chariol, S. Chevillard, A. Braun, P. Rat, G. Lacroix. 2015. Specific Uptake and Genotoxicity Induced by Polystyrene Nanobeads with Distinct Surface Chemistry on Human Lung Epithelial Cells and Macrophages. *PLoS One*. 10:e0123297. https://doi.org/10.1371/journal.pone.0123297

Powell, J. J., V. Thoree, and L. C. Pele. 2007. Dietary microparticles and their impact on tolerance and immune responsiveness of the gastrointestinal tract. *British Journal of Nutrition*. 98(S1):S59–S63. https://doi.org/10.1017/S0007114507832922

Prata, J. C. 2018. Airborne microplastics: Consequences to human health? *Environmental Pollution*. 234:115–126. https://doi.org/10.1016/j.envpol.2017.11.043

Prata, J. C. J. P. da Costa, A. V. Girão, I. Lopes, A. C. Duarte, T. Rocha-Santos. 2019. Identifying a quick and efficient method of removing organic matter without damaging microplastic samples. *Sci Total Environ*. 686: 131-139.

Prietl, B., C. Meindl, E. Roblegg, T. R. Pieber, G. Lanzer, and E. Fröhlich. 2014. Nano-sized and micro-sized polystyrene particles affect phagocyte function. *Cell Biology and Toxicology.* 30:1–16. https://doi.org/10.1007/s10565-013-9265-y

Radke, E. G., B. S. Glenn, J. M. Braun, and G. S. Cooper. 2019. Phthalate exposure and female reproductive and developmental outcomes: a systematic review of the human epidemiological evidence. *Environ Int.* 130: 104580. doi: 10.1016/j.envint.2019.02.003.

Rainieri, S., and A. Barranco. 2019. Microplastics, a food safety issue? *Trends in Food Science and Technology.* 84:55–57. https://doi.org/10.1016/j.tifs.2018.12.009

Reinholz J., C. Diesler, S. Schöttler, M. Kokkinopoulou, S. Ritz, K. Landfester, V. Mailänder. 2018. Protein machineries defining pathways of nanocarrier exocytosis and transcytosis. *Acta Biomater.* 71: 432-443.

Renner, G., T.C. Schmidt and J. Schram. 2018. Analytical methodologies for monitoring micro(nano)plastics: which are fit for purpose? *Environ. Sci. Health*. 1:55–61. doi: 10.1016/j.coesh.2017.11.001

Revel, M., A. Châtel, and C. Mouneyrac. 2018. Micro(nano)plastics: A threat to human health? *Current Opinion in Environmental Science & Health*. 1:17–23. https://doi.org/10.1016/j.coesh.2017.10.003

Rist, S., B. Carney Almroth, N. B. Hartmann, and T. M. Karlsson. 2018. A critical perspective on early communications concerning human health aspects of microplastics. *Science of the Total Environment*. 626:720–726. https://doi.org/10.1016/j.scitotenv.2018.01.092

Rubio, L., J. Bach, R. Marcos, and A. Hernández. 2017. Synergistic role of nanoceria on the ability of tobacco smoke to induce carcinogenic hallmarks in lung epithelial cells. *Nanomedicine*. 12:2623–2635. https://doi.org/10.2217/nnm-2017-0205

Sanders, E., and C. T. Ashworth. 1961. A study of particulate intestinal absorption and hepatocellular uptake. *Experimental Cell Research*. 22:137–145. https://doi.org/10.1016/0014-4827(61)90092-1

Santana, M. F. M., F. T. Moreira, and A. Turra. 2017. Trophic transference of microplastics under a low exposure scenario: Insights on the likelihood of particle cascading along marine food-webs. *Marine Pollution Bulletin*. 121:154–159. https://doi.org/10.1016/j.marpolbul.2017.05.061

Schirinzi, G. F., I. Pérez-Pomeda, J. Sanchís, C. Rossini, M. Farré, and D. Barceló. 2017. Cytotoxic effects of commonly used nanomaterials and microplastics on cerebral and epithelial human cells. *Environmental Research*. 159:579–587. https://doi.org/10.1016/j.envres.2017.08.043

