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Microplastics in the human digestive environment: a focus on the potential and challenges facing *in vitro* gut model development

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Cr: Chromium; GIT: Gastro-Intestinal Tract; M-ARCOL: Mucus ARtificial COLon model; MPs: microplastics; PA: polyamide; PE: polyethylene; PET: polyethylene terephthalate; PLA: polylactic acid; PLGA: poly(lactic-co-glycolic acid); PP: polypropylene; PS: polystyrene; PVC: polyvinyl chloride; M-SHIME: Mucosal- Simulator of the Human Intestinal Microbial Ecosystem; TIM-1: TNO Gastro-Intestinal Model 1

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Abstract

Plastic pollution is a major issue worldwide, generating massive amounts of smaller plastic particles, including microplastics (MPs). Their ubiquitous nature in the environment but also in foodstuff and consumer packaged goods has revealed potential threats to humans who can be contaminated mainly through air, food and water consumption. In this review, the current literature on human exposure to MPs is summarized with a focus on the gastrointestinal tract as portal of entry. Then, we discuss the vector effect of MPs, in their pristine *versus* weathered forms, with well-known contaminants as heavy metals and chemicals, or more emerging ones as antibiotics or microbial pathogens, like *Pseudomonas* spp., *Vibrio* spp., *Campylobacter* spp. and *Escherichia coli*. Comprehensive knowledge on MP fate in the gastrointestinal tract and their potential impact on gut homeostasis disruption, including gut microbiota, mucus and epithelial barrier, is reported *in vitro* and *in vivo* in mammals. Special emphasis is given on the crucial need of developing robust *in vitro* gut models to adequately simulate human digestive physiology and absorption processes. Finally, this review points out future research directions on MPs in human intestinal health.

Keywords:

microplastics; vector effect; digestion; intestinal barrier; human *in vitro* gut models

1. Introduction

Plastic production has significantly increased worldwide over the last decades, from 1.7 million tons in the 1950s to over 368 million tons in 2019 (PlasticsEurope, 2020), because of its success as a multifunctional, resistant, easy-to-process and affordable material. Due to these unique properties, plastics are central to modern living and constitute a vital source for innovation-driven growth. However, negative impacts related to their end-of-life cannot be set aside. Plastic wastes have different fates: they can be reused, recycled, energy recovered (burned), deposited at dumping sites or lost in the environment (European Parliament, 2008). Plastic debris can be classified according to their size. Concerning microplastics (MPs), the most frequently used definition considers all plastic particles <5 mm in diameter (Arthur et al., 2009) even though no international consensus has been reached yet (Hartmann et al., 2019). The possible mechanism for generation of a majority of MPs is the *in situ* weathering breakdown of plastic debris. In the marine environment, it is primarily the UV-B radiation in sunlight that leads to photo-oxidative degradation of plastics and once initiated, the degradation can proceed through thermooxidation. Other types of degradation processes, like biodegradation, are orders of magnitude slower compared to light-induced oxidative degradation and hydrolysis is usually not a significant mechanism in seawater (Andrady, 2011; Mattsson et al., 2015). MPs are then found in all environmental media from marine to ground water, lakes, sediments, soil and even in the atmosphere (Miranda et al., 2019; Wu et al., 2019a). Moreover, MPs may be directly released from domestic and industrial sources (Weinstein et al., 2016), as well as from plastic products used for food packaging, fast-food delivery, and water consumption (Fadare et al., 2020; Hernandez et al., 2019). Negative impact on the environment and organisms resulting from plastic pollution is well-established (Bucci et al., 2019). Each organism is exposed, from fish or worms to mammals (Toussaint et al., 2019). Inevitably, plastics enter the food chain and drive contaminants, probably impacting gut homeostasis (Lu et al., 2019). While more and more studies evaluate the toxicological issue of MP exposure on various organisms, a debate remains on whether the

64 associated risk is real or overrated (Backhaus and Wagner, 2020). Even though humans are also
65 exposed through inhalation and dermal contact, food ingestion remains the main exposure source
66 (Galloway, 2015). Increasing works and reviews have reported the human health issues of MP
67 exposure but only few are focused on human intestinal health at the forefront of this exposure (Hirt
68 and Body-Malapel, 2020; Lu et al., 2019; Paul et al., 2020). Based on this background, we first
69 summarize the available evidence regarding the degree of human exposure to MPs *via* oral route.
70 Next, we highlight the vector effect of MPs for well-known contaminants as heavy metals and
71 chemicals, and more emerging ones as antibiotics or microbial pathogens. Afterwards, we detail the
72 fate and impact of MPs on the digestive tract of mammals and humans, with a special emphasis on *in*
73 *vitro* approaches to determine the physico-chemical transformations of MPs during digestion and
74 their putative role on intestinal barrier disruption. Finally, we will discuss the potentialities of
75 advanced *in vitro* gut models simulating human digestive physiology for unravelling toxic and health
76 effects of MPs.
77
78 **2. Oral exposure sources to MPs in humans**
79 **2.1. Drinking water and beverages**
80 MP contamination has been reported in various beverages including bottled and tap water. In the
81 study reported by Mason and colleagues (Mason et al., 2018), samples from bottled water were
82 processed by Nile red staining, 1.5-µm filtration and analyzed using an optical microscope; they
83 were then classified in two size ranges: larger than 100 µm (polymer identity further confirmed with
84 Fourier transform infrared spectroscopy (FTIR)) and between 6.5-100 µm. In the study of Kosuth
85 and collaborators (Kosuth et al., 2018), samples from tap water were processed by a 2.5-µm
86 filtration, analyzed using a dissection microscope and only particles not stained by the Rose Bengal
87 were counted; no confirmation by FTIR was performed. Under these conditions of sample analysis,
88 bottled water seemed to have the highest particle concentration (ranging from 0 to 10,000

particles/L), compared to tap water (ranging from 0 to 61 particles/L) (Kosuth et al., 2018; Mason et al., 2018). These differences in contamination level have also been found by Danopoulos and colleagues, with concentrations of 628 MPs/L and 4,889 MPs/L for tap and bottled water, respectively (Danopoulos et al., 2020). After tap water analysis from fourteen different countries, the most abundant shapes reported were for 98.3% fibers being between 0.1 to 5 mm in size (Kosuth et al., 2018). For bottled water from various brands in nine different countries, fragments (66%) and fibers (13%) were predominant, with 95% of particles being between 6.5 and 100 µm in size. Polypropylene (PP), polyethylene terephthalate (PET) and polyethylene (PE) are the most abundant polymers (Danopoulos et al., 2020; Koelmans et al., 2019). MP contamination in tap water can derive from environmental water pollution but also from the degradation of plastic water pipes, amplified by water disinfectants commonly used as chlorine dioxide (Vertova et al., 2019). For bottled water, the presence of MPs results from bottle degradation, mainly through ultraviolet radiations or during bottling process (Mason et al., 2018). Other studies have shown the contamination of beer (from 2 to 79 fibers/L, from 12 to 109 fragments/L and from 2 to 66 granules/L), milk but also refreshments (ranging from 10 to 100 MPs/L) and white wines (2,563-5,857 particles/L) (Diaz-Basantes et al., 2020; Liebezeit and Liebezeit, 2014; Prata et al., 2020). Despite the small number of studies available to date, results are highly variable and depend on the analytical methods and experimental protocols used, notably for the minimal size of particles to be extracted and analyzed.

2.2. Air

Numerous studies have reported air contamination by MPs: PE, PP, polystyrene (PS) and PET are generally the major polymers found, with fibers as the predominant shape (more than 90%). Synthetic textiles are the main source of airborne MPs even though other sources may occur, such as from degradation of larger plastics in landfills or from incineration, from traffic or re-suspended dust. Meteorological conditions like wind, rain and snow events, and human activities affect the

distribution and deposition of airborne MPs (Chen et al., 2019). Indoor air of different apartments was investigated and all were contaminated with MPs (1.7-16.2 particles/m^3) (Vianello et al., 2019). MP fibers can be ingested by eating air-dust exposed food but also by dust ingestion particularly for young children (Dris et al., 2017). This route of exposure is important for infants with a median daily intake of inhaled PET-based MPs estimated between 4,000 and 150,000 ng/kg of body weight (bw) per day (from 360 to 150,000 ng/kg bw/day in adults depending on their living area) (Zhang et al., 2020). In fact, adults consume MPs through fiber deposition from dust fallout in a house during a meal (13,731–68,415 particles/year/capita), which may represent a higher exposure than after mussel consumption (from 123 to 4,620 MPs/year/capita) (Catarino et al., 2018). A large part of those particles could enter the digestive tract through the lung mucociliary clearance mechanisms after inhalation.

2.3. Food

In 2016, the European Food Safety Authority (EFSA) published an overview on the presence of MPs (and also smaller-sized nanoplastics) in food, with a focus on seafood (EFSA 2016). Up to now, 201 edible species of which only one terrestrial species, chicken, have been reported to be contaminated (Toussaint et al., 2019). MPs in bivalves, shrimps and fish reach average concentrations of 0.2-4 particles/g, 0.75 particles/g and 1–7 particles/g, respectively. In addition, contamination in honey (40 to 660 fibers; 0-38 fragments/kg of honey), sugar (217 ± 123 fibers and 32 ± 7 fragments/kg of sugar), salt (from 0 to 19,800 particles/kg of salt), canned sardines and sprats was reported (Bouwmeester et al., 2015; Karami et al., 2018; Peixoto et al., 2019). Currently, it remains unclear whether this contamination is already present in raw materials or comes from processing and/or food packaging steps. A recent study has reported the important release of MPs from PP feeding bottles (16,200,000 particles/L) especially during sterilization process and exposure to high-temperature water, highlighting the urgent and critical need of risk assessment in infants (Li et al., 2020b). Another study reported MP pollution ranging from 52,050 to 233,000 particles/g

depending on fruit and vegetable types, with fruits being more contaminated than vegetables. Among each group, apples were the most polluted while lettuces represented the least contaminated. This MP pollution seems to originate from soil pollution leading to root absorption and translocation in other tissues (Oliveri Conti et al., 2020). It should be underlined that an insufficient number of studies has yet investigated the presence of such particles in other very commonly consumed food items including beef, poultry, dairy and cereals.