Schmidt, C., C. Lautenschlaeger, E. M. Collnot, M. Schumann, C. Bojarski, J. D. Schulzke, C. M. Lehr, A. Stallmach. 2013. Nano- and microscaled particles for drug targeting to inflamed intestinal mucosa—A first in vivo study in human patients. *Journal of Controlled Release*. 165:139–145. https://doi.org/10.1016/j.jconrel.2012.10.019

Schneider, T., G. Burdett, L. Martinon, P. Brochard, M. Guillemin, U. Teichert, and U. Draeger. 1996. Ubiquitous fiber exposure in selected sampling sites in Europe. *Scandinavian Journal of Work, Environment & Health*. Scandinavian Journal of Work, Environment & HealthFinnish Institute of Occupational HealthDanish National Research Centre for the Working EnvironmentNorwegian National Institute of Occupational Health. https://doi.org/10.2307/40966551

Schymanski, D., C. Goldbeck, H. U. Humpf, and P. Fürst. 2018. Analysis of microplastics in water by micro-Raman spectroscopy: Release of plastic particles from different packaging into mineral water. *Water Research*. 129:154–162. https://doi.org/10.1016/j.watres.2017.11.011

Steffens, K.-J. 1995. Persorption — Criticism and Agreement as Based upon In Vitro and In Vivo Studies on Mammals. In *Absorption of Orally Administered Enzymes*. (9–21). Berlin, Heidelberg: Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-79511-4_2

Stock, V., L. Böhmert, E. Lisicki, R. Block, J. Cara-Carmona, L. K. Pack, …A. Lampen. 2019. Uptake and effects of orally ingested polystyrene microplastic particles in vitro and in vivo. *Archives of Toxicology*. 93:1817–1833. https://doi.org/10.1007/s00204-019-02478-7

Su L., H. Deng, B. Li, Q. Chen, V. Pettigrove, C. Wu, H. Shi. 2019. The occurrence of microplastic in specific organs in commercially caught fishes from coast and estuary area of east China. *J Hazard Mater*. 365: 716-724.

Talsness, C. E., A. J. M. Andrade, S. N. Kuriyama, J. A. Taylor, and F. S. vom Saal. 2009. Components of plastic: experimental studies in animals and relevance for human health. *Philosophical Transactions of the Royal Society B: Biological Sciences*. 364:2079. https://doi.org/10.1098/RSTB.2008.0281

Thompson, R. C. 2004. Lost at Sea: Where Is All the Plastic? *Science.* 304:838–838. https://doi.org/10.1126/science.1094559

Toussaint, B., B. Raffael, A. Angers-Loustau, D. Gilliland, V. Kestens, M. Petrillo, I. M. R. Echevariia, G. Van den Eede. 2019. Review of micro- and nanoplastic contamination in the food chain. *Food Additives & Contaminants: Part A*. 36:1–35. https://doi.org/10.1080/19440049.2019.1583381

United Nations Environment Programme., Emerging Issues in our Global Environment. 2012.

United States Environmental Protection Agency. Municipal Solid Waste (MSW) in the United States: Facts and Figures 2012. http://www.epa.gov/solidwaste/nonhaz/municipal/msw99.htm.

Vales, G., L. Rubio, and R. Marcos. 2016. Genotoxic and cell-transformation effects of multi-walled carbon nanotubes (MWCNT) following in vitro sub-chronic exposures.

Journal of Hazardous Materials. 306:193–202. https://doi.org/10.1016/j.jhazmat.2015.12.021

van Sebille, E., C. Wilcox, L. Lebreton, N. Maximenko, B. D. Hardesty, J. A. van Franeker, M. Eriksen, D. Siegel, F. Galgani, K. L. Law. 2015. A global inventory of small floating plastic debris. *Environmental Research Letters*. 10:124006. https://doi.org/10.1088/1748-9326/10/12/124006

Vila, L., A. García-Rodríguez, C. Cortés, R. Marcos, and A. Hernández. 2018. Assessing the effects of silver nanoparticles on monolayers of differentiated Caco-2 cells, as a model of intestinal barrier. *Food and Chemical Toxicology*. 116(Pt B), 1–10. https://doi.org/10.1016/j.fct.2018.04.008

Volkheimer, G. 1975. Hematogenous dissemination of ingested polyvinyl chloride particles. *Annals of the New York Academy of Sciences*. 246(1 Toxicity of V), 164–171. https://doi.org/10.1111/j.1749-6632.1975.tb51092.x

Volkheimer, G. The phenomenom of persorption: persorption, dissemination, and elimination of microparticles. 2001. In Old Herborn University Seminar 2001. Monograph; Heidt, P. J., Nieuwenhuis, P., Rusch, V.D., Waaij, D. V. D., Eds.; Old Herborn University.14:7−13

von Moos, N., P. Burkhardt-Holm, and A. Köhler. 2012. Uptake and Effects of Microplastics on Cells and Tissue of the Blue Mussel Mytilus edulis L. after an Experimental Exposure. *Environmental Science & Technology*. 46:11327–11335. https://doi.org/10.1021/es302332w

Walczak, A. P., E. Kramer, P. J. M. Hendriksen, R. Helsdingen, M. van der Zande, I. M. C. M. Rietjens, and H. Bouwmeester. 2015. *In vitro* gastrointestinal digestion increases the translocation of polystyrene nanoparticles in an *in vitro* intestinal co-culture model. *Nanotoxicology*. 9:886–894. https://doi.org/10.3109/17435390.2014.988664

Walczak, A. P., E. Kramer, P. J. M. Hendriksen, P. Tromp, J. P. F. G. Helsper, M. Van Der Zande, I. M. C. M Rietjens, H. Bouwmeester. 2015. Translocation of differently sized and charged polystyrene nanoparticles in in vitro intestinal cell models of increasing complexity. *Nanotoxicology*. 9:453–461. https://doi.org/10.3109/17435390.2014.944599

Walczak, A. P. 2015. Development of an integrated in vitro model for the prediction of oral bioavailability of nanoparticles. Wageningen University, Thesis publication.

Waring R. H., R. M. Harris, S. C. Mitchell. 2018. Plastic contamination of the food chain: A threat to human health? *Maturitas.* 115: 64-68.

White, J. R., and A. Turnbull. 1994. Weathering of polymers: mechanisms of degradation and stabilization, testing strategies and modelling. *Journal of Materials Science*. 29:584–613. https://doi.org/10.1007/BF00445969

Wright, S. L., and F. J. Kelly. 2017. Plastic and Human Health: A Micro Issue? *Environmental Science and Technology.* 51:6634–6647. https://doi.org/10.1021/acs.est.7b00423

Wright, S. L., R. C. Thompson, and T. S. Galloway. 2013. The physical impacts of microplastics on marine organisms: A review. *Environmental Pollution*. 178:483–492. https://doi.org/10.1016/J.ENVPOL.2013.02.031

Wu, B., X. Wu, S. Liu, Z. Wang, and L. Chen. 2019. Size-dependent effects of polystyrene microplastics on cytotoxicity and efflux pump inhibition in human Caco-2 cells. *Chemosphere*. 221:333–341.

https://doi.org/10.1016/j.chemosphere.2019.01.056

Xia, T., M. Kovochich, M. Liong, J. I. Zink, and A. E. Nel. 2008. Cationic Polystyrene Nanosphere Toxicity Depends on Cell-Specific Endocytic and Mitochondrial Injury Pathways. *ACS Nano*. *2:*85–96. https://doi.org/10.1021/nn700256c

Yang, D., H Shi, L. Li, J. Li, K. Jabeen, and P. Kolandhasamy. 2015. Microplastic Pollution in Table Salts from China. *Environmental Science & Technology*. 49: 13622–13627. https://doi.org/10.1021/acs.est.5b03163

Zhou X. X., L. T. Hao, H. Y. Wang, Y. J. Li, J. F. Liu. 2019. Cloud-point extraction combined with thermal degradation for nanoplastic analysis using pyrolysis gas chromatography-mass spectrometry. *Anal Chem*. 91: 1785-1790.

Zweibaum, A., M. Laburthe, E. Grasset and D. Louvard. 1991. Use of cultured cell lines in studies of intestinal cell differentiation and function., in M. Field and R.A. Frizzel (eds) Handbook of Physiology: The Gastrointestinal System, Am. Physiol. Soc., Bethesda. 223-255.