2.4. Human consumption

Humans are exposed to numerous MP sources (Fig. 1) (Rist et al., 2018) but to date, only one study clearly quantifies human exposure to MPs integrating dietary and inhalation sources. A child with a typical American diet consumes per day 106 (female) and 113 (male) MPs and inhales 97 (female) to 110 (male) of these particles. Regarding adults with a typical American diet, they consume 126 (female) to 142 (male) MPs and inhale 132 (female) to 170 (male) particles per day. However, Cox and colleagues pointed out that, according to the geographical localization and dietary habits, MP exposure could vary (Cox et al., 2019). For instance, when daily water intake recommendations are achieved through ingestion of bottled water, MP intake is increased by 22-fold compared to tap water consumption (Cox et al., 2019). In addition, seafood products are among the major food contributors for MP intake known to date. As illustrated in Japanese, known to be one of the largest consumers of fish and seafood products, they could ingest up to 154 MPs per day only through seafood products. However, data from other food groups consumed daily are now required for identifying additional sources of MP exposure in humans. Schwabl and his team reported in a pilot study the presence of MPs in stool samples from healthy volunteers living in eight countries worldwide to be representative of different geographic regions and dietary habits. Several plastics with in average 20 MPs per 10 g of stool and between 50 to 500 µm in size were found in all fecal samples. Each sample had 3 to 7 different types of plastics with a relative frequency of 62.8 % of PP, 17% of PET, 11.2 % of PS and 4.8 % of PE (Schwabl et al., 2019). Although this study only

investigated MPs larger than 50 µm for a small number of subjects (n=8, 3 males, 5 females, aged 33-65 years) with only 1 stool sample provided per participant, it is still striking that MPs are found in all analyzed samples. Further studies on larger populations should now be performed to correlate types and amounts of fecal MPs to geographic area, dietary habits, other potential sources of MP ingestion, and health impacts. Recently, Senathirajah and colleagues used the current knowledge to estimate the global average rate of MP ingestion in humans, which was in the range 0.1-5 g of MPs per week (Senathirajah et al., 2021). Beyond differences in experimental conditions and analytical methods used across studies, their analysis was limited by a scarcity of data for certain food categories (honey, sugar and fish: limited available data; pasta, oil, milk, bread, rice, meat and wheat: absence of data at the time of analysis), which led to only investigate four types of consumables with the most robust data (e.g. drinking water, salt, beer and shellfish). Similarly, the probable contribution of MPs from plastic utensils/food packaging was excluded due to data limitations (Senathirajah et al., 2021). Nevertheless, this pioneering study constitutes a first reliable step towards health risk assessment in humans. .

3. MPs as vectors for contaminants and microorganisms: consequences on the physico-chemical transformations of MPs and contaminants during human digestion

3.1. Sorption/desorption of chemical contaminants onto/from MPs

3.1.1 Sorption of chemical pollutants and heavy metals onto MPs

One major concern about MP health effects is related to their association with different chemicals (Fig. 2), and notably pollutants adsorbed from the surrounding environment and thereby having their environmental fate altered (Hahladakis et al., 2018; Wang et al., 2018). This “vector effect” is a potential concern facing the extent of MP pollution (Syberg et al., 2015) and little is known regarding the consequences on intestinal health.

Sorption of chemical pollutants (e.g. organochloride pesticides and polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and other benzene-ring derivatives) onto MPs has been widely investigated, as recently reviewed (Yu et al. 2019). Sorption is highly governed by physico-chemical characteristics (e.g. polymer matrix structure combining highly ordered crystalline regions and less structured amorphous regions, particle size, surface roughness) as well as environmental conditions (Velez et al., 2018). Lowering pH protonates the surface of MPs, leading to enhanced sorption of anionic pollutants. Likewise, when salinity increases, cation competition increases, inducing sorption or desorption of contaminants (Yu et al., 2019). The presence of ionic surfactant may also influence adsorption of hydrophilic pollutants. Indeed, MP adsorption capacity could increase from three to twenty-six times according to the co-existing surfactants (Xia et al., 2020). Adsorption typically occurs in the crystalline regions whereas internal partition is more likely to happen in the amorphous regions (Velez et al., 2018). Pristine MPs that means in their original conditions adsorb and concentrate contaminants such as polycyclic aromatic hydrocarbons, organochloride pesticides, polychlorinated biphenyls, perfluoroalkylated substances (PFASs) and several types of antibiotics, but also heavy metals as chromium (Cr), zinc or lead (Godoy et al., 2019; Guo et al., 2019; Heskett et al., 2012; Li et al., 2018; Razanajatovo et al., 2018; Rochman et al., 2013; Wang et al., 2015; Yu et al., 2020).

Weathering of MPs is furthermore important for the association of pollutants. During fragmentation, chemicals are susceptible to migrate to the plastic surface and then be released (Schrank et al., 2019). The migration rate closely depends on the molecular weight, initial concentration of the chemical substance present in the plastic, as well as the thickness, crystallinity and surface structure of the plastic (Hahladakis et al., 2018; Teuten et al., 2009). Weathering affects the physico-chemical properties of MPs as size, crystallinity or oxygen-containing groups (Fig. 2) and, thus, change contaminant sorption profile onto MPs (Liu et al., 2020a). For example, weathered MPs contain more surface oxygen-containing groups than pristine ones, leading to greater

contaminant sorption especially for the more hydrophilic ones. Furthermore, after etching and/or UV radiation, specific surface area and pore volume of MPs are increased, by formations of nanovoids and larger cracks in the surface, which also favors contaminant adsorption process (Li et al., 2018; Velez et al., 2018).

Sorption of heavy metals onto MPs is generally due to electrostatic interactions between metal ions and polar sites on the plastic surface. As for other contaminants, weathered MPs contain more heavy metals than their pristine counterparts (Holmes et al., 2012; Vedolin et al., 2018; Wang et al., 2019). Adsorption is enhanced in waters with high chemical and biological oxygen demands as urban wastewater and irrigation water. However, after maximum adsorption, metals tend to slowly desorb from MPs (Godoy et al., 2019). Acidic conditions, like those encountered in gastric juice during digestion (see section 3.1.2), may promote the leaching of metals bound to MPs (Teuten et al., 2009).

The environmental importance of the "vector effect" is still debated. A study of the interaction between biota and silver-contaminated MPs in zebrafish showed that MPs altered the pollutant fate but not necessarily by increasing its uptake, illustrating that plastics can bind contaminants, which are further less available for uptake (Khan et al., 2015). Similarly, Kleinteich and colleagues found that contaminant bioavailability in soil decreased as a function of binding to MPs (Kleinteich et al., 2018). MPs can thus both serve as a source and a sink for other contaminants, in a case-by-case specific manner. The process is complex and, as described above, depends on the pollutant, the composition and state of the MPs, as well as the conditions surrounding the particles, such as pH in the gut. Koelmans and colleagues argued that the vector effect might be insignificant in an environmental context, due to the ratio of MPs to other particles in the environment such a natural particles (Koelmans et al., 2016). Concentrations of MPs are numbers of magnitude lower than natural particles, which implies that the MP vector effect might not have large importance on a global scale. However, efforts to quantify MPs have been limited by sampling techniques and

238 especially smaller particles, with higher surface to volume ratio, are typically underestimated. This
239 implies that data are still needed in order to determine the impact of the vector effect, especially in
240 "hot spot" areas where MP concentrations are significantly higher than the average.

241 *3.1.2. Desorption of heavy metals from MPs during their in vitro human digestion and physico-*
242 *chemical transformations*

243 To date, studies on *in vitro* human digestion are scarce and mainly focused on simple batch
244 models exposing MPs, alone or with adsorbed chemical contaminants (only heavy metals studied so
245 far), to simulated digestive fluids. The main characteristics of currently used models, including their
246 advantages and limitations, are given in Table 1. A high desorption under acidic conditions was
247 reported in the specific case of Chromium in a human static *in vitro* digestion model, including
248 mouth, gastric, small intestinal, and large intestinal digestive phases (Liao and Yang, 2019). The
249 digestion was performed at 37°C on glass tubes for each compartment independently with adapted
250 juices composition, digestion time and pH. The large intestine phase lasting 18 hours only included
251 some enzymes in its juice, but no microbiota was implemented. Bioaccessibility was then determined
252 as the percentage of bioaccessible Cr content in digested MPs to the total Cr content of the Cr-loaded
253 MPs. Cr adsorbed on various MP types (PE, PP, PVC, PS and PLA, 150 µm) was more bioaccessible
254 in the gastric environment than in the small or large intestinal phases, due to acidic conditions
255 favoring desorption of anionic Cr species (e.g. CrO_4^{2-} and $HCrO_4^-$) from the MP surface, while no
256 release was observed in the oral phase. Differences were noted between Cr(VI) and Cr(III),
257 characterized by a higher bioaccessibility in the gastric phase for Cr(VI). Moreover, PLA exhibited
258 the highest oral bioaccessibility of Cr(VI) and Cr(III) in comparison to other polymer types, probably
259 due to its degradation enhanced by the action of enzymes present in simulated digestive juices (Liao
260 and Yang, 2019).