Figure legends

Figure 1: representation of Caco-2/HT29/Raji-B model. Adapted from García-Rodríguez et al., 2018.

Figure 2: Human sources of exposure and Biomonitoring scheme.

Figure 3: *In vitro* low-dose long-term studies.

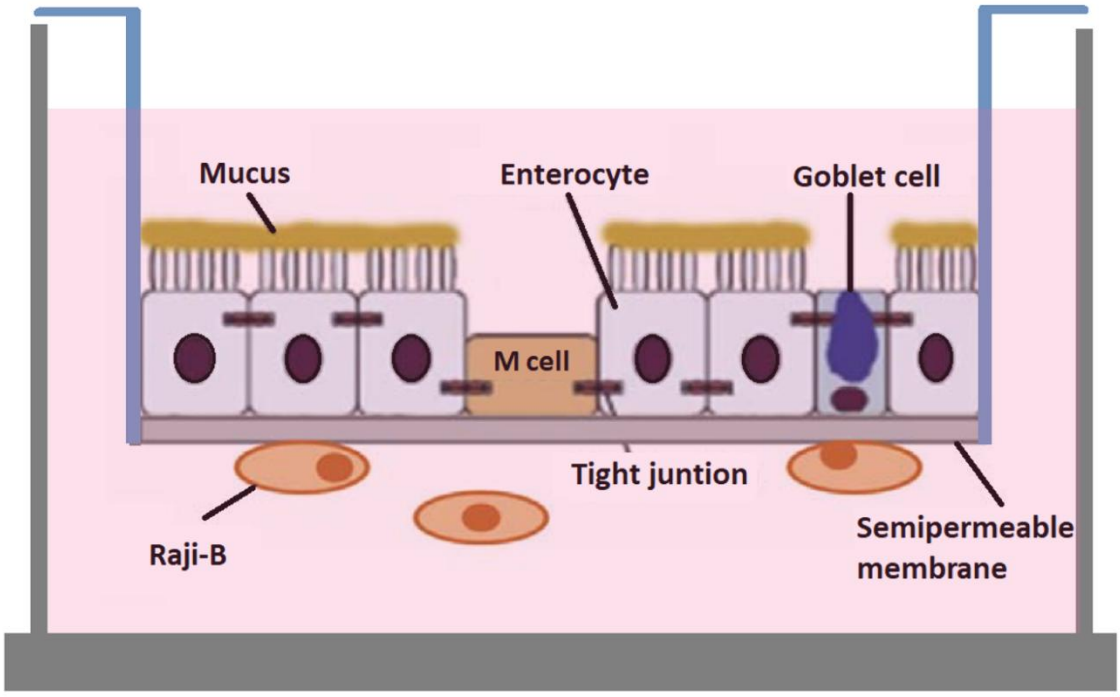
Mucus
Enterocyte
Goblet cell
M cell
Tight juntion
Raji-B
Semipermeable
membrane

Figure 1.

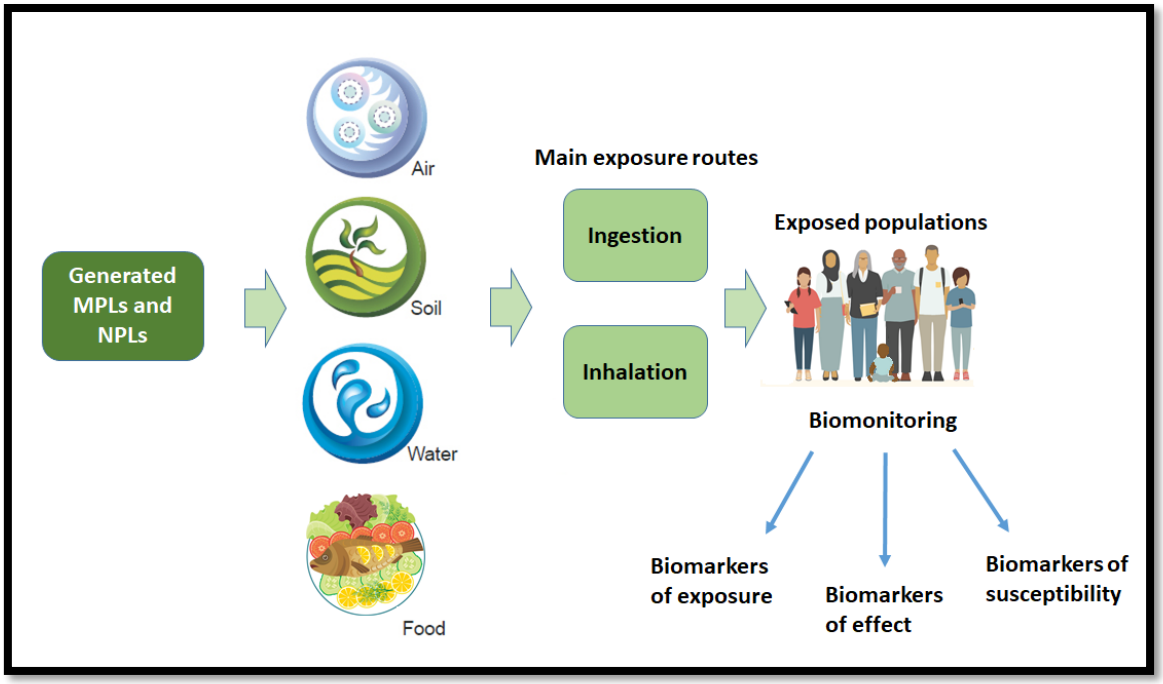
Generated MPLs and NPLs
Air
Soil
Water
Food
Main exposure routes
Ingestion
Inhalation
Exposed populations
Biomonitoring
Biomarkers of exposure
Biomarkers of effect
Biomarkers of susceptibility

Figure 2.

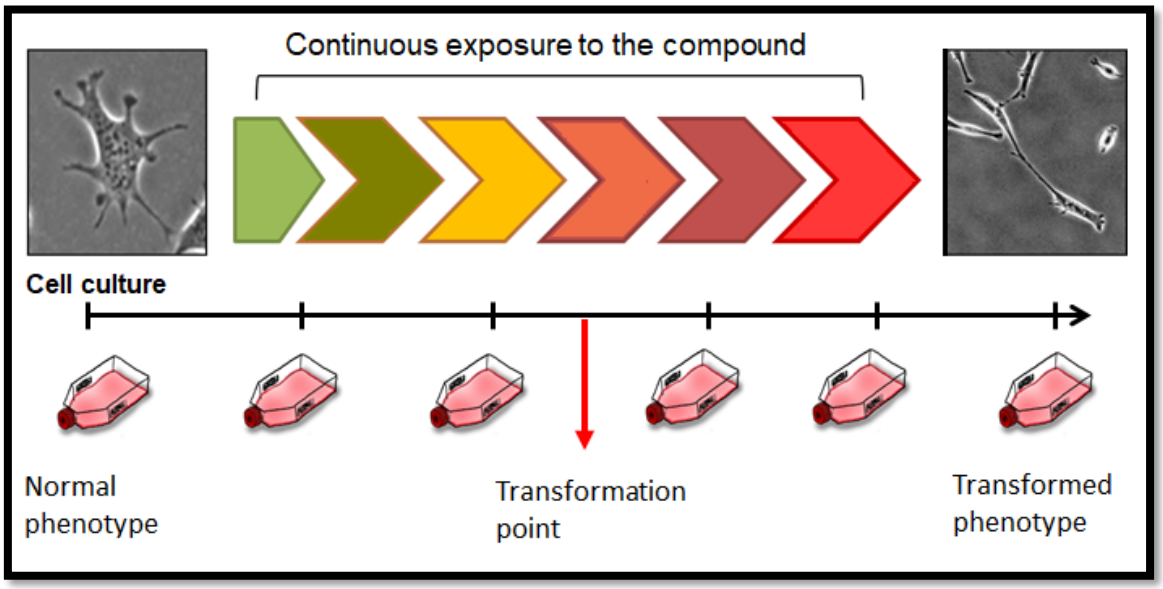
Continuous exposure to the compound
Cell culture
Normal phenotype
Transformation point
Transformed phenotype

Figure 3.