261 *3.1.3. Physico-chemical transformations of MPs during in vitro human digestion*

To support the aforementioned differences in desorption of heavy metals, and more generally chemical contaminants, during *in vitro* human digestion, it is important to unravel the physico-chemical transformations of MPs themselves (size, shape, surface properties, formation of a biomolecular corona etc.). The study of Stock and colleagues revealed no striking alteration of the physico-chemical characteristics of five types of MPs (PE, PP, PVC, PET and PS) by artificial digestive juices, mimicking the saliva, gastric, and intestinal phases of human digestion (Stock et al., 2019a). All digestion steps were successive in the same vessel. MPs were digested during 5 min at 37°C in presence of synthetic saliva, containing α-amylase, mucin and various electrolytes, under agitation at pH 6.7. Gastric juice, composed of pepsin, mucin, electrolytes and hydrochloride acid, was then added, and the pH adjusted at 2 for the gastric phase for 2 hours. Finally, the intestinal digestion began by addition of artificial intestinal juice, containing trypsin, pancreatin, bile extract and electrolytes, with pH value set at 7.5 for 2 hours. The same authors emphasized the importance to consider corona formation onto MPs surface due to the adsorption of organic compounds such as proteins, mucins and lipids during digestion process (Fig. 1). Owing to its biological impact (Monopoli et al., 2012), the characteristics of the biomolecular corona formed around the MPs during their transit in the digestive tract should be more deeply studied to determine the behavior of the MPs in the gut, and particularly their uptake rate.

3.2. Pathogenic bacteria and antibiotics on MPs, potential role in spread of antimicrobial resistance in the gut

Microorganisms, including bacteria, are able to aggregate and adhere to biological or non-biological surfaces, in complex communities commonly referred to as biofilms (Lopez et al., 2010). Common environmental surfaces for biofilm formation include rocks, weeds and woods, but also plastic particles as they offer a suitable support for the formation of biofilm communities. In contrast to other floatable materials, like wood and weeds, plastic particles provide a long-lasting surface preserving their inhabitants from influence of environmental variations (De Tender et al., 2017;

287 Lobelle and Cunliffe, 2011). Accordingly, in recent years, marine plastic particles have been
288 reported to harbor diverse microbial species associated in dense multi-species biofilm structures
289 (Kirstein et al., 2016; Zettler et al., 2013) (Fig. 2). The microbial inhabitants associated with MPs
290 include potentially pathogenic bacterial species, like *Pseudomonas* spp., *Vibrio* spp., *Campylobacter*
291 spp. and *Escherichia coli* (Bryant et al., 2016; Curren and Leong, 2019; Kirstein et al., 2016;
292 McCormick et al., 2014; Wu et al., 2019a; Zettler et al., 2013). These observations have led to the
293 hypothesis that MPs colonized by enteric pathogens may constitute a novel vector for transmission of
294 infectious diseases, possibly indirectly through contamination of food and drinks, although the dose
295 transmitted by this route would most likely not reach infectious levels. A more concerning aspect is
296 the association of antibiotics on MPs as mentioned in the above section. For some antibiotics,
297 concentrations selecting for resistance have been reported. Specifically, levofloxacin
298 (fluoroquinolone) can be adsorbed in concentrations up to 1.2 mg/g (Yu et al., 2020) onto PVC,
299 whereas ciprofloxacin (fluoroquinolone), amoxicillin (penicillin), and tetracycline can be adsorbed to
300 polyamide (PA) in concentrations ranging from 1 to 3 mg/g (Li et al., 2018). Finally,
301 sulfamethoxazole was found to be strongly adsorbed onto PA and PE MPs in concentrations of 0.4
302 and 0.1 mg/g, respectively (Guo et al., 2019; Razanajatovo et al., 2018). These results point out that
303 some of the most common MP particles are not only vectors for potential pathogenic microbes, but
304 also favour hitch-hiking of microbes that are resistant to antimicrobials. In support of this, recent
305 studies have shown a significantly higher prevalence of antimicrobial resistance genes in
306 microbiomes isolated from MPs than from seawater (Wang et al., 2020; Xue et al., 2020; Yu et al.,
307 2020) whereas other studies have highlighted the ability of MPs to act as habitats for increased gene
308 exchange. Thus, high concentrations antibiotics and bacteria on MP surfaces could drive the spread
309 and sharing of antimicrobial resistance genes (Arias-Andres et al., 2018; Wang et al., 2020; Xiang et
310 al., 2019; Yang et al., 2019). Accordingly, apart from enhancing the development of antibiotic
311 resistant bacteria, ingestion of MPs may facilitate transfer of antimicrobial resistance genes to the

312 intestinal microbiome. However, to our knowledge, no study has been performed so far to unravel
313 the consequences of oral exposure to pathogen- and/or antibiotic resistance gene-contaminated MPs
314 during *in vitro* human digestion or *in vivo* in mammals.
315
316 **4. Impact of MPs in the digestive tract: lessons from mammalian model organism**
317 **4.1. Uptake and tissue accumulation**
318 As a first site for MPs after ingestion, the gastro-intestinal tract (GIT) is of utmost importance
319 as the gatekeeper for their bioavailability. The first studies investigating the fate of MPs in the GIT
320 upon ingestion in mammals (rats and mice) have used (fluorescent) PS beads, usually 2 µm diameter
321 size, as they are convenient for detection through diverse microscopy techniques, such as scanning
322 electron microscopy, transmission electron microscopy or fluorescence/confocal microscopy (Carr et
323 al., 2012). After administration to rats, it was demonstrated that MP uptake is a fast process, as
324 particle absorption in intestinal tissue already took place after 5 min of ingestion, and significantly
325 higher amounts of MPs were found in the epithelia of the proximal part of the small intestine
326 compared to the distal one (Carr et al., 2012). Accumulation of these MPs was visible at the level of
327 the microvilli of enterocytes, in the intercellular spaces, in the epithelial-stromal boundary and in the
328 blood vessels. Interestingly, accumulation in Peyer's patches was much lower. Nevertheless, 5 weeks
329 after a single-dose ingestion, the few MPs that were monitored in this region persisted while at the
330 level of enterocytes, MPs had disappeared. In mice, Stock and co-workers reported that only few
331 MPs were detected by fluorescence microscopy in the intestinal walls of animals after oral exposure
332 three times per week for 28 days by oral gavage with a mixture of 1 µm, 4 µm and 10 µm PS MPs,
333 corresponding to an administered dose of 1.25 mg/kg bw, 25 mg/kg bw, and 34 mg/kg bw,
334 respectively (Stock et al., 2019b). No histological lesion nor inflammatory response was observed. In
335 contrast, in mice fed 600 µg/day (corresponding to 30 µg/kg bw/day for a mouse weight of
336 approximately 20 g) of PE MPs (10–150 µm) for 5 consecutive weeks, the histological scores of

337 colon and duodenum were significantly higher than the ones determined for non-treated animals (Li
338 et al., 2020a). In mammals, it is thus not clear whether MP exposure leads to gut inflammation.
339 Results depicted yet are highly dependent on treatment conditions (duration, doses) but also on the
340 polymer type and size of MPs and potentially on their shape and biomolecular corona. In addition,
341 doses administered *in vivo* have to be compared to the human daily intake between 0.2 and 10 mg/kg
342 bw/day (considering an adult human body weight of 70 kg), derived from the values recently
343 reported by Senathirajah and colleagues (Senathirajah et al., 2021), even though it is clearly a first
344 estimation of the amount of potentially ingested MPs, which will serve as a basis for future
345 investigations in humans.

346 There are different routes of MP uptake in the GIT due to their size range, as reviewed by
347 EFSA (EFSA, 2016). Cells involved in microparticle (and also nanoparticle) transport are notably
348 specialized M-cells overlying Peyer's patches, which belong to the mucosa-associated lymphoid
349 tissues, and transport large structures, amongst which antigens, bacteria and viruses, to the immune
350 system. MP particles of less than 10 µm could be trapped into the intestine by M-cells according to
351 the adherence on the mucus, high adherence to the loosely adherent mucus layer leading to a quick
352 clearance and fewer absorption time (Ensign et al., 2012). A marked decrease in uptake efficiency by
353 M-cells of particles between 200 and 500 nm diameter was reported compared to smaller sizes
354 (Powell et al., 2010). Generally, small-sized particles seem to better translocate across the gut than
355 larger ones (Wright and Kelly, 2017). For MPs between 5 and 110 µm diameter, persorption, a
356 paracellular transport, especially in desquamation zones and between the villi, appears to be the most
357 important uptake mechanism (Wright and Kelly, 2017). Not only the size, but also the
358 hydrophobicity, surface functionalization and charge, and biomolecular corona, influence MP uptake
359 ability. The role of mucus as a first barrier, with size- and charge-exclusion properties, is also of
360 uppermost importance, as reviewed by Gillois and colleagues (Gillois et al., 2018). Mucus may trap
361 MPs, thereby decreasing their toxicity to the host. However, it is possible that MPs more easily cross

362 the mucus layer because of their physico-chemical transformations after contact with the intestinal
363 content, as reported for dietary microparticles (Powell et al., 2007). Conversely, MPs may affect
364 mucus characteristics all along the GIT. Recent studies in mice highlighted the impact of MPs on
365 mucus homeostasis pathways (Fig. 1). After exposure to 0.5, 5 and 50 µm PS MPs at 100 µg/L and
366 1000 µg/L for 6 weeks, mucus secretion in the colon was significantly decreased as shown by Alcian
367 blue-periodic acid Schiff staining, which was supported by the down regulation of genes involved in
368 mucus synthesis/secretion pathways (*Muc2*, *Muc1*, *Retnlb* and *Klf4*) in the colon (Jin et al., 2019; Lu
369 et al., 2018). This dysregulation of the protective mucus barrier function, probably acting in concert
370 with an impaired epithelial barrier and a gut microbiota dysbiosis (see section 4.2), was already
371 depicted for pesticides; persistent organic pollutants and food additives (Gillois et al., 2018), even
372 though the underlying molecular mechanisms remain to be fully unveiled.

373 Most studies highlight MP presence in the gut and other tissues of many species, including
374 rodents, in a MP size-dependent manner (Lu et al., 2019). In rats, 6% of MPs (PS, 870 nm) were
375 found in the circulation 15 min after oral administration (Eyles et al., 1995) while nano-sized
376 particles (PS, 50 nm) led to 34 % of absorption, with a main accumulation in the liver (Jani et al.,
377 1990). Following a 4-week exposure period, 20 µm and 5 µm PS MPs accumulated in the gut, liver
378 and kidney of mice in a MP size-dependent localization. The biggest particles were consistently
379 distributed in the three tissues while the smallest ones accumulated more in the gut of exposed mice
380 (Deng et al., 2017). However, the relevance of this work has been recently discussed by others
381 (Braeuning, 2019), notably due to the fact that the quantity of particles detected in the organs
382 massively exceeded the quantity of particles administered during the study.

383 **4.2. Bidirectional relationship with gut microbiota**

384 The intestinal microbiota, i.e. the huge community of microorganisms present in the GIT, raises
385 an increasing interest the past few years, with a recent focus on the potential impact of MPs on this
386 ecosystem (Fig. 1) (Fackelmann and Sommer, 2019). To date, no study has been carried out on the

387 human gut microbiota. However, studies already conducted on the cecal or fecal contents of mice
388 orally exposed to MPs revealed microbiota composition modifications (Jin et al., 2019; Lu et al.,
389 2018). Mice exposed to PS MPs (0.5 µm and 50 µm) in drinking water at two different
390 concentrations (100 µg/L or 1000 µg/L) during 5 weeks exhibited microbiota alterations. Impaired α
391 and β-diversities and a significant reduction in the abundance of Firmicutes and α, β and γ-
392 Proteobacteria were reported in the cecal content of treated mice in all conditions using a PCR-based
393 quantification assay. The Actinobacteria abundance was reduced only after an exposure to 1000 µg/L
394 of PS MPs (Jin et al., 2019; Lu et al., 2018). Analysis of 16S rRNA sequencing of cecal content
395 demonstrated a decrease in the relative abundance of Firmicutes, Bacteroidetes and Verrucomicrobia
396 at the highest dose for both MP sizes (Lu et al., 2018) and of Actinobacteria after exposure to 5 µm
397 PS MPs at a concentration of 1000 µg/L (Jin et al., 2019) while Proteobacteria proportion increased
398 (Jin et al., 2019; Lu et al., 2018). In addition, at the genus level, 15 types of bacteria were
399 significantly affected; in particular, *Bifidobacterium*, *Prevotella* and *Veillonella* decreased while
400 *Coprococcus* and *Anaeroplasma* increased in the cecal content following exposure with 1000 µg/L
401 of PS MPs (Jin et al., 2019). After exposure to 6, 60, and 600 µg/day of PE MPs present in the diet
402 during 5 weeks in mice, an increase in gut microbial abundance and diversity from fecal samples was
403 reported by 16S metagenomics data. Treated-mice showed a higher abundance of *Staphylococcus* as
404 well as a reduction in *Parabacteroides* abundance compared to untreated animals (Li et al., 2020a).
405 Finally, co-exposure of PE MPs (45–53 µm) bound to di-(2-ethylhexyl) phthalate (DEHP) during 30
406 days led to gut microbiota composition modification in mice, characterized by a lower β-diversity
407 and an increase in the relative abundance of Actinobacteria in the fecal content (Deng et al., 2020).
408 Such effect was stronger for the di-(2-ethylhexyl) phthalate -contaminated MPs than for the virgin
409 MPs.

410 Concerning the bidirectional relationship between MPs and gut microbiota, little is known about
411 the microbial degradation ability of MPs in mammals. However, some microorganisms from the gut

412 microbiota of soil waxworms are able to degrade MPs. The gut of the larvae *Plodia interpunctella*
413 contains two bacterial strains, *Enterobacter asburiae YT1* and *Bacillus sp. YP1*, able to degrade PE.
414 Indeed, researchers observed the formation of pits and cavities on the PE surface and addition of
415 carbonyl groups (Yang et al., 2014). Bacteria extracted from the gut of *Lumbricus terrestris*,
416 members of the phyla Actinobacteria (*Microbacterium awajiense*, *Rhodococcus jostii*,
417 *Mycobacterium vanbaalenii* and *Streptomyces fulvissimus*) and Firmicutes (*Bacillus simplex* and
418 *Bacillus spp*), were able to reduce the size of low density PE MPs (from 150 µm to an average of
419 53.1–41.3 µm) in a gamma sterilized soil culture after 21 days (Huerta Lwanga et al., 2018).
420 According to the huge and diverse microbial communities in the human gut, it seems plausible
421 that some members could harbour this degradation ability. Indeed, after comparison between bacteria
422 identified as able to degrade MPs (Jacquin et al., 2019) and bacteria found in the human gut
423 microbiota, according to the database of Plaza Oñate and his team (Plaza Oñate et al., 2019), bacteria
424 belonging to Proteobacteria, Actinobacteria, Bacteroidetes and Firmicutes phyla were present in
425 both; however, to date, no rigorous study has been carried out on the possible plastic degradation
426 metabolism by the human gut microbiome.
427 As presented above, the few existing studies focused on the bacterial fraction but none
428 investigated the impact of MP exposure on the composition and activity of the fungal population
429 ("mycobiome") in the gut (Sánchez, 2019), which is increasingly recognised as a key player in
430 intestinal health and disease (Richard and Sokol, 2019). Furthermore, a limitation for many of
431 studies reported for gut bacteria is that they mostly rely on 16S rRNA gene sequence analysis, which
432 does not allow species level resolution. To fully resolve microbial issues, including the prevalence of
433 pathogenic bacteria attached to MPs and the risks associated with this (see section 3.2), there is a
434 need to go beyond community profiling by employing high-resolution molecular analysis of species
435 genotypes in combination with cultivation dependent phenotypic characterizations.
436

5. *In vitro* gut models: current uses and new developments for addressing the fate and toxicity of MPs

Research on the fate, transformation and effects of MPs upon human intake remains limited, in part due to challenges of system complexity, analytical methods and varied forms of ingested plastic particles (size, shape, polymer type). Studies in humans, mainly through clinical studies, would obviously be the ideal strategy but remain also hampered by ethical, regulatory and cost reasons. *In vivo* studies in mammals cannot address to all the questions raised, due to major gut characteristics differences depending on species (Hugenholtz and de Vos, 2018). Furthermore, animal experiments are acknowledged by the 3R principle rules which widely encourage the development of alternative *in vitro* approaches to reduce the number of animals used in research. Then, *in vitro* models of the digestive tract are of uppermost importance for human gut research. A wide range of *in vitro* gut models has been already developed, including cellular cultures and digestion systems, from simple static mono-compartmental models to the most complex dynamic multi-compartmental ones. All those models, and their coupling, have a huge potential for toxicological investigations related to MPs, and current developments and achievements are detailed below. Future directions are also proposed.

5.1. *In vitro* upper gut models

Complementarily to the studies conducted by Liao and Yang (Liao and Yang, 2019) and by Stock and colleagues (Stock et al., 2019a) (see sections 3.1.2 and 3.1.3), Tan and co-workers recently conducted an *in vitro* experiment in a gastrointestinal system reproducing the main phases of the mouth, stomach and small intestinal human digestion (Table 1). Briefly, lipid-MP emulsion was incubated during 10 min at pH 6.8 at 37°C in simulated saliva. Then, the oral digesta was mixed with simulated gastric fluid at pH 2.5 for 2 hours. Finally, the gastric digesta was added to simulated intestinal fluids for 2 hours at pH 7. Five MP types (PE, PET, PVC, PLGA and PS) were tested and showed an inhibitory effect on lipid digestion, especially for PS, due to an interaction of these

462 hydrophobic particles with both lipid droplets and lipases. Laser Scanning Confocal Microscopical
463 Imaging and Nile Red staining were used to identify the PS MPs, the lipid droplets and the digested
464 lipid. The formation of such heteroaggregates decreased the bioavailability of lipids droplets by
465 reducing the lipid availability to be digested by lipases but also reduced the activity of lipases by
466 changing their secondary structure (Tan et al., 2020). Despite these first steps forward, the systems
467 currently used remain simplified, operating under static conditions and are only composed of
468 different gut-simulating vessels with the corresponding digestive juices adjusted for pH and
469 temperature. Some important parameters are absent, such as dynamic digestion processes (i.e. transit
470 between successive digestive compartments or time-effect on gastric and intestinal pH and digestive
471 secretion concentrations and flow rates), the presence of compounds from food matrices, the
472 influence of gut microbiota (mainly in the large intestine, but also in the small intestine) (see section
473 5.2), and lastly interactions with intestinal epithelial, immune cells and/or mucus (see section 5.4).
474 More physiologically relevant *in vitro* gut models could be highly valuable for a better understanding
475 of the fate and effects of MPs in the human digestive environment. The main characteristics of these
476 models, including advantages but also potential limitations, are given in Table 2. In particular, the
477 physico-chemical transformations of MPs (e.g. formation of biomolecular corona, particle
478 degradation), as well as the potential desorption of particle-adsorbed pollutants in the upper part of
479 the digestive tract, could be investigated by using the complex *in vitro* TNO gastrointestinal system 1
480 (TIM-1). This model is currently the most complete simulator of the upper human GIT reproducing
481 the main physico-chemical parameters of the stomach and small intestine, i.e. body temperature,
482 kinetics of gastric and intestinal pHs, transit time, gastric, pancreatic and biliary digestive secretions
483 and passive absorption of nutrients and water (Cordonnier et al., 2015). Compared to static model,
484 TIM-1 also enables a better evaluation of bioaccessibility, closer from *in vivo* situation. Absorption
485 of small molecules (such as particle-associated contaminants) is modelled in TIM-1 *via* the use of
486 circulating dialysis fluid through hollow fibers. Moreover, TIM-1 can be used to investigate the

487 influence of food matrices (including lipid-rich matrices) on MP physico-chemical transformations,
488 degradation and/or bioaccessibility of associated contaminants (Helbig et al., 2013; Larsson et al.,
489 2016; Minekus et al., 2014; Miszczycha et al., 2014). Different results related to the bioaccessibility
490 of some heavy metals, previously obtained in a simple static model compared to the dynamic TIM-1
491 (Torres-Escribano et al., 2011), clearly underlines the importance to investigate the behavior of MPs,
492 in their virgin *versus* contaminated (pollutants, heavy metals, antibiotics, pathogens etc.) forms, in
493 the human gut through more complex *in vitro* systems.

494 **5.2. *In vitro* lower gut models – interactions with gut microbiota**

495 No study on the fate of MPs in the lower GIT, and in particular their potential bidirectional
496 interactions with a gut microbiota from human origin, has been reported so far using *in vitro* models
497 of the small intestine and colon (Table 2). As already shown for pollutant bioaccessibility (Reygner
498 et al., 2016), effect of antibiotics on gut microbiota (El Hage et al., 2019; Ichim et al., 2018;
499 Marzorati et al., 2020), pathogen survival and virulence (Roussel et al., 2020), and transfer of
500 resistance genes to human gut microbiota (Lambrecht et al., 2019), these studies, including the role
501 of mucus as an ecological niche for the gut microbiota, could be performed in a mono-
502 compartmental model simulating the mean physico-chemical and microbial parameters of the human
503 colon, such as the Mucus Artificial Colon model (M-ARCOL) (Deschamps et al., 2020) or in a more
504 complex system simulating the entire GIT (stomach, small intestine and three parts of the colon), the
505 Mucosal Simulator of the Human Intestinal Microbial Ecosystem (M-SHIME) (Lambrecht et al.,
506 2019). Impact of MPs and associated-contaminants on human gut microbiota can be monitored both
507 at the level of microbiota composition and metabolic activity (e.g. production of short chain fatty
508 acids, gases). Inversely, it is possible to investigate the gut microbiota capacity to degrade and
509 metabolize MPs, in particular specific microbial metabolic pathways activated by these particles.
510 Interestingly, inter-individual variabilities in gut microbiota structure and function can be envisioned
511 *in vitro* by inoculating the models with fecal samples issued from different donors. Of interest, all the

512 aforementioned *in vitro* models of the upper and lower GIT have already been adapted to simulate
513 the digestive conditions found in different aged populations, such as infant, adult or elderly people
514 (De Boever et al., 2001; Denis et al., 2016; Roussel et al., 2016; Van den Abbeele et al., 2019). This
515 could allow to decipher if the fate and effects of MPs would be affected in specific populations
516 compared to adults.

517 **5.3. Models for uptake, fate and effects of MPs on the intestinal barrier**

518 Complementarily to *in vitro* models of the upper and lower GIT, cell models, like Caco-2 cell
519 monolayers, are useful to investigate uptake, fate and effects of MPs on the gut barrier (Table 1). The
520 majority of studies reported so far are focused on virgin particles without taking into account the
521 impact of the digestive processes and/or the influence of chemical or microbial contaminants. The
522 Caco-2 cell line was originally isolated from a human adenocarcinoma, and spontaneously
523 differentiates upon confluence to a monolayer of polarized enterocytes, expressing microvilli,
524 transporters and brush border enzymes. Exposure to MPs was shown to disturb the intestinal
525 epithelial barrier, as measured by a decreased transepithelial electrical resistance (TEER), and
526 transport of MPs through the Caco-2 cell model was regulated by tight and adherent junctions (Carr
527 et al., 2012). Wu and colleagues showed an up-regulation of the cell inflammatory pathways, such as
528 NF-κB and MAPK signalling, and a downregulation of proliferation pathways in a concentration-
529 (from 10^{-8} to 10^{-1} mg/mL) and time- (24 and 48 hours) dependent manner on Caco-2 cells (Wu et al.,
530 2019b). In addition, on the same cell line, increasing the MP exposure concentration (12.5, 25, 50
531 mg/L) and duration (24 and 48 hours) led to an increased cytotoxicity. Limitations of the Caco-2 cell
532 model are the lack of a mucus barrier and the simulation of a single cell type. Hesler *et al.* (2019)
533 studied the impact of carboxy-modified PS MPs (500 nm), as well as smaller-sized particles (50 nm)
534 on a coculture of the Caco-2 cell line with mucus-producing HT29-MTX-E12 cells, but found no
535 significant cytotoxicity unless applied at very high concentrations, thereby pointing out a possible
536 protective effect of the mucus layer (Hesler et al., 2019). Des Rieux and co-workers were one of the

first research groups studying particle uptake in an inverted combined Caco-2/Raji-B model (des Rieux et al., 2007). They demonstrated that the uptake of 200 nm carboxylated PS particles was significantly increased in the co-culture and inverted model compared to the control Caco-2 monoculture or the non-inverted model through a non-specific absorption endocytosis. Interestingly, in another study where larger PS MPs (2 µm) were applied to Caco-2 and Caco-2/Raji-B models, no difference in absorption was observed, thereby highlighting the importance of microplastic size in the assessment of bioavailability and toxicity (Carr et al., 2012). Monocytic THP-1 cells were also used in combination with Caco-2 cells for the study of MP toxicity (Carr et al., 2012), and the intestinal barrier function was found to decrease upon co-culture (Carr et al., 2012). Considering Caco-2/Raji-B (M-cell model) and Caco-2/HT29-MTX (mucus model) co-cultures *versus* Caco-2 monoculture exposed to PS MPs of three different sizes (1, 4 and 10 µm), a significantly higher uptake rate was observed for both the 1 µm and 4 µm particles in the co-cultures compared to the monoculture (no significant differences with the 10 µm particles). Furthermore, no differences were depicted between the M-cell and the mucus models for the 4 µm MPs whereas for the 1 µm MPs a higher uptake was found in the M-cell model compared to the other co-culture (Stock et al., 2019b). A three-dimensional *in vitro* intestinal cell model, composed with a mixture of human intestinal epithelial Caco-2 and HT29-MTX cells coupled to human blood monocyte-derived macrophages and dendritic cells, was designed to evaluate MP cytotoxicity (PP and PA, 50-500 µm) after 6, 24 and 48 hours of an aerosolized exposure to a concentration of 823.5–1380.0 µg/cm^2 (Lehner et al., 2020). Interestingly, MPs were aerosolized directly onto the cells in order to avoid floating of those hydrophobic particles in the cell culture medium, thus ensuring a better control of particles interacting with cells. Contrary to previous studies, no significant toxicity effects were reported in terms of inflammatory response and barrier integrity disruption. Technical improvements were also introduced by Stock and colleagues to counteract the problem of floating particles in the cellular culture system by developing an inverse cell culture model, which was successfully tested on liver

562 HepG2 cells (Stock et al., 2019c). MP impact on gut cells thus highly depends on MP characteristics
563 (size, polymer type), concentration applied and duration of exposure. In addition, cell line used, and
564 experimental design are important to consider when investigating MP uptake and impact on the gut
565 in regard to the discrepancies of results previously described.

566 Overall, these cell models have shown merit in increasing knowledge on the mode-of-action of
567 MP uptake and cellular responses. Nevertheless, the current models still suffer from limitations.
568 First, almost all described cell cultures are from cancer origin, and therefore, effects on cell
569 signalisation, immune responses, oxidative stress and toxicity need to be carefully interpreted as
570 cancer cells show distinct differences in basal metabolism and reactive oxygen species (ROS) levels
571 for instance. Furthermore, the Caco-2 cell line is a model for small intestinal enterocytes, and
572 therefore doesn't take into account the spatial differences of the intestinal mucosal morphology and
573 functioning depending on the location to the GIT, which may be of importance such as seen *in vivo*
574 (Carr et al., 2012). In addition to this, colonic models – despite the longer residence time compared
575 to small intestine – were, as far as we know, not applied yet for MP research.

576 **5.4. Coupling *in vitro* gut models with cell cultures**

577 To date, the very few existing studies on MPs have only considered simple systems. In particular,
578 Liu and colleagues used a dynamic three-stage *in vitro* digestion system mimicking the mouth,
579 stomach and small intestinal human conditions to investigate the differences between pristine and
580 digested MPs (PS, 5 µm *versus* 100 nm) on Caco-2 cells (Liu et al., 2020b) (Table 1). Each
581 simulated digestive juice was reproduced by mixing salt solutions, organic compounds, enzymes and
582 proteins. The pH was set at 6.8 for the saliva, 2.5 for the gastric compartment and 6.5 for the
583 simulated small intestine. During the oral phase, the salivary digestion was reproduced as well as a
584 mastication process *via* vibration for 5 minutes. The digestive treatment induced an increase in the
585 average size of the particles and of their zeta potential, thus favoring particle agglomeration. Caco-2
586 cells were exposed either to the digested MPs or to pristine ones. For undigested particles, alterations

587 of transport function were observed with an increased epithelium permeability reported through the
588 para-cellular marker Lucifer Yellow (LY) and a downregulation of gene expression associated with
589 the intestinal barrier functions (*zonula occludens* ZO-1 and occludin). In addition, alteration of
590 transmembrane transport through the intestinal epithelium was also shown by a down-regulation of
591 *ABCC2* and *ABCG2* gene expression. However, for digested MPs, a reduction in the cytotoxicity and
592 impairment of the transport function was obtained. On the contrary, an increase in the pro-
593 inflammatory effects was observed but only for digested small-sized particles (100 nm) compared to
594 pristine ones by cytokine release assay targeting interleukin-8 (IL-8) and monocyte chemoattractant
595 protein-1 (MCP1). Those differences between digested and pristine particles could be attributed to
596 the formation of a biomolecular corona (Liu et al., 2020b).

597 Further studies on interaction of MPs with the intestinal barrier could be performed by a more
598 complex coupling between *in vitro* gut models and cell cultures (Table 2), as previously done with
599 arsenic in the SHIME system and subsequent exposure of food digests to Caco-2/HT29-MTX cells
600 (Calatayud et al., 2018), or even more advanced cell models including immune cells. As a next step,
601 a relevant approach could be a continuous input of the digestive medium from *in vitro* gut models on
602 cell cultures, as done with the Host-Microbiota Interaction (HMI^{TM}) module, primarily adapted to be
603 coupled to the SHIME model (Marzorati et al., 2014). Of interest, this HMI module integrates Caco-
604 2 cells combined with a mucus layer, maintained under relevant shear forces and microaerophilic
605 conditions that can impact both commensal microbes and pathogens (De Weirdt and Van de Wiele,
606 2015; Marzorati et al., 2011). Other more 3D complex models could be used in the future (Table 2),
607 such as multicellular models which are currently developed for MPs (Lehner et al., 2018, 2020) or
608 intestinal organoids (Kim et al., 2020), not explored yet. Likewise, bioengineered human gut-on-chip
609 devices such as HuMiX (Shah et al., 2016), Intestine-Chip (Kim et al., 2015) and Colon-Chip
610 systems (Sontheimer-Phelps et al., 2020) would enable to take into account the influence of
611 mechanical forces (peristalsis, intestinal flow), and ultimately commensal microbes and mucus, on

612 MP fate and toxicity in the gut. All these combined models mimicking human intestinal physiology
613 *in vitro* are undoubtedly highly helpful to unravel fate, physico-chemical transformations,
614 degradation, metabolism and toxicity-related mechanisms, which would be useful for future hazard
615 and risk assessment in the context of MPs.
616
617 **6. Conclusions**
618 This review reports the different sources of oral exposure to MPs and focuses on the
619 gastrointestinal route and the potential adverse effects on human intestinal health, with a special
620 emphasis on the relevance of *in vitro* gut models for an in-depth study of associated mechanisms. In
621 all environments, the presence of MPs increases drastically, and to tackle this critical issue shared
622 science, policy, economy and society, governments are increasingly implementing regulatory laws
623 related to plastic pollution. In the European Union (EU), the European Strategy for Plastics in a
624 Circular Economy (European Commission 2018a) stressed the need to tackle the challenges related
625 to plastics, which have been identified as one of the five priority areas addressed by the EU Action
626 Plan for the Circular Economy (European Commission 2015). In such context, the current COVID-
627 19 sanitary crisis is forcing to rethink plastic concern worldwide. With the pandemic contributing to
628 increased plastic use in healthcare, and large volumes of waste being unfit for recycling due to
629 potential biohazards, medical plastic waste could grow at an unprecedented scale. A similar situation
630 might arise in the food industry and packaging, textiles and other services that have decided to
631 temporally limit reusable. Recently, the use of disposable masks has been added to the list of MP
632 sources (Aragaw, 2020). All these practices will contribute to the increasing plastic pollution and MP
633 issues (Patrício Silva et al., 2021),
634 More and more studies on human health are regularly published but due to the lack of
635 standardized detection and quantification methods for complex matrices and the self-contamination
636 during sampling and experimentation due to plastic devices, results are often hardly comparable. An

637 adaptation of the experimental set-ups, consumables and practices seems crucial to provide firm
638 assessments on the potential health impact of MPs. Up to now, studies have shown that oral intake is
639 a major route of human exposure. Drinking water, especially from plastic bottles, and seafood are
640 among the greatest sources identified to date even though MP contamination is increasingly reported
641 in many other food items, including milk, white wines, fruits and vegetables. Increasing attention is
642 then paid to the GIT as the first barrier but also portal of entry and target for MPs. Complementarily
643 to the action of virgin particles, the effects of adsorbed contaminants, including but not restricted to
644 chemical pollutants, have to be considered in view of the vector effect hypothesis. In particular, MPs
645 harbor a unique microbiome, which is shaped by polymer type and environmental factors. MPs then
646 provide a stable support for widespread and specific microbial hitchhiking. Furthermore, the findings
647 of several human pathogenic microbial species associated with MPs represent a potential risk for
648 human exposure to these pathogens, either directly through ingestion of MPs or indirectly by cross-
649 contamination of food or water. In addition, ingestion of MPs may enhance the release of antibiotics
650 and the development of antibiotic-resistant bacteria in the human gut, act as vehicle for transfer of
651 antimicrobial resistance genes to the intestinal microbiome and favour human infection related to
652 resistant bacteria. From the physico-chemical point of view, particle weathering is also a critical,
653 albeit poorly explored, player in the field; it leads to strong modifications of MPs due to oxidation,
654 fragmentation and degradation and surface coverage by a complex mixture of organic and inorganic
655 materials, thus leading to different sorption and toxicity profiles.

656 Current studies on the impact of MPs on intestinal health are mainly based on rodents (mice) and
657 cellular cultures and physiologically-relevant *in vitro* models of the human gut, allowing to study the
658 influence of digestive physico-chemical processes (pH, digestive secretions), mechanical forces
659 (peristalsis, intestinal flow), as well as mucus and gut commensals in a regionalized manner, are
660 needed to better evaluate the impact of such exposure on disruption of the human gut homeostasis.
661 As endpoints of adverse effects, oxidative stress, genotoxicity, mitochondrial function and alterations

in gene expression linked to inflammatory process and oxidative stress, as well as cross-talks with immune system and other organs such as liver and brain should be further studied (Yong et al., 2020). Due to its growing role in health and disease (Mathieu et al., 2018), the gut-lung axis could also be simulated.

In conclusion, as the adverse effects on the gut may strongly depend on the shape, size and surface properties of MPs, more representative particles, including weathered and/or contaminant-associated ones, than pristine PS microbeads as currently used, as well as dedicated analytical methods should be investigated in the future. Based on the first estimations of the amount of MPs potentially ingested by humans, more realistic exposure scenarios (i.e. relevant MP doses rather than overload conditions, chronic long-term exposure) should also be considered for better health hazard and risk assessment. Reliable *in vitro* gut models could then be of highly relevance to mimic such exposure scenarios taking also into account the consequences on at-risk populations (e.g. children, elderly) and ultimately pathophysiological conditions (e. g. obese patients, Inflammatory Bowel Disease patients).

677 **Figures legends**

678

679 **Fig 1. MPs and the human gastrointestinal tract: an overview**

680 Humans are primarily exposed to MPs by the gastrointestinal tract through the air inhaled, the food
681 and the water consumed. After ingestion, some physico-chemical transformations, with notably the
682 formation of a biomolecular corona, occur triggered by the gut environment; inhibitory effect on
683 lipid digestion may be observed. Disruption of intestinal homeostasis affects gut microbiota, mucus
684 and epithelial barriers.

685 *MPs: microplastics*

686

687 **Fig 2. MPs: vectors for contaminants and microorganisms**

688 MPs, in their pristine and weathered forms, interact with various compounds as chemicals, heavy
689 metals, antibiotics and even microorganisms. MPs act then as vectors that could favour the
690 appearance of antibiotic-resistant bacteria by concentrating antibiotics and bacteria onto their
691 surfaces.

692 *MPs: microplastics; spp: species; PAHs: polycyclic aromatic hydrocarbons; PCBs: polychlorinated*
693 *biphenyls; PFASs: perfluoroalkylated substances*

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Table 1 *In vitro* gut models already used for studies on MPs

Numerous cell models of the intestinal barrier are used, from monocultures to co-cultures of various cell types. Limited simple batch *in vitro* systems reproducing the digestive environment are also utilized.

Models	Simulated compartment	Characteristics	Applications	Results	References
Cellular culture					
Caco-2 cells	Gut epithelium (enterocytes) barrier	Expressed microvilli, transporters and brush border enzymes No mucus layer, only one cell type and no other cell interactions	Epithelium barrier integrity (TEER) and uptake	PS MPs (5.9×10^6 non-ionic yellow/green fluorescent microspheres, 2 µm in diameter) transport regulated by tight and adherent junctions Decrease in TEER Ethanol impact on tight junction integrity and increase in MP transport through Caco-2 cells	Carr *et al*., 2012
			Cytotoxicity	PS MPs (5 µm) Upregulation in the cell inflammatory and cytotoxicity (NF-κB and MAPK signaling) pathways and downregulation of proliferation pathways in a concentration- (from 10^{-8} to 10^{-1} mg/mL) and time- (24 and 48 h) dependent manner	Wu *et al*., 2019b
Co-culture of Caco-2/HT29-MTX cells	Gut epithelium (enterocytes) barrier + mucus layer	Expressed microvilli, transporters and brush border enzymes + mucus layer Very thin mucus layer, only two types of cells	Cytotoxicity	Carboxy modified PS MPs (500 nm) and 50 nm particles: no significant cytotoxicity unless applied at very high concentrations (higher than 50 µg/mL) Protective effect of mucus	Hesler *et al*., 2019

Co-culture Caco-2/HT29-MTX cells coupled to human blood monocyte-derived macrophages and dendritic cells	Gut epithelium (enterocytes) barrier + mucus layer + immune cells	Expressed microvilli, transporters and brush border enzymes + mucus layer + immune cells	Cytotoxicity	PP and PA, 50-500 µm, 6, 24 and 48 h exposure period, concentration of 823.5–1380.0 µg/cm^2 No significant cytotoxicity, inflammatory response (release of IL-8, IL-1β, TNFα) and barrier integrity disruption	Lehner *et al*., 2020
Inverted insert culture system combining Caco-2/Raji-B cells	Gut epithelium (enterocytes) barrier and interaction with immune cells	Expressed microvilli, transporters and brush border enzymes Interaction of enterocytes with B lymphocytes Inserts inverted to make the Caco-2 cell monolayer more accessible to B lymphocytes leading to Caco-2 differentiation in M-cells (15–30% of the Caco-2 cells)	Uptake	Uptake of 200 nm carboxylated PS MPs (4.5 × 10^9 particles/mL) significantly increased in the co-culture and inverted model compared to the control Caco-2 monoculture or the non-inverted model through a non-specific absorption endocytosis	Des Rieux *et al*., 2007
Caco-2/Raji-B cells leading to Caco-2 differentiation in M-cells		Expressed microvilli, transporters and brush border enzymes Interaction of enterocytes with B lymphocytes Raji-B cells leading to Caco-2 differentiation in M-cells	Uptake	No difference in absorption of PS MPs (2 µm) between Caco-2 and Caco-2/M-cell models	Carr *et al*., 2012
Caco-2/HT29-MTX cells (mucus co-culture model) or Caco-2//Raji-B cells (M-cell model)	Gut epithelium (enterocytes) barrier and interaction with mucus or immune cells	Expressed microvilli, transporters and brush border enzymes Interaction of enterocytes with mucus or B lymphocytes	Uptake	Higher uptake rate of PS MPs (1 µm and 4 µm (1 × 10^8/mL)) but not PS MPs (10 µm (3 × 10^6/mL)) with both co-cultures compared to Caco-2 cells only No differences between the M-cell and the mucus models for the 4 µm MPs Higher uptake in the M-cell model compared to the mucus model for 1 µm MPs	Stock *et al*., 2019a
***In vitro* gut models**					

Batch model of upper GIT	3 digestive compartments mouth, stomach and intestine	Only one vessel Simple models easy to use reproducing digestive juices (pepsin, trypsin, pancreatin, bile extract), pH and temperature Batch conditions Lack of digestion dynamics, microbiota in the large intestine, interactions with epithelial cells and/or mucus	Impact of the gastrointestinal passage on the physico-chemical MP characteristics	High stability of all MPs (100 mg/mL (PE 90.1 µm, PET 60 µm, PVC 136.5 µm), 50 mg/mL (PP 67.1 µm) or 10 mg/mL (PS 3.8 µm)) in artificial digestive juices No degradation of MPs Adsorption of organic compounds on MP surface (corona)	Stock *et al.*, 2019b
Whole digestive system *in-vitro* method (WDSM)	Mouth, gastric, small intestinal, and large intestinal digestive phases	Various tubes Transfer of digesta from a tube to another after the digestion step ended		150 µm MPs (PE, PP, PVC, PS, PLA) Highest Cr adsorption capacity for PS Weakest Cr adsorption capacity for PLA Intermediate Cr adsorption capacity for PE, PP and PVC Highest bioavailability of Cr(VI) for PLA No Cr release from MPs in the mouth phase In gastric phase: bioaccessibility of Cr(VI) > Cr(III) but = in the intestinal phases.	Liao and Yang, 2019
Batch model of upper GIT			Impact of MP-lipid co-ingestion on lipid digestion	PE (10 µm), PET (50 µm), PVC (1 µm), PLGA (10 µm) and PS MPs (10 µm versus 1 µm and 50 nm) Inhibitory effect on lipid digestion (high inhibition for PS MPs: size-independent but concentration-dependent) Interaction of MPs with both lipid droplets and lipases (hetero-aggregates) Decrease in the bioaccessibility of lipids droplets and reduced activity of lipases by changes in their secondary structure	Tan *et al.*, 2020

Dynamic and multi-compartmented model of upper GIT	3 digestive compartments mouth, stomach and intestine	3 vessels for mouth, the stomach and the intestine compartments respectively	Influence of the digestive process on intestinal toxicity of MPs	5 µm PS MPs lower intestinal toxicity than 100 nm PS particles Digestive treatment: alleviation of cytotoxicity and transport function disorder of the Caco-2 monolayer induced by the non-digested PS MPs Combined toxicities of PS MPs and arsenic decreased by digestive treatment *in vitro* digestion of 100 nm PS particles: increase in their proinflammatory effects Formation of a corona on the PS-MP surface during digestion: changes in size, Zeta potential, and adsorbed compounds	Liu *et al.,* 2020b

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707 *Cr: chromium; GIT: gastrointestinal tract; IL-1β: interleukin 1β; IL-8: interleukin 8; MAPK:*
708 *mitogen-activated protein kinases MPs: microplastics; NF-κB: nuclear factor-κB; PA: polyamide;*
709 *PE: polyethylene; PET: polyethylene terephthalate; PLA: polylactic acid; PLGA: poly(lactic-co-*
710 *glycolic acid); PP: polypropylene; PS: polystyrene; PVC: polyvinyl chloride; TEER: Transepithelial*
711 *electrical resistance, TNFα: tumor necrosis factor α*

712

713 **Table 2 Potential of *in vitro* gut models for future studies on MPs.**

714 These *in vitro* models include dynamic multi-compartmental models coupled or not to cellular
715 cultures, 3D models like intestinal organoids and gut-on-a-chip microfluidic devices.

716

Model	Simulated compartment	Advantages	Limits	Potential applications for MPs	References
Complex cellular culture models					
3D models	Gut epithelium (culture of Caco-2 cells, HT29-MTX cells, human Raji-B lymphocytes and immune cells)	Under development		Impact of MPs on a complex gut barrier	Lehner *et al.*, 2018
Intestinal organoids	Gut wall (enteroids = derived from adult stem cells isolated from the crypt of small intestine; colonoids = derived from adult stem cells isolated from colonic tissue)	- 3D organization - Cells derived from human biopsies - Personalization (e.g. infant/adult/elderly)	GIT microenvironment not fully taken into account (e.g. pH, anaerobic, intestinal media, physical constraints or microbiota interactions) Difficult access to the lumen Highly expensive, required specialized expertise and lack of standardization	Impact of MPs on a complex gut barrier	Kim *et al.*, 2020
Microfluidic systems					
HuMiX (Human–microbial crosstalk) organ on a ship	Gut epithelium	- Culture of human cell lines (Caco-2) with or without bacteria (trial with *Lactobacillus rhamnosus* GG) - Conditions representative of the human GI–microbe interface (micro-anaerobic environment, oxygen gradient, epithelium absorption)	DMEM medium used to support Caco-2 cell and microbial growth (not representative of intestinal media) Lack of several cellular types characteristic of the gut epithelium (e.g. goblet cells, M-cells) and 3D structure Limited range of flow rates (low) No peristalsis reproduced, no complete mucus layer	Impact of MPs on the gut epithelium and specific species of gut microbiota	Shah *et al.*, 2016

Intestine-chip	Gut epithelium	- 3D structure of the gut epithelium with mechanical forces (peristalsis, intestinal flow) - Co-culture of human cell lines (Caco-2; HT29-MTX, endothelial cells, M-cells) with or without bacteria (trial with *Lactobacillus rhamnosus* GG) - Reproduced passive absorption - Formation of local anoxic microenvironment - Possibility to analyze contributions of individual cellular, chemical, and physical control parameters one-at-a-time	Limited range of flow rates (low) No complete mucus layer No true anaerobic conditions or oxygen gradient Missing muscle and neuronal system cells Possible adsorption of hydrophobic molecules by PDMS matrix	Impact of MPs on the gut epithelium and specific species of gut microbiota	Kim *et al.*, 2015 Bein *et al.*, 2018
Colon Chip	Colon epithelium	Primary human colonic epithelial cells including spontaneously differentiated goblet cells and a complete mucus layer corresponding to that reported in humans	Expensive and required dedicated expertise and instrumentation Stem cell differentiation difficult to achieve No input from immune and nervous system No reproduction of the full complexity of the human gut microbiota	Impact of MPs on the colonic epithelium, the mucus layer	Sontheimer-Phelps *et al.*, 2020
***In vitro* gut models**					
TNO Gastrointestinal model (TIM-1)	Upper GIT (stomach & small intestine)	- Most complex model of the human upper GIT - Main physico-chemical parameters of the stomach and small intestine (temperature, pH kinetics, transit time, digestive secretions and passive absorption) - Adapted to simulate infant, adult or elderly digestive conditions	No interaction with the intestinal epithelial cells, no oral phase, no intestinal microbiota, no mucus, no active absorption	Physico-chemical transformations/ degradation of MPs in the upper GIT Biomolecular corona evolution during the fate of MPs in the upper GIT. Effect of food matrices	Cordonnier *et al.*, 2015 Roussel *et al.*, 2016 Denis *et al.*, 2016
Mucus Artificial Colon model (M-ARCOL)	Lower GIT (colon)	- Main physico-chemical but also microbial (luminal and mucus-associated microbiota) parameters of the human colon - Anaerobiosis maintained by the sole activity of gut microbiota - Possible long-term experiments (chronic exposure)	Average colonic conditions simulated (not the three compartments) No epithelium interaction Expensive and required specific expertise and instrumentation	Bidirectional interactions of MPs with human luminal and mucosal gut microbiota (composition and activity)	Deschamps *et al.*, 2020

Mucosal Simulator of the Human Intestinal Microbial Ecosystem (M-SHIME)	Physico-chemical parameters of the stomach, small intestine and the three parts of the colon and presence of gut microbiota in the colon	- The only *in vitro* model simulating the entire human GIT. Reproduction of the three parts of the colon including gut microbiota and mucus specific microenvironment - Adapted to simulate infant or adult digestive conditions - Possible long-term experiments (chronic exposure) - Possible coupling with cell culture and HMI module)	Upper GIT less complex than in the TIM-1 model No epithelium interaction, continuous flow of N_2 to maintain anaerobic environment Expensive and required specific expertise and instrumentation	Physico-chemical transformations/ degradation of MPs in the entire GIT, biomolecular corona modification and luminal and mucosal microbiota interactions (composition and activity)	Lambrecht *et al*., 2019 De Boever *et al*., 2001 Van den Abbeele *et al*., 2019
Coupling *in vitro* gut models with cellular culture models					
Culture of intestinal cells exposed to supernatant from M-SHIME or M-ARCOL	Gut epithelium	- Easy way for prospective studies on the crosstalk between digested MPs /microbiota/epithelium	No direct interaction of microbiota with the intestinal epithelium No complete intestinal cell wall represented	Impact of digested MPs on the crosstalk between gut microbiota metabolites and epithelium	/
Host-Microbiota Interaction (HMI) module	Gut epithelium and mucus layer (first adapted for the SHIME model)	- Simulation of bacterial adhesion to the gut wall - Reproduction of physiological shear forces and microaerobiosis - Continuous coupling with the M-SHIME	No all the cell types encountered in the intestinal wall (immune cells, M-cells…). Due to cytotoxicity, studies restricted to a 48-h period.	Effects of chronic exposure to digested MPs on the gut epithelium and mucus layer	Marzorati *et al*., 2014

GIT: gastrointestinal tract; HMI: Host-Microbiota Interaction module; HuMiX: human–microbial crosstalk; MPs: microplastics; M-SHIME: Mucosal- Simulator of the Human Intestinal Microbial Ecosystem; PDMS: poly(dimethyl siloxane) polymer; TIM-1: TNO gastrointestinal system 1

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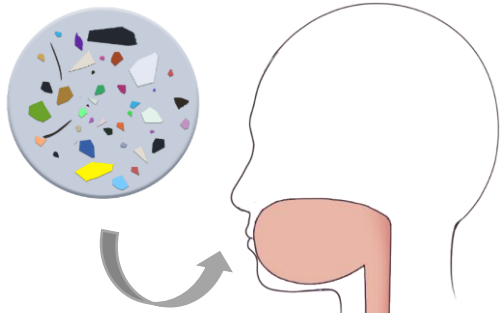
MPs & EXPOSURE SOURCES

Air
From 97 to 170 MPs/day
Drinking water
Bottled water: from 174 to 349 MPs/day
Tap water: from 8 to 16 MPs/day
Food
106 - 142 MPs/day

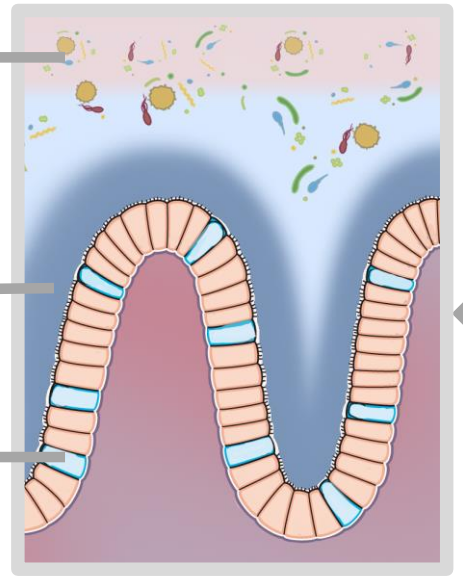


MPs & GUT BARRIER

MICROBIOTA
Gut microbiota dysbiosis

MUCUS
↘ Mucus secretion
↘ Expression of mucus genes

EPITHELIUM
Low absorption
No histological lesion
Inflammatory effect/cytotoxicity
(depending on the studies)



MPs & DIGESTION

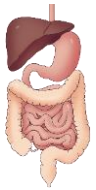
PHYSICO-CHEMICAL MODIFICATIONS
Corona formation
Adsorption of organic compounds
Modification of MP properties
Lipid digestion impairment
Interaction MPs/lipid droplets and lipases

MPs & STOOLS

20 MPs per 10 g of stool
50-500 µm diameter

WEATHERING

Modifies MP properties →
size, shape, hydrophobicity, salinity, crystallinity



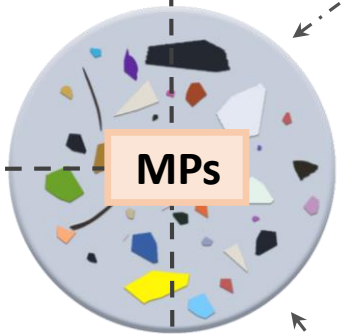
Influences the formation of a corona
onto the surface of MPs

VECTOR EFFECT

Physico-chemical interactions MPs/pollutants depending on
- Polymer/MP characteristics
- External factors: pH, salinity, temperature

CHEMICAL CONTAMINANTS

PAHs, organochloride pesticides, PCBs, PFASs

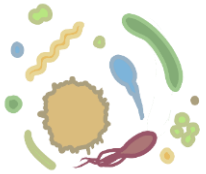


HEAVY METALS

Chromium, zinc, lead

MULTI-SPECIES BIOFILMS

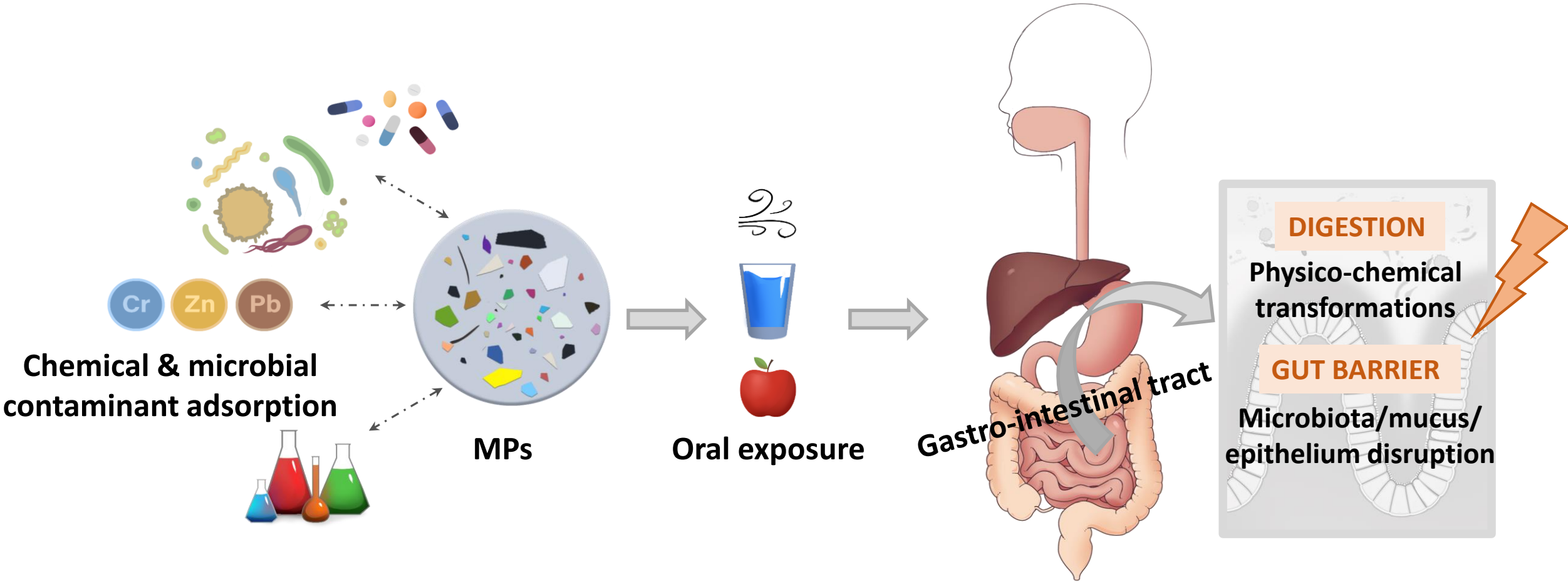
Potential pathogenic strains
Pseudomonas spp., *Vibrio* spp.,
Campylobacter spp., *Escherichia coli*



ANTIBIOTICS

Fluoroquinolones, tetracyclines,
sulfonamides, glycopeptide antibiotics

Higher prevalence of antibiotic-resistant bacteria


Cr
Zn
Pb
Chemical & microbial contaminant adsorption
MPs
Oral exposure
Gastro-intestinal tract
DIGESTION
Physico-chemical transformations
GUT BARRIER
Microbiota/mucus/ epithelium disruption